Role of volatile infochemicals in snail - periphyton interactions

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„Hinterher ist man immer schlauer“

Table of contents

Abstract

The fitness of consumers varies widely in aquatic and terrestrial habitats depending on resource quantity and quality. In aquatic food webs, herbivores are most affected by a variable resource quality, which is caused by considerable spatial and seasonal variations of nutrient availability in an ecosystem. Herbivores underlie a particularly high pressure in terms of their ability to acquire a sufficient nutrient supply in order to maintain high rates of growth and reproduction in heterogeneous environments. Particularly for organisms with limited motility such as gastropods, food searching is a very cost-intensive process. The effectiveness of food searching could be increased through the perception of diet-derived infochemicals that convey information about a food resource's quality over a certain distance. Chemical information transfer is a major agent in the regulation of interspecific and intraspecific interactions in natural ecosystems. The information transmission via chemical cues, like volatile organic compounds (VOCs) would clearly help to optimize foraging processes of herbivores and it would be adaptive for them to have efficient chemoreceptive mechanisms to locate food resources over distances. Despite the importance of the interaction of primary producers and grazers for the structure of a benthic natural system there is little knowledge about factors and mechanisms that allow the communication of these organisms.

This study aimed to elucidate important aspects and mechanisms of a snail-periphyton interaction, mediated by the transmission of volatile infochemicals from algae and this study highlights the natural relevance. As model organisms, the common pond snail *Lymnaea stagnalis* and the benthic green alga *Uronema/ Ulothrix fimbriata* were used for investigations like growth and behavioural assays but also for GC-MS analyses. I was able to show that the availability of essential macro-elements Nitrogen and Phosphorous in benthic algae lead to reduced fitness of juvenile *L. stagnalis* and additionally to qualitative and quantitative changes in the algal VOCs bouquet. The results of the behavioural assays revealed that VOCs extracted from *U. fimbriata* serve as foraging cues for *L. stagnalis.* Further, I was able to demonstrate for the first time that snails are able to differentiate between high and low quality food sources just by the perception of food odours released from benthic green algae after cell wounding. In field experiments I tested whether this foraging strategy is relevant on a larger spatial scale. The data of the field experiments showed that *L. stagnalis* is able to recognise algal odour bouquets as foraging infochemicals under natural conditions. Further findings gave also strong evidence that the feeding style of snails (radular cell damage) leads to VOC release under natural conditions because the grazing of *L. stagnalis* caused an aggregation of conspecifics under natural conditions. Furthermore, *L. stagnalis* appear to be able to distinguish between high and low quality food resources based on resource-quality specific odour bouquets under natural conditions. My results suggest that the perception of volatile cues is a process relevant on environmental scales and thus a possible mechanism to explain the frequently observed patchy distribution of grazers in ecosystems. The results of a VOCs liberation experiment demonstrated that gastropod grazing indeed leads to VOCs release. Further, I was able to show that a certain threshold of VOCs level is necessary for *L. stagnalis* which induces a directed foraging behaviour towards the odour. Finally, a mass balance model demonstrated that the grazer mediated VOC release is able to yield signal concentrations sufficient for the recognition by other lymnaeids which then utilize these cues as foraging infochemicals. The emission of ecologically relevant volatiles through snail grazing with subsequent attraction of other gastropod grazers to algal biofilms indicates an important but so far understudied chemical signalling mechanism of ecological importance.

Zusammenfassung

In aquatischen wie auch in terrestrischen Ökosystemen variiert die Fitness von Konsumenten in Abhängigkeit der Ressourcenquantität und -qualität. In aquatischen Nahrungsnetzen werden Herbivore besonders von variabler Ressourcenqualität beeinträchtig, die durch starke räumliche und saisonale Schwankungen der Nährstoffverfügbarkeit im Ökosystem verursacht wird. Herbivore Grazer unterliegen einem besonders hohen Druck, eine ausreichende Nährstoffversorgung für hohe Wachstums- und Reproduktionsraten in heterogenen Umgebungen sicherzustellen. Insbesondere für Grazer mit begrenzter Beweglichkeit, wie Gastropoden (Schnecken), ist die Nahrungssuche ein sehr kostenintensiver Prozess. Die Effektivität der Nahrungssuche von herbivoren Gastropoden könnte durch die Wahrnehmung von Signalsubstanzen erhöht werden, die nicht nur Auskunft geben über An- und Abwesenheit von Futter, sondern gleichzeitig die Futterqualität anzeigen. Die Übertragung von Informationen mittels chemischer Signale ist ein wichtiger Aspekt bei der Regulation von inter-und intraspezifischen Interaktionen in natürlichen Ökosystemen. Die Wahrnehmung nährstoffabhängiger Infochemikalien, zu denen flüchtige organische Verbindungen (volatile organic compounds, VOCs) gehören, wäre für Grazer hoch adaptiv und würde dazu führen, ihre Nahrungssuche deutlich zu optimieren. Trotz der Bedeutung der Interaktion von Primärproduzenten und Konsumenten und deren Einfluss auf die Struktur von aquatischen Ökosystemen gibt es kaum Wissen über Faktoren und Mechanismen, die diese Kommunikation ermöglichen.

Diese Studie untersucht wichtige Aspekte und Mechanismen einer durch Infochemikalien von Algen vermittelten Grazer/ Algen Interaktion und zeigt vor allem die ökologische Bedeutung dieser Prozesse. Als Modellorganismen wurden die Gemeine Spitzschlammschnecke (*Lymnaea stagnalis*) und die benthische Grünalge *Uronema/ Ulothrix fimbriata* für Untersuchungen zu Wachstum und Verhalten, sowie auch für die GC-MS-Analysen verwendet. Ich konnte nachweisen, dass eine geringe Nährstoffverfügbarkeit in benthischen Algen zu reduzierter Fitness von juvenilen *L. stagnalis* sowie zu quantitativen und qualitativen Veränderungen im Algen-VOCs-Bouquet führen. Die Ergebnisse der Verhaltenstests zeigten, dass aus *U. fimbriata* extrahierte VOCs von *L. stagnalis* als Fouragierkairomone wahrgenommen werden.

Desweiteren konnte ich zum ersten Mal beweisen, dass *L. stagnalis* in der Lage ist, zwischen VOCs aus Algen mit hoher und niedriger Qualität zu unterscheiden. Die Relevanz dieser Futtersuch-Strategie in größeren räumlichen Skalen wurde in Freilandversuchen getestet. Die Daten der Freilandversuche zeigten, dass *L. stagnalis* auch unter natürlichen Bedingungen in der Lage ist, VOCs aus Algen als Fouragierkairomone wahrzunehmen. Weitere Untersuchungen unter natürlichen Bedingungen stellten dar, dass die Beweidung eines Biofilms von *L. stagnalis,* Artgenossen herbeilockt. Das deutete stark darauf hin, dass der Fraßstil von *L. stagnalis* (raspeln mittels Radula verursacht Zelllyse) zur VOC-Freisetzung führt. Darüber hinaus ist *L. stagnalis* in der Lage, auch unter natürlichen Bedingungen Nahrungsressourcen von hoher und niedriger Qualität basierend auf ressourcenspezifischen Biofilmbouquets zu unterscheiden. Meine Ergebnisse deuten darauf hin, dass die Wahrnehmung von flüchtigen Signalen ein relevanter Prozess in Ökosystemen ist. Das könnte somit ein möglicher Mechanismus sein, um die häufig beobachtete heterogene Verteilung von herbivoren Grazern in Ökosystemen zu erklären. Die Ergebnisse von VOC-Freisetzungs-Experimenten zeigten, dass Grazing von *L. stagnalis* tatsächlich zur Freisetzung von VOCs führt. Weiterhin konnte ich den Schwellenwert von VOC-Konzentrationen bestimmen, der notwendig ist, um eine gerichtete Verhaltensreaktion von *L. stagnalis* in die Richtung der Signalquelle auszulösen. Schließlich konnte mittels eines Massenbilanz-Modells dargestellt werden, dass die grazing-induzierte VOC Emission ein Signal in ausreichender Konzentration freisetzt, welches andere Lymnaeide als Futtersignal wahrnehmen können. Die grazing vermittelte Emission von ökologisch relevanten flüchtigen Stoffen aus Algen die dann zu Anlockung von Artgenossen führt, deutet auf einen wichtigen, aber bisher wenig erforschten chemischen Signalmechanismus von ökologischer Bedeutung hin.

General introduction and aim of the study

Chemical communication plays a fundamental role for the regulation of inter- and intraspecific interactions of organisms and the understanding of chemical signal transfer is of great importance to explain the ecologically relevant interactions (Dicke and Sabelis 1988, Vos et al. 2006). Many substances can be active as infochemicals for example proteins, amino acids, or aldehydes, lipids and terpenes (Klaschka 2008). Infochemicals that affect interspecific interactions, are so called semiochemicals (behaviour-modifying chemicals) (Dicke and Sabelis 1988). Semiochemicals are divided into three categories: allomones, kairomones or synomones, the classification depends on the organisms which benefit from the interaction (Dicke and Sabelis 1988). One major group of semiochemicals that serves as chemical cues are volatile organic compounds (VOCs). VOCs are substances of low molecular weight which possess a low to medium hydrophilicity, which makes them functional as chemical cues in both aquatic and terrestrial environments (Fink 2007).

Numerous studies extensively investigated the ecological functions of VOCs in terrestrial ecosystems and their importance for interspecific interactions, especially plant herbivore/pollinator interactions (Laothawornkitkul et al. 2009, Figure 1). For example VOCs liberated from flowers are well known as floral scents. The primary ecological importance of floral scents is the extent to which it mediates interactions between flowers and their animal visitors (Dudareva and Pichersky 2010). Other VOCs released from leaves and roots have been reported to protect plant organs from infection by plant pathogens (Croft et al. 1993, Shiojiri et al. 2006) and thus maintain the fitness of plants. A very special infochemial-mediated interaction occurs among plants, insect herbivores and their natural enemies such as insect carnivores. Here plants emit VOCs as response to an herbivorous attack which recruits the natural enemies of the herbivores that damage the plant, thus facilitate an indirect plant defense strategy (Ode 2006, Arimura et al. 2009). These so-called tritrophic interactions are not restricted to aboveground plant parts, but occur also belowground. For example, insects that feed on maize roots initiate the liberation of volatiles, which attracts nematodes that prey on insect larvae (Rasmann et al. 2005). Finally, volatile emission does not only affect herbivores and pathogens but can also trigger defense responses in neighbouring plants (Baldwin et al. 2006). Plants are able to ´eavesdrop´ on VOCs liberated from herbivore-attacked neighbors to activate defenses before they are attacked themselves. This interaction might help to enhance the fitness of plants in natural communities.

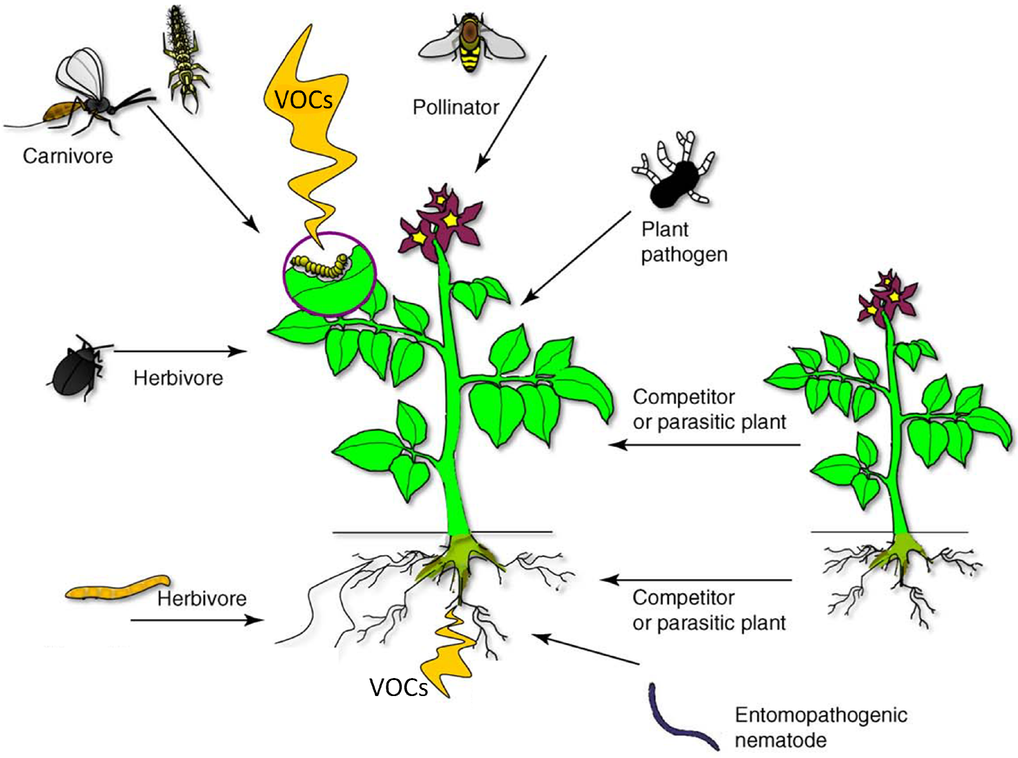

Figure 1: Volatiles released from plants above and below ground (modified after Dicke and Baldwin 2010)

Most common volatiles in higher plants are the so called green-leaf volatiles (GLVs) that are known to induce inter- and also intraspecific interactions. The name derives from the characteristic scent that is produced when green leaves are disrupted or injured (Hatanaka 1993). GLVs typically consist of a blend of saturated and unsaturated C_6 alcohols, aldehydes and esters. Intact and healthy plants usually release very small amounts of GLVs, but the GLV production increases dramatically after plant tissue is damaged by herbivory (Rose et al. 1996, Van den Boom et al. 2004). GLVs are so called oxylipins (oxygenase-derived derivatives of fatty acids) which are produced when the fatty acids within the membrane lipids are oxidized by the action of enzymes such as lipoxygenases (LOX, Andreou and Feussner 2009). The involved metabolic pathway is called oxylipin pathway. In higher plants, exclusively the C_{18} polyunsaturated fatty acids (PUFAs) linolenic and linoleic acid are used for the synthesis of GLVs (Blée 2002). Besides the lipoxygenase pathway, volatiles are also synthesised via the isoprenoid or the shikimic acid pathways (reviewed in Laothawornkitkul 2008). All these pathways have been relatively well studied in terrestrial plants but the biochemical regulation and function of most of these compounds are not clearly known.

In comparison to the interactions that have been found to be regulated by VOCs terrestrial habitats, there is only little knowledge about the role of volatile infochemicals in aquatic environments interactions (Fink 2007). One of the first volatile infochemical that was identified in an aquatic environment was the pheromone Ectocarpene, secreted from settled female gametes of the marine brown algae *Ectocarpus siliculosus* to attract motile male gamets (Müller et al. 1971). The most commonly studied example regarding infochemicals is an activated defence strategy of marine diatoms against herbivorous zooplankton. This interaction gained special attention, because diatoms provide the bulk of the food resources in oceans that sustains the marine food chain to the top predator. It was reported that copepods that extensively fed on diatoms possessed a high egg production but the hatching rate was really low even though the diatoms were rich in proteins, vitamins and essential fatty acids (Ianora and Poulet 1993). In 1999 researchers could identify PUAs from the diatom *Thalassiosira rotula* and demonstrated that they inhibited embryonic development of copepods and sea urchin embryos (Miralto et al. 1999). Due to the production of cytotoxic volatiles, diatoms potentially sabotage the future generations of their predators by the inhibition of the growth of the copepod population. Similar compounds were earlier isolated from freshwater diatoms (Wendel and Jüttner 1996) but the biological function of these VOCs was unknown at this time. Later, it could be demonstrated that the volatiles released from the freshwater diatoms are not only active in the regulation of defense mechanisms but can be instead attractive to gastropod grazers (*Radix ovata*) and serve as foraging cues (Fink et al. 2006a). These freshwater pulmonates were also shown to use algal VOCs bouquets liberated from damaged benthic green algae as food-finding signals (Fink et al. 2006b). For the green algae it was shown that multicomponent odour is necessary to elicit the foraging behaviour of the gastropods whereas single components of the VOC bouquet induced no behavioural response (Fink et al. 2006b).

Other studies emphasised the beneficial role of volatiles from cyanobacterial biofilms as habitat finding cues for freshwater nematodes (Höckelmann et al. 2004) and aquatic insects (Evans 1982). Cyanobacterial biomats which are known to produce a variety of volatile odours (Jüttner 1984) serve in this interaction as habitat and offer shelter and food for the free living organisms. Since cyanobacteria are known to emit a broad variety of volatiles other aquatic organisms also use these infochemicals most likely for interaspecific interactions. Thus, volatile semiochemicals play also important and different roles in structuring diverse interspecific interactions in aquatic food webs.

Typically these volatiles liberated from algae and cyanobacteria are produced via oxylipin or carotene oxidase pathways in both marine and freshwater systems (Jüttner 1995, Pohnert and Boland 2002). Several studies investigated the biosynthesis of oxylipins in marine diatoms (reviewed in Pohnert 2005) and showed that volatile PUAs are, like in higher plants, break-down products from oxidative transformation of polyunsaturated fatty acids (PUFAs). In diatoms glycoclipids (D'Ippolito et al. 2004, Cutignano et al. 2006) and phospholipids (Pohnert 2002, Fig. 2) are efficiently cleaved upon cell damage via lipolytic activity. This involves lipoxygenase (LOX) and hydroperoxide lyase and thereby the transformation of PUFAs is induced (Pohnert 2005). The free C_{16} and the C_{20} PUFAs eicosapentaenoic (EPA) and arachidonic acid serve as precursors and are converted by lipoxygenases and lyases to PUAs to produce volatile aldehydes (D'Ippolito et al. 2004, Ianora and Miralto 2010). The synthesis of aldehydes and other oxylipins in marine diatoms is caused by cell damage and that leads to mixing of substrates and enzymes (Cutignano et al. 2006). Beside diatoms, it was also shown for the benthic green alga *U. fimbriata* to liberate a multicomponent odour mix of C_5 and C_7 lypoxygenase products and a variety of volatile nor-carotenoids after cell damage (Fink et al. 2006b). Due to high diversity of primary producers many substances and pathways provide the option to release varying and ecological relevant infochemicals into the surrounding that could give information about the presence of food resources.

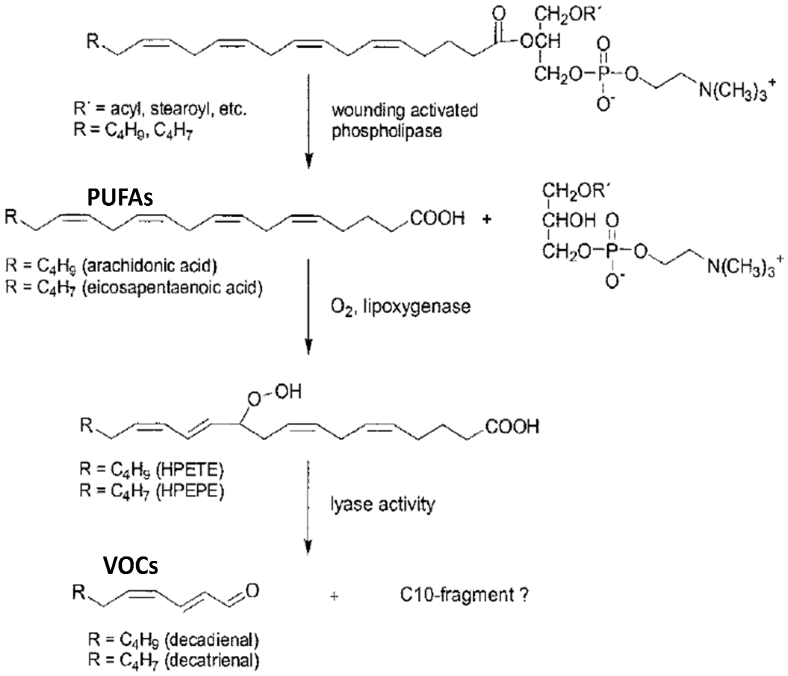

Figure 2: Proposal of the biosynthesis of volatiles of *T. rotula*. The hydrolysis of EPA from phospholipids enables the release of volatiles from diatoms (modified from Pohnert 2002).

