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Organic matter cycling in the York River estuary, Virginia: An analysis of potential sources and sinks

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Organic Matter Cycling in the York River Estuary, Virginia: An Analysis of Potential Sources and Sinks

A Dissertation
Presented to
The Faculty of the School of Marine Science
Virginia Institute of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Leigh McCallister
2002
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

S. Leigh McCallister

Approved, October 2002

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University of Quebec, Montreal, Canada
To my high school science teachers, for instilling curiosity
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Approach

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ABSTRACT

A study of the sources of organic matter (OM) and the biogeochemical and physico-chemical sinks was undertaken in the York River estuary, Virginia. The reactivity of dissolved organic carbon (DOC) was enhanced from -25 to as much as -68% by the combined effects of exposure to natural sunlight and bacterial decomposition. In contrast, sunlight exposure decreased the bioreactivity of DOC in the higher salinity lower York by a factor of five. The combined effects of photochemical and bacterial processing was found to modify both the bioavailability and metabolic fate of OM (e.g. respiration vs. biomass). Although bacterial decomposition of DOC was frequently about two times greater in sunlight-exposed incubations compared to non-irradiated controls, there were no parallel enhancements in the microbial utilization of corresponding DON and DOP fractions.

Stable isotopic (δ13C, δ15N) and radiocarbon (Δ14C) values of bacterial nucleic acids were used to estimate the sources and ages of OM assimilated by bacteria in the York and Hudson River estuaries. Bacterial production in freshwater regions of the York was fueled by OM of young (decadal in age), terrigenous origin and it was estimated to account for 42-89% of OM assimilated. The remainder (11-58%) of OM supporting bacterial growth was derived from freshwater algae. In the mid-salinity York, bacterial production was supported primarily by phytoplankton-derived OM in the spring and summer (93-100%) and marsh derived OM in the fall (73-100%). Isotopic values of bacteria in higher salinity regions of the estuary suggested that production was supported by phytoplankton-derived OM (86-100%) in July and November whereas a more balanced mixture of algal and marine-like OM (50-69%) was assimilated in October. In contrast to the young (10-20 yr) OM supporting bacterial production in the York, production in the Hudson River estuary was subsidized by a substantial portion of old (~1200 BP) terrigenous OM.

Differences in the sources and cycling of particulate organic matter (POM) and high molecular weight dissolved organic matter (HMW DOM (≥ 3kDa)) were assessed along the York using lipid biomarkers, C:N ratios, and stable isotopes (δ13C and δ15N) analyses. Higher C:N ratios, lower δ13C and δ15N values and depletions of total lipid and lipid compound classes in HMW DOM relative to POM, suggested differences in the reactivity and cycling of these two OM fractions. Within the dissolved pool, polyunsaturated fatty acids (FA) were a good predictor of DOC decomposition in bioassays. Concentrations of sterols, diagnostic for terrigenous sources, were enhanced in the HMW DOM relative to the POM in more saline regions of the estuary. Ternary plots based on FA biomarkers show that POM is dominated by FA derived from phytoplankton/zooplankton sources, while HMW DOM FA have bacterial and vascular plant signatures. Thus, the physical form of OM (particulate vs dissolved) may affect both the distribution and biogeochemical processing of OM such that terrigenous DOM appears to be exported from rivers while POM is retained within the estuary.
DISSOLVED ORGANIC MATTER CYCLING IN THE YORK RIVER ESTUARY, VIRGINIA: AN ANALYSIS OF POTENTIAL SOURCES AND SINKS
Chapter I
PROJECT OVERVIEW

Background and Justification

The relative importance of allochthonous and autochthonous organic matter (OM) sources and their respective biological and physico-chemical sinks in rivers and estuaries is critical for a fundamental understanding of these biogeochemical gateways linking land and sea. Rivers and estuaries may also be in close proximity to human population centers and thus integrate the effects of both natural and anthropogenic factors during their transport and discharge to adjacent coastal seas. Currently however, the degree to which rivers and estuaries may moderate anthropogenic changes remains unclear. The reason for this ambiguity arises from intense physical, chemical, and biological processing in rivers and estuaries which homogenizes the varied inputs of organic matter and obscures source appropriations (Cloern et al., 2002; Findlay et al., 1998). Consequently the sources and quantities of autochthonous and allochthonous OM fueling bacterial metabolism within estuarine systems and the origins and bioreactivity of material subsequently exported remain largely unresolved.

The effects of long-term changes in land use patterns (clear cutting, agriculture, run-off etc.) are also focused in the coastal zone (Houghton et al., 1999). Organic matter delivery to rivers and estuaries has changed as a result of alterations in freshwater delivery, water diversions, proliferation of river catchments as well as modifications in land use. At present it is difficult to predict the consequences of this unprecedented
alteration in the water and elemental cycles of these systems. Even more ambiguous are
the long-term impacts of these changes on biogeochemical processes in rivers and
estuaries. Undoubtedly these system-wide shifts have fundamentally altered both the
magnitude and composition of riverine organic matter exported to rivers and estuaries
thereby potentially shifting the balance between production and respiration in the water
column (Smith and Hollibaugh, 1993; Hopkinson and Vallino, 1995; Howarth et al.,
1996). The results of basin-wide environmental manipulations converge in estuaries and
underscore the necessity for a mechanistic understanding of factors establishing present-
day ecosystem metabolic balance as a result of both natural and human-related activities
(Ver et al., 1999).

Bulk organic matter pools entering an estuary may potentially be modified in a
number of ways, including: chemically through flocculation (Fox, 1983; Sholkovitz et al.,
1978) and photolytic reactions (Miller and Moran, 1997; Amon and Benner, 1996; Kieber
1989); biologically through autochthonous production (Peterson et al., 1994; Fisher et al.,
1998; Raymond and Bauer, 2001), and heterotrophic modifications (Brophy and Carlson,
1989) and respiration (Findlay et al., 1992; Moran et al., 1999); and physically through
homogenization of spatially distinct sources (Cloern et al., 2002; Findlay et al., 1998) and
sedimentation (Hedges et al., 1992; Prahl et al., 1994). These dynamic and interacting
biogeochemical and physico-chemical processes act on numerous allochthonous
(terrestrial vascular plant and soils, oceanic dissolved organic matter) and autochthonous
(phytoplankton, benthic algae, salt marsh macrophytes, fresh and salt water grasses) OM
sources in estuaries whose magnitude and relative contributions vary in both space and
time. Consequently estuaries remain one of the most challenging ecosystems in which to trace the origins, transformations and fates of organic matter.

The transport and fate of terrigenous OM, which comprises the vast majority of riverine material, is in particular ultimately modulated by these biogeochemical gateways. The efficiency of rivers and estuaries in retaining terrestrial derived OM (particulate and dissolved) is a critical parameter for constraining global C budgets (Stallard 1998; Hedges et al., 1997). Globally, rivers deliver 0.25 Pg of dissolved organic carbon (DOC) per year to the ocean (Meybeck, 1982; Hedges, 1992; Hedges et al., 1997). Several studies have reported conservative transport of DOC through estuaries (e.g. Laane and Koole, 1982; Sharp et al., 1982; Mantoura and Woodward, 1983; Ittekot 1989) suggesting a lack of significant removal by microbial and higher food webs. The highly condensed polymeric nature of allochthonous DOM in riverine and estuarine systems has perpetuated this view (Thurman, 1985). One of the great paradoxes in marine biogeochemistry, however, is the apparent absence of a terrestrial signature in open ocean DOM (Meyers-Schulte and Hedges, 1986; Druffel et al., 1992; Hedges et al., 1992; Opsahl and Benner, 1997; Hedges et al., 1997). Likewise, reports of net system heterotrophy in estuarine ecosystems (Smith and Hollibaugh, 1993; Heip et al., 1995; Frankignoulle et al., 1998; Raymond et al., 2000) require the utilization of some portion of this allochthonous material on time scales of estuarine mixing.

As the principal consumers of dissolved organic matter (DOM) (Pomeroy, 1974; Azam et al. 1983), heterotrophic bacteria are pivotal in apportioning its fates between assimilation into biomass, respiration (Findlay et al., 1992; Moran et al., 1999) and transformation to more recalcitrant forms (Brophy and Carlson, 1989) with subsequent
export to coastal seas. Although a clear delineation of OM cycling from its initial biological fixation to eventual respiration or export from a riverine/estuarine system is fundamental to a mechanistic knowledge of C flow in estuaries, our understanding has been hampered by numerous practical and analytical factors including: overlapping isotopic signatures of OM; mixing of co-equal sources of organic matter; seasonal variability in the inputs of autochthonous and allochthonous sources; and physical homogenization of OM sources (Canuel et al., 1995; Cloern et al. 2002).

**Study Objectives and Goals**

The primary objective of this research was to evaluate the sources and transformations of organic matter along a temperate coastal plain estuarine continuum. Understanding the role of estuaries in mediating seaward fluxes of significant quantities of allochthonous and autochthonous organic matter would benefit from a better understanding of 1) the fraction of total DOM utilized and/or added within an estuary 2) the sources of DOM supporting bacterial production and 3) the origin and diagenetic state of material exported to the coastal seas. Currently all of our available techniques to resolve OM cycling within estuaries have methodological limitations. Hence resolution of these aforementioned parameters with a *single* approach in estuaries has often proven equivocal.

The explicit goal of this research was to resolve both the sources of OM and the potential photochemical and microbial sinks for DOM along the estuarine salinity gradient, through the use of several complementary approaches including: measurements of bacterial ectoenzyme activity; bulk DOM stoichiometry; natural abundance stable and
radioisotope analyses of bacterial nucleic acids; and lipid biomarker composition of both particulate and dissolved OM. The system chosen for study was the York River estuary (VA, USA). Moreover given the recent recognition that biological, chemical, and physical processes act in concert rather than individually during the decomposition of organic matter in aquatic systems (Miller and Moran, 1997; Anderson & Williams, 1999; Moran et al., 1999), the effects of sunlight exposure on DOM bioavailability, bacterial growth and subsequent estuarine C cycling was also assessed.

**APPROACH**

**Study Site and Sampling Locations**

The York River estuary is a moderately stratified sub-estuary of Chesapeake Bay, created by erosion of a drowned river valley during the Pleistocene, and formed by the convergence of the Pamunkey (~80% of total freshwater input) and Mattaponi (~20% of total freshwater input) Rivers (Figure 1). Discharge from the York ranges from ~10-500 m$^3$ sec$^{-1}$ (Sin et al., 1999); however 1999-2000, when the majority of sampling for this study was conducted, were relatively dry years. The annual mean streamflow in 1999 (13.9 m$^3$ sec$^{-1}$) represented the 10 year minimum from 1990-2000. The Pamunkey is considerably narrower than the estuary proper and has extensive freshwater marshes, some greater than 1,000 acres in size (Silberhorn, 1987), in the upper reaches. Phytoplankton biomass in the York displays large seasonal and temporal fluctuations, with maximal chlorophyll a concentrations of ~ 30 μg L$^{-1}$ in the spring at the low salinity transitional area of the estuary (Sin et al., 1999). Previously the estuary has shown a continuous increase in bacterial growth rate toward the freshwater end with opposing
gradients of bacterial abundance (increasing downstream) and production (increasing upstream), (Schultz, 1999).

Surface water (~0.2 m depth) was collected from three sites in the York River estuary (Figure 1): 1) the mouth of the estuary where it enters Chesapeake Bay (S = ~20), 2) the Pamunkey River (S = ~0), and 3) a site which approximated an equal mixing of the two end-members (S = ~10). The mid-salinity site was located in close proximity to the estuarine chlorophyll maximum. Sampling spanned the period of June 1998 to November 2000 for all three components (I-III below) of the study.

**Estuarine DOM Cycling: A Stoichiometric and Enzymatic Approach**

Recent advances in methods to chemically characterize DOM (Benner et al., 1992; McCarthy et al., 1997; Boon et al., 1998) can often not distinguish between biologically labile and refractory material. Separation of DOM into high molecular weight (HMW) and low molecular weight (LMW) classes has yielded conflicting results with respect to lability. Amon and Benner (1994) estimated a high turnover rate for the HMW material in contrast to Jensen (1980) and Sundh (1992) who demonstrated rapid utilization of LMW components. This discrepancy may be reconciled in part by the consideration that LMW compounds such as amino acids, organic acids and, simple sugars are readily assimilated by bacteria as are HMW polysaccharides and proteins. Such findings reflect our ignorance of the chemical composition of DOM and hence our inability to predict the lability of various components. Consequently, bioassays remain a primary tool for determining the reactivity of heterogeneous pools such as bulk DOM.
Studies of estuarine organic matter cycling have historically focused on DOC, while fewer studies have considered dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP). If DOM dynamics are to be viewed in the context of net system metabolism and ecosystem function, it is important to investigate both DON and DOP turnover coincident with DOC metabolism, since DON and DOP may be spatially and temporally decoupled from DOC cycling. Preferential remineralization of N and P relative to C may result in: (1) retention of nutrients in systems where heterotrophic production may be limited by N (Kirchman 1994) and P (Zweifel et al., 1995); (2) export of recycled, C-enriched DOM to the coastal sea (Hopkinson et al., 1997); and (3) amelioration of the competition between phytoplankton and bacteria for limiting nutrients (Bratbak and Thingstad, 1985). Studies directed toward estuarine ecosystem function thus require a stoichiometric evaluation of DOM elemental decomposition.

Bacteria may only transport small molecular weight (<600 Daltons) monomeric units across their cell membranes. HMW material, therefore, requires a step-wise depolymerization and transformation into smaller subunits prior to uptake (Chrost, 1991; Hoppe, 1991; Chrost and Rai, 1993). Extracellular enzymes are inducible hydrolyases regulated by sensitive repression/derepression control of synthesis (Chrost, 1991; Hoppe, 1991). Specific enzyme activities have been positively correlated with both depletion of readily assimilable substrates or influx of polymeric materials (Chrost, 1991; Chrost and Rai, 1993). The close coupling between enzyme activities and the uptake of semi-labile substrate pools can serve as indices of nutrient limitation and the major compound groups

(Servais, 1989; Amon and Benner, 1996; Carlson and Ducklow, 1996; Cherrier et al., 1996; Carlson et al., 1999).
subsidizing bacterial production (Chrost and Overbeck, 1987; Sinsabaugh et al., 1997; Findlay et al., 1998; Hopkinson et al., 1998; Foreman et al., 1998).

The York River estuary was used as a model system for temperate estuaries with low-relief headwater regions. The York is pristine amongst the sub-estuaries of the Chesapeake Bay with much of the watershed land-use dominated by forest (Bender, 1986). In contrast to estuaries with high relief, mechanically weathered headwaters, the low relief soils of the York headwaters are dominated by chemical weathering. Thus the relative contribution of deeper/older soils is minimal and riverine derived OM in the York is young (~15-20 yrs mean Δ¹⁴C age) and enriched in bomb radiocarbon (Δ¹⁴C ~ +230 ‰) (Raymond and Bauer 2001a,b). Consequently, OM cycling in the York River estuary is uncomplicated by inputs of older, terrestrial derived OM and represents a more simplified system in which to delineate pathways of OM cycling.

The potential of the York as a model estuarine system in which to study the cycling of allochthonous and autochthonous DOM (primary research objective) hinged on along system gradients in source, cycling, age and diagenetic state of OM. Thus, the stoichiometry of bacterial DOM decomposition and ectoenzyme activity measurements were employed to initially evaluate DOM cycling along the salinity gradient in the York River estuary. Some of these results are presented here as a background to the following chapters (see Appendix I for complete dataset). A comprehensive body of previous work evaluating dissolved inorganic carbon (DIC) (Raymond et al., 2000) and DOC (Raymond et al., 2001a), nutrients and Chl a (Sin and Wetzel, 1996), bacterial abundance and production (Schultz, 1999), and δ¹³C and Δ¹⁴C values of DIC, DOC, and particulate
organic carbon (Raymond and Bauer 2001a,b) along the full salinity gradient was invaluable to the interpretation of data throughout the present study.

The York River estuary exhibits clear differences in both the sources and lability of OM along the freshwater to saltwater continuum. These differences are reflected in the bulk DOM stoichiometry between the estuarine endmembers. Freshwater DOM (C:N:P = 2150:92:1) is depleted in both N and P relative to the mouth of the estuary (C:N:P = 550:38:1) (Figure 2). In addition, bacterial decomposition studies indicated significant differences in the total OM remineralization both within the C, N, and P containing constituents of the DOM as well as (Figure 2) between the low and high salinity endmembers. The total DOM remineralized at the mouth relative to the freshwater endmember was elevated by 26% and 86% for DOC and DON, respectively. Approximately 46% of the DOP pool was remineralized at the mouth while no detectable DOP decomposition was measured in the fresh river waters. This disparity in reactivity suggests variability in the sources and lability of DOC, DON, and DOP supporting bacterial production at the two sites.

Potential ectoenzymatic hydrolysis rates corroborate the conclusions from stoichiometric data. The decline in potential maximum hydrolysis rates ($V_{max}$) of $\alpha$-glucosidase and $\beta$-glucosidase from the freshwater endmember to the mouth (Figure 3) suggests that bacteria rely on C-rich compounds to fuel production at the head of the estuary. The opposing trend in the $V_{max}$ of leucine aminopeptidase implies a greater dependence on N-enriched compounds at the mouth. The high C:N:P ratio of the freshwater DOM coupled with increased glucosidase $V_{max}$ values point to a larger terrigenous influence, while lower C:N:P ratios and a greater leucine aminopeptidase
$V_{\text{max}}$ suggest more reactive DOM at the mouth of the estuary. The elevated alkaline phosphatase $V_{\text{max}}$ at the freshwater site suggests inorganic P limitation there (Chrost and Overbeck, 1987). The spatial differences in bulk DOC, DON, and DOP concentrations, the initial lability of these DOM pools, and contrasts in the sources of DOM supporting bacterial production along the salinity gradient point to variable sources of allochthonous and autochthonous OM along the York River estuarine continuum.

Isotope ($\delta^{13}\text{C}, \Delta^{14}\text{C}$) mixing curves along the salinity gradient of the York (Raymond and Bauer, 2001b), suggest both the persistence of a terrigenous DOM signature throughout the estuary and a significant source of autochthonous OM within the estuary (Raymond and Bauer, 2001b). Furthermore, DIC data indicate that a substantial portion of heterotrophic production must be supported by allochthonous (i.e. terrestrial) sources (Raymond et al., 2000). Collectively, these prior findings provoke several key questions which comprised the framework for this dissertation:

- What are the combined effects of sunlight exposure and bacterial decomposition on DOC, DON, and DOP bioavailability and bacterial growth?
- What are the sources and ages of OM supporting bacterial production along the estuarine continuum?
- How do the relative inputs of autochthonous (e.g., diatom, dinoflagellate, zooplankton, bacterial) and allochthonous (e.g., vascular plant, soils) OM vary along the salinity gradient over the annual cycle?

The three chapters comprising the core of the dissertation are briefly outlined below.

The balance between the photoproduction of bioavailable and biorefractory components of DOM and their subsequent effects on bacterial growth was assessed along the salinity gradient in the York River estuary. The approach was to conduct experimental incubations in space and time from stations along the estuarine salinity gradient, measure the chemical and elemental changes in the DOM pool throughout the time course, and evaluate the impact of sunlight-exposed DOM on microbial bioenergetics through measures of bacterial growth rate, production, and growth efficiency.

II. Identifying the Sources and Ages of Organic Matter Supporting Estuarine Bacterial Production: A Novel Multiple Isotope ($\delta^{13}$C, $\delta^{15}$N, $\Delta^{14}$C) Approach.

A unique multiple isotope ($\delta^{13}$C, $\delta^{15}$N, and $\Delta^{14}$C) approach was used to constrain the ages and relative contributions of allochthonous and autochthonous DOM supporting in situ bacterial production in the York River estuary and the Hudson River. Bacterial nucleic acids were extracted and analyzed for isotopic composition as a representative biomarker for bacterial biomass. The isotopic fidelity between DOM sources and bacterial nucleic acids has previously been determined in laboratory experiments (Coffin et al., 1990). Additionally, bioassays were performed to assess the isotopic signature of biomass produced during short-term (36-60 hours) incubations of surface water collected along the estuarine gradient.
IV. Comparison of Sources and Reactivity of Estuarine Dissolved and Particulate Organic Matter using a Multi-Tracer Approach

Lipid distributions and stable ($\delta^{13}$C, $\delta^{15}$N) isotopes were analyzed concomitantly in both particulate and dissolved OM fractions as a means of resolving the cycling of allochthonous and autochthonous OM along the salinity gradient of the York River estuary. Both lipid and stable isotope data were used to evaluate the extent of diagenetic processing within each OM fraction. Findings from this portion of the study suggest that biogeochemical and physico-chemical estuarine processes may maintain the observed gradient in source distribution and diagenetic state between particulate and dissolved OM fractions along the estuarine continuum.
LITERATURE CITED


Figure 1. Map of the York River estuary. Inset shows the York's location relative to the Chesapeake Bay proper. Sampling locations are designated with an "X" and marked with the approximate salinity.
Figure 2. Comparison of DOC (Panels a,b), DON (Panels c,d) and DOP (Panels e,f) decomposition in bioassay incubations from the head (Panels b,d,f) and mouth (Panels a,c,e) of the York River estuary in Sept./Oct. 1998. The % of total DOC, DON, and DOP utilized over the duration of the incubation is indicated in each panel with the exception of Panel f where utilization was not measurable. Error bars indicate ± 1 S.D. of the mean (n=3).
Figure 3. Calculated potential maximum hydrolysis rates ($V_{\text{max}}$) for bacterial ectoenzymes at 3 salinities (0.5, 10, 21) in the York River estuary (Sept. 1998). $V_{\text{max}}$ was calculated by nonlinear regression (rectangular hyperbole) from incubations at 8 substrate concentrations. Error bars indicate $\pm 1$ S.D. of mean (n=3).
Chapter II

The Effects of Natural Sunlight Exposure on Decomposition of Estuarine Dissolved Organic C, N and P and Bacterial Growth
ABSTRACT

The interaction between photochemical and microbial processes in the decomposition of DOC, DON, and DOP and the subsequent impact of sunlight-irradiated DOM on bacterial metabolism was investigated along the salinity gradient of a temperate east coast estuary, York River estuary, Virginia. Water samples were collected, exposed to natural sunlight in quartz tubes, and subsequently monitored for DOC, DON, and DOP decomposition and bacterial response in regrowth incubations. In situ light exposure yielded no detectable photochemical production of NH$_4^+$ or PO$_4^{3-}$ or loss of DOC due to abiotic remineralization. Although bacterial decomposition of DOC was frequently about two times greater in sunlight-exposed incubations compared to non-irradiated controls, there were no parallel enhancements in the microbial utilization of corresponding DON and DOP fractions. Significant differences in bacterial decomposition of sunlight-irradiated DOC were observed only after prolonged incubation (>7 days), suggesting that enhanced DOC lability may have been attributable to photochemical modification of higher molecular weight organic matter. Low concentrations of ambient inorganic nutrients in August 1999 resulted in a significant reduction in the remineralization of N and P in sunlight-exposed treatments and coincided with both an increase in the bacterial utilization of DOC and a subsequent decrease in bacterial growth efficiency (BGE). Conversely, during nutrient replete conditions (April 2000), enhanced DOC bioavailability resulted in the net immobilization of inorganic N and P by bacterial biomass production, but without a corresponding impact on BGE. These results suggest
that the combination of photochemical and microbial decomposition may increase bacterial demand for inorganic nutrients, alter BGE, and influence the partitioning of C between bacterial biomass and respiration.

**INTRODUCTION**

Dissolved organic matter (DOM) in estuaries is derived from diverse allochthonous and autochthonous sources. The cycling of this DOM is controlled by various biological, chemical and physical processes. Although bacteria have long been recognized as a primary control on DOM cycling (Pomeroy, 1974; Azam et al. 1983), photolytic effects have recently been found to both stimulate and inhibit DOM turnover (Benner and Biddanda, 1998; Obernosterer et al., 1999; Obernosterer et al., 2001). To date, the influence of photochemical reactions on DOM cycling has been inferred primarily by following the production of low molecular weight (LMW) organic compounds (Kieber et al., 1989; Mopper et al., 1991; Moran and Zepp, 1997; Bertilsson and Tranvik, 1998) and bacterial biomass and production as proxies for C cycling (Lindell et al., 1995; Wetzel et al., 1995; Benner and Biddanda, 1998; Bushaw et al., 1996; Obernosterer et al., 2001). The majority of studies was originally conducted in blackwater rivers, streams, and lakes with light-limited primary production (De Haan, 1993; Lindell et al., 1995; Bano et al., 1998) or, alternatively, humic material was isolated for UV exposure (Wetzel et al., 1995; Bushaw et al., 1996; Kieber et al., 1990; Miller and Moran, 1997). Consequently, stimulatory effects of photochemistry were observed in many of the earliest studies. Recently, however, photochemical work has moved beyond high humic/high dissolved organic carbon (DOC) environments.
studies in sub-tropical lagoon and temperate rivers show that exposure to UV light may have little impact on DOC bioavailability (Ziegler and Benner, 2000; Wiegner and Seitzinger, 2001).

Enzymatic hydrolysis has been recognized as the rate limiting step in polymeric DOM degradation (Billen and Fontigny, 1987; Chrost, 1990; Chrost, 1991). The term "refractory" was thus applied to DOM that was impervious to enzymatic degradation. This simplistic view has also recently undergone revision. Photochemical oxidation offers an alternative pathway for the decomposition and remineralization of polymeric DOM (Kieber et al., 1989; Mopper et al., 1991; Moran and Zepp, 1997; Bertilsson and Tranvik, 1998), a pathway whose importance may be intensified by the increasing penetration of ultraviolet light (UV) light to the earth's surface (Crutzen, 1992; Madronich, 1994). The effects of UV on DOM cycling in estuaries have been difficult to discern and are complicated by the diversity of autochthonous and allochthonous sources. Variations (both natural and anthropogenically induced) in the initial bioreactivity, source, and aromaticity of DOM affect its biological and photochemical fate during seaward transit. In general the balance between photoinhibition and photostimulation of DOM cycling is thought to hinge on the initial bioreactivity of various subcomponents of DOM.

It has been traditionally assumed that organic matter supplied to estuaries from terrigenous sources was more refractory than "fresh" DOM originating from in situ production (Mantoura and Woodward, 1983; Ittekot 1989). Terrestrial organic matter may be "hidden" from UV exposure for years to millennia sorbed to soils and sedimentary rocks (Keil et al., 1994; Mayer 1994a, Mayer 1994b, Petsch et al, 2000).
However, once exposed in the estuarine "window" (Cole, 1999), biological and diagenetic alteration of once-refractory terrestrial DOM may occur. Photochemical transformations thus may provide both a potential sink for allochthonous DOM and a mechanism for the preservation and transport of autochthonous DOM to the coastal ocean.

The influence of photochemistry on DOM dynamics centers on the initial bioreactivity of the substrates (Tranvik and Kokalj, 1998; Benner and Biddanda, 1998; Obernosterer et al., 1999; Benner and Ziegler, 1999; Tranvik, 1999). The York River estuary exhibits clear differences in the sources and lability of DOM (Raymond and Bauer, 2001, Chapters 3 and 4). These differences are reflected in the changing stoichiometry of bulk DOM during estuarine transport and mixing. Freshwater DOM (C:N:P = 2150:92:1) is depleted in both N and P relative to the mouth (C:N:P = 550:38:1) (Chapter 1). Striking differences were also found in the decomposition rates both within the C, N, and P containing constituents of the DOM as well as between the York River endmembers (Raymond and Bauer 2000, Chapter 1). While the importance of photochemical processes in the abiotic and microbial decomposition of DOC are well documented, the effects of photochemistry on dissolved organic nitrogen (DON) and dissolved organic phosphorous (DOP) have not been evaluated previously. Furthermore, the influence of photochemical DOM alteration on bacterial growth efficiency (BGE) has largely been overlooked, despite the critical role it might play in partitioning the flow of C between bacterial biomass and respiration.

This chapter examines the effects of sunlight on DOM bioavailability and its consequences for bacterial growth. By determining BGE, changes in the total carbon
utilization by bacteria are also evaluated (Jahnke and Craven, 1995) thus allowing the effects of interacting physical and biological processes on estuarine carbon flow to be better differentiated.

METHODS

Experimental Approach

This study was designed to evaluate the effects of natural sunlight irradiation both on the bioavailability of DOC, DON, and DOP, and its concomitant effects on bacterial metabolism. Experimental designs for sunlight exposure treatments and subsequent bioassays are shown in Figure 1 and described below. Although exposure to sunlight may have direct detrimental affects on bacterial growth and survival, this study was not designed to elucidate those potential impacts. The primary approach was to conduct seasonal experimental incubations on large volume samples taken along the salinity gradient in the York River estuary, VA. Similar incubations were performed using leachates from two potential end-member sources (estuarine/marine phytoplankton and riverine vascular plants). Short-term measurements of bacterial growth parameters such as production and growth rate alone are not sufficient indices for fully quantifying DOM utilization on scales of estuarine residence times. Furthermore, the abiotic release of inorganic nutrients and LMW organic photoproducts may stimulate bacterial production in the short term, yet may not reflect the overall lability of the bulk DOM pool due to the concomitant formation of biorefractory compounds. Therefore longer incubation times, approximating water residence time (1-2 months) in the estuary, were used, and the
bioavailability of DOC, DON, and DOP was monitored over the time course of incubation.

**Sample Collection and Leachate Preparation**

Sampling periods encompassed extremes in monthly mean streamflow and productivity (Figure 2a). Surface water was collected in August 1999, April 2000 and July 2000 from three sites in the York River estuary: 1) the mouth of the estuary where it enters the Chesapeake Bay ($S = -20$), 2) the Pamunkey River ($S = 0$), and 3) a site which approximated an equal mixing of the two end-members ($S = -10$). The mid-salinity site was in close proximity to the estuarine chlorophyll maximum. August 1999 was the yearly low for streamflow ($2.5 \text{ m}^3 \text{ sec}^{-1}$) whereas April 2000 was the annual high for streamwater run-off ($52.2 \text{ m}^3 \text{ sec}^{-1}$). The annual mean streamflow in 1999 (13.9 m$^3$ sec$^{-1}$) represented the 10 year minimum from 1990-2000 (Figure 2b). (streamflow data retrieved from http://waterdata.usgs.gov).

Bulk DOM from along the York salinity gradient contains a mixture of allochthonous and autochthonous sources (Chapters 3 and 4) presumably with varying susceptibility to photolytic reactions. Thus, leachates of algal and freshwater marsh plants were also used to examine potential photochemical impacts on individual autochthonous DOM sources to the York as previous research has suggested exposure to sunlight may impact fresh/labile and humic/riverine organic matter differentially (Benner and Biddanda, 1998; Obernosterer et al., 2001). In order to assess the effects of photochemistry on a potential autochthonous DOM source to the freshwater/brackish region of the York, leaves from the most prominent freshwater emergent macrophyte in
the York river, *Peltandra virginica*, were collected from a tidal freshwater marsh along the Pamunkey River, rinsed with deionized (DI) water, and leached in an aerated carboy in the dark for 10 days. To determine potential effects of concentration on photoreactivity, high (~1800 µM) and low (~100 µM) DOC concentrations of *Peltandra* leachate were prepared. For the phytoplankton leachate, an axenic mixed phytoflagellate culture, obtained from the VIMS phytoplankton culture facility, was sonicated on ice with a probe for 15 minutes to lyse algal cells.

