Drivers and Food Web Effects of *Gonyostomum semen* Blooms

Karin S.L. Johansson
Faculty of Natural Resources and Agricultural Sciences
Department of Aquatic Sciences and Assessment
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2013

Acta Universitatis agriculturae Sueciae
2013:52

Cover: Lake Hagasjön
(photo: C. Trigal)

ISSN 1652-6880
ISBN 978-91-576-7842-3 (printed) and 978-91-576-7843-0 (digital)

Print: SLU Service/Repro, Uppsala 2013

Drivers and Food Web Effects of *Gonyostomum semen* Blooms

Abstract

The flagellate *Gonyostomum semen* forms dense late-summer blooms in humic lakes and is a nuisance to swimmers because it forms a slimy coat on the skin, causing irritation in sensitive individuals. Increasing occurrence and bloom incidence of *G. semen* has been reported during recent decades, but it is not clear which factors affect the distribution and bloom formation of this alga. Large cell size, ejection of long, slimy threads (trichocysts), and nighttime migration to the hypolimnion may limit grazing on *G. semen* by herbivorous zooplankton, resulting in a decreased coupling between phytoplankton and higher trophic levels during blooms. The studies included in this thesis investigate which factors affect *G. semen* occurrence and bloom formation and how *G. semen* blooms affect the community composition and trophic interactions in boreal, humic lakes.

The occurrence of *G. semen* has increased between 1995 and 2010, especially in southern Sweden. Bloom incidence and total biomass did not increase continually, but fluctuated among years and peaked in the middle of the study period. Temperature and length of the growing season affected the occurrence and, to a lesser extent, bloom formation of *G. semen*, but local factors such as pH and water colour were more important for bloom formation. More lakes may become suitable habitats with the ongoing increase in water colour and increasing temperatures may result in a more frequent occurrence and bloom formation of *G. semen*.

Blooms resulted in a shift in zooplankton assemblages toward predominance by small cladocerans, which were not able to feed on *G. semen* but instead fed more on heterotrophic food resources, supporting the hypothesis of a reduced coupling between phytoplankton and zooplankton. Zooplankton assemblages predominated by small animals feeding on low-quality resources may reduce the food quality for planktivorous fish. Instead, the invertebrate predator *C. flavicans* appeared to benefit from *G. semen* blooms, as indicated by its high abundance in bloom-lakes. Calanoid copepods and a large cladoceran fed efficiently on *G. semen* in the laboratory, indicating that there is, however, some trophic coupling between *G. semen* and higher trophic levels. This supports the use of biomanipulation of fish communities for controlling *G. semen* blooms.

Keywords: Algal blooms, Raphidophyceae, trophic interactions, food webs, PUFA, bacteria, heterotrophic protists, zooplankton, benthic invertebrates

Author's address: Karin S.L. Johansson, SLU, Department of Aquatic Sciences and Assessment, P.O. Box 7050, 750 07 Uppsala, Sweden
E-mail: karin.johansson@slu.se

Sammanfattning på svenska

Algen gubbslem (*Gonyostomum semen*) bildar intensiva blomningar i humösa sjöar under sensommaren, då den kan utgöra över 95 % av den totala växtplanktonbiomassan. Gubbslem orsakar besvär för personer som badar eftersom den bildar ett slemmigt lager på huden som kan orsaka hudirritation hos känsliga individer. Flera studier har rapporterat om ökande förekomst och blomningsfrekvens av gubbslem under de senaste årtiondena, men det är inte klarlagt vilka miljöfaktorer som gynnar förekomst och blomning av algen. Gubbslemmets stora cellstorlek i kombination med att cellerna kan skicka ut långa slemmiga trådar (trichocyster) och att algen migrerar till hypolimnion på natten kan minska betningstrycket från djurplankton och leda till att flödet av energi och näringsämnen till högre trofinivåer minskar. Studierna som ingår i den här avhandlingen har undersökt vilka miljöfaktorer som bäst förklarar förekomst och blomningar av gubbslem och hur algen påverkar samhällsstruktur och födointeraktioner i boreala, humösa sjöar.

Resultaten visar att antalet sjöar där gubbslem förekommer har ökat under perioden 1995–2010, speciellt i södra Sverige. Antalet blomningar och den totala biomassan av gubbslem ökade däremot inte utan varierade mellan åren och nådde en topp under mitten av perioden. Temperatur och tillväxtsäsongens längd var viktiga faktorer för förekomsten av gubbslem och i mindre utsträckning för bildandet av blomningar. Lokala faktorer som pH och vattenfärg var dock viktigare för att blomningar skulle uppstå, vilket tyder på att gubbslem har vissa habitatkrav som måste vara uppfyllda för att blomningar ska kunna bildas. Den pågående ökningen av vattenfärg och stigande temperaturer kan göra att fler sjöar blir lämpliga habitat för gubbslem, att vi oftare kommer att hitta gubbslem i våra sjöar och att antalet blomningar kommer att öka.

Blomningar av gubbslem ledde till att djurplanktonsamhällena dominerades av små cladocerer. De kan inte äta gubbslem utan åt istället mer heterotrofa födokällor (bakterier eller heterotrofa protister), vilket stöder hypotesen om en minskad koppling mellan växt- och djurplankton under blomningar av gubbslem. Ett djurplanktonsamhälle dominerat av små djur med en diet som till stor del består av lågkvalitativa födoresurser kan utgöra en dålig resursbas för planktivora fiskar. Däremot tyder stora antal av den rovlevande tofsmygglarven *Chaoborus flavicans* i blomningssjöar på att denna predator gynnas av gubbslemsblomningar.

Det fanns trots allt några djurplanktonarter som kunde äta gubbslem – calanoida copepoder och en stor cladocerart. Detta tyder på att blomningar av gubbslem skulle kunna kontrolleras genom biomanipulation av fisksamhällen.

Though a straight line appears to be the shortest distance between two points,
life has a way of confounding geometry.
Gordon Livingston

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Trigal C, Hallstan S, Johansson KSL, Johnson RK (2013). Factors controlling occurrence and bloom formation of the nuisance flagellate *Gonyostomum semen* in boreal lakes. *Harmful Algae* In press.

II Johansson KSL, Trigal C, Vrede T, Johnson RK (2013). Community structure in boreal lakes with recurring blooms of the nuisance flagellate *Gonyostomum semen*. *Aquatic Sciences* DOI 10.1007/s00027-013-0291-x

III Johansson KSL, Vrede T, Lebret K, Johnson RK (2013). Zooplankton feeding on the nuisance flagellate *Gonyostomum semen*. *PLOS ONE* 8(5)

IV Johansson KSL, Trigal C, Vrede T, van Rijswijk P, Goedkoop W, Johnson RK. Algal blooms induce shifts in basal food resources for consumers in boreal lakes – evidence from fatty acid biomarkers. (manuscript).

Papers I–II are included with the permission of the publishers.

The contribution of Karin S.L. Johansson (KJ) to the papers included in this thesis was as follows:

I KJ was partly involved in the selection of predictor variables, in the interpretation of the results and in the writing of the paper

II KJ and CT were responsible for the field sampling, KJ analyzed the data and wrote the paper

III KJ designed the study together with TV, collected the experimental animals in the field, conducted the experiment, analyzed the data and wrote the paper

IV KJ was partly involved in the design of the study, prepared and extracted the samples for fatty acid analysis, analyzed the data and wrote the manuscript

Abbreviations

DOC	Dissolved organic carbon
POC	Particulate organic carbon
PUFA	Polyunsaturated fatty acids
EFA	Essential fatty acids
LA	Linoleic acid
ALA	Alpha-linolenic acid
ARA	Arachidonic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
OOB observations	Out-of-bag observations
AUC	Area under receiver operator curve
NMDS	Non-metric multidimensional scaling
NPMANOVA	Non-parametric multivariate analysis of variance
SIMPER	Similarity percentage
ANOVA	Analysis of variance
Tukey's HSD test	Tukey's honestly significant difference test
GC-FID	Gas chromatography-flame ionization detection
GC-MS	Gas chromatography-mass spectrometry

1 Introduction

Algal blooms constitute a societal and ecological problem that has rendered increasing attention from scientists and policymakers during recent decades. Bloom incidence appears to have increased, although increased research focus and monitoring of algal blooms may also have resulted in a higher detection probability (Anderson et al., 2002). Algal blooms are, however, not a recent phenomenon; discoloration and foul smell of the water in the Nile followed by a fish kill is mentioned in the Bible (Exodus 7:20–21) and Charles Darwin reported results from microscopic examination of an algal bloom in the 19th century (Granéli & Turner, 2006). Perhaps the most well-known consequences of algal blooms are fish kills and shellfish poisoning caused by toxin-producing species, but blooms of non-toxic algae may also have large socio-economic and ecological impacts. The effects of non-toxic blooms include anoxia, food web decoupling, fish mortality through suffocation, shading of other primary producers, restricted recreational use of aquatic ecosystems, and decreasing property value (Brett & Müller-Navarra, 1997; DeMott *et al.*, 2001; Anderson *et al.*, 2002; Granéli & Turner, 2006; Hoagland & Scatasta, 2006; Dodds *et al.*, 2008). The economic costs related to algal blooms have been estimated to $800 million in the European Union and $80 million in the United States of America (Hoagland & Scatasta, 2006).

1.1 Drivers of algal blooms

The apparent increase in bloom incidence has often been attributed to anthropogenic environmental change. Increased nutrient levels, primarily phosphorus and nitrogen, have been shown to increase productivity and algal biomass through both experimental nutrient additions and correlative studies (Schindler, 1977; Smayda, 1990). Global warming is expected to cause an increased strength and duration of water column stratification and decreased viscosity of surface waters, favouring algae that can regulate their position in the water column, including many bloom-forming flagellates and cyanobacteria (Edwards *et al.*, 2006; Paerl & Huisman, 2009). In addition, higher water temperatures and longer growing seasons provide extended periods of optimal growth conditions for thermophilic taxa (e.g. cyanobacteria), resulting in higher bloom incidence and a poleward range expansion of bloom-forming taxa from warmer areas (Paerl & Huisman, 2009). Extreme weather events such as hurricanes as well as human-mediated transport via e.g. ship ballast water may also facilitate the range expansion of bloom-forming taxa (Hallegraeff, 2010). Human alterations of aquatic food webs through overfishing of piscivores may also lead to increased bloom incidence by increasing the predation pressure from planktivorous fish on grazing zooplankton and thus reducing grazing on phytoplankton (Brett & Goldman, 1996).

