LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 61, 2016, 1563–1573

doi: 10.1002/lno.10296

Algal blooms increase heterotrophy at the base of boreal lake food webs-Evidence from fatty acid biomarkers

Karin S. L. Johansson,[a,*1] Cristina Trigal,[2] Tobias Vrede,[1] Pieter van Rijswijk,[3] Willem Goedkoop,[1] Richard K. Johnson[1]

[1]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden
[2]Swedish Species Information Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden
[3]Department of Estuarine and Delta Systems, Royal Netherlands Institute for Sea Research, Yerseke, The Netherlands

Abstract

Physical defenses and grazer avoidance of the bloom-forming microalga *Gonyostomum semen* may reduce the direct coupling between phytoplankton and higher trophic levels and result in an increased importance of alternative basal food resources such as bacteria and heterotrophic protozoans. To assess the importance of algal and heterotrophic food resources for zooplankton during *G. semen* blooms and the effects of zooplankton diets on a higher consumer, we analyzed the fatty acid composition of zooplankton and the invertebrate predator *Chaoborus flavicans* from eight lakes along a gradient in the predominance of *G. semen* relative to other algae and the duration of *G. semen* blooms. The proportion of fatty acids of bacterial origin increased significantly along the *G. semen* gradient in all consumers studied. In addition, the proportion of polyunsaturated fatty acids (PUFA) decreased in cladocerans. These results suggest that heterotrophic pathways can compensate for a reduced trophic coupling between phytoplankton and filter-feeding zooplankton. The lower PUFA content in cladoceran prey from lakes at the higher end of the *G. semen* gradient did not affect the PUFA content of the predator *C. flavicans*, suggesting selective assimilation and retention of PUFA and/or feeding on other, more PUFA-rich prey.

Increased occurrence and spatial distribution of the bloom-forming microalga *Gonyostomum semen* during recent decades (Lepistö et al. 1994; Rengefors et al. 2012; Trigal et al. 2013) has raised concerns in northern Europe, as it is a nuisance to swimmers and could cause a reduction of the energy flow to higher trophic levels in the pelagic food web during blooms (Johansson et al. 2013a). *G. semen* is a large (length 36–92 μm, diameter 23–69 μm; Figueroa and Rengefors 2006), flagellated raphidophyte that forms dense blooms in late summer, when it can constitute more than 95% of the total phytoplankton biomass (Pithart et al. 1997). The naked, fragile cells release slimy threads (trichocysts) upon physical stimulation, which can cause skin irritation for swimmers and thereby reduce the recreational value of lakes with large populations of the alga (Sörensen 1954; Cronberg et al. 1988). In addition, the presence of trichocysts in combination with large cell size may limit the edibility of *G. semen* for filter-feeding zooplankton, as large and filamentous algae (net phytoplankton) may interfere with filter feeding and result in energy losses and rejection of other food particles (Gliwicz and Lampert 1990; Lebret et al. 2012; Johansson et al. 2013b).

In an earlier study, we found significant differences in zooplankton assemblage composition between lakes with and without recurring blooms of *G. semen*, while the total biomass of zooplankton was similar in both lake categories (Johansson et al. 2013a). In lakes with blooms, the small cladoceran *Ceriodaphnia* spp. was predominant, whereas the larger *Daphnia cristata* predominated in lakes without blooms. Calanoid copepods occurred in all lakes, but their total biomass was lower in bloom-lakes. As small cladocerans cannot feed on *G. semen* (Lebret et al. 2012; Johansson et al. 2013b), *Ceriodaphnia* likely uses other food resources during blooms of *G. semen*. Experiments have shown that *Ceriodaphnia* is superior at utilizing bacteria as a food source compared with other daphniids (Pace et al. 1983; Iwabuchi and Urabe 2010). In contrast to small cladocerans the copepod *Eudiaptomus gracilis*, which usually is the most abundant calanoid copepod in

*Correspondence: karin.johansson@bioenv.gu.se

[a]Present address: Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

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Table 1. Morphometric and physico-chemical characteristics of the lakes sampled in this study. Water chemistry values represent spring (April–May) turnover conditions.

	Surface area (km^2)	Mean depth (m)	TOC ($mg\ L^{-1}$)	pH	Total N ($\mu g\ L^{-1}$)	Total P ($\mu g\ L^{-1}$)
Holmeshultasjön	0.69	5.0	12	6.6	540	11
Brunnsjön	0.10	5.3	19	5.6	600	13
St Skärsjön	0.30	3.8	5	6.6	510	12
Hagasjön	0.12	3.6	10	6.4	390	10
Älgarydssjön	0.33	1.3	15	5.3	470	20
Bäen	0.50	3.4	10	5.4	780	17
Storasjö	0.40	1.8	12	5.5	430	27
Harasjön	0.54	2.1	14	5.3	520	23

Swedish temperate lakes (Pejler 1965), can feed on *G. semen* at high rates (Johansson et al. 2013b), suggesting that *G. semen* is an important food resource for calanoid copepods during blooms. However, *G. semen* cells migrate vertically and occur in the hypolimnion at night, reducing the predation pressure from zooplankton (Salonen and Rosenberg 2000). This suggests that alternative food resources could become more important during *G. semen* blooms, even for calanoid copepods. As suspension-feeding copepods do not capture and consume bacteria efficiently (Vrede and Vrede 2005), bacterivorous protozoa could instead be used as an alternative food resource during blooms of *G. semen* (Wiackowski et al. 1994; Jürgens et al. 1996).

