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Temperature as a driver for the expansion of the microalga *Gonyostomum semen* in Swedish lakes

Karin Rengefors [a,*], Gesa A. Weyhenmeyer [b,c], Ina Bloch [c]

[a] Aquatic Ecology, Dept. of Biology, Ecology Building, Lund University, 22362 Lund, Sweden
[b] Dept. of Ecology and Genetics/Limnology, Uppsala University, Norbyvägen 18 D, 75236 Uppsala, Sweden
[c] Dept. of Aquatic Sciences and Assessment, SLU/Swedish University of Agricultural Sciences, Box 70 50, 750 07 Uppsala, Sweden

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ABSTRACT

Gonyostomum semen (Ehrenb) Diesing is a bloom-forming and noxious phytoplankton species, that usually occurs in brown-water lakes and which is often referred to as an invasive species. The aim of our study was to analyze changes over time in the occurrence and distribution of blooms, and to find possible drivers of this change. We also performed spatial analyses to identify environmental factors coupled to *Gonyostomum*'s distribution. The effect of temperature on key processes in the *Gonyostomum* life cycle was further investigated experimentally to determine potential mechanistic causes. Our results show that *G. semen* has expanded in Swedish lakes since 1988. At the turn of the Millennium it was present in more than a quarter of the lakes included in the Swedish national lake monitoring program. *Gonyostomum*-lakes have significantly higher DOC, higher nutrient levels, and lower pH than non-*Gonyostomum* lakes. Trend analyses show a significant increase in the number of lakes with *Gonyostomum*, as well as in biomass and occurrence in samples. One explanation is that we more often find water temperatures exceeding 6 °C, which is also the threshold for positive growth in our laboratory experiments. Moreover, according to our partial least square regression model (PLS) analysis in one lake, we find that the increase in biomass is a function of temperature in combination with other factors. Thus, we conclude that an increase in water temperature resulting in longer growth season may be a driver of the expansion of *Gonyostomum*. However, temperature alone cannot explain why the species has expanded to new lakes within the same climatic region. Possibly an interplay between DOC and temperature can explain the patterns observed.

1. Introduction

The threat of biological invaders to ecosystems has been the focus of much research in recent decades (Richardson and Pysek, 2008). Invaders can influence freshwater systems at all levels of ecological organization (Simon and Townsend, 2003) and can also cause extensive economic problems. Well known examples for tremendous socio-economic and ecological effects of invasive species are the introduction of the Nile perch *Lates nilotica* (L) (Witte et al., 1992; Goldschmidt et al., 1993; Balirwa et al., 2003) and the expansion of the zebra mussel *Dreissena polymorpha* (Pallas) in rivers and the Great Lakes of North America (Strayer et al., 1999; Schloesser and Nalepa, 1994; Ricciardi, 2003). According to a definition given by Valery et al. (2008) a species has to gain a competitive advantage due to disappearance of natural obstacles and it has to become dominant in the newly conquered areas to be considered invasive. These criteria are fulfilled by several algal species. The most well-documented example is that of the spreading of the periphyton *Caulerpa taxifolia* (Bryopsidophyceae) in the Mediterranean Sea after being introduced from Australia (Stam et al., 2006). Among the marine harmful planktonic species, several dinoflagellates could be considered invasive, e.g. the toxic *Alexandrium minutum*, which most likely has been dispersed via ballast water and established in new areas (Lilly and Halanych, 2005). In freshwaters, the toxic cyanobacteria *Cylindrospermopsis raciborskii* is an example of invasive harmful microalgae (Neilan et al., 2003).

Another presumably invasive species is the large (50–100 μm) flagellated freshwater species *Gonyostomum semen* (Ehrenberg) Diesing. *G. semen* (hereafter referred to *Gonyostomum* only) belongs to the class Raphidophyceae and is world-wide the most common freshwater representative of this class (Bourelly, 1985). Like the marine raphidophytes, *Gonyostomum* alternates between a flagellated planktonic phase and a benthic resting cyst stage (Figueroa and Rengefors, 2006). In the laboratory cells undergo sexual reproduction and form cysts in response to nutrient

* Corresponding author. Tel.: +46 46 222 8427; fax: +46 46 222 4536.
E-mail address: Karin.Rengefors@biol.lu.se (K. Rengefors).

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limitation (Figueroa and Rengefors, 2006), but in the field cysts are observed when the lake temperature drops below 10 °C (personal observation). The factors that regulate cyst germination have not been investigated in detail to date, although preliminary findings indicate that temperature affects germination (Figueroa and Rengefors, 2006). In marine raphidophytes, *Chatonella* cysts are known to undergo a mandatory dormancy period (Imai et al., 1991), while *Heterosigma akashiwo* resting cells do not require a dormancy period before germinating (Han et al., 2002). Both species have a temperature range optimum for germination and viability (Imai and Itakura, 1999; Imai et al., 1991).

Gonyostomum cells contain numerous trichocysts that discharge mucilaginous threads upon mechanical disturbance or physical contact with other organisms (Sörensen, 1954). The slime threads are up to 500 μm long (Sörensen, 1954) and it has been suggested that a toxic substance may be excreted in connection with the expulsion of trichocysts, although this has not been investigated further (Cronberg et al., 1988). However, *Gonyostomum* trichocysts were shown to cause lysis of co-occurring *Rhodomonas* (Cryptophyecae) cells (Rengefors et al., 2008). Blooms adversely affect lakes used for recreation, as the mucilaginous strands are discharged upon contact with bathers, thereby covering them with a slimy layer, often causing itching and other skin reactions (Sörensen, 1954). The first report is from 1948, when a popular bathing site was closed down as a result of a *Gonyostomum* bloom. Swimmers did not return for several years, which had a great economic impact for the beach and café business (Sörensen, 1954). Since then, nuisance to bathers has been reported in newspapers and county management reports but they have not been verified by medical examination or scientific studies. Moreover, management reports from Norway and Sweden suggest that the mucilage from blooms of *Gonyostomum* may clog filters when lakes are used for drinking water supply, and that the blooms cause bad smell and taste of drinking water (e.g. Berge, 1991). Recent studies also suggest that *Gonyostomum* blooms create a bottleneck in the energy transfer to higher trophic levels since most *Gonyostomum* lakes lack the large grazers that are able to feed on it (Lebret et al., 2012). However, no such coupling was shown between *Gonyostomum* and fish and invertebrate biomass (Trigal et al., 2011). Because of its negative impact on recreation and because *Gonyostomum* blooms clog filters of drinking water plants, the Swedish Environmental Protection Agency has termed it as a noxious species.

