



# A hypothesis linking chrysophyte microfossils to lake carbon dynamics on ecological and evolutionary time scales



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## ARTICLE INFO

### Article history:

Received 23 October 2012

Received in revised form 30 August 2013

Accepted 23 September 2013

Available online 1 October 2013

### Keywords:

chrysophytes

siliceous microfossils

paleolimnology

lake carbon dynamics

global environmental change

Eocene

## ABSTRACT

Chrysophyte algae are common in the plankton of oligotrophic lakes and produce a rich microfossil record of siliceous cysts and scales. Paleolimnological investigations and phytoplankton records suggest that chrysophyte populations are increasing in a wide range of boreal and arctic lakes, ultimately representing one component of the limnological response to contemporary global changes. However, the exact mechanisms responsible for widespread increases of chrysophyte populations remain elusive. We hypothesize that recent increases in chrysophytes are related to rising  $p\text{CO}_2$  in lakes, in part because these algae lack carbon concentrating mechanisms and therefore rely on diffusive entry of  $\text{CO}_2$  to Rubisco during photosynthesis. We assessed the abundance of modern sediment chrysophyte microfossils in relation to summer  $\text{CO}_2$  relative saturation in 46 New England (USA) lakes, revealing significant positive relationships for both cysts and scales. These observations imply that correlations between chrysophytes and limnological conditions including low pH, oligotrophy, and elevated dissolved organic matter are ultimately underscored by the high  $p\text{CO}_2$  associated with these conditions. In lakes where chrysophyte populations have expanded over recent decades, we infer that increasingly heterotrophic conditions with respect to  $\text{CO}_2$  have stimulated production by these organisms. This linkage is supported by the remarkable abundance and diversity of chrysophytes from middle Eocene lake sediments, deposited under atmospheric  $\text{CO}_2$  concentrations significantly higher than present. The Eocene assemblages suggest that any chrysophyte- $\text{CO}_2$  connection borne out of results from modern and sub-recent sediments also operated on evolutionary time scales, and thus the absence of carbon concentrating mechanisms appears to be an ancient feature within the group. Chrysophyte microfossils may potentially provide important insights concerning the temporal dynamics of carbon cycling in aquatic ecosystems.

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## 1. Introduction

Chrysophytes are primarily photosynthetic, occasionally mixotrophic, heterokont algae with silica metabolism. Although chrysophytes are widely distributed, they are most commonly associated with oligotrophic, low pH, or humic lake, pond, and river ecosystems (Siver, 1995; Kristiansen, 2005). Chrysophytes include two Classes, Chrysophyceae and Synurophyceae, for which all taxa produce endogenous siliceous cysts as a resting stage, sometimes referred to as stomatocysts or statospores. Some members of the Chrysophyceae and all synurophytes also produce siliceous scales. The number of scales sheathing an individual cell varies tremendously, ranging from 25 to 200 (Siver, 1991). Together, cysts and disarticulated scales form a robust fossil record for the chrysophyte algae, from which past environmental conditions can

be inferred, most notably lake water pH and covariates (Smol, 1995; Paterson et al., 2002; Siver, 2002).

Paleolimnological analyses (Wolfe and Perren, 2001; Paterson et al., 2004; Köster et al., 2005; Laird and Cumming, 2008; Ginn et al., 2010; Thienpont et al., 2013) and multi-decadal phytoplankton monitoring records (Findlay et al., 2001; Paterson et al., 2008) indicate marked recent increases in chrysophyte abundance across many arctic and boreal lake regions. These authors have advanced several hypotheses to explain this trend, including the effects of climate warming on lake stratification patterns, changes in nutrient availability, diminished grazing pressures, and altered ultraviolet radiation regimes associated with dissolved organic carbon (DOC). However, none of these explanations is entirely satisfactory because most chrysophytes are highly motile flagellates that are able to migrate opportunistically to the most suitable microhabitats in the water column (Nicholls, 1995). Furthermore, many taxa, particularly within the Chrysophyceae, supplement photosynthesis with saprotrophic or phagotrophic nutritional strategies, implying a diminished role for dissolved nutrient concentrations in these instances (Raven, 1995). Furthermore, although these changes are geographically widespread, they are not necessarily expressed in

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all lakes of a given region. This implies that the underlying mechanism is somewhat decoupled from the direct effects of climate change on limnological regimes, and instead relates to processes that may differ considerably between individual lakes, such as those underpinned by biogeochemical cycling.

A conspicuous trait of chrysophyte physiology is the lack of any known enzymatic carbon concentrating mechanism (CCM; usually one or more carbonic anhydrases) to convert  $\text{HCO}_3^-$  to  $\text{CO}_2$  when accessing ribulose biphosphate carboxylase–oxygenase (Rubisco; Saxby-Rouen et al., 1998; Bhatti and Colman, 2005; Raven et al., 2005). During photosynthesis, chrysophytes are therefore metabolically dependent on direct diffusive entry of  $\text{CO}_2$  to Rubisco, which is ultimately modulated by ambient  $p\text{CO}_2$ . This biochemical pathway appears to be shared among all photosynthetic chrysophytes and likely represents a plesiomorphic character for the group as a whole (Maberly et al., 2009). This contrasts sharply with other clades of aquatic algae that rely strongly upon CCMs, including cyanobacteria, chlorophytes, diatoms, and most rhodophytes (Giordano et al., 2005). Although the exact reason for the absence of CCMs in chrysophytes remains unspecified, it may suggest that they initially evolved during high  $\text{CO}_2$  climatic regimes (Raven et al., 2012). Accordingly, chrysophytes are predicted to be favored under conditions of high limnetic  $p\text{CO}_2$  because they are not metabolically encumbered with the cost of expressing CCMs, as are the co-occurrent photosynthetic algae with which they compete.

From the above observations, we develop and evaluate an alternate hypothesis for the documented increase of chrysophyte populations over recent decades. We propose that rising lake water  $p\text{CO}_2$  is the proximate cause for these observations, thereby bridging studies of chrysophyte physiology, performed on axenic cultures under controlled laboratory conditions, with detectable responses at the lake ecosystem scale. While we do not deny the potential importance of any of the limnological factors mentioned above, or of their myriad interactions in both space and time, our objective is to provide a unifying framework that is broadly applicable to the range of lake ecosystems where chrysophyte populations are expanding. In doing so, we evaluate

chrysophytes as potential indicators of  $\text{CO}_2$  dynamics in lakes, which is a topic of interest in limnology and biogeochemistry for several reasons.

It has been established that  $\text{CO}_2$  supersaturation is very common in lakes worldwide, leading to the condition of net heterotrophy with regard to carbon cycling (Kling et al., 1991; Cole et al., 1994; Duarte and Prairie, 2005). However, it remains uncertain to what extent this condition represents a natural limnological state, or one that has been enhanced, directly or indirectly, by anthropogenic interference with the global carbon cycle (Cole et al., 2007). Two principal mechanisms may lead to increased  $p\text{CO}_2$  in lakes: (1) continuous re-equilibration with the rising  $\text{CO}_2$  content of the atmosphere owed to fossil fuel combustion and land-use changes (currently  $\sim 2$  ppm  $\text{CO}_2$  per year; NOAA, 2012); and (2) enhanced mineralization of organic carbon in lakes. Contributions from the latter are necessary for supersaturation to develop, and for lakes to become net sources of  $\text{CO}_2$  to the atmosphere. It is now recognized that organic carbon mineralization rates are highly temperature dependent and therefore predicted to increase in the future (Bergström et al., 2010; Gudasz et al., 2010; Kosten et al., 2010). While the resulting increase of lake water  $p\text{CO}_2$  can enhance whole-lake primary production (Sobrinho et al., 2009; Jansson et al., 2012), it is not clear how the community structure of primary producers is affected. For these reasons collectively, it is of considerable interest to evaluate whether sediment proxies exist that track the status of lake water  $\text{CO}_2$  through time.

