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Received: 10 August 1995
Accepted: 1 October 1996

Morphological changes in *Scenedesmus* induced by infochemicals released in situ from zooplankton grazers

Abstract—Biochemical substances released from *Daphnia galeata* induced colony formation in the green alga *Scenedesmus acutus*. Normally this strain consisted mainly of single cells in cultures. However, if exposed for 48 h to either water with live *Daphnia* or to 0.1- μ m filtered water from a culture with *Daphnia* present, these unicellular “Chodatella” stages were induced to form colonies (coenobia). Colony induction was not unique to *Daphnia*; other zooplankters (rotifers and copepods) were able to induce colonies in *S. acutus* as well. This morphological response could also be evoked when *Scenedesmus* was exposed to 0.1- μ m filtered lake water during high zooplankton abundances. Especially during early spring, a clear relationship was found between rotifer abundance and colony formation in our test alga in the laboratory. Filtered lake water, incubated nonaxenically for at least 2 d at 20°C, did not induce colony formation, possibly due to microbial degradation. The morphological changes in *Scenedesmus* could promote grazing resistance in small zooplankters and can be interpreted as an adaptive antipredator strategy.

Information transfer by chemicals in aquatic systems has gained attention recently but has mainly focused on the response of zooplankton to the presence of potential predators (Larsson and Dodson 1993). Infochemicals (for terminology, see Dicke and Sabelis 1988) exuded by carnivorous zooplankton (DeBeauchamp 1952; Gilbert 1966, 1967), by planktivorous fish (e.g. Ringelberg 1991; Loose et al. 1993; Tollrian

1994), and by invertebrates (Dodson 1989; Hanazato 1990, 1991; Tollrian 1993) have been reported to induce defenses in zooplankton. These predator-induced defenses are widespread among the protozoa, rotifers, and cladocera (Havel 1987; Larsson and Dodson 1993) but are not universal (Kuhlmann and Heckmann 1985; Havel 1987). While predator-induced defenses in aquatic systems are well documented, hardly any information on herbivore-induced defenses exists. Studies on herbivore-induced defenses are mainly focused on chemical defenses in individual terrestrial plants to protect the same individual against herbivory. However, some evidence has been reported on plants responding to chemical cues emitted from attacked individuals of the same species (see Havel 1987). In the aquatic macrophyte *Potamogeton*, reduced palatability was observed after the plants had been attacked by caddis larvae (Jeffries 1990). Havel (1987) suggested that it would be worth examining grazer-induced defenses in macrophytes and algae. There is, however, little information regarding grazer-mediated antiherbivore responses in phytoplankton. The different success of herbivores feeding on algae is mainly due to structural properties of algal species such as size, rigid cell walls, mucous sheets, spines, and colony formation, which increase grazing resistance (De Benardi and Guissani 1990). Also, production of toxins or repellent chemicals by cyanobacteria promote grazing resistance (Lampert 1981, 1982; DeMott and Moxter 1991).

Grazer-induced changes in palatability of algae would augment the seasonal species succession (Sommer et al. 1986) and allow some species to persist longer.

Recently, Hessen and Van Donk (1993) discovered *Daphnia magna*-induced phenotypic plasticity in the green alga *Scenedesmus subspicatus* Chodat. This alga formed numerous large four- to eight-celled coenobia and more rigid and longer spines when exposed to water in which daphnids had been cultured. Lampert et al. (1994) found comparable results for coenobia formation in the spineless *Scenedesmus acutus* Meyen mediated by chemicals released from *D. magna*. This "Daphnia kairomone" appeared to be a nonvolatile, heat-stable, and pH-resistant (in the range from 1 to 12) organic substance of small molecular mass (<500 MW). The substance was not affected by treatment with pronase E, an enzyme that reacts with peptides (Lampert et al. 1994).

