

Infochemical-mediated trophic interactions between the rotifer *Brachionus calyciflorus* and its food algae

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Abstract

We studied how chemicals obtained as filtrates from algal monocultures (algal chemicals) and from rotifer cultures with or without algae (rotifer chemicals) affected feeding rates of the rotifer *Brachionus calyciflorus* on its food algae, both directly and indirectly (through chemical-induced changes in algal morphology). Algal chemicals had a strong stimulating effect on the feeding rate of *B. calyciflorus*, but these effects were counteracted by rotifer chemicals. In functional response experiments, rotifer chemicals lowered maximum ingestion rates and had strong effects on assimilation rates and assimilation efficiencies of *B. calyciflorus*, probably due to the release of unspecific (auto)toxic metabolites. Furthermore, rotifer chemicals induced colony formation in the food alga *Scenedesmus obliquus*. Above the optimum particle size for ingestion by *B. calyciflorus*, larger algal colony sizes increased the food-handling time, thus lowering ingestion and assimilation rates. Through their effects on trophic interactions, infochemicals may play a role in structuring and the functioning of aquatic food webs.

Aquatic organisms continuously excrete a rich mixture of all kinds of chemicals that can be used by other individuals as information chemicals. These infochemicals may affect the behavior and physiology of aquatic organisms, and as such alter the structure, functioning, and evolution of food webs. Despite the potential importance of this “infochemical web” for aquatic food webs, there is scant information on what kinds of infochemically mediated feedbacks may occur in aquatic food webs, particularly in comparison with the complex feedback mechanisms that have been described for terrestrial food webs (e.g., Dicke and Grostal 2001). Therefore, we need more insight into how and to what extent the infochemical web is capable of affecting aquatic trophic interactions. We investigated this by using a simple bitrophic system of one rotifer species and its phytoplankton food.

The cosmopolitan herbivorous rotifer *Brachionus calyciflorus* Pallas is a typical inhabitant of many eutrophic freshwater systems (e.g., Jeppesen et al. 1990; Gosselain et al. 1998), and is widely used as food organism in freshwater aquaculture. Although the feeding of *B. calyciflorus* has

been the subject of study for decades (e.g., Starkweather 1980b; Rothhaupt 1990b; Fussmann et al. 2005), the chemical ecology of its feeding is less well understood, in particular with respect to the chemicals and causal mechanisms involved (Snell 1998). Our study investigated the direct and indirect effects of the chemicals that are involved in the feeding interaction between *B. calyciflorus* and its algal food (Fig. 1). Potential interactions include the effects of algal chemicals on rotifer feeding (Fig. 1A), effects of rotifer chemicals on rotifer feeding (Fig. 1B), and effects of rotifer chemicals on algae, which alter algal morphology (Fig. 1C) and hence indirectly affect rotifer feeding.

In the complex mixture of suspended organic and inorganic particles, feeding starts with locating food and recognizing it as being edible. To do that the corona of *B. calyciflorus* is surrounded by many chemo- and mechanosensory neurons (Clément et al. 1983). *B. calyciflorus* and congeners change their swimming and feeding behavior in response to food type and density in the environment (Starkweather 1980b; Snell et al. 1987). Individual *B. calyciflorus* have been reported to attach more frequently, swim more slowly, and increase their turning rates in algal suspensions compared with control medium (Charoy and Clément 1993). To select its algal prey, *B. calyciflorus* is largely dependent on tactile information (mechanoreception), and shows a clear size preference (DeMott 1986; Rothhaupt 1990a,b). Nevertheless, *B. calyciflorus* is able to detect algal patches in the absence of physical contact (Charoy 1995), which suggests that algal odors (Fig. 1A) could be involved in the foraging behavior of *B. calyciflorus*.

Chemicals released by conspecifics (crowding chemicals) induce shifts in life history. Medium from crowded cultures induces mixis in *Brachionus* species (Gilbert 2003; Stelzer and Snell 2003), and negatively affects several demographic parameters in various rotifer species (Kirk 1998; Yoshinaga et al. 1999). To understand the variety of crowding

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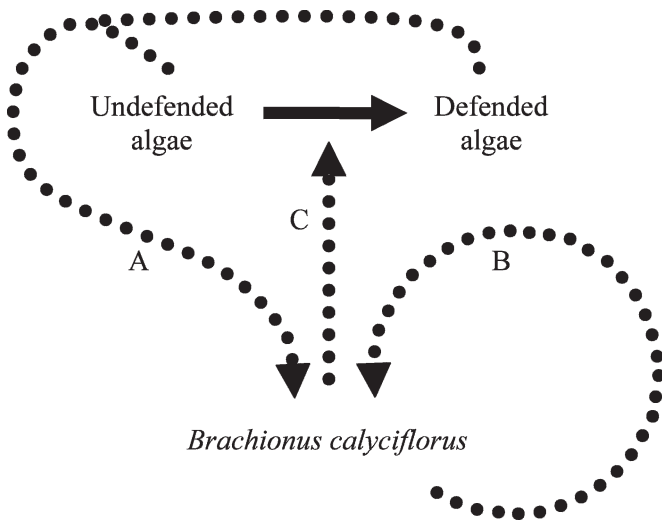


Fig. 1. Scheme of potential effects of algal- and rotifer-released chemicals on the feeding of *Brachionus calyciflorus* on its food algae. Several chemically mediated pathways (dotted) are involved in these interactions: (A) algae-released chemicals may affect feeding; (B) rotifer-released chemicals may affect feeding, and (C) rotifer-released chemicals may induce colony formation in algae (solid arrow), which in turn may affect feeding.

responses described in zooplankton literature, at least two types of crowding-related chemicals need to be distinguished. The first type comprises species-specific infochemicals (pheromones), which may affect reproductive parameters of *Brachionus* species (e.g., Yoshinaga et al. 1999; Gilbert 2003; Serra et al. 2005). The second type comprises species-unspecific metabolites, which have autotoxic effects (e.g., reduced feeding rates, reduced enzyme activities) when exceeding certain threshold concentrations. Both types of chemicals might affect the feeding of *B. calyciflorus* (Fig. 1B), since *B. calyciflorus* could actively reduce its feeding activity in high-density patches of conspecifics, or experience (auto)toxic effects on feeding because of unspecific metabolites.

In addition to these direct effects on feeding, indirect infochemically mediated effects may also alter food intake rates. *B. calyciflorus* excrete chemicals (Fig. 1C, dotted arrow) that may induce defenses in their algal food (Fig. 1C, solid arrow). In the natural, eutrophic habitat of *B. calyciflorus*, green algae such as *Scenedesmus* are often dominant (e.g., Jeppesen et al. 1990; Gosselain et al. 1998), and may constitute a large part of the diet of *B. calyciflorus*. *B. calyciflorus* infochemicals induce colony formation in many strains of the chlorophyte genera *Scenedesmus* and *Desmodesmus*, and also for these species, colony formation is proportional to the infochemical concentration in the test medium (Verschoor et al. 2004a).