The quantity and the quality of food resources are decisive for the fitness of consumers and both can vary widely in aquatic and terrestrial habitats. The consequences of the uptake of low quality resources are reduced growth and reproduction, as it has been shown for several invertebrates (Trichilo and Leigh 1988, Stelzer and Lamberti 2002, Stiling and Moon 2005). The resource quality depends on different factors (Sperfeld et al. 2012), in particular on the availability of the essential macro-elements nitrogen (N) and phosphorous (P), related to carbon (C) as energy source (Sterner and Elser 2002). The balance of these elements in living systems is expressed as C:N:P stoichiometry. In this context, several studies reported that resource quality was even more important than food quantity (Boersma and Kreutzer 2002, Fink and von Elert 2006). Usually, there is a heterogeneous nutrient availability in both aquatic and terrestrial ecosystems because the N and P content of food resources can vary both spatially and seasonally (Elser et al. 2000). Such heterogeneity in nutrient content (C:N:P ratio) of resources can lead to reduced fitness of consumers (Elser et al. 2000). However, most animals are homeostatic and maintain constant body elemental composition actively even while externally imposed variation (Sterner and Elser 2002). Thus, animals perform best when the biochemical composition of the food is completely balanced with respect to the animal's requirements. Herbivores are homeostatic, while primary producers typically show a flexible stoichiometry (Persson et al. 2010). Therefore, especially herbivores highly need a balanced supply of nutrients in order to maintain growth and reproduction in heterogeneous environments (Sterner and Elser 2002). For herbivores like snails with low motility, food searching is even more cost-intensive because they move slow and their locomotion is based on secretion of a polysaccharide mucus (Lauga and Hosoi 2006). Therefore, it would be highly adaptive for such slowly moving consumers to be able to identify and locate high-quality resources over certain distances by perceiving food quality dependent signals like VOCs.

Therefore I hypothesised in **chapter I**, whether herbivorous freshwater gastropods are able to use VOCs not only as food finding signals (Fink et al. 2006b) but also as indicators for resource quality.

As model organism the great pond snail *Lymnaea stagnalis* (L.) was chosen. It is a common benthic grazer and inhabits a wide range of freshwater habitats and was shown to feed on filamentous algae (Bovbjerg 1968). By manipulating the nutrient content of the filamentous green alga *Uronema/ Ulothrix fimbriata,* I aimed to obtain a food resource of defined algal food quality. In a laboratory growth experiment, I investigated the impact of nutrient content of the algal food on the fitness of juvenile pond snails. Using behavioural assays in the laboratory, I examined in **chapter I** the foraging behaviour of *L. stagnalis* towards VOCs released from *U. fimbriata* grown under high and low nutrient availability.

Several studies on natural ecosystems documented that heterogeneous resource availability plays a major role in the spatial variation of animal abundance (Wiens 1976, McNaughton 1988, Morgan et al. 1997). The patchiness at both levels of primary producers and herbivores is believed to be a major cause for non-equilibrium processes in trophic interactions, though the mechanisms leading to heterogeneity at the consumer level are poorly understood. To investigate and to quantitatively assess behavioural responses of consumers to changes in resources that lead to their patchy distribution, movement models have been developed (Focardi et al. 1996, Farnsworth 1998). Theoretical considerations already predicted directed walks (Fronhofer et al. 2013), but mechanisms that could explain such directed movements are rare.

Since many species live in patchy environments where food resources are often heterogeneously distributed, it would be adaptive for them to have efficient chemoreceptive mechanisms to locate food resources over distances. The recognition of such diet derived infochemicals could lead to a directed foraging behaviour. A directed movement of consumers through the heterogeneous landscape based on behavioural decisions would be advantageous in comparison to random movement. In contrast, random searching for suitable food patches would require increased activity, which causes costs and leads to reduced fitness. In the laboratory experiments on the freshwater grazer *L. stagnalis* (**chapter I**), it was demonstrated that these freshwater gastropods are attracted by algal VOCs, but the environmental relevance under natural conditions of this chemically mediated interaction was not yet clear. From previous studies it was known that algal cells release VOCs after cell wounding (Fink et al. 2006a, b). But it was not clear how volatiles could be released under natural conditions.

Therefore I hypothesised in **chapter I** that detection of algal foraging cues should be relevant on larger spatial scales and that the grazing of *L. stagnalis*, which is based on radular cell damage, could lead to the release of volatile lipoxygenase products. *L. stagnilis* recognise the algal volatiles as foraging cues and that leads to attraction of conspecifics and similarly to a patchy distribution pattern. In **chapter II** I analysed in field experiments the foraging behaviour of *L. stagnalis* to volatiles and tested whether snails´ grazing on natural biofilm leads to attraction of conspecifics which would indicate that volatiles are released.

In a further experimental approach within **chapter II**, I analysed the adaptive behavioural response of snails to conspecifics that graze simultaneously on high or low quality biofilm to see if the snails recognise volatiles as resource quality indicators on a large spatial scale.

From previous studies it is known that cell disruption leads to the release of algal volatiles. However, until now it is not yet clear how the cell disruption occurs that leads to algal VOCs release under natural conditions. Interestingly, the feeding mode of aquatic gastropods involves the rasping of substrate-attached algal biofilms via their radular tongue, which most likely causes massive algal cell damage during the snails’ ingestion process that lead to the liberation of volatiles. During movement, gastropods could then compare the intensity of chemical stimuli in their proximity and use the concentration gradients for orientation (Chase 1982). Therefore, it was reasonable to hypothesise that feeding style of the grazers leads to cell disruption in biofilm algae. The cell damage leads to the activation of the enzyme cascade that causes the liberation of VOCs and subsequently to the attraction of other gastropod grazers from the surroundings. Probably a certain concentration of volatiles is necessary to induce foraging behaviour of the snails.

Therefore I hypothesized in **chapter III**, that while *L. stagnalis* scrapes over the substrate, algal cells are damaged and this initiate the formation of VOCs. Further I hypothesized that a certain threshold concentration of VOCs is necessary to initiate a directed foraging behaviour of *L. stagnalis* towards a VOC source. The threshold value is the concentration level above which an odorous signal can be perceived and/or a response measured (Lawless and Heymann 1999).

To investigate these hypotheses, I conducted experiments in the laboratory and tested whether grazing of the freshwater gastropod *L. stagnalis* on benthic algae causes the release of VOCs **(chapter III)**. To determine the threshold concentration of volatiles I analysed the behavioural response of *L. stagnalis* towards a gradient of different VOC concentrations.

Using experimental data sets collected within **chapter I-III,** I developed a simple mass balance model to evaluate that grazing-mediated cell disruption can cause the liberation of sufficient amounts of VOCs to elicit foraging behaviour in freshwater gastropods.

With the present study I aim to give new insights in the functions and mechanisms that are involved in the infochemical-mediated interaction of primary producers and consumers. Additionally I want to demonstrate that the emission of volatiles is an important chemical signaling mechanism of ecological importance.

Chapter I:
The smell of good food: volatile infochemicals as resource quality indicator

Summary

1. Foraging success generally depends on various environmental and physiological factors. Particularly for organisms with limited motility such as gastropods, food searching is a very cost-intensive process. As energy gain through foraging is dependent on both resource quality and quantity, consumers have to be able to differentiate between varying resource items.
2. The effectiveness of food searching could be increased through the perception of diet-derived chemical signals that convey information about a food resource’s quality over a certain distance. This strategy would clearly help to optimize movement decisions.
3. In this study, we investigated the foraging behaviour of a freshwater gastropod towards volatile signal substances released from benthic algae grown under high and low nutrient availability, representing high and low food quality, using behavioural assays in the laboratory.
4. Our results demonstrate that volatile organic compounds (VOCs) serve as foraging kairomones for these aquatic, benthic herbivores. Further, we were able to show for the first time that snails are able to differentiate between high and low quality food sources only by the perception of food odours alone (volatile infochemicals)**.**
5. Gas chromatography coupled with mass spectrometry demonstrated quantitative as well as qualitative differences in the chemical composition of the VOC’s bouquet, dependent on algal nutrient content.
6. Our results suggest that the recognition of resource quality via the reception of signal substances is likely to be adaptive for consumers with low mobility to maximize ingestion of high quality resources.

Introduction

The fitness of consumers varies widely in aquatic and terrestrial habitats depending on resource quantity and quality. Frequently, resource quality was found to be even more important than food quantity (Boersma & Kreutzer 2002; Fink & von Elert 2006). The ingestion of low quality resources leads to reduced growth, reproduction and altered foraging behaviour, as has been shown for several invertebrates (Trichilo & Leigh 1988; Stelzer &Lamberti 2002; Stiling & Moon 2005). Multiple factors have been found to determine resource quality (Sperfeld, Martin-Creuzburg & Wacker

2012) in particular the availability of the essential macro-elements nitrogen (N) and phosphorous (P), related to carbon (C) as energy source (Sterner & Elser 2002). The balance of these elements in living systems is expressed as C:N:P stoichiometry. The N and P content of food resources can vary both spatially and seasonally, leading to a heterogeneous nutrient availability for consumers in both aquatic and terrestrial ecosystems (Elser *et al.* 2000). Heterogeneity in nutrient content (C:N:P ratio) of resources can lead to nutritional constraints on consumer fitness (Elser *et al.* 2000). This is of particular importance for herbivores, as they maintain a homeostatic regulation of body elemental composition, while primary producers typically show a flexible stoichiometry (Persson et al. 2010). Herbivores thus underlie a particularly high pressure in terms of their ability to acquire a balanced supply of nutrients in order to maintain high rates of growth and reproduction in heterogeneous environments (Sterner & Elser 2002).Hence, the ability to locate food from distance based on environmental cues and to move towards high-quality food resources in a habitat with patchy resource quality distribution would be highly advantageous (Fronhofer, Hovestadt & Poethke 2013). This is particularly true for homeostatic gastropod grazers, as they are characterized by a slow, cost-intensive locomotion based on the secretion of a polysaccharide mucus (Denny 1980). Information about the quality of food resources could be transmitted via chemical signals similar to pollinator attraction by plant chemical signals (reviewed by Raguso, 2009). The most common signals in terrestrial ecosystems are volatile organic compounds (VOCs) (Baldwin *et al.* 2006; Gershenzon 2007). VOCs are substances of low molecular weight which possess a low to medium water solubility, which makes them accessible as chemical cues in both aquatic and terrestrial environments (Fink 2007). Decades of research has focused on the ecological functions of VOCs in terrestrial ecosystems (e.g. Kessler & Baldwin 2001; Baldwin *et al.* 2006;Kessler & Halitschke 2009). However, until recently, only very few studies have investigated the ecological functions of VOCs in aquatic environments (Fink 2007). For instance, wounded microalgal cells are known to liberate volatile lipoxygenase products which are involved in an activated chemical defense strategy against grazers (Miralto *et al.* 1999; Pohnert & Boland 2002;Ianora *et al.* 2004). Freshwater gastropods were demonstrated to utilize VOC mixtures liberated from damaged cells of both diatoms (*Achnanthes biasolettiana*) and green algae (*Ulothrix fimbriata*) as food-finding signals (Fink, von Elert & Jüttner 2006a; Fink, von Elert & Jüttner 2006b). It would be highly adaptive for grazers to not only be able to detect the presence of food sources but quality differences between them via olfactory cues.

The goal of our study therefore was to investigate whether herbivorous freshwater gastropods are able to perceive VOCs as indicators of resource quality. The common pond snail *Lymnaea stagnalis* (L.) which feeds on filamentous algae (Bovbjerg 1968) was chosen as a model consumer species. By manipulating the nutrient content of the benthic green alga *U. fimbriata* in continuous cultures, we created a defined resource for the assessment of algal food quality in a growth experiment with juvenile pond snails. In these experiments, the pond snails' behavioural response to VOCs released from these algae with different nutrient content was investigated. Specifically, we addressed the following hypotheses: 1) The somatic growth rate of juvenile *L. stagnalis* is dependent on the C:N:P stoichiometry of the resource. 2) The snails are able to perceive volatile signal released from benthic algae. 3) Nutrient limitation in terms of P and N changes the algal VOC bouquet and therefore 4) provokes an adaptive behavioural response of the gastropods towards VOCs from high quality resources.

Materials and methods

Cultures

Juveniles of the freshwater gastropod *L. stagnalis* were hatched and reared from eggs laid by adult individuals originally collected in a pond in Appeldorn, Germany, and kept in a climate chamber at 20 ± 0.5° C under constant dim light in aerated tap water. The snails were fed Tetra PlecoMin™ fish food pellets (Tetra, Melle, Germany) *ad libitum*. The filamentous green alga *U. fimbriata* (strain SAG 36.86 from the Göttingen Algal Culture Collection, SAG) was continuously cultivated in chemostats on Cyano medium (Von Elert & Jüttner 1997) with a light (PAR) intensity of 80 µmol photons s^{-1} m^{-2}. Cyano medium was originally designed for cyanobacteria and is thus particularly rich in dissolved phosphorus (400 µM K_2PO_4) and nitrogen (8000 µM $NaNO_3$). Nutrient-limited algae were obtained by culturing *U. fimbriata* on Cyano medium with either reduced phosphorus (8 µM K_2PO_4) or reduced nitrogen (400 µM $NaNO_3$) concentrations. The different algal nutrient treatments will be further referred to as follows: Nutrient-limited algae reduced in P (-P+N) or in N (+P-N), nutrient saturated treatment (+P+N). Carbon concentrations of the algal suspensions were estimated from photometric light extinction at 480 nm using carbon-extinction equations.

Elemental analyses

For nutrient analyses, aliquots of the respective *U. fimbriata* cultures were filtered on pre-combusted glass fibre filters (Whatman GF/F, 25 mm Ø). Filters for C/N analysis were dried at 60℃ for 24 h and packed in tin capsules (HekaTech) for subsequent analysis using a Thermo Flash EA 2000 Analyser. For analysis of the particulate phosphorus, the filters were directly transferred into a solution of potassium peroxodisulfate and 1.5% sodium hydroxide and then autoclaved for 60 min at 120℃. Subsequently, soluble reactive phosphorus was analysed using the molybdate-ascorbic acid method (Greenberg, Trussel & Clesceri 1985). For C:N:P analysis of the algae, three aliquots per sampling day of each algal culture were filtered and analysed. For the nutrient analyses of the snails, soft bodies were extracted from the shells under a dissecting microscope, dried at 60℃, ground to a powder and analysed as described above. The C:N:P ratios of the experimental snails were calculated for each single snail. In the case of the gastropods fed with P-limited algae, the individual soft-body dry masses were too low for be analysed separately. Hence, replicates had to be pooled to yield enough biomass for C/N analysis.

Growth experiment

The initial shell length (i.e. the distance from the apex to the most distal part of the shell's aperture) of a cohort of approx. 10-day-old *L. stagnalis* juveniles was determined to the nearest 0.02 mm using a calliper. In the growth experiment, the snails were kept individually in glass containers with 200 ml of aerated tap water at 20 ± 0.5℃ under dim light. The experiment consisted of three treatments (+P+N, -P+N, +P-N) with eleven replicates each and a food biomass equivalent to 2 mg particulate organic carbon per individual. Water and food were renewed daily. The snails were transferred to new containers every other day to avoid accumulation of biofilm and waste products. On day 33, the snails fed +P+N algae consumed almost 100 % of the provided food; the experiment was then terminated in order to avoid a growth limitation caused by food quantity. At the end of the experiment, the snails' shell length was measured and the soft-bodies were removed from the shells under a dissecting microscope, frozen at -80℃ and subsequently freeze dried to determine the softbody dry mass (dm). Because the initial softbody dry mass of the snails in the experiment could not be determined directly, the lengths of 20 juvenile snails within the same size range as the experimental animals were measured; the snails were then removed from their shells and their soft bodies were freeze dried. The dry mass was determined with a microbalance (Mettler UTM2) to the nearest microgram. A regression curve was calculated with the soft body mass and shell length data. Using the measured shell lengths and the regression function, the initial soft body mass of the snails at the beginning if the experiment could be estimated. The dry masses of the animals on day 1 and day 33were used to calculate the somatic growth rate. The somatic growth rate [d^{-1}]was calculated as:

$$g = \frac{\ln dm_{final} - \ln dm_{start}}{t[d]}$$

where dm_{start} is the estimated dry mass (calculated from the regression function) of the animals at the beginning of the experiment and dm_{final} is that of the juvenile snails after 33 days (t = 33).

Food choice assays

Algal VOCs for food choice assays were extracted from batch cultures of *U. fimbriata* as described by Fink, von Elert and Jüttner (2006b). To initiate the release of VOCs, an algal biomass equivalent to 10 mg particulate organic carbon of *U. fimbriata* was extracted with 25 % sodium chloride by closed-loop stripping for 45 min and adsorbed onto Tenax TA (Chrompack, 150 mg/tube) as described by Jüttner(1988). The VOC extract was obtained as described by Fink, von Elert and Jüttner (2006b) by eluting the adsorbent material with diethyl ether. The ether was carefully evaporated to dryness under a gentle stream of nitrogen gas and the residue directly re-dissolved in 100 µl ethanol. Control samples were prepared in the same way (25 % sodium chloride in 40 ml ultrapure water) but without algae. Sodium chloride and all solvents were analytical grade and were obtained from VWR, Darmstadt, Germany.

The food-choice assay was based on the setup developed by Fink *et al*. (2006b). Prior to the food choice experiments, the juvenile snails were starved for 24 hours to increase their food searching motivation. The food choice assays were conducted in aquaria (320 x 170 x 180 mm) filled with 1 L of filtered (0.2 µm) and aged tap water. To release VOCs from a defined position, two containers (Fink *et al*. 2006b) were placed at opposite sides of each aquarium. The samples (VOC extracts from *U. fimbriata* +P+N, -P+N, or +P-N) and the extraction control were each dissolved in 7 ml of filtered tap water, gently filled into the respective containers and released by opening the containers. The position of the extraction control and VOC container was regularly exchanged between replicate assays toavoid a directional bias. In a series of control assays (n = 12), both containers were filled with filtered tap water only.

At the beginning of the experiment, five individually labelled *L. stagnalis* of equal size (15 ± 5 mm) were placed into the centre of the aquarium. The snails were given five minutes to acclimate to the experimental setup before the experiment was started by opening the VOC source containers. The position of the snails was then recorded every minute using a coordinate system placed beneath the aquarium. The coordinate system was composed of concentrical rings (1 cm apart from each other) starting from two VOC source containers at the opposite ends of the aquarium (score 0). Hence, the position of each individual snail could be determined as the relative distance to both VOC point sources at any time point (see supplementary figure S1). The position reading of all snails at the start of the experiment was 0 due to the equal distance to both containers. The snails' position readings ranged from -27 cm (closest to the VOCs source 1) to +27 cm (closest to the VOCs source 2). The position of each snail was recorded every 60 seconds for 45 minutes in each assay; from these 45 minutes, the interval from 5 to 35 minutes was defined as the active search phase based on preliminary experiments. To avoid pseudoreplication, the mean of the relative distances of the five individuals from one assay was considered as one replicate. Since the relative preference for VOCs from algae with high or low nutrient (P or N) content was to be determined in the further assays, a gastropod preference index (GPI) was calculated as:

$$\mathrm{GPI} = \frac{\Delta_{(\mathrm{VOC\ source\ or\ control})}\ [\mathrm{cm}]}{\Delta_{\mathrm{total}}[\mathrm{cm}]}$$

where $\Delta_{(\text{VOC source or control})}$ is the mean sum of the position readings in the respective half of the choice arena, Δ_{total} is the total sum of the position readings from both sides of the choice arena. The GPI thus gives the proportion of snail positions in the respective half of the aquarium. For example, a GPI of 0.8 for the +P+N VOC extract treatment indicates that 80 % of the snail positions were recorded on the side with the +P+N VOCs, whereas 20 % of the positions were recorded in the half with the +P-N VOCs.Hence, observed GPIs range from -1 (maximum preference for VOCs from nutrient depleted algae) to +1 (maximum preference for VOCs extract from nutrient saturated *U. fimbriata*).

