Master's thesis

Factors affecting the distribution and abundance of the flagellated alga *Gonyostomum semen* (Ehrenberg) Diesing in Finland

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ABSTRACT

The flagellated alga *Gonyostomum semen* has become a widespread and abundant species in the phytoplankton communities of lakes in Scandinavia. The alga occurs particularly in humic lakes, and can form dense blooms that can cause problems for water treatment plants and can be a nuisance to swimmers. Small lake area, high water colour, slight acidity and behavioural characteristics of the alga have all been suggested as possible explanations for its increasing distribution and abundance. This thesis evaluates the possible role of various environmental factors in the abundance and distribution of *G. semen* in Finland. Physicochemical variables from typical lakes where *G. semen* was present in epilimnetic samples are evaluated, as are the surface water type classes of these lakes. Correlation analysis between the physicochemical factors and the density of *G. semen*, and logistic regression analysis between the physicochemical and morphological factors of very low abundance *G. semen* lakes and bloom-forming *G. semen* lakes were made. The phytoplankton composition during *G. semen* blooms mainly consisted of flagellates. The alga thrives in high total phosphorus concentration, small, shallow, dark-water humic lakes in Finland. Maybe the most important single environmental change causing abundance of *G. semen* in Finland is the darkening of lake water colour.

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TIIVISTELMÄ

Limalevä *Gonyostomum semen* (Ehrenberg) Diesing on nykyisin laajalle levinnyt ja runsas siimalevä Pohjoismaiden järvien planktonleväyhteisöissä. Levää esiintyy etenkin humuspitoisissa järvissä ja se voi muodostaa runsaita kukintoja, jotka voivat haitata vedenpuhdistuslaitoksia ja uimareita. Järven pieni koko, tummavetisyys, vähän hapan vesi ja tyypilliset ravinnepitoisuudet ja lajin käyttäytymisen ominaispiirteet ovat olleet selityksinä lajin runsastumiselle. Tässä tutkimuksessa arvioidaan järvien vedenlaatutekijöitä jotka voivat mahdollisesti selittää lajin esiintymistä ja runsastumista Suomessa. Tässä työssä esitetään tyypilliset olosuhteet ja pintavesityyppiluokat kun pintavesinäytteissä esiintyy limalevää. Tutkimuksessa suoritettiin korrelaatioanalyysi vedenlaatutekijöiden ja *G. semen* -runsauden välillä ja logistinen regressioanalyysi hyvin vähäisen *G. semen* -määrän järvien ja kukintoja muodostavien *G. semen* -järvien vedenlaatu- ja morfologisten tekijöiden välillä. *G. semen* -kukintoja muodostavien järvien kasviplanktonlajisto koostui pääasiassa siimallisista levistä. Suomessa laji esiintyy runsaana korkean fosforipitoisuuden omaavissa, tummavetisissä, pienialaisissa ja matalissa humusjärvissä. Järvien veden värin tummuminen on ehkä tärkein yksittäinen muutos joka luultavasti on lisännyt limalevän määrää Suomessa.

Contents

1. INTRODUCTION **5**
2. MATERIAL AND METHODS **8**
3. RESULTS **9**
3.1. Harmful algal bloom reports with *G. semen* 9
3.2. Lakes with *G. semen* 9
3.3. *G. semen* and physicochemical factors 12
3.4. Bloom-forming lakes and lakes with very low *G. semen* abundance 16
4. DISCUSSION **18**
REFERENCES **20**

1. INTRODUCTION

The flagellated alga *Gonyostomum semen* (Ehrenberg) Diesing is a unicellular raphidophyte (*Raphidophyceae*) and is part of the freshwater phytoplankton. The intact cell (Figure 1) is 40-65 µm long (Tikkanen 1986) but according to Cronberg *et al.* (1988) up to 100 µm long. It is usually egg-shaped, yellowish-green in color and dorsoventrally flattened. It has two flagella arising from an apical pit (Figure 1) and contains rod-like slime bodies (trichocysts) (Tikkanen 1986, Cronberg *et al*. 1988, Figueroa & Rengefors 2006).

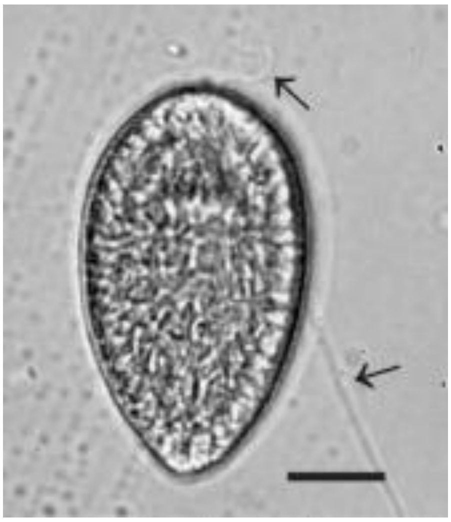

Figure 1. Intact cell of *Gonyostomum semen*. Arrows show the two flagella pointing in opposite directions. Scale bar is 10 *µ*m (Figueroa & Rengefors 2006).

G. semen lacks a real cell wall and is therefore very fragile (Cronberg *et al*. 1988). One cell has numerous (200 to 500) chloroplasts which are plano-convex in shape. They are approximately 2-3 µm wide, 3-4 µm long, and form a single layer immediately inside the cell membrane (Figure 2) (Coleman & Heywood 1981).

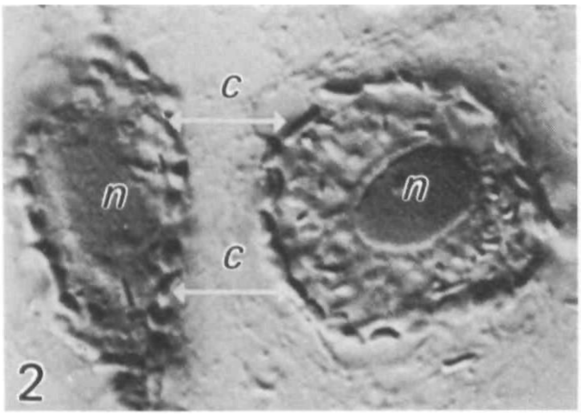

Figure 2. Two cells of *G. semen* photographed using Nomarski differential interference microscopy with magnification of 1000 times. The cell on the left is in oblique longitudinal section while the cell on the right is in transverse section. The chloroplasts (c) can be seen immediately interior to the cell membrane and the nucleus (n) is also present (Coleman & Heywood 1981).

Reports of blooms in Scandinavian lakes caused by this alga have increased since the 1980s (Cronberg *et al*. 1988, Hongve *et al*. 1988). During large blooms *G. semen* can represent 95 % of the total phytoplankton biomass of a lake (Findlay *et al*. 2005). Mucilaginous strands discharged from the numerous trichocysts of the *G. semen* cells contacting skin of swimmers during blooms can cause skin irritation and probably even allergic reactions (Tikkanen 1986, Hongve *et al*. 1988, Lepistö *et al*. 1994). Clogging of filters in water treatment plants and bad smell and taste in drinking water due to the discharged mucus, have been reported in Sweden and Norway (Cronberg *et al*. 1988, Berge 1991). This negative impact on recreation and drinking water has caused the Swedish Environmental Protection Agency to term *G. semen* a noxious species (Rengefors *et al.* 2012).

No reports of *G. semen* nuisance effects to swimmers were reported in Finland before 1978 (Lepistö *et al*. 1994). Before the 1980s, mass occurrences of the alga were rare, but it is important to note that *G. semen* cells are typically counted from epilimnetic (0-2 m depth) phytoplankton samples so the abundance of *G. semen* below 2 m depth is poorly reported. In 1982, samples started to be preserved with acetic Lugol's iodine solution in Finland, making the preservation and later identification of *G. semen* cells reliable. Before this samples were preserved with formaldehyde which destroys the fragile *G. semen* cells, making the later identification unreliable. In the samples of 1982, *G. semen* was found from 11 lakes in central Finland, and in 1986 from 42 lakes, including lakes in northern Finland (Lepistö *et al*. 1994).