## 2. Materials and methods

### 2.1. Recent paleolimnological records of chrysophyte microfossils

Recent trends in chrysophyte microfossil abundance are illustrated by stratigraphic profiles from the sediments of nine lakes, three each from the following regions: the High Arctic (northern Greenland, Ellesmere Island, and western Spitsbergen), the middle Arctic (Baffin Island and southwestern Greenland), and the boreal forest of northeastern Alberta (Fig. 1). Details concerning the various sites have been presented elsewhere (Wolfe and Perren, 2001; Michelutti et al., 2005; Hazewinkel

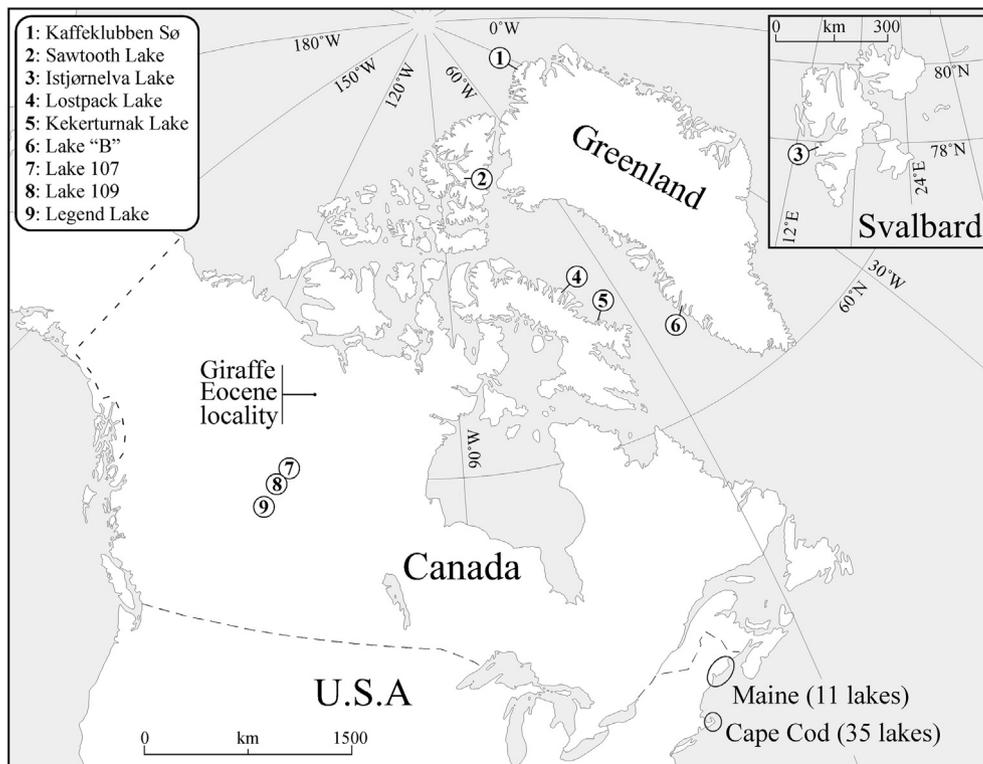


Fig. 1. Location map of the sites investigated for modern and fossil chrysophyte microfossils.

et al., 2008; Perren et al., 2009, 2012; Holmgren et al., 2010). All cores are of the gravity type, preserving the mud–water interface intact (Glew et al., 2001). Chronologies are based on excess sediment  $^{210}\text{Pb}$  activities and associated age–depth modeling (Appleby, 2001), with the exception of Kekerturnak Lake (Baffin Island), where  $^{14}\text{C}$  dates were extrapolated to the core top. In all cores, chrysophyte microfossils were enumerated and expressed as concentrations per g dry sediment by spiking acid-digested slurries with a known concentration of external markers (Wolfe, 1997).

## 2.2. Modern samples from New England lakes

Water samples and surface sediments (0–1 cm) were collected from 35 lakes on Cape Cod, Massachusetts, and an additional 11 lakes in Maine, as part of a larger survey of chrysophyte biogeography across the eastern USA (Siver and Lott, 2012). Site details and water chemistry are presented in Table 1. Lake water pH, alkalinity, and temperature were used to estimate  $p\text{CO}_2$  using standard relations for inorganic carbon equilibria (Kling et al., 1992; Stumm and Morgan, 1996). Mean values from three summer collections were used to characterize  $p\text{CO}_2$  for each lake; between-sample differences were small. Relative saturation (RS) was obtained by dividing  $p\text{CO}_2$  by the mean northern hemisphere atmospheric  $\text{CO}_2$  concentration for the year of sampling (NOAA, 2012).

Chrysophyte microfossils were prepared from sediment samples by oxidation of organic matter with a solution of  $\text{H}_2\text{SO}_4$  and  $\text{K}_2\text{Cr}_2\text{O}_7$ , followed by rinsing and settling into Battarbee chambers (Battarbee, 1973). This technique yields microfossil concentration estimates of similar precision to the spike method. Total chrysophyte cysts and synurophyte scales were enumerated per field of view in light microscopy under oil immersion ( $1000\times$ ) to tallies  $\geq 500$  microfossils, and the area counted related back to the initial dry mass of processed sediment to yield total concentrations for both microfossil groups.

Generalized linear models (GLM, McCullagh and Nelder, 1989) were used to test the significance of relationships between surface-sediment chrysophyte concentrations and lake water  $p\text{CO}_2$ , in order to relax assumptions concerning normal distributions. A first GLM was fitted to chrysophyte cyst and scale concentrations from lake populations with  $\text{CO}_2$  RS  $> 1$  ( $n = 36$ ) and RS  $< 1$  ( $n = 10$ ), in order to assess the significance of chrysophyte responses towards supersaturation versus undersaturation with respect to  $\text{CO}_2$ . A second GLM was fitted similarly but using microfossil concentrations from lake populations with alternately RS  $> 2$  ( $n = 16$ ) and RS  $< 2$  ( $n = 30$ ), thus evaluating the significance of higher degrees of  $\text{CO}_2$  saturation. These analyses were conducted using SPSS v. 9.0.1 (SPSS, 1999).

Additionally, multivariate associations between sediment chrysophyte concentrations and lake water properties were assessed using principal component analysis (PCA), an indirect ordination technique that models linear responses between variables and orthogonalized synthetic gradients, or axes (ter Braak and Prentice, 1988). The PCA included the following limnological parameters, for which measurement details are presented elsewhere (Siver and Lott, 2012): pH,  $p\text{CO}_2$ , color, conductivity, temperature, Secchi depth, and concentrations of total phosphorus (TP), total nitrogen (TN), and chlorophyll *a* (Table 1). Sediment chrysophyte microfossil concentrations (total cysts and scales per g dry mass sediment) were included as supplemental (passive) variables that plot on the ordination space defined by the environmental variables without influencing the structure of the PCA in any way. Variables were square-root transformed prior to analysis, and results were centered and standardized to variance. PCA was conducted using CANOCO v. 4.5 (ter Braak and Šmilauer, 2002).

