



Seasonal abundance, community composition, and silica content of diatoms epiphytic on *Cladophora glomerata*

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ARTICLE INFO

Article history:

Received 17 May 2008

Accepted 8 December 2008

Communicated by Hunter Carrick

Index words:

Benthos

Cladophora

Diatom

Epiphyte

Periphyton

Silica

ABSTRACT

The establishment of dreissenid mussels in the Great Lakes has been implicated in causing a resurgence of benthic macroalgae, notably *Cladophora glomerata* (L.) Kützing. The seasonal proliferation and nutrient retention of epiphyton on *Cladophora* may be important to littoral zone processes. In this study, peak diatom epiphyte density (601 diatoms μg^{-1} dry mass) co-occurred with peak *Cladophora* biomass. The exceptionally adhesive *Cocconeis pediculus* came to dominate the epiphyton to the near exclusion of all other diatoms by early summer, indicating powerful constraints on the epiphyte assemblage. There was a strong relationship between epiphytic silica content and epiphyte abundance, but the relationship was different between seasons. In the spring and fall when *Cladophora* was growing vigorously, silica content of the diatom epiphytes was 20.1–25.6 $\text{pmol Si diatom}^{-1}$. In the summer, during *Cladophora* senescence, silica content was 6.5–10.0 $\text{pmol Si diatom}^{-1}$ and valves were visibly thinner. These observations suggest that diatom epiphytes may be limited by Si during peak *Cladophora* biomass. Areal estimates of silica content of *Cladophora* epiphyton was strongly related with *Cladophora* biomass: at 2 m depth, silica content increased from 22.7 mmol Si m^{-2} in the spring to 490 mmol Si m^{-2} during peak *Cladophora* biomass. Silica content can be a valuable proxy for diatom epiphyte abundance if the vitality of the *Cladophora* substrate is considered. Future work needs to assess the seasonality of pelagic versus benthic silica demand and the interannual variability of epiphyte silica content to assess how changes in *Cladophora* biomass may affect nearshore Si cycling.

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Introduction

Epiphytes often have high biomass-specific production rates and can achieve high biomass, allowing them to be major contributors to littoral or coastal production (Allen, 1971; Hickman, 1971; Cattaneo and Kalff, 1980; Moncreiff et al., 1992). The nature and magnitude of the interactions between epiphytes and their macrophyte substrates are complex, but have been investigated extensively along gradients of nutrient availability and invertebrate grazer assemblage and abundance (McCormick and Stevenson, 1989; Power, 1991; Dodds, 1991; Dudley, 1992). As a consequence of their high biomass and high productivity, epiphyton may be important reservoirs of sequestered nutrients in nearshore environments, an area of research that has received comparatively much less investigative attention. Although epiphyton generally appear to be important vectors for transferring energy to higher trophic levels (Wetzel, 2001; Moncreiff and Sullivan,

2001), few generalities on their role in nutrient retention, especially with seasonal resolution, have emerged.

In littoral zone areas of the lower Laurentian Great lakes underlain by hard substrate, *Cladophora glomerata* (L.) Kützing, a filamentous green alga, grows profusely each year commencing in the late spring (Higgins et al., 2005; Malkin et al., 2008). This macroalga is a nuisance from early summer through to the autumn when it detaches and decays, fouling the nearshore environment (Wilson et al., 2006; Higgins et al., 2005), clogging water intakes, potentially creating local areas of hypoxia (Turner et al., 1995), and promoting (potentially pathogenic) bacterial growth (Byappanahalli et al., 2003). In the 1960s and 1970s, *Cladophora* was abundant in the lower Great Lakes at densities considered to be a nuisance. The implementation of lakewide phosphorus abatement programmes was largely successful in remediating the magnitude of this nuisance (reviewed in Higgins et al., 2008). However, since the invasion and the prolific expansion of dreissenid mussels (i.e., the zebra mussel, *Dreissena polymorpha*, and the quagga mussel, *D. bugensis*) in the lower Great Lakes since the late 1980s, a resurgence in *Cladophora* biomass in the lower Great Lakes has been observed (Higgins et al., 2008). Due to increased water clarity associated with efficient filtration of the water column by dreissenid mussels, and potentially due to increased nutrient retention in the benthos due to mussel feeding and excretion, the

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dreissenid mussels have been credited with promoting greater benthic primary production, especially of the fast-growing *Cladophora* (Hecky et al., 2004; Zhu et al., 2006; Higgins et al. 2008). In recent years, this process of benthification (*sensu* Zhu et al., 2006) has promoted *Cladophora* biomass in the Great Lakes to reach densities as high as 940 g m^{-2} at shallow well-lit depths in Lake Erie (Higgins et al. 2005), although peak biomass in Lake Ontario in an area distant from wastewater treatment plants and tributary runoff was found to reach a maximum closer to 120 g m^{-2} at shallow depths (Malkin et al. 2008).