Fatty acid biomarkers are increasingly used in food-web studies; based on the principle that certain prey organisms produce specific fatty acids, some of which are incorporated into the lipids of consumers without being altered (Desvilettes et al. 1997; Brett et al. 2006). For example, most algae are rich in polyunsaturated fatty acids (PUFA), whereas branched and odd-length fatty acids are typical of bacteria (Desvilettes et al. 1997). Accordingly, concentrations of specific fatty acid biomarkers in the lipids of animals provide a semi-quantitative estimate of the relative importance of different food sources in their diet (Desvilettes et al. 1997; Goedkoop et al. 2000; Lau et al. 2014). In addition, the fatty acid composition of food resources provides information about their nutritional quality. PUFA cannot be synthesized de novo by most animals and are considered essential components of their diet (Brett and Müller-Navarra 1997). Three PUFA in the ω3 and ω6 families are particularly important and generally considered essential in the aquatic literature: eicosapentaenoic acid (EPA, 20:5ω3) and arachidonic acid (ARA, 20:4ω6), which are precursors to hormones involved in reproduction and immunological responses, and docosahexaenoic acid (DHA, 22:6ω3), which regulates cell membrane properties and the development and functioning of brain and eyes (Brett and Müller-Navarra 1997; Parrish 2009). Due to the importance of EPA, ARA, and DHA for the growth and reproduction of most animals, the content of these fatty acids in zooplankton lipids can be used as a measure of nutritional quality for higher consumers. Aquatic consumers have been shown to selectively incorporate and retain essential PUFA, resulting in an accumulation of these compounds with trophic level (Persson and Vrede 2006; Gladyshev et al. 2011).

In this study, we analyzed the fatty acid composition of mesozooplankton from eight lakes with a gradient in the predominance of *G. semen* relative to other algae and the duration of *G. semen* blooms to assess the importance of algal and microbial food resources for zooplankton during blooms. In addition, we analyzed the fatty acid composition of late-instar phantom midge larvae (*Chaoborus flavicans*) to study the influence of zooplankton fatty acid composition on a predator. We hypothesized: (1) that fatty acids of cladocerans from lakes at the higher end of the *G. semen* gradient would contain a larger proportion of bacterial markers (BAFA) and a smaller proportion of PUFA, reflecting higher feeding rates on bacteria and/or heterotrophic protozoans, (2) that *E. gracilis* from all lakes would contain a high proportion of PUFA, while *E. gracilis* from lakes at the higher end of the *G. semen* gradient would contain more BAFA due to feeding on heterotrophic protozoans when *G. semen* is not accessible due to migration to the hypolimnion, and (3) that the proportion of BAFA in *C. flavicans* would increase and the proportion of PUFA would decrease along the *G. semen* gradient due to feeding on small cladocerans with a higher proportion of BAFA and a lower proportion of PUFA.

Material and methods

Study sites

Eight small, humic lakes located in forested catchments (>45% of catchment area) in southern Sweden were sampled in 2009 (Table 1). The lakes showed a gradient of *G. semen* bloom duration and intensity, ranging from no detection of *G. semen* to *G. semen* constituting > 80% of the total phytoplankton biomass already in June. Historically, four of the lakes (Bäen, Harasjön, Storasjö, and Älgarydssjön) experienced repeated late-summer blooms of *G. semen*, whereas

Table 2. Percentage *G. semen* of total phytoplankton biomass on three sampling occasions in 2009 and ranking of the study sites based on the extent and duration of *G. semen* dominance.

Lake	Date	% *G. semen*	Date	% *G. semen*	Date	% *G. semen*	Rank
Holmeshultasjön	06 Jun	0	20 Aug	0	25 Aug	0	1
Brunnsjön	07 Jun	0	18 Aug	0	29 Aug	0	1
St Skärsjön	03 Jun	2	25 Aug	14	27 Aug	8	2
Hagasjön	05 Jun	0	17 Aug	13	26 Aug	34	3
Älgarydssjön	05 Jun	7	13 Aug	15	25 Aug	88	4
Bäen	03 Jun	29	18 Aug	91	28 Aug	88	5
Storasjö	06 Jun	87	19 Aug	92	26 Aug	92	6
Harasjön	04 Jun	92	30 Jul	93	27 Aug	96	7

blooms were not observed in the other four lakes (except for one bloom in St Skärsjön in 2005). The lakes are all reference lakes in Swedish national and regional environmental monitoring programs and are not affected by point source pollution. Based on data from sampling occasions in June, early- and late August, 2009 (Table 2), the eight lakes were ranked by their *G. semen* dominance (% of total phytoplankton biomass) and bloom duration. Lakes with no occurrence of *G. semen* were ranked the lowest, intermediate ranks were given to lakes that had low levels of *G. semen* (< 50% of total phytoplankton biomass) at all sampling occasions, followed by lakes that had low percentages of *G. semen* in the beginning and were later dominated (> 50% of total phytoplankton biomass) by *G. semen*. The highest ranks were given to lakes that were dominated by *G. semen* at all sampling occasions.

Sampling and determination of phytoplankton

Phytoplankton samples were taken from epilimnetic water column samples that were collected using an acrylic glass tube sampler from three to five locations over the deepest part of the lake and mixed to form a composite sample. Subsamples (250 mL) from the composite sample were preserved with Lugol's iodine solution. Phytoplankton were determined, counted and measured under an inverted microscope according to a modified Utermöhl technique commonly used in the Nordic countries (Olrik et al. 1998).

