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Published in:
Limnology and Oceanography

DOI:
10.4319/lo.2012.57.3.0727

2012

Link to publication

Citation for published version (APA):
Lebret, K., Fernández Fernández, M., Hagman, C. H. C., Rengefors, K., & Hansson, L.-A. (2012). Grazing resistance allows bloom formation and may explain invasion success of Gonyostomum semen. *Limnology and Oceanography*, *57*(3), 727-734. https://doi.org/10.4319/lo.2012.57.3.0727

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Limnol. Oceanogr., 57(3), 2012, 727–734
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doi:10.4319/lo.2012.57.3.0727

Grazing resistance allows bloom formation and may explain invasion success of *Gonyostomum semen*

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Abstract

The nuisance alga *Gonyostomum semen* (Raphidophyceae) has expanded in the Nordic countries during the last decades and can dominate lake phytoplankton communities almost completely. A possible explanation to its dominance could be limited grazing by zooplankton. We investigated the potential grazing pressure on *G. semen* using an experimental approach supported by field data. We determined the grazing rate by cladocerans, calanoid copepods, and *Chaoborus* larvae to determine which were able to feed on *G. semen*. Only the large cladoceran *Daphnia magna* was able to feed successfully on *G. semen*. The large cell size of *G. semen* was likely a limiting factor for the filtering apparatus of smaller cladocerans. The copepod *Eudiaptomus gracilis* did not graze on *G. semen*, although the mechanism behind this selective feeding is still unknown. In addition to the experimental study, we quantified the zooplankton and phytoplankton communities in 40 lakes to determine the composition and abundance of the zooplankton communities co-occurring with *G. semen*, suggesting that large cladoceran species were not present in lakes where *G. semen* occurred. Hence, the growth of *G. semen* is not significantly controlled by grazing in natural systems, which likely facilitates bloom formation and invasion success of *G. semen*.

Algal blooms are a recurrent problem in both marine and freshwater environments (Anderson 1989). These blooms often have negative effects on the ecosystem function and on human health, as several bloom-forming species can produce toxins associated with human illness or fish poisoning episodes (Anderson 1989). Algal blooms also have important negative economical effects, reducing the use of aquatic systems for recreational purposes, or restricting the consumption of aquaculture products during toxic events. Bloom-forming species may have one or both of two complementary strategies to form dense populations: higher growth rate (linked to bottom-up control or competition) and/or lower mortality rate (often associated with top-down control) than other phytoplanktonic species.

Numerous adaptations have evolved in algal species that increase their competitive ability over other algal species, such as higher growth rate (Smayda 1997); production of allelopathic compounds that can reduce the growth of competitive species (Legrand et al. 2003); higher nutrient uptake rates (Smayda 1997); diel vertical migration, allowing them to reach the nutrients in the deep layer at night and the light at the surface during daytime (Cullen and Horrigan 1981); and uptake of organic nutrients and mixotrophy (Anderson et al. 2002). Mortality in phytoplankton can occur due to attacks by parasites and viruses but most predominantly due to grazing by zooplankton (Calbet and Landry 2004). Defense against grazers can involve numerous strategies, such as large cell size (Stoyneva et al. 2007); colony formation (Van Donk and Hessen 1993); complex cell structure formation of, for example, spines; grazer avoidance by limited or delayed germination of cysts in the sediment during periods of high zooplankton abundance (Hansson 1996); production of toxic substances (Gustafsson and Hansson 2004); or allelopathic compounds against grazers (Legrand et al. 2003; Hansson et al. 2007). Bloom-forming species may have one or several of these adaptations that contribute to the formation of dominant populations. In natural systems, grazer avoidance is an important process to limit the loss of biomass, limiting the number of cells that potentially could divide and produce new biomass during the formation of a bloom. Grazing avoidance is therefore likely an important component in the success of bloom-forming species. Hence, the effect of grazers is presumably larger on slow-growing species, as the growth rate may not compensate for the rapid loss of biomass due to grazing.

Biological invasions represent a major ecological perturbation having important environmental as well as economical effects (Davis 2009). An invasive species spreads rapidly, colonizes new sites, and forms dominant populations (Valery et al. 2008). As for bloom-forming phytoplankton species, the success of invasive species in their new environment may be tightly linked to grazing or predation pressure (Davis 2009). Absence or restriction of top-down control after establishment in a new environment is one of the key factors characterizing invasive populations. Invasive phytoplankton have been understudied. Very few phytoplankton species are considered to be invasive, including the diatom *Didymosphenia geminata* in New Zealand (Kirkwood et al. 2007), the cyanobacterium *Cylindrospermopsis raciborskii* in temperate regions (Neilan et al. 2003), and *Alexandrium tamarense* (Lilly et al. 2002). To our knowledge, only one study explored the potentiality of herbivores to limit a phytoplankton invasion (Sperfeld et al. 2010). However, the mechanisms involved in the invasion process are still unclear, although aquatic systems

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are expected to be highly affected by invasive species in the future (Sala et al. 2000).