1.2 The pelagic food web

In the classical view on pelagic food webs, phytoplankton constitutes the base of the food web and nutrients and energy are transferred to higher trophic levels through direct consumption by metazoan zooplankton (multicellular zooplankton, hereafter referred to as zooplankton). However, other basal food resources may also be used by zooplankton. Thirty years ago, Azam et al. (1983) described an additional pathway for carbon transfer to higher trophic levels, called the *microbial loop*. This pathway involves bacterial recycling of dissolved organic carbon (DOC) that has been leached from growing and senescing algal cells and excreted by animals. The organic matter re-enters the pelagic food web as bacteria are consumed by zooplankton, either through direct ingestion or via consumption of heterotrophic protists. In addition to within-lake carbon fixation, there is a substantial input of terrestrial (allochthonous) carbon in many lakes. Numerous studies have shown a significant contribution by allochthonous carbon to aquatic consumers (reviewed by Cole et al., 2011), although it is not completely clear how this organic matter is transferred to higher trophic levels. Most of the allochthonous carbon reaches aquatic ecosystems in the dissolved phase (Ivarsson & Jansson, 1994) and Tranvik (1992) suggested that bacteria introduce this dissolved terrestrial carbon into the pelagic food web as they do with aquatic (autochthonous) DOC. Other authors have suggested that particulate forms of terrestrial carbon (POC) are more important for higher trophic levels, despite their smaller contribution to the total carbon pool (Cole et al., 2011).

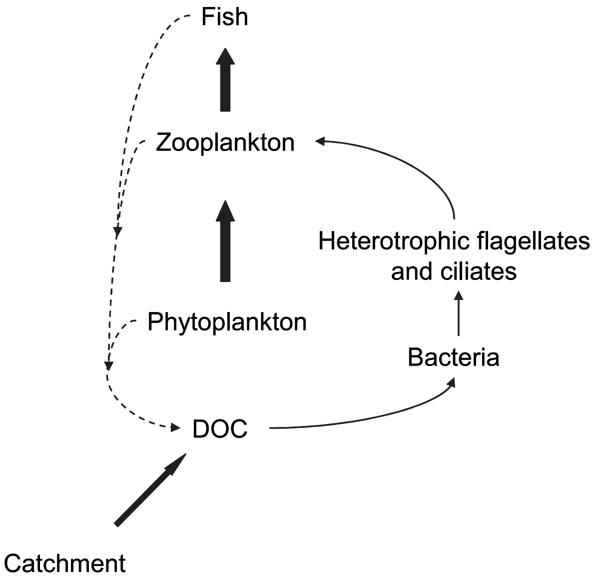

Figure 1. Schematic illustration of a pelagic food web

1.3 Food quality

The nutritional quality of terrestrial POC, bacteria and bacterivorous protozoa is, however, low. For example, these food resources are generally devoid of or contain very low quantities of polyunsaturated fatty acids (PUFA) (Napolitano, 1999). Certain PUFA are necessary for normal growth and reproduction of zooplankton as well as vertebrates, and need to be acquired from the diet because they cannot be synthesized *de novo* by animals (Brett & Müller-Navarra, 1997). These fatty acids are often referred to as essential fatty acids (EFA). Although there is some disagreement among researchers regarding exactly which fatty acids should be termed essential, five ω3 and ω6 PUFA are commonly classified as EFA in aquatic studies. These are the long-chain fatty acids ARA (arachidonic acid, 20:4ω6), EPA (eicosapentaenoic acid, 20:5ω3) and DHA (docosahexaenoic acid, 22:6ω3), and their precursors LA (linoleic acid, 18:2ω6) and ALA (alpha-linolenic acid, 18:3ω3) (Smyntek *et al.*, 2008). ARA and EPA are precursors of hormones involved in reproductive and immunological processes and DHA serves important functions in the regulation of membrane properties and the development and maintenance of brain and visual functions (Brett & Müller-Navarra, 1997; Parrish, 2009).

Eukaryotic algae generally have a high PUFA content, and the difference in nutritional quality between algae and terrestrial POC, bacteria and bacterivorous protists has been demonstrated in several experiments. Some of the alternative food resources could not even support survival of the study organisms. For example, *Daphnia* fed peat soil POC as a single food source died after two days (Wenzel *et al.*, 2012a) and those fed pure bacterial cultures survived only 5–12 days (Taipale *et al.*, 2012; Wenzel *et al.*, 2012b). Terrestrial POC in the form of ground, senesced alder leaves, as well as bacterivorous protists supported some growth and reproduction when provided as the sole food resource, but at much lower rates than supported by pure algal diets (DeBiase *et al.*, 1990; Ederington *et al.*, 1995; Brett *et al.*, 2009). However, when alternative food resources are provided together with high-quality algae, the mixtures may support a similar level of growth and reproduction as a pure algal diet (Taipale *et al.*, 2012). Brett *et al.* (2009) found that when combined with high quality phytoplankton, terrestrial POC contributed more to *Daphnia* production than would be expected based on its low quality as a single food resource. This suggests that the contribution of a certain proportion of high quality algae in the diet may facilitate simultaneous utilization of alternative food resources. In the experiment by Brett *et al.* (2009), algal fatty acids had a disproportionately large impact on the fatty acid composition of *Daphnia* when the animals were feeding on the mixed diet, suggesting that high quality food resources are used for production whereas the lower quality components of the diet are catabolized (Brett *et al.*, 2009).

Despite the demonstrably low food quality of alternative food resources and the importance of algae in zooplankton nutrition, populations of cladocerans and cyclopoid copepods still occurred in lake water from a humic lake after it had been kept in darkness for 1.5 years (Daniel *et al.*, 2005). The authors of this experimental study suggested that trophic upgrading of microbial food resources by heterotrophic protists could explain the survival of zooplankton in the absence of phytoplankton production. Although trophic upgrading in protists consuming heterotrophic bacteria, to my knowledge, has not been reported from freshwater systems, an experiment by Bec *et al.* (2010) in which the heterotrophic chrysophyte *Paraphysomonas* was fed cyanobacterial strains lacking PUFA synthesis suggests that some heterotrophic protozoa are capable of *de novo* synthesis of PUFA. How common this is among different taxa, however, remains to be investigated.

1.4 Zooplankton feeding behaviour

Among pelagic zooplankton there are two major feeding modes: suspension feeding and raptorial feeding. Suspension feeders collect small particles suspended in the water by creating a water current that brings particles close to feeding appendages by which they are collected (Kiørboe, 2011). Herbivorous cladocerans, calanoid copepods and most rotifers are suspension feeders, but their mode of particle capture differs. In cladocerans, the feeding current brings food particles into the space between the carapace valves and the animals collect them using the comb-like structures on their thoracic appendages. The particles are transferred to the so called food groove between the thoracic limbs and transported forward to the mouth (McMahon & Rigler, 1963). Suspension-feeding rotifers and calanoid copepods both collect individual particles from the feeding current: rotifers by using cilia close to the mouth and calanoid copepods by grasping particles with their maxillae (Price *et al.*, 1983; Wetzel, 2001).

Food selectivity varies among suspension-feeding zooplankton. Cladocerans and rotifers may show some selectivity by rejecting unwanted particles (Starkweather, 1980; Kirk, 1991; Ghadouani *et al.*, 2004) and studies of *Bosmina* suggest that some herbivorous cladocerans may search more actively for a certain type of food (DeMott & Kerfoot, 1982). In general, however, suspension-feeding cladocerans and rotifers are considered rather unselective grazers and the type of food particles consumed is mainly regulated by their availability in the feeding suspension and by their size (Burns, 1968; Wetzel, 2001; Kiørboe, 2011). In contrast, suspension-feeding copepods are selective and ingest particles based on quality. For example, feeding experiments have shown that calanoid copepods prefer flavoured plastic beads to untreated ones, algae to plastic beads, alive to dead algae, digestible to digestion-resistant algae, and non-toxic to toxic algae (Starkweather & Bogdan, 1980; DeMott, 1986; DeMott, 1988). The degree of selectivity is, however, influenced by the presence of high-quality food resources and the copepods tend to be less choosy if their preferred foods are scarce (DeMott, 1988; DeMott, 1989). The lower size limit of particles that can be ingested is generally higher in suspension-feeding copepods than in rotifers and cladocerans and copepods cannot feed efficiently on bacteria, in contrast to many cladocerans and rotifers (Bogdan & Gilbert, 1987; Brendelberger, 1991; Arndt, 1993; Vrede & Vrede, 2005).

Raptorial feeders detect individual, larger particles and catch them by attacking them and grasping them with their mouth parts (Wetzel, 2001). Cyclopoid copepods and some large rotifers are raptorial feeders and most calanoid copepods use both suspension feeding and raptorial feeding in parallel (Wetzel, 2001; Brönmark & Hansson, 2005). Copepods are capable of remote detection of particles and lunge at them by a jumping movement, whereas *Asplanchna* needs to come in physical contact with the prey to initiate an attack (Sarma, 1993; Kiørboe, 2011). There are both carnivorous and herbivorous species of cyclopoid copepods, whereas most calanoid copepods are predominantly herbivorous. Cyclopoid copepods prefer motile prey, whereas calanoids do not differ in their preference for motile and non-motile food particles (Demott & Watson, 1991; Wetzel, 2001). Raptorial rotifers are omnivores, feeding on both phytoplankton and animal prey (Chang *et al.*, 2010).

1.5 Food web effects of algal blooms

Many bloom-forming phytoplankton species have adaptations to reduce losses from grazing. Such adaptations may include e.g. elongation in one or more dimensions (Vanderploeg *et al.*, 1988), large cell size or colony formation (Burns, 1968; Gliwicz & Lampert, 1990; Hessen & Van Donk, 1993), mucus production (Porter, 1976), production of toxins or allelochemicals (Wolfe, 2000; Selander *et al.*, 2012), and avoidance of grazers through jumping behaviour (Jakobsen, 2002) or vertical migration (Salonen & Rosenberg, 2000; Strom *et al.*, 2013). In addition, some bloom-forming taxa are nutritionally inadequate for zooplankton (Brett & Müller-Navarra, 1997; Martin-Creuzburg & von Elert, 2009).