## 2.3. Middle Eocene lake sediments

Lake sediments from the Giraffe fossil locality in northern Canada ( $64^\circ 48'\text{N}$ ,  $110^\circ 04'\text{W}$ , Fig. 1) provide the oldest known record of

freshwater chrysophytes with modern morphological and taxonomic affinities, as detailed elsewhere (Siver and Wolfe, 2005). These lacustrine sediments comprise organic shales that are middle Eocene in age, deposited between 48 and 38 Ma (millions of years before present) in the post-eruptive crater of a kimberlite intrusion. Stomatal indices from *Metasequoia* (Coniferae: Cupressaceae) foliage preserved in these sediments suggest that atmospheric  $\text{CO}_2$  concentrations were between 700 and 1000 ppm at the time of deposition, fostering a warm temperate climate regime (Doria et al., 2011). Using field-emission scanning electron microscopy (SEM), we investigated chrysophyte microfossils from unprocessed fractures and surfaces of shale bedding planes, as well as from slurries after oxidation of the organic matrix.

## 3. Results

### 3.1. Recent stratigraphic trends in chrysophyte microfossils

Paleolimnological records demonstrate that several-fold increases of chrysophyte microfossil concentrations are recorded in recently deposited sediments from many arctic and boreal lakes (Fig. 2). These trends are unrelated to biogenic silica dissolution, given that well preserved diatom floras exist below the initial increase of chrysophyte cysts and scales. In the arctic sites, this trend is primarily, but not exclusively, expressed by cyst concentrations, whereas in the boreal lakes scales of the genera *Mallomonas* and *Synura* increase markedly. Because of differences in sediment accumulation rates, chrysophyte concentrations are not directly comparable between these lakes and vary by several orders of magnitude. However, similar stratigraphic trends are obtained if the data is presented as the ratio of chrysophyte microfossils to diatom frustules (Smol, 1985). However, normalization to diatom numbers may introduce artifacts that do not relate wholly to the chrysophytes, favoring the use of sediment absolute abundances (concentrations) in the current analysis.

Several specifications are required concerning the illustrated paleolimnological profiles. First, the depicted increases in chrysophytes are not taxonomically specific, but rather reflect increased production from whichever taxa represent the group as a whole within the lake in question. Second, the upward inflection of chrysophyte microfossil concentrations in these cores is highly time-transgressive, beginning in the late 19th century in some lakes, but much later in others (e.g., AD 1950–1990, Fig. 2). This asynchrony suggests that these stratigraphic changes are not mediated directly by rising atmospheric  $\text{CO}_2$  and attendant warming, but instead are driven by biogeochemical processes that have evolved in a lake-specific manner.

In each of these regards, the illustrated trends (Fig. 2) are entirely consistent with observations from southern Ontario, New Brunswick, and Nova Scotia (Paterson et al., 2004; Ginn et al., 2010), as well as unpublished data from lakes in the Peruvian Andes and the Hudson Bay lowlands (N. Michelutti and W.O. Hobbs, personal communications). Taken together, the paleolimnological data indicate that increased chrysophyte production is a widespread and important recent ecological change in a broad spectrum of lakes.

### 3.2. Modern chrysophyte microfossils and lake $p\text{CO}_2$

The  $p\text{CO}_2$  values obtained from the 46 New England lakes range from 92 to 3136  $\mu\text{atm}$  (mean:  $868 \pm 803 \mu\text{atm}$ ; median: 577  $\mu\text{atm}$ ). Of these, 36 lakes (that is, 78%) were supersaturated relative to atmospheric  $\text{CO}_2$  at the time of sampling, many strongly so. For example, 16 lakes had RS  $> 2$ , and five had RS  $> 5$ . The distribution of RS values from these 46 lakes (Fig. 3A) parallels compilations from other regions globally (Cole et al., 1994; Duarte and Prairie, 2005), and is therefore deemed unexceptional.

Chrysophyte microfossil concentrations in the surface sediments of these lakes are highly variable, an expected result given between-lake differences in sedimentation rates, herbivory, dispersal mechanisms,