In the northern part of Europe, the freshwater raphidophyte *Gonyostomum semen* has spread, increased its distribution range, and colonized numerous lakes during the last four decades (Cronberg et al. 1988; K. Rengefors unpubl.). This raphidophyte is now considered to be an invasive species, with a special concern in Scandinavia and Finland. Recently, *G. semen* was found to form dense invasive populations in Poland (Poniewozik et al. 2011), suggesting that the species is now spreading to southern countries of Europe. *G. semen* is a bloom-forming species that can dominate the phytoplankton community to up to 97% during extended periods of time despite a low growth rate (Cronberg et al. 1988; Findlay et al. 2005; K. Lebret unpubl.). Despite the increasing number of studies on *G. semen*, very little is known about the factors involved in the invasion process and bloom formation of *G. semen*. To our knowledge, *G. semen* does not produce toxins, in contrast to the marine raphidophyte species, which produce compounds that are highly toxic for fish (Landsberg 2002). *G. semen* possesses mucocysts and trichocysts that are responsible for the production of mucilage, which may cause skin irritation, thereby reducing the recreational value of the lakes (Cronberg et al. 1988). The trichocysts can expel slime threads following a mechanical stress. They have been observed in numerous phytoplanktonic species and may be used as a defense mechanism (Ukeles and Sweeney 1969; Tillmann and Reckermann 2002). In addition, Rengefors et al. (2008) have shown that *G. semen* is able to induce lysis of other phytoplanktonic species (i.e., *Rhodomonas* sp.). This strategy was suggested to be involved in their capacity to develop dense blooms by reducing the competition for nutrients. Furthermore, *G. semen* is mixotrophic, as it showed increased growth rates in culture medium supplemented with humic acids or with *Rhodomonas* sp. (Rengefors et al. 2008). Nevertheless, little is known about the top-down control on *G. semen*. Their large cell size may be a limitation for feeding by zooplankton, but no clear evidence has been shown. Havens (1989) suggested that *G. semen* is an inedible species because of its large size and the presence of trichocysts but did not test his hypothesis. On the contrary, Cronberg et al. (1988) and Findlay et al. (2005) suggested that large cladocerans and rotifers may have a negative effect on the abundance of *G. semen* due to grazing. However, no experiments were carried out that directly assess the capacity of potential grazers to feed on *G. semen*.

In our study, we aimed at determining if *G. semen* biomass could be controlled by top-down factors using laboratory experiments supported by field data. Grazing experiments with *G. semen* were conducted using four different potential grazer species (*Daphnia magna*, *Daphnia pulex*, the calanoid copepod *Eudiaptomus gracilis*, and *Chaoborus* larvae). We hypothesized that because of the large size of *G. semen* and possibly their mucilage production, zooplankton used in the experiment would not be able to feed on *G. semen*. *Chaoborus*, known as a predator, was used in the grazing experiment because it has been recently observed that *Chaoborus* abundances were high in lakes with high cell concentration of *G. semen* (Trigal et al. 2011). We hypothesized that predatory *Chaoborus* larvae do not graze on *G. semen*, allowing us to exclude the possibility that *Chaoborus* benefits directly from high abundance of *G. semen*. In addition, using data on the zooplankton and phytoplankton composition from a lake survey (40 Swedish lakes), we further hypothesized that grazer species able to feed on *G. semen* do not co-occur with *G. semen* in most natural systems. Finally, correlating the zooplankton and phytoplankton data, we investigated how zooplankton abundance is affected in lakes with a high abundance of *G. semen*.

Methods

Two sets of experiments were performed. First, grazing experiments were conducted to determine if the potential grazers were able to feed on *G. semen* and a known edible species (*Pseudokirchneriella subcapitata*, Chlorophyceae) simultaneously (referred to as the grazing experiment). A second experiment was performed to determine the maximum grazing rate of *D. magna* on *G. semen*. Thus, *D. magna* was allowed to feed on a monoculture of *G. semen* (referred to as the maximum grazing rate experiment).