**Treatments and Sampling Strategy**

Water for ambient DOM and leachate-amended incubations were filtered sequentially through combusted (500 °C, 4.5 hr) GF/D (2.7 µm) filters, GF/F (0.7 µm) filters and a 0.2 µm acid-soaked (10% HCl) Gelman capsule filter to remove POM and bacteria. To ensure bacteria-free filtrates, water and leachate samples were stored at 4 °C when necessary. Immediately prior to the start of each incubation, these were filtered an additional time through a 0.2 µm capsule to exclude bacteria which may have grown during the storage period (less than 2 days). Bacterial counts were performed on both ambient DOM leachate preparation. Before exposure to sunlight, samples were taken for DOC, NH₄⁺ and PO₄³⁻ analyses (Figure 1). The filtrate was subsequently distributed between triplicate dark (foil-wrapped polycarbonate bottles) and sunlight-exposed (quartz tubes) treatments. Following 9 hours of sunlight exposure on the VIMS pier on a cloudless day, all controls and treatments were again sub-sampled for DOC, NH₄⁺ and PO₄³⁻ to assess the impact of natural sunlight on DOC loss and inorganic nutrient release (Figure 1). Degradation incubations were then initiated by inoculation with a 1% (v:v)
bacterial (0.7μm filtrate) inoculum to assess photolytic changes in organic matter availability and bacterial metabolic parameters. Incubations were kept in the dark at in situ temperatures for the duration of the incubation (14, 28 days). All samples were frozen at -20°C until analysis for DOM and inorganic nutrient concentrations.

Incubations were conducted using both short (<7 days) and long (7-28 days) time scales to assess potential differences in the microbial response to sunlight exposed DOM between these periods. The various methodologies employed with each sampling period are outlined in Table 1.

**Short-Term Measurements**

Bacterial production, growth rate, ectoenzyme activity, and growth efficiency were the parameters chosen in order to assess potential photochemical transformations of DOM on shorter time scales (<7 days). Ectoenzyme activity was measured 4 days after inoculation. The lag in sampling prior to this measurement allowed for a maximal bacterial response to potential sunlight-induced changes in DOM. Incubations conducted in April 2000 to address bacterial growth were sampled for bacterial production and abundance at days 0, 4, and 7 (additional time point (t = 2 days) added for July 2000 sampling). DON, dissolved inorganic nitrogen (DIN), DOP and dissolved inorganic phosphorous (DIP) were measured at days 0, 2 and 7 (August 99) and at days 0 and 7 (April 2000). DOC measurements were made at t = 0, 1.5, and 7 days (August 1999), t = 0, 4, and 7 days (April 2000) and t = 0, 2, 4, and 7 days (July 2000).
**Long-Term Measurements**

To assess DOM bioavailability (August 1999), incubations were additionally subsampled for DOP/DIP and DON/DIN, at day 14 in addition to the aforementioned time points (short term measurements). During the April 2000 sampling the harvest times were modified to days 0, 7, 21, and 28 to better approximate time scales of estuarine mixing. DOC and bacterial production were sampled more frequently at days 0, 4, 7, 21, and 28 for the April 2000 sampling period.

Data from later time points (days 14-28) were used to evaluate the bioavailability of DOC, DON and DOP, on time scales of estuarine mixing. Additionally, the later time points assessed potential photochemical modifications of DOM, resulting from a delayed bacterial response and/or DOM decomposition (e.g. not immediate photochemical release of LMW moieties).

**Bacterial Production and Growth Efficiency**

Bacterial growth efficiency (BGE) is the efficiency at which bacteria convert DOC into bacterial biomass (BB) and is expressed as:

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\text{BGE} = \frac{\Delta BB}{\Delta DOC} \times 100\% \quad (\text{Eq. 1})
\]

\(\Delta BB\) was estimated from time dependent changes in bacterial abundance and measurements of bacterial production integrated over the time course of incubation. The conversion of cell abundance to BB was estimated using a cell-specific C content of 20 fg C cell\(^{-1}\) (Lee and Fuhrman, 1987). The conversion factor 1.5 kg C mol\(^{-1}\) leucine (Simon and Azam, 1989) was applied to convert \(^3\)H-leucine incorporated to units of C assimilated.
into cell biomass. Changes in DOC concentration (ΔDOC) were measured directly (see below).

**Analytical Methods**

**DOM and Inorganic Nutrients**

Thawed samples were analyzed for DOC, total dissolved N and P (TDN and TDP respectively), DIN (NO₃⁻ + NO₂⁻ + NH₄⁺) and DIP (PO₄³⁻). Phosphate (8 ml) was analyzed spectrophotometrically using a 5-cm pathlength cell and the acidified blue molybdenum method (Koroleff, 1983) with an analytical precision of ± 0.03 μM. Inorganic nitrogen species (3 ml each) were analyzed on a Technicon Autoanalyzer and the analytical precision associated with these measurements was 0.2 μM for both NO₃⁻ (NO₃⁻, NO₂⁻) and NH₄⁺, respectively. TDN and TDP (10 ml each) were analyzed by alkaline persulfate oxidation (Koroleff, 1983) with an analytical precision of ± 2.2 and 0.2 μM, respectively, and the organic fraction (DON and DOP) determined by the difference between the total and inorganic fractions. DOC (4 ml) was determined by high temperature combustion on a Shimadzu TOC-5000 with an analytical precision of ± 7.5 μM.

**Bacterial Biomass and Activity**

Samples (10 ml) for bacterial abundance were preserved with 0.2 μm filtered 25% glutaraldehyde diluted to a final concentration of 2%. Preserved samples were stored at 4 °C until slide preparation (within 7 days of sample collection). Bacterial abundance (August 1999 only) was determined by acridine orange epifluorescence microscopy.
(Hobbie et al., 1977) with a Zeiss Axiophot microscope. Duplicate slides were counted for each treatment at each sampling time, with a minimum of 350 cells counted per slide. Bacterial cell numbers for April and July 2000 samples were determined using a Becton-Dickinson FACS caliber benchtop flow-cytometer and the nucleic acid stain Syto-13 following the methods of del Giorgio et al. (1996). Bacterial cells and microspheres were separated in a log-log cytogram of green fluorescence intensity and side scatter. Samples were run for 30 seconds or until a minimum of 20,000 events were counted. Bacterial cell concentrations in the sample were calculated using microspheres as an internal standard.

Bacterial productivity was measured by $^3$H-leucine (Kirchman et al., 1985) and $^3$H-thymidine (Fuhrman and Azam 1982) incorporation as modified by Smith and Azam (1992). A sample aliquot (1.7 ml) was added to screw-top microcentrifuge tubes, followed by the addition of $^3$H-leucine and $^3$H-thymidine at saturating concentrations (40 nM and 25 nM respectively (Schultz, 1999)). Procedural blanks consisted of simultaneous additions of sample water, $^3$H-leucine, and 100 $\mu$l 100% trichloroacetic acid (TCA). Triplicate live samples and a single blank were run for each assay. Tubes were incubated in the dark at in situ temperatures for approximately 1 hour at which time the incubation was terminated by the addition of 100 $\mu$l of 100% TCA to sample tubes. The centrifuge, vortex, mixer and wash sequence as outlined in Schultz (1999) was followed, where microcentrifuge tubes were placed in scintillation vials and radioassayed in a liquid scintillation counter (Wallac, model 1409). Integrated production estimates were based on $^3$H-leucine incorporation and integrated by trapezoidal integration. In April 2000 samples were integrated over a 28 day time course ($t = 0, 4, 7, 14, 21$ and 28 days)
(Table 1). For the July 2000 time course samples were integrated over the first 7 days of incubation (t = 0, 1, 2, 4 and 7) days.

Potential ectoenzyme activity was determined through the use of model fluorogenic substrates according to the protocol outlined in Hoppe (1993). Water samples collected along the York River estuary transect were analyzed for the activity of the ectoenzymes: β-1,4-glucosidase, leucine aminopeptidase and alkaline phosphatase (all Sigma) which correspond to C, N and P acquiring enzymes respectively. Enzyme substrates were added at a 1% dilution (at predetermined saturating concentrations) to water (~ 8 mls) harvested from both dark controls and UV-exposed treatments and incubated in the dark at in situ temperatures. Flourescence resulting from enzyme activity was read on a TK Hoefer 1000 fluorometer, preset with excitation and emission characteristics of 364 and 445 nm respectively.

**Chlorophyll a**

Approximately 100 ml of surface water were collected from each sampling location in an amber polycarbonate bottle and stored on ice (maximum 8 hours) until analysis. Triplicate aliquots of 8 ml were filtered through GF/F (Whatman) filters. Filters were placed in 8 ml test tubes filled with DMSO/acetone/water solution (45:45:10) sealed and kept in the dark for a minimum of 48 hours (Burnison, 1980). Sample flourescence was read on a Turner fluorometer (model 10-AU).
Data Analysis

Data were imported into MiniTab and analyzed using a Student's t-test to compare differences between light and dark treatments only. Potential daily and seasonal variations in the total sunlight irradiation precluded statistical comparisons between seasons and stations. Unless otherwise noted, all confidence intervals are expressed at the 95% (p = 0.05) level.

RESULTS

Water Column Characteristics

Maximal Chl a biomass (29.2 μg l⁻¹) measured at the mid-salinity site corresponded with the greatest streamwater discharge (52.2 m³ sec⁻¹) in April 2000 (Table 2). Elevated Chl a levels at the mouth (17.9 μg l⁻¹) corresponded with the late July 2000 sampling period and may have resulted from a red tide event typical of the time and location of sampling. Chl a concentrations at the freshwater endmember were uniformly low (~ 4-5 μg l⁻¹) (Table 2). Samples collected in late August 1999 and July 2000 were characterized by elevated water temperatures (27-30°C), and low to moderate streamwater discharge, (2.5 and 9.4 m³ sec⁻¹), respectively. The concentration of NH₄⁺ and PO₄³⁻, the primary inorganic nutrients subsidizing estuarine bacterial production, varied across all sampling periods and hydrologic regimes (Table 2). Minimum NH₄⁺ and PO₄³⁻ concentrations for the freshwater endmember coincided with the lowest streamwater discharge in August 1999. Concentration of NH₄⁺ and PO₄³⁻ at the same site in April 2000 were more than double the August 1999 values. Maximum levels of NH₄⁺ were measured at the mid-salinity site in April 2000 (3.1 μM), and were greater than...
three times the August 1999 concentrations (Table 2). In July 2000, NH$_4^+$ concentrations decreased with increasing salinities and maximal PO$_4^{3-}$ concentrations were measured in the mid-estuary.

DOC concentrations varied between ~240 and 520 μM and generally decreased seaward, where concentrations at the mouth were approximately half that of the freshwater endmember. C:N ratios calculated from DOC and DON concentrations ranged from ~16-22 and were lowest at the mouth in April 2000.

**Abiotic Photochemical Impacts on DOC, NH$_4^+$ and PO$_4^{3-}$ Concentrations**

Concentrations of DOC, NH$_4^+$ and PO$_4^{3-}$ measured along the York River estuary did not show significant differences (p > 0.05) between dark and sunlight-exposed treatments (Figure 3). Similarly, no detectable loss of DOC due to photomineralization between the dark and sunlight exposed treatments was found in either the phytoplankton leachate or the low concentration *Peltandra virginica* leachate. However, a significant (p < 0.05) decrease in DOC concentrations was observed in the high DOC *Peltandra* leachate sunlight exposed treatments (Figure 3). There was a loss of 160 μM C in this treatment corresponding to a depletion of 9% of the starting DOC concentration. The photochemically mediated release of NH$_4^+$ from humic complexation (Bushaw et al., 1996) and of PO$_4^{3-}$ from associations with iron complexes (Francko and Heath, 1982) was not detected in any of the river water or leachate samples (Figure 3).
Effect of Phototransformation of DOM on Bacterial Ectoenzyme Activity

Potential ectoenzyme activities were measured 4 days after inoculation to assess the potential influence of photochemically induced transformations in DOM on subsequent bacterial hydrolytic activity. Ectoenzyme activities in the sunlight exposed groups were normalized to the activity measured in the dark controls (Figure 4). The only significant impact ($p < 0.05$) of sunlight exposure on relative ectoenzyme activity was noted for the freshwater endmember (Figure 4) where activities of all three enzymes were consistently reduced in sunlight exposed treatments relative to dark controls.

Net DOC Utilization

Neither high nor low concentrations of *Peltandra* leachate yielded significant differences in the loss of DOC between dark treatments and sunlight exposed treatments (Figure 5). However, exposure of phytoplankton-derived DOM to natural sunlight resulted in a 90% reduction in the subsequent bioavailability of DOM to bacterial utilization (Figure 5). Sunlight exposure of unamended water samples from the various salinities in the York both enhanced and impaired the utilization of DOC relative to dark controls (Figure 6). Total DOC utilization was consistently enhanced at the freshwater endmember at all sampling times following sunlight exposure. DOC utilization was 58% higher in light vs dark treatments (significant at $p < 0.05$) in July 2000 at the low salinity site.

In general DOC utilization at the mid-salininty site was enhanced in sunlight exposed relative to dark treatments despite its association with the Chl $a$ maximum, a potential source of labile phytoplankton derived material (Figure 6). Enhanced net DOC
utilization in the light vs dark treatments was greatest in April 2000, where DOC loss was
~35% greater in water previously exposed to sunlight. Inclusive of all 14 and 28 day
light and dark incubations, DOC utilization ranged from 10 - 65 µM (Table 3) at the
freshwater and mid-salinity sites. DOC utilization was markedly different at the mouth
of the estuary where bacterial utilization of DOM was only 2-20 µM (Table 3) in both
dark and light incubations over 28 days.

The effect of sunlight-exposure on DOC utilization at the mouth of the York
yielded conflicting results based on the April and July 2000 experiments. Loss of DOC
in sunlight exposed treatments was enhanced by 65% in April 2000 over dark control
levels. However, DOC utilization in July 2000 was impaired by 60% at the same site.
The disparity in these results may be reconciled in part by the elevated Chl a levels (18
µg l⁻¹) measured in July 2000 (Table 2) thus providing a potential source of labile DOM
(relatively undiluted by aromatic, riverine DOC) to be photolytically modified to a more
bio-recalcitrant form (see Figure 5).

\textbf{NH}_4^+ and PO_4^{3-} Production During DOM Utilization

A representative time course incubation from the freshwater location in August
1999 showed enhanced (approximately double) DOC utilization in light exposed relative
to dark treatments (Figure 7a). DOC loss between light and dark incubations was
indistinguishable until after day 7. Between days 7 and 14 no further change in DOC
concentration was measured in the dark incubations but ~8 µM C was consumed in the
light treatments. DOC was utilized at a rate of 0.7 (± 0.3) µM day⁻¹ and 1.4 (± 0.3) µM
day⁻¹ over 14 days for dark and light exposed treatments, respectively (Table 3).
Differences in DON and DOP metabolism between sunlight exposed and dark treatments were not detected within the analytical error (Figure 7 b,c). Likewise no significant differences in (NO₃) concentrations were observed between light and dark incubations over the time course (Figure 7e). Dark controls displayed a net production of NH₄⁺ (0.7 μM), and a small but significant (0.02 μM) (p < 0.05) production of PO₄³⁻ (Figure 7 d,f). In comparison there was a significant 80% decrease in NH₄⁺ production in the sunlight exposed treatments (Figure 7 d). Similar to DOC utilization, differences in net NH₄⁺ production between light exposed and dark treatments were not detectable until after 7 days of incubation. In sunlight exposed treatments inorganic phosphate was immobilized in the organic phase, and there was no net production of PO₄³⁻.

The August 1999 incubations at the mid-salinity site yielded results similar to the freshwater site (Figure 8). Decomposition of DOC was enhanced in the light relative to dark treatments by approximately 10 μM or 3% (Table 3). No significant differences (p > 0.05) were again noted in the concentrations of DON, DOP or NO₃ (Figure 8 b,c,e) between dark and sunlight exposed treatments. In contrast to the freshwater site, there was an absence of net PO₄³⁻ production (Figure 8f). However, similar to the freshwater endmember (Figure 7) NH₄⁺ displayed a 50% decrease in net NH₄⁺ production in the light exposed treatment (Figure 8d).

A 40% increase in DOC utilization was observed at the same mid-salinity site during the April 2000 sampling (Table 3, Figure 9). There was no significant difference between light exposed and dark treatments in the amount of DOC remineralization prior to day 7 of the incubation (Figure 9). Though DON and DOP showed no significant differences between light and dark treatments, an increase in both DON and DOP was
observed over the time course in both treatments (Figure 9 b,c). Concomitant with the increase in DON and DOP was a decline in all inorganic nutrients (Figure 9 d,e,f).

**Effects of Photoaltered DOM on Bacterial Production and Growth Efficiency**

Measurements of bacterial production by both $^3$H-leucine and $^3$H-thymidine incorporation and increases in cell numbers (employed as a proxy for DOC bioavailability) did not always yield results consistent with DOC utilization (compare Figure 9 and 10). A time course (28 d) of bacterial production ($^3$H-leucine and $^3$H-thymidine) from the mid-salinity site in April 2000 did not show significant differences between light exposed and dark treatments suggesting no discernible photolytic impact on DOC bioavailability (Figure 10 a,b). This contrasts with results based on direct measurement of DOC utilization (Figure 9, Table 3). Similarly, estimates of DOC loss derived from increases in bacterial cell numbers suggest no selective impact of sunlight exposure on subsequent DOC lability (Figure 10 c).

Relative bacterial production estimates based on $^3$H-leucine incorporation rates in light normalized to dark treatments show differences in the response to sunlight exposure between the two sampling periods (Figure 11). Production rates integrated over the duration of the incubation for sunlight-exposed treatments in April 2000 were not significantly different from dark, though the mid-salinity site showed decreased production (~37% lower than dark treatments). Differences between the effect of light exposure were also observed when production rates ($^3$H-leucine) were integrated over the entire 28 days of the experiment (Figure 11 inset). In July 2000, integrated bacterial production in the light treatments ranged from 1.75 to 2.5 times the production measured...
in the dark over the initial 7 day incubation period and was significant (p < 0.05) at all sites (Figure 11, Table 4). In two of the six experiments a concomittant increase in bacterial growth rates was observed with the increase in integrated production (Table 4). Although a significant increase in integrated production was measured at the mouth of the York in July, there was not a similar response in bacterial growth rates (Table 4). During the April sampling period, no significant differences were found in integrated production, nor were growth rates significantly altered at the freshwater and mid-salinity sites. However, a significant reduction in bacterial growth rates was measured at the most saline site (Table 4).

Estimates of BGE (Table 4) were calculated based on estimates of integrated production (based on \(^{3}\)H-leucine incorporation) and DOC consumption. The impact of sunlight exposure on BGE varied between sites, sampling dates and duration (7 vs 28 days). Despite significant increases in both integrated production and bacterial growth rates in sunlight exposed samples, there was no significant difference in BGE (7 d) between light and dark treatments (July 2000). In contrast a significant reduction in BGE (28 d) in sunlight-exposed relative to dark treatments was calculated for both the freshwater and mid-salinity sites in April. In April 2000, BGE (28 d) at the mouth showed no significant difference between light and dark treatments. However, when BGE was calculated over a shorter duration (7 d), a significant increase in BGE in light exposed treatments was calculated thus suggesting an influence of incubation time on BGE (Table 4). In water from the freshwater site, there was no difference in the effect of sunlight-exposure on BGE when calculated for either 7 or 28 days. Conversely for the high salinity endmember, when BGE was calculated over longer time scales (28 days).
there was an increase in the BGE in sunlight exposed relative to dark treatments which was not detected on shorter time scales (7 days) (Table 4). These results suggest that bacteria in the York may alter their growth efficiency throughout the course of an incubation. In general, the absence of consistent trends between sites and sampling times highlights the dependence of bacterial responses on organic matter composition and lability which itself varies in time and space.

**DISCUSSION**

**Role of Sunlight in DOM Cycling**

Over the past decade there has emerged a recognition that biological, chemical, and physical processes work in concert (Figure 12) rather than as isolated pathways during the decomposition of organic matter in aquatic systems (Miller and Moran, 1997; Anderson & Williams, 1999; Moran et al., 1999). Biological utilization of allochthonous DOM is facilitated by photochemical processes in a number of systems. Photochemical reactions involving DOM may generate four possible terminal products (Table 6): 1) inorganic end-products (CO, CO$_2$) (Mopper et al., 1991; Valentine and Zepp, 1993; Miller and Zepp, 1995); 2) LMW carbonyl products (Kieber and Mopper, 1987; Kieber et al., 1989; Kieber et al., 1990; Wetzel et al., 1995); 3) photobleached DOM (Miller and Zepp, 1995; Miller and Moran, 1997; Moran et al., 1999) and 4) inorganic nutrients (Franko and Heath, 1982; Bushaw et al., 1996) (Table 6). Microbial decomposition of DOM may also be enhanced by the photochemical fragmentation of polymerized forms of DOM into monomeric carbonyl groups such as acetate, pyruvate, etc. (Kieber et al., 1989; Mopper et al., 1991) or DFAA (Amador et al., 1989). Bacterial production is
stimulated by the uptake of these photoproducts (Mopper and Stahovec, 1986; Kieber et al., 1989; Mopper et al., 1991; Wetzel et al., 1995). Additionally, photochemical sensitization of humic material may render it more susceptible to microbial attack (Geller, 1986; Lindell et al., 1995; Miller and Moran, 1997). Thus it has been hypothesized that photochemical reactions may cause conformational changes in DOM, distinct from the photoproduction of LMW organic compounds, which may accelerate utilization of photoaltered DOM by bacteria (Miller and Moran, 1997 (Table 6). Studies neglecting photochemical effects may underestimate the fraction of DOM that is potentially degraded within an estuary by a factor of three or more (Miller and Moran, 1997; Bano et al., 1998; Moran et al., 1999).

The influence of photochemistry on DOM cycling is twofold. Though photochemical reactions may stimulate the decomposition of DOM on the one hand, UV light may simultaneously catalyze the formation of biorefractory substrates on the other hand. Labile DOM moieties may be photochemically complexed with natural DOM thorough polymerization and condensation reactions and rendered unavailable for microbial utilization (Benner and Ziegler, 1999; Tranvik et al., 1999; Obernosterer et al., 1999). The current state of our understanding indicates that photochemical reactions generate both bioreactive as well as biorefractory compounds (Anesio et al., 1999; Benner and Biddanda, 1998; Benner and Ziegler, 1999; Tranvik et al., 1999; Herndl et al., 1999; Miller, 1999; Obernosterer et al., 2000) (Table 6). Figure 12 depicts the various potential positive and negative feedbacks of sunlight exposure on DOM cycling and bacterial metabolism in an estuarine environment. Though the role of sunlight in DOM metabolism is complex, the often contrasting effects may be partially resolved by
evaluating the concurrent roles of photochemistry and microbial decomposition in the flow of carbon through a particular system. Bacterial DOC utilization integrates the positive and negative feedbacks of photochemistry on bacterial metabolism and ultimately reveals the dominant pathway (i.e. inhibitory vs stimulatory) for a given environment. This study examines both potential abiotic photochemical impacts on DOC and inorganic nutrient cycling and biotic influences (e.g. bacterial DOC utilization, bacterial production and BGE).

Photomineralization of DOM and Inorganic Nutrient Production

Organic carbon may be photochemically oxidized and lost from a system as one of more inorganic gases (CO and CO₂) (Farjalla et al., 2001; Bertilsson et al., 1999, 2000; Amon and Benner, 1996; Graneli et al., 1996; Moran and Zepp, 1997). Abiotic photochemical mineralization of DOC in surface waters may dominate (sevenfold greater than bacterial DOC utilization (Amon and Benner, 1996)) in aquatic systems with high humic content (De Haan, 1993; Miller and Zepp, 1995; Amon and Benner, 1996) and results in a complete bypass of the microbial loop. Direct photochemical mineralization in water from the salinity continuum of the York River estuary was negligible in the present study (Figure 3) as estimated from no measurable loss of DOC in the absence of bacteria. However, 8% of the total DOC in a concentrated Peltandra virginica leachate was photomineralized, no loss was measured in a less concentrated leachate (Figure 3). As photomineralization was calculated as a DOC loss rather than by more sensitive CO₂ analysis, our methodology may have lacked the sensitivity to detect small DOC variations (< 1.5 μM) in the low concentration leachate.
The photochemical liberation of limiting nutrients such as dissolved inorganic nitrogen (DIN) (Amador et al., 1989; Bushaw et al., 1996) and dissolved inorganic phosphate (DIP) (Francko and Heath, 1982) has also been well-documented though primarily in freshwater high humic environments. These potential abiotic pathways are depicted in Figure 12 by the dotted arrows. However in the York River estuary (Figure 3) and other riverine/estuarine environments (Wiegner and Seitzinger, 2001; Reche et al., 1998) the potential photolytic subsidy of NH$_4^+$ and PO$_4^{3-}$ appears minimal.

**Effects of Sunlight-Exposed DOM on Potential Enzyme Activity**

Bacteria only transport small molecular weight (<600 Daltons) monomeric units across their cell walls (Alexander, 1973). Polymeric material, however, requires a stepwise depolymerization and transformation into smaller subunits prior to uptake. Extracellular enzymes are inducible hydrolyases regulated by sensitive repression/derepression control of enzyme synthesis (Chrost, 1991; Hoppe, 1991). Enzymatic activities have been correlated positively with both a depletion of readily assimilable substrates or an influx of polymeric material (Chrost, 1991; Chrost and Rai, 1993). Thus, the intimate links between enzyme activities and the availability of semi-labile substrate pools can serve as indices of nutrient, carbon and energy limitation and help identify qualitatively the major compound groups subsidizing bacterial production (Chrost and Overbeck, 1987; Sinsabaugh et al., 1997; Findlay et al., 1998; Hopkinson et al., 1998; Foreman et al., 1998).

Bacterial exploitation of the by-products of radiative energy transfer in the coastal environment offers an alternative means for C and nutrient acquisition whereby the
energetically more expensive pathway of enzyme synthesis can be circumvented. The sunlight-mediated liberation of ammonium (Bushaw et al., 1996) and orthophosphate (Francko and Heath, 1982) may provide an additional source of limiting nutrients for bacterial growth. The potential biological uptake of photochemically produced inorganic nutrients is illustrated in Figure 12. The photochemically induced release of inactive phosphatase from its association with dissolved humic material (Wetzel, 1992) may also defray the cost of additional enzyme synthesis. Though significant decreases in the activity of all three enzymes (leucine amino peptidase, alkaline phosphatase, and beta glucosidase) at the freshwater endmember (Figure 4) were observed, it was not possible to attribute this exclusively to an abiotic release of \( \text{NH}_4^+ \) or \( \text{PO}_4^{3-} \) (Figure 3). Instead, reduced enzyme activity may be due to sunlight-induced changes in DOM itself. The photolytic production of LMW carbon compounds (Kieber et al., 1989; Mopper et al., 1991) and the release of DFAA from humic material (Amador et al., 1989) may subsidize bacterial growth without incurring the expense of enzyme synthesis. However, we were not able to detect concurrent changes in bulk DON concentrations within the analytical sensitivity of DON measurements.

Similarly, the measurement of bulk DOC does not differentiate between the contributions of LMW and HMW components. A significant decrease in all ectoenzyme activities was observed in the low salinity samples (Figure 4), where higher concentrations of humic materials are expected. Humics are condensed organic compounds which are highly colored due to their high degree of aromaticity and strongly light adsorbing relative to their autochthonous counterparts (Morris et al., 1995; Reche et al., 1999). This capacity for light absorption drives the photochemical reactivity of these
organic molecules (Miller 1999). The observed decreases in enzyme activity cannot be
ascribed to photochemically induced releases of inorganic \(\text{NH}_4^+\) or \(\text{PO}_4^{3-}\). Deviations
may have been mediated by photolytically induced changes in organic pools such as
dissolved combined and free amino acids (Amador et al., 1989) as well as the photolytic
cleavage of HMW DOC to LMW carbon moieties (Kieber et al., 1989; Bertilsson and
Tranvik, 1998).

**Photochemical Effects on DOM Bioavailability**

Exposure of water samples and individual source leachates to sunlight both
stimulated and inhibited microbially mediated DOC decomposition as has been observed
by other studies (Table 6). The initial lability of the phytoplankton leachate was reduced
by 90% compared to non-irradiated controls (Figure 5). In previous experiments,
sunlight-initiated condensation reactions were thought to be responsible for a \(\sim\) 40% increase in the formation of refractory DOM from the exposed protein ribulose 1,5-
bisphosphate carboxylase as compared to that aged in the dark (Keil and Kirchman,
1994). Naganuma et al. (1996) noted the decreased bioavailability of UV-exposed
peptone to cultured bacteria. A unifying characteristic for DOM photoalteration is the
initial bioreactivity of the irradiated material. Approximately 70% of the initial
phytoplankton derived DOM in dark treatments was metabolized within a 14-day period,
suggestive of the inherent lability of the leachate (Figure 5). Sunlight exposed,
umamended water samples collected from the mouth of the York River in late July 2000
were the only ones to demonstrate a significant decrease in bacterial DOC degradation
(Figure 6). The July sampling period coincided with the peak chlorophyll a
measurements at this station, which were approximately 2.5 times greater than the spring sampling (Table 2). Decreased DOC decomposition may also reflect photoalteration of labile phytoplankton derived DOC to refractory forms. In other samples DOC bioavailability was ~2 times greater in sunlight exposed treatments than non-irradiated controls (Figure 6, Table 3). The dominant influence of sunlight on DOC decomposition in the present study was through enhanced susceptibility to microbial decomposition, rather than direct photochemical oxidation to an inorganic form.

The rates of DOC decomposition were significantly greater in irradiated DOM as compared to dark treatments (Table 3; Figure 6), however there were no corresponding differences in the microbial utilization of the associated DON and DOP fractions (Figures 7, 8 and 9 panels b,c). These results suggest that the susceptibility of DON and DOP pools to photochemical alteration may be uncoupled from that of DOC. In general, photochemical effects on DON and DOP lability have been only cursorily examined. Wiegner and Seitzinger (2001) determined light exposure did not alter the biological availability of DON. In agreement with the present study, Bertilsson et al., (1999) found irradiation increased the bioavailable pool of DOC, while DON remained unaffected. To my knowledge, there is only one study on the UV enhanced availability of DOP to bacteria (Tranvik et al., 1999) which based its conclusions on increased bacterial biomass in a P-limited incubation exposed to light relative to a dark control, rather than direct chemical detection.

A summary of studies examining the dual impact of photochemical and microbial interaction on the bioavailability of DOM in a variety of environments and in potential source leachates is compiled in Table 6. The majority of the studies conclude that
bioavailability is enhanced after exposure to sunlight, though this finding is based primarily on bacterial biomass and production as a proxy for enhanced C cycling. Though fewer in number, studies which suggest decreased bioavailability of DOC were conducted in the open ocean, and are based solely on bacterial production measures (Table 6). Although microbially mediated changes in DOC from deep waters (open ocean) are most likely not discernible given current instrumentation, concomitant measures of respiration may more accurately portray the fate of C as a consequence of photochemical and bacterial interactions. This study is the first to my knowledge to examine the interaction between photochemical and microbial interaction in the bioavailability of bulk DOM (DOC, DON, and DOP) in a temperate estuary using combined bacterial production and DOM utilization approaches. Overall the bioavailability of DOC appears enhanced, though changes in bulk DON and DOP were not discernible. The fate of this C appears to be variable, in part because bacteria may modulate their growth efficiencies throughout organic matter decomposition. Consequently C flows may be photochemically directed either through the microbial loop (increased BGE) or via a bypass of the microbial loop (decreased BGE) (Figure 12). This study highlights an unexamined aspect of photochemistry: photochemically enhanced DOC bioavailability increases bacterial demand for inorganic nutrients and may result in decreased remineralization or immobilization of inorganic nutrients.

