

The Effects of System-Level Nutrient Enrichment on Bacterioplankton Production in a Tidally-Influenced Estuary*

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ABSTRACT

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We describe the use of Monie Bay 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 subestuary of the Chesapeake Bay, consisting of a shallow semienclosed 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 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.

ADDITIONAL INDEX WORDS: *Bacterioplankton, bacterial production, nutrient loading, agricultural land use, estuary, Monie Bay.*

INTRODUCTION

Nonpoint 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 nutri-

ent 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 US 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.

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Coastal eutrophication is traditionally evaluated within the context of phytoplankton abundance (SMITH, LEFFLER, and MACKIERNAN, 1992), increases in ambient nutrient concentrations and organic matter loading (NIXON, 1995), and general increases in heterotrophic activity (TUTTLE, JONAS, and MALONE, 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 (DUCKLOW *et al.*, 1986; SHERR and SHERR, 1988; BRUSSAARD and RIEGMAN, 1998). 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 (TUTTLE, JONAS, and MALONE, 1987; SMITH and KEMP, 2001). The transfer of algal-derived organic matter to top consumers and the extent to which it is lost via respiratory processes are 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 are 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) are 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, MABERLY, and COOPER, 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 studied extensively, 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 might 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, FINDLAY, and PACE, 1988; DEL GIORGIO and COLE, 1998) may identify differences in the bacterioplankton community along an enrichment gradient, these data typically are 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 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 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 nonpoint sources of anthropogenic nutrient loading have been used successfully to evaluate the response of estuarine bacterioplankton to system-level nutrient enrichment (HOPPE, GIESENHAGEN, and GOCKE, 1998; REVILLA, IRIARTA, and ORIVE, 2000).

The Monie Bay component of the Chesapeake Bay National Estuarine Research Reserve in Maryland (CBNERRMD) 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, semienclosed 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, STRIBLING, and STEVENSON, 1994; JONES, MURRAY, and STEVENSON, 1997). Additional predictable variability in nutrient enrichment is introduced by the timing and nature of agricultural practices in each creek basin (CORNWELL, STRIBLING, and STEVENSON, 1994; JONES, MURRAY, and STEVENSON, 1997; FIELDING, 2003). Thus, nutrient concentrations, land use, and salinities in the three tidal creeks represent a broad range of conditions on relatively small spatial scales, making Monie Bay an ideal system for comparative investigations of the effect of agricultural nutrient enrichment on salt-marsh communities

(JONES, MURRAY, and STEVENSON, 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 article we describe the use of Monie Bay 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 *vs.* 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 (OB); and (4) evaluating conditions before, during, and after pulsed inputs of agriculturally derived nutrients.

METHODS

Site Description

Monie Bay is a tidally influenced subestuary located on the eastern shore of Chesapeake Bay (38°13'30" N, 75°50'00" W). The reserve consists of a relatively small (*i.e.*, 1- to 2-kilometers wide and 4-kilometers long) open bay (OB) and three tidally influenced creeks varying in size and agricultural land use (Figure 1). Monie Creek (MC) has the largest of the three creek watersheds (45 square kilometers), covering approximately 2.5 times and 5 times more area than those of Little Monie Creek (LMC; 17.9 square kilometers) and Little Creek (LC; 9.4 square kilometers), respectively. The linear reach of MC from headwaters to the OB is 6.5 kilometers, compared with 3.7 kilometers for LMC and 2.9 kilometers for LC. The creek channels in these systems are the result of tidal scouring, with no significant fluvial input (WARD, KEARNEY, and STEVENSON, 1998). Monie Creek experiences year-round inputs of fresh water, whereas LMC and LC have salinities driven entirely by tidal flushing from the OB and seasonal or episodic freshwater inputs occurring predominantly in the spring or following major rain events

(JONES, MURRAY, and STEVENSON, 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 (Figure 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, MURRAY, and STEVENSON, 1997) that are ascribed to agricultural activities (*i.e.*, crop farming, livestock and poultry operations) within the watershed (CORNWELL, STRIBLING, and STEVENSON, 1994). In comparison, LC watershed is dominated by tidal marsh; approximately one-third of the watershed is forested. Residential development in the LC watershed is minimal and similar to that of other creeks (*i.e.*, $\leq 3\%$), 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 that experiences less frequent flooding (KEARNEY, STEVENSON, and WARD, 1994; STRIBLING and CORNWELL, 1997; WARD, KEARNEY, and STEVENSON, 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, MURRAY, and CORNWELL, 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 OB