Various environmental conditions have been suggested to be linked to the increasing distribution and abundance of *G. semen* in Scandinavia. In Norway from 1982 to 1986, in epilimnetic samples taken in August and September each year, total phosphorus (P) concentrations in samples dominated by *G. semen* varied between 10-50 µg l^{-1}, and total nitrogen (N) concentrations were less than 100 µg l^{-1}. Inorganic N (NO_3+NH_4) concentrations were always very low (< 10 µg l^{-1}) in these samples. The alga never dominated the deeper lakes (medium depth > 15 m) and usually dominated in acid to circumneutral lakes with pH ranging from 5.0-7.0 (Hongve *et al*. 1988).

In Sweden, *G. semen* is most common in small lakes in forested areas, with moderate to high humic content (50-60 mg Pt l^{-1} or approximately 10 mg DOC l^{-1}) and slight acidity. The alga was reported to be able to develop high biomasses in total P concentrations typical of mesotrophic forest lakes and to have a wide range of tolerance to water colour and acidity (Cronberg *et al*. 1988).

Lepistö *et al*. (1994) found a very significant linear correlation between the density of the alga and water colour in Finnish lakes. The median water colour in lakes where *G. semen* was present was 55 mg Pt l^{-1} and total P and chlorophyll *a* concentrations had significant correlations when using non-linear rank correlation models. High nutrient (especially P) concentrations and slightly acidic to neutral conditions appeared to favour *G. semen*, so it seemed to prefer dystrophic and eutrophic lakes in Finland.

In a laboratory and field study on the effect of light on the vertical distribution of *G. semen*, Eloranta & Räike (1995) found that the cells started to migrate toward the surface if the water column was undisturbed. In the laboratory the migration stopped when the photon flux density at the water surface in the acrylic tubes containing the cells reached circa 75-95 µmol m^{-2} s^{-1}. This corresponded well to the observations from the field studies. The authors suggested that the alga migrates towards the light, but avoids light intensities typical of the epilimnion in oligotrophic waters. In lakes where Secchi disc transparency is 1-2 m and water colour is high, light is at the red part of the spectrum and the migrating algae reach the epilimnion. This light intensity-limit and stratified non-mixing conditions limit the occurrence of *G. semen* in the routine lake monitoring phytoplankton samples, usually taken up to 2 m below the water surface. Eloranta & Räike (1995) concluded that the occurrence and abundance of *G. semen* depends mostly on weather conditions, lake morphometry, sampling depth and timing of sampling. High numbers of *G. semen* cells have been found when sampling has hit an undisturbed, dense layer of the alga in dark brown-coloured lakes, so correlations between cell densities and water quality factors are unreliable. In other words, *G. semen* can be caught only on calm, warm summer conditions, during the strongest stratification period. They also mentioned peat processing and general eutrophication as probable causes for increased *G. semen* distribution.

According to the trait-differentiated functional grouping of phytoplankton outlined by Reynolds *et al.* (2002), if the genus *Gonyostomum* is dominating frequently it forms its own group (coda Q**)**. In this group the habitat is described as follows: high colour, generally productive, humic, small forest lake, usually in high-latitudes with low calcium content and pH on the acid side of neutrality. How *G. semen* in Finland fits to this functional group has not been studied, but in a study of 15 Swedish forest lakes Willén (2003) allocated lakes dominated by *G. semen* to a distinct group which was consistent with the criteria of the coda Q. Also Findlay *et al*. (2005) agreed with the coda Q for *G. semen* in their study of experimental Lake 979 during flooding seasons, when large *G. semen* blooms occurred.

In addition to suitable environmental conditions, adaptations of *G. semen* help it to survive and compete. Diel vertical migration (DVM) between the illuminated surface water and the hypolimnion with its high concentrations of inorganic and organic compounds, may induce rapid growth of *G. semen* in strongly stratified small humic lakes (Eloranta & Järvinen 1991, Salonen & Rosenberg 2000). Formation of benthic resting cysts when environmental conditions are unfavourable (Cronberg 2005, Figureoa & Rengefors 2006), and the large cell size and trichocysts which help to reduce grazing by zooplankton (Lebret *et al*. 2012), can also contribute to the increased abundance of *G. semen* populations. Trichocyst expulsion has also been suspected to cause cell lysis to other algae and the released organic compounds from this lysis are apparently used by *G. semen*, giving it a competitive advantage in the phytoplankton community, both through eliminating its competitors and using them for energy (Rengefors *et al*. 2008). The exact mechanisms behind the onset of blooms and increasing distribution are still unknown, but multiple drivers acting within suitable conditions, and allowing benefits from the different adaptations of *G. semen,* are needed, not just a single or few environmental factors (Findlay *et al*. 2005).

This thesis set out to evaluate environmental factors that may affect the abundance and distribution of *G. semen* in Finland, using long time series of data collected from lakes all around Finland by the regional authorities and stored in to the national phytoplankton database of the Finnish Environment Institute (SYKE). Physicochemical variables from typical lakes where *G. semen* is present in samples are evaluated, as are the surface water type classes of these lakes. The number of *G. semen* blooms reported to the authorities is also presented and typical phytoplankton species composition in the samples where *G. semen* dominates the phytoplankton biomass is described briefly. The main questions addressed in the thesis are: 1) what are the most important physicochemical variables that may explain the expanding distribution of *G. semen* in Finland and its abundance in Finnish lakes; 2) how do "bloom forming *G. semen* lakes" differ from lakes where *G. semen* abundance is minimal; and 3) has the invasion of the taxa, reported in the 1990s (Lepistö *et al*. 1994) for lakes in Finland, continued during recent decades?

2. MATERIAL AND METHODS

The harmful algal bloom database of SYKE was searched for *G. semen* bloom reports, the search time frame being from 1970 to 2013. Each of the four bloom abundance classes used in the database was included in the search: unnoticeable, noticeable, abundant and very abundant. In each reported bloom each dominant phytoplankton species is numbered according to their abundance as a part of the bloom from 1 onwards. If numbered 1 the species is the most dominant species in the bloom.

The phytoplankton samples in which *G. semen* has been found (1460 samples covering 480 lakes) and the water quality data associated with them, from 1980 to 2010 in Finland, were downloaded from the national phytoplankton database of SYKE. Each sample has been taken from a depth of 0 to 2 m, between May and October. The number of samples varied from 1 to 50 per lake and not all lakes were sampled every year. Of the years with 20 or more samples with *G. semen* in 1980-2010 (1352 samples in 21 separate years), lakes with 4 or more samples with *G. semen* (99 lakes, 827 samples) were chosen for correlation analysis. Mean cell number of *G. semen* in each lake was correlated with mean values of 10 different water quality factors (total N and total P concentrations, water temperature, chlorophyll *a*, Secchi disc transparency, pH, colour, alkalinity, turbidity and total N / total P ratio) for the same lake. Because the data differed from normal distribution for all the variables used, the nonparametric Spearman's rank-order correlation analysis was used.