The phenotypic changes could promote resistance of algae to grazing by small animals because colonies might exceed the maximum size of grazable particles, which is directly related to the size of the grazer (Burns 1968). Spherical algae >45 μm cannot be ingested by even the largest *Daphnia* species (Porter 1977).

Until now, the induction of colony formation has only been studied with "Daphnia" water from laboratory cultures. We were interested if colony formation could be induced with natural lake water (Lake Zwemlust, The Netherlands) during high zooplankton abundances. In a first experiment, the colony-inducing ability of *Daphnia galeata* was examined. We used this animal because it is the dominant (~90% of total numbers of cladocera) daphnid in the lake. To address whether the observed colony formation in *Scenedesmus* is a unique *Daphnia* effect or a general herbivore effect, a second experiment was performed with zooplankters other than *Daphnia*. After we had ensured that morphological changes in our algae could be induced, we tested in a third series of experiments the colony-inducing effect of filtered lake water. Biodegradability of the inducing factor was examined in a fourth experiment. As all defense mechanisms may have costs (Dodson 1989), growth rates were carefully examined to reveal whether metabolic costs associated with colony formation are reflected in growth rates.

The green alga *S. acutus* Meyen was obtained from the chemostat culture of the Max-Planck Institute (Plön, Germany). This test alga was cultured axenically in a 1.0-liter chemostat on 20% Z8 medium (Skulberg and Skulberg 1990) at 20°C, continuously illuminated with an irradiance of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and with a growth rate of 0.7 d^{-1} . An inoculum of the exponentially growing algae was transferred to 100-ml Erlenmeyer flasks containing 50 ml medium and sealed with Parafilm. Each batch culture contained 43 ml autoclaved Z8 (20%) medium, 2 ml algal inoculum, and either 5 ml additional Z8 medium filtered through a 0.1- μm membrane filter (Schleicher & Schuell, Germany) or 5 ml membrane-filtered (0.1 μm) test water. The batches were incubated at 20°C on a shaking table and continuously illuminated from above by fluorescent cool-white tubes at 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The tests were run in triplicate for at least 48 h.

The production and release of infochemicals by daphnids in the laboratory was tested by using a clone of *D. galeata* isolated from Lake Zwemlust. The animals were cultured in 1-liter

jars at 18.5°C in 20% Z8 medium without trace elements and with *Scenedesmus* as food. To produce zooplankton exudates, animals were transferred into sterile 100-ml Erlenmeyer flasks containing *S. acutus* (~10⁵ cells ml⁻¹) and allowed to graze for 24 h in the dark at 20°C. Water from these incubations was filtered through a 0.1- μm membrane filter before it was added to the algal test cultures.

Hessen and Van Donk (1993) and Lampert et al. (1994) performed experiments with *D. magna*, and we repeated these experiments with *D. galeata* to test if colony formation in *S. acutus* could be induced by exudates released from *D. galeata* (Exp. 1A). We kept 20 adult *D. galeata* (2.4 \pm 0.2 mm; length \pm 1 SD) in 100 ml 20% Z8 medium with *S. acutus* as food. After 24 h, 5 ml water from this culture was filtered through a 0.1- μm membrane filter and added to the algal cultures (10% of final solution). For the second treatment we added not only 5 ml test water, but also 1 adult *D. galeata* (~2 mm) to the algae. Algal cultures in 20% Z8 medium served as controls.

After we had ensured that colony formation could be induced in our algae, an experiment (Exp. 1B) was carried out with *D. galeata* varying in concentration range from 0 to 200 ind. liter⁻¹ to investigate the relationship between colony formation and *Daphnia* density.