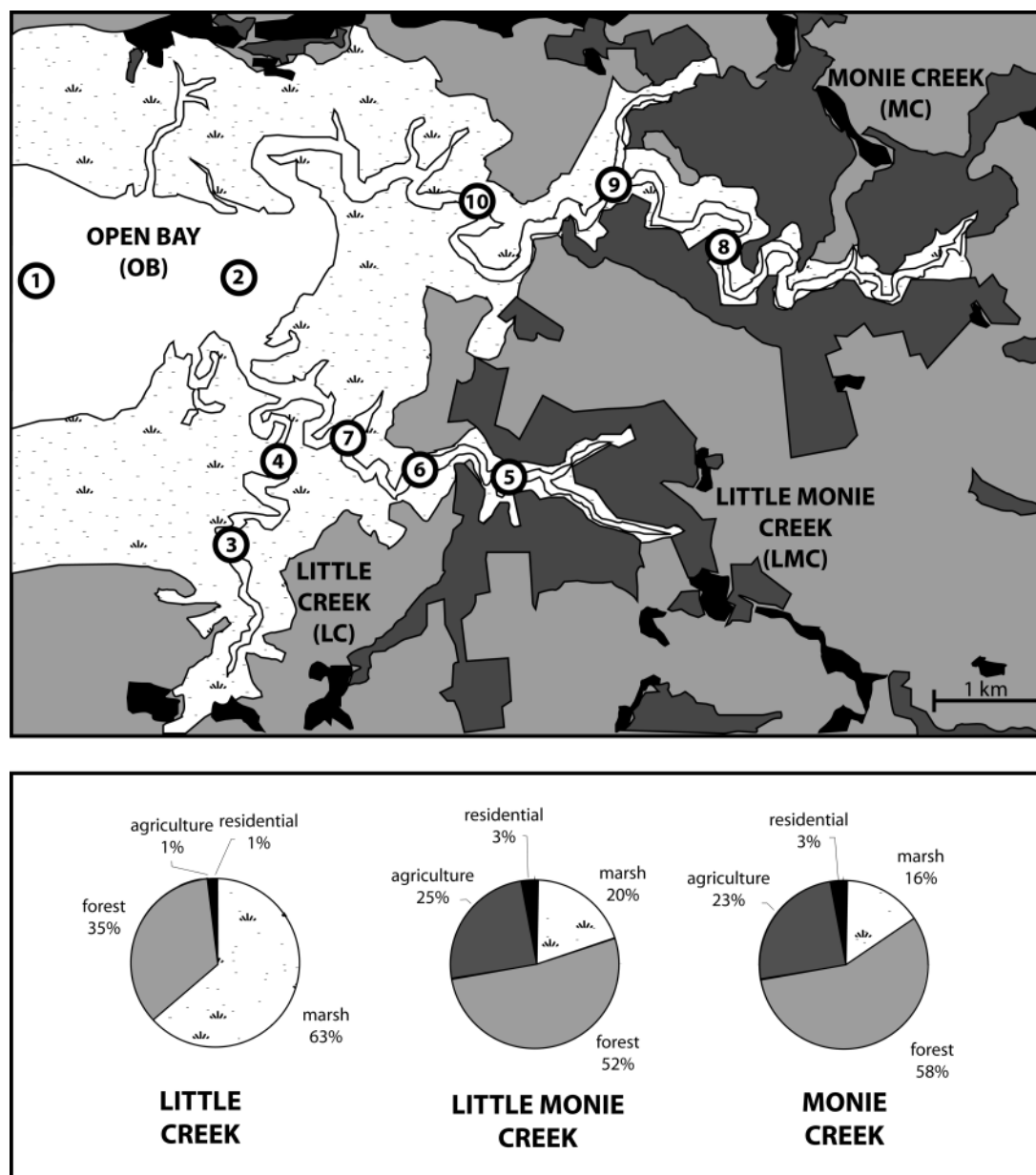


Figure 1. Monie Bay National Estuarine Research Reserve System with location and number of each sampling station (upper panel) and proportion of each watershed attributed to one of four land-use categories (lower panel).

waters and associated bacterioplankton communities. The resulting changes in the bacterioplankton community in each tidal creek relative to the OB are assumed to be a response to the environ-

mental 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 pos-

sible that top-down effects of grazers may also have an impact on estuarine bacterioplankton communities (GONZALEZ, SHERR, and SHERR, 1990; RIEMAN *et al.*, 1990; DEL GIORGIO *et al.*, 1996b). By sampling each creek system on the ebb tide, we were able to capture the metabolic response of bacterioplankton to these changing conditions, given that 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, STRIBLING, and STEVENSON, 1994; JONES, MURRAY, and CORNWELL, 1997) to define a range of comparisons (Figure 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 1). Differences between creek systems were identified using analysis of variance (ANOVA), results from which were used to classify the status of the three creeks (Figure 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, MURRAY, and CORNWELL, 1997)—were used to investigate the roles 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 OB. Changes in environmental conditions and the bacterioplankton community along this axis were identified by regressions of salinity *vs.* 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 *vs.* 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 (Figure 2), and can be in the form of pulsed enrichment events associated with the timing of agricultural nutrient applications within the watershed (CORNWELL, STRIBLING, and STEVENSON, 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 postenrichment 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 as model effects.

Sample Collection and Estimates of Bacterial Abundance and Production

We established 10 sites in the OB and tidal tributaries of Monie Bay (Figure 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 mon-

