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Environmental and algal community influences on benthic algal extracellular material in Lake Opeongo, Ontario

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Abstract: Extracellular material can play a key role in benthic ecosystems, but most of our current knowledge about extracellular material comes from marine intertidal systems. Data from lakes are lacking. We worked in Lake Opeongo, a 5800-ha dystrophic lake on the Canadian Precambrian Shield, and investigated changes in extracellular material as a function of light intensity, sediment characteristics, and disturbance regime and algal biomass, community composition, and primary production, all factors important in marine intertidal studies. We used permutation-based path analysis to test alternative models. We found a negative effect of in situ primary production on loosely bound, colloidal extracellular material, indicating that extracellular material may be released under stressful conditions. The effect of algal community composition on colloidal extracellular material also was significant. Total extracellular material was affected only by time of year, indicating that tightly bound, capsular extracellular material is more refractory than colloidal extracellular material. We found no significant effects of environmental factors (light, nutrients, or wind-driven disturbance) on either colloidal or total extracellular material, despite their importance in marine intertidal systems.

Key words: extracellular material, benthic algae, lake, environment, algal community, path analysis, Lake Opeongo

Benthic algae excrete copious amounts of extracellular material (EM), sometimes in quantities equivalent to >70% of their total fixed C (Underwood and Paterson 2003). EM is composed primarily of carbohydrates, and contains proteins, lipids, and deoxyribonucleic acid. In marine intertidal zones, EM can stabilize sediments, affect nutrient and toxin recirculation, and provide a C source for bacteria, fungi, and benthic macroinvertebrates (Decho 1990, Underwood and Paterson 2003). Benthic algae are thought to excrete EM for several reasons (Decho 1990, Underwood and Paterson 2003). EM is used for attachment to sand grains and hard surfaces (Kilroy and Bothwell 2011); vertical migration through the sediment (Saburova and Polikarpov 2003, Consalvey et al. 2004, Apoya-Horton et al. 2006); protection from stressors, such as desiccation or exposure to contaminants (Decho 1990); and as a way to remove excess C fixed under nutrient limitation or high-light intensities (Staats et al. 2000, Underwood and Paterson 2003).

Several environmental and community factors affect the amount of EM produced in marine systems. These factors include light, nutrients, disturbance, time of year, algal biomass and community composition, biomass and activity of nonphotosynthesizers, and primary production (Decho 1990, Smith and Underwood 1998, Underwood and Paterson 2003, Kilroy and Bothwell 2011). However, whether these factors affect the amount of EM present in freshwater lake systems is not known. We investigated production of EM in spring and summer in a lake on the Canadian Precambrian Shield. These lakes are typically oligotrophic, with granite bedrock, thin soils, and little development in their watersheds. They often are surrounded by coniferous forest and have high dissolved organic matter concentrations and brown water. These lakes differ from lakes in other regions of the world, but hundreds of thousands of them exist on the Canadian Shield. We used path analysis to explore relationships among the amount of benthic EM and factors (light intensity, sediment characteristics, disturbance regime, algal biomass and community composition, and primary production) that marine studies indicated might influence its production.

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METHODS

Site description

Lake Opeongo is a dystrophic lake in Algonquin Provincial Park, Ontario, Canada (lat 45°42′N, long 78°22′W). All sites were in South Arm (Fig. 1), a 7-shaped basin with a maximum length of 5.8 km, an area of 22.1 km^2, and maximum depth of 50 m. We selected sites based on their presumed degree of exposure to the prevailing winds. Three sites were in protected areas, and 4 sites were more exposed. At each site, we sampled multiple depths. Samples spanned the entire depth of the littoral zone. The shallowest sites were ≥ 1m deep (depth set by the length of the corer) or where soft sediments began, and the deepest sites were at the photosynthetically active radiation (PAR) compensation depth (~6 m). We sampled 16 site–depth combinations between 24 May and 31 August 2007 (n = 26 samples; Table 1).

Environmental factors

Light Just before sampling, we measured PAR profiles from the water surface to just above the sediment surface, making sure to avoid disturbing the sediment. Cloud cover was variable, so we expressed profiles as percentages of deck-cell readings (%I_0). We plotted profiles to determine attenuation coefficients and the light intensity at the sampling depth.