In Exp. 2 the ability to induce colony formation in *S. acutus* by zooplankters other than *Daphnia* was investigated. The commercially available rotifer *Brachionus calyciflorus* (0.23 \pm 0.03 mm [length \pm 1 SD]; AquaSense, The Netherlands) was incubated at a density of 1,000 animals liter⁻¹ for 24 h in the dark at 21°C on *Chlorella vulgaris* (8.2 \times 10⁵ cells ml⁻¹; 2.7 \times 10⁷ $\mu\text{m}^3 \text{ml}^{-1}$). The copepod *Eudiaptomus gracilis* (330 ind. liter⁻¹) and the small cladoceran *Bosmina longirostris* (1,000 ind. liter⁻¹; 0.39 \pm 0.09 mm) were isolated from Lake Zwemlust and incubated on *S. acutus* (4.2 \times 10⁵ cells ml⁻¹; 3.0 \times 10⁷ $\mu\text{m}^3 \text{ml}^{-1}$). An incubation of *D. galeata* (200 ind. liter⁻¹; 2.07 \pm 0.05 mm) served as positive control. In Exp. 2, 5 ml water from these incubations was filtered through a 0.1- μm membrane filter and added to a 45-ml suspension of *S. acutus* in 100-ml Erlenmeyer flasks. In the control, 5 ml membrane-filtered 20% Z8 medium was added, while in an additional control, 5 ml unfiltered 20% Z8 medium was added to the Erlenmeyer flasks to test the effect of filtration on colony formation. Initial algal density was 1.4 \times 10⁵ cells ml⁻¹.

In Exp. 3 the lake was monitored from February to June 1995 by using *S. acutus* from the chemostat as a test alga. Lake water was passed through a 0.1- μm membrane filter and 5 ml (10% of final solution) was immediately added to algal suspensions in 100-ml Erlenmeyer flasks. Tests were analogous to Exp. 1.

Biodegradability of the colony-inducing factor was examined in Exp. 4. Fresh lake water was filtered through a 3- μm filter (Schleicher & Schuell, Germany) to remove zooplankton and algae, then stored in the dark at 5, 15, and 25°C. After 0, 1, and 2 d storage, samples of 5 ml were filtered through a 0.1- μm membrane filter to remove bacteria and added to test flasks to examine the colony-inducing ability of the water. To test for the role of bacteria in an additional experiment, lake water was passed through a 3- μm and a 0.1- μm membrane filter to remove plankton without and with bacteria, respectively, and stored for 1 week before use.

Algal densities and particle size distributions were deter-

Table 1. Exp. 1A: Effect of filtrate (5 ml through 0.1 μm) from an incubation of 200 *Daphnia galeata* liter⁻¹ with and without one live *Daphnia* on mean particle volumes (± 1 SD) and average numbers of cells per colony (± 1 SD) of *Scenedesmus acutus*. Different symbols (a, b, c) sharing the same vertical column indicate differences at a 95% level (Tukey's test).

Incubation	Mean particle volume (μm^3)	Mean number of cells per colony
Control	98.0 \pm 5.3 a	1.76 \pm 0.06 a
Filtrate	292.9 \pm 4.0 b	5.05 \pm 0.05 b
1 <i>Daphnia</i>	455.1 \pm 17.6 c	5.83 \pm 0.03 c

mined routinely in the size range from 3.0 to 20.0 μm equivalent spherical diameter (100 μm capillary) for *S. acutus* using a Coulter Multisizer II. For each incubation at least 200 aggregates (i.e. single cells as well as coenobia) of *Scenedesmus* were counted microscopically and the number of cells per colony determined. Mean particle volumes and mean cells per aggregate of the different incubations were compared statistically by applying one-way ANOVA. The incubations were distinguished for significant differences applying the Tukey test (Fowler and Cohen 1993).

To reveal whether metabolic costs associated with colony formation are reflected in decreased growth, growth rates ($= [\ln(V_t) - \ln(V_0)] \times t^{-1}$, units of d⁻¹) were calculated from the increase in algal biovolumes (V) and from increase in cell numbers. The cell numbers were computed by multiplying the number of aggregates (determined by Coulter Multisizer II) by the mean number of cells per aggregate (determined by microscopy). Growth rates were compared by one-way ANOVA (Fowler and Cohen 1993) by using the program Statistix (vers. 3.1).