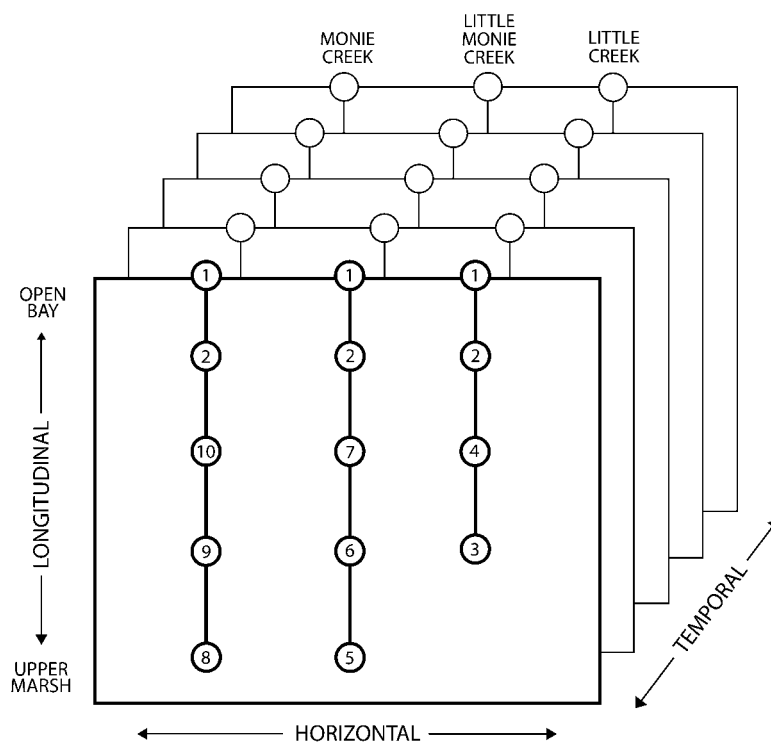


Figure 2. Diagram of experimental approach used in Monie Bay. Comparisons were made in three dimensions, including longitudinal (transects along the creek axis), horizontal (comparisons among creek systems), and temporal (seasonal or event-based comparisons).

itoring and research projects (JONES, MURRAY, and CORNWELL, 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 liters of subsurface (<0.5 meters) 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 laboratory, a small subsample 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 3H-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 AP15 Millipore (Billerica, MA) filter (~1 micrometer) using a peristaltic pump, then incubating the sample 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 kilograms 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).

Table 1. Nutrient concentrations, biological parameters, and watershed characteristics for the three tidal creeks and open bay. Table values are derived from 2-year means \pm SE (n).

	MC	LMC	LC	OB	n
Depth (m)	3.3 \pm 0.2 (29)	3.1 \pm 0.2 (25)	2.0 \pm 0.1 (18)	2.2 \pm 0.1 (14)	86
TDN (μ M)	40.6 \pm 2.1 (65)	40.1 \pm 2.8 (58)	26.8 \pm 1.6 (38)	28.1 \pm 1.7 (40)	201
TDP (μ M)	0.7 \pm 0.08 (65)	0.8 \pm 0.12 (58)	0.2 \pm 0.03 (38)	0.3 \pm 0.03 (40)	201
DON (μ M)	36.7 \pm 1.7 (65)	35.6 \pm 1.9 (58)	21.6 \pm 1.9 (38)	19.8 \pm 1.8 (40)	201
NH ₄ ⁺ (μ M)	2.3 \pm 0.314 (65)	3.0 \pm 0.428 (58)	2.1 \pm 0.2 (38)	1.7 \pm 0.178 (40)	201
NO _x (μ M)	4.5 \pm 0.859 (65)	4.8 \pm 1.045 (58)	3.1 \pm 0.64 (38)	6.6 \pm 1.299 (40)	201
PO ₄ ³⁻ (μ M)	0.2 \pm 0.05 (65)	0.3 \pm 0.065 (58)	0.2 \pm 0.1 (37)	0.0 \pm 0.006 (40)	200
Salinity	6.9 \pm 0.4 (75)	9.9 \pm 0.3 (62)	11.6 \pm 0.3 (38)	12.1 \pm 0.3 (42)	217
DOC (mg L ⁻¹)	11.5 \pm 0.5 (71)	8.9 \pm 0.4 (59)	7.7 \pm 0.3 (36)	6.0 \pm 0.2 (39)	205
DOC:TDN	24 \pm 2 (62)	20 \pm 1 (55)	27 \pm 2 (36)	20 \pm 1 (37)	190
TDN:TDP	103 \pm 22 (60)	84 \pm 8 (55)	170.0 \pm 27 (34)	130 \pm 15 (35)	184
<i>a</i> ₃₅₀ ^{§1}	20 \pm 0.7 (43)	17 \pm 0.7 (35)	15 \pm 1 (19)	12 \pm 0.9 (22)	119
BA (10 ⁶ cells mL ⁻¹)	11.8 \pm 0.7 (74)	13.3 \pm 1.0 (62)	12.8 \pm 1.4 (35)	11.5 \pm 1.0 (42)	213
BP (μ g C L ⁻¹ h ⁻¹)	1.8 \pm 0.1 (59)	2.6 \pm 0.2 (48)	1.5 \pm 0.1 (29)	1.1 \pm 0.1 (33)	169
Filtered BP (μ g C L ⁻¹ h ⁻¹) ^{§3}	1.0 \pm 0.1 (59)	1.4 \pm 0.1 (48)	1.0 \pm 0.2 (29)	0.6 \pm 0.1 (33)	169
% filtered BP ^{§2}	55.6 (59)	53.8 (48)	66.7 (29)	54.5 (33)	169
Watershed size (km ²)	45	17.9	9.4	72.3	
Agriculture ^{§4}	23	25	<1	16	

^{§1} Specific absorbance at 350 nm \times 10³.