The same analysis, with the same water quality factors, was used for lakes that had at least one sample where *G. semen* comprised 75 % or more of the total phytoplankton biomass (74 samples covering 50 lakes out of the 480 lakes of the data set). These lakes with high abundance of *G. semen* were considered as "bloom-forming lakes". Logistic regression analysis was used to find significant differences in physicochemical conditions between the bloom-forming lakes and lakes with very low abundance of *G. semen*. Lakes with at least one sample with *G. semen* comprising 1 % or less of the biomass (178 lakes out of the all 480 lakes in the data set) were considered as lakes with very low *G. semen* abundance. Eight lakes were removed from the very low *G. semen* abundance lakes since they were also among the bloom-forming lakes. So finally there were two lake groups, 50 bloom-forming lakes and 170 lakes with very low abundance of *G. semen*. Area, maximum depth and elevation of both lake groups were also included in the analysis.

The phytoplankton species compositions in bloom forming lakes were described from the 74 samples where *G. semen* comprised 75 % or more of the total phytoplankton biomass. If a species was found in at least in 50 % of the samples then it was considered as a typical species occurring during *G. semen* blooms. The identification of the species was to genus level in downloaded sample data.

The downloaded files containing the phytoplankton sample data were handled and sorted to data sets for the statistical analyses with Microsoft Excel. The *G. semen* cell numbers were transformed to natural logarithms for the analyses. IBM SPSS Statistics 20 package was used to calculate the descriptive statistics and to run the correlation and logistic regression analysis.

3. RESULTS

3.1. Harmful algal bloom reports with *G. semen*

From 1984 to 2013 a total of 608 algal blooms of which *G. semen* has been a part have been reported to the Finnish national harmful algal bloom registry. There were no reports from the 1970s; the oldest are from 1984 since when there has been no year without a report.

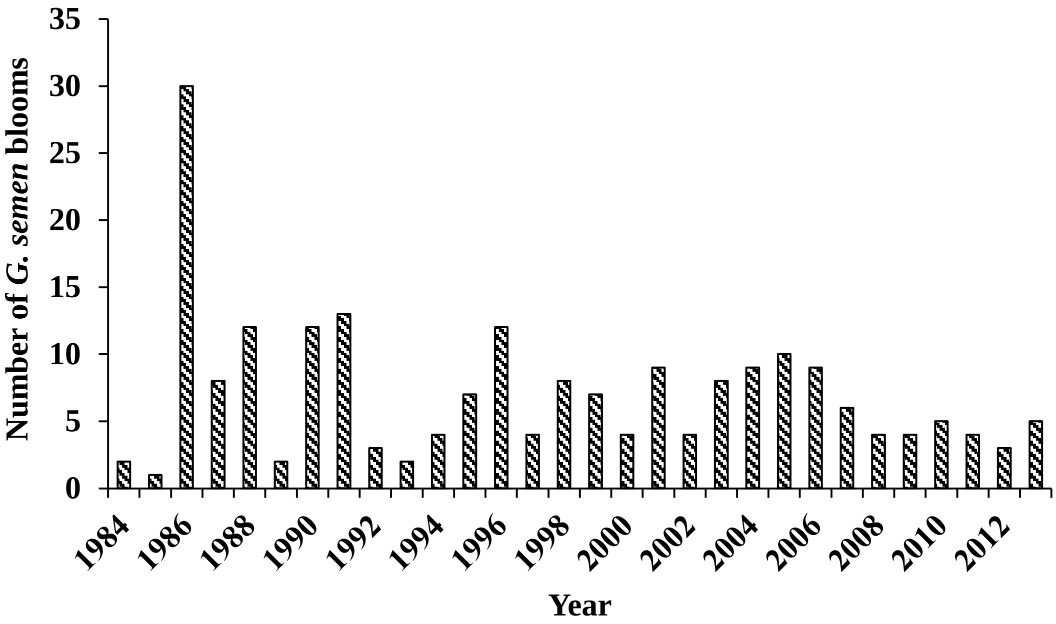

Figure 3. Number of blooms in which *G. semen* was causing the bloom (species no. 1 or 2 in all four bloom abundance classes) reported to the national harmful algal bloom database of SYKE in Finland.

A total of 211 blooms in which *G. semen* was the main species causing the bloom (category 1 or 2 in species abundance) were reported to the database (Figure 1). *G. semen* blooms were most frequent in 1986 (30 reports), since when they have remained below 15 reports annually.

3.2. Lakes with *G. semen*

From 1980-2010 *G. semen* was present in 1460 phytoplankton samples in total, covering 480 lakes in Finland. The number of samples varied from one in 1980 to 161 in 2008 (Figure 2). In the 1980s the highest number was in 1986 (85 samples) and the average was 29 samples. The highest number in the 1990s was in 1990 (46 samples), the average being 24 samples. Since 2006 the number has remained above 95 annually and the average from 2000 to 2010 was 85 samples. *G. semen* has been more frequent in phytoplankton samples in the 21st century than it was in the 1980s or the 1990s.

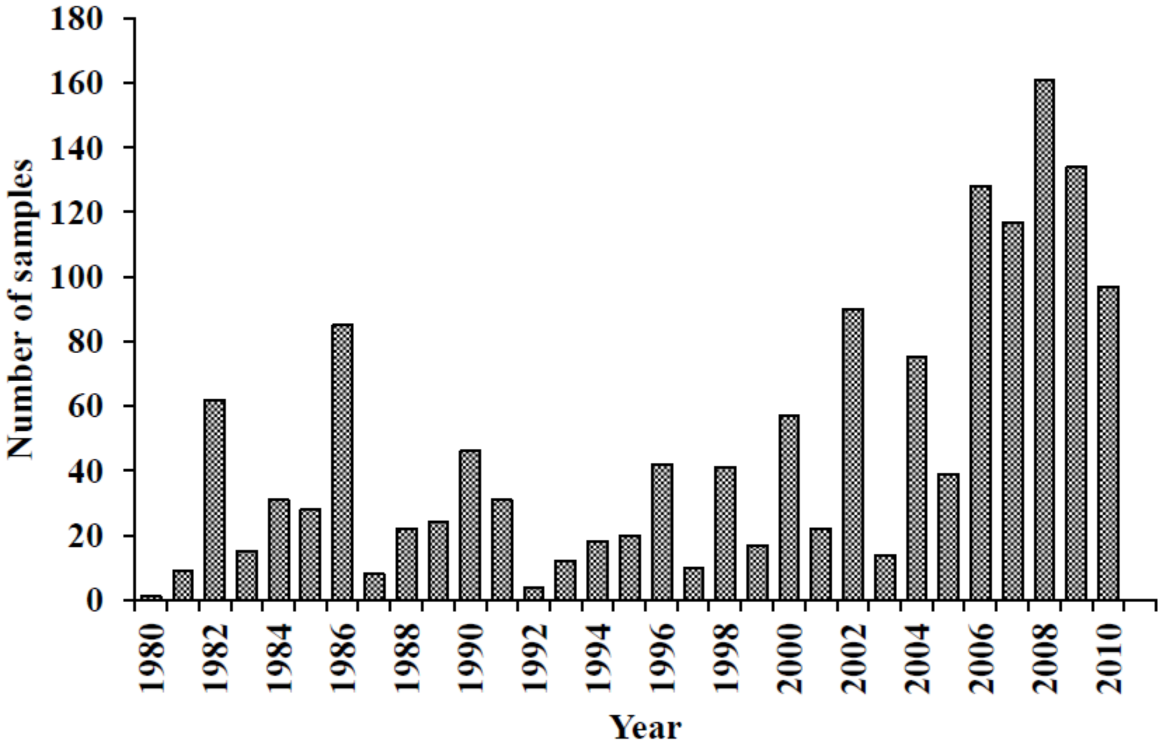

Figure 4. The 1460 phytoplankton samples that contained *G. semen* from Finnish lakes in the national phytoplankton database in 1980-2010.

