ABSTRACT

Title of Dissertation: THE REGULATION OF BACTERIOPLANKTON CARBON METABOLISM IN A TEMPERATE SALT-MARSH SYSTEM

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This study describes an investigation of the factors regulating spatial and temporal variability of bacterioplankton carbon metabolism in aquatic ecosystems using the tidal creeks of a temperate salt-marsh estuary as a study site. Differences in land-use and landscape characteristics in the study site (Monie Bay) generate strong predictable gradients in environmental conditions among and within the tidal creeks, including salinity, nutrients, and the quality and quantity of dissolved organic matter (DOM). A 2-yr study of bacterioplankton metabolism in this system revealed a general positive response to system-level nutrient enrichment, although this response varied dramatically when tidal creeks differing in salinity were compared. Of the numerous environmental parameters investigated, temperature and organic matter quality had the greatest

influence on carbon metabolism. All measures of carbon consumption (i.e., bacterioplankton production (BP), respiration (BR) and total carbon consumption (BCC)) exhibited significant positive temperature dependence, but the disproportionate effect of temperature on BP and BR resulted in the negative temperature dependence of bacterioplankton growth efficiency (BGE = BP/[BP+BR]). Dissolved organic matter also had an influence on carbon metabolism, with higher BCC and BGE generally associated with DOM of greater lability. Our exploration of factors driving this pattern suggests that the energetic content and lability of DOM may be more important than nutrient content or dissolved nutrients alone in determining the magnitude and variability of BGE. Investigations of single-cell activity revealed that BCC and BGE may be further modulated by the abundance, proportion, and activity of highly-active cells. Differences in single-cell activity among creeks differing in freshwater input also imply that other cellular-level properties (e.g., phylogenetic composition) may be an important factor. Collectively, results from this research indicate that the variability of bacterioplankton carbon metabolism in temperate estuarine systems represents a complex response to a wide range of environmental and biological factors, of which temperature and DOM quality appear to be the most important. Furthermore, this research reveals fundamental differences in both cellular and community-level metabolic processes when freshwater and marine endmembers of estuaries are compared that may contribute to the variability in bacterioplankton carbon metabolism within and among estuarine systems.

THE REGULATION OF BACTERIOPLANKTON CARBON METABOLISM IN A TEMPERATE SALT-MARSH SYSTEM

By

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2005

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DEDICATION

To my patient and beautiful wife –

for her unfaltering love and tireless support

ACKNOWLEDGEMENTS

I would like to begin by thanking my advisor, Paul del Giorgio, for bringing me into the fold of microbial ecology. His dedication to "good science", standards of excellence, and high expectations have provided a challenging yet welcome inspiration for my own work. His enthusiasm for science and strong work ethic are balanced by a passion for family and life (not to mention good food and wine) – and in this regard Paul been an exceptional role model. I would also like to give heartfelt appreciation to my advisor Mike Kemp for taking me under his wing late in my dissertation. Although our conversations seldom went where we intended, they always led to novel and valuable insight into my dissertation research – or at least science in general. Above all, Mike helped keep me in touch with the roots of classic ecology, a perspective that has undoubtedly influenced my dissertation and future research.

I would like to thank the other members of my graduate advisory committee (Tom Fisher, Dave Kirchman, and Diane Stoecker) for the time, insight, and expertise dedicated to my dissertation research, as well as Neil Blough for his expertise on CDOM analyses and valuable input as Dean's representative. I would also like to thank Roger Newell for showing me the ropes of sampling in Monie Bay, Todd Kana for assistance with MIMS respiration measurements (without which this dissertation research would not have been possible), Tom Jones for his contribution of background data and insight on Monie Bay, and Thierry Bouvier for research collaborations, companionship, guidance and expertise in flow cytometry, and for tolerating my remedial French. For their invaluable field and laboratory assistance I would like to thank Meredith Guaracci and Rob Condon, my partner in crime Kitty fielding for countless early morning sampling

adventures to Monie Bay, and W. Dave Miller for exhaustive critique of various manuscripts and for sharing late night orders to Mario's.

I would also like to acknowledge the contribution of various funding sources, including the National Estuarine Research Reserve System (NERRS) Graduate Research Fellowship program, grants from the Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET), and funding from Horn Point Laboratory in the form of graduate fellowships and various grants for travel and dissertation research.

Last but not least, I would like to thank my family. To my mother for being a role model for hard work and achievement, instilling and supporting a love of science, and reminding me that even excellence can be improved upon. To my son Gabriel, who never really grasped why writing a dissertation should take precedence over playing with trucks. To my wife Carrie, for her patience, sacrifice, and unflinching support. And finally, to our daughter Micah, who's bright blue eyes and nighttime cuddles became chicken soup for this dissertation writer's soul.

TABLE OF CONTENTS

CHAPTER I

Introduction and Background

INTRODUCTION

It is now widely accepted that heterotrophic bacterioplankton communities are an important and ubiquitous component of all natural aquatic systems and play a fundamental role in their ecological function (Azam et al. 1983; Finlay et al. 1997; Sherr and Sherr 1988). The abundance, growth, and production associated with these communities has been well studied in a wide range of systems, providing valuable information regarding the factors regulating these processes and their contribution to carbon and nutrient cycling. This is in contrast, however, to our relatively limited knowledge of the regulation and magnitude of bacterioplankton respiration (BR) in aquatic systems and those aspects of carbon metabolism which respiration influences. As a result, although bacterioplankton carbon consumption (BCC=BP+BR) and growth efficiency (BGE=BP/[BP+BR]) describe two fundamental aspects of carbon cycling – namely the magnitude of carbon processed by bacterioplankton communities and how that carbon is partitioned between growth and respiration – these measures of carbon metabolism remain less frequently studied and the factors regulating their magnitude and variability are not well understood.

A Brief History of Microbial Ecology

Our appreciation for the numerical dominance and ecological importance of bacterioplankton is a relatively recent development. Only three decades ago Pomeroy (1974) challenged the prevailing paradigm that regarded bacterioplankton as a small community of microorganisms that function as little more than decomposers of organic matter. The resulting change in perspective – coupled with an improved ability to identify the abundance of natural bacterioplankton (Hobbie et al. 1977; Staley and

Konopka 1985) – led to a more detailed description of the important role of these communities in aquatic food webs (Azam et al. 1983; Ducklow 1983). Subsequent improvements in measures of growth and biomass production (Bell 1993; Smith and Azam 1992) and studies employing these methods led to further appreciation of the role of bacterioplankton in mediating many important ecological processes, such as the cycling and regeneration of inorganic nutrients (Brussaard and Riegman 1998; Kirchman 2000b) and the transfer of organic matter to higher trophic levels (Sherr and Sherr 1988), as well as an improved understanding of their importance relative to phytoplankton production and abundance (Cole et al. 1988; White et al. 1991).

Techniques for evaluating metabolic activity on the cellular level (Li et al. 1995; Rodriguez et al. 1992) have led to the important discovery that not all of the bacterioplankton in natural assemblages are metabolically active and that dormant and/or slow-growing cells may make up a large percentage of the bacteria in these communities. In addition, it is now generally accepted that highly-active bacterioplankton are those that are responsible for the majority of growth and production in natural assemblages (Gasol et al. 1999; Sherr et al. 1999b). The abundance of highly-active cells also tends to vary in ecologically meaningful ways, with a higher proportion of highly-active cells typically reported for more eutrophic systems (del Giorgio and Scarborough 1995). Coherence of cellular and community-level metabolic processes has been reported (del Giorgio et al. 1997; Lebaron et al. 2001a; Smith 1998), although this relationship generally remains poorly understood.

It is often assumed that measures of production and growth reflect the magnitude and variability of total bacterioplankton carbon demand (i.e., BCC). However,

significant changes in BGE among and within aquatic systems (del Giorgio and Cole 1998) have led to the realization that bacterioplankton respiration (BR) represents a significant pathway of carbon flux that varies independently of BP. However, due to a number of methodological constraints, estimates of BR remain uncommon relative to those of growth and production (del Giorgio and Cole 1998; Jahnke and Craven 1995). Thus, our understanding of the regulation of BR and related metabolic processes (e.g., BCC and BGE) remains somewhat limited.

Factors Regulating Bacterioplankton Carbon Metabolism

The processing of carbon by the bacterioplankton community can be illustrated as a linear sequence of metabolic events, beginning with the consumption of dissolved organic matter (DOM) and ending with cell growth and division (Fig. 1.1). Although the sequence represented by this conceptual model may appear straightforward, each step is regulated by different environmental factors that may vary spatially and seasonally in aquatic systems. Given the variability associated with these factors, it is unrealistic to assume *a priori* that any one measure of carbon metabolism can be used to accurately predict any other, although such coherence of different measures of carbon metabolism is often expected (del Giorgio and Cole 1998; Rivkin and Legendre 2001),

The multiple factors influencing carbon metabolism in natural aquatic systems can be divided into three general categories: environmental, biological, and phylogenetic (Fig. 1.1). Environmental effects are those associated with abiotic factors and that exert an external influence on bacterioplankton metabolism (e.g., temperature dependence, nutrient limitation), whereas biological effects may be related to characteristics of bacterioplankton cells (e.g., physiology, inherent metabolic properties) or external

biological or ecological processes (e.g., grazing, competition). Phylogenetic effects are those related to the phylogenetic composition of bacterioplankton assemblages and may interact with environmental and biological effects. These three categories are by no means independent. For example, many biological properties of natural assemblages are determined by phylogenetic composition. In addition, environmental conditions may have an effect on biological processes that indirectly regulate community-level carbon metabolism. Nonetheless, the basic framework illustrated in Fig. 1.1 is suitable for summarizing the factors regulating bacterioplankton carbon metabolism and for visualizing the general hypotheses of this dissertation research.

Specific aspects of environmental, biological, and phylogenetic factors and their influence on each aspect of bacterioplankton carbon metabolism are summarized in Fig. 1.1. In general, it is believed that consumption of organic carbon by bacterioplankton communities (i.e., BCC) is regulated predominantly by characteristics of the DOM pool, such as molecular structure, size, and lability (del Giorgio and Davis 2003; Søndergaard and Middelboe 1995). Although the affinity of specific bacterioplankton for a particular substrate may influence rates of carbon consumption (Cottrell and Kirchman 2000), it is unlikely that such species-specific biological factors will have a significant effect on rates of total carbon consumption. Temperature is another environmental factor that may have an effect on BCC, for studies of DOC consumption during long-term incubations of size-fractioned samples imply a significant temperature dependence of short-term bacterioplankton carbon consumption (Raymond and Bauer 2000).

Other environmental factors may be important in the partitioning of carbon into growth versus respiration (i.e., BGE). These include the energy content of carbon

substrates (Linton and Stevenson 1978), quality and size of DOM (Amon and Benner 1996), and availability of carbon relative to dissolved nutrients (Touratier et al. 1999). In addition, a meta-analysis of data from various marine systems (Rivkin and Legendre 2001) suggests a negative temperature dependence of BGE. At this level in the metabolic sequence illustrated in Fig. 1.1, biological factors may begin to have a more pronounced effect. For example, it is hypothesized that the balance between the abundance of highly-active cells and that of inactive or slow-growing cells may influence bacterial growth efficiency, based on the assumption that highly-active cells generally have higher cellular-level BGE (del Giorgio and Cole 2000). In this regard, preferential grazing of highly-active cells by protozooplankton may contribute to shifts in BGE (Gonzalez et al. 1990; Lebaron et al. 1999).

Compared to other measures of carbon metabolism, the range and variability of production and growth – as well as the factors regulating these processes in aquatic systems – have been well described and are better understood. Production and growth may be limited by nutrient and substrate availability, temperature constraints, or some combination thereof (Shiah and Ducklow 1994a). In addition, biological factors such as grazing (Gonzalez et al. 1990; Sherr et al. 1992), the relationship between single-cell activity and growth (Cottrell and Kirchman 2003; del Giorgio et al. 1997), and viral mortality (Tuomi and Kuuppo 1999) are also important in determining BP and the transfer of this biomass into population growth.

A third factor that may play an important role in the magnitude and variability of carbon metabolism is phylogenetic composition, which may have both direct and indirect effects. Direct effects on BCC may result from the predisposition of certain phylotypes

to degrade specific substrate groups (Cottrell and Kirchman 2000) or the presence of substrate-specific enzymes (Kirchman et al. 2004). Direct effects on growth and production include inherent community and cellular-level metabolic properties (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2004; Pinhassi et al. 1999) and enzymatic activities (Kirchman et al. 2004) associated with specific phylogenetic groups. Phylotype-specific grazing may also have a significant effect on the growth rate of natural bacterioplankton communities (Langenheder and Jurgens 2001; Lebaron et al. 2001b). Although both phylogenetic composition and bacterioplankton metabolism respond to changes in environmental conditions, bacterioplankton carbon metabolism may also be influenced directly by phylogenetic composition. In this regard, the extent to which changes in bacterioplankton metabolism result from the effect of environmental conditions, intrinsic properties of the bacterioplankton community related to phylogenetic composition, or a complex interaction of both is difficult to determine. For this reason, the specific role of phylogeny in regulating carbon metabolism, growth, and other metabolic processes remains poorly defined.

Evaluating the Regulation of Natural Bacterioplankton Communities

There are numerous approaches to identifying the effect of environmental conditions on bacterioplankton metabolism. However, these analyses are seldom conducted on scales that are directly relevant to *in situ* ecological processes. Small-scale experiments, such as those using flask (Carlson and Ducklow 1996) and mesocosm (Lebaron et al. 2001b) incubations, may identify direct effects but not accurately represent the *in situ* response of natural bacterioplankton communities to resource supply or changes in other environmental conditions. Conversely, large-scale comparative

studies of multiple systems (Cole et al. 1988; del Giorgio and Cole 1998; White et al. 1991) may represent *in situ* changes in the bacterioplankton community. However, data in these meta-analyses are frequently integrated over large temporal and spatial scales, limiting the ability to explore relationships between metabolic processes that occur simultaneously. These analyses may also include climatic, regional, or systematic variability that confounds the ability to identify meaningful ecological relationships within the dataset.

In an attempt to ameliorate these problems, many studies have combined elements of both large and small-scale investigations, conducting enrichment experiments on entire systems. This approach has been implemented successfully in lakes (Pace and Cole 2000) and the open ocean (Kolber et al. 1994), although characteristically low water-residence times in tidally-flushed systems (Rasmussen and Josefson 2002) presents a challenge to this type of manipulation in most estuaries. An alternative is to conduct comparative experiments among systems characterized by strong gradients in environmental factors of interest. For example, nutrient availability, organic carbon quality and supply, and salinity tend to vary significantly yet predictably among and within estuarine sub-systems (Boynton and Kemp 2000; Fisher et al. 1988; Sharp et al. 1982). A number of studies have exploited such natural and anthropogenic gradients to investigate the metabolic response of bacterioplankton to environmental factors (Cottrell and Kirchman 2004; Findlay et al. 1996; Hoppe et al. 1998; Revilla et al. 2000).

The field sampling associated with this dissertation research was conducted exclusively at the Monie Bay component of Maryland's National Estuarine Research Reserve. This system is dominated by three tidal creek systems (Little Creek (LC), Little

Monie Creek (LMC), and Monie Creek (MC)) that drain adjacent marshes and interact tidally with the waters of an open bay (OB). Differences in agricultural land-use and associated farming practices among creek watersheds generate predictable spatial and temporal patterns in nutrient enrichment both among and within the tidal creek systems (Cornwell et al. 1994; Fielding 2002; Jones et al. 1997). Monie Bay was selected for this research because it offers steep gradients in a wide range of environmental conditions, yet within this variability are general systematic patterns that are related to ambient nutrient concentrations and DOM source, supply, and composition (Fig. 1.2). Comparisons among the four sub-systems can be used to isolate key environmental factors influencing bacterioplankton metabolism that might not be revealed in small-scale (e.g., incubations) or large-scale (e.g., meta-analyses) investigations.

RESEARCH QUESTIONS AND APPROACHES

Primary Research Objectives

The magnitude and variability of bacterioplankton production and growth in aquatic systems has been well studied and the factors regulating these processes are relatively well described (Cole et al. 1988; Vrede et al. 1999; White et al. 1991). My research focuses on the less frequently studied aspects of carbon metabolism illustrated in the upper levels of Fig. 1.1 (i.e., BR, BCC, and BGE) and the regulation of these processes in natural aquatic systems. The research described in the following dissertation pursues four primary objectives that address fundamental questions regarding the regulation of bacterioplankton metabolism. First, I describe the spatial and temporal variability of cellular and community-level bacterioplankton metabolic processes in a temperate salt-marsh dominated estuary. Second, I investigate the variability in various

environmental factors including salinity, temperature, inorganic nutrients, and the quality and quantity of DOM and the influence on community-level carbon metabolism. Third, I investigate the coupling of cellular and community-level metabolism, and fourth, I explore the metabolic response of bacterioplankton communities to system-level anthropogenic nutrient enrichment.

Chapter II: Experimental Design and Systematic Patterns in Monie Bay

This research begins with Chapter II (Apple et al. 2004) and a description of the use of Monie Bay research reserve to investigate the effect of system-level nutrient enrichment on bacterioplankton communities. The focus of this study is to: (1) describe the spatial and temporal variability in water column chemistry, temperature, and BP within and among the tidal creeks of Monie Bay, (2) investigate factors regulating the response of bacterioplankton to system-level nutrient enrichment, and (3) establish a basis for the experimental design to be used in subsequent chapters investigating other aspects of bacterioplankton metabolism. An important conclusion of this chapter is that bacterioplankton communities respond positively to increasing nutrient concentrations at the system-level, but that this metabolic response may be mediated by other environmental factors such as temperature, organic matter quality, and salinity.

Chapter III: Effect of Temperature

The apparent temperature-dependence of BP observed in Chapter II has been documented in other temperate estuaries (Lomas et al. 2002; Shiah and Ducklow 1994b). However, the extent to which the temperature dependence of bacterioplankton growth and production reflects other aspects of carbon metabolism and how these temperature dependencies might change among systems differing in their degree of resource

enrichment is not well understood. Chapter III (Apple et al. submitted) investigates how temperature affects bacterioplankton carbon metabolism using a continual dataset (>2-yr) of monthly sampling within and among the sub-systems of Monie Bay. This study identifies a significant temperature dependence of all measures of bacterioplankton carbon metabolism, as well as a negative temperature dependence of BGE (BP/[BP+BR]) driven by differences in the effects of temperature on BP and BR. Although carbon metabolism varied significantly with temperature, this relationship did not override the effects of other environmental factors, as evidenced by persistent differences in the magnitude of carbon metabolism among the different tidal creeks. I concluded that temperature and resource supply have a simultaneous yet independent influence on bacterioplankton carbon metabolism.

Chapter IV: Variability and Regulation of Carbon Metabolism

The study of temperature dependence reported in Chapter III led to the hypothesis that nutrient availability and the quality and quantity of DOM may also have a significant influence on carbon metabolism in the tidal creeks of Monie Bay. Chapter IV explores the effect of dissolved nutrients and DOM quality on bacterioplankton carbon consumption (BCC) and growth efficiency (BGE). Results from this investigation suggest that energetic content and lability of DOM are more important than nutrient (i.e., nitrogen and phosphorus) content in regulating these metabolic processes. Multivariate analysis of residuals from the temperature-dependence relationships reported in Chapter III revealed that environmental factors regulating carbon metabolism differed among the measured aspects (i.e., BGE, BCC, and BP) and confirmed the importance of organic matter quality in regulating carbon metabolism. Although temperature and organic

matter quality explain much of the variability in bacterioplankton carbon metabolism, this chapter speculates that cellular-level metabolic processes may influence community-level metabolism and help explain patterns in carbon metabolism observed among the tidal creeks.

Chapter V: Linking Cellular and Community-Level Metabolism

In Chapter V, I investigate the relationship between cellular-level metabolic activity and community-level carbon metabolism, focusing specifically on the role of single-cell activity in regulating BGE and exploring differences in single-cell activity associated with differences in salinity. Results from this study suggest that the proportion of highly-active cells and the relative intensity of their activity have an important influence on bacterioplankton carbon consumption and growth efficiency. In addition, tidal creeks differing in freshwater inputs also differed dramatically in cellular-level characteristics, including the proportion of highly-active cells, the distribution of activity within the highly-active fraction, and the relationship between cellular-level and community-level metabolism. This chapter leads to the conclusion that there is a cellular-level physiological basis for many of the patterns in carbon metabolism reported in previous chapters, specifically regarding the differences between more and less saline sub-systems.

Chapter VI: Summary and Research Conclusions

The research described in this dissertation set out to explore the variability and regulation of bacterioplankton carbon metabolism in the tidal creeks of a salt-marsh dominated estuary. C Components of this research investigating carbon metabolism (Chapters II and IV) and single-cell activity (Chapter V) revealed that, even on the

relatively small spatial scales investigated, the temporal and spatial variability in bacterioplankton metabolism is high and comparable to that found across a broad range of aquatic systems. This variability, however, is constrained by two primary environmental factors: temperature and differences in resource supply (discussed in Chapter II and Chapters II & IV, respectively). The first of these factors regulates the magnitude of carbon metabolism throughout the year, whereas the second influences the magnitude of carbon metabolism in each estuarine sub-system at any given temperature or season. Of the different aspects of resource supply investigated, the energetic quality and lability of DOM appears to have the most pronounced influence on bacterioplankton carbon metabolism. Combined with patterns in single-cell activity observed among estuarine sub-systems (Chapter V), these relationships provide valuable insight into the response of bacterioplankton to system-level nutrient enrichment when estuarine sub-systems differing in their freshwater input are compared.

LITERATURE CITED

Amon, R., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography **41:** 41-51.

Apple, J. K., P. A. del Giorgio, and W. M. Kemp. submitted. Temperature regulation of bacterial production, respiration, and growth efficiency in estuarine systems. Aquatic Microbial Ecology.

Apple, J. K., P. A. del Giorgio, and R. I. E. Newell. 2004. The effect of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. Journal of Coastal Research **45:** 110-133.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and T. F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series **10:** 257-263.

Bell, R. 1993. Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine (Chapter 56). Handbook of Methods in Aquatic Microbial Ecology**:** 495-503.

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography **47:** 453-470.

Boynton, W. R., and W. M. Kemp. 2000. Influence of river flow and nutrient loading on selected ecosystem processes and properties in Chesapeake Bay, p. 269-298. *In* J. E. Hobbie [ed.], Estuarine science: A synthetic approach to research and practice. Island Press.

Brussaard, C. P. D., and R. Riegman. 1998. Influence of bacteria on phytoplankton cell mortality with phosphorus or nitrogen as the algal-growth-limiting nutrient. Aquatic Microbial Ecology **14:** 271-280.

Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquatic Microbial Ecology **10:** 69-85.

Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross system overview. Marine Ecology Progress Series **43:** 1-10.

Cornwell, J. C., J. M. Stribling, and J. C. Stevenson. 1994. Biogeochemical studies at the Monie Bay National Estuarine Research Reserve, p. 645-655. *In* M. Lynch and B. Crowder [eds.], Organizing for the Coast: Thirteenth International Conference of the Coastal Society.

Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine proteobacteria and members of Cytophaga-Flavobacter cluster consuming low-and high-molecular weight dissolved organic matter. Applied and Environmental Microbiology **66:** 1692-1697.

---. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnology and Oceanography **48:** 168–178.

---. 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquatic Microbial Ecology **34:** 139-149.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29:** 503-541.

---. 2000. Bacterial growth energetics and efficiency in natural aquatic systems, p. 289-325. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

del Giorgio, P. A., and J. Davis. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399-424. *In* S. Findlay [ed.], Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Elsevier Science.

del Giorgio, P. A., Y. T. Prairie, and D. F. Bird. 1997. Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, enumerated using CTC reduction and flow cytometry. Microbial Ecology **34:** 144-154.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. Journal of Plankton Research **17:** 1905-1924.

Ducklow, H. W. 1983. Production and fate of bacteria in the oceans. BioScience **33:** 494-501.

Fielding, K. P. 2002. Differential substrate limitation in small tributaries of Chesapeake Bay, Maryland influenced by non-point source nutrient loading. Masters. University of Maryland.

Findlay, S., M. L. Pace, and D. T. Fischer. 1996. Spatial and Temporal Variability in the Lower Food Web of the Tidal Freshwater Hudson River. Estuaries **19:** 866-873.

Finlay, B. J., S. C. Maberly, and J. I. Cooper. 1997. Microbial diversity and ecosystem function. Oikos **80:** 209-213.

Fisher, T. R., L. W. Harding, D. W. Stanley, and L. G. Ward. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science **27:** 61-93.

Gasol, J. M., U. L. Zweifel, F. Peters, J. A. Fuhrman, and A. Hagstrom. 1999. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Applied and Environmental Microbiology **65:** 4475-4483.

Gonzalez, J. M., E. B. Sherr, and B. F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and Environmental Microbiology **56:** 583-589.

Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology **33:** 1225.

Hoppe, H. G., H. C. Giesenhagen, and K. Gocke. 1998. Changing patterns of bacterial substrate decomposition in a eutrophication gradient. Aquatic Microbial Ecology **15:** 1-13.

Jahnke, R. A., and D. B. Craven. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: A need for respiration rate measurements. Limnology and Oceanography **40:** 436-441.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A Two-Year Study of the Short-Term and Long-Term Sequestering of Nitrogen and Phosphorus in the Maryland National Estuarine Research Reserve, p. 1-100. Maryland National Estuarine Research Reserve.

Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria, p. 261-288. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

Kirchman, D. L., A. I. Dittel, S. E. G. Findlay, and D. Fischer. 2004. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. Aquatic Microbial Ecology **35:** 243-257.

Kolber, Z. S., R. Barber, K. Coale, S. Fitzwater, and R. Greene. 1994. Iron limitation of phytoplankton photosynthesis in the equatorial pacific ocean. Nature **371:** 145-148.

Langenheder, S., and K. Jurgens. 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnology and Oceanography **46:** 121-134.

Lebaron, P., P. Servais, H. Agogué, C. Courties, and F. Joux. 2001a. Does the High Nucleic Acid Content of Individual Bacterial Cells Allow Us To Discriminate between Active Cells and Inactive Cells in Aquatic Systems? Applied and Environmental Microbiology **67:** 1775-1782.

Lebaron, P. and others 2001b. Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in abundances, activity and composition. FEMS Microbiology Ecology **34:** 255-266.

Lebaron, P., P. Servais, M. Troussellier, C. Courties, and J. Vives-Rego. 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquatic Microbial Ecology **19:** 225-267.

Li, W., J. Jellett, and P. Dickie. 1995. DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnology and Oceanography **40:** 1485-1495.

Linton, J., and R. Stevenson. 1978. A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substrates. FEMS Microbiology Letters **3:** 95-98.

Lomas, M. W., P. M. Glibert, F. Shiah, and E. M. Smith. 2002. Microbial processes and temperature in Chesapeake Bay: current relationships and potential impacts of regional warming. Global Change Biology **8:** 51-70.

Pace, M. L., and J. J. Cole. 2000. Effects of whole-lake manipulations of nutrient loading and food web structure on planktonic respiration. Canadian Journal of Fisheries and Aquatic Sciences **57:** 487-496.

Pinhassi, J. and others 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquatic Microbial Ecology **17:** 13-26.

Pomeroy, L. 1974. The ocean's food web, a changing paradigm. BioScience **24:** 499-504.

Rasmussen, B., and A. Josefson. 2002. Consistent Estimates for the Residence Time of Micro-tidal Estuaries. Estuarine, Coastal and Shelf Science **54:** 65-73.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. Aquatic Microbial Ecology **22:** 1-12.

Revilla, M., A. Iriarta, I. Madariaga, and E. Orive. 2000. Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuarine, Coastal and Shelf Science **50:** 297-313.

Rivkin, R. B., and L. Legendre. 2001. Biogenic carbon cycling in the upper ocean: Effects of microbial respiration. Science **291:** 2398-2400.

Rodriguez, G. G., D. Phipps, K. Ishiguro, and H. F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Applied and Environmental Microbiology **58:** 1801-1808.

Sharp, J. H., C. H. Culberson, and T. M. Church. 1982. The chemistry of the Delaware Estuary. General considerations. Limnology and Oceanography **27:** 1015-1028.

Sherr, B. F., E. B. Sherr, and J. Mcdaniel. 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. Applied and Environmental Microbiology **58:** 2381-2385.

Sherr, E. B., and B. F. Sherr. 1988. Role of microbes in pelagic food webs: A revised concept. Limnology and Oceanography **33:** 1225-1227.

Sherr, E. B., B. F. Sherr, and C. T. Sigmon. 1999. Activity of marine bacteria under incubated and in situ conditions. Aquatic Microbial Ecology **20:** 213-223.

Shiah, F. K., and H. W. Ducklow. 1994a. Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Marine Ecology Progress Series **103:** 297-308.

---. 1994b. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnology and Oceanography **39:** 1243-1258.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using super(3)H-leucine. Marine Microbial Food Webs **6:** 107-114.

Smith, E. M. 1998. Coherence of microbial respiration rate and cell-specific activity in a coastal plankton community. Aquatic Microbial Ecology **16:** 27-35.

Søndergaard, M., and M. Middelboe. 1995. A cross system analysis of labile dissolved organic carbon. Marine Ecology Progress Series **118:** 283-294.

Staley, J. T., and A. Konopka. 1985. Measurement of in Situ Activities of Nonphotosynthetic Microorganisms in Aquatic and Terrestrial Habitats. Annual Review of Microbiology **39:** 321-346.

Touratier, F., L. Legendre, and A. Vézina. 1999. Model of bacterial growth influenced by substrate C:N ratio and concentration. Aquatic Microbial Ecology **19:** 105-118.

Tuomi, P., and P. Kuuppo. 1999. Viral lysis and grazing loss of bacteria in nutrient- and carbon-manipulated brackish water enclosures. Journal of Plankton Research **21:** 923-937.

Vrede, K., T. Vrede, A. Tisaksson, and A. Karlsson. 1999. Effects of nutrients (P,N,C) and zooplankton on bacterioplankton and phytoplankton - a seasonal study. Limnology and Oceanography **44:** 1616-1624.

White, P. A., J. Kalff, J. B. Rasmussen, and J. M. Gasol. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microbial Ecology **21:** 99-118.

FIGURES

Fig. 1.1. Summary of factors regulating different aspects of bacterioplankton carbon metabolism and growth. Factors appearing in dashed boxes represent those that are investigated as part of my dissertation research. References include (1) del Giorgio and Davis 2003, Søndergaard and Middelboe 1995; (2) Raymond and Bauer 2000; (3) Daneri et al. 1994, Rivkin and Legendre 2001; (4) Linton and Stevenson 1978, Touratier et al. 1999; (8) Shiah and Ducklow 1994; (9) Coveney and Wetzel 1992; (10) Bergstedt et al. 2004, Delaney 2003; (11) Hoppe et al. 1998, Kirchman et al. 2004 (12) Unanue et al. 1999; (13) del Giorgio and Cole 2000; (14) Middelboe and Søndergaard 1993; (15) Gonzalez et al. 1990, Sherr et al. 1992; (16) Tuomi and Kuuppo 1999; (17) Cottrell and Kirchman 2003, del Giorgio et al. 1997; (18) Cottrell and Kirchman 2000; (19) Bouvier and del Giorgio 2002; (20) Bouvier and del Giorgio 2002, Pinhassi et al. 1999; (21) Langenheder and Jurgens 2001, Lebaron et al. 2001.

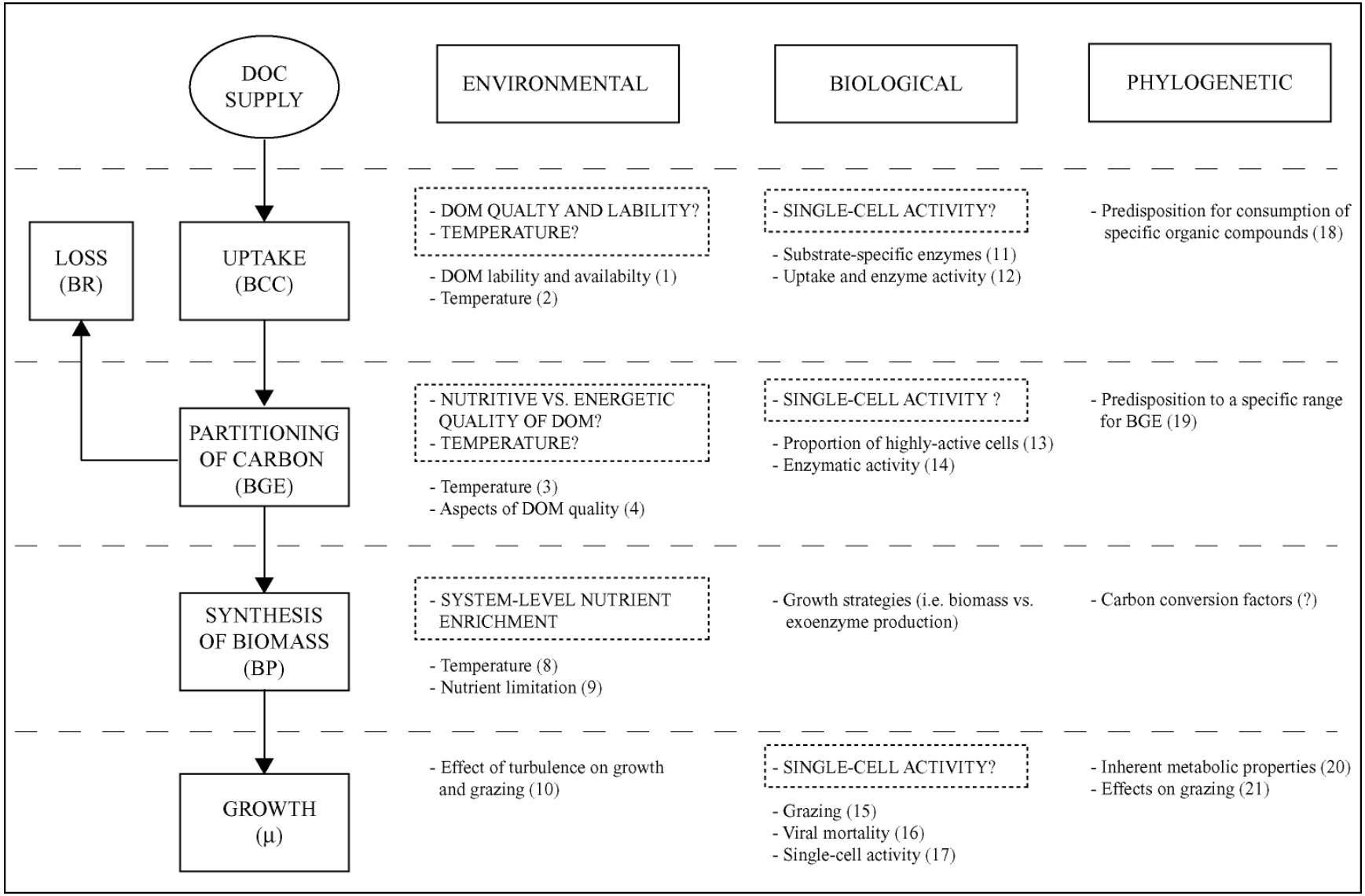
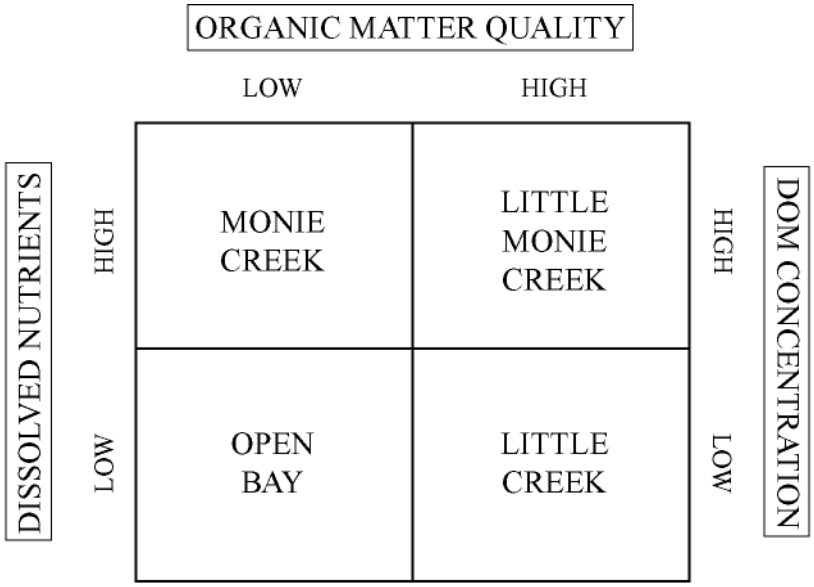

DOC SUPPLY
ENVIRONMENTAL
BIOLOGICAL
PHYLOGENETIC
LOSS (BR)
UPTAKE (BCC)
- DOM QUALTY AND LABILITY?
- TEMPERATURE?
- DOM lability and availabilty (1)
- Temperature (2)
- SINGLE-CELL ACTIVITY?
- Substrate-specific enzymes (11)
- Uptake and enzyme activity (12)
- Predisposition for consumption of specific organic compounds (18)
PARTITIONING OF CARBON (BGE)
- NUTRITIVE VS. ENERGETIC QUALITY OF DOM?
- TEMPERATURE?
- Temperature (3)
- Aspects of DOM quality (4)
- SINGLE-CELL ACTIVITY ?
- Proportion of highly-active cells (13)
- Enzymatic activity (14)
- Predisposition to a specific range for BGE (19)
SYNTHESIS OF BIOMASS (BP)
- SYSTEM-LEVEL NUTRIENT ENRICHMENT
- Temperature (8)
- Nutrient limitation (9)
- Growth strategies (i.e. biomass vs. exoenzyme production)
- Carbon conversion factors (?)
GROWTH (μ)
- Effect of turbulence on growth and grazing (10)
- SINGLE-CELL ACTIVITY?
- Grazing (15)
- Viral mortality (16)
- Single-cell activity (17)
- Inherent metabolic properties (20)
- Effects on grazing (21)

Fig. 1.2. Qualitative comparison of relative differences in ambient nutrient concentrations and the quality and concentration of DOC in the four estuarine sub-systems of Monie Bay.


ORGANIC MATTER QUALITY
LOW
HIGH
DISSOLVED NUTRIENTS
HIGH
LOW
MONIE CREEK
LITTLE MONIE CREEK
OPEN BAY
LITTLE CREEK
HIGH
LOW
DOM CONCENTRATION

CHAPTER II

The effects of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary

ABSTRACT

We describe the use of Monie Bay Research Reserve as a natural experiment to evaluate the effect of system-level nutrient enrichment on natural bacterioplankton communities. Monie Bay, a component of the Chesapeake Bay National Estuarine Research Reserve System, is a sub-estuary of the Chesapeake Bay, consisting of a shallow semi-enclosed bay and three tidally influenced creeks varying in their agricultural land use and freshwater inputs. As part of a 2-year study in this system, we identified distinct spatial and seasonal patterns in ambient nutrient concentrations, salinity, and source and quantity of organic matter that were related to differences in agricultural practices and watershed characteristics among the three tidal creeks. Principal components analysis (PCA) identified freshwater delivery of nutrients and temperature as key factors driving the overall variability of this system. Despite significant variability in nutrient concentrations and bacterioplankton production (BP) throughout the year, we observed persistent response of bacterioplankton to nutrient enrichment, as evidenced by a comparison of 2-year averages in agriculturally-developed Little Monie Creek (LMC) relative to the undeveloped Little Creek (LC), and by a comparison of the nutrient-enriched upper estuary of LMC to sites nearer the open bay. Bacterioplankton responded positively to pulsed nutrient availability, with elevated rates of BP associated with agriculturally-derived nutrient inputs to the Monie Bay system. Freshwater inputs play an important role in mediating the response of bacterioplankton to nutrient enrichment, as evidenced by relatively low estimates of BP in the freshwater-dominated, agriculturally-developed Monie Creek. This response is attributed to changes in organic matter quality in the system and the direct effect of salinity on bacterioplankton community metabolism.

INTRODUCTION

Non-point source inputs of agriculturally-derived nutrients have been unequivocally linked to nutrient enrichment and subsequent eutrophication of coastal systems (Beaulac and Reckhow 1982; Fisher 1985). Understanding the effect of nutrient inputs on coastal system function is necessary to allow agricultural production to be sustained without sacrificing water quality and integrity of estuarine resources. An integral program in this endeavor is the National Estuarine Research Reserve System (NERRS), a network of sites located throughout the coastal U.S. that have been established for long-term research, education, and stewardship of national estuarine resources. The use of these sites as "living laboratories" is a primary objective of NERRS and plays an important role in understanding and mitigating anthropogenic impacts on the health and function of estuarine resources.

Coastal eutrophication is traditionally evaluated within the context of phytoplankton abundance (Smith et al. 1992), increases in ambient nutrient concentrations and organic matter loading (Nixon 1995), and general increases in heterotrophic activity (Tuttle et al. 1987). Heterotrophic bacterioplankton (i.e., the microbial community) are seldom incorporated in these assessments, despite their widespread acceptance as an important component in ecosystem health, function, and the eutrophication process (Brussaard and Riegman 1998; Ducklow et al. 1986; Sherr and Sherr 1988). The microbial community mediates almost every important ecological process related to eutrophication of aquatic systems. Deep-water anoxia and periods of net-heterotrophy in the Chesapeake Bay are driven almost exclusively by aerobic, heterotrophic bacterial metabolism (Smith and Kemp 2001; Tuttle et al. 1987). The

transfer of algal-derived organic matter to top consumers and the extent to which it is lost via respiratory processes is dictated by the efficiency of carbon cycling by the microbial community (Azam et al. 1983; Sherr and Sherr 1988). Similarly, the availability and cycling of inorganic nutrients is regulated by their rapid utilization by bacterioplankton relative to phytoplankton (Kirchman 1994) and the variable efficiency with which dissolved organic matter (DOM) and particulate organic matter (POM) is remineralized (del Giorgio and Cole 1998). The microbial community not only drives these ecological processes, but also responds rapidly to even the most subtle changes in these processes (Finlay et al. 1997). Thus, the characteristics of this community represent a sensitive and integrative biological synthesis of environmental conditions and ecological processes that effectively integrates key ecological aspects of ecosystem function that are seldom considered in management and conservation efforts.

The response of natural bacterioplankton communities to inputs of inorganic nutrients has been extensively studied, although seldom on the spatial or temporal scale at which system-level nutrient enrichment typically occurs. Small-scale experiments, such as those using flask (Carlson and Ducklow 1996) and mesocosm (Lebaron et al. 2001b) incubations, may identify direct effects but not accurately represent the *in situ* response of natural bacterioplankton communities to system-level enrichment. Conversely, although large-scale comparative studies of multiple systems (Cole et al. 1988; del Giorgio and Cole 1998) may identify differences in the bacterioplankton community along an enrichment gradient, these data are typically integrated over large temporal and spatial scales. Consequently, they cannot be used to isolate the immediate and direct effect of system-level enrichment on the bacterioplankton community alone.

The direct effect of system-level nutrient enrichment can be identified by combining the elements of both large- and small-scale studies and conduct enrichment experiments on entire systems. This approach has been implemented successfully in lakes (Pace and Cole 2000) and the open ocean (Kolber et al. 1994), although the characteristically low water-residence time in tidally-flushed systems (Rasmussen and Josefson 2002) presents a challenge to this type of manipulation in most estuaries. An alternative is to use estuarine systems where enrichment gradients already exist to simulate a large-scale nutrient enrichment experiment. For example, steep gradients generated by point and non-point sources of anthropogenic nutrient loading have been used successfully to evaluate the response of estuarine bacterioplankton to system-level nutrient enrichment (Hoppe et al. 1998; Revilla et al. 2000).

The Monie Bay component of the Chesapeake Bay Maryland NERR is an ideal system for this approach to investigating the direct effect of system-level nutrient enrichment on estuarine systems. The research reserve is dominated by a shallow, semi-enclosed embayment and three creek systems that drain adjacent marshes and interact tidally with bay waters. Differences in agricultural land use among creek watersheds generate distinct patterns in nutrient enrichment both among and within the creek systems (Cornwell et al. 1994; Jones et al. 1997). Additional predictable variability in nutrient enrichment is introduced by the timing and nature of agricultural practices in each creek basin (Cornwell et al. 1994; Fielding 2002; Jones et al. 1997). Thus, nutrient concentrations, land-use, and salinities in the three tidal creeks represent a broad range of conditions on relatively small spatial scales, making the Monie Bay Research Reserve an ideal system for comparative investigations of the effect of agricultural nutrient

enrichment on salt-marsh communities (Jones et al. 1997). In addition, because this system exhibits a large range of conditions (i.e., nutrient concentrations, salinity, land use) over relatively small spatial scales, studies conducted in this system are not hindered by the additional variability typically imposed by differences in basin or region-level processes and conditions (i.e., rainfall, climate, irradiance, temperature, atmospheric deposition of nutrients, etc.).

In this paper we describe the use of Monie Bay NERR as a natural experiment to investigate the effect of system-level nutrient enrichment on estuarine bacterioplankton communities. We begin by identifying spatial and seasonal patterns of agricultural land use, nutrient enrichment, and water column chemistry within and among the three tidal creeks. The effect of system-level nutrient enrichment on the bacterioplankton community is subsequently explored by: (1) comparing agriculturally-impacted versus unimpacted tidal creeks; (2) comparing creeks differing in terrestrial influence but experiencing similar enrichment; (3) evaluating changes along creek axes from enriched headwaters to the relatively unenriched open bay; and (4) evaluating conditions before, during, and after pulsed inputs of agriculturally-derived nutrients.

Objectives

The present study is part of an ongoing effort to describe the variability and range of bacterioplankton metabolism, identify the environmental factors regulating these metabolic processes, and investigated the metabolic response of these communities to system-level nutrient enrichment in the tidal creeks of a temperate salt-marsh system. The research described in the present study focuses on the influence of DOM quality on the magnitude and variability of BGE and BCC. As part of this study, we investigate two

fundamental hypotheses. The first is that BGE is regulated by the nutritive quality of DOM. We investigate this hypothesis by exploring the relationship between BGE, nutrient consumption, and indices of organic matter quality. The second hypothesis is that BGE is influenced by the magnitude of carbon consumed by bacterioplankton. To test this hypothesis, we investigate the extent of coupling between paired estimates of BCC and BGE using a comprehensive and long-term (>2y) dataset describing the range and variability of BGE and BCC in the sub-systems of Monie Bay research reserve.

METHODS

Site Description

Monie Bay is a tidally influenced sub-estuary located on the eastern shore of Chesapeake Bay (38°13.50'N 75°50.00'W). The reserve consists of a relatively small (i.e., 1-2 km wide and 4 km long) open bay (OB) and three tidally influenced creeks varying in size and agricultural land use (Fig. 2.1). Monie Creek (MC) has the largest of the three creek watersheds (45 km^2), covering approximately 2.5 and 5 times more area than those of Little Monie Creek (LMC; 17.9 km^2) and Little Creek (LC; 9.4 km^2), respectively. The linear reach of MC from headwaters to the open bay is 6.5 km, compared to 3.7 for LMC and 2.9 for LC. The creek channels in these systems are the result of tidal scouring, with no significant fluvial input (Ward et al. 1998). Monie Creek experiences year-round inputs of fresh water, whereas LMC and LC have salinities driven entirely by tidal flushing from the open bay and seasonal or episodic freshwater inputs occurring predominantly in the spring or following major rain events (Jones et al. 1997). MC and LMC are quite similar with respect to land use patterns, with approximately 25% of each watershed agriculturally developed and a similar proportion

of watershed acreage attributed to marsh and forest (Fig. 2.1). As a result, MC and LMC are characterized by steep spatial gradients in nutrient availability, with low salinity regions experiencing elevated inputs of allochthonous nitrogen and phosphorus (Jones et al. 1997) that are ascribed to agricultural activities (i.e., crop farming, livestock and poultry operations) within the watershed (Cornwell et al. 1994; Fielding 2002). By comparison, LC watershed is dominated by tidal marsh with approximately one-third of the watershed being forested. Residential development in the LC watershed is minimal and similar to that of other creeks (i.e., ≤ 3%), and there is almost no agricultural land use (i.e., <1%).

The marsh macrophyte community of Monie Bay is dominated by *Spartina* spp. (*S. alterniflora* and *S. patens*), with *Juncus roemerianus* and *Phragmites australis* more prevalent in the upper marsh experiencing less frequent flooding (Kearney et al. 1994; Stribling and Cornwell 1997; Ward et al. 1998). An exception is the upper reaches of MC, which is characterized by a diverse freshwater macrophyte community and greater abundance of macrophytes that use C3 photosynthetic pathways (Jones et al. 1997; Stribling and Cornwell 1997).

Experimental Design

Our investigation of the response of bacterioplankton to system-level resource enrichment combines the manipulative aspect of a traditional small-scale nutrient enrichment experiment (Caron et al. 2000; Lebaron et al. 2001b) with the ecological relevance and larger spatial scale of *in situ* field observations. Using this approach, each tidal creek is analogous to an individual treatment in a small-scale manipulative experiment, whereby watershed characteristics (rather than the scientist) manipulate the

environmental conditions of interest. For example, nutrient and DOM concentrations and the source and quality of dissolved and particulate organic seston are determined by the extent of agricultural land use, dominant macrophyte cover, and watershed size. Tidal inundation of each creek serves as an "inoculum" of open bay waters and associated bacterioplankton communities. The resulting changes in the bacterioplankton community in each tidal creek relative to the open bay are assumed to be a response to the environmental conditions unique to each creek system. Analyses focused specifically on parameters related to resource regulation of bacterioplankton (i.e., dissolved nutrients and DOM), although it is possible that top-down effects of grazers may also have an impact on estuarine bacterioplankton communities (del Giorgio et al. 1996b; Gonzalez et al. 1990; Rieman et al. 1990). By sampling each creek system on the ebb tide, we were able to capture the metabolic response of bacterioplankton to these changing conditions, as the tidal cycle is a comparable time frame in which estuarine bacterioplankton respond to changing environmental conditions (Painchaud et al. 1996). We used well-documented patterns in agricultural land-use and nutrient availability among and within the tidal creeks of Monie Bay (Cornwell et al. 1994; Jones et al. 1997) to define a range of comparisons (Fig. 2.2); including horizontal (i.e., among creek), longitudinal (i.e. within creek), and temporal (i.e., seasonal and event-based).

Horizontal Comparisons

Environmental conditions and biological parameters in three tidal creek systems were compared using 2-year means (Table 2.1). Differences between creek systems were identified using ANOVA, results from which were used to classify the status of the three creeks (Fig. 2.3) and form the basis for subsequent comparisons. For example, a

comparison of LC and OB was used to identify the effect of the marsh alone, specifically the response of bacterioplankton to increases in substrate enrichment in the absence of an increase in nutrient concentrations. This comparison of LC and OB also provided a means by which comparisons of LMC could be normalized for the effect of the marsh. A comparison of LMC and LC, exhibiting significantly different nutrient concentrations yet similar salinities, serves as nutrient enrichment and reference, respectively. Comparisons between these two systems were used to identify the effect of system-level nutrient enrichment alone on estuarine bacterioplankton communities. Similarly, the two agriculturally impacted creeks (MC and LMC) – with similar ambient nutrient concentrations but differences in DOM source and freshwater inputs (Jones et al. 1997) – were used to investigate the role of substrate source, quality, and quantity in mediating the effect of nutrient enrichment on bacterioplankton.

Longitudinal Comparisons

Longitudinal (i.e., within system) comparisons were used to identify changes in environmental and biological parameters along the creek axis from enriched conditions in the upper marsh to unenriched conditions in the open bay. Changes in environmental conditions and the bacterioplankton community along this axis were identified by regressions of salinity versus environmental and microbial parameters of interest. This longitudinal approach allowed the comparison of disparate conditions encountered among the creeks in this system (e.g., high versus low nutrients), while also revealing the gradient between these extremes and tracking the corresponding shift in numerous environmental and biological parameters along the gradient.

Temporal Comparisons

Temporal variability of nutrient enrichment added a third dimension to the horizontal and longitudinal comparisons described above (Fig. 2.2), and can be in the form of pulsed enrichment events associated with the timing of agricultural nutrient applications within the watershed (Cornwell et al. 1994) or associated with predictable seasonal changes in environmental conditions (e.g., temperature, freshwater inputs, irradiance). Pulsed nutrient inputs were used to evaluate the effect of system-level nutrient enrichment on bacterioplankton by comparing pre- and post-enrichment conditions, and parsing the 2-year dataset by season identified general seasonal effects. The interaction between system-specific (i.e., among creek) patterns and season was identified using a full-factorial ANOVA with system (i.e., MC, LMC, LC, and OB), season (spring, summer, winter, fall), and their interaction (SYSTEM*SEASON) as model effects.

Sample Collection and Estimates of Bacterial Abundance and Production

We established 10 sites in the open bay and tidal tributaries of Monie Bay (Fig. 2. 1). Two sites were located in both OB and LC, and three in each of the two agriculturally developed creeks. Stations were located at intervals roughly proportional to the total creek length and were selected to capture existing gradients in nutrient concentrations and related water quality variables. In general, these stations coincide with those used in previous monitoring and research projects (Jones et al. 1997) (del Giorgio, University of Maryland Center for Environmental Science, personal communication). The 10 sites were visited monthly between April 2000 and February 2002. Approximately 20 L of sub-surface (<0.5 m) water were collected in Nalgene HDPE carboys (Nalge Nunc

International, Rochester, NY) immediately following high tide and transported back to the laboratory for filtration. Water temperature, salinity, Secchi depth, and water column depth were recorded at each site. Upon return to the lab, a small sub-sample was removed from each carboy for determining total bacterioplankton production and abundance, and concentrations of inorganic nutrients, and dissolved organic carbon (DOC). Bacterial production (BP) was estimated from the uptake of ^{3}H-leucine according to the centrifugation method of Smith and Azam (1992). Rates of leucine uptake were measured in all unfiltered water samples to gather an estimate of the total community production, which includes free-living and attached bacterioplankton. Estimates of filtered bacterial production were determined by gently passing several liters of sample water through an through an AP15 Millipore (Billerica, MA) filter (~1 μm) using a peristaltic pump, then incubating in the dark at *in situ* field temperature. There were three measurements of leucine uptake in the filtered fraction during the incubation, at 0, 3, and 6 h, and these individual measurements were averaged to obtain a mean rate of bacterial leucine uptake for the incubation period. Rates of leucine uptake were converted to rates of carbon production assuming a conversion factor of 3.1 Kg C mol leu^{-1} (Kirchman 1993). Bacterioplankton abundance (BA) was determined on live samples using standard flow-cytometric techniques and the nucleic acid stain SYTO-13 (del Giorgio et al. 1996a).

Nutrients and Other Analyses

Filtered samples for DOC analysis were acidified with 100 μl of 1N phosphoric acid and held at 4˚C until analysis. DOC content was determined with a Shimadzu (Shimdazu Corporation, Kyoto, Japan) high-temperature catalyst carbon analyzer (Sharp

et al. 1995). Samples for nutrient analyses were filtered through Whatman (Whatman Inc., Clifton, NJ) GF/F filter and frozen at -25˚C for later analysis of phosphate (i.e., PO_4^{3-}, soluble reactive phosphorus), nitrite (NO_2^-) and nitrate (NO_3^-) following Strickland and Parsons (1972), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) following Valderrama (1981), and ammonium (NH_4) following (Whitledge et al. 1981). Dissolved organic nitrogen (DON) was determined as the difference between TDN and dissolved inorganic nitrogen components. Absorbance of DOC was determined on GF/F filtered samples by performing absorbance scans (290-700 nanometers) using a Hitachi U-3110 spectrophotometer (Hitachi Corporation, Tokyo, Japan) and either 1- or 5-centimeter quartz cuvettes, depending upon the concentration of colored dissolved organic matter (CDOM). Absorptivity at 350 nm (a_{350}) was used as an index of CDOM concentrations (Moran et al. 2000; Blough and Del Vecchio 2001). Specific absorbance (a_{350}*) was determined by dividing a_{350} by ambient DOC concentrations. Chlorophyll *a* was determined with standard methods using a Turner 10-AU fluorometer (Turner Designs, Sunnyvale, CA) (Strickland and Parsons 1972).

Land Use and Watershed Designations

Land use within the watersheds of MC, LMC, and LC was classified as residential, agriculture, marsh, or forest (Anderson et al. 1976) using geo-referenced satellite imagery provided by the Maryland Department of Natural Resources. The watershed for each of the three tidal creeks was identified based on boundaries established by USGS for first and second order streams within the larger Monie drainage basin. The relative proportion of land use for each creek system was calculated using the

watershed designations identified above and land use polygon size relative to the entire watershed area (Lee et al. 2000).

Statistical Analyses

All statistical analyses, including standard least squares regressions, one and two-way analyses of variance (ANOVA), and principal component analysis (PCA) were performed using JMP 5.0.1 statistical software package (SAS Institute, Inc.). The entire composite dataset was used for all statistical analyses, except ANOVA's comparing system-specific means (Fig. 2.3, Table 2.3). In these instances, each system was characterized by values observed at the uppermost two sites in each creek (i.e., sites 3-4, 5-6, 8-9 in LC, LMC, and MC, respectively). Due to significant differences in rainfall and salinity among years, regression statistics for longitudinal comparisons with salinity as the independent variable (i.e., Table 2.2) were performed on data from year one only.

RESULTS

The three tidal creeks of Monie Bay differ with respect to agricultural land use, nutrient and DOC concentrations, salinity, and rates of bacterial production (Table 2.1). We observed significant differences in these measured parameters among creek systems and along longitudinal creek transects (Fig. 2.3). In addition, sampling over the 2-year duration of this study revealed considerable seasonal fluctuations in these parameters among creek systems. Our evaluation of this overlapping variability in Monie Bay and the corresponding horizontal, longitudinal, and seasonal comparisons (e.g., Fig. 2.2) are described below.

Horizontal Comparisons Among Creeks

In general, dissolved nutrient concentrations were higher in the two creeks with agriculturally developed watersheds and similar in LC and OB (Table 2.1). A statistical comparison of 2-year means (Fig. 2.3; ANOVA; Tukey-Kramer HSD; α=0.05) indicates that TDN, TDP, and DON were significantly higher in MC and LMC relative to both LC and OB. There were no statistical differences in dissolved ammonium and phosphate among the creek systems when 2-year means were considered, although concentrations in MC were significantly higher than those of OB. We observed considerable seasonal variability in nitrate, resulting in no significant differences among systems in this parameter (data not shown). In addition, dissolved nutrient stoichiometry (N:P; Table 2.1) in MC and LMC suggests that these systems are disproportionately enriched with phosphorus relative to nitrogen when compared to LC and OB. Mean salinity decreased with increasing watershed size, and there was a consistent hierarchy among the creeks (Table 2.1, Fig. 2.3; MC<LMC<LC<OB). Both colored and total dissolved organic carbon were inversely related to salinity, with highest values of DOC and CDOM in MC and lowest in OB and similar among-system hierarchy to that of salinity (i.e., MC>LMC>LC>OB). The pattern in CDOM mirrored that of salinity (Fig. 2.3), with significantly higher a_{350}* values in MC relative to all other systems and similar values when LMC and LC, as well as LC and OB, were compared.

Longitudinal Patterns Within Creeks

We explored changes in water column chemistry and biology along the estuarine axis using regressions of salinity versus other measured parameters (Table 2.2). All parameters decreased to varying degrees along the creek axis. There were significant

decreases in DOC, phosphate, TDN, TDP, and DON in both MC and LMC, and a significant decrease in total BP along the creek axis in LMC. The concurrent decrease of inorganic nutrient concentrations and BP in LMC is illustrated in Fig. 2.4. There were no significant changes in nutrients along the axis of Little Creek, although the trend of decreasing BP was significant (Table 2.3).

Seasonal and Temporal Patterns in Monie Bay

Dissolved nutrient concentrations were highly variable throughout the 2-year sampling period, with seasonal maxima of both TDP and TDN in April and July of 2000 and April and September of 2001 (Fig. 2.5). In April 2000, TDN peaked at 90 μM in both MC and LMC, and TDP was 2 and 4 μM in MC and LMC, respectively. A second peak occurred in July 2000, with TDN concentrations of 73 and 50 μM in MC and LMC and TDP concentrations of 3 and 1 μM, respectively. In 2001, April concentrations were 81 and 118 μM for TDN and 2 and 4 μM for TDP in MC and LMC, respectively. The summer peak in nutrients occurred later in 2001, with TDN concentrations of 42 and 62 μM and TDP concentrations of 1 and 6 μM in September in MC and LMC, respectively. TDN and TDP concentrations in LC and OB did not exhibit the same seasonal pattern of enrichment. Despite the considerable inter-annual variability, the pattern of nutrient concentrations among creeks persisted such that TDP and TDN were always higher in the agriculturally-impacted creeks relative to LC and OB for all dates sampled. Bacterial production was always highest in LMC, higher in all creeks than the open bay, and generally followed seasonal patterns in temperature (Fig. 2.5, lower panel).

We evaluated differences in 2-year means among seasons using ANOVA and Tukey-Kramer HSD (Fig. 2.6). Spring was characterized by lower salinity and higher

TDN and NO_x (NO_2^- + NO_3^-) concentrations. There was no significant difference in TDP or DON among seasons, and phosphate concentrations were similarly elevated in all seasons but winter. Salinity increased throughout the seasons, with lowest measurements in spring and highest measured in winter, and was generally mirrored by DOC and CDOM concentrations. Temperature and BP followed a similar seasonal pattern, with highest values in summer and lowest values in winter, and BA was significantly higher in the spring.

Given the overlapping spatial and seasonal variability in Monie Bay, we investigated the interaction of these factors by conducting a two-way ANOVA with system (creek) and season as model effects (Table 2.3). We observed significant interactions between season and system when salinity, DOC, salinity, NH_4^+, and NO_x were considered. These interactions correspond to disproportionately elevated DOC concentrations and reduced salinity in MC in the spring, lower NO_x concentrations in LC in the spring relative to other systems, and disproportionately elevated NH_4^+ concentrations in LMC in the spring.

Principal Components Analysis

Principal components analysis identified two composite variables (hereafter PC1 and PC2) that explained 75.4% of the variability within the composite dataset (n=160), with 47.6 and 27.8% attributed to PC1 and PC2, respectively. PC1 had high factor loadings (eigenvectors greater than 0.8) for DOC, TDN, TDP, and DON and was negatively correlated with salinity, whereas PC2 was strongly correlated with temperature. The distribution of sampling events from MC and LMC on PC1 and PC2 (Fig. 2.7, upper panel) identifies the similarities between these systems and indicates that

variability in these systems is dominated by freshwater delivery of dissolved nutrients and organic matter and that they experience similar nutrient loading dynamics. In contrast, the negative correlation of sampling events from LC and OB with PC1 (Fig. 2.7, lower panel) indicates higher salinities (i.e., reduced freshwater inputs) and minimal nutrient loading. Temperature and/or seasonal effects explain most of the variability in these systems, as evidenced by the distribution along PC2.

We explored seasonal patterns in water column chemistry using PCA and the same composite dataset, parsed by season in Fig. 2.8. Samples collected in spring were positively correlated with PC1 and negatively correlated with PC2, indicating elevated concentrations of dissolved nutrients and DOC, and lower temperatures during this season. Samples collected during summer and fall were positively correlated with PC2 (associated with higher water temperatures) and had a similar distribution along PC1. Samples collected in winter were negatively correlated with both PC1 and PC2.

Bacterial Production and Abundance

Two-year means of total bacterial production were highest in the agriculturally-developed creeks and lowest in LC and the open bay (Table 2.1; LMC>MC>LC>OB). Bacterial production in LMC (2.6 ± 0.2 μg C liter^{-1} hr^{-1}) was significantly higher than that of LC and OB (1.5 ± 0.1 and 1.1 ± 0.1 μg C liter^{-1} hr^{-1}, respectively). Although BP in MC (1.8 ± 0.2 μg C liter^{-1} hr^{-1}) was higher than that of LC, this difference was not significant (Fig. 2.3). BP in both MC and LMC was significantly higher than that of OB. Bacterial production in the filtered fraction was always lower than that of total bacterial production. The contribution of the filtered fraction to total production ranged from approximately 54% in MC, LMC, and OB to 67% in LC (Table 2.1). Bacterial

abundance was higher in LMC and LC and lower in MC and OB (Table 2.1), although these differences were not significant. Bacterial production decreased from the upper estuary to the open bay in all creeks, with the largest and most significant change in LMC (Fig. 2.4, Table 2.2). A similar but weaker trend was observed in the filtered fraction in both LMC and LC. There were no significant changes in BA along creek axes.

DISCUSSION

Monie Bay Research Reserve exhibits a diverse range of environmental conditions and watershed characteristics within one small estuarine system. Spatial and temporal patterns in nutrient concentrations and organic matter loading among the three tidal creeks and the open bay create conditions ideal for the use of this system as a natural experiment to investigate the effects of system-level nutrient enrichment. Our study in Monie Bay revealed consistent relationships between agricultural land-use, ambient nutrient concentrations, freshwater input, and rates of bacterial production. The bacterioplankton community responds positively to system-level nutrient enrichment, although this response appears to be mediated by nutrient and organic carbon delivery associated with patterns in freshwater input to these tidal creeks.

Patterns in Nutrient Enrichment

Nutrient enrichment in the creeks of Monie Bay is a function of both short- and long-term nutrient transport mechanisms, including baseline inputs of nitrogen from groundwater and pulsed inputs of nitrogen and phosphorus associated with fertilizer application and rainfall events. These loadings generate distinct patterns in nutrient enrichment that are apparent throughout the year both within and among creek systems. We observed a persistent hierarchy of ambient nutrient concentrations among the creeks (MC>LMC>LC) during all months sampled as well as when 2-year means were considered. Despite significant differences in salinity (Fig. 2.3) and freshwater inputs (Jones et al. 1997), MC and LMC are remarkably similar with respect to the dynamics of nutrient delivery (Figs. 2.5 & 2.7). Measured nutrient concentrations among the three creeks exhibited an identical pattern and similar magnitude to those observed by JONES

et al. (1997), reaffirming the robust nature of spatial patterns of nutrient enrichment in Monie Bay Research Reserve.

Short time-scale inputs, such as those associated with fertilizer application within the watersheds, have an episodic effect on the enrichment of the system – a phenomenon that has been well documented in other agriculturally developed watersheds of this region (Lowrance et al. 1997; Staver and Brinsfield 2001). The timing of these periods of enrichment (Fig. 2.5) coincide with fertilizer application schedules in the Monie Bay watershed, where chicken manure and/or liquid urea are applied in late March to early April, followed by the application of liquid urea in June (Williams, Sommerset County Agricultural Extension, personal communication), suggesting that the periodic acute enrichment of this system is driven by fertilizer application to fields within the drainage basins and the subsequent transport of water and associated nutrients into the tidal creeks during rain events (Norton and Fisher 2000; Speiran et al. 1998). CORNWELL *et al.* (1994) and JONES *et al.* (1997) observed a similar timing of maxima in nutrient concentrations and also attributed these to agricultural nutrient loading associated with fertilizer and manure applications. The negative loading of salinity and positive loading of dissolved nutrients on PC1 indicates that freshwater inputs drive most of the nutrient delivery to these systems. Concurrent enrichment of the two agriculturally-developed creeks (MC and LMC) with both TDN and TDP (Fig. 2.5) implicates overland flow from rainfall events as a loading mechanism, as it is well documented that phosphorus is transported with sediment via storm flow and/or erosional events (Norton and Fisher 2000). In addition, subsurface transport may also be responsible for less episodic inputs of phosphorus to these systems. Sims *et al.*(1998) observed environmentally significant

inputs of phosphorus via subsurface flow in system where excessive use of organic wastes increased soil phosphorus concentrations well above crop requirements. Long-term application of phosphorus-rich chicken manure to fields within MC and LMC may have resulted in concentrations approaching the sediment adsorption maxima (Sims and Wolf 1994), a consequence of which is an increase in the equilibrium concentration in subsurface waters and leaching of phosphorus into adjacent aquatic systems (Sims et al. 1998; Sims and Wolf 1994).

The importance of freshwater inputs in driving nutrient delivery in this system implies that there will also be distinct patterns in nutrient enrichment among seasons. We observed significantly lower salinity and significantly higher concentrations of TDN and NO_x in the spring. The lack of a similar pattern in TDP and PO_4^{3-} suggests that the delivery of nitrogen at this time was driven by a general increase in freshwater input and not necessarily by overland flow related to episodic storm events. Freshwater delivery to the creeks of Monie Bay is lowest in winter, as evidenced by elevated salinity and reduced PO_4^{3-}, NO_x, TDN, DOC, and CDOM at this time. For the most part, these seasonal effects are independent of the spatial patterns observed among the creeks, although there were certain conditions under which there was significant interaction of these effects (Table 2.3). We observed the strongest interaction in the spring, with disproportionately low salinities and elevated DOC in MC and disproportionately elevated ammonium in LMC. The pattern in MC can probably be attributed to a larger drainage basin more effectively delivering spring rainfall and stored organic matter from macrophyte senescence the previous fall. All systems except LC were disproportionately loaded with NO_x in the spring, suggesting that temporally based (i.e., not system based)

comparisons are more appropriate for identifying the effects of nitrate in Monie Bay, and that LC is indeed more pristine with respect to the impact of agricultural nutrients.

The transition from elevated nutrient concentrations in the upper estuary, where agricultural development is greatest (Fig. 2.1), to lower concentrations near the bay (Table 2.2, Fig. 2.4) was corroborated by other investigators (Cornwell et al. 1994; Jones et al. 1997). JONES *et al.* (1997) specifically report a doubling of nitrogen and phosphorus concentrations along a transect from the open bay to headwaters of LMC. These patterns observed in multiple studies clearly suggest that nutrients from agricultural land use enter each creek upstream and are measurably diluted or consumed as they pass downstream into the marsh and are subjected to tidal mixing.

In addition to patterns of acute nutrient enrichment associated with agricultural practices, we observed significant and persistent differences in nutrient concentrations among the creeks during months of little or no fertilizer application (Fig. 2.5). This indicates that acute periodic inputs augment a more chronic, background level of inputs from contaminated groundwater and surficial aquifers that have been infiltrated by agriculturally derived nutrients (Speiran et al. 1998; Weil et al. 1990). This nutrient delivery provides a relatively constant, low-level input via base flow that reflects long-term (i.e., 5-20 y) agricultural land use in the watershed (Speiran et al. 1998; Weil et al. 1990). As a result, despite extensive variability in nutrient concentrations throughout the year, monthly and annual nutrient concentrations in the impacted creeks are always significantly higher than those of the reference creek (Figs. 2.3 & 2.5). Additional evidence of the long-term effects of agricultural practices on the tidal creeks of this system is revealed by dissolved nutrient stoichiometry. JONES *et al.* (1997) report

extremely low N:P ratios in LMC, attributing this to the high phosphorus content of chicken manure (Sims and Wolf 1994) produced and applied in the LMC watershed. Despite the small areal coverage of poultry farms located in the LMC drainage basin (i.e., 0.9% of the entire Monie Bay watershed), these facilities account for the majority of nitrogen and phosphorus inputs to the Monie Bay system (81 and 68%, respectively; JONES *et al.*, 1997). It is clear that this a persistent if not long term effect, as we observed the same pattern in N:P ratios among the tidal creeks (Table 2.1; lowest in LMC) almost a decade after the original 1994 field work of JONES *et al.* (1997).

Response to System-Level Enrichment

Nutrient enrichment of the tidal creeks in Monie Bay has a pronounced effect on the productivity and functioning of these systems, driving patterns marsh macrophyte productivity and biomass (Jones et al. 1997), as well as sediment biogeochemistry and nutrient cycling (Cornwell et al. 1994; Stribling and Cornwell 2001). Our study revealed that bacterioplankton also respond to system-level nutrient enrichment, although this response differs among the creek systems and appears to be modulated by the interaction of various environmental factors. Elevated BP and DOC in LC relative to OB suggest that bacterioplankton respond positively to marsh-derived increases in organic matter supply. We observed a similar pattern in LMC, where inputs of agriculturally-derived nutrients combine with marsh-derived organic matter to produce the highest rates of BP recorded among the tidal creeks of Monie Bay. Despite comparable nutrient and organic matter enrichment in MC relative to LMC, we did not observe elevated rates of BP in this system, suggesting that additional factors mediate the response of bacterioplankton to

system-level enrichment. We predict that the muted response to enrichment observed in MC is driven by allochthonous inputs of lower-quality, terrestrially-derived DOM.

Effect of the Marsh

Higher bacterial abundance and production in LC relative to the open bay (Table 2.1) suggests that the marsh environment itself has a positive effect on the bacterioplankton community. Similar trends of increased production and abundance have been observed in other temperate estuaries (Goosen et al. 1997; Hoch and Kirchman 1993; Revilla et al. 2000) and tidal creeks of the Chesapeake Bay (Shiah and Ducklow 1995) and have generally been attributed to inputs of labile marsh detritus (Bano et al. 1997; Reitner et al. 1999). Our observation of higher rates of BP and DOC concentrations in LC relative to OB (Table 2.1), consistently higher BP in LC versus OB at all sampling events (Fig. 2.5), and a significant increase in both DOC and BP along the axis of LC (Table 2.2), corroborates these studies and further suggests that there is a positive effect of marsh detritus on BP. Similar nutrient concentrations between LC and OB (Table 2.1, Fig. 2.3) indicates that these increases in BP are driven by changes in the quality and quantity of DOM and POM substrates associated with natural marsh processes (Goosen et al. 1997; Shiah and Ducklow 1995), rather than an effect of nutrients alone. Thus, elevated rates of BP observed in agriculturally-impacted marsh systems are most likely driven by a combination of the direct effect of anthropogenic nutrient enrichment (Revilla et al. 2000) and the positive effect of natural marsh processes, although the effect of the marsh is probably minimal relative to that of system-level nutrient enrichment (Scudlark and Church 1989).

Effect of Enrichment: Little Monie Creek vs. Little Creek

Our comparison of LMC and LC was used to isolate the effect of system-level nutrient enrichment on bacterioplankton, an approach that relies on these systems being comparable in all aspects other than agricultural nutrient loading. As part of their 2-year study of these creeks, JONES *et al.* (1997) concluded that the overall similarity in physical parameters – coupled with differences in watershed practices – makes these creeks directly comparable and provides an excellent study area to assess the impact and ultimate fate of agricultural nutrients in brackish marsh systems. Given that LMC and LC are adjacent watersheds (Fig. 2.1), it is unlikely that large spatial-scale processes (i.e., climate, precipitation, atmospheric deposition of nutrients) will contribute to differences between these systems, and we predict that differences in nutrient concentrations between these systems are predominantly a function of agricultural land use, extent of marsh acreage, and/or watershed size and hydrology (Norton and Fisher 2000).

Watershed size does not appear to have an effect. Although the watershed of LMC is only twice the size of that of LC (Table 2.1), LMC has higher TDP and PO_4^{3-} during all months sampled by a factor of 4.5 and 6.5, respectively (data not shown). JONES *et al.* (1997) report similar findings, with phosphorus concentrations in LMC being four-fold higher than those of LC. Thus, based on watershed size, LMC is disproportionately enriched with phosphorus relative to LC.

With respect to dissolved nitrogen, JONES *et al.* (1997) report and we observed concentrations two- to three-fold higher in LMC than LC. This difference suggests that LMC is not as enriched with nitrogen as with phosphorus, although it is more likely that TDN concentrations in LC are influenced by the inputs of nitrogen-enriched groundwater

(Speiran et al. 1998; Weil et al. 1990) or the influx of nitrogen laden waters from the open bay during periods of nitrogen loading to the entire system. For example, when nutrient rich water from MC and LMC is transported to the open bay during ebb tide, it is then introduced to LC via tidal interactions. This phenomenon can be observed in the concurrent peaks of TDN in all three tidal creeks (Fig. 2.5). In their evaluation of nutrient concentrations over the course of a tidal cycle, JONES *et al.* (1997) found the highest nutrient concentrations in LC at high tide, further suggesting delivery of dissolved nitrogen from the open bay. Such enrichment of LC may lead to a smaller apparent difference between annual TDN concentrations observed in LMC and LC, inaccurately suggesting that LMC may not be disproportionately enriched with nutrients. Phosphorus does not experience the same effect as nitrogen, as it is transported in the particulate phase during storm and runoff events (Norton and Fisher 2000).