VOCs Analyses

VOCs were extracted from chemostat cultures of *U. fimbriata* by closed-loop stripping as described above. VOCs were then thermally desorbed from Tenax TA (Jüttner 1988) and directly transferred onto a capillary column (DB 1301, 30-m length, 0.250 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA) of an Agilent 7890A gas chromatograph combined with an Agilent 5975C single quadrupole mass spectrometer (Agilent, Waldbronn, Germany). Helium was used as the transfer and carrier gas. VOCs produced by *U. fimbriata* were separated with the temperature program 4 min at 0℃, 3° C min $^{-1}$ to 45℃, 30℃ min $^{-1}$ to 95℃, 10℃ min^{-1} to 105℃, 30℃ min $^{-1}$ to 165℃,5℃ min $^{-1}$ to 180℃, and 5 min at 230℃, and were identified by comparing the retention times and mass spectra (EI at 70 eV) with those of reference compounds (Aldrich). The compounds were quantified by using calibration curves previously determined for each compound using 3-hexanone as internal standard and compound-specific target ions. Only the four compounds 1-penten-3-ol, 1-penten-3-one, trans-2-pentenal, and E,E-2,4-heptadienal could be calibrated directly. Since the cis isomer of 2-pentenal and the E,Z isomer of 2,4-heptadienal were not available, the quantification of these two compounds was done using the calibration of the respective trans isomers.

Statistical Analyses

Prior to using parametric tests, all data were checked for normal distribution using the Shapiro-Wilks test and for homoscedasticity using Levene’s test. The growth rates of the snails kept under different food regimes were tested for differences in growth rate using one-way analysis of variance (ANOVA) with snail soft body growth rate as the dependent variable and the algal nutrient treatment as the predictor variable followed by post-hoc comparison with Tukey`s honest significant difference test (HSD) for unequal n. Not every animal survived until the end of the experiment, thus an unequal number of replicates were obtained for the different treatments. The C:N:P ratios of the samples (snails and *U. fimbriata*) were log (x) transformed to ensure homoscedasticity prior to one-way ANOVA followed by Tukey´s HSD, with the C:N:P ratio as dependent variable. The gastropods' mean relative positions in the behavioural assays were analysed via a repeated-measurement ANOVA with the mean relative position of the five individuals per assay as the dependent variable, the food nutrient treatment as the fixed factor and reading time as random factor (repeated measurement). To analyse the gastropod preference indices (GPI) of the respective VOC treatments, GPI values were arc-sin square root transformed (Underwood 1997). We statically tested the transformed GPIs of the corresponding replicates in one half of the choice arena versus the GPIs in the other half with a one-way ANOVA with GPI as the dependent variable and the food nutrient treatment as fixed factor.

The total amounts of VOCs released from the different *U. fimbriata* cultures were compared via a one-way ANOVA. Due to heteroscedasticity of the data, a non-parametric Kruskal- Wallis ANOVA on ranks followed by Dunn's post-hoc test was used to assess the differences between the amounts of each single VOC released from nutrient-saturated and nutrient depleted *U. fimbriata*. All statistical analyses were performed using Sigma Plot® v. 11 (SysStat), except for the repeated-measures ANOVA, which was calculated using Statistica® v.10 (StatSoft) and a significance level of 0.05.

Results

Somatic growth rate

The somatic growth rate of juvenile *L. stagnalis* fed +P+N algae was significantly higher than the growth rate of snails fed with algae depleted in either P or N ($F_{2,24}$ = 172.8, $p < 0.005$, Fig. 1). Snails grown on -P+N and +P-N algae had a six-fold (-P+N *U. fimbriata*) or three-fold (+P-N *U. fimbriata*) lower growth rate than snails fed nutrient saturated algae (Fig. 1).

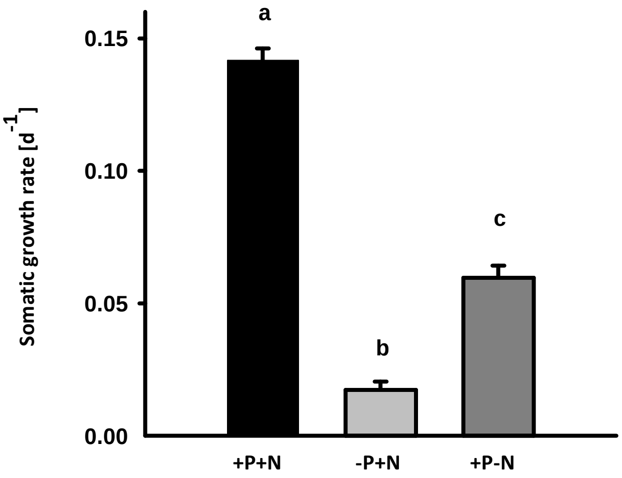

Figure 1: Somatic growth rate (mean ± SE of n(+P+N) = 9, n(-P+N) = 6, n(+P-N) = 11) of *L. stagnalis* in the laboratory growth experiment. Different letters indicate significant differences between treatments.

C:N:P ratios of U. fimbriata and L. stagnalis

An analysis of variance (ANOVA) of the C:N:P ratios of the *U. fimbriata* cultures confirmed that the P and N availability in the culture medium strongly influenced algal stoichiometry ($F_{2,6}$ = 1128.09, $p < 0.05$, Fig. 2). Algae grown under P-depletion had higher C:P and N:P ratios than algae grown at sufficient P supply in the growth medium. The C:N ratio of *U. fimbriata* grown on +P-N medium was significantly higher than in the other two algal cultures. When juvenile *L. stagnalis* were fed either nutrient-saturated or nutrient-depleted *U. fimbriata*, their soft body N:P ratio was not affected ($F_{2,12}$ = 1.73, $p = 0.218$, Fig. 2). The C:N ratio of gastropods fed with +P-N algae was slightly but significantly higher than the C:N ratio of snails fed nutrient-rich or P-limited algae ($F_{2,12}$ = 18.54, $p < 0.05$), and the C:P ratio of soft bodies was lower in snails fed +P-N algae ($F_{2,12}$ = 4.32, $p < 0.05$) than in snails fed nutrient saturated or –P+N algae.

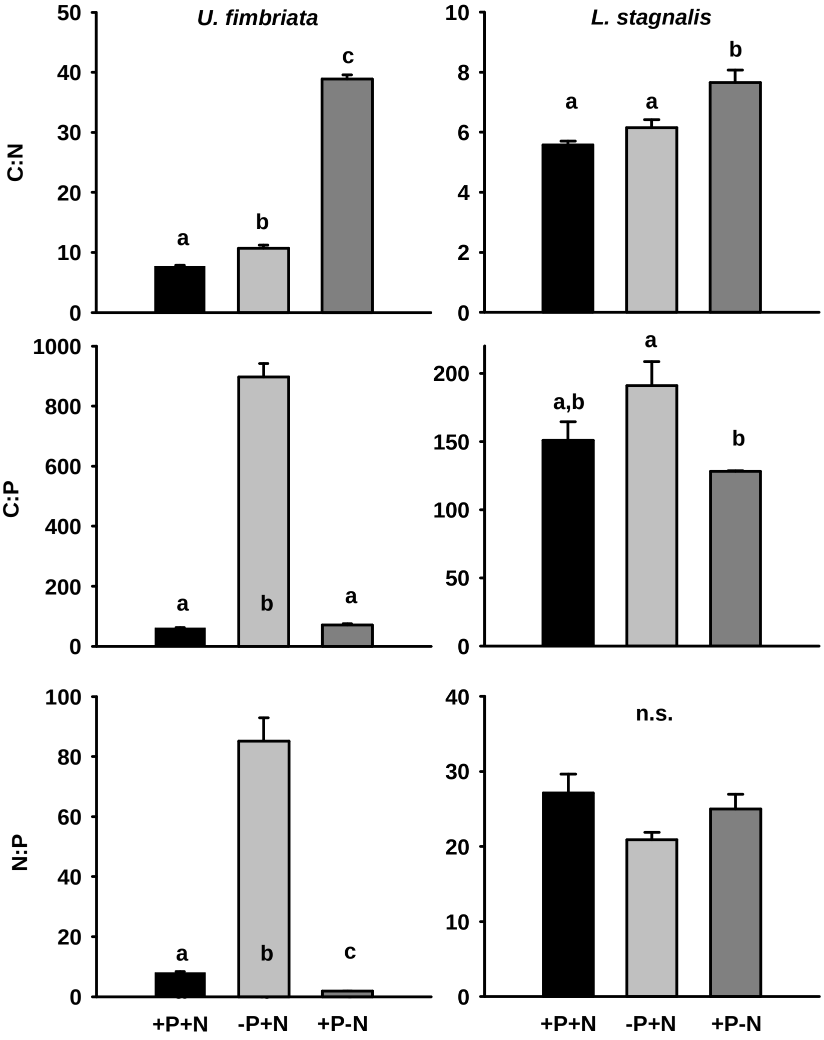

Figure 2:C:N:P ratios (mean ±SE) of *U. fimbriata* (n = 3) and *L. stagnalis* ($n_{(+P+N)}$= 7, $n_{(-P+N)}$ = 5 and $n_{(+P-N)}$ = 3) grown under different nutrient regimes; note different scaling of the y-axes; different letters indicate significant differences.

Food choice experiments

Control assays without chemical stimuli on either side of the aquarium confirmed that there was no directional bias, i.e. no preference for either side could be observed (Fig

3). In bioassays in which algal VOC extracts were applied, i.e. when snails could choose between a VOC extract from *U. fimbriata* and a control extract, juvenile *L. stagnalis* showed a clear chemical attraction towards the source of algal VOCs (Fig. 3). This preference for algal VOCs was demonstrated by a significantly higher mean relative position of the gastropods towards the VOC source in the active searching phase (repeated measures ANOVA, $F_{1,21}$= 5.12, $p < 0.05$) than towards the control source.

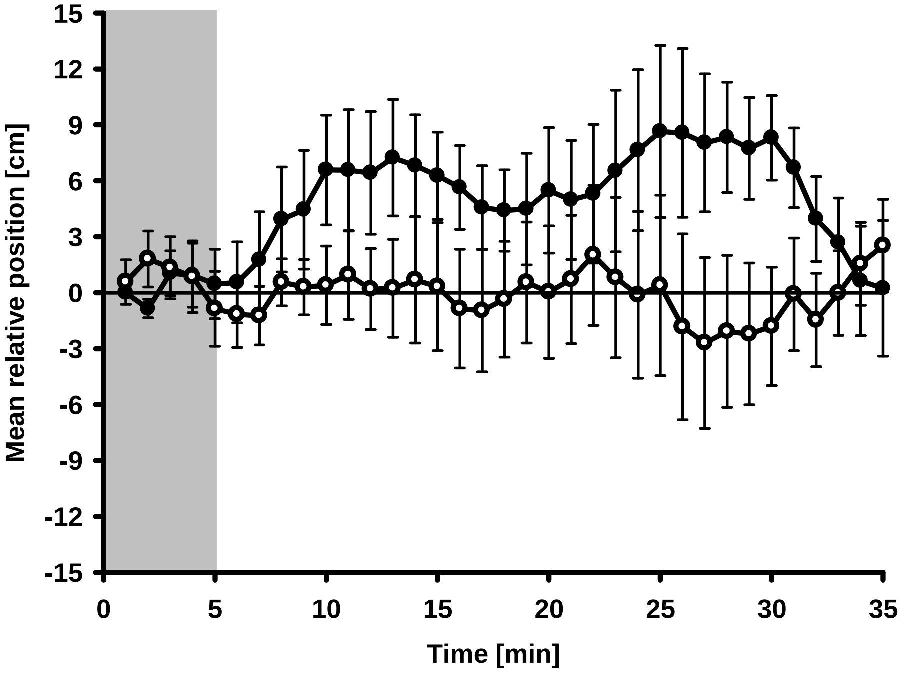

Figure 3: Mean relative position (± SE) of *L. stagnalis* to the VOC source containers in the food choice assays; test extracts (filled circles): VOCs of disrupted *U. fimbriata* cells (n = 11); control assays (open circles): both containers filled with water (n = 12); the non-shaded area indicates the phase of active search behaviour.

VOC analyses

All of the main VOC components previously reported to occur in *U. fimbriata* by Fink et al. (2006b) were found both in nutrient-saturated as well as in nutrient-depleted algae (Tab. 1).

Table 1: Results of Kruskal-Wallis one-way analyses of variance on ranks (followed by post-hoc comparisons using Dunn's test) of the mean (of n=7) amounts of the five major VOCs released from nutrient-saturated, P- and N-depleted *U. fimbriata*. Different letters indicate significant differences at p<0.05.

	H	df	+P+N	-P+N	+P-N
1-penten-3-one	15.348	2	a	b	b
1-penten-3-ol	17.121	2	a	b	a,b
cis-2-pentenal	16.196	2	a	b	a,b
(E,Z)-2.4-heptadienal	11.143	2	a	b	b
(E,E)-2.4-heptadienal	16.794	2	a	b	a,b

However, both the total quantity and the relative composition of VOCs were strongly influenced by the algal nutrient content. The total amounts of VOCs released from nutrient- saturated algae were significantly higher than the quantity of VOCs released from P- and N-limited *U. fimbriata* (ANOVA, $F_{2,18}$= 522.94, p < 0.0001, Tab. 1, Fig. 4). In total, P- and N-limited algae released 18-fold and 30-fold lower amounts of VOCs, respectively, than nutrient-saturated algae. Nutrient-limited algae released reduced amounts of C_5 components (1-penten-3-one,1-penten-3-ol,cis-2-pentenal,trans-2-pentenal) in comparison to measured C_5 VOC amounts from nutrient-saturated algae. The VOC component trans-2-pentenal could not be quantified in nutrient-depleted *U. fimbriata* because the detected amounts were below the limit of quantification. In contrast, algae limited in N and P released proportionally higher amounts of the C_7 compounds (E,Z)-2.4-heptadienaland (E,E)-2.4-heptadienal than nutrient-saturated *U. fimbriata* did (Fig. 4).

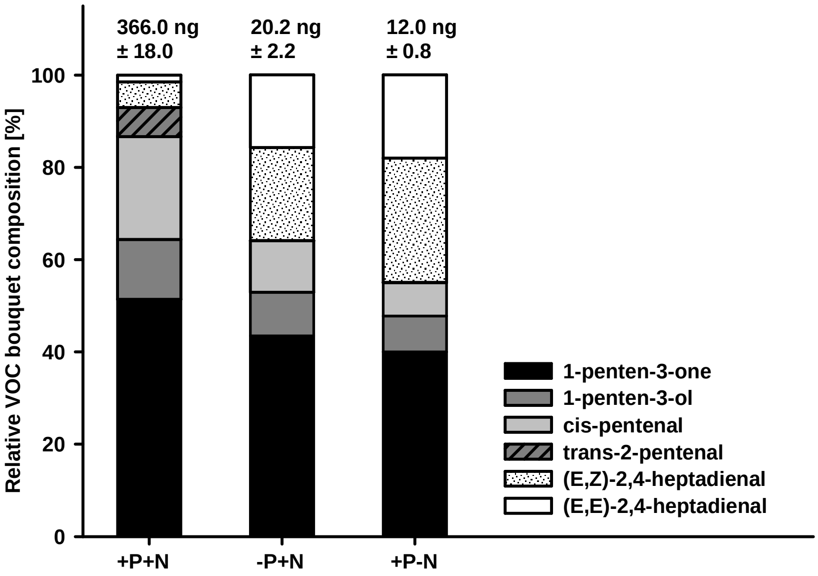

Figure 4: Relative composition of the VOCs bouquets of nutrient-saturated, P- and N-limited *U. fimbriata*; numbers above the bars give mean total amounts of VOCs (± SE of n = 7).

Food choice experiments with VOCs of nutrient-saturated and of P-and N-depleted U. fimbriata

When juvenile *L. stagnalis* were given the choice between VOCs extracted from -P+N algae versus VOCs extracted from +P+N algae, the snails clearly preferred the VOCs released from nutrient-rich algae (one-way ANOVA, $F_{1,20}$ = 159.96, $p < 0.001$, Fig.5). The same preference was observed when the snails had the choice between VOCs liberated from +P-N algae versus +P+N algae: the snails clearly preferred the VOC bouquet derived from the high-quality food (one-way ANOVA, $F_{1,20}$ = 51.11, $p < 0.001$, Fig.5). The average GPI obtained in these choice assays was significantly higher than in the control GPI: 80 % of the readings were the near VOCs source from nutrient-saturated algae.

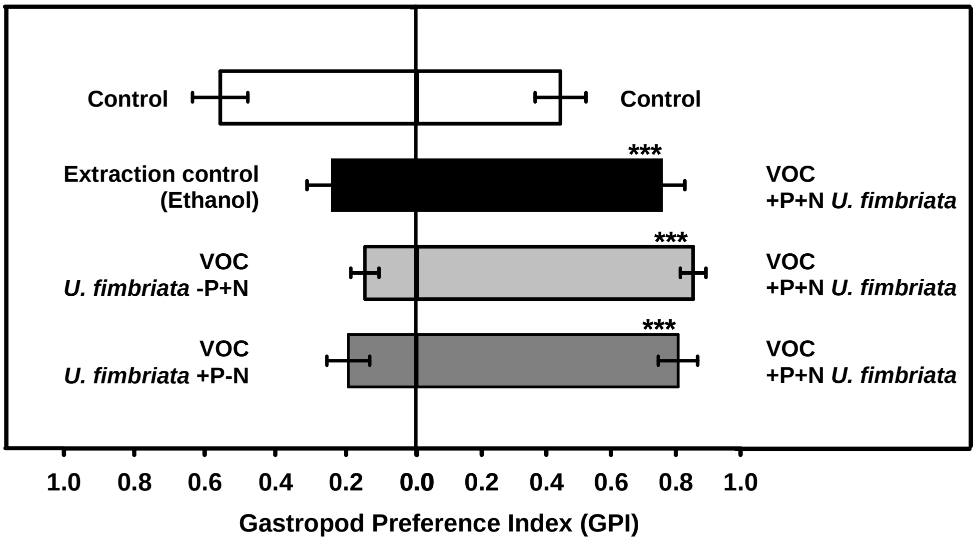

Figure 5: Gastropod position index (during the active searching period 5 - 35 min, ± SE) of *L. stagnalis* in the behavioural biotests dependent on the offered VOC extracts. White bars represent control assays (n = 12) in which both containers were filled with water; filled bars represent food choice assays (n = 11) in which one container contained VOCs extracted from nutrient-saturated *U. fimbriata*, while the respective container on the opposite end of the aquarium was filled with either an extraction control (black bars), VOCs from P-limited (light grey bars) or N-limited (dark grey bars) *U. fimbriata.* Asterisks indicate significant differences (*** $p < 0.001$) within one setup.

