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Phenotypic plasticity and differentiation in an invasive freshwater microalga

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ABSTRACT

Recent studies show that both marine and limnic microalgal species often consist of several genetically distinct populations. This is also valid for the nuisance freshwater algae *Gonyostomum semen*, which originates from acidic, brown water swamp lakes, but can nowadays also be found in clearer lakes with close to neutral pH. We hypothesized that the observed genetic differentiation among *G. semen* lake populations, reported in earlier studies, is connected to adaptation to local environmental conditions. In the present study we performed controlled laboratory experiments to test whether 12 strains originating from five lakes varied in their response to five to six different pHs, light intensities and DOC concentrations. Overall, growth (0.01–0.37 day^{-1}) was observed over a wide range of light intensities and pHs, demonstrating high potential for photoacclimation and extensive plasticity of individual strains. Moreover, we found similar growth rates and consistent growth optima for specific pHs by strains from the same lake, suggesting genetic differentiation of populations into distinct phenotypes. However, observed strain specific preferences did not always reflect environmental conditions in the lakes of origin and provided limited evidence for the hypothesized local adaptation. Instead, the observed phenotypic differentiation may indicate resilient effects of founder events. We suggest that the wide phenotypic plasticity in this species enables it to thrive in fluctuating and variable environments, and may play a role in its ability to colonize new habitats.

1. Introduction

Despite being widespread, many microalgal species are differentiated into genetically distinct populations. In fact, some widely distributed species contain both cosmopolitan and endemic clades at the same time (Godhe et al., 2006; Rynearson et al., 2009; Watts et al., 2011). Genetically diverged populations of harmful algal bloom (HAB) species have been reported in many studies of the toxic dinoflagellate genus *Alexandrium* (Casabianca et al., 2012; Nagai et al., 2007; Tahvanainen et al., 2012). Also, several well-differentiated populations of the marine, red-tide forming raphidophyte *Chattonella marina* were recently described by Demura et al. (2014). Studying the population structure of HAB species is especially important, as high genetic diversity challenges predictions of dispersal and response to environmental changes. Cryptic genetic diversity, expressed in different phenotypes, may allow HAB species to invade new habitats or thrive with global change (Kremp et al., 2012; Litchman, 2010), as they can tolerate a wide range of environmental conditions.

Although high intraspecific genetic diversity in microalgae has been frequently confirmed, the mechanisms behind the divergence is largely unknown (Figuerola and Green, 2002). Genetic differentiation may be caused by limited dispersal due to geological barriers or geographic distance (Papke and Ward, 2004). On the other hand, successful establishment in new habitats might be restricted by biological barriers like founder effects or local adaptation (Foissner, 2008; Weisse, 2008). The monopolization hypothesis, which was established for zooplankton, argues that large genetic differentiation between well-connected habitats can be explained by rapid population growth after historical founder events (De Meester et al., 2002). Low genetic diversity after colonization by only a few individuals can lead to random genetic drift during parthenogenetic growth. Newly established, fast growing populations can adapt quickly to the local environmental conditions (Allen et al., 2010), and later immigrants face difficulties

Abbreviations: AFLP, amplified fragment length polymorphism; DOC, dissolved organic carbon.

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Table 1
Location and environmental conditions of lakes of origin. TOC = total organic carbon, Pt = platinum.

Lake	Liasjön	Dammen	Mjöträsket	Torsjön	Kylänalainen
Strains	LI21, LI22	DM18, DM22	MJ12, MJ21, MJ39	TO01, TO13	KY12, KY20, KY23
Location	Southern Sweden	Southern Sweden	Northern Sweden	Southern Sweden	Southern Finland
Longitude	56.762604	56.551346	66.033538	56.762604	60.410038
Latitude	13.990871	14.320550	22.100801	14.906361	23.754930
pH	4.31	6.6	6.91	6.47	5
TOC ($mg\,l^{-1}$)	30–50	n.a.	6.8	6	5
Water colour					
Absorbance 420 nm, cm^{-1}	0.256	0.058	0.052	0.041	0.029
$mg\,Pt\,l^{-1}$	883	200	179	141	100

to establish, as they have to compete with this locally adapted resident population with higher fitness (Haag et al., 2006). Hence, bottlenecks after founder events are expected to lead to high genetic differentiation among populations (Wade and McCauley, 1988; Whitlock and McCauley, 1990). This hypothesis might also be applicable to microalgae, as most species can rapidly give rise to enormous populations by asexual reproduction.

When gene flow between habitats is restricted, each local population should evolve traits that provide an advantage in its local environmental conditions (Loeuille and Leibold, 2008; Nosil and Crespi, 2004). Adaptation can occur in response to a variety of environmental variables. As these variables structure habitats also on a very fine scale, adaptation allows closely related phytoplankton populations to coexist. Hence, local adaptation by natural selection is an important mechanism for population differentiation (Kawecki and Ebert, 2004). For example, Berge et al. (2011) found evidence for ecotype differentiation in two common dinoflagellate species based on pH adaptation along an oceanic-costal gradient. Rynearson and Armbrust (2004) identified several populations of *Ditylum brightwellii* (Bacillariophyceae) with distinct genetic and physiological characteristics in closely connected estuaries. On the east coast of the USA a high degree of toxin variability was found in 18 strains of *Karlodinium veneficum* (Dinophyceae) despite homogenous morphology, genetic data and photopigments, which suggest different functional roles among the coexisting strains (Bachvaroff et al., 2009).