^{§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 composed 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, NO_x = NO₃⁻ + NO₂⁻, DOC = dissolved organic carbon, BA = total bacterial abundance, BP = total bacterioplankton production.

Nutrients and Other Analyses

Filtered samples for DOC analysis were acidified with 100 microliters of 1 normal phosphoric acid and held at 4°C until analysis. DOC content was determined with a Shimadzu high-temperature catalytic carbon analyzer (Shimadzu Corporation, Kyoto, Japan) (SHARP *et al.*, 1995). Samples for nutrient analyses were filtered through a Whatman GF/F filter (Whatman Inc., Clifton, NJ) and frozen at -25°C for later analysis of phosphate (*i.e.*, PO₄³⁻, soluble reactive phosphorus), nitrite (NO₂⁻), and nitrate (NO₃⁻) following STRICKLAND and PARSONS (1972); total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) following VALDERRAMA (1981); and ammonium (NH₄) following WHITLEGE *et al.* (1981). Dissolved organic nitrogen (DON) was determined as the difference between TDN and DON 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). Absorptiv-

ity at 350 nm (*a*₃₅₀) was used as an index of CDOM concentrations. Specific absorbance (*a*₃₅₀^{*}) was determined by dividing *a*₃₅₀ by ambient DOC concentrations (MORAN, SHELDON, and ZEPP, 2000; HU, MULLER-KARGER, and ZEPP, 2002). 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, agricultural, 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 the US Geological Survey 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).

	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
SALINITY					<0.0001	216
DOC					<0.0001	204
a ₃₅₀ *					<0.0001	118
BP					0.005	168
filtered BP					0.02	168
BP/cell					<0.0001	137

Figure 3. Horizontal comparisons of 2-year means among systems. For each parameter, bar height represents the magnitude of the 2-year mean. Means that are statistically similar share the same bar height. (ANOVA and Tukey-Kramer HSD, $\alpha=0.05$). Parameters are defined in Table 1.

Statistical Analyses

All statistical analyses, including standard least squares regressions, one- and two-way ANOVA, and principal components analysis (PCA) were performed using the JMP 5.0.1 statistical software package (SAS Institute, Inc., Cary, North Carolina). The entire composite dataset was used for all statistical analyses, except ANOVAs comparing system-specific means (Figure 3, Table 2). In these instances, each system was characterized by values observed at the uppermost two sites in each creek (*i.e.*, sites 3 and 4, 5 and 6, and 8 and 9 in LC, LMC,

and MC, respectively). Because of significant differences in rainfall and salinity among years, regression statistics for longitudinal comparisons with salinity as the independent variable (*i.e.*, Table 3) were performed on data from year 1 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 1). We observed significant differences in these measured parameters among creek

Table 2. Probability values from two-way ANOVA tests with system and season as model effects.

Parameter	Model Effects			n
	System	Season	Interaction	
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 and 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 ($p > 0.05$).

systems and along longitudinal creek transects (Figure 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.*, Figure 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 1). A statistical comparison of 2-year means (Figure 3; ANOVA; Tukey-Kramer honest significant difference [HSD]; $\alpha=0.05$) 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 1) in MC and LMC suggests that these systems are disproportionately enriched with phosphorus relative to nitrogen when compared with LC and OB. Mean salinity decreased with increasing watershed size, and there was a consistent hierarchy among the creeks (MC<LMC<LC<OB; Table 1, Figure 3). Both colored and total DOC 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 (Figure 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 *vs.* other measured parameters (Table 3). 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 Figure 4. There were no significant changes in nutrients along the axis of Little Creek, although the trend of decreasing BP was significant (Table 2).

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 2000 and April and September 2001 (Figure 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

Table 3. Regression statistics for the relationship between salinity and biological parameters for the uppermost two sites in each creek (year one only; n = 12).

Parameter	Monie Creek			Little Monie Creek			Little Creek			
	Slope	r ²	F ratio	Slope	r ²	F ratio	Slope	r ²	F ratio	p
Total dissolved nitrogen	-4.8	0.37	7.1	-5.1	0.52	10.9	-1.5	nr	0.33	ns
Total dissolved phosphorus	-0.2	0.45	9.9	-0.1	0.44	7.7	0.03	nr	0.83	ns
Dissolved organic nitrogen	-3.1	0.39	7.7	-3.6	0.58	13.9	-0.4	nr	0.05	ns
Ammonia	-0.3	0.12	1.7	-0.4	0.16	1.8	-0.3	0.14	1.7	0.2
Nitrate and nitrite	-1.5	0.28	4.6	-1.1	0.22	2.9	-0.8	nr	0.45	ns
Phosphate	-0.1	0.50	12.0	-0.1	0.52	10.9	-0.01	0.26	3.1	0.1
Dissolved organic carbon	-1.5	0.56	15.1	-1.2	0.79	38.4	-1.0	0.48	9.4	0.01
Specific absorbance (a ₃₅₀ ^{*)}	-0.001	0.48	18.2	-0.001	0.33	11.0	-0.0004	0.15	2.9	0.1
Total bacterial production	-0.2	0.16	3.6	-0.8	0.89	71.6	-0.4	0.51	10.3	0.009
Filtered bacterial production	-0.1	nr	1.6	-0.6	0.80	32.1	-0.3	0.34	5.1	0.05
Total bacterial abundance	—	nr	—	—	nr	—	—	nr	—	ns

nr = no relationship (r² < 0.1), ns = not significant (p > 0.05).