Discussion

It has been suggested that the use of volatile signals for the evaluation of nutritional resource quality should be a highly adaptive strategy for consumers (Goff & Klee 2006) but experimental evidence is lacking. Here we demonstrate for the first time that a freshwater gastropod can evaluate a resource’s dietary quality over a distance. The observed change in foraging behaviour clearly showed that the snails are able to perceive and distinguish the chemical cues released from the resource.

Effects of nutrient availability on algae and gastropods

Our results show that a sufficient availability of the macro-elements P and N is crucial for gastropod growth: The growth rate of snails was higher when they fed on high quality food, and somatic growth was severely constrained when snails ingested –P+N algae. Furthermore, we observed mortality during the growth experiment only in the treatment where the snails had been fed P-depleted algae. This highlights the importance of dietary P for freshwater invertebrates (Stelzer & Lamberti 2002; Fink & von Elert 2006).The ingestion of +P-N *U. fimbriata* also constrained the growth of juvenile *L. stagnalis*, but not as severely as the intake of P-depleted algae. These findings are similar to those found for the somatic growth rate of the freshwater pulmonate *R. ovata* (Fink & von Elert 2006) and the lotic pleurocerid snail *Elimia livescens* (Stelzer & Lamberti 2002). Hence, the C:N:P ratio of *U. fimbriata* cultures was a good indicator of food quality. Low availability of dissolved nitrogen led to a high algal C:N ratio, whereas low amounts of dissolved phosphorus in the algal growth medium caused a high algal C:P ratio as was previously reported by Fink& von Elert(2006).In comparison to the flexible algal stoichiometry, the C:N:P analyses of juvenile snails showed more constant body C:N:P ratios which were independent of the nutrient ratios in their diet. Hence, *L. stagnalis* can be considered to be a homeostatic consumer (Persson *et al.* 2010). Our findings confirm the results of previous studies (Sterner & Elser 2002) which investigated the growth limitation of primary consumers by the elemental composition of their food resources. For primary consumers, in particular for homeostatic organisms, the unbalanced availability of the essential elements P and N can lead to severe nutrient constraints. Therefore an effective strategy for optimizing nutrient uptake would be advantageous, e.g. food recognition via signal substance perception. However, this requires that food resources release different signals depending on nutrient content and also that consumers are able to recognize these differences.

Our analysis of algal VOCs showed that the nutrient availability of dissolved nutrients (PO_4 and NO_3) had a strong impact on the bouquet of VOCs released from *U. fimbriata* upon cell damage. As also found in a previous investigation by Fink *et al*.(2006b)the C_5 components 1-penten-3-on, 1-penten-3-ol were the most abundant components in the bouquet released from lysed cells of *U. fimbriata*. The chemical analysis of the VOC extracts derived from algae grown under different nutrient regimes revealed that the nutrient-depleted algal cultures liberated the same VOC components as nutrient-saturated algae, but in substantially lower amounts. Additionally, the comparison of the VOC mixtures showed clear differences in the relative composition of the VOC's bouquet. It seems likely that the nutrient depletion affected biochemical processes within the algae which caused the observed differences in the algal VOC's bouquets. The release of volatile substances is based on cell damage which initiates an enzymatic cascade. The enzymatic pathway that leads to VOC's release in benthic green algae has not yet been explained, but Pohnert& Boland (2002) suggested a VOC release pathway in diatoms: The enzyme cascade starts with the degradation of the phospholipid membrane to unsaturated fatty acids and finally leads to the release of a volatile cleavage product called oxylipin(Pohnert & Boland 2002). Under natural conditions, multiple factors can initiate this VOC liberating cascade, including mechanical damage by hydrodynamic forces, meiofauna and grazing herbivores (Fink 2007). However, the impact of nutrient limitations on VOC production has, to our knowledge, not been investigated in any other aquatic organism so far. Hence, the ecologically relevant question thus was, whether *L. stagnalis* are able to recognize differences in algal dietary quality through changes in the VOC's bouquet and if they might therefore be able to differentiate between high and low quality food by the odour alone.

Effects on gastropod behaviour

In the present study it was clearly shown that *L. stagnalis* respond to the VOC bouquet released from damaged cells of the benthic green alga *U. fimbriata* as a cue for finding food. Such a behavioural response had previously been demonstrated for a closely related pulmonate gastropod (*Radix ovata*) by Fink *et al.*(2006b). In the study of Fink *et al.* (2006b), it was further demonstrated that the attraction of snails was clearly mediated by volatile compounds and not other, more hydrophilic algal products. The single compounds were insufficient for the attraction of snails, but only the mix (bouquet) of C_5 and C_7 volatiles mediated the attraction to the freshwater snails. At the beginning of the behavioural assays, *L. stagnalis* showed an undirected movement within the first five minutes. Presumably, the VOC mixture had not reached the middle of the experimental set yet, so that the snails first perceived thesignal after these five minutes. Afterwards the herbivores exhibited a directed movement towards the source of the algal VOCs. The preference behaviour was exhibited for approx. 30 minutes before the response started to decline. This decreased response could be explained by a lack of reward (i.e. food) for the snails or by degradation or partial evaporation of VOCs. Another more probable explanation for the decreasing response is no clear VOC concentration gradient was detectable in the choice arena (aquarium) after the experimental period. Such a concentration gradient is necessary to initiate a directed food searching response by terrestrial snails (Chase 1982). When *L. stagnalis* were offered VOC extracts from +P-N or –P+N *U. fimbriata* versus extracts from nutrient replete (+P+N) algae, the snails showed a consistent and strong preference for the VOCs from nutrient-saturated algae. Hence, these snails are able to sense food quality differences based solely on odorous dietary compounds. We are not able distinguish whether this differential behavioural response to VOCs from high and low nutrient algae is due to the higher amount or the different bouquet of nutrient rich versus nutrient poor algae. However, field experiments suggest that the qualitative change of the bouquet might be more important than the quantitative change (Moelzner unpublished data). Interestingly, a possible linkage between flavour perception and the nutritional value of food has already been suggested Goff & Klee(2006).

The observed foraging behaviour of the primary consumer *L. stagnalis* by using chemical cues is highly adaptive. We here show that the movement of snails is not random (Streit 1981); the perception of infochemicals enables the freshwater gastropods to make directed movement decisions. This should be a superior foraging strategy in comparison to random walks, as predicted by theoretical considerations in mechanical movement models (Fronhofer, Hovestadt & Poethke 2013). Contrary to the findings of Teyke (1995), our results further revealed that no prior feeding experience of the snails to *U. fimbriata* is necessary to initiate foraging behaviour. Here, the foraging behaviour is only based on the presence of algal olfactory cues. We conclude that the use of chemical cues to directly recognize a high-quality food source over distance mediates optimal foraging and could thus be an adaptive strategy for the fitness optimization of many animal species.

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Supplementary figure

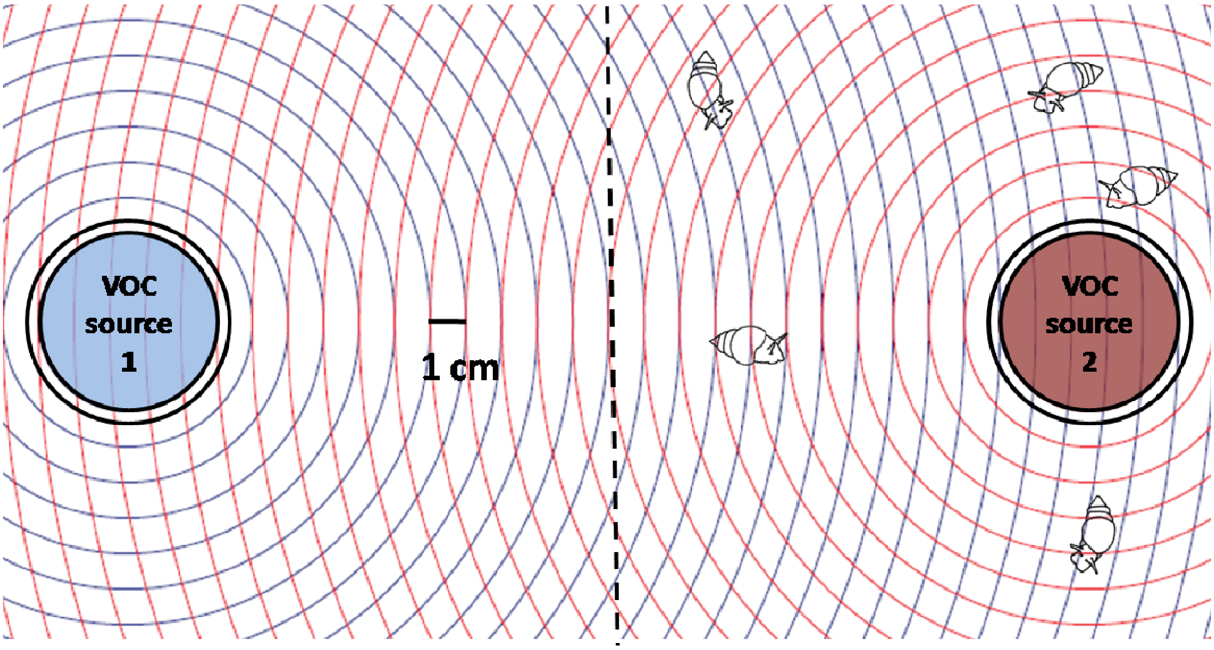

Figure S1: Schematic drawing of the choice arena used for bioassays on the preference of *L. stagnalis* towards volatile organic compounds (VOCs) extracted from the green alga *U. fimbriata.* The VOC extracts and control samples were released from VOC source containers positioned at opposite sides in the testing chamber (for details see Fink *et al.* 2006b). The position of the snails was as recorded as relative distance in cm from the VOC source using the coordinate system (blue and red lines with numbers; e.g. 0_{red} = VOC source container, 0_{blue} = control container and the difference of the distances to the respective containers for each individual snail and reading interval. The dashed line shows the center of the test chamber as well as the starting position of the snails.

Chapter II:

Consumer patchiness explained by volatile infochemicals in a freshwater ecosystem

Summary

Many animal species show considerable spatial variation in abundance within their habitats. Since they live in patchy environments where food resources are often heterogeneously distributed, it would be adaptive for them to have efficient chemoreceptive mechanisms to locate food resources over distances. In three field experiments, we investigated the foraging behaviour of freshwater herbivores to targets containing odorant stimuli. We demonstrate that freshwater gastropods are able to recognize odour bouquets as foraging infochemicals and that grazing of conspecifics causes an aggregation of grazers under natural conditions. Further, they appear to be able to distinguish between high and low quality food resources based on resource-quality specific odour bouquets. Our results suggest that the perception of volatile cues is a process relevant on environmental scales and thus a possible mechanism to explain the frequently observed patchy distribution of grazers in ecosystems.

Introduction

Across many species it is well known that organisms are unevenly distributed through the landscape (Downes, Lake & Schreiber 1993; Moens *et al.* 1999;Fryxell, Wilmshurst & Sinclair 2004). This patchiness at both the level of primary producers and herbivores is believed to be a major cause for non-equilibrium processes in trophic interactions, though the mechanisms leading to heterogeneity at the consumer level are poorly understood. Several studies documented that heterogeneous resource availability plays a major role in the spatial variation of animal abundance (Wiens 1976; McNaughton 1988; Morgan, Brown & Thorson 1997) and movement models have been developed to quantitatively assess behavioural responses of consumers to changes in resources at various spatial scales (Focardi, Marcellini & Montanaro 1996; Farnsworth 1998). Besides acquisition of sufficient resource quantity, consumers (in particular herbivores) need to acquire resources with a balanced nutrient content in order to maintain high rates of growth and reproduction in heterogeneous environments (Sterner & Elser 2002).Previous studies demonstrated that an unbalanced availability of both food quantity and quality (as mineral nutrients) severely limits the growth of freshwater herbivores (Sterner

1993; Fink & von Elert 2006;Moelzner & Fink 2014). For consumers, random searching for suitable food patches would require increased activity, which causes costs and leads to reduced fitness. A directed movement of consumers through the heterogeneous landscape based on behavioural decisions would be advantageous in comparison to random movement. Theoretical considerations already predicted directed walks (Fronhofer, Hovestadt & Poethke 2013), but mechanisms that could explain such directed movements are rare. An effective and highly adaptive mechanism would be the detection and localisation of food resources over certain distances via the perception chemical cues. For example, Höckelmann, Moens and Jüttner (2004) found aquatic nematodes to be attracted to cyanobacterial biofilms by multicomponent odours. In earlier laboratory choice experiments (Fink, von Elert & Jüttner 2006a; Fink, von Elert & Jüttner 2006b; Moelzner & Fink 2014), we have demonstrated that freshwater gastropods are attracted by algal volatile organic compounds (VOCs), but the environmental relevance of this chemically mediated interaction was not yet clear from the available laboratory studies. The benthic green alga *Ulothrix fimbriata* (Moelzner and Fink 2014) and also the diatom *Achnanthes biasolettiana* (Fink *et al.* 2006a) have been shown to release low-molecular volatile substances upon cell disruption. For diatoms, it was shown that cell damage initiates an enzyme cascade that leads to the degradation of membrane phospholipids and subsequent release of volatile cleavage products(Pohnert & Boland 2002). Under natural conditions, cell wounding can be initiated by multiple factors like mechanical damage by hydrodynamic forces or grazing herbivores (Fink 2007). When gastropods graze on a biofilm, their radula rasps over the underlying substrate (Elliott & Susswein 2002). This feeding style probably causes severe cell disruption and subsequent liberation of VOCs. Herbivores could use such grazing-induced volatiles to localize food sources. The advantage of low-molecular infochemicals is that they diffuse slowly in aquatic surrounding which enables the rapid formation of chemical gradients surrounding a source (Steinke, Malin & Liss 2002). During movement, gastropods could compare the intensity of chemical stimuli in their surroundings and detect concentration gradients that enable them to orientate to chemical signals like VOCs (Chase 1982). Moreover, it would be even more adaptive for consumers when they might use these grazing-induced odorant stimulants to distinguish resources based on their nutritional quality.

In the current study we investigated whether gastropod consumers perceive and pursue odorant signals and subsequently aggregate on the corresponding food patch which – together with increased patch residence time – should ultimately result in a patchy grazer distribution. Specifically, we hypothesised that (1) gastropods show patchy distribution patterns in a natural lake ecosystem, (2) a synthetic mix of VOCs (Fink *et al.* 2006b)designed to mimic the natural odour bouquet from a benthic alga is recognized by these gastropods and leads to local aggregation of grazers, (3) grazing of snails on a biofilm is recognized by conspecifics and induces aggregation near the corresponding grazing patch, (4) a different availability of dissolved nutrients causes changes in the VOC bouquet of natural biofilms, and (5) when snails graze simultaneously on high or low quality biofilm, their conspecifics recognize the quality difference based on volatile cues and exhibit an adaptive response by aggregating near the high quality resource. To investigate these hypotheses, we conducted three field experiments in artificial ponds using the great pond snail *Lymnaea stagnalis* (L.) as a model consumer species. The first experiment (I) investigated the behavioural response of the grazers towards a synthetic mixture of pure odour compounds, while the second experiment (II) assessed the distribution of snails dependent on the presence of grazing conspecifics. Finally, a third field experiment was designed to analyse the behaviour of gastropods that had the choice between conspecifics grazing on patches of high or low dietary quality.

Material and methods

Gastropods & biofilm

For the field choice assays, adults of the freshwater gastropod *L. stagnalis*(Fig. 1A) were collected from a pond in Appeldorn, Germany and were kept in artificial ponds in the botanical garden of the University Cologne on natural periphyton. Prior to experiment II, algal biofilms were precolonized for a month in outside aquaria on unglazed ceramic tiles (4.7 x 4.7 cm, V&B pro architectura, Germany) until they had reached a final dry mass of 10 mg tile^{-1}. The aquaria were inoculated with a 1:1 mixture of tap and pond water (pond of the Cologne Biocenter) and approx. 30 mg POC of a pure *Ulothrix fimbriata* culture (strain SAG 36.86, Culture Collection of Algae, Göttingen, Germany) to enable the establishment of semi-natural periphyton communities. A granulose slow-release fertilizer (Plantacote ^{TM} Depot 6M, Urania, Agrochem, 10 beads per aquarium) was used to constantly provide P and N for periyphyton growth (Hillebrand & Kahlert 2001). To produce biofilms with different nutrient content for experiment (III), the tiles were precolonized for four weeks as described above. Afterwards the pond/ tap water mixture was replaced by algal growth medium. Tiles with nutrient saturated biofilm were kept in a medium that was originally designed for cyanobacteria (Von Elert & Jüttner 1997)and is thus particularly rich in dissolved phosphorus (400 µM K_2PO_4) and nitrogen (8000 µM $NaNO_3$). Nutrient-depleted biofilms were obtained by culturing the tiles on algal medium with reduced phosphorus (8 µM K_2PO_4) and reduced nitrogen (400 µM $NaNO_3$) concentrations. The two different biofilm nutrient treatments will be further referred to as: Nutrient-depleted (–P–N) and nutrient saturated treatment (+P+N). Since algal biomass development is reduced under low nutrient conditions, precolonisation was started four weeks earlier for the –P–N tiles compared to the +P+N tiles.

Elemental analyses of the biofilm prior to choice assays

The biofilms were analysed prior to the field choice assays to ensure that nutrient content differed. For this, the biofilms were removed from the tiles with a scalpel and resuspended in a defined volume of tap water. The algal suspension of each replicate was divided into four aliquots of different volumes: Two aliquots were analysed for C/N and particulate P, respectively as described by Moelzner and Fink (2014). The third aliquot was preserved with Lugol´s iodine solution for the determination of the taxonomic composition and the fourth aliquot was used for VOC extraction to further analyse the algal VOC bouquets via gas chromatograph combined with mass spectrometry. VOCs produced by biofilms were identified by comparing the retention times and mass spectra with those of reference compounds and quantified using 3-hexanone as internal standard as described by Moelzner and Fink (2014).The C:N:P ratios of the biofilm samples and the amounts of VOCs released from nutrient saturated and depleted biofilm were analysed via one-way ANOVAs followed by Tukey´s HSD Tests using SigmaPlot® v. 11 (SysStat).

General setup of field choice experiments

The field food choice assays were conducted in four circular artificial ponds (diameter 1.9 m, Unique Koi, Germany) filled with 560 L of aged tap water which resulted in a water level of20 cm. The ponds were shaded with a PVC tarpaulin to avoid direct sunlight on the experimental setups. To enable the snails to select between the two targets we deployed two transparent Makrolon® plates (40 x 40 cm, Fig. 1) at the opposite sides of the artificial ponds in a distance of approx 75 cm to each other. The ponds and the position of the target areas in the ponds were randomly assigned for each replicate assays to avoid a directional bias.

Prior to the field choice assays, the experimental animals were starved for 72 hours to increase their food searching motivation and enlarge their olfactory sensitivity (Croll & Chase 1980). For each experiment, 36 starved *L. stagnalis* of similar shell length (30 ± 10 mm) were randomly distributed within the ponds. Subsequently, the prepared target areas were inserted into the choice arena. The snails were given ten minutes to acclimate to the experimental setup before the experiment was started. The number of the snails located on each of the target areas was then recorded every 30 minutes for three or six hours, depending on the experiment (see below). The mean number of snails that would be situated on one target plate with a theoretical random distribution was calculated as $(N_{snails}*A_{target})/A_{pool}$ where N_{snails} is the total amount of experimental snails, A_{target} the area of the target plate and A_{pond} the area of the artificial pond.