The invasive raphidophyte *Gonyostomum semen* (Ehrenberg) has aroused much scientific attention in the last decades in Scandinavia (Cronberg et al., 1988; Lepistö et al., 1994; Rakko et al., 2008; Rengefors et al., 2012), as it has spread significantly over the past 50 years from a few lakes in the South to the Arctic Circle. Furthermore, a significant increase in biomass, often connected to extensive blooms in summer, has been reported (Lepistö et al., 1994; Rengefors et al., 2012; Trigal et al., 2013). Cell length ranges from 50 to 100 μm and, despite its high competitiveness, this species is very fragile and grows slowly (0.02–0.08 d^{-1} observed in nature, Lebret et al., 2012). *G. semen* is considered a nuisance species, as it alters plankton communities (Angeler and Johnson, 2013) and discharges mucilaginous strands with trichocysts that can cause skin irritations to people swimming in lakes with blooms (Lepistö et al., 1994; Sörensen, 1954). This results in decreased recreational value of the lakes. *G. semen* was originally described from small lakes and ponds with low pH and high DOC concentration resulting in immediate absorption of short light wavelengths (blue and green) and little light penetration (Drouet and Cohen, 1935; Sörensen, 1954). During the last decades it has also been reported from many non-humic environments with higher pH (Cronberg et al., 1988; Rengefors et al., 2012). Despite this very recent expansion an earlier study by Lebret et al. (2013) reported weak but significant differentiation of this species into genetically distinct populations in Northern Europe.

We hypothesized that *Gonyostomum semen* strains are adapted to the local environmental conditions of their native lake. This may in turn prevent immigration of individuals from other populations and thereby restrict gene flow. To test if strains were locally adapted we performed controlled laboratory experiments, in which we monitored the growth rates of different strains in gradients of pH, DOC concentration and light intensity. These environmental variables were chosen as lakes with regular occurrence of *G. semen* vary significantly in these parameters. However, bloom formation of this species is usually correlated to low pH and high DOC concentrations (Rengefors et al., 2012; Trigal et al., 2013). Adaptation to acidic conditions and low light intensities, which are found at high DOC levels, might give *G. semen* a competitive advantage over many other algal species.

2. Materials and methods

2.1. Sampling and culturing

In these experiments we used clonal *Gonyostomum semen* cultures, which were established from several lakes in Fenno-Scandinavia in August 2010 and 2011. We chose two strains from the lakes Liasjön, Dammen and Torsjön in Southern Sweden, three strains from lake Mjöträsket in Northern Sweden and three strains from lake Kylänalainen in Southern Finland. Lake Liasjön is characterized by a low pH and high DOC concentrations (Table 1). Lake Kylänalainen has a low pH as well, but low concentrations of DOC, which results in little absorbance and deep penetration of light in this lake (Table 2). The Swedish lakes Dammen, Torsjön and Mjöträsket have intermediate pHs and DOC concentrations. The water temperature at the day of sampling varied between 18 °C and 23 °C. The microalgae were sampled with a plankton net (mesh size 20 μm) from the shore of each lake and filtered directly through 150 μm mesh to remove large zooplankton, which might feed on *G. semen*. Single cells were isolated by micropipetting under an inverted microscope (Nikon Eclipse TS100, Melville, New York, USA) and transferred into 96-well plates filled with a 300 μl mixture (1:1) of artificial medium

Table 2
Depth (cm), where experimental light intensity equals light intensity in lakes of origin.

Lake	Absorbance (420 nm, cm^{-1})	Light intensity (μmol photons $m^{-2}\,s^{-1}$)				
		250	150	25	10	5
Liasjön	0.256	5	5	8	12	17
Dammen	0.058	22	24	37	52	74
Mjöträsket	0.052	24	27	41	58	83
Torsjön	0.041	31	34	52	73	105
Kylänalainen	0.029	43	48	74	103	148

(modified Woods Hole medium, pH 7, Guillard and Lorenzen, 1972) and filtered, autoclaved lake water. The cells were cultivated in 20 °C climate chambers with a light:dark cycle of 14:10 h and a photon flux of 25 μmol photons m^{-2} s^{-1}. When the cells started dividing, they were gradually transferred to bigger volumes up to 40 ml and grown in sterile, vented, polystyrene tissue culture flasks (VWR, Radnor, Pennsylvania, USA).

