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Impacts of gelatinous zooplankton on dissolved organic matter cycling and bacterioplankton communities in the York River Estuary

Robert Howard Condon
College of William and Mary - Virginia Institute of Marine Science

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IMPACTS OF GELATINOUS ZOOPLANKTON ON DISSOLVED ORGANIC MATTER CYCLING AND BACTERIOPHAN MEKTON COMMUNITIES IN THE YORK RIVER ESTUARY

A Dissertation
Presented to
The Faculty of the School 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
Robert Howard Condon
2008
APPROVAL SHEET

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

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DEDICATION

I dedicate this dissertation to my beautiful wife, Beth, for her love, intellectual support, and everlasting encouragement over the past six years.
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ABSTRACT

Large gelatinous zooplankton (GZ) blooms of lobate ctenophores, *Mnemiopsis leidyi*, and scyphomedusae, *Chrysaora quinquecirrha*, occur throughout Chesapeake Bay and its tributaries. The mechanisms of GZ bloom formation, and the roles GZ blooms play in dissolved organic matter (DOM) and carbon (C) cycling are not fully understood. During 2003–2006, I conducted laboratory experiments and field surveys in the lower York River to determine factors controlling timing and magnitude of GZ blooms, and to evaluate their effects on C cycling. Highest biomass of *M. leidyi* occurred in early summer (May-June) and in late winter. Peaks in ctenophore biomass in the mesohaline region occurred one-month earlier than in downriver, polyhaline regions, due to higher ctenophore reproduction and larval dispersal upriver. High predation by *C. quinquecirrha* scyphomedusae on *M. leidyi* appears to cause the rapid decline in summer ctenophore blooms, and we hypothesize that subsequently medusae become C-limited. High GZ biomass coincides with peaks in microbial biomass, and as DOM is released by zooplankton but consumed by bacteria, these disparate trophic levels may be linked. I measured DOM production by GZ and the response of free-living bacterioplankton to GZ DOM, quantified in terms of bacterial metabolism, and bacteria phylogenetic community composition. Release rate of DOC by both GZ species was high relative to simultaneous release of DON and DOP, and for *M. leidyi* DOM metabolites were C-rich due to high mucus production in ctenophores. Furthermore, bacterioplankton abundance and production rapidly increased (within 6 hours) in response to uptake of GZ metabolites; however, decreases in bacterial growth efficiencies indicated that increases in bacterial C respiration were greater relative to changes in bacterial biomass. Enumeration of microbial assemblages using the fluorescence in situ hybridization (FISH) technique showed specific bacterial groups, namely *γ-proteobacteria*, are responsible for increased metabolism of GZ DOM metabolites. In the context of worldwide increases in GZ, my results have significant implications for C transfer in marine food webs, with the potential for more C to be shunted to the microbial loop away from higher trophic levels.

Robert Howard Condon

SCHOOL OF MARINE SCIENCE
THE COLLEGE OF WILLIAM AND MARY IN VIRGINIA

Dr. Deborah K. Steinberg, Professor of Marine Science
AUTHOR’S NOTE

The primary research chapters of this dissertation were written in the format of the journal under which each is in review or to be submitted. These chapters were written in the third person to represent my co-authors. The citations for the chapters are as follows:

Chapter 2


Chapter 3


Chapter 4


Chapter 5

IMPACTS OF GELATINOUS ZOOPLANKTON ON DISSOLVED ORGANIC
MATTER CYCLING AND BACTERIOPLANKTON COMMUNITIES IN THE
YORK RIVER ESTUARY
CHAPTER 1

Introduction to the dissertation
Large increases in gelatinous zooplankton populations have been observed in many coastal and estuarine ecosystems (Purcell et al. 2000, Graham 2001, Mills 2001, Uye et al. 2003), and in many cases (e.g. the Black Sea) the presence of these predators has had dramatic effects on both the planktonic species composition and the ecosystem as a whole (Purcell 1997, Purcell et al. 2001). Given current and projected worldwide increases in eutrophication and over-fishing, these trends are likely to continue in the near future (Jackson et al. 2001) with unknown consequences (Mills 2001).

In systems like Chesapeake Bay, understanding the mechanisms that influence nutrient cycling is critical to fully comprehend anthropogenic influences such as eutrophication on ecosystem functioning. Zooplankton play a key role in the cycling of organic and inorganic material (Steinberg et al. 2000, Schnetzer & Steinberg 2002, Steinberg et al. 2002), but in contrast to our understanding of the dynamics of crustacean zooplankton (e.g., copepods) in nutrient cycling, little is known about the gelatinous zooplankton (Riemann et al. 2006).

_Gelatinous Zooplankton and nutrient cycling in Chesapeake Bay_

In Chesapeake Bay and its tributaries, such as the York River estuary, a dramatic shift in zooplankton community structure takes place during the spring and summer bloom of gelatinous zooplankton. Two gelatinous zooplankton predators dominate – the scyphozoan medusa, _Chrysaora quinquecirrha_ (sea nettle), and the lobate ctenophore, _Mnemiopsis leidyi_ (Burrell & van Engel 1976, Condon & Steinberg In review). In addition, several other gelatinous species are found in these waters, including the
scyphomedusae *Aurelia* sp. (moon jelly), *Cyanea* sp. (Lion's mane jelly), and the beroid ctenophore, *Beroe* sp. though little is known of their distribution and abundance (Burrell & van Engel 1976, Calder 1983, Feigenbaum & Kelly 1984). Like other zooplankton groups, these gelatinous zooplankton may contribute to cycling of dissolved organic and inorganic matter through their excretion (Hansson & Norrman 1995, Riemann et al. 2006). While a small number of studies have investigated inorganic excretion by gelatinous zooplankton, such as ammonium ($NH_4^+$; Kremer 1977, Muscatine & Marian 1982, Kremer et al. 1986, Morand et al. 1987, Park & Carpenter 1987, Youngbluth et al. 1988, Schneider 1989, Matsakis 1992, Nemazie et al. 1993) and phosphate ($PO_4^{3-}$; Kremer 1977, Schneider 1989), there is a dearth of knowledge regarding their organic excretion processes. A few published studies demonstrate that gelatinous zooplankton excrete dissolved organic carbon (DOC), nitrogen (DON) and phosphorus (DOP) (Kremer 1977, Hansson & Norrman 1995, Carlson 2002). In addition, gelatinous zooplankton may enhance their contribution to the organic matter pool through the sloughing of mucus (Shanks & Graham 1988, Hansson & Norrman 1995). Few studies have simultaneously measured organic and inorganic excretion from gelatinous zooplankton, or simultaneously measured excretion of multiple elements (Kremer 1977).

**Gelatinous Zooplankton interactions with Bacterioplankton Communities**

Bacteria are the major consumers of dissolved organic and inorganic (particularly $NH_4^+$) pools in the sea (Kirchman 2000). In general, microbial community responses to favorable environmental conditions (in this case increases in organic and inorganic
nutrient concentrations) are increased biomass, (Shiah & Ducklow 1997) and increased metabolism (del Giorgio & Cole 1998, Carlson et al. 2007) although these trends can be variable (Shiah & Ducklow 1994, 1997). Bacterial activity and growth efficiencies may also be related to the phylogenetic composition of the bacterial community (Bouvier & del Giorgio 2002, del Giorgio & Bouvier 2002) because different phylotypes are specialized for the uptake of specific organic compounds (Alonso-Sáez & Gasol 2007).

During the summer, Chesapeake Bay and its tributaries support high microbial biomass (Shiah & Ducklow 1994), which coincides with a period where peak abundances of gelatinous zooplankton also occur (Cargo & King 1990, Kemp et al. 2005, Purcell & Decker 2005). At these times, the potential contribution of organic and inorganic matter by gelatinous zooplankton via excretion to the DOM pool likely would be greatest (Hansson & Norman 1995, Riemann et al. 2006). Consequently, gelatinous zooplankton may provide a source of biologically important compounds to bacteria, where they may be sequestered and reincorporated into the food chain via the microbial loop. This raises an interesting point particularly in the context of spatial and temporal increases in gelatinous zooplankton populations (Graham 2001, Mills 2001) and the shift to microbially dominated systems in many estuarine and coastal areas (Jackson et al. 2001). To date, very few studies have investigated the effects of DOM release by gelatinous zooplankton on microbial metabolism and community structure.

Structure of dissertation

This dissertation is separated into four main chapters and presents results from laboratory experiments and field surveys in the York River estuary conducted during
2003-2006 to examine the role of gelatinous zooplankton in affecting nutrient cycling and bacterioplankton communities in Chesapeake Bay.

In Chapter 2, I begin by detailing the major physical and biological factors controlling the timing, accumulation, and decline in *M. leidyi* ctenophore and *C. quinquecirrha* scyphomedusae populations in the York River estuary. I also discuss the impacts of gelatinous zooplankton predation on C cycling within the estuary.

In Chapter 3, I examine the production and simultaneous release rates of DOM (DOC, DON and DOP) and inorganic nutrients (NH$_4^+$ and PO$_4^{3-}$) by *M. leidyi* ctenophores and *C. quinquecirrha* medusae, and discuss the physical and biochemical causes for variations in the release of organic and inorganic constituents within and across gelatinous species. Contributions made by gelatinous zooplankton blooms to DOC, and to inorganic N and P pools are also discussed.

In Chapter 4, I explore how zooplankton metabolites affect bacterial production and diversity by examining how different phylotypes from within natural community assemblages utilize copepod and gelatinous zooplankton-derived DOM and inorganic metabolites for growth.

Finally, in Chapter 5, I look closer at the responses of microbial communities to the release of gelatinous zooplankton-derived DOM in terms of bacterial biomass, metabolism (specifically respiration and production), and bacterioplankton community composition. The large-scale implications for increased shunting of C by gelatinous zooplankton to the ‘microbial loop’ are discussed.
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CHAPTER 2

Development, Biological Regulation, and Fate of Ctenophage (*Mnemiopsis leidyi*)
Blooms in the York River Estuary, USA
ABSTRACT

Blooms of lobate ctenophores, *Mnemiopsis leidyi*, proliferate in estuaries and coastal regions worldwide. However, their role in food web structure and carbon flow between trophic levels is not fully understood. During 2003–2006, we conducted field surveys along a salinity gradient in the lower York River, a sub-estuary of the Chesapeake Bay, to determine factors controlling the timing and magnitude of *M. leidyi* blooms, and to evaluate effects of gelatinous zooplankton on carbon cycling. Samples for density, biovolume, and carbon content of ctenophores, scyphomedusae, and mesozooplankton were collected using surface net tows and quantified in the laboratory. Historical published records on ctenophores and physical data from regional databases were used to complement this data set. Highest biomass of *M. leidyi* occurred in early summer (up to 50 mg C m\(^{-3}\)), although blooms also appeared in winter and spring. Peaks in ctenophore biomass in the mesohaline York River occurred in May, one month earlier than further downriver in the polyhaline mainstem Chesapeake Bay, likely due to higher ctenophore reproductive potential and larval dispersal upriver. Copepod biomass remained low (<10 mg C m\(^{-3}\)) during ctenophore blooms but was not limiting for *M. leidyi*. Rather, high predation by *Chrysaora quinquecirrha* scyphomedusae on *M. leidyi* (scyphomedusae requirements of up to 240% of *M. leidyi* C d\(^{-1}\)) appears to cause the rapid decline in summer ctenophore blooms, and we hypothesize that subsequently medusae become carbon-limited. Long-term trends suggest *M. leidyi* blooms have shifted forward one month, possibly due to localized temperature increases over the past 40 years. These results suggest that *M. leidyi* blooms have a pivotal, and potentially changing, role in carbon transfer in estuarine and coastal food webs.
INTRODUCTION

Large increases in gelatinous zooplankton populations have occurred in many coastal and estuarine ecosystems worldwide (e.g., Brodeur et al. 2002, Uye et al. 2003). Given current and projected global increases in eutrophication and over-fishing, these trends are likely to continue into the near future with unknown consequences (Mills 2001). The lobate ctenophore, *Mnemiopsis leidyi*, is one example of a widespread and invasive species (e.g., Purcell et al. 2001, Faasse & Bayha 2006) that blooms in estuarine and coastal systems, including the Chesapeake Bay and its tributaries (Burrell & van Engel 1976, Feigenbaum & Kelly 1984, Purcell et al. 2001, Purcell & Decker 2005).