G. semen allegedly has a world-wide distribution (Bourelly, 1985; Eloranta and Räike, 1995; Alves-de-Souza et al., 2006) although no recent biogeographic studies including molecular data are available to show that they are the same species. There is evidence of increasing distribution and abundance especially in northern Europe during recent decades (Hongve et al., 1987; Lepistö et al., 1994; Rakko et al., 2008). This increase was first noted in Scandinavia (Cronberg et al., 1988; Laugaste, 1992; Lepistö et al., 1994). Blooms of the species were subsequently reported in lakes in Eastern, Central and Western Europe (Pithart et al., 1997; Le Cohu et al., 1989; Korneva, 2000; Hehmann et al., 2001). In the majority of cases, *Gonyostomum* is encountered in humic lakes (Rosén, 1981; Willén, 1990; Hörnström, 1999), although mass developments have also been found in non-colored, oligotrophic lakes (Laugaste, 1992), large, deep reservoirs (Le Cohu et al., 1989; Negro et al., 2000), rivers (Korneva, 2000), and small eutrophic floodplain pools (Pithart et al., 1997). In Sweden it was originally encountered only in oligotrophic, humus-rich lakes, and was first recorded by Sörensen (1954) in 1948. Subsequently, it has been found in more and more nutrient-rich water bodies (Cronberg et al., 1988), with mass-developments mainly in southern Sweden (Cronberg, 2005). Today it is one of the most common flagellates in Swedish humic lakes.

The factors and vectors that have promoted the expansion of *Gonyostomum* in lakes still remain inconclusive and debated. There are a variety of suggestions why *Gonyostomum* has increased in occurrence, but a clear understanding of the mechanisms are still lacking. Among the suggested drivers for the observed increased distribution and abundance are eutrophication (Rosén, 1981; Hongve et al., 1987; Lepistö and Saura, 1998), acidification (Cronberg et al., 1988), and low grazing pressure (Lebret et al., 2012). However, acidification has largely been halted in Scandinavia while *Gonyostomum* has continued to increase. Furthermore, since eutrophication and acidification often coincide with temperature change, whose signal is much more consistent among lakes (Weyhenmeyer, 2008), we aimed towards examining whether temperature per se has an effect on the occurrence and abundance of *Gonyostomum*. An example of how temperature change can promote the size of the population of an invasive algal species is the freshwater cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya & Subba Raju, which originated from tropical regions and has by now spread to the temperate zone (Wiedner et al., 2007). We hypothesized that a temperature increase causes an increase in the *Gonyostomum* biomass and occurrence. To test this hypothesis we carried out a study including both analyses of monitoring data and laboratory studies to investigate the effect of temperature. First, we performed a spatial analysis to determine which physico-chemical factors were connected to the presence of *Gonyostomum* in lakes. To this end we analyzed a set of 205 lakes sampled once in the summer during five years. Secondly, to determine what factors were correlated to the increase of *Gonyostomum* we used complete time series of monthly physico-chemical and phytoplankton data over a period of 20 consecutive years from 1988 to 2007 of thirteen lakes across Sweden. Finally, in the laboratory, we tested the effect of temperature on some key features of the planktonic phase of the *Gonyostomum* life cycle, namely cyst germination and growth rate.

2. Methods

2.1. Study sites and sampling for temporal and spatial data

We examined physical, chemical and phytoplankton data of lakes that belong to the national lake inventory program of Sweden (http://www.slu.se/aquatic-sciences).

In a spatial analysis we investigated *Gonyostomum* abundance coupled to abiotic factors on a larger data set limited to a five-year period (1997–2002) to avoid effects of changes over time. Physico-chemical data and phytoplankton species and biomass data from 205 lakes, which were small (median lake area: 0.8 km^2, median mean lake depth: 4.3 m), oligotrophic (median total phosphorus concentrations: 8 $\mu g\, l^{-1}$, median total nitrogen concentrations: 300 $\mu g\, l^{-1}$) and mostly humic (median absorbance at 420 nm of 0.45 μm filtered water in a 5 cm cuvette: 0.06, median pH: 6.9) were sampled in summer time. We evaluated August values (in the few cases where August values were not available we used July values) since according to the Swedish environmental quality criteria (Willén, 2003) these values are considered best suitable for a comparison of phytoplankton biomass and species richness among Swedish lakes that are located in very different climatic regions. The following physico-chemical data were analyzed: pH, conductivity, total nitrogen, total phosphorus, and water color (absorbance at 420 nm in 5 cm cuvette). Sampling and chemical analyses were performed as reported below.

For the temporal analysis of factors coupled to *Gonyostomum* we used data from a program of frequent sampling (monthly during ice-free season from May to October; available from 1988 to 2007, resulting in 120 water samples for each lake). Both

phytoplankton and water chemistry were available for a total of thirteen lakes that were approximately equally distributed over Sweden (Fig. 1). Of the evaluated lakes the northernmost lake is located in the Arctic region, the southernmost in the boreo-nemoral zone. All thirteen lakes are relatively small (lake area range: 0.1–2.7 km^2), shallow (mean depth range: 2–11 m), and nutrient poor (Table 1). The lakes are regarded as reference lakes, i.e. lakes that, apart from atmospheric deposition and climate change, have little or no exposure to human activities in the catchment area.

The lakes were sampled monthly during the ice-free season (May–October). In each lake, surface water samples (0.5 m) for chemical analyses were collected by taking a mid lake water sample using a Plexiglas sampler. The samples were kept cool during transport to the laboratory. Water chemical variables were determined according to international (ISO) or European (EN) standards (Wilander et al., 2003). We examined the following variables from 1988 to 2007: surface water temperature (Temp), intensity of thermal stratification (Stratification; calculated as the difference between surface water temperature and bottom temperature), pH, conductivity (Cond), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), sulfate (SO_4), alkalinity (Alk), ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), total nitrogen (TN), total phosphorous (TP), secchi-depth (Secchi), absorbance at 420 nm of 0.45 μm filtered water in a 5 cm cuvette (Water color), total organic carbon (TOC) and reactive silica (Si). Phytoplankton samples were taken at the same location with a 2 m long tube sampler, giving a representative sample from the upper 0 to 2 m water layer. The samples were preserved with Lugol's solution. Counts were made using an inverted light microscope according to Utermöhl technique, following a standardized procedure agreed upon in EU countries (EN 15204, 2006). Biovolumes of individual cells were calculated from linear dimensions of measured cells applied to appropriate stereometric formulae according to Olrik (1989). Sampling and analyses of all water chemical and biological variables have been carried out by one and the same laboratory. More detailed information on analyses and lake characteristics are available on http://www.slu.se/aquatic-sciences.