C. glomerata, and its associated epiphytes, are the dominant flora on rocky substrates in the littoral zone of Lake Ontario from late spring through autumn. With the establishment of dreissenid mussels, nutrient cycling in the nearshore has been altered, resulting in an increase in benthic production in the nearshore (Hecky et al. 2004). The potential contribution of epiphyton to total benthic primary production and to nutrient cycling in the re-engineered littoral ecosystem are not known. However, increased surface area for attachment provided by *Cladophora* suggests that epiphytic production may also be significantly higher now compared with post-nutrient reduction, pre-dreissenid times. A knowledge of the seasonality and potential biomass contributed by epiphytes is a first step towards understanding how *Cladophora* and its epiphytes interact and contribute to nearshore processes.

The first two objectives of this study were to quantify the diatom epiphyte abundance and species assemblage on *Cladophora* through the growing season in a Laurentian Great Lake. Visual examination of *Cladophora* generally finds diatoms to be the dominant epiphyte taxon at sites highly exposed to wave action. Methods to quantify diatom epiphyte abundance are typically laborious and impractical for routine assessment. A faster method to quantify diatom epiphyte abundance would allow for more measurements and comparisons between sites and through time. Therefore, our third objective was to evaluate whether epiphyte silica content can be used quantitatively as a proxy for diatom epiphyte abundance on *Cladophora*. To accomplish this, we compared direct counts of epiphyte abundance with epiphyte silica content through the growing season. Our final objective was to estimate the areal quantity of silica retained by epiphytes associated with *Cladophora* through the growing season.

Methods

Site description

Cladophora and its attendant epiphytes were collected from the north shore of Lake Ontario at Oakville, Ontario, Canada (43.44°N , 79.66°W) at 2 m depth throughout the growing season in 2005 (late spring through early autumn). The species present throughout the Laurentian Great Lakes is *C. glomerata* (L.) Kützinger (Ross, 2006). The site is underlain by boulders and cobble and supports up to 100% *Cladophora* coverage at 2 m depth during midsummer. The shoreline is exposed to the open lake near the discharge of Sixteen Mile (Oakville) Creek, which has an urbanized catchment in its lower reaches.

Sample collection

Cladophora and its attendant epiphytes were sampled by hand by a snorkeller. The total biomass (*Cladophora* plus epiphytes) will be referred to as the “*Cladophora* assemblage” throughout this study. In order to investigate the silica content and abundance of *Cladophora* epiphytes macroalgal filaments from approximately 2–5 basal hold-fasts were taken in triplicate from randomly selected rocks. To quantify areal *Cladophora* biomass, five quadrats (0.25 m^2) were harvested by snorkellers using scraping tools. Filaments were gently handled throughout their manipulation. Filaments were collected in

fine-mesh sampling bags, then transferred to sealed Whirlpak bags and transported on ice back to the lab. Samples were rinsed with de-ionized water in a strainer (mesh size approximately 1 mm) to remove debris. Invertebrates were removed using forceps. All samples were observed under dissecting microscope (magnification 4–25 \times) to ensure that each macroalgal sample was *Cladophora* and to record, semiquantitatively, the relative abundance of adnate and stalked diatom epiphyte coverage. The general appearance of *Cladophora* thalli were also recorded at this time. *Ulothrix zonata* was often present attached to hard substrate in the spring prior to rapid growth of *Cladophora*. Once water temperatures reached approximately 10°C , these habitats were overtaken with *Cladophora* and *Ulothrix* biomass became undetectable. In the first seasonal samplings, however, some replicate samples collected were predominantly *Ulothrix*, rather than the targeted *Cladophora*. Because of the difficulty in separating these two macroalgae, samples that were primarily composed of *Ulothrix* were discarded, and as such, not all samples were represented by triplicates. *Cladophora* assemblage samples for epiphyte investigations were dried in a lyophilizer (Modulyo D, Thermo Savant, Holbrook, NY) at -50°C for 48 h, while samples to quantify biomass were dried in an oven at 65°C for at least 48 h. Samples for epiphyte studies were collected on 12 dates from 17 May to 16 August, of which there were a total of 32 samples. Areal *Cladophora* biomass was estimated on 10 dates through the same season.

Water samples for analyses of suspended Si were collected from 3 depths (2, 5, 10 m) along 3 transects. The middle transect included the site from which *Cladophora* was collected, and the other transects were located 3 km to the east and 3 km to the west of this location. Samples were collected at one time each during the spring (1 June) and the summer (25 July) in 2005. Water was collected with a 6-L Niskin sampler at a depth half-way to the lake bottom. Following filtration through a $0.2 \mu\text{m}$ polycarbonate filter under low vacuum pressure, the material retained was analysed for biogenic silica and the filtrate was analysed for soluble molybdate-reactive silicate concentration.

Epiphytic diatom enumeration and species identification

Epiphytic diatoms were isolated from the *Cladophora* samples and mounted on slides following standard diatom preparation techniques (Ruhland et al. 1999), the details of which are described herein. Pre-weighed freeze-dried *Cladophora* assemblage samples were digested with a concentrated nitric-sulphuric acid solution (50:50 molar ratio) in test tubes for 4 h, in a water bath heated to 90°C . This treatment digests organic material, leaving behind siliceous material, in this case, primarily diatom frustules. Following digestion, samples were stirred then left to stand for 24 h, allowing the siliceous material to settle. These slurries were then washed once daily with de-ionized water. Washing was accomplished by aspirating the top of the solution down to 1 cm above the settled material, carefully avoiding disturbance of the material, replacing the removed solution with fresh de-ionized water and then stirring the slurry with a glass rod. The pH of the solution was measured daily and washing was considered complete once a pH of 6.0 was obtained which typically required 15 washings.