The economic impacts of *Mnemiopsis leidyi* blooms in estuarine and coastal systems are well documented. For example, the accidental introduction into the Black Sea and subsequent expansion of this ctenophore has significantly altered food web structure and heavily impacted commercial fisheries because *M. leidyi* are voracious predators on copepods and ichthyoplankton (Purcell & Decker 2005, Costello et al. 2006). Similar impacts have been observed in native habitats, where temporal shifts in *M. leidyi* blooms have occurred and consequently driven *Acartia* copepod populations to summer extinction (Sullivan et al. 2001). Despite obvious predatory impacts by this ctenophore on food web structure, we know very little regarding what impact *M. leidyi* has on large-scale ecosystem processes, such as carbon cycling.

Several studies have evaluated the seasonality, environmental cues, and biological characteristics that control *Mnemiopsis leidyi* populations, and demonstrate that ctenophores are well adapted to bloom formation. *Mnemiopsis* ctenophores are
hermaphrodites that have the ability to produce high numbers of cydippid larvae via broadcast spawning (>1500 larvae m\(^{-3}\)), but reproductive effort is restricted to temperatures >10 – 15 °C (Purcell et al. 2001, Costello et al. 2006). As a result, a major increase in ctenophore density in the Chesapeake Bay region occurs in late spring (Purcell et al. 2001), when water temperatures increase, thereby releasing thermal limitation on reproduction and growth (Costello et al. 2006). Ctenophore biomass may further accumulate at these times because ctenophores have wide salinity tolerance (i.e., they are euryhaline), copepod prey are generally abundant, reproductive output can occur multiple times (Purcell & Decker 2005), and ctenophore growth rates are high (Reeve et al. 1989).

The persistence of *Mnemiopsis leidyi* blooms is temporally and spatially dependent on various physical and biological variables. The cannibal ctenophore, *Beroë* sp., and the scyphomedusan, *Chrysaora quinquecirrha*, can consume large quantities of *M. leidyi* (Purcell & Cowan 1995, Finenko et al. 2003), and in Chesapeake Bay, declines in ctenophore biomass have been attributed to predation by these gelatinous carnivores (Burrell & van Engel 1976, Feigenbaum & Kelly 1984, Purcell et al. 2001, Purcell & Decker 2005). Other scyphomedusae are found in Chesapeake Bay, including *Aurelia* sp. and *Cyanea* sp., and these predators may also contribute to ctenophore depletion (Purcell & Decker 2005). Several fish and turtle species also consume ctenophores (Link & Ford 2006), but while some vertebrate species are abundant in Chesapeake Bay they do not consume gelatinous zooplankton (Murdy et al. 1997, D. Portnoy, pers. comm.). Low dissolved oxygen concentrations can have pronounced effects on crustacean zooplankton populations, but *M. leidyi* tolerate hypoxia (Decker et al. 2004). Food limitation
(starvation) and physical advection (e.g., currents) may also play an important role in the removal of ctenophore biomass from estuarine and coastal waters (Burrell & van Engel 1976, Feigenbaum & Kelly 1984, Purcell et al. 1994, Costello et al. 2006, J. Costello, pers. comm.).

We report results from a multi-year field program in the lower York River with the objective to determine the major physical and biological factors controlling the timing, accumulation, and decline of *Mnemiopsis leidyi* populations. Previous research on *M. leidyi* blooms in this estuary identified seasonality and regions where blooms proliferated (Burrell & van Engel 1976). Because temporal shifts in *M. leidyi* blooms have been observed in other regions (Sullivan et al. 2001, Costello et al. 2006), we evaluated whether similar shifts have occurred in the Chesapeake Bay. In addition, we determine particulate organic carbon (POC) transfers within the local food web in order to evaluate the potential impact of gelatinous zooplankton blooms on carbon cycling within estuarine ecosystems.
METHODS

Sampling location

The York River estuary is a partially-mixed, microtidal, sub-estuary of Chesapeake Bay (Lin & Kuo 2001), approximately 35 km from the mouth of the Bay (Fig. 1). The York River extends about 55 km in length from its formation at the confluence of the Pamunkey and Mattaponi rivers to the mouth. We sampled four stations along a 15 km transect in the lower York River (Fig. 1). Sampling sites ranged along a salinity gradient from mid-way upriver (Station 1) to the south-western flank of the Bay about 3 km outside the mouth of the York River (Station 4). Stations were determined based on proximity to ongoing and historical research programs monitoring gelatinous zooplankton and physical parameters within the York River estuary and Chesapeake Bay. Sampling usually occurred twice a month between May and August, and monthly between September and April, with daily sampling completed within 3 – 4 hours.

Zooplankton sampling

A variety of biological and physical parameters were measured at each of the stations on each sampling date. All gelatinous zooplankton species were collected by replicate, double oblique tows in the surface waters (0 – 3 m) using 1-m diameter plankton nets with a non-filtering cod end and attached flowmeter. After collection,
zooplankton net samples were gently emptied into buckets filled with ambient York River water, stored at *in situ* temperature, and transported to VIMS for enumeration in the laboratory. When tows contained high gelatinous biomass (>2-l by volume biomass in tow), samples were counted in the field.

A range of morphometric measurements were made on live gelatinous zooplankton in the laboratory. Each sample was poured through 500 μm mesh sieves that retained ctenophores and medusae. Ctenophores and medusae were gently separated by species, counted, and total displacement biovolumes measured according to Purcell & Decker (2005). In tows containing >50 *Mnemiopsis leidyi* individuals, sub-samples were taken and scaled up to total density and biovolume. Total density (*D*, no. m⁻³) and biovolume (*B*, ml m⁻³) of ctenophores and medusae were standardized using volume filtered by the net.

Ctenophore total length (*TL*, mm) and medusae bell diameter (*BD*, mm) were measured on up to 30 randomly selected individuals from each tow. Additional biomass measurements were made on individual ctenophores and medusae over their full size range collected at each station. Individual displacement biovolumes (ml) were measured in graduated cylinders, and weights determined on a high precision balance. For dry weights (*DW*), individuals were oven dried at 55°C for 1 – 2 weeks until weights between two successive days were ± 0.1% (mg). Linear regressions plotting individual size and biomass were derived (Table 1), and used in calculations of total ctenophore and medusae biomass in the field and of clearance rates (see below).

Ctenophores and medusae were collected in nets containing two different mesh sizes: 500 μm (June 2003 – May 2004) and 200 μm (July 2004 – May 2005). To
determine whether there were differences in catching efficiency between nets, we performed replicate tows (n = 2) using both net types at each station after May 2005. There was no significant difference in volume-specific biovolumes (F = 0.53, p = 0.71) and densities (F = 0.34, p = 0.85) of Mnemiopsis leidyi collected in the 200 μm and 500 μm nets (fully nested ANOVA with station, net, and date as factors). Supplemental tows using 1600 μm mesh nets were conducted between May – August, 2005 and 2006 to collect large scyphomedusae.

Mesozooplankton (>200 μm) and Mnemiopsis leidyi larvae were sampled from surface waters (0-2 m) at each station during 2004 – 2006 using a 0.5 m diameter net (200 μm mesh) equipped with a flowmeter and a non-filtering cod end; data for stations 1 and 4 are reported here. Following collection, samples were concentrated on a 53 μm mesh sieve and immediately fixed in 4% formaldehyde buffered with sodium borate. Sub-samples were taken using the Stempel pipette method and at least 200 non-gelatinous organisms counted under a dissecting microscope (100x magnification). Zooplankton were classified to major taxa and abundant copepod species noted. Total M. leidyi larvae in preserved samples were determined by counting tentacle bulbs (Purcell 1988). Larval growth stage was verified by first converting bulb length (TB, mm) to M. leidyi wet weight (WW_M, g), using equation 1 (Table 1), and then to M. leidyi TL (mm, Equation 2, Table 1). M. leidyi individuals >10 mm (lobed) were omitted from calculations of larval density.

Analysis of York River physical properties
We analyzed data on temperature, salinity and dissolved oxygen collected by the Chesapeake Bay National Estuarine Research Reserve System Virginia (CBNERRSVA, http://www2.vims.edu/vecos/) using monitoring stations located at Goodwin Islands (CHE 019.38), Gloucester Point/VIMS (YRK 005.40) and Claybank (YRK 015.09) (Fig. 1). Supplemental temperature data were obtained from Virginia Institute of Marine Science (VIMS) Ferry Pier (http://www.vims.edu/resources/databases.html) and VIMS buoy (http://www.vims.edu/~lbrass/vims_obs.html) monitoring sites. Total freshwater input into the York River between January – June were calculated from average daily freshwater inputs (ft^3 sec^{-1}) obtained from United States Geological Survey (USGS) monitoring stations located in the Pamunkey (site 01673000) and Mattaponi (site 01674500) rivers (http://water.usgs.gov/oss/).

Analysis of historical trends in ctenophores

We obtained historical data on *Mnemiopsis leidyi* blooms in the York River by conducting a literature and database search for sampling conducted in southern Chesapeake Bay and the York River. We only considered data that were acquired at or in the vicinity of our sampling sites and dates (Fig. 1). As ctenophore physiology and reproduction are sensitive to temperature (Purcell et al., 2001), we compared timing of ctenophore blooms to deviations from average daily temperatures recorded between 1955 – 2006 at the VIMS Ferry Pier using Hsieh (1979), and VIMS and CBNERRSVA databases. In addition, long-term trends in Pamunkey and Mattaponi River freshwater inputs were analyzed, and used as a proxy for salinity.
Measurements of POC biomass in zooplankton and phytoplankton standing stocks

Total C in ctenophore, scyphomedusae, and calanoid copepod populations (mg C m⁻³) was calculated by multiplying field measurements of biomass (mg DW m⁻³) with values of weight-specific C content (mg C g DW⁻¹). POC content of pre-weighed subsamples of ground, dried *Mnemiopsis leidyi* was measured on a Carlo Erbra EA-1108 CHN Elemental Analyzer. C biomass for *Chrysaora quinquecirrha* was calculated using a conversion factor of 0.111 mg C mg DW⁻¹ (Nemazie et al. 1993) after converting medusa size to dry biomass using empirical relationships (see results). Total copepod C biomass was calculated by multiplying copepod densities by an individual weight of 6 µg C for *A. tonsa* (Roman 1977).

