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

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**A B S T R A C T**

*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 have 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.

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1. Introduction

The threat of biological invaders to ecosystems has been the focus of much research in recent decades (Richardson and Pyse, 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; Baliwra 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 freshwater, 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 (Bourrelly, 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

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limitation (Figuerola 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 (Figuerola 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 Ikakura, 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 (Cryptophyceae) 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; Rakkö 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; Hennmann 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) Seenayya & 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/aguatic-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 km², median mean lake depth: 4.3 m), oligotrophic (median total phosphorus concentrations: 8 μg L⁻¹, median total nitrogen concentrations: 300 μg 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 km²), 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 (SO₄), alkalinity (Alk), ammonium-nitrogen (NH₄-N), nitrate-nitrogen (NO₃-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 Oličk (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.

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:

\[ \mu = \frac{\ln B_t - \ln B_0}{t} \]

(1)

where \( B_t \) represents the cell concentration (cells ml⁻¹) on the day of sampling and \( B_0 \) 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
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. Rotehögstjärnan, 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 KaleidaGraph.

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: \( p < 0.0001 \)) and was a result of Gonyostomum spreading to more lakes (Fig. 2B) as well as a more frequent occurrence within one lake (Rotehögstjärnan). 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.

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. Rotehögstjärnan, 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 KaleidaGraph.

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<table>
<thead>
<tr>
<th>Lake name</th>
<th>pH</th>
<th>Cond (ms m⁻¹)</th>
<th>Alk (meq l⁻¹)</th>
<th>TP (µg l⁻¹)</th>
<th>TN (µg l⁻¹)</th>
<th>Water color (420 nm 5 cm⁻¹)</th>
<th>TOC (mg l⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Abiskojaure</td>
<td>7.1 ± 0.17</td>
<td>3.0 ± 0.7</td>
<td>0.17 ± 0.05</td>
<td>5.5 ± 3.0</td>
<td>217 ± 70</td>
<td>0.02 ± 0.01</td>
<td>1.8 ± 1.7</td>
</tr>
<tr>
<td>Jutsajaure</td>
<td>6.7 ± 0.16</td>
<td>1.8 ± 0.1</td>
<td>0.09 ± 0.01</td>
<td>9.5 ± 2.4</td>
<td>312 ± 78</td>
<td>0.09 ± 0.02</td>
<td>6.3 ± 2.6</td>
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<tr>
<td>Remmarnsjön</td>
<td>6.3 ± 0.23</td>
<td>1.9 ± 0.2</td>
<td>0.05 ± 0.02</td>
<td>11.2 ± 4.2</td>
<td>334 ± 70</td>
<td>0.19 ± 0.04</td>
<td>9.7 ± 2.0</td>
</tr>
<tr>
<td>Stenåsjön</td>
<td>6.4 ± 0.27</td>
<td>1.8 ± 0.2</td>
<td>0.05 ± 0.01</td>
<td>7.9 ± 3.3</td>
<td>291 ± 66</td>
<td>0.11 ± 0.02</td>
<td>6.4 ± 0.8</td>
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<tr>
<td>Övre Skärsjö</td>
<td>5.7 ± 0.22</td>
<td>2.9 ± 0.4</td>
<td>0.004 ± 0.001</td>
<td>7.1 ± 2.7</td>
<td>373 ± 78</td>
<td>0.13 ± 0.04</td>
<td>7.2 ± 1.2</td>
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<td>Stora Enåvätten</td>
<td>6.6 ± 0.21</td>
<td>4.1 ± 0.4</td>
<td>0.06 ± 0.01</td>
<td>9.4 ± 4.5</td>
<td>410 ± 94</td>
<td>0.07 ± 0.02</td>
<td>9.1 ± 1.9</td>
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<tr>
<td>Rotehögstjärnan</td>
<td>5.6 ± 0.33</td>
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<td>0.01 ± 0.01</td>
<td>15.1 ± 4.1</td>
<td>438 ± 93</td>
<td>0.24 ± 0.08</td>
<td>11.9 ± 2.6</td>
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<tr>
<td>Fräcksjön</td>
<td>6.5 ± 0.20</td>
<td>7.1 ± 1.3</td>
<td>0.07 ± 0.01</td>
<td>10.2 ± 3.1</td>
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<td>-0.03 ± 0.02</td>
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<td>313 ± 81</td>
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<tr>
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<td>366 ± 116</td>
<td>0.04 ± 0.02</td>
<td>4.4 ± 1.3</td>
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<tr>
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<td>13.1 ± 4.6</td>
<td>657 ± 154</td>
<td>0.40 ± 0.16</td>
<td>18.6 ± 5.5</td>
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</table>
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 biomass. 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 biomass. 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 Allgjütern *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).

<table>
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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; μg l$^{-1}$), total phosphorus (TP; μg l$^{-1}$) and water color (Color; 420 nm, 5 cm$^{-1}$) in lakes with *Gyes* and without *(Gno)* *Gonyostomum*.

![Fig. 2](image-url)
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 GSBO2 (Southern strain) (maximum specific growth rate of 0.13 at 9 °C for GSTV1 and of 0.11 at 12 °C for GSBO2). 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).

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).
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, Rengenfors 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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