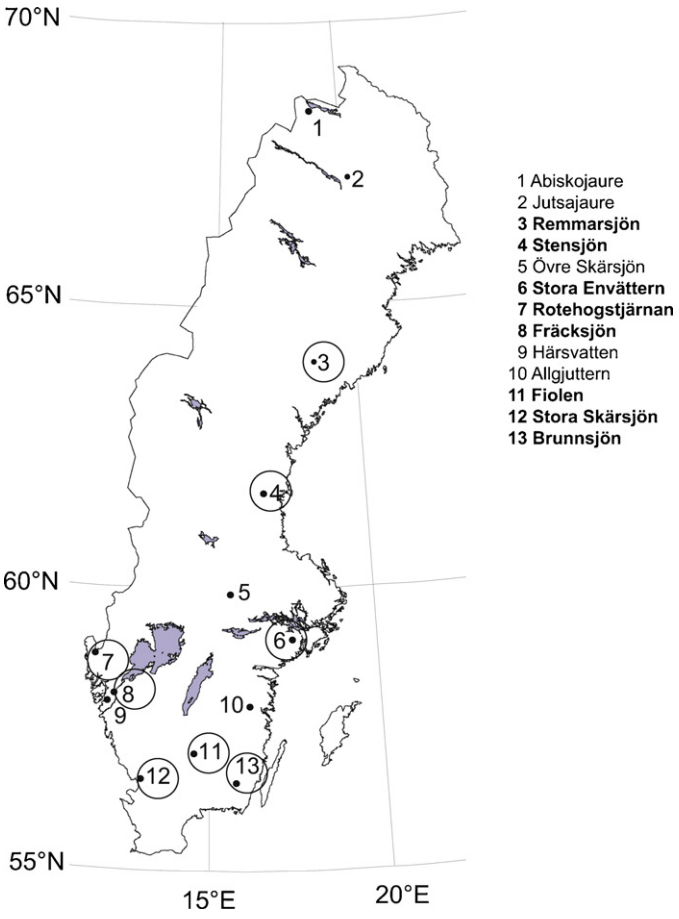

Fig. 1. Map of Sweden showing the locations of the 13 study lakes in the temporal data analysis. Lakes in which *Gonyostomum semen* occurred during 1988–2007 are in bold and circled on the map.

2.2. Laboratory experiment

The effect of temperature was tested on two important stages in the *Gonyostomum* life cycle; on growth rate during the vegetative planktonic stage and on germination of resting cysts (bloom initiation).

2.2.1. Temperature effect on growth rate

Two *Gonyostomum* strains (GSB02 and GSTV1), established from single cell isolates from the humic lakes Bökesjön in southern Sweden (55°34′N, 13°26′E) and Tvigölen (60°05′N–17°24′E) in central Sweden were used. These strains were isolated in 2004 and 2006 respectively, and were used for this study as they were known to grow well (see Rengefors et al., 2008), and because new isolation and growth of this *Gonyostomum* is slow and not always successful. Stock cultures were maintained in artificial freshwater Modified Woods Hole (MWC) medium (Guillard and Lorenzen, 1972) with selenium additions buffered at pH7 at a photon flux of 30 μmol m^{-2} s^{-1} and a 14:10 h light: dark cycle.

Temperature effects on growth rate were determined using culture tubes in an aluminum temperature gradient bar (TGB), which contained holes for the insertion of large test tubes arranged in rows of four between the two ends of the bar (Blankley and Lewin, 1976; Rengefors and Anderson, 1998). Heating one end and cooling the other resulted in a temperature range of 3–28 °C with intervals of 1 °C at lower temperatures and 2–3 °C at higher temperatures. Before starting the experiment the cultures were grown for 4 days at 10, 15, and 20 °C for temperature acclimatization. At the start of the experiment each tube (4 replicates for each temperature) was filled with 30 ml medium, which was inoculated to give an initial *Gonyostomum* concentration of 250 cells ml^{-1}. As a light source we used cool white fluorescent light bulbs and light levels were kept constant at 30 μmol photons m^{-2} s^{-1} and a light:dark cycle of 14:10 h, thus imitating daylength in southern Sweden in August. The growth rate was monitored every 5th day with in vivo fluorescence measurements. Fluorescence was converted to cell concentrations in order to be able to calculate growth rate. This was achieved by creating a standard curve of microscope counts (at least 400 cells counted at 100× magnification) versus in vivo fluorescence. The growth rate (μ; day^{-1}) was calculated as:

$$\mu = \frac{\ln B_{t1} - \ln B_{t0}}{t} \quad (1)$$

where B_{t1} represents the cell concentration (cells ml^{-1}) on the day of sampling and B_{t0} the cell concentration at the previous sampling occasion, separated by t days. The specific growth rates were compared between strains and among temperatures using repeated measures ANOVA in the JMP program, version 9.0.

2.2.2. Temperature effect on germination

Cysts were collected using a 20 μm plankton net from the water column in Lake Bökesjön in Southern Sweden at the end of the bloom (October) as the cells were encysting. Zooplankton were removed by sieving (150 μm), and the cell and cyst suspension

Table 1
Physical and-chemical characteristics of 13 studied Swedish lakes with mean values ± standard deviation; lake names are listed from north to south; for abbreviations see methods.

Lake name	pH	Cond (ms m^{-1})	Alk (mekv l^{-1}l)	TP (μg l^{-1})	TN (μg l^{-1})	Water color (420 nm 5 cm^{-1})	TOC (mg l^{-1})
Abiskojaure	7.1 ± 0.17	3.0 ± 0.7	0.17 ± 0.05	5.5 ± 3.0	217 ± 70	0.02 ± 0.01	1.8 ± 1.7
Jutsajaure	6.7 ± 0.16	1.8 ± 0.1	0.09 ± 0.01	9.5 ± 2.4	312 ± 78	0.09 ± 0.02	6.3 ± 2.6
Remmarsjön	6.3 ± 0.23	1.9 ± 0.2	0.05 ± 0.02	11.2 ± 4.2	334 ± 70	0.19 ± 0.04	9.7 ± 2.0
Stensjön	6.4 ± 0.27	1.8 ± 0.2	0.05 ± 0.01	7.9 ± 3.3	291 ± 66	0.11 ± 0.02	6.4 ± 0.8
Övre Skärsjön	5.7 ± 0.22	2.9 ± 0.4	0.004 ± 0.01	7.1 ± 2.7	373 ± 78	0.13 ± 0.04	7.2 ± 1.2
Stora Envättern	6.6± 0.21	4.1 ± 0.4	0.06 ± 0.01	9.4 ± 4.5	410 ± 94	0.07 ± 0.02	9.1 ± 1.9
Rotehogstjärnan	5.6 ± 0.33	5.1 ± 1.2	0.01 ± 0.01	15.1 ± 4.1	438 ± 93	0.24 ± 0.08	11.9 ± 2.6
Fräcksjön	6.5 ± 0.20	7.1 ± 1.3	0.07 ± 0.01	10.2 ± 3.1	454 ± 108	0.11 ± 0.03	8.9 ± 1.5
Härsvatten	4.7 ± 0.15	6.2 ± 1.4	-0.03 ± 0.02	4.7 ± 2.7	313 ± 81	0.01 ± 0.01	2.7 ± 2.3
Allgjuttern	6.7 ± 0.19	5.0 ± 0.5	0.07 ± 0.01	8.1 ± 5.8	360 ± 75	0.05 ± 0.02	7.2 ± 1.0
Fiolen	6.6 ± 0.16	5.7 ± 0.7	0.06 ± 0.01	12.6 ± 6.1	474 ± 116	0.05 ± 0.02	6.6 ± 1.9
Stora Skärsjön	6.9 ± 0.20	8.0 ± 0.7	0.12 ± 0.02	9.2 ± 3.7	366 ± 116	0.04 ± 0.02	4.4 ± 1.3
Brunnsjön	5.6 ± 0.20	6.6 ± 1.2	0.002 ± 0.01	13.1 ± 4.6	657 ± 154	0.40 ± 0.16	18.6 ± 5.5

was placed at 15 °C at 14:10 LD cycle, 10 μmol photons m^{-2} s^{-1} with filtered lake water. After one week, cells (mostly *Gonyostomum* cysts) were concentrated using a 20 μm mesh and stored in 3 mL cryovials with a hole in the lid. A 20 μm net was placed between vial and lid. The cryovials were submerged in lake sediment (from a lake lacking *Gonyostomum*) in tightly sealed plastic jars and stored at 4 °C.