Under ordinary incubation, 80 \pm 2% of the spineless *S. acutus* occurred as unicells with cell dimensions of 14 \pm 1 \times 4 \pm 1 μm . The average number of cells per colony was 1.3 with a mean volume of 103 μm^3 .

In Exp. 1A, number of cells per colony as well as mean particle volumes increased when *S. acutus* was incubated with *Daphnia* filtrate or with one live daphnid (Table 1). In the controls, >71% of the population remained unicellular, while treatments consisted of \sim 16% unicells (Fig. 1). Formation of 8-celled coenobia was clearly promoted in the treatments compared to the controls. In the controls \sim 2% 8-celled coenobia were found, while in the treatments with filtrate \sim 36% and with one *Daphnia* \sim 52% of the population consisted of 8-celled coenobia. These data correspond with the results of Lampert et al. (1994). Mean dimensions of 8-celled coenobium were 24 \pm 6 \times 19 \pm 3 μm (maximally 33 \times 25 μm).

Induction of colonies is clearly dependent on the concentration of the *D. galeata* factor (Exp. 1B). Both particle volumes and numbers of cells per colony increased with higher concentrations of *D. galeata* exudates (Fig. 2). After 48 h the one-way ANOVA indicated significant differences for the mean particle volumes ($F_{4,10} = 52.4$; $P < 0.001$) and number of cells per aggregate ($F_{4,10} = 49.4$; $P < 0.001$). A Tukey's test revealed four homogeneous groups: the control; 10, 50 and 100, and 200 *Daphnia* liter⁻¹.

Colony formation in *Scenedesmus* could also be induced by

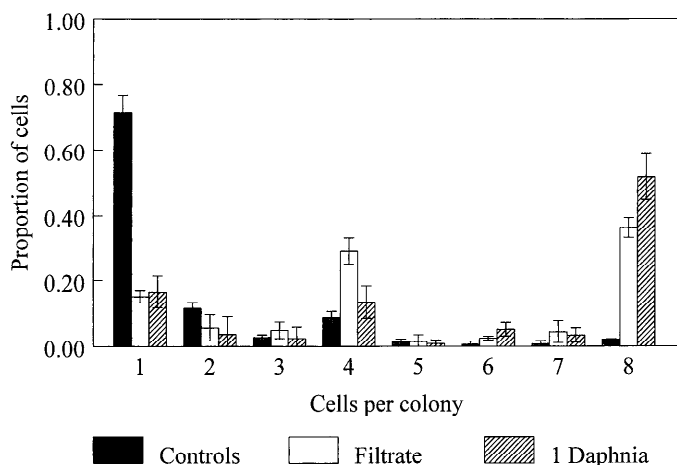


Fig. 1. Colony size in *Scenedesmus acutus* after 48 h of incubation with or without 5 ml filtrate (0.1 μm) from a *Daphnia galeata* culture and with or without one live *Daphnia* (Exp. 1A). Values are means ± 1 SD ($n = 3$).

other zooplankton (Exp. 2; Table 2). Thus, induction of colonies is not a unique response of *Scenedesmus* to *Daphnia* but the result of a general herbivorous zooplankton effect. Therefore, colony formation induced by lake water was most likely related to overall zooplankton composition and abundance rather than simply *Daphnia* abundance. Induction strength of lake water (Exp. 3) was estimated from the response in *Scenedesmus* to different zooplankters in the laboratory (Table 2). The evoked response in *Scenedesmus* by 200 adult *D. galeata* liter⁻¹ (large grazers) was arbitrarily chosen to represent an induction factor (IF_{200}) with a strength of one. Of each taxon, IF_{200} is the colony inductive strength of 200 animals liter⁻¹. Small grazers (*B. longirostris* in Exp. 3) had an IF_{200} of 0.2 (i.e. 1,000 *Bosmina* induce equal amounts of colonies in *Scenedesmus* as 200