The adaptive significance of infochemical-induced colony formation in *Scenedesmus obliquus* has generally been thought to be a defense mechanism against small herbivores such as rotifers and small cladocera (Van Donk et al. 1999). However, most evidence for this defense is circumstantial, and the real defensive benefits of colony formation (i.e., diminished grazing) have only received marginal

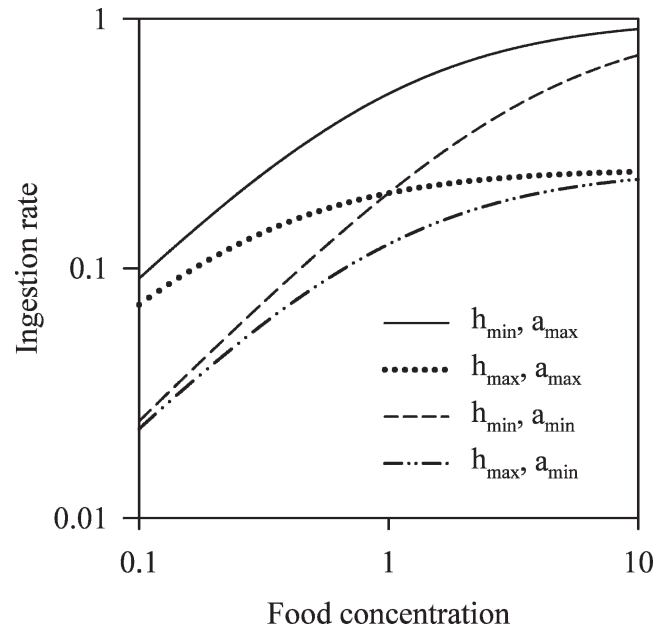


Fig. 2. Effect on feeding rates when changing the two parameters of an arbitrarily scaled type II functional response: handling time (h) and attack rate (a) to either minimum (min) or maximum values (max). There are four possible outcomes: (1) reference functional response, maximum attack rate, and minimal handling time (solid line); (2) increased handling time (and maximum attack rate) (dotted line); (3) lowered attack rate (and minimal handling time) (dashed line); (4) lowered attack rate and increased handling time (dash-dotted line). Note the logarithmic scales.

attention this far. Many of these colonies are still within a size range that could be ingested by *B. calyciflorus* (Rothhaupt 1990b), so they may not offer complete defense against ingestion. If a defense is not completely effective, the protective benefit of it depends on prey density: Defenses may affect attack rate or handling time of the prey, or both (Jeschke and Tollrian 2000). The qualitative differences between these two effects of defenses can be illustrated by incorporating them in Holling's (1959) type II functional response model (Fig. 2, solid line), which uses those two parameters (attack rate and handling time) to describe feeding rates as a function of food concentration. If colony formation would affect handling time, defenses would be mainly effective at high prey densities (Fig. 2, dotted line). If colony formation would affect attack rate (i.e., affecting the maximum clearance rate of suspension feeders), then defenses would be mainly effective at low prey densities (Fig. 2, dashed line). Obviously, defenses would be highly effective in lowering feeding rates if they would affect both handling times and attack rates (Fig. 2, dash-dotted line).

Apart from population-level effects, inducible defenses may also have effects on higher levels of organization. Parameters from the *Scenedesmus-Brachionus* system (sometimes with the rotifer *Asplanchna* as top predator) have been used in model analyses to predict the effects of inducible defenses on communities, food webs, and ecosystems (Vos et al. 2004a,b). In these models, inducible

defenses were modeled by increasing the handling time of consumers on defended algae. The analyses revealed that the average biomass of the different trophic levels in systems consisting of either undefended or constitutively defended species responds in a “classical,” stepwise fashion to enrichment. In contrast, the average biomass in systems with inducible defenses responded in a gradual and continuous manner to enrichment, which is more consistent with empirical observations (Vos et al. 2004b). Furthermore, inducible defenses are predicted to stabilize community dynamics, as compared with systems with undefended or permanently defended species (Vos et al. 2004a). The experiments in this paper provide the empirical information that is critically needed to link the described effects of infochemicals on algal colony size (Lüring and Van Donk 1997; Van Donk et al. 1999; Verschoor 2004a) with the predicted and observed effects of inducible defenses on bi- and tritrophic food chains (Verschoor et al. 2004b; Vos et al. 2004a,b).

In this paper, we address four main research questions: (1) What are the effects of algae and rotifer chemicals on the feeding rate of *B. calyciflorus* at a fixed food concentration? (2) Do rotifer chemicals alter the functional response of *B. calyciflorus*? (3) Do rotifer chemicals induce colony formation in *S. obliquus*? (4) Does increased algal size alter the functional response of *B. calyciflorus*, and what parameters are affected?

Materials and methods

A total of four experiments was carried out. Before the actual experiments were carried out, filtrates were obtained from algae monocultures, from starved *B. calyciflorus*, and from *B. calyciflorus* that had been grazing on algae. First, we compared the ingestion rates of *B. calyciflorus* at a fixed food concentration (1 mg C L⁻¹ *S. obliquus*), when exposed to these different chemicals (experiment 1). Second, we compared the functional response (both ingestion and assimilation rates) of *B. calyciflorus* when exposed to filtrate of high densities of conspecifics (rotifer chemicals; experiment 2). Third, we performed a bioassay on the morphologic response of *S. obliquus* when exposed to the algal and rotifer chemicals produced in experiment 1 (experiment 3). Finally, we compared the functional response of *B. calyciflorus* on *S. obliquus* cultured under control conditions (medium size, 149 μm³) and on *S. obliquus* cultured in medium with rotifer chemicals (induced colonies = large size, 252 μm³). We furthermore compared these functional responses with those on a small, non-inducible strain of *S. obliquus* (UTCC T7, 45 μm³) and on the large *Desmodesmus maximus* Hegewald (UTEX 614, 756 μm³) (experiment 4), as the latter two closely related Scenedesmaceae seem to lack inducible colony formation (Verschoor et al. 2004a).

The following phytoplankton species and strains have been used: *Scenedesmus obliquus* from the culture collections of the University of Toronto, Canada (UTCC T7), from the Max Planck Institute for Limnology, Germany (MPI), from the University of Texas at Austin, United States of America (UTEX 2630), and from the Norwegian

Institute for Water Research (NIVA-CHL6); and *D. maximus* (UTEX 614). *B. calyciflorus* were raised from commercially available resting eggs (MicroBiotests). COMBO medium was used as universal phytoplankton and zooplankton medium (Kilham et al. 1998).

Experiment 1. Effects of algal and rotifer chemicals on rotifer ingestion rates—*S. obliquus* UTEX 2630 were incubated in densities of 0, 10, and 100 mg C L⁻¹, combined with *B. calyciflorus* in densities of 0, 10, and 100 individuals mL⁻¹, yielding nine different algae × rotifer density combinations. Initial algal densities were calculated from regressions between absorption at 750 nm measured on a Unicam Helios δ photospectrometer and carbon content measured on a CALANUS carbon/nitrogen analyzer (UniQuant). After 24 h in the dark (100 revolutions per minute [RPM], 20°C), these suspensions were prefiltered over a 60-μm mesh screen and filtered over precombusted glass fiber filters (Whatman GF/F).