**Impact of Sunlight Exposed DOM on Bacterial Metabolism**

The efficiency with which bacteria utilize DOM is critical to the flow of C through the estuary, its potential export to the coastal ocean, the regeneration of inorganic
nutrients, and the flow of energy to higher trophic levels (Ducklow, 1990). Previous studies have relied on bacterial production as a proxy for gauging enhanced vs impaired cycling of DOM (Lindell et al., 1995; Wetzel et al., 1995; Benner and Biddanda, 1998; Bushaw et al., 1996; Obernosterer et al., 2001). Conclusions based on bacterial growth measurements and DOC decomposition may differ (see Figures 9 and 10), in part due to the timescales associated with the different types of measurements. Bacterial growth and production assays are generally conducted on short timescales (hours to days). However a significant enhancement in sunlight exposed bulk DOC decomposition is often not discernible until after approximately 7 days of incubation (Figures 7, 8 and 9, panel a). This study employed combined production and DOM measurements to gain a mechanistic knowledge of photochemical influences on organic carbon flow within a temperate estuary.

The most important variables thus far identified affecting BGE in aquatic systems (del Giorgio and Cole, 1998) are inorganic nutrients (Benner et al., 1988; Kroer, 1993), C:N of substrate (Billen, 1984; Goldman et al., 1987; Kroer, 1993), maintenance cost (Linton, 1990; Middleboe and Sondergaard, 1993; Morita, 1997), oxidation state of DOM (Meyer, 1994; Vallino et al., 1996), and temperature (Newell and Lucas, 1981; Roland and Cole, 1999). The metabolic versatility of bacteria allows for the uncoupling of anabolic and catabolic pathways. Components of the DOM may be partitioned to either pathway depending on the immediate cellular requirements. The allocation of a substrate to either energy production or biomass synthesis is controlled by substrate availability, C:N ratio, oxidation state, and the source and availability of inorganic nutrients.

Consequently, BGE is determined by this distribution in metabolic expenditures, yet
BGE has been ignored in the majority of studies assessing the impact of photochemistry on DOC cycling (Table 6).

Rates of BGE based on cell abundance, bacterial growth rate, and inorganic nutrient remineralization in the York for August 1999 and April 2000 are summarized in Table 5. Significant decreases in BGE in sunlight exposed experiments relative to dark controls for August 1999 (Table 5) coincided with a low streamflow period, and depleted stocks of NH4+ and PO4³⁻ (Table 2). The increased availability of labile DOC compounds was not balanced by a corresponding increase in DON or DOP availability. In order to build biomass, photolytic liberation of DOC compounds must be stoichiometrically balanced by available sources of N and P to meet cellular requirements (Lancelot and Billen 1986). Bacteria may have increased their respiration of DOC in the absence of the requisite N or P to construct biomass. Under conditions of mineral nutrient limitation bacteria may shift their metabolism to catabolize excess DOC, thus increasing their respiration rate and decreasing biomass synthesis or BGE (Hessen, 1992). The net impact of photochemistry in this system is to create an excess of labile DOC (Table 3, Figure 6) and diminish the role of bacteria as remineralizers of inorganic nutrients (Table 5).

In the present study, BGE was significantly lower in light exposed treatments (Table 5) suggesting that production estimates alone underestimated the flow of C through the bacterial community. Our findings support previous studies (Jahnke and Craven, 1995; Carlson et al., 1999; del Giorgio and Cole 2000) which caution against reliance on production estimates alone as a direct measure of C flow through a system. The mechanisms which control bacterial growth and production do not ultimately
regulate total DOC consumption (Kirchman and Rich, 1997). Although significant changes in bacterial production and cell abundance were not apparent in light exposed treatments (Table 4, Figure 10), corresponding bacterial decomposition of DOC demonstrated increased lability (Table 3, Figure 9) after 7 days. The lag time (7 days) prior to the divergence of DOC decomposition between light exposed and dark controls supports previous research by Miller and Moran (1997) who attributed increased DOC bioavailability to photochemical modifications in humic or HMW DOC rather than direct photochemical cleavage to LMW compounds.

In April 2000, inorganic nutrient concentrations were several times greater than August 2000 (Table 2). As shown in Figure 9, DOC was rapidly metabolized in April, and was paralleled by a corresponding uptake of inorganic nutrients. A significant difference in BGE was not discernible between light and dark incubations, as starting concentrations of inorganic nutrients were able to balance the increased availability of C in contrast to incubations in August 1999. Our results suggest bacteria may have modulated their growth efficiency in response to a depletion of inorganic nutrients. Carbon moieties may have been shunted to anabolic pathways as bacteria awaited the stoichiometric complement of N and/or P to construct biomass, resulting in higher respiration rates and decreased BGE (Table 5). There was no relationship between growth rate and BGE. Although an additional explanation for decreased BGE may have been inefficient growth (Vallino et al., 1996, del Giorgio and Cole, 1998) on highly oxidized photoproducts (Moran and Zepp, 1997) the same relationship was not found in April 2000 (Table 5). Photolytic processes may impair bacterial remineralization of inorganic nutrients by uncoupling DOC availability from that of organic N and P pools.
thereby increasing inorganic nutrient demand, and immobilizing N and P in bacterial biomass (Figures 7-9).

CONCLUSIONS

Abiotic release of inorganic nutrients due to photolytic reactions of DOM in the York River estuary was below detection. Though differences in enzyme activity were discernible we were unable to attribute them to photolytic inorganic nutrient release. Instead, decreases in enzyme activity may in fact be a result of photochemical modifications within organic pools. Photolytically induced changes in the bioavailability of bulk DON and DOP were also not significant. While the bioavailability of DOC is enhanced by photochemical and microbial interactions, the efficiency with which this carbon is incorporated into bacterial biomass (and potentially higher trophic levels) is highly variable. It appears to depend partially on inorganic nutrient availability as well as other environmental aspects. The ecological consequence of bacterial modulation of their growth efficiency is that bacterial production estimates alone may underestimate the total carbon flow through the system. In order to accurately assess photochemical impacts on C flow, estimates of both bacterial production and total C consumption are required.

This study underscores the complexity of natural aquatic ecosystems which is often masked by the inherent simplicity required for experimental study. To gain a mechanistic knowledge of the impact of photochemistry on an ecosystem basis, a comprehensive parameterization of the effect of sunlight on DOM bioavailability as well as primary production, bacterial production and inorganic nutrient demand is necessary.
Individual systems (estuarine, riverine, lake, open ocean) require a modeling approach to address implicitly and oft overlooked positive and negative feedbacks between variables.
LITERATURE CITED


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Table 1. Summary of analyses and calculations per sampling period ("X" designates parameters measured and calculations performed for different sampling times and treatments).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Aug-99</th>
<th>Apr-00</th>
<th>Jul-00</th>
<th>High Concentration</th>
<th>Low Concentration</th>
<th>Phytoflagellate Leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of incubation (days)</td>
<td>14</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abiotic impacts (DOC, NH₄⁺, PO₄³⁻)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DOC remineralization</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DON, DOP remineralization</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻, NH₄⁺, PO₄³⁻ remineralization</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ectoenzyme activity</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bacterial Production (¹H-leucine Incorporation)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Bacterial Production (¹H-thymidine Incorporation)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bacterial cell abundance (AOCD)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
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<td>X</td>
</tr>
<tr>
<td>Bacterial cell abundance (Flow cytometry)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Calculations</td>
<td></td>
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<tr>
<td>BGE (integrated production 7 day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
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<tr>
<td>BGE (integrated production 28 day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>BGE (cell abundance)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
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Table 2. Site and Water Column Characteristics of York River Estuary During Sampling Periods.

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<tr>
<th></th>
<th>Streamflow</th>
<th>Salinity</th>
<th>Water Temp.</th>
<th>Chlorophyll a</th>
<th>NH₄⁺</th>
<th>PO₄³⁻</th>
<th>DOC</th>
<th>DON</th>
<th>C:N*</th>
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<tbody>
<tr>
<td>Aug-99</td>
<td>2.5</td>
<td>0</td>
<td>30</td>
<td>5.9</td>
<td>0.4</td>
<td>0.16</td>
<td>445</td>
<td>23.2</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>29</td>
<td>26.9</td>
<td>0.9</td>
<td>0.97</td>
<td>410</td>
<td>22.4</td>
<td>18.3</td>
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<tr>
<td>Apr-00</td>
<td>52.2</td>
<td>0</td>
<td>13</td>
<td>4.5</td>
<td>2.7</td>
<td>0.34</td>
<td>438</td>
<td>20.6</td>
<td>21.3</td>
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<td>11</td>
<td>11</td>
<td>29.2</td>
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<td>0.30</td>
<td>369</td>
<td>16.5</td>
<td>22.4</td>
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<td>11</td>
<td>7.0</td>
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<td>0.08</td>
<td>250</td>
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<td>Jul-00</td>
<td>9.4</td>
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<td>28</td>
<td>5.7</td>
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<td>27</td>
<td>23.5</td>
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<td>0.61</td>
<td>518</td>
<td>n.d.</td>
<td>n.d.</td>
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<td>20</td>
<td>27</td>
<td>17.9</td>
<td>0.2</td>
<td>n.d.</td>
<td>239</td>
<td>n.d.</td>
<td>n.d.</td>
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* C:N values were calculated from DOC/DON concentrations
n.d.- not determined
Table 3. Amounts, percentages and rates of DOC decomposition in York River estuary microbial utilization assays.

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<tr>
<th></th>
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<tr>
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<td>Dark</td>
<td>Light</td>
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<td>Light</td>
</tr>
<tr>
<td>DOC Remineralized (μM)</td>
<td>10 (3.7)</td>
<td>19 (3.8)</td>
<td>16 (0.7)</td>
<td>26 (0.9)</td>
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<tr>
<td>% of total DOC Remineralized</td>
<td>2.5 (0.9)%</td>
<td>4.8 (0.9)%</td>
<td>3.9 (1.4)%</td>
<td>6.5 (0.2)%</td>
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<tr>
<td>Rate of DOC Utilization (μM/day)</td>
<td>0.7 (0.3)</td>
<td>1.4 (0.3)</td>
<td>1.1 (0.1)</td>
<td>1.9 (0.1)</td>
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<td>Dark</td>
<td>Light</td>
<td></td>
<td>Dark</td>
<td>Light</td>
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</tr>
<tr>
<td>DOC Remineralized (μM)</td>
<td>15 (2.5)</td>
<td>28 (20.1)</td>
<td>48 (3.4)</td>
<td>67 (6.9)</td>
<td>7 (2.0)</td>
<td>21 (4.4)</td>
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<tr>
<td>% of total DOC Remineralized</td>
<td>3.5 (0.6)%</td>
<td>6.6 (4.3)%</td>
<td>12.8 (0.8)%</td>
<td>17 (3.5)%</td>
<td>2.5 (0.7)%</td>
<td>7.7 (1.7)%</td>
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<tr>
<td>Rate of DOC Utilization (μM/day)</td>
<td>1.1 (0.2)</td>
<td>1.9 (1.2)</td>
<td>2.9 (0.6)</td>
<td>3.9 (0.6)</td>
<td>0.4 (0.1)</td>
<td>1.1 (0.04)</td>
<td></td>
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<th>Jul-00 S = 0</th>
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<th>Jul-00 S = 10</th>
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<th>Jul-00 S = 20</th>
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<td>Dark</td>
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<td>Dark</td>
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<td>Light</td>
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<td>Dark</td>
<td>Light</td>
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</tr>
<tr>
<td>DOC Remineralized (μM)</td>
<td>21 (1.4)</td>
<td>49 (3.1)</td>
<td>20 (15.1)</td>
<td>35 (7.2)</td>
<td>11 (2.4)</td>
<td>2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>% of total DOC Remineralized</td>
<td>4.5 (0.4)%</td>
<td>11 (0.7)%</td>
<td>3.9 (2.9)%</td>
<td>6.7 (1.3)%</td>
<td>4.7 (0.01)%</td>
<td>&lt; 0.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate of DOC Utilization (μM/day)</td>
<td>1.4 (0.2)</td>
<td>4 (0.2)</td>
<td>0.9 (0.2)</td>
<td>2.2 (0.1)</td>
<td>0.7 (0.2)</td>
<td>n.d.</td>
<td></td>
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</tbody>
</table>

\(^a\) The total amount of DOC remineralized was calculated for the duration of the experiments: August 1999 (14 days); April and July 2000 (28 days).

\(^b\) Numbers in parentheses are ± 1 S.D. of mean.

\(^c\) Boldface denotes significant differences in light vs dark treatments. Student’s t-test (p < 0.05).

\(^d\) The rate of DOC utilization was calculated over the first 14 days of all incubations.

\(^e\) n.d. indicates a rate which was not detectable.
Table 4. Bacterial metabolic parameters calculated for the York River estuary in April and July 2000.

<table>
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<td>Dark</td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>Integrated production(^a) (28 days) nmol leu (^{-1}) (± 1 S.D.)</td>
<td>S = 0</td>
<td>109.3</td>
<td>67.1</td>
<td>S = 10</td>
<td>246.4</td>
<td>489.5</td>
</tr>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>Integrated production (7 days) nmol leu (^{-1}) (± 1 S.D.)</td>
<td>S = 11</td>
<td>209.1</td>
<td>329.5</td>
<td>S = 20</td>
<td>247.2</td>
<td>119.3</td>
</tr>
<tr>
<td>Growth Rate(^b) (10(^{-10})) mol leu cell (^{-1}) (± 1 S.D.)</td>
<td>Light</td>
<td>32.7</td>
<td>3.0</td>
<td>Dark</td>
<td>16.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Direction of significant(^c) changes in growth rate (light compared to dark)</td>
<td>n.s.(^*)</td>
<td>n.s.</td>
<td>(+)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Direction of significant changes in BGE(^d) (28 day) (light compared to dark)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>n.d.(^f)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Direction of significant changes in BGE (7 day) (light compared to dark)</td>
<td>(-)</td>
<td>n.s.</td>
<td>(+)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

\(^a\) Integrated production was calculated from estimates of \(^3\)H-leucine incorporation integrated over a 28 day time course for April and a 7 day time course for both April and July 2000 by means of trapezoidal integration.

\(^b\) Growth rate was calculated by measures of \(^3\)H-leucine incorporation per cell.

\(^c\) Significance was calculated by Student's t-test (p < 0.05) and is denoted by (-) or (+) to indicate values for light exposed treatments which are either lesser or greater than dark treatments.

\(^d\) BGE was calculated from integrated production measurements (Figure 11) and DOC decline over specified time intervals (7 or 28 days).

\(^e\) n.s. designates results that are not significant.

\(^f\) Parameters not determined are indicated by n.d.

\(^g\) Bold numbers indicate significant differences in integrated production between light and dark treatments.
Table 5. Summary of bacterial DOC utilization, BGE, Growth rate, and inorganic nutrient production for the York River estuary samples collected in August 1999 and April 2000.

<table>
<thead>
<tr>
<th></th>
<th>Aug-99</th>
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<th>Apr-00</th>
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<td>S = 10</td>
<td>S = 0</td>
<td>S = 11</td>
<td>S = 22</td>
<td>S = 0</td>
<td>S = 11</td>
<td>S = 22</td>
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<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>Dark</td>
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<tr>
<td>Growth Rate* (cells/day) x 10^4</td>
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<tr>
<td>(± 1 S.D.)</td>
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<td></td>
</tr>
<tr>
<td>Light</td>
<td>1.6</td>
<td>2.3</td>
<td>2.1</td>
<td>2.6</td>
<td>4.2</td>
<td>2.8</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Dark</td>
<td>(0.04)</td>
<td>(0.02)</td>
<td>(0.3)</td>
<td>(0.1)</td>
<td>(0.9)</td>
<td>(2.1)</td>
<td>(1.4)</td>
<td>(2.2)</td>
</tr>
<tr>
<td>Light</td>
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<td>Dark</td>
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<tr>
<td>BGE</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
<td>± 1</td>
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<tr>
<td>Light</td>
<td>18.6</td>
<td>32.8</td>
<td>19.8</td>
<td>65.1</td>
<td>18</td>
<td>44.4</td>
<td>27.8</td>
<td>22.1</td>
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<tr>
<td>Dark</td>
<td>(3.9)</td>
<td>(3.9)</td>
<td>(4.2)</td>
<td>(14.0)</td>
<td>(11.1)</td>
<td>(18.6)</td>
<td>(24.5)</td>
<td>(9.7)</td>
</tr>
<tr>
<td>Light</td>
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<tr>
<td>Direction of significant changes in growth rate*</td>
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<tr>
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<td>(-)</td>
<td>(-)</td>
<td>n.s.</td>
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<td>n.s.</td>
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<tr>
<td>Direction of significant changes in BGE</td>
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<tr>
<td>Light</td>
<td>(-)</td>
<td>(-)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>Direction of significant changes in NH₄⁺ change</td>
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<tr>
<td>Light</td>
<td>(-)</td>
<td>(-)</td>
<td>n.s.</td>
<td>(•)</td>
<td>(•)</td>
<td>(•)</td>
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<tr>
<td>Direction of significant changes in PO₄³⁻ change</td>
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</tr>
<tr>
<td>Light</td>
<td>(-)</td>
<td>n.a.</td>
<td>n.s.</td>
<td>(•)</td>
<td>(•)</td>
<td>n.s.</td>
<td>(•)</td>
<td>n.s.</td>
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<tr>
<td>Dark</td>
<td></td>
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</tr>
</tbody>
</table>

*a Growth rate was calculated from increases in cell abundance over time.
*b Significance was calculated by Student's t-test (p < 0.05) and is denoted by (-) or (+) to indicate values for light exposed treatments which are either lesser or greater than dark treatments.
*c BGE was calculated from cell abundance and DOC decline over time interval.
*d n.s. designates results that are not significant.
*e n.a. designates incubations where NH₄⁺ or PO₄³⁻ production is not applicable, and where there was net loss.
Table 6. Summary of studies examining the enhanced and reduced bioavailability of DOM following sunlight or artificial light treatment.

<table>
<thead>
<tr>
<th>System or Source of DOM</th>
<th>Effect on Bioavailability</th>
<th>Method</th>
<th>Effects on DON/DOP and/or nutrient production</th>
<th>Change in DON, DOP</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Bulk DOM</td>
<td>Enhanced</td>
<td>DOC utilization</td>
<td></td>
<td></td>
<td>Geller et al., 1986</td>
</tr>
<tr>
<td>Plant Leachate</td>
<td>Enhanced</td>
<td>Bacterial production</td>
<td></td>
<td></td>
<td>Weitzel et al., 1995</td>
</tr>
<tr>
<td>Bulk DOM (humic lakes)</td>
<td>Enhanced</td>
<td>Bacterial biomass</td>
<td></td>
<td></td>
<td>Lindell et al., 1996</td>
</tr>
<tr>
<td>Humic Isolates (Boreal Pond)</td>
<td>Enhanced</td>
<td>Bacterial Production</td>
<td></td>
<td></td>
<td>Bushaw et al., 1996</td>
</tr>
<tr>
<td>Humic Isolates (Saltmarsh)</td>
<td>Enhanced</td>
<td>Bacterial Production</td>
<td></td>
<td></td>
<td>Miller and Moran, 1997</td>
</tr>
<tr>
<td>Subtropical seagrass meadow</td>
<td>No Effect</td>
<td></td>
<td>No Effect (DON)</td>
<td></td>
<td>Ziegler and Benner, 2000</td>
</tr>
<tr>
<td>Forested and Anthropogenic non-point sources to rivers</td>
<td>No Effect</td>
<td></td>
<td></td>
<td></td>
<td>Wiebe and Seitzinger, 2001</td>
</tr>
<tr>
<td>Bulk DOM (non-point sources to rivers)</td>
<td>No Effect</td>
<td>Bacterial Growth on DOM utilization</td>
<td></td>
<td>(-) with added inorganic nutrients</td>
<td>Farjalla et al., 2001</td>
</tr>
<tr>
<td>Humic lake (Colored DOC)</td>
<td>Enhanced</td>
<td>Bacterial Biomass</td>
<td></td>
<td>(+)</td>
<td>Kieber et al., 1989</td>
</tr>
<tr>
<td>Intertidal Water (bog)</td>
<td>Enhanced</td>
<td>Bacterial Biomass</td>
<td>Enhanced (DON/DOP)</td>
<td></td>
<td>Tranvik et al., 1999</td>
</tr>
<tr>
<td>Bulk DOM (chlorophyll max.)</td>
<td>Enhanced</td>
<td>Pyruvate uptake</td>
<td></td>
<td></td>
<td>Obernosterer et al., 2001</td>
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<tr>
<td>Southern Ocean</td>
<td>Reduced</td>
<td>Bacterial Production</td>
<td></td>
<td></td>
<td>Tranvik and Kokalj, 1998</td>
</tr>
<tr>
<td>Bulk DOM (deep water)</td>
<td>Enhanced</td>
<td>Bacterial Production</td>
<td></td>
<td></td>
<td>Anesio et al., 2000</td>
</tr>
<tr>
<td>Humic Isolates plus algal DOC</td>
<td>Reduced</td>
<td>Bacterial Respiration</td>
<td></td>
<td>(+)</td>
<td>Anesio et al., 2000</td>
</tr>
<tr>
<td>Leachate (Aquatic Primary Producers)</td>
<td>Reduced</td>
<td>Bacterial C Utilization (respiration + biomass)</td>
<td></td>
<td>(-)</td>
<td>Benner and Hidda, 1998</td>
</tr>
<tr>
<td>Leachate (Terrestrial Primary Producers)</td>
<td>Enhanced</td>
<td>Bacterial C Utilization (respiration + biomass)</td>
<td></td>
<td></td>
<td>Benner and Hidda, 1998</td>
</tr>
<tr>
<td>Bulk DOM (Surface Ocean)</td>
<td>Reduced</td>
<td>Bacterial Production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk DOM (Deep Ocean)</td>
<td>Enhanced</td>
<td>Bacterial Production</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Boreal Watershed (Humic waters)</td>
<td>Reduced</td>
<td>DOC Utilization</td>
<td></td>
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</tr>
<tr>
<td>Phyto-flagellate Leachate</td>
<td>Reduced</td>
<td>DOC Utilization</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Prorocentrum sp. Leachate</td>
<td>No Effect</td>
<td></td>
<td></td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>Bulk DOM (Temperate Estuary, Low Flow)</td>
<td>Enhanced</td>
<td>DOC Utilization/Bacterial Production</td>
<td></td>
<td>Decreased INP Production</td>
<td>This study</td>
</tr>
<tr>
<td>Bulk DOM (Temperate Estuary, Moderate and High Flow)</td>
<td>Enhanced</td>
<td>DOC Utilization/Bacterial Production</td>
<td></td>
<td>No effect (DON, DOP)</td>
<td>This study</td>
</tr>
<tr>
<td>Bulk DOM (Temperate Estuary, Moderate and High Flow)</td>
<td>Enhanced</td>
<td>DOC Utilization/Bacterial Production</td>
<td></td>
<td>No effect (DON, DOP)</td>
<td>This study</td>
</tr>
</tbody>
</table>

\[ a \text{ IN = Inorganic nutrient} \]
Figure 1. Experimental Design
Pre-exposure:
subsampled for
DOC, $\text{NH}_4^+\text{PO}_4^{3-}$,
AODC slides made
to ensure bacteria-
free water

Light Exposed
Dark Control

Post-exposure: water
subsampled again for
DOC, $\text{NH}_4^+\text{PO}_4^{3-}$.
Water transferred to
polycarbonate bottles,
inoculated with
bacteria, and monitored
for DOM utilization and
bacterial parameters
over incubation time
course.

Bacterial Abundance
Bacterial Production
Exoenzyme Activity
DOC, DON, DOP Decomposition
Inorganic Nutrient Production
Bacterial Growth Rate
Bacterial Growth Efficiency
Figure 2. Monthly (panel a) and annual (panel b) mean streamflow statistics for the Pamunkey River near Hanover, VA. Data retrieved from U.S. Geological Survey (http://waterdata.usgs.gov). Arrows denote sampling periods in August 1999, April 2000 and July 2000.
Figure 3. Ratios of the concentrations of DOC, NH4+, and PO4^{3-} (panels a, b and c respectively) in dark controls (filled symbols) and sunlight exposed treatments (open symbols) relative to the starting concentration before sunlight exposure. Concentration changes measured in dark controls may have resulted from microbially mediated changes from bacteria passing through the 0.2 μm capsule prefiltration. Error bars denote ± 1 S.D. of the mean (n=3). Concentrations below detection limit indicated by b.d. Significance (p < 0.05) denoted by (*) using Student’s t-test.
Figure 4. Relative activity of three ectoenzymes measured in April 2000 in the York River estuary. Values are presented as fluorescence measurements in sunlight exposed treatments normalized to dark treatments. Error bars designate ± 1 S.D. of the mean (n=3). Stars denote significance at $p < 0.05$ by Student’s t-test.
Leucine Aminopeptidase

Alkaline Phosphatase

Beta Glucosidase

(Fluorescence exposed incubations)

(Fluorescence dark incubations)

Dark

Sunlight

Salinity

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Figure 5. Total DOC utilized in leachates (low concentration *Pelatandra virginica* and phytoflagellate 14 day incubation; high concentration *Pelatandra virginica* 28 day incubation). Error bars designate ± 1 S.D. of the mean (n = 3). Stars denote significance at $p < 0.05$ Student’s t-test.
Peltandra virginica (low concentration) 100 µM
Peltandra virginica (high concentration) 1800 µM
Phytoflagellate 365 µM

µM DOC utilized

Dark
Sunlight

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Figure 6. Total DOC utilized in August 1999, April 2000 and July 2000 York River unamended experimental incubations. Error bars designate ± 1 S.D. of the mean (August 1999, n=2) (April and July 2000, n=3). Stars denote significance at $p < 0.05$ Student's t-test.
Figure 7. Representative incubation time courses from August 1999 for the York freshwater endmember. Panels a-c show DOC, DON and DOP concentrations, respectively. Panels d-f show NH4+, NOx, and PO43- concentrations, respectively. Error bars denote ± 1 S.D. of the mean (n=2).
Figure 8. Incubation time courses from August 1999 for the mid-salinity (S=10) York River sampling station. Panels a-c show DOC, DON and DOP concentrations, respectively. Panels d-f show NH4+, NOx, and PO43- concentrations, respectively. Error bars denote ± 1 S.D. of the mean (n=2).
Figure 9. Incubation time courses from April 2000 for the mid-salinity (S=11) York River estuary sampling station. Panels a-c show DOC, DON and DOP concentrations, respectively. Panels d-f show NH4+, NOx, and PO43- concentrations, respectively. Error bars denote ± 1 S.D. of the mean (n=3).
The graph shows the concentration changes of different substances over time under dark and sunlight conditions.

- **DOC (μM)**: Concentration of Dissolved Organic Carbon.
- **DON (μM)**: Concentration of Dissolved Organic Nitrogen.
- **DOP (μM)**: Concentration of Dissolved Organic Phosphorus.
- **NH₄⁺ (μM)**: Concentration of Ammonium.
- **NO₃⁻ (μM)**: Concentration of Nitrate.
- **DIP (μM)**: Concentration of Dissolved Inorganic Phosphorus.

The x-axis represents time in days, and the y-axis shows the concentration levels. The dark and sunlight conditions are represented by different markers and error bars indicating variability.
Figure 10. Bacterial growth parameters (³H-leucine incorporation, ³H-thymidine incorporation, and bacterial cell abundance in panels a, b, and c respectively) for April 2000 mid-salinity (S=11) incubations. Error bars are ± 1 S.D. of the mean (n=3).
Figure 11. Relative differences in bacterial production (integrated over 7 days) between sunlight exposed and dark treatments from three sampling stations along the York River estuary salinity gradient in April and July 2000. Inset shows estimates of bacterial production from April integrated over 28 days. Error bars designate ± 1 S.D. of the mean (n=3). Stars denote significance at $p < 0.05$ Student's t-test (within treatment dates).
Figure 12. Conceptual model depicting interactive photochemical and microbial regulation of DOM cycling in an estuary.
Abiotically Mediated \( \rightarrow \) 
Biotically Mediated \( \rightarrow \)

\[ \text{NH}_4^+; \text{PO}_4^{3-} \rightarrow \text{DOM} \] 
\[ \text{CO}_2 \rightarrow \text{Enhanced Availability } \pm \] 
\[ \text{Decreased Availability } \pm \] 

River Flow 

Bacteria
Chapter III

Identifying the Sources and Ages of Organic Matter Supporting River and Estuarine Bacterial Production: A Novel Multiple Isotope ($\delta^{13}$C, $\delta^{15}$N, $\Delta^{14}$C) Approach
ABSTRACT

The stable isotopic ($\delta^{13}$C, $\delta^{15}$N) and radiocarbon ($\Delta^{14}$C) values of organic matter (OM) assimilated by bacteria in the York and Hudson River estuaries were measured by two independent methods: 1) extraction of bacterial nucleic acids from water collected in situ and 2) production of biomass from bioassay incubations. The organic matter sources supporting bacterial production in the York River estuary could not be discerned solely through dual stable ($\delta^{13}$C, $\delta^{15}$N) isotope plots as overlaps in terrestrial, freshwater phytoplankton and marsh derived organic matter isotopic signatures prohibited unequivocal source assignments. Dual isotope plots of $\delta^{13}$C and $\Delta^{14}$C better resolved the organic matter sources assimilated by bacteria. Bacterial production in freshwater regions was fueled by OM of young (decadal in age), terrigenous origin and estimated to account for 42-89% of OM assimilated. The remainder (11-58%) of OM supporting bacterial growth was derived from freshwater algae. In the mid-salinity York, bacterial production was supported primarily by phytoplankton-derived OM in the spring and summer (93-100%) and marsh derived OM in the fall (73-100%). Isotopic values of bacteria higher salinity regions of the estuary suggested that production was supported by phytoplankton-derived OM (86-100%) in July and November whereas a more balanced mixture of algal and and marine-like OM (50-69%) was assimilated in October. In contrast to the young (10-20 yr) OM supporting bacterial production in the York, production in the Hudson River estuary was subsidized by a substantial portion of old
(-1200 BP) allochthonous (presumably soil-derived) OM. The stable and radioisotope values of bacteria produced from bioassays frequently did not reflect the isotopic signature of bacterial nucleic acids extracted from river and estuarine waters. These discrepancies may be a result of the uncoupling of a supply of fresh, reactive dissolved organic matter (DOM) or the metabolic flexibility of bacterial populations to using a range of different-aged DOM components.