Given the proportion and extent of marsh acreage in the LC watershed relative to that of LMC (Fig. 2.1; 63 vs. 30%, respectively), it is also possible that natural marsh processes may contribute to differences in nutrients between these systems. CORNWELL *et al.* (1994) and STRIBLING and CORNWELL (2001) report a significant effect of the marsh on nutrient budgets in Monie Bay. The authors observe a decrease in nutrient concentrations over the course of the growing season, attributing the decrease to consumption of nitrogen and phosphorus by marsh macrophytes and loss of nitrogen via sediment denitrification. These studies also report a significant contribution of the marshes to water-column NH_4^+ via sediment ammonification. If the extensive marsh acreage in LC is a significant sink for water-column nitrogen and phosphorus – and thus contributes to differences in nutrient concentrations between these two systems

– then it should also be a source of ammonium. However, comparisons of 2-year means (Table 2.1), creek transects (Table 2.2), and data from JONES *et al.* (1997) reveal no such enrichment of LC with ammonium, and we conclude that elevated nutrient concentrations in LMC are driven exclusively by agricultural inputs, with only negligible effects attributed to catchment size and extent of marsh coverage.

Biological Response to Enrichment

JONES *et al.* (1997) identified an effect of agricultural nutrient enrichment among the tidal creeks of Monie Bay, with elevated plant biomass, tissue nutrient concentrations, and water column chlorophyll *a* in LMC relative to that of LC. These changes in the macrophyte community were correlated with rainfall and associated runoff events, further indicating that nutrient delivery to this system is derived from agricultural practices. The positive effect of enrichment on marsh macrophytes and phytoplankton was reflected in the positive relationship between BP and system-level enrichment in LMC, indicating that elevated productivity in LMC is a robust and consistent pattern that can be observed on many levels of biological organization. Despite considerable inter-annual variability in BP and nutrient concentrations, we observed consistently higher rates of bacterial production in LMC relative to LC throughout the year (Fig. 2.5), when 2-year means from these systems were compared (Fig. 2.3), and when the nutrient-enriched upper estuary of LMC was compared to sites nearer the open bay (Fig. 2.4).

In addition, although we did not observe a significant difference in bacterioplankton abundance between these two creeks (Table 2.1), cell-specific production (i.e., bacterial production per individual cell) in LMC was significantly higher than that of all other systems (Table 2.1, Fig. 2.3; Tukey-Kramer HSD; α=0.05;

$p<0.0001$; $n=137$). We predict that the observed increases in cell-specific production in LMC were associated with nutrient-driven increases in the growth and metabolism of individual cells within the assemblage, such that small, dormant, or slow-growing cells became more active and larger in direct response to enriched conditions (Choi et al. 1999; del Giorgio and Scarborough 1995). A comparison of LC and LMC revealed that total BP in LC was dominated by the filtered fraction (< 1 μm) relative to LMC (67 vs. 54%, respectively). This difference in filtered versus total BP indicates a decrease in the relative abundance of small, free-living cells in LMC and suggests a shift of bacterioplankton to a particle-attached state associated with elevated POM in this system (Jones et al. 1997) or an increase in the abundance of larger, more rapidly growing cells that are then retained in the AP15 filter (Gasol and del Giorgio 2000). Thus, the change in total bacterial production observed in LMC not only represents a general increase in bacterioplankton metabolism, but also a shift of production from smaller, free-living cells to that of particle-associated and/or larger, rapidly growing free-living bacteria. The shift of bacterioplankton production to the attached fraction under enriched conditions may represent an important emergent property in estuarine systems (Crump and Baross 2000; Crump et al. 1998) that has far reaching implications with respect to our ability to accurately assess carbon flux in natural aquatic systems (Biddanda et al. 2001; Cotner and Biddanda 2002).

Effect of Freshwater Inputs: Monie Creek vs. Little Monie Creek

Despite consistently elevated nutrient concentrations in MC, bacterial production in this system was consistently lower than that of LMC and only marginally higher than LC when 2-year means and individual sampling events were considered (Figs. 2.3 and

2.5, respectively). Small-scale incubation experiments conducted in the fall of 2000 revealed a similar phenomenon (data not shown), namely the lack of a productive response to inorganic nutrient enrichments by bacterioplankton from MC. Relatively low rates of BP in MC suggest that there are systematic differences in environmental conditions between MC and LMC that mediate the effect of nutrient enrichment on bacterioplankton metabolism. We hypothesize that low quality terrestrial DOM – as evidenced by elevated CDOM (Table 2.1) and $\delta^{13}C$ signatures of terrestrial C3 plants (Stribling and Cornwell 1997) – drives the muted response to nutrients observed in MC, although the direct effect of salinity on bacterioplankton community metabolism and phylogeny may also be important.

Elevated DOC concentrations in MC relative to other creeks (Table 2.1, Fig. 2.3) would ostensibly suggest that bacterioplankton production should not be carbon limited in this system (Baines and Pace 1991; Vallino et al. 1996) and therefore bacterioplankton should be free to respond productively to increases in ambient nutrient concentrations. Significant inputs of fresh water to this system (Jones et al. 1997) are accompanied by an increase in the input of terrestrially-derived organic matter, as evidenced by measurements of CDOM (Table 2.1, Fig. 2.3) and stable isotope analysis (Stribling and Cornwell 1997). Although the watersheds of MC and LMC are similar with respect to the percent of forested land (Fig. 2.1), MC is characterized by more extensive forested uplands. Organic matter from terrestrial sources typically has elevated concentrations of high-molecular weight DOM (Mcknight et al. 2001) that tends to be more refractory (Sun et al. 1997) and therefore yields lower growth efficiencies (Goldman et al. 1987) and lower rates of bacterial production (Amon and Benner 1996; Moran and Hodson 1990).

It is therefore likely that BP itself is functionally carbon limited, driven by lower growth efficiencies and the dominance of low-quality, terrestrially derived substrates in this system.

Bacterial growth efficiency (BGE) is highly variable among aquatic systems (del Giorgio and Cole 1998; del Giorgio and Cole 2000) and on small spatial scales within estuarine systems (del Giorgio and Bouvier 2002). Based on the lack of coherence between BP and bacterial respiration (BR) associated with highly variable BGE, BP alone is a poor predictor of total carbon flux, and lower bacterial production in MC does not necessarily translate into a similar reduction in total carbon consumption. In fact, it is likely that bacterial respiration in MC is high relative to BP, driven by the increased metabolic demands of processing and incorporating refractory organic matter into bacterial biomass (Linton and Stevenson 1978) or the direct effect of changes in dissolved nutrient stoichiometry on BR (Cimbleris and Kalff 1998). In addition, shifts in ambient salinities that occur during tidal mixing when low salinity headwaters meet high salinity water from the bay may stress estuarine bacterioplankton communities, causing mortality and inhibiting growth (del Giorgio and Bouvier 2002). Over a distance of less than 4 km, bacterioplankton communities in MC may be exposed to a salinity range from <1 ppt in the upper estuary to >13 ppt in the open bay (data not shown), a much larger range than that of LMC and potentially generating a gradient adequate for disrupting bacterioplankton community metabolism (del Giorgio and Bouvier 2002), thereby producing lower growth efficiencies and lower rates of production. Because the rate at which organic matter is regenerated into dissolved nutrients or is available for consumption by higher trophic levels is a direct function of BGE (Jorgensen et al. 1999;

Kirchman 2000a; Sherr and Sherr 1988), it is impossible to accurately predict microbially-mediated changes in carbon flux and nutrient cycling in aquatic systems without independent assessments of both bacterial production and respiration.

The effect of substrate quality on BP in freshwater-dominated MC may be accompanied by the direct effect of salinity itself on the phylogenetic composition of bacterioplankton communities. Recent studies have identified dramatic shifts in phylogenetic composition of natural bacterial assemblages along salinity gradients, with the dominance of specific phylogenetic groups associated with certain salinity regimes (Crump et al. 1999; del Giorgio and Bouvier 2002). In turn, phylogenetic composition of natural bacterial assemblages has been linked to bacterioplankton metabolic properties (Bouvier and del Giorgio 2002; Pinhassi et al. 1999) and even the utilization of specific organic substrates (Cottrell and Kirchman 2000). Thus, we predict that differences in the metabolic response of bacterioplankton to nutrient enrichment of MC versus LMC may be driven by differences in organic matter quality, as well as changes in the phylogenetic composition of resident bacterioplankton assemblages and the unique metabolic capacities associated with these phylotypes.

Response to Pulsed Nutrient Inputs

We investigated the response of bacterioplankton to temporal changes in nutrient enrichment by comparing estimates of BP during and following periods of nutrient input from this watershed. LMC was selected for these comparisons because this system has demonstrated a strong response to nutrient enrichment, as evidenced by field observations (Tables 2 and 3, Figs. 2.3, 2.4 & 2.5), manipulative nutrient enrichment experiments (unpublished data), estimates of chlorophyll a concentrations, and changes in the marsh

macrophyte community associated with episodic nutrient delivery (Jones et al. 1997). Pulsed nutrient inputs in July 2000 resulted in elevated nutrient concentrations (50 and 1 μM for TDN and TDP, respectively) relative to those in September (Fig. 2.5). Similarly, BP was significantly higher in July relative to September (6.8 vs. 2.6 μg C liter^{-1} hr^{-1}, respectively). The same comparison of BP in MC during these summer months revealed a muted response of bacterioplankton to nutrient enrichment that has become characteristic of this particular creek system (Table 2.1, Fig. 2.3). Despite higher nutrient concentrations in July than in September for MC (73 vs. 41 μM and 3.0 vs. 0.6 μM for TDN and TDP, respectively) there was not a significant difference in BP (Fig. 2.5; 2.2 vs. 2.0 μM). Intermediate concentrations of TDN and TDP (55 and 1.7 μM, respectively) in August may have stimulated the marginally higher BP at this time (3.1 μg C liter^{-1} hr^{-1}), although this was probably an effect of higher temperature (28.0ºC) on BP (Shiah and Ducklow 1994a). As a result of sampling difficulties, estimates of BP in LMC during August were not available.

During April 2000, the bacterioplankton community in LMC was exposed to elevated TDN and TDP (Fig. 2.5; 90 and 4 μM, respectively), delivered as a result of spring fertilizer applications and runoff events. The following month, nutrient concentrations in LMC were much lower and well below the overall mean for this system (Table 2.1). Although a comparison of BP between April and May (2.2 and 4.3 μg C liter^{-1} hr^{-1}, respectively) does not initially indicate a positive response to nutrients, SHIAH and DUCKLOW (1994a) conducted a series of studies in marshes similar to those of Monie Bay and found that BP is regulated predominantly by temperature in small estuarine systems during non-summer months. The authors report an average Q_{10}

value of 2.7 (± 0.3) for bacterial growth in the temperature range of 3 to 25°C. We hypothesized that the bacterioplankton community in April may have been constrained by temperature and therefore less responsive to system-level nutrient enrichment. This is further supported by the general coherence of temperature and BP when monthly sampling (Fig. 2.5) and comparison of seasonal means (Fig. 2.6) are considered. Using the reported Q_{10} value of SHIAH and DUCKLOW (1994a) and a difference in ambient water temperature for April and May of 10°C (Fig. 2.5; 13 vs. 23°C, respectively), we calculated temperature corrected estimates of BP for these two months and estimated that BP in April would have been elevated relative to that of May (6.0 vs. 4.3 μg C liter^{-1} hr^{-1}, respectively). Based on the comparisons of BP during summer months and temperature-corrected estimates of BP in April and May, we conclude that although bacterioplankton respond positively to increases in ambient nutrient concentrations, temperature is an important environmental factor mediating the magnitude of this response and should be considered in seasonal comparisons of BP in temperate systems.

Concluding Remarks

We observed a persistent response of bacterioplankton to agriculturally-driven enrichment of the tidal creeks, a conclusion that corresponds with generally held paradigms regarding the effect of nutrients on natural heterotrophic bacterioplankton communities (Kirchman 2000a). However, not all systems responded to nutrient enrichment in a similar manner, and freshwater inputs and/or salinity plays an important role in mediating the effect of nutrients on estuarine bacterioplankton communities. We attribute the muted response to enrichment observed at lower salinities to an abundance of refractory, terrestrially-derived organic matter and/or the direct effect of salinity on

bacterioplankton, which in turn drive changes in substrate quality, assemblage phylogenetic composition, and ultimately bacterioplankton community metabolic processes.

The metabolic response of bacterioplankton to nutrient enrichment is extremely complex, occurring at both the cellular (Choi et al. 1999; del Giorgio and Scarborough 1995) and community (Pace and Cole 2000; Vrede et al. 1999) levels. As a result, we recognize that estimates of BP and BA alone cannot accurately capture subtle changes in bacterioplankton metabolism and together are inadequate to unequivocally identify mechanisms underlying the disparate response of MC and LMC to nutrient enrichment. When coupled with BP and BA, estimates of single-cell activity such as DNA content (Gasol and del Giorgio 2000; Lebaron et al. 2001a) or the abundance of actively respiring cells (Rodriguez et al. 1992) may provide a more sensitive index of bacterioplankton metabolism. Similarly, combining estimates of BP and BR not only yields an estimate of bacterial growth efficiency, but also a measure of the total carbon consumed by the bacterioplankton community. A comprehensive suite of cellular and community-level indices of bacterioplankton metabolism is essential for accurate assessment of microbially-mediated carbon and nutrient cycling in aquatic systems and the investigation of these parameters in Monie Bay may ultimately lead to important insight regarding mechanisms underlying the response of bacterioplankton to system-level nutrient enrichment of estuarine systems.

The effect of system-level enrichment on estuarine systems is reflected in numerous aspects of marsh ecology (Cloern 2001), including phytoplankton abundance (Anderson and Taylor 2001), macrophyte community diversity and production (Day et al.

1989; Valiela 1995), and sediment biogeochemical processes (Cornwell et al. 1996; Dauer et al. 2000). The metabolic response of bacterioplankton to system-level nutrient enrichment is not as well documented (Hoppe et al. 1998; Lebaron et al. 1999), although it may represent a more sensitive and integrative assessment than other traditional indices of ecosystem function and eutrophication. The bacterioplankton community responds rapidly (i.e., hours to days) to changes in environmental conditions and is well suited for investigations of tidally-influenced systems where episodic and pulsed nutrient inputs are common. However, the persistence of system-specific patterns in bacterial production among the creeks of Monie Bay over our 2-year study period also suggests an integration of conditions over much longer time periods. Combining multiple aspects of bacterioplankton metabolism in investigations of estuarine systems may provide an extremely comprehensive and multi-faceted index of ecosystem function that reflects changes in system-level processes on multiple spatial and temporal scales.

LITERATURE CITED

Amon, R., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography **41:** 41-51.

Anderson, J., E. Hardy, J. Roach, and R. Witmer. 1976. A Land Use and Land Cover Classification System For Use With Remote Sensor Data. United States Department of the Interior.

Anderson, T. H., and G. T. Taylor. 2001. Nutrient pulses, plankton blooms, and seasonal hypoxia in western Long Island Sound. Estuaries **24:** 228-243.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and T. F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series **10:** 257-263.

Baines, S. B., and M. L. Pace. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnology and Oceanography **36:** 1078-1090.

Bano, N. and others 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. Marine Ecology Progress Series **157:** 1-12.

Beaulac, M. N., and K. H. Reckhow. 1982. An examination of land use - nutrient export relationships. Water Resources Bulletin **18:** 1013-1024.

Biddanda, B., M. Ogdahl, and J. B. Cotner. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnology and Oceanography **46:** 730-738.

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography **47:** 453-470.

Brussaard, C. P. D., and R. Riegman. 1998. Influence of bacteria on phytoplankton cell mortality with phosphorus or nitrogen as the algal-growth-limiting nutrient. Aquatic Microbial Ecology **14:** 271-280.

Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquatic Microbial Ecology **10:** 69-85.

Caron, D. A., E. L. Lim, R. W. Sanders, M. R. Dennett, and U. G. Berninger. 2000. Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. Aquatic Microbial Ecology **22:** 175-184.

Choi, J., E. B. Sherr, and B. F. Sherr. 1999. Dead or alive? A large fraction of ETS-inactive marine bacterioplankton cells, as assessed by reduction of CTC, can become ETS-active with incubation and substrate addition. Aquatic Microbial Ecology **18:** 105-115.

Cimbleris, A. C., and J. Kalff. 1998. Planktonic bacterial respiration as a function of C:N:P ratios across temperate lakes. Hydrobiologia **384:** 89-100.

Cloern, J. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series **210:** 223-253.

Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross system overview. Marine Ecology Progress Series **43:** 1-10.

Cornwell, J. C., D. J. Conley, M. Owens, and J. C. Stevenson. 1996. A sediment chronology of the eutrophication of Chesapeake Bay. Estuaries **19:** 488-499.

Cornwell, J. C., J. M. Stribling, and J. C. Stevenson. 1994. Biogeochemical studies at the Monie Bay National Estuarine Research Reserve, p. 645-655. *In* M. Lynch and B. Crowder [eds.], Organizing for the Coast: Thirteenth International Conference of the Coastal Society.

Cotner, J. B., and B. Biddanda. 2002. Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems. Ecosystems **5:** 105-121.

Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine proteobacteria and members of Cytophaga-Flavobacter cluster consuming low-and high-molecular weight dissolved organic matter. Applied and Environmental Microbiology **66:** 1692-1697.

Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Applied and Environmental Microbiology **65:** 3192-3204.

Crump, B. C., and J. A. Baross. 2000. Characterization of the bacterially-active particle fraction in the Columbia River estuary. Marine Ecology Progress Series **206:** 13-22.

Crump, B. C., J. A. Baross, and C. A. Simenstad. 1998. Dominance of particle-attached bacteria in the Columbia River estuary, USA. Aquatic Microbial Ecology **14:** 7-18.

Dauer, D. M., S. B. Weisberg, and J. A. Ranasinghe. 2000. Relationships Between Benthic Community Condition, Water Quality, Sediment Quality, Nutrient Loads, and Land Use Patterns in Chesapeake Bay. Estuaries **23:** 80-96.

Day, J. W. J., C. A. S. Hall, W. M. Kemp, and A. Yanez-Arancibia [eds.]. 1989. Estuarine Ecology. John Wiley and Sons.

del Giorgio, P. A., D. F. Bird, Y. T. Prairie, and D. Planas. 1996a. Flow cytometric determination of bacterial abundance in lake plankton using the green nucleic acid stain SYTO 13. Limnology and Oceanography **41:** 783-789.

del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnology and Oceanography **47:** 471-486.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29:** 503-541.

---. 2000. Bacterial growth energetics and efficiency in natural aquatic systems, p. 289-325. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

del Giorgio, P. A., J. M. Gasol, D. Vaque, P. Mora, S. Agusti, and C. M. Duarte. 1996b. Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. Limnology and Oceanography **41:** 1169-1179.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. Journal of Plankton Research **17:** 1905-1924.

Ducklow, H. W., D. A. Purdie, P. J. L. B. Williams, and J. M. Davies. 1986. Bacterioplankton: A sink for carbon in a coastal marine plankton community. Science **232:** 863-867.

Fielding, K. P. 2002. Differential substrate limitation in small tributaries of Chesapeake Bay, Maryland influenced by non-point source nutrient loading. Masters. University of Maryland.

Finlay, B. J., S. C. Maberly, and J. I. Cooper. 1997. Microbial diversity and ecosystem function. Oikos **80:** 209-213.

Fisher, T. R. 1985. Nitrogen and phosphorus inputs to Chesapeake Bay, p. 58-62. The Chesapeake Bay Symposium. National Marine Educators Conference.

Gasol, J. M., and P. A. del Giorgio. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina **64:** 197-224.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by C:N ratio. Limnology and Oceanography **32:** 1239-1252.

Gonzalez, J. M., E. B. Sherr, and B. F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and Environmental Microbiology **56:** 583-589.

Goosen, N. K., P. Van Rijswijk, J. Kromkamp, and J. Peene. 1997. Regulation of annual variation in heterotrophic bacterial production in the Schelde Estuary (SW Netherlands). Aquatic Microbial Ecology **12:** 223-232.

Hoch, M. P., and D. L. Kirchman. 1993. Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Marine Ecology Progress Series **98:** 283-295.

Hoppe, H. G., H. C. Giesenhagen, and K. Gocke. 1998. Changing patterns of bacterial substrate decomposition in a eutrophication gradient. Aquatic Microbial Ecology **15:** 1-13.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A Two-Year Study of the Short-Term and Long-Term Sequestering of Nitrogen and Phosphorus in the Maryland National Estuarine Research Reserve, p. 1-100. Maryland National Estuarine Research Reserve.

Jorgensen, N., N. Kroer, R. B. Coffin, and M. P. Hoch. 1999. Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. Aquatic Microbial Ecology **18:** 247-261.

Kearney, M. S., J. C. Stevenson, and L. G. Ward. 1994. Spatial and Temporal Changes in Marsh Vertical Accretion Rates at Monie Bay - Implications for Sea-Level Rise. Journal of Coastal Research **10:** 1010-1020.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria (Chapter 58), p. 776. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole [eds.], Handbook of Methods in Aquatic Microbial Ecology. CRC Press.

---. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. Microbial Ecology **28:** 255-271.

--- [ed.]. 2000. Microbial Ecology of the Oceans. Wiley-Liss.

Kolber, Z. S., R. Barber, K. Coale, S. Fitzwater, and R. Greene. 1994. Iron limitation of phytoplankton photosynthesis in the equatorial pacific ocean. Nature **371:** 145-148.

Lebaron, P., P. Servais, H. Agogué, C. Courties, and F. Joux. 2001a. Does the High Nucleic Acid Content of Individual Bacterial Cells Allow Us To Discriminate between Active Cells and Inactive Cells in Aquatic Systems? Applied and Environmental Microbiology **67:** 1775-1782.

Lebaron, P. and others 2001b. Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in abundances, activity and composition. FEMS Microbiology Ecology **34:** 255-266.

Lebaron, P., P. Servais, M. Troussellier, C. Courties, and J. Vives-Rego. 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquatic Microbial Ecology **19:** 225-267.

Lee, K. Y., T. R. Fisher, T. E. Jordan, D. L. Correl, and D. E. Weller. 2000. Modeling the hydrochemistry of the Choptank River basin using GWLF and Arc/Info: 1. Model calibration and validation. Biogeochemistry **49:** 143-173.

Linton, J., and R. Stevenson. 1978. A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substrates. FEMS Microbiology Letters **3:** 95-98.

Lowrance, R. and others 1997. Water quality functions of Riparian forest buffers in Chesapeake Bay watersheds. Environmental Management **21:** 687-712.

Mcknight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Anderson. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnology and Oceanography **46:** 38-48.

Moran, M. A., and R. E. Hodson. 1990. Bacterial production on humic and non-humic components of dissolved organic matter. Limnology and Oceanography **35:** 1744-1756.

Nixon, S. W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia **41:** 199-219.

Norton, M. M., and T. R. Fisher. 2000. The effects of forest on stream water quality in two coastal plain watersheds of the Chesapeake Bay. Ecological Engineering **14:** 337-362.

Pace, M. L., and J. J. Cole. 2000. Effects of whole-lake manipulations of nutrient loading and food web structure on planktonic respiration. Canadian Journal of Fisheries and Aquatic Sciences **57:** 487-496.

Painchaud, J., D. Lefaivre, J. C. Therriault, and L. Legendre. 1996. Bacterial dynamics in the upper St. Lawrence Estuary. Limnology and Oceanography **41:** 1610-1618.

Pinhassi, J. and others 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquatic Microbial Ecology **17:** 13-26.

Rasmussen, B., and A. Josefson. 2002. Consistent Estimates for the Residence Time of Micro-tidal Estuaries. Estuarine, Coastal and Shelf Science **54:** 65-73.

Reitner, B., A. Herzig, and G. Herndl. 1999. Dynamics in bacterioplankton production in a shallow, temperate lake (Lake Neusiedl, Austria): evidence for dependence on

macrophyte production rather than on phytoplankton. Aquatic Microbial Ecology **19:** 245-254.

Revilla, M., A. Iriarta, I. Madariaga, and E. Orive. 2000. Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuarine, Coastal and Shelf Science **50:** 297-313.

Rieman, B. and others 1990. Carbon budgets of the microbial food web in estuarine enclosures. Marine Ecology Progress Series **65:** 159-170.

Rodriguez, G. G., D. Phipps, K. Ishiguro, and H. F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Applied and Environmental Microbiology **58:** 1801-1808.

Scudlark, J. R., and T. M. Church. 1989. The Sedimentary Flux of Nutrients at a Delaware Salt-Marsh Site - a Geochemical Perspective. Biogeochemistry **7:** 55-75.

Sharp, J. and others 1995. Analyses of dissolved organic carbon in sea water: the JGOFS EqPac methods comparison. Marine Chemistry **48:** 91-108.

Sherr, E. B., and B. F. Sherr. 1988. Role of microbes in pelagic food webs: A revised concept. Limnology and Oceanography **33:** 1225-1227.

Shiah, F. K., and H. W. Ducklow. 1994. Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Marine Ecology Progress Series **103:** 297-308.

---. 1995. Multiscale variability in bacterioplankton abundance, production, and specific growth rate in a temperate salt-marsh tidal creek. Limnology and Oceanography **40:** 55-66.

Sims, J. T., R. R. Simard, and B. C. Joern. 1998. Phosphorus loss in agricultural drainage: historical perspective and current research. Journal of Environmental Quality **27:** 277-293.

Sims, J. T., and D. C. Wolf. 1994. Poultry waste management: agricultural and environmental issues. Advances in Agronomy **52:** 1-83.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using super(3)H-leucine. Marine Microbial Food Webs **6:** 107-114.

Smith, D. E., M. Leffler, and G. Mackiernan [eds.]. 1992. Oxygen dynamics in the Chesapeake Bay: A synthesis of recent research. Maryland Sea Grant.

Smith, E., and W. Kemp. 2001. Size structure and the production/respiration balance in a coastal plankton community. Limnology and Oceanography **46:** 473-485.

Speiran, G. K., P. A. Hamilton, and M. D. Woodside. 1998. Natural processes for managing nitrate in ground water discharged to the Chesapeake Bay and other surface waters: more than forest buffers, p. 6. USGS.

Staver, K. W., and R. B. Brinsfield. 2001. Agriculture and Water Quality on the Maryland Eastern Shore: Where Do We Go from Here? BioScience **51:** 859-868.

Stribling, J. M., and J. C. Cornwell. 1997. Identification of important primary producers in a Chesapeake Bay tidal creek system using stable isotopes of carbon and sulfur. Estuaries **20:** 77-85.

---. 2001. Nitrogen, phosphorus, and sulfur dynamics in a low salinity marsh system dominated by *Spartina alterniflora*. Wetlands **21:** 629-638.

Strickland, J. D., and T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Bulletin of the Fisheries Research Board of Canada **167:** 1-310.

Sun, L., E. Perdue, J. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnology and Oceanography **42:** 714-721.

Tuttle, J., R. Jonas, and T. Malone. 1987. Origin, development and significance of Chesapeake Bay anoxia, p. 442-472. *In* S. Majumdar, L. Hall, , Jr. and H. Austin [eds.], Conference 152. National Meeting AAAS: "Chesapeake Bay Fisheries and Contaminant Problems".

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry **10:** 109-122.

Valiela, I. 1995. Marine Ecological Processes. Springer-Verlag.

Vallino, J. J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. Limnology and Oceanography **41:** 1591-1609.

Vrede, K., T. Vrede, A. Tisaksson, and A. Karlsson. 1999. Effects of nutrients (P,N,C) and zooplankton on bacterioplankton and phytoplankton - a seasonal study. Limnology and Oceanography **44:** 1616-1624.

Ward, L. G., M. S. Kearney, and J. C. Stevenson. 1998. Variations in sedimentary environments and accretionary patterns in estuarine marshes undergoing rapid submergence, Chesapeake Bay. Marine Geology **151:** 111-134.

Weil, R. R., R. A. Weismiller, and R. S. Turner. 1990. Nitrate contamination of groundwater under irrigated coastal plain soils. Journal of Environmental Quality **19:** 441-448.

Whitledge, T. C., S. C. Mallory, C. J. Patton, and C. D. Wirick. 1981. Automated Nutrient Analysis in Seawater. Department of Energy and Environment.

Table 2.1. Nutrient concentrations, biological parameters, and watershed characteristics for the three tidal creeks and open bay. Table values are derived from 2-yr means ± SE (n).

	MC		LMC		LC		OB		*n*
Depth (m)	3.3 ± 0.2	(29)	3.1 ± 0.2	(25)	2.0 ± 0.1	(18)	2.2 ± 0.1	(14)	86
TDN (μM)	40.6 ± 2.1	(65)	40.1 ± 2.8	(58)	26.8 ± 1.6	(38)	28.1 ± 1.7	(40)	201
TDP (μM)	0.7 ± 0.08	(65)	0.8 ± 0.12	(58)	0.2 ± 0.03	(38)	0.3 ± 0.03	(40)	201
DON (μM)	36.7 ± 1.7	(65)	35.6 ± 1.9	(58)	21.6 ± 1.9	(38)	19.8 ± 1.8	(40)	201
NH_4^+ (μM)	2.3 ± 0.3	(65)	3.0 ± 0.4	(58)	2.1 ± 0.2	(38)	1.7 ± 0.2	(40)	201
NOx (μM)	4.5 ± 0.8	(65)	4.8 ± 1.1	(58)	3.1 ± 0.64	(38)	6.6 ± 1.3	(40)	201
PO_4^{3-} (μM)	0.2 ± 0.05	(65)	0.3 ± 0.07	(58)	0.2 ± 0.1	(37)	0.0 ± 0.006	(40)	200
Salinity	6.9 ± 0.4	(75)	9.9 ± 0.3	(62)	11.6 ± 0.3	(38)	12.1 ± 0.3	(42)	217
DOC (mg L^{-1})	11.5 ± 0.5	(71)	8.9 ± 0.4	(59)	7.7 ± 0.3	(36)	6.0 ± 0.2	(39)	205
DOC:TDN	24 ± 2	(62)	20 ± 1	(55)	27 ± 2	(36)	20 ± 1	(37)	190
DON:DOP	103 ± 22	(60)	84 ± 8	(55)	170 ± 27	(34)	130 ± 15	(35)	184
a350*[1]	20 ± 0.7	(43)	17 ± 0.7	(35)	15 ± 1	(19)	12 ± 0.9	(22)	119
BA (10^6 cells mL^{-1}	11.8 ± 0.7	(74)	13.3 ± 1.0	(62)	12.8 ± 1.4	(35)	11.5 ± 1.0	(42)	213
BP (μgC L^{-1} h^{-1})	1.8 ± 0.1	(59)	2.6 ± 0.2	(48)	1.5 ± 0.1	(29)	1.1 ± 0.1	(33)	169
Filtered BP (μgC L^{-1} h^{-1})[2]	1.0 ± 0.1	(59)	1.4 ± 0.1	(48)	1.0 ± 0.2	(29)	0.6 ± 0.1	(33)	169
% filtered BP[3]	55.6 %	(59)	53.8 %	(48)	66.7 %	(29)	54.5 %	(33)	169
Watershed $(km)^2$	45		17.9		9.4		72.3		
Agriculture[4]	23 %		25 %		<1 %		16 %		

[1]specific absorbance at 350nm x 10^3
[2]BP for the AP15 filtered fraction
[3]Percentage of total BP attributed to the AP15 filtered fraction
[4]Percentage of agricultural land use within each watershed. The open bay watershed is comprised of adjacent marshes and the watershed from each creek.
MC = Monie Creek, LMC = Little Monie Creek, LC = Little Creek, OB = open bay, TDN = total dissolved nitrogen, TDP = total dissolved phosphorus, DON = dissolved organic nitrogen, NOx = NO_3^- + NO_2^-, DOC = dissolved organic carbon, BA = total bacterial abundance, BP = total bacterioplankton production.

Table 2.2. Probability values from two-way ANOVAs with system and season as model effects

	Model Effects			
Parameter	System	Season	Interaction	*n*
Temperature	ns	<0.0001	ns	176
Total dissolved nitrogen	<0.0001	<0.0001	ns	162
Total dissolved phosphorus	<0.0001	ns	ns	162
Dissolved organic nitrogen	<0.0001	ns	ns	162
Ammonium	0.001	<0.0001	0.003	162
Nitrate & Nitrite	0.05	<0.0001	0.05	162
Phosphate	ns	ns	ns	160
Dissolved organic carbon	<0.0001	<0.0001	<0.0001	166
Salinity	<0.0001	<0.0001	0.02	176
Specific absorbance	<0.0001	0.001	ns	97
Total bacterial production	<0.0001	0.01	ns	137
Total bacterial abundance	ns	<0.0001	ns	172

ns = not significant ($p > 0.05$).

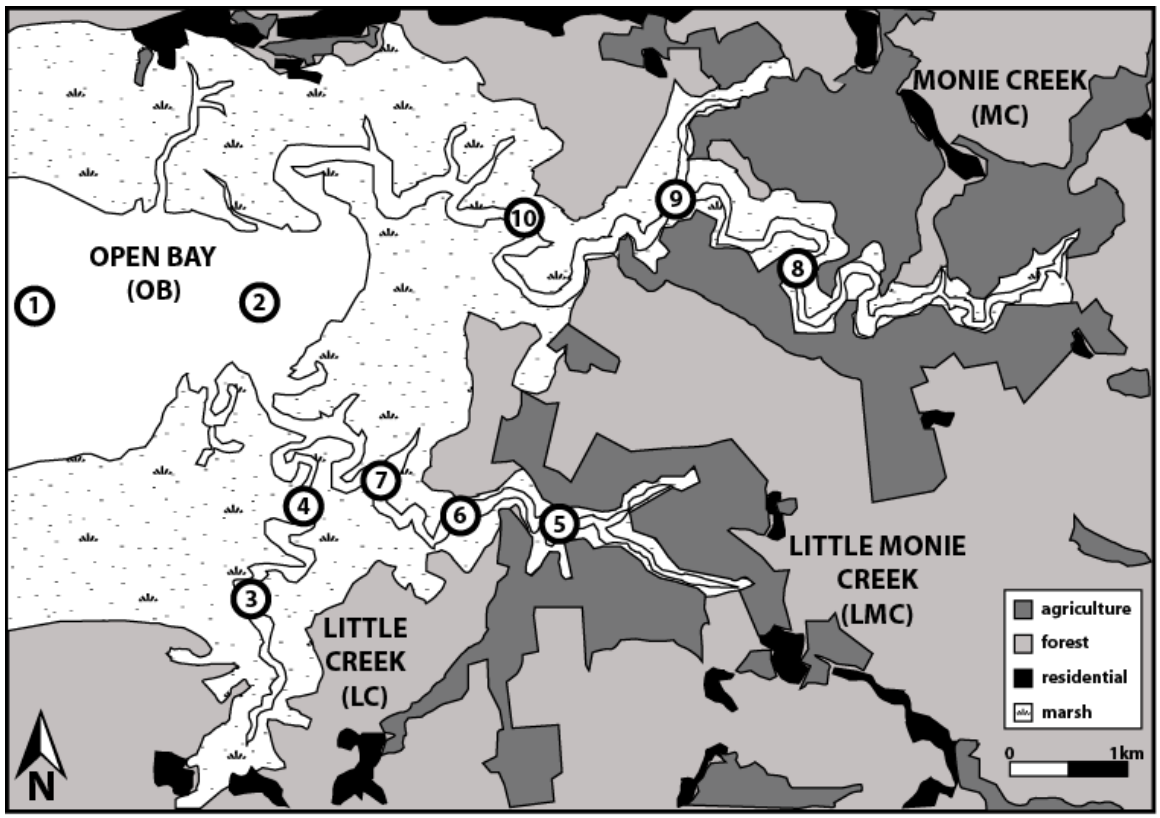
Table 2.3. Regression statistics for the relationship between salinity and physical and biological parameters for the uppermost two sites in each creek (year one only; n=12).

	Monie Creek				Little Monie Creek				Little Creek			
Parameter	slope	r^2	F ratio	p	slope	r^2	F ratio	p	slope	r^2	F ratio	p
Total dissolved nitrogen	-4.8	0.37	7.1	0.02	-5.1	0.52	10.9	0.008	-1.5	nr	0.33	ns
Total dissolved phosphorus	-0.2	0.45	9.9	0.009	-0.1	0.44	7.7	0.02	0.03	nr	0.83	ns
Dissolved organic nitrogen	-3.1	0.39	7.7	0.02	-3.6	0.58	13.9	0.004	-0.4	nr	0.05	ns
Ammonia	-0.3	0.12	1.7	ns	-0.4	0.16	1.8	0.2	-0.3	0.14	1.7	0.2
Nitrate and nitrite	-1.5	0.28	4.6	0.05	-1.1	0.22	2.9	0.12	-0.8	nr	0.45	ns
Phosphate	-0.1	0.50	12.0	0.005	-0.1	0.52	10.9	0.008	-0.01	0.26	3.1	0.1
Dissolved organic carbon	-1.5	0.56	15.1	0.002	-1.2	0.79	38.4	0.0001	-1.0	0.48	9.4	0.01
Specific absorbance (a350*)	-0.001	0.48	18.2	0.0004	-0.001	0.33	11.0	0.003	-0.0004	0.15	2.9	0.1
Total bacterial production	-0.2	0.16	3.6	0.075	-0.8	0.89	71.6	0.0001	-0.4	0.51	10.3	0.009
Filtered bacterial production	-0.1	nr	1.6	0.23	-0.6	0.80	32.1	0.0005	-0.3	0.34	5.1	0.05
Total bacterial abundance	-	nr	-	ns	-	nr	-	ns	-	nr	-	ns

nr = no relationship ($r^2 < 0.1$), ns = not significant ($p > 0.05$).

FIGURES

Fig. 2.1: Monie Bay NERR site with location and number of each sampling station (upper panel) and proportion of each watershed attributed to one of four land-use categories (lower panel).


MONIE CREEK
(MC)
OPEN BAY
(OB)
LITTLE MONIE
CREEK
(LMC)
LITTLE
CREEK
(LC)
1
2
3
4
5
6
7
8
9
10
N
agriculture
forest
residential
marsh
0
1km

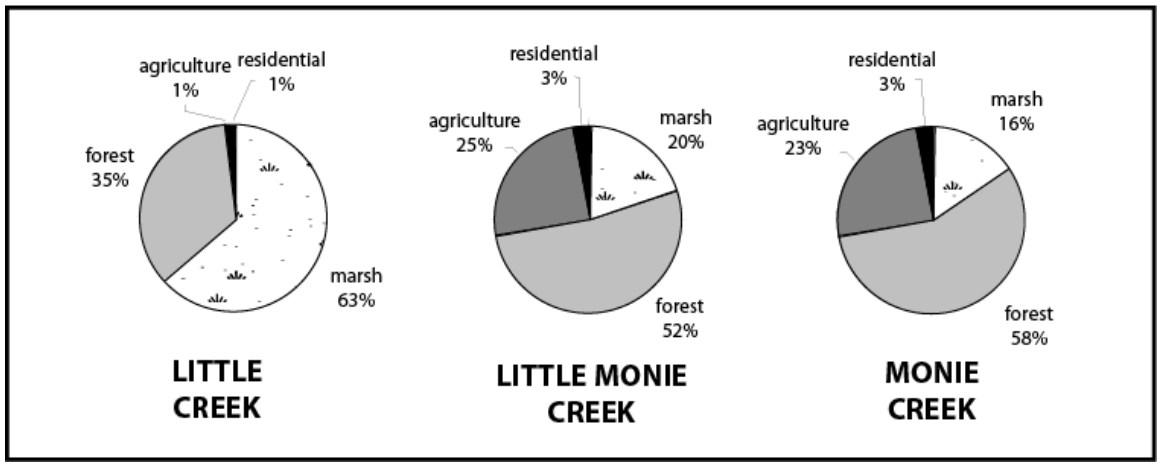

agriculture
1%
residential
1%
forest
35%
marsh
63%
LITTLE
CREEK
residential
3%
agriculture
25%
marsh
20%
forest
52%
LITTLE MONIE
CREEK
residential
3%
marsh
16%
agriculture
23%
forest
58%
MONIE
CREEK

Fig. 2.2. Diagram of experimental approach used in Monie Bay. Comparisons were made in three dimensions, including longitudinal (transects along the creek axis), horizontal (comparisons among creek systems), and temporal (seasonal or event-based comparisons).

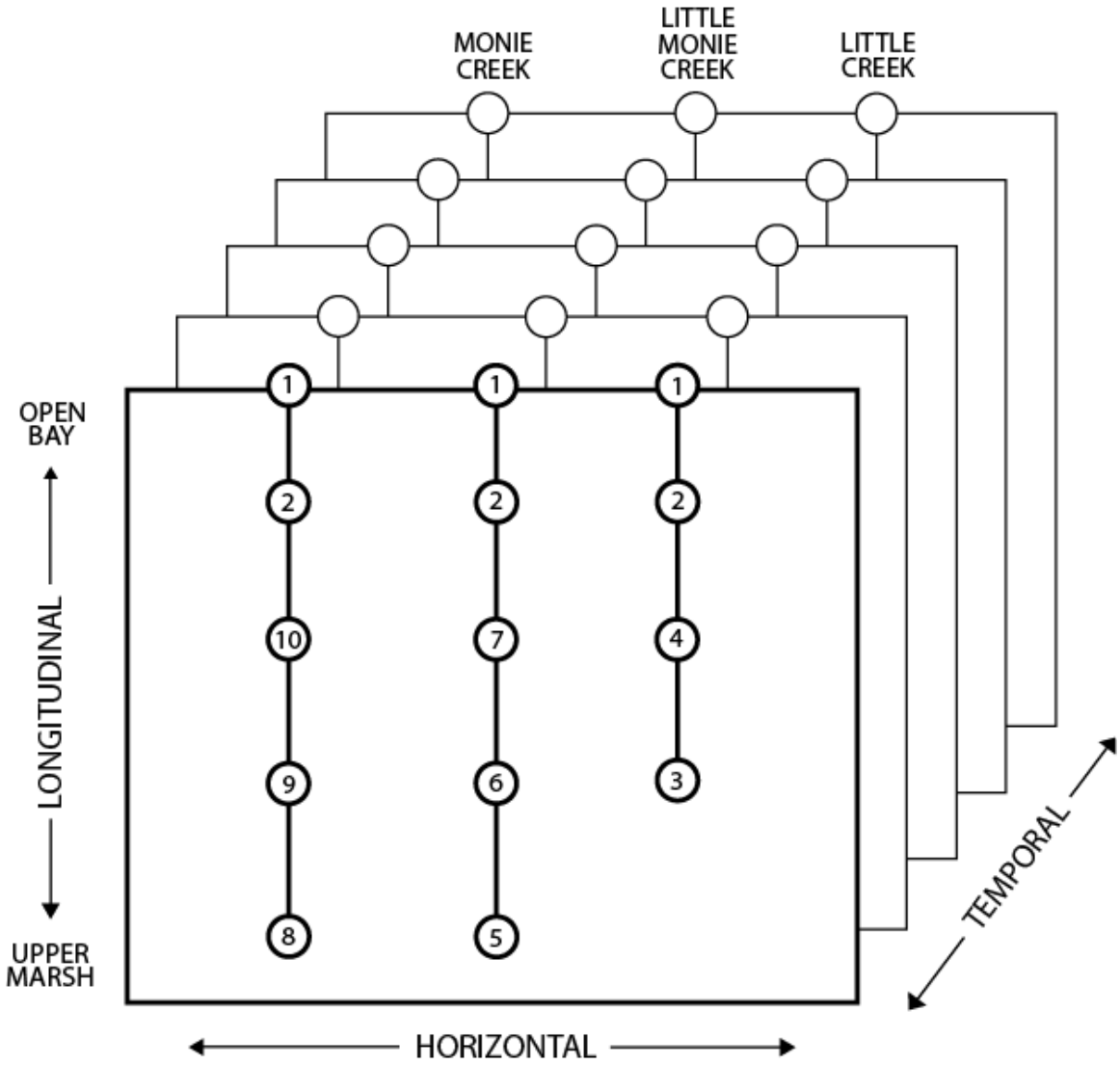

MONIE CREEK
LITTLE MONIE CREEK
LITTLE CREEK
OPEN BAY
LONGITUDINAL
UPPER MARSH
HORIZONTAL
TEMPORAL
1
1
1
2
2
2
10
7
4
9
6
3
8
5

Fig. 2.3. Horizontal comparisons of 2-yr means among systems. For each parameter, bar height represents the magnitude of the 2-yr mean. Means that are statistically similar share the same bar height. (ANOVA and Tukey-Kramer HSD, □=0.05). Parameters are defined in Table 2.1.

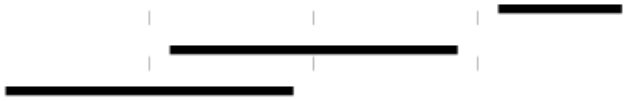
	MONIE CREEK	LITTLE MONIE CREEK	LITTLE CREEK	MONIE BAY	p-value	n
TDN					<0.0001	200
TDP					0.0003	200
DON					<0.0001	200
PO_4^{3-}					0.006	199
NH_4^+					0.01	200
SALINTY					<0.0001	216
DOC					<0.0001	204
a350*					<0.0001	118
BP					0.005	168
filtered BP					0.02	168
BP/cell					<0.0001	137

Fig. 2.4. Transect of ambient nutrient concentrations (TDP ■ and TDN □) and total bacterial production (BP) along the axis of agriculturally-impacted LMC and open bay (each point represents 2-yr mean ± SE, n=21).

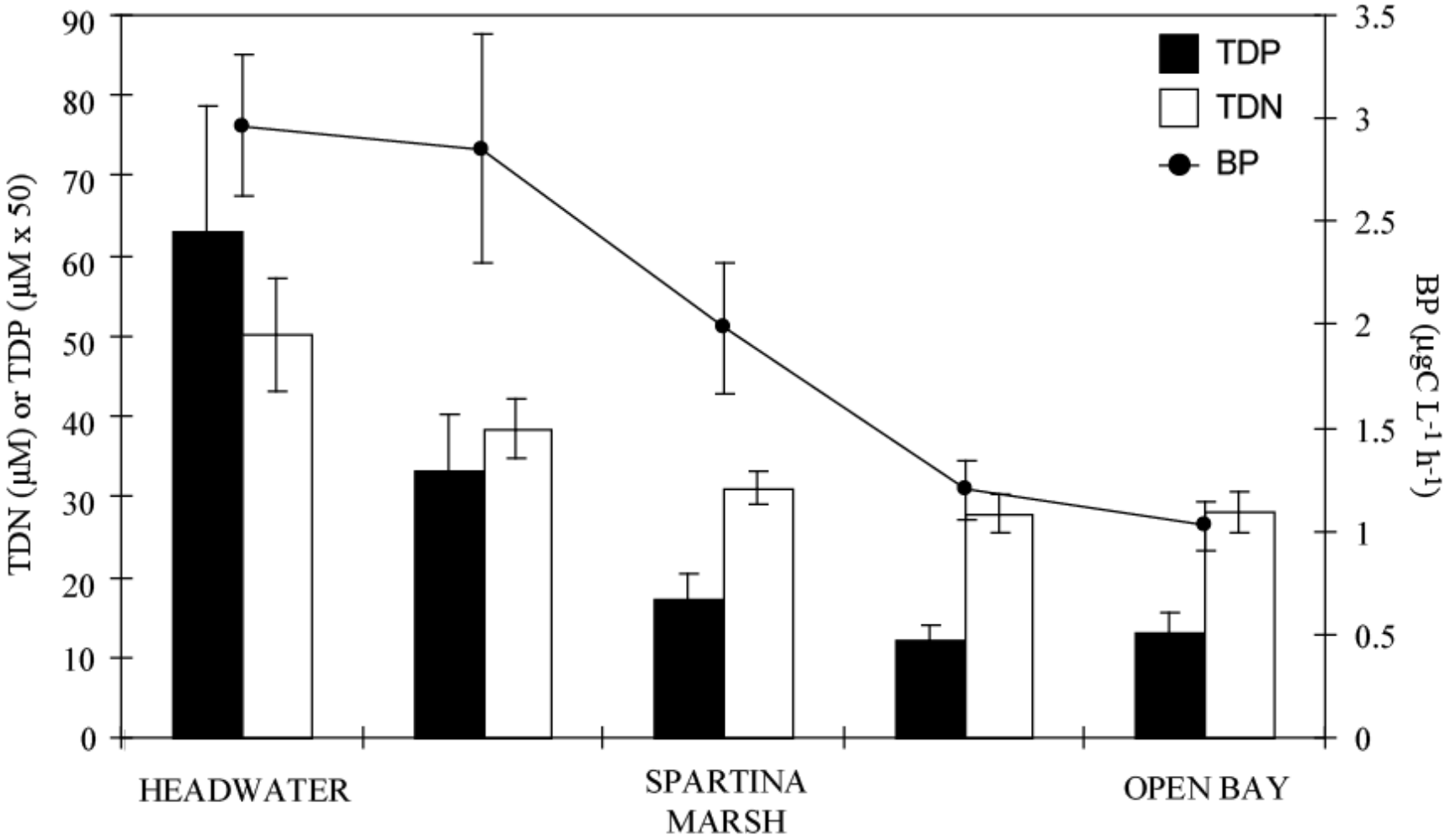

TDP
TDN
BP
TDN (µM) or TDP (µM x 50)
BP (µgC L-1 h-1)
0
10
20
30
40
50
60
70
80
90
0
0.5
1
1.5
2
2.5
3
3.5
HEADWATER
SPARTINA MARSH
OPEN BAY

Fig. 2.5. Two-year seasonal variability in total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), total bacterial production (BP) and temperature (TEMP) among the creeks and open bay.

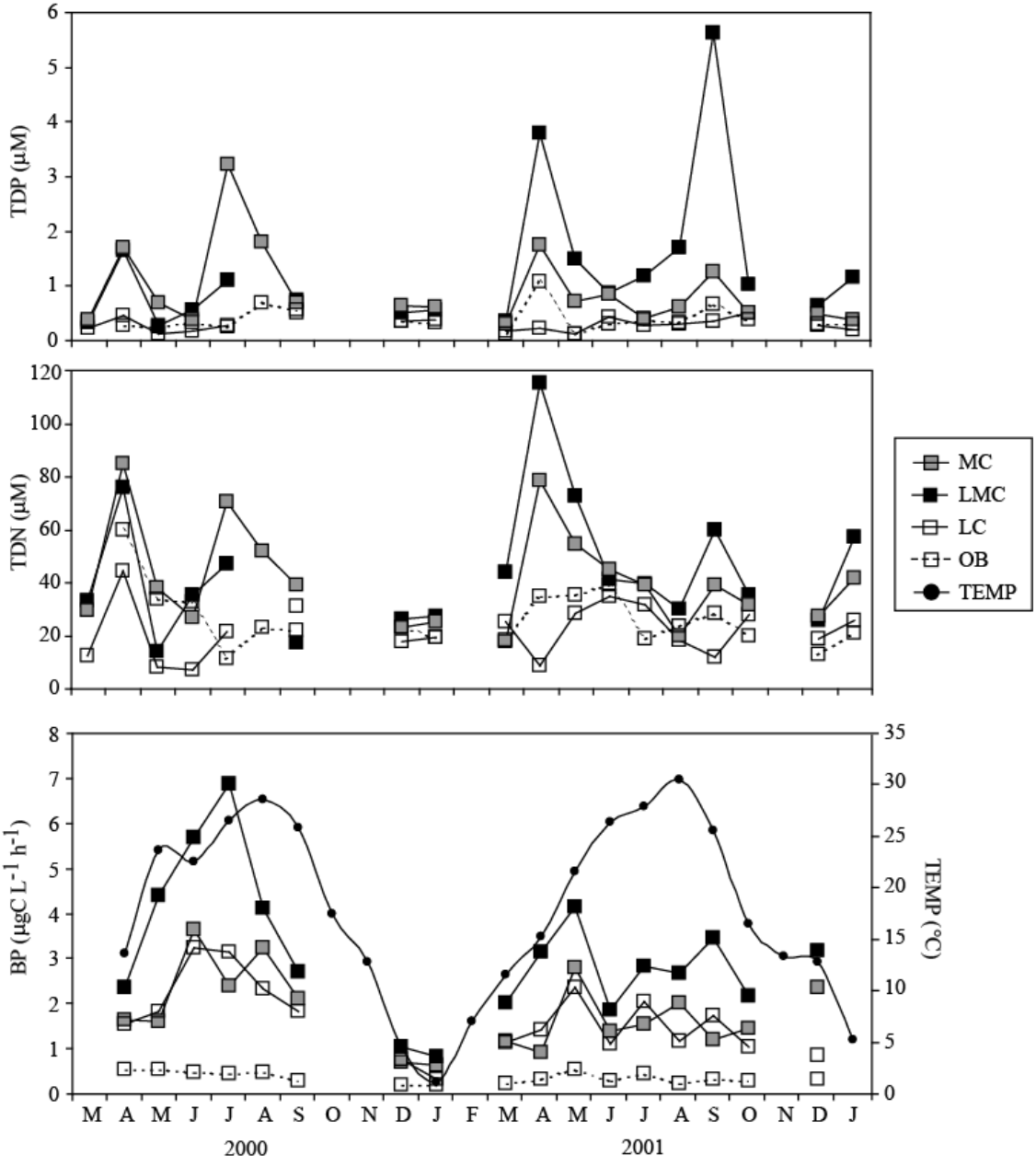

TDP (µM)
TDN (µM)
BP (µgC L-1 h-1)
TEMP (°C)
MC
LMC
LC
OB
TEMP
M A M J J A S O N D J F M A M J J A S O N D J
2000
2001

Fig. 2.6. Comparisons of seasonal means for environmental and biological parameters measured over the 2-yr sampling period. For each parameter, bar height represents the magnitude of the 2-yr mean. Means that are statistically similar share the same bar height. (ANOVA and Tukey-Kramer HSD, □=0.05). Parameters are defined in Table 2.1.

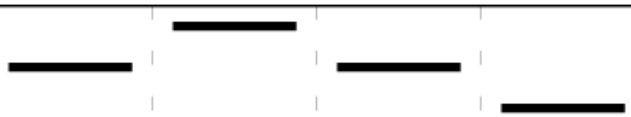
	SPRING SUMMER FALL WINTER	p-value	n
TDN		0.0003	200
TDP	no significant differences among seasons	ns	200
DON	no significant differences among seasons	ns	200
NH_4^+		<0.0001	200
NOx		<0.0001	200
PO_4^{3-}		0.04	199
SALINTY		<0.0001	216
DOC		<0.0001	204
a350*		0.003	118
TEMP		<0.0001	216
BP		0.008	168
BA		<0.0001	168

Fig. 2.7. Principal components analysis of each sampling event with loadings on PC1 and PC2 (n=160). Sampling events are separated by system: Monie Creek and Little Monie Creek (upper panel) and Little Creek and the open bay (lower panel).

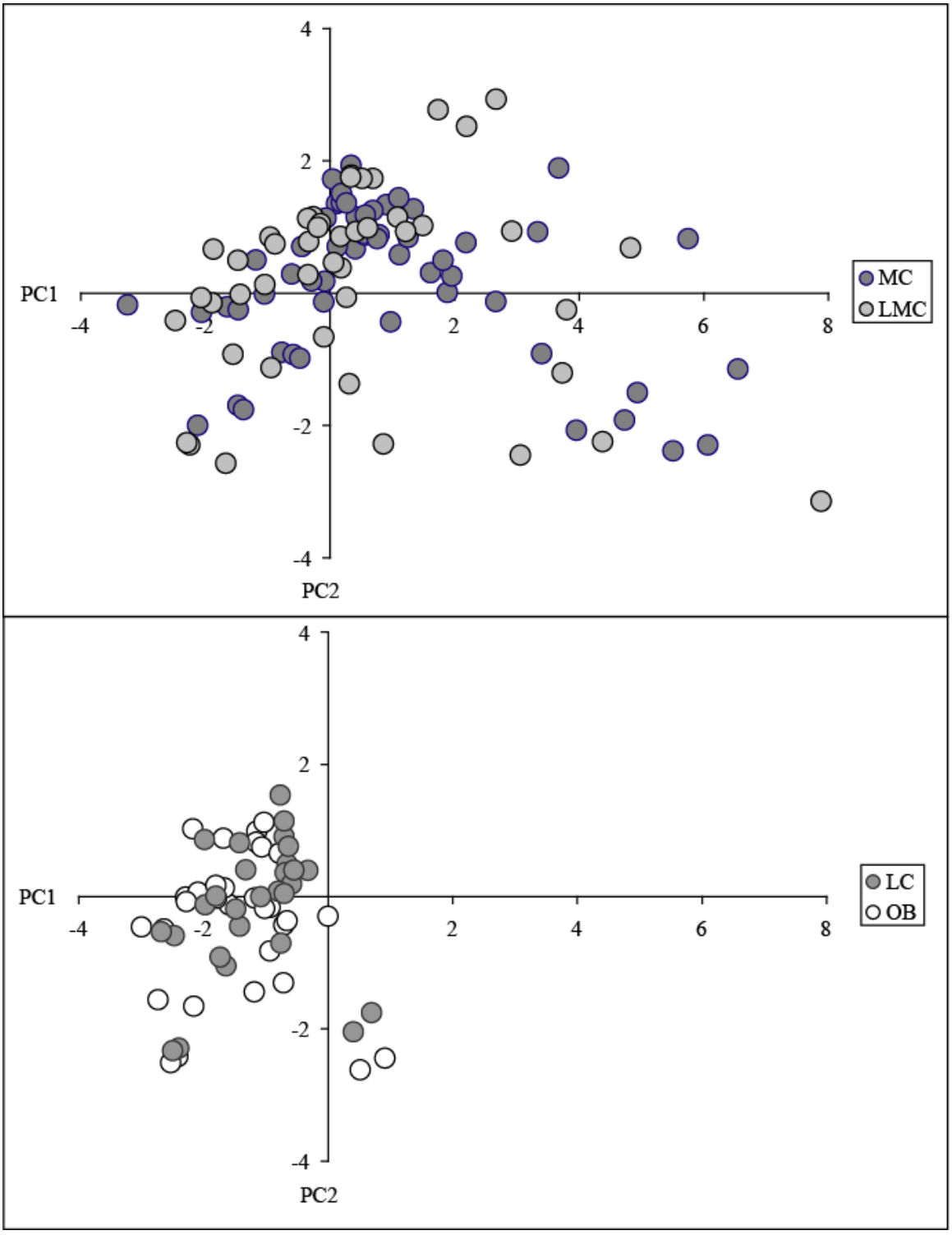
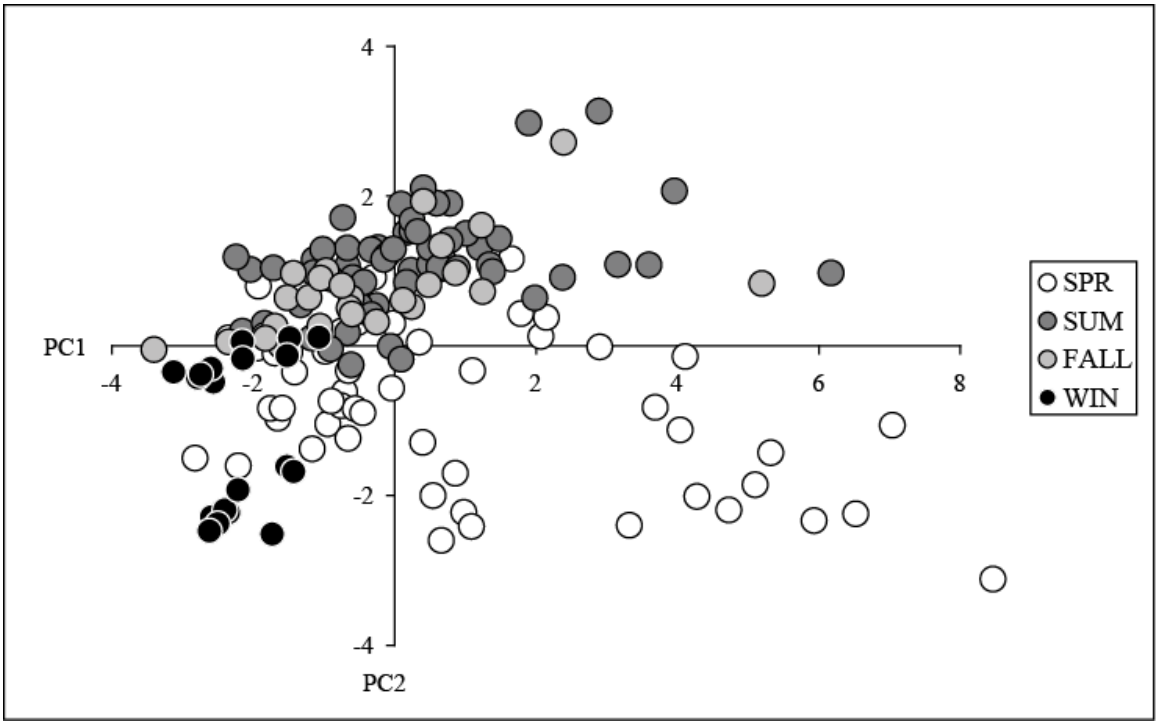

4
2
-2
-4
PC1
PC2
-4
-2
2
4
6
8
MC
LMC
4
2
-2
-4
PC1
PC2
-4
-2
2
4
6
8
LC
OB

Figure 2.8. Principal components analysis of each sampling event with loadings on PC1 and PC2. Sampling events are identified by season (n=160).


4
2
-2
-4
PC1
PC2
-4
-2
2
4
6
8
SPR
SUM
FALL
WIN

CHAPTER III

Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary

ABSTRACT

There is consensus that temperature plays a major role in shaping microbial activity, but there are still questions as to how temperature influences different aspects of bacterioplankton carbon metabolism under different environmental conditions. In this paper we examine the temperature dependency of different measures of bacterioplankton carbon metabolism (i.e., growth, production, respiration, carbon consumption, and growth efficiency) and whether this temperature dependency changes at different temperatures. We further explore if the relationship between temperature and carbon metabolism varies among systems differing in their degree of enrichment. Two years of intensive sampling in a temperate estuarine system (Monie Bay, Chesapeake Bay, USA) revealed significant differences in the temperature dependence of bacterial growth, production (BP) and respiration (BR), which resulted in a strong non-linear temperature regulation of bacterial growth efficiency (BGE), with generally lower values in summer (< 20%) and higher in winter (> 50%). We also observed significant differences in the temperature response of all measures of carbon metabolism investigated at different temperature ranges, with the most pronounced effects at lower temperatures. Despite significant differences in nutrient and organic carbon availability, both the temperature dependence and magnitude of BR and of bacterioplankton carbon consumption (BCC) were remarkably similar. Although temperature dependencies of BP and BGE were also similar among all sub-systems, the magnitude of BP and BGE differed significantly, with highest values in the nutrient-enriched sub-system and lowest in the open bay. This pattern in carbon metabolism among sub-systems was present throughout the year and was confirmed in temperature manipulation experiments, suggesting the temperature effects on BP and BGE did not override the influence of resource availability. We conclude that temperature is the dominant regulating factor in this estuarine systems, whereas other environmental factors such as nutrient availability and the quality and quantity of organic carbon resources play a much larger role in regulating the magnitude of BP, BGE and thus of bacterial growth.

INTRODUCTION

It is well established that temperature plays a fundamental role in regulating the activity and growth of all microorganisms (Madigan et al. 2003; Rose 1967). The effect of temperature on cellular processes in cultured bacteria has been well documented, with a general consensus that metabolic rates approximately double for each 10ºC increase in temperature (Morita 1974). This general rule often masks the fact that the temperature dependencies of different biochemical processes can vary greatly. Disparate effects of temperature have been documented for the uptake of various forms of inorganic nitrogen and different amino acids (Crawford et al. 1974; Reay et al. 1999), enzymatic activity, and variability in the coupling of cellular respiration to ATP production (Rose 1967). Furthermore, temperature manipulation experiments conducted on bacterial cultures reveal a difference in the response of cellular growth versus respiration (Rose 1967), indicating that differences in temperature dependence are evident at multiple levels of cellular organization.

Although there is no reason to think that bacterioplankton should respond to temperature any differently than cultured bacteria, the results obtained from single bacterial cultures are often difficult to extrapolate to complex microbial communities. The effects of temperature on bacterioplankton carbon metabolism has been the subject of numerous studies (Felip et al. 1996; Hoch and Kirchman 1993; Pomeroy et al. 1995; Raymond and Bauer 2000; Sampou and Kemp 1994; Shiah and Ducklow 1994b). The overwhelming majority of these have focused on the temperature dependence of bacterial growth and production (BP) alone. In general, these studies share two fundamental conclusions. First, that the temperature dependency of bacterial growth and production is

stronger at lower temperatures, and second, that the effect of temperature is often modulated by other environmental conditions, namely the availability of inorganic nutrients and the quality and quantity of organic matter substrates.

There are fewer studies that have investigated the effect of temperature on total community and bacterioplankton community respiration in coastal and marine systems (Jahnke and Craven 1995). These generally report a positive temperature-respiration relationship that is often more robust than that of BP and less susceptible to the influence of other environmental conditions (Iturriaga and Hoppe 1977; Pomeroy et al. 1995; Sampou and Kemp 1994). Strong temperature dependence of BR has been observed in cold water (<4ºC) systems (Griffiths et al. 1984; Pomeroy and Deibel 1986; Pomeroy et al. 1991), although temperature adaptation of psychrophilic bacterioplankton suggest that these relationships may not accurately represent the temperature dependency of microbial communities in temperate systems (Rose 1967). Furthermore, studies of the effect of temperature on total carbon consumption suggest that patterns in the temperature dependence of BCC may reflect that of BR (Raymond and Bauer 2000). Although these studies collectively indicate that temperature exerts a strong positive effect on respiration, the few available empirical estimates vary greatly and it is unclear if there is a regular pattern in t temperature dependence of BR in across coastal or estuarine systems.

Differences in the shape of the temperature dependency of BP and BR suggested by these studies further imply an inherent temperature dependency of BGE (i.e., BGE = BP/(BP+BR)). However, direct investigations of the effect of temperature on BGE in aquatic systems are very few and have not come to any consensus. Some manipulative experiments suggest that BGE decreases with increasing temperature (Griffiths et al.

1984; Iturriaga and Hoppe 1977; Roland and Cole 1999; Tison and Pope 1980), although other similar studies report no such temperature effect (Crawford et al. 1974). Surveys of seasonal variability have also yielded conflicting results, reporting negative (Bjørnsen 1986; Daneri et al. 1994), positive (Lee et al. 2002; Roland and Cole 1999), and little or no effect of temperature (Kroer 1993; Ram et al. 2003; Reinthaler and Herndl 2005; Toolan 2001) on BGE. It is unclear to what extent these discrepancies are due to differences in methodology, lack of sufficient observations, or reflect a true diversity in the effects of temperature on microbial carbon metabolism in different aquatic ecosystems.

In summary, the influence of temperature on bacterioplankton carbon metabolism is both complex and diverse, and in spite of an abundant literature, there are still major gaps in our understanding. These gaps are in part due to the scarcity of longer-term studies that have simultaneously measured bacterial growth, production and respiration (Jahnke and Craven 1995; Reinthaler and Herndl 2005), so that truly comparable rates can be derived that are also appropriate for estimating BGE and identifying its temperature dependence. In this paper we present results from an intensive two-year study carried out in a temperate salt marsh-estuary that contains steep environmental gradients. We investigate three fundamental questions regarding the temperature dependency of bacterioplankton carbon metabolism. First, do different aspects of carbon metabolism (i.e., BP, BR, BCC, and BGE) exhibit similar temperature dependence? Second, is the temperature dependence of each aspect of carbon metabolism the same for all temperature ranges? And third, does the relationship between temperature and carbon

metabolism vary among estuarine sub-systems differing in their degree of nutrient and organic carbon enrichment?

METHODS

Our study was conducted in the Monie Bay component of Maryland's National Estuarine Research Reserve (NERR), a tidally-influenced temperate salt-marsh estuary located on the eastern shore of Chesapeake Bay (38°13.50'N, 75°50.00'W), consisting of an open bay (OB) and three tidal creeks varying in size and watershed characteristics (Fig. 3.1). Monie Creek (MC) and Little Monie Creek (LMC) are characterized by elevated nutrient concentrations attributed to predominant agricultural land-use, whereas Little Creek (LC) is a relatively pristine tidal-creek system with an undeveloped watershed dominated by marsh and forest (Apple et al. 2004; Jones et al. 1997). These tidal creeks offer a broad range of environmental conditions, including salinity, quality and quantity of dissolved organic matter, and dissolved nutrient concentrations that change on relatively small spatial scales (Fig. 3.1, Table 3.1). The utility of this system for investigating the effect environmental conditions on bacterioplankton community metabolism has been described (Apple et al. 2004).

Thirteen stations within the four sub-systems of Monie Bay research reserve were visited monthly between March 2000 and January 2002, with biweekly sampling during summer months (June – August). Approximately 20 L of near-surface (<0.5 m) water were collected in the morning (between 0800h and 1000h) immediately following high tide. Water temperature and salinity were recorded at each station. Water samples were transported back to the laboratory for filtration within approximately 1 h. Upon return to

the lab, a small sub-sample was removed from each carboy for determining total bacterioplankton production and abundance.

Estimates of filtered bacterial production, respiration, and abundance were determined by gently passing several liters of sample water through an AP15 Millipore filter (~1 μm) using a peristaltic pump, then incubating in the dark in a 8 liter incubation assembly at *in situ* field temperature (see Appendix B). Total BP was also determined directly using unfiltered water samples. Incubations were sub-sampled at 0, 3, and 6 h. Bacterial production (BP) was estimated using ^{3}H-leucine incorporation rates following modifications of Smith and Azam (1992) and assuming a carbon conversion factor of 3.1 kg C · mol leu^{-1} (Kirchman 1993). Bacterial respiration (BR) was determined by measuring the decline of oxygen concentration over the course of the 6 h incubation, with longer incubations (8 h) used at lower ambient water temperatures (<15°C). Dissolved oxygen concentrations were measured using membrane-inlet mass spectrometry (Kana et al. 1994). Bacterioplankton carbon consumption was calculated by adding contemporaneous measurements of filtered BP and BR. Bacterial growth efficiency was calculated as the ratio of filtered BP and BCC (BGE = BP/(BP + BR). Bacterial abundance (BA) was determined on live samples using standard flow-cytometric techniques and the nucleic acid stain SYTO-13 (del Giorgio et al. 1996a). Estimates of BA, BR, and BP were used to calculate cell-specific production (BP_{sp}) and respiration (BR_{sp}).

The direct effect of temperature on carbon metabolism was investigated further using temperature manipulation experiments. In the spring of 2004, samples were collected from each estuarine sub-system and incubated at both ambient (18ºC) and

manipulated (7ºC) temperatures. Rates of bacterioplankton carbon metabolism associated with these changes in temperature were determined following the methods described previously and compared to regressions describing the temperature response of natural bacterioplankton communities as identified by our field data.

Simple least-squares regression analysis was used to identify the relationship between temperature and measured metabolic rates, where bacterial rates were log-transformed to meet requirements for normal distribution and regressed against in situ temperatures. Type I regressions were used because local scale (<100m) variations in diel-mean water temperature and measurement errors were small (Jones et al. 1997). The temperature dependence of different aspects of BCM was identified using the slope of least-squares regression. For each measured metabolic rate or efficiency, differences in the effect of temperature (slope) and the effect attributed to each estuarine sub-system (*y*-intercept) were identified using ANCOVA with temperature and creek system as model effects (JMP 5.0; SAS Institute) and Student's *t*-test (Zar 1984). Environmental Q_{10} values were derived from in situ water temperatures and measured or calculated parameters using the following equation:

$$Q_{10} = (R_1/R_2)^{10/(T1-T2)}$$

in which R_1 and R_2 are rates or efficiencies at two temperature extremes (i.e., T_1 and T_2, respectively), where $T_1 > T_2$ (Caron et al. 1990; Sherr and Sherr 1996). R_1 and R_2 were predicted using the equation derived from linear regression of observed rates of carbon metabolism (or efficiencies) and the corresponding ambient water temperature in ºC.

RESULTS AND DISCUSSION

Temperature dependence differs among measures of carbon metabolism

Bacterioplankton production (BP), respiration (BR), and growth represent the measured endpoints of numerous biochemical and physiological processes. Based on previous evidence, we hypothesized that these community-level metabolic processes would differ in their temperature dependence. Arrhenius plots (Fig. 3.2) revealed a highly-significant positive effect of temperature on bacterial respiration (BR) and production (BP) when the 30ºC *in situ* temperature range was considered. The slope describing the relationship between BP and temperature was significantly lower than that of BR (ANCOVA; $r^2 = 0.49$; $n = 277$; $F = 87.7$; $p < 0.0001$) and characterized by greater variability at higher temperatures. Relationships between all investigated measures of bacterioplankton carbon metabolism versus temperature are reported in Table 3.2. Bacterioplankton carbon consumption (BCC) exhibited a positive slope intermediate to those of BR and BP. Of these aspects of carbon metabolism, BR exhibited the strongest temperature dependence ($r^2 = 0.66$), followed by BCC ($r^2 = 0.60$; regression not shown) and BP ($r^2 = 0.16$).

Significantly different slopes in Arrhenius plots indicate that BP and BR respond differently to changes in temperature and suggest that this may be attributed to differences in the activation energy associated with these metabolic processes (Zumdahl 1989). For example, the lower slope of the BP regressions indicates a lower activation energy required for this process relative to that of BR. This difference in activation energy is not surprising, as anabolic growth and production processes are subsidized in part by energetic input from catabolic respiratory processes. Because of this metabolic

link with BR, the temperature dependence of BP is a combination of the effect of temperature on specific anabolic processes and on BR itself. Thus the temperature regulation of BP – and ultimately BGE – appears to be more variable and complex than that of BR.

The significant difference in the temperature response of BP and BR resulted in a negative temperature dependence of BGE when the full annual temperature range was considered (Fig. 3.3, Table 3.2). This relationship (Fig. 3.3A) is similar to that reported by Daneri *et al*. (1994) in their study of BGE in marine enclosures (BGE = -0.017*TEMP + 0.52; $r^2 = 0.35$; $p < 0.0001$) and similar to that calculated by Rivkin and Legendre (2001) in their review of BGE in marine systems (BGE = -0.011*TEMP + 0.37; $r^2 = 0.54$; $p < 0.0001$). Studies conducted in sub-arctic marine sediments (Griffiths et al. 1984) and on both mixed seawater and pure cultures (Bjørnsen 1986; Tison and Pope 1980) also report a negative effect of temperature on bacterial growth efficiencies.

The primacy of temperature in regulating BGE is not revealed in the findings of all studies of growth efficiency, many of which identify organic matter supply and quality (del Giorgio and Cole 1998; Jorgensen et al. 1999; Reinthaler and Herndl 2005) and dissolved nutrient stoichiometry (Goldman et al. 1987; Kroer 1993) as the most important factors responsible for the regulation of BGE in coastal and estuarine systems. These conclusions do necessarily not contradict the significant effect of temperature reported in other studies, rather provide insight into the simultaneous influence of both temperature and resource supply on the magnitude of BGE. Whereas temperature drives changes in the magnitude of BGE throughout the year, resources account for differences in magnitude at any given temperature and between systems differing in their degree of

enrichment. In addition, our results provide evidence that temperature and resource supply may interact at higher temperatures, with a non-linear decrease in BGE that may represent a combined effect of resource limitation and adverse effects of elevated temperatures.