The first step of the experiment was to determine the maturation or mandatory dormancy period of the cysts. Cysts were removed from storage monthly for seven months, starting at two weeks of age. At least 30 cells were isolated into separate wells of a 96-well Nunc plate (Nunclon, Denmark) containing a 200 μl mixture of 50% MWC medium and 50% sterile-filtered lake water. Plates were incubated at 15 °C at a 14:10 light:darkness cycle at 1 μmol photons m^{-2} s^{-1} (low light) or in complete darkness. Cysts were checked every two days for two weeks in the low light treatment, and weekly in the dark treatment. Empty cysts were denoted as a sign of germination (excystment) and are easily distinguished from dead cysts.

Mature cysts (as determined above) from the same batch of cysts were used to determine the effect of temperature on cyst germination. They cysts had at that point been stored for seven months. A temperature range was set up from 0 to 22 °C in the TGB as described above. Using 17 rows in the bar, 17 different temperatures with four replicates were established. Each replicate consisted of a tube filled with 15 ml consisting of 50:50 autoclaved MWC medium and sterile-filtered Bökesjön lake water. Tubes were stored overnight at 4 °C in the dark before proceeding. To each tube, a cells suspension of approximately 70 cysts was added and the tubes were dispensed randomly in the TGB. The day-light cycle was set to 14:10 and the light level to 1 μmol photons m^{-2} s^{-1}. After 14 days the suspension from each row was dispensed into 20 ml glass vials, rinsed with water to recover all cells, and preserved with 20 μl Lugol's solution. Vials were stored in the dark until counted in an inverted microscope (Nikon Eclipse TS100) using 25 ml glass settling chambers (manufactured in-house).

2.3. Statistical analyses

For spatial statistical analyses we divided our 205 lakes into two groups: one group comprising *Gonyostomum* lakes and another group with lakes without *Gonyostomum*. As a next step we tested whether water chemical characteristics of the two groups were significantly different with help of a non-parametric Wilcoxon-test. A non-parametric test was chosen due to our non-normally distributed data. p-Values < 0.05 were considered as statistically significant.

For temporal statistical analyses we first used a seasonal Mann–Kendall test to analyze the statistical significance of trends in physical, chemical and biological variables in each of our 13 lakes. The Mann–Kendall test is an approved tool to examine non-normal distributed data (Helsel and Hirsch, 1992). For one of the lakes, i.e. Rotehogstjärnan, we additionally used a partial least square regression model (PLS) for predicting temporal variations in the *Gonyostomum* biomass. PLS was chosen because of the method's insensitivity to the *X*-variable's interdependency and the insensitivity to deviations from normality (Eriksson et al., 1999). PLS is commonly used to find fundamental relations between two matrices (*X* and *Y*) where the variance in *X* is taken to explain the variance in *Y*. Here we used PLS with all available 19 physical and chemical variables as *X*. In PLS, *X*-variables are ranked according to their relevance in explaining *Y*, commonly expressed as VIP-values (Eriksson et al., 1999). The higher the VIP values are the higher is the contribution of an *X*-variable to the model performance. VIP-values exceeding 1 are considered as important *X*-variables. All statistical analyses were performed in JMP, version 9.0. A linear regression for cyst germination versus temperature was calculated in Kaleida Graph.

3. Results

3.1. Spatial distribution and physico-chemical characteristics of *Gonyostomum* lakes

In the analysis of 205 lakes distributed all across Sweden we found *Gonyostomum* in 27% of the lakes (55 lakes). The *Gonyostomum* lakes were located at significantly lower altitudes and latitudes than the non-*Gonyostomum*- lakes (non-parametric Wilcoxon-test: $p < 0.0001$). Moreover, they showed significantly lower pH, higher conductivity, higher total nitrogen, higher total phosphorus, and higher water color (non-parametric Wilcoxon-test: $p < 0.0001$) (Table 2).

3.2. Temporal variation of *Gonyostomum*

At the beginning of the time series in 1988 we found *Gonyostomum* in less than 10% of the 78 phytoplankton samples that were taken in the 13 lakes during a year. At the end of the time series, in 2007, *Gonyostomum* occurred in more than 20% of the 78 phytoplankton samples (Fig. 2A). The increase in the occurrence of *Gonyostomum* in Swedish lakes was highly significant (Mann–Kendall: $p < 0.001$) and was a result of *Gonyostomum* spreading to more lakes (Fig. 2B) as well as a more frequent occurrence within one lake (Rotehogstjärnan). Currently, *Gonyostomum* is most often present in May, September, and October samples. *Gonyostomum* was never observed in samples when water temperature was below 6 °C.

Table 2
Analyses of 205 lakes. 10, 50 and 90 percentiles of altitude (Alt; in meter above sea level), pH, conductivity (Cond; ms m^{-1}), total nitrogen (TN; $\mu g\,l^{-1}$), total phosphorus (TP; $\mu g\,l^{-1}$) and water color (Color; 420 nm, 5 cm^{-1}) in lakes with (*Gyes*) and without (*Gno*) *Gonyostomum*.

	Alt			pH			Cond			TN			TP			Color		
	10	50	90	10	50	90	10	50	90	10	50	90	10	50	90	10	50	90
Gyes	49	140	250	5.6	6.7	7.2	2.5	4.8	9.8	224	443	646	8	12	28	0.04	0.12	0.28
Gno	74	457	1089	5.8	7.0	7.7	0.9	2.6	9.9	53	250	655	4	6	20	0.002	0.03	0.10

We found one lake, Rotehogstjärnan in southwestern Sweden, in which *Gonyostomum* occurred in almost all samples. Out of the eight lakes where *Gonyostomum* was observed at least once during 1988–2007, Rotehogstjärnan showed the highest TP concentrations, the lowest pH, the strongest thermal stratification and the second highest water color. Rotehogstjärnan was also the lake with the highest *Gonyostomum* biomass with values of over 4 $mm^3\,l^{-1}$ representing up to 95% of the total phytoplankton biovolume. In Fiolen and St. Skärsjön *Gonyostomum* occurred for the first time in 2002 with values of up to 1.3 $mm^3\,l^{-1}$, representing 85% of the total phytoplankton biovolume. In Fräcksjön *Gonyostomum* appeared in the samples for the first time in 1997. Here it comprised 18% of the total biomass with similar amounts in the two following years and a slight decrease until 2007. In the other lakes *Gonyostomum* was detected in very low concentrations, but was nevertheless recorded at a few occasions even in the northern lakes. In the lakes Abiskojaure, Jutsajaure, Övre Skärsjön, Härsvatten and Allgjuttern *Gonyostomum* was not detected during the time period 1988–2007.