INTRODUCTION

As the principal consumers of dissolved organic matter (DOM) (Pomeroy, 1974; Azam et al. 1983), heterotrophic bacteria are pivotal in apportioning its fate in aquatic systems between assimilation into biomass, respiration (Findlay et al., 1992; Moran et al., 1999) and transformation to more recalcitrant forms (Brophy and Carlson, 1989) and subsequent export to coastal seas. A clear delineation of C cycling from its initial biological fixation to eventual respiration or export of organic matter (OM) from riverine/estuarine systems is therefore needed. However, our understanding of OM sources and sinks in these systems has been hampered by such factors as overlapping isotopic signatures, mixing of co-equal sources of organic matter, seasonal variability in the inputs of autochthonous and allochthonous sources, and physical homogenization of OM sources (Canuel et al., 1995; Hedges and Keil, 1999; Cloern et al. 2002). While bioassays are a primary method for determining the bioavailability of bulk pools such as DOM (Servais 1989; Amon and Benner 1996; Carlson and Ducklow 1996; Cherrier et al., 1996; Carlson et al., 1999; Sobczak et al., 2002) they are limited in the information
they can provide about the specific (i.e., in terms of biochemical composition and age) potential sources of OM supporting heterotrophic production.

Globally, rivers deliver 0.25 Pg of DOC per year to the ocean (Meybeck, 1982; Hedges, 1992; Hedges et al., 1997) usually via estuaries. Several studies have reported conservative transport of dissolved organic carbon (DOC) through estuaries (e.g. Laane and Koole, 1982; Sharp et al., 1982; Mantoura and Woodward, 1983; Ittekot 1989) suggesting a lack of removal by the foodwebs there. The highly condensed polymeric nature of allochthonous DOM in riverine and estuarine systems has perpetuated this view. Still one of the great paradoxes in marine biogeochemistry is the apparent absence of a terrestrial signature in open ocean DOM (Meyers-Schulte and Hedges, 1986; Druffel et al., 1992; Hedges et al., 1992; Opsahl and Benner, 1997; Hedges et al., 1997). Reports of net system heterotrophy in coastal ecosystems (Smith and Hollibaugh, 1993; Heip et al., 1995; Frankignoulle et al., 1998) also require the \textit{de facto} utilization of some portion of this allochthonous material on time scales of estuarine mixing.

The application of natural isotopic tracers to trace OM inputs and cycling has helped resolve the primary sources of OM contributing to secondary production and subsequently aided in the establishment of trophic level linkages (Peterson et al., 1985; Currin et al., 1995; Deegan and Garritt 1997). Although stable isotope analysis has proven a useful means for inferring these sources and pathways both in freshwater and marine systems (Hedges et al., 1988; Canuel et al., 1995; Currin et al., 1995), ambiguities often arise in identifying the relative contributions of multiple sources to bulk OM pools because the isotopic signature of a given primary producer is frequently not unique and distinguishable from the others.
Both $\delta^{13}$C and $\delta^{15}$N have also been applied with varying degrees of success for identifying the sources of OM assimilated by bacteria (Coffin et al., 1989; Coffin et al., 1990; Kelley et al., 1998; Cifuentes and Coffin, 1999. Nevertheless, in dynamic riverine/estuarine environments with variable organic and inorganic nutrient sources, stable isotope signatures alone may not allow for unequivocal source identifications (Cloern et al., 2002). However, the simultaneous use of multiple isotopic tracers may help overcome some of these limitations (Peterson et al., 1985). Natural abundance radiocarbon measurements ($\Delta^{14}$C) have the potential to provide additional resolution in discerning the relative importance of allochthonous and autochthonous sources to bacterial production (Cherrier et al., 1999). The greater dynamic range of $\Delta^{14}$C (~ -1000 to +250 $^\circ$/oo) compared to $\delta^{13}$C sources (~ -32 to -12 $^\circ$/oo) in rivers and estuarine environments, may allow it to be better resolved between multiple autochthonous sources, and autochthonous and allochthonous OM may be better differentiated and traced by their dual $\Delta^{14}$C and $\delta^{13}$C signatures (Raymond and Bauer, 2001; Bauer et al., 2002).

The overall objective of this study was to identify the sources and ages of DOM supporting bacterial production in the York and Hudson River estuaries, where published findings suggest that a substantial portion of heterotrophic production (i.e., bacterial) must be supported by allochthonous (i.e. terrestrial) sources. A unique stable isotopic ($\delta^{13}$C, $\delta^{15}$N) and natural radiocarbon ($\Delta^{14}$C) and approach was thus employed to examine the sources of DOM fueling the microbial food web in these two systems. The strong contrast in mean DOM ages, between these two systems provides a unique opportunity to
employ multiple isotopes to trace the natural sources and ages of DOM fueling bacterial metabolism in them.

**METHODS**

**Study Sites and Sampling Locations**
Details regarding the setting of the York River estuary component of this study have been documented previously (Chapter 1) of this dissertation.

The Hudson River basin (33,500 km$^2$) encompasses parts of Vermont, eastern New York, Massachusetts, Connecticut and New Jersey. By established convention, sampling sites are designated by distance upriver from the Battery in New York City (Figure 1). The river is divided into upper non-tidal and a lower tidal freshwater section. The tidally influenced, freshwater Hudson extends south from the head of tide at Green Island, NY (240 km) approximately 130 km before encountering saline waters in the Hudson River estuary in the vicinity of Newburgh, NY. The tidal amplitude is ~1-2 m over this entire distance. The present study concentrated on the tidal freshwater Hudson; however, seawater intruded into the lower reaches of the study area in June 2001. The Hudson River averages ~ 1 km across with an average depth of ~ 10 m, and is well mixed with essentially no vertical temperature gradients (Raymond et al., 1997). Phytoplankton biomass in the Hudson River, while historically high (maximum Chl $a$ ~17 mg L$^{-1}$), has been decimated in recent years by the zebra mussel invasion which drastically reduced phytoplankton standing stock (Chl $a$ 1-5 $\mu$g L$^{-1}$) (Caraco et al., 1997). Approximately 93% of the freshwater inputs to the study area can be attributed to flow over the Green Island dam (83%) and Roundout Creek (10%) (Findlay et al., 1998).
Sampling and Experimental Design

Water for bacterial nucleic acid extractions and bioassays from the York River estuary was collected from three sites (see Chapter 1 this dissertation) from along the salinity gradient throughout various seasons and flow regimes (Table 1). In the tidal freshwater Hudson River, bacteria for nucleic acid extractions were collected from Poughkeepsie at km 122 and Corning Preserve at km 240 along the tidal freshwater Hudson River in October 2000. Water samples for bacterial nucleic acid extraction were collected again in June 2001 from the previous two sites as well as an additional location further downstream (George Washington Bridge, salinity 3.2 at km 25).

Table 1 outlines the different methodologies employed for each sampling in order to determine the stable and radio-isotope signatures of DOM assimilated by bacteria. In general, samplings combined bacterial collection for nucleic acid extraction with one of several different bioassay approaches. Based on initial estimates of cell abundances (Schultz, 1999; Findlay et al., 1999), an assumed nucleic acid content per cell of 6-35 fg cell\(^{-1}\) (Coffin and Cifuentes, 1993) and assumed C content of nucleic acids ~45% (Coffin and Cifuentes, 1993), it was estimated that between 100 – 200 L would be necessary for extraction of sufficient bacterial C for radiocarbon analysis.

Stable isotopic ($\delta^{13}$C and $\delta^{15}$N) and radiocarbon ($\Delta^{14}$C) natural abundances were used to estimate the relative sources and ages of DOM assimilated by bacteria via one of two independent methods: (1) direct bacterial nucleic acid extractions, and (2) microbial biomass produced from bioassay re-growth incubations. Bacterial nucleic acids represent a source-specific (i.e., bacterial) biomarker which allows for in situ estimates of the source and age of DOM supporting bacterial production. An alternative approach to
estimating the isotopic signatures of DOM incorporated by bacteria is through bioassay incubations where the isotopic signature of the bacterial biomass produced should reflect the sources and ages of DOM supporting bacterial production. The stable and radioisotope signatures of microbially assimilated DOM may be measured by allowing bacteria to grow exponentially on the ambient DOM pool, harvesting the biomass produced and analyzing it for stable and radio-isotopic analysis (Coffin et al., 1990; Kelly et al., 1998). For both approaches, surface water was collected from sites along the York and Hudson River estuaries and returned to VIMS and IES respectively for immediate processing. Figure 2 illustrates the experimental design and sampling methodologies.

Sample collection and processing for natural abundance radiocarbon required meticulous attention in order to avoid contamination. In all cases, samples for $\Delta^{14}C$ were processed in lab space which had never been exposed to radioisotopes. All incubation bottles, filtration apparati, tubing, etc. were purchased for the sole purpose of natural abundance $\Delta^{14}C$ isotopic analysis and were kept in complete isolation from any sources of tracer $^{14}C$.

Sample Collection and Concentration of Bacteria

Surface water samples were collected in acid-leached (10% HCl) Nanopure rinsed polycarbonate bottles (~20 L). Water samples were pre-filtered through 0.7 μm combusted glass fiber filters (GF/F) to remove macrozooplankton, POM, protozoans and larger phytoplankton (Coffin et al., 1990; Cherrier et al., 1999) and subsequently concentrated by tangential flow ultrafiltration (Table 1). The Amicon DC-10 ultrafiltration unit was fitted with a single polysulfone hollow fiber cartridge (0.1 μm).
Particles (<0.1 μm) were concentrated to ~1-L final volume. The bacterial concentrate was harvested onto acid soaked (10% HCl), Nanopure-rinsed Gelman microculture capsules (0.2 μm pore-size). Once the bacteria were concentrated, the filter capsule was purged of water, sealed with combusted aluminum foil, and stored at -80 °C until extraction. Alternatively, after the previously outlined pre-filtration, bacteria were also concentrated directly on 0.2 μm Gelman microcapsules as opposed to concentration by tangential flow filtration (Table 1). Gelman microcapsules were placed in-line and downstream of the 0.7 μm pre-filter. Microcapsules were kept on ice throughout the concentration process and stored as described above until extraction.

**Nucleic Acid Extraction**

Bacterial nucleic acid extractions were performed according to the modified method of Coffin et al. (1993) as outlined in Cherrier (1997). In brief, bacterial cells collected in the microcapsules were lysed by adding a detergent/buffer solution (20 mM TRIS, 2 mM EDTA, and 2% sodium dodecyl sulfate (SDS)) and heating the sealed capsule in a 100 °C water bath for 15 min. Following the precipitation and removal of the SDS from the lysate, nucleic acids were isolated and purified by dialysis, followed by ethanol, phenol, and isoamyl alcohol/chloroform precipitations. Twice capsules were re-extracted by the above protocol to serve as methodological blanks to assess potential contamination by solvents and processing. Possible contamination of the extract by protein was assessed by spectrophotometric absorbance (A_{260}/A_{280}) ratios (Sambrook et al., 1989). Approximately 10% of the extract was used to verify purity and was then
retained for subsequent $\delta^{13}$C and $\delta^{15}$N stable isotope analysis. Extracts were stored at (-80 °C) until isotopic analysis (<1 month).

**Nucleic Acid Purification and Humic Correction**

Humic materials may co-extract with nucleic acids when present in high concentrations (Coffin and Cifuentes, 1993; Jackson et al., 1997; Edgcomb et al., 1999) and are detectable as an amber tinge to an otherwise opaque extract. Co-extraction of humics occurred at the freshwater York River estuary station and during the June 2001 sampling of Hudson River (Corning Preserve and Pougkeepsie). DNA has been purified for molecular amplification by various methodologies, most employing gels to bind humics. Two methods used successfully for the removal of humics from DNA extracts for subsequent PCR amplification (MicroSpin Sephacryl S-300 columns, Edgcomb et al., 1999 and Sepharose 4B pre-loaded in spun columns, Jackson et al., 1997) were marginally successful in the removal of humic compounds in this study (gels were used according to manufacturers specification and procedures as outlined in Edgcomb et al., 1999 and Jackson et al., 1997). Columns retained a fraction of humic material although eluent remained amber colored despite several passes of sample through columns. As complete removal of contaminating humic material was not possible, a correction was applied to adjust $\delta^{13}$C and $\Delta^{14}$C isotopic values for the contribution of humics to the overall extract. The separate contributions of humic material and nucleic acids contributing to the measured isotopic signatures was estimated as:

$$^h \text{C}_{(\text{humics + NA})} = ^h \text{C}_{(\text{humics})} (x) + ^h \text{C}_{(\text{NA})} (y)$$
where \( h_{C_{\text{humics} + \text{NA}}} \) is the measured isotopic value (\( \delta^{13}C, \Delta^{14}C \)) for the total extract.

\( h_{C_{\text{humics}}} \) is the isotopic signature of humics, measured as described below, and \( x \) and \( y \) are the relative contributions from humic and nucleic acid carbon, respectively (and \( x + y = 1.0 \)). The values of \( x \) and \( y \) were estimated independently from C:N values as:

\[
C:N_{\text{(humics + NA)}} = C:N_{\text{humics}}(x) + C:N_{\text{NA}}(y)
\]  

(2)

The C:N values of the total extract (\( C:N_{\text{(humics + NA)}} \)) were measured on a FinniganMAT Delta\text{plus} dual-inlet continuous flow isotope ratio mass spectrometer. A literature value of \( \sim 2.25 \) was used for C:N values for nucleic acids (Coffin and Cifuentes, 1993). Humic compounds from both the York and Hudson River estuaries were isolated as described below and the C:N ratio measured on a Finnigan MAT Delta\text{plus} dual-inlet continuous flow isotope ratio mass spectrometer. Equation (2) was then solved for the relative contribution of humics and nucleic acids in the total extract value. Values for \( x \) and \( y \) were then substituted into equation (1) and solved for \( h_{C_{\text{NA}}} \), the isotopic composition of nucleic acids.

**Isolation of Humic Materials**

In order to correct for potential contamination by freshwater humics, surface water (2-L) for humic isolation was collected in May 2002 from the York River (freshwater end-member) and in July 2002 from the Hudson River (Corning Preserve) estuaries. In the Hudson River estuary the relative contribution of humics to bulk DOC may vary in space but it was assumed the source and \( \delta^{13}C \) and \( \Delta^{14}C \) signature would not. Water samples were passed sequentially through combusted GF/F and 0.2 \( \mu \)m Gelman microcapsules to remove particles and then acidified to pH 2.0 with 6 N HCl. The
sample was then passed through a column of Supelco Superlite DAX-8 that had previously been cleaned with ether, acetonitrile, and methanol, and rinsed with HCl, NaOH, and DI water (Moran and Hodson, 1994). Humic substances were eluted from the resin with 0.1 N NaOH, dried by vacuum evaporation (Savant Speedvac SC210A) and acidified with 10% HCl.

**Bacterial Bioassay Incubations**

The particle-free filtrate (0.1-0.2 μm) was retained from the above described collection of bacteria for bacterial bioassays. The filtrate from either the Amicon tangential flow filtration unit (0.1μm) or from the Gelman 0.2 μm microcapsules was collected in acid cleaned (10% HCl) polycarbonate containers (~20 L) (Figure 2). Subsamples of the filtrate were taken for bacterial abundance and DOC concentration to ensure both the effectiveness of prefiltration and the absence of DOC contamination from hollow fiber and Gelman capsule filtrations. The filtrate was subsequently inoculated with a 1% (v:v) water sample that had been previously passed through a GF/F (0.7 μm) filter to remove most grazers and autotrophs (Figure 2) but which allows bacteria to pass. Small (~16 L) and large (~90 L) volume bioassays were conducted to assess both the stable and radio-isotope signatures of DOM incorporated by bacteria.

Incubations were carried out in the dark at room temperature (~23 °C). Bacterial abundance was monitored throughout the incubation in order to maximize bacterial biomass, and minimize the impact of grazing. Upon termination (36-60 hours), incubation water was passed through a precombusted (500 °C, 4.5 hours) in-line glass fiber filter (see Table 1 for details) to collect the bacterial size fraction. Small volume
bioassays were performed in duplicate and analyzed for both $\delta^{13}$C and $\delta^{15}$N of bacterial biomass. Both the volume required (90 L) and the expense of $\Delta^{14}$C analysis precluded duplicate samples within a given sampling timeframe. Blanks were collected to estimate the fraction of DOM which may adsorb to filters as opposed to that which is incorporated by bacteria by passing particle-free filtrate (approx. 0.2 μm) directly through glass fiber filters. Water volumes used for blanks were the same as sample volumes (16 or 90 L). Samples and blanks were stored at −80 °C until isotopic analysis.

A variation of the bioassay approach was employed during during the August 2000 sampling of the York River estuary. Surface water was collected from three stations along the York and passed through a 0.7 μm glass fiber filter and a 0.2 μm Gelman capsule. Approximately 1 L of particle-free filtrate was dispensed into acid leached (10% HCl) polycarbonate incubation bottles. Triplicate incubations were conducted for each sampling site. Incubations were inoculated and monitored as in the above larger volume incubations. However, after ~48 hours bacteria from approximately 400 ml of incubation water were harvested by vacuum filtration onto 0.2 μm pre-combusted (500 °C, 4.5 hours) Anodiscs. Anodiscs were acid fumed overnight, dried at 60 °C, and stored at room temperature until stable isotope analysis. Methodological blanks consisted of ~400 ml of inoculated incubation water passed through a 0.2 μm Anodisc filter immediately following the addition of the 1% (v:v) bacterial inoculum. Blanks were then handled in an analogous manner to biomass samples in subsequent preparation for mass spectrometry analysis.
DOM Isolation for Isotopic Analysis

High molecular weight (> 3 kD) dissolved organic matter for stable ($\delta^{13}$C, $\delta^{15}$N) and $\Delta^{14}$C radio-isotope analysis was concentrated by tangential flow filtration of York River water (~ 75-125 liters) at 3 sampling locations (0, 10, and 20 salinity). Particle-free water (0.2 µm) was retained after bacteria were isolated and concentrated for nucleic acid extractions in March and October 2000. An Amicon DC-10 concentrator equipped with two spiral-wound polysulfone cartridges was used to concentrate the HMW DOM to a ~1-L volume. The sample was further reduced to ~ 50 ml final volume by turbo-evaporation and then lyophilized. Lyophilized DOM was reconstituted in ~10 ml of DI water and desalted overnight in Pierce 3.5 kD slide-a-lyzers according to manufacturer's specifications. Desalted HMW DOM was again lyophilized and prepared for isotope analysis as outlined above.

Macrophyte Leachate Preparation for Isotopic Analysis

A leachate was prepared for stable ($\delta^{13}$C) isotopic analysis from *Peltandra virginica*, the most prominent freshwater grass common to the York. Although the particulate carbon from *Peltandra* leaves could have been analyzed as a potential source, leachates themselves may differ isotopically from parent material (Coffin et al., 1990; Hullar et al., 1996) and may better represent the DOM source material available for riverine bacterial uptake. Leaves were collected in July prior to senescence, rinsed with DI water and allowed to leach in the dark in an aerated acid washed carboy (20 L) with DI water for 10 days. A sub-sample (2 liters) was subsequently reduced in volume by turbo-evaporation to approximately 50 ml. The concentrated 50 ml leachate was
lyophilized to remove all associated water. After acidification with 10% HCl, samples were analyzed for $\delta^{13}C$ (see below).

**Sample Preparation and Isotopic Analyses**

Glass fiber filters containing bacteria from bioassay incubations were thawed and dried overnight at 60 °C. Anopore filters were dried immediately following filtration of the bioassays. Both filter types were then acid-fumed overnight with concentrated HCl and subsequently dried overnight at 60 °C. Lyophilized *Peltandra* leachate and HMW DOM were transferred to baked (500 °C) 2 ml vials, acidified with 10% HCl, dried overnight and analyzed with a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer (Stable Isotope Facility, University of California, Davis). Filter samples and *Peltandra* leachate were combusted to CO$_2$ and N$_2$ gas by Dumas combustion and analyzed using a Sira Series II isotope ratio mass spectrometer (Colorado Mass Spectrometry Company).

Aliquots of nucleic acid extracts (~ 1/10 initial sample) were thawed, transferred (3 DI rinses) to combusted (500 °C) centrivap tubes. Samples were reduced in volume to approximately 100 µl by vacuum evaporation (Labconco Centrivap Model 78100-00D), transferred quantitatively (3 DI rinses) to acetone rinsed tin foil CHN capsules and dried overnight at 60 °C. Samples were analyzed using a FinniganMAT Delta$^{+}$plus dual-inlet continuous flow isotope ratio mass spectrometer (G.G. Hatch Isotope Laboratories, University of Ottawa).

As a precautionary measure, following $\Delta^{14}C$ accelerator mass spectrometric (AMS) analysis, sample targets from the nucleic acid extracts were immediately (within
1-2 hours) removed from atmospheric CO$_2$ (placed in a sealed evacuated plastic bag).

Graphite targets were subsequently drilled out and residual graphite was placed in a pre-combusted 6 mm quartz tube with added CuO and Ag. Quartz tubes were evacuated, sealed, combusted at 900 °C with CuO/Ag metal catalyst and evolved CO$_2$ was analyzed for $\delta^{13}$C.

Stable isotope values are reported in standard del ($\delta$) notation as

$$\delta X = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 10^3 \quad (3)$$

where X is $^{13}$C or $^{15}$N and R is $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N. The recognized standards are PeeDee Belemnite (NBS-1) and atmospheric N$_2$ for $\delta^{13}$C and $\delta^{15}$N, respectively.

For natural abundance $\Delta^{14}$C measurements, nucleic acid extracts (~1 ml) were thawed, transferred (3 DI rinses) to pre-baked (500 °C) 13 mm diameter Pyrex tubes and reduced in volume to ~ 2 ml by vacuum evaporation. Samples were subsequently acidified overnight at 4 °C with 1 ml % H$_3$PO$_4$, reduced in volume by vacuum evaporation, and transferred to combusted (500 °C) quartz tubes (6 mm diameter) and vacuum evaporated until all water was removed (minimum 14 hours). Samples were then processed by sealed quartz-tube combustion (900 °C using a CuO/Ag metal catalyst) to produce CO$_2$ (Sofer 1980). The CO$_2$ was then reduced to graphite in an atmosphere of H$_2$ over cobalt catalyst (Vogel et al., 1987). Graphite targets were analyzed at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory. $\Delta^{14}$C is defined as the $^{0}/_{oo}$ (per mil) deviation of a sample from the $^{14}$C activity of 19th century wood. All reported $\Delta^{14}$C values were corrected for fractionation using the $\delta^{13}$C values of the samples and the conventions of Stuiver and Pollach (1977).
Blank Corrections

Whenever possible, bioassay samples were corrected for filter $\delta^{13}C$ and $\Delta^{14}C$ blanks by:

$$ (I_{\text{meas}})(\text{mass}_{\text{meas}}) = [(I_{\text{biomass}})(\text{mass}_{\text{biomass}})] - [(I_{\text{blank}})(\text{mass}_{\text{blank}})] $$  \hspace{1cm} (4)

where the mass of total sample (mass$_{\text{meas}} =$ biomass (+ blank C)), mass of corresponding blank filters (mass$_{\text{blank}}$), isotopic signature ($\delta^{13}C$ or $\Delta^{14}C$) of sample ($I_{\text{meas}}$), and isotopic signatures of blank filter ($I_{\text{blank}}$) were measured. The carbon content of actual bacterial biomass (mass$_{\text{biomass}}$) was calculated as the difference between (mass$_{\text{meas}}$) and (mass$_{\text{blank}}$). The equation was solved for the $\delta^{13}C$ or $\Delta^{14}C$ isotopic signature of bacterial biomass ($I_{\text{biomass}}$).

Bacterial Abundance

Samples (10 ml) for bacterial abundance were preserved with 0.2 μm filtered 25% glutaraldehyde diluted to a final concentration of 2%. Preserved samples were stored at 4°C until slide preparation (within 7 days of sample collection). Bacterial abundance was determined by acridine orange epifluorescence microscopy (Hobbie et al., 1977) with a Zeiss Axiophot microscope. Duplicate slides were counted per treatment with a minimum of 350 cells counted per replicate.

Chl a and Phaeopigments

Approximately 100 ml of surface water was collected from each sampling location in an amber polycarbonate bottle and stored on ice until analysis. Chl a was determined by DMSO/acetone extraction according to Burnison (1980). Triplicate
aliquots of 8 ml were filtered through GF/F (Whatman) filters. Filters were placed in 8 ml test tubes filled with DMSO/acetone/water solution (45:45:10), sealed and kept in the dark for a minimum of 48 hours. Sample fluorescence was read on a Turner fluorometer (model 10-AU). After determination of Chl a, samples were acidified with HCl and analyzed fluorometrically as above for phaeopigments.

RESULTS

Water Column Characteristics

Samples for bacterial nucleic acids were collected from the York and Hudson River estuaries across several seasons and differing hydrologic regimes (streamflow data retrieved from http://waterdata.usgs.gov). Maximal streamflow (30.5 m$^3$ s$^{-1}$) in the York occurred in the spring (March 2000) and decreased to a low in the fall (3.5 m$^3$ s$^{-1}$) (Table 2). Temperatures ranged from a low of 11 °C (March) to 27 °C (July) in the York River estuary. Chl a and phaeopigments varied in space and time with maximal concentrations of each consistently associated with the mid-salinity station (Table 2). In general, phaeopigment (Chl a degradation products) concentrations were approximately half or greater than the total Chl a. Chl a at the mid-salinity location was elevated by 5 and 2.5 times over the head and mouth of the estuary, respectively. Peak Chl a concentrations of 34.4 μg L$^{-1}$ in the York coincided with the spring (March) sampling and concentrations declined by 65 % over time to a low of 12.3 μg L$^{-1}$ in the fall (Table 2). Chl a concentrations at the freshwater endmember were low ranging from 3.5 to 5.7 μg L$^{-1}$ throughout the sampling period, and were 7.4 μg L$^{-1}$ at the mouth of the York, with the
notable exception of July where concentrations were a factor of ~2.5 times greater (17.9 μg L⁻¹).

Samples were collected from the Hudson River estuary during moderate to high streamflow periods (Table 1). Monthly averaged streamflow rates for the Hudson River estuary (USGS Green Island, NY) were almost double during June 2001 (457.3 m³ s⁻¹) as compared to October 2000 (263.4 m³ s⁻¹) (Table 2). Chl a concentrations were consistently higher (50 to 90 %) at km 122 (Poughkeepsie) compared to the upriver station (km 140, Albany). Elevated Chl a (~ 24 μg L⁻¹) was measured in mid-June from a sampling station just north (~25 km) of the Poughkeepsie location (Data courtesy of N. Caraco and J. Cole).

**Isotopic Signatures of Bacterial Biomass: Large Volume Incubations**

Large volume bioassays were conducted at sampling locations in both the York River estuary and the Hudson River to determine the δ¹³C and Δ¹⁴C signatures of organic C assimilated by heterotrophic bacteria. The blank C (most likely DOC) sorbed to filters ranged from 0.2 to as much as 2 times the sample biomass (Table 3). Although a relationship between quantity of blank C and salinity was not discernible for the July 2000 sampling period, the amount of C sorbed to filters was negatively correlated with salinity (r² = 0.96) during the November 2000 sampling (Table 3). The Δ¹⁴C values of sorbed C blanks were consistently more depleted at the freshwater endmember of the York and became subsequently more enriched seaward (Table 3, Figure 3 a). The Δ¹⁴C of blank C in the York River freshwater endmember varied by only 24.5 ‰ between samplings, whereas in the Hudson River it varied by ~ 160 ‰ between the October 2000
and June 2001 samplings (Table 3, Figure 3a). The $\delta^{13}$C values for blank C varied from -25.1 to -35.2 $\%_{oo}$ and -26.8 to -33.9 $\%_{oo}$ in the York and Hudson river estuaries respectively (Table 3).

The $\Delta^{14}$C and $\delta^{13}$C values of bacterial biomass produced in bioassays were corrected for blank contributions according to equation 2. Corrections were not possible when blank carbon exceeded biomass C in samples, and these samples were omitted from blank-corrected plots (Figure 3 b,c,d). The corrected $\Delta^{14}$C signatures for bacterial biomass from bioassays in the York ranged from +32 to -536 $\%_{oo}$ (Table 3, Figure 3b). The $\Delta^{14}$C of biomass from incubations from the mouth was modern in both July and November 2000 whereas at the mid-salinity station and freshwater endmember it was more depleted, -536 $\%_{oo}$ and -411 $\%_{oo}$, respectively (Table 3, Figure 3b). Corresponding $\delta^{13}$C values of bacterial biomass were available for only the mouth and mid-salinity station where values averaged approximately -22.8 $\%_{oo}$ ($\pm$ 2.4 $\%_{oo}$, n = 3) (Table 3, Figure 3c).

The bioassay results for the Hudson River yielded distinctly different bacterial $\Delta^{14}$C signatures of biomass produced between the two sampling periods, October 2000 and June 2001. The organic C assimilated by bacteria in October 2000 was greatly depleted in $\Delta^{14}$C while that in June 2001 was enriched (-11 $\pm$ 30 $\%_{oo}$ , n=2) and modern in origin (Table 3, Figure 3b). Similarly, the $\delta^{13}$C value of bacterial biomass during the October 2000 sampling was depleted (-34.1 $\%_{oo}$) compared to June 2001. The $\delta^{13}$C values corresponding with modern $\Delta^{14}$C signatures (June 2001) in bacterial biomass were more enriched (-27.7 and -24.7 $\%_{oo}$) for Corning Preserve and Albany, respectively (Table 3, Figure 3c).
Dual isotope plots ($\Delta^{14}C$, $\delta^{13}C$) of bacterial biomass produced in bioassays showed differences in space and time in the sources and ages of organic C assimilated (Figure 3d). The isotopic values of organic C incorporated into biomass in the York River were significantly more $\Delta^{14}C$ enriched at the mouth during both sampling periods than the mid-salinity station where the radiocarbon signature of bacterial biomass was depleted and suggested the assimilation of older OM. The Hudson River incubations displayed large temporal differences in the isotopic signatures of C assimilated (Figure 3d). Bacterial biomass produced during the June 2001 sampling was $\Delta^{14}C$ enriched and modern in age, though dual isotope plots (Figure 3d) showed it to be most similar in its signatures to that of bacterial biomass from the mouth of the York River estuary (Figure 3d). Hudson River (Poughkeepsie) bacterial biomass from the October 2000 incubations was greatly $\Delta^{14}C$ and $\delta^{13}C$ depleted and was distinct from that sampled in June 2001 as well as from the isotopic signatures of biomass from the York (Figure 3d).

**Isotopic Signatures of In Situ Bacterial Nucleic Acids**

Isotopic signatures of *in situ* bacterial biomass were determined by extraction of nucleic acids from a pre-concentrated bacterial size fraction. Although the extraction procedure purifies nucleic acids from proteins and other cellular components, humic acids may be co-extracted. Humic material co-extracted with nucleic acids during both the York freshwater samplings and at the Corning Preserve and Poughkeepsie locations in the Hudson River during the June 2001 sampling. Bacterial nucleic acid isotopic values were corrected for the contribution of humic material using equations 3 and 4. Humic material was discernible from nucleic acids by its greater C:N ratios, 18.5 and
34.7 for the York and Hudson, respectively (Table 4). The $\delta^{13}$C values of humic material for the York (~27.5 $^\circ$/oo) and Hudson (~27.2 $^\circ$/oo) river estuaries were similar to that of the uncorrected nucleic acid extracts, and thus $\delta^{13}$C corrections were less than 1 $^\circ$/oo for both systems (Table 4). In the York River estuary, isolated humics were depleted in $\Delta^{14}$C relative to uncorrected nucleic acid extracts. Once the contribution of humics was subtracted, nucleic acid extracts were enriched in $\Delta^{14}$C by approximately 35 $^\circ$/oo (Table 4) relative to uncorrected values. On the other hand, humic isolates in the Hudson River were slightly enriched in $\Delta^{14}$C (15 and 5 $^\circ$/oo for Corning Preserve and Poughkeepsie respectively) relative to uncorrected extracts (Table 4).