As zooplankton differ in their feeding mode and their ability to feed on, or avoid, different types of particles, algal blooms may result in changes in zooplankton assemblage composition. Mesocosm experiments and field observations have shown shifts in zooplankton assemblage composition during blooms of large or filamentous algae (often referred to as net phytoplankton) towards predominance of small cladoceran taxa (Xie *et al.*, 1998; DeMott *et al.*, 2001; Ghadouani *et al.*, 2003). Normally, large cladocerans are competitively superior to smaller ones due to feeding on a broader size range of food particles, more efficient particle collection and lower metabolic costs per unit body mass (Brooks & Dodson, 1965; Gliwicz, 1990). However, larger species also have wider carapace gapes and large particles are therefore more likely to enter their food groove. Particles too large to be ingested need to be rejected from the food groove, which results in energy losses and the simultaneous rejection of other food particles. Consequently, larger species become more inefficient feeders with increasing biomass of net phytoplankton and the competition pattern between large and small cladocerans is reversed (Gliwicz & Lampert, 1990). Because fish generally are visual predators, a zooplankton assemblage predominated by small cladocerans may be less favourable for zooplanktivorous fish due to the lower visibility of small prey. Instead, tactile, gape-limited invertebrate predators may be favoured (Brooks & Dodson, 1965; Hanazato & Yasuno, 1989).

Highly toxic algal blooms may have very dramatic impacts on pelagic food webs, disrupting trophic interactions by poisoning zooplankton and fish (Sunda *et al.*, 2006; Michaloudi *et al.*, 2009). Some toxic algae may instead cause sublethal responses in zooplankton, as shown in numerous experimental studies (reviewed by Landsberg, 2002). The effects include e.g. reduced feeding, reduced activity, impaired reproduction, and delayed development in juveniles (Forsyth *et al.*, 1991; Bagøien *et al.*, 1996; Frangópulos *et al.*, 2000). There have been reports of negative effects on higher trophic levels from toxin accumulation in zooplankton feeding on toxic algae (Kelly *et al.*, 1992; Scholin *et al.*, 2000), but other food web effects of interactions between toxic algae and zooplankton at sublethal concentrations appear to be quite unexplored and merit further study (Landsberg, 2002; Turner, 2006).

1.6 *Gonyostomum semen*

During recent decades, several studies have reported an increase in the occurrence and bloom frequency of *Gonyostomum semen* in northern Europe (Cronberg *et al.*, 1988; Lepistö *et al.*, 1994; Rengefors *et al.*, 2012), which has raised concerns among the public and freshwater managers. *G. semen* blooms negatively affect the recreational qualities of freshwater ecosystems by producing a mucus that attaches to the skin of swimmers, forming an unpleasant, slimy coat that may cause skin irritation in sensitive individuals. It has also been suggested that the mucus may clog filters in water treatment plants (Cronberg *et al.*, 1988).

G. semen is a large, flagellated phytoplankton species belonging to the class Raphidophyceae. Many raphidophytes are marine bloom-forming species that produce potent toxins and have substantial impacts on fisheries and ecosystem functioning (Landsberg, 2002), but no toxin production by *G. semen* has, to my knowledge, been reported. Ecological effects of *G. semen* blooms may instead be related to its physical defences and grazer avoidance behaviour. Cells of *G. semen* are large (up to 100 µm long), fragile and burst upon physical stimulation, releasing slimy threads called trichocysts – properties that likely makes them difficult for zooplankton to ingest. In addition, *G. semen* cells migrate to the hypolimnion at night whereas zooplankton avoid this cold and often anoxic part of the water column or show the opposite migration pattern, resulting in a spatial separation that may reduce encounters with grazers (Zaret & Suffern, 1976; Salonen & Rosenberg, 2000; Wissel *et al.*, 2003). During blooms, *G. semen* can constitute more than 95 % of the total phytoplankton biomass (Pithart *et al.*, 1997), suggesting that zooplankton that cannot feed on it must seek alternative food resources, such as bacteria or heterotrophic protists. Fatty acid analyses of suspended particles (seston) in lakes dominated by *G. semen* showed high levels of EPA, suggesting that the nutritional quality of this alga may be quite high (Gutseit *et al.*, 2007). However, any grazers of *G. semen* may also be reduced to using other food resources part of the time due to the vertical migration of the alga.

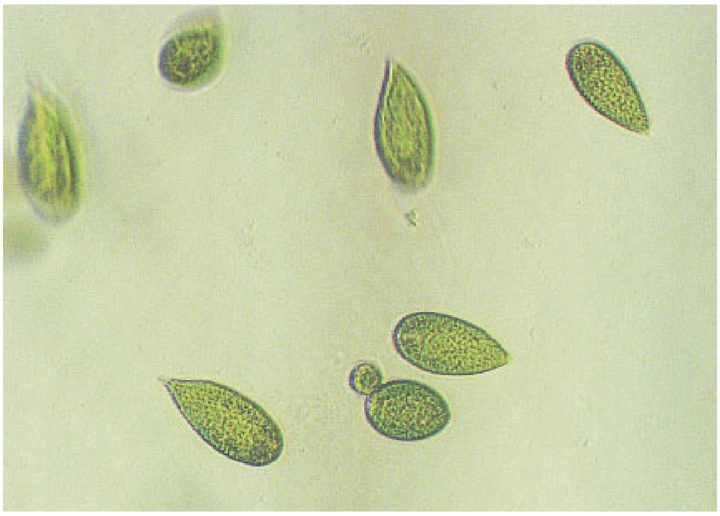

Figure 2. Cells of *G. semen* (photo: Karin Rengefors)

G. semen occurs primarily in humic lakes, but has also been reported from other lake types and reservoirs. The increased occurrence of the alga has been linked to increasing concentrations of DOC in freshwaters of northern Europe and increasing temperatures (Rengefors *et al.*, 2012). Experiments have shown that *G. semen* grows better after addition of humic substances and the presence of another phytoplankton species also enhanced its growth, probably because *G. semen* induced cell lysis and used the organic compounds released by its competitor (Rengefors *et al.*, 2008). In addition, the chloroplast arrangement of

G. semen may provide a competitive advantage at the low light conditions of humic systems (Peltomaa & Ojala, 2010). Higher temperatures also enhance the growth rate of *G. semen*, although only up to a certain threshold (19 °C, Rengefors *et al.*, 2012). Moreover, the life cycle of *G. semen* includes a benthic resting stage in which it stays dormant during unfavourable conditions, and hatching from these resting cysts is temperature-dependent (Rengefors *et al.*, 2012).

2 Objectives of the thesis

The overall objectives of this thesis were to increase our understanding of the drivers of *Gonyostomum semen* blooms and the effects of blooms on aquatic food webs. The specific objectives were to:

1. Study trends in occurrence and bloom formation of *G. semen* and investigate which variables are the best predictors of *G. semen* occurrence and bloom incidence in boreal lakes (I)

2. Study the effects of *G. semen* blooms on the taxonomic composition of phytoplankton, zooplankton and profundal benthic assemblages (II)

3. Study the ability of naturally occurring zooplankton to feed on *G. semen* (III)

4. Assess the contribution of bacterial versus algal food resources to the diet of zooplankton in lakes dominated by *G. semen* (IV)

3 Material and methods

3.1 Paper I

In this study we investigated trends in *G. semen* prevalence, geographical distribution and bloom incidence and assessed the importance of a number of predictor variables for the occurrence and bloom formation of *G. semen*. We used a set of 146 lakes distributed across three ecoregions (Borealic Uplands, Fennoscandian Shield and Central Plains) in Sweden, which had been sampled for phytoplankton at least once during the period 1992–2010. As predictors we used water chemistry variables (total and dissolved macronutrients, water colour, pH, and the major cations and anions), climatic variables (monthly averages of air temperature in April, May, June, July and August, length of the growing season, and an index of extreme temperatures), land use variables (proportion of deciduous forest, coniferous forest, water and wetlands) and morphometric variables (lake size, depth and slope of catchment).

Phytoplankton samples were collected once between July 15th and September 15th, generally during the early part of this period in southern Sweden and later in the north. In small lakes (<1 km^2) samples were collected at five locations in the epilimnion (0–4 m) over the deepest part of the lake with an acrylic plastic tube sampler (Ø30 mm) and pooled to form a composite sample, in order to cover the spatial variation expected in small, wind-sheltered lakes (Willén, 2003). In larger lakes (>1 km^2) one sample was taken over the deepest part of the lake. Subsamples of 250 mL were collected and preserved with Lugol's iodine solution. Phytoplankton were determined, counted and measured using a modified Utermöhl technique (Olrik *et al.*, 1998).

For investigation of trends in the prevalence, average biomass, geographical distribution and bloom incidence of *G. semen* we used a subset of

45 lakes that had been sampled annually during the period 1995–2010. These lakes were located in the Fennoscandian Shield and Central Plains ecoregions. As a measure of the geographical distribution of *G. semen* we used the centroid of its distribution and blooms were defined as *G. semen* biovolume exceeding 1 mm^3 L^{-1} and *G. semen* constituting >50% of the total phytoplankton biovolumes. The biovolumes threshold of 1 mm^3 L^{-1} is above the median of the total phytoplankton biovolume in lakes of this study in which *G. semen* did not dominate and where biomass peaks in the time series were not due one single algal species. Since criteria for defining blooms are often subjective, we also performed the analysis using > 75% and 90% *G. semen* of total phytoplankton biovolume. Trends were analyzed using Kendall tau regression.

For the analysis of the importance of different predictor variables for the occurrence *G. semen* we used a subset of 124 lakes, which were assigned to the following categories: Observations preceded and/or followed by at least five years of no detection of *G. semen* were coded as 0 (no detection), observations from lakes in which *G. semen* was always detected were coded as 1, and for lakes in which *G. semen* was detected occasionally (more frequently than every 5th year) the years when *G. semen* was detected were coded as 1. Twenty-two lakes were excluded from the dataset because they could not be assigned to any of these categories. For the analysis of bloom predictors we used the 84 lakes in this subset in which *G. semen* had been detected at least once. The observations were divided into bloom (1), i.e. *G. semen* biovolume > 1 mm^3 L^{-1} and *G. semen* constituting >50 % of total phytoplankton biovolumes, and no bloom (0). The lakes in these two datasets were located in all three ecoregions, although the bloom dataset only included one lake in the Borealic Uplands.