At each station, surface water (0-2 m) was collected in polycarbonate bottles for determination of chlorophyll-α (Chl-α) and total suspended POC concentrations. Chl-α (Parsons 1984) and total suspended POC were measured using particulates retained on pre-combusted Whatman GF-F filters. POC samples were dried at 55°C and desiccated with 6N HCl prior to measurement. Average C: Chl-α was determined using slopes of linear plots regressing Chl-α against total POC (Equation 3, Table 1). Total phytoplankton POC was determined by multiplying Chl-α by estimated C: Chl-α.

Calculation of Chrysaora and Mnemiopsis predation
We determined ingestion rates by gelatinous zooplankton on copepods, and by

*Chrysaora quinquecirrha* on *Mnemiopsis leidyi* (Purcell & Cowan 1995, Purcell &

Decker 2005) to evaluate effects of food limitation and predation, respectively, on *M.

leidyi* populations during April – August. Calculations of population ingestion rates were

based on C biomass of standing stocks, published empirical relationships linking predator

and prey type, and mean size of *Mnemiopsis* and *Chrysaora* on each sample date.

*Chrysaora* clearance rates on *Mnemiopsis* ($CR_{CM}$) were calculated using the

regression (Equation 4, Table 1) relating *C. quinquecirrha* medusa size ($BD_{C}$, mm) to *M.

leidyi* cleared (Purcell & Decker 2005). $CR_{CM}$ (1 medusa$^{-1}$ d$^{-1}$) were converted to C

ingested by *Chrysaora* populations ($I_{CM}$, mg C m$^{-3}$ d$^{-1}$) as follows:

$$I_{CM} = CR_{CM} \times POC_{M} \times D_{C}$$

where $POC_{M}$ is *M. leidyi* C biomass (mg C l$^{-1}$) and $D_{C}$ is *C. quinquecirrha* density (no. 

medusae m$^{-3}$).

*Mnemiopsis* size was converted to wet biomass using equation 2 (Table 1), and

clearance rates on copepods ($CR_{MA}$) were determined using equation 5 (Table 1). 

Clearance rates (1 medusa$^{-1}$ d$^{-1}$) were converted to potential copepod C ingested by *M.

leidyi* populations ($I_{MA}$, mg C m$^{-3}$ d$^{-1}$) as follows:

$$I_{MA} = CR_{MA} \times POC_{A} \times D_{M}$$
where $POC_A$ is calanoid copepod C biomass (mg C l$^{-1}$) and $D_M$ is $M$. leidy$^i$ density (no. ctenophores m$^{-3}$).

$Chrysaora$ ingestion rates on copepods (mostly $Acartia$) were calculated using equation 6 (no. copepods medusa$^{-1}$ d$^{-1}$, Table 1) (Purcell 1992), and converted to copepod C ingested ($CI_{CA}$, mg copepod C medusa$^{-1}$ d$^{-1}$) by multiplying by 6-µg C copepod$^{-1}$ (Roman 1977). $Chrysaora$ population C ingestion rates on copepods ($I_{CA}$, mg C m$^{-3}$ d$^{-1}$) were calculated as follows:

$$I_{CA} = CI_{CA} \times D_C$$

where $D_C$ is $C$. quinquecirrha density (no. medusae m$^{-3}$).

To assess the potential for predator C-limitation and predation on gelatinous zooplankton, population C ingestion rates were converted to C-based daily prey consumption ($DC$, no. prey m$^{-3}$ d$^{-1}$) for $Mnemiopsis$ leidy$^i$ and $Chrysaora$ quinquecirrha as follows:

$$DC = I \div POC_p$$

where $I$ is population ingestion rate for each predator and $POC_p$ is average C biomass for each prey (ctenophores or copepods, mg C indiv.$^{-1}$). $DC$ was then converted to daily population predation pressure ($DPP$, % ingested d$^{-1}$) for both gelatinous predators using the formula:
\[ DPP = (DC \div D_P) \times 100 \]

where \(D_P\) is the density of prey (no. prey m\(^3\)).

**Statistical analyses**

Data were analyzed using ANOVA, two-sample t-tests, and linear regressions using Minitab statistical software. Data were checked for normality and homogeneity of variance primarily using box plots and histograms of data and residuals. Where data did not conform to the assumptions of the statistical test, data were either \(\log_{10}\), or square- or fourth-root transformed following Quinn & Keough (2002). We assumed a level of significance of \(\alpha = 0.05\).
RESULTS

**Seasonal periodicity of *Mnemiopsis* blooms**

During this study, *Mnemiopsis leidyi* was the most common gelatinous species in surface waters throughout the lower York River estuary. The seasonal cycle of *M. leidyi* was bi-modal, with high biomass and density peaks observed during mid-winter (December - February) and early summer (May - June) (Fig. 2 & 3). The only exception to this pattern is the lack of high biomass in winter 2005-2006. Time periods of high *M. leidyi* biomass were relatively brief persisting for 2 - 4 weeks (Fig. 2 & 3a – d).

Major differences were observed between the four stations in *M leidyi* biomass, density, and timing of the spring-summer bloom. The highest *M. leidyi* biomass and density consistently occurred upriver at station 1 (Fig. 2, 3a, c), and occasionally at station 2 (biomass: $F = 9.85, p < 0.0001$, density: $F = 18.25, p < 0.0001$; two-way ANOVA with station and year as fixed variables) (Fig. 2). At these sites, *M. leidyi* biomass peaks first appeared in May with some secondary peaks in June and July (Fig. 2, 3a, c). May biomass ($97 - 135 \text{ ml m}^{-3}$) was consistent between years ($2004 - 2006, F = 3.08, p = 0.072$) but higher densities were recorded in 2004 ($221.5 \pm 67.6, F = 6.15, p = 0.010$) than in 2005–2006 at station 1 (Fig. 3a). Compared to station 1, the timing of spring – summer biomass peaks in the polyhaline region (stations 3 and 4) was offset by a month with annual peaks occurring in June (Fig. 3b, d). High biomass peaks were not observed in May at the downriver sites (Fig. 3b, d). We focus the remainder of our analyses primarily to the two distinct end-member stations (stations 1 and 4).
Potential biological & physical controls on *Mnemiopsis leidyi* blooms

*Reproduction potential and larval production*

The potential for mature ctenophores to seed high ctenophore densities through egg and larval production was higher at station 1 due to higher biomass and density of mature ctenophores (Fig. 3). The size distribution of *Mnemiopsis leidyi* differed with month between the two sites (Fig. 3e, f). Preceding summer peaks (April 2004 – 2005, no data for station 1 in April 2006), *M. leidyi* sizes were larger upriver at station 1 (F = 7.37, p = 0.008, Fig. 3e), where they also occurred at higher density (F = 27.3, p = 0.002, Fig. 3a) and biomass (F = 35.8, p = 0.001, Fig. 3c). In contrast, during summer maximum abundances (May and June), mean ctenophore size was larger at station 4 compared to station 1 (Fig. 3e, f), except in May 2005 when *M. leidyi* were rare downriver (F = 174.4, p < 0.0001, Fig. 3a, c). However, biovolume and density of total (Fig. 3a, c) and >30-mm sized ctenophores were higher upriver in all years, so the potential for greater egg production would have likely remained high at this site during May and June (May: F = 55.5, p < 0.0001, June: F = 7.13, p = 0.011). Time-averaged May biovolumes at upriver were significantly higher than June biovolumes downriver in 2005 (F = 4.95, p < 0.05) and 2006 (F = 4.89, p < 0.05). No comparisons were made for 2004 because no sampling was conducted during June.

In all years surveyed, the highest density of cydippid larvae (ctenophores <10mm) occurred upriver at station 1 (F = 12.7, p = 0.001, Fig. 3a). Here large peaks in larval density were observed during and after maximum biovolume peaks in adults in both
summer and non-summer periods (Fig. 3a). In contrast, larval density peaks at station 4 preceded *Mnemiopsis leidyi* maximum biovolume peaks (e.g., peaks in larvae May 2005 & 2006, Fig. 3b), and larval density was significantly related to bloom size (linear regression, $r^2 = 0.33$, $t = 4.48$, $p < 0.0001$).

*Distribution of potential prey*

The most common mesozooplankton sampled were calanoid copepods (primarily *Acartia tonsa*), cladocerans (*Podon* sp.) and barnacle nauplii. Copepod density was higher downriver at station 4, with annual peaks (>5000 copepods m$^{-3}$) comprised mostly of *Acartia* between June and September (Fig. 4a), but also in December, 2004. In contrast, calanoid copepod density remained low (<1000 copepods m$^{-3}$) at station 1, except in August 2004 and 2006 where peaks of *Eurytemora affinis* occurred (Fig. 4a). Total calanoid copepod densities were significantly inversely related to *Mnemiopsis leidyi* biomass (data combined from station 1 & 4, linear regression on log$_{10}$ transformed data, $r^2 = 0.34$, $F = 37.9$, $p < 0.0001$), inferring control of copepod populations via predation by lobate ctenophores throughout the York River (Fig. 4b). High densities of cladocerans (up to 10201 ± 554 m$^{-3}$) were also observed during winter 2004 and spring 2005 and 2006.

*Predation by large scyphomedusae and Beroë on Mnemiopsis leidyi populations*

Four potential gelatinous consumers of *Mnemiopsis leidyi* occurred in the York River (Fig. 5). In June and July, relatively large (50 – 150 mm bell diameter) *Chrysaora quinquecirrha* scyphomedusae occurred at stations 1 and 2 in all years (Fig. 5a), and were
mostly absent from stations 3 and 4, except in 2006 (Fig. 5b). Peaks in *C. quinquecirrida* were associated with water temperatures $>25^\circ C$ and were restricted to salinities ranging 12 – 20 psu. In 2006, large *Aurelia* scyphomedusae (50 – 320 mm) occurred at all stations during June and July (Fig. 5c, d), and large aggregations of *Aurelia* were observed in the region of stations 2 and 3 during this time (not sampled). Cannibal ctenophores, *Beroë* sp., were present during July - August in some years, and in winter 2004 (Fig. 5e, f). In general, *Mnemiopsis* populations were depleted when scyphomedusae and *Beroë* populations increased (Fig. 5a - f). In 2006, *Cyanea* sp. scyphomedusae occurred in high numbers throughout the York River during January – May (Fig. 5g, h). High densities of *M. leidyi* did not occur when *Cyanea* was present, but occurred in 2004 and 2005 when *Cyanea* was absent or at low levels (Fig. 3a – d, Fig. 5g, h).