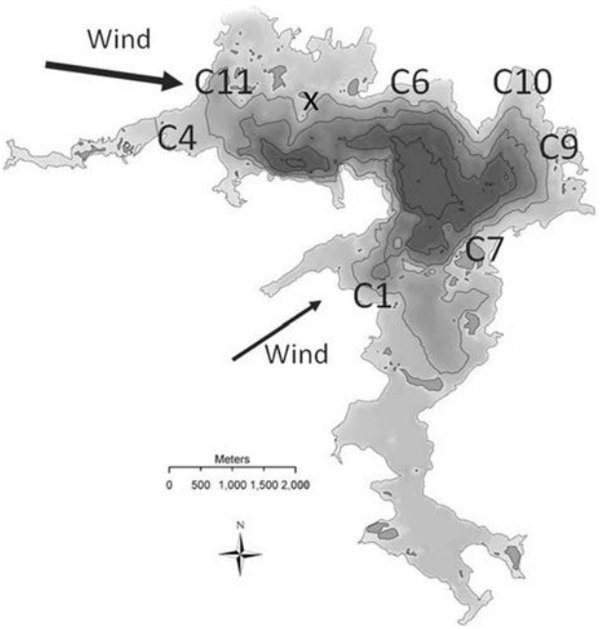

Figure 1. South arm of Lake Opeongo showing sampling sites (C1 1 and 2 m; C4 1 and 2 m; C6 2 m, C7 2, 4, and 6 m; C9 2 and 3 m; C10 1, 2, and 4 m; and C11 2, 4, and 6 m) and the weather station (×) where wind data were collected. Map provided by the Harkness Laboratory of Fisheries Research.

Table 1. Sample dates, sites, and depths. There were 16 locations (combinations of site and depth) that were sampled 1 to 3 times each.

Site	Depths (m)	Dates
C1	1	24 August
	2	29 May, 14 June, 29 August
C4	1	15 June, 30 August
	2	28 May, 31 August
C6	2	12 June
C7	2	06 June, 02 July, 21 August
	4	03 July
	6	04 July
C9	2	02 June
	3	18 June
C10	1	24 May, 05 June
	2	29 Jun, 23 August
	4	07 June
C11	2	31 May, 22 August
	4	01 June, 06 July
	6	05 July

Sediment type We used a 4.5-cm-diameter core tube to collect 5 random samples within a 1-m^2 quadrat at each site. We sliced off and retained the top 0.5 cm of each core (the photic layer of the sediments; MacIntyre et al. 1996), and we pooled the 5 slices to create a spatially integrated sample. We used one 2-g aliquot of this sediment for pigment analysis (see below). We used 3 aliquots for estimation of water content. We dried samples for 2 d at 60°C, reweighed them, and expressed water content as % sediment wet mass.

Disturbance We approximated physical disturbance from measurements of wind speed and direction taken every 10 min at the weather station on an island in the center of the South Arm (Fig. 1). Wind data were provided by the Harkness Laboratory of Fisheries Research. The prevailing winds blow from the south or west, so we assumed that sites on these shores (C1, C4, and C11) were more protected than sites on the opposite shores (C6, C7, C9, and C10) (Fig. 1). We quantified disturbance as wind speed multiplied by effective fetch (Håkanson and Jansen 1983) for the direction from which the wind was blowing at each site. We averaged this wind-weighted fetch over the 24 h before sample collection to create a proxy for short-term storm-driven disturbance. We also averaged wind-weighted fetch over the study duration (9 May–6 September) to estimate seasonal exposure.