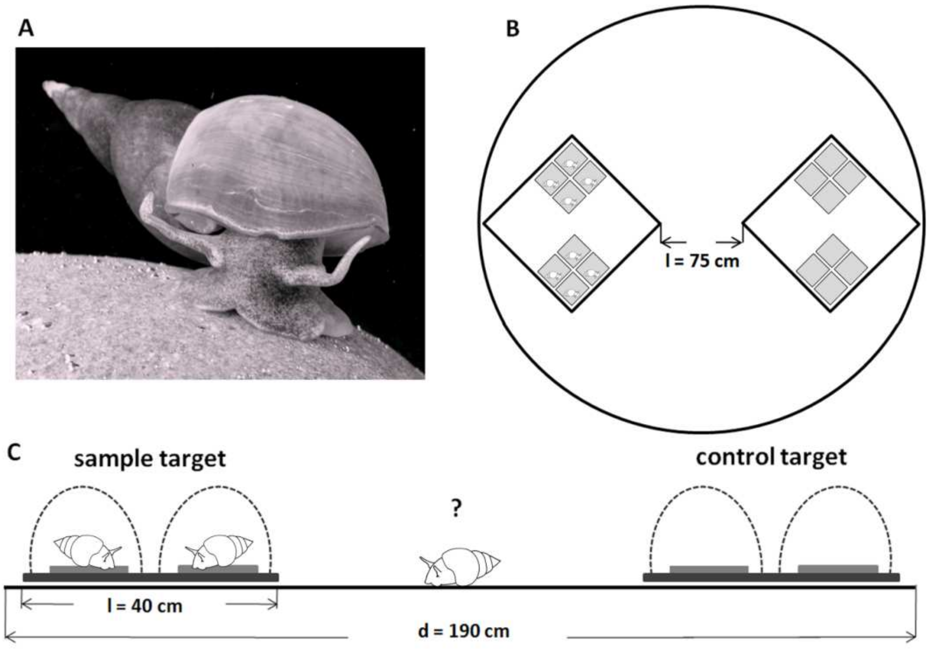

Figure 1: The great pond snail, *Lymnaea stagnalis* grazing on periphyton (A); experimental design for field choice assays with *L. stagnalis* grazing on biofilm on the sample target and biofilm only on the control target (field choice assay (II)) in top (B) and frontal view (C); the targets were placed into the circular artificial ponds as far as possible from each other (B); eight tiles with one snail per tile for the sample target and eight biofilm tiles for the control target were place on the polycarbonate plate and covered with a net (C) to exclude the experimental snails.

Field choice experiment (I) with a synthetic VOC bouquet

During experiment (I) the preference of the snails towards a synthetic VOC bouquet was investigated. Earlier laboratory studies (Fink et al. 2006b, Moelzner unpublished) have demonstrated that freshwater gastropods are attracted not only by natural, algae-borne odour bouquets, but also to a synthetic bouquet made from pure components and designed to mimic the VOC mixture liberated from benthic green algae. To test whether snails respond similarly to the presence of synthetic bouquets under natural conditions, we designed a VOC mix based on the known odour bouquet of the green alga *Ulothrix fimbriata* (Fink *et al.* 2006b). By using the synthetic VOC bouquet, we were able to offer identical VOC concentrations in all replicates and could easily produce large amounts of samples necessary for the field assays. All VOCs were dissolved in ethanol p.a. and diluted with distilled water (final volume: 30 mL) whereas the control consisted of ethanol and distilled water only. To mimic the hypothesized VOCs gradient through gastropod grazing, we continuously pumped the VOC mix using peristaltic pumps (Minipuls 3, Gilson) and Tygon tube

(T3601-13, Saint Gobain) into the artificial ponds. During the field experiments, the odour reservoirs were stored on ice. The tube opening was located in the middle of the target plates from which a volume of 10 mL h^{-1} VOCs (2.56 µg h^{-1}, concentration in the pond: 4.57 ng L^{-1} h^{-1}) was pumped onto each of the target areas. Every 30 minutes, the number of snails on each of the plates was counted for total experimental duration of 3 h.

Field choice experiment (II) with grazing snails

In the field choice experiment (II), we analysed whether snails that graze on an algal biofilm attract conspecifics through the release of VOCs. For this experiment, we used the same artificial ponds but a different setup for the target areas (Fig. 1B, C). On each target plate, two metal frames were mounted and covered by 1 mm mesh. The nets were tightly attached on the plate with Velcro strips (Hillebrand & Kahlert 2001). Four snails and four tiles were positioned within each cage, whereas the 36 experimental snails were excluded from the food patches by the cages' mesh. The cages allowed that VOCs which were released through snail grazing on the algal biofilm could be liberated into the surrounding water.

The field choice experiment (II) consisted of two variants. In the first variant, eight empty tiles (four per cage) with one snail per tile were placed on the control target plate whereas eight biofilm tiles (four per cage) with one snail per tile were placed on the opposite target area. This was intended to control for the attraction of conspecifics (e.g. by pheromones) independent of their foraging activity. In the second variant of field choice assay (II) we wanted to control whether the biofilm alone attracts snails. Therefore, eight biofilm tiles without snails were placed on the control target plate and eight biofilm tiles with one snail on each tile were placed on the other target area. Every 30 minutes for six hours the snails which were located on the target areas were counted.

Field choice experiment (III)

In field choice experiment (III), we analysed whether snails get attracted by conspecifics grazing on a nutrient rich (+P+N) biofilm in comparison to conspecifics grazing on a nutrient depleted biofilm (–P–N). The experimental setup was similar to the one above. Two cages were attached per target plate. Since we were limited in the number of biofilm tiles, we had to reduce the tile number from four to two tiles per cage, to conduct sufficient assays and biofilm analyses. For the assays, two biofilm tiles and two snails per tile were placed within each cage. For statistical analyses of all three field experiments, the data were tested for homogeneity of variances. The data set of the field assays I and II (control biofilm) were ln transformed to obtain homogeneity of variances. Then, the mean number of gastropods on the respective target areas was compared via a repeated-measurement ANOVA with the treatment or control as the fixed factor and reading time as random factor (repeated measurement). The repeated-measurement ANOVA was used to detect any possible difference between the mean numbers of gastropods on the different target areas, as previously described by Fink *et al.* (2006b) for similar purposes. The statistical analyses were performed with Statistica® v.10 (StatSoft) and a significance level of 0.05. We assumed that not all 36 experimental snails would be equally motivated for food searching, but also searching for e.g. mating partner or spawning grounds. Therefore, a preference index (PI) for all actively choosing snails (i.e. that demonstrated attraction to either of the target/control plates) was calculated. The preference index was calculated as (S / S + C) where S is the number of gastropods on the target plate with the synthetic VOC mix (in expt. I), snails grazing on a natural biofilm (in expt. II) and snails grazing on nutrient saturated biofilm (in expt. III), C is the number snails on the control target plates (in expts. I, II) and snails grazing on nutrient depleted biofilm (in expt. III). The PI was calculated at minute 180, to obtain comparable PI values for all bioassays despite different total duration. Hence, a PI of 1 indicates that 100 % of the snails that have selected one of the target plates with synthetic VOC mix or grazing conspecifics whereas a PI of 0 means that all gastropods were situated on the control target plates.

Results

Field choice experiments

In field choice experiment (I), where snails could choose between a synthetic VOC mix and a solvent control, the animals significantly preferred the source of the VOC bouquet over the control source (repeated measures ANOVA, $F_{1,14} = 14.83$, $p < 0.05$, Fig. 2, Tab. 1). The PI was 0.78 ± 0.12 SE, demonstrating that almost 80 % of the animals were located on the target plate with the infochemicals.

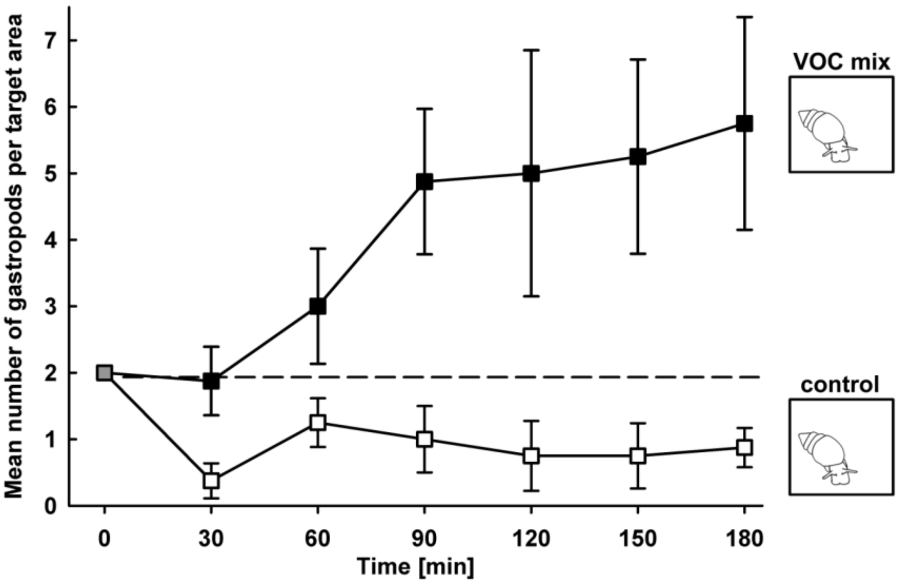

Figure 2: Mean number (± SE) of *L. stagnalis* on the target area in the first field choice experiment with a synthetic VOC bouquet (n = 8), corresponding to the VOCs liberated from wounded green algae (Fink 2006b); filled squares: numbers of snails on the target area with synthetic VOC bouquet; open squares: numbers of *L. stagnalis* on the control target; the grey square and the dashed line indicate the mean number of snails which would be situated on the plate at a random distribution (start condition).

In field choice experiment (II), we investigated whether actively grazing *L. stagnalis* attract conspecifics, presumably through the release of VOCs. The experimental snails were significantly attracted by conspecifics grazing on biofilm tiles compared to a control with conspecifics on empty tiles (repeated measures ANOVA, $F_{1,10} = 5.84$, $p < 0.005$, Fig. 3 A, Tab. 1). The PI was 0.74 ± 0.06 SE. Hence, approximately 75 % of the experimental snails were located on the target plate with conspecifics grazing on biofilm. In the second variant of the field choice assay (II) it was investigated whether the biofilm emits attractive infochemicals in the absence of gastropod grazers (Fig. 3 B). Here, the experimental snails showed a significant preference for the conspecifcs grazing on a biofilm versus biofilm alone as shown by the significantly higher mean number of snails on the sample target plate (repeated measures ANOVA, $F_{1,14} = 6.57$, $p < 0.05$, Fig. 3 B, Tab. 1) as also shown by the PI of 0.63 ± 0.02 SE.

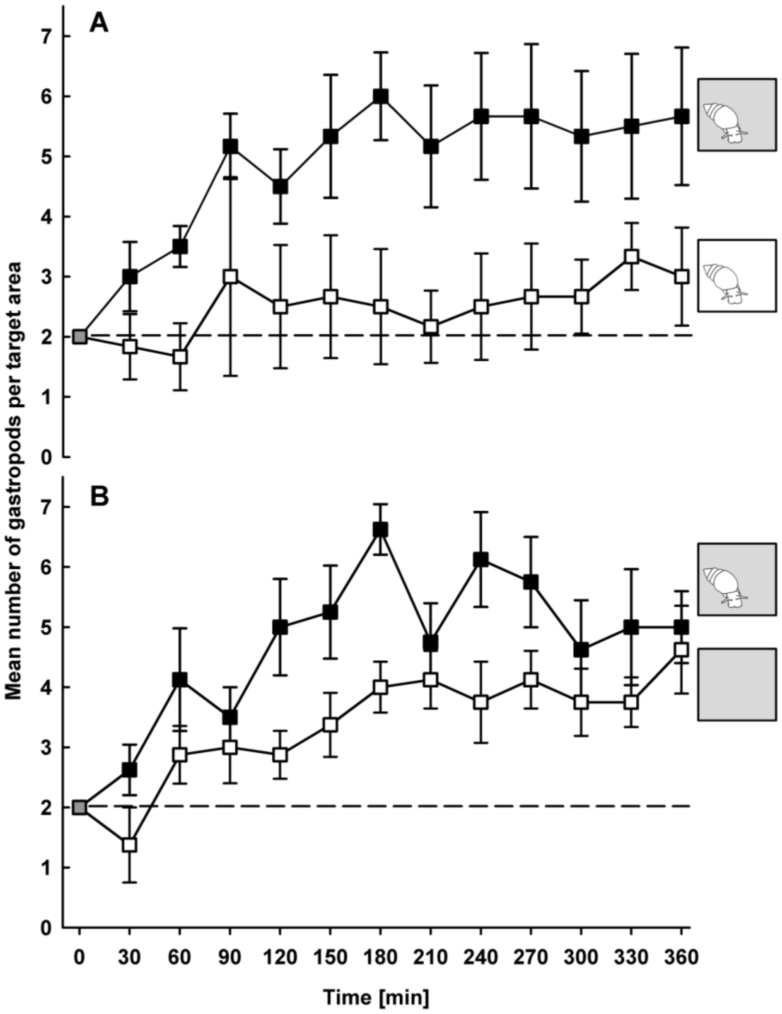

Figure 3: Mean number (± SE) of *L. stagnalis* on the target areas in the second field choice experiment depending on the presence of grazing conspecifics; A: number of snails on the target area with conspecifics grazing on biofilm (filled squares) versus the number of snails on the control target area with conspecifics on empty tiles (open squares, n = 6);B:number of snails on the sample target area with conspecifics grazing on biofilm versus the number of snails on the control target area where tiles with biofilm only were offered (open squares, n = 8); the grey square and the dashed line indicate the mean number of snails which would be situated on the plate at a random distribution (start condition).

Prior to field choice assay (III), we conducted analyses of the biomass, molar C:N:P ratios and VOCs of the nutrient depleted and nutrient saturated biofilms (Fig. 4). The analyses of the C:N:P ratios of the biofilms grown on the tiles confirmed that the algal stoichiometry was highly influenced by the availability of dissolved P and N in the algal growth medium. Biofilms grown under P and N depleted conditions had significantly higher C:P ratios ($F_{1,4} = 83.51$, $p < 0.001$) and C:N ratios ($F_{1,4} = 239.50$, $p < 0.001$) in comparison to biofilms on tiles grown under nutrient saturated conditions (Fig. 4 A,B). The dry mass of the biofilm grown under nutrient depleted conditions was significantly higher than in the P and N saturated biofilm ($F_{1,4} = 12.97$, $p < 0.05$), due to a longer precolonisation period prior to the experiments. However, microscopic analysis showed very similar algal community compositions irrespective of the fertilisation treatment and all samples contained *U. fimbriata*. The VOCs analysis demonstrated that all main VOCs previously reported to occur in *U. fimbriata* (Fink *et al.* 2006b; Moelzner & Fink 2014) were also present in our biofilm samples. The total amounts of VOCs released from nutrient- saturated biofilms were not significantly higher than the quantity of VOCs released from –P–N biofilms (one-way ANOVA, $F_{1,4} = 0.087$, $p > 0.05$, Fig. 4C). However, the relative composition of the bouquets differed between the nutrient treatments: Nutrient-depleted biofilms released significantly lower amounts of the C_5 component 1-penten-3-olin comparison to nutrient-saturated biofilm (one-way ANOVA, $F_{1,4} = 0.087$, $p > 0.05$, Fig. 4C). In contrast, the –P–N biofilm released proportionally, but not significantly higher amounts of the C_7 compounds (E,Z)-2,4-heptadienal (one-way ANOVA, $F_{1,4} = 2.437$, $p = 0.193$, Fig. 4C) and (E,E)-2,4-heptadienal than the nutrient-saturated biofilm (one-way ANOVA, $F_{1,4} = 1.289$, $p = 0.320$, Fig. 4C).

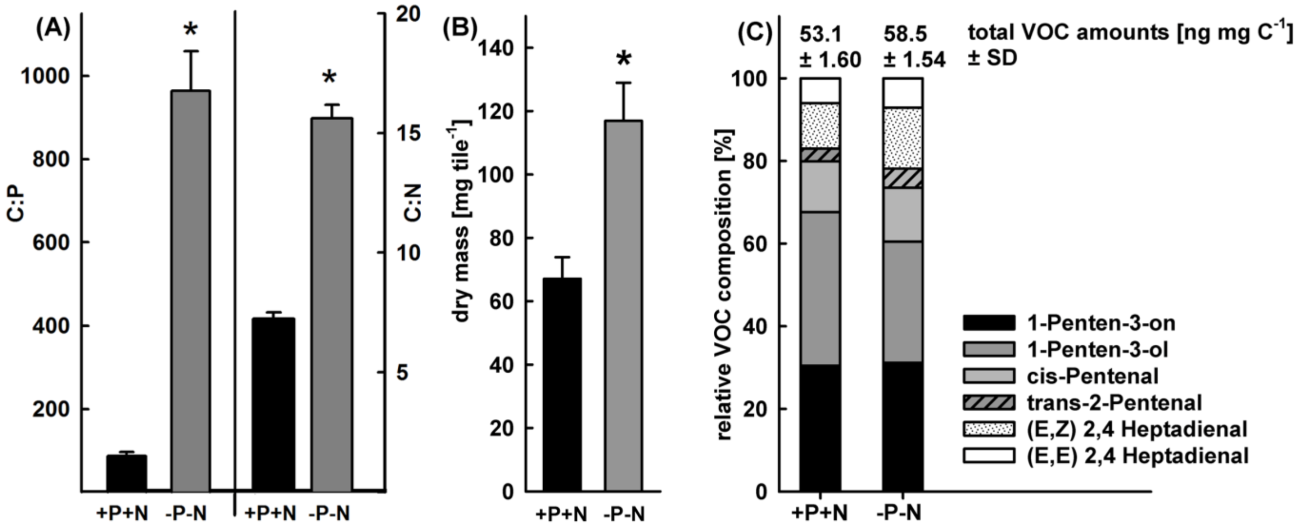

Figure 4: Analyses of the biofilm grown on tiles under different nutrient regimes in the field; A: biofilm stoichiometry, molar C:P and C:N ratios (mean ± SE, $n_{(+P+N)} = 3$, $n_{(-P-N)} = 3$); note the different scaling of the y-axes; B: biofilm dry mass (mean ±SE, $n_{(+P+N)} = 3$, $n_{(-P-N)} = 3$); asterisks indicate significant differences; C: relative composition of the volatile bouquets of nutrient-saturated, P- and N-depleted biofilms (± SE of n = 3).

Subsequently, the tiles covered with biofilm with either high or low nutrient content were offered in field choice assay (III). Snails were attracted by conspecifics that grazed on biofilm with high P and N content as demonstrated by a significant higher mean number of snails on the target plate with the +P+N biofilm versus the target plate with nutrient-depleted biofilm (repeated measures ANOVA, $F_{1,12} = 6.40$, $p < 0.05$, Fig. 5, Tab. 1). The PI was 0.66 ± 0.11 SE, thus demonstrating that two thirds of attracted snails were situated on the plate with conspecifics grazing on nutrient-rich biofilm.