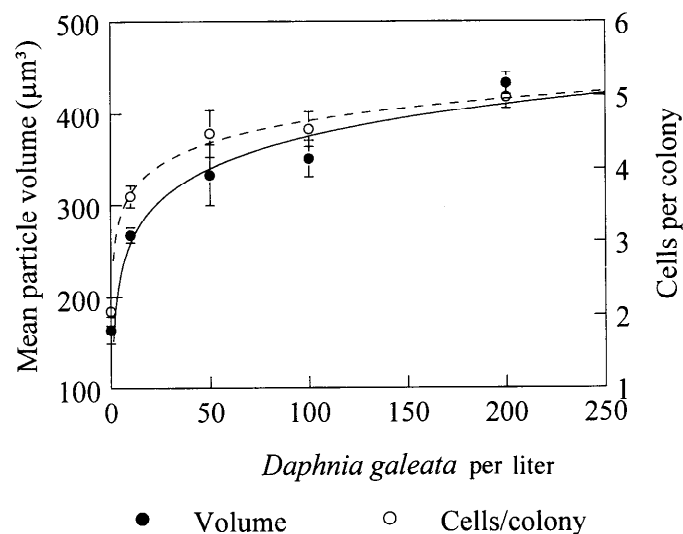


Fig. 2. Effect of 5 ml filtrate from a *Daphnia galeata* cultures varying in density from 0 to 200 animals liter⁻¹ on mean particle volume (solid line) and average number of cells per colony (dotted line) of *Scenedesmus acutus* (Exp. 1B). Values are means ± 1 SD ($n = 3$).

Table 2. Exp. 2: Effect of 5 ml water, filtered through 0.1 μm , from incubations of different zooplankters on colony formation in *Scenedesmus acutus*, expressed as mean particle volumes (± 1 SD) and as mean number of cells per colony (± 1 SD). Different symbols (a, b) sharing the same vertical column indicate differences at a 95% level (Tukey's test).

Incubation	Animals liter ⁻¹	Mean particle volume (μm^3)	Mean No. cells per colony
Control	—	58.6 \pm 6.4 a	1.14 \pm 0.01
Z8 filtered	—	87.6 \pm 6.7 a	1.78 \pm 0.09
<i>Brachionus calyciflorus</i>	1,000	208.3 \pm 12.0 b	3.17 \pm 0.55
<i>Eudiaptomus gracilis</i>	330	189.5 \pm 34.5 b	2.61 \pm 0.88
<i>Bosmina longirostris</i>	1,000	220.3 \pm 27.8 b	2.79 \pm 0.36
<i>Daphnia galeata</i>	200	211.0 \pm 47.6 b	2.85 \pm 0.22

D. galeata), herbivorous copepods an IF_{200} of 0.5, and rotifers an IF_{200} of 0.2. By using these IF_{200} s and zooplankton composition data of 1995, rough estimates for total zooplankton induction strength (TIF) were calculated. Colony formation data, as increase in number of cells per colony (=cells/colony of treatment minus cells/colony of control), are highly variable during the observed period (Fig. 3). A weak relationship between colony formation and TIF based on zooplankton numbers was observed ($r^2 = 0.25$) during this period (based on estimated zooplankton biomass, the relationship was even weaker: $r^2 = 0.02$). However, in the early spring (February–April 1995) a clear relationship between TIF and colony formation was observed. This was mainly due to the calculated inductive ability of rotifers, the most abundant zooplankter during this period (Fig. 4). However, during *Daphnia* dominance (April–June) the relationship between TIF and colony formation was less strong.