All nine different filtrates were used immediately to prepare suspensions of labeled *S. obliquus* UTEX 2630 of 1 mg C L⁻¹, which were applied in 10-min feeding experiments. Because of nonnormal and heteroscedastic data (Bartlett’s test), a two-way analysis of variance (ANOVA) was applied on ranked data using the Scheirer–Ray–Hare extension of the Kruskal–Wallis test (Sokal and Rohlf 2000), followed by pairwise comparisons using the Mann–Whitney *U*-test. The remainder of the filtrates was frozen (−18°C) immediately until use in experiment 3, and three of nine filtrates yielded sufficient additional material for a preliminary chemical analysis (see Discussion).

Experiment 2. Effects of rotifer chemicals on the rotifer functional response—For this experiment, we used filtrates of 10 mg C L⁻¹ *S. obliquus* MPI with *B. calyciflorus* densities of 0 (control medium) or 100 individuals mL⁻¹ (crowding water), produced as in experiment 1. Labeled algae were prepared in suspensions of 0.1, 0.2, 0.5, 1, 2, 5, and 10 mg C L⁻¹ *S. obliquus* MPI, with either control medium or crowding water, and feeding experiments were performed with *B. calyciflorus* to determine ingestion and assimilation rates.

Experiment 3. Effects of algal and rotifer chemicals on algal size—*S. obliquus* UTEX 2630 were harvested from log-phase batch cultures, centrifuged (2,500 RPM, 10 min), and suspended in the nine different filtrates that had been produced in experiment 1. Algal inoculum densities were 2 × 10⁶ μm³ mL⁻¹ in final volumes of 50 mL that were incubated in cellulose-stoppered 100-mL Erlenmeyer bottles. Incubations were done in triplicate for 48 h in an incubator at 20°C, 100 RPM, and receiving continuous light (120 μmol photons photosynthetically active radiation [PAR] m⁻² s⁻¹, cool white fluorescent tubes). Colony sizes (mean particle volumes) were measured at *t* = 0 and *t* = 48 h using a CASY cell counter (Schärfe System). Because of heteroscedasticity of the data (Bartlett’s test), we analyzed log-transformed final particle volumes using two-way ANOVA, followed by pairwise comparisons between treatments using Tukey’s honestly significant differences (HSD) test.

Experiment 4. Effects of algal size on the rotifer functional response—This experiment investigated the functional response of *B. calyciflorus* on differentially sized strains of Scenedesmaceae: the small *S. obliquus* UTCC T7, which has little or no inducible colony formation in response to zooplankton infochemicals in previous experiments (Lürling 1999; Verschoor et al. 2004a), two sizes of the larger *S. obliquus* UTEX 2630 (control and infochemically induced), and on the very large and spiny *D. maximus* UTEX 614. Differently sized *S. obliquus* UTEX 2630 were obtained by incubating 10 mg C L⁻¹ algae with 100 *B. calyciflorus* mL⁻¹ (treatment), or with algae alone (control) in the dark for 24 h, and filtering as previously described. *S. obliquus* UTEX 2630 were either incubated with 20% (vol) treatment or control medium, and all algal strains were labeled during 24 h. After that, algae were prepared in food concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10 mg C L⁻¹ (all in standard COMBO medium), and feeding experiments were performed with *B. calyciflorus* to determine both ingestion and assimilation rates.

Methods used to label algae and to determine ingestion and assimilation rates—Algae, in a concentration of 10 mg C L⁻¹, were incubated for 24 h in COMBO medium with additional 5.0 mg L⁻¹ NaH¹³CO₃ at 20° ± 0.5°C, 120 μmol photons (PAR) m⁻² s⁻¹, and 100 RPM. After incubation with the ¹³C tracer, algae were centrifuged and washed twice in C-free medium (without any NaHCO₃, NaH¹³CO₃, or organic buffer added), and measured on a CASY cell counter, and samples were taken to determine carbon content afterward on the carbon analyzer. For each food concentration (i.e., 0.1, 0.2, 0.5, 1, 2, 5, and 10 mg C L⁻¹), algal suspensions were prepared in quadruplicate in Erlenmeyer flasks. To obtain uniformly sized *B. calyciflorus*, we used animals that passed through a 120-μm filter but that were retained on an 80-μm filter; this proved to be effective to exclude both juveniles and large (often egg-bearing) individuals. *B. calyciflorus* were acclimatized to the algal concentrations that would be used in the experiment in unlabeled algae suspensions on a rotating table (40 RPM) at 20° ± 0.5°C in dim light for at least 1 h. Per experimental flask, approximately 200 *B. calyciflorus* were added to the labeled algae suspensions, final volumes being 50 mL, and using the same conditions as under acclimatization. The animals were allowed to feed for 10 min, which is half the gut residence time. After feeding, the animals were removed immediately, and all animals were rinsed immediately in C-free medium. For the determination of ingestion rates, half of these animals were temporarily preserved in petri dishes using 30 g L⁻¹ NaCl solution. NaCl was used to kill and temporarily preserve these animals while preventing addition of carbon to the medium. To determine assimilation rates, the remaining half of the animals (which already had ingested algae) was washed into Erlenmeyer flasks with fresh C-free medium, and left to defecate for 1 h. After this period, these animals were removed, rinsed again with C-free medium, and then preserved in petri dishes with NaCl solution, similarly to the animals used for the determination of ingestion rates. From these dishes (either used for ingestion or assimilation), at

least 50 animals were picked and put into 5 × 8 mm pressed tin cups (Elemental Microanalysis). These tin cups had been prerinced in 50 : 50 (vol) methanol : chloroform to remove lipid traces and dried. Once filled with animals, these cups were dried for 24 h at 70°C and then measured in a Carlo Erba 1106 elemental analyzer (EA) coupled online with a Finnigan Delta-S isotope ratio mass spectrometer (IRMS). We calculated corrected carbon-specific ingestion and assimilation rates on the basis of delta values; for more details on methods and calculations see Verschoor et al. (2005). Assimilation efficiencies were determined per replicate (bottle) from the ratio between assimilation and ingestion rates of the samples from that replicate.

Functional response estimates and comparisons—Carbon-specific uptake rates (CSR, mg C mg C⁻¹ h⁻¹), either ingestion (CSIR) or assimilation rates (CSAR), were fitted to the curvilinear Michaelis–Menten model

$$\text{CSR} = \text{FC} \times \frac{\text{CSR}_{\text{max}}}{(\text{FC}_h + \text{FC})} \quad (1)$$

with FC being the food concentration (mg C L⁻¹), CSR_{max} the maximum carbon-specific uptake rate (mg C mg C⁻¹ h⁻¹), and FC_h the half-saturation food concentration (mg C L⁻¹). This model is mathematically equivalent to a Holling type II model (Real 1977), but has parameters that are easier to interpret when using carbon-specific rates. Fitting was done by iterative nonlinear regression of the functional response model on CSIR and CSAR, which were log₁₀-transformed because of heteroscedasticity. We also fitted type I and type III functional response models (Holling 1959), but the type II model gave better model fits for all CSIR and for most CSAR.