Sampling and sample preparation of animals

Zooplankton and *C. flavicans* for fatty acid analyses were sampled in late August. Zooplankton were collected by vertical hauls with a 180-μm plankton net over the deepest part of the lake, and late-instar *C. flavicans* larvae were sampled by taking Ekman grabs of profundal sediments. Zooplankton samples were collected on 65-μm nylon mesh and shock-frozen in dry ice in the field. *C. flavicans* samples were sieved (0.5-μm mesh), kept dark and cool during transportation, sorted into cryovials on the same day, and shock-frozen in dry ice. Samples were stored under N_2 atmosphere at −20°C in the lab. Prior to analysis, samples were freeze-dried and sorted under a dissecting microscope. Of the predominant zooplankton taxa in the net haul samples, i.e., *Ceriodaphnia* spp., *Daphnia* spp. (*D. cristata* and *Daphnia longispina*), *Bosmina* spp., and *E. gracilis*, ~0.6 mg of dry weight was transferred to methanol-rinsed tin capsules (one sample per taxon and lake). For *C. flavicans* samples, three individuals were ground with a pestle and ~0.6 mg dry weight of the homogenate was weighed into methanol-rinsed tin capsules. The weight of each sample was recorded and later used for normalization of fatty acid concentrations to sample dry weight.

Algal cultures

Cultures of *G. semen* were established from single cell isolates from three lakes in the southern parts of Sweden and Finland: Dammen (N56.553 E14.318, strain Dm18), Liasjön (N56.447 E13.988, strain Li22), and Kylänalanen (N60.409 E23.754, strain Ky23). The cultures were grown in modified Wright's cryptophyte (MWC) medium (Guillard and Lorenzen 1972) with an addition of Se (final concentration 1.2 $\mu g\ L^{-1}$) at 20°C in a light intensity of 20 $\mu mol\ m^{-2}\ s^{-1}$ under a 12:12h light:dark cycle. Culture densities were determined by counting cells in a Sedgewick-Rafter chamber and average cell size was determined by measurements of length and width under an inverted microscope. Samples of 15–20 mL from each algal culture were collected in triplicate on pre-combusted GF/C filters, frozen and subsequently freeze-dried. The dry weight of algae was estimated from biovolumes, a carbon content of 11% of wet weight and a dry weight two times the carbon content (Lundgren 1978).

Lipid extraction and fatty acid analysis

Total lipids of zooplankton and cultured *G. semen* were extracted with chloroform, methanol and water by a modified Bligh and Dyer method (Bligh and Dyer 1959; Boschker 2004). The resulting lipid extract was derivatized to fatty acid methyl esters (FAMEs) by mild alkaline transmethylation (Boschker 2004). Samples were stored frozen (−80°C) until analysis. FAMEs were determined by gas chromatography-flame ionization detection (GC-FID) on a Trace Ultra GC (Thermo Scientific) with a fused silica pre-column (5 m*0.32 mm, SGE