Algal community and biomass

We assessed the algal community through pigment analysis of sediments. We froze the sediment aliquots in liq-

uid N_2 until they could be transported to the laboratory where they were held at −80°C until analysis. We ground each aliquot in 2 mL of 90% acetone and held them in the refrigerator overnight. We filtered the resulting extract through a 0.45-μm PFTE filter (Cole Parmer, Vernon Hills, Illinois) and analyzed the pigments by high-performance liquid chromatography (HPLC) on a unit consisting of a Perkin–Elmer series 200LC quaternary pump and a series 785A programmable ultraviolet-visible light absorbance detector (Perkin–Elmer, Woodbridge, Ontario). We used the ramping procedure of Jeffrey et al. (1997) to run the extract through a 25-cm Simplicity LC-18 column (Supelco Corp., a Sigma–Aldrich Company, Oakville, Ontario), and detected absorbance at 436 nm. We estimated algal biomass as chlorophyll (chl) *a*, standardized against known chl *a* concentrations (Sigma–Aldrich Canada Co., Oakville, Ontario, Canada). Chl *a* concentrations are not the same as algal biomass, and chl content/cell can change with light or nutrient conditions (MacIntyre et al. 2002, Falkowski and Raven 2007), but chl *a* is the most widely used method of estimating algal biomass (e.g., Underwood and Smith 1998, van Duyl et al. 1999, Blanchard et al. 2000).

Community composition We used HPLC to measure 12 additional algal pigments. We used chl *b*, fucoxanthin, and echinenone, calibrated with commercial standards (chl *b*: Sigma Chemical Co.; fucoxanthin and echinenone: DHI Lab Products, Hoersholm, Denmark), to represent chlorophytes, bacillariophytes, and cyanophytes, respectively. Elution times of identified pigments were: chl *c* 6.4 min, fucoxanthin 7.9 min, chl *b* 15.2 min, chl *a* 16.2 min, and echinenone 16.8 min. We quantified additional pigments as peak area/g sediment wet mass but did not identify them. We labeled these pigments by their elution times.

We used correspondence analysis (CA) of pigment relative abundances to reduce the number of community-composition variables (Jackson 1997). Only the 1st axis, which explained 38% of the variance, explained more variance than would be expected by chance, so we reference community composition as a value along the 1st CA axis (CA1). Sites were arranged along CA1 mostly on the basis of pigments that have not been identified. Therefore, scores on CA1 cannot be related to specific changes in community composition.

Primary production

We used a modified Winkler protocol (Roland et al. 1999) to measure primary production at each site based on the change in O_2 concentration in each of eight 7-cm-diameter cores. We collected cores from the same 1-m^2 quadrats as above. A boundary layer can decrease the rate of photosynthesis (e.g., Mass et al. 2010), so we situated the cores in an incubator in which water was circulated with a peristaltic pump. We circulated lake water through the incubation cooler to maintain the in situ temperature during incubation. We produced a photosynthesis vs irradiance (P–I) curve for each site by incubating the chambers under 7 light intensities, ranging from full darkness (in duplicate) to ~1000 μmol photons m^{-2} s^{-1}, slightly higher than the highest PAR at the sediment surface of the brightest site (~700 μmol photons m^{-2} s^{-1}). We modeled each P–I curve as a hyperbolic tangent, which provides the best fit for experimental photosynthesis measurements especially in the light-saturated portion of the curve (Jassby and Platt 1976, Falkowski and Raven 2007). We estimated 3 parameters from the P–I curves: 1) P_{MAX}: the maximum photosynthesis that occurred under saturating light intensities, 2) alpha: the initial increase in photosynthesis with increased light at subsaturating intensities, and 3) Pz: estimated in situ production (rate of photosynthesis at the light level at the sampling depth).

Extracellular material

We measured the amount of EM at each site by the method of Underwood et al. (1995) as modified by Cyr and Morton (2006). Samples were collected, sliced, pooled, subsampled, and frozen as described above for pigment analysis. We extracted samples in distilled water on a continuous shaker for 20 min, and centrifuged them at 30,000 *g* for 30 min. We decanted and retained the supernatant, which contained the loosely bound colloidal EM. We extracted the pellet, which contained the more tightly bound capsular EM (and cells and sediment), in 15 mmol/L ethylenediaminetetraacetic acid (EDTA) on a continuous shaker for 4 h and then centrifuged the mixture at 30,000 *g* for 30 min. We added 0°C ethanol to the 2 supernatants to precipitate the relatively large extracellular polymeric substances (EPS) from the low-molecular-weight (LMW) compounds. We analyzed the carbohydrate content of all 4 fractions by the phenol/sulfuric acid assay (DuBois et al. 1956). We added the EPS and LMW fractions together to yield 2 fractions: colloidal EM and total (colloidal plus capsular) EM.