**Table 1**  
Locations and raw data from the 46 New England lakes.

Lake name	Latitude	Longitude	Elevation (m)	pH	Alkalinity ( $\mu\text{eq L}^{-1}$ )	Temperature ( $^{\circ}\text{C}$ )	DIC ( $\mu\text{Mol}$ )	$\text{CO}_2$ ( $\mu\text{Mol}$ )	$\text{pCO}_2$ ( $\mu\text{atm}$ )	$\text{CO}_2$ RS	Cysts ( $\times 10^5 \text{ g}^{-1}$ )	Scales ( $\times 10^5 \text{ g}^{-1}$ )	Secchi (m)	Cond. ( $\mu\text{S cm}^{-1}$ )	Chl. a ( $\mu\text{g L}^{-1}$ )	Color (Pt units)	Total P ( $\mu\text{g L}^{-1}$ )	Total N ( $\mu\text{g L}^{-1}$ )
Ashumet	N 41° 38' 03.05"	W 70° 32' 25.87"	13	7.52	121.0	22.9	129.3	8.5	235	0.6	2.73	0.92	3.6	93	4.38	10	21	308
Baker	N 41° 45' 58.60"	W 70° 00' 03.76"	6	6.61	25.7	25.5	39.8	14.1	423	1.2	6.96	7.95	5.7	94	1.08	10	7	191
Clapps	N 42° 03' 09.24"	W 70° 12' 25.00"	1	5.21	4.7	23.8	70.4	65.7	1870	5.1	7.01	4.96	4.6	102	4.23	5	11	209
Cliff	N 41° 45' 35.66"	W 70° 01' 07.78"	8	7.03	15.3	23.1	18.6	3.3	92	0.3	2.12	4.39	4.7	69	12.50	10	15	298
Crooked	N 41° 36' 30.10"	W 70° 34' 59.20"	9	6.92	77.3	23.6	98.7	21.4	605	1.7	4.19	10.87	3.7	82	2.34	20	9	211
Crystal-O	N 41° 46' 33.98"	W 69° 59' 09.54"	5	6.44	14.5	25.3	26.2	11.7	349	1.0	2.39	2.60	4.3	111	1.62	10	9	251
Deep	N 41° 37' 34.15"	W 70° 34' 50.27"	11	6.87	68.7	24.3	89.5	20.8	602	1.6	2.28	6.66	5.3	79	1.29	10	9	425
Depot	N 41° 49' 51.75"	W 69° 58' 40.10"	3	6.64	42.7	25.4	64.5	21.9	654	1.8	1.50	2.63	3.5	120	2.03	15	11	291
Flax-B	N 41° 45' 57.85"	W 70° 01' 23.26"	7	6.58	29.7	24.0	47.3	17.7	505	1.4	2.96	12.88	4.4	91	2.18	10	12	186
Great-E	N 41° 49' 38.11"	W 69° 59' 01.41"	2	7.12	124.7	24.6	146.0	21.4	626	1.7	1.00	0.89	3.0	143	4.38	28	17	328
Grews	N 41° 34' 05.85"	W 70° 36' 44.96"	3	6.77	44.7	23.9	62.0	17.3	494	1.4	1.81	3.86	4.8	54	1.42	10	16	358
Gull	N 41° 57' 19.34"	W 70° 00' 42.99"	2	6.80	55.7	21.8	76.6	21.0	560	1.5	0.00	3.40	5.2	146	0.92	3	18	261
Hamblin	N 41° 39' 47.65"	W 70° 24' 36.94"	13	7.13	82.7	22.8	97.0	14.3	395	1.1	0.73	4.69	4.7	76	3.07	12	9	228
Herring-W	N 41° 57' 37.20"	W 70° 00' 35.53"	2	6.59	94.7	21.4	153.4	58.7	1548	4.2	5.58	0.47	3.0	147	4.05	10	17	261
Higgins-B	N 41° 45' 32.97"	W 70° 28' 55.42"	7	6.43	10.8	25.0	19.7	8.9	264	0.7	2.46	4.20	6.2	85	1.68	5	8	161
Horseleech	N 41° 58' 10.38"	W 70° 00' 15.08"	2	6.31	27.0	21.8	58.5	31.5	838	2.3	1.30	0.39	3.8	206	1.39	4	8	222
Hoxie	N 41° 43' 47.34"	W 70° 26' 20.78"	9	6.49	131.0	24.2	226.4	95.4	2753	7.5	2.23	30.63	4.4	101	1.55	20	10	303
Jenkins	N 41° 35' 58.92"	W 70° 34' 51.02"	6	6.95	89.3	43.2	105.0	15.7	837	2.3	1.77	21.42	5.6	83	1.40	5	8	215
Little Cliff	N 41° 45' 34.14"	W 70° 00' 49.90"	8	6.03	6.9	24.0	21.6	14.7	421	1.2	1.10	0.31	6.0	75	0.70	2	8	161
Lover's	N 41° 41' 48.39"	W 69° 58' 58.28"	4	7.96	204.0	25.4	208.1	5.0	149	0.4	0.58	0.45	2.6	154	5.28	25	18	267
Mares	N 41° 35' 02.02"	W 70° 36' 05.85"	4	6.64	24.0	24.1	36.5	12.5	359	1.0	0.00	7.42	5.1	64	3.45	10	7	196
Mashpee	N 41° 39' 08.33"	W 70° 28' 55.42"	17	7.33	125.3	22.8	139.0	13.8	379	1.0	1.39	0.80	4.1	81	2.61	8	15	242
Minister	N 41° 50' 24.88"	W 69° 58' 29.38"	4	6.28	91.0	24.5	198.8	107.8	3136	8.6	3.15	11.98	1.9	206	5.82	20	30	416
Mystic	N 41° 41' 03.34"	W 70° 24' 51.16"	13	7.06	151.0	24.8	180.2	29.3	859	2.4	0.73	0.54	3.9	89	2.38	9	18	229
Peters	N 41° 41' 07.60"	W 70° 29' 07.63"	20	7.06	159.7	24.9	191.0	31.4	924	2.5	1.59	29.49	4.8	92	1.93	10	10	222
Scargo	N 41° 44' 35.19"	W 70° 11' 00.19"	4	7.20	108.0	25.7	122.9	15.0	454	1.2	0.95	0.00	5.2	114	2.17	6	9	261
Schoolhouse	N 41° 41' 40.97"	W 69° 59' 34.37"	4	7.09	79.7	25.4	93.9	14.2	426	1.2	0.00	0.00	4.1	91	1.88	10	11	291
Sheep	N 41° 44' 00.20"	W 70° 04' 46.07"	10	6.64	15.8	24.7	23.9	8.1	236	0.6	2.22	1.01	9.4	71	1.09	1	6	163
Shubael	N 41° 40' 22.42"	W 70° 23' 25.62"	13	6.92	61.7	23.8	78.5	16.8	479	1.3	3.84	8.48	5.0	98	2.60	5	10	280
Snake	N 41° 40' 44.41"	W 70° 31' 05.13"	21	6.51	29.3	23.8	50.2	20.9	595	1.6	1.39	12.97	4.5	61	3.95	15	11	212
Spectacle-F	N 41° 35' 11.44"	W 70° 35' 48.66"	9	6.55	33.0	24.4	54.0	21.0	610	1.7	0.81	10.19	4.4	74	1.51	15	8	272
Spectacle-S	N 41° 41' 59.23"	W 70° 27' 34.17"	19	6.44	21.3	24.1	38.8	17.5	502	1.4	0.85	3.79	5.3	61	4.68	6	10	213
Triangle	N 41° 41' 50.83"	W 70° 27' 19.91"	19	6.52	14.3	25.7	23.9	9.6	289	0.8	3.41	3.14	4.3	64	1.53	15	11	180
Wakeby	N 41° 40' 18.50"	W 70° 28' 38.11"	17	7.37	132.7	23.0	145.6	13.0	361	1.0	0.78	0.40	3.5	83	3.62	9	16	243
White	N 41° 41' 25.25"	W 69° 39' 00.74"	4	7.29	137.7	25.4	153.3	15.8	472	1.3	0.92	4.27	3.8	186	2.37	20	14	373
Boyd	N 43° 55' 41.54"	W 69° 30' 08.27"	99	6.30	103.0	18.3	234.3	131.4	3114	8.3	6.95	52.11	0.8	44	3.24	31	<1	271
Bubble	N 44° 20' 51.03"	W 68° 14' 40.26"	112	6.65	50.6	14.3	81.7	31.1	649	1.7	8.12	4.17	7.0	30	1.01	2	<1	92
China	N 44° 23' 43.96"	W 69° 34' 36.16"	64	7.25	36.7	15.3	42.2	5.6	120	0.3	0.97	4.35	0.9	81	2.89	9	<1	230
Cranberry	N 45° 04' 01.95"	W 67° 18' 03.65"	66	6.36	119.0	17.2	254.0	135.0	3089	8.3	2.70	6.07	2.3	23	1.99	87	<1	375
Crystal	N 44° 17' 36.02"	W 69° 23' 56.89"	124	6.24	48.9	17.3	121.9	73.0	1675	4.5	14.35	29.32	0.8	34	2.54	20	<1	209
Hogdon	N 44° 18' 49.68"	W 68° 24' 04.50"	13	6.17	44.0	16.0	123.1	79.1	1742	4.7	7.90	8.51	4.7	46	3.59	55	<1	240
James	N 45° 01' 55.39"	W 67° 16' 45.97"	66	6.30	43.0	16.1	100.2	57.2	1263	3.4	9.05	31.04	3.1	21	2.54	45	<1	186
Ross	N 43° 55' 13.50"	W 69° 29' 58.10"	40	6.10	35.0	18.6	105.3	70.3	1683	4.5	14.58	32.91	1.0	34	1.90	24	<1	262
Vose	N 45° 07' 31.12"	W 67° 15' 10.31"	45	6.84	115.0	17.6	157.8	42.9	993	2.7	4.32	9.65	3.0	24	1.76	23	<1	157
Witch Hole	N 44° 23' 53.38"	W 68° 14' 57.62"	59	6.21	13.3	16.7	34.8	21.5	485	1.3	3.73	3.34	0.9	25	1.00	42	<1	302
Wood	N 44° 24' 30.67"	W 68° 16' 25.35"	57	6.49	41.0	18.2	74.8	33.8	799	2.1	4.80	3.60	3.8	22	0.83	7	<1	109