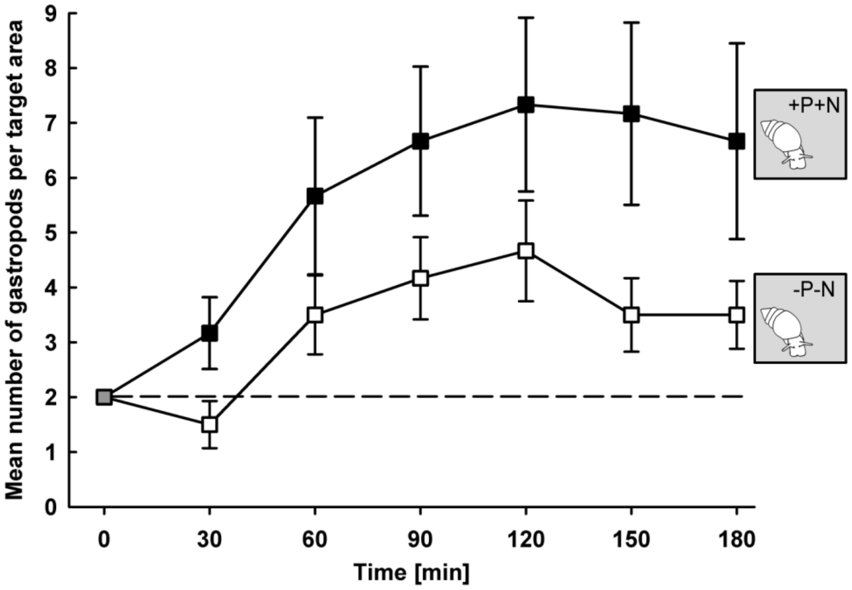

Figure 5: Mean number (± SE) of *L. stagnalis* on the target areas in the third field choice experiment (n = 7) dependent on the nutrient content of the grazed biofilms in the enclosures; depicted are the number of snails on the target area with conspecifics grazing on biofilm grown under nutrient saturated conditions (filled squares, +P+N) versus the number of snails on the target plate with conspecifics grazing on nutrient depleted biofilm (open squares,–P–N); the grey square and the dashed line indicate the mean number of snails which would be situated on the plate at a random distribution (start condition).

Table 1: Results of repeated-measurement analyses of variances on the mean number of gastropods in the field choice assays

	SS	***df***	***F***	***P***
I. Experiment (choice between synthetic VOCs target and control target, n = 8)				
Treatment	21.592	1	14.836	0.0017
Error	20.375	14		
Time	2.826	5	2.142	0.0704
Time x treatment	1.649	5	1.249	0.2955
Error	18.474	70		
II. Experiment [a] (choice between snails grazing on natural biofilm or empty tiles, n = 6)				
Treatment	225.000	1	5.840	0.0362
Error	385.250	10		
Time	56.917	11	2.704	0.0040
Time x treatment	14.167	11	0.673	0.7610
Error	210.417	110		
II. Experiment [b] (choice between snails grazing on natural biofilm or biofilm only, n =8)				
Treatment	93.521	1	6.569	0.0225
Error	199.313	14		
Time	145.417	11	5.949	0.0000
Time x treatment	24.229	11	0.991	0.4568
Error	342.188	154		
III. Experiment, choice between snails grazing on +P+N or –P-N biofilm, n = 7)				
Treatment	198.107	1	6.400	0.0264
Error	371.429	12		
Time	91.488	5	7.446	0.0000
Time x treatment	18.250	5	1.485	0.2080
Error	147.429	60		

Discussion

Herbivores are generally known to be heterogeneously distributed in their habitats (Sandulli & Pinckney 1999; Fratini, Cannicci & Vannini 2001;Kie *et al.* 2002). The patchy distribution patterns demonstrate that within one region, some localities contain dense animal abundances, while other nearby habitats are not occupied by herbivores. Several studies suggest that animals respond to local heterogeneity of food supplies (McNaughton 1988; Focardi *et al.* 1996;Fryxell *et al.* 2004). Resource patchiness can occur both in terms of resource quantity or nutrient availability (i.e. resource quality). The nutrient supply can have tremendous influence on the nutrient content of food resources and thus may affect the foraging behaviour of animals because these individuals have to yield high rates of energy intake in order to increase their fitness (Hill & Grossman 1993; Moelzner & Fink 2014).

Field choice experiment (I) with a synthetic VOC bouquet

When we offered a synthetic VOC bouquet in field choice experiment (I), the grazers were clearly attracted by these infochemicals even on a naturally relevant spatial scale. This resulted in a heterogeneous snail distribution pattern similar those commonly observed in the field (Brown 1985; Smith, Vaala & Dingfelder 2003).The synthetic VOC mix was designed to mimic the *b*ouquet of the benthic green alga *U. fimbriata*. Such a synthetic bouquet has already been shown to be attractive for the freshwater gastropod species *Radix ovate* (Fink *et al.* 2006b)and *L. stagnalis* (Moelzner, unpublished data). Gastropods possess efficient chemoreceptive mechanisms which enable them to orientate towards chemical signals(Croll 1983). The sensory mechanisms that lead to the detection of volatiles are not yet known. The chemosensory organs that are known to be involved in chemoreception of gastropods are the paired tentacles and the unpaired osphradium, a sensory epithel located in the mantle cavity (Townsend 1974; Wedemeyer & Schild 1995). An analysis of chemosensory neurons of *L. stagnalis* indicated that the lip and tentacle nerves play a crucial role in appetitive reception and feeding behaviour in response to chemical stimuli (Nakamura *et al.* 1999).

Field choice experiment (II) with natural biofilm and grazers

In experiment (II), we investigated whether snails that graze on a natural biofilm attract conspecifics presumably through the release of lipoxygenase products after radular cell damage. From prior studies it is known that cell disruption is required for VOC release from algae (Pohnert & Boland 2002). We clearly demonstrate thatthe experimental snails were attracted to the target with conspecifics grazing on a biofilm in comparison to the control targets which again resulted in a patchy gastropod distribution in the experimental ponds. During the experiment, the grazing snails were caged under a net. Therefore, the experimental snails had no access to the food patch and this allows us to separate infochemical effects from patch residence effects. The results of the assays where control targets with snails on empty tiles were present demonstrated that conspecifics without biofilm were not attractive to other *L. stagnalis* individuals. In contrast, the results of the assay with the control target with biofilm only demonstrated that more snails were located on the control target than it would have been expected with a random distribution. This suggests that the control tiles with biofilm alone (i.e. without snail), also liberated VOCs that were attractive to snails but not as attractive as grazing conspecifics. Presumably, meiofauna, bacteria, fungi and micorgrazers feeding on biofilm, caused cell disruption which may cause a release of VOCs (Fink 2007). Similar to our findings, a previous study demonstrated that mangrove snails get attracted by conspecifics feeding on leaves, while intact leaves and non-feeding snails were not attractive (Fratini *et al.* 2001). This supports the idea that the grazing activity itself leads to the release of chemical stimuli. The results of experiment (II) clearly indicate that the experimental snails were attracted from their feeding conspecifics after they perceived volatiles as foraging kairomones over distance and as a consequence exhibited a patchy distribution pattern. This is further supported by the observation that the bouquet of VOCs released from this natural biofilm was similar in composition to the artificial bouquet designed to mimic the odour of the green alga *U. fimbriata* (Fink *et al.* 2006b; Moelzner & Fink 2014).

Biofilm analyses

The analysis of biofilm C:N:P ratios clearly demonstrated that the nutrient depleted biofilms exhibited a lower C:P ratio than the nutrient saturated biofilm. High C:P ratios result in low resource quality and causes reduced growth of the consumers (Fink & von Elert 2006; Moelzner & Fink 2014). The chemical analysis of the VOCs extracted from the biofilm samples revealed that nutrient-saturated and nutrient-depleted biofilms released similar total amounts of VOCs, independent from the availability of the essential nutrients P and N. However, the relative composition of the algal VOC bouquet was altered with different nutrient supply, as we had also observed in an earlier study (Moelzner & Fink 2014). Since the tiles for the experiments were pre-colonized in natural lake water with addition of the green alga *U. fimbriata*, the analysed biofilm consisted partly of *U. fimbriata* and a natural algal community. Thus we expected similar but not equal VOCs bouquets to the known bouquet of *U. fimbriata.* In earlier publications, it was shown that diatoms which were also part of the analysed biofilm, do also release volatiles after cell disruption (Fink et al 2006a). These volatiles were shown to function as food finding cues but are chemically different from the VOC bouquet of *U. fimbriata.* We thus assume that the specific VOCs bouquets of different freshwater algae can differ considerably in their chemical composition and VOC profiles from many more algae are required to understand more about their chemical diversity and ecological functions(Fink 2007). Hence, our analytical approach was limited to the known VOCs bouquet of *U. fimbriata*. The different nutrient content could have also have led to a changes in the algal and/or bacterial biofilm composition (Stoodley *et al.* 1998; Stelzer &Lamberti 2001) and subsequent changes in VOC bouquet. However, this seems unlikely at least for the algae based on our microscopic investigations of fixed biofilm samples.

Field choice experiments (III) with biofilms of different nutrient content

Eventhough the differences in the VOC bouquet of the biofilms with different nutrient quality were small, the snails were attracted by the target where conspecifcs grazed on biofilm with high nutrient content. Snails are known to be very sensitive to chemical cues (Croll 1983) and even subtle deviations in an odour can be recognized and lead to altered behaviour. In a previous study, it has been shown that resource availability has a strong influence on the volatile production of freshwater chrysophytes(Watson & Satchwill 2003), whereas nutrient-depleted marine diatoms were shown to increase the release of polyunsaturated short-chain aldehydes (PUAs, (Ribalet *et al.* 2007).

Interestingly, thesnails were attracted by conspecifics grazing on high quality versus low quality biofilm even though the biomass of the high nutrient biofilm was lower than thatof the low nutrient biofilm. This supports our earlier findings that food quality (in terms of nutrient content) is even more important for freshwater gastropods than food quantity (Fink & von Elert 2006; Moelzner & Fink 2014). Our findings indicate that adaptive behavioural decisions driven by chemical cues can influence processes at coarser-scales and that fine-scale structural patchiness is important for freshwater herbivores. Furthermore, the structural patchiness we examined is caused by a biological process (grazing), and thus emphasizes the natural relevance of this ecological interaction.

Ecosystem consequences

Although herbivory is often viewed as negative interaction from the perspective of the primary producer, it has been demonstrated that grazers play an important role for primary production in terms of nutrient recycling (McNaughton, Banyikwa & McNaughton 1997; Vanni 2002).Consumers indirectly fertilize primary producers through their dissolved and particulate excretion products and can therefore alter nutrient cycling and affect producers´ elemental composition (McNaughton *et al.* 1997; Liess & Haglund 2007). Several studies revealed that grazer presence can increase the nutrient content of periphyton (Hillebrand & Kahlert 2001; Frost *et al.* 2002). Thus, the feedback interaction among grazing, nutrient cycling and primary production might be affected by the release of VOCs. The liberation of volatile infochemicals by primary producers would attract consumers and conspecifics to the respective food patch and ensures sufficient nutrient supply through grazing mediated nutrient regeneration.

The ability of many animals to select food patches that maximize their fitness is dependent upon their ability to recognize and assess resource patches over a range of spatial scales. Therefore, consumers have to optimise the length of time spent on each patch to maximise the number of resource items harvested per time unit(Wajnberg, Hoffmeister & Coquillard 2013). It is known that environmental patchiness can strongly influence the outcome of interspecific competition by favouring specific foraging strategies (Chase, Wilson & Richards 2001). The current study suggests that the perception of infochemicals enables straighter movements to food patches and increase foraging efficiency. This should lead to increasing net displacement of consumers, and thereby improve their chances of finding new resources.

Obviously, foraging infochemicals are not the only possible determinant of patchy grazer distributions. A heterogeneous distribution of herbivores may also be related to patchiness in predator pressure (Turner, Fetterolf & Bernot 1999), temperature fluctuations, water depth (Smith *et al.* 2003) or habitat heterogeneity (Doak 2000). Other factors, in particular increased patch residence time on high quantity and/or quality patches will further increase the patchiness in herbivore occurrence patterns(Nonacs 2001). Nevertheless, we here show convincing evidence that volatile infochemicals, liberated through the grazing activity of conspecifics, can cause patterns in grazer patchiness similar to those frequently observed in natural ecosystems.

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Chapter III:

Gastropod grazing on benthic algae leads to liberation of food-finding infochemicals

Abstract

Chemical information transfer is a major agent in the regulation of interspecific and intraspecific interactions in natural ecosystems. One important group of such infochemicals both in terrestrial and aquatic ecosystems are so-called volatile organic compounds (VOCs) that can evoke behavioural or physiological responses like predator avoidance and mate or host location. In previous work, we have demonstrated that freshwater gastropods utilize VOCs released from benthic algae as food finding cues, although the specific nature of the VOC release and perception were not yet clear. Therefore we tested whether gastropod grazing on biofilms leads to algal cell damage and a subsequent liberation of wounding-associated VOCs. In bioassays we investigated the algal VOC bouquet level which is necessary to elicit a behavioural response of freshwater gastropods. The results of the liberation experiment showed that gastropod grazing leads to VOCs release. We also found that a certain threshold level of volatiles is necessary for snails to recognise the volatile infochemicals and subsequently respond with a directed foraging behaviour towards the odour. Finally, a calculated mass balance model demonstrated that the grazer mediated VOC release produced a signal concentration that is sufficient to be recognized by conspecifics and utilized as foraging infochemicals. The emission of ecologically relevant volatiles through snail grazing with subsequent attraction of other gastropod grazers to algal biofilms indicates an important but so far understudied chemical signalling mechanism of ecological importance.

Introduction

Within and across ecosystems infochemicals mediate inter- and intraspecific in (Dicke and Sabelis 1988). In particular intraspecific information exchange, via chemical signals (allomones), is crucial for the regulation of many predator-prey interactions in both terrestrial and aquatic ecosystems (Kats and Dill 1998). Hence, an understanding of chemical signal transduction and emission is of critical importance for the study of consumer-resource interactions. One important group of signal substances are volatile organic compounds (Baldwin, et al. 2006, Gershenzon 2007). VOCs are small molecular compounds with a low to medium water hydrophilicity, which makes them functional as chemical cues in both terrestrial and aquatic habitats (Fink 2007). In terrestrial ecology, there are numerous studies that investigated the role of volatiles. For example, odour cues from nectar and pollen in flowers attract insect pollinators and structure plant pollinator interactions. Herbivorous insects were found to be repelled by chemicals released from plants damaged by herbivory (Bernasconi, et al. 1998, De Moraes, et al. 2001). Other studies on terrestrial plant-herbivore interactions reported that herbivory can induce the emission of VOCs from plants which attract parasitoids of the herbivores and thus lead to an indirect plant defence strategy (Arimura, et al. 2009, Ode 2006). Unfortunately, there is considerably less knowledge available on the role of VOCs in aquatic environments (Fink 2007). The most commonly studied example is an activated chemical defence strategy of marine diatoms against herbivorous zooplankton, which is mediated by antimitotic polyunsaturated aldehydes (Miralto, et al. 1999, Pohnert and Boland 2002). There, volatile aldehydes were found to inhibit the embryonic development of the brood of marine copepods and thus effect in an indirect defence of algal blooms on the population level (Ianora, et al. 2004). Further, it was reported that wound-activated substance from marine diatoms can act simultaneously as toxins and infochemicals also for benthic invertebrates (Maibam, et al. 2014). In previous studies (Fink, et al. 2006a, Fink, et al. 2006b, Moelzner and Fink 2014), we have demonstrated that freshwater gastropods use volatile organic compounds released from green alga as foraging infochemicals. In a field study we showed that grazers are attracted to a synthetic VOC mix that mimicked the natural algal VOC bouquet as well as when an algal biofilm is grazed upon by conspecifics (Moelzner and Fink in review).What is common to the studies on VOCs from marine and freshwater microalgae is that the liberation of VOCs appears to depend on algal cell damage and a wounding-activated enzyme cascade (Fink, et al. 2006b, Pohnert 2000). For diatoms, it was shown that the enzyme cascade starts with the degradation of phospholipid membranes to liberate polyunsaturated fatty acids and subsequent oxygenation of these fatty acids via a lipoxygenase. Finally, a lyase causes the release of a volatile lipoxygenase product called oxylipin(Pohnert and Boland 2002). Although the enzymatic mechanisms have been studied in detail under laboratory conditions, it is not yet clear how algal VOCs are released into the environment under a natural and ecologically relevant scenario. Interestingly, the feeding mode of aquatic gastropods involves the rasping of substrate-attached algal biofilms via their radular tongue, which most likely causes massive algal cell damage during the snails' ingestion process. Therefore, it was reasonable to hypothesise that radular scraping leads to cell disruption in biofilm algae which activates the enzyme cascade that leads to the liberation of VOCs and subsequently to the attraction of other gastropod grazers from the surroundings. The property of low-molecular volatiles is that they diffuse slowly in aquatic surroundings which enables the formation of chemical gradients (Steinke, et al. 2002) and gastropods are known to utilize concentration gradients to orientate towards chemical signals (Chase 1982). Presumably, a certain concentration of infochemicals in the proximity is required for the animals to recognize the signal substances and show behavioural response towards the volatile algal cues. To investigate these hypotheses, we conducted VOC liberation experiments in the laboratory and tested whether grazing of the freshwater gastropod *Lymnaea stagnalis* on a biofilm-forming alga caused the release of VOCs. To determine how different VOC levels influence the foraging behaviour of the gastropods, we used behavioural bioassay to investigate the odour threshold of the snails' behavioural response, and grazing experiments to quantify *L. stagnalis*' feeding rates dependent on body size. Using these experimental data sets, we developed a simple mass balance model to demonstrate that grazing-mediated cell disruption can cause the liberation of sufficient amounts of VOCs to elicit foraging behaviour in freshwater gastropods.

Materials and methods

Cultures

Juveniles of the freshwater gastropod *Lymnaea stagnalis* were hatched and reared from eggs laid by adult individuals originally collected in a pond in Appeldorn, Germany, and kept in a climate chamber at 20 ± 0.5°C under constant dim light in aerated tap water. The snails were fed Tetra PlecoMin™ fish food pellets (Tetra, Melle, Germany) *ad libitum*. The filamentous green alga *Uronema fimbriata* (strain SAG 36.86 from the Göttingen Algal Culture Collection, SAG , formerly classified as *Ulothrix fimbriata*, but reassigned to the genus Uronema by (Caisová, et al. 2011) was continuously cultivated in chemostats on Cyano medium (Von Elert and Jüttner 1997) with a light (PAR) intensity of 80 µmol photons s^{-1} m^{-2}. Particulate organic carbon (POC) concentrations of the algal suspensions were estimated from photometric light extinction at 480 nm using previously determined carbon-extinction equations from POC analyses of filtered algae in a Thermo Flash EA 2000 elemental analyzer.

VOC liberation experiment

Prior to the VOC liberation experiment, ten adult *L. stagnalis* (3 – 4 cm) were starved for 24 h prior to the experiment to increase their feeding motivation. A biomass of approx. 170 mg POC of a *U. fimbriata* suspension harvested freshly from the chemostat culture was transferred into round bottom flask and adjusted to a final volume of 250 mL with aged tap water. Subsequently, the preconditioned *L. stagnalis* were gently slipped into the flask and 100 ng of 3-Hexanone were added as internal standard. Subsequently, liberated VOCs were extracted 4 hours via ‘closed loop stripping’ (Jüttner 1988). With this technique, the VOCs are transferred from the aqueous phase into the headspace above the sample and subsequently trapped on 150 mg of the adsorbent Tenax TA (Chrompack). Control extractions with the same algal biomass, but without snail grazers were conducted to test whether ungrazed algae also liberate VOCs. The VOCs were thermally desorbed from the adsorbent and analysed qualitatively and quantitatively via GC-MS (Agilent Technologies) as described previously (Moelzner and Fink 2014). The amounts of VOCs (as ng mg C^{-1}) were log (x+1) or √x transformed to ensure homoscedasticity prior to one-way ANOVA followed by Tukey´s HSD. The VOC amounts were analysed as the dependent variable to assess the difference between the release of each single VOC component using the treatment (with our without grazers) as the predictor variable.