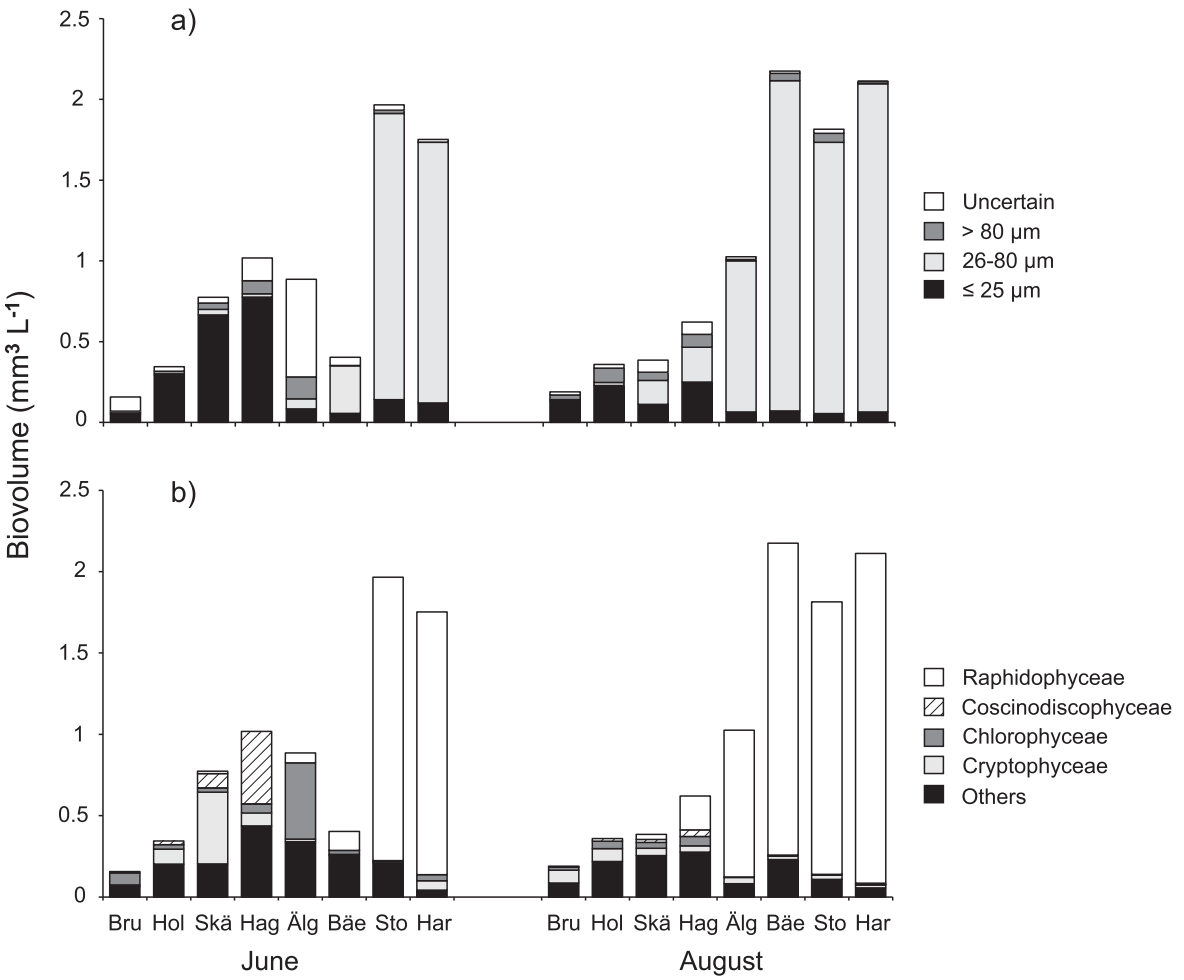

Fig. 1. Biovolume of (**a**) phytoplankton size classes and (**b**) phytoplankton taxonomic groups in the study lakes in June and late August. Size classification was based on the cell or colony length.

Analytical Science) and a polar capillary BPX70 analytical column (50 m*0.32 mm*0.25 μm, SGE Analytical Science). Samples were injected using splitless mode. The methyl esters were identified based on their retention times on the GC column relative to internal standards (added 12:0 and 19:0), and naturally occurring 16:0. Relating the retention times to three compounds with different chain length increases the precision of the identification compared with using only one internal standard. Relative retention times had previously been calibrated using commercially available standards. FAMEs were quantified by comparison of their peak areas to that of the internal standard 19:0, which was added in a known quantity. Some FAMEs that could not be identified by GC-FID were determined by gas chromatography-mass spectrometry (GC-MS, Agilent model 5975C). In total, 56 FAMEs (excluding internal standards) were analyzed in the animal samples (Supporting Information Table S1). Slightly fewer (47) methyl esters were analyzed in the algal samples (Supporting Information Table S2) due to the less clear separation on the older column used for those analyses and because we did not do any complementary GC-MS analyses. Odd-numbered, saturated fatty acids with a chain length of 13–17 carbons and branched (*iso*, *anteiso* and 10Me) fatty acids were classified as bacterial biomarkers (Desvilettes et al. 1997; Napolitano 1999).

Peak area normalization was used to calculate percentages of total FAME, i.e., individual FAME peaks were divided by the total peak area in the chromatogram (excluding added internal standards). Fatty acid weights were calculated by relating the peak area of individual FAMEs to the peak area and weight of the internal standard 19:0. Fatty acid weights were corrected for the methyl group added during transmethylation and the fraction of chloroform recovered during extraction (Boschker 2004), and normalized to mg dry weight of sample. For unidentified peaks, we estimated the ratio between the molecular weight of fatty acid and FAME using equivalent chain length of the unknown compounds and the FA/FAME weight ratio of known fatty acids with similar chain length. In cases where one peak may represent two FAMEs that could not be separated we used the average of the ratio for both compounds.