We performed pairwise comparisons between treatments that were directly adjacent, and decided on the basis of these results that it was not necessary to test for differences between the nonadjacent species pairs. For these comparisons, our null hypothesis (H₀) was that the separate functional response models were not significantly better predictors than when the model was fitted to the pooled observations. This hypothesis was compared against the alternative hypothesis (H₁) that the separate models were better predictors, using the significance of the maximum likelihood ratio statistic *G* (also known as *G*², Bishop et al. 1975),

$$G = 2N \times \ln \left(\frac{\text{MS}_{\text{among}}}{\text{MS}_{\text{within}}} \right) \quad (2)$$

with *N* being the total number of observations (i.e., samples), MS_{among} the total variance of the residuals between the log₁₀-transformed predicted and observed values of the alternative model (H₁), and MS_{within} the variance of the residuals of the null model (H₀). *G* has a chi-square distribution with degrees of freedom corresponding to the difference between the numbers of parameters of the two models being compared.

For analysis of assimilation efficiencies, food concentrations and assimilation efficiencies were first log-transformed. We tested our data for linearity within treatments,

which was decided if only the first-order term of a polynomial regression was significant. Then data were tested for homoscedasticity (Hartley F_{max}), and we applied an analysis of covariance model (ANCOVA), for which we first compared the slopes of the regressions. Since slopes of the compared regressions were not significantly different, the interaction terms were removed from the model for further tests on treatment effects.

Except for the comparisons of the functional response models, which were done manually with a spreadsheet program, all analyses were done using Statistica 6.1 (Statsoft, Inc., Tulsa, Oklahoma).

Results

Experiment 1. Effects of algal and rotifer chemicals on rotifer feeding—Neither algal density nor rotifer density alone had significant effects on the CSIR of *B. calyciflorus* (Fig. 3), but they had a significant interaction effect ($\chi^2 = 22.15$, $df = 4$, $p = 0.0002$). The highest algae density, filtrate of 100 mg C L⁻¹ algae without rotifers (hN), yielded remarkably high ingestion rates in all replicates, and was significantly different from all other treatments (Table 1). The second-highest algae density had effect in the absence of rotifers (mN > nN), and regression of CSIR against the concentration of “algal smell” in the absence of rotifer chemicals is significant (CSIR = 0.0007 × algae concentration [mg C L⁻¹] + 0.1074, $r^2 = 0.80$, $p < 0.0001$).

The interaction between algal and rotifer chemicals is complex (Fig. 3, Table 1): in the absence of other food cues (i.e., algal chemicals), the highest concentration of rotifer chemicals (nH) had a slightly stimulating and significant effect, but no significant effects of rotifer chemicals were present at intermediate algal chemical levels (compare mN, mM, and mH). At the highest concentration of algal chemicals, rotifer chemicals had a significant inhibiting effect on the stimulating effect of algal chemicals (hH < hM < hN).

Experiment 2. Effects of rotifer chemicals on rotifer functional response—When *B. calyciflorus* was exposed to rotifer chemicals, both ingestion (Fig. 4A) and assimilation rates (Fig. 4B) decreased. Maximum ingestion and assimilation rates were lowered by 23% and 39%, respectively; half-saturation food concentrations for ingestion and

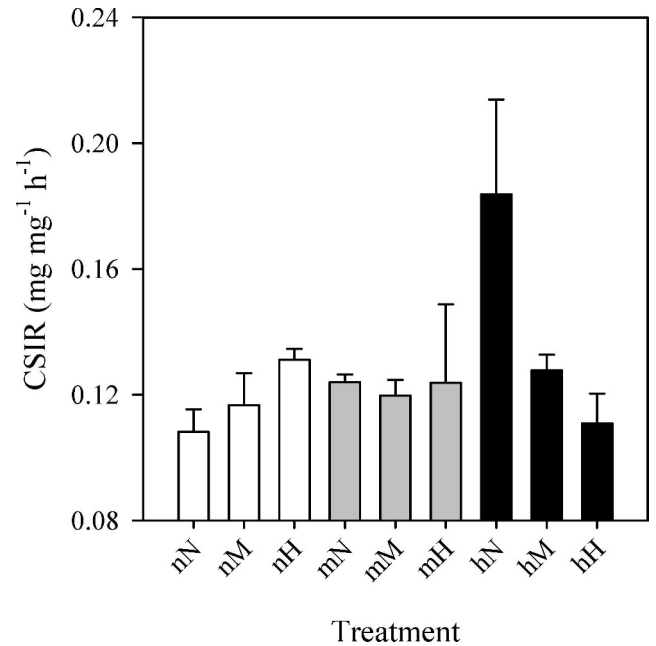


Fig. 3. Effect of chemicals released by *S. obliquus* and *B. calyciflorus* on averages of carbon-specific ingestion rates (CSIR) of *B. calyciflorus* on 1 mg C L⁻¹ *S. obliquus* MPI. Different treatments are filtrates of different densities of *S. obliquus* and *B. calyciflorus* before 24-h incubation. Treatment codes: first letter stands for *S. obliquus* density: n = no algae (white), m = moderate density (10 mg C L⁻¹, gray), h = high density (100 mg C L⁻¹, black); second (capital) letter stands for *B. calyciflorus* density: N = 0, M = moderate density (10 individuals mL⁻¹), H = high density (100 individuals mL⁻¹). Error bars represent +1 SD.

assimilation rates were 6% and 10% lower, respectively (Table 2). Effects of rotifer chemicals on the functional response were significant (Table 2). Also, ANCOVA revealed that when exposed to rotifer chemicals, assimilation efficiencies were significantly lower (Fig. 4C, Table 3).

Experiment 3. Effects of algal and rotifer chemicals on algal colony formation—Mean particle volumes (MPVs) of *S. obliquus* exposed to the same chemicals as in experiment 1 show a quite different pattern (Fig. 5) as compared with CSIR (Fig. 3). Both algal density and rotifer density had significant effects on MPVs (algae, $F_{2,26} = 4.94$, $p = 0.020$;

Table 1. Significance levels of the pairwise comparisons (Mann–Whitney *U* test) among the different treatments applied in experiment 1. Treatment codes are as in Fig. 3. Bold values indicate significantly different carbon-specific ingestion rates (all $U=0.00$, $df=4,4$, $p=0.0286$). N.S., not significant.

	<i>p</i>	Treatment code							
		nN	nM	nH	mN	mM	mH	hN	hM
Treatment code	hH	N.S.	N.S.	<0.05	<0.05	N.S.	N.S.	<0.05	<0.05
	hM	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.	<0.05	
	hN	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05		
	mH	N.S.	N.S.	N.S.	N.S.	N.S.			
	mM	<0.10	N.S.	<0.05	N.S.				
	mN	<0.05	N.S.	<0.05					
	nH	<0.05	<0.05						
	nM	N.S.							