The corrected $\Delta^{14}$C signatures of bacterial nucleic acids were most enriched at the freshwater endmember of the York where values averaged 214 ± 29 $^\circ$/oo (n=2) (Table 4). Values became more depleted (i.e., older) with increasing salinity (Figure 4a) and averaged 62 ± 20 $^\circ$/oo (n=4) and 15 ± 43 $^\circ$/oo (n=3) for the mid-salinity and mouth locations respectively (Table 5). The corresponding $\delta^{13}$C values were lightest at the freshwater endmember (~28.9 ± 0.9 $^\circ$/oo, n=2)) and increased seaward with the exception of May 2000 where bacterial nucleic acids at the mouth displayed an anomalously light $\delta^{13}$C of ~27.6 $^\circ$/oo. (Figure 4b).

Dual isotope ($\Delta^{14}$C, $\delta^{13}$C) plots of bacterial nucleic acids in the York River estuary show distinct separations between the fresh and saltwater sites/regions (Figure 5). The freshwater region was enriched in $\Delta^{14}$C and depleted in $\delta^{13}$C relative to the mouth. An isotopic distinction was not always discernible in bacterial nucleic acids between the mid-salinity station and the mouth as signatures overlapped (July 2000) (Table 5, Figure...
5. Bacterial nucleic acid $\Delta^{14}C$ signatures in May 2000 at the mouth were the most depleted (-35.4 \text{‰}) of all $\Delta^{14}C$ values in the York River estuary.

The $\delta^{13}C$ signatures of bacterial nucleic acids in the Hudson River estuary ranged from -28.2 to -25.0 \text{‰} and fell within the range of average freshwater (-28.9 \text{‰}) and mid-salinity (23.5 \text{‰}) York River bacterial nucleic acid values (Table 5). However the $\Delta^{14}C$ of bacterial nucleic acids in the Hudson River (16.2 to -153.1 \text{‰}) was more depleted than those of the York River (234.3 to -35.4 \text{‰}) (Table 5).

**Comparison of Isotopic Signatures of Bacteria by In Situ and Bioassay Approaches**

A comparison of the $\delta^{13}C$, $\delta^{15}N$ and $\Delta^{14}C$ isotopic values of bacteria biomass measured *in situ* (i.e., nucleic acids) vs. using the bioassay (i.e., biomass production) approach yielded different results. At all sampling locations along the salinity gradient of the York, the $\delta^{13}C$ signatures of “total” bacterial biomass collected on 0.2 $\mu$m Anopore disks was consistently enriched (by ~4 to 8 \text{‰}) over those of bacterial nucleic acids (Figure 6a). At the freshwater site, the $\delta^{13}C$ of bacterial biomass collected by 0.7 $\mu$m glass fiber filters (16 L) was approximately 5 \text{‰} heavier than bacterial nucleic acid values. In contrast the bacterial biomass produced in bioassays from the mid- and high-salinity locations were depleted in $\delta^{13}C$ by 5 and 2 \text{‰}, respectively. $\delta^{13}C$ values measured by large volume (90 L) bacterial bioassays most closely resembled the bacterial nucleic acids.

The two bioassay filtration types (Anopore and glass fiber filters) used to estimate the $\delta^{15}N$ signature of bacterial biomass yielded comparable results to the $\delta^{13}C$, but both were depleted by 4 to 13 \text{‰} compared to the $\delta^{15}N$ signatures of bacterial nucleic acids.
assimilated by bacteria at the mouth of the York River estuary were not significantly different from those of bacterial nucleic acids (+14.8 ± 43 ‰ (n=3); Table 5). However, $\Delta^{14}C$ values of biomass produced in bioassays from the freshwater and mid-salinity locations (-411 ‰ and -536 ‰, respectively) were strongly $\Delta^{14}C$ depleted relative to average bacterial nucleic acids from the same locations (+213 ± 29 ‰ and +62 ± 20 ‰). Discrepancies were also apparent in the Hudson River between the two methods, with strong $\Delta^{14}C$ depletions in biomass produced in bioassays in October 2000. The bacterial biomass produced in October 2000 was significantly depleted in $\Delta^{14}C$ (-718 from km 240; -846.2 ‰ from km 122) relative to bacterial nucleic acids (-153.1 ‰ from km 122). In June 2001 the $\Delta^{14}C$ signature of bacterial biomass produced from the same locations (+10.2, -32.1 ‰) more closely resembled that of bacterial nucleic acids (+5.6, +16.2 ‰) (Table 2, Table 5).

**Comparison of Bacterial Nucleic Acid Isotopic Signatures in the York and Hudson River Estuaries**

Dual isotope ($\Delta^{14}C$ vs. $\delta^{13}C$) plots of bacterial nucleic acids demonstrate intersystem variations in the isotopic signatures of DOC assimilated (Figure 7). Isotopic signatures for bacterial nucleic acids along the fresh to saltwater continuum are included for the York River estuary whereas nucleic acids from only freshwater and oligohaline (salinity 3.2) reaches of the Hudson River estuary were measured. $\delta^{13}C$ signatures of bacterial nucleic acids in the Hudson River estuary are approximately 2.7 ‰ heavier than their freshwater counterparts in the York (Figure 7). Bacterial nucleic acids
collected in fall 2000 from the freshwater portions of both rivers differ in $\Delta^{14}C$ of organic matter assimilated by $-370 \, ^\circ\text{o}$ with Hudson River bacteria $\Delta^{14}C$ depleted ($-153 \, ^\circ\text{o}$) and York River bacteria $\Delta^{14}C$ enriched ($+214 \, ^\circ\text{o}$). Nucleic acid $\Delta^{14}C$ values from the freshwater (140 km, 122 km) Hudson River in June 2001 were modern in age and were most similar isotopically to nucleic acids from the mouth of the York (Figure 7).

**DISCUSSION**

**Comparison of Methods for Estimating Isotopic Signatures of DOM Assimilated by Bacteria**

The $\Delta^{14}C$ and $\delta^{13}C$ values from bioassay experiments often do not agree with the isotopic signatures of nucleic acids *in situ* (Figure 6). The interpretation of bioassay results may thus be confounded by bacterial community shifts during the timecourse incubations, the uncoupling of *in situ* processes and the difficulties in correcting for DOC sorbed to filters rather than that which is assimilated into bacterial biomass. Despite these and other potential artifacts, previous studies have found close agreement between the $\delta^{13}C$ signatures of bacterial biomass in bioassays and nucleic acids extracted directly from bacteria (Coffin et al. 1990; Coffin and Cifuentes 1993; Kelly et al., 1998). However, in the present study a concurrent assessment of $\delta^{15}N$ in bioassays and nucleic acids was undertaken. Furthermore this study is the first to apply $\Delta^{14}C$ to bacterial bioassays. There is no *a priori* reason to assume that $\Delta^{14}C$ will replicate the behavior of $\delta^{13}C$. Lastly, these results indicate that the sorbed blank C (Table 3, Figure 3) may differ isotopically from that assimilated by bacteria, even though prior studies did not consider such a potential bias.
Bioassays suffer from several other potential artifacts including the induced utilization of more refractory DOM compounds (Coffin et al., 1993; del Giorgio and Cole, 1998) because they may isolate bacteria from a continuous supply of fresh DOM. Should in situ bacterial production be tightly coupled to a steady supply of DOM, bioassays would uncouple that supply and likely promote the use of compounds which would not have been assimilated in situ. Previous studies suggest that dissolved free amino acids (DFAA), which are rich energy sources, and potential building blocks for bacteria and are relatively more important in fulfilling estuarine bacterial N demand than inorganic sources (Kirchman 1994). Ambient concentrations of DFAA in the Chesapeake Bay plume remain low due to the close coupling between release and uptake with turnover times averaging an hour or less (Fuhrman 1990). Rapid exhaustion of this labile organic pool of N in a bioassay may force bacteria to fulfill their N demand with inorganic sources. When inorganic N is abundant (as in estuaries), bacteria may fractionate N by 5 to 15% (Peterson and Fry, 1987). The depleted δ15N signatures of bioassay derived biomass relative to bacterial nucleic acids may reflect an artificially depleted N source as a result of inorganic subsidies (Figure 6b).

Sorption of DOM to glass fiber filters may also increase the organic matter (OM) presumed to be of particulate origin and result in an inflated estimate of particulate organic matter (POM). The contribution of sorbed DOM is particularly significant when POM (or POM normalized to surface area of filter) concentrations are low (Moran et al., 1999). Discrepancies in the δ15N signature of POM (Altabet 1992) may be partially reconciled by the unaccounted contribution of sorbed DOM to the particulate isotopic measurement (Moran et al., 1999). In the past, the use of bioassays to discern the δ13C
signature of bacterial biomass has neglected the potential contamination by sorbed DOM to the measured total isotopic signature (Coffin 1990; Coffin and Cifuentes 1993; Kelley et al., 1998). The bioassay approach itself may be highly sensitive to sorbed DOM since particulate OM concentrations are low. Total organic C varied from ~ 0.1 µg to 1 mg (Table 3). Although blanks were processed concurrently with all large volume bioassays it was difficult to apportion OM between biomass derived and adsorbed contributions.

Mid-salinity and freshwater bioassays in the York River (October 2000) resulted in blank C values greater than those in the subsequent biomass collection (Table 3). Though at first these results appear implausible, they in actuality underscore shortcomings inherent in the methodology.

Water for blanks was filtered through glass fiber filters immediately after the removal of all particles (0.2 µm) and prior to bacterial inoculation. Bacterial biomass (i.e., plus sorbed DOM) was harvested ~ 60 hours after inoculation. Microbial processing of OM undoubtedly altered the DOC available to bind to the filter blank by respiration and subsequent loss of lipophilic compounds and bacterial transformation of original organic compounds to those less particle reactive. Microbial processing decreases the aliphatic character of DOM, increases the aromaticity of DOM (Sun et al., 1997) and subsequently decreases its affinity for surfaces. Following microbial modification, the concentration of particle reactive compounds may have been reduced by both respiratory losses as well as bacterial transformations.

Surprisingly, the Δ¹⁴C of sorbed blank DOC was highly aged (~ 1900 to 16,560 years B.P.) (Table 3), and bore no resemblance to bulk DOC ages (Raymond and Bauer 2001b), thus suggesting the selective sorption of an old surface reactive or lipophilic
fraction. The $\Delta^{14}C$ signatures of sorbed C varied linearly with salinity in the York (Figure 3a). This observation is puzzling as it is in direct opposition to the trend in ages of the bulk DOC pool which is $\Delta^{14}C$ enriched at the head of the estuary and decreases seaward (Raymond and Bauer 2001b). Initially it was suspected that contamination by the sea surface microlayer may have occurred. The microlayer may be enriched with organic matter by $10^3$ over water column concentrations (Liss and Duce 1997) and dominated by lipid components (Baier et al., 1974; Williams et al., 1986); however, the linearity and duplication of the $\Delta^{14}C$ trend in blanks suggests additional causes. Another possibility is that the depleted $\Delta^{14}C$ signature landward is a result of phenolic materials which though common to terrestrial and freshwater environments, are limited in their marine distribution. Although phenolic materials may be derived naturally from algae (Steinberg 1989), fresh and marine sediments (King 1986, 1988), soil and freshwater humic material (Meyers-Schulte and Hedges 1986, Malcom 1990), and marine microlayers (Carlson and Mayer 1980), the highly depleted $\Delta^{14}C$ signatures of sorption blanks suggests an anthropogenic origin.

Of the potential anthropogenic sources of phenols to riverine/estuarine environments (public owned treatment works, air releases, water disposal and recreational boating), recreational and commercial boating is the most likely origin of $\Delta^{14}C$ depleted phenols to the York and Hudson river estuaries. Turnover times and microbial utilization rates of a representative phenol, p-cresol, were rapid and averaged between 1.7 to 37 hours (Boyd and Carlucci 1996) suggesting microbial decomposition may play a pivotal role in mediating the distribution of phenols along the fresh to marine continuum. Further, bacteria in Prince William Sound, Alaska preferentially assimilated
indigenous organic compounds, using crude oil as a substrate only after labile substrates had been exhausted (Coffin et al., 1997). Since bioassays are closed systems and are artificially removed from inputs of new DOM, the signatures of bacterial biomass produced in them may be reflective of bioreactive constituents of DOM, but not necessarily compounds of the same source and age as those metabolized in situ.

A potential criticism of the nucleic acid extraction technique and one which a bioassay approach circumvents might be the inclusion of inactive bacteria (Zweifel et al., 1993; Sherr et al., 1999) from upstream locations to the selected sampling site. The net result of this would be a bacterial isotopic signature biased by OM assimilated at a separate location rather than in situ. Bacteria from the Hudson River are capable of modifying enzyme activities on short (hourly) time scales with minimal metabolic cost to adapt to new OM sources (Findlay et al., 1998). Thus it is likely that bacteria collected at any given location along the Hudson have adapted their enzyme capabilities to the existing supply of organic matter. Bacteria in the York showed metabolic differentiation by both temperature and salinity, indicating a similar adjustment to in situ OM supply (Schultz and Ducklow, 2000). Another potential caveat with the in situ nucleic acid extraction approach is the methodological dependence on an initial size fractionation (0.1 μm to 0.7 μm) which presumably isolates bacteria from primary producers and microheterophic grazers. The efficacy of this separation has been questioned as overlaps in size classes occur. However, through the use of 16S rRNA oligonucleotide probes, Coffin et al. (1990) have confirmed that greater than 90% of the <1.0 μm filtrate was bacterial, partially dispelling this criticism.
In summary, the interpretation of bioassay isotopic signatures is confounded by the contribution of blank derived OM. Furthermore, bioassays are closed systems which uncouple bacterial metabolism from fresh supplies of OM potentially restricting the availability of compounds assimilated in situ. As a result of the artifacts associated with the bioassay methodology, only bacterial nucleic acid isotopic values will be used subsequently evaluate the sources supporting bacterial production in the York and Hudson River estuaries.

**Use of Natural Abundance Isotopes for Assessing Bacterial OM Cycling**

$\delta^{13}C$ analysis of bacterial nucleic acids has proven a powerful tool for tracing the sources of carbon assimilated by bacteria (Coffin et al., 1989; Coffin et al., 1990; Cherrier et al., 1999; Kelly et al., 1998). The isotopic signature of potential parent substrate is preserved in bacterial biomass (Coffin et al., 1990; Hullar et al., 1996). Further, the specificity to bacteria of nucleic acids in 0.2-0.8 μm filtered water was demonstrated with 16S RNA analysis (Coffin et al., 1990). Simultaneous measurements of $\delta^{13}C$ and $\delta^{15}N$ in bacterial biomass (Coffin et al., 1997; Hopkinson et al., 1998) may help to further identify sources.

Bacteria may assimilate and fractionate $\delta^{13}C$ and $\delta^{15}N$ isotopes differently. The fractionation of $\delta^{13}C$ by heterotrophic metabolism is minimal (~1-2‰, Coffin et al., 1989). Conversely bacteria can be 5-15‰ lighter in $\delta^{15}N$ when N is plentiful (Peterson and Fry, 1987). The use of $\delta^{15}N$ in bacteria is further confounded by the numerous sources of N available (Wheeler and Kirchman, 1988). While amino acids are the preferred N source in estuaries, ammonium can account for a significant fraction (5-60%)
of total N uptake (Keil and Kirchman, 1991; Kirchman et al., 1989). The subsidization of N from inorganic sources may, however, compromise the use of N isotopes in tracing organic matter sources.

An alternative and novel tracer for examining the sources of organic matter supporting bacterial production is the use of natural abundance radiocarbon ($\Delta^{14}C$). Cherrier et al. (1999) used complimentary $\delta^{13}C$ and $\Delta^{14}C$ measurements of bacterial nucleic acids to discern the sources of DOM supporting bacterial production in a Florida estuary. $\Delta^{14}C$ is a sensitive tracer for small changes in organic matter due to the very large dynamic range in $\Delta^{14}C$ of DOC source material. In the York River estuary, the DOC at the freshwater end-member is enriched by the presence of bomb $\Delta^{14}C$ and contrasts with the highly $\Delta^{14}C$ depleted DOC at the marine end-member yielding an effective range (>1000 $\permil$) two orders of magnitude greater than $\delta^{13}C$ (~10 $\permil$) (Raymond, 1999).

**Isotopic Signatures of Potential DOM Sources to Estuarine Bacteria**

Potential sources of DOM in estuaries may arise from a large number of allochthonous and autochthonous sources which span a broad range of $\delta^{13}C$, $\delta^{15}N$ and $\Delta^{14}C$ values. Limited stable and radio-isotope datasets are available with which to decipher possible contributions to the isotopic signatures of bacteria. This is especially true of $\delta^{15}N$ and $\Delta^{14}C$. Compilations of existing literature values for the isotopic values of estuarine OM sources were used to compare the bacterial isotopic signatures and constrain the importance of potential allochthonous and autochthonous sources to bacterial production (Table 6). The measured $\delta^{13}C$-DIC and $\Delta^{14}C$-DIC values were used
to constrain the predicted isotopic signatures of phytoplankton in the York and Hudson River estuaries assuming kinetic fractionations of 20 \( ^\circ \text{o}_\text{o} \) for \( \delta^{13}\text{C} \) values (Chanton and Lewis 1999). As \( \Delta^{14}\text{C} \) values have been normalized to \( \delta^{13}\text{C} \) according to the principles of Stuiver and Polach (1977) no additional correction was applied. The predicted phytoplankton isotopic signatures for both the York River estuary and Hudson River are summarized in Table 6.

**Potential Contributions of Different OM Sources to York River Estuarine Bacteria**

Previous work in the York River estuary (Raymond, 1999; Raymond and Bauer 2001b) constrained the important allochthonous and autochthonous OM sources to riverine/estuarine phytoplankton, marsh derived organic matter, and terrestrially derived (i.e., soil/forest litter) material. The boxes in Figure 8 demarcate the ranges of published \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) values of potential OM sources to estuaries. In addition to the \( \delta^{13}\text{C} \) and \( \delta^{15}\text{N} \) of bacterial nucleic acids, discrete measurements of various sources indigenous to the York River estuary are included. Phytoplankton were assigned an average \( \delta^{15}\text{N} \) of 6.5 and 7.5 \( ^\circ \text{o}_\text{o} \) (median of published range Table 6) for freshwater and saline regions of the York, respectively as in situ measurements were not available.

The relative importance of potential sources in the York River estuary may be a function of the proximity of a given sampling location to a particular source. Hence the freshwater reaches of the York River estuary are most likely dominated by OM derived from freshwater phytoplankton, terrestrial/soil derived material and marsh OM; the mid-salinity location influenced by oligohaline phytoplankton, marsh OM, terrestrial/ soil, and estuarine phytoplankton; and the mouth reflective of a mixture of marine/estuarine...
phytoplankton, OM advected from the Bay, and remnant terrestrial/marsh OM. The above distributions of OM are consistent with $\delta^{13}C$ and $\Delta^{14}C$ isotopic mixing curves in the York River estuary which suggested similar distributions and relative importance of potential sources along York River estuary (Raymond 1999; Raymond and Bauer 2001).

The overlapping $\delta^{13}C$ and $\delta^{15}N$ of freshwater phytoplankton, terrestrial/soil DOM, oligohaline and marsh OM confound the ability to distinguish amongst the dominant sources supporting bacterial production at the head of the York River estuary (Figure 8). Previous work in the York by Sin et al. (1999) concluded that phytoplankton in the upper York are limited by low light and high flow rates. Chl $a$ concentrations at the time of nucleic acid collection averaged only 4.4 $\mu$g L$^{-1}$ (Table 2). In addition, radiocarbon values of bulk DOC from the freshwater York suggested little input of phytoplankton derived OM to the bulk dissolved pool (Raymond and Bauer 2001b). Thus, the importance of algal derived OM to the upper York is thought to be minimal. However, recent characterization of freshwater particulate and dissolved OM with a sensitive lipid biomarker approach suggests a bioreactive, algal fraction undetectable by bulk water column and OM analyses (Chapter 4). Nonetheless, dual isotope ($\delta^{15}N$ vs $\delta^{13}C$) plots (Figure 8) alone do not distinguish the potential sources of OM assimilated by freshwater bacteria.

The $\delta^{13}C$ and $\delta^{15}N$ signatures of bacteria relative to the potential sources at the mouth of the York River estuary suggest that they may be explained solely by marine/estuarine phytoplankton (Figure 8). In contrast, bacterial isotopic signatures in the mid-salinity region fall outside the primary sources of OM due to their elevated $\delta^{15}N$ values. The enriched $\delta^{15}N$ may be partially explained as N derived from higher trophic
level metabolism. Overall, $\delta^{15}N$ in organisms usually reflects the nitrogen isotope ratios of primary producers plus an enrichment of $\sim 3-4$ $\%_\infty$ per trophic level (Michener and Schell 1994). Enhanced concentrations of lipid biomarkers in the mid-salinity York River estuary which are diagnostic for zooplankton inputs (Chapter 4) support the interpretation that bacterial N may be derived from higher trophic levels. However the November 2000 value is anomalously high ($\delta^{15}N \sim 17$ $\%_\infty$), and may be an artifact of mass spectrotometric measurements of low amounts of N (<5 $\mu$g) in these samples.

The application of natural abundance $\Delta^{14}C$ provides an additional isotopic dimension with which to discern the relative importance of OM sources to bacterial biomass production. There is a paucity of $\Delta^{14}C$ measurements in estuarine systems, yet Raymond (1999), Raymond and Bauer (2001a), Raymond and Bauer (2001b), and Bauer et al. (unpublished) provide not only the most comprehensive study of $\Delta^{14}C$ in an estuarine system, but furnish $\Delta^{14}C$ values for potential sources in both the York River estuary and the Hudson River (Table 6). Unlike Figure 8, which defined potential sources based on literature values, Figure 9 plots only discrete values of $\Delta^{14}C$ and $\delta^{13}C$ as indices of allochthonous and autochthonous OM sources since literature values other than those listed in Table 6 are virtually non-existent.

In contrast to the $\delta^{15}N$ vs. $\delta^{13}C$ plot (Figure 8), the $\Delta^{14}C$ vs. $\delta^{13}C$ plot (Figure 9) shows a differentiation of the relative contributions of potential OM sources assimilated by bacteria. In particular, terrestrial/soil DOM and freshwater phytoplankton DOM are isotopically distinct (Figure 9). A two-source isotopic mixing model was therefore employed to estimate the contributions of potential sources to bacterial nucleic acids.

The generalized mixing equation is:
\[ X_{\text{NA}} = f_1 X_{\text{OM-f1}} + f_2 X_{\text{OM-f2}} \]  

where \( X_{\text{NA}} \) is the isotopic composition (\( \Delta^{14}C \) or \( \delta^{13}C \)) of bacterial nucleic acids, \( X_{\text{OM}} \) the isotopic signature of the two major OM sources, and \( f \) is the relative contribution of each source. On the basis of this model, bacteria in the freshwater region of the York were estimated to assimilate a mixture of terrigenous and freshwater algal derived OM (Table 7). Recently fixed terrestrial OM will reflect present-day atmospheric \( \Delta^{14}C \) values of \(-100 \% \) (Raymond and Bauer 2001b) and consequently our average \( \Delta^{14}C \) bacterial nucleic acid value of \( 215 \% \) requires OM from CO\(_2\) fixation \(-15-20\) years ago when atmospheric inventories of \( \Delta^{14}C\) -CO\(_2\) were \(-115 \% \) greater than today (Levin and Krommer 1997). Unlike phytoplankton which should reflect current \( \Delta^{14}C\)-DIC values (143 \%), bacterial nucleic acids are more \( \Delta^{14}C \) enriched (\(-215 \% \)) and consistent with contemporary terrestrial soil OM (Trumbore et al., 1992) and forest floor OM (Richter et al., 1999) (Table 6) as a primary source of their biomass. Solving Equation 5 using the isotopic signatures (\( \Delta^{14}C \) and \( \delta^{13}C \)) for terrigenous and freshwater algae, suggests bacteria \(-42-89\% \) of organic carbon assimilated by bacteria is of terrestrial origin while the remainder (11-58\%) is derived from freshwater algae (Table 7). Thus, despite relatively low Chl \( a \) (Table 2) concentrations and a predominant riverine signature for DOC (Raymond and Bauer, 2001b), algal derived OM is of substantial importance to bacterial production in the freshwater regions of the York. The importance of algal derived OM to bacterial production is further supported by lipid biomarker analysis of OM in the dissolved pool which links the presence of labile algal biomarkers (polyunsaturated fatty acids) to the relative bioavailability of bulk DOC (Chapter 4). Surprisingly, the \( \Delta^{14}C \) signature of freshwater POC (\(-68 \% \)) suggests it contributes
little to freshwater bacterial carbon demand despite greatly elevated C-acquiring (α,β glucosidase) exoenzyme activities (Chapter 1) and the implied lability (based on lipid signatures) of the particulate vs dissolved fractions (Chapter 4).

Figure 9 also suggests that the sources of OM supporting bacterial production in the mid-estuary may originate from either estuarine phytoplankton or freshwater marshes and may vary across seasons. Bulk OM derived from marshes is ~2 to 4 ⁰/∞ depleted in δ¹³C relative to phytoplankton sources and is of similar age (Table 6, Figure 9). Bacteria may assimilate OM almost entirely of phytoplankton origin in the spring and summer (May and July 2000) with algal derived OM accounting for 93-100% of the bacterial nucleic acid signature (Table 7). In contrast, marsh derived OM is of greater importance to bacterial production in the fall (October and November 2000) and accounts for an estimated 73-100% of the OM assimilated by bacteria (Table 7). This finding is consistent with previous work in the York which suggests that marshes in the low salinity reaches are sites of intense OM recycling and export ~60 g C m⁻² yr⁻¹ (Neubauer et al., 2000; Neubauer and Anderson, in press) of which a significant portion is thought to subsidize bacterial production (Raymond and Bauer 2001b). The heavier δ¹³C signatures of bacterial nucleic acid which coincide almost exclusively with OM of phytoplankton origin, may conceivably also be the result of preferential utilization of an isotopically heavy component of marsh derived OM. Furthermore, bacteria may preferentially assimilate more labile components of DOM, and discriminate amongst individual compounds derived from the same origin (Benner 1987; Coffin et al., 1990). For example, Coffin et al. (1990) suggested that bacteria preferentially selected cellulose and
hemicellulose components of *Spartina alterniflora* and discriminated against the more refractory and isotopically lighter lignin component.

Isotopic values of bacterial nucleic acids from the York mouth of the York (e.g. high salinity) may be explained by contributions from *in situ* algal production and the landward advection of Chesapeake Bay OM (Figure 9). During July and November, bacteria were estimated using equation 5 to assimilate organic matter almost exclusively (86-100%) of estuarine phytoplankton origin with nominal (0-14%) contributions from advected Chesapeake Bay DOC (Table 7, Figure 9). During October 2000, bacteria from the lower York appeared to assimilate a more balanced mixture of the two potentials sources with allochthonous OM (Bay) comprising the greater portion of assimilated OM (50-69%).

**Potential OM Sources to Bacterial Production in the Hudson River Estuary**

In contrast to the York River estuary, fewer stable and radioisotope values of potential sources are available for Hudson River. Table 6 lists the $\delta^{13}$C, $\delta^{15}$N, and $\Delta^{14}$C of POM, DOC, humic material and phytoplankton for sampling stations along the freshwater Hudson River. Corning Preserve, the northward extent of sampling, is primarily influenced by large inputs of allochthonous carbon from the surrounding watershed which spills over the Green Island Dam at Troy. In contrast, the furthest downstream sampling location (Poughkeepsie) may be influenced by export from freshwater wetlands (i.e., near Tivoli Bays), release from shoals and fine sediments (i.e., near Kingston) and input from additional tributaries (near Roundout Creek) (Findlay et al., 1998). Terrestrial inputs to the Hudson River are large and approximated as 650 g C
m² yr⁻¹ (Howarth et al., 1996). In contrast, combined phytoplankton and macrophyte production accounts for only ~5% of the OM supplied by allochthonous sources (Howarth et al., 1996; Caraco et al., 1997).

The measurement of depleted bacterial nucleic acids (Δ¹⁴C = -153 ‰) from the mid-Hudson (122 km) in October 2000 indicates that contributions from recently fixed autochthonous production (Δ¹⁴C = -50 ‰) are not significant. Possible sources of old (~1280 years BP) C to the Hudson River are essentially limited to allochthonous OM, since *in situ* production will reflect assimilation of modern C (~ +100 ‰; Raymond and Bauer 2001b). OM desorption from sediments may also provide a mechanism for the introduction of depleted Δ¹⁴C classified as autochthonous in origin, however, compared to the flux of organic C from tributaries this input would be of minor consequence (Komada and Reimers 2001).

Bacterial nucleic acid Δ¹⁴C values in June 2001 were variable in the Hudson River. Bacteria at both Corning Preserve (240 km) and Poughkeepsie (122 km) were significantly Δ¹⁴C enriched (+5 ‰ and +16 ‰ respectively) as compared to the nucleic acids collected further downstream (-144.2 ‰ 25 km) as well as from the previous October 2000 sampling. The upstream nucleic acid values were substantially more Δ¹⁴C enriched than those predicted by the use of OM derived from deeper/older soil horizons (Petsch et al., 2000) or phytoplankton/submerged macrophyte OM (-50 ‰). Instead the nucleic acid Δ¹⁴C values suggest the assimilation of OM derived from recent vascular plant production (terrestrial/marsh) concurrent with a portion of older Δ¹⁴C depleted material (soil). Of the two potential sources of recently fixed Δ¹⁴C enriched OM (forest litter or emergent macrophyte) to the Hudson River emergent macrophytes are the more
probable source of C assimilated by bacteria. Humics isolated from the water column during similar seasonal and hydrologic conditions (May and July 2002) reflect the C:N (~35) of soil derived organic matter implying the younger, C:N depleted forest litter (70; Caraco et al., 1998) is of minor importance to bulk DOM. Furthermore the heavier (~1.5 °/oo) δ13C values of nucleic acids from June 2001 sampling suggest emergent macrophytes to be the more likely isotopic source (Tables 5 and 6).

The Δ14C age (1200 BP) of nucleic acids further downstream are considerably older than those from either Corning Preserve or Poughkeepsie which are modern in age. Potentially the younger OM source was either 1) utilized prior to reaching the sampling location 2) was microbially transformed such that it was rendered unavailable to subsequent bacterial decomposition or 3) undesirable to the bacterial community as the older DOC component was preferentially assimilated.