2.2. Molecular fingerprinting

Each culture was genotyped to assure that strains represented genetically distinct clonal lineages. Cultures were harvested by centrifugation at 500 × *g* for 15 min. Pellets were frozen at −80 °C until DNA extraction. DNA was extracted from all cultures following a modified CTAB protocol (Lebret et al., 2012). Final DNA concentrations were measured spectrophotometrically with a NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). For each sample, the quality of the DNA was determined using 260/280 ratios. Each strain was genotyped by amplified fragment length polymorphism (AFLP) following protocols from Vos et al. (1995) and Lebret et al. (2012) using 100 ng extracted DNA. The M and E-primers 5′-GACTGCGTACCAATTCNNN-3′, and 5′-GATGAGTCCTGAGTAANNN-3′ were used for selective amplification. Specifically, the following six primer combinations were chosen: $E_{TCT} \times M_{CGA}$, $E_{TCT} \times M_{CCG}$, $E_{TAG} \times M_{CGG}$, $E_{TCG} \times M_{CAG}$, $E_{TCG} \times M_{CGG}$ and $E_{TCG} \times M_{CGA}$. PCR products from three primer combinations labelled with different dyes (Ned, Fam and Hex) were combined in single wells of a 96-well plate. All samples were analyzed by ABI3730XL capillary electrophoresis using a MapMarker 1000 bp size standard at the Uppsala Genome Centre, Sweden. The software GeneMapper (version 4.0, Applied Biosystems) was used to detect peaks in the electropherograms and these raw data were evaluated and filtered with AFLPscore, version 1.4b (Whitlock et al., 2008). After the scoring, a data set based on presence and absence of fragments was generated. Expected heterozygosity within lake groups was calculated in the programme GeneAlex, version 6.4 (Peakall and Smouse, 2006). The software FAMD, version 1.25 (Schluter and Harris, 2006), was used to create a genetic distance matrix based on Jaccard's similarity coefficients and an UPGMA (unweighted pair group method using arithmetic averages) dendrogram of the 12 strains.

2.3. pH experiment

In order to determine differences in growth rate between *Gonyostomum semen* strains in response to different pHs, the 12 strains were cultivated for approximately two weeks (3–4 generations) in five distinct pHs (pH 8, 7, 6, 5 and 4) corresponding to the natural range of pH in Northern European lakes (Rengefors et al., 2012). The experiment was set up in two batches, which were conducted one after the other. Each batch consisted of six strains, replicated four times, at four or five different pH levels. The experimental growth conditions did not differ from culturing conditions except for the pH. The MWC medium was prepared with MilliQ and lake water without adding any buffer to be able to realize different pHs by bubbling with CO_2. Final adjustments were made with a few drops of 0.2 M HCl or 0.1 M NaOH. Prior to the start of the experiment, the pH in all cultures was gradually changed every second day by one unit, starting from pH 7, by adding acidic medium (pH 3.5) to the culture until it reached the new pH. The strains were acclimated for at least 3 days in the final experimental conditions. The pH was measured to the nearest 0.01 unit with a pH meter (Mettler-Toledo GoFive, Greifensee, Switzerland). The pH sensor was calibrated on a daily basis using IUPAC buffers pH 4.01 and 7.0 (International Union of Pure and Applied Chemistry). All culturing flasks were filled to capacity (80 ml) with the appropriate medium to avoid gas exchange with the air. The experiment ran on benches that were isolated by black foil or cardboard from the surrounding light climate in a walk-in incubator. An initial cell concentration of 25 cells ml^{-1} was used to avoid strong fluctuations in pH due to photosynthesis and respiration. During the experiment we sampled 3 ml every second or third day, depending on the growth rate, from all cultures for pH measurement, measuring of chl *a* autofluorescence and cell counts. The sample volume was replaced by fresh medium with lower pH to adjust the pH in the cultures.

Every time at least 100 cells, fixed with Lugol's solution, were manually counted in a 1 ml Sedgewick-Rafter chamber with an inverted microscope (Nikon Eclipse TS100, Melville, New York, USA) with 400× magnification from at least two replicates. The cell concentration from the uncounted cultures was calculated based on the linear correlation between chl *a* autofluorescence and cell number. The raw fluorescence was measured with a TD-700 Fluorometer (TURNER DESIGNS, Sunnyvale, California, USA) after running a single point calibration with a culture close to the highest expected experimental cell concentration (1000 cells ml^{-1}) and a blank consisting of the culture medium.

Total inorganic carbon (TIC) was measured with the total organic carbon analyzer TOC-V_{CPN} (Shimadzu, Kyoto, Japan) in the different culture media before the experiment and in one replicate of each strain in all pH treatments at the end of the experiment.

2.4. DOC experiment

The 12 strains (replicated as above) from the pH experiment were additionally used to test the response of growth rate to different DOC concentrations. All strains were acclimated prior to the experiment to five different DOC concentrations (0, 10, 20, 30, 40 mg C l^{-1}) for two weeks (app. three cell divisions). These DOC concentrations were chosen to cover the range of natural concentration in humic lakes with regular *Gonyostomum semen* blooms. Nordic Reservoir Natural Organic Matter (International Humic Substance Society), extracted from the Norwegian lake Vallsjøen, was used as a DOC source and dissolved in artificial MWC medium. The experimental growth conditions did not differ from culturing conditions in the other experiments. The experiment started with 100 cells ml^{-1} in 35 ml and each culture was sampled every third day for cell counts. Cell densities were estimated solely by counting as above.