To quantify diatoms, well-mixed 2 mL sub-samples of the slurries were added to 10 mL of de-ionized water along with 0.3 mL of a microsphere solution (concentration 4.7×10^6 spheres mL^{-1}). Well-mixed aliquots of these samples were mounted onto coverslips in dilution series, allowed to dry overnight on slide warmers, then mounted onto glass microscope slides using Naphrax mounting solution. Species were identified using Krammer and Lange-Bertalot (1991). Between 250 and 350 diatom valves were identified and counted together with the number of microspheres observed, facilitating the calculation of absolute diatom valve density. Diatom abundance was obtained by dividing the number of valves counted in half.

Si analyses

A subsample of each of the digested diatom epiphyte slurries was analysed for silica content (SiO_2 ; expressed as moles of Si). A hot NaOH digestion was performed to hydrolyse biogenic silica to molybdate-reactive silicates. A well-mixed 100 μL subsample of the washed slurry was digested with 8.0 mL of 0.2 M NaOH solution in an oven at 105 °C for 2 h (modified from Paasche 1973). This relatively harsh treatment to digest biogenic silica (compared with heated sodium bicarbonate digestion; APHA 1998) was justifiable because the lithogenic silica content in the slurry was negligible. Following digestion, samples were analysed for molybdate-reactive silicates.

The silica content of cultured, epiphyte-free *C. glomerata* isolated from Lake Ontario by the Canadian Phycological Culture Centre (CPC; formerly the University of Toronto Culture Collection) was also measured, following the same procedures. Cultures were grown in Chu-10 media containing 0.21 mM dissolved Si (Chu 1942). This concentration is expected to be in excess of the *Cladophora* demand for growth (Moore and Traquair 1976).

Molybdate-reactive silicates were measured using the standard heteropoly-blue method (APHA 1998), with the modifications that phosphate interference was prevented by the addition of tartrate and the reducing agent used was stannous chloride (Stainton et al. 1977). The absorbance of the final blue solution was measured at 820 nm using a spectrophotometer (Cary 100 Bio) and a 1-cm pathlength cuvette (Stainton et al. 1977). Sodium metasilicate nonahydrate was used to prepare silicate standards (APHA 1998). To reduce the potential for contamination by glassware, polyethylene containers were used to hold samples and reagents in all steps of the procedures.

The silica content of lake water particulates retained on filters were measured by first digesting samples with the hot NaOH methods described above, followed by performing the reactive silicates assay. This digestion treatment may have resulted in an overestimate of suspended biogenic silica given the possible presence of resuspended non-living siliceous material that could have been digested by these procedures. Therefore, the values reported should be considered as maximum possible biogenic silica concentrations. Dissolved reactive silicates in filtered lake water were also measured following the above assay procedures.

Statistical analyses

Epiphytic diatom abundance and epiphyte silica content data were grouped by the vitality of their *Cladophora* substrate, based on visual examination. Young, bright green *Cladophora* samples were collected on all dates up to 16 June, inclusive, and then again on 16 August. These samples were combined to form the group, “vigorous *Cladophora*”. *Cladophora* samples collected during the summer were distinctly more yellow and filaments tended to break easily during sampling. Samples collected from this season were labelled “senescent *Cladophora*”.

The relationship between silica content (per dry mass *Cladophora* assemblage) and diatom epiphyte abundance (per dry mass) was tested by analysis of covariance (ANCOVA) with *Cladophora* vitality as the independent variable and silica content as the covariate. Diatom epiphyte abundance was log-transformed to achieve homogeneity of variances (Levene's test, $F_{(1,30)} = 0.456$, $p = 0.505$). Our dataset was amenable to ANCOVA because there was no correlation between the independent variable and the covariate (i.e., *Cladophora* vitality and silica content; $r = -0.009$, $p = 0.960$) and there was a significant correlation between the dependent variable and the covariate (i.e., epiphyte abundance and silica content; $r = 0.413$, $p < 0.05$; Owen and Froman 1998). Because we were interested in whether there were any differences in silica content between epiphytes from the two *Clado-*

phora groups, both the equality of slopes (epiphyte abundance * *Cladophora* vitality), and the equality of intercepts (*Cladophora* vitality) were tested for significance using the General Linear Model routine of the software package SPSS (version 16, 2007). If either of these terms were significant, then linear regression equations using ordinary least squares for each group were calculated separately.