Temperature dependencies are non-linear

The temperature dependence of a metabolic process is conventionally identified by a linear relationship on an Arrhenius plot (Pomeroy et al. 2000). However, not all *in situ* metabolic processes responded to annual temperature changes in this manner. Unlike the strong and highly-significant log-linear relationship of BR (Fig. 3.2A) and BCC (Table 3.2), we observed an almost asymptotic shift in the temperature dependence of BP, with a highly significant linear relationship ($\log(BP) = -6863*1/K + 24.2$; $r^2 = 0.37$; $F = 38.7$; $n = 68$; $p < 0.0001$) at lower temperatures (i.e., <20ºC) and no apparent relationship at higher temperatures (Fig. 3.2B, Table 3.2). As a result, the temperature dependence of BP was curvilinear across the 0 to 30ºC temperature range and more accurately described by a 2nd order polynomial equation (Figs. 3.2B & 3.4). Setting the first derivative of this polynomial equation equal to zero, we estimated the inflection point at which BP no longer increases and begins to decrease with temperature to be approximately 22ºC (Fig. 3.4). This value is strikingly similar to the optimum temperature of 20ºC reported by Autio (1992) for specific growth rate of temperate brackish water bacterioplankton and of 20-25ºC for cold-water isolates.

Patterns in the temperature dependence of cell-specific production (BP_{sp}) and respiration (BR_{sp}) were similar to those of their community-level counterparts, with a significant difference in temperature response of both BP_{sp} and BR_{sp} (ANCOVA; r^2 =

0.30; $n = 308$; $F = 32.8$; $p < 0.0001$), resulting in higher cell-specific respiration than production at temperatures above approximately 20°C (Fig. 3.5B). A comparison of mean abundance at low and high temperatures revealed a small increase from 9.2 to 9.5 x $10^6$0 cells ml^{-1}, although the contribution of this change in abundance to total variability in BP_{sp} or BR_{sp} was relatively small (i.e., 24% and 36%, respectively).

The significant negative linear relationship between BGE and temperature documented by our study (Fig. 3.3A) and others (Daneri et al. 1994; Rivkin and Legendre 2001) implies that there is a consistent and predictable decrease in BGE with increasing temperature. This, however, may be misleading and not accurately represent more subtle changes in BGE throughout the year in temperate estuarine systems. As observed with BP (Fig. 3.4), we found a non-linear change in BGE across the annual temperature range. Most striking was the precipitous decrease in BGE at temperatures above ~22ºC (Fig. 3.3B). At temperatures above this inflection point, the negative temperature dependence of BGE was stronger and highly significant ($r^2 = 0.23$; $p < 0.0001$), whereas at lower temperatures it was weak and marginally significant ($r^2 = 0.09$; $p = 0.02$; Fig. 3.3B). This pattern in BGE is driven by the combination of a strong exponential temperature dependence for BR (Fig. 3.2A) and the curvilinear temperature response for log-transformed BP (Fig. 3.4).

Several explanations exist for such non-linear responses of growth efficiency over wide temperature ranges. Rose (1967) observed substantive increases in cell-specific respiration relative to cell-specific production at temperatures above approximately 20ºC, suggesting that a direct and disproportionate effect of temperature on cellular-level growth and respiration may be the mechanism driving changes in BP and BGE that we

have observed at warmer temperatures. Other studies conducted in lakes (Carlsson and Caron 2001; Coveney and Wetzel 1995) and temperate estuaries (Hoch and Kirchman 1993; Raymond and Bauer 2000; Shiah and Ducklow 1994a), however, offer a different explanation. Direct and indirect evidence from these studies suggests that there is a weakening of temperature dependence above 15 to 20ºC that is attributable to a shift from temperature- to resource-limitation. In this case, bacterioplankton metabolism would be released from the physiochemical constraints imposed by temperature and subsequently subjected to limitation attributed to other environmental conditions, such as nutrient and carbon availability (Coveney and Wetzel 1995; Felip et al. 1996; Shiah and Ducklow 1994a).

It is difficult to determine if the inflection point of 22ºC (Fig. 3.4) represents a physiological optimum temperature for BP in this system – above which elevated temperatures have an adverse effect on bacterioplankton growth – or is simply the result of growth limitations imposed by other environmental factors encountered during warmer months. It is likely that a decrease in BP during summer months may be attributed in part to seasonal fluctuations in nutrient or substrate availability (Pomeroy et al. 1995) characteristic of this and other estuarine systems (Apple et al. 2004; Fisher et al. 1988). The consistent increase in BR across the entire temperature range (Fig. 3.2A) eliminates carbon limitation as a factor driving the decline in BP. This, in turn, leads to the hypothesis that seasonal variability in the availability of dissolved nutrients and/or changes in the quality of dissolved organic matter may play an important role. Reduction in ambient nutrient concentrations during summer months as a result of uptake by tidal-marsh communities during the plant growing season has been documented locally in this

system of tidal creeks (Jones et al. 1997) and may be associated with decreases in nutrient availability and DOM quality. In addition, regional-scale changes in nutrient cycling have been observed for Chesapeake Bay, with a shift from nitrate as the dominant form of dissolved nitrogen in the spring to ammonium and DON in the summer and fall. These changes represent a shift from a predominantly autotrophic system in the spring that becomes progressively more heterotrophic as the year progresses (Bronk et al. 1998). Collectively, these studies provide evidence that there may be fundamental changes in nutrient cycling and availability among seasons that would possibly limit bacterioplankton growth and production during summer months.

Effect of resources on seasonality of carbon metabolism

We addressed the hypothesis that environmental conditions other than temperature have an impact on seasonal variability of carbon metabolism by comparing rates observed at similar temperatures (i.e., 14-16ºC and 21-22˚C) in spring and fall. Although temperatures were similar during these two seasons, nutrient concentrations were consistently higher in spring than at comparable temperatures in fall. There was no significant difference in the magnitude of BR among seasons when these similar *in situ* temperature ranges were considered (n = 20 and 40, respectively; data not shown), suggesting that temperature may be the main factor regulating BR in DOM rich systems such as this marsh-dominated estuary. However, a similar comparison of BP in spring and fall revealed significant differences among seasons. Bacterioplankton production was always higher in spring than fall when samples of similar temperature were compared. In the 21 to 22˚C temperature range, mean BP of samples collected in June 2000 and May 2001 was significantly higher than that of September 2000 (Student's *t*-

test; $t = 1.7$, df = 28; $p < 0.1$) and samples in the 14 to 16ºC range collected during April 2001 were also significantly higher when compared to October 2001 (Student's *t*-test; $t = 1.7$, df = 18; $p < 0.07$). These observations support the idea that differences in nutrient concentrations tend to regulate BP but not BR, introducing a source of variability in BGE when narrow temperature ranges are considered.

Although the above comparison indicates that environmental factors other than temperature influence seasonal patterns in BP, this was not consistently reflected in the patterns of BGE. Significant between-season differences in BGE were only observed within the 14 to 16ºC range (Student's *t*-test; $t = 4.2$, df = 13; $p < 0.05$), suggesting that the effect of other environmental factors on BGE may be more pronounced or apparent at lower temperatures. This is consistent with the higher degree of variability in the temperature dependence of BGE at a lower temperature range (Fig. 3.3B). Bacterial respiration is typically the larger of the two components that make up BGE. As a consequence of the strong temperature dependence of BR, however, respiration and production rates are comparable at lower temperatures (Fig. 3.5A), allowing temperature-independent environmental factors to influence BP and, in turn, BGE. In contrast, when temperatures are high, BGE is lower and less variable as a result of the increased magnitude of BR. Ultimately, the annual range in BGE is driven at lower temperatures by the variability in BP and by a combination of elevated BR and limited production during warmer months.

The decrease in BGE observed during summer months is in striking contrast to results reported in a recent study of bacterial growth efficiency in coastal waters of the North Sea (Reinthaler and Herndl 2005). The authors observed highest growth

efficiencies during summer months and lowest in winter, attributing this seasonal pattern to dependence of bacterioplankton carbon metabolism on labile DOM production associated with algal productivity. Studies conducted in Chesapeake Bay, however, observed a decrease in bacterioplankton carbon metabolism during the summer and attribute this to a decline in the availability of labile organic matter (Cowan and Boynton 1996; Smith and Kemp 1995). If seasonal variability of BGE in Monie Bay is influenced by resource supply, it is more likely that these studies most accurately represent dynamics of bacterioplankton carbon metabolism in Monie Bay, where a turbid water column and elevated concentrations of allochthonous DOM reduce the reliance of bacterioplankton carbon demand on algal production

Although resource limitation offers a plausible explanation for the decrease in BP and BGE that we have observed at elevated temperatures, it is important to recognize that there may be direct physiological effects of temperature on bacterioplankton growth and production. The almost identical shapes of temperature response functions, with decreasing BP at temperatures >20ºC, together with the persistent rank-order of BP among the four sub-systems of Monie Bay (Fig. 3.4) suggests the influence of an environmental factor more general than resource supply and quality, as these factors varied greatly among the four sub-systems (Table 3.1). The converging polynomial regressions that show a similar decrease in BP at higher temperatures in all four sub-systems suggest that growth and production of estuarine bacterioplankton in these tidal creeks may be adversely impacted by the direct effect of temperature during summer months – or by some other environmental stressor that was not measured during the course of our study. Direct physiological effects of temperature on bacterioplankton

might include a disproportionate increase in energetic demands of anabolic processes at higher temperatures (Caron et al. 1990) or physiological stress associated with super-optimal ambient water temperatures (Sherr and Sherr 1996). In addition, there is evidence suggesting that leucine-to-carbon conversion factors may be temperature dependent, possibly resulting in apparent changes in leucine-based estimates of BP that are driven by temperature rather than growth or protein synthesis (Tibbles 1996).

Temperature coefficients change in different temperature ranges

The log-linear relationship between bacterioplankton carbon metabolism and temperature reveals differences in temperature dependence when different temperature ranges are considered. In general, the effect of temperature on bacterioplankton carbon metabolism was greatest at lower temperatures, as evidenced by higher correlation coefficients and steeper slopes for the 0-15 ºC temperature range (Table 3.2). These differences in temperature dependence were reflected in estimates of temperature coefficients (i.e., Q_{10} values; Table 3.4). The temperature dependence of BR was much stronger at lower temperatures, with an almost two-fold higher effect of temperature at colder versus warmer temperatures for both BR and cell-specific respiration. Similar temperature coefficients have been reported for respiration of marine bacterioplankton (Pomeroy and Deibel 1986) and lake bacterioplankton and sediments (Carignan et al. 2000; Den Heyer and Kalff 1998), suggesting that this may be a transferable property of respiration in aquatic bacterioplankton communities. The temperature dependence of BP also decreased with increasing temperature, although the shift was not as dramatic and the relationship was weaker than that exhibited by respiration. The temperature dependence of cell-specific metabolism also changed dramatically when low and high

temperatures ranges were compared, with a much stronger effect of temperature observed at the lower temperature range (Table 3.2). Increases in bacterioplankton abundance and carbon metabolism with temperature are characteristic of estuarine bacterioplankton communities (Lomas et al. 2002) and the increase in cell-specific metabolism that we observed was to be expected. The temperature dependence of BP_{sp} in the lower temperature range was almost identical to that reported for non-summer months in similar temperate estuaries (Hoch and Kirchman 1993; Shiah and Ducklow 1994a; Shiah and Ducklow 1994b).

Changes in temperature dependence at different temperature ranges indicates that assuming a uniform temperature response (i.e., constant Q_{10}) for all measures of carbon metabolism may not accurately reflect *in situ* metabolic processes throughout the year. Although we estimated a collective mean Q_{10} for all measured rates of carbon metabolism across the annual temperature range (i.e., $Q_{10} = 2.2$; Table 3.4) that was similar to the commonly used Q_{10} of 2 (del Giorgio and Davis 2003; Toolan 2001), a comparison of the collective mean Q_{10} for high and low temperature ranges (i.e., 1.1 and 3.0, respectively) indicates that assuming a constant temperature dependence would tend to underestimate the effect of temperature on carbon metabolism at low temperatures and overestimate the effect at higher temperatures. This discrepancy becomes even more pronounced when different aspects of carbon metabolism are considered individually, with the assumption of a constant Q_{10} potentially producing as much as a three-fold difference in predicted versus *in situ* rates. Thus, although estimates of carbon metabolism based on a constant Q_{10} of 2 may be adequate for low-precision predictions of the temperature dependence of carbon metabolism on annual time scales, they may fall

short when accurate predictions of the inter-annual variability in temperature dependence of multiple aspects of carbon metabolism are desired.

The uncertainty surrounding patterns in the temperature response of aquatic microbes has profound implications with respect to our capacity to model effectively the functioning of bacterioplankton communities in natural aquatic systems. Despite the substantial annual and diel variability in water temperatures of most temperate estuaries, models of microbial communities in these systems seldom address the temperature dependence of bacterioplankton carbon metabolism (Davidson 1996; Ducklow 1994; Eldridge and Sieracki 1993; Painchaud et al. 1987) or account for temperature effects with approaches that may mask the actual biological response (Lomas et al. 2002). Understanding the temperature dependence of BGE is of particular importance, as it describes the partitioning of carbon into biomass or respiratory losses by the bacterioplankton community, and it is likely that models assuming a fixed value for BGE across all temperatures may provide inaccurate estimates of microbially mediated carbon flux in aquatic systems.

Temperature dependence is similar among different systems, but magnitudes differ

As detailed in previous work in this system (Apple et al. 2004), the tributaries of Monie Bay exhibit significant systematic differences in many environmental conditions (Table 3.1). Despite this variability, we found no significant difference in the effect of temperature (i.e., slope of temperature-response function) on measures of carbon metabolism among the four sub-systems (Table 3.3). We did observe, however, significant differences in the magnitude of most measured metabolic processes (i.e., function intercepts). The *y*-intercepts for BP, BCC, and BGE differed significantly

among the sub-systems (Table 3.3), with highest values consistently observed in the nutrient enriched tidal creek (LMC), lowest in the open bay, and intermediate in the less enriched LC and freshwater-influenced MC (Fig. 3.4). In particular, BP had significantly ($p < 0.0001$) higher and lower *y*-intercepts for LMC and OB, respectively, when compared to MC and LC. Intercepts for MC and LC were statistically similar and not different than the overall *y*-intercept for the composite dataset. Significant but independent effects of both sub-system and temperature were also observed with BCC ($p < 0.0001$; r^2 =0.66; df = 146; F = 38.2) and BGE ($p < 0.0001$ and $p = 0.004$, respectively; r^2 =0.41; df = 146; F = 1.8). The temperature response of BR was the least variable, with statistically similar slopes and *y*-intercepts among all sub-systems (ANCOVA; $r^2 = 0.65$, $n = 139$, $p < 0.0001$). Although one might predict that temperature and environmental conditions interact to regulate the seasonal patterns in bacterioplankton growth efficiency and carbon consumption (Pomeroy and Wiebe 2001), we found no significant interaction of these parameters when BCC and BGE were considered ($p = 0.8$ and 0.3, respectively).

The robust nature of temperature dependencies and systematic patterns in the magnitude of carbon metabolism was confirmed by our temperature manipulation experiments, where bacterioplankton production and respiration were measured at ambient (18ºC) and reduced (7ºC) water temperatures (see Appendix A). This experiment was designed to investigate the direct effect of temperature on bacterioplankton carbon metabolism. Not only did rates of BP and BR from incubations at ambient and manipulated temperatures conform to the temperature dependencies expected based on regression models, but rates also exhibited the same rank-order among

sub-systems observed previously in this system that corresponds to system-level enrichment (Figs. 3.2 & 3.4; Table 3.1; Apple et al. 2004). In this regard, temperature appears to regulate the magnitude of carbon metabolism on a relatively coarse scale throughout the year, while finer scale variability at any given temperature is attributed to local environmental conditions. In turn, this system-specific variability in carbon metabolism is probably driven by environmental factors related to differences in resource enrichment, including nutrient availability or the quantity and quality of dissolved organic matter.

If bacterioplankton carbon metabolism in estuarine systems were regulated exclusively by temperature, one could expect all aspects of bacterial carbon metabolism to converge at low temperatures, regardless of the sub-system in question. As temperatures increase, metabolism would become less constrained by temperature and environmental differences among the sub-systems would become more apparent and be reflected in the magnitude of their respective metabolic rates, resulting in a pattern of diverging lines of differing slopes from a common baseline in the low temperature region of the metabolism versus temperature plot. However, significant differences in *y*-intercepts and near perfectly parallel lines for each sub-system would suggest that there is a strong environmental component regulating bacterioplankton growth and production that persists throughout the year and is independent of temperature. In contrast, the lack of significant differences in either the slopes or the intercepts of the BR versus temperature relationship would suggest that temperature is the main overriding control of respiration in these systems. This pattern also suggests that the environmental factors varying among these tidal creek systems either do not have a strong regulatory effect on

BR or are at levels that do not result in limitation. The importance of temperature in regulating respiration regardless of other environmental conditions has also been observed for the main stem Chesapeake Bay (Sampou and Kemp 1994), where effects of temperature on respiration of both bacterioplankton and total community respiration were identical for field measurements plotted versus ambient water temperatures and for rates measured in temperature manipulation experiments, despite seasonal changes in nutrient status.

The independent effects of temperature and resource supply generate a unique pattern bacterioplankton growth and production, with a generally curvilinear response throughout the year and a hierarchy in magnitude that appears to be a function of resource enrichment (Fig. 3.4). The decrease in temperature dependence at higher temperatures has been attributed to the effects of resource limitation on bacterioplankton metabolism (Coveney and Wetzel 1995; Shiah and Ducklow 1994a), although the nature of this change in temperature response has not been well described. In an effort to address the change in the temperature response of bacterioplankton growth and production, Felip et al. (1996) propose a conceptual model depicting changes in growth as a function of temperature throughout the year (Fig. 3.6). The authors acknowledge that the nature of changes in growth and production at higher temperatures remains undocumented and poorly understood. Our results not only confirm the conceptual model of Felip *et al* (1996), but also provide additional insight into the nature of these changes in the temperature response of bacterioplankton growth and production at higher temperatures. As suggested by Felip et al. (1996), we observed a threshold below which bacterial production (i.e., 22.0ºC) and growth (i.e., 20.1ºC; cell-specific production;

regression not shown) are strongly temperature regulated. However, unlike the conceptual model (Fig. 3.6), the temperature response of bacterioplankton below this threshold appears to be similar among different sub-systems, with near parallel lines and little or no interaction between resource supply and temperature (e.g., Fig. 3.3). In addition, production and growth may actually decline above this threshold. Although the mechanism of this response cannot be determined (e.g., resource limitation or adverse effects of temperature), the pattern remains consistent among all sub-systems studied and among multiple measures of carbon metabolism. Thus, the weakening of temperature dependence reported by others may actually represent the approach of a functional or physiological optimum temperature, below and above which bacterioplankton growth declines.

Concluding Comments

The difference in temperature dependence of BR and BP that we have observed has important implications with respect to identifying not only the magnitude of carbon processed by the bacterioplankton community (i.e., BCC), but also the way in which this carbon is processed (i.e., BGE). The temperature dependence of BR is strong (high r^2) and has a relatively steep slope, log-linear response across the annual temperature range, and similar slope and intercept among the different estuarine sub-systems. The relationship between BP and temperature, on the other hand, is characterized by much lower r^2 values, curvilinear response, and significantly different intercepts for sub-systems differing in degree of resource enrichment. These results would suggest that respiration is the metabolic process that is most directly influenced by temperature, whereas environmental factors such as nutrient and organic carbon resources play a much

larger role in regulating the magnitude of BP. Thus, although the basic temperature control of carbon consumption appears to be similar in all systems, the influence of temperature on bacterial production and growth appears to be strongly modulated by local environmental conditions. Collectively, our results indicate that the annual variability of carbon metabolism is regulated predominantly by the direct effect of temperature, which in the case of BP and BGE is then further modulated by the secondary effect of resource availability and/or quality. As a consequence, systems that follow the same basic seasonal progression in respiration and carbon consumption may differ substantially in terms of bacterial biomass production, growth, and growth efficiencies and thus differ in how organic matter is processed by the bacterioplankton community.

What remains unclear is the extent to which reduced rates of BP and lower BGE in summer months are driven by resource limitation versus the direct and adverse effect of elevated temperatures. Other studies conducted in the Chesapeake Bay have reported a decrease in benthic and plankton community respiration at high summer temperatures, attributing this to a decline in the availability of labile organic matter (Cowan and Boynton 1996; Smith and Kemp 1995). We did not observe a similar decrease in BR during summer months, suggesting that the lability or availability of organic matter was not limiting carbon metabolism at this time. Because the rank-order of the four sub-systems cannot be attributed to nutrient concentrations alone, there may be characteristics of the organic matter pool related to the nutrient content and quality of DOM that change seasonally and contribute to the precipitous decline in BP and BGE in warmer months. Further investigations into the direct effects of organic matter quality and elevated

temperatures are necessary to determine the mechanisms behind this decrease in BP and BGE.

The patterns in bacterioplankton metabolism observed in our study may provide evidence for the strategies that bacteria employ to maximize growth. The relatively strong temperature dependence of BR and BCC would suggest that within the constraints of temperature bacteria maintain the highest possible rates of organic carbon consumption at all times. In winter, when carbon consumption is kept low by temperature, BGE is generally higher as a result of weaker temperature constraints on growth. Thus the decline in total carbon consumption imposed by lower temperatures in winter may be offset by higher BGE, such that growth does not really decline proportionately to the decline in carbon consumption. As temperature increases, bacterioplankton may continue to maximize the consumption of organic matter, and BGE decreases simply because the effects of resource availability and other factors limit a commensurate increase in BP. Ultimately, bacteria may attain higher overall growth rates at higher temperatures by maximizing carbon consumption rather than growth efficiency. We observed the same basic pattern of a strong BR-temperature relationship and of declining BGE with temperature in all the different estuarine sub-systems we studied, and the fact that others have reported similar patterns in BGE (Bjørnsen 1986; Cowan and Boynton 1996; Griffiths et al. 1984; Tison and Pope 1980) would suggest that this may be a general strategy of aquatic bacterioplankton communities.

LITERATURE CITED

Apple, J. K., P. A. del Giorgio, and R. I. E. Newell. 2004. The effect of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. Journal of Coastal Research **45:** 110-133.

Autio, R. M. 1992. Temperature regulation of brackish water bacterioplankton. Hydrobiologia **37:** 253-263.

Bjørnsen, P. K. 1986. Bacterioplankton Growth-Yield in Continuous Seawater Cultures. Marine Ecology Progress Series **30:** 191-196.

Bronk, D. A., P. M. Glibert, T. C. Malone, S. Banahan, and E. Sahlsten. 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. Aquatic Microbial Ecology **15:** 177-189.

Carignan, R., D. Planas, and C. Vis. 2000. Planktonic production and respiration in oligotrophic Shield lakes. Limnology and Oceanography **45:** 189-199.

Carlsson, P., and D. A. Caron. 2001. Seasonal variation of phosphorus limitation of bacterial growth in a small lake. Limnology and Oceanography **46:** 108-120.

Caron, D. A., J. C. Goldman, and T. Fenchel. 1990. Protozoan respiration and metabolism, p. 307-322. *In* G. Capriulo [ed.], Ecology of Marine Protozoa. Oxford University Press.

Coveney, M. F., and R. G. Wetzel. 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an oligotrophic lake. Limnology and Oceanography **40:** 1187-1200.

Cowan, J. L. W., and W. R. Boynton. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors, and ecological significance. Estuaries **19:** 562-580.

Crawford, C. C., J. E. Hobbie, and K. L. Webb. 1974. The utilization of dissolved free amino acids by estuarine microorganisms. Ecology **55:** 551-563.

Daneri, G., B. Riemann, and P. J. L. B. Williams. 1994. In situ bacterial production and growth yield measured by thymidine, leucine and fractionated dark oxygen uptake. Journal of Plankton Research **16:** 105-113.

Davidson, K. 1996. Modeling microbial food webs. Marine Ecology Progress Series **145:** 279-296.

del Giorgio, P. A., D. F. Bird, Y. T. Prairie, and D. Planas. 1996. Flow cytometric determination of bacterial abundance in lake plankton using the green nucleic acid stain SYTO 13. Limnology and Oceanography **41:** 783-789.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29:** 503-541.

del Giorgio, P. A., and J. Davis. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399-424. *In* S. Findlay [ed.], Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Elsevier Science.

Den Heyer, C., and J. Kalff. 1998. Organic matter mineralization rates in sediments: A within- and among-lake study. Limnology and Oceanography **43:** 695-705.

Ducklow, H. W. 1994. Modeling the microbial food web. Microbial Ecology **28:** 303-319.

Eldridge, P. M., and M. E. Sieracki. 1993. Biological and hydrodynamic regulation of the microbial food web in a periodically mixed estuary. Limnology and Oceanography **38:** 1666-1679.

Felip, M., M. L. Pace, and J. J. Cole. 1996. Regulation of planktonic bacterial growth rates: The effects of temperature and resources. Microbial Ecology **31:** 15-28.

Fisher, T. R., L. W. Harding, D. W. Stanley, and L. G. Ward. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science **27:** 61-93.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by C:N ratio. Limnology and Oceanography **32:** 1239-1252.

Griffiths, R., B. Caldwell, and R. Y. Morita. 1984. Observations on microbial percent respiration values in arctic and subarctic marine waters and sediments. Microbial Ecology **10:** 151-164.

Hoch, M. P., and D. L. Kirchman. 1993. Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Marine Ecology Progress Series **98:** 283-295.

Iturriaga, R., and H. G. Hoppe. 1977. Observations of heterotrophic activity on photoassimilated organic matter. Marine Biology **40:** 101-108.

Jahnke, R. A., and D. B. Craven. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: A need for respiration rate measurements. Limnology and Oceanography **40:** 436-441.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A Two-Year Study of the Short-Term and Long-Term Sequestering of Nitrogen and Phosphorus in the Maryland National Estuarine Research Reserve, p. 1-100. Maryland National Estuarine Research Reserve.

Jorgensen, N., N. Kroer, R. B. Coffin, and M. P. Hoch. 1999. Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. Aquatic Microbial Ecology **18:** 247-261.

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennet, and J. C. Cornwell. 1994. Membrane inlet mass-spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. Analytical Chemistry **66:** 4166-4170.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria (Chapter 58), p. 776. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole [eds.], Handbook of Methods in Aquatic Microbial Ecology. CRC Press.

Kroer, N. 1993. Bacterial-Growth Efficiency on Natural Dissolved Organic-Matter. Limnology and Oceanography **38:** 1282-1290.

Lee, C. W., I. Kudo, T. Yokokawa, M. Yanada, and Y. Maita. 2002. Dynamics of bacterial respiration and related growth efficiency, dissolved nutrients and dissolved oxygen concentration in a subarctic coastal embayment. Marine and Freshwater Research **53:** 1-7.

Lomas, M. W., P. M. Glibert, F. Shiah, and E. M. Smith. 2002. Microbial processes and temperature in Chesapeake Bay: current relationships and potential impacts of regional warming. Global Change Biology **8:** 51-70.

Madigan, M. T., J. M. Martinko, and J. Parker. 2003. Brock Biology of Microorganisms, 10th ed. Prentice Hall.

Morita, R. Y. 1974. Temperature Effects on Marine Microorganisms, p. 75-79. *In* R. R. Colwell and R. Y. Morita [eds.], Effect of the Ocean Environment on Microbial Activities. University Park Press.

Painchaud, J., D. Lefaivre, and J. C. Therriault. 1987. Box model analysis of bacterial fluxes in the St. Lawrence Estuary. Marine Ecology Progress Series **41:** 241-252.

Pomeroy, L. R., and D. Deibel. 1986. Temperature Regulation of Bacterial-Activity During the Spring Bloom in Newfoundland Coastal Waters. Science **233:** 359-361.

Pomeroy, L. R., J. E. Sheldon, W. M. Sheldon, J. O. Blanton, J. Amft, and F. Peters. 2000. Seasonal changes in microbial processes in estuarine and continental shelf waters of the southeastern USA. Estuarine, Coastal and Shelf Science **51:** 415-428.

Pomeroy, L. R., J. E. Sheldon, W. M. Sheldon, and F. Peters. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. Marine Ecology Progress Series **117:** 259-268.

Pomeroy, L. R., and W. J. Wiebe. 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquatic Microbial Ecology **23:** 187-204.

Pomeroy, L. R., W. J. Wiebe, D. Deibel, R. J. Thompson, G. T. Rowe, and J. D. Pakulski. 1991. Bacterial Responses to Temperature and Substrate Concentration During the Newfoundland Spring Bloom. Marine Ecology Progress Series **75:** 143-159.

Ram, A. S. P., S. Nair, and D. Chandramohan. 2003. Bacterial growth efficiency in the tropical estuarine and coastal waters of Goa, southwest coast of India. Microbial Ecology **45:** 88-96.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. Aquatic Microbial Ecology **22:** 1-12.

Reay, D. S., D. B. Nedwell, J. Priddle, and J. C. Ellis-Evans. 1999. Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Applied and Environmental Microbiology **65:** 2577-2584.

Reinthaler, T., and G. J. Herndl. 2005. Seasonal dynamics of bacterial growth efficiencies in relation to phytoplankton in the southern North Sea. Aquatic Microbial Ecology **39:** 7-16.

Rivkin, R. B., and L. Legendre. 2001. Biogenic carbon cycling in the upper ocean: Effects of microbial respiration. Science **291:** 2398-2400.

Roland, F., and J. J. Cole. 1999. Regulation of bacterial growth efficiency in a large turbid estuary. Aquatic Microbial Ecology **20:** 31-38.

Rose, A. H. 1967. Thermobiology. Academic Press Inc.

Sampou, P., and W. M. Kemp. 1994. Factors regulating plankton community respiration in Chesapeake Bay. Marine Ecology Progress Series **110:** 249-258.

Sherr, B. F., and E. B. Sherr. 1996. Temporal offset in oceanic production and respiration processes implied by seasonal changes in atmospheric oxygen: The role of heterotrophic microbes. Aquatic Microbial Ecology **11:** 91-100.

Shiah, F. K., and H. W. Ducklow. 1994a. Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Marine Ecology Progress Series **103:** 297-308.

---. 1994b. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnology and Oceanography **39:** 1243-1258.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using super(3)H-leucine. Marine Microbial Food Webs **6:** 107-114.

Smith, E. M., and W. M. Kemp. 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. Marine Ecology Progress Series **116:** 217-231.

Tibbles, B. J. 1996. Effects of temperature on the incorporation of leucine and thymidine by bacterioplankton and bacterial isolates. Aquatic Microbial Ecology **11:** 239-250.

Tison, D. L., and D. H. Pope. 1980. Effect of temperature on mineralization by heterotrophic bacteria. Applied and Environmental Microbiology **39:** 584-587.

Toolan, T. 2001. Coulometric carbon-based respiration rates and estimates of bacterioplankton growth efficiencies in Massachusetts Bay. Limnology and Oceanography **46:** 1298–1308.

Zar, J. H. 1984. Biostatistical Analysis, Second ed. Prentice-Hall, Inc.

Zumdahl, S. S. 1989. Chemistry, 2nd ed. D. C. Heath & Company.

Table 3.1. Two-year means for watershed land use and environmental conditions in each of the sub-systems of Monie Bay National Estuarine Research Reserve.

	Little Monie Creek	Monie Creek	Little Creek	Open Bay
Agricultural land use	25%	23%	<1%	3%
Salinity	9.9	6.9	11.6	12.1
Total dissolved nitrogen (μM)	40.1	40.6	26.8	28.1
Total dissolved phosphorus (μM)	0.78	0.65	0.21	0.25
Dissolved organic matter (mg L^{-1})	8.9	11.5	7.7	6.0
Colored DOM (a_{350})	17	20	15	12

Table 3.2. Regression statistics for the relationship between temperature and bacterioplankton metabolic processes. All biological parameters are log transformed except BGE.

	All Data					0 to 15°C					15 to 30°C				
Parameter	slope	r^2	*F*	*p*	*n*	slope	r^2	*F*	*p*	*n*	slope	r^2	*F*	*p*	*n*
BR	0.105	0.66	277.6	<0.0001	147	0.126	0.45	40.9	<0.0001	52	0.087	0.33	53.8	<0.0001	113
BP	0.036	0.16	33.0	<0.0001	177	0.073	0.25	21.1	<0.0001	65	0.009	0.004	0.5	0.05	131
BCC	0.081	0.60	212.9	<0.0001	147	0.112	0.53	52.6	<0.0001	49	0.057	0.195	26.8	<0.0001	113
BGE	-0.014	0.34	73.3	<0.0001	147	-0.011	nr	4.4	0.05	52	-0.026	0.37	60.5	<0.0001	105
BR_{sp}	0.079	0.35	74.8	<0.0001	139	0.137	0.34	21.7	<0.0001	45	0.036	0.04	4.2	0.043	109
BP_{sp}	0.015	0.03	4.1	0.04	169	0.091	0.27	20.9	<0.0001	58	-0.030	0.03	4.1	0.046	127
BA	0.018	0.06	8.6	0.004	139	0.002	<0.001	<0.01	0.9	43	0.054	0.2	27.0	<0.0001	109

BR = bacterial respiration, BP = bacterial production, BCC = bacterial carbon consumption (BP+BR), BGE = bacterial growth efficiency, BR_{sp} = cell-specific respiration, BP_{sp} = cell-specific production, BA = total bacterial abundance.

Table 3.3. Probability values from analysis of covariance (ANCOVA) tests with temperature (0 to 30ºC) and sub-system (LMC, MC, LC, OB) as model effects.

	Model Effects				
Parameter*	Temperature	Sub-system	Interaction	r^2	*n*
BR	<0.0001	0.08	0.9	0.65	129
BP	<0.0001	<0.0001	0.8	0.34	169
BP_{filt}	<0.0001	0.0004	0.7	0.23	169
BCC	<0.0001	0.0002	0.8	0.63	139
BGE	<0.0001	0.004	0.3	0.39	129
BR_{sp}	<0.0001	0.8	0.7	0.36	139
BP_{sp}	<0.0001	0.002	0.7	0.17	129

*Table parameters defined in Table 3.2.

Table 3.4. Estimates of Q_{10} values for measures of bacterial metabolism calculated at different temperature ranges ($Q_{10} = (R_1/R_2)^{10/(T1-T2)}$).

Parameter	0 to 15ºC	15 to 30ºC	0 to 30ºC
BR	3.8	2.2	1.6
BP	1.3	1.10	1.4
BCC	3.0	1.8	2.3
BR_{sp}	3.9	1.4	4.4
BP_{sp}	2.5	-1.0	1.2
MEAN	3.0	1.1	2.2
BGE	-1.3	-3.6	-1.5

FIGURES

Fig. 3.1. Study site (Monie Bay NERR) with location and number of each sampling station. Land use is designated as agriculture, forest, residential, and marsh.

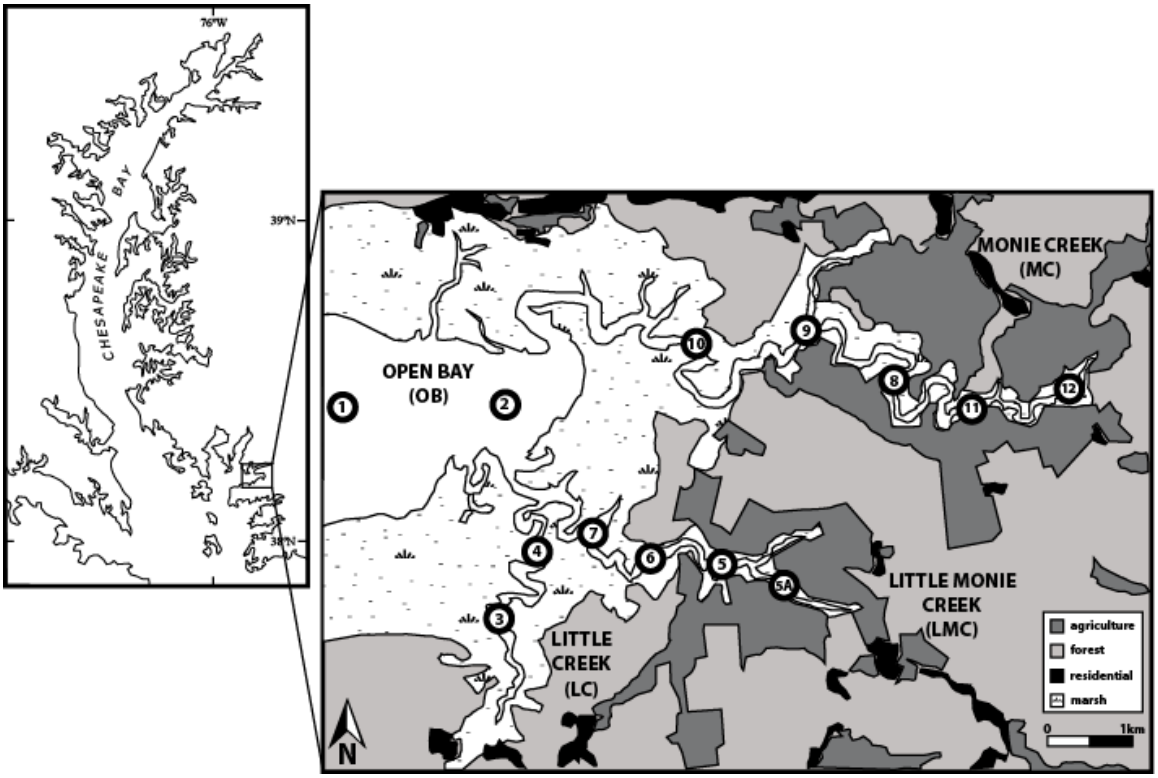

76°W
39°N
38°N
CHESAPEAKE BAY
OPEN BAY
(OB)
MONIE CREEK
(MC)
LITTLE MONIE
CREEK
(LMC)
LITTLE
CREEK
(LC)
1
2
3
4
5
5A
6
7
8
9
10
11
12
N
agriculture
forest
residential
marsh
0
1km

Fig. 3.2. Arrhenius plots illustrating the temperature dependence of A) bacterial respiration (BR) and B) bacterial production. Regression statistics are reported in Table 3.2.

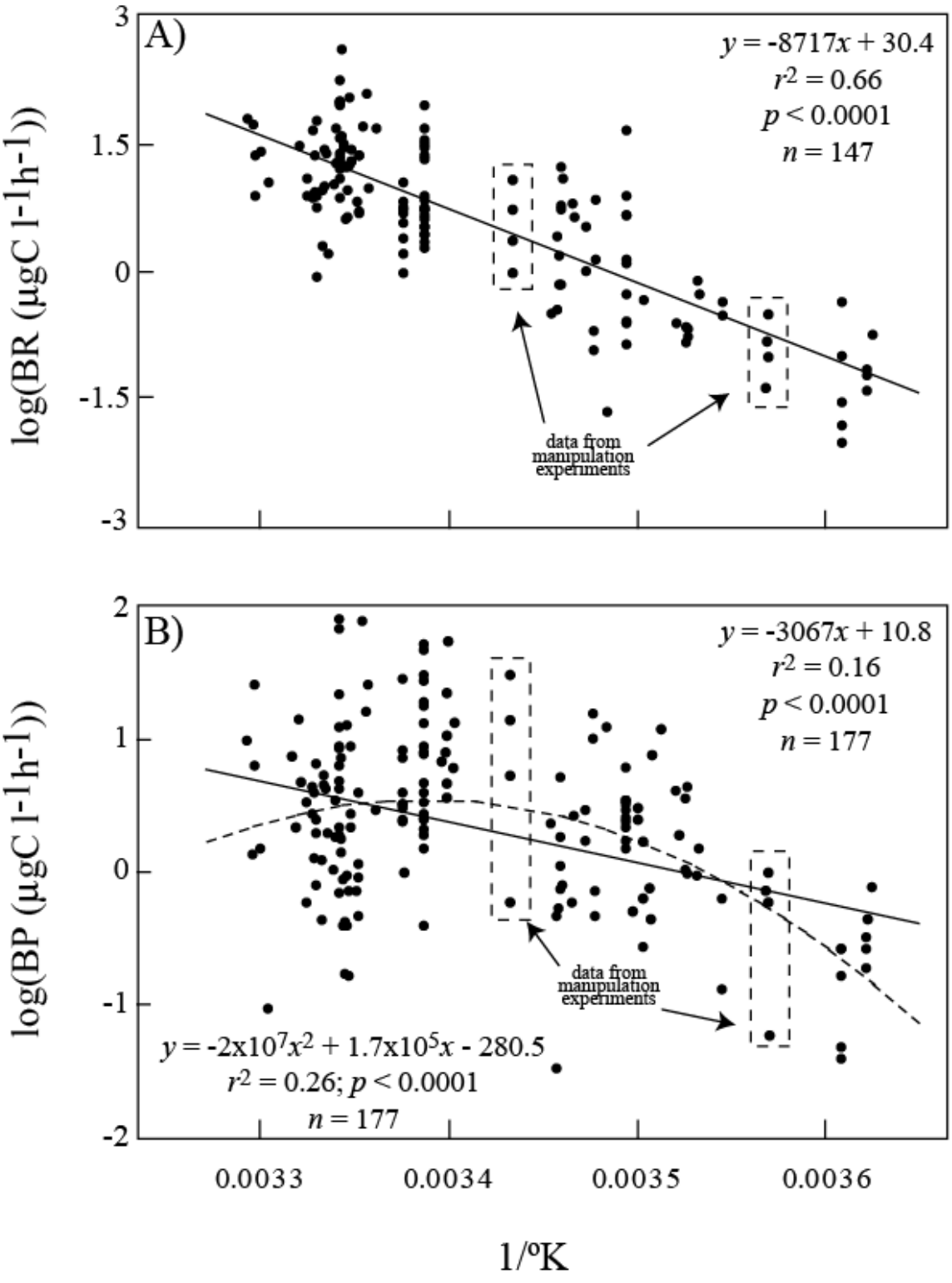


Fig. 3.3. Linear relationships between bacterial growth efficiency and temperature for A) the entire dataset and B) warmer versus colder ambient water temperatures. Regression statistics are reported in Table 3.2.

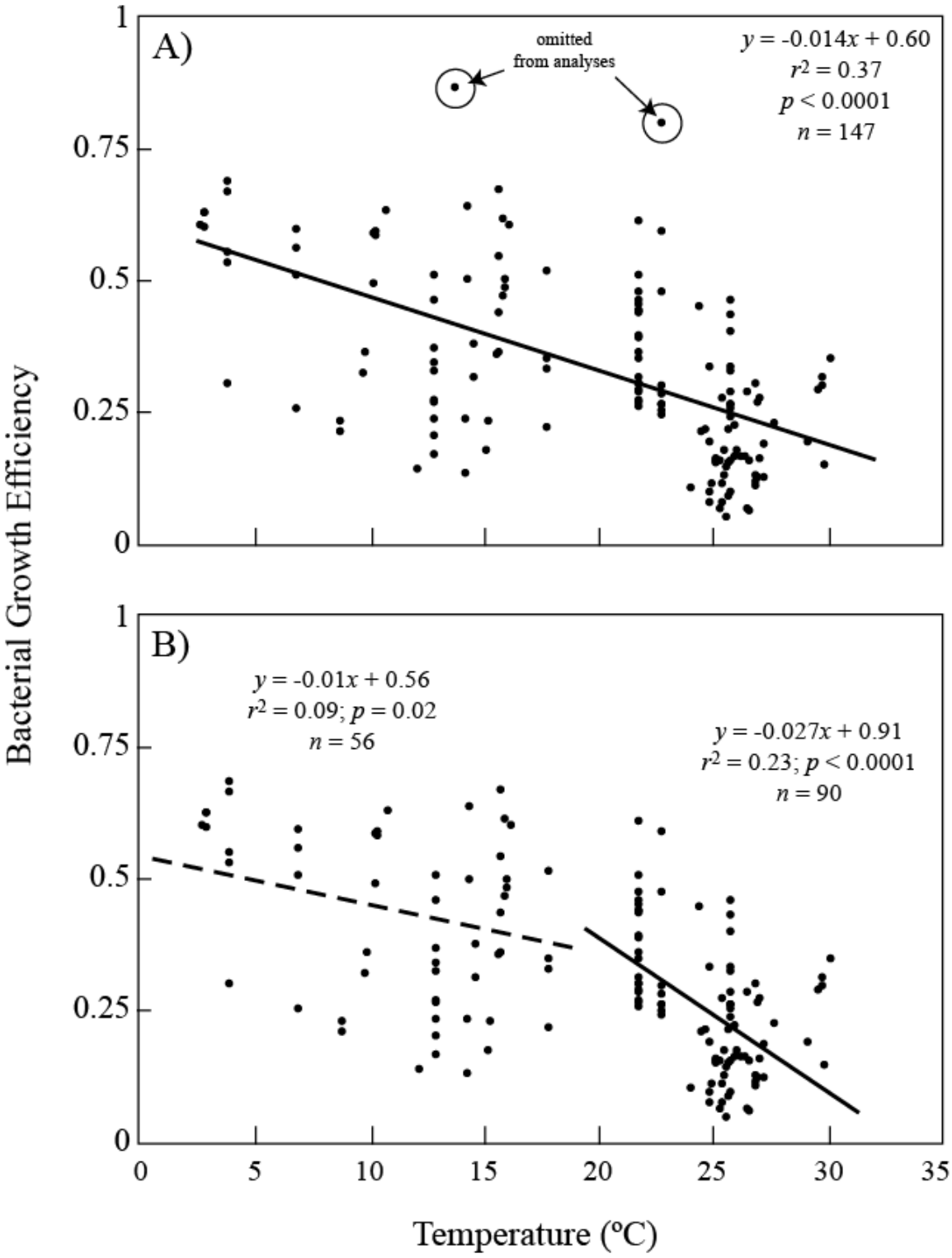

A)
omitted
from analyses
y = -0.014x + 0.60
r2 = 0.37
p < 0.0001
n = 147
B)
y = -0.01x + 0.56
r2 = 0.09; p = 0.02
n = 56
y = -0.027x + 0.91
r2 = 0.23; p < 0.0001
n = 90
Bacterial Growth Efficiency
Temperature (°C)
0
0.25
0.5
0.75
1
0
5
10
15
20
25
30
35

Fig. 3.4. Relationship between bacterioplankton production (BP) and temperature in each of the estuarine sub-systems of Monie Bay (i.e., LMC, MC, LC, OB). The inflection point of the second order polynomial describing the temperature response of the entire dataset (i.e., 22°C) is indicated by the vertical dotted line. Rates of BP from temperature manipulation experiments are indicated by boxes.

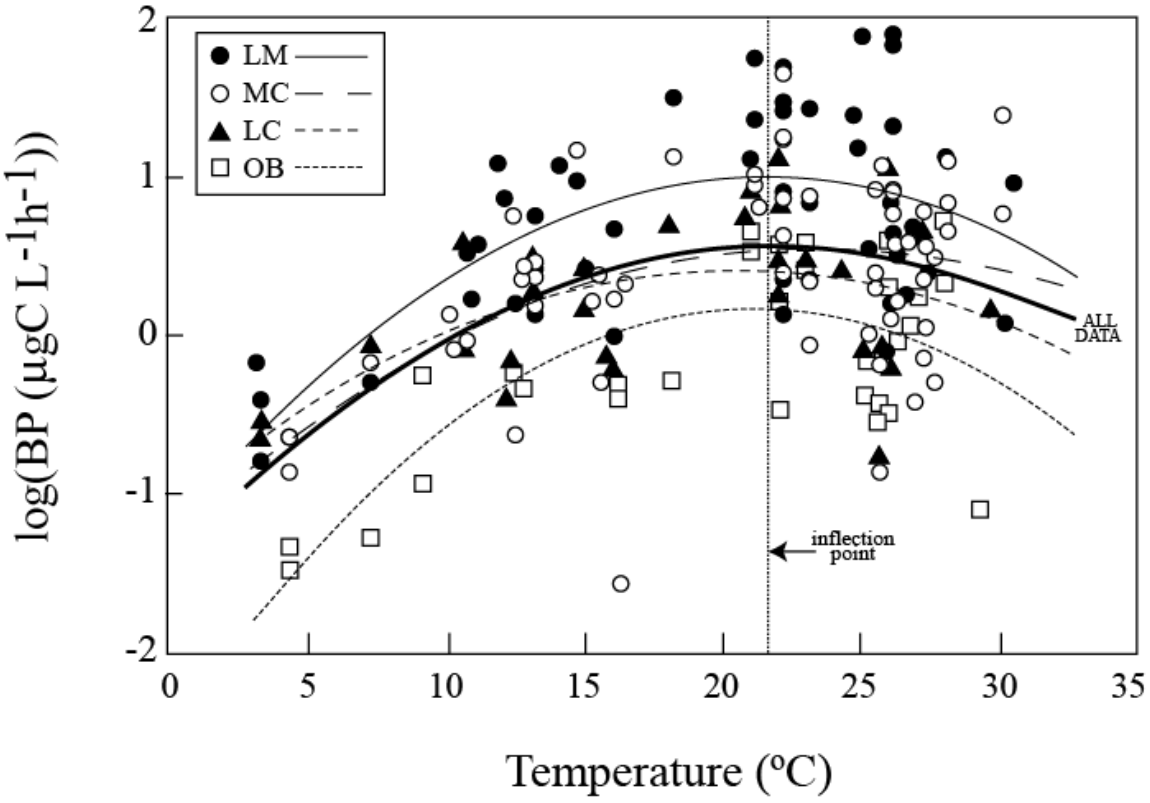

LM
MC
LC
OB
ALL DATA
inflection point
log(BP (µgC L-1h-1))
Temperature (ºC)
2
1
0
-1
-2
0
5
10
15
20
25
30
35

Fig. 3.5. Comparison of the temperature dependence of A) bacterioplankton respiration (BR) versus production (BP) and B) cell-specific respiration (BRsp) versus cell-specific production (BPsp). Dotted lines represent 95% confidence intervals.

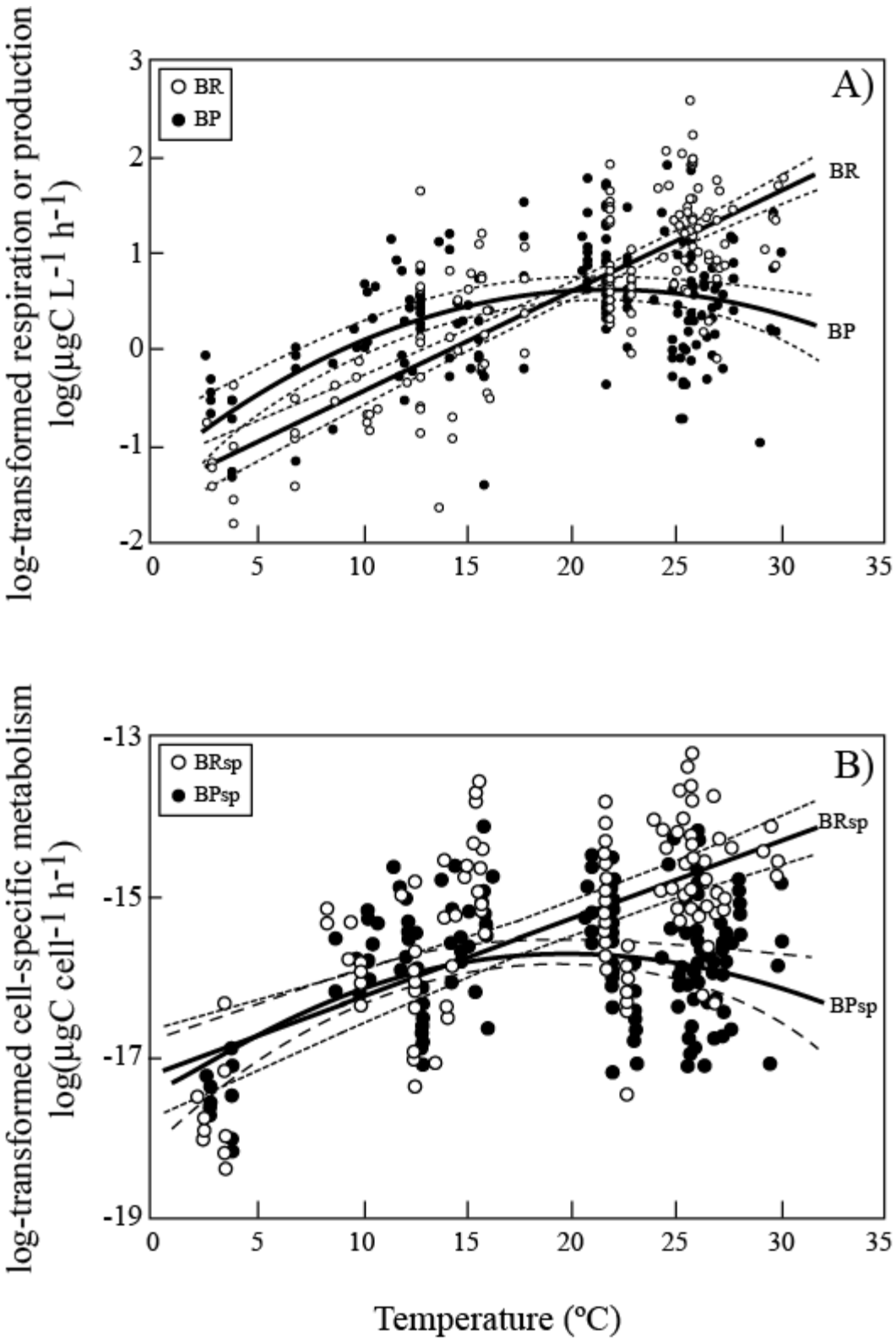
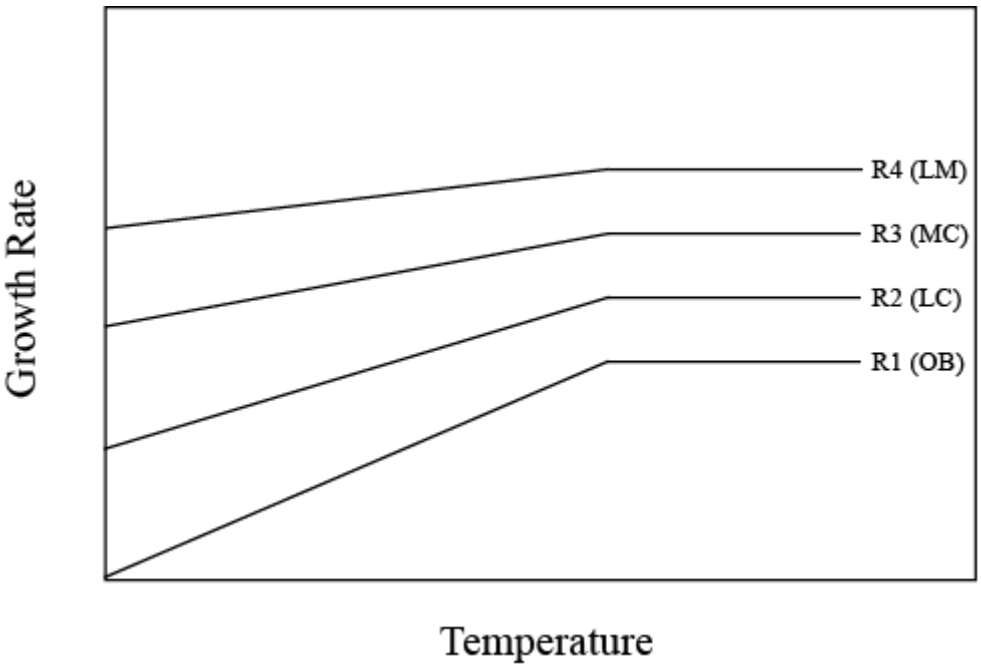

A)
BR
BP
log-transformed respiration or production
log(µgC L-1 h-1)
3
2
1
0
-1
-2
0
5
10
15
20
25
30
35
B)
BRsp
BPsp
log-transformed cell-specific metabolism
log(µgC cell-1 h-1)
-13
-15
-17
-19
Temperature (°C)

Fig. 3.6. Conceptual model of the temperature response of bacterioplankton growth in systems differing in resource supply, where R1-R4 represent increasing resource concentrations such as those encountered among the estuarine sub-systems of Monie Bay (from Felip et al. 1996).


Growth Rate
R4 (LM)
R3 (MC)
R2 (LC)
R1 (OB)
Temperature

CHAPTER IV

The variability and regulation of bacterioplankton carbon metabolism in the tidal creeks of a small estuarine system

ABSTRACT

In this paper we report results from a 2-yr study of bacterial production (BP), respiration (BR), growth efficiency (BGE), and carbon consumption (BCC) in the open bay and tidal creeks of a salt-marsh system in Chesapeake Bay, USA. During the course of our study, BP and BR ranged from 0.2 to 6.8 and 0.1 to 13.5 μg C L^{-1} h^{-1}, with overall means of 1.8 ± 0.1 and 2.7 ± 0.2 μg C L^{-1} h^{-1}, respectively (n = 139). Total bacterial carbon consumption ranged from 0.4 to 15.9 μg C L^{-1} h^{-1}, with an overall mean of 3.75 ± 0.2 μg C L^{-1} h^{-1}. Mean BGE for the 2-yr sampling period was similar to that reported for other estuarine systems (0.32 ± 0.02) and of comparable range (i.e., 0.06 to 0.68). Despite extensive variability in growth efficiency relative to carbon consumption and the disparate effect of temperature on these measures of carbon metabolism, a positive coupling of BGE and BCC emerged from our data. Analyses indicate that the coherence of BCC and BGE as well as their magnitude may be regulated by dissolved organic matter (DOM) lability. The effects of DOM quality on carbon metabolism were investigated further by measuring the uptake of dissolved nutrients by bacterioplankton during short-term incubations. Results from these analyses suggest that nutrient availability and the nutrient content (e.g., C:N ratios) of DOM may not be as important as frequently believed in the regulation of BGE, and aspects of organic matter quality related to lability, energetic content, or chemical structure may prove to be a more important determining factor.

INTRODUCTION

The range and variability of bacterial growth efficiency (BGE) in natural waters (del Giorgio and Cole 1998) indicates that bacterial production (BP) and respiration (BR) are frequently uncoupled. Thus, it is unlikely that any single measure of bacterioplankton carbon metabolism can be used to predict the magnitude or variability of any other. The lack of consistent coherence among aspects of carbon metabolism may be attributed to the wide range of environmental factors that have significant, but often disproportionate, effects on these processes. Manipulative experiments and field studies alike have identified significant effects of temperature (Apple et al. submitted), dissolved organic carbon (Shiah and Ducklow 1994a), inorganic nutrients (Smith and Kemp 2003), nutrient stoichiometry (Goldman et al. 1987), salinity (del Giorgio and Bouvier 2002), and organic substrate source and quality (Amon et al. 2001; Revilla et al. 2000) on bacterioplankton metabolism, with evidence that these parameters influence different pathways of carbon metabolism differently. For example, both bacterioplankton growth (μ) and production tend to respond positively to increases in inorganic nutrients and organic carbon concentrations (Carlson and Ducklow 1996; Caron et al. 2000), whereas BR, and thus total bacterioplankton carbon consumption (BCC = BP+BR), may be regulated predominantly by the availability of dissolved organic carbon alone. The regulation of BGE is more complex, possibly involving dissolved nutrient stoichiometry and organic matter quality in addition to nutrient availability. Given the varied response of bacterioplankton to their environment, there is no reason *a priori* to assume that all aspects of metabolism will respond similarly to changes in environmental conditions or that they are regulated by the same environmental factors. There is, however, general

agreement that nutrient availability and dissolved organic matter (DOM) quality are important in regulating both the magnitude of carbon consumed (i.e., BCC) as well as the way in which this carbon is processed (i.e., BGE).

Of the numerous environmental factors that regulate the magnitude and variability of bacterioplankton metabolism in temperate systems, temperature effects have received the greatest attention and are probably the easiest to predict accurately (Apple et al. submitted; Pomeroy et al. 1995; Raymond and Bauer 2000; Rivkin and Legendre 2001; Sampou and Kemp 1994; Shiah and Ducklow 1994b). Although bacterioplankton metabolism is broadly dependent upon temperature, these effects do not necessarily override the effects of other environmental conditions (Chapter III, Pomeroy and Wiebe 2001). For example, regressions of temperature versus carbon metabolism reported in Chapter III generated similar slopes but significantly different intercepts when systems differing in extent of resource enrichment were compared. The authors concluded as have others (Pomeroy and Wiebe 2001) that temperature and resource supply have simultaneous but different effects on carbon metabolism. Having already described the temperature-dependence of bacterioplankton carbon metabolism in Monie Bay in Chapter III, an investigation of temperature-independent environmental factors and their effect on the regulation of different aspects of bacterioplankton carbon metabolism is a natural continuation of this previous work.

Organic matter quality is frequently estimated by evaluating the elemental composition of organic matter available for consumption, with the underlying assumption that the energetic cost of growth should decrease (i.e., BGE increases) as DOM becomes enriched with nitrogen (N) and phosphorus (P) and the stoichiometry more closely

resembles that of bacterioplankton biomass (Goldman et al. 1987; Jørgensen et al. 1994). This is the most commonly studied and well described aspect of DOM quality (Kirchman 2000b). In contrast, an alternate measure of the quality of organic matter pertains to its chemical structure, composition, and energetic content, whereby higher quality organic matter is that which provides the greatest energy yield, which is in turn a function of the strength of molecular bonds (e.g., lability) or the oxidation state of the molecules being consumed (Linton and Stevenson 1978). Although these measures of quality represent fundamentally different properties of DOM, heterogeneous mixtures of DOM that occur in natural aquatic systems make it extremely difficult to discriminate between the two and identify their respective influence on bacterioplankton carbon metabolism.

This study focuses on the influence of DOM quality on the magnitude and variability of BGE and BCC and addresses two fundamental hypotheses. The first is that BGE changes as a function of the magnitude of carbon consumed by the bacterioplankton community. To test this hypothesis, we investigate the coupling between paired estimates of BCC and BGE using a comprehensive two-year dataset of BGE and BCC in a tidally-influenced salt marsh system. The second hypothesis is that BGE is regulated by the relative availability of dissolved nutrients and nutrient content of DOM. This hypothesis was tested using the relationship between BGE, ambient nutrient concentrations, dissolved nutrient stoichiometry, and the uptake of dissolved nutrients by bacterioplankton.

METHODS

Sample Collection

Our study was conducted in the Monie Bay component of Maryland's National Estuarine Research Reserve System (MDNERRS), a temperate salt-marsh system located on the eastern shore of Chesapeake Bay (38°13.50'N, 75°50.00'W) and consisting of an open bay and three tidally-influenced creeks (Fig. 4.1). Conditions in the tidal creeks and the utility of this reserve as a model system for investigating estuarine bacterioplankton communities have been described in detail by Apple et al. (2004).

In addition to the original sites of previous studies (Apple et al. submitted; Apple et al. 2004), we established three additional sites located in the upper reaches of the two agriculturally developed creeks (Fig. 4.1). Each of the ten original sites was visited monthly between March 2000 and January 2002, with biweekly sampling during summer months (June – August). More extensive transects including the additional sites were conducted periodically throughout the sampling period. Water temperature and salinity were recorded at each site. Approximately 20 L of near-surface (<0.5 m) water were collected from each site between 0800h and 1000h immediately following high tide and transported in 20L HDPE Nalgene carboys back to the laboratory for filtration. Elapsed time from sampling to filtration rarely exceeded 2 h.

Water Column Analyses

Samples for DOC analysis were filtered through a Whatman GF/F filter, acidified with 100 μl of 1N phosphoric acid, and held at 4˚C until analysis. DOC content was determined with a Shimadzu high-temperature catalyst carbon analyzer (Sharp et al. 1995). Samples for nutrient analyses were filtered through Whatman GF/F filter and

frozen at -25˚C for later analysis of phosphate (i.e. PO_4^{3-}, soluble reactive phosphorus), nitrite and nitrate (NO_x) following (Strickland and Parsons 1972), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) following (Valderrama 1981), and ammonium (NH_4^+) following (Whitledge et al. 1981). Photospectral absorbance of DOC was determined on GF/F filtered samples by performing absorbance scans (290-700 nanometers) using a Hitachi U-3110 spectrophotometer and either 1- or 5-centimeter quartz cuvettes, depending upon the relative concentration of CDOM. Absorptivity at 350 nm (a_{350}) was used as an index of CDOM concentrations (Moran et al. 2000; Blough and Del Vecchio 2001). Specific absorbance (a_{350}*) was determined by dividing a_{350} by ambient DOC concentrations (Hu et al. 2002; Moran et al. 2000). Chlorophyll *a* was determined with standard methods using a Turner 10-AU fluorometer (Strickland and Parsons 1972).

Estimates of Bacterioplankton Carbon Metabolism

Upon return to the lab, a small sub-sample was removed from each carboy for determining total BP, colored dissolved organic matter (CDOM), and concentrations of inorganic nutrients, dissolved organic carbon (DOC), and chlorophyll-*a*. Estimates of filtered BP and BR were determined by gently passing several liters of sample water through an AP15 Millipore filter (~1 μm) using a peristaltic pump and incubating in the dark at *in situ* field temperature. Water samples were contained in a flow-through incubation assembly consisting of two 4L Erlenmyer flasks and sub-sampled at 0, 3, and 6 h. In addition, changes in dissolved nutrient concentrations from which estimates of nutrient uptake would be derived were determined in a subset of these incubations (n = 93) by calculating the difference in dissolved nutrient concentrations between 0 and 18h

(see Appendix B). Bacterial production was estimated using incorporation of ^{3}H-leucine following modifications of Smith and Azam (1992) and assuming a carbon conversion factor of 3.1 Kg C · mol leu^{-1} (Kirchman 1993). Bacterial respiration was determined by measuring the decline of oxygen concentration over the course of the 6 h incubation, with longer incubations (8 h) used at lower ambient water temperatures (<15°C). Dissolved oxygen concentrations were measured using membrane-inlet mass spectrometry (Kana et al. 1994). A respiratory quotient (RQ) of 1.0 was used to convert oxygen measurements to carbon values (del Giorgio, L'Université du Québec à Montréal, personal communication). Rates of BP and BR were reported as μg C L^{-1} h^{-1}. Bacterioplankton carbon consumption was calculated by adding simultaneous measurements of filtered BP and BR, and BGE was calculated as the ratio of filtered BP and BCC (BGE = BP/(BP + BR)). Lability of DOC was determined on a sub-set of the samples (n = 14) by filtering approximately 1L of sample water through 0.2μm Sterivex filters into duplicate 500ml borosilicate glass flasks and inoculating each with 10ml of AP15 filtered sample water (see Appendix B). Consumption of DOC was determined by measuring DOC concentrations in each flask every few days for 24 days. Consumption of DOC was reported as lability (μg C L^{-1} d^{-1}) and percent labile DOC (i.e., DOC consumed/initial [DOC]).

Statistical Analyses

All statistical analyses, including standard least squares regressions, step-wise multiple regressions, and analyses of variance (ANOVA) and covariance (ANCOVA) were performed using JMP 5.0.1 statistical software package (SAS Institute, Inc.). The effect of temperature was eliminated from our data by calculating residuals of the

temperature dependencies described previously for this system (Chapter III) for each measured aspect of bacterioplankton carbon metabolism. The effect of temperature-independent environmental conditions were then explored using step-wise multiple regressions, with temperature residuals for BCC, BGE, and BP as dependent variables and salinity, log-transformed absorbance and nutrient concentrations (a_{350}, NH_4^+, NO_X, PO_4^{3-}, TDN, DON, TDP, DOC), and dissolved nutrient stoichiometry (C:N, N:P, C:P) as independent variables. Data were log-transformed to meet requirements for normal distribution for subsequent statistical analyses.

RESULTS

Water Column Chemistry

Two-year means of measures of water column chemistry generally reflected patterns reported previously in Monie Bay (Apple et al. 2004), with significantly higher nutrient concentrations in the two agriculturally impacted creeks (LMC and MC) relative to the other sub-systems (Fig. 4.2A,B). Total dissolved nitrogen, DON, and TDP were significantly higher in LMC and MC than in both LC and OB (Fig. 4.2A; Tukey-Kramer HSD; $n = 166$; $\alpha = 0.05$; $p<0.0001$). Phosphate concentrations were similar among the three creeks and significantly higher than OB (Fig. 4.2B; Tukey-Kramer HSD; $n = 166$; $\alpha = 0.05$; $p=0.005$). Ammonium concentrations were also similar among the three creeks, although LMC was the only sub-system in which ammonium concentrations were significantly higher than that of OB (Fig. 4.2A;Tukey-Kramer HSD; $n = 166$; $\alpha = 0.05$; $p=0.01$). There was no significant difference in 2-yr means for NO_X among all sub-systems.

The lowest salinities were consistently recorded in MC, with a significantly lower 2-yr mean (5.8) relative to all other sub-systems (Fig. 4.2C). Salinities in LMC were intermediate and ranged from 2 to 15, with a 2-yr mean (9.2) significantly lower than LC and OB and significantly higher than MC. Mean salinities in LC and OB (11.2 and 12.0, respectively) were statistically similar (Tukey-Kramer HSD; $n = 178$; $\alpha = 0.05$; $p<0.0001$), higher than that of LMC or MC, and ranged from 4 to 16 in LC and 10 to 16 in OB.

Dissolved organic carbon, absorbance of DOC at 350 nm, and specific absorbance among the sub-systems exhibited an inverse hierarchy to that observed for salinity (Fig. 4.2D, Table 4.1). The highest DOC concentrations were observed in MC (12.5 mg L^{-1}), intermediate in LMC (9.6 mg L^{-1}), and lowest in LC and OB (7.7 and 6.0 mg L^{-1}, respectively). Both absorbance and specific absorbance were highest in MC (0.23 and 0.020, respectively) and lowest in OB (0.07 and 0.012, respectively). Although absorbance of DOC was significantly different in LC and LMC, specific absorbance in LC was similar to that of both LMC and OB (Tukey-Kramer HSD; $n = 119$; $\alpha = 0.05$; $p<0.0001$). Mean chlorophyll-*a* concentrations among the sub-systems ranged from 7.4 to 15.7 μg L^{-1} and were highest in OB, lowest in LC, and statistically similar among the three tidal creeks (Table 4.1).

Other characteristics of DOM that differed systematically included measures of lability and dissolved nutrient stoichiometry (Table 4.1). Both lability and percent labile were highest in LMC. Lability was lowest in OB and similar in both LC and MC. The percentage of labile DOC in MC was lower than all other systems despite the highest concentrations of DOC. Little Creek had the highest C:N and N:P ratios of all systems

(Tukey-Kramer HSD; $n = 183$; $\alpha = 0.05$; $p<0.0001$). C:N ratios were similar among the other sub-systems and N:P ratios were similar and significantly lower in the two agriculturally developed creeks (Tukey-Kramer HSD; $n = 183$; $\alpha = 0.05$; $p<0.0001$).

Spatial and Seasonal Patterns in BCC and BGE

Bacterial respiration and production were quite variable, ranging from 0.1 to 13.5 and 0.1 to 5.8 μg C L^{-1} h^{-1}, respectively (Table 4.2), with a relatively weak but significant positive correlation between log-transformed values (Fig. 4.3; $r^2 = 0.17$; $n = 138$; $p<0.0001$). The two-year mean for BR (3.7 ± 0.2 μg C L^{-1} h^{-1}; $n = 139$) was higher than that of both total and filtered BP (Table 4.2). Mean BP (1.8 ± 0.1 μg C L^{-1} h^{-1}; $n = 138$) was always higher than that of the filtered fraction (1.0 ± 0.1 μg C L^{-1} h^{-1}; $n = 139$), although the range of these two measures of production was similar (0.2 to 6.8 versus 0.1 to 5.8 μg C L^{-1} h^{-1}). On average, bacterial production attributed to the filtered fraction accounted for 63% of total BP and ranged from 47 to 90%. The 2-yr mean for bacterial carbon consumption (BCC) was 3.75 ± 0.2 μg C $liter^{-1}$ hr^{-1} ($n = 139$) and ranged from 0.4 to 15.9 μg C L^{-1} h^{-1}, while the overall mean growth efficiency (BGE) for the entire system was 0.32 ± 0.02 (n = 139) and ranged from 0.06 to 0.68.

Carbon Metabolism Among Sub-Systems

Values for all measures of bacterioplankton carbon metabolism were consistently higher in LMC and lowest in OB (Fig. 4.4A), with intermediate values measured in MC and LC. This hierarchy was observed for 2-yr means as well as at each individual sampling event. Bacterial carbon consumption was highest in LMC and similar among the other three sub-systems. Bacterial respiration exhibited a similar pattern to that of

BCC, although differences among the three creek systems were not significant. Both filtered and total BP were significantly higher in LMC than all other systems. As observed with BCC, there was a distinct pattern in BGE among the sub-systems, with highest BGE recorded in LMC and lowest in OB (Fig. 4.4B). Two-year mean BGE in the tidal creeks was significantly higher than OB (Tukey-Kramer HSD; $p = 0.09$) and highest in LMC and LC.

Carbon Metabolism Among Seasons

Bacterial carbon consumption was highest in summer (Jun – Aug) and fall (Sep – Nov) and lowest in winter (Dec – Feb) and spring (Mar – May; Fig. 4.5A). Two-year means for BCC were similar in summer (5.1 ± 0.3; $n = 61$) and fall (4.1 ± 0.4; $n = 31$), as well as in spring (2.1 ± 0.4; $n = 38$) and winter (0.8 ± 0.7; $n = 9$). The differences among seasons were highly significant (Fig. 4.5A; Tukey-Kramer HSD; $n = 139$; $p < 0.0001$). The pattern in BGE among seasons was the opposite of that observed for BCC (Fig. 4.5B), with the lowest mean efficiencies recorded for summer months (0.23 ± 0.02; $n = 61$), highest in winter (0.57 ± 0.05; $n = 9$), and intermediate in spring (0.37 ± 0.02; $n = 38$) and fall (0.35 ± 0.03; $n = 31$). Mean BGE was significantly higher in winter and significantly lower in summer, and similar in spring and fall (Tukey-Kramer HSD; $n = 139$; $p < 0.0001$).

Nutrient Uptake and Carbon Metabolism

Changes in dissolved nutrient concentrations during respiration incubations were highly variable. Consumption of NO_X and DON was observed in almost all incubations (90 out of 93), with mean uptake of -0.07 ± 0.01 μM h^{-1} and -0.37 ± 0.05 μM h^{-1} for NO_X and DON and maximum uptake -0.47 and -1.5 μM h^{-1}, respectively. In contrast, we

observed both uptake and production of ammonium, with a maximum uptake of -0.26 $\mu M\ h^{-1}$ and maximum production of 0.18 $\mu M\ h^{-1}$. The overall mean of -0.001 ± 0.005 $\mu M\ h^{-1}$ and the similarity in the number of incubations in which uptake of NH_4^+ versus production was observed (Table 4.3) suggests a general balance between uptake and production of ammonium in this system. The balance between uptake and production was observed in all sub-systems but LC, where the majority of incubations (i.e., 13 out of 18) exhibited net uptake of ammonium. Changes in phosphorus concentrations during incubations were variable, ranging from -0.076 to 0.083 $\mu M\ h^{-1}$ for TDP and -0.028 to 0.029 $\mu M\ h^{-1}$ for PO_4^{3-}. There was an overall balance between uptake and remineralization of all forms of dissolved phosphorus, although phosphate consumption was observed in the majority (66%) of the incubations.

Ambient nutrient concentrations, estimates of BGE, and the uptake of various constituents of the dissolved nutrient pool were combined to explore the influence of various aspects of the nutritive quality of DOM on growth efficiency (Fig. 4.6). There was no relationship between BGE and dissolved nutrient stoichiometry (DOC:DON; Fig. 4.6A), uptake ratios of total carbon and nitrogen (BCC:TDN uptake; Fig. 4.6B), and estimates of the C:N ratio of DOM consumed by bacterioplankton (BCC:DON uptake; Fig. 4.6C). Identical results were observed when arcsine-transformed values for BGE and nutrient ratios were evaluated. Similarly, we observed no correlation between BGE and the proportion of N or P that was derived from organic vs. inorganic sources (Fig. 4.7). In general, most of the N consumed by bacterioplankton in all sub-systems appeared to be derived from DON, although the contribution of inorganic to total nitrogen uptake was significantly higher in OB than the three tidal creeks (Fig. 4.8A).

Phosphorus uptake exhibited a similar pattern among sub-systems (Fig. 4.8B). Uptake in LC and LMC was generally balanced between organic and inorganic sources, although P-uptake in MC was dominated by organic sources and that in OB was almost exclusively phosphate.