Use of EDTA as the extractant could have caused some contamination from intracellular carbohydrates, but Cyr and Morton (2006) found little intracellular contamination when they used the protocol we followed (concentrations < 20 mmol/L). Moreover, the concentration of EDTA used in our study was 15 mmol/L, a concentration much lower than that used in studies of marine intertidal systems (Underwood et al. 1995) and even some freshwater streams (Battin and Sengschmitt 1999).

Statistical analyses

We conducted a path analysis with the *lavaan* package in R (version 2.13.2; R Project for Statistical Computing, Vienna, Austria). Path analysis is similar to multiple regression but has the advantages that hypothesized causal relationships can be modeled among explanatory variables and that the variance in the response variable can be par-

titioned into direct and indirect effects of the explanatory variables (Li 1981, Bassow and Bazzaz 1998, Mitchell 2001). These advantages are important because each explanatory variable may affect extracellular material directly or indirectly. For example, light affects photosynthesis, which affects extracellular material. Path analysis is more robust than multiple individual comparisons because it can account for the variation caused by all the other explanatory variables and can be used with an unbalanced design. For example, time of year would be a significant variable if, after accounting for the variation from all other variables, sites had higher EM early than late in the season.

We developed separate models for colloidal and total EM. We developed the full model (Fig. 2) from a redundancy analysis that related environmental variables to benthic algal primary production (Scott 2013), and we hypothesized links from these variables to the amount of EM. We sequentially removed each of the 5 environmental variables from the model (Table 2). The links among the environmental, community, and productivity variables, remained unchanged in these models. Each new model was evaluated with Akaike's Information Criterion (AIC). If AIC decreased by >2 (Anderson and Burnham 2002), the variable was removed from the model. If AIC increased or decreased by <2, the variable was retained in all subsequent models.

When sample size is small, as in our study (n = 26), the p-values associated with each path may be unreliable when estimated by traditional methods. Therefore, we ran a permutation test (based on 1000 permutations) on our best model. Because the data were structured (by depth, date, etc.) and unbalanced, we preserved the associations among environmental variables for each sample and randomized only the EM. After each randomization, we ran a new path analysis. Thus, the p-value associated with each path leading to EM is the probability of finding an estimate equal to or more extreme than the observed estimate in the 1000 permutations plus the original estimate (a total of 1001). We used $\alpha = 0.1$ to reduce the possibility of type II errors that might be caused by the small sample size. The other paths remained unchanged in these permutations, so no p-values are associated with these paths. Model selection and permutation tests were done for both colloidal and total EM.

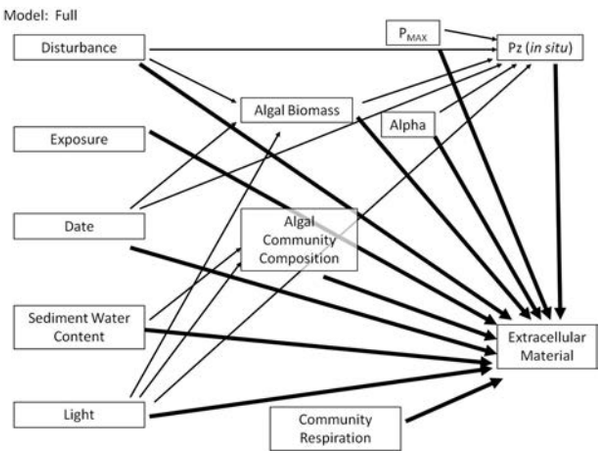

Figure 2. The full path model for colloidal and total extracellular material. Thin arrows represent paths that came from previous research on the effect of the environment on benthic primary production and remained constant throughout the model selection. P_{MAX} = maximum photosynthesis, Pz = estimated in situ production.

RESULTS

Sediment water content ranged from 24.6 to 70.6%, and PAR at the sampling depth ranged from 4.0 to 658.2 μmol photons m^{-2} s^{-1}. Algal biomass ranged from 9.1 to 136.3 mg chl/m^2, and gross in situ primary production ranged from 13.9 to 42.8 mg O_2 m^{-2} h^{-1}. In all but one case, respiration exceeded photosynthesis, a result indicating that the sediments of Lake Opeongo were net heterotrophic.

