

Defenses in phytoplankton against grazing induced by nutrient limitation, UV-B stress and infochemicals

Ellen Van Donk

Department of Water Quality Management and Aquatic Ecology, Agricultural University Wageningen, P.O. Box 8080, 6700 DD Wageningen, The Netherlands; Present address: Netherlands Institute of Ecology, Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands

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Abstract

It is becoming increasingly evident that the efficiency of zooplankton grazing on algae is not only a matter of quantity of the grazer relative to its food. Planktonic primary producers are not defenseless food-particles that are easily harvested by the consumers. Several algal species are able to adjust their phenotype (colony formation, spines, size) in such a way that it results in a reduced grazing pressure. It was recently demonstrated that morphological changes in the cell wall of green algae, induced by nutrient limitation and UV-B stress, may reduce their digestibility. A high fraction of induced cells pass intact and viable through the gut of the zooplankters, such that the grazing impact on the population is strongly reduced. It was also found that the presence of exudates (infochemicals) released by daphnids may change the morphology of algae. Unicellular green algae of the genus *Scenedesmus* were induced to form eight-cell coenobial types, heavily armed with spines, within three to five days after adding filtered water from an algal culture with *Daphnia* present. Both defence mechanisms may play an important role in zooplankton production and competition, and may serve as an example of highly efficient strategies to resist heavy grazing pressure.

Introduction

Primary producers have evolved a set of defence mechanisms against grazing, involving both structural, behavioural and chemical traits. This is particularly known from terrestrial systems (e.g., Futuyma, 1983). In aquatic, pelagic systems, numerous studies have documented the grazing pressure from herbivorous zooplankton on phytoplankton, and it is well known that zooplankton feed with highly different success on various phytoplankton species. This is primarily related to parameters like size and shape of the cells and structure of the cell wall (e.g., Burns, 1968; Porter, 1975; De Bernardi & Guissani, 1990). Production of endo- or extracellular toxins is also a common property of several strains of pelagic primary producers, serving as an efficient grazer defence (e.g., Lampert, 1987; Fulton & Paerl, 1987; Demott & Moxter, 1991).

While it is well known that grazing resistance may also vary among different strains of the same algal species, there is hardly any knowledge on the factors inducing these grazing repellants. In the present paper I will review induced defences in phytoplankton from recent literature.

Induction by nutrient limitation and UV-B stress

Morphological changes in algae and reduced digestion by zooplankton

Van Donk & Hessen (1993) observed that both *Daphnia pulex* and *Daphnia magna* exerted lower grazing pressure on P-starved green algae (*Selenastrum capricornutum* and *Scenedesmus subspicatus*) relative to nutrient saturated algae. They found that P-starved cells passed largely intact through the daphnid's gut. In

a follow-up study with *D. magna* feeding on *S. capricornutum*, Van Donk & Hessen (1995) confirmed this mechanism and demonstrated the same effect in UV-B irradiated algae. The observation that most cells remained intact after gut passage led Van Donk & Hessen (1993) to conclude that the effect could be largely attributed to resistance of the algae to the digestive enzymes of the grazers. They observed an increase in cell volume and wall thickness of nutrient-limited and UV-B stressed algae, which may reflect a storage of photosynthates, perhaps due to delayed cell division. These morphological changes may be adaptive mechanisms, i.e., anti-grazer defences, induced by bad environmental conditions. Increased cell volumes and a granular appearance have also been noticed in other algae that were cultured under conditions of nutrient limitation (Mitchell et al., 1992; Sterner et al., 1993; Van Donk & Hessen, 1993) and UV-B stress (Veen et al., 1995; De Lange & Van Donk, 1997). In a recent study, the use of a mutant clone of the green alga *Chlamydomonas reinhardtii*, that lack a cell wall, allowed a direct test of the role of algal cell wall properties on *Daphnia* grazing and growth (Van Donk et al., 1997a; Lüring & Van Donk, 1997a). The results confirmed the idea that the cell wall of the alga, especially under nutrient limitation, forms a barrier for the digestion by zooplankton. Cells of the wall-deficient mutant of *Chlamydomonas*, grown under both nutrient-limited and nonlimited conditions, were efficiently ingested and digested by *Daphnia* (Van Donk et al., 1997a).