Results

C. glomerata in 2005 was first detectable at 2 m depth at the end of May when surface water temperatures were between 5 °C and 10 °C. The *Cladophora* assemblage grew rapidly in early June, reaching biomass of 97 ± 17.5 (SE) g dry mass m^{-2} at 2 m depth (Fig. 1A). Peak biomass of the *Cladophora* assemblage was observed between 10 and 23 June. Following this peak, *Cladophora* biomass began to slough and became visibly more senescent. Two seasonal growth cohorts were observed. During the initial growth of the second seasonal cohort, *Cladophora* filaments were again bright green and were less fragile to touch. The second cohort was dominant by 16 August.

Seasonal epiphytic diatom community assemblage

Seventeen species of diatom epiphytes were identified from 12 dates of *Cladophora* collection (Table 1). The earliest samples of

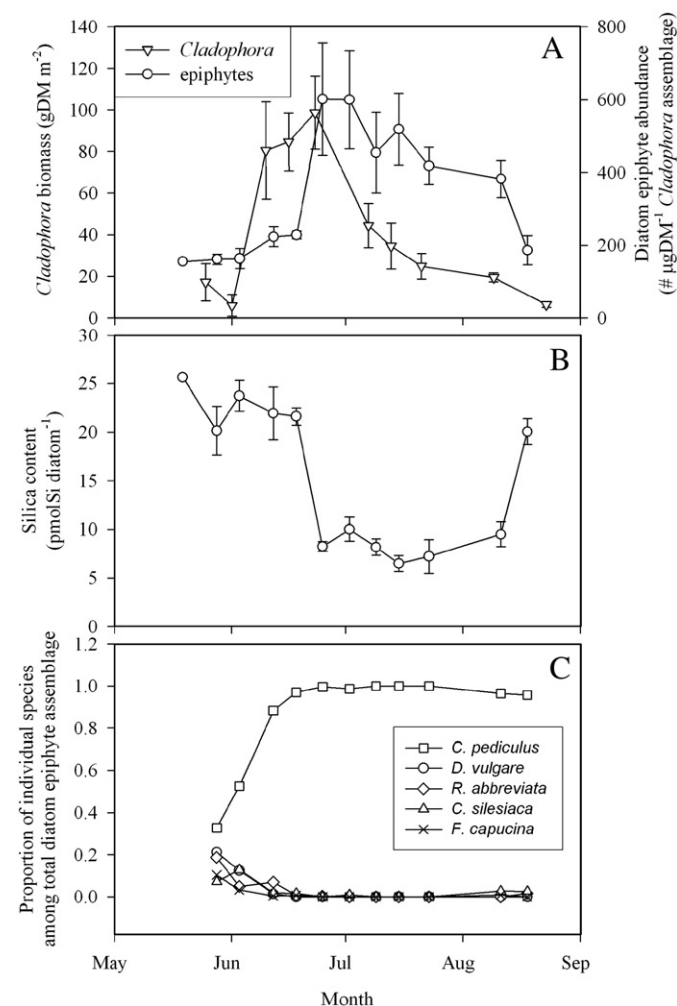


Fig. 1. (A) Seasonal changes in *Cladophora* biomass, diatom epiphyte abundance (per biomass of the *Cladophora* assemblage), (B) silica content per diatom, and (C) relative proportion of the dominant diatom epiphytes that were present on 3 or more sampling dates, *Cocconeis pediculus*, *Diatoma vulgare*, *Rhoicosphenia abbreviata*, *Cymbella silesiaca* and *Fragilaria capucina*. Error bars represent the standard error of the estimate.

Table 1
Diatom species present on *Cladophora* in Lake Ontario near Halton at 2 m depth.

Species	Range of diatom epiphyte density (diatoms μg^{-1} DM <i>Cladophora</i>)
<i>Amphora pediculus</i> Kützing	1.2–3.3
<i>Cocconeis neothumensis</i> Krammer	0.4
<i>Cocconeis pediculus</i> Ehrenberg	54–485
<i>Cocconeis placentula</i> Ehrenberg	2.1
<i>Cymbella silesiaca</i> Bleisch	3.8–14
<i>Cymbella sinuata</i> Gregory	0.4
<i>Diatoma tenuis</i> Agardh	1.3–2.0
<i>D. vulgare</i> Bory	6.2–35
<i>Fragilaria capucina</i> Desmazieres	1.2–17
<i>F. construens</i> Ehrenberg	2.4–2.6
<i>F. neoproducta</i> Lange-Bertalot	1.2–1.3
<i>Gomphonema olivaceum</i> Hornemann	2.1
<i>Navicula cryptocephala</i> Kützing	0.7–0.8
<i>Navicula tripunctata</i> Müller	1.3–2.4
<i>Nitzschia palea</i> Kützing	3.7–4.6
<i>Rhoicosphenia abbreviata</i> Agardh (also known as <i>R. curvata</i>)	1.2–31
<i>Surirella brebissonii</i> Krammer, Lange-Bertalot	0.7

Diatom epiphytes were enumerated on eleven sampling dates starting on 26 May.