Comparison of Bacterial Isotopic Signatures Between the York River Estuary and Hudson River

Reports of system net heterotrophy for the York (Raymond, 1999; Raymond et al., 2000) and Hudson (Findlay et al., 1991a; Howarth et al., 1996) rivers, and of rivers and estuaries in general (Cole and Caraco, 2001; Raymond and Bauer 2001c) necessitates the utilization of allochthonous DOM within these systems. Enzymatic and stoichiometric data (unpublished) for the York further corroborate this conclusion (Chapter I). Although the York and Hudson appear fundamentally similar in both their uncoupling of bacterial and phytoplankton production and the suggested bacterial reliance on allochthonous DOM sources (both estuaries net heterotrophic), the two
systems differ with regard to the mean age of bioavailable DOC and the relative importance of autochthonous production to total bioavailable DOC concentrations.

The majority of the DOC in the Hudson River is supplied by inputs from tributaries (Howarth et al., 1996; Findlay et al., 1998). In contrast the York River estuary maintains approximately three times the standing stock of phytoplankton compared to the Hudson and may be subsidized with DOC from extensive freshwater marshes. The striking differences in radiocarbon ages of bulk DOC and POC in both systems (Raymond and Bauer 2001a) are consistent with this allocation of allochthonous terrestrial material. Whereas bulk DOC at the head of the York is modern in age, Hudson River DOC was fixed on average ~1385 years BP (Raymond, 1999; Raymond and Bauer 2001a). Similarly POC ages in the York span from modern to 1690 BP, while Hudson River POC (4610 BP) is considerably older (Raymond, 1999; Raymond and Bauer 2001).

$\Delta^{14}C$ values of bacterial nucleic acids at the head of the York River ($\sim +214^{\circ}/_{oo}$, Table 5) correspond to DOC of decadal age which is predominately derived from recently deposited soils and degraded modern vegetation (Richter et al., 1999). Conversely the significantly older $\Delta^{14}C$ signatures of Hudson River bacteria (Table 5) may be derived from OM associated with deeper soils thousands of years ago. What remains perplexing is how material that has resisted terrestrial microbial decomposition for millennia is capable of supporting high levels of estuarine heterotrophic production in the matter of hours to days.

Two potential mechanisms exist for altering the bioavailability of recalcitrant OM. First, OM association with mineral grains in soils and sediments may convey resistance to microbial decomposition (Keil et al., 1994; Mayer 1994a, Mayer 1994b). However
these associations are rarely permanent (Thimsen and Keil 1998) and once OM is
dissociated from mineral surfaces (through changes in redox, pH, solute concentration or
resuspension) it may be rapidly degraded (Keil et al., 1994; Hedges and Keil 1999).
Alternatively, allochthonous OM is composed of highly condensed humic compounds
which are extremely photoreactive. Once released into rivers and estuaries these
allochthonous compounds are exposed to UV light and may be photochemically
transformed into OM which is bioavailable (Wetzel et al., 1995; Kieber et al., 1990;
Miller and Moran 1997; Cole 2000).

In the context of measurements traditionally employed to characterize the
metabolic state of an estuary (DIC, bacterial abundance and production, Chl a, primary
production) the Hudson and York River estuaries appear deceptively similar. Only
through the application of Δ14C natural abundance analysis on bacterial nucleic acids do
we realize the two systems diverge entirely with respect to the age of C fueling secondary
(bacterial) production. Intensive agriculture and urban/residential land use in the Hudson
River watershed (Howarth et al., 1991) delivers deeper/older soil horizons to the surface
and is thus likely to be a principal source of old OM to the Hudson River estuary.

CONCLUSIONS

The findings from this study have implications with regard to fundamental
methods and concepts currently accepted in biogeochemistry. This study is the first to
measure the Δ14C of bacterial biomass produced in bioassays as a means of estimating the
age of DOC assimilated by bacteria. Not only is it the first comprehensive attempt to
constrain the contribution of filter adsorbed DOM to the resultant biomass isotopic
signature, but it underscores the widely overlooked influence of filter adsorbed DOC to
the more universal measurements of $\Delta^{14}$C and $\delta^{13}$C POC. In addition, allochthonous
organic matter was traditionally thought recalcitrant and unavailable to support bacterial
production. Although this paradigm has undergone revision (Tranvik and Hofle, 1987;
Moran and Hodson, 1990; Leff and Meyer, 1991; Moran and Hodson, 1994) the term
"old" still remains synonymous with refractory. However, $\Delta^{14}$C values of bacterial
nucleic acids in the Hudson River estuary suggest that the terms "refractory" and "old"
are not necessarily interchangeable.
LITERATURE CITED


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Table 1. Summary of methods used to measure the stable and radioisotope signatures of bacteria in the York and Hudson River estuaries.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mar-00</th>
<th>May-00</th>
<th>Jul-00</th>
<th>Oct-00</th>
<th>Nov-00</th>
<th>Jun-01</th>
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<td></td>
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<tr>
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<td>High-Moderate</td>
<td>Moderate-Low</td>
<td>Low</td>
<td>Low</td>
<td></td>
</tr>
<tr>
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<td>75 (GM)</td>
<td>115 (GM)</td>
<td>125 (GM)</td>
<td>215 (GM)</td>
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<tr>
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<td>215 (HF)</td>
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<tr>
<td>Discharge</td>
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<td>High-Moderate</td>
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<td></td>
</tr>
<tr>
<td>Nucleic Acid Collection and Extraction (vol.)</td>
<td>240 km (Corning Preserve)</td>
<td>220 (HF)</td>
<td>185 (GM)</td>
<td></td>
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</tr>
<tr>
<td>Distance (Site)</td>
<td>122 km (Poughkeeps)</td>
<td>200 (HF)</td>
<td>185 (GM)</td>
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<td>25 km (Palisades)</td>
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<td>95 (GM)</td>
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<td>Bioassay (90 L)</td>
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<tr>
<td>122 km (Poughkeeps)</td>
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<td>0.45</td>
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</table>

*a* Volume of water filtered for bacterial nucleic acid extraction indicated in Liters.

*b* (GM) designates concentration of bacteria directly onto Gelman microcapsules (0.2 μm).

*c* (HF) designates samples concentrated by tangential flow filtration (hollow fiber cartridge (0.1 μm) prior to Gelman micro-capsules.

*d* Denotes pore size of filter used to harvest bacterial biomass produced in bioassays. Glass fiber filters (0.45 or 0.7 μm). Anopore filters (0.2 μm).
Table 2. Site and Water Characteristics in the York River estuary during the present study.

<table>
<thead>
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<th></th>
<th>Streamflow (m³/sec)</th>
<th>Salinity (°C)</th>
<th>Water Temp. (°C)</th>
<th>Chlorophyll a (µg/l)</th>
<th>Phaeopigments (µg/l)</th>
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<td>n.d.</td>
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<tr>
<td></td>
<td></td>
<td>25 km</td>
<td>3.2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>


b n.d. – Not determined
c Data courtesy of Drs. Nina Caraco and Jon Cole IES.
d June 2001 Chlorophyll a and phaeopigments concentrations are the average of data collected May 16, 2001 and July 17, 2001 (data courtesy of Drs. Nina Caraco and Jon Cole IES).
Table 3. $\Delta^{14}C$ and $\delta^{13}C$ values for filter blanks, biomass samples and blank corrected samples from large volume (90L) bioassays.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Salinity/Distance</th>
<th>Total Sample</th>
<th>Blank</th>
<th>Blank Corrected</th>
<th>Ratio</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$\mu g$ C $\Delta^{14}C (7_{%})$</td>
<td>$\delta^{13}C (7_{%})$</td>
<td>$\mu g$ C $\Delta^{14}C (7_{%})$</td>
<td>$\delta^{13}C (7_{%})$</td>
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<td>373</td>
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<tr>
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<td>11</td>
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<td>-637</td>
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<td>Hudson River</td>
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<td>479</td>
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<td>n.d.</td>
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<tr>
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<td>984</td>
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<tr>
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<td>122 km</td>
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\(^a\) n.d.- Not determined.

\(^b\) Boldface indicates C of bacterial biomass less than C mass adsorbed to blanks. No blank correction was applied.
Table 4. Bacterial nucleic acid $\Delta^{14}C$ and $\delta^{13}C$ values corrected for humic contributions.

<table>
<thead>
<tr>
<th></th>
<th>Date</th>
<th>Salinity/River km</th>
<th>Uncorrected</th>
<th>Corrected</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C:N</td>
<td>$\Delta^{14}C$ ($^\circ/oo$)</td>
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<td>Oct-00</td>
<td>0</td>
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<tr>
<td></td>
<td>Nov-00</td>
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</tr>
<tr>
<td></td>
<td>Humic</td>
<td>0</td>
<td>18.5</td>
<td>111</td>
</tr>
<tr>
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<td>240 km</td>
<td>7.5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Jun-01</td>
<td>122 km</td>
<td>6.2</td>
<td>16</td>
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<td></td>
<td>Humic</td>
<td>240 km</td>
<td>34.7</td>
<td>21</td>
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$a$ n.a. – Not applicable.
Table 5. Stable ($\delta^{13}$C, $\delta^{15}$N) and radio ($\Delta^{14}$C) isotopic values of extracted bacterial nucleic acids.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Salinity/River km</th>
<th>$\delta^{13}$C ($^{o}$/oo)</th>
<th>$\delta^{15}$N ($^{o}$/oo)</th>
<th>$\Delta^{14}$C ($^{o}$/oo)</th>
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<td>-25.6</td>
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$^a$ Corrected for isotopic contribution of humics.

$^b$ Not applicable; sample lost at graphitization.
Table 6. Published ranges of isotope values of potential organic matter sources to estuaries.

<table>
<thead>
<tr>
<th>Literature isotope signatures</th>
<th>$\delta^{13}C$ ($\delta_{C}^{13}$)</th>
<th>$\delta^{15}N$ ($\delta_{N}^{15}$)</th>
<th>$\Delta^{14}C$ ($\Delta_{C}^{14}$)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terrestrial (vascular plant)</td>
<td>-26 to -30</td>
<td>-2 to +2</td>
<td>+152 to +310</td>
<td>Fry and Sherr, 1984, Hedges and Garritt, 1997</td>
</tr>
<tr>
<td>Terrestrial soils (surface)</td>
<td>-23 to -27</td>
<td>2 to 6.4</td>
<td>+152 to +310</td>
<td>Glazer et al., 2002, Richter et al., 1999</td>
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<tr>
<td>Freshwater phytoplankton</td>
<td>-24 to -30</td>
<td>5 to 8</td>
<td>-</td>
<td>Anderson and Arthur, 1983, Sigleo and Macko, 1985</td>
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<tr>
<td>Marine/estuarine phytoplankton</td>
<td>-18 to -24</td>
<td>6 to 9</td>
<td>Fry and Sherr, 1984, Gurnu et al., 1995</td>
<td></td>
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<tr>
<td>C-4 saltmarsh plants</td>
<td>-12 to -14</td>
<td>3 to 7</td>
<td>Fry and Sherr, 1984, Gurnu et al., 1985</td>
<td></td>
</tr>
<tr>
<td>Benthic macroalgae</td>
<td>-12 to -18</td>
<td>0 to 5</td>
<td>Gordon et al., 1995</td>
<td></td>
</tr>
<tr>
<td>C-3 Freshwater/brackish marsh plants</td>
<td>-23 to -26</td>
<td>3.5 to 5.5</td>
<td>Fry and Sherr, 1984, Sullivan and Montecorto, 1990</td>
<td></td>
</tr>
</tbody>
</table>

Isotopes specific to York River Estuary

| Freshwater grass leachate (Pelamis virginiensis) | -29.6 | This study |
| Marsh OM (0-6 cm) | -22.3 to -26.4 | +45 to +58 | Raymond and Bauer, 2001b |
| Marsh macrophytes | -23.3 to -28.9 | 5.3 to 11.0 | Neubauer, 2000 |
| Marsh macroalgae (benthic) | -23.7 to -27.7 | 8.4 to 11.3 | Neubauer, 2000 |
| Phytoplankton (freshwater endmember) | -25.5 to -34.6 | +110 to +164 | Raymond and Bauer, 2001b |
| Phytoplankton (mid salinity) | -21.8 to -24.2 | +56 to +72 | Raymond and Bauer, 2001b |
| Phytoplankton (York River mouth) | -20.1 to -22.8 | +47 to +62 | Raymond and Bauer, 2001b |
| Chesapeake Bay DOM | -23.7 | -77 | Raymond and Bauer, 2001b |
| Terrestrial (leaf OM) | -27.8 to -28.1 | 4.0 to 4.7 | +434 | This study |
| HMW DOM (0 salinity) | -27.8 to -28.1 | 4.0 to 4.7 | +434 | This study |
| HMW DOM (10 salinity) | -24.0 to -24.5 | 5.5 to 7.5 | +434 | This study |
| HMW DOM (20 salinity) | -22.3 to -22.7 | 7.8 to 9.2 | +434 | This study |
| FW DOM | -28.2 to -30.0 | 6.4 to 7.9 | +24 to 190 | Raymond and Bauer, 2001a, This study |
| Humics (resin-extracted) | -27.5 | +111 | This study |

Isotopes specific to Hudson River

| POM (240 km) | -29.0 | 6.0 | -101 to 156 | This study, Raymond and Bauer, 2001a |
| POM (122 km)* | -27.1 to -27.4 | 2.8 to 3.2 | +96 | This study, Raymond and Bauer, 2001a |
| DOC (240 km) | -27.0 to -27.2 | 7.3 to 13.7 | Bauer et al., unpublished |
| DOC (152 km) | -27.0 | 7.3 to 13.7 | Bauer et al., unpublished |
| Phytoplankton (240 km) | -30.0 to -31.1 | 8.0 | -44 to 50 | Bauer et al., unpublished, Caraco et al., 1998 |
| Phytoplankton (152 km) | -30.5 | 8.0 | -52 | Bauer et al., unpublished, Caraco et al., 1999 |
| Submerged macrophytes | -22.0 | 8.0 | +22 | Caraco et al., 1998 |
| Emergent macrophytes | -20.0 | 8.0 | +22 | Caraco et al., 1998 |
| Terrestrial (leaf OM) | -27.0 | -2.0 | +22 | Caraco et al., 1998 |
| Humics (resin-extracted) | -27.2 | +22 | +22 | This study |

* $\Delta^{14}C$ from km 152
Table 7. Estimates of the relative contributions of potential OM sources assimilated by bacteria along the York River salinity gradient. See text for details.

<table>
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<tr>
<th>Date</th>
<th>Salinity</th>
<th>N. a.</th>
<th>N. a.</th>
<th>100°</th>
<th>0</th>
<th>N. a.</th>
<th>N. a.</th>
</tr>
</thead>
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<td>10</td>
<td>n.a.</td>
<td>n.a.</td>
<td>100°</td>
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<td>N. a.</td>
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<tr>
<td>Jul-00</td>
<td>20</td>
<td>n.a.</td>
<td>n.a.</td>
<td>86-100</td>
<td>n.a.</td>
<td>0-14</td>
<td></td>
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<tr>
<td></td>
<td>10</td>
<td>n.a.</td>
<td>n.a.</td>
<td>93</td>
<td>7</td>
<td>N. a.</td>
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<tr>
<td>Oct-00</td>
<td>20</td>
<td>n.a.</td>
<td>n.a.</td>
<td>31-50</td>
<td>n.a.</td>
<td>50-69</td>
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<tr>
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<td>n.a.</td>
<td>n.a.</td>
<td>27</td>
<td>73</td>
<td>N. a.</td>
<td>N. a.</td>
</tr>
<tr>
<td>Nov-00</td>
<td>20</td>
<td>n.a.</td>
<td>n.a.</td>
<td>87-100</td>
<td>n.a.</td>
<td>0-13</td>
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<td></td>
<td>10</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0-25</td>
<td>75-100</td>
<td>N. a.</td>
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</tbody>
</table>

a Isotopic values for potential sources as shown in Table 6.
b n.a. applicable (end member not used in mass balance).
c Values without a range were calculated based on a single isotope.
Figure 1. Map of the Hudson River Watershed. The map shows the tidal Hudson River (heavy line) that runs from river km 240 south to New York City, and is formed by the confluence of the Upper Hudson River and the Mohawk River. Darkened areas on the map show regions of intense agriculture; light cross-hatching denotes dominantly forested lands. Other land (clear) is areas of mixed land use and includes forest, agriculture and developed lands.
Figure 2. Experimental design for nucleic acid collection and bioassay incubations.
1% (v:v) 0.7 \mu m inoculum

Filtrate (0.2 \mu m)

Filtrate (0.1 \mu m)

Bioassay Sample Incubations: \(~ 36 \text{ to } 60 \text{ Hours}\)

Blank: Immediately Collected

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Figure 3. $\Delta^{14}C$ and $\delta^{13}C$ isotopic results for large volume (90L) bioassays in both the York and Hudson River estuaries. a) The $\Delta^{14}C$ values of DOC sorbed to glass fiber filter blanks. b) Blank corrected $\Delta^{14}C$ values of bacterial biomass produced. c) Blank corrected $\delta^{13}C$ values of bacterial biomass. d) Blank corrected dual isotope ($\Delta^{14}C$, $\delta^{13}C$) plot of bacterial biomass. Values above the dashed line indicate samples containing bomb $^{14}C$. Panel a inset shows linear regressions for the $\Delta^{14}C$ value of DOC sorbed to filter blanks along the York River estuary salinity gradient material.
Figure 4. $\Delta^{14}C$ (a) and $\delta^{13}C$ (b) signatures of bacterial nucleic acids along the salinity gradient of the York River estuary.
Figure 5. Dual isotope plot (Δ^{14}C, δ^{13}C) of extracted bacterial nucleic acids along the salinity gradient of the York River estuary.
Figure 6. Comparison of methods used to estimate isotopic signatures of bacterial biomass. a) $\delta^{13}C$ and b) $\delta^{15}N$ measurements of bacterial biomass along the York River estuary salinity gradient.
- Glass Fiber (16L)
- Anodiscs (400 mLs)
- Glass Fiber Filters (90 L blank corrected)
- Bacterial Nucleic Acids
Figure 7. Dual isotope ($\Delta^{14}C$ vs $\delta^{13}C$) plot of bacterial nucleic acids from the York and Hudson. Sample locations are as follows: **York**: freshwater (FW); mid-salinity (Mid), Mouth; **Hudson**: Corning Preserve (km 140); Poughkeepsie (km 122); Palisades (km 25).
Figure 8. Comparison of dual plots of δ15N vs. δ13C of bacterial nucleic acids and potential sources of organic matter for bacteria within the York River estuary. Rectangles are published isotopic ranges (Table 6) for each potential estuarine source group and are not unique to the York River estuary. Isotopic values for bacterial nucleic acids, POM and HMW DOM were measured in this study. Multiple points represent different sampling sites and times. Symbols with cross-hairs are published literature values from other isotopic studies in the York and are plotted as discrete points (see Table 6 for ranges). The δ15N values for phytoplankton OM and marsh sediment (0-6 cm) OM were assumed to be the median of published values where only the δ13C was measured in situ (Table 6).
FW Phytoplankton

Terrestrial (vascular plant)

Terrestrial Soils

Fresh/Brackish Marsh

Marine/Estuarine Phytoplankton

δ¹³C (‰)

δ¹⁵N (‰)

-32 -30 -28 -26 -24 -22 -20 -18

0 3 6 9 12 15 18 21

Bacterial nucleic acids

Phytoplankton

POM

HMW DOM

Marsh sediment (0-6 cm)

Marsh macrophytes

Marsh microalgae (benthic)
Figure 9. Comparison of $\Delta^{14}C$ vs. $\delta^{13}C$ of bacterial nucleic acids and potential sources within the York River estuary. $\delta^{13}C$ and $\Delta^{14}C$ values for HMW DOM were measured in this study and are the average of humic material extracted from water collected at the freshwater endmember and HMW DOM concentrated by tangential flow ultrafiltration (see Table 6 for values). Previously published potential OM sources measured within the York River estuary are plotted as discrete points and differentiated by cross haired symbols (see Table 6 for values). $\Delta^{14}C$ values of DOC from Raymond and Bauer (2001b) were considered representative of total riverine DOC. The error bars designate ± 1 S.D. of the mean when n=2 or greater. The month and location of sampling are designated by month: Location (freshwater (FW), mid-salinity (Mid), or Mouth).
Chapter IV

Comparison of Sources and Reactivity of Estuarine Dissolved and Particulate Organic Matter using a Multi-Tracer Approach
ABSTRACT

In estuaries, organic matter (OM) can originate from a complex array of autochthonous and allochthonous sources. Removal of OM from an estuary can occur by heterotrophic metabolism, flocculation, sedimentation, photo-oxidation and discharge to the coastal seas. Discerning the cycling and fate of OM in estuaries is challenging due to the myriad of source inputs, diagenetic processing, and physical removal pathways. System-wide shifts in land use patterns have altered both the magnitude and composition of OM exported from rivers. Although compositional differences between particulate and dissolved OM pools may be generally reflected in C:N ratios and stable isotopic composition, biomarker analyses may further resolve disparities in sources, reactivities, and cycling in these pools.

Differences in the sources and cycling of particulate organic matter (POM) and high molecular weight dissolved organic matter (HMW DOM (> 3kDa)) were assessed along the York River, VA estuary using lipid biomarker, C:N ratios, and stable isotope (δ13C and δ15N) analyses. Riverine HMW DOM exhibits a unique isotopic signature distinct from both its POM counterpart and HMW DOM from more saline regions of the estuary. C:N ratios were higher in HMW DOM relative to POM, revealing potential differences in the reactivity and cycling of these two pools of OM. Total lipid and lipid compound classes were depleted in the HMW DOM relative to POM. The strongest depletions were evident for the fatty acids (FA), the most reactive pool of lipids, suggesting that HMW DOM may be more refractory than POM. However, within the dissolved pool, polyunsaturated FA (% of total FA) were a good predictor of dissolved organic carbon (DOC) decomposition in bioassays, thus providing a bioreactivity index.
directly linking DOC decomposition to HMW DOM chemical composition.

Concentrations of sterols, diagnostic for allochthonous (i.e., terrestrial) sources, were enhanced in the HMW DOM relative to the POM in more saline regions of the estuary. Saturated FA dominated the dissolved organic pools while monosaturated and polyunsaturated acids were the primary FA classes in the POM, suggesting that HMW DOM has undergone more diagenetic processing than POM. Ternary plots based on FA biomarkers show that POM is dominated by FA derived from phytoplankton/zooplankton sources, while HMW DOM FA have bacterial and vascular plant signatures. Differences in FA abundance and composition suggest enhanced reworking of the dissolved relative to the particulate OM pools. Thus, the physical form of OM (particulate vs dissolved) may affect both the distribution and biogeochemical processing of OM such that terrigenous DOM appears to be exported from rivers while POM is retained within the estuary. These observations may have implications for how different sources and physico-chemical forms of terrestrial OM are processed in rivers and estuaries during transit.

INTRODUCTION

Although rivers have been termed “arteries of the continent” (Degens et al. 1991) they do not transport OM passively from land to sea. Instead rivers and their associated estuaries are the vital hydrologic links between the continents and oceans, functioning as unique biogeochemical reactors. Bulk organic matter (OM) pools entering an estuary may potentially be modified in several ways: 1) chemically through flocculation (Fox, 1983; Sholkovitz et al., 1978) and photolytic reactions (Miller and Moran, 1997; Amon
and Benner, 1996a; Kieber 1989); 2) biologically through autochthonous production
(Peterson et al., 1994; Fisher et al., 1998; Raymond and Bauer, 2001), heterotrophic
modifications (Brophy and Carlson, 1989), and respiration (Findlay et al., 1992; Moran et
al., 1999); and 3) physically through homogenization of spatially distinct sources (Cloern
et al., 2002; Findlay et al., 1998) and sedimentation (Hedges et al., 1992; Prahl et al.,
1994). These dynamic biogeochemical and physico-chemical estuarine processes act on
numerous allochthonous (terrestrial vascular plant and soils, the landward advection of
marine dissolved organic matter (DOM)) and autochthonous (phytoplankton, benthic
algae, salt marsh macrophytes, fresh and salt water grasses) OM sources whose absolute
magnitude and relative contributions vary in both space and time. Consequently estuaries
remain one of the most challenging systems in which to trace the origins, transformations
and fates of OM (Hedges and Keil, 1999).

The transport and fate of terrigenous materials in particular are ultimately
modulated by these biogeochemical gateways to the ocean. The efficiency of estuaries in
retaining and transforming terrestrial derived OM (both particulate and dissolved) is
critical for constraining global C budgets (Stallard 1998; Hedges et al., 1997). Although
terrestrial OM in the particulate phase is primarily deposited within the coastal zone
(Hedges et al., 1992; Prahl et al., 1994; Shi et al., 2001), adsorptive/desorptive exchange
between particulate and dissolved reservoirs (Henrichs and Sugai, 1993; Wang and Lee,
1993; Hedges and Keil, 1999; Komada and Reimers, 2001) simultaneously dispels our
once simplistic view and complicates our ability to delineate the seaward flow of
terrigenous OM.
Globally, rivers deliver ~0.25 x 10^{15} g of dissolved organic carbon (DOC) per year to the ocean (Meybeck, 1982; Hedges, 1992; Hedges et al., 1997), of which the terrestrial contribution to the oceanic reservoir is uncertain (Hedges et al., 1997). Despite past and recent reports that the estuarine transport of DOC is conservative, at least in certain systems (e.g. Laane and Koole, 1982; Sharp et al., 1982; Mantoura and Woodward, 1983; Ittekot 1989), there is a marked absence of a terrigenous signal in open ocean DOM (Meyers-Schulte and Hedges, 1986; Druffel et al., 1992; Hedges et al., 1992; Opsahl and Benner, 1997; Hedges et al., 1997). The disparity in these findings underscores the shortcomings inherent in tracing select constituents of OM strictly through bulk OM concentrations.

Stable isotope analyses have proven useful as a tool for inferring sources and following pathways of OM cycling in aquatic systems (Hedges et al., 1988; Canuel et al., 1995; Currin et al., 1995) and are frequently coupled with elemental information to provide additional resolution. Estuaries typically span both fully freshwater and marine environments with a correspondingly large range of δ^{13}C dissolved inorganic carbon (DIC) values (-29 to 2 °/oo) (Coffin et al., 1994) and overlapping δ^{13}C values for individual sources of OM (Cloern et al., 2002). δ^{15}N analysis is frequently a powerful complement to δ^{13}C isotopes in resolving terrestrial and marine OM sources (Fry and Sherr 1984). This is primarily due to the comparably narrower range of δ^{15}N vs. δ^{13}C in vascular plant and estuarine primary producers (Gearing 1988; Fry and Sherr 1984) and significant δ^{15}N depletion in terrestrial (~0 ± 2 °/oo) vs. estuarine OM (Fry and Sherr 1984; Michener and Schell 1994). Nonetheless, unequivocal OM source assignments are frequently compromised by overlapping isotopic signatures, mixing of co-equal sources.
of OM, seasonal variability in the inputs of autochthonous and allochthonous sources, and physical homogenization (Canuel et al., 1995; Cloern et al., 2002).

Stable isotope signatures can be more successful in discerning the origins and cycling of OM when coupled with source specific biomarker compounds (Cloern et al., 2002). The application of lipid biomarkers to further differentiate sources of OM within complex aquatic ecosystems is a powerful and complementary approach to natural abundance isotopic measurements and provides additional power to: 1) distinguish between potential primary autochthonous sources (e.g. diatoms, dinoflagellates) 2) differentiate between OM of primary (e.g. algal) and secondary (e.g. zooplankton, bacteria) autochthonous origin, and 3) further resolve the relative importance of terrestrial sources to bulk OM pools along the estuarine gradient.

Resolving the molecular level detail of dissolved OM (DOM) has not been commensurate with analyses of particulate OM (POM), owing in part to the limitations imposed by the required sample size. However the advent of tangential flow ultrafiltration (Benner et al., 1992; Bianchi et al., 1995; Santschi et al., 1995; Bauer et al., 1996) coupled with lowered detection thresholds for many molecular tracers now allow for greater insights into the composition of and relationships between PM and DOM. The primary objective of this study was to employ a suite of bulk (C:N ratios, \(\delta^{13}C\), \(\delta^{15}N\)) OM parameters concurrently with two classes of lipid biomarker compounds (fatty acids, alcohols and sterols) to assess the relative contribution of terrestrial and autochthonous sources to both POM and DOM throughout a temperate estuary. In addition, fatty acid and sterol distributions were simultaneously used to infer bioreactivity and diagenetic states of DOM and POM. Previous studies in the York River estuary, Virginia, USA
suggested that biogeochemical processing along the estuary significantly alters the character and reactivity of terrigenous DOM during its seaward transport (Raymond and Bauer 2001a; Chapter 3). Furthermore, significant autochthonous OM from both algal and marsh sources has been observed in the York (Raymond and Bauer 2001a; Neubauer 2000; Sin et al., 1999). This study further elucidates the biogeochemical and physico-chemical processes responsible for the gradients in observed sources and diagenetic state between POM and DOM fractions along the estuarine continuum.

METHODS

Study Sites and Sampling Locations

The York River estuary is a sub-estuary of Chesapeake Bay, created by erosion in the Pleistocene, and considered pristine amongst Chesapeake Bay sub-estuaries (Bender, 1986). It is moderately stratified and formed by the convergence of the Pamunkey (~80% of total freshwater input) and Mattaponi (~20% of total freshwater input) Rivers. Discharge from the York ranges from ~10-500 m$^3$ sec$^{-1}$ (Sin and Wetzel, 1996). Sampling extended from the mouth of the estuary where it enters the Chesapeake Bay (salinity ~20), into the Pamunkey River to the ~0 salinity end-member, a distance of ~100 km. The Pamunkey is considerably narrower than the estuary proper and has extensive freshwater marshes (some greater than 1000 acres in size (Silberhorn, 1987) in the upper reaches. Phytoplankton biomass in the York displays large seasonal and temporal fluctuations, with maximum chlorophyll $a$ (Chl $a$) concentrations attaining ~ 30 μg L$^{-1}$ in the spring at the low salinity transitional area of the estuary (Sin and Wetzel, 1996). The estuary has been observed to have opposing gradients of bacterial abundance.
(increasing downstream) and production (increasing upstream), with a consequent increase in cell-specific growth rate toward the freshwater end (Schultz, 1999).

**Sample Collection and Filtration**

York River estuary surface water samples (57 to 125 L) were collected from three sites along the salinity gradient (S = ~0, 10, 20) during both high flow (March 2000) and low (October 2000) flow regimes in acid leached (10% HCl) Nanopure-rinsed polycarbonate bottles (~20 L). Water samples were peristaltically pumped through combusted (525 °C for 4h) Whatman GF/F filters (0.7 μm nominal pore size) to concentrate particles for isotopic and lipid analyses. The 0.7 μm filtrate was subsequently passed through an in-line Gelman filter capsule (0.2 μm) to remove bacteria, and the high molecular weight (> 3 kD) DOM concentrated with an Amicon DC-10L tangential flow ultrafiltration unit equipped with two spiral-wound polysulfone cartridges to a 1-L volume. The sample was further reduced in volume (~ 50 ml) by Turbo-evaporation and lyophilized. Both POM and DOM samples were stored at ~80 °C until further analysis. Between samples, ultrafiltration cartridges were cleaned sequentially with Micro detergent, HCl, NaOH and deionized water per manufacturers specifications.