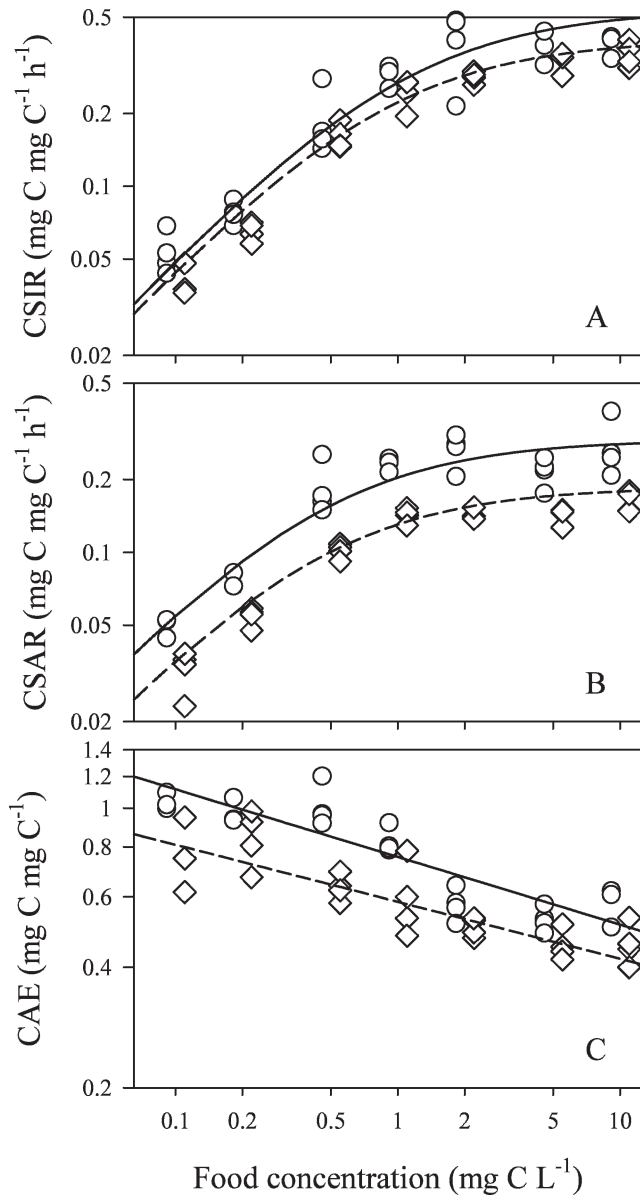


Fig. 4. (A) Carbon-specific ingestion rates (CSIR), (B) carbon-specific assimilation rates (CSAR), and (C) carbon assimilation efficiencies (CAE) of *B. calyciflorus* feeding on different concentrations of *S. obliquus* UTEX2630 in control medium (open circles) and rotifer-conditioned medium (open diamonds). For CSIR and CSAR, we plotted the fitted functional response models for both control medium (solid line) and rotifer-conditioned medium (dashed line), and for CAE we plotted the resulting power functions derived from the linear regressions on the log-transformed data. Both scales are logarithmic, and symbols for rotifer-conditioned medium (diamonds) have been offset to the right for visibility.

rotifers, $F_{2,26} = 20.37, p < 0.0001$), and had a significant interaction effect ($F_{4,26} = 4.09, p = 0.016$). The treatment with most intensive grazing (highest initial algae and rotifer densities, hH) had significantly larger MPVs than all other treatments (all Tukey HSD test, $p < 0.05$), except from the treatment with moderate algae and high rotifer densities (mH, filled circles in Fig. 5). The lack of significant

Table 2. Pairwise comparison of the fitted functional response curves within the feeding experiments on the effects of rotifer-released (crowding) chemicals on the feeding of *B. calyciflorus* (Experiment 2) and on the effects of different mean particle volumes (MPVs) of Scenedesmeaceae on the feeding of *B. calyciflorus* (Experiment 4). Given are the MPVs of the different algae (μm^3), parameter estimates for maximum carbon-specific ingestion and assimilation rates (CSIR_{max} resp. CSAR_{max}; $\text{mg C mg C}^{-1} \text{h}^{-1}$), the half-saturation food concentration (FC_h; mg C L^{-1}), and the numbers of observations (*n*). Between rows, the results of the pairwise comparisons of the models are given by the maximum likelihood ratio statistic *G* (all being chi-square distributed, *df*=2) and its significance (*p*). Abbreviations used for algal strains: MPI = *S. obliquus* MPI, UTCC7 = *S. obliquus* UTCC T7, UTEX2630 = *S. obliquus* UTEX 2630 (with Unind = uninduced, and Ind = induced colonies), DM = *D. maximus* UTEX 614; for treatments: SM = standard COMBO (control) medium, RM = rotifer-conditioned medium.

	Strain	Treatment	MPV	Ingestion				Assimilation					
				CSIR _{max}	FC _h	<i>n</i>	<i>G</i>	<i>p</i>	CSAR _{max}	FC _h	<i>n</i>	<i>G</i>	<i>p</i>
Experiment 2	MPI	SM	59	0.538	1.010	22	28.86	<0.0001	0.292	0.437	22	122.07	<0.0001
	MPI	RM	59	0.414	0.848	25			0.178	0.394	26		
Experiment 4	UTCC7	SM	45	0.432	1.500	25	2.73	0.26	0.312	1.222	25	45.22	<0.0001
	UTEX 2630- Unind	SM	149	0.364	1.116	22	53.51	<0.0001	0.198	0.645	22	102.00	<0.0001
	UTEX 2630- Ind	SM	252	0.207	0.686	22	47.68	<0.0001	0.094	0.224	22	31.02	<0.0001
	DM	SM	756	0.113	0.188	23			0.062	0.099	21		

Table 3. Results of the ANCOVA tests on log-transformed assimilation efficiencies, determined from feeding experiments on the effects of conspecific (crowding) chemicals on the feeding of *B. calyciflorus* (experiment 2) and on the feeding of *B. calyciflorus* on Scenedesmaceae of different mean particle volumes (experiment 4). Neither experiments 2 nor 4 revealed significant differences between slopes (exp. 2: $MS=0.000065$, $F_{1,42}=0.0018$, $p=0.97$; exp. 4: $MS=0.0206$, $F_{3,76}=0.18$, $p=0.69$), so the interaction terms (log food concentration \times treatment) were removed for further testing on treatment effects. Log-transformed data were used to assure homoscedasticity and a linear correlation between dependent and independent variables.

Test	Source	df	Mean square	F value	p
Experiment 2	Log food concentration	1	0.2680	7.48	0.0090
	Treatment (rotifer chemicals)	1	0.2692	7.51	0.0089
	Error	43	0.0358		
Experiment 4	Log food concentration	1	0.4776	12.94	0.0006
	Treatment (algae size)	3	0.0593	1.61	0.19
	Error	79	0.0369		

response of algae exposed to either filtrate from high algal densities alone (hN) or from high rotifer densities alone (nH) indicates that algal MPV was only affected by filtrate in which sufficient grazing had taken place.