2.5. Light experiment

To test adaptations of strains to different light conditions, we used the same 12 strains as in the previous experiments (replicated as above) and cultivated them for approximately two weeks (3–4 generations) in six distinct light climates. A pilot experiment with one strain had shown that the growth rate of *Gonyostomum semen* does not differ significantly between light intensities from 12 to 100 μmol photons m^{-2} s^{-1} (unpublished data). Hence, we cultivated the 12 strains at 5, 10, 25, 150 and 200 μmol photons m^{-2} s^{-1} (AURA Luminette 36 W 840). Additionally red light at an intensity of 25 μmol photons m^{-2} s^{-1} was used, as red light penetrates deepest in brown water lakes (Eloranta, 1978). The red light climate was achieved by filtering out all other wavelength (<600 nm) with a Rosculux filter (#19, Port Chester, NY, USA). The light intensities and spectra were measured with an Ocean Optics Inc. USB 2000 spectrometer, Dunedin, Florida, USA (software OOIBase32) and a LI-COR light meter (Model LI-189, Lincoln, Nebraska, USA). The chosen light intensities correspond to a range of 4–150 cm depth in the original lakes of the tested strains (Table 2), which is a distance covered by diel vertical migration of *G. semen* (Salonen and Rosenberg, 2000). All strains were acclimated to the experimental

light conditions by gradually changing the light intensity (20 μmol photons m^{-2} s^{-1} per day) starting from 20 μmol photons m^{-2} s^{-1} and grew in each light treatment for at least 10 days (two generations) prior to the start of the experiment. The experiment started with 500 cells ml^{-1} and was sampled every four days. Cell densities were estimated by counting as above.

2.6. Statistical analysis

Growth rates were calculated from the obtained growth curves (cell concentration as a function of time). We calculate the maximum growth rate per day during the exponential growth phase between all measured time points after the lag phase ($\mu = (\ln N_1 - \ln N_0) * t^{-1}$) and calculated an average growth rate for each replicate.

We tested for differences in growth rates among strains, groups of strains pooled by their lake of origin and effects of environmental variables using a mixed model ANOVA (environmental variable = fixed, within subject factor; lake = random, between subject factor) with the factor strain nested in the factor lake. Single main effects were investigated by pairwise comparison of lake groups in each treatment and treatment levels in each lake group using one-way ANOVAs. Linear correlations between growth rates and light intensity and DOC concentrations were analyzed by Spearman rho correlations. All statistical tests were performed in the programme IBM SPSS Statistics, version 21 (Armonk, New York, USA).

3. Results

3.1. Molecular fingerprinting

After evaluating and filtering the AFLP raw data in AFLPscore, 302 loci were retained for downstream analysis. On average 30% of these loci were polymorphic among lake groups. All 12 strains showed a unique AFLP profile featuring between three and 24 private loci and therefore represented unique genotypes. Jaccard-distance-coefficients ranged from 0.6 to 0.925 between strains and expected heterozygosity from 0.009 to 0.136 within lake groups. In the UPGMA dendrogram all strains were clearly differentiated from each other, but did not cluster by lake of origin, except for the strains DM18 and DM22, which formed a monophylum (Fig. 1).

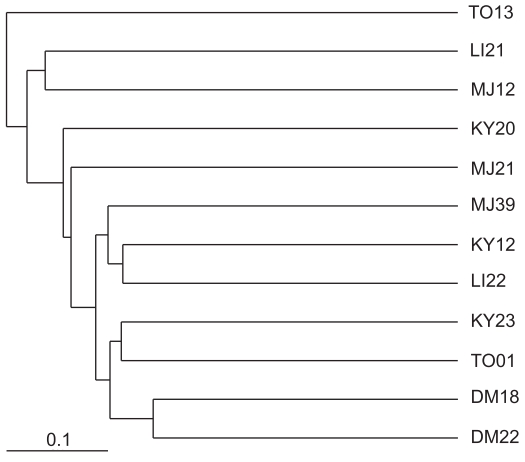

Fig. 1. UPGMA dendrogram based on Jaccard's similarity coefficient of AFLP loci of the 12 *G. semen* strains used in the three experiments. Each lake is represented by either two or three strains. Dammen = DM18, DM22; Kylänalainen = KY12, KY20, KY23; Liasjön = LI21, LI22, Mjöträsket = MJ12, MJ21, MJ39, Torsjön = TO01, TO13. The scale bar shows approximate Jaccard's similarity coefficient of 0.1.

3.2. pH experiment

The growth rates differed significantly among strains and lake groups depending on the specific pH (mixed model ANOVA, pH × lake, $p < 0.001$, Table 3). These differences were especially pronounced at pH 4, as only strains from lake Kylänalainen were able to grow under these conditions (Fig. 2). The strains LI21, DM18, DM22, MJ12, MJ21 and MJ39 did not survive the acclimation to pH 4 and were therefore not used in that experimental treatment. A simple main effect test between lake groups at single pHs showed that there were group specific differences between growth rates. At pH 5 and 6 strains from Mjöträsket grew 70% slower than all other strains, while strains from Dammen were on average 75% faster than all other strains at pH 6 ($p < 0.01$). Strains from Liasjön grew significantly faster than all other strains at pH 7 ($p < 0.04$). However, there were no significant differences in growth rates among lake groups at pH 8. Strains from the lakes Mjöträsket, Liasjön and Dammen showed a significant growth optimum at one or two pHs (pH 7 and pH 6/7 respectively, Fig. 2), while strains from Kylänalainen and Torsjön grew evenly over a range of pHs (pH 5–8 and pH 5–7 respectively, Fig. 2). The pH in the lake of origin was reflected by the pH at the growth optima of the strains DM18, DM22, KY12, MJ12, MJ21, MJ39 and TO13. The strains LI21, LI22, KY20, KY23 and TO01 grew faster at a different pH then present in their lake of origin (Fig. 3).