Analysis of the development of the *Gonyostomum* biomass from 1988 to 2007 showed significant trends over time for *Gonyostomum* biomass in Fräcksjön and St Skärsjön (Mann–Kendall: $p < 0.05$) and increasing biomass in the other lakes. Differentiation into months revealed that the largest increase of *Gonyostomum* biomass was in October (seasonal Mann–Kendall, $p < 0.01$). Such an increase could neither be explained by TP concentrations as these generally decreased in the study lakes nor by Si concentrations as they did not show significant changes over time. NO_3-N concentrations decreased significantly over time in our *Gonyostomum* lakes but these decreases were rarely observed in October but rather during summertime. Variables that changed most significantly over time in the *Gonyostomum* lakes, especially in October, were surface water temperatures and the intensity of stratification (seasonal Mann–Kendall, $p < 0.01$, Fig. 2C).

Using a PLS model with 19 different physical and chemical input variables to predict temporal variations in *Gonyostomum* biomass in the lake that had *Gonyostomum* present throughout the growing season, i.e. Rotehogstjärnan, confirmed that water temperature was one of the most important variables driving *Gonyostomum* biomass temporal variations (Table 3). However, other factors also contributed to explaining temporal variations, including water color, TP, pH, and Si.

3.3. Temperature effect on Gonyostomum germination

The dormancy experiment showed that *Gonyostomum* cysts were able to germinate after approximately 11 weeks in both weak light (1 μmol photons $m^{-2}\,s^{-1}$) and complete darkness (Fig. 3A). The maximum excystment occurred after 24 weeks (in May), when 80% of the cysts germinated. At the time of maximum excystment, *Gonyostomum* cysts germinated at all temperatures tested (0–22 °C), and the proportion of germinated cysts was positively correlated ($R^2 = 0.87$) with temperature within the two-week test period (Fig. 3B).

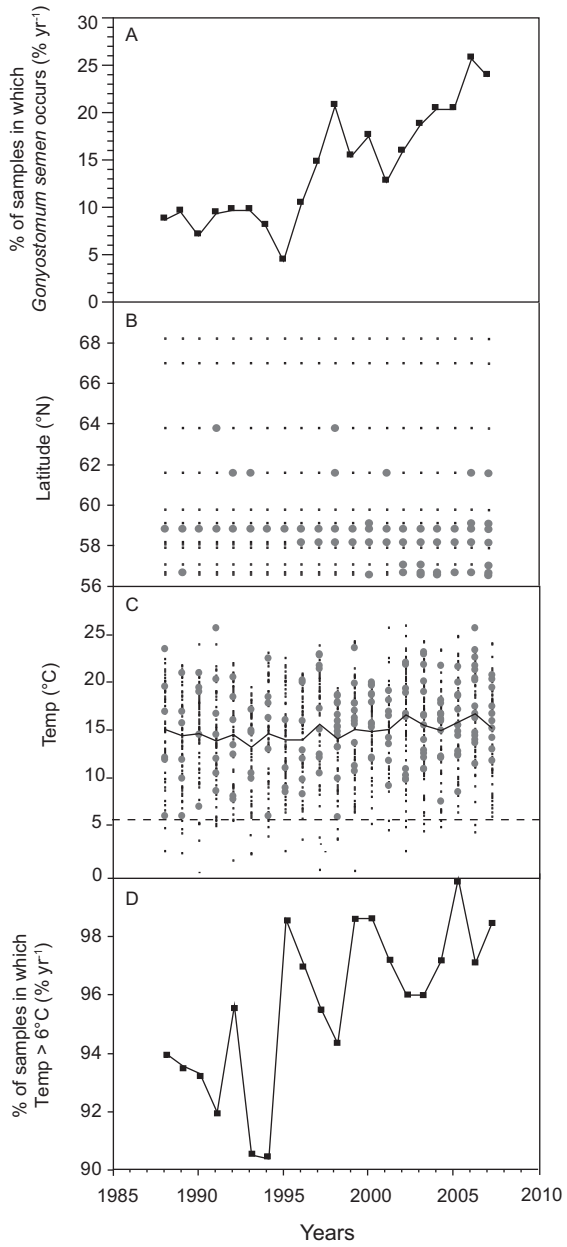

Fig. 2. *Gonyostomum semen* occurrence and surface water temperatures in October from 1988 to 2007. Panel (A) shows the percentage of samples in which *Gonyostomum* occurred in relation to the total number of samples taken in 13 lakes distributed all over Sweden on a monthly interval in the growing season from May to October during 1988–2007. Panel (B) shows the latitude and year when *Gonyostomum* was detected in a water sample (large gray dots; the small black dots are water samples without *Gonyostomum*). Panel (C) Occurrence of *Gonyostomum* (gray dots) at all measured water temperatures from 1988 to 2008. The black line represents yearly mean water temperatures over Sweden, the dashed black line represents the threshold surface water temperature at which *Gonyostomum* no longer occurs in the lakes. Panel (D) Number of water samples in which surface water temperatures exceed 6 °C.

Table 3
VIP values of a PLS model for the prediction of temporal variations in the *Gonyostomum* biomass in Rotehogstjärnan. VIP values above 1 (marked in bold) are considered as important for the model performance. For abbreviations see methods. The PLS model explained 43% of the temporal variations in *Gonyostomum* biomass.

X-variable	VIP value
Temp	**1.85**
Stratification	**1.14**
Secchi	**1.53**
Alk	**1.01**
pH	**1.45**
SO_4	0.72
Cond	0.85
Ca	0.82
Mg	0.93
Na	**1.06**
K	0.50
Cl	0.94
NH_4-N	0.95
NO_3-N	**1.03**
TN	0.42
TP	**1.61**
TOC	0.87
Water color	**1.14**
Si	**1.71**

3.4. Temperature effect on Gonyostomum growth

Testing a direct temperature effect on *Gonyostomum* under controlled conditions in the laboratory showed that both *Gonyostomum* strains started growing at temperatures above 6 °C and reached highest cell densities (2146 ± 138 cells ml^{-1} for GSBO2 and 2573 ± 183 cells ml^{-1} for GSTV1) after 25 days. Using repeated measures ANOVA we found significant differences in the temperature response of the specific growth rates between the two strains ($p < 0.0001$), where GSTV1 (Northern strain) reached its growth optimum at a lower temperature than strain GSB02 (Southern strain) (maximum specific growth rate of 0.13 at 9 °C for GSTV1 and of 0.11 at 12 °C for GSB02). A sharp decrease in the growth of *Gonyostomum* was notable below 9 °C with no positive growth at temperatures below 6 °C. Above 19 °C the growth rate decreased rapidly with increasing temperature (Fig. 4).