*Hydrological effects on Mnemiopsis leidyi summer biovolume and density peaks*

Throughout the year, maximum abundances of *Mnemiopsis leidyi* were observed over wide salinity (10 – 22 psu) and temperature (2 – 29°C) ranges, and during the summer at salinities 12 – 21 psu and at temperatures $>17^\circ C$. Salinity differed between the two sites during April – July, with higher salinity recorded at station 4 (>20 psu) compared to station 1 (12 – 18 psu, $F = 13.81$, $p < 0.0001$ with station, year, and month as fixed variables). There was no significant difference in monthly temperature (April – July) between stations ($F = 2.48$, $p = 0.116$), and water temperatures were $>17^\circ C$ by May in all years sampled. Spring – summer salinity and temperature were within the range found for ctenophore bloom formation.
Total monthly average daily freshwater discharge during January–June was similar to the 50-yr mean (254,532 ± 19,767 ft³ sec⁻¹) in 2004 and 2005 (207,780 and 240,296 ft³ sec⁻¹, respectively) but lower in 2006 (133054 ft³ sec⁻¹). Low dissolved oxygen was not a factor controlling *Mnemiopsis leidyi* distributions with concentrations remaining above 4 mg O₂ l⁻¹ in surface waters at both sites.

**Comparison between current and historical *Mnemiopsis leidyi* distributions**

Time-series studies of gelatinous zooplankton in the in the southern Chesapeake Bay are limited, and Burrell (1972) is the only such study available for the York River estuary. Monthly *Mnemiopsis leidyi* biovolumes recorded during this (2004 – 2006) and Burrell’s (1968 – 1969) study, collected on similar days (± 2) of the year and location (Fig. 1), indicate that current day ctenophore biovolume peaks appear earlier seasonally than 40 years prior (Fig. 6). In the mesohaline region upriver at stations 1 and 2, historical (1968 – 1969) peaks in *M. leidyi* occurred in June and July, and were not observed in May (Fig. 6a). In contrast, during 2004 – 2006, large ctenophore biovolume occurred in May upriver (Fig. 6a). Similar trends were observed downriver at stations 3 and 4 but offset by about a month, with peaks occurring in July and August during 1968 – 1969, and in June during 2004 – 2006 (Fig. 6b).

During 1955 – 1974, the number of winter – spring days <10°C per year (the minimum temperature for ctenophore reproduction, Purcell et al. 2001) were consistently above the 50-year mean (i.e., more days <10°C, Fig. 7a). However, there was an increase in mean water temperature during the post-1974 period which resulted in significantly
fewer number of days <10°C each winter and spring (F = 14.6, p < 0.001, Fig. 7a). In addition, there was a significant positive relationship between the day of the year in which the temperature reached and remained above the 10°C threshold and increases in winter – spring water temperature (linear regression, r² = 0.47, F = 44.8, p < 0.001, Fig. 7b). There were no significant trends in freshwater discharge from the Pamunkey and Mattaponi Rivers during 1955 – 2006, suggesting neither flow nor salinity was a driving factor in these shifts.

**Food web dynamics & carbon transfer**

*Carbon standing stocks*

As there was significantly higher biomass and density of gelatinous zooplankton in the upriver, mesohaline region, we focus our analysis on station 1. Water column particulate C: Chl-α was 80:1 at station 1 (Equation 3, Table 1). Phytoplankton C biomass was high throughout the year, particularly in the summer (>1000 mg C m⁻³, Fig 8a) when annual peaks in Chl-α were observed in the surface waters. C content in copepod standing stock was generally <10 mg C m⁻³ (Fig. 8b), with some peaks (>60 mg C m⁻³) in autumn 2004 and August 2006 when *Mnemiopsis leidyi* were virtually absent.

C per individual *Mnemiopsis leidyi* was 1.7 ± 1.0 % of dry weight. *Mnemiopsis* C biomass was highest during summer blooms (up to 50.2 mg C m⁻³) and ctenophore C biomass often exceeded copepod C biomass at these times (Fig. 8c). Maximum ctenophore densities in winter and spring (except March 2005) contained relatively low carbon biomass (<2 - 3 mg C m⁻³, Fig. 8c). C content in *Chrysaora quinquecirrha* varied
annually and was highest in July when large medusae were present (20 - 30 mg C m\(^{-3}\)), and directly following ctenophore blooms (Fig. 8d) (See Table 1 for regressions relating *M. leidyi* and *C. quinquecirrha* size to dry weight and C).

*Carbon transfers between gelatinous zooplankton & copepods*

Predation potential of *Chrysaora quinquecirrha* on *Mnemiopsis leidyi* (*I\(_{CM}\)) was high (consuming <1 - 242% d\(^{-1}\), and up to 73 mg C m\(^{-3}\) d\(^{-1}\) of available ctenophore C), but low for copepods (<1 - 26 % d\(^{-1}\), <1 mg C m\(^{-3}\) d\(^{-1}\), Table 2). DPP for *M. leidyi* consuming copepods were high (0 - 208% d\(^{-1}\), up to 2.5 mg C m\(^{-3}\) d\(^{-1}\)), but were usually lower than medusae consuming lobate ctenophores across sample dates (Table 2).

*Mnemiopsis* potential population ingestion (\(I_{MC}\)) and daily population predation pressure (\(DPP_{MC}\)) rates on copepods before (0.5 - 21 % d\(^{-1}\), 8 - 84 copepods m\(^{-3}\) d\(^{-1}\)) and during (typically 0 - 58% d\(^{-1}\), 0 - 697 copepods m\(^{-3}\) d\(^{-1}\)) ctenophore bloom periods were within the range of available standing stocks of copepods (81 - 1775 copepods m\(^{-3}\), Table 2, Fig. 4). In general, *Chrysaora quinquecirrha* C-based ingestion rates of *M. leidyi* (*I\(_{CM}\)) were initially lower than ctenophore C standing stocks in June (no data for 2004), but were at least an order of magnitude higher than available ctenophore C in July and August when ctenophore populations were greatly reduced (Table 2, Fig. 3, 5a & 8c, d). DPP of *C. quinquecirrha* consuming copepods (\(DPP_{CA}\)) was low (<20 copepods m\(^{-3}\) d\(^{-1}\)) throughout the summer (Table 2), and copepod densities were highest (>18000 copepods m\(^{-3}\)) at times when *M. leidyi* C requirements were low for these prey in July and August (Fig. 4).
DISCUSSION

Factors influencing timing and magnitude of *Mnemiopsis leidyi* blooms

*Bloom formation: Reproductive timing and larval dispersal*

The largest summertime *Mnemiopsis leidyi* blooms consistently occurred in the upriver, mesohaline region with ctenophore density and biomass decreasing along the increasing salinity gradient. In addition, mesohaline bloom peaks (animals $>10$ mm TL) appeared about one month earlier than those downriver. We attribute this spatial and temporal lag in the accumulation of *M. leidyi* biomass to the reproductive timing and effort associated with seasonal increases in water temperature upriver, coupled to larval dispersal downriver.

Temperature plays an important role in bloom formation because growth, metabolism, maturation, and the initiation of reproduction are thermally dependent (Purcell et al. 2001, Costello et al. 2006). Generally, *Mnemiopsis* initiate sexual reproduction when temperatures reach 10°C, and larger animals ($>30$ mm TL) produce appreciable quantities of eggs at temperatures $>10$°C (Purcell et al. 2001, Costello et al. 2006). While we did not measure egg production in this study, ctenophore maturity and population reproductive potential were inferred from field densities of potentially mature individuals ($>30$ mm TL). Preceding mesohaline ctenophore blooms in April, higher densities of large *M. leidyi* were located upriver at station 1 (Fig. 3). As a result, ctenophores may have a greater potential for reproductive output in the mesohaline region, and this process would be accelerated during late spring (April – May) because of
removal of temperature limitation on egg production. In turn, high growth rates exhibited
by smaller *M. leidy i* at warmer temperatures (Reeve et al. 1989) would contribute to rapid
bloom formation in May upriver.

High mesohaline reproductive potentials are supported by cydippid larvae
distribution data. Highest larval densities (up to 500 larvae m⁻³) occurred at station 1,
where larval density peaks co-occurred in space and time with ctenophore bloom peaks,
implying that larvae originated locally from blooms in the mesohaline region. In
contrast, low densities of larvae (< 100 larvae m⁻³) occurred downriver at station 4, and
while *Mnemiopsis leidy i* individuals were generally larger at station 4, total biomass of
potentially reproductively mature ctenophores was low.

Costello et al. (2006) postulated that source/sink characteristics of metapopulation
dynamics are an inherent reproductive strategy for maintaining *Mnemiopsis leidy i*
populations worldwide. Metapopulations are localized, subunit populations that originate
and are maintained through larval dispersion and recruitment from isolated, reproductive
populations (Hanski 1999). We suggest that reproductively active populations of *M.
leidy i* in the mesohaline region produce high quantities of larvae which are then
transported downriver and seed ctenophore blooms in the polyhaline region. The average
residence times for surface water in the lower York River ranges 25 – 40 days (Shen &
Haas 2004), and corresponds to observed time lags between summer biomass peaks up-
and downriver.

*Bloom decline: Mortality due to predation, not food limitation*
*Chrysaora quinquecirrha* is a major predator of *Mnemiopsis leidyi* (Purcell & Cowan 1995, Purcell & Decker 2005), and studies have correlated high predation by this gelatinous predator with the bulk removal of ctenophore biomass (Feigenbaum & Kelly 1984, Purcell & Decker 2005, Breitburg & Fulford 2006). The decline in *M. leidyi* blooms in the York River estuary appears to be due primarily to predation by *C. quinquecirrha* scyphomedusae, and not to bottom-up food limitation of *M. leidyi*. *Chrysaora* medusae appeared in surface waters early to mid-summer (June – July) at water temperatures >25°C and salinities ranging 16 – 20 (Fig. 6, Decker et al. 2007). In conjunction with the arrival of *C. quinquecirrha*, we observed sharp declines in *M. leidyi* biomass, which implies swift removal of ctenophores (2 – 4 weeks) by scyphomedusan predators. Predation rates would be highest in the mesohaline region where medusae and ctenophores co-occur and highest densities of both species were consistently recorded. Scyphomedusae C ingestion rates on ctenophores support these conclusions, as predicted daily rations for *C. quinquecirrha* populations typically exceeded prey biomass (Table 2). In addition, predation pressure by other gelatinous species may accelerate ctenophore depletion in certain years. For example, high densities of large *Aurelia* medusae, with relatively high predation potentials (e.g., Martinussen & Bamstedt 1995), were distributed throughout the York River during summer 2006 (Fig. 5). Furthermore, while we did not measure or estimate predation by *Beroë*, which was occasionally abundant during late summer, Burrell & van Engel (1976) suggested this cannibal species (not *Chrysaora*) was responsible for the removal of *Mnemiopsis leidyi* blooms in the York River. Similar predation effects were observed in the Black Sea following the introduction of *Beroë* to this system (Kideys 2002, Finenko et al. 2003).
Broad comparisons between predation rates and standing stocks do not incorporate growth of predator and prey. *Mnemiopsis* growth (C) rates range 0.8 d\(^{-1}\) for small ctenophores and 0.34 d\(^{-1}\) for larger ctenophores at prey concentrations comparable to York River densities (Reeve et al. 1989, Purcell et al. 2001). It is improbable that ctenophore growth (<100% d\(^{-1}\)) could compensate for overall high predation by scyphomedusae rates (up to 240% d\(^{-1}\)). In addition, while growth rates are high, ctenophore blooms upriver comprise smaller-sized individuals that are less efficient at avoiding predation than larger ctenophores (Purcell & Cowan 1995).