Collection of grazers and algal cultures—For the grazing experiment, four species were tested, including three zooplankton species (*D. magna*, *D. pulex*, and the calanoid copepod *E. gracilis*), and *Chaoborus*. The grazer individuals were sampled a few days before the beginning of each experiment. *D. magna* and *Chaoborus* sp. were collected from ponds and lakes close to Lund, Sweden (55°33′N, 13°38′E). *D. pulex* was collected from lake Bysjön, Sweden (55°40′N, 13°32′E). The calanoid copepods were sampled from Lake Krankesjön, Sweden (55°42′N, 13°28′E). All the potential grazer individuals were collected from environmental samples and had a wide size spectrum to mirror a lake population. The grazing experiments were carried out with cultures of two algal species: *G. semen* GSBO182 (isolated in July 2009 from Lake Bökesjön, Sweden (55°34′N, 13°26′E) and *P. subcapitata* NIVA-CHL1 obtained from the Norwegian Institute for Water Research (NIVA) culture collection. The algal cultures were grown with artificial modified Wright's cryptophyte (MWC) medium (Guillard and Lorenzen 1972) modified by an addition of selenium (1.84 mg L^{-1}) at 20°C with a 14 : 10 light : dark (LD) cycle and a light intensity of 20 μmol m^{-2} s^{-1}.

For the maximum grazing rate experiment, *D. magna* (clone 4, originating from Sheffield, UK) individuals were isolated from continuous cultures with M7 medium with *P. subcapitata* NIVA-CHL1 as a food source. The *G. semen* strain used in the experiment was *G. semen* NIVA-7/05, isolated from Lake Vansjø, Østfold, Norway (59°43′N, 10°67′E) in 2005. Prior to this experiment, it was grown at 20°C under continuous light condition with an intensity of 5–8 μmol m^{-2} s^{-1} and in a 20% modified Z8 medium (Kótai 1972) with addition of vitamins (100 μg L^{-1} thiamin, 1 μg L^{-1} biotin, and 1 μg L^{-1} vitamin B12) and soil extract.

Grazing experiments—The grazing experiments were carried out with the four different grazer species independently

in 100 mL of MWC medium containing both *G. semen* and *P. subcapitata*. *P. subcapitata* was used to allow the grazers to actively select their food. The general design of the grazing experiment was based on Lehman and Sandgren (1985), a method that has been widely applied (Hansson 1996). The initial algal cell concentrations were chosen to obtain equal biovolume of both algal species during the experiment. The respective concentration of *G. semen* and *P. subcapitata* was 40 cells mL^{-1} and 1100 cells mL^{-1}, respectively. The *G. semen* cell concentration was determined to correspond to a pre-bloom concentration (i.e., 40,000 cells L^{-1}), when the influence of grazers to limit the formation of a bloom should be the highest. The experiments included six treatments with zooplankton abundances of one, two, three, four, five, or six individuals, respectively, corresponding to a concentration of 10–60 individuals per liter. The experiments were conducted for 24 h at 20°C, with a 14 : 10 LD cycle with a light intensity of 20 μmol m^{-2} s^{-1}. As the growth rate of *G. semen* is low (approximately 0.08 cell d^{-1}; K. Lebret et al. unpubl.; Rengefors et al. 2008), the change in the *G. semen* abundance during the experiment was considered negligible. To determine the initial abundance of *G. semen* and *P. subcapitata*, 25-mL samples were collected before addition of the grazers and preserved with Lugol's solution. After 24 h, the experiments were stopped by addition of a few drops of Lugol's solution to each flask. The samples were settled overnight in 25-mL counting chambers, and the cells were counted using a inverted microscope (Nikon Eclipse TS100) at ×100 magnification for *G. semen* and ×400 for *P. subcapitata*.

Maximum grazing rate experiment—To determine the maximum grazing rate of *D. magna* on *G. semen*, a grazing experiment was carried out on a monospecific culture of *G. semen* (NIVA-7/05). Prior to this experiment, young *D. magna* individuals were kept in M7 medium with no addition of food for 2 d. At the day of experiment, they were 13 d old with no egg sacs and approximately 2 mm long. Immediately before the experiment, the *D. magna* were transferred to fresh M7 medium to wash off any residue of other algae used as food source. The experiment was carried out in six-well plates (Nunc) with 10 mL of M7 medium with a concentration of *G. semen* of 105 cells mL^{-1}. Six treatments were performed with, respectively, zero, one, two, three, four, and five *D. magna*. Four replicates were performed for each treatment. The experiment was carried out for 6 h in a climate-controlled room at 20°C during light conditions (5–8 μmol m^{-2} s^{-1}). The experiment was terminated by adding one drop of Lugol's solution to each well. The plates were then left in darkness to sediment overnight. For determination of *G. semen* abundance at the end of the experiment, all wells were counted using an inverted microscope (Olympus IX71) at ×40 and ×100 magnification.

Net growth rate and ingestion rate determination—To determine the effect of each grazer species on *G. semen* and *P. subcapitata*, the changes in each phytoplankton abundance were determined according to Lehman and Sandgren (1985). The changes in phytoplankton abundance were then expressed for each algal species and grazer abundance as net growth rate (r) calculated using Eq. 1:

$$r = \ln(N_t/N_0)/\Delta t \quad (1)$$

where N_t is the final cells concentration (cells mL^{-1}) of the respective algal species, N_0 the initial cell concentration (cells mL^{-1}), and Δt the running time of the experiment in days.