Costs and impact on grazers

There may be a trade-off between growth rate and the metabolic costs associated with morphological changes produced for grazing protection. With mineral nutrient supply in excess, fast growth rates may to some extent compensate for grazing losses. In nutrient deficient and UV-B stressed systems, however, growth rate slows and some morphological means of grazer protection would be more beneficial, even at the expense of growth rate. For some algal species, increased cell wall thickness under unfavourable conditions may be seen as a preparation for encystment. In this species it may not be appropriate to claim that thickening of the cell wall is a primary strategy to depress growth of grazers, yet it may have exactly that effect. *Daphnia* grazing on nutrient-limited algae (Van Donk et al., 1997a) and UV-B stressed algae (De Lange & Van Donk, 1997) exhibited reduced growth rates and reduced fecundity. Mitchell et al. (1992) also found that *Daphnia* survived

better, grew faster, and had larger broods when fed on nonlimited *Chlamydomonas* than on N- or P-limited cells. They suggested that the effect might be related to the nutritional inadequacy of the algae, but they were not able to find a direct relation to the nutrient content of the cells. Other studies have also reported decreased growth and reproduction in *Daphnia* that were fed P- or N-limited algae, even though food was abundant (Hessen, 1990; Watanabe, 1990; Groeger et al., 1991; Sommer, 1992; Sterner et al., 1993). The observed differences in life history parameters of *Daphnia* grazing on nonlimited and nutrient-limited algae are probably due to both a reduced food availability, because of decreased assimilation of nutrient-limited algae, and a reduced nutritional content of the algae. This finding is strikingly analogous to the well-known reduced palatability of older leaves in many terrestrial plants due to their poor nutrient quality (Mattson, 1980).

If these observations also apply to natural plankton populations, they might constitute an important mechanism contributing to the stability of open-water ecosystems. Thus at times when phytoplankton growth rates are reduced by nutrient deficiency or UV-B stress, grazing pressure on the phytoplankton would also be reduced by the depression of assimilation rates and by the reduced population growth rates of the zooplankton. Moreover, viable gut passage may be beneficial for the nutrient-limited algae, as it allows them to take up nutrients from the *Daphnia* gut when pools of dissolved nutrients are depleted (Porter, 1973). In this way the algae may become less nutrient-limited after repeated ingestion and egestion, i.e., after several gut passages. As the algae become increasingly less nutrient-limited, they will become available again to the zooplankton. It is clear that nutrient limitation and UV-B stress of the phytoplankton can alter trophic interactions, reducing transfer of energy to herbivorous zooplankton (Table 1).

Induction by infochemicals from herbivores

Most of the literature on herbivore-induced defences has concentrated on the response of terrestrial plants. These plants are subject to attack by a variety of herbivores and pathogens, and their chemical defences have received much attention (e.g., Futuyma, 1983). The signals activating many of the induced defences are endogenously produced in plants in response to injury, and the formed secondary compounds (e.g., alkaloids,

Table 1. Summary of factors inducing defences in phytoplankton and indirect effects on zooplankton. Presence of a feed-back mechanism is also indicated

Inducing factor	Defenses in phytoplankton	Indirect effects on zooplankton	Feedback mechanism
Nutrient limitation	Increase in size Thicker cell wall	Assimilation of algae ↓ Growth rate ↓ Fecundity ↓	Yes, nutrient uptake by algae during viable gut passage
UV-B stress	Increase in size Thicker cell wall	Assimilation of algae ↓ Growth rate ↓ Fecundity ↓	No
Infochemicals from zooplankton	Colony formation Spines	For small zooplankton: Ingestion rate ↓ Growth rate ↓ Fecundity ↓	Yes, food-limited zooplankton forms less infochemicals

phenolics) protect the same individual later against herbivory.

In aquatic ecosystems, defences similar to those found in terrestrial vegetation were observed for some aquatic macrophytes, that were grazed upon by caddis larvae (Jeffries, 1990). Until recently, however, there was a general lack of information regarding herbivore-induced responses in phytoplankton. Most research in aquatic ecosystems concerning induced defences has focused on the response of zooplankton to the presence of potential predators (Ringelberg, 1991; Larsson & Dodson, 1993). These defences are activated by exogenous cues derived from predators with a broad taxonomic distribution.