Food limitation is an alternative hypothesis for bloom depletion (Purcell et al. 2001). Copepods are primary prey for *Mnemiopsis leidyi* (Purcell et al. 1994, Purcell & Decker 2005), and voracious predation by ctenophores can deplete copepod stocks (Purcell & Decker 2005, Costello et al. 2006). However, in this study estimated daily rations for *M. leidy*i on copepods were comparable with copepod standing stocks, and despite remaining very low in abundance, copepods were not depleted during ctenophore blooms. In addition, ctenophores may be able to reduce resource limitation by supplementing their diet with alternative prey including cladocerans, barnacle nauplii, and microzooplankton (Stoecker et al. 1987, Purcell et al. 1991, Sullivan & Gifford 2004). We thus conclude that *M. leidy*i bloom declines in the York River are not a result of food limitation.

**Potential role of seasonal refuge for overwintering populations**

For blooms to occur annually, mature *Mnemiopsis leidyi* must develop within or be transported to the regions they occur. Overwintering populations may be important in
maintaining *M. leidyi* biomass that will ultimately contribute to bloom formation during the ensuing summer (Costello et al. 2006b). In the York River, overwintering populations could adopt several strategies. First, low winter temperatures are not lethal to *Mnemiopsis* ctenophores but limit egg production, so blooms could develop directly during non-summer periods within the mesohaline region (Fig. 3, Purcell et al. 2001, Costello et al. 2006). We did observe high winter and spring densities of ctenophores (>400 indiv. m$^{-3}$) in some years at station 1, which were comprised of small-sized ctenophores that were likely not reproductive at those temperatures. Second, through larval dispersal and recruitment downriver during the previous summer, smaller populations could overwinter and mature ctenophores transported upriver the following spring. This would be an advantageous survival strategy because low densities of scyphomedusae and *Beroë*, but higher copepod abundances, were observed downriver.

Gelatinous zooplankton exhibit unique behavioral adaptations that allow them to utilize certain environmental conditions for movement (Graham et al. 2001, Dawson & Hamner 2003). We suggest two possible physical mechanisms by which ctenophores could be transported upriver. One way would be to use bottom waters of the normal two-layered estuarine circulation within the tributary (Hayward et al. 1982). Transportation upriver would be dependent on *Mnemiopsis leidyi* ctenophores actively swimming to depth. In general, larger ctenophores, with presumably higher swimming speeds, were found downriver and would likely be able to adjust their vertical position in the water column (J. Costello, pers. comm.). Another potential transport process for ctenophores is tidally based. Haas (1977) demonstrated the breakdown of estuarine circulation during spring tides caused by the intrusion of relatively freshwater from Chesapeake Bay into
the York River. Due to the resultant homogeneous conditions, and because the net vertical tidal flux is landward (Haas 1977, Hayward et al. 1982), *M. leidy* may use these physical conditions to redirect their distribution upriver.

**Influence of climate on *Mnemiopsis leidy* blooms**

Comparisons with historical data for *Mnemiopsis leidy* in the lower York River suggest that biomass peaks have shifted over the past 40 years. Present day peaks in biomass in the upriver (May) and downriver (June) surface waters appear approximately one month earlier than in the late 1960's (Burrell, 1972). Chesapeake Bay climate and water temperature have undergone major changes during this time frame (Austin 2002), and we hypothesize that shifts observed in *M. leidy* blooms are associated with these increases in water temperature. Similarly, dramatic 2-month shifts were observed in *M. leidy* blooms of Narragansett Bay and these were attributed to increases in average monthly surface water temperatures (Sullivan et al. 2001, Costello et al. 2006).

Austin (2002) suggested warmer temperatures can initiate reproductive effort through early spawning of fish larvae. From 1974 – 2006, numbers of consecutive winter and spring days <10°C decreased, and springtime water temperatures reached and remained above 10°C approximately one month earlier (Fig. 7). As a result, pre-bloom spawning and larval dispersion by *Mnemiopsis leidy* could occur earlier (April), because of release of temperature limitation on egg production. It is unlikely that salinity was a factor in causing changes in *M. leidy* blooms because there were no trends in freshwater river discharge and lobate ctenophores are euryhaline (Purcell et al. 2001).
This early appearance in *Mnemiopsis leidyi* could also be fostered by decreased predation by *Chrysaora quinquecirrha*, which are absent in April and May. It is unknown whether parallel shifts in the appearance of these gelatinous medusae have occurred, as there is no long-term historical data. Parallel shifts in scyphomedusae would be related to changes in environmental stimuli affecting the timing of asexual reproduction by benthic scyphopolyps (Condon et al. 2001). This is unlikely for *C. quinquecirrha* because salinity and temperature have remained stable during historical periods of asexual reproduction (Austin 2002), and medusae densities may in fact have decreased in northern Chesapeake Bay (Cargo & King 1990, Breitburg & Fulford 2006).

Higher winter and spring temperatures may increase the likelihood of blooms of other scyphomedusan species. For example, in 2006 during spring (Feb – April) high densities of *Cyanea* sp. medusae were recorded when water temperatures were well above average. During the same time, spring blooms of *Mnemiopsis leidyi* were not observed. As *Cyanea* medusae consume gelatinous prey (Bamstedt et al. 1997), the prevalence of these scyphomedusae with increased temperature may impact winter/spring *M. leidyi* populations through ‘top-down’ control, thereby limiting further temporal shifts in ctenophore blooms.

Other studies have recently correlated climatic factors with gelatinous zooplankton populations. In particular, the North Atlantic Oscillation Index (NAO) has been used as an environmental variable to explain increases in medusae (Brodeur et al. 2002, Lynam et al. 2004), including Chesapeake Bay scyphomedusae (Purcell & Decker 2005). Our limited time series restricts similar comparisons, although *Mnemiopsis leidyi* blooms could be connected with NAO as York River water temperature is positively
correlated to positive phases of NAO (Austin 2002). While the strength of positive phase of the NAO has waned in recent years (Austin 2002), anthropogenic influences (e.g., greenhouse gas emissions) may counteract declines in natural climate cycles, forcing temperatures to increase above current levels (Preston 2004). It is unknown how *M. leidy* blooms will react to future changes in climate for the Chesapeake Bay region.

**Consequences of gelatinous zooplankton blooms for carbon cycling**

*Increased C assimilation by gelatinous zooplankton linked to temporal bloom shifts*

High predation rates by *Mnemiopsis leidy* blooms during late spring – summer convert large quantities of C fixed by primary and secondary producers into gelatinous biomass, which is not consumed by most pelagic organisms. In this regard, gelatinous zooplankton potentially have a negative impact on C transfer within the planktonic food web by limiting C bioavailability to higher trophic levels (Berdnikov et al. 1999, Hagy 2002). In estuaries, this shunting of C into gelatinous biomass may have critical costs for fisheries production, especially commercial and anadromous fish that depend on mesozooplankton production for reproduction and growth (Austin 2002, Hagy 2002).

C bioavailability for tertiary production may hinge on the timing of blooms of *Chrysaora* medusae, and potentially *Beroë* ctenophores (Table 2, Burrell & van Engel 1976, Finenko et al. 2003, Purcell & Decker 2005). For example, Purcell and Decker (2005) noted that when *C. quinquecirrha* predominated, *Mnemiopsis leidy* biomass was suppressed, releasing ‘top-down’ control on copepods. However, as *M. leidy* blooms have shifted earlier seasonally, the transformation of copepod C into gelatinous form
would now also occur one month earlier. Furthermore, declines in *M. leidyi* by *C. quinquecirrha* predation occurs mid-summer, when a large amount of copepod C standing stock has already been consumed and converted into gelatinous biomass. Thus temporal shifts in *M. leidyi* bloom peaks may simply prolong the residence time in which C is ‘locked up’ in gelatinous form.

C transfer within the summertime York River planktonic food web probably culminates with *Chrysaora quinquecirrha*. However, temporal shifts in *Mnemiopsis leidyi* blooms in the York River estuary may in turn limit *C. quinquecirrha* medusae populations. Following rapid declines of *M. leidyi* in June, estimated C requirements for *C. quinquecirrha* consuming *M. leidyi* were much greater than available in ctenophore standing stocks. Based on these estimates, we suggest the rapid decline in *C. quinquecirrha* scyphomedusae is the result of C-limitation. This hypothesis is supported by stable isotope data with elevated δ¹⁵N values in *Chrysaora* medusae related to periods of starvation in summer (Rosen, 1994, Hagy, 2002). At these times, *Beroe* sp. may be important alternative prey for *C. quinquecirrha*, although *Beroe* sp. may also become C-limited due to bulk *M. leidyi* removal.

*Potential fate of C assimilated by gelatinous zooplankton blooms*

We suggest the potential enhancement of two C pathways associated with ctenophore and scyphomedusae blooms. First, live gelatinous zooplankton could accelerate regeneration of assimilated C as dissolved inorganic and organic C via respiration, excretion and mucus production (Kremer 1977, Hansson & Norrman 1995). This process may be prominent during starvation when scyphomedusae can survive on
stored C reserves (Hamner & Jenssen 1974). In turn, recycling of gelatinous zooplankton metabolites could augment the summertime dominance of bacterioplankton by fueling their metabolism (Hansson & Norrman 1995). Second, moribund gelatinous zooplankton could sink from surface waters and ultimately contribute to benthic production (Billett et al. 2006). While phytoplankton cells generally contribute the majority of the annual C export from surface waters in Chesapeake Bay (Kemp et al. 2005), gelatinous zooplankton could enhance summertime C flux (Billett et al. 2006) because carcasses may penetrate strong vertical stratification barriers that exist throughout the estuary during summer (Haas 1977, Hayward et al. 1982). All of these suggested pathways of C transfer, in conjunction with temporal shifts and worldwide spatial increases in gelatinous zooplankton blooms, indicate gelatinous species have the potential to significantly alter C availability and transfer within planktonic food webs.
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Fig. 1. Map of stations sampled in current (stars) and historical (squares) studies, and other monitoring sites (circles) in the York River estuary. Y15, Y10, Y00 and CB10 denote historical monitoring sites from Burrell (1972). Chesapeake Bay National Estuarine Research Reserve System Virginia (CBNERRSVA) monitoring stations are located at Claybank (YRK 015.09), Virginia Institute of Marine Science – VIMS (YRK 005.40), and Goodwin Islands (CHE 019.38). The position of United States Geological Survey (USGS) monitoring stations located in the Pamunkey (site 01673000) and Mattaponi (site 01674500) rivers are indicated by arrows inside Chesapeake Bay insert (top right).
Fig. 2. *Mnemiopsis leidy*. Contour plots of average (a) *M. leidy* biomass (biovolume, ml ctenophore m$^{-3}$) and (b) density (no. ctenophores m$^{-3}$) for all four stations (plotted up-to downriver) sampled in the York River estuary. Plots generated with Surfer © software using triangulation data interpolation. Inverted triangles (black) denote sample dates.
Mnemiopsis Biovolume (ml m$^{-3}$)

Mnemiopsis Density (No. m$^{-3}$)

[Graphs showing temporal and spatial variations of biovolume and density over the years 2003 to 2006]
Fig. 3. *Mnemiopsis leidyi*. Comparison of population and individual size of *M. leidyi* blooms between stations 1 and 4. (a, b) Mean density (mean ± 1 SD, no. ctenophores m$^{-3}$) of lobed (>10mm TL) and cydippid larval *M. leidyi*. Note difference in density scales (y-axes). (c, d) Mean biomass (mean ± 1 SD, biovolume, ml ctenophore m$^{-3}$) of lobed *M. leidyi*. (e, f) Box plots of *M. leidyi* size range (mm). Horizontal dashed line = minimum size for mature ctenophores (30mm TL). Box and whiskers represent 10$^{th}$, 25$^{th}$, median, 75$^{th}$ and 90$^{th}$ percentiles.
Fig. 4. *Mnemiopsis leidyi*. (a) Mean density (± 1 SD) of calanoid copepods at station 1 (white circles) and station 4 (black circles). (b) Scatter plot comparing mean calanoid copepod and *M. leidyi* density at stations 1 and 4. Insert is a blow-up of lower values. Error bars are omitted.
Figure a: Copepod density (No. copepods m⁻³) over the years 2004 to 2006.