Also, to compare the two experiments with *D. magna*, the ingestion rate (I) of *G. semen* by *D. magna* was calculated using Eq. 2:

$$I = (n_0 - n_t)/(n_g{}^x dt) \quad (2)$$

where n_0 is the initial *G. semen* cell abundance, n_t the final *G. semen* abundance, n_g the number of *D. magna* individuals, and dt the running time of the experiment in hours.

Table 1. Physicochemical characteristics of the 40 Swedish lakes sampled for the lake survey.

Parameters (unit)	Range observed in the 40 lakes
Lake size (km^2)	0.03–1.50; 3.30 for one lake
pH	5.06–7.41
Total phosphorus ($\mu g\ L^{-1}$)	2.78–16.40
Dissolved phosphorus ($\mu g\ L^{-1}$)	1.03–7.24; 13.5 for one lake
Total nitrogen ($\mu g\ L^{-1}$)	263.6–1112.0
Dissolved organic carbon (DOC; $mg\ L^{-1}$)	3.71–26.85

Field sampling—In July 2007, 40 lakes were sampled in southern Sweden to determine the zooplankton composition and *G. semen* abundance. The lakes were selected to cover a wide range of *G. semen* abundances from very dense populations to an absence of cells. The choice of the lakes was also made to obtain a reduced range of physicochemical parameters (Table 1). Neither the zooplankton nor the *G. semen* abundances were correlated with the physicochemical characteristics of the lakes. The zooplankton samples were collected by filtering and concentrating 10 liters of epilimnetic water through a 100-μm net. The concentrated samples were then preserved using Lugol's solution. For each sample, the zooplankton abundance was determined by analyzing a 2-mL subsample using an Olympus CK2 inverted microscope. The identification and counting of the large zooplankton were done at ×10 magnification and the small species at ×20 magnification. For each species, the length of up to 10 randomly chosen individuals was measured. For the determination of *G. semen* abundance, a 50 mL aliquot of epilimnion water was collected using a 2-m-long Plexiglas tube sampler and preserved with Lugol's solution. Twenty-five milliliters of each sample was sedimented overnight in a 25-mL counting chamber; *G. semen* cells were then counted using an inverted microscope (Nikon Eclipse TS100) at ×40 magnification.

Statistics—To determine the effect of the individual grazer species on each algal species for each experiment, Pearson correlations were calculated between the number of grazer individuals and the growth rate (r) of the respective algal species. The correlations were considered as significant at $p < 0.05$. To determine the effect of the

