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INVESTIGATING EXUDATE- AND HABITAT-MEDIATED EFFECTS OF PHYTOPLANKTON ON LAKE BACTERIAL COMMUNITY DYNAMICS

BY

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THESIS

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ABSTRACT

Correlated patterns of abundance, activity, and composition are commonly observed between phytoplankton and bacterial communities. There are a number of potential explanations for correlated dynamics. Recent observations of temporal succession in lake microbial communities provide evidence for phytoplankton populations acting as biological drivers structuring bacterial communities. The following thesis addresses two potentially important connections between phytoplankton and bacteria: resource-mediated linkage through production of labile carbon exudates and habitat-mediated linkage. To investigate phytoplankton exudates as a potential linkage, the first investigation focused on bacteria able to use the photorespiration-specific exudate glycolate. Diversity and dynamics of glycolate-utilizing bacteria were characterized in six lakes using functional gene glycolate oxidase subunit D (*glcD*). Freshwater glycolate-utilizing populations exhibited a range of taxonomic diversity and contained many sequences clustering with a *glcD* sequence from the cosmopolitan freshwater *Polynucleobacter* genus. Glycolate-utilizing and total bacterial community-level variation was largely explained by dynamics of phytoplankton populations (35-40%) and the interaction between these phytoplankton populations and the environment (17-18%). Population-level correlations between specific phytoplankton and glycolate-utilizing bacteria were also detected. These observations support the hypothesis that algal exudates are resource-based drivers of bacterial community composition and identify bacterial taxonomic groups with members capable of responding directly to a specific exudate. Contribution of epiphytic bacteria inhabiting algal cells to correlated community dynamics was investigated by comparing temporal community patterns in the particle-associated bacteria to those of whole bacteria and phytoplankton in Crystal and South Sparkling Bogs. Regular patterns of succession, in addition to correlations between phytoplankton and whole bacterial communities (ρ=0.514, p=0.001) and phytoplankton and particle-associated bacterial communities (ρ=0.739, p=0.001), were detected in Crystal, but not South Sparkling Bog (ρ=0.265, p=0.038; ρ=0.167, p=0.103, respectively). Attached and free-living bacterial assemblages were compared to classify bacterial taxa based on presence in attached and/ or free-living fractions. Despite mixed results for community-level correlations, bacterial populations were positively correlated to abundance of specific phytoplankton in both lakes. Algal-correlated bacteria ranged from primarily particle-associated bacteria in Crystal Bog to a mix of particle-associated and free-living bacteria in South Sparkling Bog. These

observations provide support for habitat-mediated linkages, while also indicating the importance of other mechanisms that affect free-living and habitat generalist populations. Taken together, these investigations contribute to a growing body of research demonstrating the importance of biological interactions in shaping microbial community structure. Microbial community composition and function have implications for energy flow, carbon flux, and biogeochemical transformations with ecosystem-level consequences. Understanding biological interactions that structure bacterial communities may facilitate the building of a predictive framework for understanding compositional and functional responses of bacteria and microbially-mediated processes to changing environmental conditions.

To my uncle, Nels “Nick” Thorgesen

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CHAPTER 1

Introduction

Bacterial processes greatly influence energy flow and biogeochemical transformations in lakes (e.g., nutrient cycling, mercury methylation) (e.g., Cole *et al.*, 1988; Roden and Emerson, 2007; Ward, 2007). Much has been learned about aquatic bacteria from aggregate community measurements, including abundance, biomass, and production (e.g., Cole *et al.*, 1982; Coveney *et al.*, 1977). Notably, aggregate measures regard all bacteria and bacterial processes as components of a 'black box,' principally considering inputs and outputs with the assumption that bacteria are functionally equivalent. However, bacterial populations differ in their metabolic capabilities and physiological tolerances (Madigan *et al.*, 2003) and respond differentially to ecological forces, such as grazing pressure (Kent *et al.*, 2006; Pernthaler *et al.*, 1997). Rates of bacterial processes including decomposition and production can be partially attributed to bacterial community composition (Bertilsson *et al.*, 2007; Strickland *et al.*, 2009). Looking into the 'black box' and understanding forces structuring bacterial communities, as well as the capabilities of populations within them may facilitate prediction of biogeochemical transformation rates as well as responses of microbially-mediated ecosystem processes to environmental change. Further, bacterial communities provide highly malleable, adaptive, and diverse systems with which to test ecological theory.

Information gained from microbial community analyses has enabled a shift from primarily descriptive studies to those seeking to determine mechanisms structuring communities. Molecular investigations of bacteria in lakes have demonstrated that certain types of bacteria are typical freshwater inhabitants (Zwart *et al.*, 2002) and that similar communities exist among lakes of similar productivity, dissolved organic carbon concentration, and dissolved organic matter source (Jones *et al.*, 2009; Yannarell and Triplett, 2004). Changes in bacterial communities have been documented across multiple years, with correlations between compositional shifts and water temperature, mixing status, and seasonal variation in organic matter source (Crump *et al.*, 2003; Nelson, 2009; Shade *et al.*, 2007). Biological drivers also appear to play an important role in structuring bacterial communities. A study investigating factors influencing temporal shifts in bacterial communities determined that variation was largely explained by phytoplankton community composition and the interaction between

phytoplankton and local environmental factors, indicating that phytoplankton are important in structuring bacterial communities (Kent *et al.*, 2007).

The following thesis consists of a literature review examining community-level correlations between phytoplankton and bacterioplankton and potential explanations; an investigation of resource-mediated control of bacterial communities using *glcD*, a functional gene encoding an enzyme linked to utilization of the photorespiration-specific exudate glycolate; an investigation of the contribution of particle-associated bacteria to correlated temporal patterns between bacterial and phytoplankton communities; and a brief summary integrating the research conducted into the larger context of understanding aquatic algal-bacterial interactions.

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CHAPTER 2

Literature Review: Correlated temporal variation between phytoplankton and bacteria in lakes and potential explanations

Bacterial and phytoplankton community correlations

Positive correlations between algal biomass or primary production and bacterial abundance, production, or activity have been reported across diverse aquatic systems (Azam *et al.*, 1983; Cole, 1982; Cole *et al.*, 1988; White *et al.*, 1991). In addition to these aggregate measures, compositional changes in the phytoplankton community have been related to shifts in bacterial community composition in both mesocosm experiments (Kent *et al.*, 2006; Pinhassi *et al.*, 2004) and field observations (Kent *et al.*, 2004; Kent *et al.*, 2007; Rooney-Varga *et al.*, 2005). Distinct bacterial communities develop in mesocosms with diatom-dominated communities compared to phytoflagellate-dominated phytoplankton communities (Pinhassi *et al.*, 2004) and when incubated with green algal detritus compared to cyanobacterial detritus (van Hannen *et al.*, 1999). At the population level, 24 out of 65 identified bacterial populations were strongly correlated to the dynamics of at least one phytoplankton species in Crystal Bog (Newton *et al.*, 2006). Correlated patterns of phytoplankton and bacterial community succession have also been documented in Crystal Bog over multiple years (Kent *et al.*, 2006; Kent *et al.*, 2007). Similarly strong correlations were observed in five additional humic lakes (Kent *et al.*, 2007).

Particle-associated bacterial response to phytoplankton

Bacteria in the water column may be free-living or associated with particulate matter. The particulate fraction includes bacteria attached to organisms, such as phytoplankton and zooplankton. Investigations of particle-associated and free-living bacteria commonly use size fractionation to isolate and characterize assemblages within respective fractions (e.g., Allgaier and Grossart, 2006; Riemann *et al.*, 2000; Riemann and Winding, 2001). Depending on the specific focus of the study, particle-association has been operationally defined as retention on filters with pore sizes ranging from 1.0 to 10 μm with the filtrate containing free-living bacteria (e.g., Allgaier and Grossart, 2006; Riemann *et al.*, 2000; Riemann and Winding, 2001).

Free-living bacteria are typically more numerous than particle-associated bacteria, often by greater than an order of magnitude (Allgaier *et al.*, 2008; Friedrich *et al.*, 1999). However, a shift in numerical dominance from the free-living to the particle-associated fraction of the

bacterial community has been observed during freshwater mesocosm phytoplankton blooms (Worm *et al.*, 2001). The particle-associated fraction has been observed to have higher cell specific rates of growth (Riemann *et al.*, 2000) and bacterial protein production (Allgaier *et al.*, 2008), which may be due to a higher proportion of active cells (Friedrich *et al.*, 1999). As a result, particle-associated bacterial activity, measured by [^{3}H] thymidine uptake, accounted for 44-57% of total activity in four lakes despite abundances of less than 10% of the total bacteria (Friedrich *et al.*, 1999).

Distinct bacterial community composition has been observed in free-living compared to particle-associated fractions within a lake using both community fingerprints and 16S rRNA-based taxonomic composition (Allgaier and Grossart, 2006; Riemann and Winding, 2001). A number of taxa are unique to free-living or particulate fractions. In particular, *Actinobacteria* composed a large proportion of free-living but not particle-associated bacteria in four lakes of varying trophic status (Allgaier and Grossart, 2006). The community composition of *Actinobacteria* within the free-living and particle-associated fractions was also different, with members of certain lineages (e.g., *Mycobacteraceae*) only found in the attached fraction (Allgaier *et al.*, 2007). Particulate and free-living fractions also share a number of common taxa (e.g., Besemer *et al.*, 2005; Riemann and Winding, 2001; Rooney-Varga *et al.*, 2005). This observed overlap may indicate that certain bacteria alternate between free-living and particle-associated lifestyles. Alternatively, phylogenetic resolution of the molecular methods used may be too coarse to differentiate distinct populations (Allgaier *et al.*, 2007; Hunt *et al.*, 2008; Jaspers and Overmann, 2004).

Free-living and particle-associated bacteria have been shown to differ in their responses to phytoplankton. Phytoplankton community composition was strongly correlated with temporal variation in the particle-associated fraction, but not the free-living fraction in a coastal marine environment (Rooney-Varga *et al.*, 2005). Algal blooms have been observed to cause a shift in production, abundance, and enzyme activity from free-living to particle-associated bacteria (Riemann *et al.*, 2000). Additionally, bacterial secondary production has been coupled to phytoplankton bloom development in attached and free-living fractions of the bacterial community (Allgaier *et al.*, 2008). Peaks in production of particle-associated bacteria suggest that they are able to utilize resources from both living and senesced algal cells (Allgaier *et al.*, 2008; Middelboe *et al.*, 1995).

Explanations for observed patterns

Community level correlations between phytoplankton and bacteria may be explained by a number of potential mechanisms. Environmental factors including temperature and nutrients have been shown to influence bacterial (Jardillier *et al.*, 2005; LaPara *et al.*, 2000; Wu and Hahn, 2006) and phytoplankton (Strecker *et al.*, 2004; Tilman *et al.*, 1982) populations. Bacteria and phytoplankton may respond similarly to changes in a shared physical environment. Alternatively, correlated community dynamics may be explained by interactions that influence the abundance of certain community members. Algal-bacterial interactions in aquatic systems have been reviewed by Cole (1982). Briefly, phytoplankton and bacteria can influence each other by contributing to or depleting a shared nutrient pool. Bacteria may shape phytoplankton communities by causing certain taxa to lyse (e.g., Kim *et al.*, 2007), influencing algal toxicity (e.g., Hold *et al.*, 2001), and releasing compounds with algicidal activity against certain taxa (e.g., Hasegawa *et al.*, 2007; Imai *et al.*, 1993; Lovejoy *et al.*, 1998; Yoshinaga *et al.*, 1997). In addition, bacteria may release stimulatory compounds, such as B vitamins, and allow certain algal taxa to persist (e.g., Bertrand *et al.*, 2007).

There are also a number of ways that phytoplankton may shape bacterial communities including food-web interactions and providing a microhabitat to colonize. The importance of phytoplankton communities to shaping total bacterial community composition was suggested by Kent and colleagues (2007) who examined the relative contribution of lake specific environmental (e.g., temperature, nutrients), regional meteorological (e.g., precipitation, photosynthetically active radiation), and biological (phytoplankton community composition, chl *a*, and heterotrophic nanoflagellate abundance) factors in explaining temporal variation of bacterial communities in lakes. Biological factors, principally phytoplankton community composition, explained about 40% of variation in the bacterial community with an added 25% of variance explained by the biological-environmental interaction (Kent *et al.*, 2007). Phytoplankton may have both top-down and bottom-up influences on bacterial communities. Many phytoplankton in humic lakes are mixotrophs (e.g., *Gymnodinium* sp., *Cryptomonas* sp.) able to carry out photosynthesis and also consume bacteria (Stoecker, 1999; Urabe *et al.*, 2000). Phytoplankton may also provide a source of dissolved organic matter for bacteria or provide surfaces to colonize. The remaining sections explore the potential for phytoplankton to act as an

evolving resource base, specifically addressing the bacterial exudate glycolate, and explore evidence for coupled abundance patterns resulting from bacterial attachment to phytoplankton.

Phytoplankton as an evolving resource base

Algal derived organic matter can become available to biota following cell senescence and death, grazer 'sloppy feeding' (Jumars *et al.*, 1989), auto or viral lysis (Bratbak *et al.*, 1994; Brussaard *et al.*, 2005), or as exudates from living cells (Baines and Pace, 1991). Algal detritus can have significant impacts on microbial communities. Availability of detritus following algal blooms appears to stimulate peaks in microbial activity and biomass (Cole, 1982). Bacterial production and hydrolytic activity increases following bloom collapse, suggesting that bacteria colonize and decompose senesced cells (Cole, 1982). Further, bacterial community composition has been shown to differ in incubations with green algal detritus compared to cyanobacterial detritus (van Hannen *et al.*, 1999).

Exudates constitute a substantial fraction of photo-assimilated carbon in natural phytoplankton communities (Baines and Pace, 1991) as well as phytoplankton cultures (Hellebust, 1965). Extracellular release in lakes ranges between 0.01 and 10.2 μg C $liter^{-1}$ hr^{-1}, averaging 5-41% of primary productivity (see Baines and Pace, 1991 for review). Exudate quantity and composition is dependent on a variety of factors including phytoplankton species (Fogg, 1983; Hellebust, 1965; Myklestad, 1995), nutrient availability (Hama and Honjo, 1987; Parker and Armbrust, 2005; Urbani *et al.*, 2005), light (Morris and Skea, 1978; Panzenbock, 2007; Parker and Armbrust, 2005) and temperature (Parker and Armbrust, 2005).