Relationship Between Carbon Consumption and Growth Efficiency

We observed a significant negative relationship between BGE and BCC that was relatively weak when the entire dataset was considered ($r^2 = 0.18$) but improved dramatically when sub-systems were considered individually (Fig. 4.9A). Stronger relationships were observed for data from LC ($r^2 = 0.50$; $n = 25$; $p<0.0001$; regression not shown) and OB ($r^2 = 0.38$; $n = 27$; $p = 0.0006$; lower hatched line) when compared to the nutrient enriched LMC ($r^2 = 0.21$; $n = 40$; $p = 0.003$; upper hatched line) and MC ($r^2 = 0.18$; $n = 47$; $p = 0.003$; regression not shown). The highest and lowest *y*-intercepts were observed for regression of data from OB and LMC, respectively, and these were statistically different (ANCOVA; $r^2 = 0.30$; $n = 147$; $F = 15.5$; $p < 0.0001$). Regressions of data from LC and MC (not shown) had *y*-intercepts that were intermediate relative to those of OB and LMC and similar to that of the entire dataset (solid line).

The negative relationship between BGE and BCC disappeared when residual values from their temperature dependence were considered (Fig. 4.9B). Despite the apparent lack of relationship between temperature residuals, among-system differences in the magnitude of BGE and BCC persisted and appeared to be related to degree of enrichment, with data from the OB located predominantly in the lower left quadrant and those from LMC in the upper right quadrant. Data from MC and LC were dispersed uniformly around the central axes. Although this pattern was not readily evident when

the entire dataset was considered (Fig. 4.9B), regression of mean residuals from each sub-system positive coupling of BGE and BCC (Fig. 4.10A) that appeared to be related to the mean lability of DOM in each sub-system (Fig. 4.10B).

Multiple Regression Analyses

Stepwise multiple regressions models of temperature residuals accounted for 41 and 32 percent of the variability in BCC and BGE, respectively, while the variability in BP was not well described (Table 4.3). Specific absorbance was an important component in models for both BCC and BGE, explaining over 36% of the variability in each. Specific absorbance was positively correlated with BCC and negatively with BGE. Both BGE and BP were positively correlated with dissolved phosphate, which accounted for most of the variability in each (i.e., 61 and 58%, respectively). Dissolved inorganic nitrogen (i.e., DIN or NH_4^+) was positively correlated with BCC and BGE, although it explained less of the variability than other model components. Multivariate analyses indicated that all measures of carbon metabolism were to some extent negatively correlated with ambient NO_X concentrations.

DISCUSSION

Organic Matter Regulates Carbon Metabolism

Environmental factors such as nutrient availability, organic carbon quality and supply, and salinity tend to covary in estuaries (Fisher et al. 1988), making it challenging to identify which is more important in regulating bacterioplankton carbon metabolism in these systems. As a result, it is difficult to determine the extent to which changes in metabolism reported along estuarine gradients (Apple et al. 2004; Revilla et al. 2000; Smith and Kemp 2003) are the direct effect of one factor, the interaction of multiple factors, or simply a general response to resource enrichment that is too complex to elucidate. The Monie Bay system provides a useful venue for investigating factors because it offers steep gradients in a wide range of environmental conditions and systematic patterns in nutrient and DOM concentrations and composition (Apple et al. 2004). In this regard, well-orchestrated comparisons among the four Monie Bay sub-systems can be used to isolate the key environmental factors influencing BCC and BGE that might otherwise not be apparent. Because the range and variability of environmental conditions in Monie Bay are similar to those reported for many temperate estuaries (Fisher et al. 1988; Sharp et al. 1982), these findings may be applicable to a wide range of aquatic systems.

In our preliminary study of BP in Monie Bay (Apple et al. 2004), we identified a general positive response of bacterioplankton to nutrient enrichment and hypothesized that differences in carbon metabolism among sub-systems may be attributed to the source and quality of DOM. In the present study, we continue this line of research and use similar comparisons among systems to determine if other aspects of bacterioplankton

carbon metabolism respond positively to enriched conditions and investigate further the influence of organic matter quality. Results from the present study suggest that bacterioplankton carbon consumption, respiration, and growth efficiency increase in response to system-level nutrient enrichment, and that this response is indeed modulated by the quality of organic matter. Our comparisons among sub-systems also indicate that not all aspects of carbon metabolism respond similarly to enriched conditions and that each may be regulated by different environmental factors.

Bacterioplankton Carbon Consumption

Increases in carbon consumption by bacterioplankton are frequently associated with increases in inorganic nutrients and DOM (Carlson and Ducklow 1996). However, patterns in 2-yr mean BCC among the four sub-systems suggest that in eutrophic carbon-rich systems such as Monie Bay, enrichment alone may not be as important as organic matter quality or composition in regulating carbon consumption. For example, a comparison LMC and LC suggests that BCC may be regulated by either dissolved nutrients or organic carbon availability, because BCC, DOC, and dissolved nutrients are all significantly higher in LMC than in LC (Figs. 4.2 & 4.4A). Similarly, although LC and OB both have relatively low nutrient concentrations (Fig. 4.2A,B), BCC is higher in LC. In addition, BCC in nutrient enriched MC was significantly lower than that of LMC and similar to that of unenriched LC, further suggesting that nutrient availability is not the determining factor. These qualitative comparisons suggest that the difference in carbon consumption among these sub-systems may be associated more with changes in the composition of DOM.

What then are characteristics of organic matter that would drive such patterns in carbon consumption? We observed a lower percentage of labile organic matter in MC than any other system and lower rates of BP, BR, and BCC compared to similarly enriched LMC (Table 4.1). Thus, although DOC concentrations in MC were higher than that of LMC (Fig. 4.2D), the lability of this organic matter was apparently lower, which probably drove the lower rates of carbon consumption that we observed. A factor contributing to the relatively high lability of organic matter in LMC may be the effect of nutrient inputs, which fuel a highly productive marsh macrophyte community that produces plant biomass – and ultimately detrital DOM – with higher nitrogen and phosphorus content (Jones et al. 1997). In this regard, elevated rates of carbon metabolism in LMC may result from increases in the concentration, lability, and nutrient content of DOM (Bano et al. 1997; Reitner et al. 1999). Comparisons of MC and LC lend credence to the importance of DOM quality in predicting the magnitude of BCC, for although ambient DOC concentrations in MC were twice that of LC (Fig. 4.2), rates of carbon consumption and indices of lability between the two sub-systems were strikingly similar (Table 4.1). This confirms our previous hypothesis that freshwater inputs to MC deliver refractory, low-quality organic matter that despite relatively high nutrient and DOC concentrations compromise bacterioplankton carbon consumption (Apple et al. 2004). Collectively, these patterns among sub-systems suggest carbon consumption is influenced predominantly by the quality of organic matter.

Bacterial Growth Efficiency

Similar comparisons among sub-systems suggest that organic matter source and quality is also important in determining the magnitude of BGE. For example, LMC and

LC are characterized by extensive Spartina alterniflora marshes (Jones et al. 1997) and lower inputs of terrestrial DOC (Fig. 4.2), suggesting that DOM in these two creeks is derived predominantly from marsh detritus. Previous studies suggest that such substrate sources are of higher quality and capable of supporting higher rates of bacterioplankton production and growth efficiencies than terrestrially derived DOM (Bano et al. 1997; Reitner et al. 1999). Accordingly, we observed almost identical values of BGE in LMC and LC (0.34 and 0.35, respectively; Student t-test; tcalc = 2.7, df = 68, $p < 0.005$;) that were also higher than those of all other sub-systems (Fig. 4.4B) despite significant temporal and spatial variations in ambient nutrient and dissolved carbon concentrations (Fig. 4.2). The fact that efficiencies were lower in MC (0.29) further supports the importance of DOM quality as opposed to nutrients in regulating BGE. Unlike the two more saline tidal creeks, MC experiences significant inputs of terrestrially-derived, refractory organic matter (Apple et al. 2004) that probably account for the systematically lower growth efficiencies recorded in MC (Goldman et al. 1987; Moran and Hodson 1990). This effect persisted despite high nutrient and DOC concentrations. Our results are consistent with those of other studies suggesting that the quality and composition of organic matter is more important than nutrients alone in regulating growth efficiency (Kroer 1993; Middelboe and Søndergaard 1993; Ram et al. 2003).

Coherence of Carbon Consumption and Growth Efficiency

Comparisons among sub-systems revealed a general similarity in the pattern of BCC, BGE, and lability (Fig. 4.4; Table 4.1), with highest values in LMC, lowest in OB, and intermediate in MC and LC. This coherence in pattern suggests that although these measures of carbon metabolism are influenced by different components of the organic

matter pool and different aspects of quality, there is general coherence of the effect of DOM quality on both short-term and long-term carbon consumption (i.e., BCC and lability) as well as the way in which this organic matter is processed (i.e., BGE). To further explore these relationships, we investigated the hypothesis that BGE increases with increasing carbon consumption and that this coherence is related to the influence of organic matter quality on each.

Initial analysis of the entire dataset revealed a negative relationship between BCC and BGE that was not expected (Fig. 4.9A), as we had anticipated a positive coupling of these two measures of carbon metabolism. However, this negative relationship was driven in part by the temperature dependence of bacterioplankton carbon metabolism, which produces low BGE and high BCC at elevated temperatures (Chapter III). Although this effect of temperature on the relationship between BGE and BCC was highly significant, it was not so strong as to override system-specific effects on the magnitude and coupling of BGE and BCC, as evidenced by significant differences in the *y*-intercepts when the sub-systems were considered individually. The hierarchy of the four sub-systems with respect to *y*-intercepts was similar to that observed for mean values of BCC and BGE, with highest values in LMC and lowest in OB (Fig. 4.9A) and intermediate in LC and MC (regressions not shown). This persistent hierarchy suggested that there are systematic variations in environmental conditions among the four sub-systems that regulate carbon consumption and growth efficiency.

In an effort to remove the confounding effect of temperature and identify other environmental factors contributing to the coupling of BGE and BCC, residuals from the temperature dependence of BGE and BCC (Chapter III) were regressed (Fig. 4.9B).

Although initial analyses revealed no relationship between these two measures of carbon metabolism, we observed that the systematic distribution of data followed a similar hierarchy to that observed previously (e.g., Figs. 4.4 & 4.9A), with data from LMC being of greater magnitude and generally associated with the upper right quadrant and those from OB associated with the lower left (Fig. 4.9B). Data from MC and LC were dispersed uniformly around the central axes. The general positive relationship between the magnitude of BCC and BGE, which was originally obfuscated by the effects of temperature (Fig. 4.9A) and the variability of the entire dataset (Fig. 4.9B), became evident in correlations of mean residual values for each system (Fig. 4.10A). We concluded from this series of analyses that there is a general positive coupling of carbon consumption and growth efficiency in this salt-marsh system. Moreover, we predict that the magnitude of these measures of carbon metabolism is regulated by those environmental factors that exhibit a similar hierarchy among sub-systems as was observed for BCC and BGE, such as DOM quality, source, or composition. Our investigation of the relationship between mean lability and mean residual BGE for each sub-system revealed a strong positive correlation (Fig. 4.10B), as did the correlation between lability and mean residual BCC ($r = 0.95$; $p<0.0001$; not shown), suggesting that energetic or structural characteristics of DOM are important environmental factors regulating the magnitude of both BGE and BCC. Ultimately, these measures of carbon metabolism may be inherently coupled in many natural aquatic systems as a result of their regulation by a very specific aspect of organic matter quality, such as lability or energetic content.

The positive relationship between BGE and BCC that we have isolated in our data was far from obvious, offering an explanation why this coherence is not frequently reported for other aquatic systems. The significant and substrate-independent effect of temperature on the magnitude of carbon metabolism, combined with the significant and independent variability of both BGE and BCC, produces a wide range of efficiencies for any given magnitude of carbon consumption that would tend to obscure their coupling as it is related to resource supply and quality. The positive relationship between BGE and BCC that emerged from our data is due in part to the scope of our study, which encompasses adequate temperature range and systematic variability to identify both the temperature dependence and systemic patterns in carbon metabolism. Given the variability of temperature, nutrient supply, and organic matter quality encountered on small spatial and temporal scales in most estuarine systems, there is no reason to assume that any individual estimate of BGE or BCC can predict the other or that they will exhibit the same coherence observed in our long-term dataset.

Indices of DOM Quality and the Influence on BGE

Based on the general assumption that BGE is regulated by substrate stoichiometry, specifically the relative concentrations carbon and nitrogen (Goldman et al. 1987; Kirchman 2000b; Touratier et al. 1999), we explored the relationship between the relative nutrient content of DOM and the magnitude of BGE. This investigation focused predominantly on the nitrogen content of DOM as an index of quality because bacterioplankton tend to be extremely plastic with respect to cellular phosphorus content (Kirchman 2000b) and thus we did not expect robust or meaningful relationships to emerge between phosphorus content of DOM and BGE. Our exploration of the

relationship between BGE and DOM quality examined three different indices, including (1) carbon and nitrogen stoichiometry of dissolved organic matter (i.e., DOC:DON), (2) the molar ratio of carbon consumed by bacterioplankton (i.e., BCC) to nitrogen consumed, and (3) the extent to which the nutrient content of organic matter was subsidized by the uptake of inorganic nutrients

Carbon and Nitrogen Stoichiometry

Many studies investigating the quality of DOM as it relates to nutrient content have focused on the relative availability of carbon and nitrogen in the water column (e.g., DOC:DON), relating this measure to the stoichiometric demands bacterioplankton growth (Goldman et al. 1987; Sun et al. 1997). Estimates of dissolved nutrient stoichiometry are compared to that of bacterial biomass and serve as an index of DOM quality based on the assumption that similarity between these two will result in more efficient growth. A number of studies rely on this assumption and employ theoretical models using substrate C:N ratios as indices of bioavailability and quality (Kirchman 2000b; Rodrigues and Williams 2001; Sun et al. 1997; Touratier et al. 1999; Vallino et al. 1996). Our analyses, however, provided no such evidence that dissolved nutrient stoichiometry or the nutrient content of DOM influences BGE (Fig. 4.6A).

Despite the widespread use of dissolved nutrient stoichiometry as an index of organic matter quality, the lack of relationship that we observed between DOC:DON ratios and BGE was not surprising. These ratios represent the stoichiometry of organic matter to which bacterioplankton are exposed rather than the carbon and nitrogen content of the organic matter that they actually consume. It is imperative that an investigation of the role of dissolved nutrient stoichiometry in regulating BGE focuses on the organic

matter that is consumed by bacterioplankton consume. In this regard, an important aspect of our study was the measurement of nutrient uptake by bacterioplankton and the relationship with concurrent measurements of short-term carbon consumption. These paired measurements allowed us to estimate the uptake stoichiometry (i.e., the molar ratio of carbon consumption and nutrient uptake) of bacterioplankton. The resulting ratios (i.e., BCC:TDN uptake and BCC: DON uptake) offer a more accurate approximation of the C:N ratio organic matter consumed by bacterioplankton than is provided by DOC:DON ratios alone. We anticipated that uptake stoichiometry would reveal meaningful relationships between the nutrient content of DOM and BGE. However, despite the improved insight into the consumption of organic matter and nutrients by bacterioplankton, we found no evidence that either total uptake stoichiometry (BCC:TDN uptake) or that of DOM (BCC:DON uptake) had any effect on the growth efficiency of bacterioplankton (Fig. 4.6B,C).

Organic vs. Inorganic Nutrient Sources

Another factor that may influence BGE is the extent to which the nutrient content of DOM is supplemented by the active uptake of dissolved inorganic nutrients. For example, DOM that is N or P limited relative to the demands of bacterioplankton growth may require the expenditure of additional energy for uptake and assimilation of inorganic nutrients, ultimately resulting in lower growth yield and BGE (Kirchman 2000b). Using direct measurements of the uptake of dissolved inorganic nutrients (i.e., DIN and PO_4^{3-}) relative to total uptake (i.e., TDN and TDP), we estimated the proportion of N and P derived from inorganic sources and explored the relationship between these values and BGE (Fig. 4.7). We hypothesized that lower growth efficiencies would be associated

with higher DIN:TDN and PO4:TDP uptake ratios, as these represent circumstances where most of the nutrient acquisition is derived from the inorganic fraction. Similarly, we expected higher growth efficiencies when the contribution of inorganic nutrients to total nutrient uptake was relatively low. The patterns in Fig. 4.7 suggest that the first part of this hypothesis may be true, as lower growth efficiencies were generally encountered when a greater proportion of nutrient uptake was of the inorganic fraction. However, conditions in which nutrients appeared to be derived predominantly from the DOM did not necessarily result in higher growth efficiencies, for although relatively high growth efficiencies occurred at both low DIN:TDN and PO4:TDP uptake ratios, there was considerable variability in BGE (<0.1 to >0.6) and low values were often recorded. These observations suggest that BGE may be adversely affected by the metabolic costs associated with either the uptake of organic nutrients or the consumption of DOM with low nutrient content, yet the presence of higher nutrient content DOM does not necessarily result in higher BGE. The high degree of variability of BGE in the presence of what appears to be relatively nutrient-rich organic matter may be attributed to the influence of other characteristics of the DOM pool, including chemical composition, energetic content or lability.

Combining the systematic patterns in organic matter source and quality that exist among the sub-systems of Monie Bay (Table 1; Apple et al. 2004) with those observed DIN:TDN and PO4:TDP uptake ratios (Fig. 4.8) provided additional insight into the means by which DOM quality may regulate BGE and carbon metabolism. Despite elevated nutrient concentrations and evidence of nutrient rich DOM (i.e., low DIN:TDN and PO4:TDP uptake ratios) in MC (Figs. 4.2 and 4.8), BGE in this system was

characteristically low (Fig. 4.4B) and probably driven by the refractory, terrestrially-derived organic matter that dominates this system (Apple et al. 2004). In this regard, adequate or even elevated N and P content in either the water column or DOM may not always produce higher growth efficiencies if other chemical characteristics of DOM (e.g., energy content, lability) require additional energy expense for its consumption and utilization. The effect of this later aspect of organic matter quality is evident when LC and LMC are considered, for although more energy may be spent by bacterioplankton on nutrient uptake in these systems relative to MC (Fig. 4.8), BGE in these systems is actually higher (Fig. 4.4B) as a result of the less refractory DOM in this system. These two systems are quite different with respect to ambient nutrient concentrations (Fig. 4.2), and we suspect that the similarity between these systems with respect to nutrient uptake ratios and BGE is driven by DOM with similar nutrient and energy content. Finally, systems such as the open bay in which organic matter comes from multiple creek sources may experience intermediate growth efficiencies (Fig. 4.4), but increases in the uptake of inorganic nutrients (Fig. 4.8) as a result of the DOM being overworked and stripped of N and P as it travels down estuary. Ultimately, BGE is a function of both the nutrient and energetic content of DOM consumed by bacterioplankton. Although the relative importance of each of these measures of quality in regulating BGE is not entirely clear, low growth efficiencies coupled with evidence of refractory yet nutrient rich organic matter in MC suggests that energy content may be the more important regulating factor.

Our study provides valuable insight into the relative importance of nutrient versus energetic content of DOM in regulating BGE, yet our conclusions regarding nutrient uptake stoichiometry may need further validation. In particular, lack of replication in

nutrient uptake experiments compromises our ability use the absence of a relationship between BGE and indices of DOM stoichiometry (Fig. 4.6) as definitive proof that DOM nutrient content is not an important factor regulating growth efficiency. Changes in nutrient uptake during the course of incubations were statistically significant relative to the error associated with the measurement of each nutrient (Strickland and Parsons 1972; Valderrama 1981; Whitledge et al. 1981), yet the error associated with the experimental manipulations and incubations themselves could not be determined and the lack of pattern between BGE and nutrient uptake may simply result from a high degree of variability. In addition, assuming a linear consumption of nutrients by bacterioplankton in incubations, intermediate measurements from time series of nutrient concentrations would either support or reject the validity of our estimates of total nutrient uptake. Unfortunately, no such measurements were made. However, there is is some evidence of temperature dependence of uptake rates for various nutrient forms (e.g., DON and PO_4^{3-}), supporting the assumption that these values may represent *in situ* processes (see Appendix B). Clearly this aspect of our study should be subject to further and more intensive experimental investigations.

Assessing the Energetic Content of DOM

Ultimately, accurate assessment of the quality of organic matter consumed by bacterioplankton relies on the ability to measure the characteristics of the short-lived, rapid turnover pool of DOM, or consumption of this DOM on very short time scales. Lability and spectral characteristics of DOM are indirect measures of structure and quality, identifying the rate at which organic matter is degraded by bacterioplankton and the presence of aromatic compounds which are presumed to be recalcitrant to microbial

degradation (Søndergaard and Middelboe 1995), respectively. However, these may be poor indices of quality because they target the longer-lived, refractory component of DOM rather than the short-lived organic matter which bacterioplankton actually utilize. Estimates of lability are typically derived from relatively long (i.e., days to weeks) incubations (del Giorgio and Davis 2003) – a length of time that is far too coarse to resolve differences in consumption of short-lived labile fractions that account for most of the bacterioplankton carbon demand in natural aquatic systems (Bano et al. 1997; Raymond and Bauer 2000; Søndergaard et al. 1995). Similarly, measurable degradation of CDOM is slow (>1 wk) relative to other sources of organic carbon (Moran and Hodson 1990) and may only represent a small percentage (e.g., <2%) of the DOM pool (Bano et al. 1997). Thus, although lability and optical properties are certainly measures of one aspect of organic matter quality, they may not be representative of the organic matter utilized by bacterioplankton *in situ*. The extent to which characteristics of one DOM fraction (i.e., that which is consumed on shorter time scales) represent the characteristics of another (i.e., that which is consumed on longer time scales) is not known, although the existence of such a relationship would serve to validate the use of lability and CDOM as effective measures of DOM quality. Until such a parameter or methodology can be isolated that measures the characteristics of the short-lived DOM pool, measurements of *in situ* BGE and BCC will remain the most accurate index of the quality and quantity, respectively, of organic matter utilized by bacterioplankton in natural aquatic systems.

Multivariate Analyses

Multivariate analyses of temperature residuals provided another means by which factors influencing BGE were identified (Table 4.3). The positive correlation of BGE with phosphate and ammonium suggests the effect of dissolved nutrients, whereas negative correlation with specific absorbance (i.e., a_{350}*) indicates the influence of DOM composition and structure that is unrelated to nutrient content and suggests that BGE is reduced in the presence of DOM that is more refractory. Our analyses were limited by the absence of any term representing the nutrient content of DOM other than dissolved nutrient ratios (i.e., DOC:TDN, DOC:TDP, TDN:TDP), which as discussed previously are questionable in their ability to represent the nutrient content of DOM that is utilized by bacterioplankton. The limited ability of these multivariate models to predict the variability in BGE or BCC (i.e., r^2 = 0.32 and 0.41, respectively) may have resulted from the absence of terms that accurately represent the nutrient or energetic content of DOM, or that represent other aspects of DOM quality that we failed to measure. Results from these multivariate analyses and our investigations of the relationship between nutrient uptake, DOM stoichiometry, and BGE described above lead to the conclusion that organic matter with relatively low energetic content (e.g., terrestrially-derived refractory DOM) has an adverse effect on growth efficiency, which is further modulated by the influence of DOM nutrient content. Observation of relatively low BGE in the presence of elevated nutrients in MC and elevated BGE in unenriched LC provides evidence that the chemical composition and energetic content of DOM may be the more important of these measures of quality in regulating BGE in this eutrophic salt marsh system.

Collectively, qualitative comparisons among-systems and multivariate analyses remind us that patterns of BCC and BGE in eutrophic estuarine systems represent a complex metabolic response to multiple environmental factors, each of which has a unique effect on the different aspects of bacterioplankton carbon metabolism. Although it is understood that multiple limiting factors interact in natural aquatic systems, there remains a tendency to seek a single limiting factor for growth or metabolism (Pomeroy and Wiebe 2001). Our investigations indicate that such simplicity does not exist for the regulation of estuarine bacterioplankton. Not only do the factors that regulate bacterioplankton carbon metabolism vary among the different aspects, but also vary for any given aspect of carbon metabolism when different systems and seasons are considered. Despite the complex response of BGE to environmental conditions and the absence of one regulating factor, we propose that DOM quality as it relates to energetic rather than nutrient content is among the most important.

Growth Efficiency, Uptake Stoichiometry, and Nitrogen Mineralization

In a review of the role of bacterioplankton in nutrient cycling, Kirchman (2000b) describes a theoretical relationship between BGE, substrate C:N ratios, and ammonium mineralization (Fig. 4.11, lower panel). Based on estimates of bacterioplankton cellular stoichiometry, the author uses this conceptual model to identify an interface between NH_4^+ uptake and excretion and thus a means by which the flux of ammonium in marine systems can be predicted. We tested the applicability of this conceptual framework in Monie Bay using measurements of ammonium flux (i.e., uptake vs. production), BGE, and estimates of substrate stoichiometry (i.e., BCC:DON uptake) collected during our study. The application of these empirical values to the conceptual model of Kirchman

(2000b) is illustrated in Fig. 4.11 (upper panel). Using the lower value for bacterial biomass stoichiometry that would be expected in coastal and estuarine systems (i.e., bacterial C:N = 4.5) as the line for zero ammonium flux, we found that the conceptual model accurately predicted net production of NH_4^+, as all of the incubations in which we observed NH_4^+ production fell below this theoretical threshold. In contrast, ammonium uptake was poorly predicted. The overwhelming majority of incubations in which production of ammonium would have been expected actually exhibited ammonium uptake, with consumption of NH_4^+ accurately predicted in only two of the 52 samples in which consumption was measured.

One of the striking differences between the two panels in Fig. 4.11 is the range in both BGE and substrate C:N, with generally higher substrate C:N ratio and narrower range in BGE suggested by the conceptual model than was observed in our study. Based on our understanding of bacterioplankton cellular stoichiometry, our estimates of substrate C:N seemed almost unrealistically low relative to the carbon and nitrogen demands of bacterioplankton, and the uptake of NH_4^+ observed in many of these incubations seemed counterintuitive. However, Kirchman (1994) reported that short-term (i.e., <19h) incubations of natural bacterioplankton may exhibit uptake of dissolved nitrogen in excess bacterioplankton carbon demand, which may produce extremely low estimates of C:N ratios for DOM. Release of dissolved nitrogen during size-fractionation filtration, which stimulates even more the uptake of dissolved nitrogen uptake, may contribute to this effect. However, even if our estimates of substrate C:N were unrealistically low, shifting the data to the right would not improve the efficacy of the conceptual model, as there is extensive overlap of incubations exhibiting NH_4^+ uptake

and production (Fig. 4.11, upper panel). Collectively, these results lead us to conclude that the conceptual framework of Kirchman (2000b) may be useful for estimating ammonium production, but limited in predicting uptake. We believe that this may be attributed to the fact that BGE and substrate stoichiometry are simply poor predictors of nitrogen demand in these eutrophic tidal creeks, or that BGE is regulated by environmental factors other than the nutrient content of dissolved organic matter consumed by bacterioplankton.

Concluding Remarks

Most studies of bacterioplankton in aquatic systems have focused on measurements of growth and production, providing valuable information regarding the regulation of these communities by environmental factors (Coveney and Wetzel 1992; Felip et al. 1996), their role plankton dynamics (Ducklow 1983; Gonzalez et al. 1990; Vrede et al. 1999) and carbon and nutrient cycling (Ducklow et al. 1986; Hoch and Kirchman 1995; Sherr et al. 1988), and their response to system-level nutrient enrichment (Apple et al. 2004; Revilla et al. 2000). Our study has focused on the somewhat less studied bacterioplankton carbon consumption and growth efficiency and the poorly understood regulation of these measures of carbon metabolism in aquatic systems. Because carbon consumption and growth efficiency describe two fundamental aspects of carbon cycling in aquatic systems – namely the magnitude of carbon processed by bacterioplankton communities and how that carbon is partitioned between growth and respiration – factors regulating the magnitude and variability of these processes will add to our growing understanding of the role of bacterioplankton communities in nutrient and carbon cycling in aquatic systems.

We observed that BGE is quite variable in this salt marsh system, exhibiting a range and overall mean that are remarkably similar to those reported by del Giorgio and Cole (1998) in their survey of over 40 studies representing lakes, rivers, estuaries, and the open ocean. Thus, even under the eutrophic conditions encountered in our study, the variability of BGE among the sub-systems of Monie Bay may be comparable to that of all aquatic systems. We also observed tremendous variability in BGE relative to simultaneous measures of bacterial carbon consumption, which in conjunction with the effect of temperature initially obfuscated the positive coupling of BGE and BCC that eventually emerged from our data. We attribute the ability to identify such patterns to the unique nature of our long-term dataset, which includes a broad range of environmental conditions that are predictably constrained when the four different sub-systems are considered. This allows for systematic relationships between carbon metabolism and environmental conditions to emerge that might not be identified in studies of smaller scope and scale, yet that may represent transferable ecosystem-scale properties of aquatic systems.

Another important conclusion of our study is that the nutrient content of organic matter may not be as important in regulating growth efficiency as is frequently assumed. Although numerous models of bacterioplankton growth rely on organic matter stoichiometry as predictors of BGE (Cajal-Medrano1 and Maske 1999; Goldman et al. 1987; Touratier et al. 1999), we found little evidence supporting this as a valid measure of organic matter quality that is effective at predicting the variability of growth efficiencies in salt-marsh systems. We hypothesize that in nutrient rich systems such as the tidal creeks of Monie Bay and in estuaries in general that the chemical composition

and energetic content of organic matter may have a greater influence on carbon metabolism than the relative content of nitrogen and phosphorus. In this regard, although the use of simple stoichiometric models may be appropriate for long-term or large-scale studies, they fail to describe the dynamics of short-term interactions between bacteria and the dissolved pools of nutrients and DOM (Kirchman 2000b).

We also concluded that organic matter quality influences the magnitude of carbon consumed by bacterioplankton and the efficiency with which carbon is processed. However, it is difficult if not impossible to determine the extent to which the coupling of BGE and BCC is driven by the direct metabolic coupling of BGE to BCC, the independent regulation of both processes by the same aspect of DOM quality, or simply a general response to multiple factors that covary along enrichment gradients. The positive relationships between BCC, BGE, and DOM lability would suggest that carbon consumption and growth efficiency are regulated by a specific aspect of DOM quality that is related to its energy content and chemical structure. Unfortunately, our measurements of DOM lability are few (n = 14) when compared to paired measures of BCC and BGE (n = 138) and analytical investigations of DOM quality limited, preventing a more comprehensive exploration of the direct effect of organic matter quality on carbon metabolism. Future research endeavors should focus on identifying assays of organic matter quality that target the substrates that bacterioplankton utilize and pair these with measures of *in situ* carbon metabolism to investigate the role of DOM quality in regulating the variability, magnitude, and coupling of different aspects of bacterioplankton carbon metabolism.

Although our study has provided insight into the relationship between carbon metabolism and different aspects of organic matter quality, the underlying mechanisms driving these relationships on the cellular level were not investigated and remain poorly understood. Shifts in both phylogenetic composition and the abundance of highly-active cells have been recorded along salinity, nutrient, and resource gradients (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2003; Crump et al. 1999; del Giorgio and Scarborough 1995), which have in turn been linked to the variability and magnitude of community-level metabolic processes (Cottrell and Kirchman 2003; Yokokawa et al. 2004). Thus, an investigation of the phylogenetic and metabolic structure of these communities may provide additional insight into mechanisms driving patterns of carbon metabolism we observed among the sub-systems of Monie Bay, especially when differences between freshwater- and saltwater-dominated tidal creeks are considered.

LITERATURE CITED

Amon, R., H. P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnology and Oceanography **46:** 287-297.

Apple, J. K., P. A. del Giorgio, and W. M. Kemp. submitted. Temperature regulation of bacterial production, respiration, and growth efficiency in estuarine systems. Aquatic Microbial Ecology.

Apple, J. K., P. A. del Giorgio, and R. I. E. Newell. 2004. The effect of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. Journal of Coastal Research **45:** 110-133.

Bano, N. and others 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. Marine Ecology Progress Series **157:** 1-12.

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography **47:** 453-470.

Cajal-Medrano1, R., and H. Maske. 1999. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton--revisiting the Pirt model. Aquatic Microbial Ecology **19:** 119-128.

Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquatic Microbial Ecology **10:** 69-85.

Caron, D. A., E. L. Lim, R. W. Sanders, M. R. Dennett, and U. G. Berninger. 2000. Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. Aquatic Microbial Ecology **22:** 175-184.

Cottrell, M. T., and D. L. Kirchman. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnology and Oceanography **48:** 168–178.

Coveney, M. F., and R. G. Wetzel. 1992. Effects of Nutrients on Specific Growth Rate of Bacterioplankton in Oligotrophic Lake Water Cultures. Applied and Environmental Microbiology **58:** 150-156.

Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Applied and Environmental Microbiology **65:** 3192-3204.

del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnology and Oceanography **47:** 471-486.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29:** 503-541.

del Giorgio, P. A., and J. Davis. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399-424. *In* S. Findlay [ed.], Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Elsevier Science.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. Journal of Plankton Research **17:** 1905-1924.

Ducklow, H. W. 1983. Production and fate of bacteria in the oceans. BioScience **33:** 494-501.

Ducklow, H. W., D. A. Purdie, P. J. L. B. Williams, and J. M. Davies. 1986. Bacterioplankton: A sink for carbon in a coastal marine plankton community. Science **232:** 863-867.

Felip, M., M. L. Pace, and J. J. Cole. 1996. Regulation of planktonic bacterial growth rates: The effects of temperature and resources. Microbial Ecology **31:** 15-28.

Fisher, T. R., L. W. Harding, D. W. Stanley, and L. G. Ward. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science **27:** 61-93.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by C:N ratio. Limnology and Oceanography **32:** 1239-1252.

Gonzalez, J. M., E. B. Sherr, and B. F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and Environmental Microbiology **56:** 583-589.

Hoch, M. P., and D. L. Kirchman. 1995. Ammonia uptake by heterotrophic bacteria in the Delaware Estuary and adjacent coastal waters. Limnology and Oceanography **40:** 886-897.

Hu, C., F. E. Muller-Karger, and R. G. Zepp. 2002. Absorbance, absorption coefficient, and apparent quantum yield: A comment on common ambiguity in the use of these optical concepts. Limnology and Oceanography **47:** 1261–1267.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A Two-Year Study of the Short-Term and Long-Term Sequestering of Nitrogen and Phosphorus in the Maryland National Estuarine Research Reserve, p. 1-100. Maryland National Estuarine Research Reserve.

Jørgensen, N. O. G., N. Kroer, and R. B. Coffin. 1994. Utilization of dissolved nitrogen by heterotrophic bacterioplankton: Effect of substrate C/N ratio. Applied and Environmental Microbiology **60:** 4124-4133.

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennet, and J. C. Cornwell. 1994. Membrane inlet mass-spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. Analytical Chemistry **66:** 4166-4170.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria (Chapter 58), p. 776. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole [eds.], Handbook of Methods in Aquatic Microbial Ecology. CRC Press.

---. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. Microbial Ecology **28:** 255-271.

---. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria, p. 261-288. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

Kroer, N. 1993. Bacterial-Growth Efficiency on Natural Dissolved Organic-Matter. Limnology and Oceanography **38:** 1282-1290.

Linton, J., and R. Stevenson. 1978. A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substrates. FEMS Microbiology Letters **3:** 95-98.

Middelboe, M., and M. Søndergaard. 1993. Bacterioplankton growth yield: Seasonal variations and coupling to substrate lability and beta -glucosidase activity. Applied and Environmental Microbiology **59:** 3916-3921.

Moran, M. A., and R. E. Hodson. 1990. Bacterial production on humic and non-humic components of dissolved organic matter. Limnology and Oceanography **35:** 1744-1756.

Moran, M. A., J. E. Sheldon, and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnology and Oceanography **45:** 1254-1264.

Pomeroy, L. R., J. E. Sheldon, W. M. Sheldon, and F. Peters. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. Marine Ecology Progress Series **117:** 259-268.

Pomeroy, L. R., and W. J. Wiebe. 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquatic Microbial Ecology **23:** 187-204.

Ram, A. S. P., S. Nair, and D. Chandramohan. 2003. Bacterial growth efficiency in the tropical estuarine and coastal waters of Goa, southwest coast of India. Microbial Ecology **45:** 88-96.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. Aquatic Microbial Ecology **22:** 1-12.

Reitner, B., A. Herzig, and G. Herndl. 1999. Dynamics in bacterioplankton production in a shallow, temperate lake (Lake Neusiedl, Austria): evidence for dependence on macrophyte production rather than on phytoplankton. Aquatic Microbial Ecology **19:** 245-254.

Revilla, M., A. Iriarta, I. Madariaga, and E. Orive. 2000. Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuarine, Coastal and Shelf Science **50:** 297-313.

Rivkin, R. B., and L. Legendre. 2001. Biogenic carbon cycling in the upper ocean: Effects of microbial respiration. Science **291:** 2398-2400.

Rodrigues, R. M., and P. J. L. B. Williams. 2001. Heterotrophic bacterial utilization of nitrogenous and nonnitrogenous substrates, determined from ammonia and oxygen fluxes. Limnology and Oceanography **46:** 1675–1683.

Sampou, P., and W. M. Kemp. 1994. Factors regulating plankton community respiration in Chesapeake Bay. Marine Ecology Progress Series **110:** 249-258.

Sharp, J. and others 1995. Analyses of dissolved organic carbon in sea water: the JGOFS EqPac methods comparison. Marine Chemistry **48:** 91-108.

Sharp, J. H., C. H. Culberson, and T. M. Church. 1982. The chemistry of the Delaware Estuary. General considerations. Limnology and Oceanography **27:** 1015-1028.

Sherr, B. F., E. B. Sherr, and C. S. Hopkinson. 1988. Trophic interactions within pelagic microbial communities: Indications of feedback regulation of carbon flow. Hydrobiologia **159:** 19-26.

Shiah, F. K., and H. W. Ducklow. 1994a. Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Marine Ecology Progress Series **103:** 297-308.

---. 1994b. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnology and Oceanography **39:** 1243-1258.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using super(3)H-leucine. Marine Microbial Food Webs **6:** 107-114.

Smith, E. M., and W. M. Kemp. 2003. Planktonic and bacterial respiration along an estuarine gradient: responses to carbon and nutrient enrichment. Aquatic Microbial Ecology **30:** 251-261.

Søndergaard, M., B. Hansen, and S. Markager. 1995. Dynamics of dissolved organic carbon lability in a eutrophic lake. Limnology and Oceanography **40:** 46-54.

Søndergaard, M., and M. Middelboe. 1995. A cross system analysis of labile dissolved organic carbon. Marine Ecology Progress Series **118:** 283-294.

Strickland, J. D., and T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Bulletin of the Fisheries Research Board of Canada **167:** 1-310.

Sun, L., E. Perdue, J. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnology and Oceanography **42:** 714-721.

Touratier, F., L. Legendre, and A. Vézina. 1999. Model of bacterial growth influenced by substrate C:N ratio and concentration. Aquatic Microbial Ecology **19:** 105-118.

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry **10:** 109-122.

Vallino, J. J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. Limnology and Oceanography **41:** 1591-1609.

Vrede, K., T. Vrede, A. Tisaksson, and A. Karlsson. 1999. Effects of nutrients (P,N,C) and zooplankton on bacterioplankton and phytoplankton - a seasonal study. Limnology and Oceanography **44:** 1616-1624.

Whitledge, T. C., S. C. Mallory, C. J. Patton, and C. D. Wirick. 1981. Automated Nutrient Analysis in Seawater. Department of Energy and Environment.

Yokokawa, T., T. Nagata, M. T. Cottrell, and D. L. Kirchman. 2004. Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. Limnology and Oceanography **49:** 1620-1629.

FIGURES

Fig. 4.1. Map of Monie Bay Research Reserve and location of sampling sites.

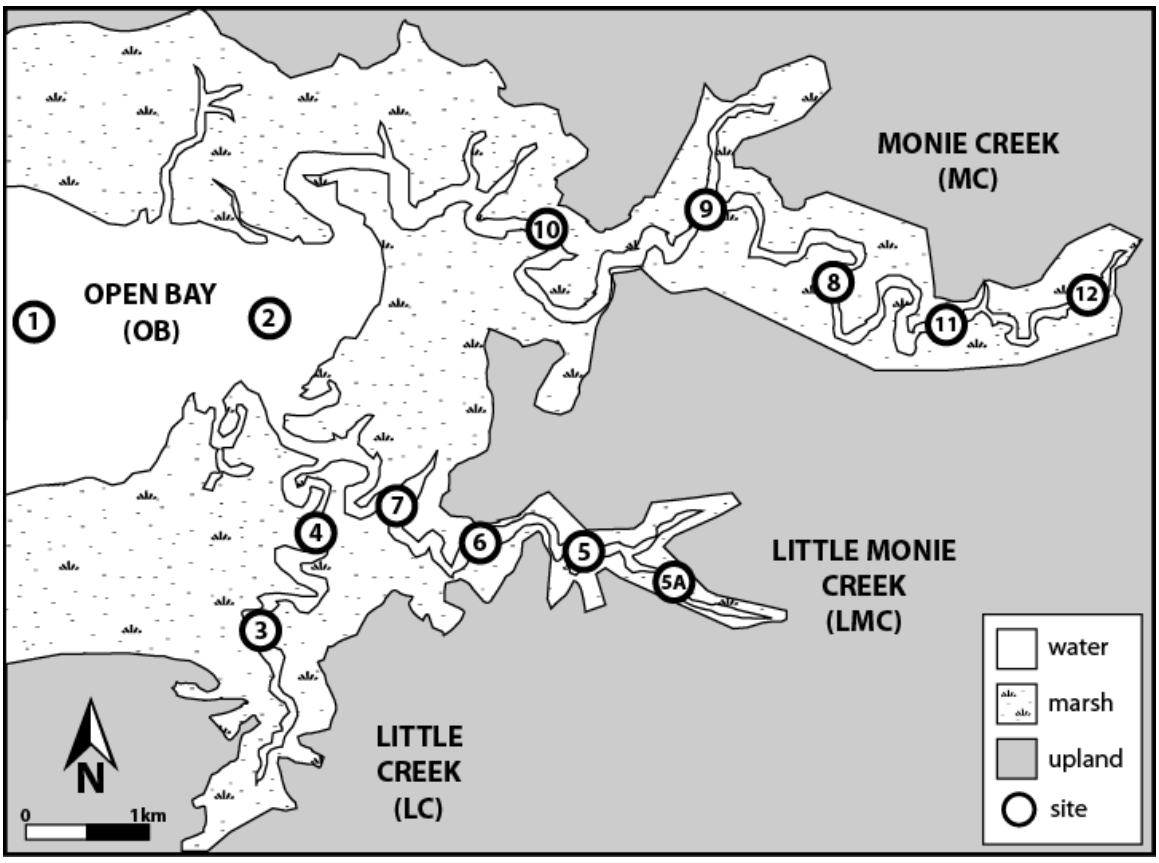

MONIE CREEK
(MC)
OPEN BAY
(OB)
LITTLE MONIE
CREEK
(LMC)
LITTLE
CREEK
(LC)
1
2
3
4
5
5A
6
7
8
9
10
11
12
N
0
1km
water
marsh
upland
site

Fig. 4.2. Two-year means for parameters of water column chemistry in each of the estuarine sub-systems: (A) nitrate + nitrite (NO_X), ammonium (NH_4^+), and dissolved organic nitrogen (DON); (B) phosphate (PO_4^{3-}) and dissolved organic phosphorus (DOP); (C) salinity; and (D) dissolved organic carbon DOC and specific absorbance (a_{350}* (m^{-1} · μM DOC^{-1})). Error bars represent the standard error of the mean.

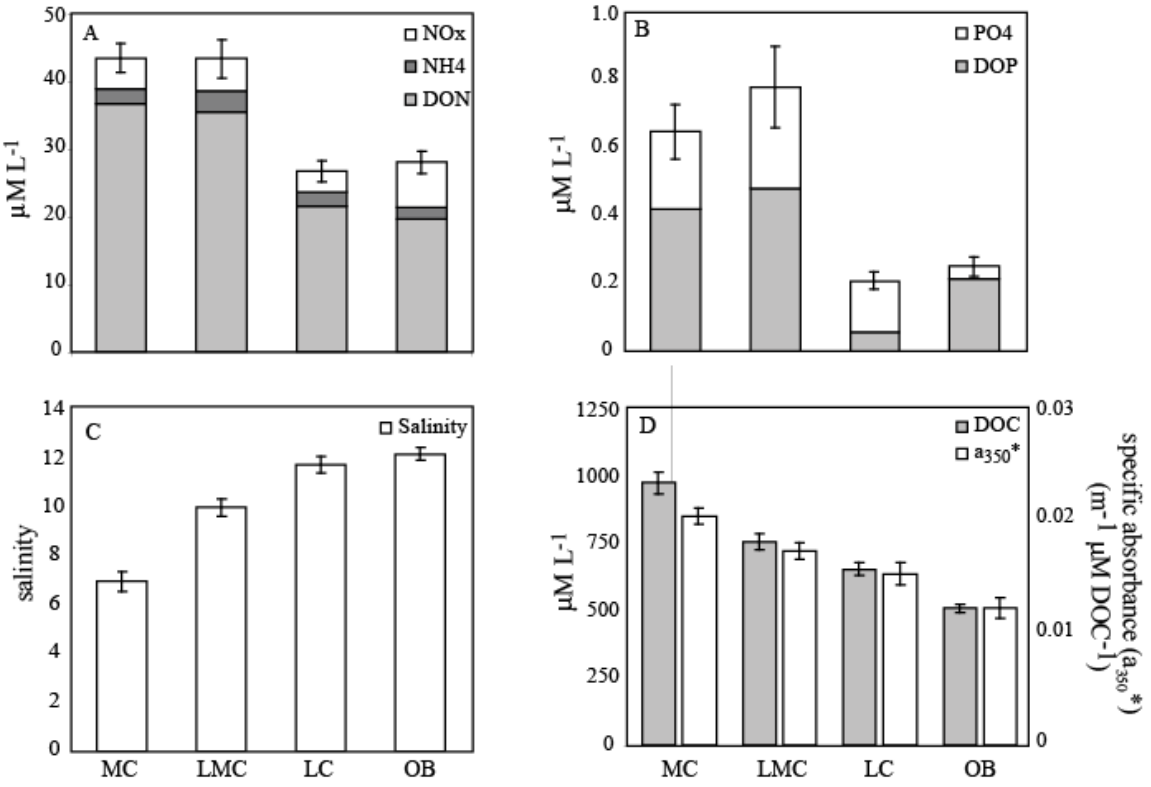

A
NOx
NH4
DON
µM L-1
B
PO4
DOP
µM L-1
C
Salinity
salinity
D
DOC
a350*
µM L-1
specific absorbance (a350*) (m-1 µM DOC-1)
MC
LMC
LC
OB

Fig. 4.3. Paired measures of bacterial production (BP) and bacterial respiration (BR) from the entire dataset (n = 138). Rates of carbon metabolism were reported as μgC L^{-1} h^{-1} and log-transformed.

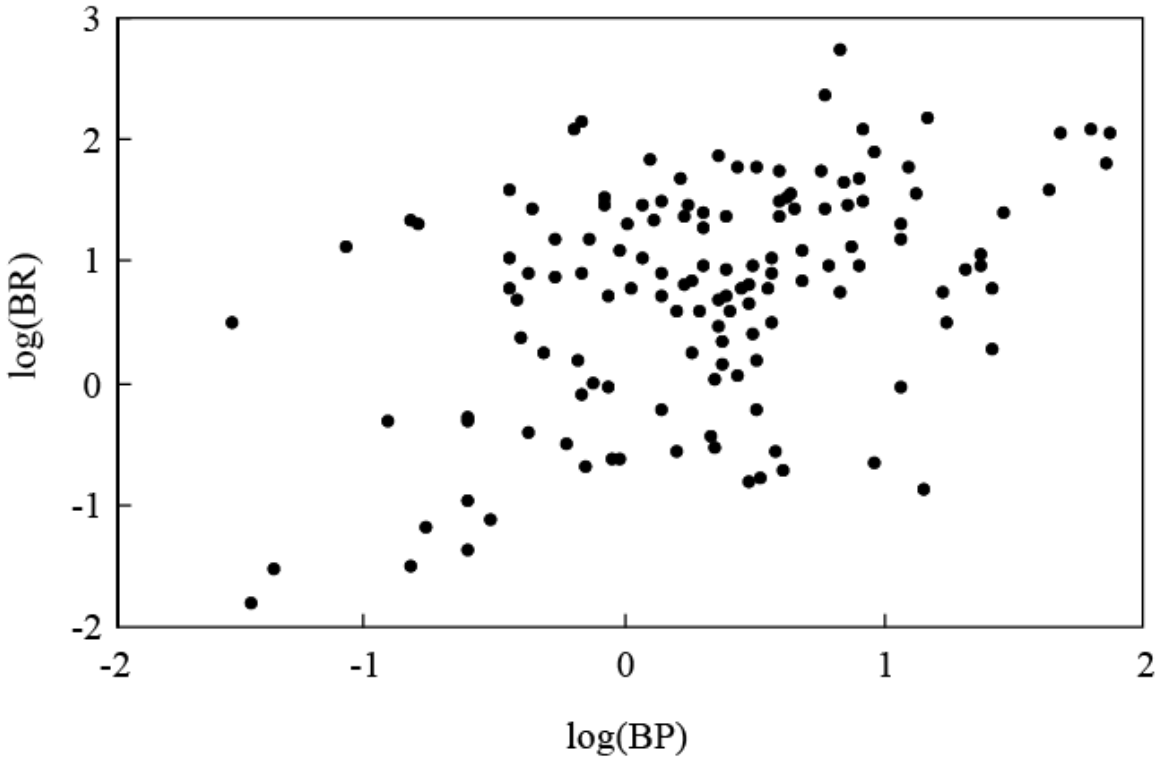

3
2
1
0
-1
-2
log(BR)
-2
-1
0
1
2
log(BP)

Fig. 4.4. Two-year means ± standard error for (A) measured rates of bacterioplankton carbon metabolism and (B) bacterial growth efficiency (BGE) among estuarine sub-systems. Columns sharing the same letter within each panel are statistically similar (Tukey-Kramer HSD, $p < 0.0001$(upper panel), $p = 0.09$ (lower panel)).

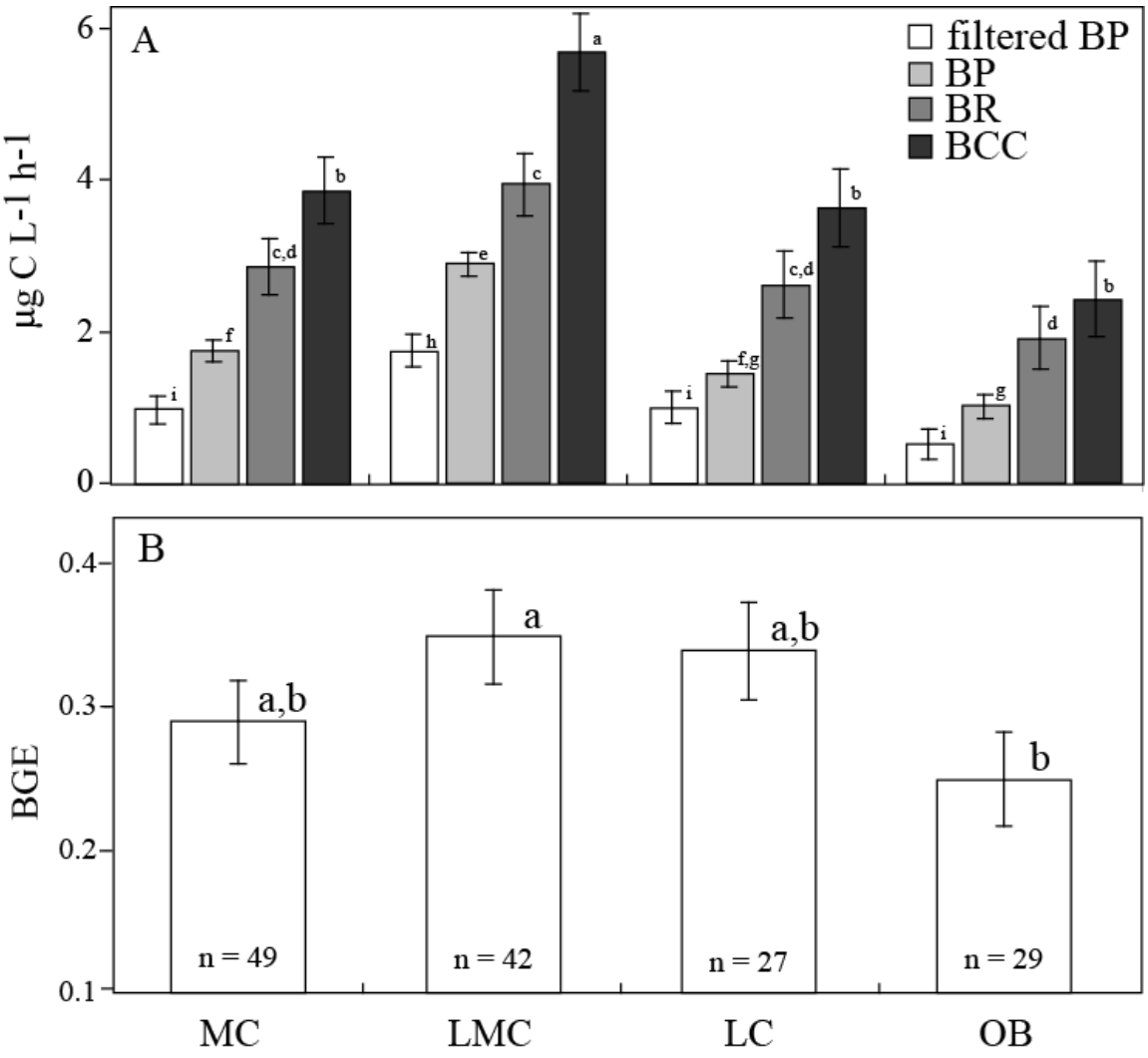

A
filtered BP
BP
BR
BCC
µg C L-1 h-1
6
4
2
0
i
f
c,d
b
h
e
c
a
i
f,g
c,d
b
i
g
d
b
B
BGE
0.4
0.3
0.2
0.1
a,b
a
a,b
b
n = 49
n = 42
n = 27
n = 29
MC
LMC
LC
OB

Fig. 4.5. Two-year seasonal means ± standard error for (A) bacterial carbon consumption (BCC) and (B) bacterial growth efficiency (BGE). Columns sharing the same letter within each panel are statistically similar (Tukey-Kramer HSD; $p < 0.0001$). Sample sizes are given in the lower panel.

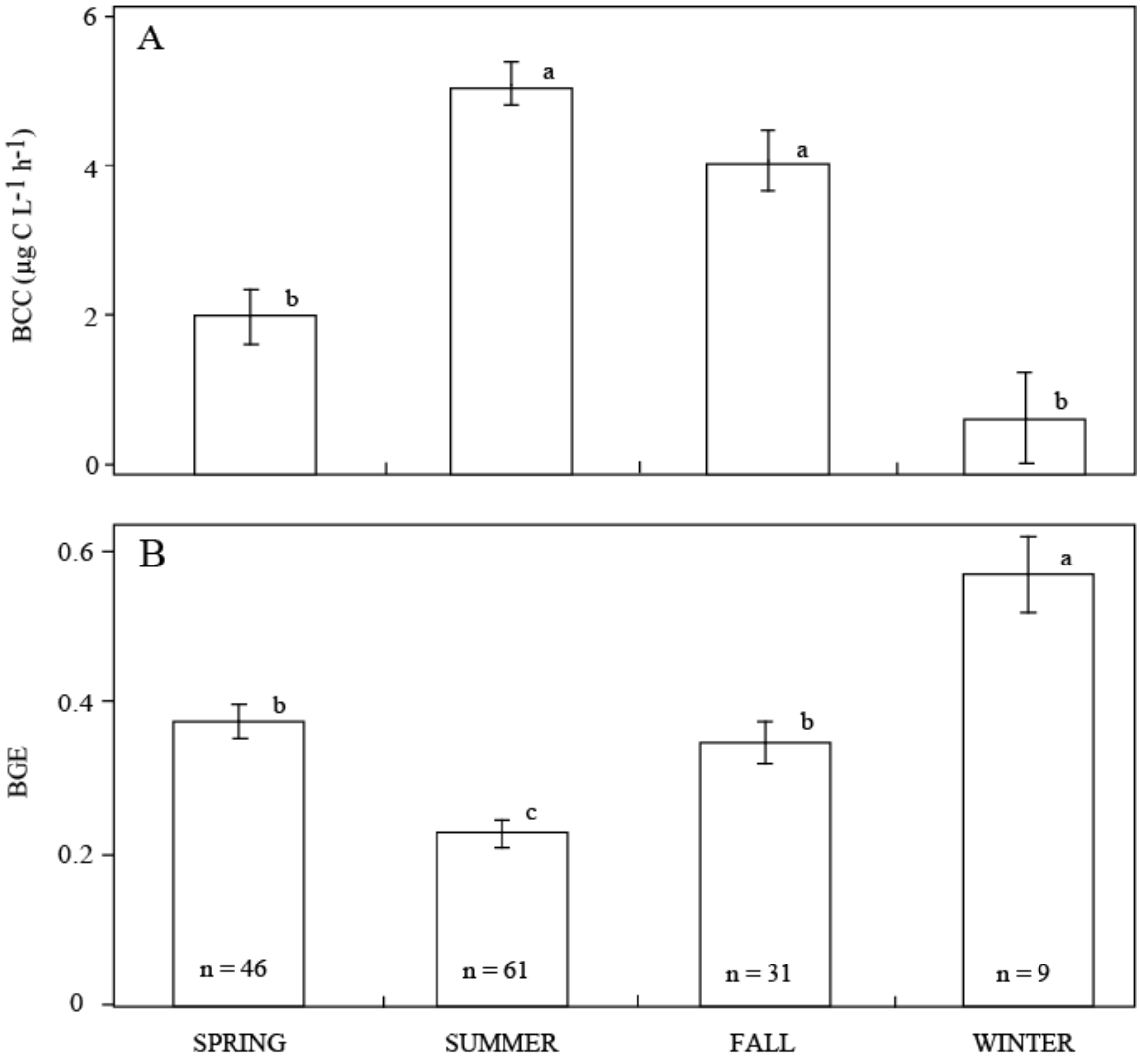

A
B
BCC (µg C L-1 h-1)
BGE
6
4
2
0
0.6
0.4
0.2
0
a
a
b
b
b
b
c
a
n = 46
n = 61
n = 31
n = 9
SPRING
SUMMER
FALL
WINTER

Fig. 4.6. Relationship between bacterioplankton growth efficiency (BGE) and (A) molar ratios of ambient dissolved carbon and nitrogen (DOC:DON), (B) total carbon and nitrogen uptake by bacterioplankton (BCC:TDN uptake), and (C) dissolved organic matter consumed by bacterioplankton (BCC:DON uptake). Carbon and nitrogen uptake ratios were derived from molar rates ($\mu M\ h^{-1}$).

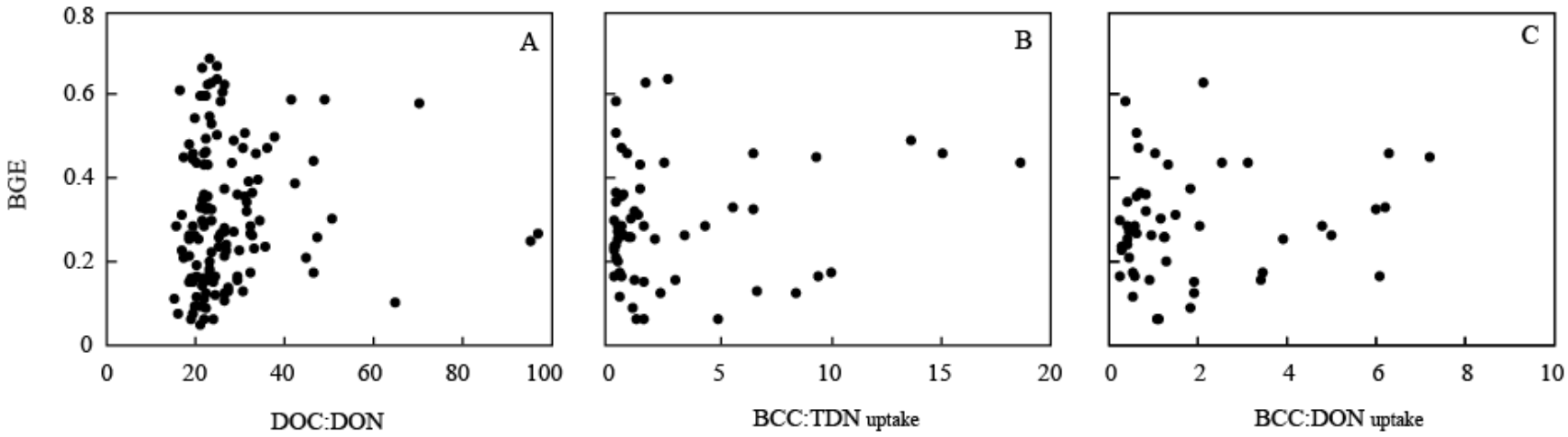

BGE
0.8
0.6
0.4
0.2
0
A
0
20
40
60
80
100
DOC:DON
B
0
5
10
15
20
BCC:TDN uptake
C
0
2
4
6
8
10
BCC:DON uptake

Fig. 4.7. Relationship between bacterial growth efficiency (BGE) and the relative contributions of (A) dissolved inorganic nitrogen (DIN) to total dissolved nitrogen (TDN) uptake (n = 35) and (B) dissolved phosphate (PO4) to total dissolved phosphorus (TDP) uptake (n = 37).

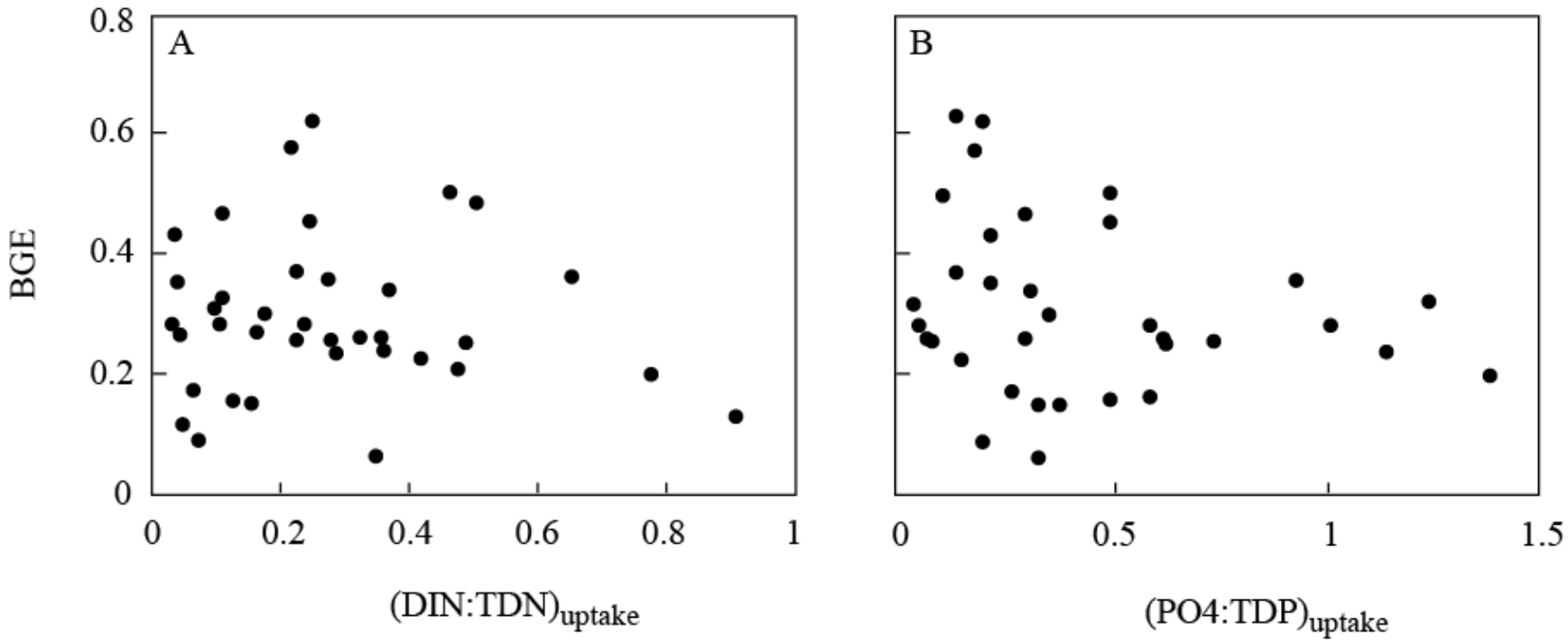

A
B
BGE
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
0.5
1.5
(DIN:TDN)uptake
(PO4:TDP)uptake

Fig. 4.8. Systematic differences in the relative contribution of (A) dissolved inorganic nitrogen (DIN) to total dissolved nitrogen (TDN) uptake and (B) dissolved phosphate (PO4) to total dissolved phosphorus (TDP) uptake.

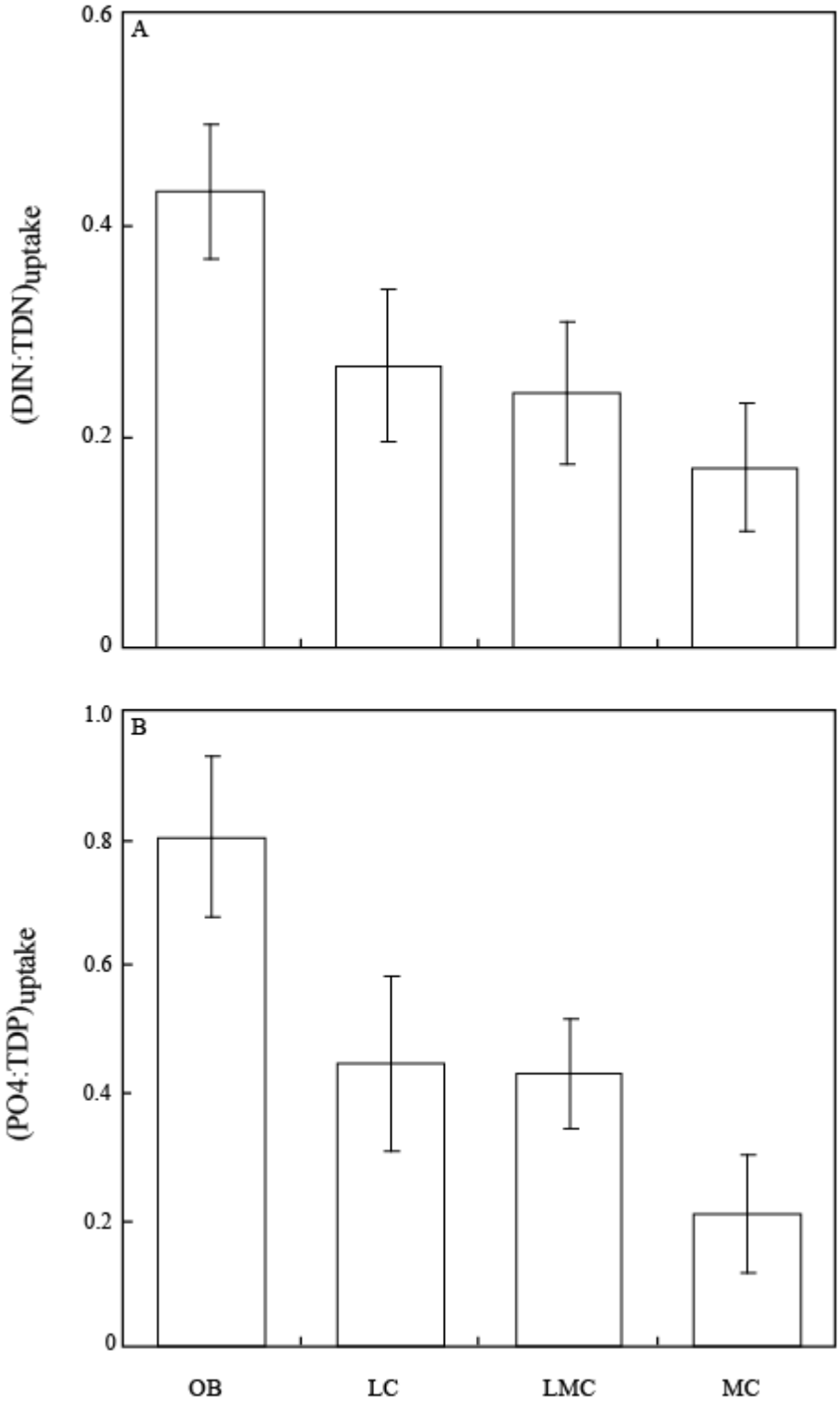
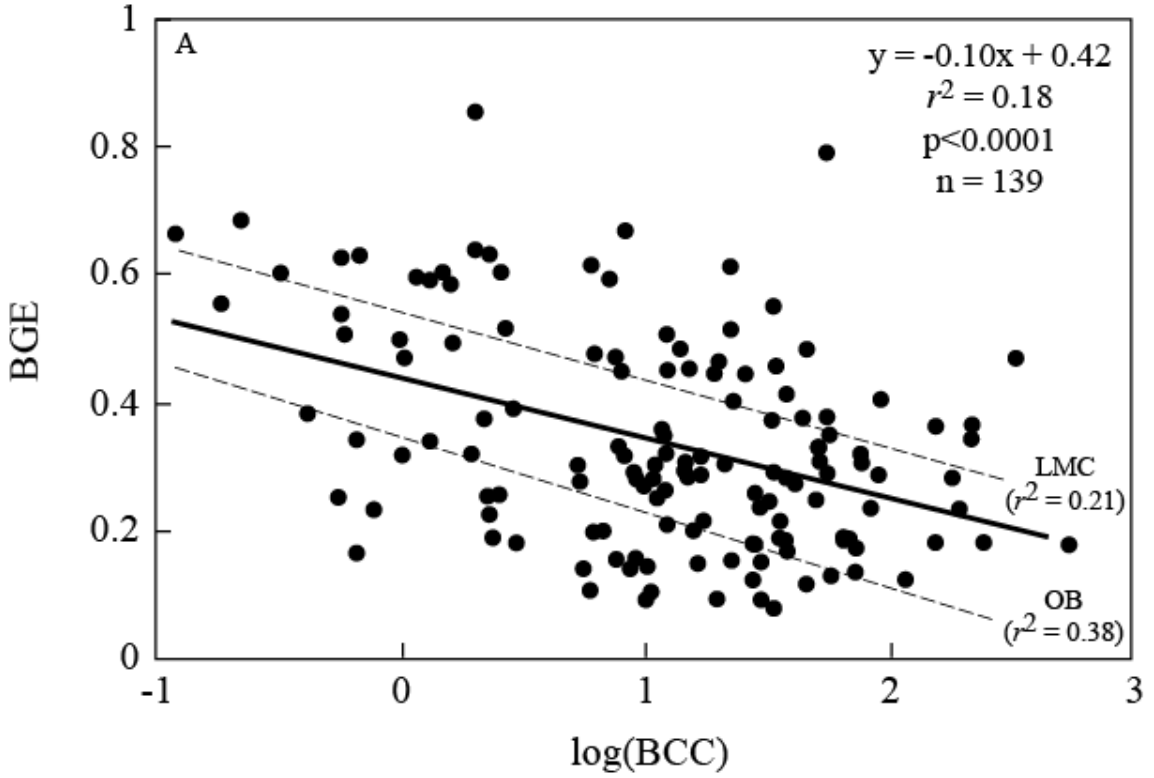

A
B
(DIN:TDN)uptake
(PO4:TDP)uptake
0.6
0.4
0.2
0
1.0
0.8
0.6
0.4
0.2
0
OB
LC
LMC
MC

Fig. 4.9. Relationship between (A) paired estimates of bacterial growth efficiency (BGE) and log-transformed values of bacterial carbon consumption (BCC; μgC L^{-1} h^{-1}) and (B) residuals from regressions of BGE and BCC versus temperature.


A
y = -0.10x + 0.42
r^2 = 0.18
p<0.0001
n = 139
LMC
(r^2 = 0.21)
OB
(r^2 = 0.38)
BGE
log(BCC)
1
0.8
0.6
0.4
0.2
0
-1
0
1
2
3

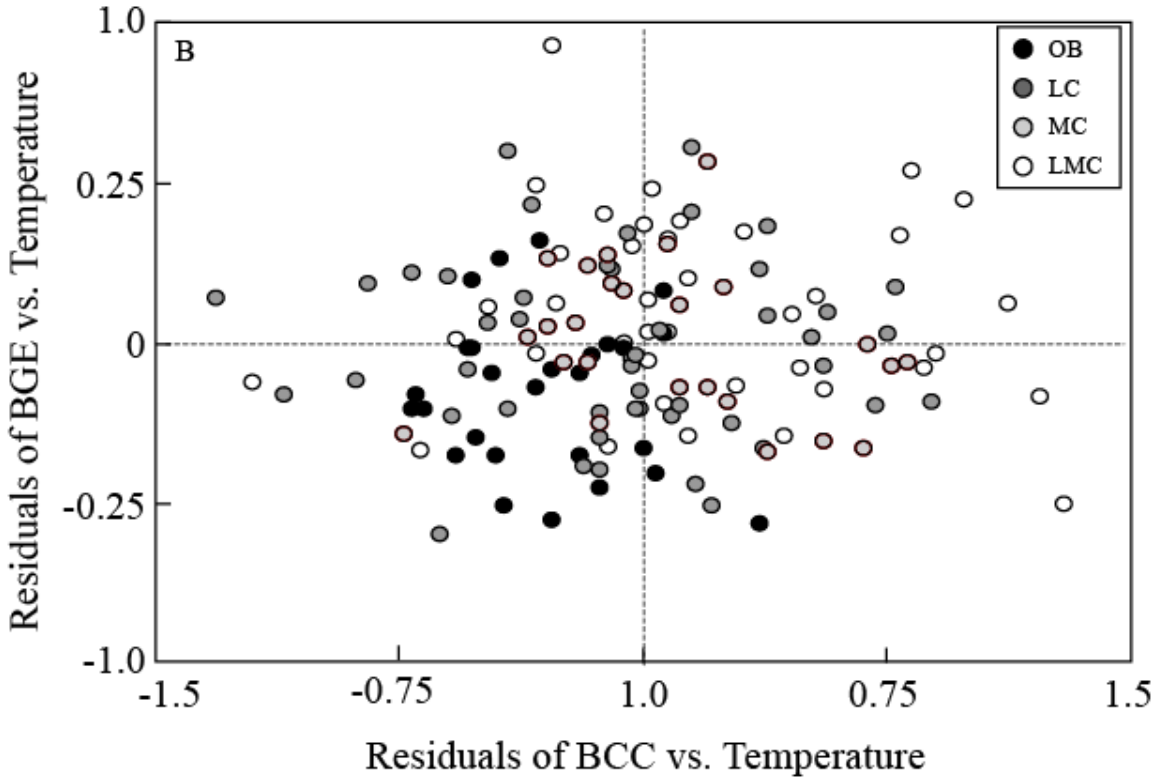

B
OB
LC
MC
LMC
Residuals of BGE vs. Temperature
Residuals of BCC vs. Temperature
1.0
0.25
0
-0.25
-1.0
-1.5
-0.75
1.0
0.75
1.5

Fig. 4.10. Correlations among sub-systems for (A) mean temperature residuals for BGE versus those for BCC and (B) mean temperature residuals for BGE versus mean lability.