Of the 480 lakes, 429 had been assigned a surface water type classification (Figure 3). *G. semen* was present in all of the 14 surface water type classes, but 5 classes included fewer than 10 lakes; these 5 classes represented lakes with short retention (Lv), rich in calcium and/or nutrients (Rk, RrRk), located in N-Lapland (PoLa) and shallow (z_{mean} < 3 m) clear water lakes (MVh),. The northernmost lake with *G. semen* was Iijärvi in Inari, Lapland, only 70 km from the northernmost point of the national border. The taxon was recorded more (66 %) in humic (Ph, MRh, Mh, Kh and Sh) than in clear water lakes. Small (Ph) and shallow humic lakes (MRh and Mh) were the most frequent classes (Figure 5).

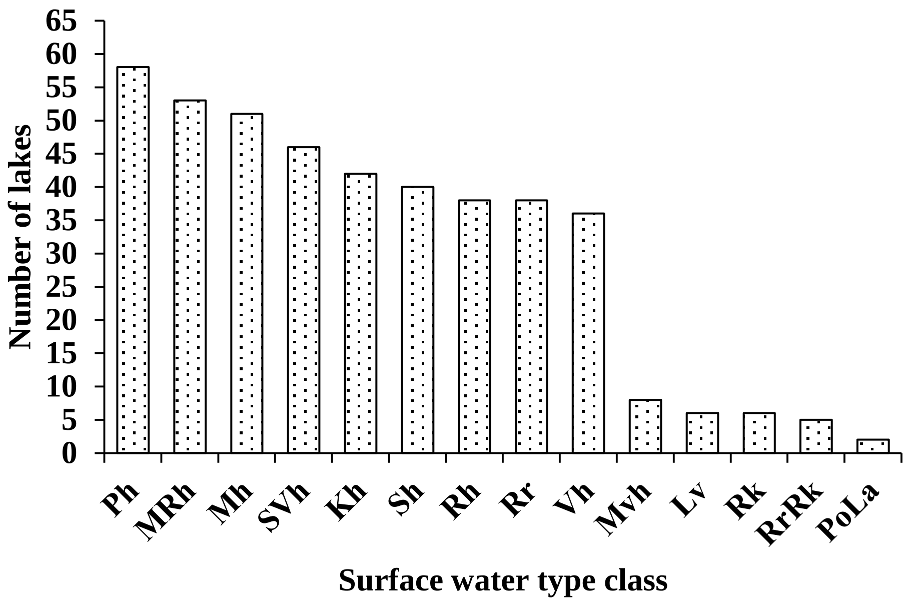

Figure 5. Number of lakes (total 429) in which *G. semen* has been found that have a surface water type classification. Ph: small humic lakes, MRh: shallow highly humic lakes, Mh: shallow humic lakes, SVh: large oligohumic lakes, Kh: medium sized humic lakes, Sh: large humic lakes, Rh: highly humic lakes, Rr: naturally nutrient-rich lakes, Vh: small and medium sized oligohumic lakes, Mvh: shallow oligohumic lakes, Lv: lakes with short retention time, Rk: calcium-rich lakes, RrRk: naturally nutrient-rich lakes and calcium-rich lakes (Note that class RrRk not valid anymore. These lakes now separated to Rr or Rk), PoLa: lakes located in N-Lapland.

Of the 50 bloom forming lakes, 36 had been assigned a surface water type classification and of these 30 were humic lakes belonging to classes MRh, Mh, Rh and Ph. Two lakes were naturally nutrient-rich (Rr) and classes Kh, MVh, RrRk and Vh had one lake each (Figure 6).

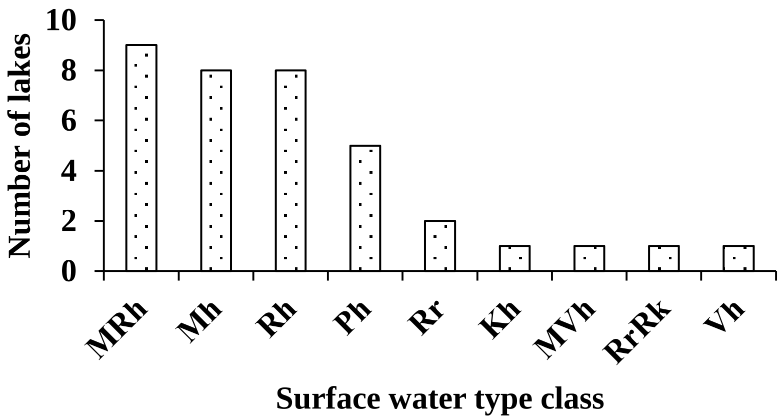

Figure 6. Number of *G. semen* -bloom forming lakes (total 36) according to their surface water type class. Surface water type abbreviations as in Figure 5.

3.3. *G. semen* and physicochemical factors

The mean total N concentration in lakes with *G. semen* was 567 µg l^{-1} and TN varied from 133 to 1448 µg l^{-1} (Table 1). Mean total P concentration was 26 µg l^{-1} and TP varied between 5.4 and 109 µg l^{-1}. Mean water temperature was 16.5 °C and varied from 11.7 °C to 20.3 °C. Mean value for pH was 6.9 and for water colour 77. pH varied between 5.4 and 7.7 and colour between 13.8 and 288.

Table 1. Average physicochemical conditions when *G. semen* was present in samples from Finnish lakes. Mean values of 99 lakes where *G. semen* was present in 4 or more samples, in the years with 20 or more samples containing *G. semen* from 1980 to 2010.

	n (lakes)	Max. / Min.	Mean ± SD	Median
Total N (µg l^{-1})	99	1447.5 / 132.5	567 ± 226	527.5
Total P (µg l^{-1})	98	109 / 5.4	26 ± 18	19.3
Temperature (°C)	99	20.3 / 11.7	16.5 ± 1.9	17.0
Chlorophyll *a* (µg l^{-1})	99	60 / 2.5	139 ± 10	8.8
Transparency (m)	98	5.0 / 0.4	2.0 ± 0.9	1.9
pH	98	7.7 / 5.4	6.9 ± 0.4	6.9
Colour (mg Pt l^{-1})	98	288 / 13.8	77 ± 48	65
Alkalinity (meq l^{-1})	91	0.58 / 0.03	0.18 ± 0.11	0.16
Turbidity (FTU)	98	36.3 / 0.3	2.9 ± 4.3	1.7
Total N / Total P	98	102 / 11.0	27 ± 12	25

Correlations (Spearman's rank-order correlation, r_s) between the density of *G. semen* (natural logarithm of mean cell number of each lake) and temperature and alkalinity were not significant (Figures 7 and 8) but the positive correlation between total N and the alga (Figure 7) was near significant ($r_S = 0.19$, $p = 0.059$). Significant positive correlations were found between the density of *G. semen* and mean values of total P ($r_s = 0.42$, $p < 0.01$), chlorophyll *a* ($r_s = 0.54$, $p < 0.01$), colour ($r_s = 0.47$, $p < 0.01$) and turbidity ($r_s = 0.44$, $p < 0.01$) (Figures 7 and 8). Significant negative correlations were found between the density of the alga and pH ($r_s = -0.28$, $p < 0.01$), transparency (Secchi depth) ($r_s = -0.55$, $p < 0.01$) (Figure 7) and total N / total P ratio ($r_s = -0.45$, $p < 0.01$) (Figure 8). When total P concentration increases, water colour darkens, water becomes more turbid and less transparent, the abundance of *G. semen* increases. Also acidity and low total N / total P ratio favours the alga.