It is well established that carbon source has a substantial effect on bacterial community composition (e.g., Jones *et al.*, 2009; Judd *et al.*, 2006; Nelson, 2009). Quantity and quality of algal exudates have been shown to alter bacterial community composition. Exudates produced under phosphorus limitation by the diatom *Cylindrotheca closterium* caused a decrease in bacterial growth rate and carbon demand, as well as a shift in bacterial community composition, compared to exudates from the same species grown in balanced phosphorus conditions (Puddu *et al.*, 2003). A change in composition of bacteria has also been observed when algal cultures shift from exponential to stationary growth (Grossart *et al.*, 2005).

Temporal variation of phytoplankton communities (e.g., Anneville *et al.*, 2002; Graham *et al.*, 2004) coupled to seasonal changes in nutrient and light availability (Magnuson *et al.*,

1990) make photosynthetically-derived organic carbon a dynamic resource (Charpin *et al.*, 1998; Morris and Skea, 1978). For example, the proportion of carbon excreted as protein in the Gulf of Maine changed seasonally, comprising 10-20% in the winter, 22-35% in the summer, with a high value of 37-47% coinciding with a spring phytoplankton bloom (Morris and Skea, 1978).

Phytoplankton exudate glycolate

Examining the effect of phytoplankton exudates on bacteria in the environment presents a suite of potential challenges. Different species of phytoplankton release unique combinations of exudates (Fogg, 1983; Hellebust, 1965; Myklestad, 1995). For example, axenic cultures of *Chlorella* sp. were observed to release mainly proline, *Olisthodiscus* sp. mainly mannitol, and *Coccolithus hexleyi* a mixture of mannitol, aspartic acid, arabinose, and an unknown compound (Hellebust, 1965). Excreted compounds have even been shown to differ between cultures of the algae *Cyclotella nana* isolated from different locations (Hellebust, 1965). Common algal exudates include amino acids (e.g., lysine, proline), organic acids (e.g., glutamic and glycolic acids), sugars (e.g., glucose, arabinose) and sugar-alcohols (e.g., mannitol, glycerol) (Hellebust, 1965; Myklestad, 1995). While exudate composition has been characterized for a range of algal taxa under controlled, and often axenic, growth conditions (e.g., Hellebust, 1965; Myklestad, 1995), it is difficult to know what compounds phytoplankton are releasing in the lake environment. Additionally, even when a particular exudate is known to be produced by phytoplankton in a given system, there may be multiple potential sources of this compound, confounding interpretation of data and making it difficult to draw conclusions specifically about exudates.

The algal exudate glycolate has several properties that make it of interest for studying algal-bacterial interactions. Glycolate is produced during photorespiration when oxygen, instead of carbon dioxide, is incorporated by the enzyme rubisco (Oliver, 1998). All organisms with rubisco from photosynthetic anaerobic bacteria (e.g., Storro and McFadden, 1981) to plants (e.g., Oliver, 1998) are able to produce glycolate. Glycolic acid is considered to be the most common algal excretory product (Fogg, 1983; Hellebust, 1965). Whether glycolate produced by photorespiration is released or metabolized differs among species and environmental conditions (Fogg, 1983). Because glycolate is a photorespiration-specific compound, phytoplankton are the predominant sources of glycolate when they are the primary photosynthesizing organisms.

In the environment, glycolic acid flux accounts for 0.5% of phytoplankton primary production, which is approximately as much as any other single substrate (Wright and Shah, 1977). Glycolate concentrations are typically between 20 and 60 μg l^{-1} (Fogg, 1983). Significant correlations have been observed between phytoplankton biomass and glycolate concentration (Leboulanger *et al.*, 1997) and percent extracellular release and glycolate concentration (Alhasan and Fogg, 1987). Glycolate concentration has also been shown fluctuate diurnally, increasing during the day and peak primary production and decreasing at night due to heterotroph consumption (Coughlan and Alhasan, 1977; Leboulanger *et al.*, 1997). Pronounced seasonal changes in glycolate concentration have also been observed (Wright and Shah, 1977).

A subset of bacteria is capable of using glycolate. Some bacteria can grow with glycolate as the only carbon source (e.g., *Pseudomonas* (Kornberg and Gotto, 1961)). Culture-based studies have shown that 63% of bacterial colonies cultured from seawater were capable of respiring glycolic acid, but were unable to use it for growth when it was the sole carbon source (Wright and Shah, 1975). These cultures, however, did show enhanced growth in the presence of glycolic acid and lactic or acetic acid, compared to lactic or acetic acid alone (Wright and Shah, 1975). Mineralization rates for glycolate are typically high, 75% and 69% of glycolate carbon was respired in Upper Klamath Lake (southern Oregon) and Gravelly Pond (Illamilton, MA), respectively compared to 26% of glucose, 29% of acetate, and 30% of glycine, indicating that metabolism of glycolate primarily involves respiration (Wright, 1975). Glycolate is considered to be a major energy source, potentially driving uptake of other compounds in aquatic systems (Wright, 1975).

The molecular basis for glycolate utilization has been well characterized. Glycolate oxidase, a multiple subunit, membrane-bound enzyme encoded by the *glc* locus enables bacteria to use glycolate (Pellicer *et al.*, 1996). Primer sets have been developed to amplify the D subunit of glycolate oxidase (*glcD*) and have been shown to amplify this locus from DNA derived from isolates capable growth on M9 media supplemented with glycolate (Lau and Armbrust, 2006). Sequences of *glcD* obtained from marine environments belonged to six major bacterial phylogenetic groups, with most forming novel clades (Lau and Armbrust, 2006; Lau *et al.*, 2007). Abundance of six dominant phylotypes of glycolate-utilizing populations followed different trajectories (i.e., increased, decreased, and remained constant) over the course of a phytoplankton bloom (Lau *et al.*, 2007). Additionally, higher levels of g*lcD* transcripts have been

detected during the day than at night, corresponding to glycolate concentration patterns (Lau *et al.*, 2007). While glycolate is only one exudate, tracking bacteria able to use glycolate facilitates the study of a subset of the bacterial community able to respond specifically to phytoplankton exudates. Thus, the glycolate oxidase gene can serve as a functional gene for algal exudate utilization.

Phytoplankton as a habitat

Phytoplankton provide a habitat for epiphytic bacterial populations attached to cells as well as endosymbiotic bacteria living within cells (Cole, 1982). Cells attached to phytoplankton likely have increased access to labile carbon exudates (Fogg, 1983). Differences observed between the responses of particle-associated and free-living bacteria to algae may be attributed, in part, to bacterial populations that live in association with algae. Electron microscopy and fluorescence microscopy using the nucleic acid stain DAPI and fluorescent *in situ* hybridization (FISH) probes have shown that bacteria are commonly associated with various algal taxa, including filamentous desmids and dinoflagellates (e.g., Biegala *et al.*, 2002; Fisher and Wilcox, 1996). Molecular surveys of epiphytic bacterial populations have shown that some epiphytic bacteria are commonly associated with different types of algae while others appear to be species specific (Fisher *et al.*, 1998; Hold *et al.*, 2001; Sapp *et al.*, 2007). Selection by algae for distinct bacterial populations is supported by the observation that bacterial populations on cultured phytoplankton cells were more similar among *Alexandrium* cultures from different geographic regions than between *Alexandrium* and other non-toxic phytoplankton in the Gulf of Maine (Hold *et al.*, 2001).

Summary

Community- and population-level correlations are frequently observed between bacteria and phytoplankton. While there are a number of potential algal-bacterial interactions that may explain such correlations, previous work conducted in north temperate lakes suggests that phytoplankton communities are influential drivers of bacterial community composition. Two potential mechanisms to explain the influence of phytoplankton bacterial populations include providing a source of organic matter and a microenvironment suitable for colonization. Phytoplankton derived organic matter may become available to bacteria through a number of pathways. The role of algal exudates in carbon flow to bacteria is of interest due to ubiquity of

exudate production, lability (relative to other C sources), and variability. Glycolate is a photorespiration-specific compound released by phytoplankton, and the glycolate utilization gene *glcD* can be used to study bacteria responding to algal exudates. Phytoplankton may also influence the structure of bacterial communities through interactions with specific attached and endosymbiotic bacterial taxa.

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CHAPTER 3

Dynamics of glycolate-utilizing bacteria in lakes suggest exudate-mediated linkage between phytoplankton and bacterial communities

Abstract

Observations of algal-bacterial community dynamics in lakes suggest that phytoplankton populations act as resource-mediated drivers of bacterial community composition. To investigate the potential for phytoplankton exudates to link algal and bacterial communities, the diversity and dynamics of heterotrophic bacterial populations able to use glycolate, a photorespiration-specific exudate, were characterized in six lakes using functional gene glycolate oxidase subunit D (*glcD*). Freshwater glycolate-utilizing bacteria exhibited broad phylogenetic diversity including *Alpha*-, *Beta*-, *Gamma*- and *Delta-proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia.* The majority of *glcD* gene sequences were betaproteobacterial, with 48% clustering with *Polynucleobacter necessarius*. T-RFLP fingerprinting of glycolate-utilizing populations revealed temporal patterns similar to those observed for total bacteria and phytoplankton. Variation in both glycolate-utilizing and total bacterial communities was largely explained by dynamics of phytoplankton populations (35-40%) and the interaction between these phytoplankton populations and the environment (17-18%). Population-level correlations between individual phytoplankton and glycolate-utilizing bacteria were also detected. These observations support the hypothesis that algal exudates are resource-based drivers of bacterial community composition and identify bacterial lineages capable of responding directly to a specific exudate.

Introduction

Processes carried out by phytoplankton and bacteria are fundamental determinants of energy flow and nutrient cycling in lakes (Cole *et al.*, 1988; Cotner and Biddanda, 2002). Correlations between phytoplankton and bacterial communities are commonly observed with aggregate community measures, such as abundance and activity (e.g., Azam *et al.*, 1983; Cole *et al.*, 1988; Cole *et al.*, 1982; White *et al.*, 1991) as well as community composition. Compositional changes in the phytoplankton community have been related to shifts in bacterial community composition in mesocosm experiments (Kent *et al.*, 2006; Pinhassi *et al.*, 2004) and observations of natural patterns of succession (Kent *et al.*, 2004; Kent *et al.*, 2007; Rooney-Varga *et al.*, 2005). Trophic interactions that influence bacterial communities have implications for bacterial functions, and potential for ecosystem level consequences (Bertilsson *et al.*, 2007; Strickland *et al.*, 2009). Accordingly, it is important to understand interactions linking phytoplankton and bacterial communities.

There are a number of potential explanations for correlated community dynamics between bacteria and phytoplankton in aquatic ecosystems. In the absence of biological interactions, correlations could result from both communities independently responding to changes in the lake environment, such as water temperature or nutrient availability. Kent and colleagues (2007) examined the relative contribution of lake-specific environmental (e.g., temperature, nutrients), regional meteorological (e.g., precipitation, photosynthetically active radiation), and biological (i.e. bacteria: phytoplankton and heterotrophic nanoflagellate measures, phytoplankton: cladoceran zooplankton populations) factors in explaining temporal variation of bacterial and phytoplankton communities in lakes. While the phytoplankton community appeared to be strongly influenced by environmental parameters, the bacterial community did not. Instead, biological factors, principally phytoplankton community composition, explained about 40% of variation in the bacterial community with an added 25% of variance explained by the biological-environmental interaction (Kent *et al.*, 2007).

Observations made by Kent et al. (2007) suggest an alternative explanation for coupled community dynamics; bacterial communities change in response to phytoplankton-derived dissolved organic matter. Organic compounds can be released from algae following cell senescence and death, as a consequence of grazer 'sloppy feeding' (Jumars *et al.*, 1989), due to cell lysis (Bratbak *et al.*, 1994; Brussaard *et al.*, 2005), and as exudates from living cells (Baines

and Pace, 1991). Algal detritus has a pronounced effect on microbial communities. The death phase of algal blooms often coincide with peaks in total microbial activity and biomass (Cole, 1982) as well as an increased proportion of particle associated bacterial production and hydrolytic activity (Middelboe *et al.*, 1995). Additionally, bacterial community composition changes in response to incubation with detritus from different algal species (van Hannen *et al.*, 1999).

Labile carbon compounds released as exudates by actively growing cells may also shape bacterial community composition. Extracellular release can comprise up to 40% of primary production (Baines and Pace, 1991). Amount and type of exudates released varies by species, and can include amino acids (e.g., lysine, proline), organic acids (e.g., glutamic and glycolic acids), sugars (e.g., glucose, arabinose) and sugar-alcohols (e.g., mannitol, glycerol) (Fogg, 1983; Hellebust, 1965; Myklestad, 1995). Environmental factors including nutrient availability (Hama and Honjo, 1987; Parker and Armbrust, 2005), light (Morris and Skea, 1978; Panzenbock, 2007; Parker and Armbrust, 2005) and temperature (Parker and Armbrust, 2005) also affect exudate composition. Seasonal succession in phytoplankton community composition (Anneville *et al.*, 2002; Graham *et al.*, 2004) and fluctuating environmental conditions make algal exudates an evolving resource base.

We hypothesize that correlated temporal patterns of phytoplankton and bacterial communities result, in part, from the response of bacterial populations to shifts in the composition and quantity of phytoplankton exudates. To characterize bacterial community response to exudates, this study focuses on bacteria capable of using glycolate, a photorespiration-specific compound excreted by actively photosynthesizing algae (Fogg, 1983; Oliver, 1998). Glycolate is one of the most common algal exudates (Fogg, 1983; Hellebust, 1965). Bacteria that possess the enzyme glycolate oxidase are able to use glycolate (Lord, 1972; Ornston and Ornston, 1969). The glycolate oxidase subunit D (*glcD)* gene has been developed as a marker to detect glycolate-utilizing bacteria and characterize population dynamics in marine environments (Lau and Armbrust, 2006; Lau *et al.*, 2007). While glycolate is only one exudate, tracking bacteria capable of using glycolate enables analysis of a subset of bacteria that may be directly responding to phytoplankton exudates. Essentially, the *glcD* gene serves as a functional gene for exudate-mediated bacterial interactions with phytoplankton. Our specific objectives were to characterize *glcD* gene diversity and compare temporal patterns of glycolate-utilizing

bacteria to phytoplankton in six humic lakes where coupled microbial community dynamics have previously been observed (Kent *et al.* 2007).