Random forest analysis was used to assess the importance of different predictor variables. This analysis is based on a combination of a number of classification trees. In the classification trees, a given predictor variable is split into two groups so that the most homogenous groups possible are formed (i.e. mainly detections in one group and non-detections in the other). These groups may then be split again using another predictor variable, and the process is repeated until the groups consist of only detections and non-detections, or until a maximum number of splits have been made. In random forest analysis, a number of classification trees are created with different, randomly selected predictor variables as their starting point and bootstrap samples of the dataset (one year per lake selected at random). We based our analyses on 500 bootstrap samples and subsets of five predictor variables. In the analyses, approximately one third of the dataset, called out-of-bag (OOB) observations, was set aside and used for cross-validation of model accuracy and estimation of the importance of each predictor. The accuracy of the model was evaluated by calculating the area under the receiver operator curve (AUC) of the OOB observations, which compares predicted probabilities of occurrence with actual observations.

3.2 Paper II

In this semi-controlled field study, we sampled eight small brown-water lakes situated in forested catchments in southern Sweden monthly during the summer in 2008 (May–September). The lakes were selected based on their physico-chemical characteristics (water chemistry and morphometry) and *G. semen* biomass from the previous 10-year period, using data from national and regional monitoring programmes. Lakes were selected so that their physico-chemical characteristics would be as similar as possible. In four of the lakes, there had been repeated blooms of *G. semen* during the last 10-year period (hereafter referred to as high-*G. semen* lakes), whereas no blooms had been recorded in the remaining four lakes, with the exception of one bloom in one lake (referred to as low-*G. semen* lakes).

We collected phytoplankton and zooplankton by taking water column samples of the epilimnion with a Ruttner sampler at three locations over the deepest part of the lake, which were pooled to form a composite sample. For phytoplankton, the composite sample was subsampled in a 250 mL bottle. For zooplankton, 3–5 L of the composite sample was concentrated and collected in a bottle. Plankton samples were preserved with Lugol's iodine solution. Profundal benthic invertebrates were sampled by taking five Ekman grabs in the deepest part of the lake. The samples were sieved through a 500 µm mesh and preserved with 70 % ethanol.

Phytoplankton samples were determined using a modified Utermöhl technique common in the Nordic countries (Olrik *et al.*, 1998). Biovolumes were calculated based on the length of phytoplankton and corresponding geometrical shapes, as described by Blomqvist and Herlitz (1998). Benthic invertebrates were sorted under 10x magnification and determined under light and dissecting microscopes. Preserved specimens were weighed.

Data was analyzed by comparing assemblages in low-*G. semen* lakes and high-*G. semen* lakes during the pre-bloom period (May) and the bloom period (August), using NMDS (non-metric multidimensional scaling) ordination to visually assess differences and NPMANOVA (non-parametric multivariate analysis of variance) to test for differences between lake groups. The contribution of individual taxa to the overall differences between assemblages in low-*G. semen* and high-*G. semen* lakes was tested using SIMPER (similarity percentage). We also tested for differences in total biovolume of phytoplankton and zooplankton, and total biomass of benthic invertebrates using Welch's *t*-test.

3.3 Paper III

In this laboratory experiment we used radioactively labelled algae and zooplankton naturally occurring in boreal, humic lakes. Zooplankton were collected from two lakes that usually have blooms of *G. semen* and two lakes where *G. semen* occurs but normally does not bloom. The four lakes were a subset of the ones used for the field study in Paper II. Zooplankton were collected with a 200 µm mesh in the pelagic zone of the lakes and transported back to the lab in 5 L of lake water that had been filtered through a 45 µm mesh. Zooplankton were kept dark and cool during transportation and stored overnight in darkness at 15 °C. The next day, we used a pipette to transfer the predominant crustaceans to beakers with GF/C-filtered lake water. The taxa used were *Daphnia cristata*, *Ceriodaphnia* spp., *Diaphanosoma brachyurum* *Eudiaptomus gracilis* and *Holopedium gibberum*. Two–three taxa were isolated and added to the same beaker.

The algae used in the experiment were a *G. semen* culture isolated from lake Liasjön in southern Sweden and a culture of the small chlorophyte *Pseudokirchneriella subcapitata* from the culture collection at the Norwegian Institute for Water Research (NIVA, Oslo). *P. subcapitata* was expected to be grazed by all zooplankton taxa in the experiment and was used for assessing the condition of the animals. Algal cultures were labelled with $H^{14}CO_3$ for 28–30 hours before the experiment. The experiment started by adding labelled algae to the beakers containing zooplankton in filtered lake water. The final concentration of *G. semen* was 2 mgC L^{-1} and the concentration of *P. subcapitata* was 18 mgC L^{-1}. The difference in concentrations was due to a calculation error. Incubations were run in triplicate for most taxa. An additional replicate of all zooplankton taxa was frozen prior to the experiment for use as blanks. The experiment was conducted in a water bath at 20 °C for 10 minutes (shorter than gut passage time for *Daphnia*, Murtaugh, 1985) and was terminated by collecting the animals on a nylon mesh mounted on an acrylic plastic cylinder and rinsing them repeatedly in carbonated water to kill them and remove any labelled algae adhering to the outside of their bodies. The animals were frozen at -20 °C. Samples of labelled and unlabeled algal cultures were collected on 1 µm polycarbonate filters and frozen in scintillation vials.

Zooplankton were freeze-dried, sorted and measured under a dissecting microscope, and placed in scintillation vials. Animals and filters with algae were dissolved using a tissue solubilizer containing a strong organic base and toluene at 60 °C. After the samples had cooled to room temperature, scintillation cocktail was added to the vials, the samples were kept in darkness at room temperature for 24 h, and the amount of radioactive label in the samples was determined using a liquid scintillation counter.

The ingestion rates (cells min^{-1} ind^{-1}) of zooplankton on *G. semen* and *P. subapitata* were calculated by dividing the blank-corrected specific activities in animals by the blank-corrected activity per algal cell and the number of animals per replicate. Differences in ingestion rates between taxa and lakes were tested using one way ANOVA (analysis of variance) and Tukey's HSD (honestly significant difference) test for post hoc comparisons.

3.4 Paper IV

In this fatty acid study we collected zooplankton and the invertebrate predator *Chaoborus flavicans* from eight small, humic forest-lakes in southern Sweden. Four of the lakes had a history of repeated blooms of *G. semen* and blooms were generally not detected in the remaining four lakes. The lakes sampled in this study were the same lakes as in paper II. Zooplankton were sampled by vertical hauls with a 200 µm plankton net over the deepest part of the lake, collected on pieces of 65 µm nylon mesh and shock-frozen in dry ice. Samples of late-instar *C. flavicans* larvae were collected with an Ekman sampler at the deepest part of the lake. The samples were sieved through a 500 µm mesh, brought back from the field in 500 mL plastic jars, and sorted into cryovials and shock-frozen in dry ice the same day. Samples were stored under N_2 atmosphere at -20 °C.

Prior to fatty acid analysis, the samples were freeze-dried. Zooplankton samples were sorted under a dissecting microscope and weighed into methanol-rinsed tin capsules. For *C. flavicans* samples, three individuals were ground with a pestle and the homogenate was weighed into methanol-rinsed tin capsules.

Total lipids of the samples were extracted with chloroform, methanol and water using a modified Bligh and Dyer-method and the lipids were derivatized to fatty acid methyl esters using mild alkaline transmethylation (Boschker, 2004). The samples were stored at -80 °C prior to analysis.

Fatty acids were analyzed using gas chromatography-flame ionization detection (GC-FID). Methyl esters were determined based on their retention times on the polar GC column, compared to internal standards (added 12:0 and 19:0, and naturally occurring 16:0). Concentrations were determined by comparison with a known amount of added internal standard (19:0). In addition, some methyl esters that could not be identified by this method were analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 49 fatty acids were analyzed and 39 of these were detected in the samples.

4 Results and discussion

4.1 *G. semen* occurrence and blooms – trends and drivers

Analysis of temporal trends of the occurrence of *G. semen* showed that this species has become more common during the study period (1995–2010) (**paper I**). The number of lakes in which *G. semen* was detected and the frequency of detection increased mainly in southern Sweden. Hence, there was no evidence of a geographical range expansion of the alga. Overall, *G. semen* was more common in the southern parts of the country and its biomass in southern lakes was higher. Surprisingly, we did not observe an increase in the total biomass or bloom frequency of *G. semen*. Instead, biomass and bloom incidence fluctuated among years, with peak biomass in the middle of the study period.

The increased occurrence of *G. semen* during our study period agrees with the results of previous studies (Lepistö *et al.*, 1994; Rengefors *et al.*, 2012). However, our finding that neither biomass nor bloom incidence increased somewhat contradicts the results of Rengefors *et al.* (2012), who found an increase in *G. semen* biomass. Differences in sampling intensity between the two studies may partially explain this discrepancy. For example, Rengefors *et al.* observed the largest change in biomass in October; hence August sampling in our study may have underestimated some trends.

Notably, the highest biomass and number of blooms was recorded in 2003, which was the year with the warmest spring and one of the longest growing seasons during the study period. Indeed, our random forest analyses showed that extreme temperatures, spring temperatures and the length of the growing season are important factors for the occurrence of *G. semen*. Spring temperatures and the length of the growing season also affected blooms, but the greater importance of local predictors such as pH and water colour indicates that certain habitat requirements need to be fulfilled in order for *G. semen* to reach high biomasses.

Higher temperatures increase the growth rate of *G. semen* and its recruitment from benthic resting stages (Rengefors *et al.*, 2012). Both recruitment and growth are generally important factors for the formation of blooms by algae that shift between benthic and pelagic life forms (Steidinger & Garccés, 2006). Hence, warmer spring temperatures and longer growing seasons may benefit *G. semen* by inducing a high recruitment and providing a longer time for the alga to build up its biomass. Extreme temperatures were found to be beneficial only to a certain extent, i.e. up to 19 °C, which agrees with results from laboratory experiments showing that the growth rate of *G. semen* decreases rapidly above 19 °C (Rengefors *et al.*, 2012). Temperature may also have indirect effects on *G. semen* by affecting the intensity and duration of thermal stratification. Prolonged and intensified stratification may favour *G. semen* due to its ability to migrate through the thermocline and access nutrients in the hypolimnion that other species cannot reach (Salonen & Rosenberg, 2000).