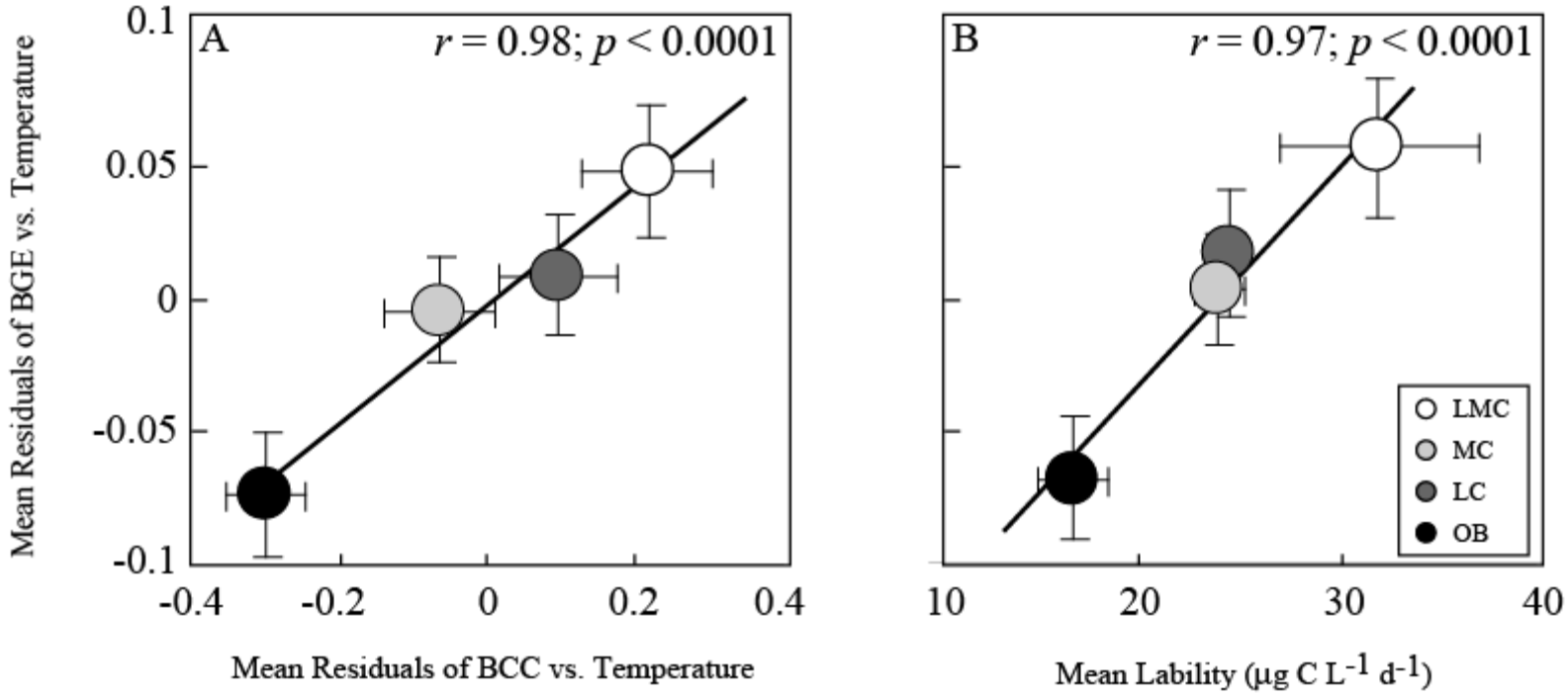
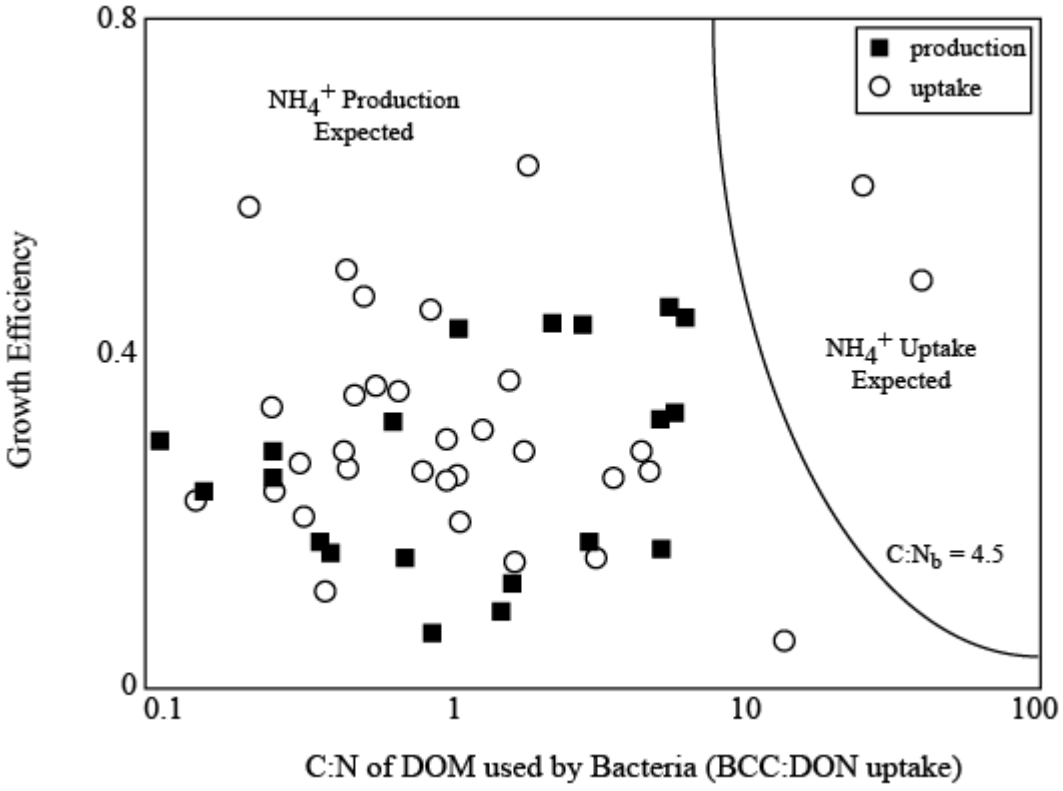
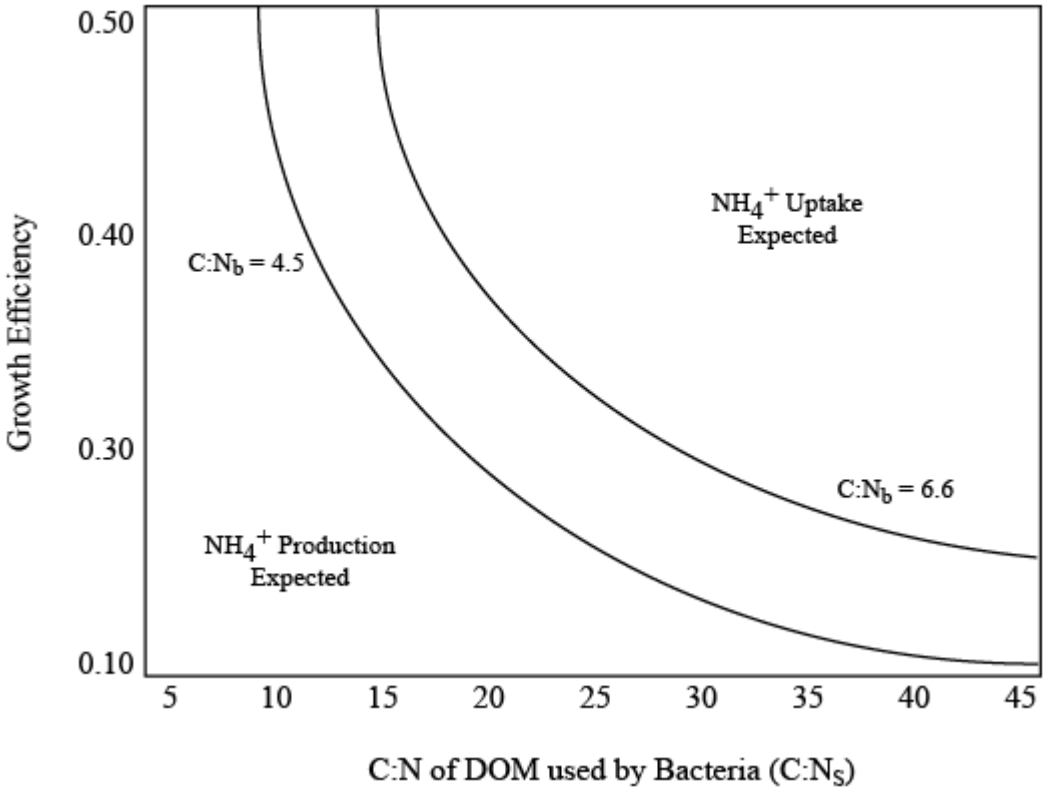

A
r = 0.98; p < 0.0001
Mean Residuals of BGE vs. Temperature
Mean Residuals of BCC vs. Temperature
0.1
0.05
0
-0.05
-0.1
-0.4
-0.2
0
0.2
0.4
B
r = 0.97; p < 0.0001
Mean Lability (µg C L-1 d-1)
10
20
30
40
LMC
MC
LC
OB

Fig. 4.11. The relationship between growth efficiency, C:N of DOM used by bacteria (BCC:DON uptake), and flux of ammonium (upper panel). Figure is adapted from Kirchman (2000; lower panel) to include empirical data from the present study. According to Kirchman (2000), the curved lines indicate zero NH_4^+ flux for C:N of bacterial biomass ($C:N_b$), with values of BGE above the curved lines resulting in net NH_4^+ uptake and those below the line resulting in net production of NH_4^+.


0.8
0.4
0
Growth Efficiency
production
uptake
NH_4^+ Production Expected
NH_4^+ Uptake Expected
$C:N_b = 4.5$
0.1
1
10
100
C:N of DOM used by Bacteria (BCC:DON uptake)


0.50
0.40
0.30
0.10
Growth Efficiency
NH_4^+ Uptake Expected
$C:N_b = 4.5$
$C:N_b = 6.6$
NH_4^+ Production Expected
5
10
15
20
25
30
35
40
45
C:N of DOM used by Bacteria ($C:N_S$)

CHAPTER V

Linking cellular and community-level metabolism in estuarine bacterioplankton communities

ABSTRACT

Cellular-level metabolic processes and community-level metabolism has been observed for natural bacterioplankton assemblages, yet the nature of this relationship remains poorly understood. it is not clear to what extent changes in community-level metabolism result from a proportional increase in the metabolism of all cells or a disproportionate increase in the metabolism of a specific subset of the bacterioplankton assemblage. We explored this question in the in the tidal creeks of a small temperate estuary, using the fluorescent stains CTC and SYTO-13 to identify characteristics of the highly-active fraction of bacterioplankton communities. We used these indices of single-cell activity to investigate their relationship with empirical estimates of bacterial production (BP), growth efficiency (BGE), and total carbon consumption (BCC). Single-cell activity was quite variable and activity within the highly-active fraction was often distributed unequally. Increases in BGE were coupled to increases in both the proportion and intensity of highly-active cells and there was a general coherence of measures of total community-level and cellular-level metabolism. We also found that freshwater and saltwater-dominated systems differ dramatically in the proportion of highly-active cells, the distribution of activity within the highly-active fraction, and the relationship between cellular-level and community-level metabolism. Our results provide insight into the role of single-cell activity in regulating bacterioplankton carbon metabolism in estuarine systems.

INTRODUCTION

The relationship between community and cellular-level metabolism in bacterioplankton communities is of fundamental interest, as cellular-level processes are the mechanism by which most ecologically relevant community-level metabolic processes are mediated (Sherr et al. 1999a). It is generally accepted that highly-active cells in natural bacterioplankton assemblages are those that are responsible for the majority of growth and production (Sherr et al. 1999b), and studies of single-cell activity from a wide range of aquatic systems have reported a general coherence of the abundance and/or proportion of highly-active cells with various community-level processes, including total bacterial abundance, production, growth, and respiration (Berman et al. 2001; del Giorgio and Scarborough 1995; Sherr et al. 1999b; Smith 1998). In addition, del Giorgio and Cole (1998) suggest that bacterial growth efficiency may be influenced by the proportion of highly-active cells. It is not clear, however, to what extent changes in metabolic activity within this highly-active fraction are manifested in the magnitude and variability of metabolic processes on the community-level.

Studies of single-cell activity in natural bacterioplankton assemblages typically use a discrete highly-active vs. inactive classification and report only the abundance or proportion of highly-active cells (Choi et al. 1999; del Giorgio and Scarborough 1995; Sherr et al. 1999a). In their review of studies investigating single-cell activity, del Giorgio and Scarborough (1995) found significant differences in the proportion of highly-active cells among a diverse array of aquatic systems, with higher values reported for estuaries and lower for lakes and oceans. Other investigators have reported similar changes in single-cell activity along enrichment gradients (Bouvier and del Giorgio 2002;

Choi et al. 1999; Cottrell and Kirchman 2003). Although these patterns in the abundance of highly-active cells may result from the direct effect system-level nutrient enrichment, they may also reflect inherent metabolic properties associated with dominant phylogenetic groups that result from changes in salinity or the supply and quality or organic matter substrates (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2003). Regardless of the mechanism driving changes in metabolism on the cellular level, these investigations have been limited to the abundance and proportion of highly-active cells. What has yet to be determined is the nature of change within the highly-active fraction associated with such changes in the proportion or abundance of highly-active cells. For example, do these result from a proportional increase in the metabolism of all bacterioplankton cells or a disproportionate increase in the metabolism of a specific subset of the bacterioplankton assemblage? In short, are increases in metabolism distributed equally among all assemblage constituents? Although the presence vs. absence criteria for identifying highly-active cells is an important and ecologically relevant measure (Sherr et al. 1999a), it overlooks the considerable metabolic diversity associated with the highly-active fraction itself (Smith and del Giorgio 2003) and cannot be used to address these questions and characterize what has been described as a continuum from relatively low to very highly-active cells in natural bacterioplankton populations (Gasol et al. 1999; Servais et al. 2003; Sieracki et al. 1999).

The proportion and abundance of highly-active cells is commonly determined using fluorescent stains that serve as indices of cellular-level activity. The redox dye CTC (5-cyano-2,3-ditolyl tetrazolium chloride) has frequently been used as an index of electron transport system (ETS) activity and thus a measure of actively respiring cells and

cellular-level activity (Rodriguez et al. 1992). In its oxidized form, CTC is a non-fluorescent water-soluble molecule, which forms the fluorescent precipitate formazan when transported into cells and reduced by ETS activity. Cells that accumulate enough reduced CTC granules (i.e., CTC+ cells) can then be enumerated using either epifluorescent microscopy or flow cytometry (Joux and Lebaron 2000; Sherr et al. 1999a). In general, the abundance of CTC+ cells in natural samples tends to be lower than that measured using other assays of single-cell activity, presumably because these cells represent the most highly-active cells in the assemblage (Sherr et al. 1999a; Smith and del Giorgio 2003). Another commonly used measure of single-cell activity is the nucleic acid stain SYTO-13 (Gasol and del Giorgio 2000; Gasol et al. 1999; Servais et al. 2003). Natural bacterioplankton assemblages that are stained with SYTO-13 and visualized using flow-cytometry typically exhibit a bimodal distribution, with cells partitioned into sub-populations of relatively high and low DNA content (Li et al. 1995). These groups of high-DNA (HDNA) and low-DNA (LDNA) bacteria are assumed to represent the highly-active and inactive fractions of the bacterioplankton community, respectively (Lebaron et al. 2001a). This bimodal distribution of bacterioplankton cells has been consistently observed in studies representing a wide range of aquatic systems (Gasol et al. 1999; Jellet et al. 1996; Marie et al. 1997; Massana et al. 2001).

The variability in activity within the highly-active fraction can be investigated using the relative intensity (i.e., fluorescence) of highly-active cells to determine the distribution of metabolic activity within this fraction (Cook and Garland 1997; Sieracki et al. 1999; Smith and del Giorgio 2003), an approach which may be more successful when cells identified with CTC are used (Berman et al. 2001; Sherr et al. 1999a; Smith and del

Giorgio 2003). However, the development of suitable analytical approaches for investigating the distribution of highly-active cells has been limited, with only a few studies exploring the variability in metabolic activity within the highly-active fraction itself (Cottrell and Kirchman 2004; Lebaron et al. 2001a; Servais et al. 2003; Sieracki et al. 1999). In this regard, even the most basic characterization of changes in the distribution of activity among the highly-active fraction would provide valuable insight into how single-cell activity changes in response to environmental conditions and how these changes might influence community-level metabolic processes. Ultimately, such information regarding cellular-level metabolism is essential if we are to fully understand the linkages between cellular-level characteristics (e.g., phylogeny, physiology, cellular metabolism), bacterioplankton carbon metabolism, and ultimately carbon flux in aquatic systems (Cottrell and Kirchman 2003).

Our investigations to date have focused exclusively on community-level measures of carbon metabolism, the environmental factors that regulate the variability and magnitude of these processes, and their response to system-level nutrient enrichment. The present study represents a shift in focus to cellular-level metabolic processes, investigating three hypotheses regarding the relationship between cellular-level metabolic characteristics and community-level metabolism processes, as well as the proportion, abundance, and distribution of activity within the highly-active fraction. Our first hypothesis is that changes in community-level carbon metabolism are associated with changes in the metabolism of a subset of the community, rather than being shared equally among all constituents. The second addresses the relationship between BGE and the proportion of highly-active cells, and we hypothesize that community-level measures of

growth efficiency are influenced by the balance between highly-active versus slow-growing or inactive cells, with higher BGE expected when the proportion of highly-active cells is greater. Finally, based on recent studies linking salinity, phylogeny, and single-cell activity in estuarine systems, we predict that the distribution of highly-active cells will differ when freshwater and saltwater-dominated systems are compared.

METHODS

Sample Collection

Our study was conducted in the Monie Bay component of Maryland's National Estuarine Research Reserve System (MDNERRS), a temperate salt-marsh system located on the eastern shore of Chesapeake Bay (38°13.50'N, 75°50.00'W) and comprised of three tidal creeks and a shallow bay that collectively represent a range in environmental conditions. Monie Creek (MC) is the largest of the three tidal creeks, receives inputs of fresh-water throughout the year, has lower overall salinity, and elevated nutrient concentrations as a result of agricultural activity within the watershed. Little Monie Creek (LMC) is also agriculturally-impacted and experiences nutrient enrichment comparable to that of MC, but experiences reduced freshwater inputs and thus higher mean salinity. The smallest of the three tidal creeks is the relatively pristine Little Creek (LC), which is not influenced by episodic nutrient inputs and has salinities comparable to that of LMC. All three creeks empty into and interact tidally with the a shallow open bay (OB). Ten different sites within these four sub-systems were visited monthly between March 2000 and December 2001. The location of sampling sites, environmental conditions in the tidal creeks, and the utility of this reserve as a model system for

investigating estuarine bacterioplankton communities have been described in detail in Apple et al. (2004).

Bacterial Enumeration and Single-Cell Characteristics

Bacterial abundance (BA) and the proportion and abundance of metabolically-active cells was determined on live samples using a Becton-Dickinson FACSCaliber bench top sheath flow cytometer. Total abundance and that of high-DNA (HDNA) cells was determined using the nucleic acid stain SYTO-13 (Molecular Probes) following del Giorgio et al. (1996a) and Gasol and del Giorgio (2000). Working stock solutions of SYTO-13 were prepared by dissolving concentrated stock with DMSO for a final concentration of 0.5mM. Two microliters of the working stock were combined with 500μl of sample in a 7ml flow cytometer Falcon tube, vortexed well, and incubated in the dark for 5 minutes. Ten microliters of reference bead stock solution containing 1μm green fluorescent microspheres (Molecular Probes) at a concentration of approximately 3000 beads μl^{-1} was added to the sample and vortexed again. Bacterial cells were visualized in a cytogram of light side scatter (SSC) versus green fluorescence (FL1) and enumerated based on the number of intact bacterial nuclei relative to the total number of reference beads counted. Each sample was run in the flow-cytometer until a minimum of 20,000 events were counted. We also determined the abundance and characteristics of cells in each of the sub-populations typically observed in natural bacterioplankton communities (Li et al. 1995), the first consisting of cells with high green fluorescence and side scatter (HDNA cells) and a second with lower green fluorescence and side scatter (LDNA cells). Regions for identifying HDNA and LDNA cells were the same for all

analyses. The proportion of HDNA and LDNA cells was calculated using the abundance of each relative to the total bacterial counts obtained by SYTO-13 staining.

The abundance of actively respiring cells (CTC+) was determined using the redox dye CTC (5-cyano-2,3-ditolyl tetrazolium chloride) following Sieracki et al. (1999) and del Giorgio et al. (1997). Prior to analyses, a stock solution of 50mM CTC (PolySciences, PA, USA) was prepared using distilled water, filtered through 0.1 μm, and stored in the dark at 5^{o}C until use. 55.5μl of this stock CTC solution was added to 500μl of live sample for an approximate final concentration 5mM, vortexed well, and incubating in the dark at room temperature for 1.5 hours. At the end of the incubation, 10μl of reference bead stock were added to the sample and vortexed. Each sample was run in the flow-cytometer until a minimum of 10,000 events were counted. CTC+ cells were identified and enumerated using orange (FL2) and red (FL3) fluorescence. The proportion of CTC+ cells (%CTC) was calculated using the abundance of each relative to the total bacterial counts obtained by SYTO-13 staining.

Mean orange fluorescence (FL2) of each sample was used as an index of activity within the highly-active fraction, with higher values representing greater metabolic activity associated with the highly-active fraction. The total number of highly-active cells was combined with mean fluorescence (i.e., FL2*CTC+) as an integrative measure of total activity for each sample. This analysis identifies both the abundance and intensity of single-cell activity, allowing the discrimination between populations that have a similar number of highly-active cells yet differences in the intensity of metabolic activity associated with each highly-active fraction.

Bacterioplankton Carbon Metabolism

Estimates of community-level carbon metabolism reported in this paper are derived from previous studies in which the methodology is thoroughly described (Apple and del Giorgio in prep.; Apple et al. 2004). Briefly, bacterial production (BP) was estimated using incorporation of ^{3}H-leucine following modifications of Smith and Azam (1992) and assuming a carbon conversion factor of 3.1 Kg C · mol leu^{-1} (Kirchman 1993). Bacterial respiration (BR) was determined by measuring the decline of oxygen concentration over the course of 6 h incubations. Bacterioplankton carbon consumption was calculated by adding simultaneous measurements of filtered BP and BR, and growth efficiency was calculated as the ratio of filtered BP and total carbon consumption (i.e., BGE = BP/(BP + BR)). Cell-specific production (BP_{sp}) was calculated by dividing rates of BP by total bacterioplankton abundance and used as a measure of bacterioplankton growth (Kirchman 2002).

Statistical Analyses

All statistical analyses, including standard least squares regressions and analyses of variance (ANOVA) and covariance (ANCOVA) were performed using JMP 5.0.1 statistical software package (SAS Institute, Inc.). Mean values derived from flow cytometric analyses were determined using CellQuest flow-cytometry software (BD Biosciences).

RESULTS

Patterns In Single-Cell Activity

Overall means for the proportion of HDNA and CTC+ cells for all tidal creeks during the 2000 sampling season were 51 and 13%, respectively (Fig. 5.1). The largest

proportion of HDNA and CTC+ cells was consistently recorded in MC (54.7 and 15.4%, respectively), with significantly higher values than the other two tidal creeks. The same general hierarchy was observed among the three tidal-creeks for both HDNA and CTC+ cells, with the highest proportion of metabolically-active cells in MC, intermediate in LMC, and lowest in LC. The proportion of both HDNA and CTC+ cells in enriched MC was significantly higher than that of the other two creeks (Tukey-Kramer HSD; $\alpha = 0.1$; $p = 0.01$ and 0.05, respectively;). Although the proportion of HDNA and CTC+ cells was also higher in enriched LMC (46.1 and 10.9%) relative to unenriched LC (40.6 and 9.5%), these differences were not significant ($p = 0.3$ and 0.6).

The abundance of highly-active cells was well correlated with total abundance (Fig. 5.2), although the relationship between HDNA abundance and total bacterioplankton abundance (Fig. 5.2A; $r^2 = 0.72$; $n = 193$; $p < 0.0001$) was much stronger than that of CTC+ abundance (Fig. 5.2B; $r^2 = 0.12$; $n = 190$; $p < 0.0001$). Total abundance of HDNA and CTC+ cells were well correlated (Fig. 5.3; log(CTC+) = 0.6*log(HDNA) + 4.6; $r^2 = 0.35$; $n = 180$; $p < 0.0001$). We observed a significant negative relationship between CTC+ abundance and mean fluorescence of CTC+ cells, with lower mean fluorescence associated with higher abundances of highly-active cells ($r^2 = 0.25$; $n = 182$; $F = 59.3$; $p < 0.0001$; data not shown).

Coherence of Cellular and Community-Level Metabolism

Long-term means of total cellular-level activity (i.e., CTC*FL2) and total carbon metabolism (i.e., BCC) exhibited a similar pattern among the four sub-systems, with the highest values recorded in LMC, lowest in the open bay, and intermediate values in MC and LC (Fig. 5.4). The positive relationship between BCC and FL2*CTC implied by

these corresponding patterns was not observed in regressions of the entire dataset (data not shown). The apparent coupling of community-level and cellular-level metabolic characteristics (Fig. 5.4) was also observed between mean fluorescence of CTC+ cells (i.e., FL2) and BGE (Fig. 5.5), with highest values recorded in the two salt-water dominated creeks (i.e., LC and LMC) and lower values in MC and OB. Analysis of the entire dataset revealed a positive relationship between BGE and single-cell activity (Fig. 5.6), despite the lack of coherence between the proportion of CTC+ cells and BGE when long-term (Figs. 5.1 & 5.5A),. The variability associated with these regressions differed among sub-systems, with the strongest relationships observed in LC and OB (i.e., r^2 = 0.21 and 0.17, respectively) and weaker in LMC and MC (i.e., r^2 = 0.12 and 0.05, respectively).

Cell-specific production (BP_{sp}; i.e., growth) was negatively correlated with the abundance of HDNA cells (Fig. 5.7A) and exhibited no relationship with the proportion of HDNA cells (data not shown). In contrast, BP_{sp} was positively correlated with the proportion of CTC+ cells (Fig. 5.7B) but had no relationship with CTC+ cell abundance (data not shown).

Comparison of Freshwater and Saltwater-Dominated Tidal Creeks

We observed differences between freshwater and saltwater-dominated tidal creeks (i.e., MC and LMC) when a number of aspects of single-cell activity were considered. Although the proportion of highly-active cells was consistently higher in MC (Fig. 5.2), mean fluorescence (Fig. 5.6A) and FL2*CTC (Fig. 5.5A) were significantly higher. The relationship between BGE and %CTC was stronger and the slope of this relationship more positive in LMC relative to MC. We also observed significant differences in the

relationship between BP and the proportion of CTC+ cells when these two creeks were compared (ANCOVA; $n = 39$; $p = 0.07$), with higher BP in LMC for any given proportion of CTC+ cells than observed in MC (Fig. 5.9). This relationship was only observed during the first year of sampling (i.e., 2000), and regressions of the entire dataset failed to identify similar patterns (data not shown). Finally, frequency distributions of CTC+ fluorescence (i.e., FL2) associated with the highly-active fraction revealed different patterns in the distribution of highly-active cells in samples from OB, LMC, and MC. These differences in the distribution of highly-active cells were quantified as mean values of bead-normalized fluorescence, with lowest values recorded in OB (0.0049), higher in LMC (0.0056), and highest in MC (0.006).

DISCUSSION

Relationship Between Total Abundance and that of Highly-Active Cells

A primary objective of our study was to investigate the relationship between total bacterioplankton abundance (BA), the abundance of highly-active cells, and the distribution of activity within the highly-active fraction. Our investigation began by examining the relationship between total bacterioplankton abundance and that of both HDNA and CTC+ cells. A positive linear relationship between total and HDNA abundance and slope of unity (Fig. 5.2A) indicates that the proportion of HDNA cells remains relatively constant as one moves from low to high abundance or from less to more productive systems. In this regard, changes in community-level metabolism along such gradients of activity are probably associated with changes in the distribution or intensity of activity within the highly-active fraction rather than changes in the abundance or proportion of highly-active cells alone. Accordingly, we observed small yet significant increases in mean green fluorescence (FL1) with increasing HDNA abundance (i.e., log(HDNA) = 1.6* log(FL1) + 12.7; $r^2 = 0.19$; $n = 193$; $p < 0.0001$; regression not shown), which implies that there is a shift in the distribution of activity associated with the highly-active fraction when communities with elevated numbers of highly-active cells are considered. This increase in fluorescence indicates more intense staining of HDNA cells and is most likely represents substantial increases in cellular nucleic acid content associated with protein synthesis and cell division.

We observed a different pattern when the abundance and fluorescence of CTC+ cells was considered. Although BA and CTC+ cell abundance were positively correlated (Fig. 5.2B), this relationship was much weaker and exhibited almost three orders of

magnitude variability in CTC+ abundance for any measure of BA (Fig. 5.2B). In addition, the slope of this regression (i.e., 0.4) revealed a decreasing exponential relationship, suggesting that the abundance of CTC+ cells may approach an asymptotic maximum that represents an upper limit of CTC+ activity sustainable by the assemblage. The positive yet divergent relationship between HDNA and CTC+, in which the abundance of CTC+ cells does not increase proportionately with that of HDNA cells (Fig. 5.3), provides additional evidence of this asymptotic response in CTC+ abundance. These observations may be attributed to the fact that these two assays of single-cell activity (i.e., HDNA and CTC) represent fundamentally different but inherently coupled cellular-level metabolic processes (Gasol and del Giorgio 2000; Rodriguez et al. 1992), which may also help explain the generally lower abundance of CTC+ cells relative to other indices of single-cell activity (Smith and del Giorgio 2003).

Influence of Single-Cell Activity on Community-Level Carbon Metabolism

Previous research by our group (e.g., Apple et al. 2004) has focused on the effect of environmental conditions on bacterioplankton communities, with minimal attention dedicated to investigating the direct and inevitable effect of single-cell activity on the magnitude and variability of bacterioplankton carbon metabolism. We are particularly interested in the role of single-cell activity in determining the magnitude of carbon consumed by the bacterioplankton community (i.e., BCC) and the way in which this carbon is processed (i.e., BGE). In this regard, we explore two hypotheses addressing the relationship between single-cell activity and these measures of community-level carbon metabolism. First, we predict that BGE is influenced by the proportion of highly-active cells, with higher growth efficiencies expected when the proportion of metabolically-

active cells is also high, and secondly, that measures of total cellular-level metabolism (i.e., FL2*CTC) will generally be coherent with measures of total community-level carbon metabolism (i.e., BCC).

Growth Efficiency

Although previous work by our group identified organic matter quality as an important factor regulating growth efficiency (e.g., Chapter IV), we predict that the proportion of highly-active cells may also have a significant influence on BGE. The rationale for this hypothesis is based on the differences in growth efficiency associated with cells at varying levels of metabolic activity, whereby highly-active cells tend to have higher growth efficiencies than those of dormant or slow-growing cells (del Giorgio and Cole 1998). This coupling of growth and cellular-level BGE comes as a result of the respiratory demands of basal metabolism associated with maintaining membrane integrity, polarization, and osmotic gradients in bacterial cells (del Giorgio and Bouvier 2002) and the fact that these energetic demands make up a much smaller proportion of the total energy flux in cells that are growing more rapidly (Berman et al. 2001; del Giorgio and Cole 1998). Thus, higher metabolic activity produces higher BGE. Similarly, slow-growing or dormant cells tend to have lower rates of production relative to these energetic demands (i.e., lower BP relative to BR) and lower BGE. In this regard, the balance between the abundance of highly-active cells and that of inactive cells may play an important role in determining the BGE of the entire assemblage, forming the basis for our hypothesis that higher efficiencies will occur in communities where highly-active cells are of higher abundance or of elevated activity.

Our investigation of the relationship between growth efficiency and single-cell activity began with a comparison of long-term means among the four sub-systems, revealing a strikingly similar pattern in mean CTC+ fluorescence (FL2) and BGE (Fig. 5.5), yet no such similarity in pattern between BGE and the proportion of highly-active cells (Figs. 5.1 & 5.5). This would suggest that contrary to our original hypothesis, the intensity of activity within the highly-active fraction rather than the proportion of highly-active cells may have a greater effect on BGE. As mentioned previously, the highly-active fraction represents a gradient in activity from barely above threshold to very highly-active (Gasol et al. 1999; Sieracki et al. 1999), thus we would expect a similar range in BGE within the highly-active fraction that is driven by changes in the cellular-level BGE of individual cells at varying levels of metabolic activity. In this manner, cells that are promoted from the inactive fraction to the highly-active fraction may tend to have lower growth rates, lower mean fluorescence, and thus lower BGE than other highly-active cells by virtue of their proximity to the threshold for enumeration. Likewise, changes in the distribution of highly-active cells that favor an increase in the activity of a more highly-active subset of the population would result in an increase in overall fluorescence, growth, and BGE associated with the highly-active fraction. We believe that such changes in activity and BGE within the highly-active fraction may describe the coherence observed between FL2 and BGE, providing insight into the distribution of highly-active cells among the tidal creeks.

The similarity in pattern of FL2 and BGE observed in our study (Fig. 5.5) implies that changes in the intensity or growth of highly-active cells may in fact be a more important determining factor than proportion or abundance alone, supporting the

conclusion of Smith and del Giorgio (2003) that the presence/absence criteria for evaluating single-cell activity is of little use when the continuum of activity within the highly-active fraction is considered. Studies identifying a high degree of variability within the highly-active fraction using other measures of single-cell activity (i.e., HDNA, microautoradiography) also conclude that there are changes in single-cell activity that influence community-level metabolic processes that are independent of changes in the proportion or abundance of highly-active cells (Cottrell and Kirchman 2003; Gasol et al. 1999; Servais et al. 2003). Thus, focusing on the distribution of growth and activity within the highly-active fraction rather than the proportion and abundance of highly-active cells alone may reveal a relationship between cellular-level metabolism and community-level metabolic processes that may otherwise not be apparent.

We continued our investigation of the hypothesis that BGE is influenced by single-cell activity using paired measurements of BGE and the proportion of CTC+ cells (Fig. 5.6). We observed a highly-significant but very weak ($r^2 = 0.12$; $n = 138$; $p < 0.0001$) positive relationship between BGE and %CTC when the entire dataset was considered that improved when the sub-systems were considered individually. Given the environmental variability within and among the tidal creeks of Monie Bay, it is difficult to determine if the weak relationships between BGE and the proportion of CTC+ cells are actually strong and meaningful relationships that are obfuscated by environmental variability, or simply weak relationship that provide little additional insight into the regulation of BGE by the proportion of highly-active cells. Assuming the former, although our results do not provide unequivocal evidence that BGE is driven by the relative abundance of highly-active cells alone, collectively they indicate that cellular-

level processes may have an important influence on the balance between production and respiration in natural bacterioplankton assemblages. Ultimately, more detailed investigations of the relationship between single-cell activity, intensity, and BGE in more controlled settings or part of manipulative experiments will be necessary to determine if the proportion of highly-active cells dictates the magnitude of bacterioplankton growth efficiency.

Total Carbon Consumption

Just as BGE is a measure of the balance between community-level production and respiration, bacterioplankton carbon consumption (i.e., BP+BR) serves as a proxy for total community-level metabolic activity. Accordingly, we hypothesized that estimates of BCC would be coherent with measures of total cellular-level activity. Furthermore, because CTC+ cells represent the most highly-active cells (Choi et al. 1999; Sherr et al. 1999a; Smith 1998), are responsible for the majority of growth and production (Sherr et al. 1999b), and are an estimate of the abundance of actively respiring cells (Rodriguez et al. 1992), we expected indices of single-cell activity in this fraction to be the most coherent with total community-level carbon consumption. Although the proportion of CTC+ cells and mean fluorescence alone do not necessarily represent a comprehensive assay of cellular-level metabolism, collectively the two (i.e., FL2*CTC) serves as an integrative and effective measure (Sherr et al. 1999a). In this regard, although we found no correlation between FL2 or the proportion of CTC+ cells and measures of carbon consumption (i.e., BP, BR, BCC), comparisons of long-term mean BCC and FL2*CTC revealed a strikingly similar pattern among sub-systems (Fig. 5.4). The greatest magnitude of both cellular- and community-level metabolism were observed in LMC,

similar and intermediate values in LC and MC, and lowest values in OB. This hierarchy among systems reinforces that which has been consistently observed in other measures of community-level carbon metabolism in Monie Bay (e.g., Chapters II & III). Although the abundance and proportion of highly-active cells was consistently higher in MC (Fig. 5.1), these cells were not as highly-active as those of LC and LMC (Fig. 5.5A), thus had lower overall FL2*CTC values. This may explain the apparent decoupling of measures of carbon metabolism and single-cell activity observed in MC.

Measures of the proportion or abundance of highly-active cells are limited in what they can reveal regarding the distribution of metabolic activity within the highly-active fraction. However, taking into consideration mean fluorescence (i.e., FL2) may be an effective way to normalize measures of highly-active cell abundance for differences in the distribution of single-cell activity. Ultimately, this may provide a more realistic assay of total cellular-level metabolism and given the variability in both the abundance and activity of CTC+ cells observed in our study (Figs. 5.2B & 5.9) and others (Cottrell and Kirchman 2004; Gasol et al. 1999; Servais et al. 2003), community-level metabolic processes will probably be coupled more tightly to such indices of total cellular-level activity (e.g., FL2*CTC) than either measures alone. Studies investigating the coherence of single-cell activity and community-level carbon metabolism take into consideration changes in the abundance of highly-active cells, as well changes in the intensity and distribution of activity within the highly-active fraction.

Specific Production

Although HDNA and CTC+ cells may represent similar or at least coupled metabolic processes (e.g., Fig. 5.3), indices of single-cell activity used in our study

differed strikingly in their relationship with estimates of bacterioplankton growth (i.e., cell specific production; Kirchman 2002), with a general negative relationship observed with HDNA abundance and positive with the proportion of CTC+ cells. The apparent decrease in growth at higher abundances of HDNA cells (Fig. 5.7A) was unexpected, as we anticipated that increases in nucleic acid content would be reflected directly in bacterioplankton growth and production (Gasol et al. 1999; Servais et al. 2003). Because total abundance and that of HDNA cells are well correlated (Fig. 5.2A) the proportion of HDNA cells is relatively constant in this system. Assuming that DNA content and BP remain well coupled (Lebaron et al. 2001a), increases in the abundance of HDNA cells should be reflected in estimates of specific production. One explanation of the apparent decoupling of the abundance of HDNA cells and BP that we observed is suggested by Cottrell and Kirchman (2004) is that such contemporaneous measurements of cellular-level and community-level metabolism fail to account for the time lag that probably exists between the ramping up of cellular-level metabolism and corresponding increases in production and growth. Thus, although production and single-cell activity were measured simultaneously, the growth associated with increases in HDNA abundance may not be manifested as growth or production for several hours. This conclusion applies to all relationships derived from paired measures of growth, production, and single-cell activity.

Another explanation is that there is a disproportionate temperature limitation of BP relative to single-cell activity, resulting in a negative relationship between the two when metabolically-active cells from colder waters are considered in our analyses. For example, the highest abundance of HDNA cells was recorded on 6 April 2000 and appear

to the furthermost right in Fig. 5.7A (n = 12). This sampling date coincided with the largest episodic nutrient loading event recorded during our two-year study (Apple et al. 2004) where relatively low ambient water temperatures were recorded (i.e., 11ºC). Based on the temperature dependence of BP recorded previously for this system (Chapter III) and the typically rapid response of highly-active cells to nutrient enrichment (Choi et al. 1999; Gasol et al. 1999), we predict that temperature had a limiting effect on community-level production that did not constrain single-cell activity. Removal of these data points significantly weakened the original relationship (r^2 = 0.07; data not shown), suggesting that growth and abundance of HDNA cells may not be well-coupled when simultaneous measurements are considered and that the negative relationship observed in Fig. 5.7A was a product of conditions during one sampling event when growth was limited relative to single-cell activity.

In contrast to the negative relationship observed between specific production and the abundance of HDNA cells, there was a weak but positive correlation between specific production and the proportion of CTC+ cells (Fig 7B). This general positive coherence of cell-specific production (BP_{sp}) and the proportion of CTC+ cells was not surprising, as other studies have documented a similar relationship (Sherr et al. 1999b). However, this relationship is driven in part by a single outlier and indicates that as with HDNA abundance the coupling of single-cell activity with bacterioplankton growth is very weak if not non-existent in this system of tidal creeks.

Many studies have reported that the highly-active fraction of bacterioplankton communities is selectively grazed when both CTC+ and HDNA cells are considered (e.g., del Giorgio et al. 1996c; Vaqué et al. 2001). Although enumeration of grazer populations

or estimates of grazing rates were not a part of this study, it is likely that grazing pressures play an important role in the relationships between bacterioplankton growth and both the abundance and proportion of highly-active cells. For example, bacteria that are attached to aggregates or detrital particles tend to be more active (Crump and Baross 2000) and may also be resistant to size-selective grazing pressures that typically impact larger, more active cells (Langenheder and Jurgens 2001). This size refuge effect also applies to small dormant cells, which are typically not subjected to heavy grazing pressure (Gonzalez et al. 1990). The potential disproportionate grazing of bacterioplankton of intermediate size and growth that might result from these circumstances would produce a bimodal distribution such as that observed between HDNA and LDNA cells (Lebaron et al. 1999; Li et al. 1995), with the bacterioplankton community divided into fractions of rapidly-growing, particle-attached cells and small, dormant cells – both of which represent grazer-resistant populations. This would maintain relatively high bacterioplankton abundance with production limited to the rapidly-growing fraction, resulting in a decrease in production per bacterioplankton cell (i.e., cell-specific production). Such a scenario is more likely in particulate rich environments such as estuaries and may offer an alternate explanation to the negative relationship observed between growth and the abundance of HDNA cells.

Differences Between Freshwater vs. Saltwater Dominated Systems

A final objective of our study was to investigate the hypothesis that bacterioplankton assemblages in saltwater and freshwater-dominated systems differ significantly in the distribution, activity, and abundance of highly-active cells. The rationale for this hypothesis is two-fold. First, studies of single-cell activity in estuarine

systems have reported differences in cellular-level metabolism when freshwater and saline endmembers are compared (Cottrell and Kirchman 2003; del Giorgio and Bouvier 2002). Secondly, previous studies in Monie Bay conducted by our group have observed consistently higher rates of carbon metabolism and higher growth efficiencies in saltwater-dominated LMC and significantly lower BCC and BGE in MC (Chapter IV). In this previous work, we suggest that DOM quality has an important but not exclusive influence on BGE in these systems and speculate that differences in carbon metabolism between LMC and MC may be attributed in part to inherent cellular-level characteristics of the bacterioplankton communities.

Given consistently lower production and growth efficiency recorded in MC, elevated proportions of highly-active cells in this system were surprising (Fig. 5.1). Assuming that highly-active cells are those responsible for the majority of growth and production in bacterial assemblages (Sherr et al. 1999b), we would have expected that either the highest proportion of highly-active cells would have been recorded in LMC or the highest rates of carbon metabolism in MC. We believe that differences in the distribution of activity within the highly-active fraction may explain this discrepancy, for although the proportion of highly-active cells was greater in MC (Fig. 5.1), the mean fluorescence (Fig. 5.5A) and total cellular-level activity (i.e., FL2*CTC; Fig. 5.4B) was lower. Thus, although the abundance and proportion of highly-active cells in MC may have been elevated, these highly-active cells were on the lower end of the continuum of activity within the highly-active fraction and thus less capable of elevated rates of carbon metabolism.

To investigate further the differences in the coherence between single-cell activity and community-level carbon metabolism in freshwater and saltwater-dominated tidal creeks, we considered the relationship between paired measures of BP and the proportion of CTC+ cells in MC and LMC (Fig. 5.8). There was a striking difference in the coupling of BP and single-cell activity when the two tidal creeks were compared, such that bacterioplankton in MC appeared to be much less productive than those in LMC for any given proportion of highly-active cells. These results suggest that either BP is limited or single-cell activity is enhanced in MC relative to LMC. Because ambient nutrient concentrations are similar between these two sub-systems (Apple et al. 2004), the direct and disproportionate effect of nutrient limitation was eliminated as a determining factor. However, Chapter IV provides evidence of low quality organic matter in MC that may limit bacterioplankton production, resulting in the discrepancy in the relationship between BP and the proportion of CTC+ cells evident in Fig. 5.9.

An alternative explanation to the limitation of BP is the enhancement of single-cell activity in MC relative to LMC. Recent studies of diversity along estuarine gradients (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2004) reveal shifts in phylogenetic composition, single-cell activity, and community-level metabolism when saltwater and freshwater-dominated endpoints were compared. Although this may be driven by the direct effect of salinity on bacterioplankton phylogeny (Barcina et al. 1997), others suggest that it may be attributed to those environmental factors that tend to covary with salinity in estuarine systems, including degree of enrichment, nutrient quantity and form, and DOM source and quantity (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2004). Of these factors, the quality and quantity of DOM may be the most important

(Cottrell and Kirchman 2004; Yokokawa et al. 2004). Given the significant differences in salinity and DOM quality and quantity between LMC and MC (Apple et al. 2004) and corresponding differences in community-level and cellular-level metabolism observed in the present study, it is likely that these two systems differ markedly with respect to the dominant phylotypes of the resident bacterioplankton assemblages. Analyses of mean fluorescence between these systems (Fig. 5.5A) as well as frequency distributions of fluorescence from individual sampling events (Fig. 5.9) provide compelling evidence that the distribution of highly-active cells is indeed different. Furthermore, our results suggest that these changes community and cellular-level metabolism are associated with the disproportionate contribution of a subset of the highly-active fraction. For example, we observed differences in the abundance of highly-active cells in the upper range of the activity continuum when CTC+ cells were compared among systems (Fig. 5.9). These changes in distribution were quantifiable, as evidenced by significant changes in bead-normalized fluorescence, with lowest values recorded in OB (0.0049), higher in LMC (0.0056), and highest in MC (0.006).

Ultimately, differences in single-cell activity and the coupling of this activity to community-level carbon metabolism between freshwater and saltwater-dominated systems may be driven by a combination of factors, including the direct effect of salinity on bacterioplankton metabolism (del Giorgio and Bouvier 2002), inherent metabolic and physiological properties associated with different phylogenetic groups (del Giorgio and Bouvier 2002; Yokokawa et al. 2004), the predisposition of certain phylotypes to the reduction or retention of reduced CTC, or the direct effect environmental conditions such as substrate quality and availability on both carbon metabolism and phylogenetic

composition (Cottrell and Kirchman 2003). In this regard, we predict that the pronounced difference in the relationship between single-cell activity and community-level carbon metabolism observed between MC and LMC (Fig. 5.8) is attributed a number of factors, including the limiting effect of low-quality DOM on BP (Chapter IV), the more pronounced effect of DOM quality on the limitation of bacterioplankton growth and production in low-salinity systems (Yokokawa et al. 2004), and the intrinsic cellular-level metabolic properties of bacterioplankton from different phylogenetic groups (del Giorgio and Bouvier 2002). Clearly freshwater and saltwater-dominated systems differ fundamentally in cellular-level metabolic properties. Without analyses of phylogenetic composition, however, it is impossible to unequivocally determine the extent to which changes in community or cellular-level metabolism are attributed to external environment factors or intrinsic characteristics of resident bacterioplankton assemblages. Although such investigations are essential in understanding the mechanisms driving the relationship between cellular and community-level metabolism, they are beyond the scope of the present study.

Conceptual Models of the Distribution of Single-Cell Activity

Estimates of the abundance and proportion of highly-active cells, although ecologically relevant, provide little information regarding the diversity of metabolic activity that exists within the highly-active fraction (Servais et al. 2003; Sieracki et al. 1999; Smith and del Giorgio 2003). As most studies of single-cell activity in natural aquatic systems focus on estimates of abundance and proportion alone, little is known regarding the distribution of activity within the highly-active fraction, how this may change in response to different stimuli, and how it is manifested in changes in

community-level carbon metabolism. In particular, it is difficult to determine the extent to which growth (μ) and production (BP) result from an equal contribution of all assemblage constituents versus the disproportionate contribution of a subset of the population. Given the absence of empirical data to identify specific changes in single-cell activity, we are in need of conceptual models which attempt to describe the continuum of possible single-cell activities that exist in natural bacterioplankton assemblages (Smith and del Giorgio 2003).

Using the relationship between the fluorescence and abundance of highly-active cells, we present four hypothetical scenarios that describe changes in the distribution of single-cell activity that might be expected in natural bacterioplankton communities (Fig. 5.10). In each model, the solid curve represents the distribution of activity within a "normal" community while the dashed curves represent a hypothetical response to some environmental stimuli. Vertical dotted lines indicate the analytical threshold for enumeration of cells as highly-active. Although an over-simplification, these hypothetical scenarios provide a framework for describing the dynamics of single-cell activity in natural bacterioplankton assemblages and for generating hypotheses regarding changes in the metabolic structure of the highly-active fraction.

One of the most straightforward relationships between cellular- and community-level metabolism is that which involves a proportional increase in activity throughout the assemblage, such that increases in any given measure of community-level metabolism (i.e., BP, BR, growth) are accompanied by an increase in single-cell activity that is distributed equally throughout the assemblage (Fig. 5.10A). In this scenario, the magnitude of increases in single-cell activity is similar among all cells, resulting in

dormant cells becoming highly-active and highly-active cells becoming even more highly-active. A change in single-cell activity of this nature would result in an increase in the number of cells enumerated as highly-active and an increase in mean fluorescence, yet minimal change in the overall distribution. As illustrated, such a response could be identified by concurrent increases in both mean fluorescence and the proportion of highly-active cells. Changes in single-cell activity of this nature may characterize the "ramping-up" of bacterioplankton metabolism prior to population growth and represent the type of response that is frequently assumed to be that of many natural bacterioplankton communities (Massana et al. 2001).

A second scenario is that in which increases in total abundance are accompanied by increases in the abundance of highly-active cells, such that the proportion and activity (i.e., fluorescence) of highly-active cells does not change (e.g., Fig. 5.10B). This model may describe the relationship between total abundance and that of HDNA cells (Fig. 5.2A), which indicates that proportional increases in the abundance of both total and highly-active cell abundance are not accompanied by changes in mean fluorescence. The consistently observed distribution of natural bacterioplankton communities into two fractions (i.e., HDNA and LDNA; Gasol et al. 1999; i.e., HDNA and LDNA; Li et al. 1995; Servais et al. 2003) may explain the lack of change in distribution (i.e., mean fluorescence) when increases in the abundance of highly-active cells was recorded.

Recent studies of single-cell activity provide evidence that the distribution of activity within the highly-active fraction frequently changes (Cottrell and Kirchman 2004; Gasol et al. 1999; Servais et al. 2003), suggesting that models of a proportional response (i.e., Fig. 5.10A,B) may not represent single-cell activity in all natural

bacterioplankton communities. A disproportionate increase in the activity of a subset of the highly-active fraction may often occur (e.g., Fig 10C) and describe the response of a specific opportunistic group or phylotype that is well-adapted to prevailing environmental conditions (Bouvier and del Giorgio 2002; Cottrell and Kirchman 2003; del Giorgio and Bouvier 2002). Such a scenario is not unlike that observed in the distribution of highly-active CTC+ cells as bacterioplankton communities are transported into nutrient-rich tidal creeks, where we observed increases in the abundance of highly-active cells at higher fluorescence suggesting the response of a specific subset of the highly-active fraction (Fig. 5.9). Depending on changes in the relative abundance of this subset of the population, such a response may not necessarily result in a change in the proportion of highly-active cells, thus measures of the intensity and abundance of highly-active cells alone would not be adequate to discriminate between this type of response (i.e., Fig. 5.10C) and that illustrated in Fig. 5.10A.

The three scenarios depicted in Figs. 5.10A,C share an underlying assumption that changes in single-cell activity are influenced predominantly by resource supply or intrinsic characteristics of the assemblage itself. However, numerous studies have reported top-down regulation of the proportion and abundance of highly-active cells resulting from selective grazing by protozooplankton (del Giorgio et al. 1996c; Gonzalez et al. 1990; Lebaron et al. 1999). One example of such an effect of grazing on single-cell activity is illustrated in Fig. 5.10D and derived from a study conducted by Lebaron et al. (1999) in which they report the preference of protozoan grazers for bacterioplankton of intermediate cell size and metabolic activity. Such selective grazing pressure would result in an increase in the abundance of cells with both low and high metabolic activity

and probably generate a drastic change in their overall distribution. The bimodal distribution of this change in single-cell activity may produce no apparent change in mean fluorescence, underscoring the need to further investigate the distribution of activity within the highly-active fraction to describe and fully interpret single-cell activity in natural bacterioplankton assemblages.

Concluding Remarks

Our study has led to several important conclusions regarding activity within the highly-active fraction of bacterioplankton communities and the relationship between this activity and community-level carbon metabolism. As reported by other recent studies, we found evidence that the distribution of activity within the highly-active fraction is quite variable and that estimates of the proportion and abundance of highly-active cells alone provides limited information regarding single-cell activity in natural bacterioplankton communities. Additionally, although among-system patterns in the proportion of HDNA and CTC+ cells were similar, the distribution of activity within the highly-active fraction differed when the two assays were compared. It logically follows that the cellular-level metabolic processes characteristics associated with these measures of single-cell activity (e.g., DNA content and cellular respiration) may also be distributed differently within the highly-active fraction, such that cells making a disproportionate contribution to bacterioplankton respiration may not necessarily be those that are undergoing the greatest rates of protein synthesis or cell division. Our research also suggests that the balance between production and respiration may be influenced by balance between highly-active versus inactive cells. Collectively, these observations imply that single cell-activity may play an important role in the regulation of

bacterioplankton growth efficiency. Thus, studies of the regulation of BGE in natural aquatic systems may need to take into consideration not only the environmental factors that influence the balance between respiration and production (e.g., DOM quality, temperature), but also the proportion and relative intensity of highly-active cells and how respiration and production is distributed among this fraction.

We observed additional linkages between single-cell activity and other measures of community-level carbon metabolism. Patterns among the four sub-systems suggest that the link between single-cell activity and community-level carbon metabolism may be more effectively captured using indices of the distribution of highly-active cells rather than abundance alone, for systems in which total cellular-level activity (i.e., FL2*CTC) was elevated also had higher rates of bacterioplankton carbon consumption (i.e., BCC). A similar coherence was observed between fluorescence of CTC+ cells and BGE. Although the mechanisms driving such patterns are not known, these observations demonstrate that fluorescence is an ecologically relevant measure of metabolic activity within the highly-active fraction.

Numerous studies have identified fundamental differences between bacterioplankton communities from freshwater and marine endmembers of estuarine systems. Our research suggests that such differences may also apply to aspects of single-cell activity. We observed systematic differences in single-cell activity among the tidal creeks indicating that freshwater and saltwater-dominated systems differ fundamentally in the proportion of highly-active cells, distribution of activity within this highly-active fraction, and the relationship between cellular-level and community-level metabolism. Although these differences appear to be related to freshwater input, it is unlikely that they

are driven by the direct effect of salinity alone, rather represent the complex interactions of multiple factors that regulate single-cell activity, including phylogenetic composition, organic matter quality, nutrient availability, salinity, and other environmental factors that tend to covary in estuaries.

The distribution of metabolic activity among bacterioplankton cells and how this changes under different environmental stimuli is not well understood. We present four conceptual models that describe how the distribution of activity within the metabolically-active fraction changes. Although these models may represent an oversimplification of the single-cell dynamics, they provide a framework that can be used to generate hypotheses regarding single-cell activity in natural bacterioplankton communities. The eventual testing of such hypotheses will provide valuable insight into the nature of changes in single-cell activity and linkages to community-level metabolic processes.

LITERATURE CITED

Apple, J. K., and P. A. del Giorgio. in prep. The variability and regulation of bacterioplankton carbon metabolism in a tidally-influenced estuary. Aquatic Microbial Ecology.

Apple, J. K., P. A. del Giorgio, and R. I. E. Newell. 2004. The effect of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. Journal of Coastal Research 45: 110-133.

Barcina, I., P. Lebaron, and J. Vives-Rego. 1997. Survival of allochthonous bacteria in aquatic systems: a biological approach. FEMS Microbiology Ecology 23: 1-9.

Berman, T., B. Kaplan, S. Chava, Y. Viner, B. F. Sherr, and E. B. Sherr. 2001. Metabolically active bacteria in Lake Kinneret. Aquatic Microbial Ecology 23: 213-224.

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography 47: 453-470.

Choi, J., E. B. Sherr, and B. F. Sherr. 1999. Dead or alive? A large fraction of ETS-inactive marine bacterioplankton cells, as assessed by reduction of CTC, can become ETS-active with incubation and substrate addition. Aquatic Microbial Ecology 18: 105-115.

Cook, K. L., and J. L. Garland. 1997. The relationship between electron transport activity as measured by CTC reduction and CO2 production in mixed microbial communities. Microbial Ecology 34: 237-247.

Cottrell, M. T., and D. L. Kirchman. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnology and Oceanography 48: 168–178.

---. 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquatic Microbial Ecology 34: 139-149.

Crump, B. C., and J. A. Baross. 2000. Characterization of the bacterially-active particle fraction in the Columbia River estuary. Marine Ecology Progress Series 206: 13-22.

del Giorgio, P. A., D. F. Bird, Y. T. Prairie, and D. Planas. 1996a. Flow cytometric determination of bacterial abundance in lake plankton using the green nucleic acid stain SYTO 13. Limnology and Oceanography 41: 783-789.

del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnology and Oceanography 47: 471-486.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29: 503-541.

del Giorgio, P. A., J. M. Gasol, D. Vaque, P. Mura, S. Agusti, and C. M. Duarte. 1996b. Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. Limnology and Oceanography 41: 1169-1179.

del Giorgio, P. A., Y. T. Prairie, and D. F. Bird. 1997. Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, enumerated using CTC reduction and flow cytometry. Microbial Ecology 34: 144-154.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. Journal of Plankton Research 17: 1905-1924.

Gasol, J. M., and P. A. del Giorgio. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina 64: 197-224.

Gasol, J. M., U. L. Zweifel, F. Peters, J. A. Fuhrman, and A. Hagstrom. 1999. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Applied and Environmental Microbiology 65: 4475-4483.

Gonzalez, J. M., E. B. Sherr, and B. F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and Environmental Microbiology 56: 583-589.

Jellet, J. F., W. K. W. Li, P. M. Dickie, A. Boraie, and P. E. Kepkay. 1996. Metabolic activity of bacterioplankton communities assessed by flow cytometry and single carbon substrate utilization. Marine Ecology Progress Series 136: 213-225.

Joux, F., and P. Lebaron. 2000. Use of fluorescent probes to assess physiological function of bacteria at a single-cell level. Microbes and Infections 2: 1523-1535.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria (Chapter 58), p. 776. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole [eds.], Handbook of Methods in Aquatic Microbial Ecology. CRC Press.

---. 2002. Calculating microbial growth rates from data on production and standing stocks. Marine Ecology Progress Series 233: 303-306.

Langenheder, S., and K. Jurgens. 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnology and Oceanography 46: 121-134.

Lebaron, P., P. Servais, H. Agogué, C. Courties, and F. Joux. 2001. Does the High Nucleic Acid Content of Individual Bacterial Cells Allow Us To Discriminate between Active Cells and Inactive Cells in Aquatic Systems? Applied and Environmental Microbiology 67: 1775-1782.

Lebaron, P., P. Servais, M. Troussellier, C. Courties, and J. Vives-Rego. 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquatic Microbial Ecology 19: 225-267.

Li, W., J. Jellett, and P. Dickie. 1995. DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnology and Oceanography 40: 1485-1495.

Marie, D., F. Partensky, S. Jacquet, and D. Vaulot. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology 63: 186-193.

Massana, R., C. Pedrós-Alió, E. O. Casamayor, and J. M. Gasol. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnology and Oceanography 46: 1181-1188.

Rodriguez, G. G., D. Phipps, K. Ishiguro, and H. F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Applied and Environmental Microbiology 58: 1801-1808.

Servais, P., E. O. Casamayor, C. Courties, P. Catala, N. Parthuisot, and P. Lebaron. 2003. Activity and diversity of bacterial cells with high and low nucleic acid content. Aquatic Microbial Ecology 33: 41-51.

Sherr, B. F., P. A. del Giorgio, and E. B. Sherr. 1999a. Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye CTC. Aquatic Microbial Ecology 18: 117-131.

Sherr, E. B., B. F. Sherr, and C. T. Sigmon. 1999b. Activity of marine bacteria under incubated and in situ conditions. Aquatic Microbial Ecology 20: 213-223.

Sieracki, M. E., T. L. Cucci, and J. Nicinski. 1999. Flow cytometric analysis of 5-cyano-2,3-ditolyl tetrazolium chloride activity of marine bacterioplankton in dilution cultures. Applied and Environmental Microbiology 65: 2409-2417.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using super(3)H-leucine. Marine Microbial Food Webs 6: 107-114.

Smith, E. M. 1998. Coherence of microbial respiration rate and cell-specific activity in a coastal plankton community. Aquatic Microbial Ecology 16: 27-35.

Smith, E. M., and P. A. del Giorgio. 2003. Low fractions of active bacteria in natural aquatic communities? Aquatic Microbial Ecology 31: 203-208.

Vaqué, D., E. O. Casamayor, and J. M. Gasol. 2001. Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bacteria with different DNA content. Aquatic Microbial Ecology 25: 163-177.

Yokokawa, T., T. Nagata, M. T. Cottrell, and D. L. Kirchman. 2004. Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. Limnology and Oceanography 49: 1620-1629.

FIGURES

Fig. 5.1. Proportion of HDNA and CTC+ cells among the tidal creeks. Overall two-year means for the proportion of HDNA cells (51%) and CTC+ cells (13%) are indicated by solid and hatched lines, respectively. Asterix indicates that the means in MC are significantly higher than the other creeks.

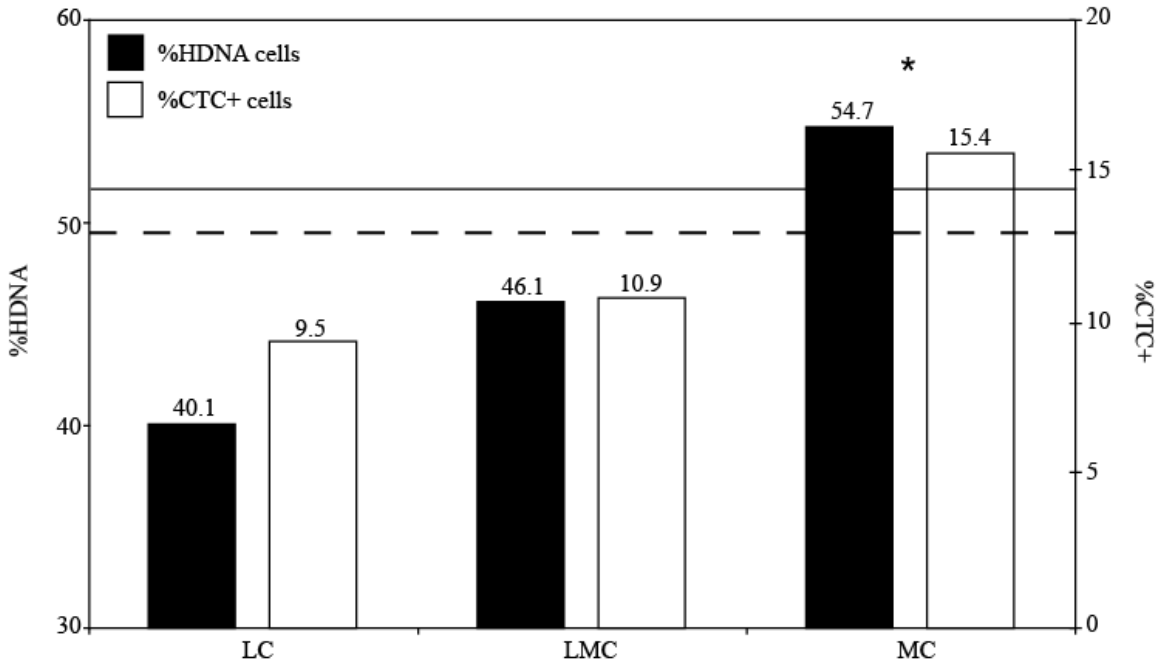

%HDNA cells
%CTC+ cells
*
54.7
15.4
46.1
10.9
9.5
40.1
%HDNA
%CTC+
60
50
40
30
20
15
10
5
0
LC
LMC
MC

Fig. 5.2. Relationship between total bacterioplankton abundance and abundance of (A) CTC+ and (B) HDNA cells.

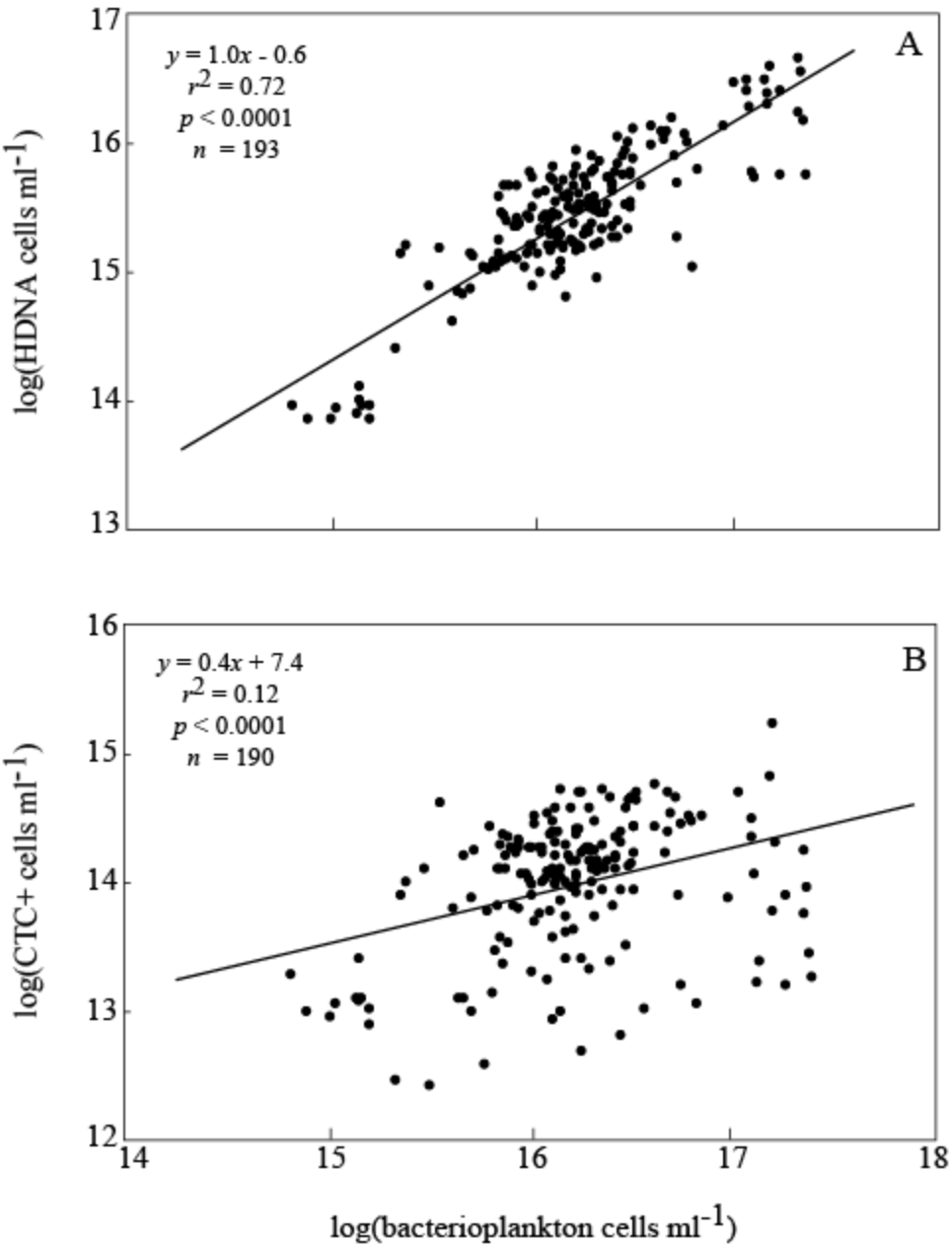

A
y = 1.0x - 0.6
r2 = 0.72
p < 0.0001
n = 193
log(HDNA cells ml-1)
17
16
15
14
13
B
y = 0.4x + 7.4
r2 = 0.12
p < 0.0001
n = 190
log(CTC+ cells ml-1)
16
15
14
13
12
14
15
16
17
18
log(bacterioplankton cells ml-1)

Fig. 5.3. Relationship between the abundance of CTC+ and HDNA cells.

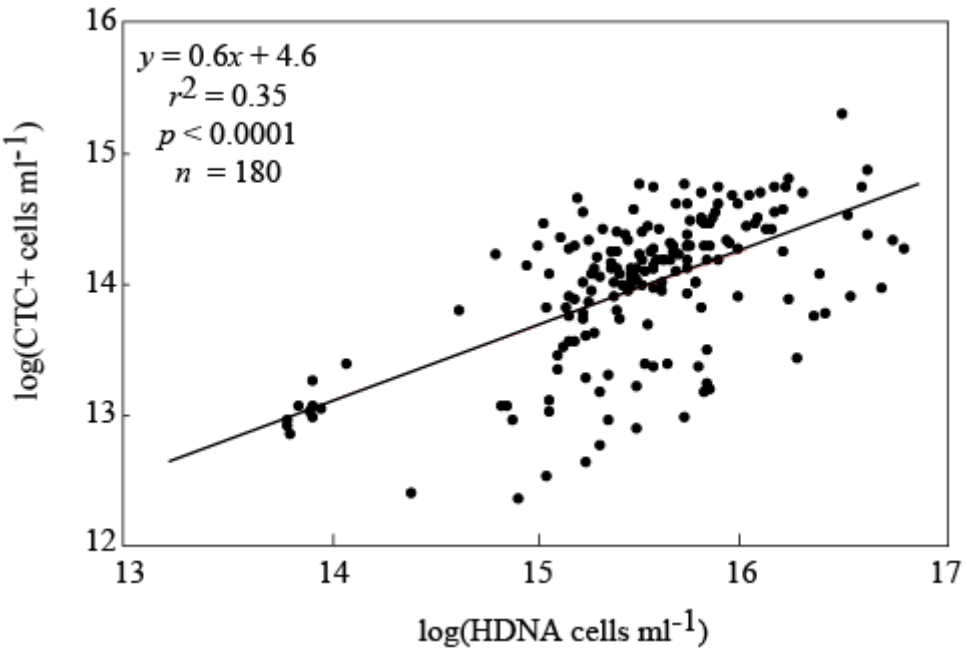

y = 0.6x + 4.6
r2 = 0.35
p < 0.0001
n = 180
log(CTC+ cells ml-1)
log(HDNA cells ml-1)
16
15
14
13
12
13
14
15
16
17

Fig. 5.4. Among-system patterns in two-year means for (A) the product of CTC abundance and intensity (FL2*CTC) and (B) bacterioplankton carbon consumption (BCC).

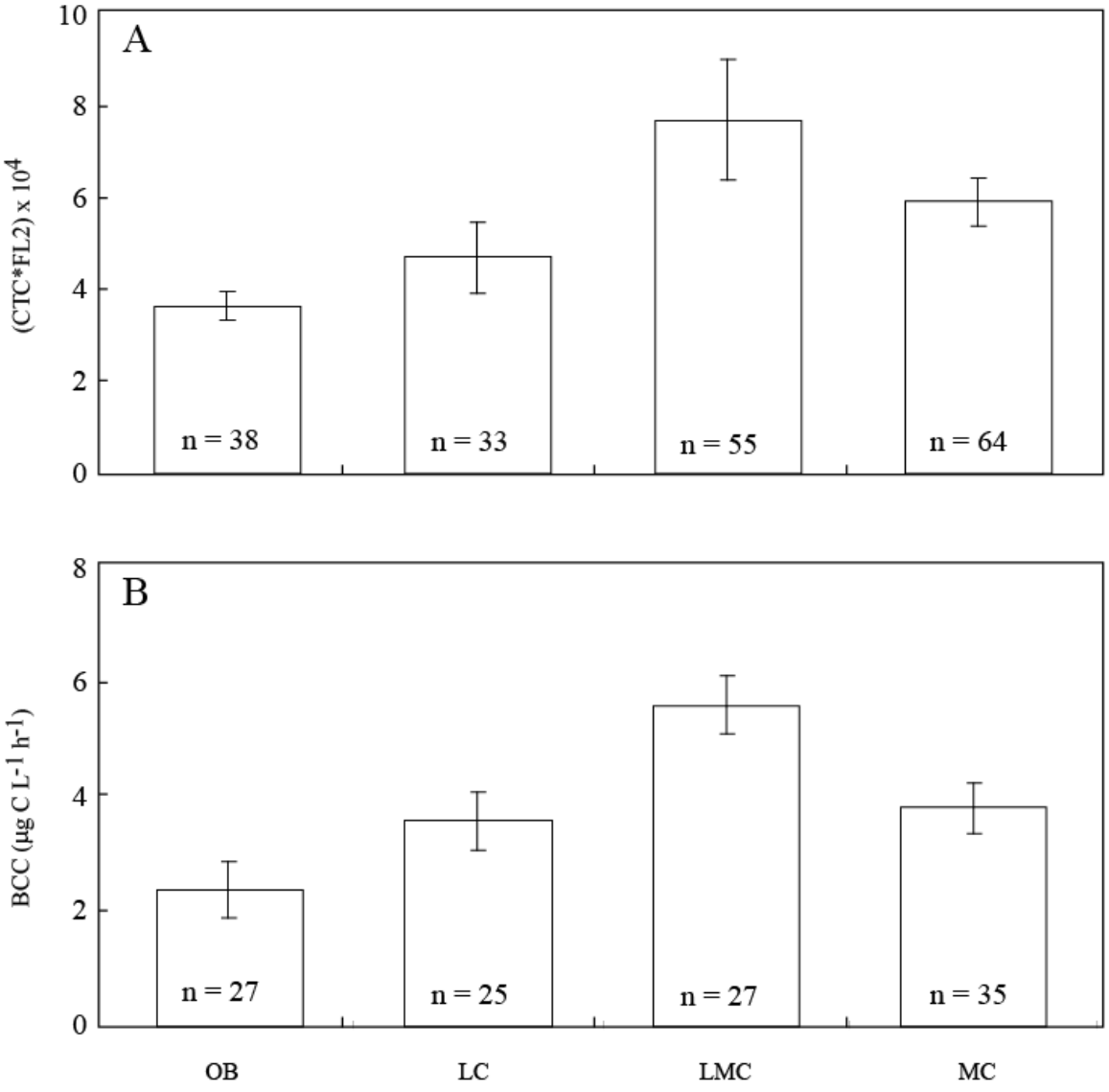

A
(CTC*FL2) x 10^4
10
8
6
4
2
0
n = 38
n = 33
n = 55
n = 64
B
BCC (µg C L-1 h-1)
8
6
4
2
0
n = 27
n = 25
n = 27
n = 35
OB
LC
LMC
MC

Fig. 5.5. Among-system patterns in (A) mean fluorescence of CTC+ cells (FL2) and (B) bacterioplankton growth efficiency (BGE).