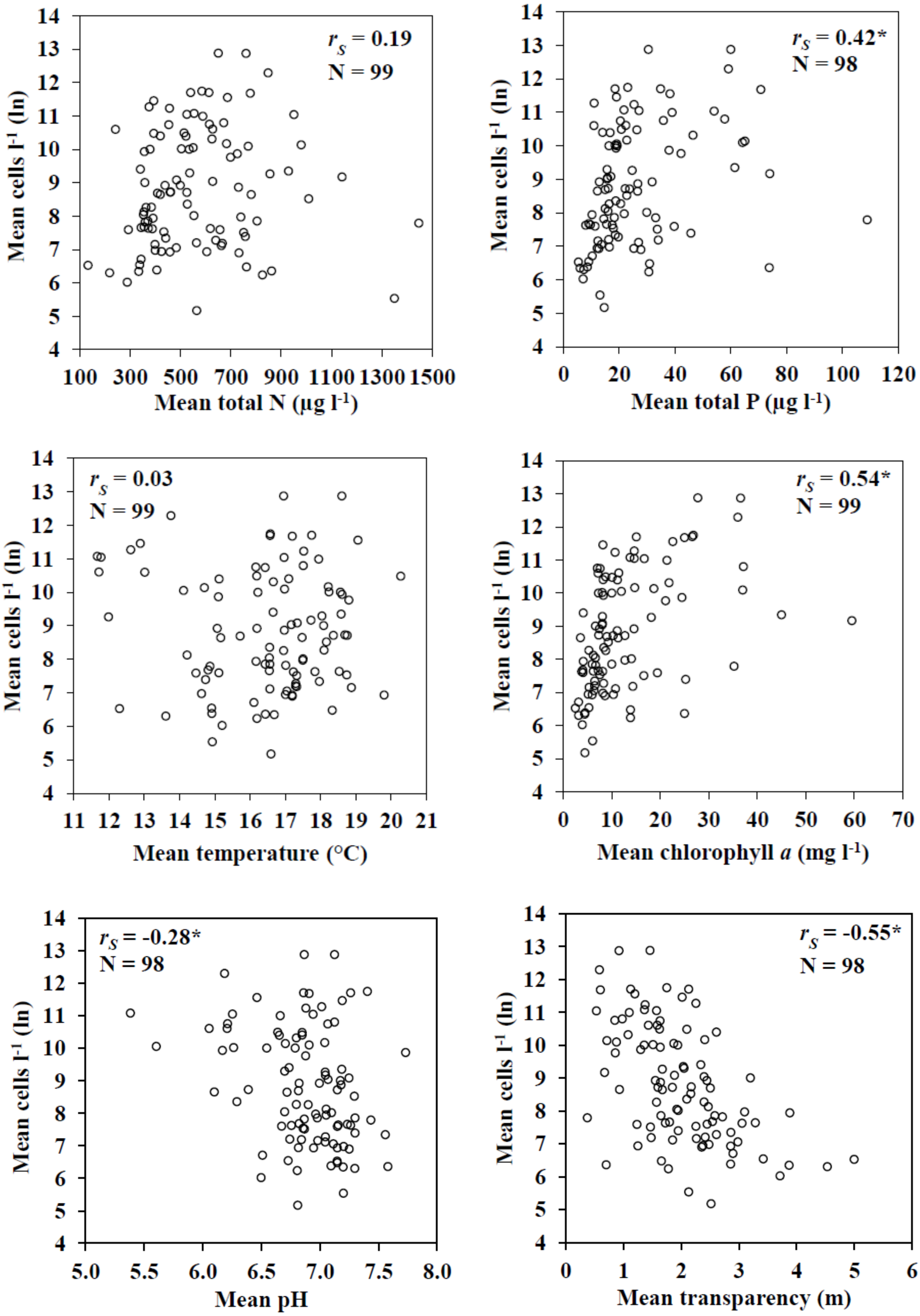

Figure 7. Relationships between mean cell number (logarithmic) of *G. semen* and mean values of six water quality factors from each lake. r_S = Spearman's rank-order correlation coefficient. * $p <$ 0.01. N = number of lakes.

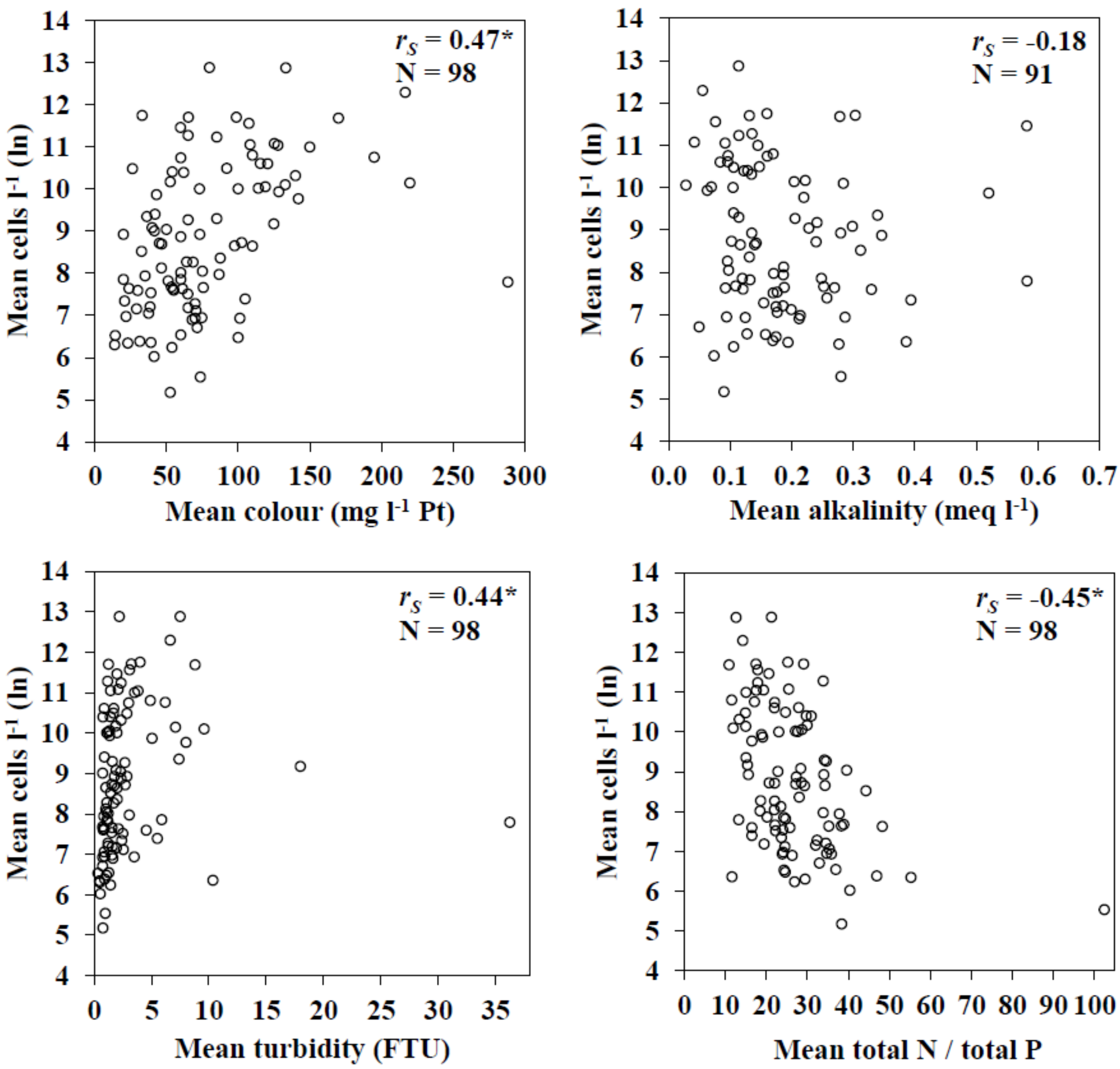

Figure 8. Relationships between mean *G. semen* cell number (logarithmic) and mean values of four water quality factors of each lake. r_S = Spearman's rank-order correlation coefficient. * $p < 0.01$. N = number of lakes.