Harvell (1984) proposed that predator-induced defences should evolve especially in clonal taxa because they often suffer intermittent non-fatal encounters with predators. This is true with bryozoans, cladocerans, rotifers and protozoans, for which numerous cases of predator-induced defences have been reported (Harvell, 1990; Larsson & Dodson, 1993). Mortality of some but not all individuals of a clone can be considered non-fatal to the genotype, and induced defences in clone-mates would improve the inclusive fitness of the prey (Havel, 1987). From this perspective, Havel (1987) suggested that it would be worth examining induced responses in polymorphic species of phytoplankton taxa.

Morphological changes and grazing resistance

Recently, it has been discovered that an infochemical released from grazing *Daphnia* induced the formation of colonies in the green alga *Scenedesmus* (Hessen & Van Donk, 1993; Lampert et al., 1994). When exposed to water in which *Daphnia* had been cultured, the green alga *Scenedesmus subspicatus* formed large four to eight celled coenobia and more rigid and longer spines (Hessen & Van Donk, 1993). Lampert et al. (1994) found comparable results for coenobia induction in spineless *Scenedesmus acutus* mediated by chemicals released from *Daphnia magna*. The colony-inducing factor is probably not a constituent of the algae themselves, because algal homogenate was not effective (Lampert et al., 1994).

The effect of *Daphnia* infochemicals on the morphology of fifteen strains of Chlorophyceae, two strains of Bacillariophyceae and three strains of Cyanobacteria was studied by Van Donk et al. (1997b). Morphological changes were only observed in the Chlorophyceae. However, the *Daphnia*-induced colony formation appeared not to be unique for *Scenedesmus* species, since *Coelastrum* strains have showed a similar response. It was also shown that *Daphnia*-induced coenobia formation is not universal for *Scenedesmus*, as *S. quadricauda* did not show a shift in morphology in response to the presence of *Daphnia* (Van Donk et al., 1997b). Lampert et al. (1994) have developed a reliable biotest for zooplankton-induced colony formation in algae. The experimental set-up used by these

authors enables to investigate of the ability of different zooplankton taxa to induce colonies in the test alga *S. acutus*. Many of the animals studied so far (Cladocera, Copepoda and Rotifera) appeared capable to induce colonies at ecological relevant densities (Van Donk et al., 1997b). Only Ostracoda and some Cladocera (e.g., *Chydorus sphaericus*) and Copepoda (e.g., *Cyclops agilis*) were not able to change the phenotype of *Scenedesmus*. Of course, it remains unclear whether the response in *Scenedesmus* is a general zooplankton effect or more unique to herbivorous zooplankton. Experiments with carnivorous zooplankters, like *Polypheumus*, *Bythotrephes* and *Leptodora* are necessary to check for that. It is also unclear whether the broad taxonomic array of zooplankton species to which the alga responds reflects the capacity of the algae to recognise the presence of a wide array of infochemicals, or whether only one or a few very generalised infochemicals are involved.

Several factors may influence colony induction by zooplankton. Actively feeding *Daphnia* induce more colonies than starved ones. Similarly, animals grazing on less edible algae, like the filamentous cyanobacterium *Oscillatoria*, are less effective in colony induction. Food quality of grazable particles, however, seems not to influence the excretion of the colony inducing substance. There are indications that the inducing factor may originate from the digestive system of the zooplankton. Bacteria present in the gut may be involved, since the ability to produce colony inducing chemicals can be blocked by exposing the animals to strong antibiotics like carbenicillin or cefotaxime. As a control needed for this experiment colonies were produced by algae exposed to these antibiotics and to chemicals produced by *Daphnia* cultured in the absence of antibiotics (Lürding & Van Donk, unpubl. data). A clear positive relationship was found between the length of the inducing animals and the degree of colony formation in *Scenedesmus* (Van Donk et al., 1997b). The linear shape of this relationship may indicate direct association with grazing activity of the animals. Smaller taxa like *D. cucullata* do not induce colonies at densities below 400 l⁻¹ probably due to the relatively low biomass.