and resource competition among co-occurring algal groups. However, the highest concentrations of both cysts and scales consistently arise in CO<sub>2</sub>-supersaturated lakes (Fig. 3B–C). For example, none of the sediments from CO<sub>2</sub>-undersaturated lakes produced cyst concentrations >4 × 10<sup>5</sup> g<sup>-1</sup> or scale concentrations >8 × 10<sup>5</sup> g<sup>-1</sup>, although considerably higher concentrations were commonly encountered in supersaturated lakes. Both cyst and scale concentrations are significantly different between populations of CO<sub>2</sub>-undersaturated and supersaturated lakes, as determined by the corresponding GLM ( $P = 0.025$  for cysts;  $P = 0.018$  for scales). The level of significance rises dramatically when the GLM is fitted between lake populations above and below a threshold CO<sub>2</sub> RS of 2 ( $P = 0.001$  for cysts;  $P < 0.001$  for scales).

Ordination by PCA places these observations in the broader context of other limnological variables (Fig. 4). The first axis explains 51.8% of variance and captures a gradient that separates lakes of high TP, pH, temperature, transparency (as Secchi depth), and total dissolved solids (as conductivity) from those that are strongly colored, have low pH, and high pCO<sub>2</sub>. The second axis (28.6% variance explained) captures primarily the influences of TN and total primary production (as chlorophyll *a*). Chrysophyte cyst and scale concentrations plot strongly on PCA axis 1 (Fig. 4A), confirming their recognized association with dilute, oligotrophic, colored and low pH environments (Siver, 1995, 2002; Smol, 1995). Importantly, elevated pCO<sub>2</sub> is directly associated with these same conditions. Furthermore, the correlation between water color and pCO<sub>2</sub> ( $r^2 = 0.30$ ; Welch's  $t = 5.69$ ,  $P < 0.001$ ) strongly suggests that DOC is the primary driver of lake CO<sub>2</sub> in the New England lakes, as elsewhere (Cole et al., 2007; Gudas et al., 2010; Kosten et al., 2010). Accordingly, lakes from Maine, many of which are strongly colored by DOC, produce consistently higher PCA axis 1 scores relative to sites from Cape Cod (Fig. 3B). However, axis 1 does not merely differentiate lakes from either region: the underlying structure of the PCA, where chrysophyte microfossils are consistently associated with low pH, pronounced color, and high CO<sub>2</sub>, is maintained when the regions are analyzed independently.

Taken together, these data suggest that pCO<sub>2</sub> has the capacity to influence chrysophyte populations at the ecosystem scale, irrespectively of the exact taxa represented in the lake in question. We view the observed patterns as a first-order expression of the ecophysiology of photosynthetic chrysophyte, namely that, in absence of CCMs, growth of these algae is stimulated under conditions of elevated pCO<sub>2</sub>. The fossil record of chrysophytes afforded by their siliceous components thus becomes a potential paleobarometer for limnetic pCO<sub>2</sub>.

### 3.3. Middle Eocene chrysophyte assemblages

We augment this proposal with observations from the middle Eocene Giraffe fossil locality (Fig. 5). Here, cysts and scales of both extinct and extant chrysophyte taxa are profuse across tens of meters of core, in particular within sediments representing the upper and final lake stage. Over this interval, the lake was shallowing as the crater became progressively infilled. In sediments deposited prior to the final terrestrialization of the aquatic ecosystem and directly overlain by volcanic ash dated to 37.84 ± 1.99 Ma (Doria et al., 2011), chrysophyte microfossils frequently outnumber diatoms by several hundred to one. To date, the genera *Chrysophaerella*, *Mallomonas*, *Paraphysomonas*, *Spiniferomonas*, and *Synura* have been documented (Siver and Wolfe, 2005). Approximately 50 distinct synurophyte morphotaxa are present in these sediments, strongly dominated by *Mallomonas* (45 morphotypes). Over 100 cyst types are present. By comparison to individual modern lakes, this diversity is truly remarkable (Siver, 1991; Siver and Lott, 2012). The range of chrysophyte microfossils observed, coupled to their sheer abundance in both *in situ* and disaggregated preparations (Fig. 5), indicates that these occurrences are far more than episodic bloom events recorded in discrete sediment laminae. Rather, chrysophytes strongly dominated the phytoplankton of this ancient ecosystem, at a time when atmospheric pCO<sub>2</sub> was between two-

and three-fold higher than present, and furthermore the lake was progressing towards a dystrophic state for which elevated DOC concentrations are envisaged. While it remains impossible to apportion the pCO<sub>2</sub> of the Eocene lake between atmospheric and DOC influences, we infer the system to have been highly supersaturated with respect to CO<sub>2</sub>, and that the proliferation of chrysophytes was at least partially in response to this condition.

Maar sediments from the Giraffe locality are not the sole example of the apparent ecological success of chrysophytes during the middle Eocene. Marine sediments deposited in the central Arctic Ocean 45–50 Ma, inferred to represent low salinity intervals, contain abundant and diverse chrysophyte cyst assemblages, but no synurophyte scales (Stickley et al., 2008). Although the ecologies of the cyst-producing organisms remain cryptic at best, these microfossils are unambiguously chrysophyte in origin, lending support to the contention that, in a general sense, chrysophytes as a group were highly successful during Paleogene greenhouse climate episodes.

## 4. Discussion

### 4.1. Recent increases of chrysophyte populations

The survey of modern chrysophyte abundances in New England lake sediments (Figs. 3 and 4) implies that lake water pCO<sub>2</sub> has the potential to shape phytoplankton community structure in important ways, thereby linking predictions from algal physiology (Giordano et al., 2005; Maberly et al., 2009) with lake biogeochemistry (Cole et al., 1994, 2007; Duarte and Prairie, 2005). If this assertion is correct, the recent paleolimnology of chrysophyte microfossils (Fig. 2) suggests that transients towards higher lake water pCO<sub>2</sub> have occurred in numerous lakes and lake regions since the Industrial Revolution, but especially in the latter half of the 20th century. While rising atmospheric CO<sub>2</sub> is possibly involved in these changes to some extent, it is noteworthy that only under conditions of CO<sub>2</sub> supersaturation do modern chrysophyte abundances rise sharply (Fig. 3). This implies that processes beyond the re-equilibration to rising atmospheric CO<sub>2</sub> levels are involved, namely that enhanced CO<sub>2</sub> subsidies from organic matter mineralization are implicated in the observed stratigraphic changes. This inference is supported by the New England data, which show that pCO<sub>2</sub> and lake color, a direct surrogate for DOC in these lakes (Siver and Lott, 2012), are closely correlated both to each other and to chrysophyte microfossil abundance (Fig. 4A). Ultimately, the limnological changes implied by the stratigraphic data are likely under some degree of climatic control, given that the microbial processes that drive organic matter mineralization are temperature dependent (Bergström et al., 2010; Gudas et al., 2010). In forested temperate and tropical catchments, the supply of terrigenous organic matter is able to sustain mineralization rates sufficient for CO<sub>2</sub> supersaturation to prevail (Kosten et al., 2010). We also note that DOC concentrations have increased sharply in many mid-latitude lakes as the result of declining atmospheric deposition of anthropogenic sulfate, in ways that appear largely unrelated to recent warming (Monteith et al., 2007).