Threshold experiment

For the determination of the threshold concentration of the snails' behavioural response to the algal VOC bouquet, the behavioural assay developed by Fink, et al. (2006b) was employed. As a positive control,VOCs extracted from a 10 mg POC of a chemostat culture of *U. fimbriata* were used, while ethanol was tested as a negative (solvent) control. To test the snails´ respond to different VOC concentrations, we used a previously developed synthetic VOC mix comprised of pure compounds (dissolved in ethanol) and designed to mimic the natural odour bouquet of *U. fimbriata* (Fink, et al. 2006b). By using a synthetic VOC bouquet, we were able to offer identical VOC concentrations in all replicates and could easily produce large amounts of samples necessary for the odour threshold assays. The food choice assays were conducted in aquaria (320 x 170 x 180 mm) filled with 1 L of aged tap water each, in which VOC release containers were placed at the opposite sides of each aquarium (see Fink et al. 2006b). At the beginning of the experiment, five juvenile *L. stagnalis* with a shell length of 15 ± 5 mm were placed into the centre of an aquarium. The snails were given five minutes to acclimate to the experimental setup before the experiment was started by opening the VOC source containers. The response of the snails to the corresponding the VOC source was recorded every minute for 20 min as the relative distance of the five snails to the respective VOC sources. The closer a snail was situated to the VOC source, the higher was its relative position score. Seven different concentrations ranging from 25.6 ng L^{-1} to 256 µg L^{-1} were offered in 8 – 21 replicate choice assays per concentration. By calculating the mean relative positions of the five individual snails within each 20 minutes assay period, we derived a single preference score for each replicate assay, which could then be statistically compared to the mean relative position of the negative control (without VOCs) using analysis of variance. The threshold concentration was defined as the lowest concentration for which a significant difference in the snails' mean position was found compared to the negative control.

Ingestion experiment

The ingestion rate was measured for *L. stagnalis* with shell heights of 12.6 – 35.0 mm at non-food limiting conditions using again the culture of *U. fimbriata* as food source. The algae were collected from the chemostat culture, shock-frozen at -80℃, freeze-dried (Christ, Alpha 1-4 LSC) and finally stored frozen until the experiments to avoid changes in the cells' composition. Prior the ingestion experiment, juvenile *L. stagnalis* (preconditioned for 48 h in aerated tap water) were placed individually in circular glass jars (diameter: 12 cm) filled with 100 ml aerated tap water each. In the centre of the jar, a glass ring (diameter 3.5 cm) was positioned, in which 14 mg of freeze dried were placed and allowed to sediment and to re-hydrate in the container, before the glass ring was gently removed to allow the snails to access the resource spot. 20 containers were stocked with algae and snails to determine grazing, while another 14 containers received algae but no gastropods to serve as controls. After 3 h of grazing, the snails were removed and the remaining algae were filtered onto GF/A glass fibre filters (Whatman). Filters were dried for 24 hours at 60℃ before determination of algal dry mass on a microbalance.

Results

VOC liberation experiment

When *L. stagnalis* grazed on *U. fimbriata*, significantly higher amounts of 1-penten-3-on ($F_{1,14} = 8.35$, $p < 0.013$), 1-penten-3-ol ($F_{1,14} = 38.33$, $p < 0.001$) and E,Z-heptadienal ($F_{1,14} = 6.47$, $p = 0.024$) were released into the surrounding medium compared to the control samples without snails (Fig. 1). The release of the polyunsaturated C_7-aldehyde E,E-heptadienal was not significantly increased by gastropod grazing. The C_5-aldehyde pentenal which is commonly observed in the bouquet from artificially damaged *U. fimbriata* (Fink, et al. 2006b, Moelzner and Fink 2014), was not detectable in both experiments, neither with nor without gastropod grazers.

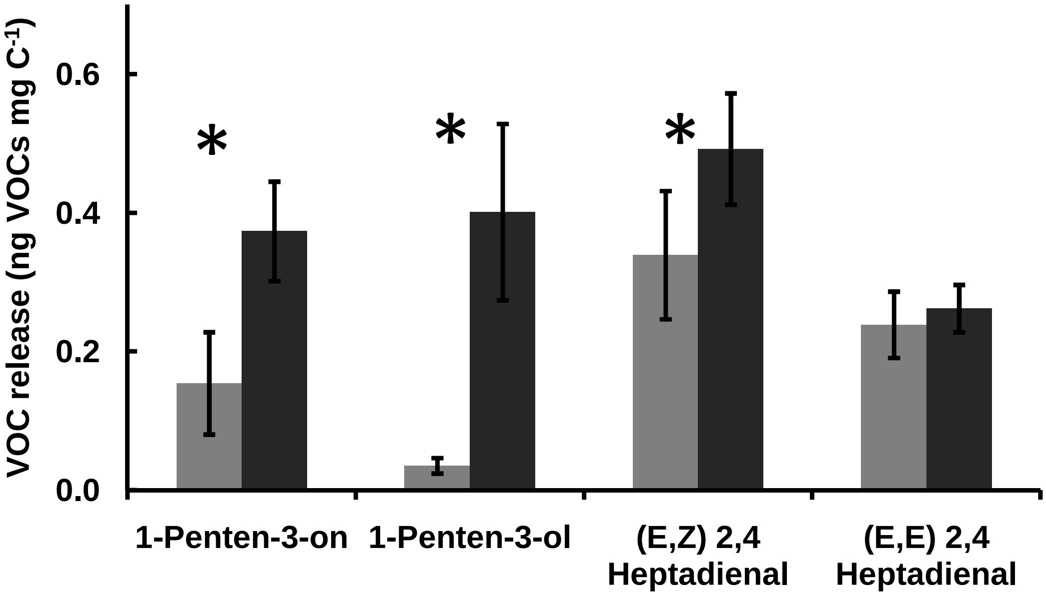

Figure 1: VOCs release (ng VOC mg C^{-1}) in grazer-free controls (grey bars, n = 8) or with actively feeding gastropod grazers (black bars, n = 6); asterisks indicate significant difference between treatments according to one-way ANOVA with $p < 0.05$.

Threshold experiment

The series of negative controls without chemical stimuli on either side of the aquarium confirmed that there was no directional bias, i.e. no preference for either side of the experimental arena, as demonstrated by a mean (± SE) gastropod position of -0.14 ± 1.05 (Fig 2). In the series of positive controls, *L. stagnalis* showed a significant preference for the natural VOC extract from *U. fimbriata* with a mean (± SE) gastropod position of 6.21 ± 1.85 ($F_{1,34}$= 8.17, $p < 0.01$). When a synthetic VOC bouquet with a concentration of 26 ng L^{-1} or 128 ng L^{-1} were offered in the choice assays, the snails´ position score was not significantly different from these in the solvent control assays. The mean position score increased significantly (4.07 ± 1.20 SE) when a VOC concentration of 0.26 µg L^{-1} was offered ($F_{1,34}$= 4.71, $p < 0.05$). The highest mean relative position of the snails towards the VOC source (5.49 ± 2.88 SE) was obtained when a VOC concentration of 1.28 µg L^{-1} was offered in the choice arena ($F_{1, 47}$ = 6.81, $p < 0.05$). When VOCs concentrations higher than 1.28 µg L^{-1} were tested, the mean relative position of the snails decreased again and the snails appeared to move away from the VOCs source, although the position readings were not significantly different to those of the solvent control assays.

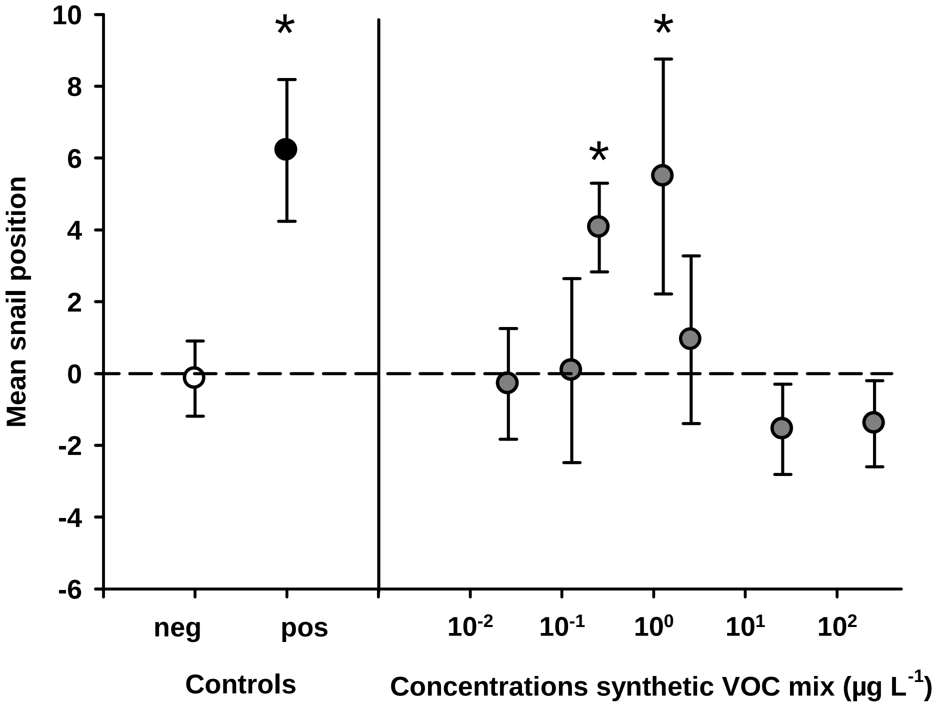

Figure 2: Mean position (± SE; 5 - 25 min) of *L. stagnalis* in the behavioural assays with a synthetic VOC bouquet corresponding to the VOCs liberated from wounded green algae (Fink 2006b); open symbol: mean position of snails in the control assays with water only (n = 28); filled symbol: positive controls (n = 8) with a natural VOC bouquet liberated from a benthic green alga; grey circles: mean position of *L. stagnalis* in the threshold assays with synthetic VOC bouquet at various concentrations (n = 8 - 21).

Ingestion experiment

As expected, the ingestion rate of *L. stagnalis* increased with increasing shell length (Fig. 3). For example, a snail with a shell height of 15 mm ingested approx. 0.25 mg C h^{-1}, whereas a conspecific of the double size ingested approx. five times more algal biomass (1.14 mg C h^{-1}). A power function ($y = 2E\text{-}0.5x^{3.22}$) was fitted to the data with an R^2 of 0.89, which corresponds to reports from the literature. Using this equation, the ingestion rate of *L. stagnalis* could be calculated for each shell height in a range from 12.6 mm to 35 mm.

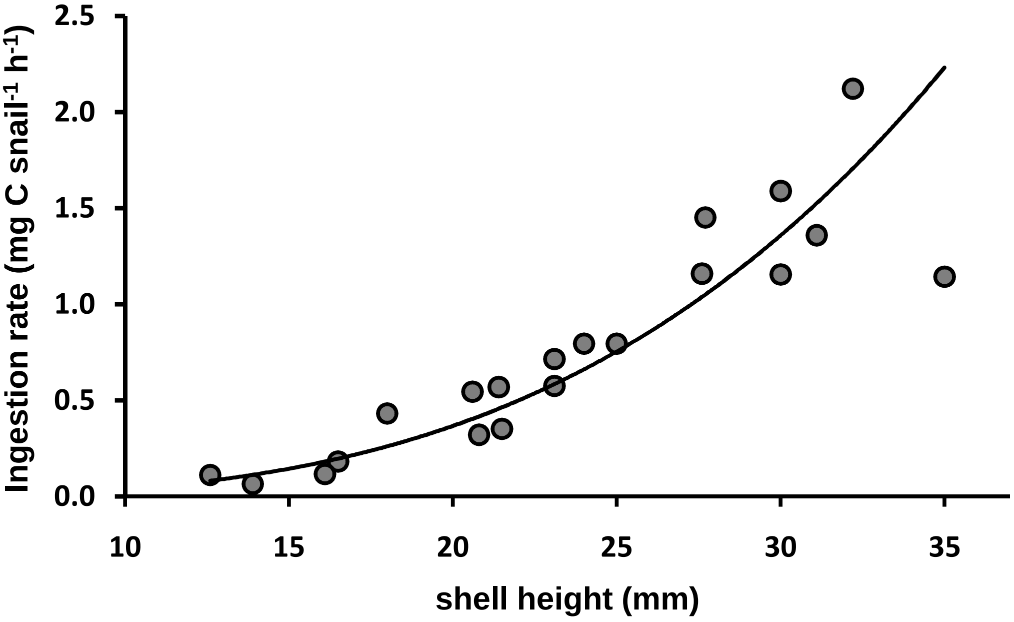

Figure 3: Relation between ingestion rate (algal mg C snail^{-1} h^{-1}) of *L. stagnalis* and their shell height; snails (n = 20) of different size were fed for 3 h on saturating amounts of freeze-dried *U. fimbriata* filaments; the fitted curve is a power function with y = 2E-0.5x$^{3.22}$, R^2 = 0.89.

Discussion

VOC liberation experiment

In prior studies it as shown that after cell lysis algal release volatiles and that gastropods were attracted from these chemical stimuli , but if active grazing on a biofilm mediates VOC liberation was not been demonstrated. The results of the present study now yield the missing piece in the puzzle – the grazing-mediated release of volatile foraging infochemicals. When snails graze on a biofilm, their radula rasps over the underlying substrate (Elliott and Susswein 2002). This feeding style causes cell disruption and apparently a subsequent liberation of VOCs. The analysis of algal VOCs from the liberation experiment showed that higher amounts of C_5 volatiles and E,Z heptadienal were released when snails grazed on *U. fimbriata* in comparison to the grazer-free controls. In prior investigations when algal cells were artificially damaged by freeze-thawing and addition of sodium chloride (NaCl, (Fink, et al. 2006b, Moelzner and Fink 2014)), the C_5 VOCs 1-penten-3-on, 1-penten-3-ol were also found to be the most abundant components in the bouquet released from damaged cells of *U. fimbriata*. In contrast to the artificially induced VOC release

(Fink, et al. 2006b), the other components of the VOC bouquet of *U. fimbriata* (namely the C_5 aldehydes cis- and trans-pentenal and the C_7 aldehydes E,E–heptadienal) were found only in minor concentrations. Until now, the enzymatic pathway that leads to release of VOCs from green algae has not yet been elucidated. It is plausible however, that the enzyme cascade in green algae resembles the one described for diatoms (Pohnert 2000).

Threshold experiment

The determination of odour thresholds is a common approach in sensory physiology, but also in applied research on food and fragrances. Odour thresholds are required to evaluate the sensory level above which an odorous signal can be perceived and/or a response measured(Lawless and Heymann 1999). Here we demonstrated that snails are able to recognize the signal substances and could identify an active concentration that is preferred by the snails. A minimum VOC concentration of 0.26 µg L^{-1} was required for attraction of the snails towards the VOCs source. This was the lowest concentration, namely the threshold concentration at which the snails showed a clear attraction towards the VOC source in comparison to the mean position of the snails in the negative controls. When the VOC concentration was increased to 1.28 µg L^{-1}, the VOC source was even more attractive, as indicated by a higher mean relative position of the experimental snails. A similar behavioural response was found in behavioural experiments with honey bees, where odorants became progressively easier to discriminate when the concentration increased (Wright and Smith 2004). With increasing VOC concentration, the snails´ positions values decreased and the snails demonstrated the tendency to crawl into the opposite direction of the VOCs source. It might be that an increasing odour intensity affects quantitative and qualitative perceptional properties as shown for honey bees (Wright, et al. 2005). The bees use the information of the odour concentration as separate stimulus dimension which means that the odour concentration is a part of the overall odour identity (Wright, et al. 2005). Another possibility for the observed snail behaviour is that the VOC concentration was too high. Thus, no odour gradient could be used by the snails for orientation in their surrounding (Chase 1982).In the positive control assays, the attraction of the snails towards the natural algal bouquet corroborates previous studies in which freshwater snails recognised algal VOCs as foraging kairomones (Fink, et al. 2006a, Fink, et al. 2006b, Moelzner and Fink 2014). However, the magnitude of the behavioural response towards chemical stimuli can vary and depending on sex, reproductive condition or the presence/absence of parasites as it was shown for marine gastropods (Curtis 1985). Or for bumblebees it was found that their sensitivity to odours increased with body size, since larger individuals have larger antennae and thus more olfactory receptors for chemical cues from the surrounding (Spaethe, et al. 2007).

Ingestion experiment

The ingestion experiment was designed to investigate the amount of algal matter that can be ingested by snails of a given body size. This allows the calculation of the amounts of VOCs that could potentially be liberated via snail grazing. As expected, the experiment demonstrated that the ingestion rate increased with body size (shell height). This body size dependence is coincident with prior findings for the same species (Zonneveld and Kooijman 1989). When the ingestion rate (h^{-1}) of a snail is divided by the snail´s shell height the specific ingestion rate per mm shell is obtained. This calculation shows that one mm of a 15 mm snail ingests 0.0167 mg C whereas one mm of the 30 mm snails ingest 0.038 mg C. For the grazer mediated VOC release, this means that larger animals graze much higher amounts of biofilm than smaller individuals, which leads to liberation of elevated VOC amounts. However, it should be noted that in natural environments, the ingestion rate of organisms is not only dependent on body size. The feeding rate of gastropods can be affected by numerous factors. For example, limiting resource quality can lead to compensatory feeding in freshwater gastropods (Fink and von Elert 2006), and marine snails (*Hydobiaulvae*) reduce their ingestion rate even under non- limiting food conditions when the population density is too high (Blanchard, et al. 2000).

Ecological relevance

The relevant question to evaluate the ecological importance of the grazing-mediated release of volatile infochemicals of course is whether the grazing of natural snail densities and the concomitant cell lysis are sufficiently large to lead to the liberation of signal concentrations that surpass the perception threshold determined here. To evaluate whether grazing-mediated cell damage could lead to the liberation of sufficient amounts of VOCs, we here aim a simple mass balance model based on the findings from this and previous studies: In an earlier lab study, we had quantified the VOCs released from an exponentially growing culture of *U. fimbriata* and found that approximately 366 ng VOCs were liberated per mg particulate algal carbon following cell disruption (Moelzner and Fink 2014). In behavioural choice assays, we demonstrated the preference of snails to this bouquet extracted from 10 mg POC of *U. fimbriata* (corresponding to 3.66 µg VOCs,(Moelzner and Fink 2014)). In our threshold assays with a synthetic VOC bouquet (designed to mimic the natural bouquet of *U. fimbriata*), a VOCs concentration of 1.28 µg L^{-1} lead to a clear significant behavioural response of the snails. This amount would be liberated after lysis of an algal biomass corresponding to 3.49 mg POC. As demonstrated in our ingestion experiment, a third of this biomass (1.14 mg POC h^{-1}) is ingested by a medium-sized *L. stagnalis* with a shell length of 30 mm in one hour. Thus, approximately three *L. stagnalis* of 30 mm shell length are able to consume the algal biomass within one hour that is necessary to liberate a VOCs signal (1.28 µg) which can be recognized by consepecifics and elicit chemotactic food-finding behaviour. This *L. stagnails* population density is commonly exceeded in the field, sometimes by several orders of magnitude (Moelzner, pers. observation). This is further corroborated by field experiments in which we could demonstrate that four adult snails grazing on a natural biofilm attracted conspecifics from the surroundings in a semi-natural ecosystem (Moelzner& Fink in review). Hence, our mass balance model plausibly explains that gastropod grazing can liberate an odorant signal at concentrations that can be recognized by conspecifics and utilized as foraging infochemicals.