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 interannual variability, the pattern of nutrient concentrations among creeks persisted such that TDP and TDN were always higher in the agriculturally influenced creeks relative to LC and OB for all dates sampled. Bacterial production was always highest in LMC, higher in all creeks than in the OB, and generally followed seasonal patterns in temperature (Figure 5, lower panel).

We evaluated differences in 2-year means among seasons using ANOVA and Tukey-Kramer HSD (Figure 6). Spring was characterized by lower salinity and higher TDN and NO_x ($\text{NO}_2^- + \text{NO}_3^-$) 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 measurements 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). 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 >0.8) for DOC,

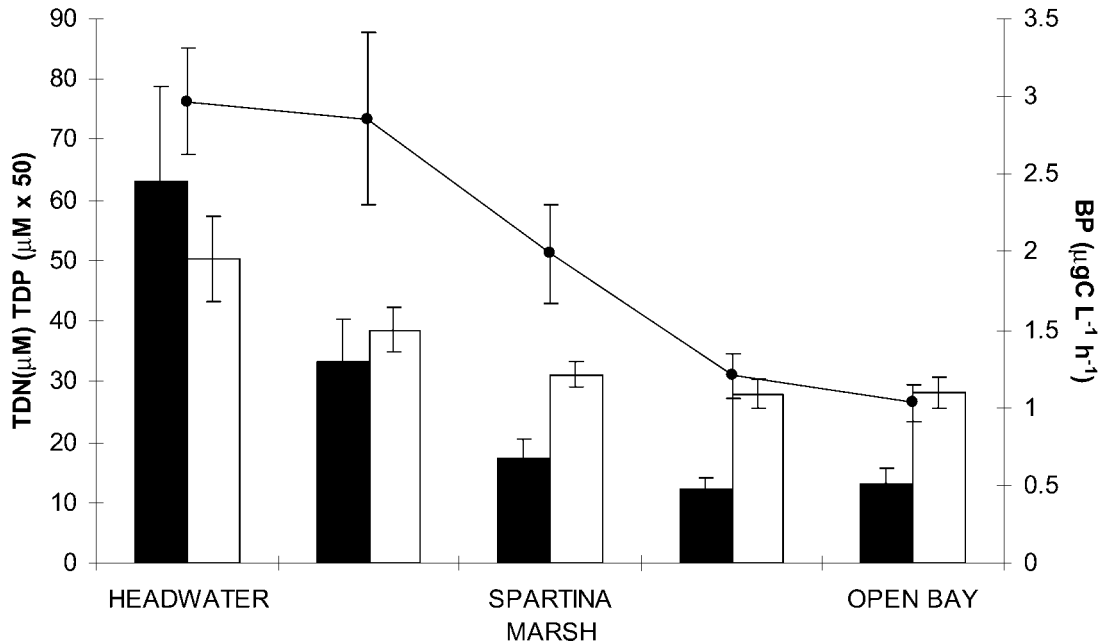


Figure 4. Transect of ambient nutrient concentrations (total dissolved phosphorus [TDP] and total dissolved nitrogen [TDN]) and total bacterial production (BP) along the axis of agriculturally impacted Little Monie Creek (LMC) and open bay (OB; each point represents 2-year mean \pm SE, $n=21$).

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 (Figure 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 (Figure 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 Figure 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 OB (LMC > MC > LC > OB; Table 1). Bacterial production in LMC (2.6 ± 0.2 micrograms C liter⁻¹ h⁻¹) was significantly higher than that of LC and OB (1.5 ± 0.1 and 1.1 ± 0.1 micrograms C liter⁻¹ h⁻¹, respectively). Although BP in MC (1.8 ± 0.2 micrograms C liter⁻¹ h⁻¹) was higher than that of LC, this difference was not significant (Figure 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 1). Bacterial abundance was higher in LMC and LC and lower in MC and OB (Table 1),