Materials and methods

Study sites and sample collection

Microbial communities were examined in six shallow humic lakes in Northern Wisconsin (Table 3.1). The sample sites and collection protocol were described previously by Kent and colleagues (2007). Briefly, integrated water column samples spanning the depth of the epilimnion were collected at the deepest point in each lake twice weekly from May 28 to August 22, 2003. Organisms present in these samples were concentrated onto 0.2 µm filters (Supor-200; Pall Gelman, East Hills, NY, USA). Filters were frozen immediately and stored at -80°C prior to DNA extraction using FastPrep DNA purification kits (MP Biomedicals, Solon, OH, USA). Samples were preserved in 2% glutaraldehyde for phytoplankton identification.

Microbial community analysis

Microbial community data were generated by Kent *et al.* (2007). Briefly, bacterial community composition was assessed using automated ribosomal intergenic spacer analysis (ARISA). Dominant phytoplankton species were identified from glutaraldehyde preserved samples. Microscopic counts of phytoplankton populations were transformed using biovolume estimates generated for species common to these lakes (Graham *et al.*, 2004).

glcD gene sequencing

Extracted DNA was amplified with primers glcD-1f, 5'-GACCCAGACAATCGGAGTGCCGTGGTTSARCCNGGNGT-3' and glcD-1r, 5'-CATAAAATTGCGCTTCATAACGCCGATGCCRTGYTCNCC-3' (Lau and Armbrust, 2006). The polymerase chain reaction contained 0.8 mM deoxynucleoside triphosphates (Promega, Madison, WI, USA), 0.3 µM of each primer, PCR buffer with 2.0 mM $MgCl_2$ (Cat# 1771; Idaho Technology Inc., Salt Lake City, Utah, USA) and 0.05 U μl^{-1} GoTaq (Promega). PCR cycles consisted of a 2 min initial denaturation at 94°C, followed by 35 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 90s and a final extension of 72°C for 5 min (MasterCycler Gradient, Eppendorf AG, Hamburg Germany). PCR products from several reactions were concentrated and purified using QIAquick PCR purification followed by gel extraction according to manufacturer

instructions (Quiagen, Valencia, CA, USA). Purified product was incubated with deoxyadenosine triphosphate to ensure the presence of a poly-a tail at the 3' end, ligated into a pGEM-T Easy Vector, and transformed into JM109 *E. coli* competent cells (Promega).

Template for cycle sequencing was generated through PCR amplification of plasmid inserts with M13 primers. Unincorporated primers, nucleotides, and salts were removed using the AMPure system (Agencourt, Beverly, MA, USA). Purified product was sequenced by the W. M. Keck Center for Functional Genomics at the University of Illinois using ABI Prism BigDye terminator sequencing kit (Applied Biosystems Inc., Foster City, CA, USA) with standard PCR sequencing conditions in two reactions, one containing the M13 forward primer and other the M13 reverse primer.

Sequence and phylogenetic analysis

Forward and reverse sequences were assembled into contigs, edited, and trimmed to contain the region between glcD-1F and glcD-1R primers using Geneious Pro 4.7.4 (Drummond *et al.*, 2009). Sequences were translated and searched against the NCBI database of Clusters of Orthologous Groups of proteins (COGs) (Marchler-Bauer *et al.*, 2009). Non-sense sequences and sequences not containing the conserved domain for *glcD*, COG0277, were removed from subsequent analyses. DNA sequences from each clone library were aligned using Translation Align with default parameters of the MUSCLE protein alignment option in Geneious Pro 4.7.4 (Drummond *et al.*, 2009). An uncorrected-P distance matrix calculated from each alignment (SplitsTree version 4.8, (Hudson and Bryant, 2006)) was analyzed by DOTUR (Schloss and Handelsman, 2005) to estimate diversity and group sequences into operational taxonomic units (OTUs) at the 97% similarity level (Lau *et al.*, 2007).

A *glcD* genealogy was constructed from amino acid sequences translated from a representative DNA sequence for each OTU, the top two BLASTX hits for each OTU, and *glcD* sequences spanning bacterial phylogenetic groups. Amino acid sequences were aligned using the MUSCLE align option with default parameters in Geneious Pro 4.7.4 (Drummond *et al.*, 2009). Columns with gaps present in the majority of sequences were removed. Maximum likelihood trees were constructed in PROML (Phylip version 3.65) using the Jones-Taylor-Thornton probability model, constant rate of change, and randomized sequence addition with 100 bootstrap replicates (Felsenstein, 2005). The majority rule algorithm was used to construct the

consensus tree. The R Statistics Package (R Development Core Team 2007) was used to visualize the relative abundance of each OTU in each clone library.

Glycolate-utilizing bacterial community profiling

Freshwater *glcD* sequences were digested *in silico* using Cleaver (http://cleaver.sourceforge.net/) to determine which restriction enzymes provided optimal differentiation among OTUs. Two primer sets were used for amplification of *glcD* for terminal restriction fragment length polymorphism (T-RFLP) analysis: glcD-1f with glcD-2r, 5'-TGCATGTTTCCATCTCCTGCGTGRAANACRTT-3', and glcD-2f, 5'-GTTGCCCCGCACGGCTTCTACTACGCNCCNGAYCC-3', with glcD-1r (Lau and Armbrust, 2006). The glcD-2f and glcD-2r primers were labeled at the 5' end with phosphoramidite dyes HEX and 6-FAM, respectively. PCR conditions were identical to those used for amplifying *glcD* for sequencing with the following modification: 32 cycles of 94°C for 30s, X°C for 30s, and 72°C for 90s. Annealing temperature (X) was 60°C for glcD-1f, -2r primer combination and 67°C for glcD-2f, 1r.

PCR products from both primer sets were combined for each sample. Excess primers, nucleotides, and salts were removed using the MinElute PCR purification kit (Qiagen). Cleaned PCR products were digested in single-enzyme digests containing *Alu*I and *Rsa*I (New England BioLabs Inc., Ipswich, MA, USA). Digested samples were analyzed via denaturing capillary electrophoresis using an ABI 3730XL Genetic Analyzer (Applied Biosystems Inc.). Electrophoresis conditions were 63°C and 15 kV with a run time of 120 min using POP-7 polymer. The ABI GeneScan ROX 1000 size standard was used as the internal size standard for the *glcD* T-RFLP fingerprints. Size-calling and profile alignment were carried out using GeneMarker version 1.75 (SoftGenetics, State College, PA, USA). To include the maximum number of peaks while excluding background fluorescence, a threshold of 50 fluorescence units was used. The signal strength of each peak was normalized to account for run-to-run variations in signal detection by dividing the area of individual peaks by the total fluorescence (area) detected in each profile.

Data analysis

The Bray-Curtis similarity coefficient was calculated for all sample pairs based on *glcD* T-RFLP profiles (Legandre and Legandre, 1998). Difference in glycolate-utilizing bacterial

assemblages among lakes was tested using the analysis of similarity (ANOSIM) procedure on pairwise Bray-Curtis similarity values (Clarke and Warwick, 2001). The magnitude of the R test statistic generated by ANOSIM indicates the degree of separation between groups of samples with scores ranging from 0 (no separation) to 1 (complete separation). Similarity and ANOSIM calculations were carried out using PRIMER 6 (PRIMER-E Ltd, Plymouth, UK).

Correspondence analysis was conducted on glycolate utilizing bacterial community composition data generated by *glcD* T-RFLP, bacterial community composition data generated by ARISA, and phytoplankton community biovolume data. Variance partitioning using partial canonical correspondence analysis was used to determine the relative importance of environmental variables, meteorological variables, and phytoplankton community composition for explaining temporal patterns in glycolate utilizing and total bacterial community composition in each lake (ter Braak and Smilauer, 2002). Environmental variables (pH, total P, total N, DOC, water temperature) and meteorological variables (mean daily air temperature, photosynthetically active radiation, and precipitation) were collected by Kent and colleagues (2007). The unique contribution of each set of variables was determined by making that set ‘explanatory variables’ and removing the influence of the other two sets of variables (covariables). Variance explained by each partial ordination was then compared to the total variance explained when all categories were included in the analysis. All correspondence and canonical correspondence analyses were conducted using Canoco 4.5.1 (Biometris-Plant Research Internationa, Wageningen, The Netherlands) (ter Braak and Smilauer, 2002).

To identify glycolate utilizing bacteria influenced by specific phytoplankton populations, a general additive model was used to fit the response of individual glycolate-utilizing populations to each phytoplankton population from a correspondence analysis of each lake using CanoDraw (ter Braak and Smilauer, 2002). Only the responses of terminal restriction fragments (T-RFs) predicted from sequence data to represent a single lineage were compared to phytoplankton biovolume. A single variable correspondence analysis was then used to determine the percent variance of each glycolate-utilizing population explained by a single phytoplankton population (ter Braak and Smilauer, 2002). Relationships were reported if they were positive, significant ($p<0.001$), and explained at least 20% of variance in bacterial population dynamics.

Results

Taxonomic diversity of glcD

The 314 *glcD* sequences retained for analysis formed fifty operational taxonomic units (OTUs) at the 97% similarity level. OTUs clustered with *Alpha*-, *Beta*-, *Gamma*- and *Delta-proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia glcD* sequences in the *glcD* amino acid genealogy (Figure 3.1). Two OTUs formed a cluster with two marine *glcD* sequences that did not include sequences from any described bacteria. Twenty-five of the OTUs formed two large clusters within the *Betaproteobacteria*. The first cluster contained fourteen OTUs and *Polynucleobacter necessarius,* with a sister group composed of *Ralstonia sp*. These OTUs represented 48% of analyzed sequences across all clone libraries and were detected in five of the six lakes. The second cluster formed a sister group with *glcD* sequences from *Bordetella sp.* and contained OTUs representing 17.5% of sequences with detection in all six lakes. Twenty-six OTUs were unique to a given clone library (Figure 3.2). A subset of sequences was detected across multiple lakes, with eight OTUs found in at least three lakes. Five OTUs were detected only in August clone libraries, while other OTUs were observed in all months. To increase temporal resolution of glycolate-utilizing assemblages, T-RFLP fingerprints were generated from biweekly samples.

Dynamics of glycolate-utilizing bacteria

T-RFLP analysis was completed for samples from five lakes, because amplification of *glcD* gene fragments from Forestry Bog samples was not sufficient to generate T-RFLP profiles. Different glycolate-utilizing assemblages were observed in each lake (ANOSIM $R=0.687$, $p<0.001$) with among-lake differences related to dissolved organic carbon, total nitrogen, and total phosphorus (Figure 3.3a). Glycolate-utilizing populations within each lake were variable through time, with changes correlating to shifts in dominant phytoplankton populations (Figure 3.3b-f). Variance partitioning was carried out in order to assess and compare the amount of temporal variation in glycolate-utilizing and bacterial populations explained by phytoplankton populations, environmental variables, and meteorological variables. Percent of variation explained by each category was highly similar between glycolate-utilizing and total bacterial communities within a lake, and among glycolate utilizing and total bacterial communities across lakes (Figure 3.4). Phytoplankton community composition explained the most variance in both

glycolate-utilizing (39%) and bacterial (37%) communities. The interaction between phytoplankton community and environmental variables also accounted for a substantial amount of variance in glycolate-utilizing (17%) and bacterial (18%) communities.

Because correlated patterns in community composition are expected to result from population level interactions, a general additive model was used to detect glycolate-utilizing populations correlated with specific phytoplankton species in each lake (Table 3.2). Correlations were detected in all five lakes. Terminal restriction fragments clustering within *Bacteroidetes*, betaproteobacterial *Bordetella*-like sequences, betaproteobacterial *Polynucleobacter*-like sequences, and *Verrucomicrobia* were correlated to various phytoplankton species found in the lakes. Phytoplankton biovolume explained up to 71% of variance in a given glycolate-utilizing population.

Discussion

Algal exudate-mediated drivers of bacterial community composition

Bottom-up, resource mediated drivers have potential to shape both bacterial community composition and activity (Comte and del Giorgio, 2009; Eiler and Bertilsson, 2007; Judd *et al.*, 2006; Kent *et al.*, 2006; Lennon and Cottingham, 2008). Correlated dynamics between phytoplankton and bacterial communities may result from exudate-mediated linkages between algal and bacterial populations. Carbon exudates released by different algal species vary in composition and quantity (Fogg, 1983; Hellebust, 1965). Exudate composition is also influenced by light, temperature, nutrient availability, and phytoplankton growth stage (Grossart *et al.*, 2005; Hama and Honjo, 1987; Morris and Skea, 1978; Panzenbock, 2007; Parker and Armbrust, 2005). As phytoplankton community composition and environmental conditions change through time (Anneville *et al.*, 2002; Graham *et al.*, 2004), the available exudate pool is expected to shift (Charpin *et al.*, 1998; Morris and Skea, 1978). It is well established that organic matter composition and origin have a substantial effect on bacterial community composition (e.g., Crump *et al.*, 2003; Jones *et al.*, 2009; Judd *et al.*, 2006; Nelson, 2009). Thus, it is probable that an evolving exudate resource base would be a central driver of pelagic lake bacterial community composition.

To investigate the potential for exudates to link phytoplankton and bacterial communities, this study focused on a subset of bacteria able to use glycolate. Glycolate is an ideal substrate

for examining algal-bacterial linkages because it is photorespiration-specific (Oliver, 1998) and one of the most common algal exudates (Fogg, 1983; Hellebust, 1965). In pelagic waters of the humic lakes studied, no populations of *Cyanobacteria* have been detected and there are no macrophytes, making phytoplankton, principally microalgae, the primary source of glycolate (Graham *et al.*, 2004; Newton *et al.*, 2006). Pelagic food webs in the lakes lack fish and are microbially dominated, reducing the complexity of trophic interactions (Kent *et al.*, 2004). Additionally, correlated community patterns between phytoplankton and bacteria in these lakes have been observed over the time period studied (Kent *et al.*, 2007) as well as over multiple years in Crystal Bog (Kent *et al.*, 2006), making them well suited for investigating algal-bacterial interactions.

Taxonomic diversity of glcD

Freshwater glycolate-utilizing bacteria exhibited broad phylogenetic diversity including *Alpha*-, *Beta*-, *Gamma*- and *Delta-proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia*, similar to that observed in total bacterial communities from humic lakes (Burkert *et al.*, 2003; Newton *et al.*, 2006). Previously available environmental *glcD* gene sequences were generated in surveys of marine systems conducted by Lau and Armbrust (2006) and Lau *et al.* (2007). Freshwater sequences from the present study increase diversity of *glcD* genes described in environmental samples, especially with respect to *Polynucleobacter*- and *Bordetella*- like sequences, which have not previously been detected in environmental samples. Differences between glycolate-utilizing bacteria in freshwater and marine systems closely match differences observed between total bacteria based on 16S rRNA gene sequences (Lau and Armbrust, 2006; Newton *et al.*, 2006).