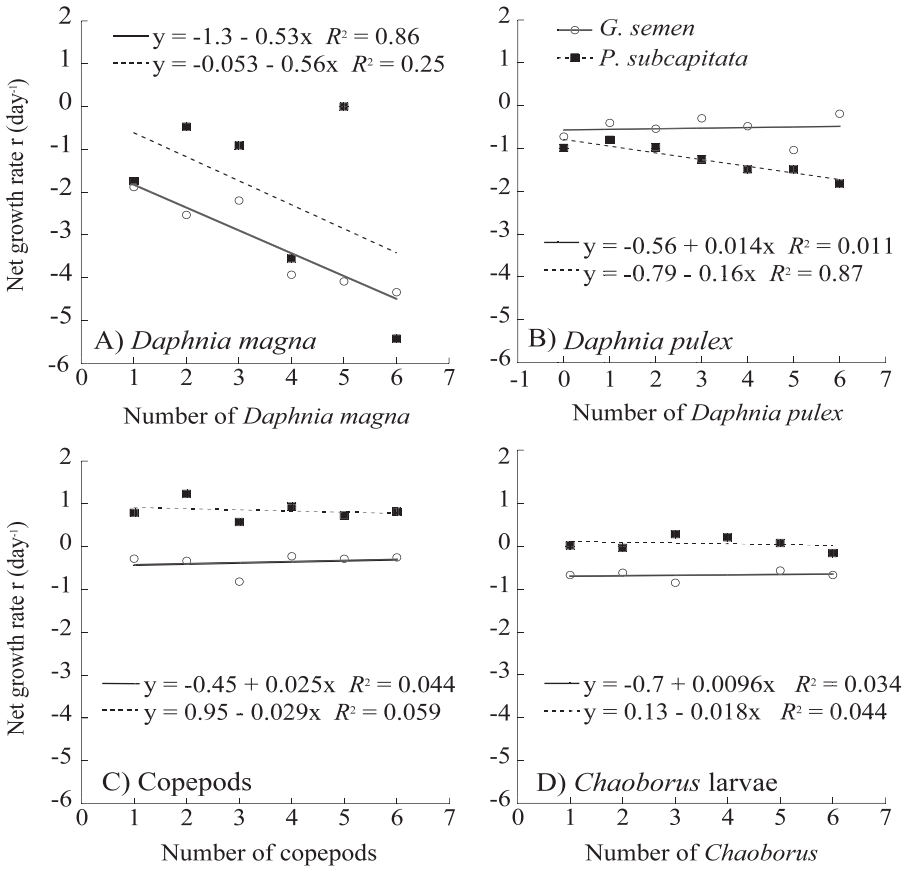

Fig. 1. Effect of the number of the different grazers on the two phytoplankton species: *Gonyostomum semen* and *Pseudokirchneriella subcapitata* expressed as net growth rate (r) between the beginning and end of the grazing experiment. (A) *Daphnia magna*; (B) *Daphnia pulex*; (C) copepods; (D) *Chaoborus* larvae.

main factor (grazer species) on net growth rate, an ANCOVA was carried out for each algal species separately, with the number of grazer individuals as a covariate. To identify the grazer species causing the significance in the ANCOVA test, a pairwise comparison using the Bonferroni method was carried out. For the field survey, the data were log transformed, and then Pearson correlations were calculated to determine the presence of any potential correlation between the abundance of *G. semen* and the abundance of the different groups of potential zooplanktonic grazers (Rotifers, cladocerans, and copepods). The cladoceran species were grouped in size classes. The three size classes were small, medium, and large cladocerans, with respective average length from eye to end of the carapace of 0–500, 500–1000, and $>$ 1000 μm. All the statistics were preformed in the Statistical Package for the Social Science version 19 for Macintosh.

Results

Grazing experiments—The number of grazers did not have a significant effect on *G. semen*'s net growth rate. However, grazer species had a significant effect on the growth rate of *G. semen* ($F_{3,19} = 34.499$, $p = 0.000$). The Bonferroni test revealed that only *D. magna* significantly reduced the growth rate of *G. semen* (Fig. 1), as the grazing by *D. magna* treatment was significantly different from the grazing by *D. pulex* ($p = 0.000$), *Chaoborus* larvae ($p = 0.000$), and *E. gracilis* ($p = 0.000$). *G. semen* growth rate was significantly reduced with increasing number of *D. magna*, suggesting that *D. magna* successfully feed on *G. semen* ($r = -0.927$, $p = 0.008$; Fig. 1A). Each *D. magna* individual, on average, ingested between 29 and 83 *G. semen* cells per hour. The ANCOVA detected significant effects of the number of grazers ($F_{1,18} = 6.327$, $p = 0.022$) and of the grazer species on the growth rate ($F_{3,18} = 16.358$, $p = 0.000$) for the grazing on the control species *P. subcapitata*, and *D. magna* had a significantly different effect on *P. subcapitata* growth rate in contrast to *Chaoborus* larvae ($p = 0.003$) and the calanoid copepod *E. gracilis* ($p = 0.000$; Fig. 1A,C,D). The growth rate of *P. subcapitata* showed a tendency to be reduced by *D. magna*; however, the correlation was not significant ($p = 0.309$). The effect of *D. pulex* on *P. subcapitata* growth rate was significantly different from the effect by copepods ($p = 0.007$; Fig. 1 B, C). The growth rate of *P. subcapitata* was significantly reduced with increasing abundances of *D. pulex* ($r = -0.983$, $p = 0.000$; Fig. 1 B).

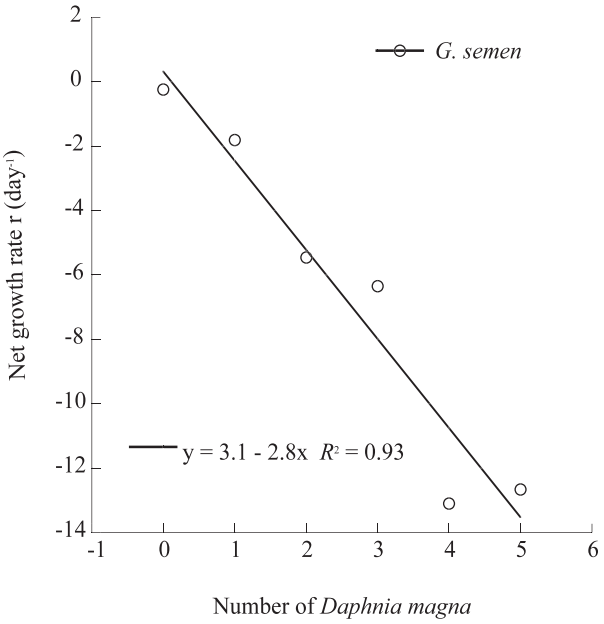

Fig. 2. Effect of the number of *Daphnia magna* on *Gonyostomum semen* expressed as net growth rate (r) between the beginning and end of the maximum grazing rate experiment.