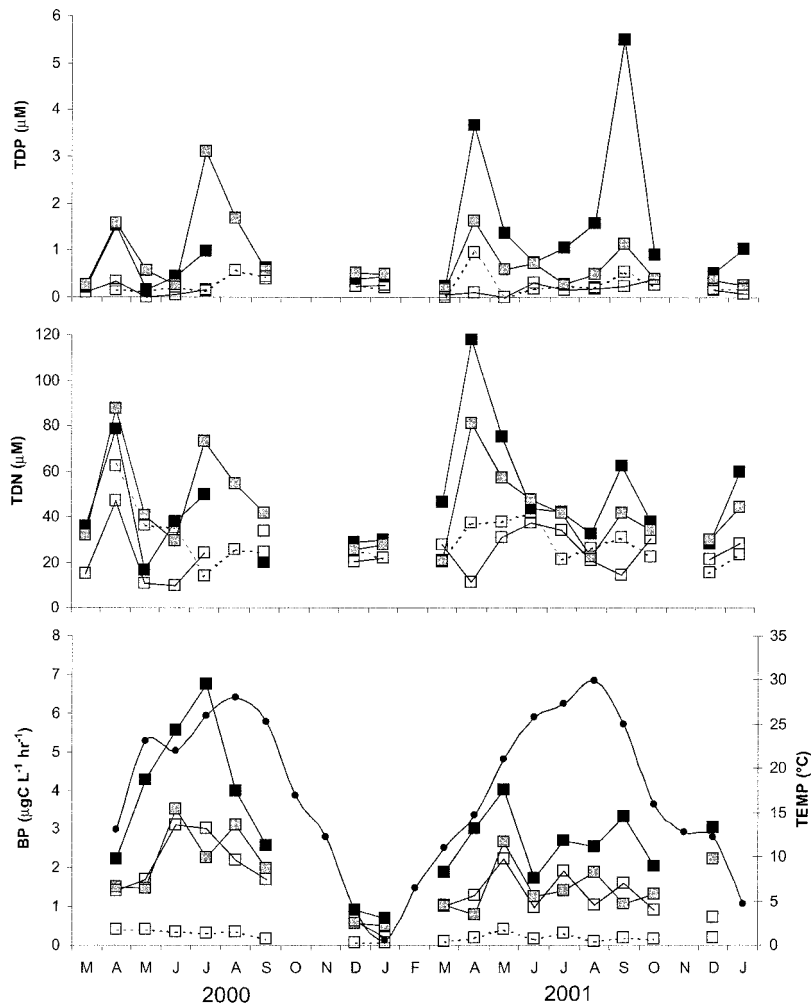


Figure 5. Two-year seasonal variability in total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), total bacterial production (BP), and temperature (TEMP) among the sub-systems of Monie Bay.

although these differences were not significant. Bacterial production decreased from the upper estuary to the OB in all creeks, with the largest and most significant change in LMC (Figure 4, Table 3). 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 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 OB 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.

	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
NO _x					<0.0001	200
PO ₄ ³⁻					0.04	199
SALINITY					<0.0001	216
DOC					<0.0001	204
a350*					0.003	118
TEMP					<0.0001	216
BP					0.008	168
BA					<0.0001	168

Figure 6. Comparisons of seasonal means for environmental and biological parameters measured over the 2-year sampling period. For each parameter, bar height represents the magnitude of the 2-year mean. Means that are statistically similar share the same bar height. (ANOVA and Tukey-Kramer HSD, $\alpha=0.05$). Parameters are defined in Table 1.

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 of 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 (Figure 3) and freshwater inputs (JONES, MURRAY, and CORNWELL, 1997), MC and LMC are remarkably similar with respect to the dynamics of nutrient delivery (Figures 5 and 7). Measured nutrient concentrations among the three creeks exhibited an identical pattern and similar magnitude to those observed by JONES, MURRAY, and CORNWELL (1997), reaffirming the robust

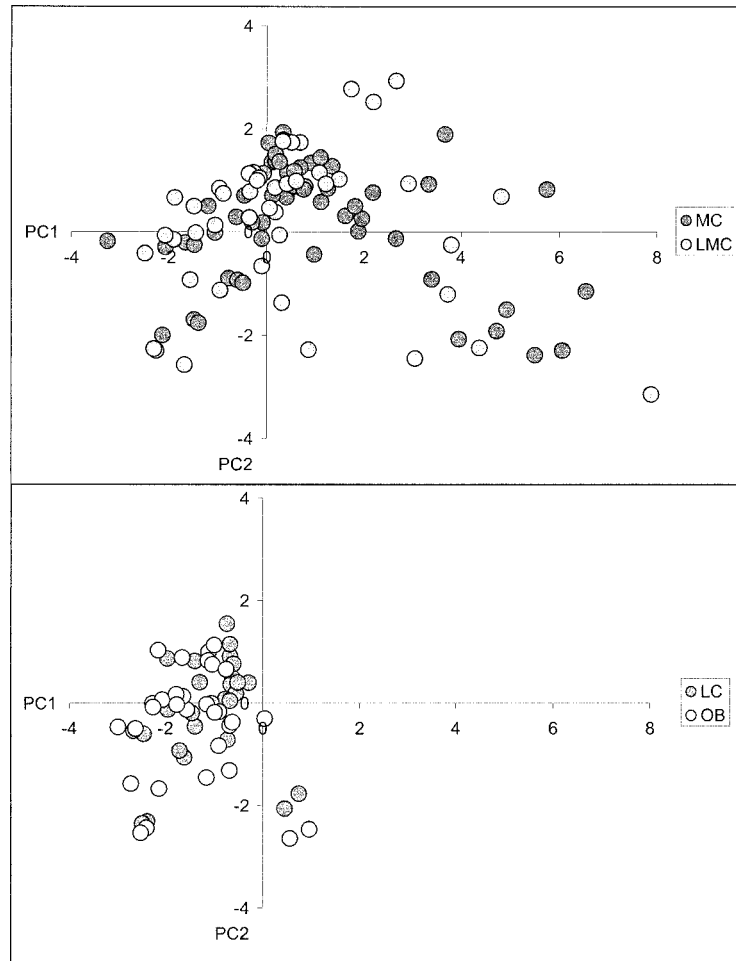


Figure 7. Principal components analysis of each sampling event with loadings on PC1 and PC2. Sampling events are separated by system: Monie Creek and Little Monie Creek (upper panel) and Little Creek and the open bay (lower panel; $n=160$).

nature of spatial patterns of nutrient enrichment in Monie Bay.