Figure b: M. leidyi density (No. ctenophore m⁻³) plotted against M. leidyi density.
Fig. 5. *Chrysaora quinquecirrha*, *Mnemiopsis leidyi*, *Aurelia* sp., *Beroë* sp. and *Cyanea* sp. Mean biomass (ml 10m\(^{-3}\), black circles) and density (no. 10m\(^{-3}\), white circles) of (a-d) *C. quinquecirrha*, (e-h) *Aurelia* sp., (i-l) *Beroë* sp., and (m-p) *Cyanea* sp. up- (stations 1 and 2) and downriver (stations 3 and 4). Grey shaded area in (a) and (b) represents mean *M. leidyi* densities (no. ctenophore m\(^{-3}\), error bars omitted), data from Fig. 3a and b.
Stn 1

- **a. Chrysaora**
- **e. Aurelia**
- **i. Beroe**
- **m. Cyanea**

Stn 2

- **b. M. lutea**
- **f.**
- **j.**
- **n.**

**Biovolume (ml 10m$^{-3}$)**

**Density (No. 10m$^{-3}$)**

**Sample Date**

2003 2004 2005 2006
Fig. 6. *Mnemiopsis leidyi*. Monthly (May – August) comparisons of mean (± SE) *M. leidyi* biomass (biovolume) between 1968 – 69 (data from Burrell, 1972) and 2004 – 2006 (this study). (a) Upriver, mesohaline region represented by stations Y10, and Y15 (Burrell 1972) and stations 1 and 2 (this study). (b) Downriver, polyhaline region represented by stations CB10 and Y00 (Burrell 1972) and stations 3 and 4 (this study). See Fig. 1 for station locations. Zeros indicate *M. leidyi* were absent from net collections and nd = no data. Sample size (n) is given for entire May – August period.
This study

May
June
July
Aug

May
June
July
Aug

Mesohaline York River
Upriver

Polyhaline Chesapeake Bay
Downriver

Burrell (1972)
This study
Fig. 7. Increase in water temperature in the York River estuary. (a) Comparison of water temperature anomaly between 1955 – 2006 against 50 year mean. York River water temperature anomaly defined as the number of days per year winter – spring water temperatures were < 10°C, minus the 50-yr. annual mean (adapted from Austin, 2002). Negative anomalies reflect increased water temperatures over the winter – spring period.

(b) Water temperature anomaly against day of year when water temperature increased to and remained above 10°C threshold (x-axis). Positive, significant linear relationship indicates present day increase in winter – spring water temperatures earlier in the year. Grey circles are 1955-1974 and black circles are 1975-2006. Dashed line is the 50-yr mean of the water temperature anomaly (see Fig. 7a).
Fig. 8. *Mnemiopsis leidyi* and *Chrysaora quinquecirrha*. Average POC (± 1 SD) standing stocks of (a) phytoplankton, (b) calanoid copepods, (c) *M. leidyi* ctenophores, and (d) *C. quinquecirrha* scyphomedusae. Off-scale values are in parentheses.
Table 1. *Mnemiopsis leidyi* and *Chrysaora quinquecirrha*. List of linear regressions calculated from size and biomass measurements of individual *M. leidyi* and *C. quinquecirrha* collected in net tows. All linear regressions were significant (p < 0.0001) and presented in the form in which they were applied to data in this study. 

- \( B \) = mean biovolume (ml m\(^{-3}\)) of paired tows collected in the 200-\(\mu\)m \((B_{200})\) and 500-\(\mu\)m \((B_{500})\) mesh net, 
- \( D \) = mean density (No. ctenophores m\(^{-3}\)) of paired tows collected in the 200-\(\mu\)m \((D_{200})\) and 500-\(\mu\)m \((D_{500})\) mesh nets, 
- \( WW \) = wet weight (g) of *M. leidyi* \((M)\), 
- \( TB \) = tentacle bulb length (mm), 
- \( TL \) = *M. leidyi* total length (mm), 
- \( CR_{CM} \) = clearance rates for *C. quinquecirrha* \((C)\) consuming *M. leidyi* \((1\ \text{medusa}^{-1}\ \text{d}^{-1})\), 
- \( BD \) = *C. quinquecirrha* bell diameter (mm), 
- \( D_A \) = copepod density (no. \(1\)\(^{-1}\)), 
- \( CR_{MA} \) = clearance rate for *M. leidyi* consuming copepods \((A)\) \((1\ \text{ctenophore}^{-1}\ \text{d}^{-1})\), 
- \( CI_{CA} \) = ingestion rate for *C. quinquecirrha* consuming copepods (no. copepods medusa\(^{-1}\) d\(^{-1}\)), 
- \( T \) = temperature (\(^\circ\)C), 
- \( TC_{Mnem} \) = total organic carbon of *M. leidyi* (mg), 
- \( POC \) = particulate organic carbon (mg), 
- \( Chl - a \) = chlorophyll - \(a\) (mg), and 
- \( DW \) = dry weight (g). 

NA = not available.
<table>
<thead>
<tr>
<th>Eq</th>
<th>Linear Regression</th>
<th>$r^2$</th>
<th>n</th>
<th>Utilization of equation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$WW_M = 0.81 \ T B_M^{1.913}$</td>
<td>0.98</td>
<td>67</td>
<td>Wet weight as a function of tentacle bulb length</td>
<td>Purcell (1988)</td>
</tr>
<tr>
<td>2</td>
<td>$TL_M^{0.5} = 3.774 \ WW_M^{0.25} + 0.996$</td>
<td>0.93</td>
<td>660</td>
<td>Total length as a function of wet weight</td>
<td>This study</td>
</tr>
<tr>
<td>3</td>
<td>$POC_{SI} = 80.2 \ Chl-a + 1517$</td>
<td>0.52</td>
<td>39</td>
<td>C: Chl—a conversions for upriver at station 1</td>
<td>This study</td>
</tr>
<tr>
<td>4</td>
<td>$\log CR_{CM} = 2.026 \ \log BD_C - 0.756$</td>
<td>0.93</td>
<td>NA</td>
<td>$C. quinquecirrha$ clearance rates on $M. leidy$</td>
<td>Purcell &amp; Cowan (1995)</td>
</tr>
<tr>
<td>5</td>
<td>$CR_{MA} = 11.22 \ WW_M^{0.5413}$</td>
<td>NA</td>
<td>NA</td>
<td>$M. leidy$ clearance rates on copepods</td>
<td>Purcell et al (2001)</td>
</tr>
<tr>
<td>6</td>
<td>$\log CiCA = 0.85 \ \log BD_C + 1.43 \ \log D_A + 3.96 \ \log T - 6.43$</td>
<td>NA</td>
<td>240</td>
<td>$C. quinquecirrha$ ingestion rates on copepods</td>
<td>Purcell (1992)</td>
</tr>
<tr>
<td>7</td>
<td>$\log ClC_{A} = 0.101 \ TL_M^{0.5} - 0.111$</td>
<td>0.85</td>
<td>617</td>
<td>Dry weight as a function of total length</td>
<td>This study</td>
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<td>8</td>
<td>$TC_{Mnem}^{0.25} = 300 * \ DW_M^{0.25} + 0.127$</td>
<td>0.92</td>
<td>91</td>
<td>Carbon content as a function of dry weight for $M. leidy$</td>
<td>This study</td>
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<tr>
<td>9</td>
<td>$BD_C = 63.291 \ DW_C^{0.5} + 19.367$</td>
<td>0.82</td>
<td>43</td>
<td>Carbon content as a function of dry weight for $C. quinquecirrha$</td>
<td>This study</td>
</tr>
</tbody>
</table>
Table 2. *Mnemiopsis leidyi* and *Chrysaora quinquecirrha*. Predicted monthly C ingestion rates for *M. leidyi* ctenophores and *C. quinquecirrha* scyphomedusae populations for upriver, mesohaline York River (Station 1). Ingestion rates based on mean sized predator (mm) and, predator and prey C standing stocks (mg C m\(^{-3}\)). Presented as upper and lower monthly range estimates for April – August, 2003 – 2006. Calculations were made using equations listed in table 1. \(B_p\) = biomass of prey, \(D_p\) = density of prey, \(I\) = population ingestion rates, \(DPP\) = daily population predation pressure rates, and \(DC\) = C-based daily prey consumption rates.
<table>
<thead>
<tr>
<th>Predator</th>
<th>Month</th>
<th>Size (mm)</th>
<th>Copepod prey</th>
<th>Population ingestion rates on copepods</th>
<th>M. leidyi prey</th>
<th>Population ingestion rates on ctenophores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$B_p$ (mg C m$^{-3}$)</td>
<td>$D_p$ (No. prey m$^{-3}$)</td>
<td>$I$ (mg C m$^{-3}$ d$^{-1}$)</td>
<td>$DPP$ (% prey C m$^{-3}$ d$^{-1}$)</td>
</tr>
<tr>
<td>C. quinquecirrhia</td>
<td>April</td>
<td>0</td>
<td>0.7 – 10.7</td>
<td>119 – 1775</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>0</td>
<td>0.7 – 9.1</td>
<td>110 – 1520</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>23 – 133</td>
<td>0 – 6.4</td>
<td>81 – 1065</td>
<td>0.01 – 0.02</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>76 – 152</td>
<td>2.9 – 7.6</td>
<td>477 – 1271</td>
<td>0.01 – 0.2</td>
<td>0 – 2</td>
</tr>
<tr>
<td></td>
<td>Aug</td>
<td>80 – 134</td>
<td>3.6 – 113.6</td>
<td>594 – 18929</td>
<td>0.2 – 0.7</td>
<td>&lt; 1 – 26</td>
</tr>
<tr>
<td>M. leidy</td>
<td>April</td>
<td>42 – 43</td>
<td>0.7 – 10.7</td>
<td>119 – 1775</td>
<td>0.05 – 0.5</td>
<td>0.5 – 21</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>10 – 39</td>
<td>0.7 – 9.1</td>
<td>110 – 1520</td>
<td>0.7 – 2.5</td>
<td>27 – 127</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>12 – 13</td>
<td>0 – 6.4</td>
<td>81 – 1065</td>
<td>0.2 – 1.8</td>
<td>24 – 208</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>22 – 63</td>
<td>2.9 – 7.6</td>
<td>477 – 1271</td>
<td>0 – 1.2</td>
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<tr>
<td></td>
<td>Aug</td>
<td>0 – 34</td>
<td>3.6 – 113.6</td>
<td>594 – 18929</td>
<td>0 – 0.1</td>
<td>0 – 2</td>
</tr>
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</table>
CHAPTER 3