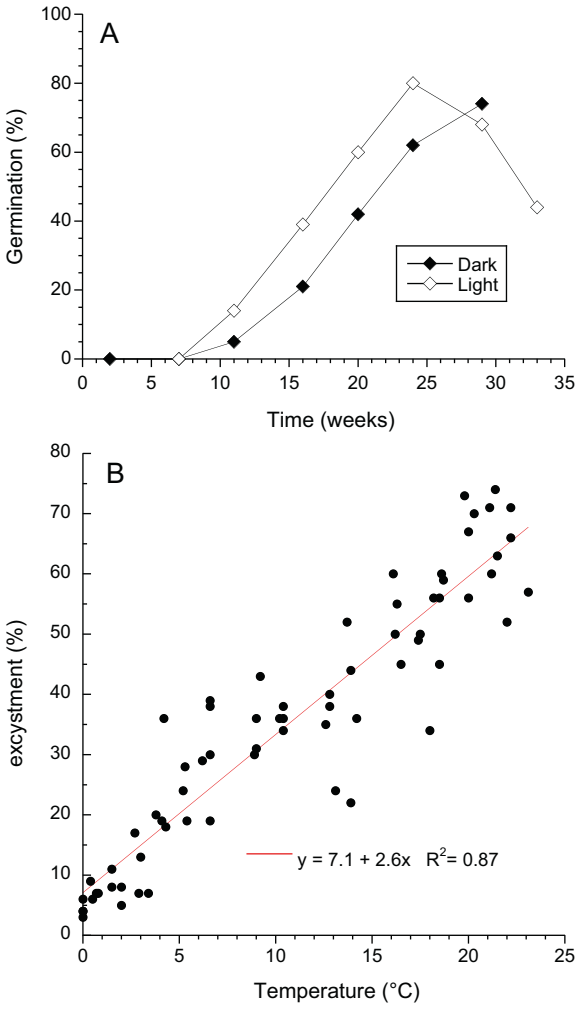

Fig. 3. (A) Dormancy period and germination percentage of *Gonyostomum semen* cysts in the dark (filled diamonds) and in low light (open diamonds) at 15 °C. (B) Percent germination of total number of cysts after two weeks exposure to low light and different temperatures during the time period of maximum germination (May).

4. Discussion

Our results provide evidence that the nuisance alga *G. semen*, has significantly increased in its occurrence and abundance in Swedish lakes since 1988. Indeed, it was present in more than a quarter of the lakes included in the Swedish national monitoring program during 1997–2002. We found that *Gonyostomum* spread to more lakes and increased in occurrence and biomass within lakes during the two decades analyzed. Our analyses identified increased surface water temperatures as a potential driver of this expansion. We suggest that this effect is coupled to an extended growth period, which is indicated by their growth and cyst germination temperature windows.

Determining the factors that explain distribution and expansion of phytoplanktonic species is very challenging. Most species tolerate a range of temperature, pH, and nutrient levels, and although factors that regulate biomass are well-known, predictions are difficult. To untangle the factors that were favorable for *Gonyostomum* growth from those causing its expansion, we performed both a spatial and temporal study. Like previous studies (Cronberg et al., 1988; Lepistö et al., 1994) on *Gonyostomum*'s expansion during the 1970s and 1980s, we identified high water color, moderate nutrient levels, and low pH as significant characteristics of *Gonyostomum* lakes. These results simply show where *Gonyostomum* is found most often, but do not explain its expansion.

In contrast, our temporal analyses based on monthly samples in 13 lakes during 20 years showed that the variables that best explained the observed increase in *Gonyostomum* biomass and expansion to new lakes were increases in surface water temperature and the intensity of thermal stratification. First of all, the seasonal Mann–Kendall test showed a significant trend of increasing *Gonyostomum* occurrence along with a significant increase in temperature. Second, no samples with a water temperature below 6 °C contained *Gonyostomum*. The PLS analysis of a single lake (Rotehogstjärnan) showed that temperature was an important factor, and last, but not least, the laboratory studies showed that positive growth was not observed below 6 °C. Together, these results provide evidence that changes in temperature may in part be responsible for the expansion of *Gonyostomum*.

Nevertheless, temperature alone cannot explain *Gonyostomum*'s expansion, as that cannot explain expansion to new lakes within the same climatic region. Other factors that have earlier been suggested to influence the growth of *Gonyostomum* are high TP concentrations (>30 μg l^{-1}; Cronberg et al., 1988; Findlay et al., 2005) as well as high DOC concentrations (>10 mg DOC l^{-1}; Rengefors et al., 2008). However, TP concentrations exceeding 30 μg l^{-1} were not detectable in the data set of 13 lakes (Table 1).

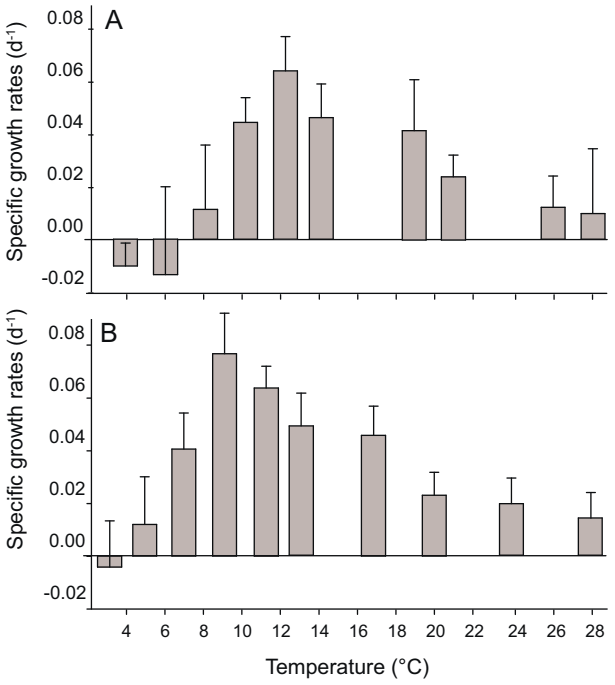

Fig. 4. Specific growth rates of *Gonyostomum semen* for the strains GSB02 from southern Sweden (A) and GSTV1 from central Sweden (B). Shown are mean values and standard deviations of four replicates at five sampling days that were grown at each temperature for 25 days.

Both water color (a proxy for DOC) and TP emerged as significant in the PLS analysis (Table 2) and the spatial study indicating that these are also important factors coupled to *Gonyostomum* occurrence. Moreover, Rengefors et al. (2008) showed that *Gonyostomum* grew better in the laboratory when provided with medium levels of humic acids. Considering that DOC concentrations are increasing over large regions in the Northern Hemisphere (Monteith et al., 2007) including Sweden (Weyhenmeyer and Karlsson, 2009), more lakes might reach DOC levels that are favorable to the growth of *Gonyostomum*.

Temperature is an important determinant of seasonal changes in phytoplankton abundance, especially for those that have overwintering resting stages (Anderson and Rengefors, 2006) such as *Gonyostomum*. A restricted temperature "window" often determines the temperature range within which a species germinates (Anderson and Rengefors, 2006), even though a wider window sets the limits for growth. *Gonyostomum* is a typical example of a species that switches between a planktonic vegetative phase and an overwintering resting stage (Figueroa and Rengefors, 2006). The resting cyst is considered as an adaptation to survive during unfavorable environmental conditions (Fryxell, 1983), including suboptimal abiotic growth conditions (e.g. Anderson et al., 1983), as well as predators and parasites (Rengefors et al., 1998; Hansson, 1996; Toth et al., 2004). Our germination studies show that *Gonyostomum* cysts can germinate at all temperatures above zero after a mandatory dormancy period, but that the germination rate increased at higher temperatures. The data also show a phenotypic variation among cysts, in that only a small proportion of the cysts germinate at low temperatures (20% germinate below 6 °C). This means that an earlier warming of the water in the spring could increase the length of the *Gonyostomum* growth season. However, we observed maximum germination (in percent) in May, after seven months storage in the cold and dark, indicating the presence of an endogenous clock partly regulating germination. The month of May corresponds to when cells are usually first observed in the field in Southern Sweden (Lebret et al., in press) and is one of the months with most frequent occurrence of *Gonyostomum* in the monitoring data.