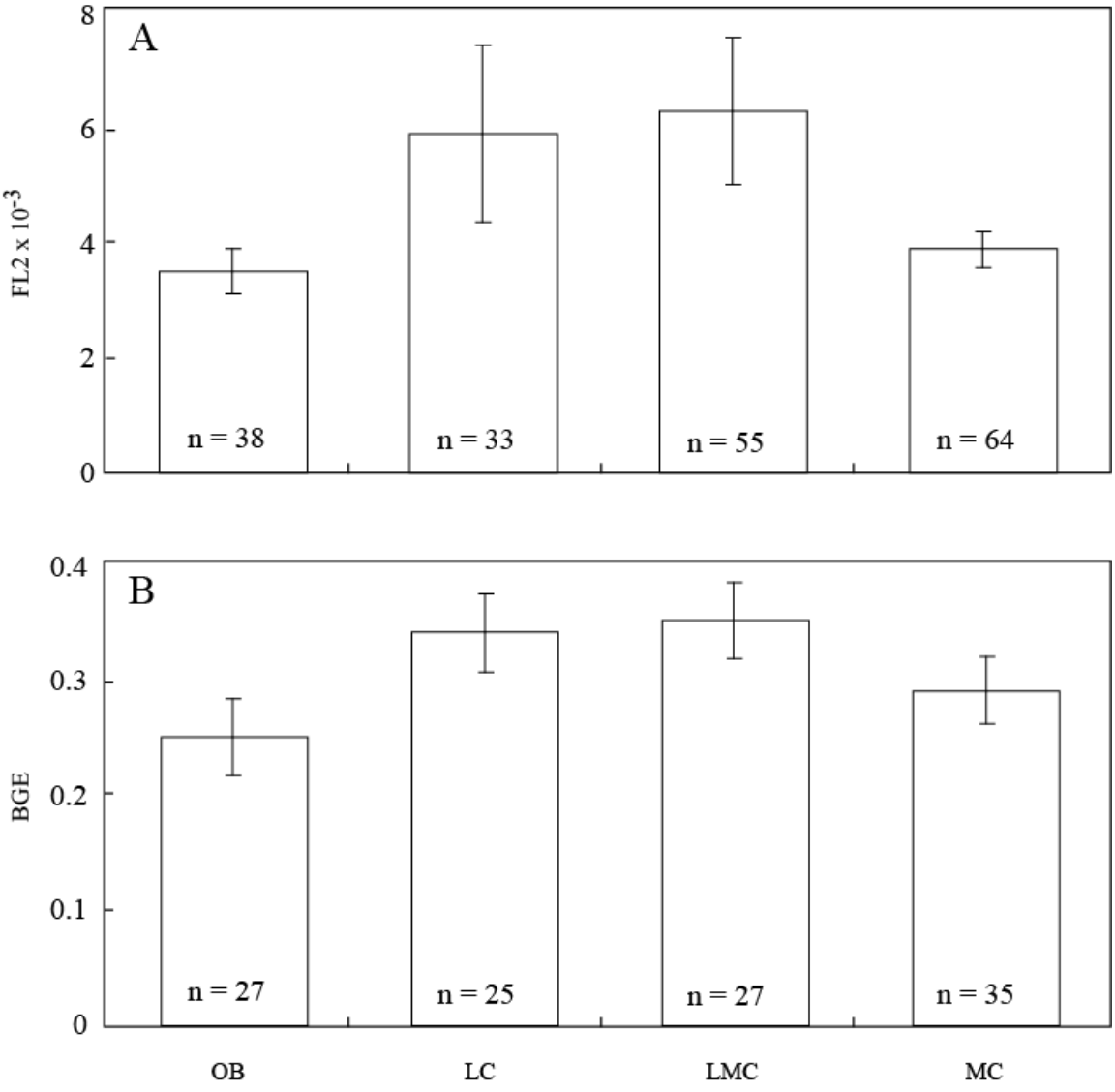

A
FL2 x 10-3
8
6
4
2
0
n = 38
n = 33
n = 55
n = 64
B
BGE
0.4
0.3
0.2
0.1
0
n = 27
n = 25
n = 27
n = 35
OB
LC
LMC
MC

Fig. 5.6. Relationship between the proportion of CTC+ cells in the filtered fraction and BGE. Best fit lines of linear regression are illustrated for each system. (ALL DATA: $y = 0.2 + 0.011x$; $r^2 = 0.12$; $p > 0.0001$; $n = 138$; LMC: $y = 0.2 + 0.018x$; $r^2=0.12$; $p = 0.03$; $n = 40$; OB: $y = 0.15 + 0.018x$; $r^2 = 0.17$; $p = 0.036$; $n = 27$; LC: $y = 0.25 + 0.01x$; $r^2 = 0.21$; $p = 0.02$; $n = 25$; MC: $y = 0.27 + 0.005x$; $r^2 = 0.05$; $p = 0.36$; $n = 46$.

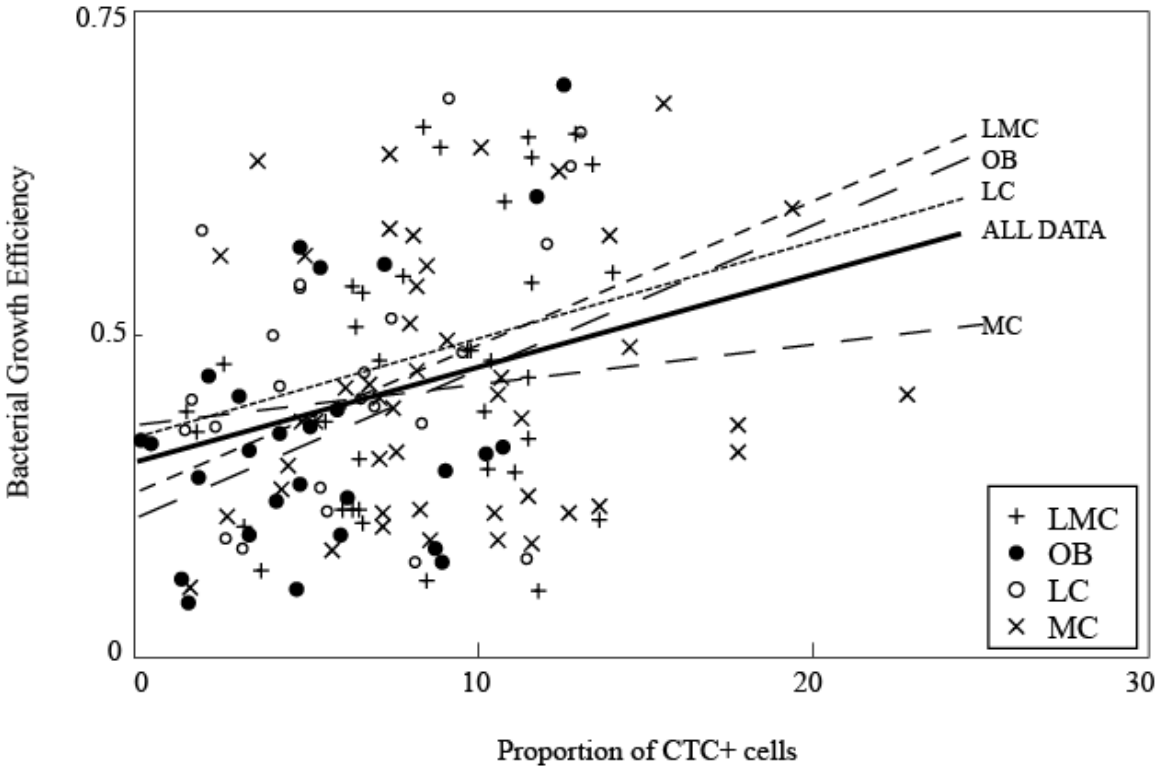

0.75
0.5
0
0
10
20
30
Bacterial Growth Efficiency
Proportion of CTC+ cells
LMC
OB
LC
ALL DATA
MC
+ LMC
● OB
○ LC
× MC

Fig. 5.7. Relationship between specific production (BPsp) and (A) the proportion of CTC+ cells and (B) the abundance of HDNA.

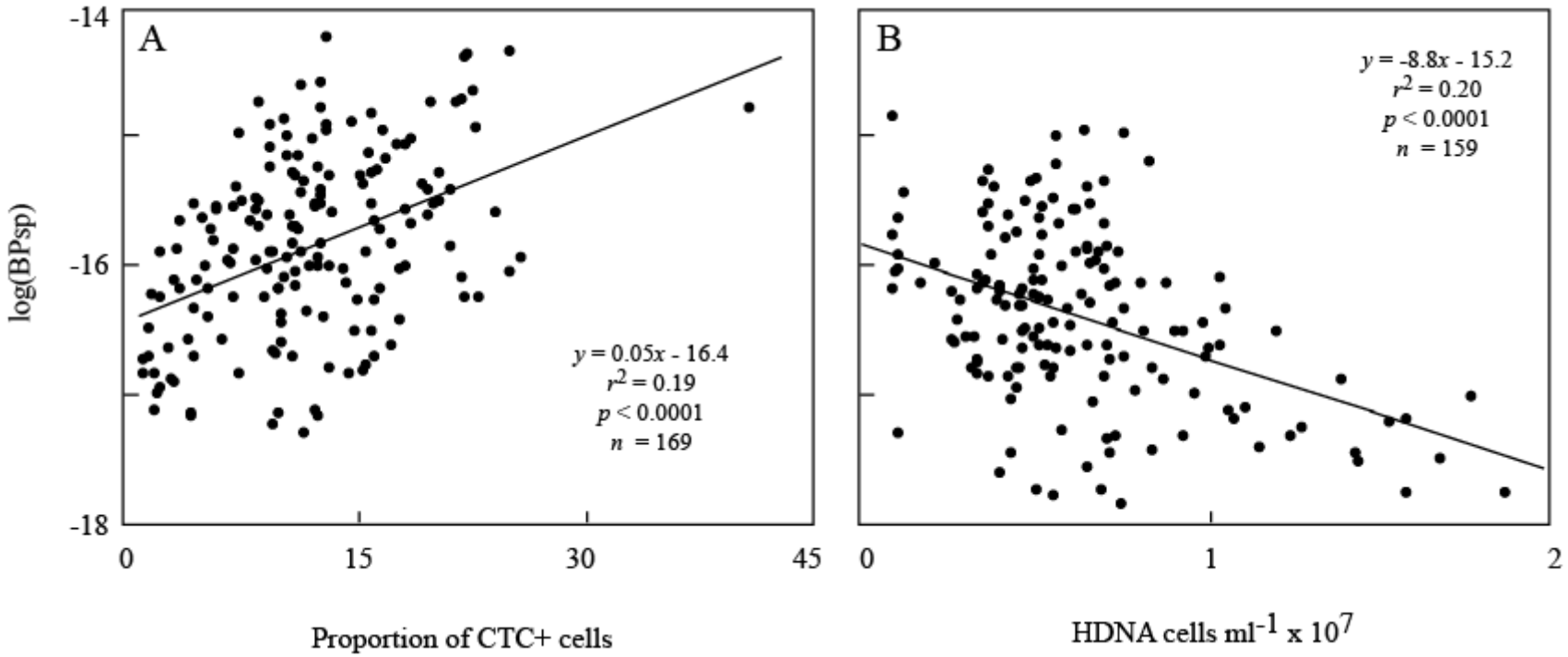

A
B
-14
-16
-18
log(BPsp)
0
15
30
45
Proportion of CTC+ cells
y = 0.05x - 16.4
r2 = 0.19
p < 0.0001
n = 169
y = -8.8x - 15.2
r2 = 0.20
p < 0.0001
n = 159
0
1
2
HDNA cells ml-1 x 107

Fig. 5.8. Comparison of the relationship between between bacterial production (BP) and the proportion of CTC+ cells (%CTC) in LMC (open circles) and MC (closed circles).

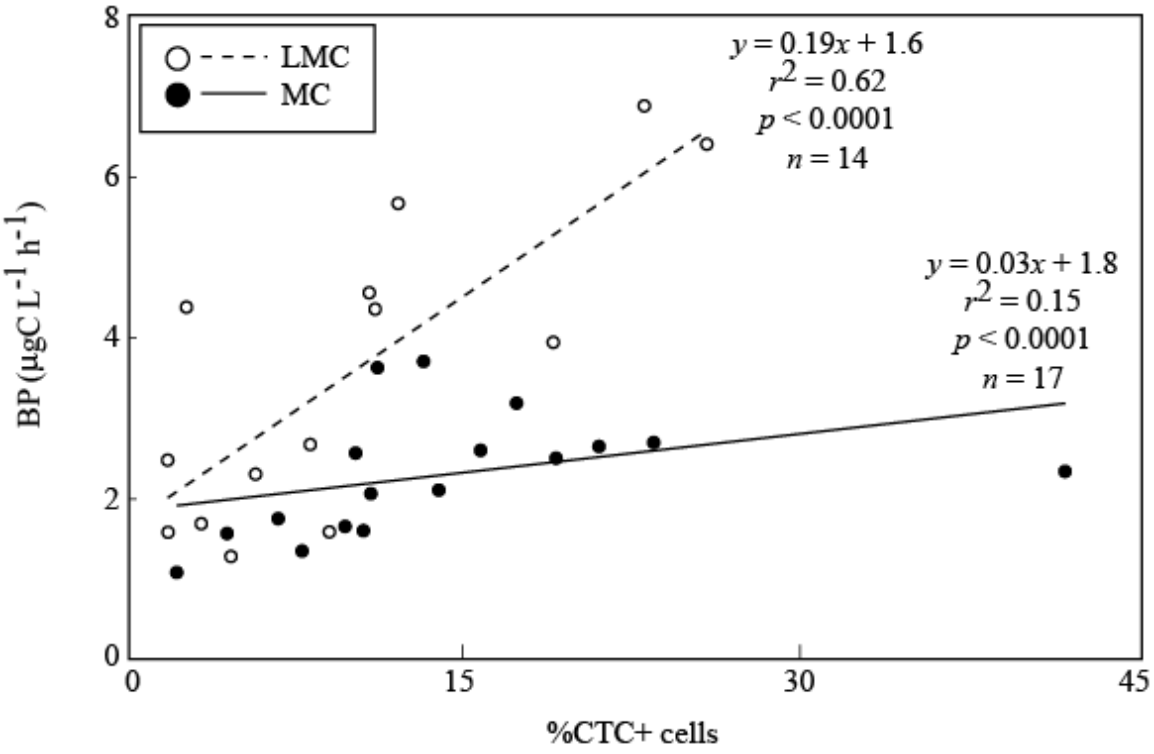

LMC
MC
y = 0.19x + 1.6
r2 = 0.62
p < 0.0001
n = 14
y = 0.03x + 1.8
r2 = 0.15
p < 0.0001
n = 17
BP (µgC L-1 h-1)
%CTC+ cells
0
2
4
6
8
0
15
30
45

Fig. 5.9. Frequency distributions of CTC+ fluorescence (FL2) in (A) the open bay (OB), (B) Little Monie Creek (LMC), and (C) Monie Creek (MC) during nutrient enriched conditions in spring 2000. Bead-normalized values of mean fluorescence for each sample are indicated at the top of each vertical axis.

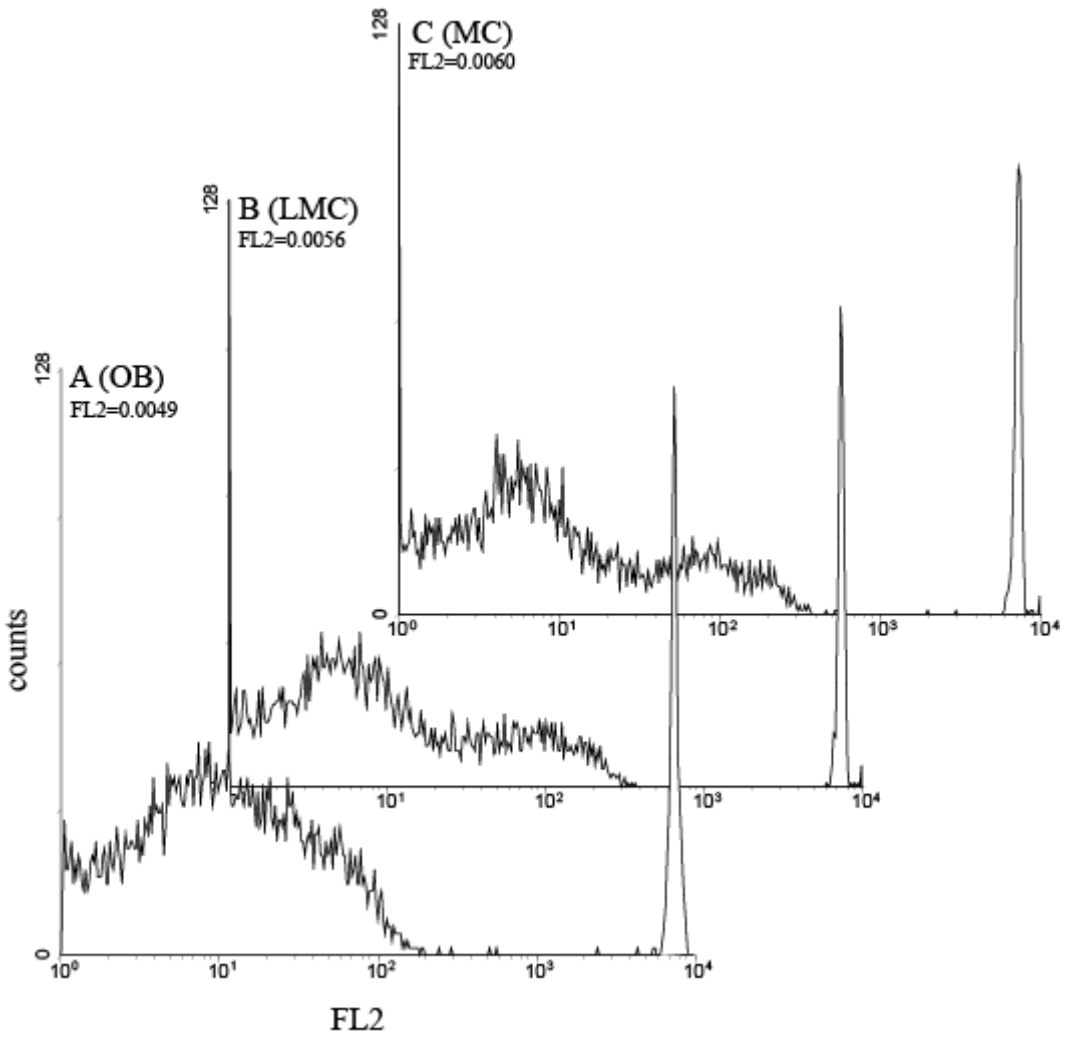
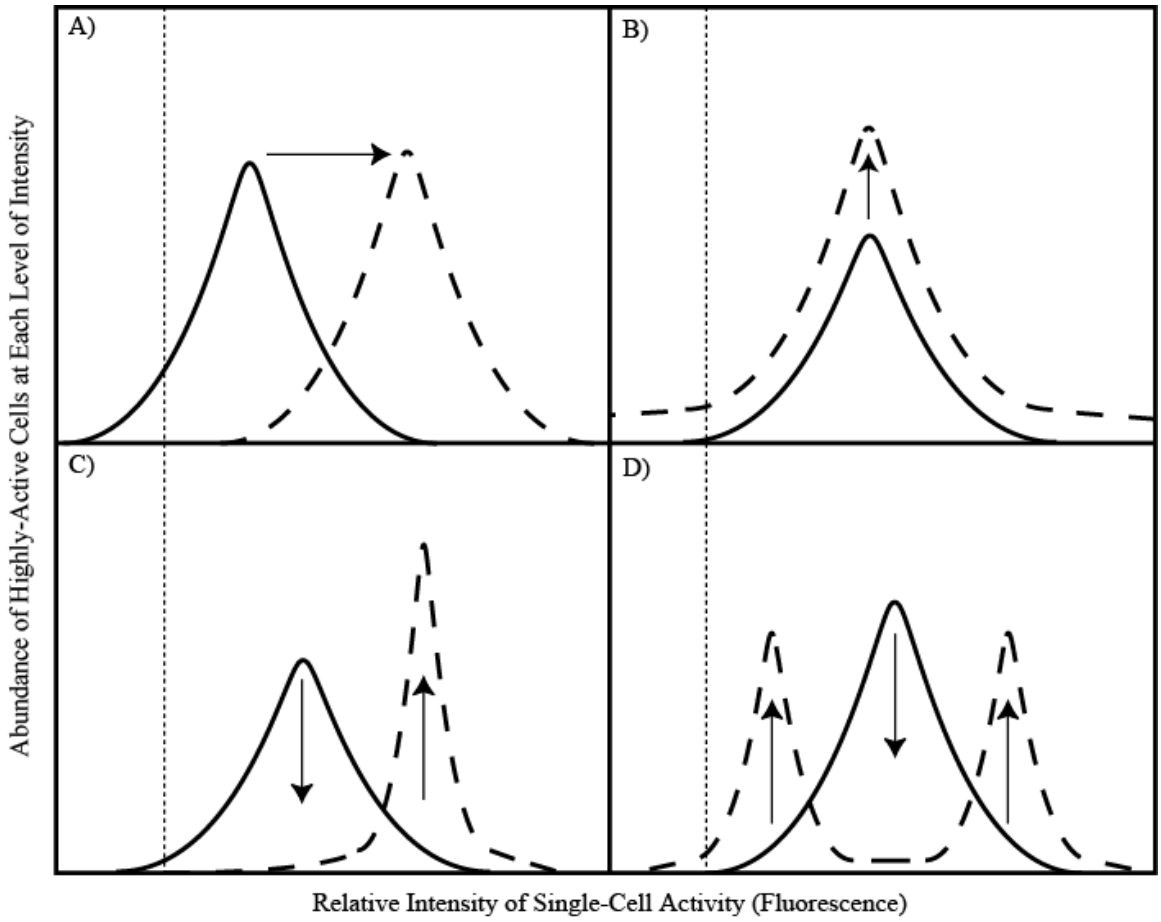

C (MC)
FL2=0.0060
B (LMC)
FL2=0.0056
A (OB)
FL2=0.0049
128
0
counts
10^0
10^1
10^2
10^3
10^4
FL2

Fig. 5.10. Conceptual diagram of changes in the distribution of highly-active cells in natural bacterioplankton communities. In each model, the solid curve represents the distribution of activity within a "normal" community while the dashed curves represent a hypothetical response to some environmental stimuli. Vertical dotted lines indicate the analytical threshold for enumeration of cells as highly-active. Four scenarios include (A) proportional increases in the abundance of highly-active cells at each level of activity, with no change in the distribution and mean intensity; (B) proportional increases in the intensity of activity throughout the highly-active fraction, such that there is no change in the distribution of highly-active cells but mean intensity increases; (C) disproportionate increase in the metabolic activity of a subset of the highly-active population, resulting in a change in both distribution and mean intensity - but not necessarily a change in abundance; and (D) an increase in the abundance of cells at relatively high and low activity (and decrease in the abundance of those at intermediate activities), resulting in a change in distribution but no change in mean intensity.


A)
B)
C)
D)
Abundance of Highly-Active Cells at Each Level of Intensity
Relative Intensity of Single-Cell Activity (Fluorescence)

CHAPTER VI

Summary and Research Conclusions

The research described in this dissertation set out to explore the variability and regulation of bacterioplankton carbon metabolism in the tidal creeks of a salt-marsh dominated estuary. These studies revealed that, even on the relatively small spatial scales investigated, the temporal and spatial variability in bacterioplankton metabolism is high and comparable to that found across a broad range of aquatic systems. This variability, however, is constrained by two primary environmental factors: temperature and differences in resource supply. The first of these regulates the magnitude of carbon metabolism throughout the year, whereas the second influences the magnitude of carbon metabolism in each estuarine sub-system at any given temperature or season. Of the different aspects of resource supply investigated, the quality of dissolved organic matter (DOM) appears to have the most pronounced influence on bacterioplankton carbon metabolism. In particular, aspects of DOM quality related to lability and energetic content appear to affect both bacterioplankton growth efficiency (BGE) and carbon consumption (BCC). When combined with differences in single-cell activity observed among the tidal creeks, these relationships offer an explanation for differences in the response of bacterioplankton to system-level nutrient enrichment when estuarine sub-systems differing in their freshwater input are compared.

Collectively, the components of this dissertation represent a relatively comprehensive and long-term investigation. Although there have been a number of studies of similar spatial and temporal scope investigating single aspects of bacterioplankton carbon metabolism in estuarine systems (Findlay et al. 1996; Hoch and Kirchman 1993), the present study includes a comprehensive assessment of cellular and community-level metabolism based on contemporaneous estimates of *in situ* rates and

properties. The merit of this study, however, lies not in the unique nature and scope of the research itself, rather in the patterns of bacterioplankton metabolism that the dataset reveals. In addition, this study and others like it offer the statistical and investigative power of a larger scale field study or meta-analysis (del Giorgio and Cole 1998; del Giorgio and Duarte 2002; White et al. 1991), while minimizing the variability associated with regional, watershed, climatic, and methodological differences. As a result, this research has revealed patterns in carbon metabolism that have not been identified in studies of smaller scope but that may ultimately represent transferable ecosystem-scale relationships.

Systematic Variability in Bacterioplankton Metabolism

Despite the relatively small size of Monie Bay research reserve, the magnitude and variability in measures of cellular and community-level metabolism recorded as part of this research are similar to those reported for a wide range of aquatic systems. Although growth efficiency is quite variable, it exhibits a range and overall mean that is remarkably similar to those reported by del Giorgio and Cole (1998) in their survey of over 40 studies representing lakes, rivers, estuaries, and the open ocean. Similarly, estimates of single-cell activity are comparable in magnitude and range to those reported collectively for temperate lakes, estuaries, and coastal systems (del Giorgio and Scarborough 1995), although slightly higher than those reported for marine systems (Sieracki et al. 1999). Thus, even though conditions in Monie Bay are at the more eutrophic end of the enrichment spectrum, the range and magnitude in cellular and community-level carbon metabolism may be comparable to that of all natural aquatic systems. In addition, systematic patterns in BGE and single-cell activity among the tidal

creeks appear to represent a scaled-down version of the systematic differences observed among entire ecosystems reported in these meta-analyses (del Giorgio and Cole 1998; del Giorgio and Scarborough 1995). In this regard, Monie Bay provides a practical and unique means by which factors driving these large-scale patterns can be investigated.

Factors Regulating Bacterioplankton Carbon Metabolism in Estuarine Systems

The variability of bacterioplankton metabolism in estuarine systems represents a complex response to a wide range of environmental conditions (e.g., temperature, salinity, DOM quality and quantity, inorganic nutrients). The tendency for many of these parameters to covary in estuarine systems (Fisher et al. 1988; Sharp et al. 1982) makes it extremely difficult to identify which, if any, is the more important determining factor. However, comparisons among the diverse sub-systems of Monie Bay have presented a means by which the individual effect of different factors can be discerned, providing valuable insight into those environmental factors that are most important in regulating bacterioplankton carbon metabolism in temperate estuarine systems. Although there is a tendency to seek a single limiting factor for growth or metabolism in natural aquatic systems (Pomeroy and Wiebe 2001), my investigations indicate that such simplicity does not exist for the regulation of estuarine bacterioplankton. The various factors regulating bacterioplankton carbon metabolism identified as part of this dissertation research are outlined in Figure 1 and discussed below.

Temperature

Of the numerous environmental factors investigated, temperature is the most important when the annual variability in carbon metabolism is considered. This study reveals that all measures of carbon metabolism exhibit some degree of temperature

dependence, although this varies with the particular aspect of carbon metabolism (e.g., BP, BGE, BR) and temperature range being considered. One of the most important findings is that temperature has a disproportionate positive effect on bacterial production (BP) and respiration (BR), resulting in the negative temperature dependence of growth efficiency. As a result, BGE in temperate aquatic systems changes predictably throughout the year in a manner that may be independent of other environmental factors that are assumed to regulate its magnitude, such as inorganic nutrients and DOM quality. Despite its regulating effect on microbially-mediated aspects of carbon flux and nutrient cycling, temperature dependence of bacterioplankton carbon metabolism is seldom considered in models of BGE (Cajal-Medrano and Maske 1999; del Giorgio and Cole 1998; Touratier et al. 1999) or water quality (Lomas et al. 2002). Given the range in water temperatures throughout the year in temperate systems (0 to 30ºC), where water temperatures are frequently below 20˚C for over two-thirds of the year (Fisher et al. 1998; Hoch and Kirchman 1993; Shiah and Ducklow 1994b), the potential effect of temperature on microbially mediated carbon flux in the water column of these systems is clearly significant. Strategies to model more effectively the role of microbial communities in aquatic systems should take into consideration the strong temperature dependence of bacterioplankton carbon metabolism, especially if they include disparate temperature ranges or seasonal comparisons in temperate systems.

Although temperature has a significant effect on all measures of bacterioplankton carbon metabolism, this effect is not so strong as to override the influence of other environmental conditions. For example, comparisons of regressions of ambient water temperature and *in situ* carbon metabolism among the sub-systems show that there is no

change in slope when each measure of carbon metabolism is considered, yet reveal significant differences in the intercepts associated with these regressions. This underscores the important influence of resource supply in determining the magnitude of carbon metabolism at any given temperature. The similarity in the slope of these regressions also indicates that temperature and resource supply have simultaneous yet independent effects on carbon metabolism, challenging the conclusion of recent studies that temperature and resources are interacting limiting factors (Pomeroy and Wiebe 2001).

DOM Quality

Contrary to currently held paradigms regarding factors that influence bacterioplankton carbon metabolism, my research reveals that inorganic nutrients and the nutrient content of DOM may not be as important as energetic content and lability in regulating bacterioplankton carbon consumption and growth efficiency. Although models of bacterioplankton growth frequently rely on organic matter stoichiometry as predictors of BGE (Cajal-Medrano1 and Maske 1999; Goldman et al. 1987; Touratier et al. 1999), I found little evidence supporting this as a valid measure of organic matter quality with respect to predicting the variability of growth efficiencies in salt-marsh systems. I hypothesize that in nutrient rich systems such as the tidal creeks of Monie Bay and in estuaries in general that the chemical composition and energetic content of organic matter may have a greater influence on carbon metabolism than the relative content of nitrogen and phosphorus. In this regard, although the use of simple stoichiometric models may be appropriate for long-term or large-scale studies, they fail to describe the

dynamics of short-term interactions between bacteria and the dissolved pools of nutrients and DOM (Kirchman 2000b).

It is difficult if not impossible to measure accurately the quality of organic matter as it relates to consumption and growth, as there appear to be no analytical characterizations of DOM that have been linked directly to *in situ* carbon metabolism. Thus, although the characteristics of DOM used in this dissertation research (i.e., lability and CDOM) are measures of one aspect of organic matter quality, the extent to which they represent the quality of organic matter actually utilized by bacterioplankton *in situ* is not clear. Although many studies assume such a correlation exists, the relationship between characteristics of DOM consumed by bacterioplankton on relatively short time scales (e.g., BCC) and that which is consumed during long-term incubations (e.g., lability experiments) or presumed to be recalcitrant (e.g., CDOM) has not been established. Until such a parameter or methodology can be isolated that measures the characteristics of the short-lived DOM pool, measurements of *in situ* BGE and BCC will remain the most accurate indices of the quality and quantity, respectively, of organic matter utilized by bacterioplankton in natural aquatic systems. Future research endeavors should focus on identifying assays of organic matter quality that target the substrates utilized directly by bacterioplankton and pairing these with measures of *in situ* carbon metabolism.

Cellular-Level Effects

Although DOM quality and temperature have a significant influence on bacterioplankton carbon metabolism, cellular-level processes may modulate the effect of these environmental factors. For example, changes in the proportion and activity of highly-active cells may have a direct effect on BGE, with higher efficiencies recorded

when the proportion and activity of highly-active cells is greater (del Giorgio and Cole 1998). This pattern is probably attributed to a shift in the mean BGE of the assemblage, as more active, rapidly growing cells tend to have higher growth yields. This study also provides evidence that single-cell activity has a direct effect on total community-level metabolism, although this effect may vary among estuarine sub-systems differing in their freshwater input. Thus, predictions of the magnitude of BGE and BCC in natural aquatic systems may need to take into consideration not only the environmental factors that influence these measures of carbon metabolism (e.g., DOM quality, temperature), but also those that determine the abundance, proportion, and relative activity of highly-active cells. Ultimately, such information regarding cellular-level metabolism is essential if we are to understand the effect of bacterioplankton communities on carbon flux in aquatic systems.

Differences Between Freshwater and Saltwater-Dominated Systems

Another important finding of this research is that tidal creeks differing in their freshwater input may differ fundamentally in both cellular and community-level metabolism. My research provides evidence that the proportion of highly-active cells, the distribution of activity within the highly-active fraction, and the relationship between single-cell activity and community-level carbon metabolism differs among tidal creeks, offering an explanation for the consistently muted response of bacterioplankton to system-level nutrient enrichment in MC relative to that of the more saline LMC. Although the factors regulating single-cell activity were not identified as part of this research, it is likely that there are appreciable shifts in phylogenetic composition among these tidal creeks that explain in part changes in the activity and proportion of highly-

active cells (Cottrell and Kirchman 2004; del Giorgio and Bouvier 2002). Ultimately, differences in single-cell activity and community-level carbon metabolism between freshwater and marine endmembers of estuarine systems probably result from the combined effect of multiple factors, including DOM supply and quality, nutrient availability, and phylogenetic composition, as well as other environmental factors that were beyond the scope of the present study.

Monie Bay as a Model Estuarine System

This dissertation research was conducted almost exclusively at the Monie Bay component of the Chesapeake Bay National Estuarine Research Reserve System (NERRS). One of the primary objective of the NERRS program is to use the research reserves as "living laboratories" to improve our understanding of estuarine systems and help mitigate adverse anthropogenic impacts on their health and function. Although the Monie Bay research reserve has been historically underutilized in this capacity (Julie Bortz, Maryland Department of Natural Resources, personal communication), my dissertation research in Monie Bay makes a valuable contribute to helping achieve these objectives. First, it documents the response of bacterioplankton to the system-level nutrient enrichment typically associated with agricultural development of coastal and estuarine systems, highlighting the value of bacterioplankton communities as integrative measures of ecosystem health and function and improving our understanding of the role of bacterioplankton in the eutrophication process. Secondly, my dissertation research establishes the experimental design for using Monie Bay research reserve as a large-scale comparative study of the effect of system-level nutrient enrichment on estuarine systems. The incorporation of natural and anthropogenic gradients (e.g., salinity, DOM source and

quantity, dissolved nutrient concentrations) into this experimental design results in a straightforward and powerful means by which the effects of multiple environmental variables can be identified. Finally, the publication of Chapter II (Apple et al. 2004) in a special issue of Journal of Coastal Research focusing specifically on studies conducted at NERRS sites will improve the visibility of this system as a research location and encourage future use of Monie Bay as a living laboratory in NERRS related research endeavors.

The utility of Monie Bay as an experimental system (e.g., as described in Chapters I & II) relies on the existence of persistent gradients and systematic differences within and among the tidal creeks and open bay that are related to landscape and watershed characteristics, such that the relative magnitude of any environmental or biological parameter can be predicted with some certainty throughout the year. The studies included in this dissertation repeatedly reveal systematic patterns that support the interpretation of Monie Bay as a large-scale nutrient enrichment experiment. Chapter II reveals systematic differences in environmental conditions (e.g., salinity, ambient nutrient and DOC concentrations, optical characteristics of DOM) and bacterial production among the four sub-systems that persist throughout the year, corroborating spatial and systematic patterns observed in studies conducted in this system over a decade earlier (Jones et al. 1997). The same systematic differences among sub-systems emerged in regressions of temperature versus measures of carbon metabolism (Chapter III), which revealed a persistent hierarchy in the magnitude of bacterioplankton production, growth efficiency, and carbon consumption among the four sub-systems that reflected previously observed patterns in dissolved nutrients and bacterioplankton production (e.g., Chapter

II). An investigation of the environmental factors driving these persistent patterns in carbon metabolism (Chapter IV) revealed that lability of DOM followed the same predictable hierarchy among sub-systems as that of BCC and BGE. Finally, I observed fundamental differences in single-cell activity between LMC and MC that, although not as predictable as other environmental and biological parameters, are probably driven by the robust systematic differences in environmental factors and contribute to systematic patterns in carbon metabolism.

The remarkable persistence (i.e., >10 yrs; Jones et al. 1997) of patterns among and within the tidal creeks of Monie Bay highlights the utility of this system for conducting long-term studies of the effects of system-level nutrient enrichment. Although my research in this system has focused specifically on the bacterioplankton community, unique patterns in environmental conditions within and among the three tidal creeks are suitable for investigating the response of a wide range of biological communities. These have included macrophytes (Jones et al. 1997) and phytoplankton assemblages (Fielding 2002), but could be extended to wide range of estuarine organisms or geochemical processes or even landscape-level investigations. In this regard, the incorporation of the experimental design and results reported in my dissertation will help ensure that future research endeavors in Monie Bay will continue to make an important contribution to our understanding of estuarine processes and the effects of anthropogenic nutrient loading on these valuable aquatic resources.

LITERATURE CITED

Apple, J. K., P. A. del Giorgio, and R. I. E. Newell. 2004. The effect of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. Journal of Coastal Research **45:** 110-133.

Cajal-Medrano1, R., and H. Maske. 1999. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton--revisiting the Pirt model. Aquatic Microbial Ecology **19:** 119-128.

Cajal-Medrano, R., and H. Maske. 1999. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton--revisiting the Pirt model. Aquatic Microbial Ecology **19:** 119-128.

Cottrell, M. T., and D. L. Kirchman. 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquatic Microbial Ecology **34:** 139-149.

del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnology and Oceanography **47:** 471-486.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29:** 503-541.

del Giorgio, P. A., and C. M. Duarte. 2002. Respiration in the open ocean. Nature **420:** 379-384.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. Journal of Plankton Research **17:** 1905-1924.

Fielding, K. P. 2002. Differential substrate limitation in small tributaries of Chesapeake Bay, Maryland influenced by non-point source nutrient loading. Masters. University of Maryland.

Findlay, S., M. L. Pace, and D. T. Fischer. 1996. Spatial and Temporal Variability in the Lower Food Web of the Tidal Freshwater Hudson River. Estuaries **19:** 866-873.

Fisher, T. R., L. W. Harding, D. W. Stanley, and L. G. Ward. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science **27:** 61-93.

Fisher, T. R., K. Y. Lee, H. Berndt, J. A. Benitez, and M. M. Norton. 1998. Hydrology and chemistry of the Choptank River Basin. Water, Air, and Soil Pollution **105:** 387-397.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by C:N ratio. Limnology and Oceanography **32:** 1239-1252.

Hoch, M. P., and D. L. Kirchman. 1993. Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Marine Ecology Progress Series **98:** 283-295.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A Two-Year Study of the Short-Term and Long-Term Sequestering of Nitrogen and Phosphorus in the Maryland National Estuarine Research Reserve, p. 1-100. Maryland National Estuarine Research Reserve.

Kirchman, D. L. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria, p. 261-288. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

Lomas, M. W., P. M. Glibert, F. Shiah, and E. M. Smith. 2002. Microbial processes and temperature in Chesapeake Bay: current relationships and potential impacts of regional warming. Global Change Biology **8:** 51-70.

Pomeroy, L. R., and W. J. Wiebe. 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquatic Microbial Ecology **23:** 187-204.

Sharp, J. H., C. H. Culberson, and T. M. Church. 1982. The chemistry of the Delaware Estuary. General considerations. Limnology and Oceanography **27:** 1015-1028.

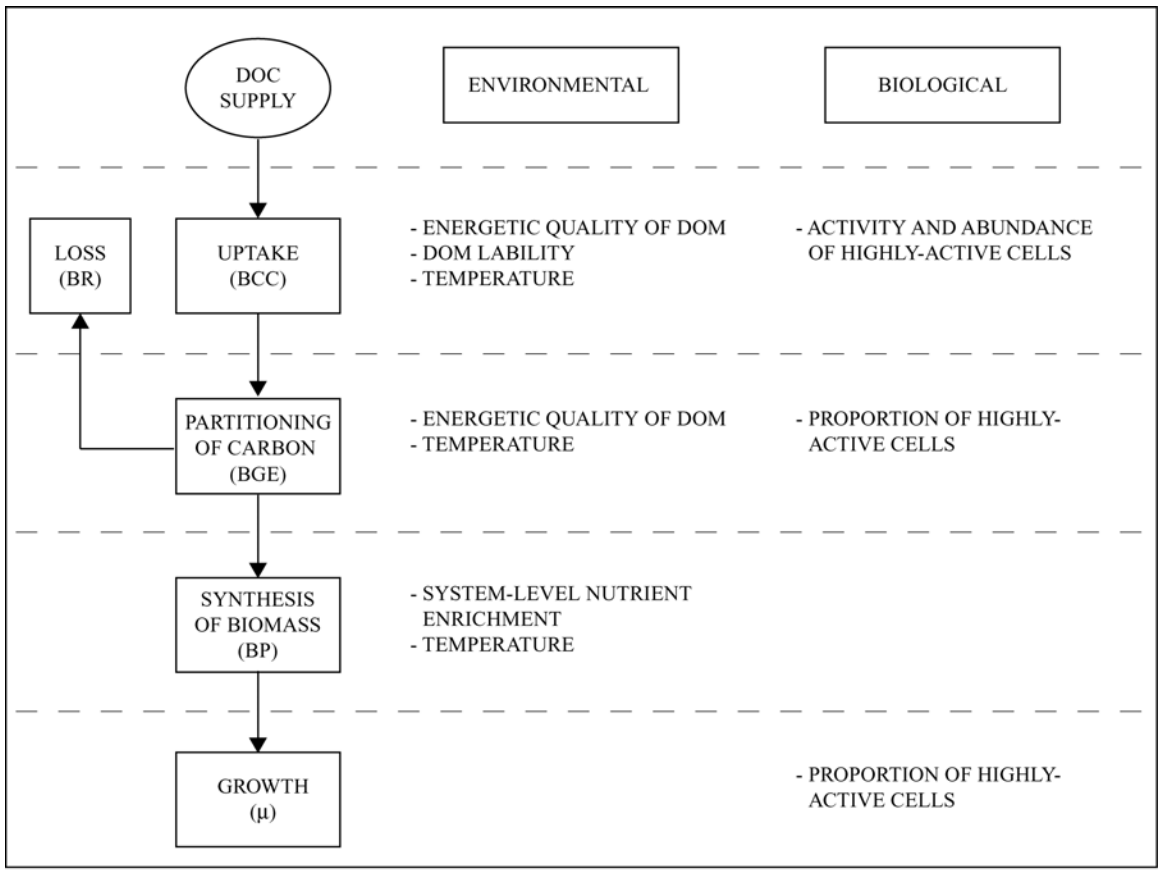
Shiah, F. K., and H. W. Ducklow. 1994. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnology and Oceanography **39:** 1243-1258.

Sieracki, M. E., T. L. Cucci, and J. Nicinski. 1999. Flow cytometric analysis of 5-cyano-2,3-ditolyl tetrazolium chloride activity of marine bacterioplankton in dilution cultures. Applied and Environmental Microbiology **65:** 2409-2417.

Touratier, F., L. Legendre, and A. Vézina. 1999. Model of bacterial growth influenced by substrate C:N ratio and concentration. Aquatic Microbial Ecology **19:** 105-118.

White, P. A., J. Kalff, J. B. Rasmussen, and J. M. Gasol. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microbial Ecology **21:** 99-118.

Fig. 1. Summary of conclusions from this dissertation research regarding the factors regulating various aspects of bacterioplankton carbon metabolism.


DOC SUPPLY
ENVIRONMENTAL
BIOLOGICAL
LOSS (BR)
UPTAKE (BCC)
- ENERGETIC QUALITY OF DOM
- DOM LABILITY
- TEMPERATURE
- ACTIVITY AND ABUNDANCE OF HIGHLY-ACTIVE CELLS
PARTITIONING OF CARBON (BGE)
- ENERGETIC QUALITY OF DOM
- TEMPERATURE
- PROPORTION OF HIGHLY-ACTIVE CELLS
SYNTHESIS OF BIOMASS (BP)
- SYSTEM-LEVEL NUTRIENT ENRICHMENT
- TEMPERATURE
GROWTH (μ)
- PROPORTION OF HIGHLY-ACTIVE CELLS

APPENDIX A: Complete Dataset

The following section includes raw data collected as part of the studies described in the chapters of this dissertation. These data include dissolved nutrients and water column chemistry (Table A-1), measures of bacterioplankton carbon metabolism (Table A-2), cellular-level characteristics (A-3), and nutrient uptake experiments (A-4).

Table A-1. Dissolved nutrients and water column chemistry.

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (μM)	NOx (μM)	DIN (μM)	PO_4^{3-} (μM)	TDN (μM)	TDP (μM)	DON (μM)	DOP (μM)	DOC (μM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
4/6/2000	1	OB	13	10	27.1	5.31	29.50	34.81	0.12	62.5	0.16	27.7	0.04	654	10.5	390.6	4089	-
4/6/2000	2	OB	13	11	28.5	1.78	29.20	30.98	0.11	57.8	0.16	26.8	0.05	639	11.1	361.3	3995	-
4/6/2000	3	LC	13	10	8.6	4.55	9.60	14.15	0.12	46.8	0.33	32.7	0.21	1079	23.1	141.8	3270	-
4/6/2000	4	LC	13	11	16.4	3.13	18.10	21.23	0.06	48.9	0.23	27.7	0.17	876	17.9	212.6	3808	-
4/6/2000	5	LM	13	5	3.8	9.94	20.30	30.24	0.81	78.6	1.53	48.4	0.72	1434	18.2	51.4	937	-
4/6/2000	6	LM	13	7	5.2	9.39	14.65	24.04	0.52	69.3	1.00	45.3	0.48	1318	19.0	69.3	1318	-
4/6/2000	7	LM	13	11	12	4.75	16.40	21.15	0.11	52.3	0.32	31.2	0.21	991	18.9	163.4	3096	-
4/6/2000	8	MC	13	4	3.9	7.89	24.10	31.99	0.72	87.7	1.59	55.7	0.87	1870	21.3	55.2	1176	-
4/6/2000	9	MC	13	3	4.5	7.71	23.30	31.01	0.68	91.4	1.58	60.4	0.90	2009	22.0	57.8	1272	-
4/6/2000	10	MC	13	7	4.4	7.76	15.00	22.76	0.50	74.2	1.12	51.4	0.62	1749	23.6	66.3	1562	-
5/8/2000	1	OB	23	11	17	2.23	13.80	16.03	0.15	36.1	0.13	20.1	-0.02	547	15.1	277.7	4205	-
5/8/2000	2	OB	23	12	10	1.10	7.19	8.29	0.04	13.7	nd	5.4	-0.04	517	37.7	-	-	-
5/8/2000	3	LC	23	10.5	3.6	2.27	1.63	3.90	0.01	10.5	nd	6.6	-0.01	709	67.5	-	-	-
5/8/2000	4	LC	23	11	6	2.68	3.96	6.64	0.02	20.9	0.03	14.3	0.02	679	32.5	696.7	22639	-
5/8/2000	5	LM	23	8	7.5	1.94	1.34	3.28	0.07	16.7	0.16	13.4	0.09	1063	63.6	104.4	6641	-
5/8/2000	6	LM	23	10	5.6	1.59	3.22	4.81	0.01	38.3	0.30	33.5	0.29	855	22.3	127.7	2850	-
5/8/2000	7	LM	23	11	2.2	2.29	3.00	5.29	nd	19.6	0.07	14.3	0.07	383	19.6	280.0	5476	-
5/8/2000	8	MC	23	5	3.9	3.44	4.53	7.97	0.26	40.4	0.58	32.4	0.32	1603	39.7	69.7	2763	-
5/8/2000	9	MC	23	6.5	13.4	1.66	4.03	5.69	0.19	45.3	0.51	39.6	0.32	1444	31.9	88.8	2832	-
5/8/2000	10	MC	23	11	8.3	1.11	2.24	3.35	0.02	15.5	0.07	12.2	0.05	813	52.5	221.4	11619	-
6/7/2000	1	OB	22	11	15.1	3.28	9.36	12.64	0.05	35.2	0.21	22.6	0.16	448	12.7	167.6	2135	-
6/7/2000	2	OB	22	12	13.5	3.26	9.42	12.68	0.05	33	0.19	20.3	0.14	427	12.9	173.7	2246	-
6/7/2000	3	LC	22	10.5	2.9	2.46	1.08	3.54	nd	9.9	0.04	6.4	0.04	620	62.6	247.5	15500	-
6/7/2000	4	LC	22	11	3.6	3.44	3.76	7.20	0.03	23.6	0.18	16.4	0.15	571	24.2	131.1	3171	-
6/7/2000	5	LM	22	8	3.6	3.52	2.70	6.22	0.17	37.9	0.45	31.7	0.28	696	18.4	84.2	1546	-
6/7/2000	6	LM	22	10	2.4	5.13	2.99	8.12	0.19	30.6	0.31	22.5	0.12	672	21.9	98.7	2167	-

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (µM)	NOx (µM)	DIN (µM)	PO_4^{3-} (µM)	TDN (µM)	TDP (µM)	DON (µM)	DOP (µM)	DOC (µM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
6/7/2000	7	LM	22	11	3.3	3.28	2.93	6.21	0.02	35	0.27	28.8	0.25	511	14.6	129.6	1892	-
6/7/2000	8	MC	22	5	4.5	0.61	1.31	1.92	0.01	29.1	0.25	27.2	0.24	681	23.4	116.4	2723	-
6/7/2000	9	MC	22	6.5	6.6	1.27	0.22	1.49	nd	27.8	0.25	26.3	0.25	850	30.6	111.2	3400	-
6/7/2000	10	MC	22	11	7.6	1.43	0.14	1.57	0.29	38.3	0.37	36.7	0.08	972	25.4	103.5	2626	-
7/3/2000	1	OB	26	11	16.7	0.94	0.13	1.07	0.02	13.9	0.12	12.8	0.10	462	33.3	115.8	3854	-
7/3/2000	2	OB	26	10.5	20	0.47	0.16	0.63	0.02	25.1	0.48	24.5	0.46	468	18.7	52.3	976	-
7/3/2000	3	LC	26	10	11.8	0.93	0.18	1.11	0.04	24	0.16	22.9	0.12	788	32.8	150.0	4927	-
7/3/2000	4	LC	26	10.5	10	0.53	0.24	0.77	-	31.2	0.22	30.4	-	700	22.4	141.8	3182	-
7/3/2000	5	LM	26	6	42.3	0.89	0.14	1.03	0.38	49.7	0.98	48.7	0.60	1085	21.8	50.7	1107	-
7/3/2000	6	LM	26	7	37.6	0.80	0.19	0.99	0.15	43.5	0.58	42.5	0.43	963	22.1	75.0	1659	-
7/3/2000	7	LM	26	9	21.2	0.56	0.14	0.70	0.02	35.5	0.36	34.8	0.34	798	22.5	98.6	2215	-
7/3/2000	8	MC	26	1.5	6.3	2.81	13.30	16.11	1.96	73.3	3.11	57.2	1.15	1701	23.2	23.6	547	-
7/3/2000	9	MC	26	4	8.2	2.37	11.30	13.67	1.18	61.2	2.14	47.5	0.96	953	15.6	28.6	445	-
7/3/2000	10	MC	26	8	9.3	0.90	1.07	1.97	0.14	37.5	0.57	35.5	0.43	568	15.2	65.8	997	-
8/3/2000	1	OB	28	10.6	13.8	2.88	0.24	3.12	0.04	25.6	0.58	22.5	0.54	565	22.1	44.1	974	-
8/3/2000	2	OB	28	10.6	16.2	2.88	0.13	3.01	0.01	15.4	0.07	12.4	0.06	-	-	220.0	-	-
8/3/2000	3	LC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	4	LC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	5	LM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	6	LM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	7	LM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	8	MC	28	2.8	17.7	1.99	1.44	3.43	0.71	54.5	1.68	51.1	0.97	1485	27.2	32.4	884	-
8/3/2000	9	MC	28	4.6	16.2	2.21	0.10	2.31	0.26	15.4	0.40	13.1	0.14	1271	82.5	38.5	3177	-
8/3/2000	10	MC	28	7.4	10.4	1.24	0.25	1.49	0.12	28.5	0.47	27.0	0.35	1028	36.1	60.6	2186	-
9/5/2000	1	OB	22	12.5	9.3	0.77	0.75	1.52	0.06	24.6	0.43	23.1	0.37	513	20.9	57.2	1194	-
9/5/2000	2	OB	22	12.5	10.4	1.88	0.54	2.42	0.05	17.4	0.33	15.0	0.28	764	43.9	52.7	2316	-
9/5/2000	3	LC	22	11.6	7.2	1.25	0.58	1.83	0.02	33.5	0.39	31.7	0.37	657	19.6	85.9	1686	-
9/5/2000	4	LC	22	11.9	6.7	0.89	0.53	1.42	0.04	30.4	0.43	29.0	0.39	822	27.1	70.7	1913	-

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (µM)	NOx (µM)	DIN (µM)	PO_4^{3-} (µM)	TDN (µM)	TDP (µM)	DON (µM)	DOP (µM)	DOC (µM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
9/5/2000	5	LM	22	10.8	9.4	0.83	0.48	1.31	0.24	19.7	0.61	18.4	0.37	788	40.0	32.3	1292	-
9/5/2000	6	LM	22	11.2	10.5	0.71	0.72	1.43	0.20	16.5	0.61	15.1	0.41	704	42.7	27.0	1154	-
9/5/2000	7	LM	22	11.8	9.2	0.68	0.65	1.33	0.49	35.6	0.73	34.3	0.24	1122	31.5	48.8	1537	-
9/5/2000	8	MC	22	6.5	25.4	0.81	0.50	1.31	0.16	41.4	0.57	40.1	0.41	1048	25.3	72.6	1839	-
9/5/2000	9	MC	22	7.9	14	1.16	0.46	1.62	0.05	39.5	0.57	37.9	0.52	654	16.6	69.3	1148	-
9/5/2000	10	MC	22	10.2	12.3	0.86	0.43	1.29	nd	21.9	0.43	20.6	0.43	639	29.2	50.9	1486	-
12/5/2000	1	OB	4	14.7	-	1.72	7.79	9.51	nd	25.6	0.22	16.1	0.22	378	14.8	116.4	1720	-
12/5/2000	2	OB	4	14.6	-	1.09	7.57	8.66	nd	23.8	0.19	15.1	0.19	359	15.1	125.3	1890	-
12/5/2000	3	LC	3	14	-	1.41	1.13	2.54	nd	20.4	0.22	17.9	0.22	482	23.6	92.7	2192	-
12/5/2000	4	LC	3	14.4	-	1.19	1.54	2.73	nd	20.9	0.25	18.2	0.25	413	19.7	83.6	1651	-
12/5/2000	5	LM	2.8	13.2	-	4.01	3.61	7.62	nd	28.8	0.40	21.2	0.40	469	16.3	72.0	1173	-
12/5/2000	6	LM	3	14.1	-	2.04	1.51	3.55	nd	23.2	0.27	19.7	0.27	435	18.7	85.9	1610	-
12/5/2000	7	LM	3	13.1	-	1.10	1.88	2.98	nd	21.3	0.28	18.3	0.28	420	19.7	76.1	1501	-
12/5/2000	8	MC	4	10.9	-	1.57	0.59	2.16	nd	25.7	0.52	23.5	0.52	567	22.0	49.4	1089	-
12/5/2000	9	MC	4	11.9	-	2.82	1.51	4.33	0.03	29.6	0.40	25.3	0.37	607	20.5	74.0	1518	-
12/5/2000	10	MC	4	13.7	-	1.83	1.45	3.28	0.03	24.3	0.34	21.0	0.31	460	18.9	71.5	1352	-
3/15/2001	1	OB	8.9	14.1	30.1	1.22	10.80	12.02	0.04	20.6	n	8.6	-0.04	386	18.8	-	-	-
3/15/2001	2	OB	8.9	14.1	32.5	1.11	14.80	15.91	0.08	29.6	n	13.7	-0.08	373	12.6	-	-	-
3/15/2001	3	LC	10.4	11.3	1	3.71	5.14	8.85	0.05	27.5	0.04	18.7	-0.01	538	19.6	687.5	13458	-
3/15/2001	4	LC	10.4	12.3	5.7	3.47	7.77	11.24	0.04	28.1	n	16.9	-0.04	442	15.7	-	-	-
3/15/2001	5	LM	10.9	7.1	10.8	5.72	15.50	21.22	0.09	46.5	0.23	25.3	0.14	608	13.1	202.2	2642	-
3/15/2001	6	LM	10.7	9.4	4.6	3.94	9.04	12.98	0.04	32.7	0.12	19.7	0.08	574	17.5	272.5	4781	-
3/15/2001	7	LM	10.5	11.9	7.7	3.49	7.07	10.56	0.05	24.8	0.26	14.2	0.21	596	24.0	95.4	2292	-
3/15/2001	8	MC	10.5	4	30.6	0.73	8.26	8.99	0.03	20.3	0.19	11.3	0.16	798	39.3	106.8	4198	-
3/15/2001	9	MC	10	6.1	24.1	0.72	5.88	6.60	0.04	29.2	0.17	22.6	0.13	716	24.5	171.8	4209	-
3/15/2001	10	MC	9.9	9.4	14.6	1.13	7.15	8.28	0.06	28.5	1.36	20.2	1.30	646	22.7	21.0	475	-
4/12/2001	1	OB	14.4	11.3	32.6	1.67	18.50	20.17	0.08	37.6	0.95	17.4	0.87	443	11.8	39.6	467	0.0607
4/12/2001	2	OB	14.4	11.3	24.8	1.42	19.10	20.52	0.05	35.9	0.30	15.4	0.25	425	11.8	119.7	1417	0.0673

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (μM)	NOx (μM)	DIN (μM)	PO_4^{3-} (μM)	TDN (μM)	TDP (μM)	DON (μM)	DOP (μM)	DOC (μM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
4/12/2001	3	LC	14.8	4	2.4	2.92	2.73	5.65	nd	11.4	0.10	5.8	0.10	641	56.2	114.0	6408	0.1215
4/12/2001	4	LC	14.8	9.7	8.2	4.24	8.93	13.17	0.04	34.1	0.29	20.9	0.25	561	16.5	117.6	1935	0.1057
4/12/2001	5	LM	13.9	1.7	5.8	18.70	46.70	65.40	2.58	118	3.68	52.6	1.10	1241	10.5	32.1	337	0.3646
4/12/2001	6	LM	14.5	4.1	7	8.97	20.60	29.57	1.07	71.3	1.87	41.7	0.80	1050	14.7	38.1	561	0.2570
4/12/2001	7	LM	14.8	8.2	7.4	4.63	8.06	12.69	0.19	36.8	0.48	24.1	0.29	702	19.1	76.7	1463	0.1403
4/12/2001	8	MC	15.4	0.9	5.1	11.10	23.70	34.80	0.96	81.2	1.63	46.4	0.67	1397	17.2	49.8	857	0.3980
4/12/2001	9	MC	15.3	1	2.6	8.35	15.20	23.55	0.59	63.3	1.14	39.8	0.55	1301	20.6	55.5	1141	0.4017
4/12/2001	10	MC	15.1	2.8	4.4	4.92	6.97	11.89	0.18	40.6	0.42	28.7	0.24	923	22.7	96.7	2198	0.2303
4/12/2001	11	MC	14.5	0.1	4.8	11.40	24.60	36.00	1.84	85.1	2.65	49.1	0.81	1881	22.1	32.1	710	0.5016
4/12/2001	12	MC	12.3	0	0.8	7.91	18.00	25.91	1.16	79.4	1.96	53.5	0.80	1480	18.6	40.5	755	0.7094
5/30/2001	1	OB	21	11.2	6.2	4.97	19.60	24.57	0.02	37.7	nd	13.1	-0.02	450	11.9	-	-	0.0560
5/30/2001	2	OB	21	10.2	5.7	2.40	13.00	15.40	nd	32	nd	16.6	nd	511	16.0	-	-	0.0742
5/30/2001	3	LC	20.8	9	3.6	4.19	7.28	11.47	nd	31.1	nd	19.6	nd	747	24.0	-	-	0.1181
5/30/2001	4	LC	21	10	4.6	4.01	10.30	14.31	nd	32.5	nd	18.2	nd	583	17.9	-	-	0.0849
5/30/2001	5	LM	21	4.8	6.9	10.40	21.40	31.80	0.86	75	1.38	43.2	0.52	979	13.1	54.3	710	0.2743
5/30/2001	6	LM	21	7.8	4.9	5.72	12.25	17.97	0.21	48.9	0.59	30.9	0.38	745	15.2	82.9	1262	0.1751
5/30/2001	7	LM	20.8	9.7	4.6	2.90	5.82	8.72	0.04	13.4	nd	4.7	-0.04	514	38.4	-	-	0.0930
5/30/2001	8	MC	21	3.1	18.9	1.02	19.20	20.22	0.24	56.9	0.59	36.7	0.35	1212	21.3	96.4	2054	0.2858
5/30/2001	9	MC	21	5.1	9.1	2.70	11.60	14.30	0.18	47.8	0.52	33.5	0.34	1005	21.0	91.9	1933	0.2211
5/30/2001	10	MC	21.1	8.6	8.1	2.68	8.83	11.51	0.12	34.9	0.35	23.4	0.23	727	20.8	99.7	2077	0.1218
5/30/2001	12	MC	20	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6/12/2001	1	OB	25.6	10.9	7.3	1.75	14.50	16.25	0.03	41.8	0.18	25.6	0.15	417	10.0	232.2	2315	0.0591
6/12/2001	2	OB	25.8	9.7	8.3	2.08	13.00	15.08	0.03	41.9	0.76	26.8	0.73	578	13.8	55.1	761	0.0881
6/12/2001	3	LC	25.6	9.6	10.8	1.54	6.52	8.06	nd	37.6	0.32	29.5	0.32	653	17.4	117.5	2040	0.1157
6/12/2001	4	LC	25.7	9.9	12	2.50	7.88	10.38	0.07	31.9	0.66	21.5	0.59	579	18.1	48.3	877	0.0986
6/12/2001	5	LM	26.2	7	16.8	0.59	1.04	1.63	0.09	43.5	0.74	41.9	0.65	915	21.0	58.8	1236	0.1899
6/12/2001	6	LM	25.9	8.7	20.6	0.55	1.93	2.48	nd	36	0.78	33.5	0.78	756	21.0	46.2	970	0.1396
6/12/2001	7	LM	25.8	9.9	13.8	1.39	7.77	9.16	0.02	35.7	0.65	26.5	0.63	585	16.4	54.9	900	0.1037

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (µM)	NOx (µM)	DIN (µM)	PO_4^{3-} (µM)	TDN (µM)	TDP (µM)	DON (µM)	DOP (µM)	DOC (µM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
6/12/2001	8	MC	25.9	3.3	12.4	0.99	4.13	5.12	0.15	49.9	0.73	44.8	0.58	1206	24.2	68.4	1652	0.3315
6/12/2001	9	MC	25.4	5.4	6.1	2.87	5.71	8.58	0.13	46.8	1.50	38.2	1.37	939	20.1	31.2	626	0.2621
6/12/2001	10	MC	25.5	8.8	5.9	2.85	9.55	12.40	nd	40.3	0.28	27.9	0.28	679	16.8	143.9	2424	0.1287
6/16/2001	1	OB	26.5	12.5	3.1	-	-	-	-	-	-	-	-	358	-	-	-	0.0479
6/16/2001	2	OB	27.2	10.4	18.1	-	-	-	-	-	-	-	-	511	-	-	-	0.0816
6/16/2001	5	LM	27.8	7.5	4.4	-	-	-	-	-	-	-	-	842	-	-	-	0.1936
6/16/2001	5A	LM	27.8	6.8	11.5	-	-	-	-	-	-	-	-	890	-	-	-	0.2140
6/16/2001	6	LM	27.6	8.9	11.8	-	-	-	-	-	-	-	-	726	-	-	-	0.1451
6/16/2001	7	LM	27.4	10	8.5	-	-	-	-	-	-	-	-	556	-	-	-	0.1030
6/16/2001	8	MC	27.8	4.1	13.5	-	-	-	-	-	-	-	-	1051	-	-	-	0.2501
6/16/2001	9	MC	27.7	5.8	10.8	-	-	-	-	-	-	-	-	895	-	-	-	0.2946
6/16/2001	9A	MC	27.7	4.2	8.7	-	-	-	-	-	-	-	-	1107	-	-	-	0.2243
6/16/2001	10	MC	27.4	8.7	4.5	-	-	-	-	-	-	-	-	651	-	-	-	0.1289
6/16/2001	11	MC	28.2	2	16.6	-	-	-	-	-	-	-	-	1323	-	-	-	0.3800
6/16/2001	11A	MC	28.1	1.4	20.9	-	-	-	-	-	-	-	-	1395	-	-	-	0.4802
6/16/2001	12	MC	27.7	0.7	26.4	-	-	-	-	-	-	-	-	1542	-	-	-	0.5956
6/25/2001	1	OB	25.7	11.4	8.9	2.20	7.91	10.11	0.08	19	0.15	8.9	0.07	416	21.9	126.7	2771	0.0551
6/25/2001	2	OB	25.5	10.3	5.5	2.71	6.14	8.85	0.05	34.8	0.32	26.0	0.27	558	16.0	108.8	1745	0.0938
6/25/2001	3	LC	25.1	10.4	6.1	2.95	3.87	6.82	0.13	36.4	0.32	29.6	0.19	610	16.8	113.8	1905	0.1158
6/25/2001	4	LC	25.1	10.5	6.8	2.56	4.66	7.22	0.03	32.3	0.26	25.1	0.23	562	17.4	124.2	2161	0.1044
6/25/2001	5	LM	25.1	9.5	11.1	2.66	1.39	4.05	0.12	41.9	0.67	37.9	0.55	747	17.8	62.5	1114	0.1684
6/25/2001	6	LM	25.1	10.2	8	2.97	3.09	6.06	nd	35.4	0.37	29.3	0.37	638	18.0	95.7	1725	0.1292
6/25/2001	7	LM	25.1	10.5	7.4	2.83	4.57	7.40	0.02	34	0.28	26.6	0.26	571	16.8	121.4	2041	0.1105
6/25/2001	8	MC	26.2	5.9	12.8	3.22	1.43	4.65	nd	44.7	0.73	40.1	0.73	952	21.3	61.2	1304	0.2246
6/25/2001	9	MC	26.1	7.7	9	3.58	2.14	5.72	nd	41	0.45	35.3	0.45	803	19.6	91.1	1785	0.1855
6/25/2001	10	MC	25.7	9.9	6.3	3.88	5.99	9.87	nd	37.1	0.30	27.2	0.30	593	16.0	123.7	1978	0.1140
7/12/2001	1	OB	26.3	11.7	11.8	1.04	0.15	1.19	nd	20.1	0.23	18.9	0.23	448	22.3	87.4	1949	0.0623
7/12/2001	2	OB	26.8	11	12.4	0.64	0.03	0.67	nd	28	0.10	27.3	0.10	605	21.6	280.0	6054	0.0961

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (µM)	NOx (µM)	DIN (µM)	PO_4^{3-} (µM)	TDN (µM)	TDP (µM)	DON (µM)	DOP (µM)	DOC (µM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
7/12/2001	3	LC	26.3	11.1	9.6	0.83	0.12	0.95	nd	33.9	0.07	33.0	0.07	713	21.0	484.3	10192	0.1402
7/12/2001	4	LC	26.3	11.2	10.8	0.99	0.04	1.03	nd	29.6	0.28	28.6	0.28	704	23.8	105.7	2514	-
7/12/2001	5	LM	26.7	10.5	13.7	0.77	n	0.77	0.67	42.1	0.92	41.3	0.25	800	19.0	45.8	870	0.1832
7/12/2001	5A	LM	26	10.3	14.6	0.91	0.06	0.97	1.22	44.8	1.66	43.8	0.44	836	18.7	27.0	504	0.1540
7/12/2001	6	LM	26.7	10.9	12.6	0.86	0.02	0.88	0.07	37.9	0.17	37.0	0.10	735	19.4	222.9	4322	0.1122
7/12/2001	7	LM	26.5	11.1	11.7	0.81	0.01	0.82	nd	31.5	nd	30.7	nd	634	20.1	-	-	0.1974
7/12/2001	8	MC	27.2	7.1	9.8	0.73	n	0.73	nd	41.3	0.03	40.6	0.03	926	22.4	1376.7	30861	0.2022
7/12/2001	9	MC	27.1	8.3	10.6	0.84	0.18	1.02	nd	39.1	nd	38.1	nd	838	21.4	-	-	0.1747
7/12/2001	10	MC	26.8	10	12.1	0.80	n	0.80	nd	33.1	0.34	32.3	0.34	743	22.5	97.4	2186	0.1392
7/12/2001	11	MC	27.5	5.6	12.8	1.58	0.03	1.61	nd	42.1	nd	40.5	nd	998	23.7	-	-	0.2207
7/12/2001	11A	MC	27.1	4.8	14.8	1.43	0.50	1.93	nd	42.4	nd	40.5	nd	836	19.7	-	-	0.2190
7/12/2001	12	MC	26.6	4.2	28.8	0.91	0.15	1.06	nd	44.4	nd	43.3	nd	1074	24.2	-	-	0.2510
7/25/2001	1	OB	27.1	12.9	14.2	1.00	0.41	1.41	0.01	21.1	0.25	19.7	0.24	610	28.9	84.4	2439	0.0617
7/25/2001	2	OB	27.1	12	10.8	0.77	0.39	1.16	nd	26.7	0.30	25.5	0.30	655	24.5	89.0	2183	0.1051
7/25/2001	3	LC	27.3	12.4	7.6	1.28	0.57	1.85	nd	34.2	0.30	32.4	0.30	630	18.4	114.0	2098	0.1281
7/25/2001	4	LC	27.2	12.3	8.3	0.75	0.37	1.12	0.12	14.5	0.18	13.4	0.06	786	54.2	80.6	4364	0.1102
7/25/2001	5	LM	27.9	12.1	12.9	1.02	0.40	1.42	0.60	41.4	1.15	40.0	0.55	693	16.7	36.0	602	0.1630
7/25/2001	6	LM	27.6	12.2	9.6	1.23	0.50	1.73	0.19	37	0.57	35.3	0.38	661	17.9	64.9	1160	0.1433
7/25/2001	7	LM	27.3	12.3	8.9	1.05	0.52	1.57	0.05	33.1	0.87	31.5	0.82	853	25.8	38.0	980	0.1221
7/25/2001	8	MC	27.5	9.4	8.2	0.65	0.39	1.04	0.27	42.1	0.53	41.1	0.26	964	22.9	79.4	1819	0.1775
7/25/2001	9	MC	27.3	10.4	9.6	0.67	0.39	1.06	0.03	40	0.39	38.9	0.36	733	18.3	102.6	1881	-
7/25/2001	10	MC	27.2	11.5	8.2	0.97	0.40	1.37	0.06	33.7	0.29	32.3	0.23	1051	31.2	116.2	3624	0.1258
8/10/2001	1	OB	29.4	12.8	10.5	2.41	0.57	2.98	0.02	26.4	0.20	23.4	0.18	-	-	132.0	-	0.0550
8/10/2001	2	OB	30.2	12.5	5.9	1.88	0.96	2.84	0.09	20.7	0.15	17.9	0.06	-	-	138.0	-	0.0858
8/10/2001	3	LC	29.8	12.7	8.6	0.92	0.30	1.22	nd	8.4	0.04	7.2	0.04	-	-	210.0	-	0.1167
8/10/2001	4	LC	30	12.8	7.2	1.06	0.34	1.40	nd	16	0.11	14.6	0.11	-	-	145.5	-	0.0932
8/10/2001	5	LM	30.4	12.2	22.3	1.06	nd	1.06	0.29	8.8	0.35	7.7	0.06	-	-	25.1	-	0.1617
8/10/2001	6	LM	30.1	12.6	13.2	1.05	0.24	1.29	0.10	13.7	0.23	12.4	0.13	-	-	59.6	-	0.1319

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (µM)	NOx (µM)	DIN (µM)	PO_4^{3-} (µM)	TDN (µM)	TDP (µM)	DON (µM)	DOP (µM)	DOC (µM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
8/10/2001	7	LM	30.1	12.8	8.9	1.42	0.34	1.76	0.05	22.3	0.15	20.5	0.10	-	-	148.7	-	0.1038
8/10/2001	8	MC	30	9.7	6.9	1.01	nd	1.01	0.20	14.3	0.28	13.3	0.08	-	-	51.1	-	0.1674
8/10/2001	9	MC	29.8	10.7	6.7	1.50	nd	1.50	0.19	20.9	0.28	19.4	0.09	-	-	74.6	-	0.1509
8/10/2001	10	MC	30	12.1	7	1.00	0.25	1.25	0.01	21.7	0.17	20.5	0.16	-	-	127.6	-	0.1096
8/22/2001	1	OB	26.6	11.3	18.4	1.87	0.57	2.44	0.06	23.7	0.30	21.3	0.24	436	18.4	79.0	1453	0.0695
8/22/2001	2	OB	26	10.7	7.9	1.68	0.29	1.97	0.08	31.5	0.32	29.5	0.24	754	23.9	98.4	2357	0.1217
8/22/2001	3	LC	26	10.4	7.7	1.96	0.44	2.40	0.04	33.6	0.30	31.2	0.26	671	20.0	112.0	2236	0.1669
8/22/2001	4	LC	26	11	8.5	1.47	0.29	1.76	0.01	31.7	0.30	29.9	0.29	582	18.3	105.7	1939	0.1269
8/22/2001	5	LM	25.9	7.5	10.6	6.66	0.77	7.43	1.97	55.9	2.82	48.5	0.85	917	16.4	19.8	325	0.3220
8/22/2001	6	LM	25.9	9.4	9.7	3.93	0.58	4.51	0.91	46.4	1.65	41.9	0.74	793	17.1	28.1	481	0.0444
8/22/2001	7	LM	25.9	10.7	8.4	0.93	0.40	1.33	0.21	33.8	0.58	32.5	0.37	593	17.5	58.3	1022	0.1480
8/22/2001	8	MC	27.1	5.6	12.8	0.92	0.28	1.20	0.26	30.9	0.68	29.7	0.42	799	25.9	45.4	1175	0.2209
8/22/2001	9	MC	27	7.6	10.8	1.24	0.30	1.54	0.15	38.8	0.59	37.3	0.44	727	18.7	65.8	1232	0.1914
8/22/2001	10	MC	26.8	10.1	8.6	1.58	0.32	1.90	0.04	34.4	0.40	32.5	0.36	633	18.4	86.0	1583	0.1392
9/11/2001	1	OB	25.2	11.2	6.7	1.27	0.55	1.82	0.01	30.9	0.54	29.1	0.53	452	14.6	57.2	836	0.0570
9/11/2001	2	OB	25.1	10.6	11.9	1.08	0.07	1.15	nd	31.4	0.53	30.3	0.53	605	19.3	59.2	1142	0.0965
9/11/2001	3	LC	24.3	9.4	10.1	1.17	0.65	1.82	0.01	14.4	0.24	12.6	0.23	818	56.8	60.0	3410	0.1408
9/11/2001	4	LC	24.6	10.1	8.9	2.51	1.44	3.95	3.53	35.5	0.54	31.6	-2.99	656	18.5	65.7	1215	0.1171
9/11/2001	5	LM	24.7	6.9	16.2	4.90	7.81	12.71	1.09	62.4	5.51	49.7	4.42	877	14.0	11.3	159	0.2214
9/11/2001	6	LM	24.9	8.8	12.9	0.90	3.05	3.95	0.32	44.2	2.25	40.3	1.93	760	17.2	19.6	338	0.1798
9/11/2001	7	LM	24.6	9.5	8.4	0.90	1.37	2.27	0.52	35.5	0.83	33.2	0.31	654	18.4	42.8	788	0.1488
9/11/2001	8	MC	25.1	9.2	8.9	1.02	0.24	1.26	0.41	41.8	1.15	40.5	0.74	872	20.9	36.3	758	0.2285
9/11/2001	9	MC	25.4	7.4	9	1.12	0.20	1.32	0.22	41	1.22	39.7	1.00	808	19.7	33.6	663	0.2105
9/11/2001	10	MC	25.4	6	7	0.78	0.28	1.06	0.06	36.2	1.04	35.1	0.98	691	19.1	34.8	664	0.1567
9/11/2001	11	MC	25.5	4.7	10.3	-	-	-	-	-	-	-	-	887	-	-	-	0.2464
9/11/2001	12	MC	25.6	3.2	15.6	-	-	-	-	-	-	-	-	977	-	-	-	0.2881
10/11/2001	1	OB	16.1	14.2	6.2	0.50	0.08	0.58	nd	22.3	0.27	21.7	0.27	413	18.5	82.6	1531	0.0563
10/11/2001	2	OB	16	12.6	5.7	0.77	0.20	0.97	0.06	24.6	0.27	23.6	0.21	537	21.8	91.1	1988	0.0893

Date	Site	System	Temp (ºC)	Salinity	Chl-*a* (mg L^{-1})	NH_4^+ (µM)	NOx (µM)	DIN (µM)	PO_4^{3-} (µM)	TDN (µM)	TDP (µM)	DON (µM)	DOP (µM)	DOC (µM)	DOC:DON	DON:DOP	DOC:DOP	a_{350} (m^{-1})
10/11/2001	3	LC	15.8	11.6	7.7	1.46	0.35	1.81	0.01	30.5	0.40	28.7	0.39	661	21.7	76.3	1652	0.1202
10/11/2001	4	LC	15.9	12.6	5.3	0.73	0.10	0.83	0.40	23.8	0.21	23.0	-0.19	577	24.2	113.3	2746	0.0966
10/11/2001	5	LM	15.9	10.5	14.2	1.41	0.14	1.55	0.06	38	0.90	36.5	0.84	737	19.4	42.2	819	0.1446
10/11/2001	6	LM	15.9	11.2	8.6	0.76	0.04	0.80	0.03	29.9	0.37	29.1	0.34	647	21.6	80.8	1748	0.1198
10/11/2001	7	LM	16	12.6	6.6	1.88	0.46	2.34	0.05	36.1	nd	33.8	-0.05	570	15.8	-	-	0.0950
10/11/2001	8	MC	15.9	8.4	8.3	1.27	0.01	1.28	0.01	34.1	0.40	32.8	0.39	728	21.3	85.3	1819	0.1501
10/11/2001	9	MC	16.3	9.7	6.6	1.30	0.36	1.66	nd	32.8	0.34	31.1	0.34	668	20.4	96.5	1966	0.1415
10/11/2001	10	MC	16.1	11.5	7.3	0.86	0.15	1.01	nd	28	nd	27.0	nd	615	22.0	-	-	0.1109
12/6/2001	1	OB	12.3	15.9	5.57	0.40	0.09	0.49	0.01	15.4	0.18	14.9	0.17	391	25.4	85.6	2171	0.0445
12/6/2001	2	OB	12.7	15.2	1.88	0.41	0.05	0.46	nd	18.7	0.20	18.2	0.20	441	23.6	93.5	2204	0.0499
12/6/2001	3	LC	12	15.2	2.81	0.87	0.03	0.90	nd	21.1	0.16	20.2	0.16	489	23.2	131.9	3057	0.0651
12/6/2001	4	LC	12.2	15.6	3.69	0.51	0.39	0.90	0.08	18.7	0.19	17.8	0.11	438	23.4	98.4	2307	0.0571
12/6/2001	5	LM	11.6	14.9	4.01	2.21	1.27	3.48	nd	28.1	0.51	24.6	0.51	518	18.4	55.1	1015	0.0757
12/6/2001	6	LM	11.9	15.3	4.83	0.66	0.38	1.04	nd	21.7	0.40	20.7	0.40	466	21.5	54.3	1165	0.0729
12/6/2001	7	LM	12.3	15.6	2.47	0.55	0.23	0.78	0.03	20.3	0.19	19.5	0.16	452	22.2	106.8	2377	0.0593
12/6/2001	8	MC	12.2	12.1	15.73	0.79	0.10	0.89	nd	29.7	0.37	28.8	0.37	623	21.0	80.3	1685	0.0960
12/6/2001	9	MC	12.5	13.2	8.6	0.49	0.02	0.51	nd	25.8	0.54	25.3	0.54	590	22.9	47.8	1093	0.0907
12/6/2001	10	MC	12.6	14.6	2.32	0.42	0.23	0.65	nd	23.1	0.23	22.5	0.23	493	21.3	100.4	2141	0.0708
1/23/2002	1	OB	4.3	15.8	12.87	0.98	5.56	6.54	nd	23.6	0.18	17.1	0.18	475	20.1	131.1	2639	-
1/23/2002	2	OB	4.6	15.1	8.95	0.62	0.47	1.09	nd	17.4	0.16	16.3	0.16	470	27.0	108.8	2938	-
1/23/2002	3	LC	4.7	11.2	3.09	3.43	3.03	6.46	0.12	28.1	0.08	21.6	-0.04	798	28.4	351.3	9969	-
1/23/2002	4	LC	4.8	13.8	6.71	2.88	2.38	5.26	0.54	22.1	0.15	16.8	-0.39	394	17.8	147.3	2628	-
1/23/2002	5	LM	5	7.4	3.45	1.66	6.61	8.27	0.02	122	1.03	113.7	1.01	1018	8.3	118.4	988	-
1/23/2002	6	LM	4.9	11.4	5.59	5.22	1.91	7.13	nd	56.4	0.27	49.3	0.27	465	8.2	208.9	1722	-
1/23/2002	7	LM	4.8	13.2	7.69	3.07	9.39	12.46	nd	34.7	0.25	22.2	0.25	481	13.9	138.8	1923	-
1/23/2002	8	MC	4.4	8.6	18.39	0.76	1.64	2.40	nd	44.2	0.25	41.8	0.25	852	19.3	176.8	3407	-
1/23/2002	9	MC	4.4	10.3	17.83	0.75	1.18	1.93	nd	42.8	0.21	40.9	0.21	561	13.1	203.8	2671	-
1/23/2002	10	MC	4.4	12.7	13.64	0.43	1.36	1.79	nd	22.6	0.14	20.8	0.14	424	18.8	161.4	3030	-

Table A-2. Measures of bacterioplankton carbon metabolism recorded during sampling of Monie Bay.