Experiment 4. Effects of algal MPV on rotifer feeding—Similarly to experiment 3, rotifer chemicals from actively grazing rotifers increased colony size in *S. obliquus* UTEX 2630. MPVs of this strain and the other strains investigated are given in Table 2. Plateau ingestion and assimilation rates of *B. calyciflorus* decreased with increasing MPV (Fig. 6A,B), caused by lowered maximum ingestion and

assimilation rates and lower half-saturation food concentrations (Table 2). Most pairwise comparisons of the ingestion and assimilation rates yielded highly significant differences, except for the comparison of ingestion rates between *S. obliquus* UTCC-T7 ($45 \mu\text{m}^3$) and uninduced UTEX 2630 ($149 \mu\text{m}^3$).

There was no observable effect of particle size on assimilation efficiencies (Fig. 6C), and ANCOVA did not show a significant treatment effect (Table 3).

Discussion

We found empirical support for all the hypothesized infochemical effects (Fig. 1) in our *Brachionus*–*Scenedesmus* system. This implies that many trophic interactions in the aquatic environment could be modified or even regulated through infochemicals. The next paragraphs will discuss to what extent the observed effects could play a role in aquatic ecosystems.

Algal smell and rotifer feeding rates—In the absence of rotifer chemicals, algal chemicals stimulated feeding rates of *B. calyciflorus* at intermediate and high algal concentrations (10 and 100 mg C L^{-1}). Although such densities may occur in the habitats of *Scenedesmus* and *Brachionus* (e.g., 30 mg C L^{-1} , Jeppesen et al. 1990), they are within the high-density range of natural systems. To consider whether this is common or extreme, one should be aware that organisms in natural systems always occur in patches because of various physical, chemical, and biological factors. Usually, field densities have been reported as average densities, i.e., integrated samples of (a part of) the water column, and averaged over different sampling points, thereby losing the spatial resolution required to detect low- and high-density patches. During our 10-min feeding experiments, our rotifers filtered only a few cubic millimeters per individual. It is within these small temporal and spatial scales that the organisms already appeared to be sensitive to different chemical concentrations. Also, turbulent eddies, which create heterogeneity in any seemingly homogeneous type of water body, range in size from millimeters to centimeters (Mitchell and Fuhrman 1989). It is important to realize that this (micro)patch scale is exactly the scale that is relevant to the ecological interactions that

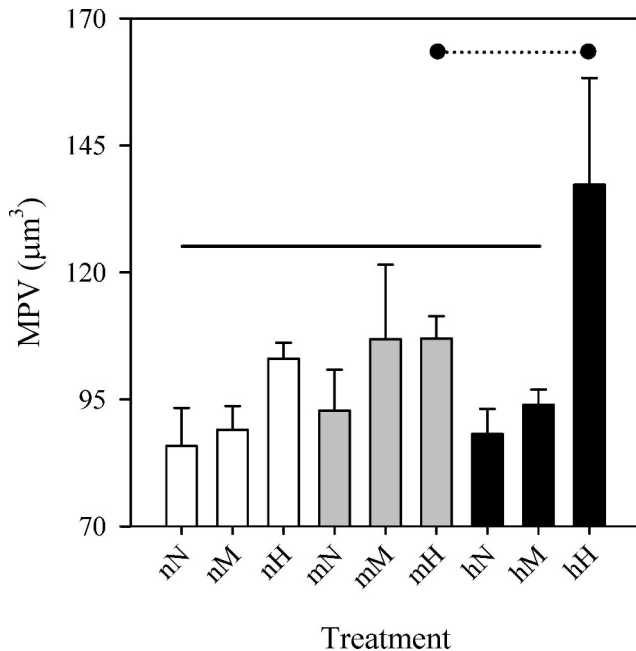


Fig. 5. Effect of chemicals released by *S. obliquus* and *B. calyciflorus* on averages of mean particle volumes (MPV) of *S. obliquus* UTEX2630. Different treatments are filtrates of different densities of *S. obliquus* and *B. calyciflorus* before 24-h incubation. Treatment codes and bar fills are as in Fig. 3; error bars represent +1 SD. Homogenous groups (treatments that were not statistically different at the 95% level, Tukey HSD test) are represented by the solid horizontal line (all except hH) and by the filled circles connected by a dotted line (mH + hH).

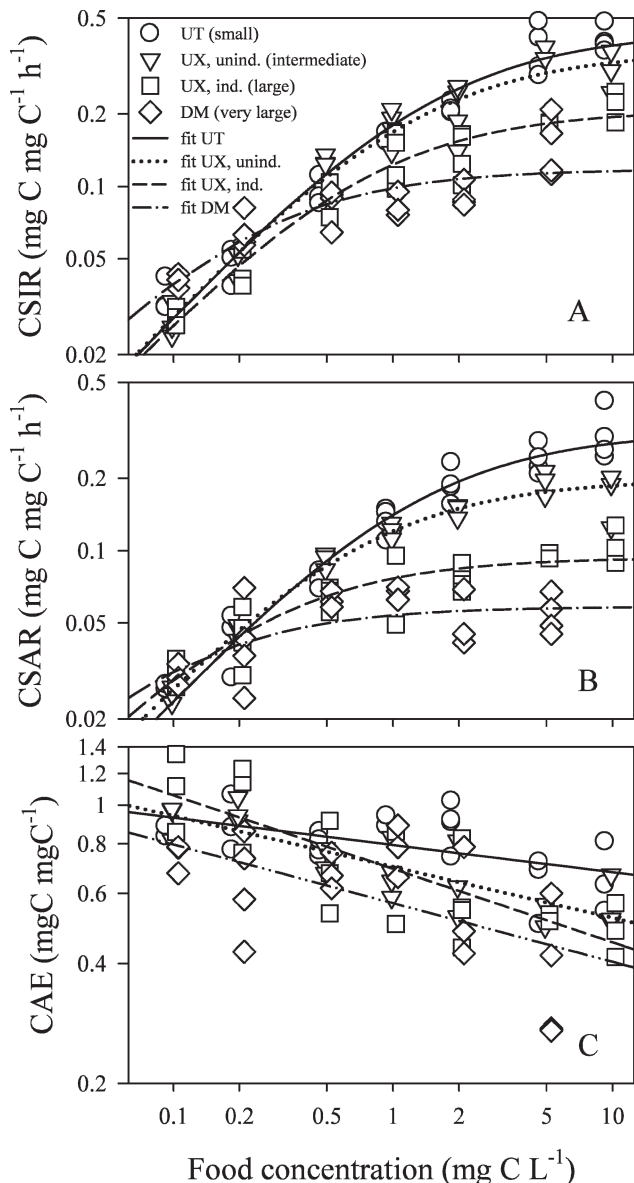


Fig. 6. (A) Carbon-specific ingestion rates (CSIR), (B) carbon-specific assimilation rates (CSAR), and (C) carbon assimilation efficiencies (CAE) of *B. calyciflorus* feeding on different concentrations of algal strains. Strains and treatments were: *S. obliquus* UTCC T7 (UT: 45 μm^3); uninduced *S. obliquus* UTEX 2630 (i.e., exposed to control medium [UX, unind.: 149 μm^3]); *B. calyciflorus*-induced colonies of UTEX2630 (UX, ind.: 252 μm^3); and *D. maximus* UTEX614 (DM: 756 μm^3). For CSIR and CSAR, we plotted the fitted functional response models for all species, and for CAE we plotted the resulting power functions derived from the linear regressions on the log-transformed data. Both scales are logarithmic.

we investigated: it is these scales at which algal and rotifer patches occur and where they have to respond to the local chemical gradients.