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; STAV-ER and BRINSFIELD, 2001). The timing of these periods of enrichment (Figure 5) coincides 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, Somerset 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 (SPEIRAN, HAMILTON, and WOODSIDE, 1998; NORTON and FISHER, 2000). CORNWELL, STRIBLING, and STEVENSON (1994) and JONES, MURRAY, and CORNWELL (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

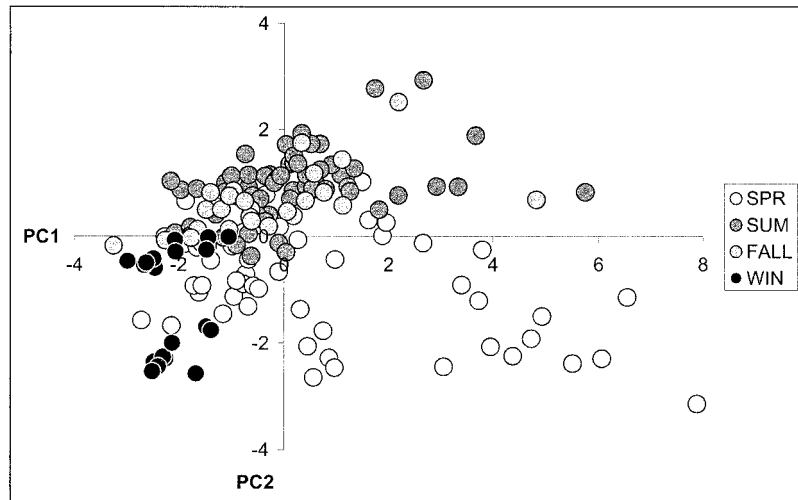


Figure 8. Principal components analysis of each sampling event with loadings on PC1 and PC2. Sampling events are identified by season ($n=160$).

loading of salinity and positive loading of dissolved nutrients on PC1 indicate 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 (Figure 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, SIMARD, and JOERN (1998) observed environmentally significant inputs of phosphorus via subsurface flow in systems 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 and WOLF, 1994; SIMS, SIMARD, and JOERN, 1998).

The importance of freshwater inputs in driving nutrient delivery in this system suggests that there will also be distinct patterns in nutrient enrichment among seasons. We observed significant-

ly 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). 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 (Figure 1), to lower concentrations near the bay (Table 3, Figure 4) was corroborated by other investigators (CORNWELL, STRIBLING, and STEVENSON, 1994; JONES, MURRAY, and CORNWELL, 1997). JONES, MURRAY, and CORNWELL, (1997) specifically report a doubling of nitrogen and phosphorus concentrations along a transect from the OB 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 (Figure 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 (WEIL, WEISMILLER, and TURNER, 1990; SPEIRAN, HAMILTON, and WOODSIDE, 1998). 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 (WEIL, WEISMILLER, and TURNER, 1990; SPEIRAN, HAMILTON, and WOODSIDE, 1998). 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 (Figures 3 and 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, MURRAY, and CORNWELL (1997) report extremely low N:P ratios in LMC, and attribute 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, MURRAY, and CORNWELL, 1997). It is clear that this is a persistent if not long-term effect, given that we observed the same pattern in N:P ratios among the tidal creeks (lowest in LMC; Table 1) almost a decade after the original 1994 field work of JONES, MURRAY, and CORNWELL (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 of marsh macrophyte productivity and biomass (JONES, MURRAY, and CORNWELL, 1997), as well as sediment biogeochemistry and nutrient cycling (CORNWELL, STRIBLING, and STEVENSON, 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 OB (Table 1) suggest 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 (HOCH and KIRCHMAN, 1993; GOOSEN *et al.*, 1997; REVILLA, IRIARTA, and ORIVE, 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, HERZIG, and HERNDI, 1999). Our observation of higher rates of BP and DOC concentrations in LC relative to OB (Table 1), consistently higher BP in LC *vs.* OB at all sampling events (Figure 5), and a significant increase in both DOC and BP along the axis of LC (Table 3), 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 1, Figure 3) indicate that these increases in BP are driven by

changes in the quality and quantity of DOM and POM substrates associated with natural marsh processes (SHIAH and DUCKLOW, 1995; GOOSEN *et al.*, 1997), 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 (JONES, MURRAY, and CORNWELL, 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, MURRAY, and CORNWELL (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 (Figure 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 size of LMC is only twice that of LC (Table 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, MURRAY, and CORNWELL (1997) report similar findings, with phosphorus concentrations in LMC being fourfold 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, MURRAY, and CORNWELL (1997) report and we observed concentrations twofold to threefold 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 (WEIL, WEISMILLER, and TURNER, 1990; SPEIRAN, HAMILTON, and WOODSIDE, 1998) or the influx of nitrogen-laden waters from the OB during periods of nitrogen loading to the entire system. For example, when nutrient-rich water from MC and LMC is transported to the OB 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 (Figure 5). In their evaluation of nutrient concentrations over the course of a tidal cycle, JONES, MURRAY, and CORNWELL (1997) found the highest nutrient concentrations in LC at high tide, further suggesting delivery of dissolved nitrogen from the OB. 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 (63% vs. 30%, respectively; Figure 1), it is also possible that natural marsh processes may contribute to differences in nutrients between these systems. CORNWELL, STRIBLING, and STEVENSON (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 1), creek transects (Table 3), and data from JONES, MURRAY, and CORNWELL (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, MURRAY, and CORNWELL (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 interannual variability in BP and nutrient concentrations, we observed consistently higher rates of bacterial production in LMC relative to LC throughout the year (Figure 5), when 2-year means from these systems were compared (Figure 3), and when the nutrient-enriched upper estuary of LMC was compared with sites nearer the OB (Figure 4).