The temperature growth experiments clearly indicate that growth does not occur below 6 °C, and that optimum temperatures for the growth of *Gonyostomum* lie between 9 and 12 °C (Fig. 4), at least in the strains tested. Since surface water temperatures during summer frequently exceed 10 °C, most pronounced changes in the *Gonyostomum* biomass in a warmer climate are expected to occur during spring and autumn. This expectation was fulfilled by our results showing most prominent changes in the *Gonyostomum* biomass in October. Growth was still positive at temperatures above 12 °C in the laboratory, indicating that *Gonyostomum* grows at higher water temperatures as well. Moreover, field studies show that *Gonyostomum* biomass increases rapidly during the summer (Lebret et al., in press).

In addition to increasing surface water temperatures we also found that increasing thermal stratification was relevant for the increases in *Gonyostomum* biomass. Strong thermal stratification reduces water column mixing and results in depletion of nutrients in the epilimnion where autotrophic organisms have their habitat. *Gonyostomum* will likely be favored by such conditions since it has the capacity to migrate vertically, resulting in a maximization of nutrient uptake in the hypolimnion and photosynthesis in the epilimnion (Eloranta and Räike, 1995; Salonen and Rosenberg, 2000).

The laboratory experiments suggest that *Gonyostomum* populations may be locally adapted to the climatic region from which it was isolated. We observed that GSTV1, the strain from a colder geographical region, showed its growth optimum at lower temperatures than GSB02, the strain from a warmer geographical region (Fig. 4). These results suggest that the growth optima of *Gonyostomum* might shift towards higher temperatures in a warmer climate due to local adaptation. Nevertheless, this conclusion is premature and must be treated with caution as phytoplankton strains are variable (Berge, 2011) and only one replicate strain was available from each location. More research is needed on the phenotypic variation among strains.

From our results we conclude that the increase in water temperature resulting in longer growth season may be a driver of the expansion of *Gonyostomum*. However, temperature alone cannot explain why the species has expanded to new lakes within the same climatic region. Possibly an interplay between DOC and temperature can explain the patterns observed. The scenario may be as follows: higher temperature resulting in a longer growth season results in more samples detected with *Gonyostomum* cells. Because the growth season is longer, the population can grow for longer resulting in higher biomass. At the end of the season, more cysts will be produced which function as dispersal propagules. In the meantime, a general increase in DOC levels have made more lakes favorable habitats for *Gonyostomum* to colonize. To date, *Gonyostomum* has been found as far north as 64°N where its mass development might still be restricted by low temperatures, but considering climate change scenarios (IPCC, 2007) it is expected that more and more lakes will experience noxious *Gonyostomum* blooms.

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References

Alves-de-Souza, C., Menezes, M., Huszar, V., 2006. Phytoplankton composition and functional groups in a tropical humic coastal lagoon, Brazil. Scielo 701–708.

Anderson, D.M., Chisholm, S.W., Watras, C.J., 1983. Importance of life cycle events in the population dynamics of *Gonyaulax tamarensis*. Marine Biology 76, 179–189.

Anderson, D.M., Rengefors, K., 2006. Community assembly and seasonal succession of marine dinoflagellates in a temperate estuary: the importance of life cycle events. Limnology and Oceanography 51 (2), 860–873.

Balirwa, J.S., Chapman, C.A., Chapman, L.J., Cowx, I.G., Geheb, K., Kaufman, L., Lowe-McConnell, R.H., Seehausen, O., Wanink, J.H., Welcomme, R.L., Witte, F., 2003. Biodiversity and fishery sustainability in the Lake Victoria Basin: an unexpected marriage? Bioscience 53 (8), 703–715.

Berge, D., 1991. Forurensningsutviklingen i drikkevannskilden Oppegårdstjernet i Frogn kommune—Årsakssammenheng. Norsk institutt for vannforskning. Report nr 2533-1991.

Berge, T., 2011. Functional Diversity of Marine Protists: Evidence from Culture Experiments. Department of Biology, University of Copenhagen, Copenhagen, 113 pp.

Blankley, F.W., Lewin, R.A., 1976. Temperature responses of a coccolithophorid, *Cricosphaera carterae*, measured in a simple and inexpensive thermal-gradient device. Limnology and Oceanography 21, 457–461.

Bourelly, P., 1985. Les algues d'eaux douce. Tom III: Les algues bleues et rouges. Les Euglèniens, Peridiniens et Cryptomonadines. N.Boubée et Cie, Paris.

Cronberg, G., 2005. The life cycle of *Gonyostomum semen* (Raphidophyceae). Phycologia 44 (3), 285–293.

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

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wold, S., 1999. Multi- and Megavariate Data Analysis using Projection Methods (PCA&PLS). Umetrics, Umeå, Sweden.

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

Figueroa, R.I., Rengefors, K., 2006. Life cycle and sexuality of the freshwater raphidophyte *Gonyostomum semen* (Raphidophyceae). Journal of Phycology 42, 859–871.

Findlay, D.L., Paterson, M., Hendzel, L.L., Kling, H., 2005. Factors influencing *Gonyostomum semen* blooms in a small boreal reservoir lake. Hydrobiologia 533, 243–252.

Fryxell, G.A., 1983. Survival Strategies of the Algae. Cambridge University Press, New York.

Goldschmidt, T., Witte, F., Wanink, J., 1993. Cascading effects of the introduced Nile perch on the detritivorous phytoplanktivorous species in the sublittoral areas of Lake Victoria. Conservation Biology 7 (3), 686–700.

Guillard, R.R.L., Lorenzen, C.J., 1972. Yellow-green algae with chlorophyllide c. Journal of Phycology 8, 10–24.

Han, M.-S., Kim, Y.-P., Cattolico, R.A., 2002. Heterosigma akashiwo (Raphidophyceae) resting cell formation in batch culture: strain identity versus physiological response. Journal of Phycology 38, 304–317.

Hansson, L.A., 1996. Algal recruitment from lake sediments in relation to grazing, sinking, and dominance patterns in the phytoplankton community. Limnology and Oceanography 41 (6), 1312–1323.

Hehmann, A., Krienitz, L., Koschel, R., 2001. Long-term phytoplankton changes in an artificially divided, top-down manipulated humic lake. Hydrobiologia 448 (1–3), 83–96.

Helsel, D.R., Hirsch, R.M., 1992. Statistical Methods in Water Resources. Elsevier, 522 p.

Hongve, D., Lovstad, O.E., Bjorndalen, K., 1987. *Gonyostomum semen*—a new nuisance to bathers in Norwegian lakes. Verhandlungen des Internationalen Verein Limnologie 23, 430–434.