The preference of *G. semen* for lakes with low pH and high water colour has been shown in several studies (Cronberg *et al.*, 1988; Willén *et al.*, 1990; Lepistö *et al.*, 1994; Rengefors *et al.*, 2012). It is not clear why *G. semen* is more common in waters with low pH. Possibly, it could be related to physiological adaptations of *G. semen* or the nature of biotic interactions in low-pH lakes. The higher incidence of blooms in humic lakes with high DOC concentrations are likely related to the mixotrophic nature of *G. semen* and its adaptation to low light conditions (Rengefors *et al.*, 2008; Peltomaa & Ojala, 2010). The increase in *G. semen* in southern Sweden may be related to the increase in water colour in this region (Kritzberg & Ekström, 2012).

At the highest dominance level (90 %), lake morphometry (i.e. shore slope) was the most important predictor of bloom formation. As recruitment from benthic resting stages mainly occurs from shallow areas, lakes with gradual slopes and large littoral zones may provide optimal conditions for a large seeding of pelagic populations, thereby facilitating intense blooms of *G. semen*.

Our results suggest that the occurrence and bloom incidence of *G. semen* may increase in the future as temperatures increase and more lakes become favourable habitats for this species as a result of the ongoing brownification of surface waters (Monteith *et al.*, 2007).

4.2 Effects of *G. semen* blooms on community structure

In **paper II**, we found that the biomass of small chrysophytes and chlorophytes in lakes with *G. semen* blooms was on average three times lower than in similar lakes without blooms and in **paper IV** we found a decrease in small, single-celled phytoplankton (≤20 µm) along a gradient of duration and intensity of *G. semen* blooms. These results indicate there is less food available for small filter feeders during *G. semen* blooms (Burns, 1968). Surprisingly, the zooplankton assemblages in both bloom-lakes and lakes without blooms were predominated by small, filter-feeding cladocerans in late summer (**paper II**). In addition, the total biomass of zooplankton was similar in lakes with and without blooms (**paper II**). There were, however, differences in the taxonomic composition of zooplankton assemblages, with *Daphnia cristata* being the predominant zooplankter in lakes without blooms and the smaller *Ceriodaphnia* spp. in bloom-lakes.

Since neither *D. cristata* nor *Ceriodaphnia* can feed on *G. semen* (**paper III**; Lebret *et al.*, 2012), the predominance of *Ceriodaphnia* during *G. semen* blooms may be related to interference of *G. semen* with filter feeding and/or the utilization of alternative food resources. The large cell size and trichocysts of *G. semen* may interfere with the feeding of cladocerans by triggering rejection when entering the food groove, in a manner similar to filamentous algae (Gliwicz & Lampert, 1990). The larger size of *D. cristata* may result in a greater effect of interference from *G. semen* because larger cladocerans have wider carapace gapes and interfering particles more frequently enter their filtering chamber (Gliwicz & Lampert, 1990). Moreover, the low biomass of small, edible algae in lakes with *G. semen* blooms may result in an increased importance of alternative food resources such as bacteria and heterotrophic protists, which *Ceriodaphnia* have been shown to utilize more efficiently than *Daphnia*. For example, a diet consisting mainly of bacteria can support positive population growth in *Ceriodaphnia* but not in *Daphnia* (Pace *et al.*, 1983) and *Ceriodaphnia* showed a higher growth rate than *Daphnia* when fed a low-concentration mixture of algae and bacteria (Iwabuchi & Urabe, 2010), despite similar ability to filter bacteria (Pace *et al.*, 1983).

The large, raptorial rotifer *Asplanchna* has been observed feeding on *G. semen* in live samples (Cronberg *et al.*, 1988), which may explain its high biomass during *G. semen* blooms (**paper II**). Rotifers and small cladocerans are preferred prey for the gape-limited invertebrate predator *Chaoborus flavicans* (Kajak & Rybak, 1979; Smyly, 1980; Havens, 1990) and the higher biomass of these prey items may partly explain the 10x higher abundance in lakes with *G. semen* blooms than in similar lakes without blooms (**paper II**). *C. flavicans* also feeds on large flagellated phytoplankton (Moore *et al.*, 1994), suggesting that *G. semen* blooms may provide *C. flavicans* with an additional food resource, particularly in early life stages. In addition, the high water colour of *G. semen*-dominated lakes may reduce fish predation pressure on *C. flavicans* (Wissel et al., 2003).

4.3 Edibility of *G. semen*

Although *G. semen* is protected from grazing by small cladocerans (**paper III**; Lebret *et al.*, 2012), some other zooplankton could graze efficiently on this alga (**paper III**). The calanoid copepod *Eudiaptomus gracilis* ingested 1.3 cells minute^{-1} individual^{-1} and the large cladoceran *Holopedium gibberum* 1.5 cells minute^{-1} individual^{-1} in our feeding experiment. These results indicate that there may be a direct link between *G. semen* and higher trophic levels despite the large cell size and physical defences of the alga. However, predominance of small cladocerans during blooms (**paper II**) suggests that the direct contribution by *G. semen* to production at higher trophic levels is limited.

Pre-bloom abundances of *E. gracilis* and *H. gibberum* in lakes with recurring *G. semen* blooms (Johansson et al., unpublished data) and the average ingestion rates in the feeding experiment (**paper III**) suggest that grazing could prevent *G. semen* from building up high biomasses. This is, however, not the case, as blooms develop in the presence of grazing zooplankton. Laboratory feeding experiments using a dense monoculture of algae tend to overestimate feeding rates, as phytoplankton in nature generally occur at a lower abundance and in a more patchy distribution. The conditions in our feeding experiment correspond to those of *G. semen* bloom when this species almost completely dominates the phytoplankton assemblage (Johansson et al., unpublished data). During the pre-bloom period, however, the diet of zooplankton may be more varied and grazing on *G. semen* less intense. In addition, *G. semen* avoids grazing by migrating to the hypolimnion at night (Salonen & Rosenberg, 2000).

The observation that some zooplankton species that occur naturally in lakes with *G. semen* blooms can feed efficiently on this alga indicates that biomanipulation by fish removal may be used as a means of controlling or delaying *G. semen* blooms. More information about bloom dynamics and the vertical distribution of zooplankton and *G. semen* in lakes with different transparencies (Eloranta & Räike, 1995; Wissel *et al.*, 2003) is, however, necessary for developing efficient management methods.

4.4 Food resources in lakes dominated by *G. semen*

The predominance of small cladocerans and the low availability of edible phytoplankton for these zooplankters during *G. semen* blooms (**paper II; paper IV**; Trigal *et al.*, 2011) suggest that the direct trophic coupling between phytoplankton and higher trophic levels may be reduced when *G. semen* dominates the phytoplankton assemblage. In addition, the vertical migration behaviour of *G. semen* probably limits grazing by those zooplankton taxa that are able to ingest it. Instead, alternative food resources such as bacteria and heterotrophic protists may become more important during *G. semen* blooms. This conjecture is supported by the increasing proportion of bacterial fatty acids (BAFA) in cladocerans, calanoid copepods and *C. flavicans* and the decreasing proportion of polyunsaturated fatty acids (PUFA) in cladocerans along a gradient of duration and dominance level of *G. semen* blooms (henceforth referred to as *G. semen* influence) (**paper IV**).

Cladocerans from lakes with high *G. semen* influence contained similar proportions of BAFA and PUFA (~15 % of total fatty acids) as opposed to cladocerans from lakes with less *G. semen*, which contained about twice as much PUFA as BAFA (~20 and 10 % of total fatty acids, respectively) (**paper IV**). Since PUFA are selectively assimilated and retained by animals (Gladyshev *et al.*, 2011; Taipale *et al.*, 2011) and other fatty acids appear to be catabolized to a larger extent, the high BAFA/PUFA ratios in small cladocerans from lakes with high *G. semen* influence show that heterotrophic food resources constitute a significant proportion of their diet.

In contrast to small cladocerans, the calanoid copepod *E. gracilis* contained a large proportion of PUFA (~60 % of total fatty acids) across the *G. semen* gradient. The composition of PUFA, however, changed with *G. semen* influence; the proportion of EPA increased and DHA decreased with increasing *G. semen* influence (**paper IV**). Fatty acid analyses of seston (suspended particles) from lakes dominated by *G. semen* suggest that *G. semen*, like other raphidophytes, contains high levels of EPA and not much DHA (Marshall *et al.*, 2002; Gutseit *et al.*, 2007). Hence, the increase in EPA and decrease in DHA along the gradient of *G. semen* influence indicates that the efficient feeding of *E. gracilis* on *G. semen* observed in laboratory experiments (**paper III**; Williamson *et al.*, 1996) also occurs in nature.

The similar biomasses of zooplankton in lakes with and without *G. semen* blooms (**paper II**) and the higher proportion of BAFA in zooplankton along the *G. semen* gradient (**paper IV**) show that heterotrophic food resources may compensate for the decreased flow from phytoplankton to zooplankton during blooms. However, a large proportion of heterotrophic food resources in the diet of primary consumers could result in lower food quality for higher consumers. Surprisingly, the proportion of PUFA in *C. flavicans* samples was similar across the *G. semen* gradient (~45 % of total fatty acids) (**paper IV**). The high proportion of PUFA in *C. flavicans* from lakes with high *G. semen* influence suggests that *C. flavicans* selectively assimilates PUFA from the diet or is feeding on other, PUFA-rich prey. Although cladocerans in lakes with high *G. semen* influence contain a lower proportion of PUFA than those from lakes with low influence, the predominant taxon in lakes with high *G. semen* influence, *Ceriodaphnia*, is an easy prey for *C. flavicans* to catch and ingest (Smyly, 1980; Hanazato & Yasuno, 1989). Therefore, the absolute quantity of PUFA available to *C. flavicans* may be large, despite the lower relative quantity of PUFA in the prey. In addition, *C. flavicans* may feed on other prey such as copepods, rotifers and even large algae (Kajak & Rybak, 1979; Moore *et al.*, 1994). For example, the large copepod *Asplanchna*, which is common during *G. semen* blooms (**paper II**) may have a high PUFA content due to feeding on *G. semen* (Cronberg *et al.*, 1988). Nevertheless, the larger proportion of BAFA in zooplankton along the *G. semen* gradient was reflected in the fatty acid composition of *C. flavicans*. Whether there are any physiological effects of a higher BAFA content in animals is not clear and merits further study.