Lake water that had been stripped of plankton larger than bacteria had lost its colony-inducing ability after 2 d of incubation at 15°C (Exp. 4). The degradation of the colony-induc-

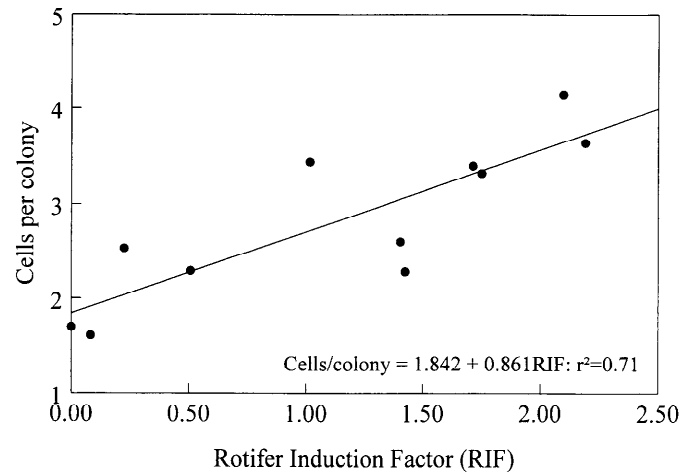


Fig. 4. Relationship between average number of cells per colony of *Scenedesmus acutus* and rotifer abundance during February through April 1995.

ing factor in lake water was temperature dependent (Fig. 5). Lake water that initially induced colonies in *S. acutus* (cells/colony, mean ± 1 SD: 3.02 \pm 0.62) remained inductive, even after 1 week storage at 22°C without bacteria (cells/colony: 3.01 \pm 0.48), but had lost its induction activity when bacteria were present (cells/colony: 1.94 \pm 0.28).

In experiments with laboratory water, growth rates (means ± 1 SD) based on total cell numbers (1.11 \pm 0.16 d⁻¹) and based on volume (1.14 \pm 0.11 d⁻¹) were not significantly different ($F_{1,28} = 0.57$; $P = 0.456$). In the experiments there was no significant difference for growth rates between controls and treatments ($F_{1,28} = 0.33$; $P = 0.570$). There was no apparent metabolic cost associated with the colonial growth as no depressed growth rates in treatments were observed.

It is well known that *Scenedesmus* shows environmentally induced phenotypic variation. Unicells as well as a variety of colonial morphologies can be found when *Scenedesmus* is cultured in either natural water or artificial media (Egan and Trai-

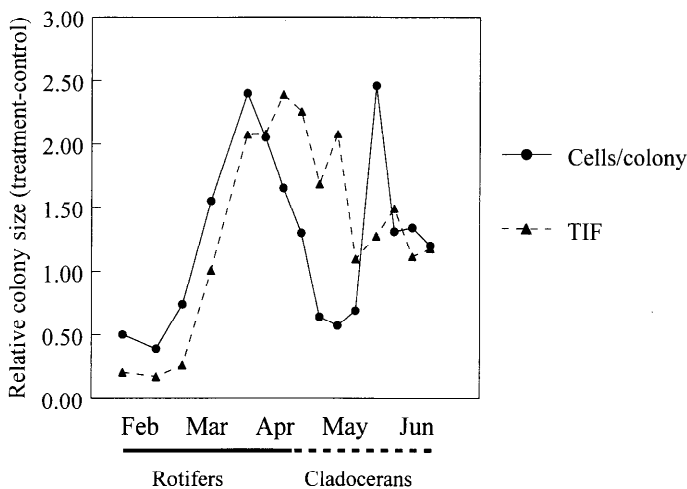


Fig. 3. Relative colony size in *Scenedesmus acutus* (treatment-control) and total zooplankton abundance represented as a total induction factor (TIF) from February through June 1995. Solid line at bottom of figure represents the period of rotifer dominance, the dotted line the period of *Daphnia* dominance.