In bloom-forming lakes (50 lakes where *G. semen* comprised 75 % or more of the total phytoplankton biomass) mean values for total N and total P concentrations were 594 and 36 µg l^{-1} respectively (Table 2). Variation for total N concentration was 130-1300 µg l^{-1} and 3-120 µg $l^{-1}$for total P. Mean surface water temperature in bloom-forming lakes was 17.2 °C and variation 11.7-21.8 °C. Respective values for transparency were 1.6 m and 0.5-2.9 m, for pH 6.5 and 5.3-7.5, for colour 117 mg Pt l^{-1} and 15-275 mg Pt l^{-1}, for alkalinity 0.13 meq l^{-1} and 0.01-0.62 meq l^{-1}, for turbidity 3.4 FTU and 0.3-21.6 FTU and for total N / total P ratio 20.7 and 8.3-47.

In bloom-forming lakes, significant positive correlations were found between the density of the alga and total N concentration ($r_S = 0.65$, $p < 0.01$), total P concentration ($r_S = 0.62$, $p < 0.01$), chlorophyll *a* ($r_S = 0.58$, $p < 0.01$), pH ($r_S = 0.40$, $p < 0.01$) (Figure 9), alkalinity ($r_S = 0.43$, $p < 0.01$) and turbidity ($r_S = 0.54$, $p < 0.01$) (Figure 10). A significant negative correlation ($r_S = -0.54$, $p < 0.01$) was found between the density of *G. semen* and total N / total P concentration ratio (Figure 10). Temperature, transparency and colour had no correlation with the density of the alga.

Table 2. Physicochemical conditions in the bloom-forming lakes (*G. semen* comprised 75 % or more of the total phytoplankton biomass in the samples) from 1980 to 2010.

	n (lakes)	Max. / Min.	Mean ± SD	Median
Total N (µg l^{-1})	47	1300 / 130	594 ± 242	547
Total P (µg l^{-1})	47	120 / 3	36 ± 26	30
Temperature (°C)	48	22 / 12	17.2 ± 2.6	17.5
Chlorophyll *a* (µg l^{-1})	41	100 / 1.3	30 ± 22	25
Transparency (m)	46	2.9 / 0.5	1.6 ± 0.6	1.5
pH	48	7.5 / 5.3	6.5 ± 0.5	6.5
Colour (mg Pt l^{-1})	44	275 / 15	117 ± 61	105
Alkalinity (meq l^{-1})	40	0.62 / 0.01	0.13 ± 0.12	0.10
Turbidity (FTU)	43	21.6 / 0.3	3.4 ± 4.0	2.2
Total N / Total P ratio	47	47.0 / 8.3	20.7 ± 9.0	19.3

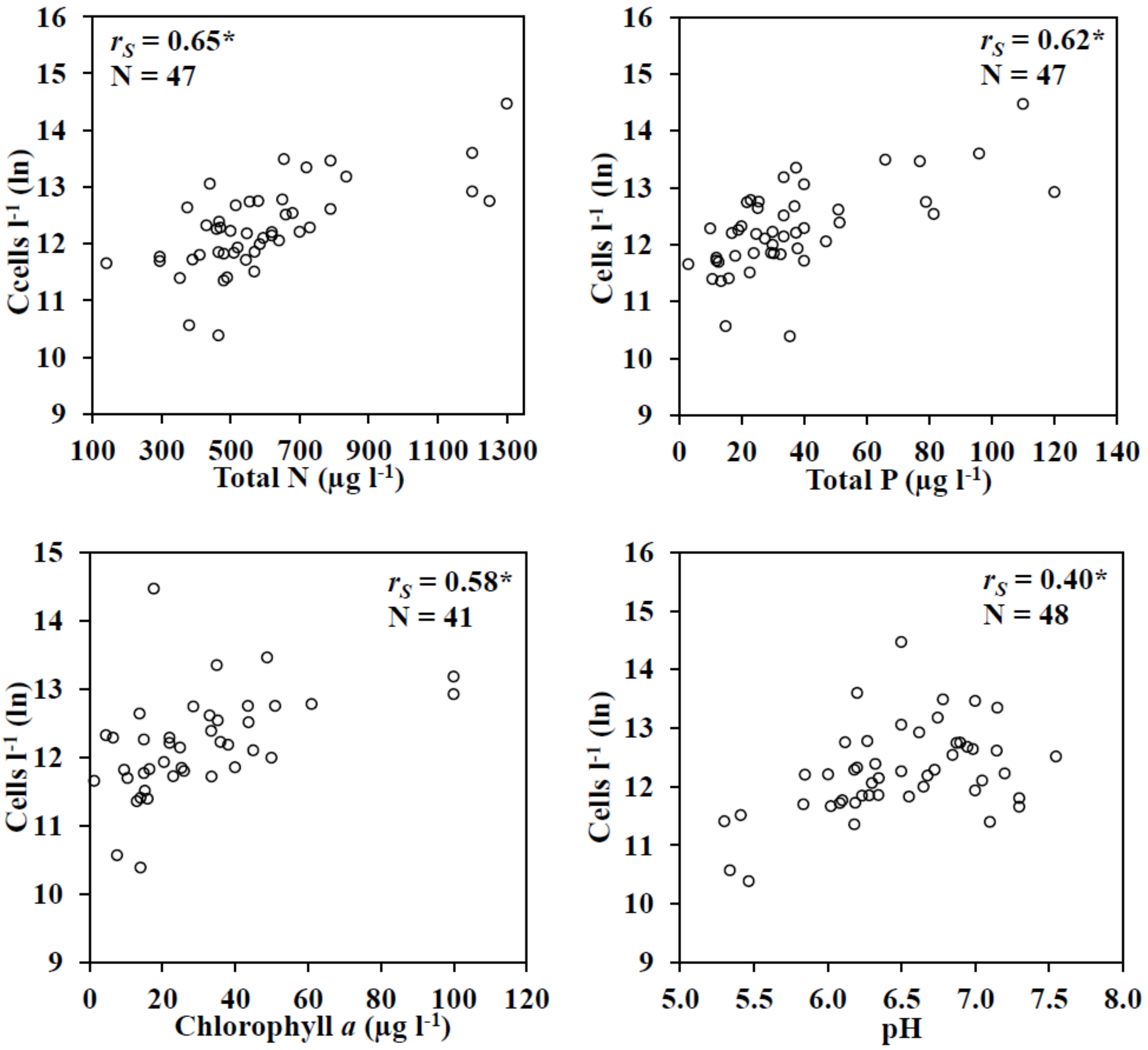

Figure 9. Relationships between *G. semen* cell number (logarithmic) and four water quality factors of each bloom-forming lake. r_S = Spearman's rank-order correlation coefficient. * $p < 0.01$. N = number of lakes.