Cladophora filaments, collected on 26 May, supported the most diverse diatom community assemblage observed. On this date, 12 species were identified, of which *Cocconeis pediculus* Ehr. (33%), *Diatoma vulgare* Bory (21%), *Rhoicosphenia abbreviata* Agardh (also known as *R. curvata*; 19%), *Fragilaria capucina* var. *vaucheriae* Des. (10%), and *Cymbella silesiaca* Bleisch (7%) were the community dominants by abundance (Table 1). With each progressive sampling date, the relative abundance of *C. pediculus* increased (Fig. 1C). From 16 June until the end of the sampling season, this species made up at least 95% of the diatom assemblage, by abundance. Epiphytes collected from the second cohort of *Cladophora* growth were also dominated by *C. pediculus*.

Seasonal epiphytic diatom abundance

Diatom epiphyte density was initially low (155 diatoms μg^{-1} DM *Cladophora* assemblage) and reached a maximum observed density of 601 diatoms μg^{-1} DM *Cladophora* assemblage on 23 June (Fig. 1A). The greatest increase in biomass of the *Cladophora* assemblage was observed between 1 June and 10 June, while the greatest increase in diatom epiphyte density was observed between 18 June and 25 June (Fig. 1A). As the *Cladophora* assemblage biomass decreased, the diatom epiphyte density (per dry mass) tended to decrease as well (Fig. 1A). Diatom epiphytes were found at relatively low density (186

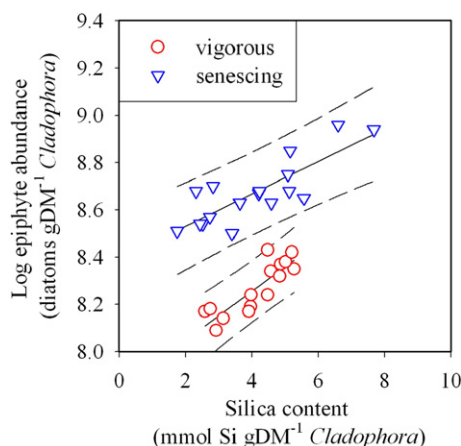


Fig. 2. Epiphytic diatom abundance as a function of silica content separated by vitality of *Cladophora* substrate. Linear regressions and 95% prediction intervals are shown. See text for regression equations.

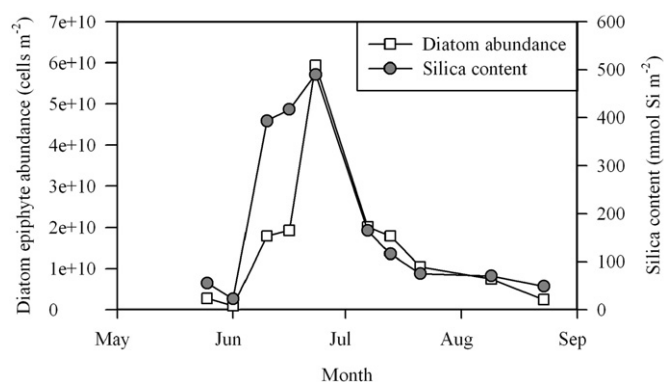


Fig. 3. Areal estimates of diatom epiphyte abundance and quantity of silica retained by epiphytes of *Cladophora* at 2 m depth.

diatoms μg^{-1} DM *Cladophora* assemblage) again during the first sampling of the second cohort of *Cladophora* growth.

Relationship between diatom epiphyte silica content and diatom epiphyte abundance

There was a strong relationship between diatom epiphyte abundance and silica content. The relationships (slopes) between these variables were not different between vigorous and senescent *Cladophora* (Fig. 2; $F_{(1,28)} = 2.23$, $p = 0.147$). However, the intercepts were different (Fig. 2; $F_{(1,28)} = 261$; $p < 0.0005$). Therefore, unique regression equations were calculated for epiphytes growing on *Cladophora* from different seasons. The regression equations solved were in the form $\log(\text{epiphyte abundance}) = a(\text{silica content}) + b$, where epiphyte abundance was expressed as cells g^{-1} DM (*Cladophora* assemblage) and silica content was expressed as mmol Si g^{-1} DM (*Cladophora* assemblage). For epiphytes growing on vigorously growing *Cladophora*, $a = 0.104$, $b = 7.840$, and $r^2 = 0.737$. For epiphytes growing on senescing *Cladophora*, $a = 0.069$, $b = 8.393$, and $r^2 = 0.677$.

Epiphyte silica content ranged from 20.1 to 25.6 $\text{pmol Si diatom}^{-1}$ and had a median of 21.8 $\text{pmol Si diatom}^{-1}$ among samples from vigorous *Cladophora*. In contrast, among samples taken from senescing *Cladophora*, silica content was 6.5 to 10.0 $\text{pmol Si diatom}^{-1}$ with a median of 8.2 $\text{pmol Si diatom}^{-1}$.

Taking into account both the silica content per diatom and the diatom density per mass of *Cladophora* assemblage, the total silica content of diatom epiphytes was in the range of 3.1 to 5.8 mmol Si gDM^{-1} (*Cladophora* assemblage), with a mean of 4.1 mmol Si gDM^{-1} .