The analysis of inorganic carbon (IC) in all cultures and treatments showed no significant influence of differences in the starting inorganic carbon concentration on the IC concentration at the end of the experiment due to different pHs (one-way ANOVA $p = 0.105$).

3.3. DOC experiment

The growth rates differed significantly among all tested strains (mixed model ANOVA, $p < 0.001$, Table 3) in the DOC experiment. In general the growth rates decreased with increasing DOC concentration (Spearman rho correlation coefficient −0.407, $p < 0.001$) (Fig. 4). The growth rates were on average twice as high at DOC 0 compared to all other treatments (one-way ANOVA $p < 0.001$) and lowest at 40 mg C l^{-1}. There were no significant

Table 3
Statistical results from mixed model ANOVA analysis. Growth rate is used as response variable.

Variable	*df*	Mean square	*F*	*p*
pH experiment				
pH	3	0.072	50.339	<0.001
Lake	4	0.076	48.093	<0.001
Strain (lake)	7	0.012	7.829	<0.001
pH × lake	12	0.017	11.679	<0.001
pH × strain (lake)	21	0.005	3.849	<0.001
Error (pH)	108	0.001		
DOC experiment				
DOC	4	0.043	85.904	<0.001
Lake	4	0.032	96.622	<0.001
Strain (lake)	6	0.16	46.405	<0.001
DOC × lake	16	0.001	2.917	<0.001
DOC × strain (lake)	24	0.002	4.415	<0.001
Error (DOC)	132	0.001		
Light experiment				
Light	4	0.117	228.827	<0.001
Lake	4	0.001	1.853	0.142
Strain (lake)	6	0.019	40.501	<0.001
Light × lake	16	0.004	8.32	<0.001
Light × strain (lake)	24	0.004	6.872	<0.001
Error (light)	132	0.001		

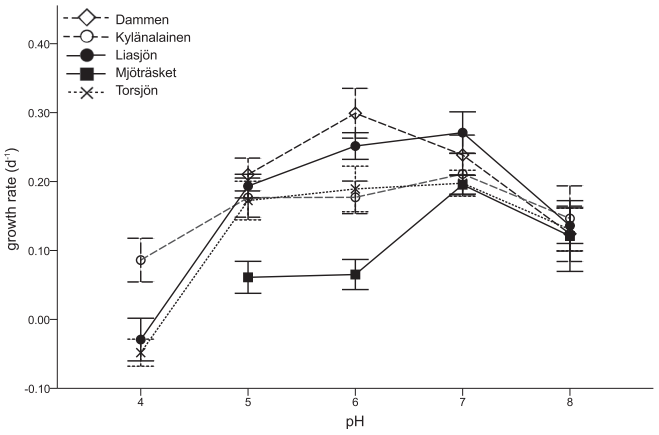

Fig. 2. Growth rates (day^{-1}) of 12 *G. semen* strains at five different pH levels. Strains are pooled by lake of origin. Error bars represent standard errors.

differences in over-all growth rates between 10, 20 and 30 mg C l^{-1} (one-way ANOVA, $p = 0.822–0.999$). Strain TO01 from lake Torsjön was excluded from all further analysis, as it did not grow in any treatments with added DOC. There were significant differences in growth rates among strains grouped by lake of origin (mixed model ANOVA, $p < 0.001$), as strains from Mjöträsket on average grew faster in all treatments ($p < 0.001$) while strains from Liasjön grew slower ($p < 0.001$) than the other strains. Furthermore, we found a significant interaction between lake of origin and DOC concentration (mixed model ANOVA, DOC × lake, $p < 0.001$). All strains showed a clear growth optimum at DOC concentration 0, but there were differences in their performance regarding higher DOC concentrations. The growth rates of strains from lake Kylänalainen, Liasjön, Mjöträsket and Torsjön did not differ significantly between higher DOC concentrations. However, strains from lake Dammen grew significantly faster at 10, 20 and 30 mg C l^{-1} than at 40 mg C l^{-1} ($p = 0.006$, $p = 0.016$, $p < 0.001$ respectively).

3.4. Light experiment

The growth rates in the light experiment differed significantly among all tested strains (mixed model ANOVA, $p < 0.001$, Table 3). However, individual variation disappeared when strains were

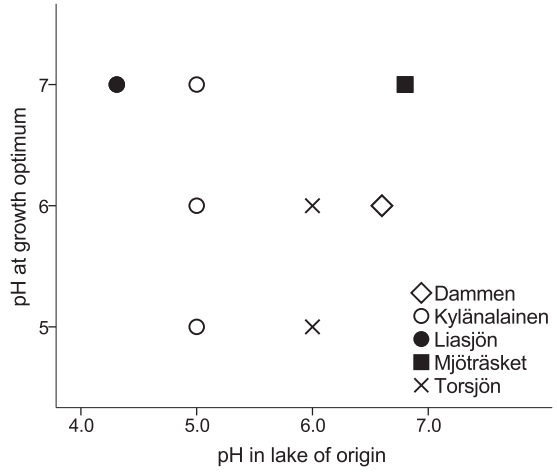

Fig. 3. pH at the growth optimum of each strain plotted against the pH in the lake of origin. Each lake is represented by either two or three strains. Dammen = DM18, DM22; Kylänalainen = KY12, KY20, KY23; Liasjön = LI21, LI22, Mjöträsket = MJ12, MJ21, MJ39, Torsjön = TO01, TO13. Line indicates 1:1 correlation between pH at growth optimum and in lake of origin.