Hörnström, E., 1999. Long-term phytoplankton changes in acid and limed lakes in SW Sweden. Hydrobiologia 394 (0), 93–102.

Imai, I., Itakura, S., 1999. Importance of cysts in the population dynamics of the red tide flagellate *Heterosigma akashiwo* (Raphidophyceae). Marine Biology 133, 755–762.

Imai, I., Itakura, S., Itoh, K., 1991. Life cycle strategies of the red tide causing flagellates *Chattonella* (Raphidophyceae) in the Seto Inland Sea. Marine Pollution Bulletin 23, 165–170.

IPCC, 2007. Summary for policymakers. In: Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E. (Eds.), Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Korneva, L.G., 2000. Ecological aspects of the mass development of *Gonyostomum semen* (Ehr), Dies. (Raphidophyta). Algologia 10, 265–277.

Laugaste, R., 1992. An alga of genus *Gonyostomum*. Eesti Loodus 12, 610–612.

Le Cohu, R., Guitard, J., Comoy, N., Brabet, J., 1989. *Gonyostomum semen*, a potential nuisance in large French reservoirs—the case of the Pareloup Lake. Archiv Fur Hydrobiologie 117 (2), 225–236.

Lebret, K., Fernandez, M., Corneliussen Hagman, C.H., 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.

Lebret, K., Kritzberg, E., Figueroa, R.I., Rengefors, K. Genetic diversity within and genetic differentiation between blooms of a microalgal species. Environmental Microbiology, in press.

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

Lepistö, L., Saura, M., 1998. Effects of forest fertilization on phytoplankton in a boreal brown-water lake. Boreal Environmental Research 3, 33–43.

Lilly, E.L., Halanych, K.M., Anderson, D.M., 2005. Phylogeny, biogeography, and species boundaries within the *Alexandrium minutum* group. Harmful Algae 6, 1004–1020.

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), 537–539.

Neilan, B.A., Saker, M.L., Fastner, J., Törökné, A., Burns, B.P., 2003. Phylogeography of the invasive cyanobacterium *Cylindrospermopsis raciborskii*. Molecular Ecology 12, 133–140.

Negro, A.I., De Hoyos, C., Vega, J.C., 2000. Phytoplankton structure and dynamics in Lake Sanabria and Valparaiso reservoir (NW Spain). Hydrobiologia 424, 25–37.

Olrik, K., 1989. Methods for quantitative assessment of phytoplankton in freshwaters. Part I. Report 4860, Swedish Environmental Protection Agency.

Pithart, D., Pechar, L., Mattsson, G., 1997. Summer blooms of raphidophyte *Gonyostomum semen* and its diurnal vertical migration in a floodplain pool. Archiv Fur Hydrobiologie Supplement Algological Studies 85, 119–133.

Rakko, A., Laugaste, R., Ott, I., 2008. Algal blooms in Estonian small lakes. In: Evangelista, V., Barsanti, L., Frassanito, A.M., Passarelli, V., Gualtieri, P. (Eds.), Algal Toxins: Nature, Occurrence, Effect and Detection. Springer, The Netherlands.

Rengefors, K., Anderson, D.M., 1998. Environmental and endogenous regulation of cyst germination in relation to seasonal succession of two fresh-water dinoflagellates. Journal of Phycology 34 (4), 568–577.

Rengefors, K., Karlsson, I., Hansson, L.A., 1998. Algal cyst dormancy: a temporal escape from herbivory. Proceedings of the Royal Society of London Series B: Biological Sciences 265 (1403), 1353–1358.

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.

Ricciardi, A., 2003. Predicting the impacts of an introduced species from its invasion history: an empirical approach applied to zebra mussel invasions. Freshwater Biology 48 (6), 972–981.

Richardson, D.M., Pysek, P., 2008. Fifty years of invasion ecology—the legacy of Charles Elton. Diversity and Distributions 14 (2), 161–168.

Rosén, G., 1981. Phytoplankton indicators and their relations to certain chemical and physical factors. Limnologica (Berlin) 13 (2), 263–290.

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. Journal of Plankton Research 22 (10), 1841–1853.

Schloesser, D.W., Nalepa, T.F., 1994. Dramatic decline of unionid bivalves in offshore waters of western Lake Erie after infestation by the Zebra Mussel, *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences 51 (10), 2234–2242.

Simon, K.S., Townsend, C.R., 2003. Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. Freshwater Biology 48 (6), 982–994.

Stam, W.T., Olsen, J.L., Zaleski, S.F., Murray, S.N., Brown, K.R., Walters, L.J., 2006. A forensic and phylogenetic survey of *Caulerpa* species (Caulerpales, Chlorophyta) from the Florida coast, local aquarium shops, and e-commerce: establishing a proactive baseline for early detection. Journal of Phycology 6, 1113–1124.

Strayer, D.L., Caraco, N.F., Cole, J.J., Findlay, S., Pace, M.L., 1999. Transformation of freshwater ecosystems by bivalves—a case study of zebra mussels in the Hudson River. Bioscience 49 (1), 19–27.

Sörensen, I., 1954. *Gonyostomum semen* (Ehrenb.) Diesing—en vattenorganism av teoretiskt och praktiskt intresse. Svensk Faunistisk Revy 2, 47–52.

Toth, G.B., Norén, F., Selander, E., Pavia, H., 2004. Marine dinoflagellates show induced life-history shifts to escape parasite infection in response to water-borne signals. Proceedings of the Royal Society of London Series B: Biological Sciences 271, 733–738.

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.

Valery, L., Fritz, H., Lefeuvre, J.C., Simberloff, D., 2008. In search of a real definition of the biological invasion phenomenon itself. Biological Invasions 10 (8), 1345–1351.

Weyhenmeyer, G.A., 2008. Water chemical changes along a latitudinal gradient in relation to climate and atmospheric deposition. Climatic Change 88 (2), 199–208.

Weyhenmeyer, G.A., Karlsson, J., 2009. Nonlinear response of dissolved organic carbon concentrations in boreal lakes to increasing temperatures. Limnology and Oceanography 54 (6), 2513–2519.
Wiedner, C., Rucker, J., Bruggemann, R., Nixdorf, B., 2007. Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia 152 (3), 473–484.
Wilander, A., Johnson, R., Goedkopp, W., 2003. National lake and stream survey 2000—a synoptic study of water chemistry and benthic fauna. Department of Environmental Assessment, Swedish University of Agricultural Sciences, p. 1.
Willén, E., 1990. Summer phytoplankton in 73 nutrient-poor Swedish lakes. Classification, ordination and choice of long-term monitoring objects. Limnologica 20, 217–227.
Willén, E., 2003. Dominance patterns of planktonic algae in Swedish forest lakes. Hydrobiologia 502 (1-3), 315–324.
Witte, F., Goldschmidt, T., Wanink, J., Vanoijen, M., Goudswaard, K., Wittemaas, E., Bouton, N., 1992. The destruction of an endemic species flock—quantitative data on the decline of the haplochromine cichlids of Lake Victoria. Environmental Biology of Fishes 34 (1), 1–28.