Production of Dissolved Organic Matter and Inorganic Nutrients by Gelatinous Zooplankton in the York River Estuary, USA
ABSTRACT

Large gelatinous zooplankton ‘blooms’ of lobate ctenophores (*Mnemiopsis leidyi*) and scyphomedusae (*Chrysaora quinquecirrha*) occur during spring and summer throughout the York River, a highly productive, sub-estuary of Chesapeake Bay. At these times, gelatinous zooplankton can influence carbon (C) and nutrient cycling through the release of dissolved organic matter (DOM), and inorganic nitrogen (N) and phosphorus (P). We conducted a series of laboratory incubations measuring simultaneous release of DOC, DON, DOP, and inorganic N (ammonium, NH$_4^+$) and P (phosphate, PO$_4^{3-}$) by *Mnemiopsis* ctenophores and *Chrysaora* medusae. Both gelatinous zooplankton species released high amounts of DOC compared to DON and DOP. For *M. leidyi* ctenophores, DOM metabolites were C-rich as indicated by higher DOC:DON (29:1) compared to canonical Redfield stoichiometry of 6.6C:1N. Daily turnover of DOC and DON in ctenophores was high (25.2% of body C and 18.3% of body N), likely due to high mucus production. In contrast, individual *Chrysaora* medusae released DOC and DON similar to Redfield ratios, and daily turnover of these elements was low (<3% of body C and N). Both gelatinous species released dissolved N and P in inorganic form but also released sizeable quantities of DON and DOP. Of the two gelatinous species examined in this study, the majority of contributions made to DOC, NH$_4^+$, and PO$_4^{3-}$ pools in the York River were by *Mnemiopsis* ctenophores during summer months (May-July). While contributions of DOC to bulk pools was low (<1% d$^{-1}$), ctenophore populations released higher amounts of DOC to labile pools, ranging between 18-29% d$^{-1}$. Contribution of excreted inorganic nutrients to NH$_4^+$ and PO$_4^{3-}$ pools was highest at times when the York River was N-limited as indicated by low inorganic N:P (5.8:1) compared to Redfield
stoichiometry (16N:1P). Despite the potential for this release to reduce nutrient limitation in phytoplankton, excretion of inorganic N and P by gelatinous zooplankton was estimated to support < 4% of primary production. Because net NH$_4^+$ released by Mnemiopsis populations exceeded standing concentrations, we hypothesize that there must be an alternative sink for inorganic N during summer. We suggest that bacterioplankton need to supplement potential uptake of ctenophore-derived DOM with inorganic N and P in order to satisfy their intracellular elemental requirements.

**INTRODUCTION**

Estuaries are dynamic ecosystems that sustain high productivity and large fluxes of organic and inorganic nutrients. Chesapeake Bay is a well-studied estuary that receives large inputs of inorganic nutrients but exports large amounts of organic nutrients on an annual basis (Kemp et al. 2005). Quantifying nutrient source-sink dynamics of inorganic and organic pools, therefore, is critical to understanding carbon (C), nitrogen (N), and phosphorus (P) cycling in this highly productive ecosystem. Zooplankton play an important role in the cycling of nutrients in planktonic food webs via their excretion of inorganic nutrients, primarily in the form of ammonium (NH$_4^+$) and phosphate (PO$_4^{3-}$) (Steinberg & Saba In Press), and by release of dissolved organic matter (DOM; Steinberg et al. 2000, Carlson 2002, Steinberg et al. 2002). Most studies of zooplankton excretion have emphasized the role of crustacean zooplankton (e.g., copepods, euphausiids), while little is known about excretion by gelatinous zooplankton (Pitt et al. In Press, Steinberg & Saba In Press) and how these common zooplankton affect nutrient dynamics.
Over the past ten years, large spatial and temporal increases in gelatinous zooplankton have occurred in coastal and estuarine systems worldwide. Gelatinous zooplankton are major predators of crustacean zooplankton and may play an equally important role in nutrient cycling (Kremer 1977). Chesapeake Bay supports high biomass (blooms) of two native species: the ctenophore *Mnemiopsis leidyi* and the scyphomedusan *Chrysaora quinquecirrha* (Purcell & Decker 2005, Condon & Steinberg In review). Because of their high biomass during blooms, gelatinous zooplankton can influence nutrient cycling at these times (Condon et al. In prep, Condon & Steinberg In review). To date, the relatively few studies examining gelatinous zooplankton excretion have focused on excretion of NH$_4^+$ and PO$_4^{3-}$, with little attention to DOM production. The excretion of inorganic N and P by *M. leidyi* ctenophores and *Aurelia* medusae can support up to 39 and 23%, respectively, of primary production in Great South Bay and Kiel Bight (Park & Carpenter 1987, Schneider 1989), but is a minor contributor (3% of microplankton production) in Chesapeake Bay (Nemazie et al. 1993). However, NH$_4^+$ and PO$_4^{3-}$ excretion by coastal and estuarine scyphomedusae might be more important for supporting primary production during times of nutrient limitation (Schneider 1989, Pitt et al. 2005). In contrast, the simultaneous release of DOM and inorganic nutrients by zooplankton may have greater influence on microbial communities in net heterotrophic Chesapeake Bay (Schultz Jr. & Ducklow 2000), because microbial production is supported by organic matter pools (Raymond & Bauer 2000). DOM excretion may also be augmented in gelatinous zooplankton by release of DOM through mucus production (Shanks & Graham 1988, Hansson & Norrman 1995).
The ctenophore *Mnemiopsis leidyi*, as well as *Aurelia* semaeostome medusae, are known to release significant quantities of their total excretia as dissolved organic carbon, nitrogen, and phosphorus (DOC, DON, and DOP) (Kremer 1977, Hansson & Norrman 1995). The response of bacterioplankton to crustacean zooplankton DOM excretia indicates that this material is labile and can support substantial bacterial production (Møller et al. 2003, Nelson et al. 2004, Steinberg et al. 2004). Because DOC concentrations in Chesapeake Bay are high but a small proportion is labile (Raymond & Bauer 2000), gelatinous zooplankton blooms could be major contributors to labile DOC pools that can support microbial production. This is in contrast to the current paradigm in which phytoplankton are viewed as the primary source of DOM in marine systems (Carlson 2002, Carlson et al. 2007).

Here, we report results from laboratory experiments measuring simultaneous release of DOM and inorganic nutrients by *M. leidyi* ctenophores and *C. quinquecirrha* medusae from the York River estuary, a southern tributary of Chesapeake Bay. The C:N:P ratios of released organic and inorganic metabolites between both gelatinous zooplankton species are compared to the canonical Redfield ratio (106C:16N:1P) (Redfield et al. 1963) in order to explore possible stoichiometric variations in the release of DOM and inorganic nutrients (Sterner & Elser 2002). Furthermore, we evaluated the contributions made by gelatinous zooplankton blooms to DOC and dissolved inorganic N and P (DIN and DIP) pools by combining results from laboratory experiments with abundance and
biometric measurements of ctenophore and medusae populations from field surveys in the York River estuary.

METHODS

Collection and preparation of zooplankton for experiments

*Chrysaora quinquecirrha* medusae were collected by dipnet or in 20 L buckets (for larger animals) from surface waters. *Mnemiopsis* ctenophores were collected during 30-second, gentle plankton tows using a 200 μm mesh net and a non-filtering cod end. Upon collection, medusae and ctenophores were immediately transported to the laboratory and incubated with field-collected copepod prey (20-100 copepods L⁻¹) at in situ temperature for 30 minutes. Prior to experimentation, gelatinous zooplankton were gently transferred individually to 20 L buckets filled with 0.2 μm filtered York River water where they remained for 15 minutes. This step rinsed the animals and provided them time to clear their guts (R. Condon, pers. obs.), reducing potential confounding effects of sloppy feeding and leaching of DOM from fecal material during the experiments.

Gelatinous zooplankton DOM and inorganic release experiments

We conducted seven laboratory experiments between July and August 2003-2007 to determine the simultaneous release rates of DOM and inorganic nutrients by *M. leidyi* ctenophores and *C. quinquecirrha* scyphomedusae in the York River estuary (Table I).
For each experiment, individual animals were incubated in the dark for 4-12 h in 1.2 L (for *M. leidyi*) or 4 L (for *C. quinquecirrha*) acid-cleaned, polycarbonate containers filled with 0.2 μm filtered (*Nucleopore*® polycarbonate filters), low-nutrient Sargasso Sea water diluted with Nanopure Diamond (*Barnstead*®) water to *in situ* York River salinity (20-22 psu). The low DOM content (e.g., 40-50 μM DOC) in the experimental media reduced methodological error and improved precision of DOM measurements. Container sizes were determined based on results from preliminary trials showing no significant difference in *Mnemiopsis* NH$_4^+$ excretion rates between 1.2 L and 4 L containers. At the start of the experiment, one animal was randomly added to each experimental container (treatment) and the release of DOM and inorganic N and P determined by measuring changes in DOC, DON, DOP, and inorganic constituents (nitrite [NO$_2^-$], nitrate [NO$_3^-$], NH$_4^+$ and PO$_4^{3-}$) in the water every 3-4 hours. Experimental controls consisted of chambers absent of animals, although the addition of gelatinous zooplankton was mimicked. These data were used to correct for the small addition of nutrients associated with transferring the animal into each chamber. At the completion of the experiment, medusae and ctenophores were removed and their wet and dry weights, and elemental composition determined according to Condon and Steinberg (*Condon & Steinberg In review*).

DOM and inorganic release rates were expressed as a function of body mass according to the allometric equation:

\[ Y = a_1 W^b \]
and expressed as a dual function of body mass and temperature by the multiple regression equation:

$$\log Y = a + a_1 \log W + a_2 T$$

where $Y$ is the release rate of organic or inorganic metabolite (μmol ind$^{-1}$ h$^{-1}$), $W$ is the dry weight (g DW), $b$ is the exponent relating excretion to body mass, and $a$, $a_1$ and $a_2$ are constants (Ikeda 1985, Nemazie et al. 1993). Release rates were further characterized by comparing C, N, and P ratios of released DOM and inorganic nutrients between the two gelatinous species and to the Redfield ratio.

DOM and inorganic release rates were normalized to gelatinous zooplankton dry weight (μmol g DW$^{-1}$ h$^{-1}$), allowing comparison to rates reported for other gelatinous zooplankton species. Elemental turnover rates were also determined on individual medusae and ctenophores (% released d$^{-1}$) by dividing excretion rates (μmol ind$^{-1}$ h$^{-1}$) by respective amounts of body C, N and P (μmol ind$^{-1}$) then multiplying by 24 h.