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP $cell^{-1}$	filtered BP $cell^{-1}$	BR $cell^{-1}$	μ (day^{-1})	filt μ (day^{-1})
4/6/2000	1	OB	13	1.72	0.28	1.13	1.41	0.2	9.5E-08	2.3E-08	9.0E-08	0.11	0.03
4/6/2000	2	OB	13	1.48	0.26	0.55	0.81	0.32	5.6E-08	1.9E-08	4.0E-08	0.07	0.02
4/6/2000	3	LC	13	1.41	0.84	2.39	3.23	0.26	4.6E-08	3.5E-08	1.0E-07	0.06	0.04
4/6/2000	4	LC	13	1.72	0.76	0.75	1.51	0.5	6.0E-08	3.9E-08	4.0E-08	0.07	0.05
4/6/2000	5	LM	13	2.21	1	5.19	6.19	0.16	7.6E-08	6.7E-08	3.5E-07	0.09	0.08
4/6/2000	6	LM	13	1.2	0.69	1.89	2.58	0.27	3.6E-08	5.2E-08	1.4E-07	0.04	0.06
4/6/2000	7	LM	13	1.6	0.98	1.92	2.9	0.34	4.7E-08	5.4E-08	1.1E-07	0.06	0.06
4/6/2000	8	MC	13	1.51	0.32	1.08	1.4	0.23	6.2E-08	2.1E-08	7.0E-08	0.07	0.03
4/6/2000	9	MC	13	1.67	0.24	0.42	0.66	0.36	6.4E-08	1.5E-08	3.0E-08	0.08	0.02
4/6/2000	10	MC	13	1.27	0.45	0.54	0.99	0.45	4.9E-08	3.3E-08	4.0E-08	0.06	0.04
5/8/2000	1	OB	23	1.83	0.7	2.24	2.94	0.24	9.2E-08	2.6E-08	8.0E-08	0.11	0.03
5/8/2000	2	OB	23	1.64	0.66	2.03	2.69	0.25	8.9E-08	8.1E-09	2.0E-08	0.11	0.01
5/8/2000	3	LC	23	1.68	0.73	1.75	2.48	0.29	5.5E-08	5.1E-08	1.2E-07	0.07	0.06
5/8/2000	4	LC	23	1.69	0.72	2.08	2.8	0.26	4.9E-08	5.5E-08	1.6E-07	0.06	0.07
5/8/2000	5	LM	23	4.27	4.56	1.21	5.77	0.79	1.3E-07	2.9E-07	8.0E-08	0.15	0.34
5/8/2000	6	LM	23	2.39	0.67	1.94	2.61	0.26	6.9E-08	3.6E-08	1.0E-07	0.08	0.04
5/8/2000	7	LM	23	1.5	0.57	1.47	2.04	0.28	5.6E-08	3.2E-08	8.0E-08	0.07	0.04
5/8/2000	8	MC	23	1.48	1.36	0.97	2.33	0.58	6.4E-08	1.0E-07	7.0E-08	0.08	0.12
5/8/2000	9	MC	23	2.51	2.49	2.82	5.31	0.47	8.8E-08	1.4E-07	1.6E-07	0.10	0.17
5/8/2000	10	MC	23	1	1.12	-	-	-	3.7E-08	7.0E-08	-	0.04	0.08
6/7/2000	1	OB	22	1.51	1.09	1.3	2.39	0.46	1.2E-07	1.8E-07	2.1E-07	0.15	0.21
6/7/2000	2	OB	22	1.83	0.52	1.54	2.06	0.25	1.7E-07	5.4E-08	1.6E-07	0.21	0.07
6/7/2000	3	LC	22	3.1	1.89	5.28	7.17	0.26	2.1E-07	2.4E-07	6.8E-07	0.25	0.29
6/7/2000	4	LC	22	2.43	1.98	4.67	6.65	0.3	1.7E-07	2.4E-07	5.5E-07	0.20	0.28
6/7/2000	5	LM	22	5.56	3.66	6.94	10.6	0.35	4.7E-07	4.9E-07	9.4E-07	0.56	0.59
6/7/2000	6	LM	22	4.45	2.07	3.71	5.78	0.36	3.6E-07	2.7E-07	4.9E-07	0.43	0.33
6/7/2000	7	LM	22	4.25	1.65	2.03	3.68	0.45	3.2E-07	2.0E-07	2.4E-07	0.38	0.23

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP cell^{-1}	filtered BP cell^{-1}	BR cell^{-1}	μ (day^{-1})	filt μ (day^{-1})
6/7/2000	8	MC	22	3.53	1.94	1.93	3.87	0.5	2.7E-07	2.5E-07	2.5E-07	0.33	0.30
6/7/2000	9	MC	22	5.31	2.8	4.44	7.24	0.39	4.0E-07	2.8E-07	4.4E-07	0.48	0.33
6/7/2000	10	MC	22	3.61	2.33	1.53	3.86	0.6	2.5E-07	2.7E-07	1.7E-07	0.30	0.32
7/3/2000	1	OB	26	1.42	1	3.28	4.28	0.23	1.7E-07	1.9E-07	6.2E-07	0.20	0.23
7/3/2000	2	OB	26	1.89	1.26	3.61	4.87	0.26	1.3E-07	1.2E-07	3.4E-07	0.15	0.14
7/3/2000	3	LC	26	3	1.93	2.95	4.88	0.4	3.0E-07	2.1E-07	3.2E-07	0.36	0.26
7/3/2000	4	LC	26	1.34	1.92	3.92	5.84	0.33	1.1E-07	2.0E-07	4.1E-07	0.14	0.24
7/3/2000	5	LM	26	6.76	5.78	6.95	12.73	0.45	6.0E-07	8.3E-07	1.0E-06	0.72	1.00
7/3/2000	6	LM	26	6.29	3.4	7.16	10.56	0.32	6.2E-07	4.6E-07	9.7E-07	0.74	0.56
7/3/2000	7	LM	26	3.84	1.76	2.35	4.11	0.43	3.0E-07	1.9E-07	2.5E-07	0.35	0.23
7/3/2000	8	MC	26	2.25	1.7	9.43	11.13	0.15	4.0E-07	3.0E-07	1.7E-06	0.48	0.36
7/3/2000	9	MC	26	2.6	2.52	7.23	9.75	0.26	3.4E-07	4.0E-07	1.2E-06	0.41	0.48
7/3/2000	10	MC	26	2.56	1.89	4.79	6.68	0.28	2.4E-07	2.3E-07	5.8E-07	0.28	0.27
8/3/2000	1	OB	28	1.46	2.21	-	-	-	1.8E-07	4.3E-07	-	0.21	0.52
8/3/2000	2	OB	28	2.01	1.92	-	-	-	2.6E-07	3.7E-07	-	0.32	0.45
8/3/2000	3	LC	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	4	LC	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	5	LM	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	6	LM	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	7	LM	-	-	-	-	-	-	-	-	-	-	-
8/3/2000	8	MC	28	3.09	1.67	-	-	-	3.3E-07	2.7E-07	-	0.40	0.33
8/3/2000	9	MC	28	2.41	2.03	-	-	-	3.1E-07	2.8E-07	-	0.37	0.34
8/3/2000	10	MC	28	2.02	1.87	-	-	-	2.3E-07	2.9E-07	-	0.28	0.34
9/5/2000	1	OB	22	0.67	0.79	2.03	2.82	0.28	3.3E-08	1.4E-07	3.5E-07	0.04	0.16
9/5/2000	2	OB	22	1.34	0.88	2.07	2.95	0.3	7.2E-08	7.1E-08	1.7E-07	0.09	0.09
9/5/2000	3	LC	22	1.7	1.06	1.39	2.45	0.43	1.3E-07	9.1E-08	1.2E-07	0.15	0.11
9/5/2000	4	LC	22	1.4	1.29	1.68	2.97	0.43	9.5E-08	1.0E-07	1.3E-07	0.11	0.12
9/5/2000	5	LM	22	2.58	1.5	2.41	3.91	0.38	1.9E-07	1.5E-07	2.4E-07	0.23	0.18

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP $cell^{-1}$	filtered BP $cell^{-1}$	BR $cell^{-1}$	μ (day^{-1})	filt μ (day^{-1})
9/5/2000	6	LM	22	1.5	1.42	1.83	3.25	0.44	1.2E-07	1.4E-07	1.8E-07	0.14	0.17
9/5/2000	7	LM	22	1.21	0.91	2.29	3.2	0.28	9.5E-08	9.3E-08	2.3E-07	0.11	0.11
9/5/2000	8	MC	22	1.97	1.54	4.25	5.79	0.27	1.0E-07	1.1E-07	2.9E-07	0.12	0.13
9/5/2000	9	MC	22	2.47	1.72	3.86	5.58	0.31	1.3E-07	1.4E-07	3.1E-07	0.15	0.17
9/5/2000	10	MC	22	1.57	1.47	1.66	3.13	0.47	1.1E-07	1.1E-07	1.2E-07	0.13	0.13
12/5/2000	1	OB	4	0.25	0.34	0.16	0.5	0.68	3.5E-08	6.1E-08	3.0E-08	0.04	0.07
12/5/2000	2	OB	4	0.27	0.25	0.21	0.46	0.54	3.0E-08	4.3E-08	4.0E-08	0.04	0.05
12/5/2000	3	LC	3	0.62	0.51	0.31	0.82	0.62	5.6E-08	6.6E-08	4.0E-08	0.07	0.08
12/5/2000	4	LC	3	0.57	0.35	0.24	0.59	0.59	5.2E-08	6.4E-08	4.0E-08	0.06	0.08
12/5/2000	5	LM	2.8	0.9	0.69	0.47	1.16	0.59	7.7E-08	8.5E-08	6.0E-08	0.09	0.10
12/5/2000	6	LM	3	0.71	0.44	-	-	-	6.8E-08	5.6E-08	-	0.08	0.07
12/5/2000	7	LM	3	0.49	0.47	0.29	0.76	0.62	4.9E-08	5.7E-08	4.0E-08	0.06	0.07
12/5/2000	8	MC	4	0.57	0.29	0.69	0.98	0.3	9.0E-08	7.9E-08	1.9E-07	0.11	0.09
12/5/2000	9	MC	4	0.57	0.4	0.36	0.76	0.53	1.1E-07	9.2E-08	8.0E-08	0.13	0.11
12/5/2000	10	MC	4	0.46	0.25	0.13	0.38	0.66	6.1E-08	4.7E-08	2.0E-08	0.07	0.06
3/15/2001	1	OB	8.9	0.42	0.18	0.69	0.87	0.21	9.0E-08	6.3E-08	2.4E-07	0.11	0.08
3/15/2001	2	OB	8.9	0.83	0.17	0.58	0.75	0.23	1.7E-07	6.1E-08	2.1E-07	0.21	0.07
3/15/2001	3	LC	10.4	1	0.47	0.5	0.97	0.48	1.3E-07	9.3E-08	1.0E-07	0.16	0.11
3/15/2001	4	LC	10.4	1.92	0.64	0.46	1.1	0.58	2.4E-07	1.7E-07	1.2E-07	0.29	0.21
3/15/2001	5	LM	10.9	1.87	0.88	0.53	1.41	0.62	2.1E-07	1.4E-07	8.0E-08	0.25	0.16
3/15/2001	6	LM	10.7	1.33	0.64	-	-	-	1.6E-07	1.4E-07	-	0.19	0.17
3/15/2001	7	LM	10.5	1.75	0.61	0.43	1.04	0.59	2.2E-07	1.5E-07	1.1E-07	0.26	0.18
3/15/2001	8	MC	10.5	1.03	0.69	0.51	1.2	0.57	1.0E-07	1.0E-07	7.0E-08	0.12	0.12
3/15/2001	9	MC	10	0.98	0.49	0.89	1.38	0.36	8.8E-08	1.2E-07	2.1E-07	0.11	0.14
3/15/2001	10	MC	9.9	1.21	0.35	0.75	1.1	0.32	1.4E-07	6.2E-08	1.3E-07	0.16	0.07
4/12/2001	1	OB	14.4	0.878	0.34	1.13	1.47	0.23	1.6E-07	6.7E-08	2.2E-07	0.20	0.08
4/12/2001	2	OB	14.4	0.72	0.33	2.27	2.6	0.13	1.0E-07	6.4E-08	4.4E-07	0.12	0.08
4/12/2001	3	LC	14.8	1.278	0.75	1.67	2.42	0.31	1.3E-07	1.0E-07	2.3E-07	0.16	0.12

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP cell^{-1}	filtered BP cell^{-1}	BR cell^{-1}	μ (day^{-1})	filt μ (day^{-1})
4/12/2001	4	LC	14.8	1.602	0.58	0.98	1.56	0.37	1.7E-07	7.3E-08	1.2E-07	0.20	0.09
4/12/2001	5	LM	13.9	3.008	1.14	0.19	1.33	0.86	3.6E-07	2.2E-07	4.0E-08	0.43	0.26
4/12/2001	6	LM	14.5	2.731	0.84	0.49	1.33	0.63	2.5E-07	1.1E-07	6.0E-08	0.30	0.13
4/12/2001	7	LM	14.8	1.6	0.88	-	-	-	1.5E-07	1.2E-07	-	0.18	0.15
4/12/2001	8	MC	15.4	0.799	0.64	2.2	2.84	0.23	8.9E-08	1.2E-07	4.2E-07	0.11	0.15
4/12/2001	9	MC	15.3	1.547	0.39	1.88	2.27	0.17	2.3E-07	7.8E-08	3.8E-07	0.28	0.09
4/12/2001	10	MC	15.1	1.311	0.69	-	-	-	1.6E-07	1.2E-07	-	0.19	0.14
4/12/2001	11	MC	14.5	3.295	0.38	0.39	0.77	0.49	4.1E-07	6.9E-08	7.0E-08	0.50	0.08
4/12/2001	12	MC	12.3	0.572	0.11	0.7	0.81	0.14	2.1E-07	4.6E-08	2.9E-07	0.25	0.05
5/30/2001	1	OB	21	1.81	0.37	-	-	-	1.6E-07	6.5E-08	-	0.20	0.08
5/30/2001	2	OB	21	2	0.37	-	-	-	1.8E-07	4.5E-08	-	0.22	0.05
5/30/2001	3	LC	20.8	2.22	0.26	-	-	-	2.2E-07	3.2E-08	-	0.27	0.04
5/30/2001	4	LC	21	2.49	0.66	-	-	-	2.4E-07	8.8E-08	-	0.28	0.11
5/30/2001	5	LM	21	4.02	0.59	-	-	-	4.1E-07	8.6E-08	-	0.49	0.10
5/30/2001	6	LM	21	5.81	1.57	-	-	-	4.8E-07	2.0E-07	-	0.58	0.24
5/30/2001	7	LM	20.8	3.13	1.18	-	-	-	3.3E-07	1.4E-07	-	0.39	0.16
5/30/2001	8	MC	21	2.67	0.58	-	-	-	1.9E-07	6.5E-08	-	0.22	0.08
5/30/2001	9	MC	21	2.85	0.61	-	-	-	2.4E-07	5.5E-08	-	0.28	0.07
5/30/2001	10	MC	21.1	2.35	0.18	-	-	-	1.9E-07	1.5E-08	-	0.22	0.02
5/30/2001	12	MC	20	-	-	-	-	-	-	-	-	-	-
6/12/2001	1	OB	25.6	0.67	0.2	2.57	2.77	0.07	4.9E-08	2.6E-08	3.4E-07	0.06	0.03
6/12/2001	2	OB	25.8	0.67	0.21	4.42	4.63	0.05	4.4E-08	2.1E-08	4.3E-07	0.05	0.02
6/12/2001	3	LC	25.6	0.98	0.23	1.86	2.09	0.11	9.6E-08	3.1E-08	2.5E-07	0.12	0.04
6/12/2001	4	LC	25.7	0.47	0.48	3.4	3.88	0.12	4.2E-08	5.2E-08	3.7E-07	0.05	0.06
6/12/2001	5	LM	26.2	1.73	1.01	5.34	6.35	0.16	1.6E-07	8.8E-08	4.6E-07	0.19	0.11
6/12/2001	6	LM	25.9	1.29	0.45	4.85	5.3	0.08	1.1E-07	4.5E-08	4.9E-07	0.14	0.05
6/12/2001	7	LM	25.8	0.96	0.68	4.22	4.9	0.14	8.2E-08	8.2E-08	5.1E-07	0.10	0.10
6/12/2001	8	MC	25.9	1.17	0.92	3.45	4.37	0.21	8.9E-08	7.0E-08	2.6E-07	0.11	0.08

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP cell^{-1}	filtered BP cell^{-1}	BR cell^{-1}	μ (day^{-1})	filt μ (day^{-1})
6/12/2001	9	MC	25.4	1.42	0.64	3.64	4.28	0.15	1.0E-07	4.5E-08	2.5E-07	0.12	0.05
6/12/2001	10	MC	25.5	0.46	0.22	3.43	3.65	0.06	3.5E-08	1.4E-08	2.1E-07	0.04	0.02
6/25/2001	1	OB	25.7	0.69	0.37	1.81	2.18	0.17	5.8E-08	6.0E-08	2.9E-07	0.07	0.07
6/25/2001	2	OB	25.5	-	-	-	-	-	-	-	-	-	-
6/25/2001	3	LC	25.1	0.97	0.89	3.86	4.75	0.19	9.7E-08	1.1E-07	4.9E-07	0.12	0.14
6/25/2001	4	LC	25.1	-	-	-	-	-	-	-	-	-	-
6/25/2001	5	LM	25.1	1.82	0.16	1.99	2.15	0.07	2.1E-07	2.7E-08	3.3E-07	0.25	0.03
6/25/2001	6	LM	25.1	-	-	-	-	-	-	-	-	-	-
6/25/2001	7	LM	25.1	-	-	-	-	-	-	-	-	-	-
6/25/2001	8	MC	26.2	1.31	1	3.54	4.54	0.22	1.1E-07	1.1E-07	3.9E-07	0.14	0.13
6/25/2001	9	MC	26.1	1.85	0.57	-	-	-	1.5E-07	6.3E-08	-	0.18	0.08
6/25/2001	10	MC	25.7	-	-	-	-	-	-	-	-	-	-
7/12/2001	1	OB	26.3	1.02	0.57	2.74	3.31	0.17	3.6E-08	4.8E-08	2.3E-07	0.04	0.06
7/12/2001	2	OB	26.8	1.11	0.16	2.55	2.71	0.06	8.0E-08	1.6E-08	2.6E-07	0.10	0.02
7/12/2001	3	LC	26.3	-	-	-	-	-	-	-	-	-	-
7/12/2001	4	LC	26.3	-	-	-	-	-	-	-	-	-	-
7/12/2001	5	LM	26.7	1.94	0.26	4.13	4.39	0.06	1.1E-07	2.3E-08	3.6E-07	0.13	0.03
7/12/2001	5A	LM	26	2	1.26	3.8	5.06	0.25	1.2E-07	1.1E-07	3.3E-07	0.14	0.13
7/12/2001	6	LM	26.7	2.07	1.06	2.71	3.77	0.28	1.3E-07	1.0E-07	2.6E-07	0.15	0.12
7/12/2001	7	LM	26.5	1.36	0.23	1.2	1.43	0.16	7.9E-08	1.6E-08	8.0E-08	0.09	0.02
7/12/2001	8	MC	27.2	1.12	0.53	3.86	4.39	0.12	6.9E-08	4.2E-08	3.0E-07	0.08	0.05
7/12/2001	9	MC	27.1	0.92	0.39	0.92	1.31	0.3	5.1E-08	3.3E-08	8.0E-08	0.06	0.04
7/12/2001	10	MC	26.8	0.7	0.24	1.34	1.58	0.15	5.0E-08	2.8E-08	1.5E-07	0.06	0.03
7/12/2001	11	MC	27.5	0.8	0.4	2.97	3.37	0.12	5.6E-08	4.0E-08	2.9E-07	0.07	0.05
7/12/2001	11A	MC	27.1	2.27	0.31	2.42	2.73	0.11	1.5E-07	3.1E-08	2.4E-07	0.18	0.04
7/12/2001	12	MC	26.6	1.89	0.76	3.98	4.74	0.16	1.4E-07	8.6E-08	4.5E-07	0.16	0.10
7/25/2001	1	OB	27.1	1.35	0.3	2.0963	2.396	0.13	1.1E-07	4.0E-08	2.8E-07	0.13	0.05
7/25/2001	2	OB	27.1	-	-	-	-	-	-	-	-	-	-

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP $cell^{-1}$	filtered BP $cell^{-1}$	BR $cell^{-1}$	μ (day^{-1})	filt μ (day^{-1})
7/25/2001	3	LC	27.3	1.9	0.97	5.2256	6.196	0.16	1.8E-07	1.1E-07	5.9E-07	0.22	0.13
7/25/2001	4	LC	27.2	-	-	-	-	-	-	-	-	-	-
7/25/2001	5	LM	27.9	3.19	1.23	4.2941	5.524	0.22	3.5E-07	1.5E-07	5.4E-07	0.41	0.19
7/25/2001	6	LM	27.6	-	-	-	-	-	-	-	-	-	-
7/25/2001	7	LM	27.3	1.55	0.86	2.3267	3.187	0.27	1.3E-07	9.1E-08	2.5E-07	0.16	0.11
7/25/2001	8	MC	27.5	1.71	0.54	2.4236	2.964	0.18	1.6E-07	5.6E-08	2.5E-07	0.20	0.07
7/25/2001	9	MC	27.3	-	-	-	-	-	-	-	-	-	-
7/25/2001	10	MC	27.2	1.83	0.9	2.515	3.415	0.26	1.6E-07	8.7E-08	2.4E-07	0.19	0.10
8/10/2001	1	OB	29.4	0.36	0.65	2.79847547	3.448	0.19	3.7E-08	1.2E-07	5.0E-07	0.04	0.14
8/10/2001	2	OB	30.2	-	-	-	-	-	-	-	-	-	-
8/10/2001	3	LC	29.8	1.2	1.6	4.00628561	5.606	0.29	1.2E-07	2.8E-07	6.9E-07	0.15	0.33
8/10/2001	4	LC	30	-	-	-	-	-	-	-	-	-	-
8/10/2001	5	LM	30.4	2.71	3.12	5.98542786	9.105	0.34	1.9E-07	3.5E-07	6.7E-07	0.22	0.42
8/10/2001	6	LM	30.1	-	-	-	-	-	-	-	-	-	-
8/10/2001	7	LM	30.1	1.15	0.94	5.59213024	6.532	0.14	1.1E-07	1.2E-07	7.0E-07	0.14	0.14
8/10/2001	8	MC	30	2.24	1.72	3.85722279	5.577	0.31	1.7E-07	2.0E-07	4.6E-07	0.20	0.25
8/10/2001	9	MC	29.8	-	-	-	-	-	-	-	-	-	-
8/10/2001	10	MC	30	4.09	1	2.41146218	3.411	0.29	3.5E-07	1.5E-07	3.7E-07	0.42	0.18
8/22/2001	1	OB	26.6	-	-	-	-	-	-	-	-	-	-
8/22/2001	2	OB	26	-	-	-	-	-	-	-	-	-	-
8/22/2001	3	LC	26	0.86	0.74	7.28460117	8.025	0.09	9.9E-08	1.1E-07	1.1E-06	0.12	0.13
8/22/2001	4	LC	26	-	-	-	-	-	-	-	-	-	-
8/22/2001	5	LM	25.9	2.39	2.37	13.5083902	15.88	0.15	1.9E-07	2.6E-07	1.5E-06	0.23	0.31
8/22/2001	6	LM	25.9	-	-	-	-	-	-	-	-	-	-
8/22/2001	7	LM	25.9	-	-	-	-	-	-	-	-	-	-
8/22/2001	8	MC	27.1	1.5	0.68	5.82877636	6.509	0.1	2.0E-07	1.2E-07	1.0E-06	0.24	0.14
8/22/2001	9	MC	27	-	-	-	-	-	-	-	-	-	-
8/22/2001	10	MC	26.8	-	-	-	-	-	-	-	-	-	-

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP cell^{-1}	filtered BP cell^{-1}	BR cell^{-1}	μ (day^{-1})	filt μ (day^{-1})
9/11/2001	1	OB	25.2	0.88	0.28	2.26	2.54	0.11	1.2E-07	5.9E-08	4.8E-07	0.15	0.07
9/11/2001	2	OB	25.1	0.73	0.39	3.85	4.24	0.09	7.4E-08	5.6E-08	5.5E-07	0.09	0.07
9/11/2001	3	LC	24.3	1.61	0.58	5.3	5.88	0.1	1.4E-07	8.3E-08	7.6E-07	0.16	0.10
9/11/2001	4	LC	24.6	-	-	-	-	-	-	-	-	-	-
9/11/2001	5	LM	24.7	3.34	2.09	7.96	10.05	0.21	2.0E-07	1.7E-07	6.6E-07	0.23	0.21
9/11/2001	6	LM	24.9	6.67	1.45	5.49	6.94	0.21	5.9E-07	1.4E-07	5.3E-07	0.70	0.17
9/11/2001	7	LM	24.6	4.11	2.06	2.61	4.67	0.44	4.3E-07	2.5E-07	3.1E-07	0.51	0.30
9/11/2001	8	MC	25.1	1.07	0.96	1.97	2.93	0.33	1.1E-07	1.5E-07	3.2E-07	0.13	0.18
9/11/2001	9	MC	25.4	2.6	0.76	4.1	4.86	0.16	2.8E-07	1.2E-07	6.5E-07	0.33	0.14
9/11/2001	10	MC	25.4	1.55	0.64	3.6	4.24	0.15	1.9E-07	9.9E-08	5.5E-07	0.22	0.12
9/11/2001	11	MC	25.5	0.88	1.39	7.7	9.09	0.15	9.5E-08	2.0E-07	1.1E-06	0.11	0.24
9/11/2001	12	MC	25.6	3.02	1.24	3.39	4.63	0.27	4.5E-07	2.8E-07	7.5E-07	0.54	0.33
10/11/2001	1	OB	16.1	0.72	0.58	0.63	1.21	0.48	1.8E-07	2.3E-07	2.5E-07	0.21	0.27
10/11/2001	2	OB	16	0.77	1.01	1.18	2.19	0.46	2.0E-07	3.5E-07	4.1E-07	0.24	0.42
10/11/2001	3	LC	15.8	0.91	1.62	2.97	4.59	0.35	2.7E-07	5.9E-07	1.1E-06	0.33	0.70
10/11/2001	4	LC	15.9	0.89	1.65	0.84	2.49	0.66	2.3E-07	5.9E-07	3.0E-07	0.28	0.71
10/11/2001	5	LM	15.9	2.05	2.49	2.13	4.62	0.54	6.9E-07	1.1E-06	9.6E-07	0.83	1.35
10/11/2001	6	LM	15.9	1.06	1.86	3.36	5.22	0.36	3.1E-07	6.4E-07	1.2E-06	0.37	0.76
10/11/2001	7	LM	16	-	1.31	0.85	2.16	0.61	-	4.2E-07	2.7E-07	-	0.50
10/11/2001	8	MC	15.9	1.32	1.55	2.06	3.61	0.43	2.2E-07	4.2E-07	5.5E-07	0.26	0.50
10/11/2001	9	MC	16.3	1.46	0.88	0.6	1.48	0.59	3.8E-07	2.8E-07	1.9E-07	0.46	0.33
10/11/2001	10	MC	16.1	0.23	1.46	1.5	2.96	0.49	5.7E-08	5.2E-07	5.3E-07	0.07	0.62
12/6/2001	1	OB	12.3	0.84	0.59	-	-	-	1.8E-07	1.2E-07	-	0.22	0.14
12/6/2001	2	OB	12.7	0.76	0.89	-	-	-	1.2E-07	1.5E-07	-	0.14	0.18
12/6/2001	3	LC	12	0.72	1.14	-	-	-	1.2E-07	2.0E-07	-	0.14	0.24
12/6/2001	4	LC	12.2	0.91	1.02	-	-	-	1.4E-07	1.5E-07	-	0.17	0.18
12/6/2001	5	LM	11.6	3.05	1.76	-	-	-	4.1E-07	4.0E-07	-	0.50	0.48
12/6/2001	6	LM	11.9	2.47	1.27	-	-	-	3.2E-07	3.0E-07	-	0.39	0.36

Date	Site	System	Temp (ºC)	BP ($\mu gC\ L^{-1}h^{-1}$)	filtered BP ($\mu gC\ L^{-1}h^{-1}$)	BR ($\mu gC\ L^{-1}h^{-1}$)	BCC ($\mu gC\ L^{-1}h^{-1}$)	BGE	BP cell^{-1}	filtered BP cell^{-1}	BR cell^{-1}	μ (day^{-1})	filt μ (day^{-1})
12/6/2001	7	LM	12.3	1.3	1.4	-	-	-	1.8E-07	2.5E-07	-	0.21	0.29
12/6/2001	8	MC	12.2	2.22	1.58	-	-	-	2.9E-07	2.4E-07	-	0.35	0.29
12/6/2001	9	MC	12.5	1.5	0.93	-	-	-	1.9E-07	1.6E-07	-	0.23	0.19
12/6/2001	10	MC	12.6	1.63	1.06	-	-	-	1.8E-07	1.7E-07	-	0.22	0.21

Table A-3. Cellular-level characteristics of bacterioplankton recorded during sampling in Monie Bay.

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
4/6/00	1	1.8E+07	8.6E+06	47	1.1E+06	5.9	4334	1.2E+07	4.1E+06	33	2.2E+05	1.8	629
4/6/00	2	2.6E+07	1.3E+07	48	1.3E+06	4.9	5570	1.4E+07	5.0E+06	37	2.9E+05	2.1	897
4/6/00	3	3.1E+07	1.5E+07	48	1.1E+06	3.6	5198	2.4E+07	1.0E+07	42	5.6E+05	2.3	2603
4/6/00	4	2.9E+07	1.3E+07	45	9.4E+05	3.3	4456	1.9E+07	7.5E+06	39	3.8E+05	1.9	1837
4/6/00	5	2.9E+07	1.8E+07	62	1.7E+06	5.9	9336	1.5E+07	8.7E+06	58	9.3E+05	6.3	5519
4/6/00	6	3.4E+07	1.9E+07	57	1.6E+06	4.7	8225	1.3E+07	7.0E+06	53	6.8E+05	5.2	3637
4/6/00	7	3.4E+07	1.7E+07	50	1.2E+06	3.4	5786	1.8E+07	8.1E+06	45	4.4E+05	2.5	2226
4/6/00	8	2.4E+07	1.6E+07	64	2.6E+06	10.7	10821	1.5E+07	9.0E+06	60	1.1E+06	7.5	4995
4/6/00	9	2.6E+07	1.6E+07	62	1.8E+06	6.8	7854	1.6E+07	8.6E+06	55	1.4E+06	9	6271
4/6/00	10	2.6E+07	1.5E+07	56	2.1E+06	7.9	9133	1.4E+07	6.4E+06	47	1.1E+06	8.4	4871
5/8/00	1	2.0E+07	3.5E+06	17	4.4E+05	2.2	1928	2.7E+07	3.2E+06	12	1.2E+05	0.5	256
5/8/00	2	1.8E+07	4.4E+06	24	5.2E+05	2.8	2449	8.2E+07	7.4E+06	9	1.4E+05	0.2	323
5/8/00	3	3.1E+07	7.3E+06	24	5.1E+05	1.6	2957	1.4E+07	2.8E+06	20	2.4E+05	1.7	1235
5/8/00	4	3.5E+07	7.4E+06	21	5.5E+05	1.6	3112	1.3E+07	3.1E+06	24	1.8E+05	1.4	813
5/8/00	5	3.4E+07	1.2E+07	36	9.4E+05	2.8	5715	1.6E+07	6.3E+06	39	5.0E+05	3.1	3356
5/8/00	6	3.5E+07	1.1E+07	33	6.7E+05	1.9	4278	1.9E+07	6.3E+06	33	3.0E+05	1.6	1404
5/8/00	7	2.7E+07	7.5E+06	28	5.2E+05	2	3446	1.8E+07	4.6E+06	26	2.4E+05	1.4	1207
5/8/00	8	2.3E+07	1.1E+07	47	1.1E+06	4.6	7495	1.4E+07	5.8E+06	43	4.7E+05	3.5	3385
5/8/00	9	2.9E+07	1.4E+07	49	4.6E+06	15.9	28104	1.8E+07	7.4E+06	42	4.2E+05	2.4	2944
5/8/00	10	2.7E+07	7.1E+06	26	6.3E+05	2.3	3934	1.6E+07	4.6E+06	29	1.9E+05	1.2	1055
6/7/00	1	1.2E+07	3.1E+06	25	1.4E+06	11.2	3886	6.2E+06	1.7E+06	27	3.4E+05	5.5	522
6/7/00	2	1.1E+07	2.7E+06	25	1.5E+06	14.2	4417	9.6E+06	2.7E+06	28	4.1E+05	4.2	615
6/7/00	3	1.5E+07	6.0E+06	40	1.2E+06	7.8	4396	7.8E+06	3.6E+06	46	6.6E+05	8.4	2183
6/7/00	4	1.4E+07	4.7E+06	33	1.4E+06	9.8	4634	8.4E+06	3.1E+06	37	5.7E+05	6.8	1680
6/7/00	5	1.2E+07	5.8E+06	48	1.5E+06	12.2	5676	7.4E+06	4.0E+06	54	7.7E+05	10.4	3050
6/7/00	6	1.2E+07	5.7E+06	46	1.4E+06	10.9	5307	7.6E+06	3.4E+06	45	7.3E+05	9.6	2792
6/7/00	7	1.3E+07	4.4E+06	33	1.5E+06	11.2	5706	8.5E+06	3.5E+06	41	6.6E+05	7.7	2161
6/7/00	8	1.3E+07	5.4E+06	42	1.5E+06	11.2	4997	7.8E+06	3.3E+06	42	5.8E+05	7.4	1916

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
6/7/00	9	1.3E+07	6.7E+06	51	1.8E+06	13.4	6139	1.0E+07	4.9E+06	49	8.1E+05	8	2466
6/7/00	10	1.5E+07	7.3E+06	50	1.9E+06	13.3	6210	8.8E+06	4.2E+06	48	8.8E+05	10.1	2815
7/3/00	1	8.4E+06	5.1E+06	61	1.7E+06	20.5	2138	5.3E+06	3.2E+06	60	5.8E+05	10.9	354
7/3/00	2	1.5E+07	8.4E+06	56	2.4E+06	16.4	3042	1.1E+07	5.7E+06	54	5.4E+05	5.2	337
7/3/00	3	1.0E+07	5.9E+06	58	1.9E+06	18.5	3038	9.1E+06	5.5E+06	60	6.9E+05	7.6	428
7/3/00	4	1.2E+07	6.7E+06	57	2.3E+06	19.1	3091	9.5E+06	5.3E+06	56	6.3E+05	6.7	393
7/3/00	5	1.1E+07	7.8E+06	69	2.6E+06	23.1	4197	7.0E+06	5.0E+06	72	9.7E+05	14	605
7/3/00	6	1.0E+07	6.6E+06	65	2.6E+06	25.9	4287	7.4E+06	4.8E+06	65	8.4E+05	11.4	522
7/3/00	7	1.3E+07	7.2E+06	56	2.5E+06	19.1	4013	9.4E+06	5.6E+06	60	6.2E+05	6.6	383
7/3/00	8	5.7E+06	4.0E+06	71	2.4E+06	41.9	3253	5.6E+06	3.7E+06	67	1.7E+06	30.8	646
7/3/00	9	7.7E+06	5.4E+06	70	1.8E+06	23.7	2731	6.3E+06	4.2E+06	68	1.1E+06	17.6	548
7/3/00	10	1.1E+07	6.3E+06	59	2.3E+06	21.2	3447	8.3E+06	4.9E+06	59	6.2E+05	7.5	308
8/3/00	1	8.2E+06	4.8E+06	58	1.6E+06	19	1736	5.1E+06	2.6E+06	51	3.0E+05	5.9	150
8/3/00	2	7.6E+06	4.3E+06	57	1.4E+06	17.7	1167	5.2E+06	2.6E+06	51	3.7E+05	7.1	181
8/3/00	3	-	-	-	-	-	-	-	-	-	-	-	-
8/3/00	4	-	-	-	-	-	-	-	-	-	-	-	-
8/3/00	5	-	-	-	-	-	-	-	-	-	-	-	-
8/3/00	6	-	-	-	-	-	-	-	-	-	-	-	-
8/3/00	7	-	-	-	-	-	-	-	-	-	-	-	-
8/3/00	8	9.3E+06	6.3E+06	67	1.6E+06	17.5	1625	6.1E+06	3.8E+06	61	1.0E+06	16.8	383
8/3/00	9	7.9E+06	5.3E+06	68	1.5E+06	19.3	1321	7.2E+06	4.4E+06	60	9.9E+05	13.7	366
8/3/00	10	8.8E+06	5.3E+06	60	1.2E+06	14	1068	6.5E+06	3.6E+06	56	7.9E+05	12.2	294
9/5/00	1	2.1E+07	7.7E+06	38	2.1E+06	10.3	2433	5.9E+06	1.7E+06	29	3.5E+05	5.9	178
9/5/00	2	1.9E+07	6.9E+06	37	2.0E+06	10.8	2321	1.2E+07	3.7E+06	30	3.8E+05	3	193
9/5/00	3	1.3E+07	4.8E+06	36	1.4E+06	10.1	1725	1.2E+07	4.0E+06	34	5.7E+05	4.8	217
9/5/00	4	1.5E+07	5.7E+06	39	1.6E+06	10.5	1391	1.3E+07	4.3E+06	35	6.0E+05	4.8	308
9/5/00	5	1.4E+07	5.1E+06	37	1.1E+06	8.2	1016	1.0E+07	3.7E+06	37	6.4E+05	6.3	329
9/5/00	6	1.3E+07	4.2E+06	34	1.2E+06	9.1	1186	1.0E+07	3.7E+06	36	6.4E+05	6.2	244
9/5/00	7	1.3E+07	4.7E+06	37	1.4E+06	10.6	1559	9.8E+06	3.4E+06	34	5.7E+05	5.8	293

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
9/5/00	8	1.9E+07	1.0E+07	53	2.1E+06	11	1624	1.5E+07	6.8E+06	47	7.4E+05	5.1	286
9/5/00	9	2.0E+07	9.5E+06	49	2.0E+06	10.3	1300	1.2E+07	5.2E+06	42	7.5E+05	6.1	290
9/5/00	10	1.4E+07	5.8E+06	41	1.4E+06	9.9	1081	1.4E+07	5.0E+06	36	6.7E+05	4.8	257
12/5/00	1	7.2E+06	-	-	9.5E+05	13.2	2080	5.6E+06	-	-	7.1E+05	12.6	1365
12/5/00	2	8.9E+06	-	-	1.1E+06	12.4	2552	5.8E+06	-	-	6.9E+05	11.9	1245
12/5/00	3	1.1E+07	-	-	1.3E+06	11.5	3432	7.8E+06	-	-	1.0E+06	13.1	2642
12/5/00	4	1.1E+07	-	-	1.8E+06	16.5	3737	5.4E+06	-	-	1.8E+06	33.5	6145
12/5/00	5	1.2E+07	-	-	1.6E+06	13.7	3928	8.1E+06	-	-	9.3E+05	11.5	2418
12/5/00	6	1.1E+07	-	-	1.7E+06	15.7	3842	7.9E+06	-	-	8.9E+05	11.3	1950
12/5/00	7	1.0E+07	-	-	1.5E+06	15.4	3356	8.3E+06	-	-	9.4E+05	11.4	1949
12/5/00	8	6.4E+06	-	-	1.5E+06	23.9	4129	3.7E+06	-	-	8.3E+05	22.7	2364
12/5/00	9	5.3E+06	-	-	1.4E+06	26	3712	4.3E+06	-	-	8.4E+05	19.3	2160
12/5/00	10	7.6E+06	-	-	1.4E+06	18.1	3350	5.3E+06	-	-	8.2E+05	15.5	1701
3/15/01	1	4.7E+06	3.8E+06	81	1.1E+06	23	5360	2.9E+06	2.4E+06	86	2.6E+05	9.1	996
3/15/01	2	4.8E+06	4.1E+06	85	1.2E+06	25.1	6173	2.8E+06	2.5E+06	88	2.9E+05	10.4	1103
3/15/01	3	7.6E+06	6.2E+06	82	1.7E+06	22	9665	5.0E+06	3.8E+06	75	6.1E+05	12.2	3184
3/15/01	4	7.9E+06	6.7E+06	85	1.4E+06	17.2	6842	3.7E+06	3.0E+06	82	4.8E+05	12.9	2397
3/15/01	5	9.0E+06	7.2E+06	80	2.0E+06	22	11166	6.5E+06	4.5E+06	69	8.3E+05	12.8	4276
3/15/01	6	8.4E+06	6.8E+06	81	1.6E+06	19.5	9171	4.6E+06	3.2E+06	71	6.6E+05	14.6	3406
3/15/01	7	8.0E+06	6.8E+06	85	1.6E+06	20.2	9100	4.0E+06	3.2E+06	80	5.3E+05	13.3	2627
3/15/01	8	1.0E+07	7.8E+06	78	2.3E+06	22.9	13676	6.8E+06	4.7E+06	69	8.4E+05	12.3	4460
3/15/01	9	1.1E+07	9.0E+06	80	1.9E+06	16.9	12109	4.2E+06	2.9E+06	69	6.1E+05	14.5	2892
3/15/01	10	8.9E+06	7.5E+06	84	1.6E+06	18	9664	5.7E+06	4.5E+06	79	6.1E+05	10.7	3207
4/12/01	1	5.4E+06	3.0E+06	55	2.2E+05	4.1	1489	5.1E+06	2.5E+06	50	1.7E+05	3.3	498
4/12/01	2	7.1E+06	3.4E+06	48	2.7E+05	3.7	4263	5.1E+06	2.5E+06	49	1.7E+05	3.3	507
4/12/01	3	9.8E+06	5.3E+06	54	3.8E+05	3.9	10094	7.4E+06	3.7E+06	50	3.1E+05	4.2	1487
4/12/01	4	9.7E+06	5.3E+06	55	5.3E+05	5.5	26370	8.0E+06	4.2E+06	53	3.2E+05	4	1586
4/12/01	5	8.4E+06	4.9E+06	58	1.3E+06	15.4	48260	5.3E+06	2.2E+06	42	8.8E+05	16.8	5928
4/12/01	6	1.1E+07	6.7E+06	61	1.1E+06	10	55188	7.6E+06	4.0E+06	53	6.3E+05	8.3	3957

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
4/12/01	7	1.1E+07	6.1E+06	58	6.3E+05	6	30445	7.2E+06	3.7E+06	51	3.2E+05	4.5	1838
4/12/01	8	9.0E+06	5.6E+06	62	8.7E+05	9.7	16327	5.2E+06	2.8E+06	53	9.2E+05	17.6	6068
4/12/01	9	6.6E+06	3.9E+06	59	1.1E+06	16	6461	5.0E+06	2.4E+06	49	5.8E+05	11.5	3800
4/12/01	10	8.4E+06	4.8E+06	58	9.8E+05	11.7	6275	5.7E+06	3.0E+06	51	3.1E+05	5.4	1989
4/12/01	11	8.0E+06	5.0E+06	63	1.8E+06	22.5	8232	5.5E+06	2.7E+06	50	7.7E+05	13.9	4438
4/12/01	12	2.7E+06	1.1E+06	41	5.6E+05	20.4	1981	2.4E+06	6.5E+05	27	1.7E+05	7.1	487
5/30/01	1	1.1E+07	5.6E+06	50	1.9E+06	17	6675	5.7E+06	2.7E+06	47	2.6E+05	4.5	695
5/30/01	2	1.1E+07	7.4E+06	66	1.5E+06	13.1	5093	8.2E+06	4.5E+06	55	3.7E+05	4.5	1308
5/30/01	3	1.0E+07	7.0E+06	70	1.2E+06	12.2	5615	8.1E+06	4.5E+06	56	5.5E+05	6.8	2473
5/30/01	4	1.1E+07	7.1E+06	67	1.2E+06	11.6	4984	7.5E+06	3.8E+06	51	4.2E+05	5.6	1910
5/30/01	5	9.8E+06	7.2E+06	74	2.0E+06	20.6	10519	6.9E+06	4.4E+06	64	9.3E+05	13.6	5826
5/30/01	6	1.2E+07	8.5E+06	71	1.6E+06	13.3	6974	8.0E+06	4.7E+06	59	8.7E+05	10.9	4529
5/30/01	7	9.6E+06	6.4E+06	67	1.3E+06	13.8	5756	8.6E+06	4.4E+06	51	4.9E+05	5.7	2199
5/30/01	8	1.4E+07	9.1E+06	63	2.4E+06	16.9	10847	9.0E+06	5.5E+06	61	1.3E+06	14.2	6932
5/30/01	9	1.2E+07	7.6E+06	63	2.0E+06	16.7	9046	1.1E+07	6.6E+06	59	1.2E+06	10.4	5779
5/30/01	10	1.3E+07	8.3E+06	66	1.7E+06	13.1	7136	1.2E+07	6.4E+06	54	7.4E+05	6.2	3510
5/30/01	12	-	-	-	-	-	-	-	-	-	-	-	-
6/12/01	1	1.4E+07	4.4E+06	32	3.3E+05	2.4	1627	7.6E+06	2.5E+06	33	1.0E+05	1.3	251
6/12/01	2	1.5E+07	6.7E+06	44	4.2E+05	2.8	1688	1.0E+07	4.2E+06	41	1.6E+05	1.6	480
6/12/01	3	1.0E+07	4.6E+06	45	4.1E+05	4	2397	7.4E+06	3.4E+06	46	2.4E+05	3.2	863
6/12/01	4	1.1E+07	4.1E+06	37	2.9E+05	2.6	1311	9.2E+06	3.5E+06	38	2.4E+05	2.6	987
6/12/01	5	1.1E+07	4.3E+06	38	1.3E+06	11.6	6908	1.2E+07	6.2E+06	54	7.5E+05	6.5	3477
6/12/01	6	1.1E+07	5.5E+06	49	6.4E+05	5.6	3498	1.0E+07	2.9E+06	29	3.5E+05	3.5	1637
6/12/01	7	1.2E+07	4.6E+06	39	5.8E+05	5	1476	8.3E+06	1.9E+06	23	2.5E+05	3	887
6/12/01	8	1.3E+07	7.2E+06	55	1.0E+06	7.6	3559	1.3E+07	3.3E+06	25	5.7E+05	4.4	3166
6/12/01	9	1.4E+07	7.4E+06	53	7.2E+05	5.1	2353	1.4E+07	3.0E+06	21	3.7E+05	2.6	1679
6/12/01	10	1.3E+07	5.7E+06	44	6.3E+05	4.8	1617	1.6E+07	2.3E+06	14	2.3E+05	1.4	841
6/16/01	1	1.0E+07	3.2E+06	32	-	-	-	6.1E+06	1.8E+06	29	-	-	-
6/16/01	2	1.2E+07	4.5E+06	39	-	-	-	8.2E+06	3.1E+06	37	-	-	-

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
6/16/01	5	1.3E+07	6.9E+06	51	-	-	-	7.2E+06	3.5E+06	48	-	-	-
6/16/01	5A	1.4E+07	7.5E+06	55	-	-	-	8.7E+06	4.5E+06	52	-	-	-
6/16/01	6	1.0E+07	4.5E+06	43	-	-	-	7.7E+06	3.3E+06	42	-	-	-
6/16/01	7	1.2E+07	4.6E+06	39	-	-	-	8.1E+06	3.0E+06	36	-	-	-
6/16/01	8	1.0E+07	3.4E+06	33	-	-	-	8.6E+06	2.5E+06	29	-	-	-
6/16/01	9	1.1E+07	3.9E+06	35	-	-	-	8.1E+06	2.8E+06	35	-	-	-
6/16/01	9A	1.0E+07	3.6E+06	35	-	-	-	1.0E+07	3.3E+06	33	-	-	-
6/16/01	10	1.1E+07	4.2E+06	39	-	-	-	9.7E+06	3.5E+06	36	-	-	-
6/16/01	11	1.2E+07	4.2E+06	34	-	-	-	9.8E+06	3.0E+06	30	-	-	-
6/16/01	11A	1.2E+07	4.0E+06	35	-	-	-	8.6E+06	2.7E+06	31	-	-	-
6/16/01	12	9.1E+06	2.9E+06	32	-	-	-	9.0E+06	2.6E+06	29	-	-	-
6/25/01	1	1.2E+07	6.0E+06	50	1.2E+06	10.3	3277	6.2E+06	2.6E+06	41	2.6E+05	4.1	662
6/25/01	2	1.2E+07	6.8E+06	57	1.5E+06	12.3	3691	-	-	-	-	-	-
6/25/01	3	1.0E+07	5.4E+06	54	1.2E+06	11.8	4078	7.9E+06	3.7E+06	47	4.3E+05	5.5	1443
6/25/01	4	9.4E+06	4.7E+06	51	1.2E+06	12.9	3625	-	-	-	-	-	-
6/25/01	5	8.8E+06	5.1E+06	58	1.2E+06	13.3	4782	6.0E+06	3.1E+06	52	5.1E+05	8.5	2374
6/25/01	6	9.5E+06	5.1E+06	54	1.3E+06	13.1	4703	-	-	-	-	-	-
6/25/01	7	1.1E+07	5.7E+06	53	1.2E+06	10.9	3884	-	-	-	-	-	-
6/25/01	8	1.2E+07	7.3E+06	63	1.6E+06	14	6110	9.2E+06	5.3E+06	58	6.5E+05	7.1	3252
6/25/01	9	1.2E+07	7.8E+06	64	1.5E+06	11.9	5684	9.1E+06	4.8E+06	53	5.8E+05	6.4	2799
6/25/01	10	1.1E+07	6.3E+06	56	1.6E+06	13.8	4491	-	-	-	-	-	-
7/12/01	1	2.8E+07	1.6E+07	57	3.0E+06	10.6	9330	1.2E+07	5.9E+06	50	7.4E+05	6.2	1910
7/12/01	2	1.4E+07	8.1E+06	59	1.7E+06	12.4	6118	9.9E+06	5.3E+06	53	4.7E+05	4.8	1482
7/12/01	3	-	-	-	-	-	-	-	-	-	-	-	-
7/12/01	4	-	-	-	-	-	-	-	-	-	-	-	-
7/12/01	5	1.7E+07	1.0E+07	60	2.6E+06	15	11072	1.1E+07	6.7E+06	59	1.3E+06	11.8	5414
7/12/01	5A	1.7E+07	1.1E+07	61	2.2E+06	12.6	8496	1.1E+07	6.9E+06	60	1.3E+06	11.5	4911
7/12/01	6	1.6E+07	9.3E+06	57	2.0E+06	12	8144	1.1E+07	5.8E+06	54	1.1E+06	10.2	4363
7/12/01	7	1.7E+07	9.9E+06	57	1.9E+06	10.7	7694	1.4E+07	7.5E+06	52	8.6E+05	6	3093

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
7/12/01	8	1.6E+07	1.1E+07	67	2.7E+06	16.8	9469	1.3E+07	8.1E+06	64	1.1E+06	8.5	4061
7/12/01	9	1.8E+07	1.2E+07	66	2.5E+06	13.9	8595	1.2E+07	7.1E+06	59	1.3E+06	10.5	5477
7/12/01	10	1.4E+07	8.6E+06	61	2.3E+06	16.3	8855	8.7E+06	4.9E+06	56	1.1E+06	12.7	4263
7/12/01	11	1.4E+07	9.6E+06	67	2.5E+06	17	7776	1.0E+07	6.7E+06	67	1.1E+06	10.6	4603
7/12/01	11A	1.5E+07	1.1E+07	72	2.6E+06	17.4	8584	1.0E+07	7.1E+06	70	1.2E+06	11.6	5061
7/12/01	12	1.4E+07	1.0E+07	73	1.9E+06	13.4	7202	8.9E+06	6.3E+06	71	1.2E+06	13.5	5691
7/25/01	1	1.2E+07	6.2E+06	50	1.4E+06	11.7	3859	7.5E+06	2.9E+06	39	4.5E+05	6	930
7/25/01	2	1.2E+07	5.5E+06	45	1.3E+06	10.8	4173	-	-	-	-	-	-
7/25/01	3	1.1E+07	4.2E+06	40	8.0E+05	7.6	2655	8.9E+06	3.2E+06	35	5.0E+05	5.6	1585
7/25/01	4	1.1E+07	4.9E+06	44	1.2E+06	10.6	3737	-	-	-	-	-	-
7/25/01	5	9.3E+06	3.8E+06	41	9.2E+05	10	3215	8.0E+06	3.1E+06	39	5.2E+05	6.5	1720
7/25/01	6	9.8E+06	3.9E+06	40	7.6E+05	7.8	2658	-	-	-	-	-	-
7/25/01	7	1.2E+07	4.9E+06	41	9.1E+05	7.6	3003	9.4E+06	3.5E+06	38	5.1E+05	5.4	1694
7/25/01	8	1.1E+07	4.1E+06	39	9.1E+05	8.6	2864	9.7E+06	3.8E+06	39	4.0E+05	4.2	1344
7/25/01	9	1.1E+07	4.3E+06	40	8.2E+05	7.5	2701	-	-	-	-	-	-
7/25/01	10	1.2E+07	4.7E+06	40	1.1E+06	9.2	3585	1.0E+07	3.8E+06	37	5.0E+05	4.8	1573
8/10/01	1	9.8E+06	5.2E+06	53	1.3E+06	13.1	2914	5.6E+06	2.6E+06	46	2.7E+05	4.8	617
8/10/01	2	1.1E+07	6.0E+06	55	1.4E+06	13.3	3491	-	-	-	-	-	-
8/10/01	3	9.8E+06	5.1E+06	52	1.3E+06	13.3	3570	5.8E+06	2.7E+06	46	4.1E+05	7.1	1252
8/10/01	4	9.8E+06	5.2E+06	52	1.4E+06	13.9	3302	-	-	-	-	-	-
8/10/01	5	1.5E+07	7.6E+06	52	2.0E+06	13.4	5943	8.9E+06	4.2E+06	47	6.3E+05	7	2294
8/10/01	6	1.2E+07	6.1E+06	51	1.5E+06	12.3	4427	-	-	-	-	-	-
8/10/01	7	1.0E+07	5.3E+06	52	1.4E+06	13.2	3441	8.0E+06	3.7E+06	46	5.3E+05	6.6	1610
8/10/01	8	1.3E+07	6.5E+06	49	1.5E+06	11.4	4142	8.4E+06	3.6E+06	42	5.7E+05	6.7	1902
8/10/01	9	1.2E+07	5.8E+06	50	1.6E+06	13.9	4114	-	-	-	-	-	-
8/10/01	10	1.2E+07	6.8E+06	57	1.6E+06	13.7	3924	6.5E+06	2.9E+06	44	4.6E+05	7.1	1313
8/22/01	1	9.0E+06	4.1E+06	45	2.1E+06	23.6	5606	8.7E+05	1.1E+05	12	5.4E+04	6.2	159
8/22/01	2	8.8E+06	3.9E+06	45	1.6E+06	18.4	4506	5.8E+06	2.0E+06	34	3.0E+05	5.2	1038
8/22/01	3	8.7E+06	3.5E+06	40	1.3E+06	15.1	5015	6.6E+06	2.5E+06	38	5.5E+05	8.2	2414

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
8/22/01	4	1.0E+07	4.4E+06	43	1.3E+06	12.5	3941	5.9E+06	1.1E+06	19	1.0E+05	1.7	211
8/22/01	5	1.3E+07	5.4E+06	43	2.7E+06	21.3	12350	9.2E+06	4.4E+06	48	1.3E+06	13.6	6753
8/22/01	6	1.0E+07	4.2E+06	42	1.7E+06	16.8	7226	6.3E+06	2.5E+06	39	5.3E+05	8.4	2416
8/22/01	7	1.0E+07	4.2E+06	41	1.0E+06	10.1	3738	6.0E+06	1.9E+06	32	3.2E+05	5.3	1112
8/22/01	8	7.5E+06	3.4E+06	46	1.0E+06	13.3	4129	5.8E+06	2.5E+06	44	3.3E+05	5.6	2237
8/22/01	9	8.1E+06	3.8E+06	46	1.0E+06	12.3	3768	5.9E+06	2.1E+06	35	4.2E+05	7.2	2146
8/22/01	10	9.7E+06	4.1E+06	42	9.5E+05	9.8	3005	5.8E+06	1.7E+06	29	3.6E+05	6.1	1393
9/11/01	1	7.2E+06	3.4E+06	47	1.9E+06	26.7	5998	4.7E+06	1.8E+06	38	4.2E+05	8.9	863
9/11/01	2	9.9E+06	4.5E+06	45	1.8E+06	18.6	5694	7.0E+06	3.0E+06	43	6.4E+05	9.1	1754
9/11/01	3	1.2E+07	5.7E+06	48	1.4E+06	11.5	6602	7.0E+06	3.4E+06	49	8.0E+05	11.5	3179
9/11/01	4	-	-	-	-	-	-	-	-	-	-	-	-
9/11/01	5	1.7E+07	1.1E+07	62	1.6E+06	9.1	6672	1.2E+07	6.6E+06	54	1.3E+06	11.1	6687
9/11/01	6	1.1E+07	5.8E+06	51	2.6E+06	22.9	12580	1.0E+07	5.0E+06	48	1.1E+06	10.3	4944
9/11/01	7	9.7E+06	5.2E+06	54	2.2E+06	22.7	9844	8.3E+06	3.6E+06	44	9.6E+05	11.5	4099
9/11/01	8	9.7E+06	4.8E+06	50	1.8E+06	18.7	8108	6.2E+06	3.0E+06	48	5.1E+05	8.2	2642
9/11/01	9	9.4E+06	4.7E+06	49	1.6E+06	16.6	6208	6.3E+06	2.9E+06	46	5.2E+05	8.2	2233
9/11/01	10	8.3E+06	3.7E+06	44	1.7E+06	20.9	6887	6.5E+06	2.9E+06	45	6.8E+05	10.4	2797
9/11/01	11	9.3E+06	3.3E+06	35	1.6E+06	17.3	6079	7.0E+06	3.4E+06	48	5.0E+05	7.2	2685
9/11/01	12	6.7E+06	3.8E+06	57	1.6E+06	23.6	6014	4.5E+06	2.4E+06	54	5.1E+05	11.2	2966
10/11/01	1	4.0E+06	1.0E+06	25	3.6E+05	9	1773	2.6E+06	4.5E+05	18	1.2E+05	4.8	735
10/11/01	2	3.8E+06	1.1E+06	28	4.6E+05	12.1	2621	2.9E+06	6.0E+05	21	2.1E+05	7.4	1521
10/11/01	3	3.3E+06	9.9E+05	30	4.0E+05	11.9	3045	2.8E+06	7.8E+05	28	2.7E+05	9.6	2263
10/11/01	4	3.9E+06	1.1E+06	29	4.5E+05	11.7	2897	2.8E+06	6.8E+05	24	2.6E+05	9.2	1917
10/11/01	5	3.0E+06	1.0E+06	34	4.1E+05	13.9	3306	2.2E+06	6.6E+05	30	2.4E+05	10.7	2214
10/11/01	6	3.4E+06	1.1E+06	32	4.4E+05	12.8	3256	2.9E+06	8.6E+05	29	2.9E+05	9.8	2420
10/11/01	7	3.8E+06	1.2E+06	31	4.5E+05	11.8	3041	3.2E+06	8.0E+05	25	2.8E+05	8.9	2199
10/11/01	8	6.0E+06	2.2E+06	37	9.8E+05	16.3	5661	3.7E+06	1.0E+06	27	3.0E+05	8.1	2321
10/11/01	9	3.8E+06	1.3E+06	34	6.4E+05	16.7	3892	3.2E+06	7.8E+05	24	2.3E+05	7.4	1739
10/11/01	10	4.0E+06	1.1E+06	28	4.2E+05	10.4	2591	2.8E+06	6.2E+05	22	2.3E+05	8.1	1756

		Whole (unfiltered) Water Sample						AP15 Filtered Fraction					
Date	Site	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cell ml^{-1}	%CTC+ cells	CTC*FL2	cells ml^{-1}	HDNA cells ml^{-1}	%HDNA cells	CTC+ cells ml^{-1}	%CTC+ cells	CTC*FL2
12/6/01	1	4.6E+06	1.8E+06	39	2.3E+05	5	632	5.1E+06	2.0E+06	39	1.6E+05	3.1	303
12/6/01	2	6.4E+06	2.8E+06	43	4.6E+05	7.2	1521	6.1E+06	2.6E+06	43	3.3E+05	5.3	629
12/6/01	3	6.2E+06	2.8E+06	45	4.6E+05	7.4	1857	5.8E+06	2.7E+06	46	3.3E+05	5.7	1141
12/6/01	4	6.6E+06	2.9E+06	44	4.1E+05	6.2	1533	6.7E+06	3.0E+06	45	3.6E+05	5.3	1086
12/6/01	5	7.4E+06	3.6E+06	49	6.9E+05	9.3	3238	4.4E+06	2.3E+06	51	3.3E+05	7.5	1415
12/6/01	6	7.7E+06	3.6E+06	47	6.1E+05	7.9	2780	4.3E+06	1.9E+06	44	2.6E+05	6.2	947
12/6/01	7	7.4E+06	3.5E+06	47	4.8E+05	6.5	1870	5.7E+06	2.5E+06	45	3.8E+05	6.7	1269
12/6/01	8	7.7E+06	3.8E+06	50	7.7E+05	10	3097	6.6E+06	3.2E+06	49	4.2E+05	6.4	1810
12/6/01	9	7.9E+06	3.7E+06	47	7.3E+05	9.3	3344	5.8E+06	2.7E+06	46	3.6E+05	6.2	1611
12/6/01	10	8.9E+06	4.1E+06	46	5.7E+05	6.4	2305	6.2E+06	2.9E+06	47	3.3E+05	5.4	874
1/23/02	1	1.5E+06	7.3E+05	48	2.3E+05	15.1	1491	9.0E+05	3.4E+05	38	8.7E+04	9.6	474
1/23/02	2	1.4E+06	8.1E+05	58	2.8E+05	19.9	1756	7.1E+05	3.2E+05	45	9.0E+04	12.7	473
1/23/02	3	1.7E+06	9.0E+05	54	1.7E+05	10.2	1170	8.9E+05	2.7E+05	30	1.2E+05	13.3	883
1/23/02	4	1.4E+06	7.6E+05	55	5.7E+05	41.2	5354	1.1E+06	5.4E+05	51	1.2E+05	11	776
1/23/02	5	3.7E+06	2.4E+06	63	6.1E+05	16.2	5731	2.5E+06	1.1E+06	42	2.9E+05	11.6	2896
1/23/02	6	2.8E+06	1.6E+06	58	3.7E+05	13	3361	1.2E+06	4.7E+05	38	1.5E+05	12.4	1168
1/23/02	7	1.8E+06	1.1E+06	58	3.1E+05	17.2	2480	9.1E+05	3.2E+05	35	1.1E+05	11.7	715
1/23/02	8	2.4E+06	1.3E+06	51	4.3E+05	17.7	2814	9.8E+05	3.3E+05	34	1.1E+05	11.4	662
1/23/02	9	2.2E+06	1.2E+06	53	4.1E+05	18.7	2729	9.4E+05	3.3E+05	35	1.1E+05	11.7	631
1/23/02	10	2.1E+06	1.1E+06	55	3.3E+05	15.9	2216	9.4E+05	3.7E+05	39	1.2E+05	12.6	755

Table A-4. Changes in nutrient concentrations ($\mu M\ h^{-1}$) during incubations designed to measure nutrient uptake and production by bacterioplankton.