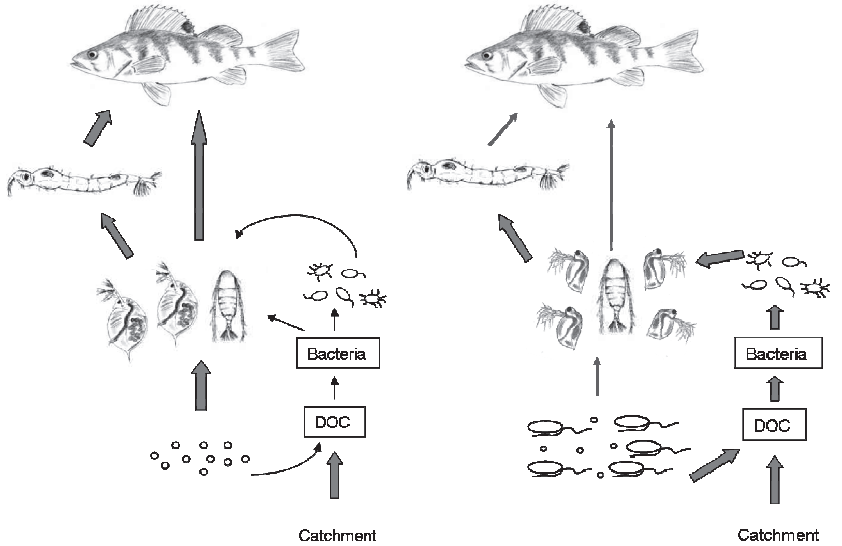

Figure 3. Hypothetical food web structure in a lake without *G. semen* (left) and a lake with a *G. semen* bloom (right)

In contrast to invertebrate predators like *C. flavicans*, planktivorous fish are generally visual foragers with a preference for larger prey (Brooks & Dodson, 1965; Hanazato & Yasuno, 1989). Hence, the predominance of small prey with a lower PUFA proportion may affect fish more than *C. flavicans*. The recruitment of fish in lakes with recurring *G. semen* blooms needs further study, as small zooplankton feeding largely on heterotrophic resources may be a poor food resource for fish and low abundances of planktivorous fish have been recorded in lakes with recurring *G. semen* blooms (Trigal *et al.*, 2011).

5 Conclusions

- The occurrence and, to a certain extent, bloom formation of *G. semen* is favoured by higher temperatures and longer growing seasons. Local factors like pH and water colour are, however, more important for the formation of blooms. Occurrence and blooms may increase when temperatures increase and the ongoing brownification of surface waters makes more lakes suitable habitats for *G.semen*.

- The taxonomic composition of zooplankton assemblages shifts toward predominance of smaller cladocerans during *G. semen* blooms. Because small cladocerans cannot feed on *G. semen* and the biomass of small, edible phytoplankton is low during blooms, the direct trophic coupling between phytoplankton and zooplankton decreases. Instead, the importance of heterotrophic food resources (bacteria and bacterivorous protists) increases. Similar biomasses of zooplankton in lakes with and without blooms suggest that heterotrophic pathways can compensate for the lower edibility of phytoplankton during blooms of *G. semen*.

- Feeding on heterotrophic resources results in a decreased proportion of nutritionally valuable polyunsaturated fatty acids (PUFA) in the predominant zooplankters during blooms and an increased proportion of fatty acids of bacterial origin (BAFA). The small size and lower nutritional quality of the predominant zooplankton during *G. semen* blooms could reduce the food quality for zooplanktivorous fish. The high abundance and PUFA content of the invertebrate predator *C. flavicans*, suggests that this species, on the other hand, is favoured by *G. semen* blooms and accumulates PUFA selectively from cladoceran prey, from other zooplankton feeding on *G. semen* (i.e. calanoid copepods and potentially the rotifer *Asplanchna*) or from feeding directly on *G. semen*.

- Despite the large cell size of *G. semen* and its ejection of trichocysts upon physical stimulation, some naturally occurring zooplankton can feed efficiently on this alga, supporting the use of biomanipulation of fish communities as a means of controlling or delaying blooms of *G. semen*.

6 Future research

Although this thesis has answered some questions regarding the ecology of *G. semen* and the effects of *G. semen* blooms, many questions about this fascinating organism and its interactions with the surrounding environment remain to be answered by future studies.

- We now know more about which environmental factors affect the occurrence and bloom formation of *G. semen*, but the process of bloom formation is still unknown. Are blooms formed by a slow build-up of biomass or by fast recruitment of a large number of cells from benthic resting stages? Do these processes interact? Do blooms form in different ways in different lakes (e.g. deeper lakes with steep slopes vs. shallow lakes with gradual slopes)?

- The observations that *G. semen* is found in an increasing number of lakes and the genetic similarity of *G. semen* populations in the Fennoscandian region (Lebret, 2012) could be interpreted as *G. semen* being an invasive species in this region. Determining the time of colonization is, however, a challenge. In addition, it is possible that *G. semen* was more common during pre-industrial times due to higher DOC concentrations, decreased to very low levels when lakes became clearer during acidification and that it is now increasing again with rising DOC concentrations (Cunningham *et al.*, 2011; Erlandsson *et al.*, 2011). Paleolimnological studies analyzing the occurrence of *G. semen* resting stages in sediment cores could potentially answer the question of when *G. semen* colonized lakes in northern Europe and provide information of the direction of *G. semen* dispersal. In addition, paleolimnological methods may give more robust information about the occurrence of *G. semen* in lakes, as the timing of *G. semen* biomass peaks may vary between lakes and years and sampling of pelagic populations hence may underestimate the distribution of *G. semen*.

- Small cladocerans are unable to feed on *G. semen*, but why? Is it because of the large cell size of the alga or the expulsion of trichocysts? If the trichocysts prevent grazing, is it by deterring grazers or by interference with the filter feeding of cladocerans?

- Some zooplankton species can feed on *G. semen*, but how much do these animals feed on *G. semen* in nature? How much time do zooplankton and *G. semen* spend in the same stratum? Does this vary between lakes and between zooplankton species? Do calanoid copepods feed on *G. semen* even when other algae are available?

- How are fish populations affected by the predominance of small cladocerans that feed on heterotrophic resources during *G. semen* blooms?

- Are there physiological implications of an increased content of bacterial fatty acids in the lipids of aquatic consumers?

References

Anderson, D.M., Glibert, P.M. & Burkholder, J.M. (2002). Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25(4), 704–726.

Arndt, H. (1993). Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) — a review. *Hydrobiologia* 255-256(1), 231–246.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyerreil, L.A. & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Marine Ecology – Progress Series* 10(3), 257–263.

Bagøien, E., Miranda, A., Reguera, B. & Franco, J.M. (1996). Effects of two paralytic shellfish toxin producing dinoflagellates on the pelagic harpacticoid copepod *Euterpina acutifrons*. *Marine Biology* 126(3), 361–369.

Bec, A., Martin-Creuzburg, D. & Von Elert, E. (2010). Fatty acid composition of the heterotrophic nanoflagellate Paraphysomonas sp.: influence of diet and de novo biosynthesis. *Aquatic Biology* 9(2), 107–112.

Blomqvist, P. & Herlitz, E. (1998). *Methods for quantitative assessment of phytoplankton in freshwaters, part 2*. Stockholm: Swedish EPA.

Bogdan, K.G. & Gilbert, J.J. (1987). Quantitative comparison of food niches in some freshwater zooplankton: A multi-tracer-cell approach. *Oecologia* 72(3), 331–340.

Boschker, H.T.S. (2004). Linking microbial community structure and functioning: Stable isotope (^{13}C) labeling in combination with PLFA analysis. In: Kowalchuk, G.A*., et al.* (Eds.) *Molecular microbial ecology manual*. pp. 1673–1688. Dordrecht: Kluwer Academic Publishers; 2).

Brendelberger, H. (1991). Filter mesh size of cladocerans predicts retention efficiency for bacteria. *Limnology and Oceanography* 36(5), 884–894.

Brett, M. & Müller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology* 38(3), 483–499.

Brett, M.T. & Goldman, C.R. (1996). A meta-analysis of the freshwater trophic cascade. *Proceedings of the National Academy of Sciences of the United States of America* 93(15), 7723–7726.

Brett, M.T., Kainz, M.J., Taipale, S.J. & Seshan, H. (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences* 106(50), 21197–21201.

Brooks, J.L. & Dodson, S.I. (1965). Predation, body size, and composition of plankton. *Science* 150(3692), 28–35.

Brönmark, C. & Hansson, L.-A. (2005). *The biology of lakes and ponds*. Second edition. ed: Oxford University Press.

Burns, C.W. (1968). The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. *Limnology and Oceanography* 13(4), 675–678.

Chang, K.H., Doi, H., Nishibe, Y. & Nakano, S. (2010). Feeding habits of omnivorous *Asplanchna*: comparison of diet composition among *Asplanchna herricki*, *A. priodonta* and *A. girodi* in pond ecosystems. *Journal of Limnology* 69(2), 209–216.

Cole, J.J., Carpenter, S.R., Kitchell, J., Pace, M.L., Solomon, C.T. & Weidel, B. (2011). Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences of the United States of America* 108(5), 1975–1980.

Cronberg, G., Lindmark, G. & Björk, S. (1988). Mass development of the flagellate *Gonyostomum semen* (Raphidophyta) in Swedish forest lakes – an effect of acidification? *Hydrobiologia* (161), 217–236.

Cunningham, L., Bishop, K., Mettävainio, E. & Rosén, P. (2011). Paleoecological evidence of major declines in total organic carbon concentrations since the nineteenth century in four nemoboreal lakes. *Journal of Paleolimnology* 45(4), 507–518.

Daniel, C., Gutseit, K., Anesio, A.M. & Graneli, W. (2005). Microbial food webs in the dark: independence of lake plankton from recent algal production. *Aquatic Microbial Ecology* 38(2), 113–123.

DeBiase, A.E., Sanders, R.W. & Porter, K.G. (1990). Relative nutritional value of ciliate protozoa and algae as food for *Daphnia*. *Microbial Ecology* 19(2), 199–210.

DeMott, W.R. (1986). The role of taste in food selection by freshwater zooplankton. *Oecologia* 69(3), 334–340.

DeMott, W.R. (1988). Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnology and Oceanography* 33(3), 397–408.

DeMott, W.R. (1989). Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. *Limnology and Oceanography* 34(1), 140–154.

DeMott, W.R., Gulati, R.D. & Donk, E.V. (2001). *Daphnia* food limitation in three hypereutrophic Dutch lakes: evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. *Limnology and Oceanography* 46(8), 2054–2060.