In the Arctic, the situation is more nuanced because terrestrial primary production is much lower, and decomposition rates are protracted by cold ambient temperatures. Here, deepening of the active layer as climate warms provides a mechanism to enhance DOC subsidies to lakes (Breton et al., 2009). Radiocarbon analyses demonstrate that legacy DOC, aged by centuries to millennia, is common in arctic lake catchments, highly mobile, and readily bio-available (Abbott and Stafford, 1996; Vonk et al., 2013). At the same time, autochthonous organic source matter must also be considered because increased primary production appears widespread in remote lakes of the Arctic (Michelutti et al., 2005; Smol et al., 2005). As this newly produced biomass is mineralized, pCO<sub>2</sub> will rise as long as lake water pH remains sufficiently low to speciate inorganic carbon primarily as CO<sub>2</sub>, and photosynthetic chrysophytes will remain competitively advantaged. Stratigraphic evidence from a variety of high-

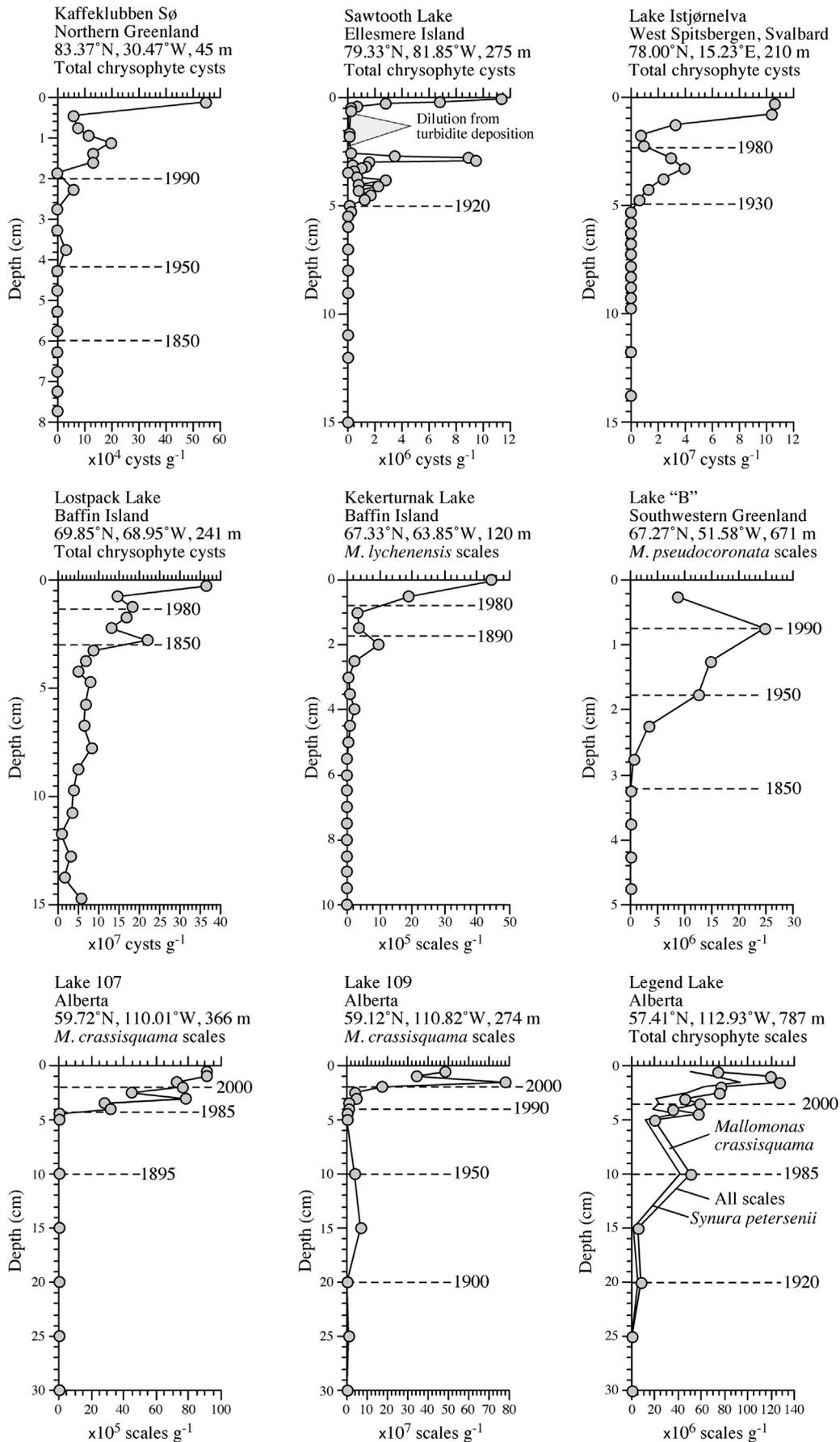
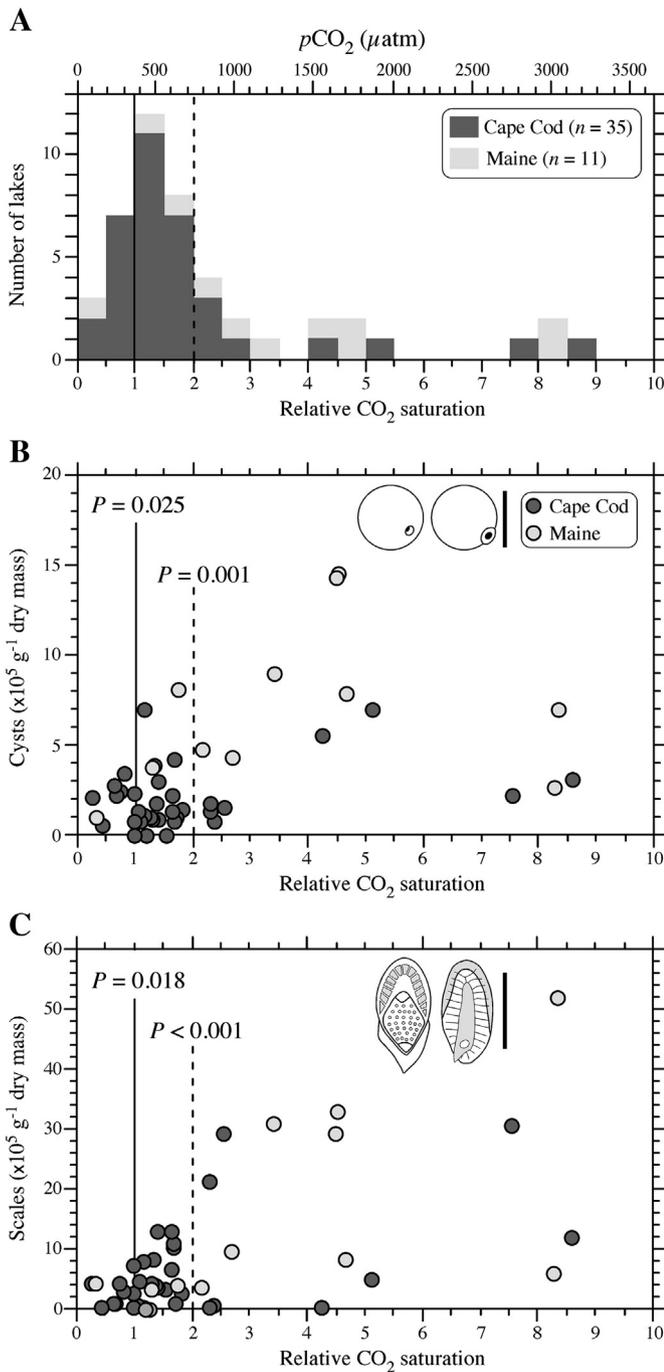