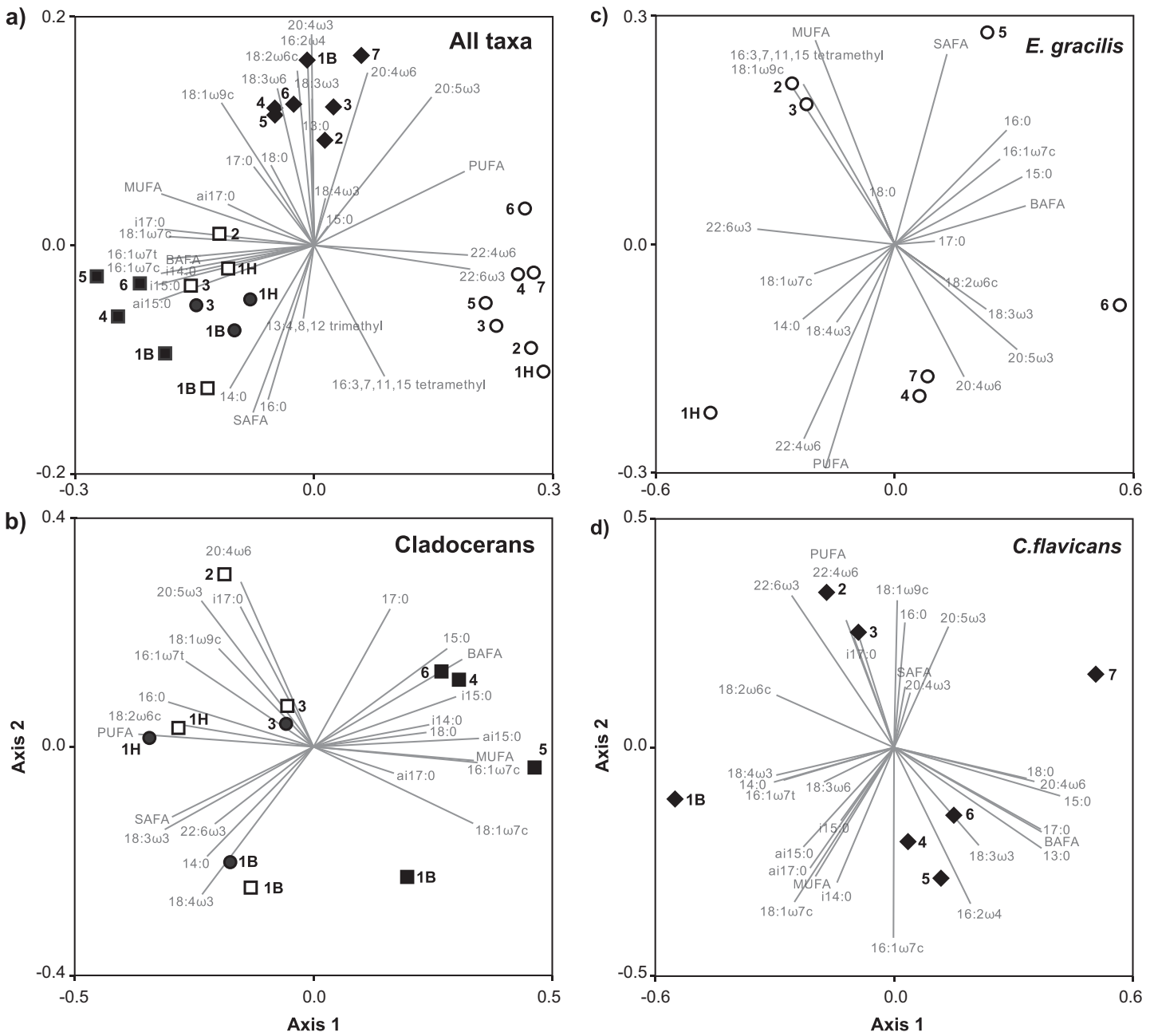

Fig. 2. NMDS ordinations based on area-normalized fatty acid data (percentage of total fatty acids) from samples of (**a**) all zooplankton taxa (stress = 0.083, R^2 axis 1 = 0.88, R^2 axis 2 = 0.049), (**b**) cladocerans (stress = 0.14, R^2 axis 1 = 0.66, R^2 axis 2 = 0.079), (**c**) *E. gracilis* (stress = 0.18, R^2 axis 1 = 0.75, R^2 axis 2 = 2.35*10^{-8}), and (**d**) *C. flavicans* (stress = 0.18, R^2 axis 1 = 0.61, R^2 axis 2 = 0.17). Filled squares represent *Ceriodaphnia* spp., open squares *Daphnia* spp., filled circles *Bosmina* spp., open circles *E. gracilis*, and diamonds *C. flavicans*. Numbers represent the ranking of lakes based on the duration and dominance level of *G. semen* blooms with higher numbers indicating longer duration and larger dominance, as presented in Table 2. The two lakes having the same rank are separated by the letters B for Brunnsjön and H for Holmeshultasjön. The R^2 value of an NMDS axis represents the coefficient of determination between the distances along the axis and the original distances, i.e., a goodness of fit measure. Trends along the second axis should be interpreted with caution due to the low R^2 values of this axis in all plots.

Data analysis

Phytoplankton were classified in three size categories based on their suitability as food for zooplankton (Burns 1968; Demott and Watson 1991): ≤ 25 μm, 26–80 μm, and > 80 μm. Taxa that could not be placed in any of these categories were categorized as "uncertain." Size classification was based on length, using measurements from samples in this study and literature values (Tikkanen and Willén 1992; John et al. 2002; Olenina et al. 2006; Guiry and Guiry 2014) for some taxa (e.g., colonial forms for which the entire

Table 3. Range of specific fatty acids or fatty acid ratios among samples and results of Spearman rank correlations (ρ) between fatty acid percentage data for key consumers and ranks of *G. semen* bloom intensity and duration. See text for further explanation.

Fatty acid group	Cladocera ($n = 11$)		*E. gracilis* ($n = 7$)		*C. flavicans* ($n = 7$)	
	Range	ρ	Range	ρ	Range	ρ
BAFA	6.9–17%	0.92***	1.1–9.0%	0.79*	3.4–8.1%	0.86*
PUFA	12–29%	−0.71*	51–63%	−0.14	40–51%	−0.36
ω3 PUFA	7.5–19%	−0.77**	41–51%	−0.57	27–35%	−0.54
ω6 PUFA	4.2–10%	−0.31	8.9–15%	0.43	11–16%	0.57
ARA	2.2–5.8%	0.18	3.8–8.5%	0.93**	4.8–8.8%	0.86*
EPA	2.9–8.2%	−0.062	12–18%	0.89*	14–20%	0.39
DHA	0–1.7%	−0.60	15–29%	−0.79*	1.5–7.7%	−0.64
BAFA/PUFA	0.30–1.4	0.85**	0.017–0.17	0.79*	0.073–0.20	0.86*
DHA/EPA	0–0.34	−0.60	0.83–2.5	−0.82*	0.083–0.46	−0.71
ω3/ω6	1.3–2.5	−0.65*	3.0–4.7	−0.61	2.0–2.9	−0.71

Asterisks indicate levels of significance: *$p < 0.05$, ** $0.01 < p < 0.05$, ***$p < 0.001$.
BAFA, bacterial fatty acid markers; PUFA, polyunsaturated fatty acids; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

colony was not measured in this study). Variation in the biomass of other phytoplankton than *G. semen* and of the three size categories along the *G. semen* gradient (lake ranks, see Table 2) was tested using Spearman rank correlation with a permutation test adapted for small sample sizes (9999 permutations) and $\alpha = 0.05$.