Colloidal EM in Lake Opeongo ranged from 50.04 to 441.34 μg glucose equivalents (gluc eq)/g sediment dry mass (DM) (mean ± 1 SE, 162.28 ± 97.7 μg gluc eq/g DM). Total EM ranged from 159.12 to 1365.48 μg gluc eq/g DM (458.48 ± 315.07 μg gluc eq/g DM). The percentage of total EM that was colloidal ranged from 12.2 to 66.1% (40.8 ± 13.4).

The full model for colloidal EM had an AIC value of 2228.3 (Table 2, Fig. 2). The best model (model 5-C; Fig. 3) had an AIC of 1653.8 when respiration, time of year, and exposure were removed. Only 2 paths leading to colloidal EM were significant, and both had negative coefficients: Pz (p = 0.054) and algal community composition (p = 0.063) (Table 3, Fig. 3). The direct effects, without taking into account the correlations with other explanatory variables as is done in the path analysis, of both Pz and community composition on colloidal EM were nonsignificant (Pz: p = 0.14, R^2 = 0.09; Colloidal EM = −2.7Pz + 205.8, Community Composition: p = 0.41, R^2 = 0.03; Colloidal EM = −14.7Community + 166). However, there was one point in the relationship between photosynthesis and colloidal EM that, given its disjunct position, was considered an atypical point (open circle, Fig. 4). If this point was removed, the relationship was significant (Fig. 4, p = 0.015, R^2 = 0.23; Colloidal EM = −5.1Pz + 230.23).

The full model for total EM had an AIC value of 2027.4 (Table 2). The best model (model 5-T, Fig. 5) had an AIC of 1505.2 when respiration and exposure were removed. Time of year was the only significant path leading to total EM, also a negative relationship (p = 0.056). Similar to algal community composition, the direct relationship between time of year and total EM was weak unless the

Table 2. Alternative path models for colloidal (C) and total (T) extracellular material, their Akaike Information Criterion (AIC) values, and the variables that were removed from the models.

Colloidal			Total		
Model	AIC	Variables removed	Model	AIC	Variables removed
Full-C	2228.3		Full-T	2027.4	
2-C	2026.0	Respiration	2-T	1847.7	Respiration
3-C	2024.6	Respiration Sediment	3-T	1846.1	Respiration Sediment
4-C	2021.6	Respiration Date	4-T	1845.9	Respiration Date
5-C	1653.8	Respiration Date Exposure	5-T	1505.2	Respiration Exposure
6-C	1652.0	Respiration Date Exposure Disturbance	6-T	1503.3	Respiration Exposure Disturbance

correlations to other explanatory variables were taken into account.

DISCUSSION

The amount of benthic EM in Lake Opeongo was slightly lower than amounts reported for other lakes. Mean colloidal EM at 27 sites in 7 lakes was 282.95 ± 138.36 μg gluc eq/g DM, mean total EM was 553.37 ± 278.89 μg gluc eq/g DM, and colloidal EM was ~53.3 ± 13.4% of the total EM (Cyr and Morton 2006). Freezing samples before analysis could have caused contamination by intracellular material, but Cyr and Morton (2006) also froze their samples, so our data are comparable with theirs.

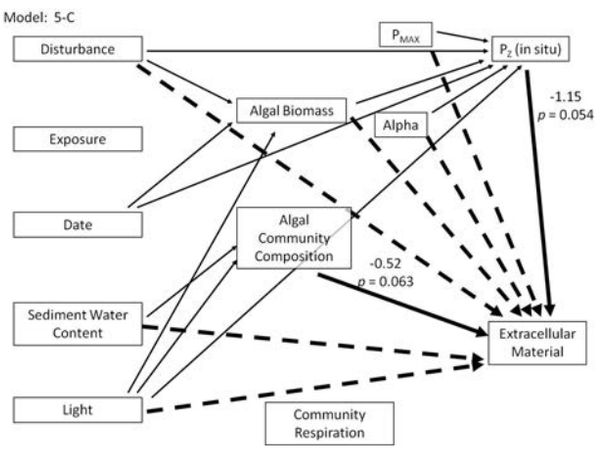

Figure 3. The best path model for colloidal extracellular material. Arrows that represent significant paths are thick and solid, with associated parameter estimates and *p*-values, whereas arrows for nonsignificant paths are dashed. Thin arrows and abbreviations are as in Fig. 2. Table 3 contains all parameter estimates.