Betaproteobacterial *glcD* sequences were prevalent in the observed lakes. Notably, betaproteobacterial population dynamics had some of the strongest correlations with individual phytoplankton populations in Crystal Bog (Newton *et al.*, 2006). Approximately 48% of *glcD* genes sequenced from humic lakes formed a cluster that included *Polynucleobacter necessarius*. Within the *Betaproteobacteria*, *Polynucleobacter* is a cosmopolitan freshwater genus containing endosymbiotic as well as free-living organisms (Vannini *et al.*, 2007; Zwart *et al.*, 2002). Abundance of PnecB, a species-like subgroup of *Polynucleobacter necessarius*, has been related to temperature as well as biomass of chrysophytes and dinoflagellates (Wu and Hahn, 2006).

Depth distribution corresponding to primary productivity and occurrence of PnecB in lakes with small fractions of allochthonous carbon further suggests a relationship of these organisms to phytoplankton (Wu and Hahn, 2006). While PnecB are not detected in acidic habitats, the subgroup PnecC is common in acidic lakes with high humic content, accounting for up to 60% of total bacterial cells (Alonso *et al.*, 2009; Hahn *et al.*, 2005). It is possible that populations from subclusters PnecB and PnecC inhabit different lake types, but have similar ecological roles responding to phytoplankton-derived carbon within their respective habitats. A second cluster of betaproteobacterial *glcD* sequences formed a sister group to *Bordetella* sequences. These bacteria likely belong to the Beta III group of *Betaproteobacteria* that contains *Bordetella sp.* (Newton *et al.*, 2006). Beta III population dynamics were previously shown to be highly correlated to *Peridiniopsis* and *Dinobryon* in Crystal Bog (Newton *et al.*, 2006).

Two OTUs appearing in three lakes were identified as *Actinobacteria*. Of ten actinobacterial populations detected by Newton and colleagues (2006), three populations were positively correlated with *Mallomonas*, and one of the three was also correlated with *Peridinium cinctum* and *Peridinium limbatum*. Four actinobacterial populations from Crystal Bog were persistent members of the bacterial community, with negative or no correlation with phytoplankton (Newton *et al.*, 2006). It has been reported that *Actinobacteria* may be able to use recalcitrant dissolved organic matter pools (Burkert *et al.*, 2003), including humic acids and would therefore be uncoupled to phytoplankton dynamics (Newton *et al.*, 2006). In fifteen lakes of varying authochonous: allochthonous carbon ratios, *Actinobacteria* from the *acIA*, *acII*, *acIV* lineages were primarily found in lakes dominated by authochonous carbon sources, potentially indicating a preference for algal-derived dissolved organic matter, while *acIB* bacteria were found in lakes with more allochthonous carbon inputs (Jones *et al.* 2009). Given the high levels of actinobacterial diversity (Newton *et al.*, 2007) and abundance in lakes (e.g., Allgaier and Grossart, 2006; Warnecke *et al.*, 2005), it is reasonable that different lineages would occupy distinct ecological niches, with some specializing on autochthonous and others on allochthonous resources. Finding freshwater actinobacterial *glcD* sequences indicates that some members of the *Actinobacteria* are capable of using algal derived dissolved organic matter.

Dynamics of glycolate-utilizing bacteria

Temporal resolution is important to studying algal-bacterial interactions as detectable changes in these communities are seen at the scale of days (Kent *et al.*, 2004). In this study, microbial communities were sampled twice weekly in replicate humic lakes over thirteen weeks from May through August. Frequent sample collection over the summer enabled observations of phytoplankton and bacterial dynamics over multiple shifts in dominance of phytoplankton species (Kent *et al.*, 2004). T-RFLP fingerprinting facilitated high temporal resolution in observing glycolate-utilizing populations. While T-RFLP presents challenges, such as masking diversity when multiple OTUs have the same fragment length, it is appropriate for characterizing broad community patterns (Hartmann and Widmer, 2008). To increase information obtained from T-RFLP, we employed two overlapping primer sets and two restriction enzymes. T-RFLP amplification was successful for five of six lakes. In Forestry Bog, inefficient amplification from both primer sets may have been due to low copy numbers of *glcD* or mismatches occurring in between environmental *glcD* and degenerate *glcD* primers.

Glycolate-utilizing bacterial populations exhibited seasonal succession similar to that observed for total bacterial and phytoplankton communities (Kent *et al*., 2007). Phytoplankton populations and the interaction between these phytoplankton populations and the environment largely explained variation in glycolate-utilizing and total bacterial communities. While organisms in bacterial and archaeal domains have *glcD* genes, no archaeal glycolate utilizing organisms were detected in the lakes studied, indicating that the glycolate utilizing organisms described in this study were essentially a subset of the total bacterial community. There are two potential interpretations for similar patterns observed between glycolate utilizing and total bacteria: glycolate-utilizing bacteria are influential in shaping the patterns of the total bacterial community, or patterns in glycolate-utilizing bacteria result from being a subset of total bacteria. The defined link between phytoplankton and glycolate-utilizing bacteria, along with the expectation that dynamics of bacteria exploiting humic acids or other allochthonous inputs should be independent of phytoplankton (Newton *et al.*, 2006), provide support for glycolate utilizing bacteria contributing to correlated patterns between phytoplankton and bacteria.

In our conceptual model of trophic interactions in this microbial food web, exudate linkages operate on the population level. A phytoplankton population releases specific exudates that stimulate growth of certain bacteria. As a result, we expect to find correlated dynamics

between pairs of phytoplankton and glycolate-utilizing populations, similar to those described between phytoplankton and bacterial populations by Newton and colleagues (2006). In the current study, relative abundance of a terminal restriction fragment (T-RF) was used to approximate glycolate-utilizing bacterial abundance. To identify T-RFs that may respond similarly to phytoplankton exudates, only T-RFs representing OTUs from a single lineage (e.g., *Betaproteobacteria*, *Polynucleobacter* cluster) were included in the analysis. While this approach does not provide the accuracy and specificity of quantitative PCR targeting individual populations (Lau *et al.*, 2007), it can generate hypotheses about bacterial populations that may be responding to specific phytoplankton species. For example, *Peridinium cinctum* population dynamics have been correlated to a glycolate-utilizing population (this study) as well as a population within the total bacterial community within the *Polynucleobacter genus* (Newton *et al.*, 2006).

Conclusion

Resource-mediated control of bacterial communities by phytoplankton has potentially important implications for aquatic carbon flow, nutrient cycling, and other bacterially-mediated biogeochemical transformations. This investigation addressed the potential for exudates to explain correlated algal-bacterial community dynamics by focusing on bacteria able to use the phytoplankton-specific exudate glycolate. Freshwater glycolate utilizer diversity provided insight into potential ecological niches for different lineages of lake bacteria. Terminal restriction fragment length polymorphism was shown to capture temporal variability in glycolate-utilizing assemblage patterns. Coupled community- and population-level dynamics between glycolate utilizers and phytoplankton provide support for the hypothesis that exudate mediated linkages connect bacteria to phytoplankton populations.

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Figures

Figure 3.1. Maximum-likelihood tree of *glcD* amino acid sequences from the current study and selected sequences from GenBank. Each OTU represents *glcD* sequences from humic lake clone libraries determined at the 97% DNA similarity level by DOTUR. Betaproteobacterial clusters B1 and B2 each contain eleven OTUs and are expanded in Figure 2. Archaeal *glcD* sequences from *Methanosarcina barkeri* strain fusaro and *Sulfolobus solfataricus* P2 were designated as outgroup sequences to root the tree. Numbers on tree branch nodes are bootstrap values based on 100 bootstraps (only values >50 are shown). Scale bar indicates 0.1 observed changes in amino acids.

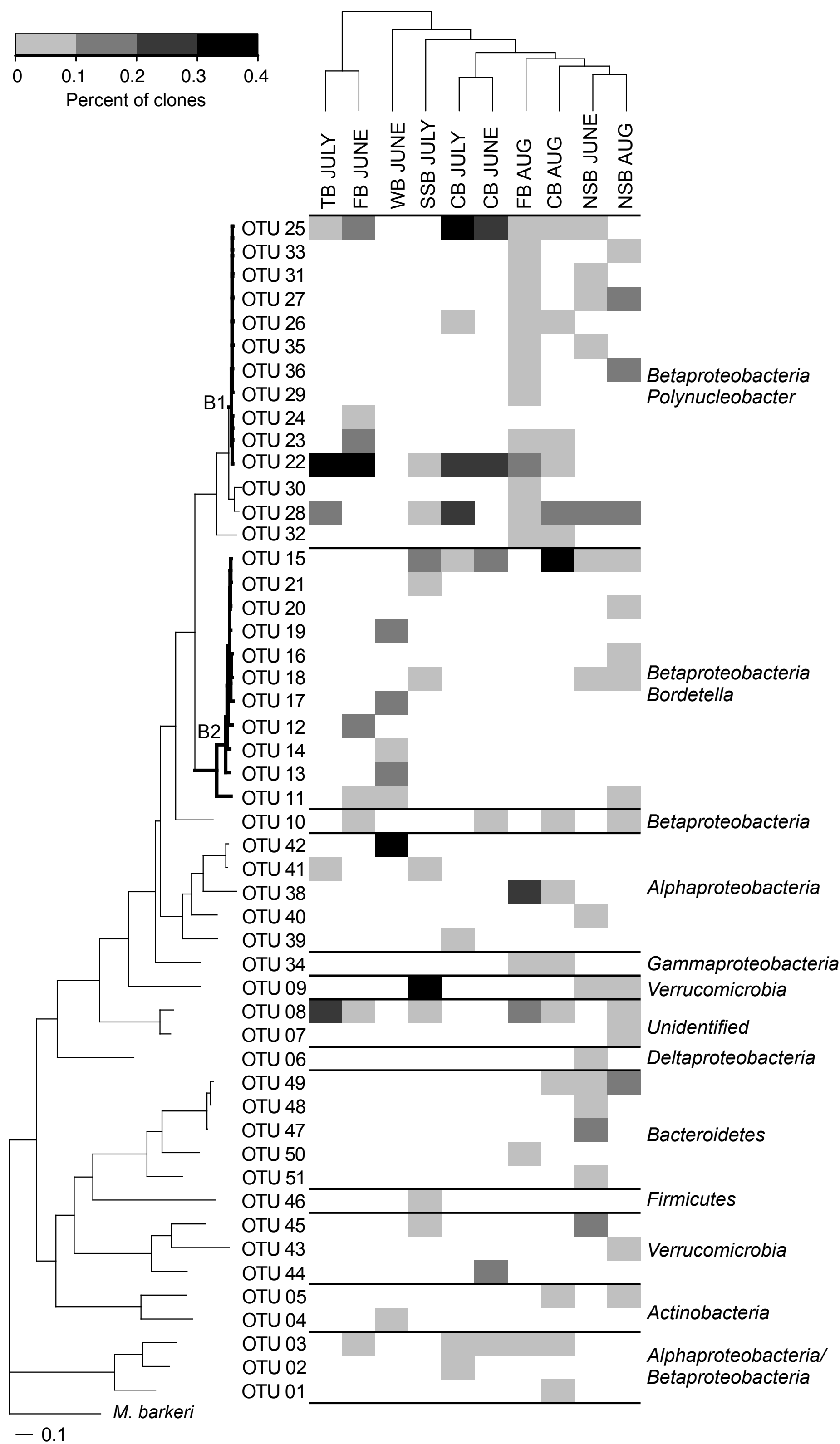

Figure 3.2. Relative abundance of OTUs in each clone library. A maximum likelihood geneology connects OTU sequences, rooted with an archaeal *glcD* sequence from *Methanosarcina barkeri*. B1 and B2 are clusters within the *Betaproteobacteria* expanded from Figure 1. Scale bar represents 0.1 amino acid changes. Clone libraries are were compared using hierarchical complete linkage clustering.