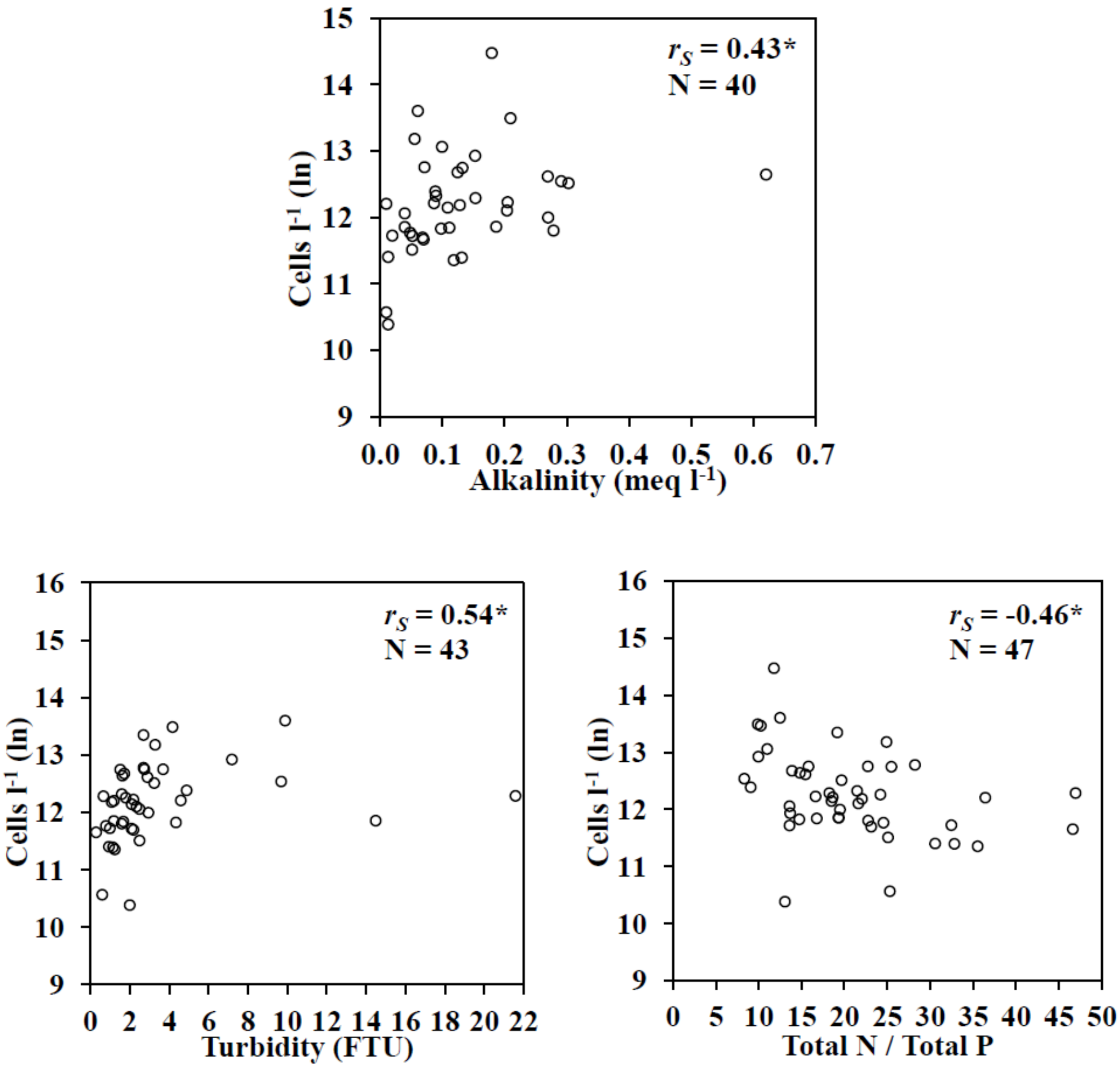

Figure 10. Relationships between *G. semen* cell number (logarithmic) and three water quality factors of each bloom-forming lake. r_S = Spearman's rank-order correlation coefficient. * $p < 0.01$. N = number of lakes.

To summarise the results of correlation analysis of typical *G. semen* lakes, total P concentration, chlorophyll *a*, colour and turbidity correlated positively whereas pH, transparency and total N / total P ratio correlated negatively with the density of the alga. Total N concentration, temperature and alkalinity had no correlation with the density of *G. semen*. In the bloom-forming lakes total N and total P concentrations, chlorophyll *a*, pH, alkalinity and turbidity correlated positively and total N / total P ratio negatively with the density of the alga.

3.4. Bloom-forming lakes and lakes with very low *G. semen* abundance

In addition to the physicochemical factors, lake area, maximum depth, mean depth and elevation were included in the logistic regression analysis. Bloom-forming lakes were smaller (mean area 4.28 km^2) and shallower (mean depth 8.2 m) than the very low *G. semen* abundance lakes (mean area 110.6 km^2, mean depth 28.7 m) (Table 3). Bloom-forming lakes were located at a slightly higher altitude (mean elevation 106.4 m) than the very low *G. semen* abundance lakes (mean elevation 100.6 m) (Table 3).

Table 3. Descriptive data for area, depth, mean depth and elevation level of the bloom-forming lakes (*G. semen* comprised 75 % or more of the total phytoplankton biomass in the samples) and very low *G. semen* abundance lakes (*G. semen* comprised 1 % or less of the total phytoplankton biomass).

	n (lakes)	Max. / Min.	Mean ± SD	Median
Area (km^2)	50**	130 / 0.02	4.3 ± 18	0.59
	170*	1377 / 0.03	1116 ± 230	18
Maximum depth (m)	50**	35.3 / 1.3	8.2 ± 7.1	6.1
	170*	93.5 / 1.6	28.7 ± 23.2	23.8
Mean depth (m)	28**	4.6 / 0.7	2.5 ± 1.1	2.0
	140*	16.3 / 0.7	5.8 ± 3.4	5.2
Elevation (masl)	31**	237 / 0.7	106 ± 54	104
	161*	290 / 3.6	101 ± 52	89

**Bloom-forming lakes *Very low *G. semen* abundance lakes.

With logistic regression analysis significant differences between physicochemical conditions of bloom-forming lakes and lakes with very low *G. semen* abundance were found in total P concentration ($p < 0.05$), chlorophyll *a* ($p < 0.001$), transparency (p < 0.01), pH ($p < 0.001$), water colour ($p < 0.001$), alkalinity ($p < 0.01$) and total N / total P concentration ($p < 0.001$). Differences in total N concentration, temperature and turbidity were not significant between the bloom-forming lakes and lakes with very low *G. semen* abundance. Significant differences were found in lake area ($p < 0.01$), maximum depth ($p < 0.001$) and mean depth ($p < 0.001$) between the bloom-forming lakes and lakes with very low *G. semen* abundance. Difference in elevation was not significant.

Table 4. Typical phytoplankton genera (n = 20) present in samples (n = 74) of the 50 bloom forming lakes during *G. semen* blooms. x = genera is a flagellate.

Genus	Flagellate	n (samples)
Asterionella		59
Aulacoseira		43
Botryococcus		47
Chrysochromulina	x	53
Cryptomonas	x	64
Dinobryon	x	61
Gonyostomum	x	44
Gymnodiniumum	x	74
Katablepharis	x	52
Mallomonas	x	53
Monomastix	x	45
Monoraphidium		55
Oocystis		40
Peridinium	x	41
Pseudopedinella	x	59
Rhodomonas	x	49
Tabellaria		49
Trachelomonas	x	44
Uroglena	x	49
Urosolenia		49

Total number of phytoplankton genera in the samples of the bloom forming lakes during *G. semen* blooms was 171 and 20 of them were found in at least half of the samples (Table 4). Of the typical genera occurring in the samples, 65 % were flagellates (Table 4).

4. DISCUSSION

The results presented here demonstrate that during the 21st century the occurrence of *G. semen* in phytoplankton samples in Finland has increased. The alga is present mainly in humic and highly humic boreal lakes (national lake types: Ph, MRh, Mh, Kh, Sh and Rh) of all size. It has been shown in experiments that *G. semen* grows better after addition of humic substances (Rengefors *et al*. 2008). Naturally nutrient-rich lakes (Rr) are also among the lakes with more frequent occurrence of *G. semen* which is consistent with previous suggestions (Lepistö *et al*. 1994, Eloranta & Räike 1995). Calcium-rich lakes (Rk) are very rare in Finland (Pilke 2012) and in Finland are probably poor environments for *G. semen* because of their quite low humic content and low water colour.