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Concluding remarks and perspectives

The interaction of primary producers and grazers is important for the structure of aquatic ecosystems (e.g. Carpenter et al. 1985). In littoral zones of lakes gastropods like *Lymnaea stagnalis* are highly abundant. They play an important role in the benthic food web because herbivorous grazer are an essential link to higher trophic levels since they serve as food source for predators (Turner *et al*., 2000) and occupy a key role in the ´top-down´ control of algal biomass (Reavell 1980, Vander Zanden et al. 1997). In lakes, benthic algae are a major source of primary production (Vadeboncoeur et al. 2001). The nutrient content of those algae varies strongly, both spatially and seasonally (Elser et al. 2000). In such heterogeneous environments it is important for herbivores to acquire a sufficient nutrient supply in order to maintain high rates of growth and reproduction (Elser et al. 2000). The foraging success could be increased through the perception of diet-derived infochemicals that convey information about a food resource's quality over a certain distance. Many interactions of organisms in ecosystems are goverened by infochemicals (Vos et al. 2006). A major group of infochemicals are volatile organic compounds (VOCs) which can induce behavioural or physiological responses like predator avoidance, mating or food source location (Laothawornkitkul et al. 2009). For freshwater gastropods (*Radix ovata*) it was shown that volatiles released from benthic green algae and diatoms upon wounding, were perceived as foraging cues (Fink et al. 2006a, b).The use of VOCs as signal substances could thus be a suitable mechanism for the communication and interaction of primary producers and grazers of a benthic natural system.

When I investigated the somatic growth rate of *L. stagnalis* depending on the nutrient content of the resource in **chapter I,** the results showed that a sufficient availability of the macro-elements P and N was crucial for the growth of the juvenile snails. The growth rate was higher when they fed on high quality food *Uronema/ Ulothrix fimbriata* (+P+N), and severely constrained when snails fed on nutrient depleted algae (–P+N or algae +P-N). These findings were similar to those observed for the somatic growth rate of the freshwater pulmonate *Radix ovata* (Fink and von Elert 2006) and the lotic pleurocerid snail *Elimia livescens* (Stelzer and Lamberti 2002). The analysis of the algal C:N:P ratio showed a flexible stoichiometry whereas the

C:N:P analyses of juvenile snails demonstrated constant body C:N:P ratios which were independent of the nutrient ratios in their diet. Therefore *L. stagnalis* can be considered to be a strict homeostatic consumer (Persson et al. 2010). These results are in line with findings of previous studies (Sterner and Elser 2002) which investigated the growth limitation of primary consumers by the elemental composition of their food resources. For primary consumers, in particular for homeostatic organisms like snails, the unbalanced availability of the essential elements P and N can strongly constrain growth and reproduction as it was shown by empirical and theoretical studies (Andersen et al. 2004, Frost et al. 2005). Accordingly an effective adaptive strategy for optimizing nutrient intake would be advantageous, e.g. food recognition via infochemical perception. However, this would imply firstly that food resources release different signals depending on nutrient content and secondly that consumers are able to recognize these differences. Therefore, I analysed the composition of the VOC bouquet released from *U. fimbriata* with high and low nutrient via GC-MS analyses. The chemical analysis of the VOC extracts revealed that the nutrient-depleted algal cultures liberated the same volatile components as nutrient-saturated algae, but in a different relative composition and also in substantially lower amounts.These findings of the present study are in contrast with those for the diatom *Skeletonema marinoi* where N- and P-limited cells in stationary phase were found to produce higher amounts of volatile aldehydes than the nutrient repleted cultures (Ribalet et al. 2007).

Until now, the enzymatic pathway that leads to release of VOCs from benthic green algae has not been elucidated. Nevertheless, researchers proposed the lipogxygenase pathway in diatoms (Pohnert 2000, 2002) that could lead to the release of volatile oxylipins. Studies on diatoms (*Skeletonema costatum)* showed that the precursor of volatile aldehydes are C_{16} and C_{20} PUFAs (D'Ippolito et al. 2004). The C_{18} fatty were expected to be the precursors for the volatiles released from the benthic green algae because green algae are closer related to the higher plants than to diatoms (Adl et al. 2012). It was shown in the present study that in *U. fimbriata* with different nutrient content the main fatty acid components are the C_{18} PUFAs alpha-linoleic (C18:3) acid and stearidonic acid (C18:4). Previous studies that analysed the fatty acid composition of freshwater microalgae also found that the C18:3 fatty acid was present in all investigated algae whereas the C18:4 was found to be species specific (Weissman 1984).The low availability of the nutrients N and P in *U. fimbriata* led to significantly lower amounts of the C_{18} PUFAs. It seemed reasonable that the lower amounts of C_{18} PUFAs caused differences in relative composition of the algal VOC's bouquets. First investigations on the biosynthetic pathway gave hints that the C_{18} PUFA alpha-linoleic acid is at least the precursor for the C_7 aldehyde heptadienal (Moelzner, Roth, Fink unpublished). Further analyses are necessary to clarify the lipoxygenase pathway of the benthic green algae into detail.

Therefore I hypothesised whether *L. stagnalis* is able to recognize differences in algal dietary quality through changes in the algal VOCs bouquet and if it might therefore be able to differentiate between high and low quality food by the odour alone. In behavioural assays I found the freshwater gastropod *L. stagnalis* to be attracted by the VOCs bouquet released from damaged cells of the benthic green alga *U. fimbriata.* A similar foraging behaviour was reported for another pulmonate gastropod (*Radix ovata)* by Fink et al. (2006b). In a further series of bioassays *L. stagnalis* showed a consistent and strong preference for the VOCs from nutrient-saturated algae versus VOC extracts from nutrient depleted *U. fimbriata.* So indeed, the observed foraging behaviour of the snails demonstrated that they are able to sense food quality differences based solely on odorous dietary compounds. Nevertheless, it was not possible to constitute, whether this differential behavioural response of the snails to the algal bouquets from high and low nutrient algae was due to the higher amount or the different relative composition of VOCs bouquets. However, the results of field experiments (**chapter II**) suggest that the foraging behaviour is based on qualitative change and not on the quantitative change. Interestingly, a possible linkage between flavour perception and the nutritional value of food has already been suggested by Goff & Klee (2006).The results of the bioassays show that the usage of infochemicals as food quality indicators enables the freshwater gastropods to make directed movement decisions. Additionally, decision making based on chemical cues is highly adaptive for such slow moving grazers. This should be a superior foraging strategy in comparison to random walks, as predicted by theoretical considerations in mechanical movement models (Fronhofer et al. 2013). Contrary to the findings of Teyke (1995), our results further revealed that no prior feeding experience of the snails in respect to *U. fimbriata* is necessary to initiate foraging behaviour. Here, the foraging behaviour is only based on the presence of algal olfactory cues.

Further I hypothesised that detection of algal foraging cues should be relevant on a larger spatial scales. It is also not clear how volatiles could be released under natural conditions. Therefore I hypothesised that the grazing of snails which based on radular cell damage, could lead to the release of volatile lipoxygenase products that are recognized as foraging cues. In **chapter II** I therefore analysed in field experiments the foraging behaviour of *L. stagnalis* to volatiles and tested whether snails´ grazing on natural biofilm leads to attraction of conspecifics which would indicate that volatiles are released.

At first I tested whether *L. stagnalis* is able to detect volatiles on a naturally relevant spatial scale (I). Therefore I used a synthetic VOC mix which was designed to mimic the bouquet of the benthic green alga *U. fimbriata*. Such a synthetic bouquet has already been shown to be attractive for the freshwater gastropod species *Radix ovata* (Fink et al. 2006b). *L. stagnalis* was clearly attracted to the VOC source even on a larger spatial scale.

In the second field experiment (II) within **chapter II**, in which *L. stagnalis* grazed on a natural biofilm it was clearly demonstrated that the conspecifics were attracted to the target with conspecifics grazing on tiles with biofilm in comparison to the control targets (biofilm tiles without grazing snails). The results also support the hypothesis that snails´ grazing initiates VOCs release: target with snails grazing on biofilm and the synthetic VOCs source similarly evoked an attraction of *L. stagnalis*. In a variant field experiment it was demonstrated that only biofilm was also attractive for the snails. This suggests that the biofilm alone (i.e. without macro grazers), also liberated VOCs that were attractive to *L. stagnalis*, but not as attractive as grazing conspecifics. Presumably, meiofauna, bacteria, fungi and micorgrazers feeding on biofilm, produced cell disruption which may cause a release of VOCs (Fink 2007). Similar to our findings, a previous study demonstrated that mangrove snails get attracted by conspecifics feeding on leaves, while intact leaves and non-feeding snails were not attractive (Fratini et al. 2001). This supports the idea that the grazing activity itself leads to the release of chemical stimuli, which functioned as distance attractants as it was demonstrated for floral scents for pollinators of patchily distributed plants (e.g., Knudsen et al. 1999).

The results of grazing experiment (II) clearly indicated that the experimental snails were attracted from their feeding conspecifics. This resulted in a heterogeneous snail distribution pattern similar to those commonly observed in the field (Brown 1985, Smith et al. 2003).

It is known that herbivores are heterogeneously distributed in their habitats (Sandulli and Pinckney 1999, Fratini et al. 2001, Kie et al. 2002). Several studies suggest that animals respond for example to local heterogeneity of food supplies (McNaughton 1988, Focardi et al. 1996, Fryxell et al. 2004). Resource patchiness can occur both in terms of resource quantity or nutrient availability (i.e. resource quality). The nutrient availability can have tremendous influence on the nutrient content of food resources and thus may affect the foraging behaviour of animals because these individuals have to yield high rates of nutrient uptake in order to increase their fitness (Hill and Grossman 1993, **chapter I**)**.** From the results presented in **chapter I** it was known that differences in algal dietary quality lead to changes in the VOC bouquet *of U. fimbriata. L. stagnalis* was thus able to recognize differences in the algal odours.

Therefore, I hypothesised that the different nutrient availability in a natural biofilm leads to changes in the VOC bouquet. Further I hypothesised that *L. stagnalis* might be able to differentiate between high and low quality food resource on a natural relevant spatial scale based on the odours released while conspecifics graze on a natural biofilm.

In a third experimental approach (III) of **chapter II,** I therefore analysed the VOC bouquet of the biofilms with different nutrient content and tested if the grazing of *L. stagnalis* on food patches with high versus low nutrient availability would lead to an attraction of *L. stagnalis* to the target with conspecifics grazing on the nutrient rich food resource. The chemical analysis of the VOCs extracted from the biofilm samples revealed that nutrient-saturated and nutrient-depleted biofilms released similar total amounts of VOCs, independent from the availability of the essential nutrients P and N. However, the relative composition of the algal VOC bouquet was altered with different nutrient supply, as I had also observed in **chapter I**. Since the tiles for the experiments were pre-colonized in natural lake water with addition of the green alga *U. fimbriata*, the analysed biofilm consisted partly of *U. fimbriata* and a natural algal community. Thus we expected similar but not equal VOCs bouquets to the known bouquet of *U. fimbriata.* In earlier publications, it was shown that diatoms which were also part of the analysed biofilm do also release volatiles after cell disruption (Fink et al 2006a). Even though the differences in the VOC bouquet of the biofilms with different nutrient quality were small, I could demonstrate in the third field experiment (III) that *L. stagnalis* was attracted by the target where conspecifics grazed on biofilm with high nutrient content. From snails it is known that they are very sensitive to chemical cues (Croll 1983) and even subtle deviations in an odour can be recognized and lead to altered behaviour. During movement, gastropods compare the intensity of chemical stimuli in their proximity and detect concentration gradients that enable them to orientate towards chemical signals (Chase 1982). In aquatic environments local concentration gradients of VOCs can develop, because seconds after cell damage high amounts of volatile aldehydes are released, as it was shown for diatoms (Pohnert 2000). Furthermore the wound activated lipoxygenase was shown to retain its activity in seawater over several minutes after wounding (Fontana et al. 2007). Altogether, the rapid onset of the aldehyde biosynthesis, the activity duration of the wound-activated lipoxygenase coupled with the slow diffusion velocity provide good conditions for the establishment of a stable chemical cue gradient.

In prior investigations, it was demonstrated that when algal cells of benthic algae were artificially damaged by freeze-thawing and addition of sodium chloride, the algae released volatiles (Fink et al. 2006b, **chapter I**). Furthermore I demonstrated in the field experiments of **chapter II** that a synthetic VOC bouquet and grazing of conspecifics on biofilm induced foraging behaviour of *L. stagnalis*. I therefore hypothesized that while *L. stagnalis* scrape over the substrate, cells are wounded and this initiate the enzyme cascade that leads to the formation of VOCs. In **chapter III,** I investigated in laboratory experiments, whether the feeding style of *L. stagnalis* cause the liberation of VOCs. The data of this liberation experiment clearly showed that higher amounts of C_5 volatiles and E,Z heptadienal were released when snails grazed on *U. fimbriata* in comparison to the grazer-free controls.

In order to further develop an idea to which extend the algal VOCs bouquet shapes or influences the foraging behaviour of the grazers, a detection threshold is required. This threshold should facilitate to evaluate the sensory level of organisms from that on, an odorous signal is recognized and leads to a response.Therefore I hypothesized in **chapter III** that a certain threshold concentration of VOCs is necessary to initiate a directed foraging behaviour of *L. stagnalis* towards a VOC source.In further bioassays I investigated the detection threshold of *L. stagnalis* for different concentrations of a synthetic VOC mix. It could be demonstrated that at very low VOC concentration no response to the VOC source occurred. With increasing VOC bouquet concentration the snails got attracted and I found the threshold concentration at which *L. stagnalis* showed a clear attraction towards the VOC source. With a two times higherVOC concentration than the threshold concentration the VOC source was even more attractive. Such a similar foraging behaviour was found in behavioural experiments with honey bees, where odorants became progressively easier to discriminate when the concentration increased (Wright and Smith 2004). When the concentration was elevated by factor 10, *L. stagnalis* showed no foraging response to the VOC source anymore. When the VOC concentration was 100 or 1000 higher than the threshold concentration, *L. stagnalis* tend to crawl into the opposite direction of the VOCs source. Most likely the increasing odour intensity affected quantitative and qualitative chemical cue perception of the snails as it was for example shown for honey bees (Wright et al. 2005). Bees used the information of the odour concentration as a separate stimulus dimension which means that the odour concentration is a part of the odour identity (Wright et al. 2005). An increased concentration changes the identity of the scent and is not recognized anymore. Another explanation for the negative response of the snails at very high VOC levels is that the VOC concentration in the choice arena was so high that no VOC concentration gradient developed. In a motionless aquatic environment chemicals are transported via molecular diffusion (Webster and Weissburg 2009). The transport of the chemicals can be described by Fickian diffusion, where the flux is proportional to the concentration. Consequently, a rapid distribution of the VOCs occurred and thus no odour gradient could be used by the snails for orientation in their surrounding (Chase 1982). However, the magnitude of the behavioural response towards chemical stimuli can vary and is depending on sex, reproductive condition or the presence/absence of parasites as it was shown for marine gastropods (Curtis 1985). For example it was found for bumblebees that their sensitivity to odours increased with body size, since larger individuals have larger antennae and thus more olfactory receptors for chemical cues from the surrounding (Spaethe et al. 2007). Like insects, gastropods do also possess efficient chemoreceptive mechanisms which enable them to orientate towards chemical signals (Croll 1983). The chemosensory organs that are known to be involved in chemoreception of gastropods are the paired tentacles and the unpaired osphradium, a sensory epithel located in the mantle cavity (Townsend 1974, Wedemeyer and Schild 1995). An analysis of chemosensory neurons of *L. stagnalis* indicated that the lip and tentacle nerves play a crucial role in appetitive reception and feeding behavior in response to chemical stimuli (Nakamura et al. 1999). These sensitive chemosensory organs enable *L. stagnalis* to recognize not only foraging cues from food resources but also i.e. kairomones from predatory fish. The primary anti-predator behaviour in *L. stagnalis* is to crawl out of the water which is even enhanced when the fish kairomone is paired with alarm cues, from crushed conspecifics (Dalesman et al. 2007). Previous studies used *L. stagnalis* to investigate cue association learning and anti-predator behaviour. It was found that *L. stagnalis* was capable to relate a potential predation risk to a recent experience (Dalesman et al. 2006). This indicates that the *L. stagnalis* might also use the mechanism of cue association learning in order to remember to the smell of the good food.

The final hypothesis of the present work was; whether the grazing of natural snail densities and the concomitant wound mediated VOCs liberation are sufficiently large enough to lead to the liberation of signal concentrations that surpass the perception threshold determined in **chapter III**.

I thus developed a simple mass balance model based on the findings from **chapter I** and **III** and previous studies: In the lab study in **chapter I**, I could quantify the amount of VOCs, extracted from 10 mg POC of *U. fimbriata* that was attractive to *L. stagnalis* in behavioural assays. In the detection threshold assays in **chapter III** with a synthetic VOC bouquet (designed to mimic the natural bouquet of *U. fimbriata*), I determined the threshold level of VOCs that lead to a clear significant behavioural response of the snails. This amount would be liberated after lysis of an algal biomass corresponding to a third of the biomass of *U. fimbriata* extracted in for the choice assays in **chapter I**. From the ingestion experiment in **chapter III,** I showed that a tenth of this biomass is ingested in one hour by a medium-sized *L. stagnalis* with a shell length of 30 mm. Thus, approximately three snails of 30 mm shell length are able to consume the algal biomass within one hour that is necessary to liberate a VOCs signal that is as strong as the threshold concentration. This can be recognized by conspecifics and elicit chemotactic food-finding behaviour. This *L. stagnalis* population density is commonly exceeded in the field, sometimes by several orders of magnitude (Moelzner, pers. observation). This is further corroborated by field experiments in which I could demonstrate that four adult snails grazing on a natural biofilm attracted conspecifics from the surroundings in a semi-natural ecosystem (**chapter II**). Hence, the mass balance model plausibly explains that gastropod grazing can liberate an odorant signal at concentrations that can be recognized by conspecifics and utilized as foraging infochemicals.

The current work clearly demonstrated that the transfer of infochemicals is important in an aquatic environment to interpret behavioural but also physiological responses of organisms to predict the resource finding success. Infochemicals distributed in the environment can provide a reliable roadmap to consumers about potential high quality resources in their proximity. From the perspective of the primary producer, herbivory is a negative interaction but it has been demonstrated that grazers play an important role for primary production in terms of nutrient recycling (McNaughton et al. 1997, Vanni 2002). Consumers indirectly fertilize primary producers like benthic algae through their dissolved and particulate excretion products and can therefore alter nutrient cycling and affect producers´ elemental composition (McNaughton et al. 1997, Liess and Haglund 2007). Several studies revealed that grazer presence can increase the nutrient content of periphyton (Hillebrand and Kahlert 2001, Frost et al. 2002). Thus, the feedback interaction among grazing, nutrient cycling and primary production might be affected by the release of VOCs. The liberation of volatile infochemicals by primary producers would attract consumers and conspecifics to the respective food patch and ensures sufficient nutrient supply through grazing mediated nutrient regeneration.

Furthermore, adaptive behavioral decisions driven by chemical cues can influence processes at coarser-scales and fine-scale structural patchiness is important for freshwater herbivores. The structural patchiness I examined is caused by a biological process (snail grazing), and thus emphasizes the natural relevance of this ecological interaction of herbivores and primary producers.

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Record of achievement

Chapter 1: The smell of good food: volatile infochemicals as resource quality indicator

Results described in this chapter were exclusively performed by me or under my direct supervision. Patrick Fink was involved in all technical discussions and discussions regarding the design and has critically read the manuscript.

Chapter 2: Consumer patchiness explained by volatile infochemicals in a freshwater ecosystem

Results described in this chapter were exclusively performed by me or under my direct supervision. Patrick Fink was involved in all technical discussions and discussions regarding the design and has critically read the manuscript.

Chapter 3: Gastropod grazing on benthic algae leads to liberation of food-finding infochemicals

Results described in this chapter were exclusively performed by me or under my direct supervision. Patrick Fink was involved in all technical discussions and discussions regarding the design and has critically read the manuscript.

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[1] entspricht chapter 1

[2] entspricht chapter 2

[3] entspricht chapter 3

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