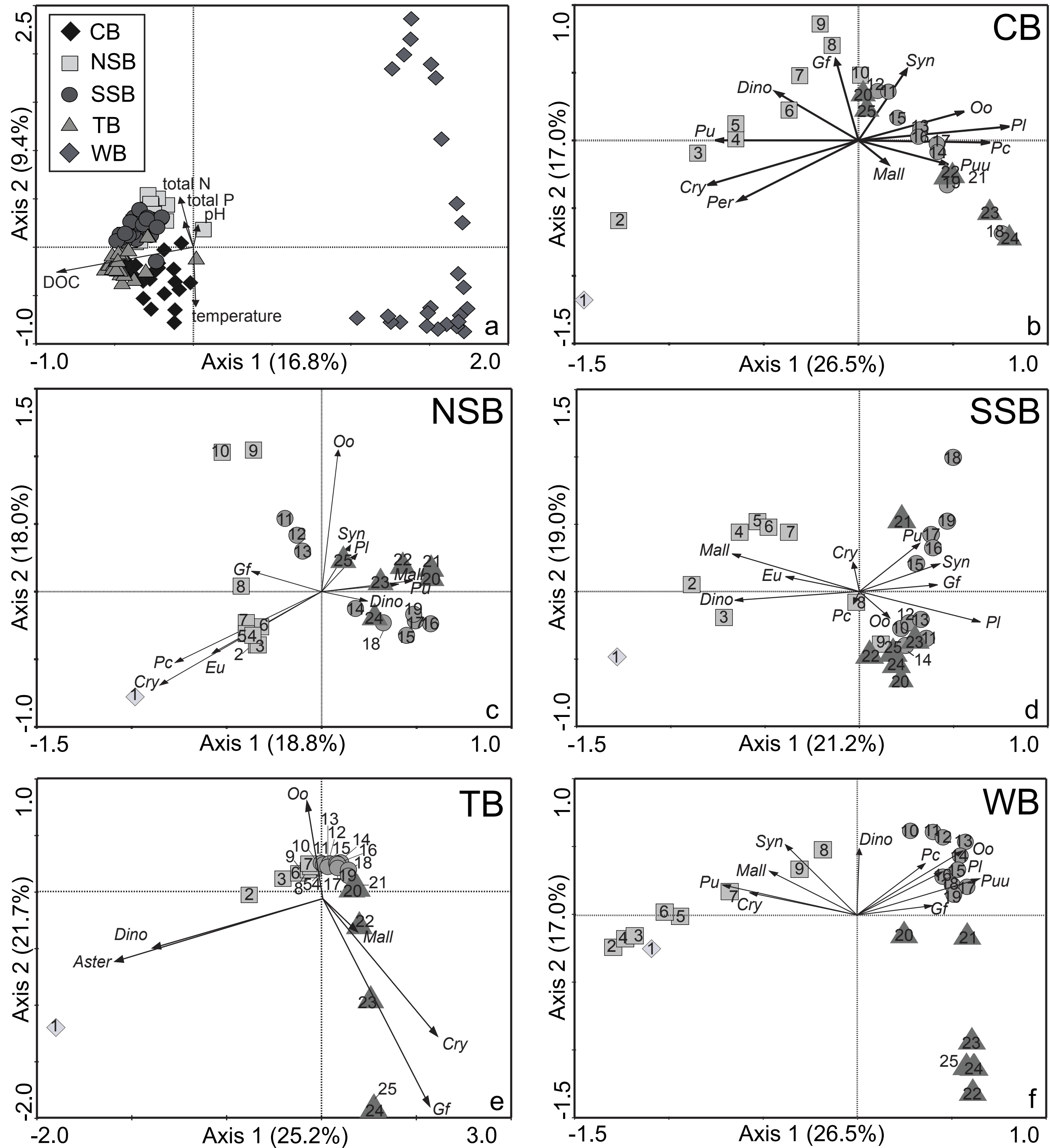

Figure 3.3. Correspondence analysis biplot of glycolate-utilizing assemblages from Crystal (CB), North Sparkling (NSB), South Sparkling (SSB), Trout (TB) and Why Not (WB) Bogs with arrows representing environmental factors (a). Correspondence analysis of glycolate-utilizing assemblages from each bog (b-f). Samples were collected twice weekly from each lake between May 28 and August 22, 2003. Points represent sample dates and are numbered consecutively with the shading and shape indicating the month sampled (diamond, May; square, June; circle, July; triangle, August). Arrows represent phytoplankton species: *Aster*, *Asterionella*; *Cry, Cryptomonas*; *Dino*, *Dinobryon*; *G. fusc*, *Gymnodinium fuscum*; *Mall*, *Mallomonas*; *Oo*, *Oocystis*, *Per*, *Peridiniopsis*; *Pc*, *Peridinium cinctum*; *Pl*, *Peridinium limbatum*; *Pu*, *Peridinium umbonatum*; *Puu*, *Peridinium umbonatum umbonatum*; *Syn*, *Synura*. Percentage of community variance explained by each axis is indicated in parentheses.

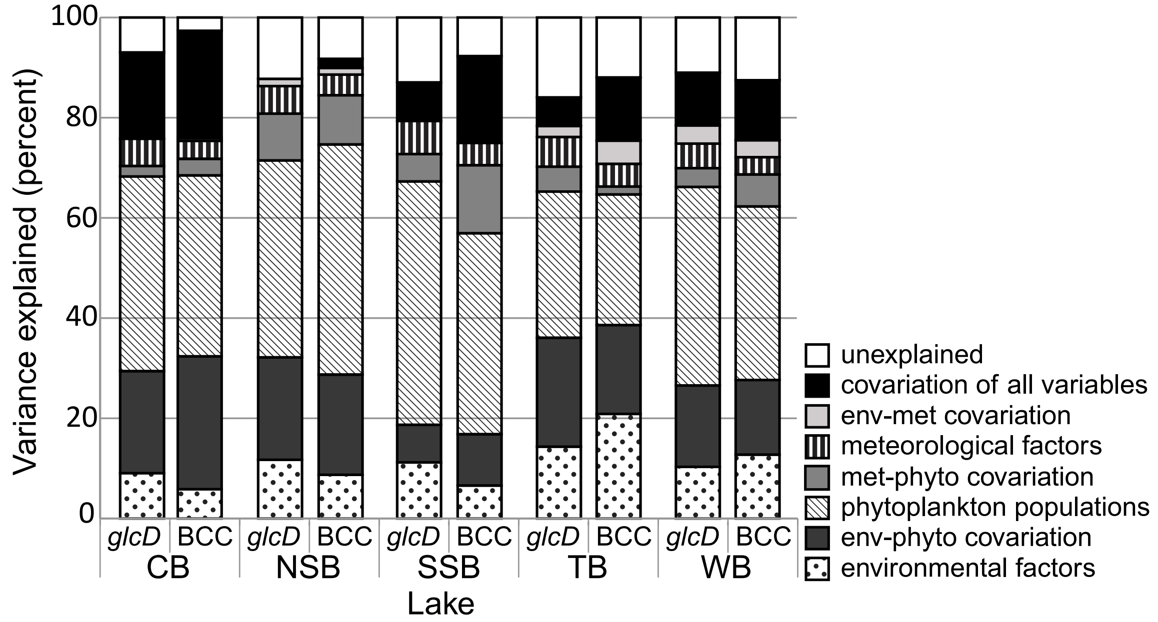

Figure 3.4. Results from glycolate-utilizing (*glcD*, assessed by *glcD* T-RFLP) and total bacterial community composition (BCC, assed by ARISA) variance partitioning for each lake. Partial canonical correspondence analysis was used to partition variance in community composition over time among environmental variables, phytoplankton populations, and meteorological variables.

Tables

Table 3.1. Lake characteristics

Lake	Latitude	Longitude	Maximum Depth (m)	Surface Area (Ha)	DOC (mg/l)	pH	Chl *a* (µg/l)
Crystal Bog (CB)	46° 00'26.8"N	89°36'22.5"W	2.5	0.56	9.5	5.1	22.4
Forestry Bog (FB)	46° 02'51.4"N	89°39'04.8"W	2.5	0.13	10.4	5.5	28.1
North Sparkling Bog (NSB)	46° 00'16.0"N	89°42'18.6"W	4.5	0.47	9.5	5.2	27.4
South Sparkling Bog (SSB)	46° 00'13.6"N	89°42'19.9"W	8.0	0.44	11.2	5.1	22.3
Trout Bog (TB)	46° 02'27.5"N	89°41'09.6"W	7.9	1.01	28.0	4.8	31.5
Why Not Bog (WB)	46° 00'17.0"N	89°37'39.9"W	6.5	1.23	6.6	5.7	8.17

Abbreviations: DOC, dissolved organic carbon; total N, total nitrogen; total P, total phosphorus; Chl *a*, chlorophyll *a*. DOC, pH and Chl *a* represent average values (Kent *et al*., 2007)

Table 3.2. Bacterial populations with significant correlation to dynamics of individual phytoplankton in the lake specified with percent variance in bacterial abundance explained by phytoplankton biovolume

Group ID	T-RF	OTUs	Phyto	Lake	% Variance Explained
Bacteroidetes	RA_312	47	*P. u. umbonatum*	WB	34.85
β- Bordetella	RA_598	12	*Cryptomonas*	TB	62.69
β- Bordetella	RA_598	12	*P. u. umbonatum*	SSB	47.02
β- Polynucleobacter	RR_678	32	*G. fuscum*	SSB	63.6
β- Polynucleobacter	RA_393	29	*Oocystis*	TB	71.25
β- Polynucleobacter	RR_496	25, 26, 27	*Oocystis*	CB	25.7
β- Polynucleobacter	FR_312	29	*P. umbonatum*	WB	58.48
β- Polynucleobacter	RR_496	25, 26, 27	*P. cinctum*	CB	50.03
β- Polynucleobacter	RR_342	31	*P. u. umbonatum*	WB	32.35
β- Polynucleobacter	RA_195	24, 32	*P. limbatum*	CB	37.74
β- Polynucleobacter	RR_496	25, 26, 27	*Synura*	CB	62.58
Verrucomicrobia	RA_156	45	*Dinobryon*	NSB	20.14

Abbreviations: *β* , denotes betaproteobacterial genera, T-RF, terminal restriction fragments coded by primer (F or R) restriction enzyme (*<u>A</u>lu*I or *<u>R</u>sa*I) and fragment length; OTUs, OTUs corresponding to T-RF. *G. fuscum, Gymnodinium fuscum; P. cinctum, Peridinium cinctum ; P. umbonatum, Peridinium umbonatum; P. u. umbonatum, Peridinium umbonatum umbonatum.* All correlations are significant at p<0.001.

CHAPTER 4

Contribution of particle-associated bacteria to correlations between phytoplankton and bacterial communities in lakes

Abstract

Bacteria commonly live in close association with algal cells. If specific bacteria attach to individual phytoplankton populations, then abundances of phytoplankton and attached bacterial populations should be tightly correlated. This interaction may contribute to correlated patterns observed between algal and bacterial communities. Algal-attached bacteria are a temporally variable subset of particle-associated bacteria. If habitat-mediated interactions between phytoplankton and attached bacteria are important for structuring the total bacterial community, particle-associated bacterial communities and populations within them should be more correlated to phytoplankton than free-living bacteria. Temporal community patterns in particle-associated bacteria were compared to those of whole bacteria and phytoplankton in Crystal and South Sparkling Bogs. Regular patterns of succession in addition to correlations between phytoplankton and whole bacterial communities (ρ=0.514, p=0.001) and phytoplankton and particle-associated bacterial communities (ρ=0.739, p=0.001) were detected in Crystal, but not South Sparkling Bog (ρ=0.265, p=0.038; ρ=0.167, p=0.103 respectively). Population-level correlations provided evidence for contribution of particle-associated bacteria to whole bacterial community dynamics, especially in Crystal Bog. Contribution of free-living and habitat generalist bacteria was also evident. Algal-attachment alone does not explain correlated algal-bacterial patterns. Instead, habitat-mediated linkages may contribute to correlated dynamics in addition to other mechanisms of algal influence, such as exudate-mediated linkages.

Introduction

In the water column, bacteria can occupy spatially distinct habitats including aggregates of inorganic and organic material as well as larger organisms, such as phytoplankton and zooplankton. Habitat specialization, or spatial resource partitioning, has implications for understanding bacterial ecology and evolution (Hunt *et al.*, 2008). Response of bacterial populations to drivers structuring bacterial communities, including water temperature, mixing status, grazing, and phytoplankton community composition (Kent *et al.*, 2006; Kent *et al.*, 2007; Shade *et al.*, 2007), is likely habitat-dependent. In particular, bacteria tightly associated with phytoplankton would be expected to respond strongly to changes in phytoplankton community composition compared to free-living bacteria.

Bacteria are commonly attached to algal cells (e.g., Biegala *et al.*, 2002; Fisher and Wilcox, 1996), which may give them a competitive advantage for accessing labile carbon exudates released by phytoplankton (Fogg, 1983). There is a certain degree of species specificity with respect to identity of bacterial epiphytes attached to particular algal taxa (Fisher *et al.*, 1998; Hold *et al.*, 2001; Sapp *et al.*, 2007). For example, bacterial communities were more similar among *Alexandrium* cultures from different geographic regions than between *Alexandrium* and other non-toxic phytoplankton in the Gulf of Maine (Hold *et al.*, 2001). If bacterial populations were attached to specific phytoplankton, abundance of these bacteria would be predicted to increase concurrently with their phytoplankton hosts.

It is possible that phytoplankton-associated bacteria disproportionately influence temporal patterns in bacterial community composition, essentially driving patterns of correlation observed between whole bacterial and phytoplankton communities. Examining temporal dynamics of particle-associated bacteria in comparison to whole bacteria and phytoplankton is one way to explore the potential influence algal-associated bacteria. Particle-associated bacteria are frequently distinguished from free-living organisms based on size fractionation (e.g., Allgaier and Grossart, 2006; Riemann *et al.*, 2000; Riemann and Winding, 2001). While algal-associated bacteria are only a subset of particle-associated bacteria, they are expected to be a highly variable component of the particle-attached fraction based on temporal variability of phytoplankton communities (e.g., Anneville *et al.*, 2002; Graham *et al.*, 2004).

Changes in phytoplankton communities have been correlated to shifts in whole bacterial community composition in mesocosm experiments (Kent *et al.*, 2006; Pinhassi *et al.*, 2004) as

well as in the environment (Kent *et al.*, 2004; Kent *et al.*, 2007; Rooney-Varga *et al.*, 2005). A large percentage of temporal variation in bacterial communities in humic lakes has been attributed to phytoplankton community composition (Kent *et al.*, 2007), suggesting that phytoplankton are able to strongly influence bacterial community structure. Phytoplankton have differential effects on attached compared to free-living bacteria. Particle-associated bacterial communities have been correlated with phytoplankton community composition and related parameters in systems where free-living bacteria were not correlated to phytoplankton (Besemer *et al.*, 2005; Rooney-Varga *et al.*, 2005). Phytoplankton blooms have been observed to coincide with shifts in abundance, production and enzyme activity from free-living to particle-associated bacteria (Riemann *et al.*, 2000; Worm *et al.*, 2001).

The objective of this study was to determine whether particle-associated bacteria contribute to correlated bacterial and phytoplankton community dynamics. Community correlations between whole bacterial, particle-associated bacterial, and phytoplankton communities were determined in two lakes where association between bacterial and phytoplankton communities in a previous year was especially strong (Kent *et al.*, 2007). Composition and dynamics of attached and free-living bacteria were compared to determine assemblage variability and bacterial population habitat specificity. Population level correlations between specific bacteria and phytoplankton were also assessed and related to bacterial habitat preference.

Materials and methods

Study sites

Bacterioplankton and phytoplankton communities were collected from two humic bog lakes in Northern Wisconsin. Crystal Bog (CB; 46° 00'26.8"N, 89°36'22.5"W) is a polymictic lake with maximum depth of 2.5 m and surface area of 0.6 ha and South Sparkling Bog (SSB; 46° 00'13.6"N, 89°42'19.9"W) is a dimictic lake with maximum depth of 8.5 m and surface area of 0.5 ha. An extensive *Sphagnum* mat surrounds each lake and is a source of humic material (Kent *et al.*, 2007). Pelagic food webs lack fish and are dominated by bacteria, algae, protozoa, and zooplankton (Kent *et al.* 2004). The phytoplankton community is typically dominated by dinoflagellates including *Peridiniopsis, Peridinium limbatum*, and *Gymnodinium fuscum*. Blooms of *Cryptomonas, Dinobryon,* and *Synura* also occur (Graham *et al.*, 2004).