The occurrence of *G. semen* in clear water lakes (SVh and Vh) is poorly presented in the data of this study since the samples are taken up to 2 m below the water surface and *G. semen* is known to prefer low-light conditions (Eloranta & Räike 1995) which also partly explains its occurrence in the epilimnion of humic lakes. The alga may be abundant in clear water lakes below 2 m depth, where the light-intensity is favourable to the alga, and its chloroplast arrangement enables it to photosynthesize despite low-light conditions (Peltomaa & Ojala 2010). Two lakes of northern Lapland (PoLa) indicate that *G. semen* has spread above the northern border of the distribution area of pine (*Pinus sylvestris*) in Finland. The distribution of the alga has continued to expand northwards and *G. semen* will probably be found more in the PoLa lake class in the future.

G. semen seems to favour lakes with total P concentration lower than 30 µg l^{-1} and in most lakes pH was from 6.5 to 7.5. The abundance of the alga seems to increase when total P concentration increases and conditions are slightly on the acidic side and transparency is low. The median colour was 65 mg Pt l^{-1}, which is a little higher than the median of 55 mg Pt^{-1} for Finnish *G. semen* lakes reported by Lepistö *et al*. (1994). Turbidity was below 5 FTU in most of the lakes and this can be carefully considered as an indicator of potential *G. semen* presence in the lake. The total N / total P ratio is clearly lower when the density of the alga is higher.

In the bloom situations the physicochemical factors that favour the alga, when they are high in concentration or in value, were total N and total P concentrations, pH, alkalinity and turbidity. The total N / total P ratio was low also in the bloom forming lakes when the *G. semen* abundance was high. Transparency and water colour were not correlated with the abundance of the alga, differing from the typical *G. semen* lakes, and another difference was the total N concentration which did not correlate with the density of the alga in the typical *G. semen* lakes. The significant correlation between the density of the alga and total N concentration in bloom forming lakes implies that the alga takes advantage also of the N for reproduction when it occurs already in high cell number. But it has to be noted that high *G. semen* biomass increases the concentrations of total P and N because of nutrients bound to the algae. This may explain the higher mean total N concentration of the bloom forming lakes in comparison to the typical *G. semen* lakes.

The lowest total P concentration for a bloom forming lake (Lake Iloittu in Lohja, Southern Finland) was only 3 µg l^{-1} when *G. semen* comprised 80 % of the phytoplankton biomass of this 0.3 km^2 sized, shallow humic lake. This suggests that total P concentration alone is not the factor responsible for the blooms, unless the wasp had been used up at the moment of sampling, while the bloom still persisted. It could be that in humic, mesotrophic, high colour lakes, *G. semen* is more effective in using the P from both external or internal loading. In addition to mixotrophy in the epilimnion, *G. semen* is known to retrieve nutrients from the hypolimnion by DVM in a small humic lake (Salonen & Rosenberg 2000).

On the basis of the logistic regression analysis, environmental conditions between the bloom forming lakes and the very low *G. semen* abundance lakes differed significantly in seven physicochemical factors; total P concentration, transparency, pH, colour, alkalinity, turbidity and total N / total P ratio. These can be considered most likely to explain the bloom forming of *G. semen* in Finnish lakes. Of these factors, total P concentration, pH, colour and alkalinity (factor whose variation is strongly linked to the variation of the pH and primary production) are in accordance with previous studies (Cronberg *et al*. 1988, Lepistö *et al*. 1994, Willén 2003, Findlay *et al*. 2005) that suggested these factors as explanations for the abundance of *G. semen*.

Bloom forming lakes differed significantly from the very low *G. semen* abundance lakes also in lake area and maximum depth. Bloom forming lakes were smaller and shallower than the very low *G. semen* abundance lakes. Small size and shallowness, together with dark water colour, are important factors in the formation of strong summer stratification. Strong summer stratification can give *G. semen* an advantage over smaller flagellates in retrieving nutrients from the often nutrient rich anoxic hypolimnion of small humic lakes (Peltomaa & Ojala 2010).

In a study on small, shallow and humic Lake Valkea-Kotinen with anoxic, nutrient rich hypolimnion, Peltomaa & Ojala (2010) observed that photosynthesis was mostly maintained by *G. semen* during the summer stratification. The phytoplankton community mainly consisted of flagellates and the large cell size and motility to retrieve nutrients from the hypolimnion were considered important characteristics of *G. semen* for dominance. In Lake Valkea-Kotinen dissolved inorganic carbon (DIC) and ammonium nitrate (NH_4N) concentrations were much higher below the 2-3 m depth than in the epilimnion, and the large cell size made the migration possible to obtain these nutrients during the strongest stratification. Peltomaa & Ojala (2010) pointed out that according to Sommer (1988), the smaller flagellates (< 5 µm) have maximum vertical migration amplitude of only 2 m and motility in strongly stratified conditions requires a lot of energy from them. This would mean that the smaller flagellates in Lake Valkea-Kotinen could not obtain nutrients from the hypolimnion, thus giving *G. semen* a competetive advantage over them. This may be true also in the shallow bloom forming lakes of this study, because phytoplankton communities in small, shallow, brown-water lakes can be dominated by flagellates during summer stagnation (Ilmavirta 1988). The phytoplankton species compositions of the bloom forming lakes, during *G. semen* blooms in this study, were also dominated by flagellates.

The results of this study support the trait-differentiated functional grouping of *G. semen* (Reynolds 2002), as characteristic of high colour, slightly acid, generally productive and small humic lakes. High colour in Finnish lakes is usually due to humic substances (Simola & Arvola 2005) and most of the high colour lakes in this thesis can be considered humic lakes. High-latitude of the lakes is also a criterion for the grouping by Reynolds (2002) and is supported by the current distribution of *G. semen* covering PoLa -class lakes in Finland.

The water colour has darkened in *G. semen* lakes during the last 20 years as shown by this study. This is probably mainly due to particulate and dissolved organic matter, P and N leached from forestry and peatland conversion for forestry, which was at its peak in the 1970s, and still affects lakes in many areas in Finland (Simola & Arvola 2005). The darkening of water gives *G. semen* advantage over the other flagellates mainly because of its ability to photosynthesize in lower light conditions than the other flagellates and its ability to reach the variety of nutrients in the hypolimnion of small humic lakes.

Because sampling is done from 0 to 2 m depth and the ability of *G. semen* to migrate and form resting cysts, its true abundance is not really known. It favours low light levels (Eloranta & Räike 1995) so in some clear water lakes it may thrive deeper in the water column than 2 m depth. It has to be noted that in this study all of the phytoplankton samples are taken from the epilimnion (0 to 2 m depth), so the abundance of *G. semen* in bloom situations below 2 m depth was not available. Future research on *G. semen* would require frequent sampling from the deeper depths of the water column of clear water lakes. If *G. semen* would be abundant in deep clear water lakes of Finland, this would support the idea that *G. semen* can survive in a wide variety of conditions. It may also well be that the increase in distribution and abundance of *G. semen* in Finland, is partly due to better recognition and counting, and improved phytoplankton sample preservation.

Active DVM to obtain nutrients from the epilimnion and the often nutrient rich hypolimnion of small humic lakes (van den Avyle *et al*. 1982, Salonen & Rosenberg 2000), total P concentration, high water colour and slight acidity are among the key factors behind the high abundance of *G. semen* in Finnish lakes. If these factors are maintained in a small (~ 4 km^2 or less in area), shallow and low total N / total P ratio -lake in Finland, it might well be a typical bloom forming *G. semen* -lake.

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