Date	Site	System	ΔNH_4^+	ΔNO_X	ΔTIN	ΔPO_4^{3-}	ΔTDN	ΔTDP	ΔDON
4/6/00	1	OB	-0.2633	-0.1000	-0.3633	-0.0039	-0.4667	-0.0028	-0.1033
4/6/00	2	OB	0.0539	-0.0944	-0.0406	-0.0028	0.0167	-0.0022	0.0572
4/6/00	3	LC	-0.0694	-0.1067	-0.1761	-0.0056	-0.5000	-0.0089	-0.3239
4/6/00	4	LC	-0.0692	-0.1639	-0.2331	-0.0022	-0.5056	-0.0044	-0.2725
4/6/00	5	LM	0.0533	-0.0222	0.0311	-0.0033	-0.0556	-0.0056	-0.0867
4/6/00	6	LM	-0.0733	-0.0528	-0.1261	-0.0022	-0.8056	0.0289	-0.6794
4/6/00	7	LM	-0.0828	-0.4372	-0.5200	-0.0028	-1.4333	-0.0089	-0.9133
4/6/00	8	MC	-0.0578	-0.0222	-0.0800	0.0056	0.3222	0.0089	0.4022
4/6/00	9	MC	-0.0722	-0.1056	-0.1778	0.0050	-0.2722	0.0017	-0.0944
4/6/00	10	MC	-0.0272	-0.0222	-0.0494	0.0000	-0.0056	0.0000	0.0439
5/8/00	1	OB	-0.0517	-0.4650	-0.5167	-0.0083	-1.4444	-0.0072	-0.9278
5/8/00	2	OB	-0.0133	0.0789	0.0656	-0.0022	0.3222	0.0011	0.2567
5/8/00	3	LC	0.0956	0.1772	0.2728	-0.0006	1.2889	0.0083	1.0161
5/8/00	4	LC	-0.0250	-0.0550	-0.0800	-0.0008	-0.2944	-0.0011	-0.2144
5/8/00	5	LM	0.0000	0.0044	0.0044	0.0011	0.1056	0.0000	0.1011
5/8/00	6	LM	-0.0539	-0.0011	-0.0550	-0.0006	-0.2500	-0.0067	-0.1950
5/8/00	7	LM	0.0103	0.0797	0.0900	0.0289	0.5222	0.0011	0.4322
5/8/00	8	MC	-0.0272	0.0156	-0.0117	0.0022	0.1500	0.0056	0.1617
5/8/00	9	MC	-0.0428	-0.0500	-0.0928	-0.0039	-0.9167	-0.0128	-0.8239
5/8/00	10	MC	0.0000	0.1256	0.1256	-0.0011	0.8833	0.0022	0.7578
6/7/00	1	OB	-0.0472	-0.0222	-0.0694	0.0078	-0.2889	-0.0011	-0.2194
6/7/00	2	OB	-0.0422	-0.0011	-0.0433	-0.0017	-0.0889	-0.0011	-0.0456

Date	Site	System	ΔNH_4^+	ΔNO_X	ΔTIN	ΔPO_4^{3-}	ΔTDN	ΔTDP	ΔDON
6/7/00	3	LC	0.0328	0.0761	0.1089	0.0000	1.2522	0.0072	1.1433
6/7/00	4	LC	-0.0172	-0.0228	-0.0400	0.0028	0.4611	0.0000	0.5011
6/7/00	5	LM	0.0133	-0.0072	0.0061	-0.0039	0.0722	0.0011	0.0661
6/7/00	6	LM	-0.1383	-0.1106	-0.2489	-0.0089	-0.9333	-0.0094	-0.6844
6/7/00	7	LM	0.0117	-0.0017	0.0100	0.0011	-0.0333	-0.0028	-0.0433
6/7/00	8	MC	0.0017	-0.0117	-0.0100	-0.0006	0.0389	-0.0050	0.0489
6/7/00	9	MC	-0.0156	-0.0061	-0.0217	0.0000	0.1611	0.0044	0.1828
6/7/00	10	MC	-0.0011	0.0028	0.0017	-0.0161	-0.0111	-0.0072	-0.0128
7/3/00	1	OB	-0.0200	-0.0011	-0.0211	-0.0011	0.5889	0.0072	0.6100
7/3/00	2	OB	-0.0033	-0.0028	-0.0061	-0.0011	0.0556	-0.0161	0.0617
7/3/00	3	LC	-0.0022	-0.0061	-0.0083	-0.0022	0.5111	0.0017	0.5194
7/3/00	4	LC	0.0017	-0.0106	-0.0089	0.0000	-0.0889	-0.0028	-0.0800
7/3/00	5	LM	0.0078	-0.0022	0.0056	-0.0017	-0.1667	-0.0033	-0.1722
7/3/00	6	LM	0.0083	0.0033	0.0117	-0.0006	-0.1389	0.0100	-0.1506
7/3/00	7	LM	0.0167	0.0150	0.0317	0.0278	-0.2611	0.0828	-0.2928
7/3/00	8	MC	-0.0217	-0.0167	-0.0383	-0.0083	-0.3222	-0.0044	-0.2839
7/3/00	9	MC	-0.0611	-0.0167	-0.0778	-0.0056	-0.2444	-0.0183	-0.1667
7/3/00	10	MC	-0.0106	-0.0028	-0.0133	-0.0006	-0.1333	-0.0100	-0.1200
8/3/00	1	OB	-0.1172	-0.0067	-0.1239	-0.0022	-0.0389	-0.0233	0.0850
8/3/00	2	OB	-0.0528	0.0011	-0.0517	0.0022	0.1056	0.0056	0.1572
8/3/00	3	LC	-	-	-	-	-	-	-
8/3/00	4	LC	-	-	-	-	-	-	-
8/3/00	5	LM	-	-	-	-	-	-	-
8/3/00	6	LM	-	-	-	-	-	-	-

Date	Site	System	ΔNH_4^+	ΔNO_X	ΔTIN	ΔPO_4^{3-}	ΔTDN	ΔTDP	ΔDON
8/3/00	7	LM	-	-	-	-	-	-	-
8/3/00	8	MC	-0.0067	-0.0028	-0.0094	-0.0044	-1.0278	-0.0428	-1.0183
8/3/00	9	MC	0.0028	-0.0022	0.0006	0.0061	1.1167	0.0228	1.1161
8/3/00	10	MC	0.0311	-0.0050	0.0261	0.0022	-0.1056	-0.0056	-0.1317
9/5/00	1	OB	-0.0189	-0.0183	-0.0372	-0.0033	-0.1611	-0.0056	-0.1239
9/5/00	2	OB	-0.0183	-0.0300	-0.0483	-0.0028	-0.2889	-0.0078	-0.2406
9/5/00	3	LC	0.0650	-0.0078	0.0572	-0.0011	-0.0111	-0.0050	-0.0683
9/5/00	4	LC	0.0022	-0.0050	-0.0028	-0.0022	-0.1056	0.0039	-0.1028
9/5/00	5	LM	-0.0172	-0.0267	-0.0439	0.0033	0.9889	0.0194	1.0328
9/5/00	6	LM	-0.0244	-0.0167	-0.0411	-0.0022	1.0778	0.0050	1.1189
9/5/00	7	LM	0.0122	-0.0361	-0.0239	-0.0272	-1.0111	-0.0267	-0.9872
9/5/00	8	MC	-0.0078	-0.0278	-0.0356	-0.0089	-1.0611	0.0361	-1.0256
9/5/00	9	MC	-0.0078	-0.0256	-0.0333	0.0150	-0.3722	0.0033	-0.3389
9/5/00	10	MC	0.0044	-0.0006	0.0039	0.0067	0.7889	0.0039	0.7850
3/15/01	1	OB	-0.7900	-2.8100	-3.6000	0.0000	-7.6000	0.0000	-4.0000
3/15/01	2	OB	-0.6000	-4.9300	-5.5300	-0.0200	-13.4000	0.0000	-7.8700
3/15/01	3	LC	-0.3600	0.3000	-0.0600	0.0100	-0.1000	0.0100	-0.0400
3/15/01	4	LC	-0.5900	-1.4750	-2.0650	-0.0300	-9.7500	0.0000	-7.6850
3/15/01	5	LM	-0.5400	0.2000	-0.3400	-0.0200	-1.4000	-0.0900	-1.0600
3/15/01	6	LM	-0.8300	-0.0800	-0.9100	-0.0300	-1.0000	-0.1000	-0.0900
3/15/01	7	LM	-0.7950	0.9450	0.1500	-0.0500	1.9000	0.0900	1.7500
3/15/01	8	MC	-0.1800	1.3500	1.1700	-0.0300	5.5000	-0.1900	4.3300
3/15/01	9	MC	-0.1900	0.0600	-0.1300	-0.0400	-4.3000	-0.1700	-4.1700
3/15/01	10	MC	0.5900	0.2100	0.8000	-0.0600	-1.6000	-1.3600	-2.4000

Date	Site	System	ΔNH_4^+	ΔNO_X	ΔTIN	ΔPO_4^{3-}	ΔTDN	ΔTDP	ΔDON
4/12/01	1	OB	0.0700	-5.5000	-5.4300	0.0850	-19.4000	-0.9000	-13.9700
4/12/01	2	OB	0.0600	-0.6000	-0.5400	0.0000	-0.6000	1.1700	-0.0600
4/12/01	3	LC	1.8800	3.2900	5.1700	0.0100	21.8000	0.0800	16.6300
4/12/01	4	LC	-0.0700	-0.3300	-0.4000	-0.0400	-1.8000	-0.2600	-1.4000
4/12/01	5	LM	-0.2000	-0.5000	-0.7000	-0.0100	-1.0000	-0.2100	-0.3000
4/12/01	6	LM	0.3300	-3.1000	-2.7700	-0.0300	-0.8000	-0.2500	1.9700
4/12/01	7	LM	6.9700	4.0400	11.0100	-0.0600	-6.1000	-0.2800	-17.1100
4/12/01	8	MC	0.2000	0.2000	0.4000	-0.0300	0.7000	-0.2300	0.3000
4/12/01	9	MC	0.4000	-0.9000	-0.5000	-0.1200	-9.2000	-0.4700	-8.7000
4/12/01	10	MC	0.0800	-0.0600	0.0200	-0.0700	-1.7000	-0.2000	-1.7200
4/12/01	11	MC	0.2000	-0.2000	0.0000	-0.0400	0.4000	0.0900	0.4000
4/12/01	12	MC	0.0300	-0.1000	-0.0700	-0.0500	0.1000	0.0000	0.1700
5/30/01	1	OB	-1.4900	0.0000	-1.4900	0.0300	1.4000	0.1300	2.8900
5/30/01	2	OB	-0.0900	-0.2000	-0.2900	0.0200	3.2000	0.2800	3.4900
5/30/01	3	LC	-0.3100	0.2000	-0.1100	0.0000	3.5000	0.3300	3.6100
5/30/01	4	LC	-0.1000	-0.6100	-0.7100	0.0000	-0.7000	0.0800	0.0100
5/30/01	5	LM	-0.4900	-0.2000	-0.6900	-0.0200	1.9000	-0.0400	2.5900
5/30/01	6	LM	-1.2000	-2.1500	-3.3500	-0.0400	-8.1000	-0.1800	-4.7500
5/30/01	7	LM	0.9100	3.6300	4.5400	0.0200	29.9000	0.1700	25.3600
5/30/01	8	MC	-0.2300	0.1000	-0.1300	-0.0400	8.9000	0.2300	9.0300
5/30/01	9	MC	-0.3000	-0.3000	-0.6000	-0.0400	1.4000	-0.1100	2.0000
5/30/01	10	MC	-0.3900	-0.3900	-0.7800	-0.1050	-1.1500	-0.3250	-0.3700
5/30/01	12	MC	-	-	-	-	-	-	-
6/12/01	1	OB	-0.3100	-0.4000	-0.7100	-0.0250	-4.2000	-0.1000	-3.4900

Date	Site	System	ΔNH_4^+	ΔNO_X	ΔTIN	ΔPO_4^{3-}	ΔTDN	ΔTDP	ΔDON
6/12/01	2	OB	-0.6500	-6.8800	-7.5300	-0.0300	-26.8000	-0.7600	-19.2700
6/12/01	3	LC	0.3500	-1.1100	-0.7600	0.0000	-5.8000	-0.2400	-5.0400
6/12/01	4	LC	-0.7000	-3.5800	-4.2800	-0.0700	-14.2000	-0.5900	-9.9200
6/12/01	5	LM	0.8200	-0.9600	-0.1400	-0.0800	-23.2000	-0.5000	-23.0600
6/12/01	6	LM	0.0900	-0.6100	-0.5200	0.0000	-2.6000	-0.5400	-2.0800
6/12/01	7	LM	-0.0500	-4.8300	-4.8800	-0.0200	-24.2000	-0.6500	-19.3200
6/12/01	8	MC	0.5400	-1.6500	-1.1100	-0.1500	-18.9000	-0.3100	-17.7900
6/12/01	9	MC	0.6600	-1.6200	-0.9600	-0.1300	-24.3000	-1.2900	-23.3400
6/12/01	10	MC	-0.1600	-0.2400	-0.4000	0.0000	0.9000	0.1800	1.3000
7/12/01	1	OB	0.5800	0.4200	1.0000	0.0800	-0.5000	-0.0900	-1.5000
7/12/01	2	OB	0.9800	0.4800	1.4600	0.0900	-2.8000	0.0600	-4.2600
7/12/01	3	LC	-0.8300	-0.1200	-0.9500	0.0000	-33.9000	-0.0700	-32.9500
7/12/01	4	LC	-0.9900	-0.0400	-1.0300	0.0000	-29.6000	-0.2800	-28.5700
7/12/01	5	LM	0.3900	0.4900	0.8800	0.0300	-5.9000	0.0300	-6.7800
7/12/01	5A	LM	0.5100	0.2900	0.8000	-0.5100	-26.4000	-0.8000	-27.2000
7/12/01	6	LM	-0.0800	0.3800	0.3000	0.1400	-12.3000	0.1900	-12.6000
7/12/01	7	LM	-0.1000	0.3100	0.2100	0.1200	-23.4300	0.1099	-23.6400
7/12/01	8	MC	0.2000	0.5000	0.7000	0.0500	-3.0000	0.3400	-3.7000
7/12/01	9	MC	0.2900	0.1800	0.4700	0.2200	-17.3000	0.2300	-17.7700
7/12/01	10	MC	0.2700	0.4300	0.7000	0.0700	-2.3000	-0.0900	-3.0000
7/12/01	11	MC	-0.0200	0.3900	0.3700	0.0000	3.9000	0.3200	3.5300
7/12/01	11A	MC	-0.3600	-0.0700	-0.4300	0.0000	-11.0000	0.4200	-10.5700
7/12/01	12	MC	0.1400	0.2500	0.3900	0.1900	-16.5000	0.3100	-16.8900
8/22/01	1	OB	-	-	-	-	-	-	-

Date	Site	System	ΔNH_4^+	ΔNO_X	ΔTIN	ΔPO_4^{3-}	ΔTDN	ΔTDP	ΔDON
8/22/01	2	OB	-	-	-	-	-	-	-
8/22/01	3	LC	0.7000	0.0800	0.7800	0.0600	5.4000	0.0600	4.6200
8/22/01	4	LC	-	-	-	-	-	-	-
8/22/01	5	LM	-1.5700	-0.7700	-2.3400	-0.3100	-15.9000	-0.7900	-13.5600
8/22/01	6	LM	-	-	-	-	-	-	-
8/22/01	7	LM	-	-	-	-	-	-	-
8/22/01	8	MC	3.2600	-0.2800	2.9800	0.2400	12.4000	0.2800	9.4200
8/22/01	9	MC	-	-	-	-	-	-	-
8/22/01	10	MC	-	-	-	-	-	-	-

APPENDIX B: Detailed Methods

Estimates of Bacterioplankton Carbon Metabolism and Abundance

Sample Collection and Filtration

Approximately 20 L of sub-surface water were collected 1-2 h following high tide by immersing 22 L Nalgene HDPE carboys ~0.25 m beneath the surface. Water was transported back to the laboratory for immediate filtration. The water collected on the ebb tide will have been subject to marsh processes for several hours and hence most indicative of changes associated with each tidal creek. Prior to filtration, a small sub-sample (~500 ml) was removed from each carboy to determine total bacterial community production and abundance (see below). Approximately 10 L of water from each site was gently filtered using a peristaltic pump and silicone tubing through 142 mm diameter AP15 Millipore filters held in a Millipore filter holder. This filtration process is necessary to remove protozooplankton grazers and their potential contribution to respiratory processes and we have found that these filters were effective in reducing the number of >3μm particles by over 90%, while allowing over 80% of the free-living bacteria to pass into the filtrate.

Filtered water was placed into a flow-through incubation assembly composed of two 4 L glass Erlenmeyer flasks connected by 0.25 inch inner-diameter Tygon tubing (Fig. B-1). A siphon was established from the top reservoir flask to permit replenishment of water as samples are drawn from the lower flask. Flasks were incubated in the dark at *in situ* field temperatures and sub-sample at 0, 3, and 6 h during summer months and 0, 4, and 8 h during colder months (i.e., when ambient water temperatures fell below 15ºC), maximizing measurable changes in oxygen content and minimizing the duration of

incubations. The total volume of water removed at any given sampling time-point was small, avoiding unnecessary dilution of the incubation flask by replacement water. We routinely used ~ 25 ml of water for estimating bacterial production and abundance and ~40 ml for oxygen analysis.

Bacterial Respiration

Sub-samples taken from incubations assemblies for determining oxygen consumption were collected in triplicate for the initial time-point and in duplicate for each subsequent time-point. Oxygen consumption was determined using membrane inlet mass spectrometry (MIMS; Kana et al. 1994). This method, based on changes in the atomic ratio of argon and oxygen in the dissolved phase, offers an extremely precise and rapid assessment of oxygen concentrations, with a reported measurement precision (CV) of 0.030% (Kana et al. 1994). We encountered slightly lower methodological precision (CV) of 0.13% for duplicate incubations and 0.08% for MIMS analysis itself.

Sub-samples were collected from each incubation assembly by siphoning water from the lower incubation flask into a 7 ml borosilicate oxygen tube fitted with a ground glass stopper. Each oxygen tube was flushed thoroughly using small bore-size Tygon tubing (0.125 inch ID). The end of the Tygon tubing was placed at the bottom of the oxygen tube and a minimum of 20 ml of sample water were allowed to pass to ensure complete flushing of water in the tube. Special care was taken to ensure that no small air bubbles were present and that a convex meniscus was visible when tube was filled, achieved by removing the Tygon tubing slowly from the borosilicate tube and arresting flow only after the tubing was removed completely. Ten microliters of half-saturated $HgCl_2$ (i.e., 3.3mg $HgCl_2$ per 100ml distilled water) were added as a fixative. Oxygen

tubes were immediately capped firmly with a ground glass stopper and stored vertically and fully immersed in water held at *in situ* temperature. Oxygen concentrations were determined within one week of sampling and typically within 2-3 days. Rates of oxygen consumption were derived from the slope of the linear regression of incubation time versus oxygen concentration and converted to carbon values (i.e., μg C L^{-1} h^{-1}) using a respiratory quotient (RQ) of 1.0 (del Giorgio, L'Université du Québec à Montréal, personal communication).

Bacterial Production

Free-living and total community bacterial production were estimated using ^{3}H-leucine incorporation rates, following modifications of Smith and Azam (1992). For each sample, 20 μl of diluted isotope working stock (40-100 Ci $mmol^{-1}$; Sigma) was added to each of four 2 ml microcentrifuge tubes (Fisher Scientific), such that the addition of 1.5 ml of sample resulted in a final concentration of 40 nM tritiated leucine. 100 μl of 100% TCA (Trichloroacetic Acid) was added to one tube that served as the blank. Tubes were capped and refrigerated until time of sample addition.

Processing of samples entailed adding 1.5 ml of sample water to the blank and three pre-loaded microcentrifuge tubes and recording start time. This was repeated for all water samples for which estimates of bacterial production were needed. Each tube was vortexed for approximately 3 to 5 s on high and then placed in a water bath in the dark at room temperature for 1 h. After approximately 55 min a pipette was prepared to dispense 100 μl of 100% TCA. Tubes were removed from the water bath and caps were removed from the three replicate tubes. After exactly 1 h, 100 μl of 100% TCA was added to the three open replicate tubes, killing all bacteria and stopping production. Stop time was

recorded. Each tube (including blanks) was vortexed well and centrifuged at 14000 rpm for 10 min. Tubes were removed from the centrifuge and the supernatant was gently aspirated using a Pasteur pipette and flexible tubing attached to a vacuum pump. Great caution was taken to avoid aspirating the bacterial pellet, which forms about ¼ inch from the bottom on the outside edge of the tube. The outside rim of each tube was marked prior to loading in the centrifuge to aid in locating the pellet.

After aspirating all samples, 1.5 ml of 5% cold TCA was added to each tube to precipitate all incorporated leucine. Tubes were vortexed well and centrifuged at 14000 rpm for 10 min. Again, tubes were removed from the centrifuge and supernatant was aspirated (as described above). 1.5 ml of scintillation cocktail (UltimaGold, Perkin-Elmer) was added to each tube and vortexed well. Tubes were then placed in uncapped glass 20 ml liquid scintillation vials and counted using a Packard Tri-Carb 2250CA scintillation counter and a protocol developed specifically for tritium and UltimaGold scintillation cocktail. Bacterial production rates were derived from leucine incorporation during the one-hour incubation using a carbon conversion factor of 3.1 Kg C · mol leu^{-1} (Kirchman 1993). Mean measurement precision was 10.7% and based on the error associated with triplicate measurements of leucine incorporation.

Bacterial Growth Efficiency

Growth efficiency was determined by dividing bacterial production by total carbon consumption

$$\frac{BP}{BP + BR}$$

where BR is the estimate of bacterial respiration (μg C L^{-1} h^{-1}), as determined by the 6 or 8 h incubation, and BP the overall mean estimate of production (μg C L^{-1} h^{-1}) derived from sub-samples collected at the three time-points during each incubation.

Bacterial Abundance and Single-Cell Activity

Bacterioplankton abundance (BA; cells ml^{-1}) and that of metabolically-active cells in free-living and whole bacterial communities was determined on live samples using standard flow-cytometric techniques and a Becton-Dickinson FACSCaliber bench top sheath flow cytometer. The nucleic acid stain SYTO-13 (Molecular Probes) was used to determine bacterial abundance and total nucleic acid content of live samples following del Giorgio et al. (1996a) and Gasol and del Giorgio (2000), respectively. SYTO-13 is a nucleic acid stain that passively diffuses through the cellular membrane of bacterial cells and binds to both RNA and DNA, fluorescing green when illuminated with UV light. Due to its extremely low intrinsic fluorescence, unbound SYTO-13 has an extremely low quantum yield and is not visible using the flow cytometric procedures we employed.

Working stock solutions of SYTO-13 were prepared by dissolving concentrated stock with DMSO (dimethlysulfate) for a final concentration of 0.5mM. Two microliters of the working stock were combined with 500μl of sample in a 7ml flow cytometer Falcon tube, vortexed well, and incubated in the dark for 5 minutes. Ten microliters of reference bead stock solution containing 1μm green fluorescent microspheres (Molecular Probes) at a concentration of approximately 3000 beads μl^{-1} was added to the sample and vortexed again. Bacterial cells were visualized in a cytogram of light side scatter (SSC) versus green fluorescence (FL1). Total cell abundance, as evidenced by the number of intact bacterial nuclei visualized by the flow cytometer, was determined by defining a

region encompassing the enumerated heterotrophic bacteria and normalizing this number for the total number of reference beads counted (del Giorgio et al. 1996a). Each sample was run in the flow-cytometer until a minimum of 20,000 events were counted to ensure suitable accuracy of abundance estimates. The coefficient of variation (CV) associated with cytometric estimates of bacterial abundance was <0.5%.

Total nucleic acid content, which serves as an index of bacterial cell size and activity (Gasol and del Giorgio 2000), was determined by identifying cells in each of the sub-populations typically observed in natural bacterioplankton communities (Li et al. 1995), the first consisting of cells with high green fluorescence and side scatter (HDNA cells) and a second with lower green fluorescence and side scatter (LDNA cells). Regions for identifying HDNA and LDNA cells were the same for all analyses. The proportion of HDNA and LDNA cells was calculated using the abundance of each relative to the total bacterial counts obtained by SYTO-13 staining.

The abundance of actively respiring cells (CTC+) was determined using the redox dye CTC (5-cyano-2,3-ditolyl tetrazolium chloride) following Sieracki et al. (1999) and del Giorgio et al. (1997). Prior to analyses, a stock solution of 50 mM CTC (PolySciences, PA, USA) was prepared using distilled water, filtered through 0.1 µm, and stored in the dark at 5^{o}C until use. 55.5µl of this stock CTC solution was added to 500µl of live sample for an approximate final concentration 5mM, vortexed well, and incubating in the dark at room temperature for 1.5 hours. At the end of the incubation, 10µl of reference bead stock were added to the sample and vortexed. Each sample was run in the flow-cytometer until a minimum of 10,000 events were counted. CTC+ cells were identified and enumerated using orange (FL2) and red (FL3) fluorescence. The

proportion of CTC+ cells (%CTC) was calculated using the abundance of each relative to the total bacterial counts obtained by SYTO-13 staining.

Mean orange fluorescence (FL2) of each sample was used as an index of activity within the highly-active fraction, with higher values representing greater metabolic activity associated with the highly-active fraction. The total number of highly-active cells was combined with mean fluorescence (i.e., FL2*CTC+) as an integrative measure of total activity for each sample. This analysis identifies both the abundance and intensity of single-cell activity, allowing the discrimination between populations that have a similar number of highly-active cells yet differences in the intensity of metabolic activity associated with each highly-active fraction.

Organic Matter Lability

Lability of DOC was determined on a sub-set of the samples (n = 14) by filtering approximately 1L of sample water through 0.2μm Sterivex filters into duplicate 500ml borosilicate glass flasks, inoculating each with 10ml of AP15 filtered sample water, and incubating in the dark at room temperature for 24 days. Dissolved organic carbon concentrations were measured in each flask every few days and consumption of DOC was determined using the slope of DOC concentrations versus time (i.e., μg C L^{-1} d^{-1}). Percent labile DOC was determined by comparing the DOC consumed to the total DOC pool (i.e., DOC consumed/initial [DOC]). We observed a linear decrease in DOC concentrations in all incubations (Fig. B-2). These regressions were highly significant ($p < 0.0001$), similar among replicates, and relatively strong (i.e., high r^2 values). Rates of DOC consumption were similar among the seven sites investigated (data from site #12 not shown), with highest values recorded for site#7.

Nutrient Uptake Experiments

Changes in dissolved nutrient concentrations from which estimates of nutrient uptake were derived were determined in 93 of the 139 incubations measuring BP and BR. Uptake rates were calculated using the difference in dissolved nutrient concentrations between initial and final (~18 h) time-points and reported in μM h^{-1}. Changes in nutrient uptake during the course of these incubations were statistically significant relative to the error associated with the measurement of each nutrient (Strickland and Parsons 1972; Valderrama 1981; Whitledge et al. 1981). However, given the lack of replication of incubations in which nutrient uptake was measured, the error associated with the measurement of nutrient concentrations in sub-samples from each incubation could not be determined. In addition, there were no intermediate samplings of nutrient concentrations during the incubations, which would have served to support or reject our estimates of total nutrient uptake.

In an effort to further validate measurements of nutrient uptake, we explored the effect of temperature, hypothesizing that if these rates represented *in situ* processes they should exhibit some form of temperature dependence (Nedwell 1999; Reay et al. 1999). Differences in the mean uptake rate of certain dissolved nutrients (i.e., DON and PO_4^{3-}) at different temperature ranges (i.e., <17ºC (n = 23) versus >20ºC (n = 33)) provided evidence that these rates may be driven in part by temperature. Mean uptake of DON was 0.41 ± 0.08 versus 0.32 ± 0.06 μM h^{-1} at high versus low temperature ranges, respectively, while uptake of phosphate was 0.007 ± 0.001 versus 0.002 ± 0.0004 μM h^{-1}. Mean uptake rates at high and low temperatures were significantly different when t-tests

accounting for differences in variance (i.e., significantly greater variance at higher temperatures) were considered (PO_4^{3-} uptake: $t = 2.06$, $df = 36$, $p < 0.04$; DON uptake: $t = 2.0$, $df = 51.2$, $p < 0.05$). Although we did not observe the expected significant positive relationship between temperature and rates of nutrient uptake, scatter plots suggests that these rates are temperature dependent, with temperature imposing an upper limit on the flux of some dissolved nutrients, including uptake of total dissolved nitrogen and total dissolved phosphorus (Fig. B-3) and production of ammonium (data not shown). In this regard, although temperature dependence may constrain uptake at lower temperatures, the influence of other environmental factors on nutrient consumption may prevent uptake rates from always being elevated at higher temperatures. The significantly higher variability in uptake rates at higher temperatures may prevent robust linear relationships between uptake and temperature from being observed. Collectively, these observations suggest that rates of nutrient uptake recorded during the course of our research may represent *in situ* processes, although this is clearly an aspect of our study that should be subject to further and more intensive experimental investigations.

Discussion of Methodological Caveats and Concerns

Variability in Estimates of Bacterioplankton Carbon Metabolism

Diel, tidal, and climatic effects may contribute to the variability in estimates of carbon metabolism, potentially obfuscating ecologically meaningful patterns or relationships. Sampou and Kemp (1994) report significant diel variability in bacterioplankton and total community respiration, concluding that meaningful patterns in respiration may be better identified when sampling takes place at the same time of day – specifically between 0800 and 1000 h. Similarly, unpublished research from our group

has identified significant variability of BP and BR throughout the tidal cycle in a temperate salt marsh. Thus, the timing of sampling alone may introduce a significant source of variability to estimates of bacterioplankton carbon metabolism and should be considered in studies conducted in estuarine and coastal systems. This tendency for this variability to obscure otherwise meaningful patterns in bacterioplankton carbon metabolism may be compounded in studies conducted over large spatial scales where variability in climatic or regional-scale conditions may have significant effect. The sampling approach used in my dissertation minimizes these additional sources of variability, as this research was conducted in a single estuarine system with samples collected between 0800 and 1000 h immediately following high tide.

Methodological approaches employed by individual investigators may also introduce variability to estimates of BP and BR that further obscure actual patterns of *in situ* BGE. Long-term incubations (e.g., days to weeks) measuring changes in particulate and dissolved organic carbon (Bjørnsen 1986; Kroer 1993; Raymond and Bauer 2000), decreases in dissolved oxygen concentrations (Lee et al. 2002), or changes in dissolved inorganic carbon (Toolan 2001) are often used to estimate *in situ* rates of carbon metabolism. However, such approaches rely on the assumption that carbon metabolism is linear over time (Daneri et al. 1994) and that rates derived over long periods of time (>12 h) are representative of *in situ* rates. However, given the rapid metabolic response characteristic of natural bacterioplankton assemblages (Choi et al. 1999) and the reliance of bacterioplankton metabolism on a small pool of high-turnover labile organic matter (Baines and Pace 1991; Rich et al. 1997), it is likely that these studies may not capture subtle changes in short-term carbon metabolism and thus fail to accurately characterize *in*

situ rates. Although many studies suggest that long-term incubations do not effect estimates of microbial metabolism (Fuhrman and Azam 1980; Hopkinson et al. 1989; Pomeroy and Deibel 1986), researchers investigating short-term variability in BP and BR (e.g., Pomeroy et al. 1994; del Giorgio, L'Université du Québec à Montréal, personal communication) report non-linear consumption of organic matter over short time periods, suggesting that measurements made from long-term incubations may not always represent short-term *in situ* rates of BR and BP.

Estimates of BP using radiolabelled substrates (Simon and Azam 1989; Smith and Azam 1992) have allowed for extremely short incubations times (<1 h), reducing artifacts associated with enclosing microbial communities in bottles and thereby improving the ability to accurately estimate *in situ* carbon metabolism. Estimates of BR, however, typically require much longer incubation times (>12 h) to detect the small changes in oxygen concentrations associated with bacterioplankton respiration. Although measurements performed on the same water samples are often reported as paired, discrepancies in incubation time between BP and BR result in the integration of these rates over different time intervals and the reporting of so-called paired measurements that are not truly simultaneous. Estimates of bacterial growth efficiency may be particularly susceptible to such discrepancies, as BGE is derived from both of these terms (i.e., BGE = BP/[BP+BR]). In this regard, studies with large differences in the incubation times for BR and BP (Daneri et al. 1994; Pomeroy et al. 1995; e.g., Pomeroy et al. 1991; Roland and Cole 1999; Sherr and Sherr 2003) may not accurately estimate *in situ* growth efficiencies. However, the use of highly-sensitive membrane inlet mass spectrometry to detect extremely small changes in oxygen concentrations (del Giorgio and Bouvier 2002;

Kana et al. 1994) and other efforts focused on improving the accuracy and precision of related measuring techniques (Carignan et al. 1998; Roland et al. 1999) has improved our ability to estimate BR in extremely short incubations ((<3h; Carignan et al. 2000) and provide results over time scales commensurate with estimates of BP. Simultaneous measurements such as these generate the paired measures of BP and BR necessary to most accurately estimate *in situ* BGE. I propose that consistency of sampling protocol, strict methodology, extended duration of sampling (>2 yrs), shorter incubations times, and relatively small spatial scale of this dissertation research has removed much of the regional, climatic, and methodological sources of variability that exist in other studies, allowing novel patterns in bacterioplankton carbon metabolism to emerge that have not been readily observed in other studies of BGE in natural aquatic systems.

Effects of Filtration

Water samples used to estimate bacterial respiration, production, and growth efficiency were filtered through 142 mm diameter AP15 Millipore filters with a retention size of approximately 1 μm. We found that this effectively reduced the number of >3μm protozooplankton grazers and allowed over 80% of the free-living bacteria to pass into the filtrate. However, removal of larger bacterioplankton cells during the filtration process may produce an artifact that contributes to the seasonal pattern in BGE observed in our study. During filtration, cells contained in the filtrate will be smaller and may disproportionately represent the slow-growing or dormant fraction of the bacterioplankton community. These cells typically have lower BGE, as baseline metabolism makes up a greater proportion of their total carbon consumption (del Giorgio and Cole 1998). Estimates of BGE derived from this free-living fraction would be lower

than *in situ* BGE at times when the bacterioplankton community is dominated by rapidly growing, attached and/or large bacterioplankton cells. Thus, lower growth efficiencies during the more productive, warmer months may in part be influenced by bacterioplankton shifting from a relatively inactive, free-living phase to an attached and/or larger, highly-active phase (Choi et al. 1999; Crump et al. 1998; Gasol et al. 1999) and the selective removal of these bacterioplankton during the filtration process.

Fig. B-1. Incubation assembly for measuring oxygen consumption in natural samples.

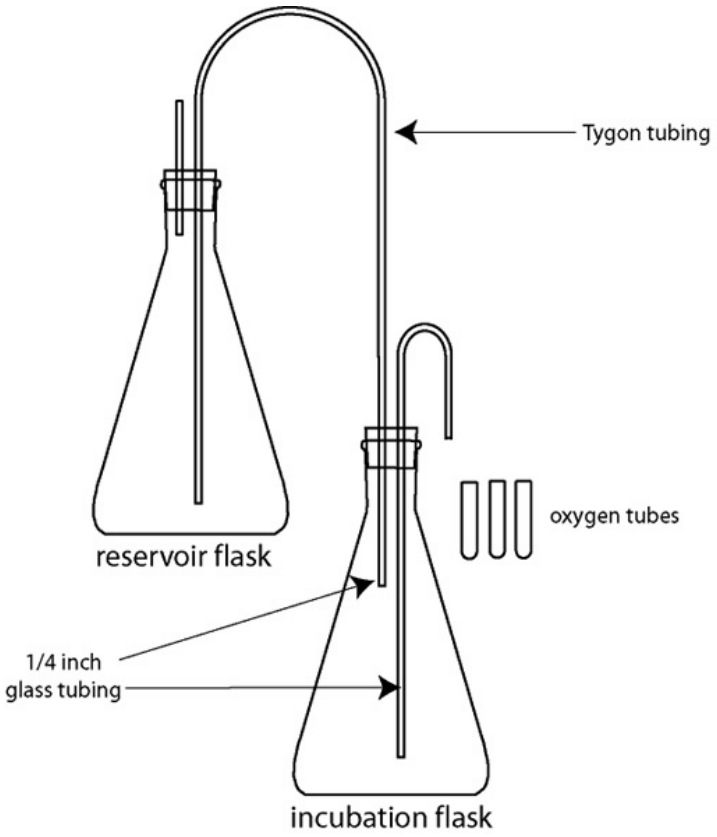

Tygon tubing
oxygen tubes
reservoir flask
1/4 inch
glass tubing
incubation flask

Fig. B-2. Consumption of dissolved organic carbon in 24-d incubations. Estimates of DOM lability (mg C L^{-1} d^{-1}) were derived from the slopes of each regression.

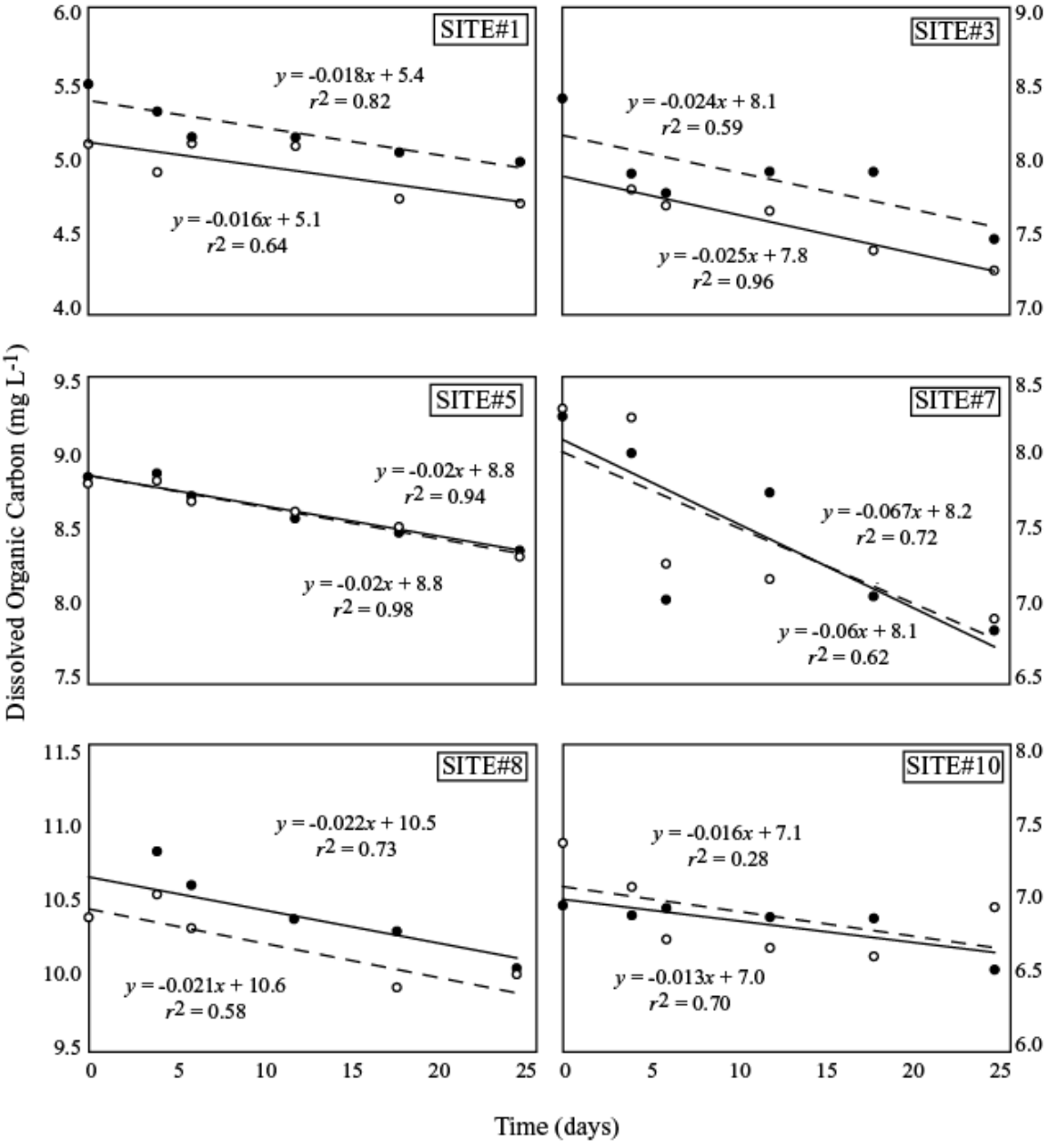
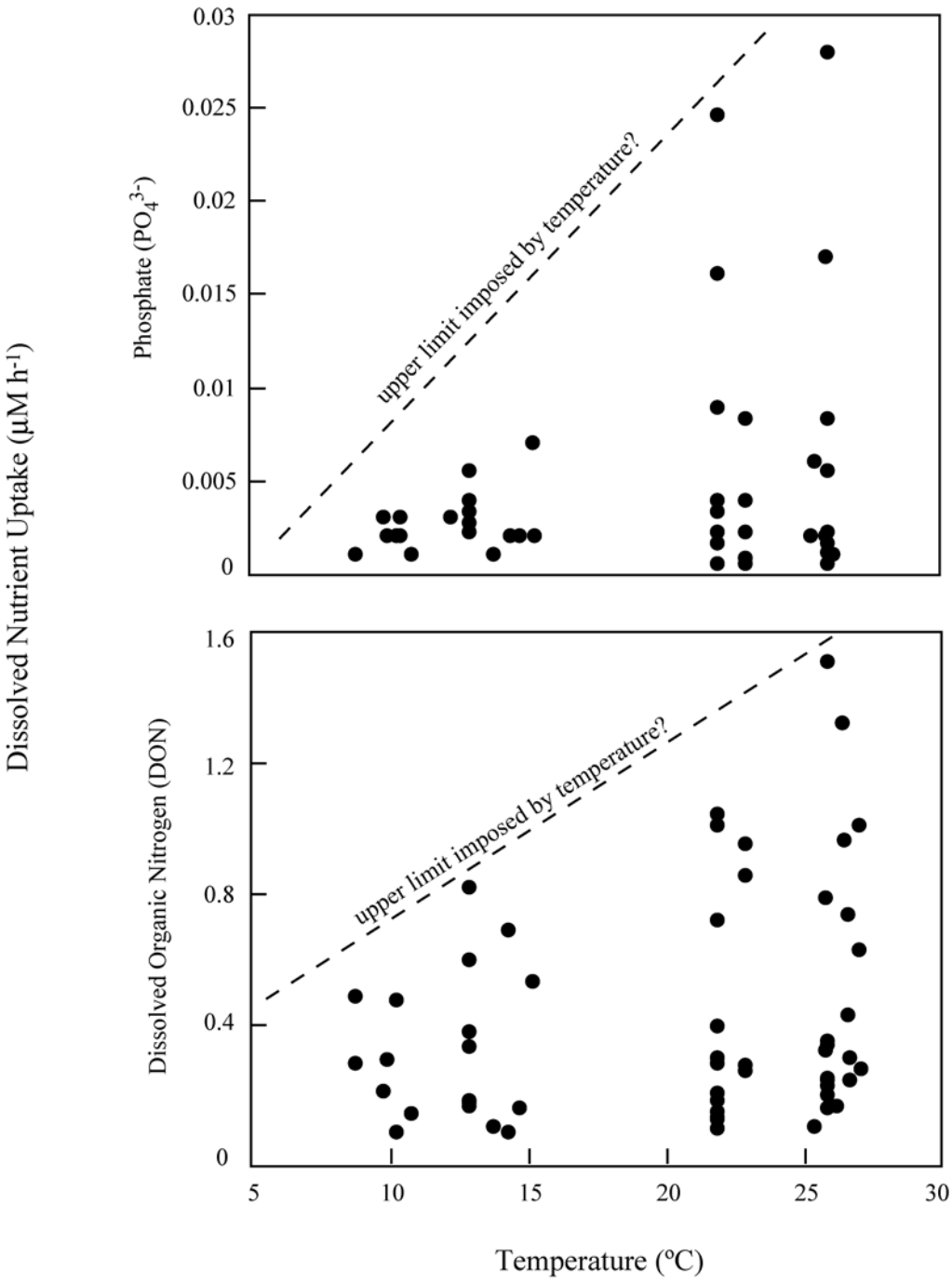

SITE#1
y = -0.018x + 5.4
r2 = 0.82
y = -0.016x + 5.1
r2 = 0.64
SITE#3
y = -0.024x + 8.1
r2 = 0.59
y = -0.025x + 7.8
r2 = 0.96
SITE#5
y = -0.02x + 8.8
r2 = 0.94
y = -0.02x + 8.8
r2 = 0.98
SITE#7
y = -0.067x + 8.2
r2 = 0.72
y = -0.06x + 8.1
r2 = 0.62
SITE#8
y = -0.022x + 10.5
r2 = 0.73
y = -0.021x + 10.6
r2 = 0.58
SITE#10
y = -0.016x + 7.1
r2 = 0.28
y = -0.013x + 7.0
r2 = 0.70
Dissolved Organic Carbon (mg L-1)
Time (days)

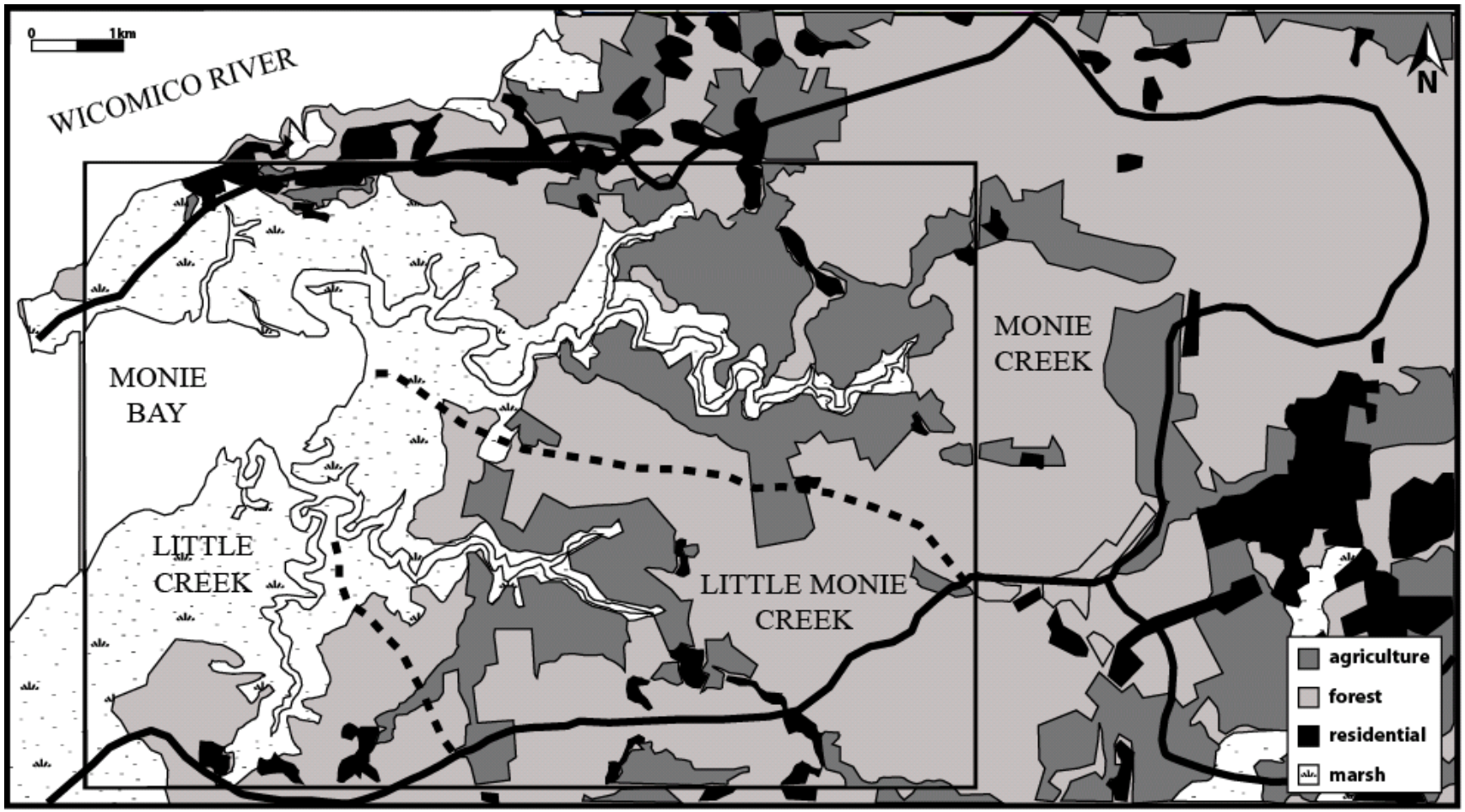
Fig. B-3. Uptake of dissolved phosphate (upper panel) and dissolved organic nitrogen (lower panel) at different temperatures. Dashed lines represent a hypothetical constraint imposed by temperature.


Dissolved Nutrient Uptake (μM h-1)
Phosphate (PO4 3-)
0.03
0.025
0.02
0.015
0.01
0.005
0
upper limit imposed by temperature?
Dissolved Organic Nitrogen (DON)
1.6
1.2
0.8
0.4
0
upper limit imposed by temperature?
5
10
15
20
25
30
Temperature (ºC)

APPENDIX C: Watershed Characteristics

Monie Bay is located in the western central part of the Lower Eastern Shore watershed, bordered on the north and south by the Lower Wicomico River and Manokin River watersheds, respectively (Fig. C-1). The Monie Bay drainage basin (USGS watershed code 02-13-03-02) covers approximately 72 km^2 and has been divided into two 3^{rd} order drainage basins – that of Monie Creek and the collective basin for Little Monie Creek and Little Creek.

Fig. C-1. Map of Monie Bay indicating drainage basins and land-use. USGS watersheds are designated by solid black lines, with sub-division of the Monie Bay drainage basin into 3rd-order catchments represented by dashed lines. The rectangle enclosing the three creeks and Monie Bay represents the area included in figures reported previously in Chapters II, III, and IV.


0
1km
WICOMICO RIVER
N
MONIE
BAY
MONIE
CREEK
LITTLE
CREEK
LITTLE MONIE
CREEK
agriculture
forest
residential
marsh

COMPLETE LITERATURE CITED

Amon, R., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography **41:** 41-51.

Amon, R., H. P. Fitznar, and R. Benner. 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnology and Oceanography **46:** 287-297.

Anderson, J., E. Hardy, J. Roach, and R. Witmer. 1976. A Land Use and Land Cover Classification System For Use With Remote Sensor Data. United States Department of the Interior.

Anderson, T. H., and G. T. Taylor. 2001. Nutrient pulses, plankton blooms, and seasonal hypoxia in western Long Island Sound. Estuaries **24:** 228-243.

Apple, J. K., and P. A. del Giorgio. in prep. The variability and regulation of bacterioplankton carbon metabolism in a tidally-influenced estuary. Aquatic Microbial Ecology.

Apple, J. K., P. A. del Giorgio, and W. M. Kemp. submitted. Temperature regulation of bacterial production, respiration, and growth efficiency in estuarine systems. Limnology and Oceanography.

Apple, J. K., P. A. del Giorgio, and R. I. E. Newell. 2004. The effect of system-level nutrient enrichment on bacterioplankton production in a tidally-influenced estuary. Journal of Coastal Research **45:** 110-133.

Autio, R. M. 1992. Temperature regulation of brackish water bacterioplankton. Hydrobiologia **37:** 253-263.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and T. F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series **10:** 257-263.

Baines, S. B., and M. L. Pace. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnology and Oceanography **36:** 1078-1090.

Bano, N. and others 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. Marine Ecology Progress Series **157:** 1-12.

Barcina, I., P. Lebaron, and J. Vives-Rego. 1997. Survival of allochthonous bacteria in aquatic systems: a biological approach. FEMS Microbiology Ecology **23:** 1-9.

Beaulac, M. N., and K. H. Reckhow. 1982. An examination of land use - nutrient export relationships. Water Resources Bulletin **18:** 1013-1024.

Bell, R. 1993. Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine (Chapter 56). Handbook of Methods in Aquatic Microbial Ecology**:** 495-503.

Berman, T., B. Kaplan, S. Chava, Y. Viner, B. F. Sherr, and E. B. Sherr. 2001. Metabolically active bacteria in Lake Kinneret. Aquatic Microbial Ecology **23:** 213-224.

Biddanda, B., M. Ogdahl, and J. B. Cotner. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnology and Oceanography **46:** 730-738.

Bjørnsen, P. K. 1986. Bacterioplankton Growth-Yield in Continuous Seawater Cultures. Marine Ecology Progress Series **30:** 191-196.

Bouvier, T. C., and P. A. del Giorgio. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography **47:** 453-470.

Boynton, W. R., and W. M. Kemp. 2000. Influence of river flow and nutrient loading on selected ecosystem processes and properties in Chesapeake Bay, p. 269-298. *In* J. E. Hobbie [ed.], Estuarine science: A synthetic approach to research and practice. Island Press.

Bronk, D. A., P. M. Glibert, T. C. Malone, S. Banahan, and E. Sahlsten. 1998. Inorganic and organic nitrogen cycling in Chesapeake Bay: autotrophic versus heterotrophic processes and relationships to carbon flux. Aquatic Microbial Ecology **15:** 177-189.

Brussaard, C. P. D., and R. Riegman. 1998. Influence of bacteria on phytoplankton cell mortality with phosphorus or nitrogen as the algal-growth-limiting nutrient. Aquatic Microbial Ecology **14:** 271-280.

Cajal-Medrano1, R., and H. Maske. 1999. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton--revisiting the Pirt model. Aquatic Microbial Ecology **19:** 119-128.

Cajal-Medrano, R., and H. Maske. 1999. Growth efficiency, growth rate and the remineralization of organic substrate by bacterioplankton--revisiting the Pirt model. Aquatic Microbial Ecology **19:** 119-128.

Carignan, R., A. M. Blais, and C. Vis. 1998. Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method. Canadian Journal of Fisheries and Aquatic Sciences **55:** 1078-1084.

Carignan, R., D. Planas, and C. Vis. 2000. Planktonic production and respiration in oligotrophic Shield lakes. Limnology and Oceanography **45:** 189-199.

Carlson, C. A., and H. W. Ducklow. 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquatic Microbial Ecology **10:** 69-85.

Carlsson, P., and D. A. Caron. 2001. Seasonal variation of phosphorus limitation of bacterial growth in a small lake. Limnology and Oceanography **46:** 108-120.

Caron, D. A., J. C. Goldman, and T. Fenchel. 1990. Protozoan respiration and metabolism, p. 307-322. *In* G. Capriulo [ed.], Ecology of Marine Protozoa. Oxford University Press.

Caron, D. A., E. L. Lim, R. W. Sanders, M. R. Dennett, and U. G. Berninger. 2000. Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. Aquatic Microbial Ecology **22:** 175-184.

Choi, J., E. B. Sherr, and B. F. Sherr. 1999. Dead or alive? A large fraction of ETS-inactive marine bacterioplankton cells, as assessed by reduction of CTC, can become ETS-active with incubation and substrate addition. Aquatic Microbial Ecology **18:** 105-115.

Cimbleris, A. C., and J. Kalff. 1998. Planktonic bacterial respiration as a function of C:N:P ratios across temperate lakes. Hydrobiologia **384:** 89-100.

Cloern, J. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series **210:** 223-253.

Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross system overview. Marine Ecology Progress Series **43:** 1-10.

Cook, K. L., and J. L. Garland. 1997. The relationship between electron transport activity as measured by CTC reduction and CO2 production in mixed microbial communities. Microbial Ecology **34:** 237-247.

Cornwell, J. C., D. J. Conley, M. Owens, and J. C. Stevenson. 1996. A sediment chronology of the eutrophication of Chesapeake Bay. Estuaries **19:** 488-499.

Cornwell, J. C., J. M. Stribling, and J. C. Stevenson. 1994. Biogeochemical studies at the Monie Bay National Estuarine Research Reserve, p. 645-655. *In* M. Lynch and B. Crowder [eds.], Organizing for the Coast: Thirteenth International Conference of the Coastal Society.

Cotner, J. B., and B. Biddanda. 2002. Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems. Ecosystems **5:** 105-121.

Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine proteobacteria and members of Cytophaga-Flavobacter cluster consuming low-and high-molecular

weight dissolved organic matter. Applied and Environmental Microbiology **66:** 1692-1697.

---. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnology and Oceanography **48:** 168–178.

---. 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquatic Microbial Ecology **34:** 139-149.

Coveney, M. F., and R. G. Wetzel. 1992. Effects of Nutrients on Specific Growth Rate of Bacterioplankton in Oligotrophic Lake Water Cultures. Applied and Environmental Microbiology **58:** 150-156.

---. 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an oligotrophic lake. Limnology and Oceanography **40:** 1187-1200.

Cowan, J. L. W., and W. R. Boynton. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors, and ecological significance. Estuaries **19:** 562-580.

Crawford, C. C., J. E. Hobbie, and K. L. Webb. 1974. The utilization of dissolved free amino acids by estuarine microorganisms. Ecology **55:** 551-563.

Crump, B. C., E. V. Armbrust, and J. A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Applied and Environmental Microbiology **65:** 3192-3204.

Crump, B. C., and J. A. Baross. 2000. Characterization of the bacterially-active particle fraction in the Columbia River estuary. Marine Ecology Progress Series **206:** 13-22.

Crump, B. C., J. A. Baross, and C. A. Simenstad. 1998. Dominance of particle-attached bacteria in the Columbia River estuary, USA. Aquatic Microbial Ecology **14:** 7-18.

Daneri, G., B. Riemann, and P. J. L. B. Williams. 1994. In situ bacterial production and growth yield measured by thymidine, leucine and fractionated dark oxygen uptake. Journal of Plankton Research **16:** 105-113.

Dauer, D. M., S. B. Weisberg, and J. A. Ranasinghe. 2000. Relationships Between Benthic Community Condition, Water Quality, Sediment Quality, Nutrient Loads, and Land Use Patterns in Chesapeake Bay. Estuaries **23:** 80-96.

Davidson, K. 1996. Modeling microbial food webs. Marine Ecology Progress Series **145:** 279-296.

Day, J. W. J., C. A. S. Hall, W. M. Kemp, and A. Yanez-Arancibia [eds.]. 1989. Estuarine Ecology. John Wiley and Sons.

del Giorgio, P. A., D. F. Bird, Y. T. Prairie, and D. Planas. 1996a. Flow cytometric determination of bacterial abundance in lake plankton using the green nucleic acid stain SYTO 13. Limnology and Oceanography **41:** 783-789.

del Giorgio, P. A., and T. C. Bouvier. 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. Limnology and Oceanography **47:** 471-486.

del Giorgio, P. A., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics **29:** 503-541.

---. 2000. Bacterial growth energetics and efficiency in natural aquatic systems, p. 289-325. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

del Giorgio, P. A., and J. Davis. 2003. Patterns in dissolved organic matter lability and consumption across aquatic ecosystems, p. 399-424. *In* S. Findlay [ed.], Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Elsevier Science.

del Giorgio, P. A., and C. M. Duarte. 2002. Respiration in the open ocean. Nature **420:** 379-384.

del Giorgio, P. A., J. M. Gasol, D. Vaque, P. Mora, S. Agusti, and C. M. Duarte. 1996b. Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. Limnology and Oceanography **41:** 1169-1179.

del Giorgio, P. A., J. M. Gasol, D. Vaque, P. Mura, S. Agusti, and C. M. Duarte. 1996c. Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. Limnology and Oceanography **41:** 1169-1179.

del Giorgio, P. A., Y. T. Prairie, and D. F. Bird. 1997. Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, enumerated using CTC reduction and flow cytometry. Microbial Ecology **34:** 144-154.

del Giorgio, P. A., and G. Scarborough. 1995. Increase in the proportion of metabolically active bacteria along gradients of enrichment in freshwater and marine plankton: implications for estimates of bacterial growth and production rates. Journal of Plankton Research **17:** 1905-1924.

Den Heyer, C., and J. Kalff. 1998. Organic matter mineralization rates in sediments: A within- and among-lake study. Limnology and Oceanography **43:** 695-705.

Ducklow, H. W. 1983. Production and fate of bacteria in the oceans. BioScience **33:** 494-501.

---. 1994. Modeling the microbial food web. Microbial Ecology **28:** 303-319.

Ducklow, H. W., D. A. Purdie, P. J. L. B. Williams, and J. M. Davies. 1986. Bacterioplankton: A sink for carbon in a coastal marine plankton community. Science **232:** 863-867.

Eldridge, P. M., and M. E. Sieracki. 1993. Biological and hydrodynamic regulation of the microbial food web in a periodically mixed estuary. Limnology and Oceanography **38:** 1666-1679.

Felip, M., M. L. Pace, and J. J. Cole. 1996. Regulation of planktonic bacterial growth rates: The effects of temperature and resources. Microbial Ecology **31:** 15-28.

Fielding, K. P. 2002. Differential substrate limitation in small tributaries of Chesapeake Bay, Maryland influenced by non-point source nutrient loading. Masters. University of Maryland.

Findlay, S., M. L. Pace, and D. T. Fischer. 1996. Spatial and Temporal Variability in the Lower Food Web of the Tidal Freshwater Hudson River. Estuaries **19:** 866-873.

Finlay, B. J., S. C. Maberly, and J. I. Cooper. 1997. Microbial diversity and ecosystem function. Oikos **80:** 209-213.

Fisher, T. R. 1985. Nitrogen and phosphorus inputs to Chesapeake Bay, p. 58-62. The Chesapeake Bay Symposium. National Marine Educators Conference.

Fisher, T. R., L. W. Harding, D. W. Stanley, and L. G. Ward. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. Estuarine, Coastal and Shelf Science **27:** 61-93.

Fisher, T. R., K. Y. Lee, H. Berndt, J. A. Benitez, and M. M. Norton. 1998. Hydrology and chemistry of the Choptank River Basin. Water, Air, and Soil Pollution **105:** 387-397.

Fuhrman, J. A., and F. Azam. 1980. Bacterioplankton Secondary Production Estimates for Coastal Waters of British Columbia, Antarctica, and California. Applied and Environmental Microbiology **39:** 1085-1095.

Gasol, J. M., and P. A. del Giorgio. 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina **64:** 197-224.

Gasol, J. M., U. L. Zweifel, F. Peters, J. A. Fuhrman, and A. Hagstrom. 1999. Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Applied and Environmental Microbiology **65:** 4475-4483.

Goldman, J. C., D. A. Caron, and M. R. Dennet. 1987. Regulation of gross growth efficiency and ammonium regeneration in bacteria by C:N ratio. Limnology and Oceanography **32:** 1239-1252.

Gonzalez, J. M., E. B. Sherr, and B. F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Applied and Environmental Microbiology **56:** 583-589.

Goosen, N. K., P. Van Rijswijk, J. Kromkamp, and J. Peene. 1997. Regulation of annual variation in heterotrophic bacterial production in the Schelde Estuary (SW Netherlands). Aquatic Microbial Ecology **12:** 223-232.

Griffiths, R., B. Caldwell, and R. Y. Morita. 1984. Observations on microbial percent respiration values in arctic and subarctic marine waters and sediments. Microbial Ecology **10:** 151-164.

Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology **33:** 1225.

Hoch, M. P., and D. L. Kirchman. 1993. Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. Marine Ecology Progress Series **98:** 283-295.

---. 1995. Ammonia uptake by heterotrophic bacteria in the Delaware Estuary and adjacent coastal waters. Limnology and Oceanography **40:** 886-897.

Hopkinson, C. S., B. Sherr, and W. J. Wiebe. 1989. Size Fractionated Metabolism of Coastal Microbial Plankton. Marine Ecology Progress Series **51:** 155-166.

Hoppe, H. G., H. C. Giesenhagen, and K. Gocke. 1998. Changing patterns of bacterial substrate decomposition in a eutrophication gradient. Aquatic Microbial Ecology **15:** 1-13.

Hu, C., F. E. Muller-Karger, and R. G. Zepp. 2002. Absorbance, absorption coefficient, and apparent quantum yield: A comment on common ambiguity in the use of these optical concepts. Limnology and Oceanography **47:** 1261–1267.

Iturriaga, R., and H. G. Hoppe. 1977. Observations of heterotrophic activity on photoassimilated organic matter. Marine Biology **40:** 101-108.

Jahnke, R. A., and D. B. Craven. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: A need for respiration rate measurements. Limnology and Oceanography **40:** 436-441.

Jellet, J. F., W. K. W. Li, P. M. Dickie, A. Boraie, and P. E. Kepkay. 1996. Metabolic activity of bacterioplankton communities assessed by flow cytometry and single carbon substrate utilization. Marine Ecology Progress Series **136:** 213-225.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A Two-Year Study of the Short-Term and Long-Term Sequestering of Nitrogen and Phosphorus in the Maryland National Estuarine Research Reserve, p. 1-100. Maryland National Estuarine Research Reserve.

Jorgensen, N., N. Kroer, R. B. Coffin, and M. P. Hoch. 1999. Relations between bacterial nitrogen metabolism and growth efficiency in an estuarine and an open-water ecosystem. Aquatic Microbial Ecology **18:** 247-261.

Jørgensen, N. O. G., N. Kroer, and R. B. Coffin. 1994. Utilization of dissolved nitrogen by heterotrophic bacterioplankton: Effect of substrate C/N ratio. Applied and Environmental Microbiology **60:** 4124-4133.

Joux, F., and P. Lebaron. 2000. Use of fluorescent probes to assess physiological function of bacteria at a single-cell level. Microbes and Infections **2:** 1523-1535.

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennet, and J. C. Cornwell. 1994. Membrane inlet mass-spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. Analytical Chemistry **66:** 4166-4170.

Kearney, M. S., J. C. Stevenson, and L. G. Ward. 1994. Spatial and Temporal Changes in Marsh Vertical Accretion Rates at Monie Bay - Implications for Sea-Level Rise. Journal of Coastal Research **10:** 1010-1020.

Kirchman, D. L. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria (Chapter 58), p. 776. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole [eds.], Handbook of Methods in Aquatic Microbial Ecology. CRC Press.

---. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. Microbial Ecology **28:** 255-271.

--- [ed.]. 2000a. Microbial Ecology of the Oceans. Wiley-Liss.

---. 2000b. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria, p. 261-288. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley and Sons, Inc.

---. 2002. Calculating microbial growth rates from data on production and standing stocks. Marine Ecology Progress Series **233:** 303-306.

Kirchman, D. L., A. I. Dittel, S. E. G. Findlay, and D. Fischer. 2004. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. Aquatic Microbial Ecology **35:** 243-257.

Kolber, Z. S., R. Barber, K. Coale, S. Fitzwater, and R. Greene. 1994. Iron limitation of phytoplankton photosynthesis in the equatorial pacific ocean. Nature **371:** 145-148.

Kroer, N. 1993. Bacterial-Growth Efficiency on Natural Dissolved Organic-Matter. Limnology and Oceanography **38:** 1282-1290.

Langenheder, S., and K. Jurgens. 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnology and Oceanography **46:** 121-134.

Lebaron, P., P. Servais, H. Agogué, C. Courties, and F. Joux. 2001a. Does the High Nucleic Acid Content of Individual Bacterial Cells Allow Us To Discriminate between Active Cells and Inactive Cells in Aquatic Systems? Applied and Environmental Microbiology **67:** 1775-1782.

Lebaron, P. and others 2001b. Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in abundances, activity and composition. FEMS Microbiology Ecology **34:** 255-266.

Lebaron, P., P. Servais, M. Troussellier, C. Courties, and J. Vives-Rego. 1999. Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquatic Microbial Ecology **19:** 225-267.

Lee, C. W., I. Kudo, T. Yokokawa, M. Yanada, and Y. Maita. 2002. Dynamics of bacterial respiration and related growth efficiency, dissolved nutrients and dissolved oxygen concentration in a subarctic coastal embayment. Marine and Freshwater Research **53:** 1-7.

Lee, K. Y., T. R. Fisher, T. E. Jordan, D. L. Correl, and D. E. Weller. 2000. Modeling the hydrochemistry of the Choptank River basin using GWLF and Arc/Info: 1. Model calibration and validation. Biogeochemistry **49:** 143-173.

Li, W., J. Jellett, and P. Dickie. 1995. DNA distributions in planktonic bacteria stained with TOTO or TO-PRO. Limnology and Oceanography **40:** 1485-1495.

Linton, J., and R. Stevenson. 1978. A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substrates. FEMS Microbiology Letters **3:** 95-98.

Lomas, M. W., P. M. Glibert, F. Shiah, and E. M. Smith. 2002. Microbial processes and temperature in Chesapeake Bay: current relationships and potential impacts of regional warming. Global Change Biology **8:** 51-70.

Lowrance, R. and others 1997. Water quality functions of Riparian forest buffers in Chesapeake Bay watersheds. Environmental Management **21:** 687-712.

Madigan, M. T., J. M. Martinko, and J. Parker. 2003. Brock Biology of Microorganisms, 10th ed. Prentice Hall.

Marie, D., F. Partensky, S. Jacquet, and D. Vaulot. 1997. Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Applied and Environmental Microbiology **63:** 186-193.

Massana, R., C. Pedrós-Alió, E. O. Casamayor, and J. M. Gasol. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnology and Oceanography **46:** 1181-1188.

Mcknight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Anderson. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnology and Oceanography **46:** 38-48.

Middelboe, M., and M. Søndergaard. 1993. Bacterioplankton growth yield: Seasonal variations and coupling to substrate lability and beta -glucosidase activity. Applied and Environmental Microbiology **59:** 3916-3921.

Moran, M. A., and R. E. Hodson. 1990. Bacterial production on humic and non-humic components of dissolved organic matter. Limnology and Oceanography **35:** 1744-1756.

Moran, M. A., J. E. Sheldon, and R. G. Zepp. 2000. Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. Limnology and Oceanography **45:** 1254-1264.

Morita, R. Y. 1974. Temperature Effects on Marine Microorganisms, p. 75-79. *In* R. R. Colwell and R. Y. Morita [eds.], Effect of the Ocean Environment on Microbial Activities. University Park Press.

Nedwell, D. B. 1999. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiology Ecology **30:** 101-111.

Nixon, S. W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia **41:** 199-219.

Norton, M. M., and T. R. Fisher. 2000. The effects of forest on stream water quality in two coastal plain watersheds of the Chesapeake Bay. Ecological Engineering **14:** 337-362.

Pace, M. L., and J. J. Cole. 2000. Effects of whole-lake manipulations of nutrient loading and food web structure on planktonic respiration. Canadian Journal of Fisheries and Aquatic Sciences **57:** 487-496.

Painchaud, J., D. Lefaivre, and J. C. Therriault. 1987. Box model analysis of bacterial fluxes in the St. Lawrence Estuary. Marine Ecology Progress Series **41:** 241-252.

Painchaud, J., D. Lefaivre, J. C. Therriault, and L. Legendre. 1996. Bacterial dynamics in the upper St. Lawrence Estuary. Limnology and Oceanography **41:** 1610-1618.

Pinhassi, J. and others 1999. Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquatic Microbial Ecology **17:** 13-26.

Pomeroy, L. 1974. The ocean's food web, a changing paradigm. BioScience **24:** 499-504.

Pomeroy, L. R., and D. Deibel. 1986. Temperature Regulation of Bacterial-Activity During the Spring Bloom in Newfoundland Coastal Waters. Science **233:** 359-361.

Pomeroy, L. R., J. E. Sheldon, W. M. Sheldon, J. O. Blanton, J. Amft, and F. Peters. 2000. Seasonal changes in microbial processes in estuarine and continental shelf waters of the southeastern USA. Estuarine, Coastal and Shelf Science **51:** 415-428.

Pomeroy, L. R., J. E. Sheldon, W. M. Sheldon, and F. Peters. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. Marine Ecology Progress Series **117:** 259-268.

Pomeroy, L. R., and W. J. Wiebe. 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquatic Microbial Ecology **23:** 187-204.

Pomeroy, L. R., W. J. Wiebe, D. Deibel, R. J. Thompson, G. T. Rowe, and J. D. Pakulski. 1991. Bacterial Responses to Temperature and Substrate Concentration During the Newfoundland Spring Bloom. Marine Ecology Progress Series **75:** 143-159.

Ram, A. S. P., S. Nair, and D. Chandramohan. 2003. Bacterial growth efficiency in the tropical estuarine and coastal waters of Goa, southwest coast of India. Microbial Ecology **45:** 88-96.

Rasmussen, B., and A. Josefson. 2002. Consistent Estimates for the Residence Time of Micro-tidal Estuaries. Estuarine, Coastal and Shelf Science **54:** 65-73.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. Aquatic Microbial Ecology **22:** 1-12.

Reay, D. S., D. B. Nedwell, J. Priddle, and J. C. Ellis-Evans. 1999. Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Applied and Environmental Microbiology **65:** 2577-2584.

Reinthaler, T., and G. J. Herndl. 2005. Seasonal dynamics of bacterial growth efficiencies in relation to phytoplankton in the southern North Sea. Aquatic Microbial Ecology **39:** 7-16.

Reitner, B., A. Herzig, and G. Herndl. 1999. Dynamics in bacterioplankton production in a shallow, temperate lake (Lake Neusiedl, Austria): evidence for dependence on macrophyte production rather than on phytoplankton. Aquatic Microbial Ecology **19:** 245-254.

Revilla, M., A. Iriarta, I. Madariaga, and E. Orive. 2000. Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. Estuarine, Coastal and Shelf Science **50:** 297-313.

Rich, J., M. Gosselin, E. B. Sherr, B. F. Sherr, and D. L. Kirchman. 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. Deep Sea Research Part II: Topical Studies in Oceanography **44:** 1645-1663.

Rieman, B. and others 1990. Carbon budgets of the microbial food web in estuarine enclosures. Marine Ecology Progress Series **65:** 159-170.

Rivkin, R. B., and L. Legendre. 2001. Biogenic carbon cycling in the upper ocean: Effects of microbial respiration. Science **291:** 2398-2400.

Rodrigues, R. M., and P. J. L. B. Williams. 2001. Heterotrophic bacterial utilization of nitrogenous and nonnitrogenous substrates, determined from ammonia and oxygen fluxes. Limnology and Oceanography **46:** 1675–1683.

Rodriguez, G. G., D. Phipps, K. Ishiguro, and H. F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Applied and Environmental Microbiology **58:** 1801-1808.

Roland, F., N. F. Caraco, J. J. Cole, and P. A. del Giorgio. 1999. Rapid and precise determination of dissolved oxygen by spectrophotometry: Evaluation of interference from color and turbidity. Limnology and Oceanography **44:** 1148-1154.

Roland, F., and J. J. Cole. 1999. Regulation of bacterial growth efficiency in a large turbid estuary. Aquatic Microbial Ecology **20:** 31-38.

Rose, A. H. 1967. Thermobiology. Academic Press Inc.

Sampou, P., and W. M. Kemp. 1994. Factors regulating plankton community respiration in Chesapeake Bay. Marine Ecology Progress Series **110:** 249-258.

Scudlark, J. R., and T. M. Church. 1989. The Sedimentary Flux of Nutrients at a Delaware Salt-Marsh Site - a Geochemical Perspective. Biogeochemistry **7:** 55-75.

Servais, P., E. O. Casamayor, C. Courties, P. Catala, N. Parthuisot, and P. Lebaron. 2003. Activity and diversity of bacterial cells with high and low nucleic acid content. Aquatic Microbial Ecology **33:** 41-51.

Sharp, J. and others 1995. Analyses of dissolved organic carbon in sea water: the JGOFS EqPac methods comparison. Marine Chemistry **48:** 91-108.

Sharp, J. H., C. H. Culberson, and T. M. Church. 1982. The chemistry of the Delaware Estuary. General considerations. Limnology and Oceanography **27:** 1015-1028.

Sherr, B. F., P. A. del Giorgio, and E. B. Sherr. 1999a. Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye CTC. Aquatic Microbial Ecology **18:** 117-131.

Sherr, B. F., and E. B. Sherr. 1996. Temporal offset in oceanic production and respiration processes implied by seasonal changes in atmospheric oxygen: The role of heterotrophic microbes. Aquatic Microbial Ecology **11:** 91-100.

---. 2003. Community respiration/production and bacterial activity in the upper water column of the central Arctic Ocean. Deep-Sea Research: Part I **50:** 529-542.

Sherr, B. F., E. B. Sherr, and C. S. Hopkinson. 1988. Trophic interactions within pelagic microbial communities: Indications of feedback regulation of carbon flow. Hydrobiologia **159:** 19-26.

Sherr, B. F., E. B. Sherr, and J. Mcdaniel. 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. Applied and Environmental Microbiology **58:** 2381-2385.

Sherr, E. B., and B. F. Sherr. 1988. Role of microbes in pelagic food webs: A revised concept. Limnology and Oceanography **33:** 1225-1227.

Sherr, E. B., B. F. Sherr, and C. T. Sigmon. 1999b. Activity of marine bacteria under incubated and in situ conditions. Aquatic Microbial Ecology **20:** 213-223.

Shiah, F. K., and H. W. Ducklow. 1994a. Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. Marine Ecology Progress Series **103:** 297-308.

---. 1994b. Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. Limnology and Oceanography **39:** 1243-1258.

---. 1995. Multiscale variability in bacterioplankton abundance, production, and specific growth rate in a temperate salt-marsh tidal creek. Limnology and Oceanography **40:** 55-66.

Sieracki, M. E., T. L. Cucci, and J. Nicinski. 1999. Flow cytometric analysis of 5-cyano-2,3-ditolyl tetrazolium chloride activity of marine bacterioplankton in dilution cultures. Applied and Environmental Microbiology **65:** 2409-2417.

Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Marine ecology progress series **51:** 201-213.

Sims, J. T., R. R. Simard, and B. C. Joern. 1998. Phosphorus loss in agricultural drainage: historical perspective and current research. Journal of Environmental Quality **27:** 277-293.

Sims, J. T., and D. C. Wolf. 1994. Poultry waste management: agricultural and environmental issues. Advances in Agronomy **52:** 1-83.

Smith, D. C., and F. Azam. 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using super(3)H-leucine. Marine Microbial Food Webs **6:** 107-114.

Smith, D. E., M. Leffler, and G. Mackiernan [eds.]. 1992. Oxygen dynamics in the Chesapeake Bay: A synthesis of recent research. Maryland Sea Grant.

Smith, E., and W. Kemp. 2001. Size structure and the production/respiration balance in a coastal plankton community. Limnology and Oceanography **46:** 473-485.

Smith, E. M. 1998. Coherence of microbial respiration rate and cell-specific activity in a coastal plankton community. Aquatic Microbial Ecology **16:** 27-35.

Smith, E. M., and P. A. del Giorgio. 2003. Low fractions of active bacteria in natural aquatic communities? Aquatic Microbial Ecology **31:** 203-208.

Smith, E. M., and W. M. Kemp. 1995. Seasonal and regional variations in plankton community production and respiration for Chesapeake Bay. Marine Ecology Progress Series **116:** 217-231.

---. 2003. Planktonic and bacterial respiration along an estuarine gradient: responses to carbon and nutrient enrichment. Aquatic Microbial Ecology **30:** 251-261.

Søndergaard, M., B. Hansen, and S. Markager. 1995. Dynamics of dissolved organic carbon lability in a eutrophic lake. Limnology and Oceanography **40:** 46-54.

Søndergaard, M., and M. Middelboe. 1995. A cross system analysis of labile dissolved organic carbon. Marine Ecology Progress Series **118:** 283-294.

Speiran, G. K., P. A. Hamilton, and M. D. Woodside. 1998. Natural processes for managing nitrate in ground water discharged to the Chesapeake Bay and other surface waters: more than forest buffers, p. 6. USGS.

Staley, J. T., and A. Konopka. 1985. Measurement of in Situ Activities of Nonphotosynthetic Microorganisms in Aquatic and Terrestrial Habitats. Annual Review of Microbiology **39:** 321-346.

Staver, K. W., and R. B. Brinsfield. 2001. Agriculture and Water Quality on the Maryland Eastern Shore: Where Do We Go from Here? BioScience **51:** 859-868.

Stribling, J. M., and J. C. Cornwell. 1997. Identification of important primary producers in a Chesapeake Bay tidal creek system using stable isotopes of carbon and sulfur. Estuaries **20:** 77-85.

---. 2001. Nitrogen, phosphorus, and sulfur dynamics in a low salinity marsh system dominated by *Spartina alterniflora*. Wetlands **21:** 629-638.

Strickland, J. D., and T. R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Bulletin of the Fisheries Research Board of Canada **167:** 1-310.

Sun, L., E. Perdue, J. Meyer, and J. Weis. 1997. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnology and Oceanography **42:** 714-721.

Tibbles, B. J. 1996. Effects of temperature on the incorporation of leucine and thymidine by bacterioplankton and bacterial isolates. Aquatic Microbial Ecology **11:** 239-250.

Tison, D. L., and D. H. Pope. 1980. Effect of temperature on mineralization by heterotrophic bacteria. Applied and Environmental Microbiology **39:** 584-587.

Toolan, T. 2001. Coulometric carbon-based respiration rates and estimates of bacterioplankton growth efficiencies in Massachusetts Bay. Limnology and Oceanography **46:** 1298–1308.

Touratier, F., L. Legendre, and A. Vézina. 1999. Model of bacterial growth influenced by substrate C:N ratio and concentration. Aquatic Microbial Ecology **19:** 105-118.

Tuomi, P., and P. Kuuppo. 1999. Viral lysis and grazing loss of bacteria in nutrient- and carbon-manipulated brackish water enclosures. Journal of Plankton Research **21:** 923-937.

Tuttle, J., R. Jonas, and T. Malone. 1987. Origin, development and significance of Chesapeake Bay anoxia, p. 442-472. *In* S. Majumdar, L. Hall, , Jr. and H. Austin [eds.], Conference 152. National Meeting AAAS: "Chesapeake Bay Fisheries and Contaminant Problems".

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry **10:** 109-122.

Valiela, I. 1995. Marine Ecological Processes. Springer-Verlag.

Vallino, J. J., C. S. Hopkinson, and J. E. Hobbie. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaces Monod growth kinetics. Limnology and Oceanography **41:** 1591-1609.

Vaqué, D., E. O. Casamayor, and J. M. Gasol. 2001. Dynamics of whole community bacterial production and grazing losses in seawater incubations as related to the changes in the proportions of bacteria with different DNA content. Aquatic Microbial Ecology **25:** 163-177.

Vrede, K., T. Vrede, A. Tisaksson, and A. Karlsson. 1999. Effects of nutrients (P,N,C) and zooplankton on bacterioplankton and phytoplankton - a seasonal study. Limnology and Oceanography **44:** 1616-1624.

Ward, L. G., M. S. Kearney, and J. C. Stevenson. 1998. Variations in sedimentary environments and accretionary patterns in estuarine marshes undergoing rapid submergence, Chesapeake Bay. Marine Geology **151:** 111-134.

Weil, R. R., R. A. Weismiller, and R. S. Turner. 1990. Nitrate contamination of groundwater under irrigated coastal plain soils. Journal of Environmental Quality **19:** 441-448.

White, P. A., J. Kalff, J. B. Rasmussen, and J. M. Gasol. 1991. The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. Microbial Ecology **21:** 99-118.

Whitledge, T. C., S. C. Mallory, C. J. Patton, and C. D. Wirick. 1981. Automated Nutrient Analysis in Seawater. Department of Energy and Environment.

Yokokawa, T., T. Nagata, M. T. Cottrell, and D. L. Kirchman. 2004. Growth rate of the major phylogenetic bacterial groups in the Delaware estuary. Limnology and Oceanography **49:** 1620-1629.

Zar, J. H. 1984. Biostatistical Analysis, Second ed. Prentice-Hall, Inc.

Zumdahl, S. S. 1989. Chemistry, 2nd ed. D. C. Heath & Company.