Pz

If the hypothesis that EM is released solely because cells are leaky and cannot contain all of their fixed C within their cell membranes were correct, we would expect a positive relationship between Pz and amount of EM. However, the negative effect of Pz on colloidal EM suggests that this hypothesis is incorrect and, thus, that the release of EM has some other function. Our study was not designed to address possible functions, but a negative relationship with Pz is consistent with the idea that EM is released in response to stress.

Primary production and the amount of EM usually are positively related (phytoplankton, Baines and Pace 1991; marine benthos, Staats et al. 2000), but negative relationships have been reported. For example, Berman (1976) and Anderson and Zeutschel (1970) found negative relationships between primary production and EM in phytoplankton. One possible explanation for a negative relationship between primary production and the amount of EM is that EM also can be derived from heterotrophic release. Sites with low primary production may have high heterotrophic biomass/activity, and heterotrophs could produce large amounts of EM. We did not find a relationship between EM and respiration (a proxy for heterotrophic biomass), but respiration may not be the best way to represent the heterotrophic community. We would expect a stronger effect of the nonphotosynthetic community than of the algal community in Lake Opeongo because the sediment in the littoral zone is net heterotrophic, especially at deeper sites. Therefore, the link between the nonphoto-

Table 3. Standardized parameter estimates for the best path model for colloidal and total extracellular material (EM). _log = log(x)-transformed, Pz = in situ photosynthesis, P_{MAX} = maximum photosynthesis, alpha = photosynthetic efficiency in subsaturating light, biomass = algal biomass as chlorophyll *a*, community = algal community composition represented by pigments, light = light intensity at sampling depth, disturbance = wind-weighted fetch averaged over the previous 24 h ($\sqrt{x}$-transformed), sediment = sediment water content ($\sqrt{x}$-transformed).

Colloidal models	Extracellular material	Estimate	Total models	Extracellular material	Estimate
Colloidal EM	P_Z	–1.15	Total EM	P_Z	–0.75
Colloidal EM	P_{MAX}	0.45	Total EM	P_{MAX}	0.35
Colloidal EM	Alpha_log	0.41	Total EM	Alpha_log	0.41
Colloidal EM	Biomass_log	–0.27	Total EM	Biomass_log	–0.19
Colloidal EM	Community	–0.52	Total EM	Community	–0.42
Colloidal EM	Light	0.56	Total EM	Light	0.30
Colloidal EM	Disturbance	–0.10	Total EM	Disturbance	–0.02
Colloidal EM	Sediment	–0.18	Total EM	Sediment	0.04
Pz	P_{MAX}	0.39	Total EM	Date	–0.40
Pz	Alpha_log	0.40	Pz	P_{MAX}	0.32
Pz	Biomass_log	–0.05	Pz	Alpha_log	0.40
Pz	Light	0.72	Pz	Biomass_log	–0.09
Pz	Date	–0.05	Pz	Light	0.72
Pz	Disturbance	–0.11	Pz	Date	0.10
Biomass_log	Light	0.37	Pz	Disturbance	–0.06
Biomass_log	Date	0.50	Biomass_log	Light	0.41
Biomass_log	Disturbance	–0.10	Biomass_log	Date	0.08
Community	Light	–0.59	Biomass_log	Disturbance	–0.33
Community	Sediment	0.06	Community	Light	–0.60
			Community	Sediment	0.08
R^2	Colloidal EM	0.40	R^2	Total EM	0.42
	Pz	0.91		Pz	0.91
	Biomass_log	0.48		Biomass_log	0.36
	Community	0.33		Community	0.33

synthetic community and EM should be explored with more-direct measures, such as bacterial activity.