Fatty acid data (percentages) were assessed visually by non-metric multidimensional scaling (NMDS) based on a Bray-Curtis similarity matrix, with fatty acid data overlaid as vectors on the ordination plot to display the characteristic fatty acids of the samples. The vectors represent the correlation coefficient between the fatty acid data and the ordination scores and the relative length of the vectors indicate the strength of the correlation. However, since the strength of a linear relationship with the ordination scores does not necessarily correspond to the importance of the fatty acid for the configuration of data points, the most important information lies in the direction of the vectors. All identified fatty acids were included in the ordinations, but for the sake of readability arrows representing fatty acids detected in three or less of the samples included in each analysis and those representing ambiguous peaks (peaks that could include either or both of two fatty acids) were removed from the plots. Fatty acid data were square-root transformed to reduce the influence of the most common fatty acids since the Bray-Curtis index inherently gives more weight to variables with high abundances.

Spearman rank correlation was used to test trends in the content of bacterial fatty acids (BAFA), polyunsaturated fatty acids (PUFA), ω3 and ω6 PUFA, and the essential fatty acids ARA, EPA, and DHA in animal samples along the gradient of *G. semen* bloom intensity and duration (lake ranks, see Table 2) on both the relative fatty acid composition (% of total FA) and concentrations (μg/mg DW). In addition, trends in the ratios BAFA/PUFA, DHA/EPA, and ω3/ω6 were tested on relative data. The ω3/ω6 ratio is a potential indicator of heterotrophy in pelagic food webs since heterotrophic freshwater protozoans often contain a larger proportion of ω6 than ω3 PUFA (Desvilettes et al. 1997; Vera et al. 2001). Relative amounts of fatty acids of consumers are semiquantitatively related to the contribution of different food resources in the diet (Napolitano 1999) and therefore these data were used to make inferences about diet. Concentration data normalized to dry weight were analyzed as a complementary measure of nutritional quality of zooplankton to higher consumers, i.e., the overall availability of nutritionally important fatty acids to predators. All statistical analyses were performed in PAST version 2.17c (Hammer et al. 2001).

Results

Assemblage composition

The size structure of phytoplankton assemblages in late August varied along the *G. semen* lake gradient, with a decreasing biomass of small phytoplankton (< 25 μm, $n = 8$, $\rho = -0.73$, $p = 0.049$) and increasing biomass of larger phytoplankton (26–80 μm, $n = 8$, $\rho = -0.73$, $p = 0.049$). Taxonomic groups and size classes of phytoplankton in the study lakes are shown in Fig. 1.

The predominant cladoceran taxa differed between lakes at the higher and lower ends of the *G. semen* gradient, with *Daphnia* and *Bosmina* being more common at the lower end and *Ceriodaphnia* more common at the higher end of the *G. semen* gradient, except in Lake Brunnsjön where all three taxa co-existed at similar abundances. The calanoid copepod *E. gracilis* and larvae of the phantom midge *C. flavicans* were isolated from seven of the eight lakes.

Fatty acid composition of animals

NMDS ordination using all data showed a clear separation between cladocerans, *E. gracilis*, and *C. flavicans*, which

Table 4. Total concentrations of fatty acids (μg FA mg^{-1} dry weight) in animal samples by lake. A dash indicates that a taxon was not found the lake.

LAKE	*Daphnia* spp.	*Bosmina* spp.	*Ceriodaphnia* spp.	*E. gracilis*	*C. flavicans*
Holmeshultasjön	25.7	24.1	–	15.3	–
Brunnsjön	15.9	20.6	39.5	–	111.9
Stora Skärsjön	14.8	–	–	14.1	72.4
Hagasjön	22.7	17.8	–	17.1	34.7
Älgarydssjön	–	–	26.6	14.9	73.7
Bäen	–	–	31.4	13.3	59.8
Storasjö	–	–	23.0	19.1	57.9
Harasjön	–	–	–	13.0	56.2

Table 5. Average quantities of the most abundant fatty acids (FA) in *G. semen* cultures (data pooled from analyses of three cultures in triplicate).

Fatty acid	% of total FA ± SD	μg FA/mg dry weight ± SD
16:0	21.9 ± 4.6	18.3 ± 4.8
16:1ω7c	16.0 ± 4.2	13.4 ± 4.4
20:5ω3	14.3 ± 4.9	13.9 ± 8.1
14:0	12.0 ± 4.5	9.5 ± 1.9
18:4ω3	10.2 ± 5.1	10.2 ± 6.7
18:3ω3	6.9 ± 2.6	6.8 ± 4.0
16:2ω4	3.8 ± 1.4	3.6 ± 2.1
20:4ω6	2.1 ± 0.6	1.9 ± 1.1
16:1ω7t	1.9 ± 0.6	1.6 ± 0.5
18:2ω6c	1.4 ± 0.1	1.3 ± 0.6

likely represents differences in diet and fatty acid incorporation that are related to taxonomy (Fig. 2a). Samples separated along a gradient from fatty acid profiles characterized by bacterial markers (BAFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SAFA) in cladocerans to fatty acid profiles characterized by long-chain polyunsaturated fatty acids (PUFA) in *E. gracilis*. Samples of *C. flavicans* were placed between cladocerans and *E. gracilis* along this gradient. The large differences between these three groups are likely due mainly to taxonomic variation in feeding strategy and fatty acid assimilation. To separate the effect of *G. semen* blooms on fatty acid composition from taxonomic differences it was necessary to analyze each taxon separately. Separate ordinations showed also within-group separation along the *G. semen* gradient. Cladocerans were separated into two groups along a PUFA–BAFA gradient with *Daphnia* and *Bosmina*, predominant in lakes at the lower end of the *G. semen* gradient, being richer in PUFA and *Ceriodaphnia*, predominant in lakes at the higher end of the *G. semen* gradient, containing more BAFA (Fig. 2b). Also *E. gracilis* samples clearly separated along the *G. semen* gradient (Fig. 2c). *E. gracilis* from lakes at the higher end of the *G. semen* gradient were characterized by high BAFA and EPA, while those from lakes at the lower end typically were higher in DHA. Samples of the predator *C. flavicans* also separated along the *G. semen* gradient. *C. flavicans* from lakes at the higher end of the gradient were characterized by larger proportions of BAFA and ARA, while those from lakes at the lower end of the gradient were richer in some 18-carbon PUFA (Fig. 2d).