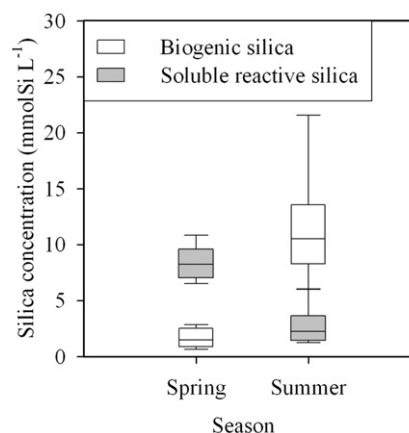


Fig. 4. Biogenic silica and soluble molybdate-reactive silicates in the water column in the late spring (1 June), and in the summer (25 July) in Halton Region, Lake Ontario.

(*Cladophora* assemblage). Cultured *Cladophora* samples contained 0.14 ± 0.06 (SE) mmol Si gDM⁻¹ ($n = 5$).

Based on the product of areal *Cladophora* biomass and diatom epiphyte abundance per mass of *Cladophora*, epiphyte density at 2 m depth increased from 9.59×10^8 cells m⁻² in the late spring to a peak of 5.93×10^{10} cells m⁻² on 23 June (Fig. 3). The amount of silica retained by diatom epiphytes at 2 m depth was calculated to range from 22.7 mmol Si m⁻² in the spring, up to 490 mmol Si m⁻² during peak *Cladophora* biomass (Fig. 3).

Water column nutrient concentrations

Biogenic silica measured from the water column (mean of 9 stations) increased from 1.7 ± 0.3 (SE) μ M in the spring of 2005 to 11.5 ± 1.5 μ M in the summer of the same year (Fig. 4). At the same time, dissolved molybdate-reactive water column silicate decreased from 8.3 ± 0.5 (SE) μ M to 2.7 ± 0.5 μ M.

Discussion

Epiphytic community abundance on *Cladophora*

In this study, we report a range from 155 to 601 diatoms μ g⁻¹ DM (*Cladophora* assemblage), increasing from late spring to a peak in early summer. These values are at the higher end of those reported for global freshwater systems, but are similar to other studies of the Great Lakes (Sheath and Morison 1982). At the lowest end of diatom epiphyte abundance on *C. glomerata*, Moore (1977) reported a maximum of 48 diatoms μ g⁻¹ DM (*Cladophora* assemblage) from a two year study involving biweekly sampling from the River Wylye, England. At the highest end, Stevenson and Stoermer (1982b) reported a seasonal average from the end of June to September of 790 diatoms μ g⁻¹ DM (*Cladophora* assemblage) collected from macroalgae exposed to nutrient-rich water originating from a wastewater treatment plant on Lake Huron.

The most rapid increase in density of *Cladophora* diatom epiphytes was observed between 16 and 23 June, about 2 weeks after the most rapid increase in *Cladophora* biomass was observed (1–10 June). As *Cladophora* biomass declined, so too did the density of epiphytes. This may indicate that during the period of *Cladophora* senescence, diatom epiphytes are removed more rapidly than they reproduce, by grazers, for example. During the decrease in epiphyte density, diatom frustules were at their thinnest (as discussed below), and therefore may have been more vulnerable to predation. Alternatively, *Cladophora* filaments that are more heavily burdened by epiphytes may slough more rapidly than less burdened filaments due to successful nutrient competition and/or shading by epiphyton, as suggested by Stevenson and Stoermer (1982a).

Epiphytic community assemblage on *Cladophora*

In contrast to other macroalgae, such as *Ulothrix*, *Spirogyra* or *Enteromorpha*, *Cladophora*'s cell walls provide a suitable substrate for a diversity of epiphyte flora (Whitton 1967; Hardwick et al. 1992; Loughheed and Stevenson 2004). In this study, a total of 17 diatom epiphyte species were observed on *C. glomerata* from 12 dates over the course of the growing season, of which 12 species were recorded from the first spring sample, collected on 26 May. In the spring, the epiphyte community was dominated by (in order of decreasing abundance): *C. pediculus*, *D. vulgare*, *R. abbreviata* (aka *R. curvata*), *F. capucina*, and *C. silesiaca*. The three most abundant species have previously been reported as dominants on *C. glomerata* (e.g., Chudyba 1965; Jansson 1967; Rönnerberg and Lax 1980; Sheath and Morison 1982; Hardwick et al. 1992; O'Connell et al. 1997). Despite the modest epiphyte species richness observed in the first seasonal samples, *C. pediculus* came to dominate the diatom epiphyte assemblage by late

spring, to the near complete exclusion of all other taxa, indicating strong constraints on the diversity of the epiphyte species assemblage.