**Lipid Extraction and Analysis**

All glassware for lipid processing was combusted at 450 °C for 4.5 hours. Prior to extraction, filters were shredded with solvent-rinsed forceps, and a lipid standard mixture (a C_{19} fatty acid methyl ester (FAME), a C_{19} alcohol, and a wax ester that yielded

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a C_{14} alcohol and a C_{20} FAME following saponification) which was added to both POM and HMW DOM fractions. Lipids were extracted from POM and HMW DOM in a mixture of MeCl_2/MeOH (2:1) by accelerated solvent extraction (Dionex ASE 200). The extraction was repeated twice, solvents were combined, and the lipid fraction partitioned by a modified Bligh and Dyer (1959). Samples were shaken, allowed to separate into two phases and the organic fraction collected. The aqueous fraction was subsequently re-extracted with hexane and the organic fraction combined with that previously collected. The combined organic phases were refrigerated overnight over pre-combusted (450°C, 4.5 hours) anhydrous Na_2SO_4 to remove residual water.

The lipid extracts were concentrated to 1 ml the following day using turbo-evaporation (Zymark Turbo Vap 500) and the total lipid contents was determined gravimetrically. The entire HMW DOM and a portion of the particulate (30 to 70%) lipid extract was saponified with 3 ml of 1 N KOH, in 15 ml glass tubes at 110°C for 2 hours (Canuel and Martens 1993). Upon cooling, the saponified sample was extracted into hexane under both basic and acidic conditions, generating neutral and acidic fractions respectively. The acidic fraction was dried under N_2 gas, converted to methyl esters by the addition of BF_3 in methanol, and purified on a silica gel column. The neutral lipids were separated into constituent classes on a silica gel column using solvents of increasing polarity. The sterols were eluted with 15% and 20% ethyl acetate in hexane, collected, concentrated to 1 ml using turbo-evaporation, and dried under N_2 gas. Bis(trimethylsilyl)trifluoro-acetamide (BSTFA) was used to convert sterols to trimethylsilyl (TMS) ethers.
Fatty acids (as methyl esters) and sterols (as TMS ethers) were analyzed by gas chromatography (Hewlet Packard 5890 Series II) and were injected directly onto a 30 m x 0.32 mm i.d. DB-5 fused silica capillary column (J&W Scientific). The carrier gas was H₂ with a flow rate of 1.5 ml min⁻¹ for FAMES and 2.3 ml min⁻¹ for sterols. Sample injection temperature was 60 °C followed by a fast ramp (30 °C min⁻¹) to 110 °C (FAMES) or 170 °C (sterols). FAMES were then ramped at 3 °C min⁻¹ to 280 °C and held for 7 minutes; sterols were ramped at 3 °C min⁻¹ to 225 °C followed by a final ramp at 2 °C min⁻¹ to 310 °C and held for five minutes. Individual peaks were identified on the basis of retention times of known standards and peak areas that were quantified relative to internal standards (methyl heneicosanoate for fatty acids and 5α-cholestane for sterols). Identifications of selected compounds were confirmed by gas chromatography-mass selective spectrometry (GC-MSD; Hewlett Packard 6890 Series Gas Chromatograph-Mass Selective Detector) operating at 70 eV with mass acquisition range of 50-550 a.m.u..

Particulate lipid concentrations and HMW DOM C₁₄ and C₁₉ alcohols were not corrected for recoveries. Factors of 1.5 to 2.5 to be applied to HMW DOM to correct these samples for C₁₉ and C₂₀ FAME standard recoveries (which ranged from 18 to 41%) are indicated in Table 3.

Elemental and Isotopic Analysis

Filters for stable isotope analysis of POM were thawed, dried at 60 °C, acid fumed overnight with HCl and subsequently dried again at 60 °C. Aliquots of lyophilized DOM were reconstituted in ~10 ml of deionized water and desalted overnight in Pierce 3.5 kD
slide-a-lyzers according to manufacturers specifications. Desalted HMW DOM was transferred to baked (500 °C) 2 ml vials, acidified with 10% HCl, and dried overnight at low heat on a hotplate (setting = 1). Organic carbon and total nitrogen contents of particulate and lyophilized OM fractions were measured via high temperature combustion on a Fisons Instruments Model EA1108 CHNS-O analyzer using acetonilide and sulfanilimide as external standards. Stable carbon and nitrogen isotope ratios were measured as CO₂ and N₂, respectively, on an isotope ratio mass spectrometer using a continuous flow system with on-line sample combustion (Europa Scientific Integra, University of California, Davis-Stable Isotope Facility). Selected samples were assessed for analytical precision and run in duplicate (relative standard deviation <0.4% per mil). Results for stable isotope values are reported in standard δ notation as:

δX = [(R_{sample}/R_{standard}) - 1] * 10^3 \quad (1)

where X is ¹³C or ¹⁵N and R is ¹³C/¹²C or ¹⁵N/¹⁴N. The standards were PeeDee Belemnite (as the secondary standard NBS-1) and atmospheric N₂ for δ¹³C and δ¹⁵N analyses, respectively.

**DOC Bioavailability Incubations**

In order to assess the bioavailability of bulk DOM (as DOC), approximately 3 L of water was retained after the initial pre-filtration (0.2 μm) from each sampling site during both sampling periods (March and October 2000). The bacterial-free filtrate was dispensed into acid cleaned (10% HCl) 1 L polycarbonate bottles. Incubations were initiated by inoculating with a 1% (v:v) bacterial inoculum (0.7 μm filtrate), maintained in the dark at room temperature (22 °C) and monitored for losses of DOC over a 28 day
period. Samples were stored frozen (-20 °C) until analysis by high temperature combustion on a Shimadzu TOC-5000. All samples were run in duplicate. Apparent first order rate constants were calculated for DOC decomposition following the equation of Berner, 1980:

$$k' = \ln \left( \frac{c_t}{c_0} \right) \frac{(t-t_0)}{(t-t_0)} \tag{2}$$

where \(C_t\) and \(C_0\) are the concentrations of DOC at the start \(t_0\) and termination \(t\) of the incubation period. Errors in \(C_t/C_0\) are estimated from duplicate and triplicate incubations.

**Additional Measurements**

Samples for bacterial abundance were preserved with 0.2 \(\mu\)m filtered 25% glutaraldehyde diluted to a final concentration of 2%. Preserved samples were stored at 4 °C until slide preparation. Bacterial abundance (August 1999) was determined by acridine orange epifluorescence microscopy (Hobbie et al., 1977) with a Zeiss Axiophot microscope.

For Chl \(a\) analysis ~ 100 ml of surface water was collected from each sampling location, in an amber polycarbonate bottle, and stored on ice until analysis. Chlorophyll \(a\) was determined by DMSO/acetone extraction according to Burnison (1980). Triplicate 8 ml aliquots were filtered through Whatman GF/F filters. Filters were placed in 8 ml test tubes filled with DMSO/acetone/water solution (45:45:10) sealed and kept in the dark for a minimum of 48 hours. Sample fluorescence was read on a Turner fluorometer (model 10-AU).
Data Analysis

Data were imported into MiniTab and analyzed statistically when possible given the limited replication in the lipid biomarker dataset. Unless otherwise noted, all confidence intervals are expressed at the 95% (p = 0.05) level. Relationships between variables were examined by Model I linear regressions. Analysis of variance (ANOVA) was used to examine variability in space and time and between dissolved and particulate organic fractions for grouped samples. March and October 2000 samples were combined to assess spatial variability. Tukey's multiple comparison test was used to conduct pairwise comparisons between sites (Underwood, 1981). Temporal variability was assessed by the grouping of particulate and dissolved samples at each salinity. Between-phase comparisons (i.e., particulate vs dissolved) were conducted by pooling particulate and dissolved samples across time (March and October 2000) and/or salinity.

RESULTS

Water Column and Bulk OM Characteristics

Higher streamflow (30.5 m$^3$ sec$^{-1}$) rates in the Pamunkey River occurred in the spring (March 2000) and decreased by an order of magnitude in the fall (3.5 m$^3$ sec$^{-1}$) (Table 1). Temperature reflected the expected seasonal patterns and ranged from 11.3 °C (March) to ~20 °C (October). Chl $a$ and phaeopigments varied spatially and temporally with maximal concentrations of each consistently associated with the mid-salinity station. In general, phaeopigment (Chl $a$ degradation products) concentrations were > ~50% of the total Chl $a$. Chl $a$ at the mid-salinity location was elevated on average by 6.5 and 3 times over the head and mouth of the estuary, respectively. Maximal Chl $a$
concentrations of 34.4 ug L\(^{-1}\) coincided with the spring (March) sampling and concentrations declined by 65% over time to a low of 12.3 ug L\(^{-1}\) in the fall (Table 1). Chl \(a\) concentrations averaged 3.6 ± 0.1 ug L\(^{-1}\) in the freshwater region and 7.6 ± 0.6 ug L\(^{-1}\) at the mouth. Bacterial abundance was ~1.7 times greater in the fall than during the spring sampling and coincided with temperatures that were ~6 °C higher. Particulate organic carbon (POC) concentrations were similar at the fresh and mid-salinity locations and were elevated by ~1.5 times in March relative to October. POC concentrations at the mouth of the estuary were consistently ~50% lower than in the mid and upper York (Table 1). In contrast to POC, concentrations of total dissolved organic carbon (TDOC) were similar during both samplings but decreased by ~175 μM seaward. The > 3 kDa HMW fraction comprised from 18 to 33% of TDOC, with higher contributions of this fraction in freshwater.

Elemental ratios expressed on a molar basis (C:N) were computed from CHN analysis of both POM and DOM fractions. The C:N of HMW DOM was consistently higher than that of the corresponding particulate fraction with values ranging from 7.4-17.9, and 5.5-9.0, respectively (Table 2). The C:N of HMW DOM decreased with increasing salinity while no discernible trend existed for POM as a function of salinity. The \(\delta^{13}\)C of both HMW DOC and POC showed similar patterns along the estuary with the most depleted \(\delta^{13}\)C (-28.1 ‰) values found at the freshwater endmember and the most enriched \(\delta^{13}\)C values at the mouth (-21.9 ‰) (Table 2, Figure 1). The \(\delta^{13}\)C of the dissolved fraction was on average ~1‰ lighter than the corresponding particulate fraction with the exception of the October sampling at the York mouth where POC was depleted by 0.6 ‰ relative to HMW DOC (Table 2). \(\delta^{13}\)C values were more variable.
along the York salinity gradient than between either dissolved or particulate OM fractions or sampling periods (Table 2, Figure 1).

Similar to $\delta^{13}$C, $\delta^{15}$N was most enriched ($+10.4\,^{\circ}\text{o/o}$) at the mouth of the York and most depleted ($+4.5\,^{\circ}\text{o/o}$) at the head of the estuary (Table 2, Figure 1b). Generally particulate $\delta^{15}$N values from the mid-estuary and mouth were $-2.2\pm0.2\,^{\circ}\text{o/o}$ heavier in March than during October. Conversely, $\delta^{15}$N of freshwater POM was enriched by $-1.5\,^{\circ}\text{o/o}$ in the fall sampling relative to the spring. The particulate fraction was on average $1.8\pm1.2\,^{\circ}\text{o/o}$ heavier than its dissolved counterpart with the exception of the fall sampling at the York mouth.

OM in the York lies along an isotopic continuum with freshwater HMW DOM and estuarine ($S=20$) POM as the endmembers (Figure 1). The freshwater HMW DOM endmember was differentiated from both its POM counterpart and from other HMW DOM samples along the estuarine gradient by a higher C:N ratio and a seaward increase in $\delta^{13}$C values respectively (Figure 1a). Similarly, freshwater POM and HMW DOM were distinguishable from other sites in the York River estuary by their depleted $\delta^{13}$C signatures averaging $-26.4\pm0.6\,^{\circ}\text{o/o}$ and $-28.0\pm0.2\,^{\circ}\text{o/o}$ respectively, corresponding to a $-3\,^{\circ}\text{o/o}$ decrease from OM fractions at the mid-salinity location (Figure 1). A $-2.6\pm1.2\,^{\circ}\text{o/o}$ increase in the $\delta^{15}$N signature of freshwater POM separated it isotopically from the associated dissolved fraction (Figure 1b). However, POM and DOM from more saline locations were not distinguishable from each other based solely on dual isotope plots (Figure 1b).
Lipid and Bulk Compound Class Contents of POM and HMW DOM

Total extractable lipid (TLE) in the particulate fraction ranged between 0.12 mg mg$_{oc}$\(^{-1}\) and 0.32 mg mg$_{oc}$\(^{-1}\) at the freshwater and high salinity locations, respectively, with a mid-salinity maximum of 0.41 mg mg$_{oc}$\(^{-1}\) (Figure 2a). Concentrations of particulate TLE between March and October sampling periods were virtually indistinguishable. TLE concentrations in HMW DOM were an order of magnitude lower than those in POM (Figure 2b). In contrast to the particulate fraction, the TLE content of HMW DOM in October was positively correlated with salinity and significantly higher in concentration relative to March (Figure 2b).

The mass of total fatty acids (FA), normalized to organic carbon, ranged from 11.1 µg mg$_{oc}$\(^{-1}\) to 100.2 µg mg$_{oc}$\(^{-1}\) for POM and 0.43 µg mg$_{oc}$\(^{-1}\) to 3.6 µg mg$_{oc}$\(^{-1}\) for HMW DOM (Figure 2c,d). The mass of total FA in the particulate fraction was positively and significantly correlated with salinity ($r^2 = 0.718$). Average FA concentrations in freshwater POM (11.3 ±0.2 µg mg$_{oc}$\(^{-1}\)) and HMW DOM (0.9 ± 0.7 µg mg$_{oc}$\(^{-1}\)) were substantially depleted relative to high salinity stations and comprised 1.1% and 0.09% of the OC, respectively. Total FA concentrations were consistently lower in the HMW DOM relative to POM with depletions through the estuary, being generally more pronounced seaward and during the spring sampling (Figure 2c,d).

Total sterol concentrations in both particulate and dissolved fractions were an order of magnitude lower than fatty acid concentrations (Figure 2e,f). Particulate sterol concentrations ranged from an average of 1.5 µg mg$_{oc}$\(^{-1}\) at the freshwater endmember to 7.2 µg mg$_{oc}$\(^{-1}\) at the York mouth and were positively correlated with salinity ($r^2 = 0.94; p < 0.001$) (Figure 2e). Sterol concentrations in the particulate fraction were 10-30%
higher in spring relative to the fall sampling. The dissolved fraction displayed sterol concentrations an order of magnitude lower than those measured in POM (Figure 2 e,f) and varied from 0.18 µg mg_{oc}^{-1} to 0.4 µg mg_{oc}^{-1}, with no consistent trends with salinity or between sampling seasons (Figure 2 f). On average, sterols accounted for between 0.45 ± 0.3 % and 0.03 ± 0.008 % of the OC for the particulate and dissolved fractions, respectively.

**Fatty Acids**

Particulate and dissolved OM fractions differed in their FA composition. Saturated FA, and C_{14}, C_{16}, and C_{18} in particular, generally comprised the bulk (45-70%) of FA in the dissolved OM pool although monounsaturated FA accounted for between 22 to 35%. (Table 3, Figure 3). Of the monounsaturated FA, 16:1ω7 and 18:1ω9 comprised between 83 to 100% of the total monounsaturated pool (Table 3). Contributions of polyunsaturated FA to HMW DOM were nominal and accounted for at most ~ 6% of total FA. Branched FA (Br-FA), of bacterial origin, were ~ 15 to 60% higher in HMW DOM during October when water column temperatures were higher and ranged from 9 to 15% of total FA (Table 3, Figure 3). In contrast to the dissolved phase, polyunsaturated FA were a substantial portion of total particulate FA in both periods. In March they accounted for ~ 34% of total FA at all locations along the estuary (Table 3, Figure 3a) whereas in October polyunsaturated FA were negatively correlated with salinity. The FA in the particulate phase were approximately evenly distributed between three main classes (saturated, monounsaturated and polyunsaturated) with minor contributions (1.7-3.5%) from branched FA (Figure 3). Despite considerable variation of Chl a in space and
time (Table 1), particulate polyunsaturated FA concentrations remained relatively constant with slight diminutions (<10%) in October at the freshwater endmember (Table 3, Figure 3).

Overall the POM displayed greater complexity in the number of individual FA present (30-40) relative to the dissolved fraction. However, the majority of FA (>70%) were comprised of six individual FA (Table 3). Four FA dominated the total bulk particulate pool across all sites and during both seasons: 14:0, 16:1w7, 16:0 and 20:5w3. While not a dominant (top 6) FA from the spring sampling at the head of the estuary, 18:4 was a principal FA at all sites other than the freshwater site in March. Freshwater POM collected in the spring contained a substantial percentage (~8%) of the 16:2 FA (Table 3). During the period of elevated spring Chl a levels at the mouth and mid-salinity York stations, 22:6w3 comprised ~ 6% of the total FA pool. Across all salinities in October, and at the freshwater location in the spring, 18:1w9 accounted for 6.5-13% of total FA (Table 3).

Similar to the POM, 16:0 and 16:1w7 were dominant fatty acids in all HMW DOM samples (Table 3). Furthermore, 18:0 was common to all DOM samples comprising, on average, 7.5–20% of total FA and the predominance of 18:1w9 was primarily confined to the spring sampling period (Table 3). Although 14:0 was absent from FA distributions at the mid-salinity station, it accounted for a substantial portion (5-10%) of FA from the two endmember sites. In contrast to the particulate phase, two FA generally ascribed to bacterial sources, i15:0 and 17:0, were amongst the six most abundant FA in the October sampling (Table 3).
Organic matter source contributions through the York River estuary and between the particulate and dissolved phases were further assessed by distinguishing between autochthonous (inclusive of both phytoplankton and zooplankton), terrestrial (i.e., vascular plant) and bacterial sources using groupings of FA that were primarily diagnostic for each of the aforementioned sources (Figure 4). Autochthonous sources consisted of both algal and zooplankton sources since the FA employed do not differentiate between the two indigenous sources. Approximately 30-35% of the particulate FA pool was derived from *in situ* algal and zooplankton derived material (Figure 4a). In contrast to the spring, freshwater site fall samples were depleted in FA diagnostic for mixed algal/zooplankton production by ~40% relative to the spring samples. Despite a mid-salinity Chl *a* maximum in both sampling seasons (Table 1), FA attributable to phytoplankton/zooplankton sources did not display a corresponding maximum (Figure 4a) in the particulate phase. In contrast to POM, FA in the dissolved pool diagnostic for mixed phytoplankton/zooplankton sources accounted for no more than 6% of the total FA pool. An authochthonous signal observed in the mid-salinity dissolved pool during the spring sampling (Figure 4a) may have resulted from elevated Chl *a* concentrations (Table 1).

FA diagnostic for bacterial inputs often accounted for ~10 fold more of the total FA in the DOM vs POM and ranged from 5-16% of the total (Figure 4b). The predominance of FA attributable to bacterial inputs was greater in the October relative to March (Figure 4b) and corresponded to higher water column temperatures (Table 1). An abrupt decline in bacterial FA occurred between the mid-estuary and mouth during
October, whereas the particulate phase showed little temporal and spatial change in the percent contribution of bacterial FA.

Even-numbered long chained fatty acids (ELCFA), primarily ascribed to terrigenous vascular plant material, generally decreased with increasing salinity for both dissolved and particulate fractions and in both seasons (Figure 4c). Overall HMW DOM collected from the head of the estuary in the spring reflected the highest (~6%) contribution of terrigenous FA.

Neutral Lipids

Alcohols ranged from 0.009-0.12 µg mg⁻¹ and 1.3-7.8 µg mg⁻¹ in the HMW DOM and POM fractions, respectively (Table 4). Maximum n-alcohol concentrations were consistently associated with peak Chl a levels in the dissolved phase, while the spring mid-salinity chlorophyll a and particulate alcohol maximum also coincided (Tables 1 and 4). Phytol, a side-chain derivative of chlorophylls a and c, comprised 0-45% and 73-96% of total alcohols for the dissolved and particulate fractions, respectively, with the springtime maxima in both fractions corresponding with the mid-salinity Chl a maximum (Tables 1 and 4). The contribution of longer chain alcohols (C₂₂-C₃₆) diagnostic for terrestrial (i.e., vascular plant) input varied from 0-14% in the particulate phase, with seasonal maxima at the freshwater endmember. The springtime maximum in long chain alcohols in the dissolved phase occurred at the head of the estuary, coinciding with maximal streamflow and accounted for ~ 30% of total alcohols.

Sterols, generally thought to be less reactive than FA (Sun and Wakeham, 1994; Sun et al., 1997), showed similar distributions within POM and HMW DOM (Figure 5).
Slight enrichments (15-65%) of C_{29} sterols were found in the dissolved relative to the particulate phases (Table 5, Figure 5). Elevated contributions of C_{29} sterols in the dissolved phase were most pronounced in the spring in both the mid and high salinity stations (Figure 5a). In addition, HMW DOM was depleted in C_{28} sterols relative to POM by 30-45% at all sites during the spring sampling and by ~30% at the head of the estuary in the fall (Table 5, Figure 5). Spatially, the contribution of C_{29} sterols was enhanced in freshwater POM and DOM whereas C_{27} and C_{28} sterols showed no consistent trend. Hopanols, diagnostic for bacteria, made up a small portion (1-2%) of freshwater POM and HMW DOM sterols in the spring.

Approximately 67 to 83% of the total sterol concentrations in both POM and HMW DOM were explained by five individual sterols characteristic of both autochthonous and allochthonous sources (Table 5). The two sterols indicative of autochthonous sources and consistently dominant across all sites and during both sampling periods in the particulate phase were cholest-5-en-3β-ol and 24-methylcholesta-5,22-dien-3β-ol, typically ascribed to zooplankton (Volkman, 1986; Killops and Killops, 1993) and diatom sources (Kates et al., 1978; Volkman, 1986), respectively. Additionally, 24-methylcholesta-5,24(28)-dien-3β-ol, also derived from diatom sources (Volkman, 1986) was a dominant sterol associated with POM with the exception of the fall sampling at the head of the estuary (Table 5). An additional autochthonous sterol, 24-norcholesta-5,22-dien-3β-ol, was significant in POM at the mid-salinity and mouth locations in spring. In contrast to the mid and lower York, three sterols (24-ethylcholesta-5,22E-dien-3β-ol, 24-methylcholesta-5-en-3β-ol and 24-ethylcholesta-5-en-3β-ol) generally diagnostic for vascular plant sources (Nichols et al., 1982; Killops and
Killops, 1993) were predominant in freshwater POM. Total particulate sterols at the mouth of the York were unique in the substantial contribution of 24-propyicholesta-5,24(28)-dien-3β-ol (Table 5).

The distributions of dominant sterols in the dissolved phase showed both general similarities and significant differences in comparison to the particulate phase in the contribution of selected sterols (Table 5). The spatial and temporal trends in the contribution of 24-norcholesta-5,22-dien-3β-ol were preserved in the dissolved phase. Overall cholest-5-en-3β-ol, 24-methylcholesta-5,22-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol were dominant and roughly equal in most HMW DOM samples with minor exceptions (Table 5). Although 24-ethylcholesta-5,22E-dien-3β-ol, 24-methylcholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol were again predominant in freshwater HMW DOM, they also showed pronounced contributions to total sterols at the mid-salinity location. In contrast to the particulate phase, 24-ethyl-5α(H)cholestan-3β-ol was important in the dissolved fraction (Table 5).

DISCUSSION

Bulk Parameters: Identification of Organic Matter Sources

Previously measured δ13C-DIC (Raymond and Bauer 2001a) values were used to constrain the predicted isotopic signatures of phytoplankton in the York assuming kinetic fractionations of 20 ‰ for δ13C values during photosynthetic CO2 fixation (Chanton and Lewis 1999). δ13C-DIC ranged from -0.2 ‰ at the head of the estuary to -14.6 ‰ at the mouth (Raymond and Bauer 2001a) and yielded predicted phytoplankton δ13C values (and hence, algal derived OM) of -30.5 ± 3.7 ‰, -22.7 ± 1.3 ‰, and -21.9 ± 1.5 ‰ for
freshwater, mid-salinity, and mouth locations, respectively (Raymond and Bauer 2001a; Chapter 3). However, the dominant source of OM to the head of the York River estuary is thought to be of primarily allochthonous origin, with minimal OM derived from algal inputs (Sin et al., 1999; Raymond and Bauer 2001a). The average $\delta^{13}$C of freshwater POM (-26.4 ± 0.6 ‰) and HMW DOM (-28.0 ± 0.2 ‰) measured in the present study was significantly lower ($p<0.001$) than that of its mid- and high-salinity counterparts (Figure 1), and was slightly more $\delta^{13}$C-enriched (2.5-4.2 ‰) than would be predicted if derived exclusively from freshwater phytoplankton. Thus these data corroborate the conclusions of Raymond and Bauer (2001a) who suggested OM in the freshwater York is predominately terrigenous in origin. Furthermore, $\delta^{13}$C values of both freshwater POM and HMW DOM (Table 2) are within the range for terrestrial vascular plant material (-26 to -28 ‰) (Fry and Sherr, 1984; Peterson et al., 1985; Canuel and Martens, 1993).

In contrast to the freshwater York, little ($\leq 1$ ‰) discrepancy existed between measured $\delta^{13}$C values in POM (Table 2) and predicted phytoplankton $\delta^{13}$C values at the mid- and high-salinity locations (see above). The suggestion of algal dominance in the mid-and lower York was further supported by substantial elevations (up to an order of magnitude) in Chl $a$ concentration relative to the freshwater site (Table 1). Furthermore, $\delta^{13}$C differences between both individual POM and HMW DOM pools and sampling periods generally varied by less than $< 1$ ‰ suggesting that spatial rather than temporal changes in the isotopic values of OM were the dominant source of variability in the York (Table 2, Figure 1).

Concurrent measurements of $\delta^{15}$N and molar C:N ratios helped to further resolve potential allochthonous and autochthonous contributions and successfully differentiate
between particulate and dissolved OM pools (Figure 1). The C:N stoichiometry of fresh phytoplankton is ~6.7 (Redfield, 1934) while that of vascular plant (20-500) material is comparatively N-depleted (Hedges et al., 1997). The calculated C:N of POM showed no consistent trend with salinity (Figure 1a); however, the spring C:N minimum (5.8) is coincident with maximum Chl a values (Table 1). Relative to the spring sampling, sub-Redfield C:N values of 5.5 and 6.9 were found at the mouth and head of the York, respectively (Table 2). These depleted ratios may be partially reconciled by greater bacterial abundance (Table 1) and biomass which has a correspondingly lower C:N value of ~4.3 (Lee and Fuhrman et al., 1987). On average the C:N ratio of HMW DOM is 40% greater than its particulate counterpart and is negatively correlated with salinity ($r^2 = 0.85, \rho < 0.01$) (Table 2, Figure 1a). C:N values (~17.4) in freshwater HMW DOM are significantly greater than either mean POM or HMW DOM C:N ratios in the mid and lower York. These N-depleted values may reflect either a greater allochthonous (vascular plant) signature or increased microbial reworking of the dissolved relative to the particulate phases. Microbially mediated diagenesis of OM may also result in increased C:N ratios due to preferential remineralization of N relative to C (Goldman et al., 1987; Hopkinson et al., 1997).

The $\delta^{15}$N signatures of terrestrial vascular plant OM and freshwater phytoplankton are generally more depleted than those of marine/estuarine phytoplankton (Sigleo and Macko, 1985; Peterson and Fry, 1987; Currin et al., 1995). Correspondingly, $\delta^{15}$N signatures of freshwater HMW DOM and lower York POM were the most depleted (4.6 ± 0.1 ‰) and enriched (4.6 ± 0.1 ‰), respectively (Table 2, Figure 1a).

Freshwater HMW DOM and POM are distinguished from their mid- and high-salinity
counterparts in dual isotope plots ($\delta^{13}$C vs $\delta^{15}$N) suggesting that the sources comprising these freshwater OM pools are unique (Figure 1b). These findings corroborate previous research in the York by Countway (1999) who likewise concluded that the sources of freshwater POM to the York are significantly different from higher salinities.

On the whole, bulk elemental and isotopic findings help distinguish freshwater POM and HMW DOM from higher salinity OM throughout the York River estuary. These results suggest a greater terrigenous signature for HMW DOM in particular. The $\delta^{13}$C and $\delta^{15}$N evidence, along with C/N, however, suggest a minor contribution of terrestrial vascular plant material to the particulate and dissolved OM in the middle to lower York River estuary. However, as a result of the relatively narrow dynamic ranges and the overlaps between potential sources, stable isotope ratios may have limited ability to resolve potential source contributions compared with more sensitive indices (e.g., specific biomarker compounds, $\Delta^{14}$C etc.) (Canuel et al., 1995; Raymond and Bauer 2001b; Cloern et al., 2002).

**Spatial and Temporal Variations in OM Sources Using Lipid and Bulk Tracers**

Fatty acid and sterol biomarkers provide a more detailed examination of spatial and temporal variations in the sources comprising OM along the estuarine continuum. Though several of the most abundant FA (e.g., 14:0, 16:0, 16:1ω7 (Table 3)) may be ascribed to a mixed planktonic (i.e., phytoplankton, zooplankton, bacterial) population, other less abundant ones are more unique to a specific source. For example, monounsaturated FA such as 16:1ω7 and 18:1ω9 and polyunsaturated fatty acids (PUFA), C20 and C22, are primarily derived from phytoplankton (Cranwell, 1982;
Volkman, 1986; Killops and Killops, 1993). The sum of $C_{18}$, $C_{20}$ and $C_{22}$ PUFA showed little variation along the estuarine salinity gradient during spring bloom conditions despite substantial changes in Chl $a$ concentrations (Figure 4, Table 1). Although freshwater OM stable isotope values (Table 2), Chl $a$ concentrations (Table 1) and previous bulk $\Delta^{14}C$-DOC measurements suggest minimal algal inputs at the head of the estuary, ~30% of the total FA were comprised of PUFA diagnostic for algal sources (Figure 4a). These results confirm previous findings of algal biomarkers in the freshwater regions of the York (Countway, 1999; Countway et al., in press) and suggest that lipid biomarkers are better able to discern individual source inputs compared to bulk OM characteristics (e.g. stable and radio-isotopes, C:N, etc.). During non-bloom conditions (October 2000), PUFA concentrations showed a strong negative, though not significant, correlation with salinity ($r^2 = 0.97; p > 0.05$). On the other hand, ELCFA ($\geq C_{24}$), predominately derived from terrestrial vascular plant inputs (Volkman, 1986; Canuel and Martens, 1993) displayed a divergent pattern, with higher relative contributions consistently found in freshwater OM (Figure 4c).

Bulk and individual sterol distributions further support the potential contribution of algal and terrestrial OM along the York River estuary (Table 5, Figure 6). Autochthonous OM sources are generally dominated by $C_{27}$ and $C_{28}$ sterols which are broadly ascribed to zooplankton and algal sources respectively (Killops and Killops, 1993). In contrast $C_{29}$ sterols predominate in higher plants (Huang and Meinschein, 1979; Volkman 1986; Killops and Killops 1993). Approximate source appropriations of OM based on sterol carbon number suggest that vascular plants are of comparatively greater importance at the head of the estuary relative to the mid-salinity and mouth.
station, respectively (Table 5, Figure 6). Sterols mainly derived from diatoms, (24-norcholesta-5,22-dien-3β-ol, 24-methylcholesta-5,22-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol (Kates et al., 1978; Volkman 1986)) comprised from 30-50% of total sterols at the York mid-salinity and mouth locations with maximum values coincident with the spring bloom (Table 5, Figure 6). Conversely, concentrations of these algal sterols in freshwater OM had significantly declined by approximately half (Figure 6).