Maximum grazing rate experiment—G. semen net growth rate was significantly reduced with increasing *D. magna* abundance ($r = -0.966$, $p = 0.002$; Fig. 2). The slope of the trend line in the maximum grazing experiment was five times higher than the slope of the grazing experiment involving two phytoplankton species (Figs. 1, 2) for the grazing on *G. semen* by *D. magna*. In the maximum grazing rate experiment, *D. magna* ingested between 38 and 78 cells of *G. semen* per hour.

Field sampling—G. semen was observed in 31 out of 40 sampled lakes, with abundances ranging from 120 to 250,200 cells L^{-1}. The copepod abundance was significantly and negatively correlated to *G. semen* abundance ($r = -0.601$, $p = 0.000$). The abundances of cladocerans and rotifers were not significantly correlated to *G. semen* abundance in the 40 lakes sampled ($p = 0.238$ and $p = 0.136$, respectively; Fig. 3A,C). However, in lakes with high cell concentration of *G. semen*, cladocerans and rotifers did not reach high abundances (Fig. 3). Large cladocerans (length > 1000 μm) were not observed in any of the 40 lakes. The cladocerans observed in the 40 lakes were all smaller than the *D. magna* individuals used in the experiment (Table 2). However, *D. pulex* individuals used in the experiment were of similar size to the largest cladocerans observed in the lakes (Table 2). The copepods from the experiments and those from the lake sampling showed similar size range (Table 2).

Discussion

In this study, we have investigated for the first time, the direct effect of grazing on the bloom-forming alga *G. semen*. Earlier studies have hypothesized that the grazing pressure by cladocerans and large rotifers such as *Asplanchna* on *Gonyostomum* might be high (Cronberg et al. 1988). Moreover, Gutseit et al. (2007) showed that *G. semen* was good-quality food for zooplankton in terms of fatty acid content. However, a limited amount of these fatty acids was transferred to the zooplankton level, suggesting that zooplankton may not successfully feed on *G. semen*. Our experiment shows that of the species tested, only large cladocerans, such as *D. magna*, were able to graze on *G. semen*. These results are consistent with the study of Burns (1968) showing that *D. magna* can ingest particles larger than 50 μm and up to 80 μm, while smaller species, such as *D. pulex*, could not ingest particles larger than 40 μm. *G. semen* commonly reaches sizes of 60–70 μm (own observations from field and culture, data not shown) and is therefore protected from the grazing pressure by small cladoceran species. In addition, daphnids are not selective feeders, and only the size of their prey can limit their feeding behavior such that particles too large to pass their filtering apparatus are rejected passively (Burns 1968). The results of our grazing experiments with *D. magna* were in accordance with the unselective feeding character of cladocerans, as their feeding rate on *G. semen* was higher when feeding on a monoculture of *G. semen* than when feeding on a mix of *G. semen* and *P. subcapitata*. Since *D. magna* feed on both strains of *G. semen* (GSBO182 and NIVA-7/05), we have no indication that potential physiological differences between the strains have altered the grazing behavior of *D. magna*.

Calanoid copepods, in contrast to daphnids, are selective grazers, selecting for tasty food (Demott 1986) or for algal cells with a high nutritional value (Butler et al. 1989). In our experiment, the calanoid copepod *E. gracilis* did not feed on either *P. subcapitata* or *G. semen*. Previous studies have shown that copepods feed selectively on algal cells larger than 20 μm (Nival and Nival 1976). Thus, the absence of grazing on *G. semen* cannot be explained by size limitation of the prey. For example, Demott and Watson (1991) showed that copepods were able to selectively feed on the green algae *Pediastrum*, which is of similar size to *G. semen* (80 μm and 60–70 μm, respectively). In contrast, the absence of grazing on *P. subcapitata* was probably the consequence of prey size selection by the calanoid copepods, as *P. subcapitata* is a small phytoplanktonic species with a diameter of approximately 7 μm (K. Lebret unpubl.).

As size limitation cannot explain the lack of grazing on *G. semen* by *E. gracilis*, it is likely that *G. semen* has some defense adaptation against predation. Some possible adaptations are grazer-deterring taste or smell, expulsion of mucilaginous trichocysts, or rapid escape behavior of *G. semen*. Although we have observed jumping behavior as well as trichocyst expulsion in the microscope, our results from the present study do not allow us to make any definitive conclusions regarding the mechanisms behind *G. semen*'s grazing avoidance. Uye and Takamattsu (1990) suggested that the marine raphidophytes *Chatonella marina* and *Fibrocapsa japonica* produce intracellular deterrent compounds, limiting the grazing by copepods. A similar adaptation may be present in *G. semen*, and their