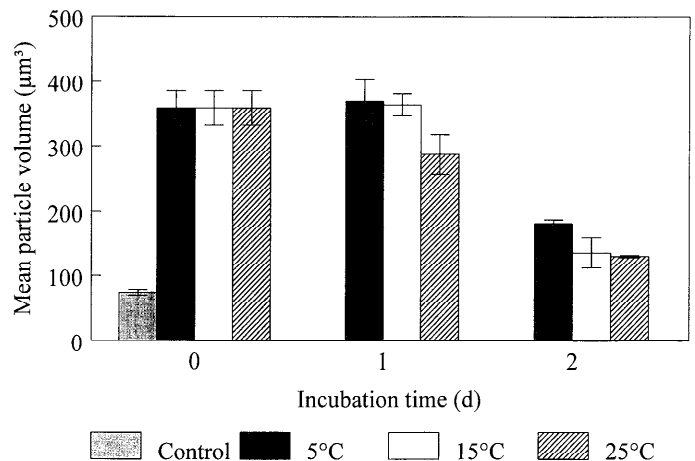


Fig. 5. Biodegradation of the infochemical(s) in Lake Zwemlust water. Effects of incubation of lake water at different temperatures on mean particle volumes of *Scenedesmus acutus*. Error bars represent 1 SD ($n = 3$).

nor 1989). In general, unicells are dominant in young, growing cultures (Trainor 1979). Expression of different *Scenedesmus* morphologies (single cells as well as coenobia) depends on environmental variables such as nutrients, pH, temperature, and age or cell density of the culture (Egan and Trainor 1989; Trainor 1993). Only recently have morphological changes in *Scenedesmus* induced by *Daphnia* infochemicals been demonstrated (Hessen and Van Donk 1993). Hessen and Van Donk (1993) and Lampert et al. (1994) found in laboratory experiments that coenobia formation in *Scenedesmus* could be induced by a biochemical cue released by *D. magna*. We found identical responses in *Scenedesmus* induced by *D. galeata* (Exp. 1A). The proportion of 8-celled coenobia (~36%) after 48 h was similar to results found by Lampert et al. (1994) (~46%). With an average length of ~25 μm , these coenobia would still be ingestible by *D. galeata* (1.1 ± 0.3 mm) in the lake but not by juveniles and smaller grazers. The relatively small size of 8-celled coenobia of *S. acutus* (maximally 33 μm) may explain why Lampert et al. (1994) found no differences in uptake of unicells and colonies by small (1.0 mm) and large (2.5 mm) *Daphnia*, while Hessen and Van Donk (1993) found lower grazing rates for small *Daphnia* when the proportion of large colonies (40–50 μm) of *S. subspicatus* was high.

In Exp. 1B a dose response of the colony formation was observed, reaching a plateau above 200 *Daphnia* liter⁻¹. This result is in contrast with the linear increase of colony formation above 50 *Daphnia* liter⁻¹ as found by Lampert et al. (1994). This difference is most probably caused by a crowding effect (e.g. Hayward and Gallup 1976) in our experiments as a result of incubation of different densities of *Daphnia* in similar volumes of water (100 ml). The production of colony-inducing chemicals seems to be related to the grazing activity of *Daphnia* (Van Donk and Lüring unpubl. data). A reduction in the clearance rate of *Daphnia* as a result of crowding may consequently result in less production of inducing chemicals per *Daphnia*. Also, it seems unlikely that the saturation point of the algal physiological response rate had been reached, because the effect can be further increased when a higher volume of incubation water is supplied (Lampert et al. 1994).

Scenedesmus responded also to the presence of the cladoceran *B. longirostris*, the copepod *Eudiaptomus gracilis*, and the rotifer *Brachionus calyciflorus*. Thus, induction of colony formation in *S. acutus* is not unique to *Daphnia* but probably widespread among herbivorous zooplankton. Interestingly, Lampert et al. (1994) found that homogenates of *Scenedesmus* or *Daphnia* did not induce colonies, indicating that the inducing chemicals are not constituents of the organisms involved but most likely the result of the interaction between the two species. The inducing chemicals most probably originate from the animals' digestive systems since starved *Daphnia* induced no colonies (Lampert et al. 1994). One interesting question is whether the observed effect is an herbivore effect resulting from grazing or a more general zooplankton effect.