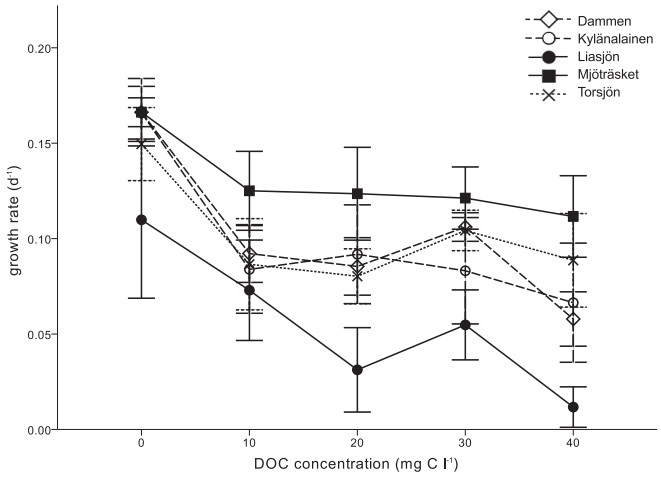

Fig. 4. Growth rates (day^{-1}) of 12 *G. semen* strains in five different DOC concentrations (mg carbon l^{-1}). Strains are pooled by lake of origin. Error bars represent standard errors.

grouped by lake of origin ($p = 0.142$). All groups showed the same trend: growth rates increased in all strains with increasing light intensity (Spearman rho correlation coefficient 0.802, $p < 0.001$). None of the strains were able to grow at 5 μmol photons m^{-2} s^{-1}. Most strains showed the lowest growth rate in the 25 μmol photons m^{-2} s^{-1} red light treatment or grew equally slow in the 10 μmol photons m^{-2} s^{-1} white light treatment. The growth rates increased significantly at 25 μmol photons m^{-2} s^{-1} white light (one-way ANOVA $p < 0.001$) and increased even farther at 150 and 200 μmol photons m^{-2} s^{-1} (Fig. 5). Growth rates increases on average eight-fold between 25 μmol photons m^{-2} s^{-1} red light and 200 μmol photons m^{-2} s^{-1} white light. Strains originating from lake Kylänanlainen were the only strains with highest growth rates at 150 μmol photons m^{-2} s^{-1} white light instead of 200 μmol photons m^{-2} s^{-1} white light, but growth rates did not differ significantly between the two treatments ($p = 0.162$). Strain LI21 from lake Liasjön was excluded from further analyses, as it did not grow in any of the treatments.

There was a significant interaction between light treatments and lake groups (mixed model ANOVA, light × lake, $p < 0.001$). Strains from lake Mjöträsket grew significantly faster at 10 μmol photons m^{-2} s^{-1} white light than strains from Kylänalainen and Liasjön, which had the slowest growth rate in this treatment ($p < 0.05$). Strains from lake Torsjön grew significantly slower than

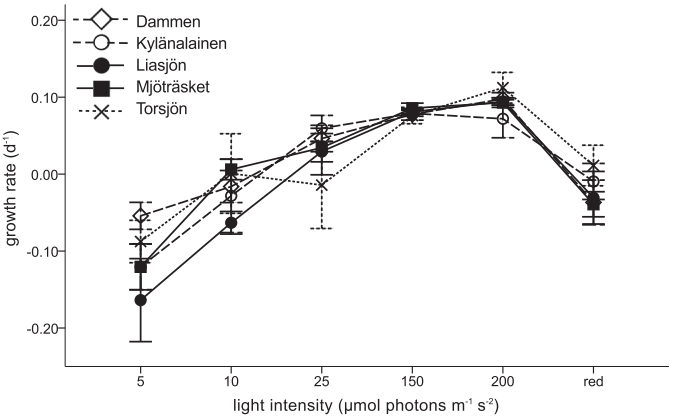

Fig. 5. Growth rates (day^{-1}) of 12 *G. semen* strains in five different light intensities: 10, 25, 150 and 200 μmol photons m^{-2} s^{-1} white light and additionally 25 μmol photons m^{-2} s^{-1} red light. Strains are pooled by lake of origin. Error bars represent standard error.

all other strains in 25 μmol photons m^{-2} s^{-1} white light ($p < 0.01$). There was no significant difference in growth rates between lake groups in the 150 μmol photons m^{-2} s^{-1} white light treatment ($p > 0.06$). At 200 μmol photons m^{-2} s^{-1} white light strains from lake Kylänalainen grew significantly slower than all other strains ($p < 0.004$) and strains from Torsjön significantly faster ($p < 0.02$). Strains from Torsjön also had a significantly higher growth rate than most other strains (except KY) in the red light treatment ($p < 0.03$).

4. Discussion

In this study we found pronounced physiological differences among strains of a single species, the nuisance microalga *Gonyostomum semen*. Our results indicate genetic differentiation in physiological traits among populations, and not only neutral genetic divergence. However, despite signatures of phenotypic differentiation among populations, the evidence for local adaptation was weak. At the same time we observed high phenotypic plasticity. Our results demonstrate the importance of using multiple strains in order to make conclusions regarding species traits.