Based on the superlative success of *C. pediculus* in this study we assert that physical or grazing pressures were important in shaping the epiphyte species assemblage. While *Diatoma* attaches by means of a short mucilaginous pad, *Rhoicosphenia* and *Cymbella* by means of mucilaginous stalks, and *Fragilaria* as filamentous threads, *Cocconeis* is an adnate raphe-bearing pennate diatom, curved to conform to macroalgal thalli (Chudyba 1965; Lowe et al. 1982). The species *C. pediculus* is exceptionally well adapted to maximize adhesive strength to substrata (Dudley 1992; Lowe et al. 1982). Whether the persistence of this single species is due primarily to the removal of other species by water motion (Luttenton and Rada 1986; Bergey et al. 1995), by selective removal of other species by grazers (Dudley 1992), or due to other pressures, cannot be determined for certain with our dataset. However, we hypothesize that the harsh physical environment (i.e., exposure to a wide range in wave action, with intermittent high intensities) at 2 m depth was an important driver in the selective removal of most species other than *C. pediculus*. Some studies of *Cladophora* epiphytes have inferred resource control of the species assemblage. For example, nitrogen limitation has been identified as a selective pressure of epiphyte species composition in some habitats, in which N-fixing taxa (e.g., the cyanobacterium *Nostoc* and endosymbionts of the diatom genus *Epithemia*) have a competitive advantage (Jansson 1966; Bergey et al. 1995; Marks and Power 2001). As discussed below, we found evidence of silica limitation of diatom epiphytes, a constraint which could select against species with low silica utilization efficiency. *C. pediculus* may hold a selective advantage over other diatom epiphytes based on silica utilization efficiency, a hypothesis which remains to be tested.

Relationship between silica content and epiphytic diatom abundance on *Cladophora*

An unanticipated outcome of this study was that the relationship between diatom epiphyte abundance and silica content differed between seasons. When *C. glomerata* was growing at maximal rates, its epiphytic diatoms had high silica content (median: 21.8 pmol Si diatom⁻¹). Conversely, when the growth rate of *Cladophora* had slowed, or its biomass was decreasing, its epiphytic diatoms had lower silica content (median: 8.2 pmol Si diatom⁻¹). The change in silica content per diatom is not explained by changes in species composition; *C. pediculus* usually accounted for more than 90% epiphytic diatoms regardless of *Cladophora* vitality. The changes in silica content per diatom were also not explained by changes in cell size or changes in surface area. With the exception of samples taken from late August, mean diatom epiphyte cell sizes were not different between seasons. The silica content of the epiphyte diatoms fell within the prediction intervals of other freshwater diatoms of the same size (Conley et al. 1989), using an estimated biovolume of 1570 μ m³ for *C. pediculus*, based on an ovoid shape with estimated dimensions 25 μ m length, 20 μ m width, and 4 μ m girdle depth.

The differences in silica content between seasons were primarily due to differences in thickness of frustules, which was visibly evident. The decrease in silicification of the diatoms epiphytic on senescing *Cladophora* was likely due to a decrease in Si availability during the summer when *Cladophora* biomass and total epiphyte abundance was high. An alternative hypothesis is that lower silicification during *Cladophora* senescence was due to higher epiphyte growth rates. Because the degree of silicification is driven by the duration of the cell wall synthesis phase, lower silicification can be a consequence of fast growth rates at non-limiting Si concentrations, which can occur under conditions of high nutrient availability, high light availability, and optimal temperatures (Martin-Jézéquel et al. 2000). However, during summer growth, N and P content of the *Cladophora* assemblage decreased to limiting levels (Malkin et al. 2008), and irradiance

decreased through the *Cladophora* canopy, likely constraining diatom epiphyte growth rates. We therefore conclude that the differences in silica content between epiphytes growing on young, vigorous *Cladophora* versus on senescing *Cladophora* were most likely due to silica limitation caused by low Si concentrations during the summer.

Within groups of *Cladophora* vitality (vigorous or senescent), diatom epiphyte silica content explained a large portion of the variability of log-transformed diatom epiphyte abundance (both expressed per mass of the *Cladophora* assemblage; $r^2_{\text{vigorous}} = 0.737$; $r^2_{\text{senescent}} = 0.677$). While Si is an essential element for *Cladophora*'s growth as a structural component of its cell wall (Moore and Traquair 1976), the silica content (mmol Si g⁻¹ DM *Cladophora*) is small relative to its diatom epiphytes. In cultured *Cladophora* grown in excess Si concentrations as part of this study, *Cladophora* contained 0.14 ± 0.06 (SE) mmol Si g⁻¹ DM, equivalent to less than 2.4–4.6% of the silica contained in diatom epiphytes. Therefore, to a first approximation, the relationship between epiphyte silica content and abundance allows for biogenic silica of a *Cladophora* assemblage to be used as a proxy for estimating *Cladophora*'s diatom epiphyte abundance. This relationship could be useful for exploring the relative abundance of diatom epiphytes at different sites and seasons around the Great Lakes for the purpose of quantifying the magnitude and flux of carbon and nutrients through the benthos. Being able to quantify the role of epiphytes may be important for solving nearshore mass balance equations, and the relationships presented herein provide a rapid means of estimating epiphyte abundance. However some caution must be advised. Our dataset was amenable to Type I model linear regression analysis, which assumes no measurement error in the independent variable. A potential consequence of this assumption is bias in the regression slope (Prairie et al. 1995). Furthermore, considerable variability exists about the regression, necessitating direct counts if accurate estimates of diatom epiphyte abundance are required.