Spearman rank correlation using relative data showed increasing trends for BAFA, decreasing PUFA and increasing BAFA/PUFA ratio in cladocerans along the *G. semen* gradient (Table 3). Specifically, ω3 PUFA and the ω3/ω6 ratio showed clear declines along the gradient. Also the calanoid copepod *E. gracilis* showed increasing BAFA percentage and BAFA/PUFA ratio along the *G. semen* gradient, but the percentage of total PUFA did not differ along the gradient for this taxon (Table 3). However, ARA and EPA in *E. gracilis* showed positive trends along the *G. semen* gradient and the percentage of DHA decreased. Hence, the DHA/EPA ratio in *E. gracilis* samples decreased along the gradient. Similar to *E. gracilis*, *C. flavicans* samples also showed an increase in the percentage of BAFA and in the BAFA/PUFA ratio along the *G. semen* gradient, but no significant trend in total PUFA (Table 3). However, the percentage of ARA in *C. flavicans* increased along the *G. semen* gradient.

Correlation analyses using fatty acid concentrations showed few significant results, probably because of the large variation in total fatty acid content of the animals (Table 4). The total fatty acid concentration showed no significant trend in any of the taxa and ranged 14.8–39.5 μg mg^{-1} dry weight among cladocerans, 13.0–19.1 μg mg^{-1} in *E. gracilis* samples and 34.7–111.9 μg mg^{-1} in *C. flavicans*. In cladocerans, the concentration of ω3 PUFA showed a significant decrease ($n = 11$, $\rho = -0.67$, $p = 0.030$) as well as DHA ($\rho = -0.70$, $p = 0.026$) along the *G. semen* gradient. In copepods, there was a significant decrease in the concentration of DHA ($n = 7$, $\rho = -0.82$, $p = 0.037$). No significant trends in fatty acid concentrations were found in *C. flavicans*.

For the full dataset on fatty acid composition of animals, see Table S1 in Supporting Information.

Fatty acid composition of *G. semen*

The most abundant fatty acids in *G. semen* were 16:0, 16:1ω7c, EPA, 14:0, and 18:4ω3 constituting on average 22%, 16%, 14%, 12%, and 10% of total fatty acids. DHA comprised less than 1% of total fatty acids. The average percentages and concentrations of the 10 most abundant fatty acids in *G. semen* (data pooled from all samples) are shown in Table 5. For the full dataset, see Table S2 in Supporting Information.

Discussion

Despite high standing stock biomass and nutritional quality, *G. semen* blooms shift pelagic consumers toward a stronger dependence of heterotrophic pathways. Cladocerans, calanoid copepods and the predator *C. flavicans* all showed an increasing proportion of bacterial fatty acid markers (BAFA) along a gradient of *G. semen* bloom intensity and duration (Table 3; Fig. 2). This shows a stronger trophic importance of bacteria and/or bacterivorous protozoans for pelagic consumers in lakes with *G. semen* blooms. In cladocerans, the increase in BAFA was paralleled by a decline in the proportion of physiologically important polyunsaturated fatty acids (PUFA). We realize that the simultaneous trends in BAFA and PUFA in cladocerans could mean that there are actual trends in both fatty acid groups or in just one of them, as a change in one variable affects the proportion of others. However, the observation of an increasing BAFA proportion without a parallel decrease in PUFA in the other taxa suggests that there is indeed an increase in the importance of heterotrophic food resources along the *G. semen* gradient. Surprisingly, the proportion of BAFA in cladocerans from two lakes at the higher end of the *G. semen* gradient was greater than the proportion of PUFA (Fig. 3). To our knowledge, this is the first time such high BAFA/PUFA ratios are reported in zooplankton. Considering that consumers selectively incorporate and retain long-chain PUFA and catabolize other fatty acids to a larger extent, heterotrophic food resources likely constitute a significant part of cladoceran diets in these lakes (Burns et al. 2011; Gladyshev et al. 2011; Taipale et al. 2011).

Mechanisms governing the observed shift toward a larger importance of heterotrophic pathways are likely related to the physical defenses and grazer-avoidance strategies of *G. semen*, in combination with the observed low biomass of small, edible phytoplankton in *G. semen*-dominated systems (Fig. 1a; Trigal et al. 2011; Johansson et al. 2013a). As small filter feeders cannot ingest *G. semen* (Lebret et al. 2012; Johansson et al. 2013b) and the availability of small, edible phytoplankton is low during blooms (Fig. 1), cladoceran taxa that are able to use alternative food resources such as bacteria will likely be favored. In line with a previous study (Johansson et al. 2013a), we found that *Ceriodaphnia* was more common in lakes with high biomasses of *G. semen*, whereas *Daphnia* and *Bosmina* were more common in lakes at the lower end of the *G. semen* gradient. Experimental studies have shown that *Ceriodaphnia* and *Daphnia* filter bacteria with similar efficiencies, but that *Ceriodaphnia* grows better and can sustain positive population growth on a bacteria-dominated diet, while *Daphnia* populations decline (Pace et al. 1983; Iwabuchi and Urabe 2010). *Ceriodaphnia* thus appears to be better adapted to low food-quality conditions where heterotrophic resources dominate the food. To our knowledge, there are no experimental studies of the effects of predominantly bacterial diets on the growth and reproduction of *Bosmina*. However, selective feeding on small flagellated algae, low feeding rates on bacteria in mixed diets and poor assimilation of bacteria (Demott 1982; Demott and Kerfoot 1982; Urabe and Watanabe 1990) suggests that *Bosmina* spp. is adapted to feeding on high-quality food resources.