In addition, although we did not observe a significant difference in bacterioplankton abundance between these two creeks (Table 1), cell-specific production (*i.e.*, bacterial production per individual cell) in LMC was significantly higher than that of all other systems (Tukey-Kramer HSD; $\alpha=0.05$; $p<0.0001$; $n=137$; Table 1, Figure 3). 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 (DEL GIORGIO and SCARBOROUGH, 1995; CHOI, SHERR, and SHERR, 1999). A comparison of LC and LMC revealed that total BP in LC was dominated by the filtered fraction (<1 micrometer) relative to LMC (67% *vs.* 54%, respectively). This difference in filtered *vs.* 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, MURRAY, and CORNWELL, 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, BAROSS, and SIMENSTAD, 1998; CRUMP and BAROSS, 2000) that has far-reaching implications with respect to our ability to accurately assess carbon flux in natural aquatic systems (BIDDANDA, OGDahl, and COTNER, 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 that of LC when 2-year means and individual sampling events were considered (Figures 3 and 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 1) and $\delta^{13}\text{C}$ 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 1, Figure 3) would ostensibly suggest that bacterioplankton production should not be carbon limited in this system (BAINES and PACE, 1991; VALLINO, HOPKINSON, and HOBBIE, 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, MURRAY, and CORNWELL, 1997) are accompanied by an increase

in the input of terrestrially derived organic matter, as evidenced by measurements of CDOM (Table 1, Figure 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 (Figure 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, CARON, and DENNET, 1987) and lower rates of bacterial production (MORAN and HODSON, 1990; AMON and BENNER, 1996). 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, 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 kilometers, bacterioplankton communities in MC may be exposed to a salinity range from $<1\text{‰}$ in the upper estuary to $>13\text{‰}$ in the OB (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 (SHERR and SHERR, 1988; JORGENSEN *et al.*, 1999; KIRCHMAN, 2000), 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, ARMBRUST, and BAROSS, 1999; DEL GIORGIO and BOUVIER, 2002). In turn, phylogenetic composition of natural bacterial assemblages has been linked to bacterioplankton metabolic properties (PINHASSI *et al.*, 1999; BOUVIER and DEL GIORGIO, 2002) and even the use of specific organic substrates (COTTRELL and KIRCHMAN, 2000). Thus, we predict that differences in the metabolic response of bacterioplankton to nutrient enrichment of MC *vs.* 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, Figures 3, 4, and 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, MURRAY, and CORNWELL, 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 (Figure 5). Similarly, BP was significantly higher in July relative to September (6.8 *vs.* 2.6 micrograms C liter⁻¹ h⁻¹, respectively). The same comparison of BP in MC during these sum-

mer months revealed a muted response of bacterioplankton to nutrient enrichment that has become characteristic of this particular creek system (Table 1, Figure 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 (2.2 vs. 2.0 μM ; Figure 5). 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 micrograms C liter⁻¹ h⁻¹), although this was probably an effect of higher temperature (28.0°C) on BP (SHIAH and DUCKLOW, 1994). 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 (90 and 4 μM , respectively; Figure 5), 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 1). Although a comparison of BP between April and May (2.2 and 4.3 micrograms C liter⁻¹ h⁻¹, respectively) does not initially indicate a positive response to nutrients, SHIAH and DUCKLOW (1994) 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 nonsummer months. The authors report an average Q_{10} value of 2.7 (± 0.3) for bacterial growth in the temperature range of 3–25°C. We hypothesized that the bacterioplankton community in April may have been constrained by temperature and therefore was less responsive to system-level nutrient enrichment. This is further supported by the general coherence of temperature and BP when monthly sampling (Figure 5) and comparison of seasonal means (Figure 6) are considered. Using the reported Q_{10} value of SHIAH and DUCKLOW (1994) and a difference in ambient water temperature for April and May of 10°C (13°C vs. 23°C, respectively; Figure 5), we calculated temperature-corrected estimates of BP for these 2 months and estimated that BP in April would have been elevated relative to that of May (6.0 vs. 4.3 micrograms C liter⁻¹ h⁻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 im-

portant environmental factor mediating the magnitude of this response and should be considered in seasonal comparisons of BP in temperate systems.

CONCLUSIONS

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, 2000). However, not all systems responded to nutrient enrichment in a similar manner, and freshwater inputs and/or salinity play 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 drives 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 (DEL GIORGIO and SCARBOROUGH, 1995; CHOI, SHERR, and SHERR, 1999) and community (VREDE *et al.*, 1999; PACE and COLE, 2000) 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 identify unequivocally 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 bacterio-

plankton 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, WEISBERG, and RANASINGHE, 2000). The metabolic response of bacterioplankton to system-level nutrient enrichment is not as well documented (HOPPE, GIESENHAGEM, and GOCKE, 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 during 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 multifaceted index of ecosystem function that reflects changes in system-level processes on multiple spatial and temporal scales.

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