The relationship between primary production and colloidal EM also should be investigated in lakes with differing amounts of benthic primary production. Lake Opeongo is a brown-water lake, and light intensities may be lower than in systems where positive relationships have been observed. We captured a wide range in primary production in Lake Opeongo (3 orders of magnitude from 0.4 to > 40 mg O_2 m^{-2} h^{-1}), but even our highest values are lower than values in many lakes.

Community composition

Algal community composition affected the amount of colloidal EM. de Winder et al. (1999) also found that the amount of EM was an order of magnitude higher in diatom than in cyanobacterial mats in a marine intertidal zone of the Wadden Sea. However, we do not know which members of the community might be producing more extracellular material in Lake Opeongo. The arrangement of pigments along CA1 could, in theory, be used to identify the algal groups that are driving the negative relationship between the community composition and extracellular material, but the pigments that best discriminated among the sites were unidentified. Nevertheless, we detected an effect of community composition despite our relatively coarse measure of community composition (pigments can differentiate major groups of algae but not species within the same group). The distinction among diatom species probably is important because some species are motile (and excrete EM in the process) and others are not (Underwood and Paterson 2003, Consalvey et al. 2004). Diatoms that cannot move, such as episammic diatoms, may secrete EM for the purpose of attachment.

Colloidal vs capsular EM

Community composition and primary production affected colloidal EM. Both factors reflected current conditions. In contrast, total EM was affected only by time of year. The difference between colloidal and total EM is

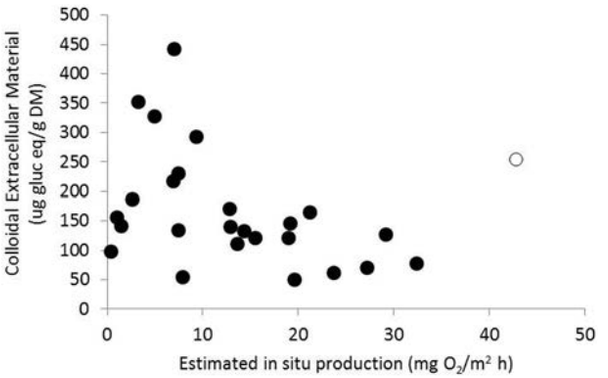

Figure 4. Scatterplot showing colloidal extracellular material vs estimated in situ primary production, with the data point on the far right (open circle, high in situ photosynthesis) removed (colloidal extracellular material = −5.1Pz + 230.23; $p = 0.015$, $R^2 = 0.23$). If all data points are included, the relationship is weaker ($p = 0.14$, $R^2 = 0.09$). Pz = estimated in situ production, gluc eq = glucose equivalents, DM = dry mass.

tightly bound capsular EM. Therefore, time of year affected capsular but not colloidal EM. This result and the lack of an effect of other factors on capsular material suggest that capsular material may be more refractory than colloidal EM. Many environmental variables covary with time of year, so we do not know to what the algae are responding. For example, water temperature increased ~10°C from early to mid-season and then remained constant to the end of the season, and day length changed throughout the season. However, in Lake Opeongo (as in other Canadian Shield lakes), phytoplankton populations do not change appreciably during the ice-free season because they are always in low abundance. Nutrient runoff from the surrounding land in response to storms probably is negligible because granite bedrock weathers little and agricultural activity is not present in the watershed. These environmental variables change in the same way across many Shield lake systems, which could mean that our results are generalizable to lakes other than Lake Opeongo.

This difference between capsular and colloidal EM has been seen in other studies. Gerbersdorf et al. (2007) found that colloidal material was correlated with current algal and bacterial biomass, whereas capsular EM was correlated with phaeopigments (indicative of past production). In other studies, bacterial activity was tightly coupled with colloidal but not capsular EM (Geesey et al. 1978, Haack and McFeters 1982, van Duyl et al. 1999). Capsular EM persists through tidal immersion, whereas colloidal EM is 0 at the start of each tidal emersion period (Orvain et al. 2003). Although the above authors have hypothesized that the amount of colloidal EM is a function of current rather than past production, little is known about the relationship between standing stock (measured in our study) and rate of production of EM.