Fresh natural lake water evoked colony formation in *Scenedesmus* grown in artificial medium in which it otherwise occurred mainly as unicells. Similar results were found with filtered lake water taken from mesotrophic Schöhsee (Germany) (Van Donk et al. 1997). Although there was a strong relationship in early spring (February–April), there was no clear relationship between the total zooplankton or *Daphnia* abundance

and the induction strength of the water during April–June 1995. Whether the weakness of the relationship in late spring was caused by low food quantity and/or quality to *Daphnia*, high microbial turnover rates of the inducing chemical, or a combination of other factors remains unclear. Our method provides only rough estimates of lake water induction strength and needs further investigation. For example, the induction strength of the lake water is based on linear interpolation from laboratory experiments, but as found in Exp. 1B zooplankton–colony formation relationships may not be linear at all. For example, 400 *Daphnia* may not equal an IF_{200} of 2 (but probably less) and 100 *Daphnia* probably have an $IF_{200} > 0.5$. The often observed patchy distribution of *Daphnia* in the field will likely result in crowding conditions inside such a patch and may consequently affect infochemical production. Despite these uncertainties, during early spring (February–April 1995) a clear relationship between zooplankton abundance and colony formation was observed. This was mainly due to strong dominance of rotifers. Therefore, the results indicate that the mechanism of grazer-induced colony formation might occur in situ.

The inducing strength of lake water weakened rapidly and had already disappeared after a 2-d incubation. Lake water stripped-off plankton including bacteria remained active, even after 1 week, suggesting an important role of bacteria in the turnover of the inducing chemicals. The evoked responses should occur only when necessary, increasing the reliability of the signal. This process is analogous to how fish exudates influence diel vertical migration of daphnids (Loose et al. 1993). These fish exudates seem also completely degraded within 2 d (Reede pers. comm.).

Scenedesmus colonies are formed when daughter cells of a recently divided cell fail to loosen (Trainor et al. 1976). Therefore, colony formation is probably not adhesion of already existing single cells. Interestingly, when incubated with one live *Daphnia*, *Scenedesmus* also formed large aggregates with dozens, sometimes even hundreds, of cells. The arbitrary arrangement of the cells suggests aggregation of existing cells and colonies. Probably two different mechanisms are involved. Besides the colony formation induced by a chemical cue released from grazing *Daphnia*, aggregation due to mucous and/or feces excretion appeared to occur.

Whether energetic/metabolic costs are associated with colony formation is not clear or at least not reflected in growth rates as no decrease in growth rates of treatments relative to controls was found. Sterner and Smith (1993) found no dependence of colony size of *S. acutus* on growth conditions. Nevertheless, grazer-induced colony formation is only beneficial during high grazing pressure as colonies might have higher sinking rates (Reynolds 1984) but may also be inedible (Hessen and Van Donk 1993). Because defense mechanisms can be costly, an on–off mechanism to mobilize defense only when necessary would therefore be advantageous (Larsson and Dodson 1993).

Recently, another mechanism inducing grazing resistance in green algae was discovered. Structural and morphological changes in P-limited cells (e.g. thicker cell wall) increased their resistance to grazing by *Daphnia* (Van Donk and Hessen 1993). Evidently, algae are not defenseless against grazers but have evolved various adaptive strategies.

Until now, grazer-induced morphological changes have only

been demonstrated in *Scenedesmus*. Further studies, however, will reveal whether the grazer-mediated response in *Scenedesmus* is a general adaptive strategy against grazing that is also present between other algae and zooplankters or unique to *Scenedesmus*.

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Received: 30 November 1994

Accepted: 14 October 1996