Field sample collection

Integrated epilimnion samples were collected twice weekly from a fixed point in the center of each bog from 22 May to 18 August 2008 (Kent *et al.*, 2004). Total bacterial community samples (100-250 ml) were concentrated onto 0.2 µm filters (Supor-200; Pall Gelman, East Hills, NY) and particle-associated bacteria (40-250 ml) were collected onto 5 µm Isopore filters (Millipore, Billerica, MA). Filters were stored at -20°C prior to DNA analysis. Once weekly, an integrated epilimnion sample was preserved in 2% glutaraldehyde for phytoplankton community analysis. For three sampling periods (4-10 July, 28 July-1 August, 18-21 August), free-living bacteria were collected from the filtrate of particle-associated 5 µm filters onto 0.2 µm filters (Supor-200; Pall Gelman, East Hills, NY).

Microbial community analysis

DNA was extracted from filters using FastDNA Spin kits (MP Biomedicals, Solon, OH). Bacterial community composition was determined using automated ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett, 1999; Yannarell and Triplett, 2005). Briefly, the 16S-23S rRNA intergenic spacer region was PCR amplified with 6-FAM labeled, universal 16S rRNA primer 1406F (5'-TGYACACACCGCCCGT-3') and 23SR (5'-GGGTTBCCCCATTCRG-3') targeting bacterial 23S rRNA. PCR cycles had an initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 35 s, 55°C for 45 s, and 72°C for 2 min, with a final extension carried out at 72°C for 2 min carried out in an Eppendorf MasterCycler Gradient (Eppendorf AG, Hamburg, Germany). PCR-amplified samples were combined with a Rhodamine X-labeled internal size standard and separated by size using denaturing capillary electrophoresis (ABI 3730x1 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). Electrophoresis conditions were 63°C and 15 kV with a run time of 120 min using POP-7 polymer. Size-calling and profile alignment was carried out using GeneMarker version 1.75 (SoftGenetics, State College, PA). To include the maximum number of peaks while excluding background fluorescence, a threshold of 1,000 fluorescence units was used. The signal strength of each peak was normalized to account for run-to-run variations in signal detection by dividing the area of individual peaks by the total fluorescence (area) detected in each profile.

Phytoplankton community analysis

Phytoplankton community composition was determined morphologically (Kent *et al.*, 2004). Glutaraldehyde preserved samples (10-25 mL) were settled in chambers for at least twenty-four hours prior to enumeration. Counts were performed on an inverted microscope at 200x and 400x magnification. Dominant phytoplankton were identified to species where possible based on Smith (1950), Prescott (1954) and Patterson (1998). Microscopic counts of phytoplankton populations were transformed using biovolume estimates generated for species common to these lakes (Graham *et al.*, 2004).

Statistical Analysis

Correspondence analysis was carried out separately for whole bacterial and particle-associated bacterial communities (ARISA relative fluorescence) and phytoplankton communities (biovolume) using Canoco 4.5.1 to visualize temporal patterns (Biometrics-Plant Research International, Wageningen, The Netherlands) (ter Braak and Smilauer, 2002). To assess correlation between two communities, rank similarities were determined from a Bray-Curtis similarity matrix and Spearman's Rank correlation was calculated using the RELATE function in Primer 6 (PRIMER-E Ltd, Plymouth, UK) (Clarke and Warwick, 2001).

Difference between particle-associated and free-living community composition was tested using the analysis of similarity (ANOSIM) procedure on pairwise Bray-Curtis similarity values in Primer 6 (Clarke and Warwick, 2001). The R test statistic generated by ANOSIM indicates the degree of separation between groups of samples with scores ranging from 0 (no separation) to 1 (complete separation). Average similarity within and between particle-associated and free-living groups and contribution of each bacterial population to group differences were determined using the similarity percentages or SIMPER routine in Primer 6. Particle-associated and free-living discrete samples and particle-associated and whole twice weekly samples were compared to using SIMPER output to determine the habitat preference for each OTU. Populations were categorized as those found exclusively in the particle-associated fraction (PA), at least two times more abundant in the particle-associated fraction (PA>FL), equally in both fractions (BOTH), at least two times more abundant in the free-living fraction (FL>PA), and not detected in the particle-associated fraction (FL).

Canonical correspondence analysis was used to visualize bacterial populations in relation to phytoplankton populations using Canoco 4.5.1 (ter Braak and Smilauer, 2002). Bacterial populations influenced by individual phytoplankton populations were identified by fitting the response of individual bacterial operational taxonomic units (OTU, defined by ARISA fragment length) to each phytoplankton population from a correspondence analysis of each lake using a general additive model using CanoDraw (ter Braak and Smilauer, 2002). A single variable correspondence analysis was then used to determine the percent variance of each bacterial population explained by a given phytoplankton population (ter Braak and Smilauer, 2002). Relationships were reported if they were positive, significant ($p<0.001$), and explained at least 10% of variance in bacterial population dynamics.

Results

Community-level correlations

Phytoplankton, whole bacterial, and particle-associated bacterial communities in Crystal Bog exhibited fairly regular temporal patterns, whereas community dynamics of phytoplankton and bacteria in South Sparkling Bog exhibited more chaotic patterns (Figure 4.1). The whole bacterial community from Crystal Bog had one noticeably atypical sampling point, due to a high abundance of a bacterial population with an ARISA fragment length of 747 bp that was not observed in other samples. The irregular pattern in South Sparkling Bog phytoplankton was largely due to the influence of *Trachelomonas*, which had two peaks in abundance 19 days apart (sample points 2 and 7). All pairs of communities in Crystal Bog were significantly correlated while only whole and particle-associated bacterial communities were correlated in South Sparkling Bog (Table 4.1).

Particle-associated and free-living bacterial populations

Particle-associated and free-living bacterial communities were distinct (ANOSIM $R=0.833$, $p=0.01$). Average similarity (over all time points) within free-living and particle-associated communities was 46% and 36%, respectively for Crystal Bog and 49% and 36%, respectively for South Sparkling Bog. Based on average similarity within each fraction, particle-associated bacteria were more temporally variable. Average similarity between particle-associated and free-living samples was 20% in Crystal Bog and 24% in South Sparkling Bog.

A number of bacterial populations were only detected in particle-associated samples while others were predominantly found in particle-associated samples, detected in both fractions, predominantly found in the free-living fraction, or not detected in the particle-associated fraction. Bacteria populations from all habitat preferences and specificities contributed to group differences between particle-associated and free-living assemblages (Table 4.2 and Table 4.3).

Population-level correlations

Bacterial OTUs were classified based on habitat and visualized using canonical correspondence analysis with phytoplankton as explanatory variables (Figure 4.2). Bacterial populations correlated with phytoplankton included both free-living and particle-associated specialists found in only one fraction, as well as generalists equally detected in both fractions. However, certain species, such as *Peridinium cinctum* in Crystal Bog, were mainly correlated with bacteria enriched in the particle-associated fraction.

Positive, significant population-level correlations between individual bacterial populations and phytoplankton populations were detected in both lakes (Table 4.4). Biovolume of a single phytoplankton species explained up to 68% of variation in bacterial population relative abundance. All seven algal-correlated populations in Crystal Bog were either exclusively or predominantly detected in the particle-associated fraction. In contrast, the ten algal-correlated populations in South Sparkling Bog included free-living, particle-associated, and generalist populations.

Discussion

Community level correlations

Correlated bacterial and phytoplankton dynamics have previously been described in six humic lakes, with particularly strong correlations in Crystal and South Sparkling Bogs (Kent *et al.*, 2007). Crystal Bog communities described in this study all showed fairly regular patterns of succession. Whole bacterial communities had one inconsistent sampling point, due to a spike in the abundance of a bacterial population that was not abundant in other samples. Since phytoplankton communities were not available for this date, it is not known whether there was a concurrent increase in any particular phytoplankton taxa. The particle-associated bacterial community was more highly correlated with the phytoplankton community than the whole

bacterial community, which is what would be predicted if particle-associated bacteria were more responsive to changes in phytoplankton populations.

Correlation was not detected between bacterial and phytoplankton communities in South Sparkling Bog. It is interesting to note that all three communities exhibited irregular patterns, indicating phytoplankton and bacteria were either interacting or responding to an external destabilizing force (e.g., temperature, nutrient fluctuations, chaotic food web interactions) (Beninca *et al.*, 2008). Irregularity in the phytoplankton population was primarily due to large peaks in *Trachelomonas* biovolume occurring in samples almost three weeks apart. Because *Trachelomonas* was only detected on three dates, it was not possible to determine if any bacterial populations were correlated with *Trachelomonas*. *Trachelomonas* is a unicellular euglenoid algal genus that has previously been detected in Crystal Bog (Graham *et al.*, 2004). Since similar regional factors (e.g., air temperature, precipitation) influence Crystal and South Sparkling Bogs, irregular patterns detected in South Sparkling Bog were probably due to within-lake factors, such as food-web interactions (Beninca *et al.,* 2008).

Particle-associated and free-living bacterial populations

Particle-associated and free-living assemblages are typically found to be distinct in terms of community-level fingerprinting (Besemer *et al.*, 2005; Riemann and Winding, 2001; Rooney-Varga *et al.*, 2005), proportion of different bacterial groups (e.g., Actinobacteria (Allgaier and Grossart, 2006), Betaproteobacteria (Lemke *et al.*, 2009)), and population-level representation of specific taxa (Allgaier *et al.*, 2007). It is also common to detect certain populations in both fractions, as was the case in this study (Besemer *et al.*, 2005; Riemann and Winding; Rooney-Varga *et al.*, 2005).

Particle-associated bacteria were more variable than free-living bacteria over time in both lakes, potentially indicating that they utilize a more variable resource base. However, since community variability aggregates the response of all populations, one fraction could appear to be more variable simply by having fewer persistent populations. Similar observations in terms of fraction variability have been made in both marine and freshwater systems. Higher spatial and temporal variability in attached bacteria have been observed in the Danube river-floodplain system (Besemer *et al.*, 2005). Attached bacteria were also more diverse and more variable over time in a coastal marine system (Rooney-Varga *et al.*, 2005).

Population level correlations

Community level correlations are likely a result of population-level interactions. Abundance of an attached bacterial population increasing with the biovolume of its algal host is an example of a population-level correlation resulting from interacting populations. In both lakes, degree of particle association did not explain correlation with phytoplankton populations. This observation suggests that phytoplankton control extends beyond algal-attached bacteria.

Bacterial populations with positive, significant correlations to individual algal populations in Crystal Bog were either exclusively or preferentially found in the particle-associated community. The particle-specialist population with an ARISA fragment length of 950, identified as being in the Alpha I clade by Newton and colleagues (2006), was highly correlated with *Crypomonas* in both this study and correlations from a previous year (Newton *et al.*, 2006). In South Sparkling Bog, particle-specialist, generalist, and free-living bacterial populations were correlated with phytoplankton. These observations provide support for the idea that particle-associated bacteria as well as free-living bacteria contribute to correlated phytoplankton and bacterial community dynamics.

Conclusion

In summary, particle-associated bacteria did not appear to disproportionately influence temporal patterns in bacterial community composition. Population-level correlations provided evidence for the contribution of particle-associated bacteria to whole bacterial community dynamics, especially in Crystal Bog. However, bacterial response to phytoplankton was not due to attached bacteria alone. Free-living bacteria as well as those found in both fractions also appear to contribute to algal-bacterial community level patterns. Algal-attachment alone does not appear to be enough to explain correlated algal-bacterial patterns. Instead, habitat-mediated correlations may act in concert with other mechanisms of phytoplankton control over bacteria, such as exudate production.

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Figures

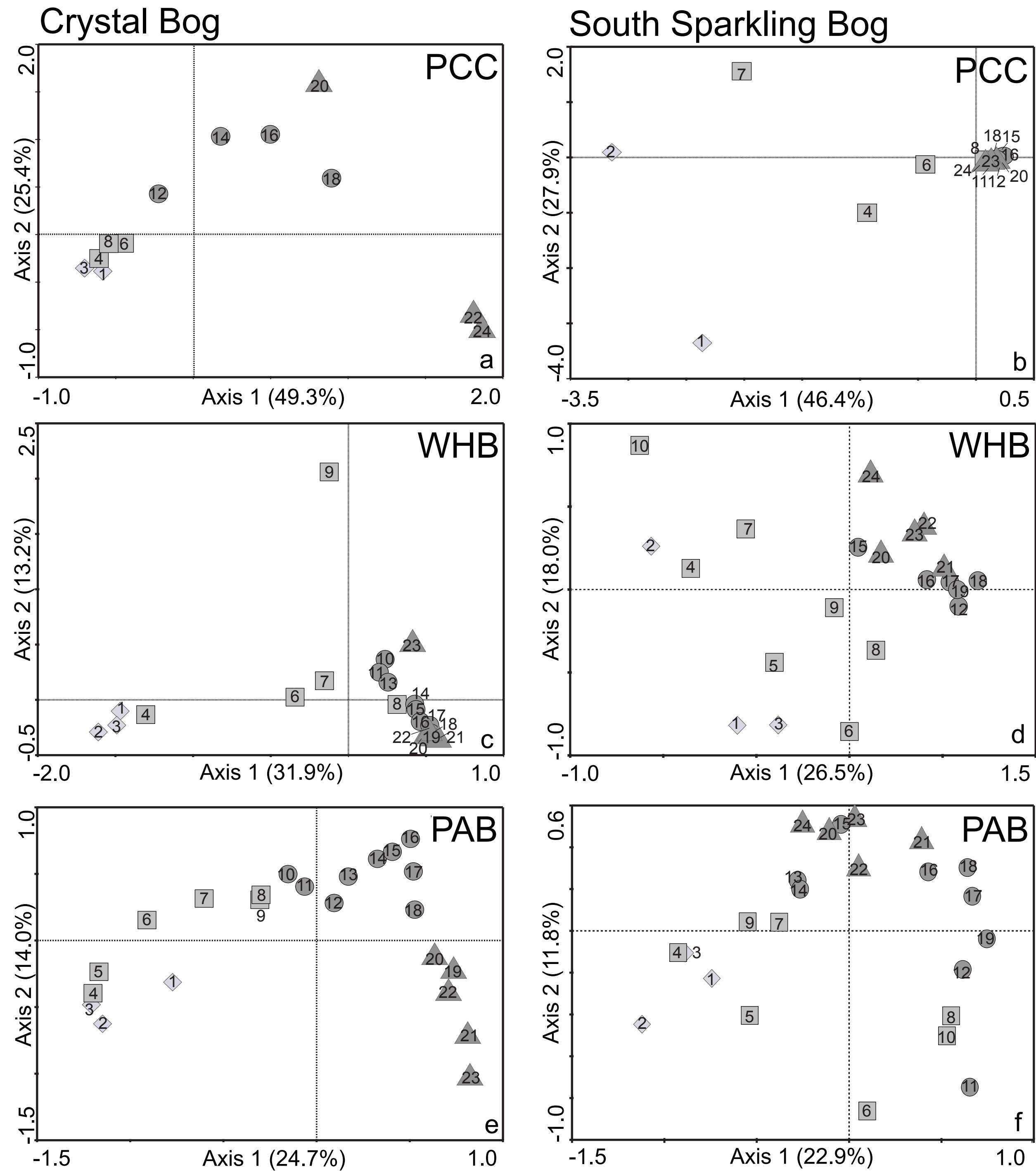

Figure 4.1. Correspondence analysis of phytoplankton (PCC), whole bacterial (WHB), and particle accociated bacterial (PAB) communities in Crystal (a, c, e) and South Sparkling Bogs (b, d, f). Samples were collected twice weekly from each lake between May 22 and August 18, 2008. Points represent sample dates and are numbered consecutively with the color and shape indicating the month sampled (diamond, May; square, June; circle, July; triangle, August). Percentage of community variance explained by each axis is indicated in parentheses.