Algae in any natural system occur in patches such as surface scums, biofilms, (micro)layers, or micropatches (e.g., Waters and Mitchell 2002). Algal patchiness will result in a strong chemical patchiness due to persistent

boundary layers around small particles (Alldredge and Cohen 1987). Within such strong chemical gradients, distance chemoreception (smell) works optimally (Atema 1996). This enables *Brachionus* to track algal patches precisely (Charoy 1995; Ignoffo et al. 2005), and to deplete such patches within a short time span (Ignoffo et al. 2005), which is in line with our own observations. Contact chemoreception (“taste”) does not seem to have a very high resolution in *B. calyciflorus* (DeMott 1986; Rothhaupt 1990a; Starkweather 1995), but *B. calyciflorus* has a clear size preference when offered differentially sized particles (DeMott 1986; Rothhaupt 1990a; this study). This suggests that distance chemoreception is the main sensory modality of *B. calyciflorus* to detect the presence of food patches, and mechanoreception is used to discriminate among food particles when they are within contact distance.

Crowding chemicals, optimal foraging, and density dependence—The stimulating effect of high levels of algal chemicals on the feeding rates in experiment 1 appeared to be counteracted by rotifer chemicals. As soon as intermediate levels of rotifer chemicals were present with the algal chemicals, the stimulating effect was reduced and was not detectable anymore at high levels of rotifer chemicals. This points to an antagonistic interaction between the stimulating effect of algal chemicals and the dampening effect of rotifer chemicals. This would fit within an optimal patch choice strategy (Charnov 1976), with maximum feeding activity in or near high-density food patches, and lower feeding activity or even evasion when high densities of competitors are present. The slightly stimulating effect of high concentrations of rotifer chemicals in the absence of algal chemicals does not seem to make sense immediately, but in this particular situation (i.e., in the absence of any cues from the algae), rotifer chemicals could be the most reliable indicator of the presence of food.

Over a larger range of food concentrations (experiment 2) we found a weak but significant inhibiting effect of rotifer chemicals on ingestion rates, and a more pronounced effect on assimilation rates. It is not very likely that the “crowding” effects were caused by pheromones; these are usually very specific and are already effective at low concentrations. For example, mixis is already induced at low densities of *B. calyciflorus* (around 70 individuals L^{-1} ; Serra et al. 2005), whereas we found negative effects on the functional response at densities that were several orders of magnitude higher (100 individuals mL^{-1}). Although this is a very high density for a field situation, patch densities in the order of several hundreds of rotifers per milliliter have already been found at a relatively coarse sampling resolution of 10 cm (Finlay and Esteban 1998). This means that at a more relevant field scale (i.e., mm–cm scale) with even more deleterious effects of conspecific patches on feeding could be present.

Since we do not think that pheromones were involved, and because the feeding behavior of *B. calyciflorus* is a sensitive proxy for toxicity (Snell et al. 1987; Snell 2005), we presume that rotifer feeding was affected by toxic, unspecific metabolites. From a preliminary chemical

analysis of our rotifer chemicals, we found that several organic and inorganic metabolites could have been responsible for the observed effects. Our crowding water contained higher levels of organic carbon, proteins, carbohydrates, dissolved phosphorus, and ammonia. Un-ionized ammonia is best studied for its rapid and deleterious effects on *Brachionus* species; it may reduce swimming, and thus feeding, activity within a 10-min exposure period (Snell et al. 1987). However, observed levels of total ammonia in our crowding water (containing filtrate of 10 mg C L⁻¹ *S. obliquus* and 100 *B. calyciflorus* mL⁻¹) were 0.33 mg L⁻¹. Of this concentration, at most a small percentage was un-ionized (pH measured was 7–8), whereas threshold concentrations for toxic effects on *Brachionus* spp. are in the order of several milligrams per liter (De Araujo et al. 2000, 2001). Probably, other chemicals than un-ionized ammonia were responsible for the observed reduction of feeding rates. Regardless of their exact chemical structure, these self-excreted chemicals apparently have significant effects on ingestion and assimilation rates of rotifers.

Empirical observations on density dependence of the functional response of *B. calyciflorus* reported adverse effects at similar densities as in our experiments (125 individuals mL⁻¹; Fussmann et al. 2005), and density-dependent reduction of population growth of different rotifer species in the laboratory also occurred at similar densities (10–100 individuals mL⁻¹; Kirk 1998; Snell et al. 2001). Our results make it at least plausible that dense rotifer populations could be regulated through autotoxic metabolites. Even so, because population processes take place at larger temporal and spatial scales, it would require extremely high (average) rotifer densities before such autotoxic effects would be observable in the field.

Inducible colony formation: adaptive significance—The significant interaction effect of algal and rotifer densities on colony formation in experiment 3 suggests that induction of colony formation in *S. obliquus* occurs through chemicals that are released by grazing *B. calyciflorus*, and not by chemicals from rotifers or algae alone. This is in line with earlier observations that MPV increases with increasing concentration of rotifer-released grazing chemicals (Verschoor et al. 2004a), which also has been found for Cladocera (Lürling 2003). There appear to be large differences in sensitivity to the infochemical concentration, even among strains of the same species. This paper shows that quite high densities of rotifers (100 mL⁻¹) are required to induce colony formation in *S. obliquus* UTEX2630, whereas for *S. obliquus* MPI, a threshold concentration for colony induction of only 0.1 rotifer mL⁻¹ has been found previously (Lürling and Van Donk 1997; Verschoor et al. 2004a). These differences in sensitivity may also explain why some strains or species do not appear to make colonies in response to herbivores: they might require a higher threshold concentration for colony induction (Verschoor et al. 2004a).

Various Scenedesmaceae strains do not only appear to make colonies in response to *Brachionus*, but also in response to information chemicals from other herbivorous

zooplankton, such as different Cladocera species (e.g., *Daphnia*, *Bosmina*), other rotifers, and copepods (Van Donk et al. 1999; Lürling 2003; Verschoor et al. 2004), but not to carnivorous Cladocera (Lürling, 2003). Also, *S. obliquus* forms colonies in response to filtrates of natural lake water during periods of high rotifer abundance (Lürling and Van Donk 1997), as well as to lake water filtrate when other herbivore species were dominant (Van Donk et al. 1999). Also, many *Scenedesmus* strains are unicellular in laboratory cultures, whereas they appear as colonies in the field (Van Donk et al. 1999), which at least suggests that herbivory could be an important factor for the phenotypes of these algae in the field. Inducible colony formation seems to be a general strategy against herbivory, with induced colony size dependent upon the actual risk of being grazed.