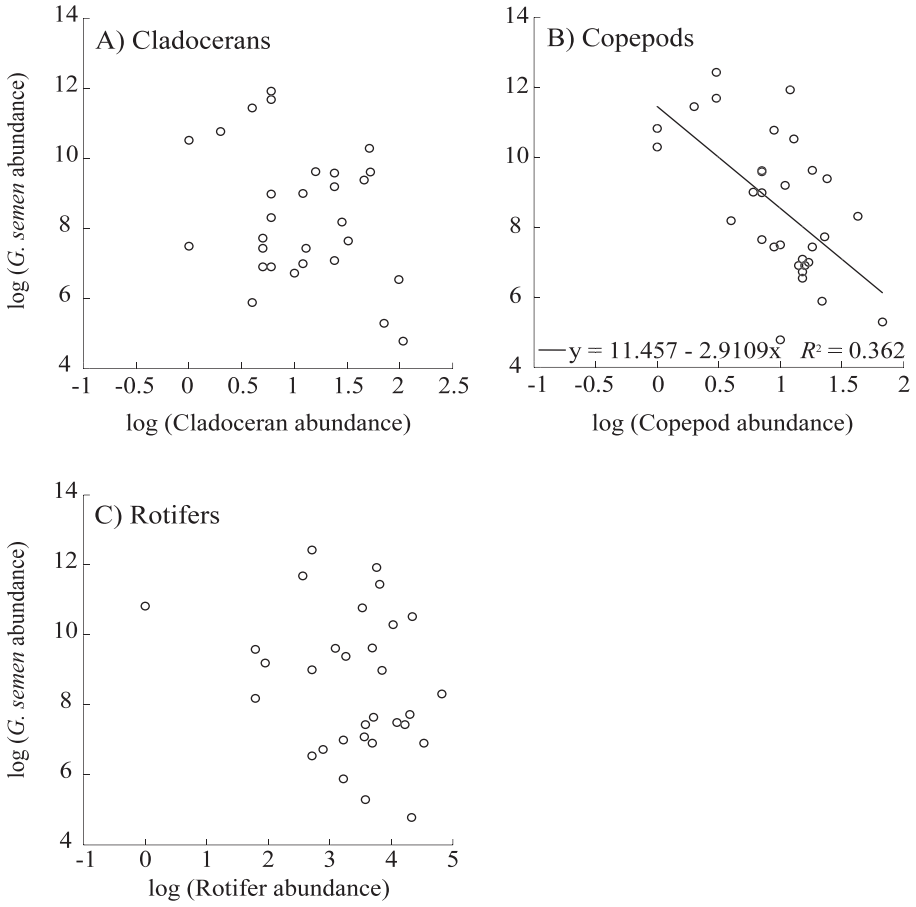

Fig. 3. *Gonyostomum semen* abundance against the abundance of the different zooplankton groups in the 40 Swedish lakes. (A) cladocerans; (B) copepods; (C) rotifers.

trichocysts may be a key factor in the protection against grazers. In dinoflagellates, Ukeles and Sweeney (1969) observed that grazing was reduced on species producing trichocysts, suggesting that trichocysts are involved in the protection against grazers, possibly clogging the feeding apparatus of the grazers. In the marine raphidophyte *Fibrocapsa japonica*, strains with trichocysts showed higher escape rates compared to strains without trichocysts during a grazing experiment with dinoflagellates (Tillmann and Reckermann 2002), again suggesting a function as grazer deterrent.

Table 2. Size range of the grazer species used in the experiment and of the grazers found in the field samples from the 40 lakes (—, no data).

Zooplankton group		Size range in μm	
		Experiment	Field
Cladocerans	All	—	230–925
	Daphnia pulex	375–1375	—
	Daphnia magna	1250–2750	—
Copepods		750–1175	300–1050
Rotifers		—	80–750
Chaoborus		5000–9250	—

Chaoborus larvae were used in our experiment since data from a recent study showed that they occurred at higher abundances in lakes with high biomass of *G. semen* (Trigal et al. 2011). However, *Chaoborus* is a predator and is not known to feed on phytoplankton. Accordingly, our data showed that *Chaoborus* larvae do not eat *G. semen* or *P. subcapitata*, which is consistent with its predatory behavior. Thus, *G. semen* cannot directly influence the abundance of *Chaoborus* despite the higher abundance in lakes with a high biomass of *G. semen*. Trigal et al. (2011) suggested that *Chaoborus* larvae may be more successful in *G. semen* lakes because of lower competition with young planktivorous fish, which were found to be less abundant in lakes with higher cell concentration of *G. semen*. Regardless of the mechanism, our study rules out the possibility that *Chaoborus* larvae may be able to feed on *G. semen* and benefit directly from *G. semen* bloom.