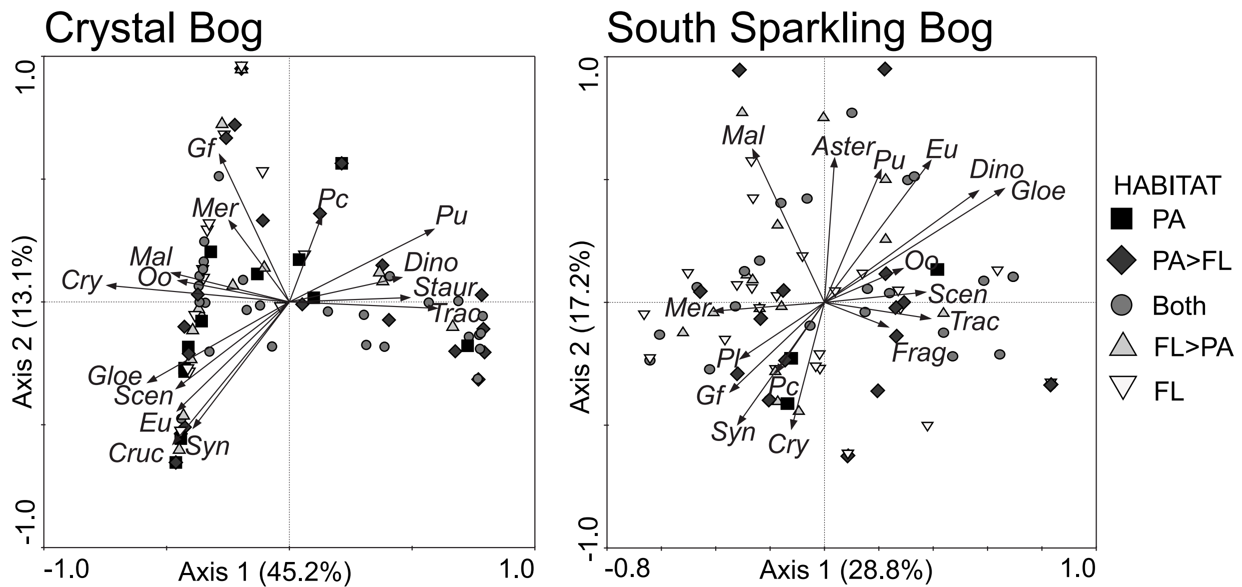

Figure 4.2. Canonical correspondence analysis biplot of individual bacterial OTUs in whole Crystal Bog samples. Symbols represent a specific bacterial OTU color coded by habitat. Arrows represent phytoplankton biovolume explanatory variables. Arrow direction indicates direction of increase and length indicates the degree of correlation with the ordination axes. *Ast, Asterionella; Cruc, Crucigenia; Cry, Cryptomonas; Dino, Dinobryon; Eu, Euglena; Frag, Fragelleria; Gf, Gymnodinium fuscum; Gloe, Gloecystis; Mall, Mallomonas; Mer, Merismopedia; Oo, Oocystis; Pc, Peridinium cinctum; Pl, Peridinium limbatum; Pu, Peridinium umbonatum; Scen, Scenedesmus; Staur, Staurastrum; Syn, Synura; Trac, Trachelomonas.* PA, only detected in the particle associated bacteria; PA>FL, more prevalent in the PA fraction; Both, detected in approximately equally in both fractions; FL>PA, more prevalent in FL fraction; FL, not detected in PA samples. PA, particle-associated; FL, free-living.

Tables

Table 4.1. Correlation between whole bacterial (WHB), particle-associated bacterial (PAB), and phytoplankton (PCC) community composition in each lake

Comparisons	rho	P
Crystal Bog		
WHB-PCC	0.514	0.001
PAB-PCC	0.739	0.001
WHB-PAB	0.864	0.001
South Sparkling Bog		
WHB-PCC	0.265	0.038
PAB-PCC	0.167	0.103
WHB-PAB	0.480	0.001

Spearman rank correlations were calculated for each set of communities and compared to results from random sample permutations. Higher rho values indicate a stronger correlation.

Table 4.2. Habitat of bacteria populations differentiating particle-associated and free-living bacterial assemblages in Crystal Bog

Habitat	AFL	Average Abundance PA	Average Abundance FL	Average dissimilarity between groups	Contribution to group differences (%)
PA	320	0.95	0.00	0.48	0.59
PA	377	1.46	0.00	0.73	0.91
PA	381	1.96	0.00	0.98	1.22
PA	385	1.34	0.00	0.67	0.84
PA	386	1.08	0.00	0.54	0.67
PA	389	0.86	0.00	0.43	0.54
PA	508	4.80	0.00	2.40	3.00
PA	565	0.97	0.00	0.48	0.61
PA	763	0.79	0.00	0.40	0.5
PA	766	1.50	0.00	0.75	0.93
PA	925	7.06	0.00	3.53	4.41
PA	946	0.88	0.00	0.44	0.55
PA	950	1.17	0.00	0.58	0.73
PA	998	1.01	0.00	0.51	0.63
PA>FL	528	0.86	0.15	0.46	0.57
PA>FL	623	1.46	0.63	0.67	0.84
PA>FL	650	4.51	0.85	2.11	2.64
PA>FL	654	2.21	0.78	0.99	1.24
PA>FL	712	0.61	0.35	0.36	0.46
PA>FL	768	1.34	0.45	0.75	0.93
PA>FL	823	2.57	0.96	1.13	1.41
PA>FL	905	1.59	0.68	0.91	1.14
PA>FL	940	5.50	0.78	2.62	3.27
PA>FL	684	16.14	0.60	7.77	9.71
BOTH	547	0.96	0.59	0.58	0.72
BOTH	618	0.66	0.46	0.41	0.51
BOTH	779	2.64	1.85	1.36	1.70
BOTH	794	1.15	1.89	1.14	1.42
BOTH	805	1.55	2.38	1.17	1.47
BOTH	888	2.58	1.67	1.57	1.96
BOTH	910	1.83	2.22	1.28	1.61
BOTH	930	3.36	5.82	2.35	2.94
FL>PA	558	0.46	6.38	2.96	3.70
FL>PA	596	0.39	5.31	2.46	3.08
FL>PA	599	1.18	4.89	2.00	2.49
FL>PA	600	0.29	1.07	0.48	0.60
FL>PA	603	0.79	2.01	1.01	1.26
FL>PA	656	3.95	1.66	2.25	2.82
FL>PA	796	4.60	15.40	5.40	6.75
FL>PA	864	2.09	4.81	2.23	2.79
FL>PA	897	1.58	4.90	1.88	2.35
FL	548	0.00	5.27	2.63	3.29
FL	583	0.00	1.31	0.66	0.82
FL	585	0.00	2.44	1.22	1.52
FL	590	0.00	1.62	0.81	1.01
FL	621	0.00	0.91	0.45	0.57
FL	675	0.00	1.29	0.65	0.81
FL	686	0.00	0.85	0.43	0.53
FL	745	0.00	0.89	0.45	0.56
FL	811	0.00	0.87	0.44	0.55
FL	816	0.00	1.21	0.60	0.75
FL	881	0.00	2.07	1.03	1.29
FL	891	0.00	0.82	0.41	0.51
FL	954	0.00	1.54	0.77	0.96
FL	959	0.00	0.86	0.43	0.54

Abbreviations: AFL, ARISA fragment length; PA, AFL observed exclusively in particle-associated fraction, PA>FL, AFL were more than twice as abundant in particle-associated fraction; BOTH, AFL detected equally in both fractions; FL>PA, AFL were more than twice as abundant in free-living fraction, FL, AFL not observed in particle-associated fraction

Table 4.3. Habitat of bacteria populations differentiating particle-associated and free-living bacterial assemblages in South Sparkling Bog

Habitat	AFL	Average Abundance		Average dissimilarity between groups	Contribution to group differences (%)
		PA	FL		
PA	508	8.16	0.00	4.08	5.33
PA	689	6.26	0.00	3.13	4.09
PA	925	2.45	0.00	1.23	1.60
PA>FL	621	1.76	10.27	4.25	5.56
PA>FL	763	15.75	3.35	6.20	8.10
PA>FL	773	1.13	0.48	0.64	0.84
PA>FL	782	1.41	0.49	0.79	1.03
PA>FL	916	3.02	1.32	1.73	2.26
PA>FL	684	17.09	0.82	8.14	10.63
BOTH	747	7.83	4.30	4.10	5.36
BOTH	754	1.00	0.91	0.65	0.85
BOTH	791	0.72	1.28	0.76	0.99
BOTH	794	7.50	7.63	3.36	4.40
BOTH	796	9.48	6.86	5.88	7.69
BOTH	813	1.00	1.34	0.84	1.09
BOTH	891	0.93	0.49	0.54	0.71
BOTH	905	3.96	5.90	1.52	1.99
FL>PA	548	0.41	2.22	0.91	1.19
FL>PA	558	0.52	3.41	1.44	1.89
FL>PA	585	0.33	2.20	0.93	1.22
FL>PA	596	1.13	5.28	2.16	2.83
FL>PA	599	0.39	3.27	1.44	1.89
FL>PA	897	0.90	2.48	1.09	1.42
FL	494	0.00	2.36	1.18	1.55
FL	545	0.00	1.02	0.51	0.67
FL	600	0.00	1.73	0.86	1.13
FL	603	0.00	1.69	0.84	1.10
FL	615	0.00	0.99	0.49	0.64
FL	626	0.00	1.80	0.90	1.17
FL	638	0.00	2.05	1.02	1.34
FL	745	0.00	1.40	0.70	0.91
FL	792	0.00	2.78	1.39	1.82
FL	805	0.00	1.41	0.71	0.92
FL	839	0.00	1.44	0.72	0.94
FL	863	0.00	1.48	0.74	0.96
FL	910	0.00	1.02	0.51	0.67
FL	930	0.00	5.45	2.73	3.56

Abbreviations: AFL, ARISA fragment length; PA, AFL observed exclusively in particle-associated fraction, PA>FL, AFL were more than twice as abundant in particle-associated fraction; BOTH, AFL detected equally in both fractions; FL>PA, AFL were more than twice as abundant in free-living fraction, FL, AFL not observed in particle-associated fraction

Table 4.4. Percent variance in bacterial OTU relative abundance explained by algal biovolume

Lake	Habitat	AFL	*Cry*	*Dino*	*Eu*	*Gym*	*P. cin.*	*Syn*
CB	PA	386				42.05		
CB	PA	950	20.11					
CB	PA>FL	393			47.06			
CB	PA>FL	528					60.41	
CB	PA>FL	532	20.11					
CB	PA>FL	858	20.11					
CB	PA>FL	972	20.11					
SSB	PA	389	67.84					
SSB	PA	998	67.84					
SSB	PA>FL	722		45.55				
SSB	PA>FL	875		45.55				
SSB	BOTH	681		45.55				
SSB	BOTH	729		45.55				
SSB	BOTH	841						53.03
SSB	FL>PA	367	67.84					
SSB	FL	483	56.05					
SSB	FL	641	67.84					

Percent variance is given only for positive relationships significant at the level of $P<0.001$, CB N=11, SSB N=14.
Cry, *Cryptomonas*; *Dino*, *Dinobryon*; *Eu*, *Euglena*; *Gym*, *Gymnodinium*; *P. cin*, *Peridinium cinctum*; *Syn*, *Synura*.

CHAPTER 5

Summary

Algal-bacterial interactions significantly impact carbon flow in aquatic systems and may more broadly influence microbially-mediated biogeochemical processes, such as denitrification (Ishida *et al.*, 2008). Correlated patterns of abundance, activity, and composition are commonly observed between phytoplankton and bacterial communities (e.g., Cole 1982, Rooney-Varga *et al.*, 2005, Kent *et al.*, 2007). Recent investigations have suggested that phytoplankton populations are important biological drivers of pelagic bacterial communities in general (Kent *et al.*, 2007) and have greater influence specifically on particle-associated compared to free-living bacteria (Rooney-Varga *et al.*, 2005). Based on these observations and others, this thesis investigated dynamics of bacteria having exudate-mediated linkages to phytoplankton as well as those in the particle-associated fraction.

To investigate phytoplankton exudates as a potential linkage, the first study characterized diversity and dynamics of bacteria with genetic potential to use glycolate, a photorespiration-specific exudate, in six lakes. Freshwater glycolate-utilizing populations, characterized using functional gene glycolate oxidase subunit D (*glcD*) exhibited a broad range of taxonomic diversity. Biovolume of phytoplankton populations and the phytoplankton-environmental interaction was able to explain 50-60% of variation in glycolate-utilizing and total bacterial communities. These observations support the hypothesis that algal exudates are a resource-mediated linkage between phytoplankton and phylogenetically diverse bacterial populations.