Potential role of epiphytes in benthic–pelagic coupling in the littoral zone

Because diatoms are the major biological sink for dissolved Si, their abundance as epiphytes may be important in nutrient cycling in the Great Lakes littoral zones. Through the 1940s and 1950s, as P loading to the lower Laurentian Great Lakes increased, phytoplankton became increasingly Si limited. Silicon was likely limiting diatom primary production throughout the year in the central and eastern basins of Lake Erie and throughout Lake Ontario during these decades (Schelske et al. 1986). Decreases in P loading from the catchment since the 1970s has resulted in lower phytoplankton production in the lower Great Lakes, and consequently, dissolved Si concentrations have increased (Guildford et al. 2005). Concentrations of Si less than 6.5 μM have been used as a threshold to define severe Si limitation (Schelske et al. 1986). Using this threshold, Si concentrations reported for the central and eastern basins for Lake Erie remain Si limited during the stratified season (Guildford et al. 2005). In the study presented here, we report that Lake Ontario dissolved Si in the water column were on average 8.3 and 2.7 μM on a spring and a summer date, respectively. The summer data indicate severe Si limitation of planktonic diatoms, and likely benthic diatoms given that their silica requirement is typically higher, in nearshore Lake Ontario, at least during part of the stratified season in 2005.

The accumulation of silica in the epiphytes of *Cladophora* occurred as *Cladophora* biomass increased, commencing in late spring and proceeding to a peak observed on 23 June in 2005. Based on measured biomass and silica content per mass of *Cladophora* on this date, the peak retention of silica by diatom epiphytes was estimated to be 490 mmol Si m⁻² at 2 m depth. Using a *Cladophora* growth model to estimate *Cladophora* biomass to deeper depths (described in Malkin et al. 2008), the biomass at 5 m and 10 m depth was estimated to be 78 and 19 gDM m⁻², respectively. If epiphyte silica per biomass of *Cladophora* at these deeper

depths is the same as at 2 m depth, then the peak epiphyte silica demand at 5 m and 10 m depth is estimated to be 319 and 75 mmol Si m⁻², respectively. The bioavailable silica measured in the spring of 2005 was 8.2 μM , which is equivalent to 17.6, 43.9, and 87.9 mmol Si m⁻² at 2, 5, and 10 m depth, respectively. The peak demand in silica by benthic epiphyton occurs later than the spring diatom bloom, typically commencing prior to lake stratification in the spring in Lake Ontario (Smith et al. 1998). Thus on a seasonal scale, the planktonic diatom silica demand appears to precede the benthic silica demand. How these demands interact on an interannual basis, in terms of silica burial and remineralisation, warrants further attention. While it is tempting to generalize the findings of silica limitation during summer stratification reported in this study, given that spring temperature warming and seasonal precipitation during the year of study were typical of the preceding decade, silica cycling can have high interannual variability, and so caution must be advised in interpreting the results too broadly. Schelske et al. (2006), for example, observed that biogenic silica storage in sediments is much more variable than phosphorus storage, both through time and between sites in the Great Lakes. Further work, involving multiple years of investigation, is clearly needed to assess the relationship between bioavailable dissolved silicates and silica retention by benthic diatoms and any possible coupling between the silica reservoirs of attached and planktonic diatoms in the Great Lakes nearshore environments.

In an investigation of epilithic and episammic periphyton from Lake Michigan in the mid-1980s, Carrick and Lowe (2007) concluded these assemblages were primarily limited by P and N and only secondarily limited by Si. In previous work, we demonstrated that peak *Cladophora* biomass accumulation at this study site is constrained by phosphorus (Malkin et al. 2008), as elsewhere in the Great Lakes. Here we add that the diatom epiphytes of *Cladophora* may be constrained by Si availability (as supported by their lower silica content, thinner frustules, and low dissolved Si concentrations) during peak areal abundance. With both planktonic and epiphytic diatoms demonstrating evidence of Si limitation, Si cycling between these pools through the growing season may be affected by changes in the growth of *Cladophora*. Our examination of *Cladophora* diatom epiphytes revealed that they make up a substantive component of the benthic flora. With the establishment of the dreissenid mussels and their prolific development in the Great Lakes, the importance of benthic secondary production has increased (Johannsson et al. 2000) and evidence is mounting to suggest that benthic primary production in the littoral zone has also increased (Malkin et al. 2008; Zhu et al., 2006). By providing structural complexity and surface area for epiphyte colonization (Lowe et al. 1982), *Cladophora* appears to facilitate nutrient retention to the littoral zone, beyond the magnitude that would be predicted based on the macroalga's growth alone.

Acknowledgments

We gratefully acknowledge Dave Depew, Ted Ozersky, and Adam Houben for suspended Si data collection, Yuri Kozlov for development of laboratory Si analysis procedures, Dusha Vukosavljevic for measuring Si content of cultured *Cladophora*, and Judy Acreman of the CPCC for assistance and advice with maintaining *Cladophora* cultures. We thank David Millie and 2 anonymous reviewers for thoughtful comments and suggestions on an earlier version of this manuscript. Funding for this research was provided by the Ontario Clean Water Agency and S. Malkin was supported by a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship and an Ontario Graduate Scholarship.

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