The results from our grazing experiment show that only large cladoceran species such as *D. magna* were able to feed on *G. semen*. The zooplankton communities sampled in the 40 lakes did not have any large cladocerans. The largest cladocerans were of similar size as the *D. pulex* used in the experiment, which were not able to feed on *G. semen*. These results suggest that there was little or no grazing pressure on the *G. semen* populations. Large zooplankton are generally

absent in lakes with fish and especially the young-of-the-year (YOY) fish, feeding intensely on zooplankton populations with a greater effect on the largest individuals (Hansson et al. 1998). Hence, during years with low recruitment of YOY fish, large zooplankton may occur in high abundance, increasing the grazing pressure on *G. semen*.

G. semen is a slow-growing species (Rengefors et al. 2008) and thus is likely to have evolved adaptations to avoid being grazed. A high grazing pressure on a slow-growing species would result in a considerable loss of biomass, which may not be compensated by growth, especially during the early stage of the bloom. Algal bloom initiation phases are critical in the bloom formation, which may be highly dependent of the interactions with zooplankton. Uye (1986) demonstrated that copepod grazing did not have any significant effect on the biomass of the marine raphidophyte *Chatonella antiqua* during bloom condition, although copepods were able to reduce the abundance of *C. antiqua* in the initial phase of the bloom. Thus, the capacity of zooplankton to control algal blooms may be greatest at the beginning of the bloom. Nevertheless, zooplankton can control the growth of a blooming species only if the algal cells are edible. An adaptation that makes algae difficult to eat or digest can thus lead to dense populations.

One reason why some species are invasive may be due to the absence or reduction of grazing or predation pressure. In several studies, it has been observed that exotic or invasive species, especially plants, are favored in the newly colonized environment by a reduction of the grazing pressure due to absence of natural enemies or a reduced grazing pressure from the local enemies (Wolfe 2002). For *G. semen*, the absence of potential grazers is an important aspect of their bloom dynamic, as *G. semen* is a slow-growing species that may not be able to compensate for biomass loss due to grazing. Thus, limited grazing can help explain why *G. semen* has successfully been able to colonize new lakes, even if it does not explain why the species has spread to more lakes.

The calanoid copepod *E. gracilis* was not able to feed on *G. semen* in our experiment, but we cannot extrapolate this conclusion to all copepod species. However, the survey data showed a negative correlation between copepods and *G. semen* abundances. Also, our data showed that in lakes with high *G. semen* abundance, cladocerans and rotifers, despite the lack of correlations, never had abundances as high as those observed in some lakes with a low abundance of *G. semen*. These results have two possible interpretations. The data suggest either that *G. semen* avoided high zooplankton abundances by avoiding recruitment to the pelagic zone and formed blooms only at low zooplankton abundances (Hansson 2000) or that the inedibility of *G. semen* negatively affected the zooplankton community through starvation. Results from our experiments support the latter interpretation, but further investigations are necessary to confirm this hypothesis as well as to highlight and understand the potential effect of *G. semen* bloom on the zooplankton community. Blooms of toxic or inedible algae have, in other studies, been observed to induce severe effects on zooplankton (Hansson et al. 2007), mostly due to lower food availability.

The results of our study suggest that measures to restore lakes affected by *G. semen* bloom might be successful only if it leads to a drastic reduction in planktivorous fish, followed by a considerable increase in the abundance of large cladocerans. Moreover, such drastic measures are unrealistic on a short-term or large-scale restoration program. Also, not only might a reduction of *G. semen* abundance following an increase in large zooplankton be caused by an increase of the grazing pressure, but the migration of newly germinated *G. semen* cells from the sediment will also be reduced when large zooplankton are abundant (Hansson 2000).

Thus, our results show that *G. semen* is little affected by grazing because of the absence of zooplankton species able to feed on it. Of the zooplankton grazers tested, only the large *D. magna*, not present in the lakes investigated, was able to feed efficiently on *G. semen*. The defense strategy of *G. semen* against copepods is not known, but we hypothesize that trichocysts may be involved. Also, the large cell size of *G. semen* is likely to protect *G. semen* from gazing by smaller daphnids. Finally, the absence of grazing pressure on *G. semen* is probably one factor explaining their success, facilitating the establishment of dense populations and being important in the bloom and invasion processes.

Acknowledgments

We thank Wilhelm Granéli for allowing us to use the field samples from the 40-lake survey. We thank Mattias Ekvall and Johan Ahlgren for the collection of the *Chaoborus* larvae and Mikael Ekvall for the constructive comments on the early version of this manuscript. The Swedish Council for Environment, Agricultural Sciences and Spatial Planning (Formas); the Crafoord Foundation (to K.R.); and the Swedish Research Council (VR, to L.A.H.) provided financial support. We also would like to thank the two anonymous reviewers for their constructive comments and suggestions to improve the manuscript.

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Associate Editor: David A. Caron

Received: 31 October 2011
Accepted: 4 February 2012
Amended: 9 February 2012