Induction of colonies in *Scenedesmus* was also observed with filtered lake water taken during spring, summer and fall, when zooplankton abundance was high (Van Donk et al., 1997b; Lürding & Van Donk, 1997b).

Only a few grazing studies were performed with algae exhibiting grazer-induced change in morphology.

Hessen & Van Donk (1993) found lower grazing rates for relatively small *D. magna* when the share of colonies of *S. subspicatus* in the food was high. They assumed that the spined colonies were larger than the maximum size that could be ingested by a 1.75-mm daphnid. However, Lampert et al. (1994) did not detect differences in the uptake of unicells and colonies of *S. acutus*. This strain of *S. acutus* does not have spines, and it may therefore be ingestible for the daphniids tested, which were above one mm in body size. Lürding & Van Donk (1996) performed grazing experiment with *D. cucullata* and two size classes of *D. pulex* feeding on *S. acutus* (unicells and colonial cells). *D. pulex* showed no differences in clearance rate when fed unicellular or colonial *Scenedesmus*, while the smaller *D. cucullata* showed a significantly depressed clearance rate when fed colonies compared to unicells. Feeding on colonial *S. obliquus* similarly depressed the clearance rate of *D. cucullata* and small *D. magna*. The degree to which colony formation in *Scenedesmus* offers protection against grazing thus seems to depend on the size of the grazer, with colony formation only being effective against small grazers (Table 1).

Costs and impact on grazers

If colony formation is only phenotypically induced in the presence of grazers, one may expect costs associated with colony growth (Dodson, 1989). These costs do not seem to be associated with algal growth rate, as no difference in population growth rates were observed between induced and non-induced cultures (Hessen & Van Donk, 1993; Lampert et al., 1994). However, large colonies probably have higher sinking rates (Reynolds, 1984). Due to the higher viscosity of the water at low temperature, the size effect on the sinking rate may be tolerable at low but not at high temperatures. The trade-off between sinking and grazer resistance would favour a direct phenotypic response to the presence of grazers, if grazing pressure varies with time (Schlichting, 1989). In warm water, when sinking losses are important, colonies will be formed only when mortality through grazing is high (Lampert et al., 1994).

The growth rate of the large-sized *D. pulex* and *D. magna* showed no differences between animals fed unicellular or colonial *S. acutus* (Lürding & Van Donk, 1996). This is in contrast with the smaller *D. cucullata*, in which a significant increase in body length occurred in animals fed unicells. The intrinsic rate of population increase of *Daphnia* on both food types was similar for *D. pulex* and *D. magna*, but significantly different

for *D. cucullata*. Age at maturity was not affected by the different food types in any of the *Daphnia* species studied. The number of live neonates released per mature female fed unicells or colonies was similar in *D. pulex*. In *D. cucullata*, however, animals feeding on unicells produced more offspring than animals feeding on colonies. These data, combined with the observations that at ecological relevant densities *D. pulex* and *D. magna* produced infochemicals whereas *D. cucullata* did not, lead to the tentative suggestion that large *Daphnia* are able to influence their algal food in such a way that the life history of smaller congeners is influenced negatively (Lürling & Van Donk, 1996). This implies that both phytoplankton-zooplankton and zooplankton-zooplankton interactions are altered by the induced colony formation. In the field, predation pressure on large *Daphnia* will be higher than on small zooplankton. The capacity to graze on larger algae and the ability to induce colony formation in some algae may contribute substantially to the competitive superiority of large over small zooplankton species. As the colonial algae will exceed the size of vulnerability to grazing by small zooplankton, total grazing pressure on the algae is likely to decrease (Table 1).

Perspectives for future research

Research on induced defences in phytoplankton has only recently started. Until now, only a few phytoplankton species have been tested for morphological changes that are inducible by nutrient limitation, UV-B stress and infochemicals produced by herbivores. To analyse whether these induced phenotypic responses in phytoplankton species are a general adaptive response against grazing, more algal species should be tested in the laboratory and *in situ*. However, other important anti-grazer properties of algae, like toxicity of Cyanobacteria and Prymnesiophytes, formation of resting spores and gelatinous sheaths, sexual reproduction, formation of zygotes and vertical migration, may be worth to examine for their inducibility. Future studies should also reveal the costs and gains for fitness, that are associated with these defences.

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