The 4-methyl sterols, derived primarily from dinoflagellates (Boon et al., 1979; Volkman, 1986), though present at all salinities, were a small fraction of the concentration of diatom derived sterols (Figure 6). Three sterols normally associated with vascular plants, (24-ethylcholesta-5,22E-dien-3β-ol (stigmasterol), 24-methylcholest-5-en-3β-ol (campesterol) and 24-ethylcholest-5-en-3β-ol (β-sitosterol) (Nichols et al., 1982; Killops and Killops, 1993)) were used as biomarkers for terrestrial plant OM.

However, assignment of these sterols exclusively to terrestrial vascular plant material is compromised by their presence in some phytoplankton (Volkman 1986). Thus, the sum of stigmasterol, campesterol and β-sitosterol is attributed to a combined vascular plant/freshwater algal source which, although not strictly terrestrial, is nonetheless unique to freshwater OM. The combined inventories of these sterols accounted for ~ 40% of total sterols at the head of the estuary and are strongly negatively correlated with salinity ($r^2 = 0.68; \rho <0.001$; Figure 6) with significant enrichments in freshwater compared to the mid and lower York.

FA typically ascribed to bacterial sources include iso- and anteiso-branched fatty acids. Although common to most heterotrophic bacteria, these lipids have most frequently been studied in sulfate reducers (Parkes and Taylor, 1983; Kaneda, 1991).
Greater inventories of Br-FA were measured in the fall and corresponded to warmer (~7 °C) water column temperatures relative to spring (Table 1, Figure 4b). Although there was a slight increase in Br-FA associated with POM toward the head of the estuary, more striking was a marked peak in Br-FA in HMW DOM at the mid-salinity spring sampling (Figure 4b). Although bacterial abundance in the middle York (October) was approximately a third of the concentration in freshwater, bacterial turnover and production may still have been greater in the mid-salinity region, thereby contributing to elevated Br-FA. Hopanols, which are sterols derived from a variety of bacteria (Cranwell 1982; Rohmer et al., 1984), were detectable only during the spring sampling at the freshwater endmember, a further indication of the unique OM sources in the freshwater region.

Zooplankton, and crustaceans in particular, contain cholesterol (cholest-5-en-3β-ol) (Volkman, 1986; Killops and Killops, 1993) which comprised a substantial (~20 ± 5.8%) portion of total sterols in both the particulate and dissolved phases at all locations along the York salinity gradient (Figure 6). A significant increase in cholesterol concentrations was observed in the fall at the York River mouth indicating that zooplankton may exert a greater impact on OM composition and suggesting more heterotrophic processing of OM in this region of the estuary during summer/fall (Figure 6).

Coprostanol (5β-cholestan-3β-ol), a sterol derived from sewage input (Kanazawa and Teshima, 1978) was detected in freshwater HMW DOM exclusively during low flow conditions (Table 5), suggesting that higher flow rates and increased terrestrial
contributions may sufficiently dilute the coprostanol signature as to render it undetectable in the spring.

In summary, spatially the contributions of diatom and higher plant lipids varied inversely. Based on stable isotope analysis, FA, and sterol distributions, OM in the mid- and high-salinity regions of the estuary displayed a more pronounced algal signature while the freshwater end-member reflected the importance of both higher plants and algal sources. In addition, the mid-estuary exhibited a strong autochthonous signature with contributions from a mixed planktonic assemblage of diatoms, dinoflagellates, bacteria and zooplankton.

**Diagenetic Implications of FA**

The biogeochemical fate of estuarine OM is ultimately determined by selective preservation or decomposition of different components along the estuarine continuum. As a general rule, FA are thought to be more reactive than sterols, and decrease in reactivity with increasing saturation (Sun and Wakeham, 1994; Canuel and Martens, 1996; Sun et al., 1997a). By examining the relative proportions of these reactive compounds and their more stable counterparts, approximations of the diagenetic state of DOM and POM can be made. As a group, FA were significantly more depleted in the dissolved relative to the particulate phase (Table 6) with greater depletion in spring. The ratio of unsaturated to saturated FA (UNSAT/SAT), a “freshness” index, was approximately four to six times greater in the particulate vs dissolved fractions and was, on average, higher in March for both fractions. An analogous gauge of OM freshness,
the ratio of 16:1ω7/16:0, corroborated the general trend of a more diagenetically modified dissolved pool and a relatively fresher particulate fraction (Table 6).

The dominance of saturated FA in HMW DOM relative to POM (Figure 3, Table 3), and its implication of a more diagenetically altered dissolved fraction, is consistent with the previous findings of Mannino and Harvey (1999). The present findings confirm the comparatively greater degree of recalcitrance of the dissolved organic pool and supports the size-reactivity hypothesis (Amon and Benner, 1994, 1996b). This hypothesis links the molecular weight of OM to its diagenetic state, thus suggesting that an increase in OM size (low molecular weight (LMW) DOM to HMW DOM to POM) corresponds to appreciable increase in bioreactivity. Independent support for this diagenetic continuum is further provided by isotopic ($\delta^{13}C$) differences in total DOC (Raymond and Bauer 2001a), HMW DOC (this study), and bacterial nucleic acids (Chapter 3) from the mid and lower York River estuary. The bulk DOC pool is depleted in $\delta^{13}C$ relative to the HMW fraction by $1.5 \pm 0.1/^\circ\text{oo}$ and $1.7 \pm 0.1/^\circ\text{oo}$ for the mid and lower York respectively (Raymond and Bauer 2001a; Table 2). Bacterial nucleic acids reflect the $\delta^{13}C$ of OM assimilated (Coffin et al., 1989, 1990). The $\delta^{13}C$ signatures of bacterial nucleic acids (Chapter 3) and HMW DOM (Table 2) from the York River estuary mouth and mid-salinity locations are not significantly different from each other ($p > 0.05$). In contrast, the $\delta^{13}C$ values differ significantly ($p < 0.05$) between the bulk DOC pool (comprised of both LMW and HMW components) and bacterial nucleic acids at the same locations. This isotopic discrepancy suggests that bacteria preferentially assimilate an isotopically heavier $\delta^{13}C$ organic component (presumably derived from $\delta^{13}C$-enriched algal material).
and select against $\delta^{13}C$-depleted terrestrially derived (e.g., lignin) OM (Benner et al., 1987; Fenton and Ritz, 1988).

The potential of OM to support bacterial production is intimately tied to its diagenetic state (i.e., the extent of its prior heterotrophic processing). Previous studies have measured dissolved free amino acids (DFAA) (Dauwe and Middleburg, 1998; Dauwe et al., 1999; Amon et al., 2001), C:N ratios (Goldman et al., 1987; Meyer et al., 1987; Kroer, 1993; Hunt et al., 2000), and proportions of aliphatic and aromatic carbon content (Sun et al., 1997b; Hopkinson et al., 1998) as indices of diagenesis and predictors of reactivity. Although lipid biomarkers have been employed to address similar questions of OM diagenesis, the traditional approach (at least in sediments) has been to monitor down-core concentrations of specific lipid compounds and/or groups of lipids concomitant with their diagenetic counterparts (Canuel et al., 1996; Zimmerman and Canuel, 2000; Azaryus 2002). Rate constants (Berner, 1980) for specific compounds and groups of compounds (generally diagnostic for specific sources e.g. algal, bacterial, vascular plant etc.) are subsequently calculated based on down-core concentration changes coupled with an approximation of elapsed time (Haddad et al., 1992; Sun and Wakeham, 1995; Canuel et al., 1996).

In contrast to previous investigations, this study calculated rate constants ($k'$) for bulk DOC decomposition using incubations and examined whether there were significant relationships with various indices of diagenesis (C:N, UNSAT/SAT FA, Chl a, % PUFA, % ELCFA) in both the dissolved and particulate pools as a means of identifying an indicator of bulk DOC reactivity. DOC utilization along the estuarine continuum was measured in 28 day bioassay incubations (Table 6) and ranged from 2.6 to 11.7% of the
total DOC. Although previous studies have noted a positive correlation between C:N ratios and DOC bioavailability (Meyers et al., 1987; Kroer 1993; Hunt et al., 2000), a significant relationship was not found in the York River estuary. In contrast to the high humic concentrations characteristic of many of the previously studied river/estuarine environments, OM from the York River estuary is primarily derived from terrestrial sources in freshwater and from a mix of marsh derived, in situ algal production and advected riverine OM at mid- and high-salinity regions (Raymond and Bauer 2001a).

The % PUFA (relative to total FA) was found to be a strong predictor of both the percent of DOC decomposed over a 28 day period and the rate constant (k') of decomposition ($r^2 = 0.87; p < 0.007$) (Figure 7). The positive correlation between % PUFA and DOC utilization suggests that in the York, labile DOM, irrespective of source, is predictable from its % PUFA distribution. However, deviations from this correlation may be seen in freshwater, especially during periods of high discharge and greater terrestrial OM influx (i.e., greater concentrations of ELCFA), and suggest a portion of the DOC decomposed by bacteria is not predictable solely by % PUFA contributions (Figure 7).

The size reactivity continuum is founded on the premise that there are differential diagenetic states for particulate and dissolved OM. $\delta^{13}$C stable isotopes and diagenetic indices (see above) generally corroborate this hypothesis, and lipid biomarkers diagnostic for discrete sources (e.g. vascular plant, algal, bacterial etc) lend further support to the size dependent continuum of bioreactivity.
Differential Cycling of Estuarine POM and DOM

Depleted $\delta^{13}C$ values and higher C:N ratios in HMW DOM relative to POM suggest different OM sources and/or diagenetic processing. These distinctions were further substantiated through employment of FA and sterol biomarkers diagnostic for specific allochthonous and autochthonous OM sources. Sterols representative of algal sources were significantly depleted in freshwater HMW DOM relative to POM (Figure 6). Similarly, autochthonous sterols ascribed to dinoflagellate sources were also significantly depleted in the DOM compared to POM fraction at the mouth of the York. In contrast, significant depletions in allochthonous (vascular plant/freshwater algae) sterols were observed in the particulate pool at the mouth of York (Figure 6). These results suggest that the dominant sources comprising particulate and dissolved OM are uncoupled especially in the high and low salinity endmembers of the York River estuary.

PUFA were observed in all HMW DOM samples (Table 3) albeit at uniformly low concentrations relative to the particulate pool and were variable in space and time (Figure 4a). In contrast to a previous study in the Delaware estuary (Mannino and Harvey, 1999), phytol, a side chain derivative of Chl $a$, was detectable in the neutral lipids in all samples with the exception of the York mouth in March 2000 (Table 4). The consistent presence of phytol and PUFA in all HMW DOM in the York reflects either a greater autochthonous or a less diagenetically altered signature than that of the DOM in the Delaware estuary. Despite this suggestion of relatively greater reactivity in the York River vs. Delaware DOM, the different OM sources and diagenetic changes in the dissolved and particulate pools in the York remain dramatic and are conceivably the greatest source of OM variation in the York (Table 6, Figures 4 and 6).
Distributions of FA attributable to mixed phytoplankton/zooplankton sources were significantly lower in the dissolved relative to particulate phases (Figure 4a). Significant enrichments in FA indicative of bacterial sources (Br-FA) were observed in all HMW DOM samples (Figure 4b), whereas FA primarily derived from terrestrial (ELCFA) sources were significantly enriched in the dissolved relative to the particulate phase in March 2000 (Figure 4c).

As a means of illustrating the principal sources comprising POM and HMW DOM in a temperate coastal plain estuary such as the York, a ternary plot was constructed with each particulate and dissolved sample, normalized to the ranges in potential sources (e.g., phytoplankton/zooplankton; bacterial; terrestrial) representative of the dataset as a whole (Figure 8). The particulate and dissolved OM fractions reflect the dominance of highly disparate sources. The particulate pool is dominated by FA primarily from a mixed autochthonous source (phytoplankton/zooplankton) with a stronger signal during March, when the spring bloom occurs. In contrast, the dissolved pool is dominated by FA derived from mixed bacterial and terrestrial origins (Figure 8). Freshwater POM and HMW DOM consistently display a greater contribution of ELCFA (terrestrial), with contributions decreasing seaward. The distinct separations in the sources of both particulate and dissolved OM along the estuarine transect are retained during low flow conditions in October 2000 (Figure 8). Conversely, increased flushing of the estuary in March promotes a more homogeneous distribution of terrigenous POM and HMW DOM. During March HMW DOM is ~ 50:50 mix of bacterial and terrestrial FA while POM is primarily planktonic. This likely reflects the effects of flow in DOM and the spring bloom in POM.
A substantial terrestrial signature persists in the dissolved phase along the estuarine salinity gradient all the way to the mouth of York River estuary. In contrast, the particulate seaward counterpart is dominated by a mixed phytoplankton/zooplankton source with a nominal terrestrial influence. The diagenetic states of DOM and POM are also reflected in each of these FA classes. For example, although PUFA are typically ascribed to mixed planktonic sources they are simultaneously suggestive of “fresher”, more reactive material. Saturated ELCFA and Br-FA, while attributable to terrestrial and bacterial sources respectively, may concurrently be indicative of a less labile, more microbially reworked OM component. The dramatic disparities in OM sources (Figure 8) and diagenetic states (Table 6) between dissolved and particulate OM phases are thus representative of the physical, chemical and biological processes operating along the estuarine continuum.

**Physico-Chemical and Biogeochemical Processes Modulating Estuarine OM Transit**

Figure 9 depicts a number of potential physico-chemical and biogeochemical processes along the estuarine continuum which may account for the observed distributions of the various particulate and dissolved OM sources in the York River estuary. The physical state of OM (i.e., particulate vs dissolved) inherently predisposes OM to select physico-chemical transformations and sinks (e.g., sedimentation/flocculation, adsorption/desorption etc.) within an estuary. However, the ultimate persistence of OM within each physical state is not predetermined, as both biogeochemical and physico-chemical processes may result in OM exchange between phases (Henrichs and Sugai, 1993; Wang and Lee, 1993; Hedges and Keil, 1999;
The uncoupling observed in the sources of allochthonous POM and HMW DOM through the estuary (Table 6, Figure 8) may result from varying susceptibilities of allochthonous particulate and dissolved OM to estuarine processing.

Sedimentation and coagulation provide a depositional sink for allochthonous POM in the upper estuary (Shi et al., 2001; Lisitsyn, 1995; Prahl et al., 1994; Hedges, 1992), thereby depleting the terrestrial signature in the particulate pool. Subsequently, autochthonous POM downstream from the turbidity maximum is likely less diluted by relic allochthonous POM (Figure 9). Similarly, allochthonous HMW DOM is subject to physico-chemical processing which may result in the flocculation of terrestrial (i.e., humic) OM at the freshwater/saltwater interface (Benner and Opsahl, 2001; Mannino and Harvey, 2000; Fox, 1983; Sholkovitz et al., 1976) although in general the efficiency of DOM removal is thought to be less than particulate OM losses in estuaries (Lisitsyn, 1995).

Sharp gradients in physical and chemical properties such as the boundary between the landward intrusion of salt and freshwater (i.e. estuarine turbidity maximum) are zones of active partitioning between the particulate and dissolved OM phases. Analogous gradients in pH, redox potential, and solute concentrations between sediments and porewaters may contribute to the adsorption/desorption of OM (Wang and Lee, 1993; Gu et al., 1995, 1996; Thimsen and Keil, 1998) and disperse formerly sediment-bound OM back to the water column (Keil et al., 1997) (Figure 9). Physical resuspension of sediments may likewise produce abrupt physico-chemical gradients and thus initiate extensive exchange between particulate and dissolved OM reservoirs (Komada and
Of critical ecological relevance are that 1) the re-equilibration of riverine sediments with overlying waters occurs on time scales less than the residence time of water (Aufdenkampe et al., 2001) and 2) the terrigenous (Bianchi et al., 1997; Mitra et al., 2000; Mannino and Harvey, 2000) and/or aged (Guo and Santschi, 2000; Komada and Reimers, 2001b) signatures of solubilized OM suggests that sediment-derived subsidies of terrigenous OM may preserve an allochthonous signature in the dissolved phase throughout estuarine transit. Estuarine bacteria may arguably provide a significant sink for terrigenous DOM (Tranvik and Hofle, 1987; Moran and Hodson, 1990; Leff and Meyer, 1991; Moran and Hodson, 1994); however, previous research in the York suggests that terrestrially derived DOM is of nominal importance to bacterial production in the mid and lower York River estuary (Chapter 3).

Hence a number of physico-chemical processes within an estuary may operate together to retain terrestrially-derived OM in the dissolved phase throughout an estuary. However, the absence of a terrigenous signature in the open ocean (Meyers-Schulte and Hedges, 1986; Druffel et al., 1992; Opsahl and Benner, 1997) suggests that in contrast to the POM pool, a major sink for terrestrial OM in the dissolved phase may occur in coastal seas and continental shelves. The susceptibility of highly colored, strongly light adsorbing (Morris et al., 1995; Reche et al., 1999) terrestrial humics to photochemical losses (Miller 1999) may be enhanced in coastal waters as a result of increased sunlight penetration into the water column (Amon and Benner, 1996a; Bushaw et al., 1996) (Figure 9). Furthermore, the photochemical removal of terrestrial OM components may generate a residual signal of photochemical processing in HMW DOM which has been detected in the waters of the Mississippi River plume and the open Pacific and Atlantic...
oceans (Benner and Opsahl, 2001). Varying degrees of susceptibility of terrigenous particulate and dissolved OM to physico-chemical processes may ultimately regulate the persistence of terrestrial-derived OM in the hydrosphere and determine the re-location of riverine OM to regions of net heterotrophy (Smith and Hollibaugh, 1993; del Giorgio et al., 1997; Duarte et al., 2001; del Giorgio and Duarte, 2002)
LITERATURE CITED


Bianchi, T.S., Lambert, C.D., Santschi, P.H. and Guo, L. (1997) Sources and transport of land-derived particulate and dissolved organic matter in the Gulf of Mexico (Texas


Korn, E.D., Greenblatt, C.L. and Lees, A.M. (1965) Synthesis of unsaturated fatty acids in the slime mold *Physarum polycephalum* and the zooflagellates *Leishmania tarentolae,*


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Table 1. Site and Water Characteristics in the York River estuary during the present study.

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<th>Sampling Date</th>
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<th>Water Temp. (°C)</th>
<th>Chlorophyll a (µg/l)</th>
<th>Phaeopigments (µg/l)</th>
<th>Bacterial Abundance (cells/l) ×10⁶</th>
<th>POC (µM)</th>
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Table 2. Bulk stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and C:N ratios of POM and HMW DOM in the York River estuary.

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Table 3. Fatty Acid distributions in POM and HMW DOM in the York River estuary.

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**Correction Factor**

|          | 1.5    | 2.0    | 2.5    | 2.5    |

---

*a Total concentrations of FA (ng mg⁻¹) in HMW DOM not corrected for recoveries.

*b Boldface indicates FA is amongst the six primary FA comprising total.
Table 4. Distributions of alcohols in POM and HMW DOM in the York River estuary.

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<td>HMW DOM</td>
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<td>404</td>
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<td>1329</td>
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</table>

*a Boldface indicates one of five dominant sterols in total distribution.*
Table 6. Diagenetic indices of POM and HMW DOM in the York River estuary.

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<tr>
<th>Date</th>
<th>Salinity</th>
<th>UNSAT/SAT FA</th>
<th>(\text{16:0}/\text{16:1})</th>
<th>(\text{% PUFA})</th>
<th>Bulk DOC utilization</th>
<th>Rate constant ((k^{10^{-1}}))</th>
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<td></td>
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<td>HMW/Particulate</td>
<td>HMW</td>
<td>HMW/POM</td>
<td>HMW DOM</td>
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<td>0.60</td>
<td>1.55</td>
<td>0.31</td>
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<td>1.62</td>
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<td>0.36</td>
<td>1.21</td>
<td>0.72</td>
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<td>1.68</td>
<td>0.36</td>
<td>0.78</td>
<td>0.41</td>
<td>0.119</td>
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<tr>
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<td>0.41</td>
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<td>1.90</td>
<td>0.42</td>
<td>1.10</td>
<td>0.44</td>
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</table>

*a* HMW DOM corrected for recoveries (see text).
*b* Numbers in parentheses represent +/-1 S.D. of the mean (n=3).
*c* Rate constants expressed as day\(^{-1}\).
Figure 1. Comparisons of the C:N ratios and stable isotopic ($\delta^{13}$C) (Panel a) and dual stable isotopic ($\delta^{13}$C, $\delta^{15}$N) (Panel b) compositions of POM and HMW DOM from the York River estuary. Symbol identifications refer to the month of collection (March = M; October = O) and the salinity.
Figure 2. Lipid content of POM and HMW DOM along the salinity gradient of the York River estuary. Panels a and b, TOC normalized concentrations (μg/mg OC) of total lipid extract (TLE), panels c and d total fatty acids (FA) and panels e and f total sterols, respectively. See text for correction factors applied to HMW DOM.
Figure 3. Distribution of major fatty acids in POM and HMW DOM along the York River estuary in a) March and b) October, 2000. Fatty acid groupings were assigned based on the number of double bonds and whether the carbon chains were normal or contained methyl branches. Saturated fatty acids contained no double bonds; monounsaturated and polyunsaturated contain one and more than one double bond, respectively.
Figure 4. Temporal and spatial variations in FAs diagnostic for phytoplankton/zooplankton, bacterial and terrestrial sources in the York River estuary: Source classification of FA in POM and HMW DOM fractions along the York River estuary. Phytoplankton/Zooplankton (Panel a): polyunsaturated C18, C20, and C22 FA. Bacterial (Panel b): *iso* and *anteiso*-C13, C15, and C17. Terrigenous (or vascular plant) (Panel c): even long chain saturated FA (C24-C30).
POC March
- POC October
- HMW DOM March
- HMW DOM October

Salinity

% of Total Fatty Acids

Salinity

% of Total Fatty Acids

Salinity

% of Total Fatty Acids

Salinity

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Figure 5. Distribution of major sterol classes in POM and HMW DOM along the York River estuary in a) March and b) October 2000. Sterols were grouped according to whether they contained 27, 28, or 29 carbon atoms. Hopanols are specific to bacteria. Other includes unidentified compounds and C30 sterols.
Figure 6. Source classifications of sterols in POM and HMW DOM fractions along the York River estuary. Sources are grouped as follows: Diatoms: 24-norcholesta-5,22-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, 24-methylcholesta-5,22-dien-3β-ol; Dinoflagellates: 4-methyl sterols; Zooplankton: cholestan-5-en-3βol; Higher Plant and/or Freshwater Algae: 24-ethylcholesta-5,22E-dien-3β-ol, 24-methylcholestan-5-en-3β-ol, 24-ethylcholestan-5-en-3β-ol; Bacterial: hopanol-3β-ol. Panel a = Freshwater; Panel b = Mid-salinity; Panel c = York mouth.
Diatom Oino-
Zoo plankton Highar Plant Bacterial

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Figure 7. Correlation between calculated rate constant of DOC decomposition (k') and % PUFA.
$r^2 = 0.87; \ p < 0.01$
Figure 9. Conceptual model identifying potential physico-chemical processes responsible for the observed distribution in FA diagnostic for allochthonous and autochthonous OM sources in HMW DOM (D), POM (P) and surficial sediments (S) (data from Azaryus, 2002) in March 2000. Numbers in boxes correspond to the % ELCFA (vascular plant) observed for each location and phase (designated above). Allochthonous sources are denoted in black; autochthonous sources in gray. CT and CA denote DOC contributions from terrestrial and autochthonous sources, respectively. Dashed arrows denote phase changes, solid arrows denote processes within the same phase. References corresponding to numbered processes give preference to literature specific to both terrestrial OM processes and molecular level (lipid) tracers when available (see text for more thorough review). Estuarine Turbidity Maximum = ETM. (1) Sedimentation (Shi et al., 2001) (2) Flocculation (Benner and Opsahl, 2001; Mannino and Harvey, 2000), (3) Sediment Adsorption (autochthonous) (Aufdenkampe et al., 2001) (4) Sediment Desorption (terrestrial) (Mannino and Harvey, 2000), (5) Resuspension Induced Desorption (of aged material) (Komada and Reimers, 2001b), (6) Photoxidation of Terrestrial OM (Benner and Opsahl, 2001).
Chapter V

Summary and Conclusions
SUMMARY AND CONCLUSIONS

A comprehensive understanding of OM cycling in rivers and estuaries is necessary for the development of accurate models to predict the metabolic state (i.e. net autotrophic vs net heterotrophic) of estuaries, coastal margin zones and the open ocean. Consequently, both the source, magnitude and diagenetic processing of OM during estuarine transit are critical factors controlling the ultimate fate of OM both within an estuarine system, and in terms of its export to the coastal ocean. The focus of this dissertation was to evaluate the sources and fates of OM in a temperate, low relief estuary, as a site of exchange and transformation of OM between terrestrial and marine reservoirs.

Due to the inherent complexity of estuaries, especially those that are biogeochemically and physically dynamic, a suite of unique yet complementary techniques may be useful for assessing the sources, processing and fates of OM there. This study used an interdisciplinary approach which combined traditional tools from the field of microbial ecology (Chapters 1 and 2) as well as geochemical methods such as the use of stable and radioisotopes (Chapters 3 and 4) and lipid biomarker compounds (Chapter 4) to assess the sources, ages and diagenetic modifications of OM along the land to sea continuum. For this study I posed and addressed three pivotal questions which define the role of estuaries in the transfer of OM between terrestrial and marine systems:

1) What are the combined effects of sunlight exposure and bacterial decomposition on
DOC, DON, and DOP bioavailability and bacterial growth?; 2) What are the sources and ages of OM supporting bacterial production along the estuarine continuum?; and 3) How do the relative inputs of autochthonous (e.g., diatom, dinoflagellate, zooplankton, bacterial) and allochthonous (e.g., vascular plant, soils) OM vary along the full salinity gradient over the annual cycle?

During the course of this study, differences in both the sources and biological processing of OM between fresh and high-salinity waters were suggested by the following: 1) the stoichiometry of the major biogenic elements (C, N and P) in OM, bacterial utilization of bulk DOC, DON, and DOP and rates of bacterial ectoenzyme activity between endmember stations in the York River estuary (Chapter 1, Appendix I); 2) the isotopic (δ¹³C, δ¹⁵N, Δ¹⁴C) signatures of bacterial nucleic acids (Chapter 3); 3) stable isotopic and lipid biomarker distributions in POM and HMW DOM (Chapter 4). These findings suggest that bacterial and physico-chemical processing throughout the estuary significantly alter the initial source signatures, bioreactivity, diagenetic state, and stoichiometry of OM from its riverine origin to export to the coastal ocean, in this case the Middle Atlantic Bight. Furthermore, lipid biomarker results suggest that the cycling and sources of estuarine particulate and dissolved organic matter are uncoupled by differences in their extents of “pre-aging”, and by biological (i.e., due to bacterial decomposition and modification) and physico-chemical (e.g., adsorption/desorption, sedimentation, flocculation etc.) processing (Chapter 4). The major findings leading to this conclusion of uncoupled cycling in the dissolved and particulate phase are discussed briefly below.
The combined effects of photochemical and bacterial processing (Chapter 2) may modify both the bioavailability and metabolic fate of OM (e.g. respiration vs. biomass). The reactivity of DOC was enhanced from ~25 to as much as ~68% by the combined effects of exposure to natural sunlight and bacterial decomposition. Consequently, this enhancement may account for at least part of the loss of terrigenous DOM in the upper, freshwater regions of the system, and as suggested by lipid biomarker distributions (Chapter 4) throughout the estuary. In contrast, sunlight exposure decreased the bioreactivity of DOC in the higher salinity lower York by a factor of five, suggesting that a portion of the non-reactive DOC exported from the York may be derived from autochthonous rather than allochthonous sources (Chapter 2). The combined effects of photochemical and bacterial processing of DOM on bacterial growth efficiency (BGE) was found to be variable. Thus in order to delineate the fate of OM-derived carbon between respiratory losses and biomass increases, future studies should examine coupled photolytic and bacterial decomposition using an approach to address both total DOC cycling and bacterial production.

A combined stable isotope and lipid biomarker approach (Chapter 4) showed that there existed a strong autochthonous signal in OM along the estuary, with enrichments of terrigenous sources in the freshwater regions in both the particulate and dissolved fractions. In contrast to previous studies employing bulk OM characteristics (Sin et al., 1999; Raymond and Bauer 2001), a substantial contribution of planktonic OM sources to the upper York (Chapter 4) was discernible in the present study. Fatty acid biomarkers diagnostic for planktonic sources were found to provide a bioreactivity index by directly linking DOC decomposition to polyunsaturated fatty acids (% of total fatty acids).
Although fatty acids may comprise only a small portion of the total OM, polyunsaturated fatty acid distributions in the York River estuary suggest algal/planktonic are a significant source of bioreactive OM. Furthermore, dual isotope ($\delta^{13}$C and $\Delta^{14}$C) signatures of bacterial nucleic acids suggest that although the bulk OM isotopic signature in the upper York is dominated by terrigenous inputs (Raymond and Bauer 2001), algal sources contribute significantly to bacterial production (11-58%) (Chapter 3).

A traditional paradigm in biogeochemistry has been both that terrigenous OM is refractory and that OM age is a reasonable first-order indicator of bioreactivity. However, recent work has suggested that coupled biogeochemical processing (e.g., photolytic, adsorption/desorption etc.) may offer both resistance to alteration for intrinsically labile biomolecules (e.g., sorptive preservation) as well as a mechanism for the enhanced cycling of recalcitrant OM (see below). Results of experiments designed to evaluate OM assimilation by bacteria during this study suggest broad classifications of OM reactivity based on source (e.g., terrigenous) or age are not entirely valid. Two-source mass balance mixing models demonstrated that up to 89% of the OM assimilated by bacteria in the freshwater regions of the York is decadal-aged material of terrigenous origin (Chapter 3) and may attribute to the corresponding loss in terrigenous biomarkers along the estuary (Chapter 4). Furthermore, the $\Delta^{14}$C age (~1,000 years old) of bacterial nucleic acids in the Hudson River estuary suggests that the terms “refractory” and “old” are not necessarily analogous (Chapter 3).

Three major conclusions have come from my research. First, although the UV component of sunlight has been established to both inhibit and stimulate the cycling of river and estuarine DOM, it also impacts the ultimate fate of carbon (i.e., in respiration
vs. biomass production) as well as the cycling of the other major biogenic elements in DOM (i.e., N and P). Second, biological and physico-chemical processing may uncouple the cycling of particulate and dissolved forms of OM in temperate coastal plain estuaries. Third, despite the historical acceptance that relic organic matter is refractory and resists utilization by bacteria in aquatic systems, this research provides the first in situ evidence for the utilization of “old” OM within estuarine systems. These findings will further enhance our understanding of estuaries as both producers and reactors of OM traversing them en route to the coastal ocean and beyond.
LITERATURE CITED


VITA

Born in Norfolk, VA, September 13, 1971. Grew up Virginia Beach, VA and attended Frank W. Cox High School. Attended University of Virginia, Charlottesville, VA, and graduated with a B.A. in Biology and Environmental Sciences in January 1993. Worked for two years at the Johns Hopkins University School of Public Health before entering the Ph.D. program of the School of Marine Science, Virginia Institute of Marine Science, College of William and Mary in August 1996.