DeMott, W.R. & Kerfoot, W.C. (1982). Competition among cladocerans: Nature of the interaction between *Bosmina* and *Daphnia*. *Ecology* 63(6), 1949–1966.

Demott, W.R. & Watson, M.D. (1991). Remote detection of algae by copepods – responses to algal size, odors and motility. *J Plankt Res* 13(6), 1203–1222.

Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T. & Thornbrugh, D.J. (2008). Eutrophication of U.S. freshwaters: Analysis of potential economic damages. *Environmental Science & Technology* 43(1), 12–19.

Ederington, M.C., McManus, G.B. & Harvey, H.R. (1995). Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnology and Oceanography* 40(5), 860–867.

Edwards, M., Johns, D.G., Leterme, S.C., Svendsen, E. & Richardson, A.J. (2006). Regional climate change and harmful algal blooms in the northeast Atlantic. *Limnology and Oceanography* 51(2), 820–829.

Eloranta, P. & Räike, A. (1995). Light as a factor affecting the vertical distribution of *Gonyostomum semen* (Ehr.) Diesing (Raphidophyceae) in lakes. *Aqua Fennica* 25(0), 15–22.

Erlandsson, M., Cory, N., Fölster, J., Köhler, S., Laudon, H., Weyhenmeyer, G.A. & Bishop, K. (2011). Increasing dissolved organic carbon redefines the extent of surface water acidification and helps resolve a classic controversy. *Bioscience* 61(8), 614–618.

Forsyth, D.J., Haney, J.F. & James, M.R. (1991). Direct observation of toxic effects of cyanobacterial extracellular products on *Daphnia*. *Hydrobiologia* 228(2), 151–155.

Frangópulos, M., Guisande, C., Maneiro, I., Riveiro, I. & Franco, J. (2000). Short-term and long-term effects of the toxic dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*. *Marine Ecology Progress Series* 203, 161–169.

Ghadouani, A., Pinel-Alloul, B., Plath, K., Codd, G.A. & Lampert, W. (2004). Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*. *Limnology and Oceanography* 49(3), 666–679.

Ghadouani, A., Pinel-Alloul, B. & Prepas, E.E. (2003). Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. *Freshwater Biology* 48(2), 363–381.

Gladyshev, M., Sushchik, N., Anishchenko, O., Makhutova, O., Kolmakov, V., Kalachova, G., Kolmakova, A. & Dubovskaya, O. (2011). Efficiency of transfer of essential polyunsaturated fatty acids versus organic carbon from producers to consumers in a eutrophic reservoir. *Oecologia* 165(2), 521–531.

Gliwicz, Z.M. (1990). Food thresholds and body size in cladocerans. *Nature* 343(6259), 638–640.

Gliwicz, Z.M. & Lampert, W. (1990). Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. *Ecology* 71(2), 691–702.

Granéli, E. & Turner, J.T. (2006). An introduction to harmful algae. In: Granéli, E.*, et al.* (Eds.) *Ecological Studies*. pp. 3–7. Berlin – Heidelberg: Springer; 189).

Gutseit, K., Berglund, O. & Granéli, W. (2007). Essential fatty acids and phosphorus in seston from lakes with contrasting terrestrial dissolved organic carbon content. *Freshwater Biology* 52(1), 28–38.

Hallegraeff, G.M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: A formidable predictive challenge. *Journal of Phycology* 46(2), 220–235.

Hanazato, T. & Yasuno, M. (1989). Zooplankton community structure driven by vertebrate and invertebrate predators. *Oecologia* 81(4), 450–458.

Havens, K.E. (1990). *Chaoborus* predation and zooplankton community structure in a rotifer-dominated lake. *Hydrobiologia* 198(1), 215–226.

Hessen, D.O. & Van Donk, E. (1993). Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. *Archiv für Hydrobiologie* 127(2), 129–140.

Hoagland, P. & Scatasta, S. (2006). The economic effects of harmful algal blooms. In: Granéli, E.*, et al.* (Eds.) *Ecology of harmful algae*. pp. 391–402. Berlin – Heidelberg: Springer; 189).

Iwabuchi, T. & Urabe, J. (2010). Phosphorus acquisition and competitive abilities of two herbivorous zooplankton, *Daphnia pulex* and *Ceriodaphnia quadrangula*. *Ecological Research* 25(3), 619–627.

Ivarsson, H. & Jansson, M. (1994). Regional variation of dissolved organic matter in running waters in central northern Sweden. *Hydrobiologia* 286(1), 37–51.

Jakobsen, H.H. (2002). Escape of protists in predator-generated feeding currents. *Aquatic Microbial Ecology* 26(3), 271–281.

Kajak, Z. & Rybak, J. (1979). The feeding of *Chaoborus flavicans* Meigen (Diptera, Chaoboridae) and its predation on lake zooplankton. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 64(3), 361–378.

Kelly, A., Kohler, C. & Tindall, D. (1992). Are crustaceans linked to the ciguatera food chain? *Environmental Biology of Fishes* 33(3), 275–286.

Kirk, K.L. (1991). Suspended clay reduces *Daphnia* feeding rate: behavioural mechanisms. *Freshwater Biology* 25(2), 357–365.

Kiørboe, T. (2011). How zooplankton feed: mechanisms, traits and trade-offs. *Biological Reviews* 86(2), 311–339.

Kritzberg, E.S. & Ekström, S.M. (2012). Increasing iron concentrations in surface waters - a factor behind brownification? *Biogeosciences* 9(4), 1465–1478.

Landsberg, J.H. (2002). The effects of harmful algal blooms on aquatic organisms. *Reviews in Fisheries Science* 10(2), 113–390.

Lebret, K. (2012). *A phytoplankton invasion: Population genetics, phylogeography, and invasion success of Gonyostomum semen.* Diss. Lund:Lund University.

Lebret, K., Fernandez, M.F., 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.

Lepistö, L., Antikainen, S. & Kivinen, J. (1994). The occurrence of *Gonyostomum semen* (Ehr.) Diesing in Finnish lakes. *Hydrobiologia* 273(1), 1–8.

Marshall, J.-A., Nichols, P.D. & Hallegraeff, G.M. (2002). Chemotaxonomic survey of sterols and fatty acids in six marine raphidophyte algae. *Journal of Applied Phycology* 14(4), 255–265.

Martin-Creuzburg, D. & von Elert, E. (2009). Ecological significance of sterols in aquatic food webs. In: Arts, M.T.*, et al.* (Eds.) *Lipids in aquatic ecosystems*. pp. 43–64. New York: Springer.

McMahon, J.W. & Rigler, F.H. (1963). Mechanisms regulating feeding rate of *Daphnia magna* Straus. *Canadian Journal of Zoology* 41(2), 321–332.

Michaloudi, E., Moustaka-Gouni, M., Gkelis, S. & Pantelidakis, K. (2009). Plankton community structure during an ecosystem disruptive algal bloom of *Prymnesium parvum*. *Journal of Plankton Research* 31(3), 301–309.

Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Hogasen, T., Wilander, A., Skjelkvale, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopacek, J. & Vesely, J. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450(7169), 537–540.

Moore, M.V., Yan, N.D. & Pawson, T. (1994). Omnivory of the larval phantom midge (*Chaoborus* spp.) and its potential significance for freshwater planktonic food webs. *Canadian Journal of Zoology* 72(11), 2055–2065.

Murtaugh, P.A. (1985). The influence of food concentration and feeding rate on the gut residence time of *Daphnia*. *J Plankt Res* 7(3), 415–420.

Napolitano, G.E. (1999). Fatty acids as trophic and chemical markers in freshwater ecosystems. In: Arts, M.T.*, et al.* (Eds.) *Lipids in freshwater ecosystems*. pp. 21–44. New York: Springer.

Olrik, K., Blomqvist, P., Brettum, P., Cronberg, G. & Eloranta, P. (1998). *Methods for quantitative assessment of phytoplankton in freshwaters, part I, Report 4860*. Stockholm: Swedish EPA.

Pace, M.L., Porter, K.G. & Feig, Y.S. (1983). Species- and age-specific differences in bacterial resource utilization by two co-occurring cladocerans. *Ecology* 64(5), 1145–1156.

Paerl, H.W. & Huisman, J. (2009). Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports* 1(1), 27–37.

Parrish, C.C. (2009). Essential fatty acids in aquatic food webs. In: Arts, M.T.*, et al.* (Eds.) *Lipids in aquatic ecosystems*. pp. 309–326. New York: Springer.

Peltomaa, E. & Ojala, A. (2010). Size-related photosynthesis of algae in a strongly stratified humic lake. *Journal of Plankton Research* 32(3), 341–355.

Pithart, D., Pechar, L. & Mattsson, G. (1997). Summer blooms of raphidophyte *Gonyostomum semen* and its diurnal vertical migration in a floodplain pool. *Archiv fuer Hydrobiologie Supplement* 119(0), 119–133.

Porter, K.G. (1976). Enhancement of algal growth and productivity by grazing zooplankton. *Science* 192(4246), 1332–1334.

Price, H.J., Paffenhofer, G.-A. & Strickler, J.R. (1983). Modes of cell capture in calanoid copepods. *Limnology and Oceanography* 28(1), 116–123.

Rengefors, K., Pålsson, C., Hansson, L.-A. & Heiberg, L. (2008). Cell lysis of competitors and osmotrophy enhance growth of the bloom-forming alga *Gonyostomum semen*. *Aquatic Microbial Ecology* 51(1), 87–96.

Rengefors, K., Weyhenmeyer, G.A. & Bloch, I. (2012). Temperature as a driver for the expansion of the microalga *Gonyostomum semen* in Swedish lakes. *Harmful Algae* 18, 65–73.

Salonen, K. & Rosenberg, M. (2000). Advantages from diel vertical migration can explain the dominance of *Gonyostomum semen* (Raphidophyceae) in a small, steeply-stratified humic lake. *J Plankt Res* 22(10), 1841–1853.

Sarma, S.S.S. (1993). Feeding responses of *Asplanchna brightwelli* (Rotifera): laboratory and field studies. *Hydrobiologia* (255/256), 275–282.

Schindler, D.W. (1977). Evolution of phosphorus limitation in lakes. *Science* 195(4275), 260–262.

Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaro, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin, R., Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V. & Van Dolah, F.M. (2000). Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403(6765), 80–84.