In contrast to small cladocerans, adults of the calanoid copepod *E. gracilis* are able to feed efficiently on *G. semen* (Johansson et al. 2013b). Indeed, the high PUFA content of all *E. gracilis* samples indicates that a substantial proportion of the diet consists of algae (Table 3). Interestingly, we found significant trends in the proportion of DHA and EPA in *E. gracilis* along the *G. semen* gradient, resulting in a range of DHA/EPA ratios between 2.5 and 0.83 (Table 3; Fig. 2c). As DHA generally is the main long-chain fatty acid found in calanoid copepods, likely due to selective incorporation and/or conversion of shorter ω3 fatty acids (Persson and Vrede 2006; Brett et al. 2009), the increase in EPA along the *G. semen* gradient indicates that *E. gracilis* feeds more on EPA-rich food resources in lakes with high *G. semen* biomasses. Our fatty acid analyses of *G. semen* cultures showed that EPA is the dominant PUFA in this alga (Table 5) and that it is very low in DHA (Table S2 in Supporting Information). Hence, the higher proportion of EPA in copepods along the *G. semen* gradient, in combination with the low biomass of other algae in lakes at the higher end of the *G. semen* gradient (Fig. 1), corroborate that the efficient feeding of diaptomid copepods on *G. semen* observed in feeding experiments (Williamson et al. 1996; Johansson et al. 2013b) also occurs in nature. Calanoid copepods thus appear to constitute an important link between primary production and higher trophic levels during blooms of *G. semen*. Diel vertical migration of *G. semen* cells may, however, restrict *E. gracilis* to using alternative food resources at night, which could explain the increasing BAFA content in *E. gracilis* samples along the *G. semen* gradient. Since suspension-feeding copepods do not feed efficiently on bacteria (Vrede and Vrede 2005), the BAFA in lipids of *E. gracilis* likely originate from consumption of heterotrophic protozoans (Wiackowski et al. 1994; Jürgens et al. 1996).

For higher trophic levels, the predominance of small bacterivorous cladocerans during *G. semen* blooms may result in a lower food quality than in similar lakes without blooms, due to their lower PUFA and higher BAFA proportion. The PUFA content of the predator *C. flavicans*, however, showed no significant trend along the *G. semen* gradient. This

suggests that *C. flavicans* larvae accumulate PUFA selectively from cladocerans or other PUFA-rich food resources. *Ceriodaphnia*, a relatively small species, is easily caught and ingested by gape-limited predators like *C. flavicans* (Smyly 1980; Hanazato and Yasuno 1989) and our results show that the total fatty acid concentration was high in this prey (Table 4). Hence, large absolute quantities of PUFA may be available to *C. flavicans*, even though their proportion in *Ceriodaphnia* was not as high as in other cladocerans. In addition, *C. flavicans* feeds on other prey than cladocerans, including phytoplankton, rotifers, copepods, and benthic invertebrates (Swüste et al. 1973; Kajak and Rybak 1979; Moore et al. 1994). Nonetheless, the increasing proportion of BAFA in *C. flavicans* along the *G. semen* gradient shows that the greater importance of heterotrophic food resources at the base of the food web is evident also in the fatty acid composition of a higher consumer.

Our results showing an increased utilization of heterotrophic resources at the base of the pelagic food web during blooms of *G. semen* and the observation of similar zooplankton biomasses in lakes with and without blooms (Johansson et al. 2013a) suggest that heterotrophic pathways may compensate for a decreased trophic coupling between primary producers and zooplankton. The importance of heterotrophic pathways during algal blooms has also been shown in an experimental study using a bloom-forming cyanobacterium, where more carbon from primary production reached filter feeders via heterotrophic pathways than through direct consumption (De Kluijver et al. 2012). However, the impact of BAFA on zooplankton health and fitness has not been studied, and lower abundances of small, zooplanktivorous perch in lakes with recurring *G. semen* blooms (Trigal et al. 2011) suggest that the effects of a zooplankton assemblage predominated by small cladocerans that feed on heterotrophic resources should be a focus of future research.

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Acknowledgments

The authors wish to thank Jan-Erik Svensson for help in zooplankton identification, Karen Lebret for providing cultures of *G. semen*, Växjö municipality and landowners by the lakes for lending us boats, staff at the Royal Netherlands Institute for Sea Research for laboratory assistance, Danny C.P. Lau for advice on data analysis, the Swedish Agency for Marine and Water Management for making data available, and the reviewers for constructive comments on a previous version of the manuscript. Funding was provided by the Swedish Environmental Protection Agency (Dnr 10/179) and the Swedish Agency for Marine and Water Management through contract for the research program WATERS, the Ramón Areces Foundation, and the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences.

Submitted 5 October 2015
Revised 4 February 2016
Accepted 25 February 2016

Associate editor: Marguerite Xenopoulos