Particle-associated community- and population-level dynamics were investigated to assess the potential contribution of attached bacteria to correlated patterns between phytoplankton and bacteria. Despite mixed results for community-level correlations, bacterial populations were positively correlated to abundance of specific phytoplankton in both lakes studied. Bacteria detected in both particle-attached and free-living fractions were found to be positively correlated to biovolume of different algal populations. It is interesting to note that bacterial populations with the strongest correlations to phytoplankton in Crystal Bog were predominantly particle-associated. These findings provide evidence for attached bacteria, including algal-associated organisms, contributing to patterns in the whole bacterial community. Free-living and habitat generalist bacterial populations also appear to respond to phytoplankton.

Seasonal patterns of glycolate-utilizing and particle-attached bacteria provide support for exudate- and habitat- mediated influence of phytoplankton on bacteria. The correlational approach employed generated a number of potentially interacting pairs of algae and bacteria, which can be used to make predictions concerning bacterial population response to changes in phytoplankton abundances. Experiments manipulating algal communities are a logical next step for testing hypotheses concerning which bacteria respond to certain algal taxa and what linkages connect algal and bacterial populations. Correlating abundance of specific bacterial ecotypes (i.e., highly related bacteria that occupy the same ecological niche) with phytoplankton in observational and experimental studies may further elucidate population-level patterns underlying community-level correlations.

Understanding interactions that structure bacteria at the level of populations and communities increases knowledge of microbial biology, ecology, and evolution. It further may facilitate the building of a predictive framework for understanding compositional and functional responses of bacteria to environmental conditions. This framework may be especially important for assessing responses to environmental change in scenarios where environmental signals (e.g., elevated CO_2; Allgaier *et al.*, 2008) may be transduced through phytoplankton to bacteria.

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APPENDIX A: Phytoplankton community data (2008)

Table A.1. Phytoplankton cells per milliliter in Crystal (CB) and South Sparkling (SSB) Bogs, average of six counts

Date	Lake	*Ast*	*Cruc*	*Cry*	*Dino*	*Eu*	*Fr*	*Gf*	*Gloe*	*Mall*	*Mer*	*Oo*	*Pc*	*Pl*	*Pu*	*Scen*	*Stau*	*Syn*	*Trac*
5/22/2008	CB	0.0	0.0	10.3	450.9	1.5	0.0	3.9	28.5	0.0	0.0	0.0	0.0	0.0	161.8	0.0	1.0	0.5	27.0
5/29/2008	CB	0.5	0.0	1.5	65.9	0.0	0.0	4.9	2.5	0.0	0.0	0.0	0.0	0.0	165.2	0.5	0.0	0.0	0.0
6/03/2008	CB	0.0	0.0	0.0	5.4	0.0	0.0	17.7	2.5	0.0	0.0	0.0	0.0	1.0	332.9	1.0	0.0	0.0	6.9
6/13/2008	CB	0.0	0.0	8.9	3.4	0.0	0.0	46.7	18.2	0.0	0.0	0.0	0.5	4.4	475.4	0.0	0.0	0.0	0.0
6/24/2008	CB	2.5	0.0	24.6	32.9	0.0	0.0	30.0	13.3	0.5	0.5	0.0	0.0	0.0	279.3	1.0	0.0	0.0	0.0
7/08/2008	CB	0.0	0.0	17.2	76.2	0.5	0.0	32.9	16.2	2.5	0.0	1.5	0.0	0.0	62.4	3.4	0.0	0.0	0.0
7/15/2008	CB	0.0	0.0	17.7	68.8	0.0	0.0	23.1	58.5	9.8	17.2	8.9	0.0	0.5	0.0	23.6	0.0	0.0	0.0
7/22/2008	CB	0.0	133.7	10.8	63.4	5.4	0.0	16.2	103.7	15.7	0.0	15.7	0.0	1.5	0.0	101.8	0.0	0.5	0.0
7/29/2008	CB	0.0	805.4	26.1	30.0	3.9	0.0	28.5	67.4	4.4	2.5	2.5	0.0	2.0	0.0	150.9	0.0	4.9	0.0
8/05/2008	CB	5.4	1277.4	24.6	12.3	3.4	0.0	14.8	159.8	1.5	0.0	1.0	0.0	0.0	0.0	3.0	0.0	0.5	0.0
8/12/2008	CB	0.0	231.1	9.3	24.6	0.5	0.0	4.9	26.6	0.0	0.0	1.5	0.0	1.0	0.0	7.4	0.0	10.8	0.0
8/19/2008	CB	0.0	163.2	14.3	70.3	1.0	0.0	4.9	9.8	0.0	0.0	3.0	1.5	0.5	0.0	0.5	0.0	9.8	0.0
8/25/2008	CB	0.0	0.0	20.2	112.1	3.4	0.0	9.8	7.9	0.0	0.0	2.0	1.5	2.5	0.0	0.0	0.0	16.7	0.0
8/31/2008	CB	3.0	0.0	43.8	23.1	3.9	0.0	10.8	11.8	0.0	0.0	1.0	0.0	2.5	0.0	2.5	0.0	64.9	0.0
9/09/2008	CB	0.0	0.0	52.1	4.9	3.4	0.0	5.4	1.0	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	69.3	0.0
9/20/2008	CB	0.0	0.0	32.5	5.4	0.0	0.0	41.3	0.0	0.0	0.0	0.5	0.0	7.4	0.0	0.0	0.0	40.8	0.0
9/28/2008	CB	0.0	0.0	7.9	0.0	1.5	0.0	13.8	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	19.2	0.0
5/23/2008	SSB	2.5	0.0	7.9	217.3	40.8	0.0	16.2	36.9	0.0	0.0	0.0	0.0	0.5	11.8	0.0	0.0	0.0	0.0
5/28/2008	SSB	0.5	0.0	9.8	206.0	24.6	0.0	15.7	60.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	174.1
6/02/2008	SSB	4.9	0.0	5.9	159.8	5.4	0.0	8.9	35.9	0.0	0.0	4.4	1.5	0.0	0.0	1.0	0.0	0.0	0.0
6/09/2008	SSB	16.2	0.0	19.7	193.7	1.0	0.0	9.3	19.2	3.0	0.0	0.5	0.0	1.0	0.0	0.5	0.0	0.0	3.4
6/17/2008	SSB	11.8	0.0	8.4	116.5	0.5	2.0	33.4	32.9	0.0	0.0	0.0	0.5	3.4	0.5	0.0	0.0	0.0	420.9
6/23/2008	SSB	6.4	0.0	15.7	108.2	0.0	0.0	77.2	5.4	2.0	0.0	2.5	0.0	4.9	0.0	0.0	0.0	0.0	0.0
7/03/2008	SSB	0.0	0.0	26.6	360.9	0.0	0.0	71.3	7.4	0.0	0.0	1.5	0.5	9.3	2.5	1.0	0.0	1.5	0.0
7/08/2008	SSB	0.0	0.0	7.4	112.1	0.0	0.0	54.1	4.4	0.0	0.5	0.0	1.0	9.3	1.0	0.0	0.0	0.0	0.0
7/18/2008	SSB	1.5	0.0	10.8	9.8	1.0	0.0	71.3	1.0	0.0	0.0	0.0	0.0	22.1	0.0	0.0	0.0	0.0	0.0
7/21/2008	SSB	0.0	0.0	21.1	25.6	0.0	0.0	328.4	2.0	0.0	0.0	0.0	1.0	104.2	0.0	0.5	0.0	3.4	0.0
7/28/2008	SSB	0.0	0.0	30.5	111.1	0.0	0.0	47.2	1.5	0.0	0.0	2.5	1.0	6.9	0.0	0.0	0.0	3.4	0.0
8/04/2008	SSB	0.5	0.0	16.2	71.3	0.5	0.0	159.3	0.0	0.0	0.0	0.0	5.9	24.1	0.0	0.5	0.0	6.4	0.0
8/14/2008	SSB	0.0	0.0	44.3	18.2	0.0	0.0	84.6	3.9	0.0	0.0	0.0	1.5	7.4	0.0	0.0	0.0	2.0	0.0
8/18/2008	SSB	0.0	0.0	75.7	18.2	0.0	0.0	69.8	5.9	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	2.5	0.0

Ast, Asterionella; Cruc, Crucigenia; Cry, Cryptomonas; Dino, Dinobryon; Eu, Euglena; Fr, Fragelleria; Gf, Gymnodinium fuscum; Gloe, Gloecystis; Mall, Mallomonas; Mer, Merismopedia; Oo, Oocystis; Pc, Peridinium cinctum; Pl, Peridinium limbatum; Pu, Peridinium umbonatum; Scen, Scenedesmus; Staur, Staurastrum; Syn, Synura; Trac, Trachelomonas

Table A.2. Phytoplankton biovolume (μm^3 per milliliter) in Crystal (CB) and South Sparkling (SSB) Bogs

Date	Lake	*Ast*	*Cruc*	*Cry*	*Dino*	*Eu*	*Fr*	*Gf*	*Gloe*	*Mall*	*Mer*	*Oo*	*Pc*
5/22/2008	CB	0	0	13423	37872	14308	0	114067	13973	0	0	0	0
5/29/2008	CB	2144	0	1918	5534	0	0	142583	1205	0	0	0	0
6/03/2008	CB	0	0	0	454	0	0	513300	1205	0	0	0	0
6/13/2008	CB	0	0	11505	289	0	0	1354542	8914	0	0	0	31958
6/24/2008	CB	10718	0	31958	2767	0	0	869758	6505	1524	897	0	0
7/08/2008	CB	0	0	22371	6402	4769	0	955308	7950	7621	0	1696	0
7/15/2008	CB	0	0	23010	5782	0	0	670142	28669	30483	31388	10178	0
7/22/2008	CB	0	44400	14062	5328	52461	0	470525	50833	48773	0	18093	0
7/29/2008	CB	0	267376	33876	2519	38153	0	826983	33006	13718	4484	2827	0
8/05/2008	CB	23580	424080	31958	1033	33384	0	427750	78298	4573	0	1131	0
8/12/2008	CB	0	76720	12144	2065	4769	0	142583	13009	0	0	1696	0
8/19/2008	CB	0	54193	18536	5906	9538	0	142583	4818	0	0	3393	95875
8/25/2008	CB	0	0	26206	9416	33384	0	285167	3855	0	0	2262	95875
8/31/2008	CB	12862	0	56886	1941	38153	0	313683	5782	0	0	1131	0
9/09/2008	CB	0	0	67752	413	33384	0	156842	482	3048	0	0	0
9/20/2008	CB	0	0	42185	454	0	0	1197700	0	0	0	565	0
9/28/2008	CB	0	0	10227	0	14308	0	399233	0	0	0	565	0
5/23/2008	SSB	10718	0	10227	18255	395841	0	462413	18069	0	0	0	0
5/28/2008	SSB	2144	0	12783	17305	238458	0	448400	29392	0	0	2637	0
6/02/2008	SSB	21437	0	7670	13423	52461	0	252225	17587	0	0	5934	95875
6/09/2008	SSB	70741	0	25567	16272	9538	0	266238	9396	14160	0	659	0
6/17/2008	SSB	51448	0	10866	9788	4769	1744	952850	16141	0	0	0	31958
6/23/2008	SSB	27868	0	20453	9086	0	0	2199963	2650	9440	0	3297	0
7/03/2008	SSB	0	0	34515	30314	0	0	2031813	3614	0	0	1978	31958
7/08/2008	SSB	0	0	9588	9416	0	0	1541375	2168	0	897	0	63917
7/18/2008	SSB	6431	0	14062	826	9538	0	2031813	482	0	0	0	0
7/21/2008	SSB	0	0	27484	2148	0	0	9360350	964	0	0	0	63917
7/28/2008	SSB	0	0	39628	9334	0	0	1345200	723	0	0	3297	63917
8/04/2008	SSB	2144	0	21093	5989	4769	0	4540050	0	0	0	0	383500
8/14/2008	SSB	0	0	57525	1528	0	0	2410150	1927	0	0	0	95875
8/18/2008	SSB	0	0	98432	1528	0	0	1989775	2891	0	0	0	0

Ast, Asterionella; Cruc, Crucigenia; Cry, Cryptomonas; Dino, Dinobryon; Eu, Euglena; Fr, Fragelleria; Gf, Gymnodinium fuscum; Gloe, Gloecystis; Mall, Mallomonas; Mer, Merismopedia; Oo, Oocystis; Pc, Peridinium cinctum

Table A.2 (cont.)

Date	Lake	*Pl*	*Pu*	*Scen*	*Stau*	*Syn*	*Trac*
5/22/2008	CB	0	1392739	0	6897	51923	72742
5/29/2008	CB	0	1422372	574	0	0	0
6/03/2008	CB	172083	2865910	1148	0	0	18516
6/13/2008	CB	774375	4093553	0	0	0	0
6/24/2008	CB	0	2404486	1148	0	0	0
7/08/2008	CB	0	537623	4016	0	0	0
7/15/2008	CB	86042	0	27541	0	0	0
7/22/2008	CB	258125	0	118771	0	51923	0
7/29/2008	CB	344167	0	176149	0	519225	0
8/05/2008	CB	0	0	3443	0	51923	0
8/12/2008	CB	172083	0	8607	0	1142294	0
8/19/2008	CB	86042	0	574	0	1038449	0
8/25/2008	CB	430208	0	0	0	1765364	0
8/31/2008	CB	430208	0	2869	0	6853765	0
9/09/2008	CB	172083	0	0	0	7321067	0
9/20/2008	CB	1290625	0	0	0	4309564	0
9/28/2008	CB	0	0	0	0	2024976	0
5/23/2008	SSB	91450	101598	0	0	0	0
5/28/2008	SSB	0	0	0	0	0	468195
6/02/2008	SSB	0	0	1148	0	0	0
6/09/2008	SSB	182900	0	574	0	0	9258
6/17/2008	SSB	640150	4233	0	0	0	1132131
6/23/2008	SSB	914500	0	0	0	0	0
7/03/2008	SSB	1737550	21166	1148	0	162250	0
7/08/2008	SSB	1737550	8466	0	0	0	0
7/18/2008	SSB	4115250	0	0	0	0	0
7/21/2008	SSB	19387400	0	574	0	378583	0
7/28/2008	SSB	1280300	0	0	0	378583	0
8/04/2008	SSB	4481050	0	574	0	703083	0
8/14/2008	SSB	1371750	0	0	0	216333	0
8/18/2008	SSB	457250	0	0	0	270417	0

Pl, Peridinium limbatum; Pu, Peridinium umbonatum; Scen, Scenedesmus; Staur, Staurastrum; Syn, Synura; Trac, Trachelomonas