Selander, E., Fagerberg, T., Wohlrab, S. & Pavia, H. (2012). Fight and flight in dinoflagellates? Kinetics of simultaneous grazer-induced responses in *Alexandrium tamarense*. *Limnology and Oceanography* 57(1), 58–64.

Smayda, T.J. (1990). Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Granéli, E.*, et al.* (Eds.) *Toxic marine phytoplankton*. pp. 29–40. New York: Elsevier.

Smyly, W.J.P. (1980). Food and feeding of aquatic larvae of the midge *Chaoborus flavicans* (Meigen) (Diptera : Chaoboridae) in the laboratory. *Hydrobiologia* 70(1), 179–188.

Smyntek, P.M., Teece, M.A., Schulz, K.L. & Storch, A.J. (2008). Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. *Freshwater Biology* 53(9), 1768–1782.

Starkweather, P. & Bogdan, K. (1980). Detrital feeding in natural zooplankton communities: Discrimination between live and dead algal foods. *Hydrobiologia* 73(1), 83–85.

Starkweather, P.L. (1980). Behavioural determinants of diet quantity and diet quality in *Brachyonus calyciflorus*. In: Kerfoot, W.C. (Ed.) *Evolution and ecology of zooplankton communities*. pp. 151–157. Hanover: University Press of New England.

Steidinger, K.A. & Garccés, E. (2006). Importance of life cycles in the ecology of harmful microalgae. In: Graneli, E*., et al.* (Eds.) *Ecology of harmful algae*. pp. 37–49. Berlin: Springer.

Strom, S.L., Harvey, E.L., Fredrickson, K.A. & Menden-Deuer, S. (2013). Broad salinity tolerance as a refuge from predation in the harmful raphidophyte alga *Heterosigma akashiwo* (Raphidophyceae). *Journal of Phycology* 49(1), 20–31.

Sunda, W.G., Graneli, E. & Gobler, C.J. (2006). Positive feedback and the development and persistence of ecosystem disruptive algal blooms. *Journal of Phycology* 42(5), 963–974.

Taipale, S.J., Brett, M.T., Pulkkinen, K. & Kainz, M.J. (2012). The influence of bacteria-dominated diets on *Daphnia magna* somatic growth, reproduction, and lipid composition. *FEMS Microbiology Ecology* 82(1), 50–62.

Taipale, S.J., Kainz, M.J. & Brett, M.T. (2011). Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in *Daphnia*. *Oikos* 120(11), 1674–1682.

Tranvik, L. (1992). Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. *Hydrobiologia* 229(1), 107–114.

Trigal, C., Goedkoop, W. & Johnson, R.K. (2011). Changes in phytoplankton, benthic invertebrate and fish assemblages of boreal lakes following invasion by *Gonyostomum semen*. *Freshwater Biology* 56(10), 1937–1948.

Turner, J.T. (2006). Harmful algae interactions with marine planktonic grazers. In: Granéli, E*., et al.* (Eds.) *Ecology of harmful algae*. pp. 259–270. Berlin: Springer.

Vanderploeg, H.A., Paffenhöfer, G.-A. & Liebig, J.R. (1988). *Diaptomus* vs. net phytoplankton: Effects of algal size and morphology on selectivity of a behaviorally flexible, omnivorous copepod. *Bulletin of Marine Science* 43(3), 377–394.

Wenzel, A., Bergstrom, A.K., Jansson, M. & Vrede, T. (2012a). Poor direct exploitation of terrestrial particulate organic material from peat layers by *Daphnia galeata*. *Canadian Journal of Fisheries and Aquatic Sciences* 69(11), 1870–1880.

Wenzel, A., Bergstrom, A.K., Jansson, M. & Vrede, T. (2012b). Survival, growth and reproduction of *Daphnia galeata* feeding on single and mixed

Pseudomonas and *Rhodomonas* diets. *Freshwater Biology* 57(4), 835–846.

Wetzel, R.G. (2001). *Limnology - Lake and river ecosystems*. 3. ed. San Diego: Elsevier.

Willén, E. (2003). Dominance patterns of planktonic algae in Swedish forest lakes. *Hydrobiologia* 502(1-3), 315–324.

Willén, E., Hajdu, S. & Pejler, Y. (1990). Summer phytoplankton in 73 nutrient-poor Swedish lakes. Classification, ordination and choice of long-term monitoring objects. *Limnologica* 20(2), 217–228.

Williamson, C.E., Sanders, R.W., Moeller, R.E. & Stutzman, P.L. (1996). Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migration theory. *Limnology and Oceanography* 41(2), 224–233.

Wissel, B., Boeing, W.J. & Ramcharan, C.W. (2003). Effects of water color on predation regimes and zooplankton assemblages in freshwater lakes. *Limnology and Oceanography* 48(5), 1965–1976.

Wolfe, G. (2000). The chemical defense ecology of marine unicellular plankton: constraints, mechanisms, and impacts. *The Biological Bulletin* 198(2), 225–244.

Vrede, T. & Vrede, K. (2005). Contrasting 'top-down' effects of crustacean zooplankton grazing on bacteria and phytoflagellates. *Aquatic Ecology* 39(3), 283–293.

Xie, P., Iwakuma, T. & Fujii, K. (1998). Changes in the structure of a zooplankton community during a *Ceratium* (dinoflagellate) bloom in a eutrophic fishless pond. *Journal of Plankton Research* 20(9), 1663–1678.

Zaret, T.M. & Suffern, J.S. (1976). Vertical migration in zooplankton as a predator avoidance mechanism. *Limnology and Oceanography* 21(6), 804–813.

Acknowledgements

I could not have done this work without the help and support of a whole lot of people. First and foremost I would of course like to thank my main supervisor Richard for believing that I could do this, for always staying positive and for somehow knowing how to give just the right reply to my confused e-mails. Many thanks also to my assistant supervisors Cristina, Tobias, and Willem for valuable help and input during different phases in my PhD project.

At the department there have also been a number of other people who have helped and encouraged me over the years. I would like to thank my fellow PhD students, especially Marcus W, Anna L, Maria K, Atlasi, Emma G, Steffi G, Mattias W, Solomon G, Simon H, and Ina B, for sharing difficult as well as joyful periods during my PhD. Hans E, thank you for all the practical help and for being such a great listener. Annika L, thank you for all your help with administrative issues and for always handling even the messiest receipts and invoices with a smile on your face. Thanks to Anders G in the workshop for quick help with sampling equipment, to Micke Ö for showing sampling methods and general support, to Jakob L for all the nice coffee breaks and much needed distraction from work, to Caroline O for being a great friend and for standing strong in our quest to make our department more environment-friendly. Thanks to the OMK ladies Åsa R, Eva L, Gunborg A, and Märit P for nice lunch discussions and for always being so supportive, to Marlen S for analyzing my phytoplankton samples, to Isabel Q, Eva H, and Anders S at the phytoplankton lab for answering my questions, and to Lars E for identifying benthic fauna, answering questions and helping with equipment. This work had not been possible without the access to high-quality environmental monitoring data, a large part of which is acquired by people at my department. I would like to give a big thanks to everyone who has been designing monitoring programs, collecting and analyzing samples, and managing the monitoring databases over the years.

During my PhD studies I have had the amazing opportunity to be a part of Geneco, the Graduate Research School in Genomic Ecology, in Lund. Many thanks to the organizers of this fantastic program and a special thanks to the PhD students in my mentor program group – it has been so good to get to know you all and I have learnt a lot from you. Through Geneco's mentor program I also got to enjoy mentorship and coaching by Lena Tranvik, who has provided invaluable help in finding my way both professionally and personally. In addition, I received financial support from Geneco to visit another institution. I chose to go to Karin Rengefors' lab at the Aquatic Ecology unit at Lund University. In this short project I had the chance to combine my experience of and passion for DNA-based methods with an interesting ecological question and also learn some new techniques. Thank you so much for inviting me, Karin, it was so much fun working in you lab! I would also like to direct a special thanks to Marie S, Katharina L, and Ashgar M for introducing me to the methods and answering an endless number of questions. In addition, I would like to thank all the staff at Aquatic Ecology for making me feel so welcome, especially the PhD students Karen L, Ingrid S, Peter L, Mattias E, Mikael E, Kim B, Johan A, Pablo U, Sara E, Melanie H, Marja B, and post docs Heather R and Sylvie T.

Jan-Erik Svensson took the time (on a Friday night!) to give me a free crash course in zooplankton identification, which was extremely useful when picking out hundreds of tiny animals for analyses. Thank you so much!

Thanks to Henric Linge at the Kronoberg County Administrative Board for help with maps and boats, to Tomas Karlsson and Åke Jonasson at Evedals' camping for help with boats and equipment and for some nice chats, and to landowners by the lakes for letting me use their boats.

I would also like to thank my friends who have stayed close despite the sometimes very large geographical distance. I would especially like to thank Solgerd E and Karin H, who helped me in the field and reminded me that it is a good idea to eat and sleep also during field work. Many thanks also to Karin N, Johanna E, Sofia S, Julia S and Leif G.

Doing a PhD project is not easy, and sometimes you really need to talk about that with someone who truly understands. Thankfully, I have had this opportunity over fika and dinner with Josefine L, Jonas R, and Karin H and over lunch with Daniel G.

Research has the potential of filling all your time if you let it. Therefore, it has been important for me to have some other activities scheduled. I have spent quite some time playing music, singing and hanging out with the lovely people in Österledskyrkan in Gamla Uppsala. Being a part of your community has meant a lot to me.

One of the reasons I got into research in the first place was that I had the opportunity to do my master's project in a research lab with Henrik Aronsson as my supervisor. I would like to thank Henrik for encouraging me to trust my ability and to apply for to apply for PhD positions even if I did not think I had exactly the right qualifications (and for the advice to give a PhD project at least a year before deciding to quit). I am also grateful for the encouragement I got from Johan Uddling during my undergraduate studies.

Last but definitely not least, I want to thank my family. Thanks to my parents for teaching me about nature as a child, for encouraging me to stay curious and question authorities, and for proofreading a lot of texts throughout my school years (including the PhD). Thanks to my brother for just being you. André, without you I think I would never have made it through this whole thing in one piece. Thanks for always being there, supporting me, comforting me when things were hard (which I admit they were quite often), celebrating when things worked out, distracting me, forcing me to do something else than working, buying sweets and generally being a fantastic person to live with. I love you to bits.