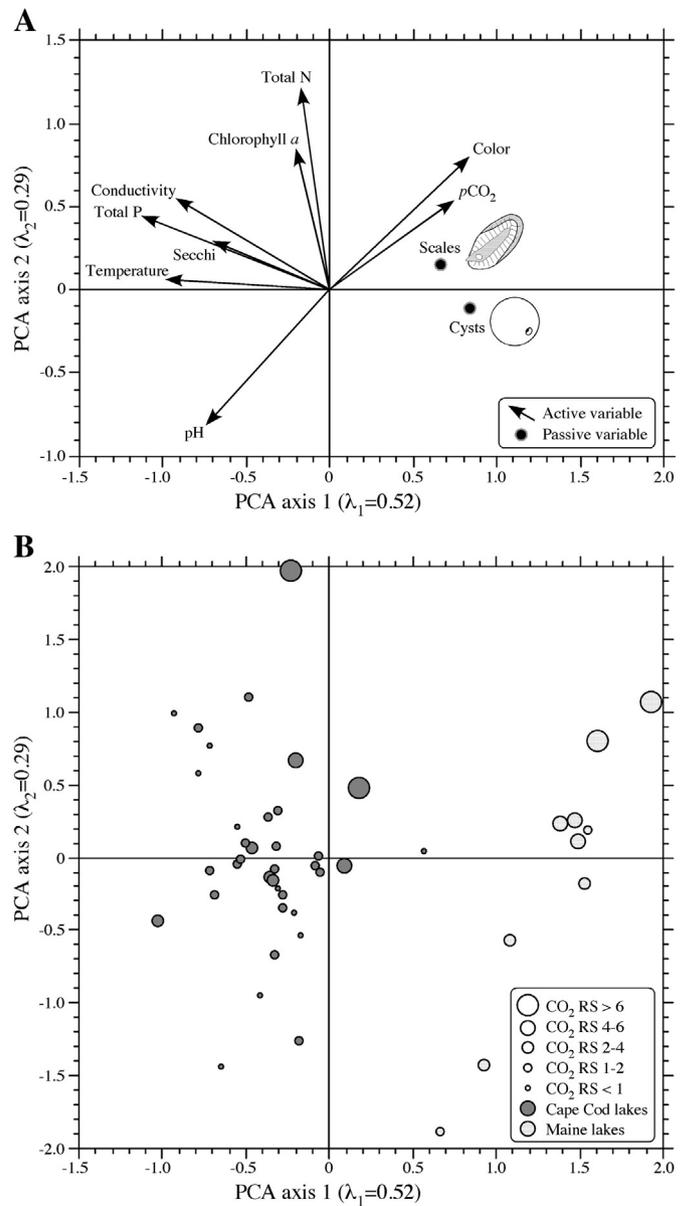
Fig. 2. Stratigraphic profiles of chrysophyte microfossil concentrations from high-arctic (upper row), mid-arctic (middle row), and boreal (lower row) lake sediment cores spanning recent centuries, with radiometric ages (in years AD) indicated by dashed lines.



**Fig. 3.** Lake water  $p\text{CO}_2$ ,  $\text{CO}_2$  relative saturation (RS), and chrysophyte microfossil concentrations from the surface sediments of 46 New England lakes. A: distribution of RS across the lake population. B: chrysophyte cyst concentrations in relation to  $\text{CO}_2$  RS. C: chrysophyte scale concentrations and  $\text{CO}_2$  RS. Vertical black lines represent  $\text{CO}_2$  saturation (RS = 1), and vertical dashed lines indicate RS = 2.  $P$ -values obtained from the various GLM analyses are provided in B and C. Insets are line diagrams of representative chrysophyte microfossils: uncollared (left) and collared (right) cysts (scale bar is 10  $\mu\text{m}$ ) in B, and *Mallomonas* (left) and *Synura* (right) scales in C (scale bar is 5  $\mu\text{m}$ ).

latitude lakes suggest that this situation has arisen in recent decades (Fig. 2), consistent with metagenomic surveys that reveal a truly impressive diversity and abundance of chrysophyte phylotypes in polar lakes (Charvet et al., 2012).

In summary, a positive feedback mechanism likely exists between lake primary production, organic matter mineralization, and limnetic  $p\text{CO}_2$ . The strength of this feedback ultimately scales with climate warming, with the ecological consequence of advantaging algae lacking