The disadvantage of a general strategy against herbivory is that it is not necessarily optimal against a single herbivore species, as different herbivores all have different feeding constraints. For *B. calyciflorus*, values of CSIR_{max} for *S. obliquus* MPI in standard medium in experiment 2 were actually higher than for the smaller *S. obliquus* UTCC-T7 (Table 2). We compared our data with estimates of CSIR_{max} derived from other (literature) data on short-term tracer-based feeding experiments with *B. calyciflorus* and the closely related (similarly sized) *B. angularis* (Fig. 7). This figure suggests a unimodal optimum in particle size, comparable with what has been found for the size preference of many rotifer species (e.g., Starkweather 1980a; Ronneberger 1999). We could not support Rothhaupt's (1990b) observation that *B. calyciflorus* has a type I functional response (with constant CSIR_{max}) below the optimum food particle size and a type II functional response above the optimum size. Our analyses always yielded higher residuals for both type I and type III functional responses for all particle sizes investigated (not shown).

An explanation for the unimodal size optimum may be found in the feeding physiology of *B. calyciflorus*. With increasing food particle size, particle retention efficiency increases. However, together with increasing retention efficiency, *B. calyciflorus* is also able to actively regulate its food uptake. One important mechanism involved in food uptake regulation is the so-called "pseudotrochal screening," which is involved in preselection/rejection of food particles, especially in dense suspensions of large particles (Starkweather 1980b). Starkweather (1980b) found a steep increase in the proportion of time spent on pseudotrochal screening when food particle sizes were larger than 4 μm equivalent sphere diameter (ESD). This was found at food concentrations where ingestion rates can be expected to approach IR_{max} (i.e., 100 mg L⁻¹ dry weight), and thus are only limited by particle handling time. The sudden increase in incidence of pseudotrochal screening matches very well with the sudden decrease in CSIR_{max} (=1/handling time) above the optimum particle size of ~5 μm ESD (Fig. 7, occurrence of pseudotrochal screens in *B. calyciflorus* individuals plotted as solid diamonds connected by a dashed line). This further implies that below the optimum particle size, an increase in size could even be

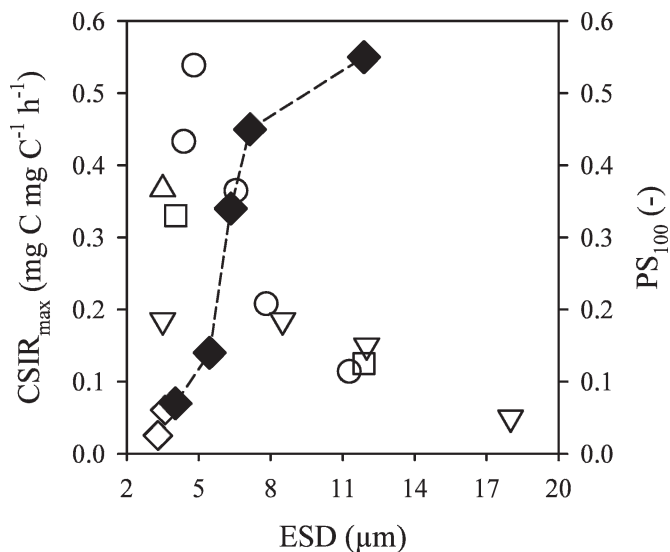


Fig. 7. Effect of particle size, here represented as equivalent spherical diameter (ESD), on maximum ingestion rates. Comparison of our estimated maximum carbon-specific ingestion rates with estimates based on literature on *B. calyciflorus* and *B. angularis* (a closely related species) feeding on differently sized food particles. For convenience and comparability with results reported in the literature, algal sizes are expressed as ESD rather than as MPV. Our data (open circles) contain maximum ingestion rates on *S. obliquus* UTCC T7 (4.4 μm ESD), on *S. obliquus* MPI in standard (control) medium (3.8 μm), uninduced *S. obliquus* UTEX 2630 (6.6 μm), induced (colonial) UTEX2630 (7.8 μm), and *D. maximus* UTEX614 (11.3 μm). Open diamonds: *B. angularis* (3.3 and 3.6 μm, Walz and Gschloessl 1988); open squares: *B. calyciflorus* (4.1 and 11.9 μm, Starkweather 1980b); open triangles down: *B. calyciflorus* (3.5, 8.5, 12, and 18 μm, Rothhaupt 1990b); open triangle up: *B. calyciflorus* (3.5 μm, Fussmann et al. 2005). Filled diamonds (and dashed line): proportion of *B. calyciflorus* observed with pseudotrochaeal screens for differently sized food items, offered at a dry weight concentration of 100 mg L⁻¹ (PS₁₀₀, given for 4.0, 5.5, 6.3, 7.1, and 11.9 μm, Starkweather 1980b).

maladaptive since it would only increase the efficiency with which that alga is caught by its herbivore. Indeed, Lüring (1999) found higher ingestion rates of *B. calyciflorus* on *S. obliquus* colonies, as compared with unicellular *S. obliquus*. To understand why this maladaptivity occurs, we have to realize that *S. obliquus* can encounter many potential herbivores that may not be distinguished on the basis of their infochemicals (Verschoor et al. 2004a). Under these conditions, a general response against herbivory, effective against most herbivores, is generally better than not responding at all.

Infochemicals in food webs—We saw that (info)chemicals are able to regulate the interaction strength between aquatic herbivores and their algal prey. This may occur through direct stimulation or inhibition, and this may in turn affect population dynamics, e.g., due to autotoxicity (Kirk 1998), although this effect is only present at very high rotifer concentrations. Indirect inhibition may occur because of defense-mediated effects on trophic interactions, and these defenses may already occur at relatively low

herbivore densities (Lüring and Van Donk 1997; Verschoor et al. 2004a). Inducible colony formation occurs frequently in many algal taxa, and as a response to many different herbivores (Van Donk et al. 1999; Tang 2003; Verschoor et al. 2004a). Such defenses, which alter predator handling times, have been predicted to alter the response of food chains to enrichment (Vos et al. 2004b) and to stabilize population fluctuations (Vos et al. 2004a). This stabilizing effect has been studied in bi- and tritrophic laboratory food chains with inducible defended *S. obliquus* or undefended *Desmodesmus bicellularis*, *B. calyciflorus*, and *Asplanchna brightwelli* (top predator). Food chains with undefended algae had indeed high fluctuations of all trophic levels, sometimes even leading to extinction of the top predator *Asplanchna*, whereas this did not occur in food chains with inducible defended algae (Verschoor et al. 2004b; Van der Stap et al. 2006). These experiments provide compelling evidence that infochemicals alter the structure and functioning of aquatic food webs through their effects on the feeding rates and functional response of the zooplankton. Studying these modifications of algae-zooplankton interactions is not only of academic interest, but will be essential for the understanding of aquatic ecosystems.

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