4.1. Differences among strains and lake groups

Strains from the same lake generally behaved similar to each other and were significantly different from other lake groups in response to both pH and DOC. These repeated findings suggest genetic differentiation into population specific phenotypes. Phenotypic differentiation of *Gonyostomum semen* was especially evident in the response to varying pHs, which may represent an important selective force in each lake. Growth optima differed significantly among lake groups, suggesting preference for a single pH in some populations and broad tolerance in other populations. The growth optima of several tested strains reflected the pH in their lake of origin, especially strains from lake Dammen and lake Mjöträsket. These strains originated from lakes with close to neutral pH and could not be acclimated to pH 4 in this experiment. Strains from more acidic lakes (especially Kylänalainen), on the other hand, could tolerate pH 4 despite the presumably high physiological cost of regulating the pH in the cytoplasm. However, other strains had their growth optimum at a pH deviating from the environmental conditions in their native lake. Furthermore, we did not observe a pattern of "local" strains outcompeting "foreign" strains at their native lake pH, which is used as a diagnostic test for local adaptation. The only exception was the treatment with pH 6, where strains from lake Dammen (pH 6.6) grew faster than all other strains. These results do not provide strong support for the hypothesized local adaptation of *G. semen* to the tested environmental conditions in each lake of origin. However, based on the limited number of strains per lake in this study we cannot completely rule out local adaptation. For instance, it is possible that this species was highly plastic in its original, acidic environments, and lost part of its tolerance for a wide range of pHs when it spread to more alkaline lakes.

Growth rates of *Gonyostomum semen* strains, grouped by lake of origin, were also significantly differentiated in the DOC experiment. In the field, *G. semen* occurrence is positively correlated to high concentrations of organic carbon (Rengefors et al., 2012; Trigal et al., 2013). Surprisingly, the DOC experiment indicated that *G. semen* does not benefit from additional DOC, but instead all strains were inhibited in their growth. However, increased DOC concentrations did not enhance this effect. Rengefors et al. (2008) suggested earlier that *G. semen* might be able to utilize humic substances as an additional carbon source (heterotrophy). They found that small amounts of fulvic acids (5.2 mg C l^{-1}) increased, but higher concentrations (9.5 mg C l^{-1}) inhibited growth rates. All treatments in the current study contained much higher DOC concentrations (10–40 mg C l^{-1}), similar to concentrations in lakes with *G. semen* blooms. One possible explanation for the inhibiting effect of DOC addition may be differences in chemical characteristics between added DOC in the experiment and humic acids in the native lakes. Although we chose DOC extracted from a Scandinavian lake in order to provide a natural source, the quality of DOC can vary significantly between different habitats depending on the catchment area (Dillon and Molot, 1997). The carbon specific absorbance (CSA = absorbance(420 nm, m^{-1}) × DOC (mg l^{-1})$^{-1}$) of the original lakes (around 0.67) was higher than the CSA of the medium used in this study (0.12) suggesting more absorbing compounds per unit carbon in *G. semen* habitats (Weishaar et al., 2003).

Cultivation of microorganisms over a long time often results in genetic changes of the cultured strain by processes like genetic drift, inbreeding and adaptation to culturing conditions (Berge et al., 2012; Lakeman et al., 2009). Lohbeck et al. (2012) reported genetic changes related to an increase in CO_2 concentration in *Emiliania huxleyi* after approximately 500 generations. The *Gonyostomum semen* strains used in this study were isolated at most 3 years prior to the experiment, which represents approximately 226 generations in this species (based on the average growth rate of 0.143 day^{-1} in these experiments under standard culturing conditions). Although, potential genetic changes in the algal strains during cultivation in the laboratory have to be considered, selection pressure has been the same on all strains due to the same culturing conditions. Subsequent adaptation to these culturing conditions should have resulted in similar growth rates instead of different growth optima as observed in the experiments. Adaptation in monoclonal cultures is also assumed to be very slow because of a lack of genetic recombination due to asexual reproduction, meaning that only mutations can give rise to genetic changes. This suggests that all observed phenotypic differences are based on genetic differences the strains established prior to cultivation.

The observed phenotypic differentiation, if not caused by local adaptation, might instead reflect resilient effects of founder events in each lake (Boileau et al., 1992). A few colonizing *Gonyostomum semen* cells were likely able to rapidly establish a large population by asexual reproduction. The growing, bottlenecked population with low genetic diversity may have experienced random genetic drift. This has been suggested as a common process after colonization of a new habitat by few individuals and is an essential part of the monopolization hypothesis (De Meester et al., 2002). Furthermore, *G. semen* forms resting stages, which could act as a genetic backup in the sediment and may have buffered the local population against the impact of new invaders. These founder effects may have prevented successful establishment of invading genotypes and resulted in slow differentiation of populations.

Several studies report similar patterns in marine phytoplankton (Casabianca et al., 2012; Godhe and Harnstrom, 2010; Rynearson and Armbrust, 2004; Watts et al., 2011), indicating that population differentiation is common in microalgae. Variability due to phenotypic differentiation into distinct populations is further enhanced by high intra-population variability in *Gonyostomum semen*. Each tested strain displayed a unique phenotypic profile with characteristic growth optimum and reaction norm to a specific range of environmental variables. AFLP analysis indicated that strain specific, phenotypic differences are based on genetic differences, although genotypes did not cluster by lake of origin like the phenotypes in the pH and DOC experiment using a very limited number of strains. However, Lebret et al. (2013) reported genetically differentiation into distinct populations using a larger data set. Freshwater habitats might provide equal or even stronger

dispersal barriers and potential for differentiation than marine habitats due to less connectivity of water bodies and large variation in environmental variables. The functional diversity of microorganisms in freshwater may therefore be considerably larger than is presently assumed based mainly upon a limited number of tested strains. Thus, using multiple algal strains in physiological experiments may be essential for evaluating species traits.