Environment

In marine systems, environmental variables strongly affect production of EM. However, except for the influence of time of year on total EM, we found no direct environmental effects on amount of EM, although environmental factors might act indirectly. For example, environmental conditions might influence which algae can live at any given site and, thus, regulate how much production could occur. Alternatively, strong relationships simply might not exist in freshwater lakes. For example, light might have little effect in Shield lakes because lake benthic algae are never exposed to full sunlight, as happens in marine intertidal zones during low tide. Reservoirs with periodic water-level fluctuations might be a promising place to study the effects of light on EM in freshwater. Nutrients often limit phytoplankton, but perhaps not benthic algae in Shield lakes because they have access to nutrients in the sediment. Furthermore, lake benthic algae may not be exposed to the high levels of disturbance experienced by marine intertidal algae.

Absence of significant effects of environmental factors on EM may not be universal in lakes. For example, highly organic, brown water may reduce the importance of light in Lake Opeongo and other Shield lakes, whereas light may be important in clearer lakes. However, we captured a range of light intensities from 4 to > 650 μmol m^{-2} s^{-1} by sampling across nearly the full range of depths in the euphotic zone (1–6 m). We focused on the potential effect of too much light, but the absence of a light effect in the brown waters of Lake Opeongo shows that too little light does not affect the amount of EM.

Another possible reason for our failure to detect an effect of light is the way we tested for this effect. In ma-

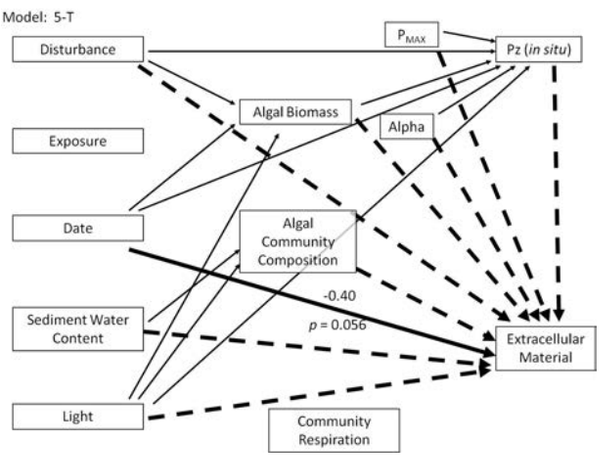

Figure 5. The best path model for total extracellular material. Arrows that represent significant paths are thick and solid, with associated parameter estimates and p-values, whereas arrows for nonsignificant paths are dashed. Thin arrows and abbreviations are as in Fig. 2. Table 3 contains all parameter estimates.

rine studies, investigators compared production of EM in light vs dark conditions (e.g., Smith and Underwood 1998, Staats et al. 2000, de Brouwer et al. 2003), whereas we sampled along a light gradient. The difference between high and no light might be greater than the difference between high and low light. Results of studies of production of EM along a light gradient in the marine intertidal zone were mixed. Perkins et al. (2001) found no difference in the amount of EM in ambient and shaded treatments, whereas Underwood (2002) found that the lowest and highest amounts of EM came from the sites with the lowest light levels (subtidal sites). Thus, use of light gradients instead of light vs dark in marine studies might decrease the apparent importance of light as a driver of the production of EM.

The physical disturbance generated by the wind is proportional to fetch length, so lake size and orientation should influence the degree of physical disturbance in the lake. The South Arm of Lake Opeongo is 5.8 km long and oriented along the primary axis of the wind. Thus, sites were either highly exposed or highly protected, so if physical disturbance influences the amount of EM, we should have been able to detect this effect in Lake Opeongo. However, we did not detect a direct effect of any level of physical disturbance on EM. Physical disturbance could have affected the amount of EM indirectly, if it acted in several opposing ways. For example, an increase in EM caused by increased migration by mobile diatoms after burial (Saburova and Polikarpov 2003, Consalvey et al. 2004, Apoya-Horton et al. 2006) might have been balanced by a decrease in EM as it is swept away by the increased water currents (Staats et al. 2000, de Brouwer and Stal 2001, Orvain et al. 2003). A detailed mechanistic approach could help elucidate the effect of disturbance on EM.

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