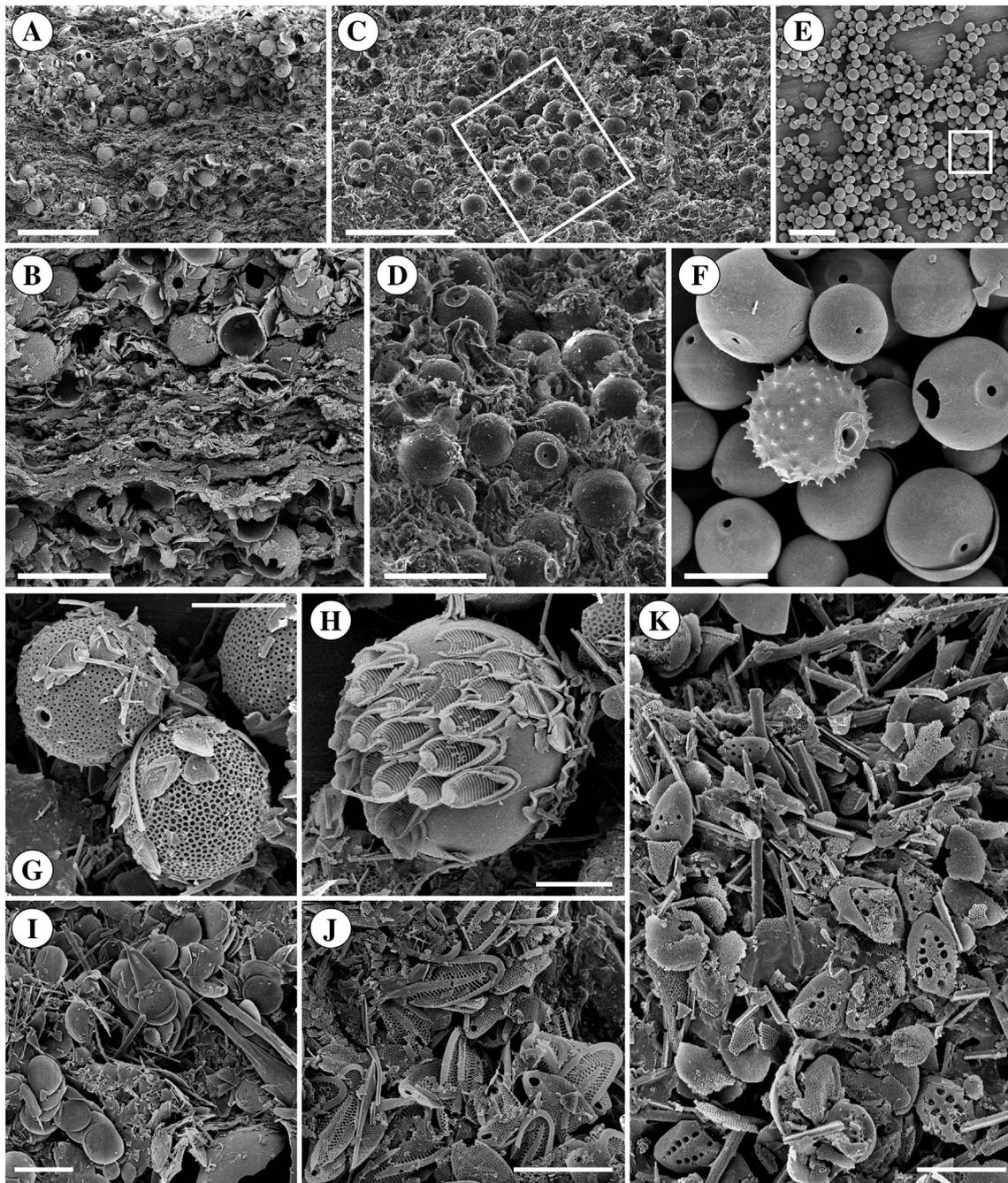


**Fig. 4.** PCA ordination results. A: scores of active (arrows) and passive (chrysophyte microfossil concentrations) parameters on the two leading axes. B: sample scores for the 46 lake population, with symbol sizes reflecting lake  $\text{CO}_2$  relative saturation.

CCMs, namely the chrysophytes, across several ecoclimatic zones. Only with attendant increases of lake alkalinity and pH, which will drive dissolved  $\text{CO}_2$  towards  $\text{HCO}_3^-$ , can this feedback be curtailed. In such a scenario, we predict that chrysophytes will be unable to remain competitive should lake  $p\text{CO}_2$  decline. However, our results to date provide no evidence for such a mechanism. For one, the inexorable rise of atmospheric  $\text{CO}_2$  guarantees that equilibrium (i.e., RS = 1) will occur at higher and higher limnetic  $\text{CO}_2$  concentrations in the future. Importantly, many of these linkages also appear to be manifested on geological and evolutionary time scales, given the apparent success of chrysophytes during Eocene greenhouse climates (Fig. 5).

#### 4.2. An evolutionary context for chrysophyte- $p\text{CO}_2$ relationships

The exceptional abundance and diversity of chrysophytes in middle Eocene sediments provide direct support for the main premise of this paper, while portending the likelihood of further expansions of these



**Fig. 5.** SEM micrographs of chrysophyte microfossils from the middle Eocene Giraffe fossil locality. A: chrysophyte cysts from an unprocessed fracture perpendicular to the bedding plane. B: higher magnification of cyst laminae separated by organic matter and clay minerals. C: unprocessed bedding plane surface. The white box represents the view magnified in D. E: cysts from an H<sub>2</sub>O<sub>2</sub>-processed sample. The white box is magnified in F, showing the range of morphotypes represented. G: Undiagnosed *Mallomonas* cysts retaining a few scales and bristles. H: *Mallomonas asmundiae* cyst with scales. I: scales of the extinct taxon *Mallomonas porifera*. J: *Synura macracantha* scales. K: *Mallomonas lichenensis* scales and bristle fragments. Scale bars are 50 μm (A, C, and E); 20 μm (B and D); 10 μm (F and G); 5 μm (H, I, J, and K).

algae in the future. Moreover, our observations also benchmark the underlying evolutionary processes accountable for the absence of CCMs in chrysophytes. On geological time scales, atmospheric CO<sub>2</sub> is likely more relevant to defining selective gradients than the limnological processes that modulate lake water pCO<sub>2</sub> over decades to centuries (Giordano et al., 2005; Raven et al., 2012). Multigene molecular clock analyses, constrained by fossil chrysophytes from the Giraffe locality, suggest important diversification events between the Triassic and Jurassic, and again during the Paleogene (Boo et al., 2010). These are precisely

intervals of elevated atmospheric CO<sub>2</sub>, at times reaching concentrations in the order of 1000–3000 ppm (Royer, 2006). As a corollary, recent phylogenetic analyses of the ribulose-bisphosphate carboxylase (*rbcl*) gene reveal strong selective pressures for Rubisco diversification in algae with CCMs during low CO<sub>2</sub> intervals, but no attendant patterns in algae lacking CCMs, including the Chrysophyceae and Synurophyceae (Young et al., 2012). This is consistent with the notion that absence of CCMs in chrysophytes is not a secondary loss but rather a plesiomorphic state. Thus, photosynthetic chrysophytes always have been, and

presumably always will be, favored by high  $p\text{CO}_2$ , irrespectively of whether it is mediated by atmospheric composition or organic matter mineralization.

#### 4.3. $\text{CO}_2$ paleobarometry and chrysophyte microfossils?

Sediment proxies sensitive to changes in lake water  $\text{CO}_2$  have hitherto been lacking. By merging clues from algal physiology, lake biogeochemistry, and paleolimnology, we offer a new proposal to explain recent trends in populations of chrysophyte algae in lakes. This integrative perspective provides a viable interpretation for currently available data, with implications not only for understanding ecological and evolutionary processes, but also for predicting future trajectories of lake phytoplankton communities. Moreover, the key premise of this paper, that chrysophytes respond sensitively to aquatic  $p\text{CO}_2$ , may be tested readily by additional analyses beyond the scope of this study. For example, we predict that recently impounded reservoirs, which are highly heterotrophic with respect to  $\text{CO}_2$  (Cole et al., 2007; Barros et al., 2011), may provide ideal habitats for chrysophyte algae. Regional analyses of changing chrysophyte populations, and by inference of lake water  $p\text{CO}_2$ , may be facilitated by exploiting paleolimnological “tops and bottoms” approaches, an efficient strategy for capturing environmental signals from a large number of lakes (Cumming et al., 1992). Establishing quantitative relationships between chrysophyte populations and lake water  $p\text{CO}_2$  remains a more challenging objective, given vicissitudes inherent to algal ecology with respect to resource competition, which are likely further complicated by the dynamic nature of carbon cycling in lakes. The present study is a first step towards assessing the temporal evolution of limnetic  $p\text{CO}_2$  using chrysophyte microfossils.

#### Acknowledgments

Funding was provided by the Natural Sciences and Engineering Research Council (Canada) and the National Science Foundation (USA; DEB-9972120, DEB-0343355, DEB-1049583 and DEB-0716606). We thank R. Hazewinkel, S. Holmgren, A.M. Lott, N. Michelutti, and B. Perren for assistance in compiling data, W. Hobbs for statistical advice and fruitful discussions, and two anonymous reviewers for their thoughtful comments.

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