4.2. Plasticity

This study demonstrated that *Gonyostomum semen* can grow over a wide range of environmental conditions, which suggests high phenotypic plasticity of this species. Plasticity is defined as variability in a phenotypic trait of the same genotype at different environmental conditions (Pigliucci, 2005; Richards et al., 2006). Heterogeneous environments select for plasticity (Hollander, 2008) and several aquatic organisms, therefore, display high tolerance to variable conditions (Strom et al., 2013). There are extensive fluctuations in light intensity during the day and between seasons (Dubinsky and Stambler, 2009; Reusch and Boyd, 2013), substantial differences in temperature, predation and nutrient concentrations in the water column and the pH in freshwater habitats varies as well (Erlandsson et al., 2008). Furthermore, organisms with high dispersal rates perceive high environmental heterogeneity also on a spatial scale (Sultan and Spencer, 2002). Menden-Deuer and Rowlett (2014) suggested that plasticity, which results from intra-specific variability, allows multiple species to coexist and explains the paradox of the plankton (Hutchinson, 1961). Individual variability maintains high functional diversity within microbial species and may open many options to persist in a habitat despite constant competition with several other planktonic species (Menden-Deuer and Rowlett, 2014).

We expected *Gonyostomum semen* to be adapted to low light intensities, as several field studies showed that *G. semen* blooms are correlated to high DOC concentrations and strong absorbance of light in the water column (Findlay et al., 2005; Trigal et al., 2013). Our data instead suggest that *G. semen* can photosynthesize over a wide range of light intensities due to photoacclimation. In comparison, several other freshwater microalgae become light saturated at irradiances between 40 and 100 μmol photons $m^{-1}\,s^{-1}$ (Kirk, 2011) while *G. semen* grew well at 200 μmol photons $m^{-2}\,s^{-1}$. However, the maximal light intensity in this experiment (200 μmol photons $m^{-2}\,s^{-1}$) did not reflect the maximal possible light intensity in lakes in Sweden (up to 1000 μmol photons $m^{-2}\,s^{-1}$ close to the surface; Hellström, 1991) and *G. semen* might actually experience photoinhibition in nature. In a previous study we showed that *G. semen* changes its pigment concentrations to acclimate to shifts in irradiance and photoprotective pigments like alloxanthin and diadinoxanthin may absorb excessive light energy (Sassenhagen et al., 2014). In addition *G. semen* has the ability to migrate in the water column and actively choose the right intensity for photosynthesis.

Most *Gonyostomum semen* strains in this study were able to grow over the wide pH range observed in its natural habitats (pH 4–7) and additionally at pH 8. So far, *G. semen* has not been found in such alkaline lakes (pH > 7), but our data suggest that it might be able to establish there, if other environmental conditions are favourable as well. Especially acidic environments are challenging for aquatic organisms, as they have to maintain a close to neutral pH in the cytoplasm despite the surrounding lower pH (Nixdorf et al., 2001). Additionally, dissolved inorganic carbon concentrations are often low in acidic waters, as it exists mainly in form of dissolved CO_2, when pH is below 6 (Wetzel et al., 1985). Some microalgal taxa like *G. semen*, freshwater Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae and Euglenophyceae can cope with these environmental conditions (Lessmann et al., 2000), but species diversity of algae has been reported to be reduced at low pH. Several cyanobacteria, diatoms, and a few green algal species can only be found in lakes with a pH above 5.0 (Blouin, 1989).

Highly plastic organisms, like *Gonyostomum semen*, usually perform well in a variety of habitats, as they can rapidly acclimate to new environmental conditions (Pigliucci, 2005; Reusch and Boyd, 2013; Schaum et al., 2013). Plastic species might have a considerable advantage over coexisting species in variable environments (Kremer and Klausmeier, 2013). For example, Stomp et al. (2008) showed that the cyanobacterium *Pseudanabaena* sp. outcompetes other cyanobacteria with stable pigmentation by chromatic adaptation during fluctuating light conditions. Several studies suggest that phenotypic plasticity might play an important role in successful invasions (Richards et al., 2006) and it could have facilitated the recent colonization of new habitats by *G. semen*, as this species can tolerate a wide range of pH and light conditions.

5. Conclusion

This study shows that *Gonyostomum semen* is highly plastic due to intra-specific variability and can grow over a wide range of environmental conditions. It might not actually prefer the acidic, humic lakes with little light penetration it originated from. Instead, *G. semen* seems to prefer habitats with neutral pH and high light intensity. However, extensive blooms of this species are still correlated to low pH and high DOC concentrations, as it probably has competitive disadvantages in phytoplankton communities of clear water lakes due to its relatively slow growth rate and high sensitivity to turbulences. At the same time, our results indicate that the genetic differentiation observed among population in Lebret et al. (2013) likely is reflected in phenotypic differentiation as well. Differential selection or founder effects probably have produced distinct populations with unique physiological characteristics.

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