The possible influence of bacterial uptake of gelatinous zooplankton DOM metabolites on measured excretion rates were investigated by measuring bacterial production in a subset of excretion chambers. Using a bacterial growth efficiency of 30% (del Giorgio & Cole 1998, Condon et al. In prep), these measurements suggest that our DOM release rates were only slightly underestimated, with bacteria potentially utilizing between 1-13% of DOC released by *M. leidy* ctenophores and *C. quinquecirrha* medusae during incubations, and thus we do not correct for bacterial uptake.
Field surveys

We combined data from laboratory experiments with field surveys to evaluate the contributions by *M. leidyi* and *C. quinquecirrha* populations to DOC, DIN, and DIP pools in the York River. Field surveys were conducted during 2004-2006 along a salinity gradient in the lower York River, measuring species composition and biomass of gelatinous zooplankton, and DOM (C, N, and P) and inorganic nutrients. Descriptions of collection and analytical protocols employed for gelatinous zooplankton and station locations are detailed in Condon and Steinberg (In review). Briefly, gelatinous zooplankton were collected during two-minute, double-oblique plankton tows in surface waters (0-2 m). Biomass of *M. leidyi* and *C. quinquecirrha* populations (g DW m$^{-3}$) were determined by converting individual ctenophore and medusae sizes to DW using empirically-derived regressions (Condon & Steinberg In review). For DOM and inorganic nutrients, bulk concentrations were determined in the laboratory on water collected from the top 1 m in 2 L, dark, acid-washed polycarbonate bottles.

Daily population release (*DPR*) of DOC and inorganic nutrients (μmol m$^{-3}$ d$^{-1}$) were determined as follows:

$$DPR = Y \times DWP \times 24$$

where *Y* is the temperature-corrected, weight-specific release rates from experiments (μmol g (DW)$^{-1}$ h$^{-1}$), *DWP* is population biomass for ctenophores and medusae (g DW m$^{-3}$) and 24 is a conversion factor for hourly into daily release rates. Daily contributions
made to bulk and labile DOC, and DIN and DIP pools (% contributed d⁻¹) were
determined by dividing daily population release rates by respective organic and inorganic
nutrient concentrations (µmol m⁻³). Labile DOC pools were estimated using a conversion
factor of 2.8% of bulk DOC (Raymond & Bauer 2000). Gelatinous zooplankton
inorganic nutrient contribution to primary production was also assessed under conditions
of phytoplankton nutrient limitation according to Sin et al. (1999). Prior studies of
zooplankton in the York River demonstrated that upriver, mesohaline waters support
significantly higher densities and biovolumes of gelatinous zooplankton as compared to
downriver, polyhaline regions near the mouth of the river. Thus, the impacts of
gelatinous zooplankton on DOC and inorganic nutrient pools were based on comparisons
between upriver and downriver locations (stations 1 and 2 for upriver and stations 3 and 4
for downriver) (Condon & Steinberg In review).

**Chemical analyses**

Water subsampled from incubation bottles was filtered through pre-combusted (500°C
for 4 h) Whatman GF/F filters, and dissolved nutrients determined in the filtrate. DOC
concentrations were measured via high temperature combustion on a Shimadzu 5000A
Total Organic Carbon (TOC) analyzer using potassium hydrogen phthalate (C₈H₅O₄K)
standard (Peltzer et al. 1996). Prior to combustion, 6N HCl was added to 5 mL sample
(pH < 3) and sparged for two minutes with C ultra free air to ensure removal of dissolved
inorganic C. DOC concentrations were based on the best three of a maximum of five
column injections within an analytical detection error set to a peak area standard
deviation ±120 or coefficient of variance of 0.8%. Samples with ±1.5 μM error were reanalyzed. In addition, data precision, instrument accuracy, and platinum catalyst efficiency were quality checked with low C (1-2 μM DOC) and deep Sargasso Sea water (44-46 μM DOC) reference standards provided by the C reference material program, University of Miami (http://www.rsmas.miami.edu/groups/biogeochem/CRM.html) (Sharp 2002).

Total dissolved N (TDN) and P (TDP) was analyzed by persulfate oxidation (Bronk et al. 2000, Sharp 2002), NO₃⁻ by the spongy cadmium (Cd) method, and NO₂⁻ and PO₄³⁻ were measured on a Lachat™ QuikChem 8500 nutrient autoanalyzer (Koroleff 1983). During analysis, the conversion of NO₃⁻ to NO₂⁻ by Cd catalyst was monitored and columns regenerated if reduction efficiency was < 97%. NH₄⁺ was measured on a Shimadzu UV-1601 spectrophotometer by manual hypochlorite method (Koroleff 1983) using standard curves corrected for sample salinity. DON and DOP were determined by calculating the difference between total dissolved and inorganic fractions (Sharp 2002).

Particulate organic C and N content of ctenophores used in experiments were measured on a Carlo Erbra EA-1108 CHN Elemental Analyzer (Condon & Steinberg In review), and C and N content in medusae were determined following Nemazie et al. (1993). Particulate organic P content of jellyfish was estimated using a literature dry weight specific conversion factor of 0.06% (Kremer 1975).
Statistical analyses

Data describing gelatinous zooplankton release rates and ratios were analyzed using single and multiple linear regressions, analysis of variance (ANOVA), and two-sample t-tests using Minitab statistical software (level of significance of $\alpha < 0.05$). Regressions were checked for outliers using Cook's $D$ statistic and, where applicable, removal of outliers in analyses are denoted in the text. Differences in York River DOM, DIN, and DIP concentrations between upriver and downriver sites were determined using nested ANOVA's with data and location as fixed factors. If ANOVA's were significant, post hoc pairwise comparison of means using Tukey's HSD tests were performed (Quinn & Keough 2002). Prior to analyses, data were checked for normality and homogeneity of variance using Kolmogorov-Smirnov tests, and box plots and histograms of data and residuals. Non-conforming data were converted using log$_{10}$ or fourth-root transformations (Quinn & Keough 2002).

RESULTS

DOM release rates

Both species of gelatinous zooplankton released relatively high amounts of their total metabolites as DOC, DON, and DOP, with higher release of DOC vs. DON and DOP ($M. leidy$: $p < 0.001$, Fig. 1; $C. quinquecirrha$: $p < 0.05$, Fig. 2). Based on data across all temperatures, weight-specific DOC release rates were similar between ctenophores and
scyphomedusae (Table II). Release per individual animal ranged $0.02 - 8.86 \mu\text{mol} \text{DOC ind.}^{-1} \text{h}^{-1}$ for *M. leidyi* (Fig. 1A) and $1.2 - 58.3 \mu\text{mol} \text{DOC ind.}^{-1} \text{h}^{-1}$ for *C. quinquecirrha* (Fig. 2A), although data were highly variable (Table II). Similarly, at $14^\circ\text{C}$ and temperatures $> 25^\circ\text{C}$ weight-specific DOC release was the same between both species (t-test, $t = 0.22, p = 0.84$, Fig. 1D and 2D). Release per individual animal ranged $0.001 - 0.8 \mu\text{mol} \text{DON ind.}^{-1} \text{h}^{-1}$ and $0.0001 - 0.07 \mu\text{mol} \text{DOP ind.}^{-1} \text{h}^{-1}$ for *M. leidyi* (Fig. 1B, C), and $0.7 - 5.0 \mu\text{mol} \text{DON ind.}^{-1} \text{h}^{-1}$ and $0.1 - 1.3 \mu\text{mol} \text{DOP ind.}^{-1} \text{h}^{-1}$ for *C. quinquecirrha* (Fig. 2B, C). Weight-specific DON release rates were higher than DOP rates for *M. leidyi* ctenophores ($t = 5.1, p < 0.001$, Fig. 1E, F,) and for *C. quinquecirrha* medusae ($t = 3.4, p < 0.05$, Fig. 2E, F, Table II). Weight-specific excretion of DON by medusae was higher than ctenophores ($t = 2.7, p < 0.05$), while DOP excretion was the same between species ($t = 0.24, p = 0.82$).

For *M. leidyi*, DOC, DON and DOP release increased with body mass, but only DOC release was significantly positively correlated with temperature ($t = 2.91, p < 0.05$, Table II, data not shown). Slopes of log-transformed data regressing body mass against DOC, DON, and DOP release rates indicated that weight-specific release rates with increasing body size decreased for DOC ($b = 0.63$), remained the same for DON ($b = 1.00$), and increased for DOP ($b = 1.28$) (Table II). Multiple regressions of body mass and temperature slightly improved predictability of DOC release by ctenophores (Table II). In contrast, release rates of DOC, DON and DOP by *Chrysaora* medusae release were not related to body size, and DOC was not related to temperature (Table II; no temperature
dependent DON and DOP release rates were measured), although these results could have been due to sample size (Table I).

Mean daily body C and N turnover rates in terms of DOC and DON were higher in *M. leidyi* compared with *C. quinquecirrha* (Table III), and ranged 0.9 – 127% DOC d\(^{-1}\) and 0.4 – 98.7% DON d\(^{-1}\) for ctenophores and 0.3 – 12.2% DOC d\(^{-1}\) and 0.5 – 6.9% DON d\(^{-1}\) for medusae. There was no significant difference between species in mean DOP turnover (*M. leidyi*: 0.8 – 79.4% DOP d\(^{-1}\), *C. quinquecirrha*: 1.6 – 24.7% DOP d\(^{-1}\); Table III, \(p = 0.19\)). DOC turnover was negatively correlated to ctenophore body mass and positively correlated to temperature, although the relationship was weak (multiple \(r^2 = 0.15\), Table III). Turnover of DON and DOP by ctenophores was not related to body mass or temperature (Table III). Similarly, DOC and DOP turnover by *C. quinquecirrha* was not related to body size or temperature, but medusa DON turnover decreased with increasing body size (27°C only; Table III).

A larger proportion of the TDN and TDP excreted by medusae and ctenophores was NH\(_4^+\) and PO\(_4^{3-}\), although sizeable proportions of DON and DOP were released by both gelatinous zooplankton species (Fig. 3). DON comprised a higher proportion of TDN released by *C. quinquecirrha* medusae (mean = 35% DON) compared to *M. leidyi* ctenophores (mean = 21% DON) (Fig. 3A). Similarly, DOP comprised a higher proportion of TDP released by medusae (mean = 46% DOP) as compared to ctenophores (mean = 34% DOP); proportions of DOP released by *Chrysaora* were similar to PO\(_4^{3-}\) released (Fig. 3B).
Excretion of inorganic nutrients

Excretion rates of NH$_4^+$ and PO$_4^{3-}$ by individual *C. quinquecirrha* scyphomedusae were typically higher than *M. leidyi* ctenophores, ranging from 0.1–22.6 μmol NH$_4^+$ ind$^{-1}$ h$^{-1}$ and 0.1–0.9 μmol PO$_4^{3-}$ ind$^{-1}$ h$^{-1}$ for medusae and 0.02–2.9 μmol NH$_4^+$ ind$^{-1}$ h$^{-1}$ and 0.003–0.1 μmol PO$_4^{3-}$ ind$^{-1}$ h$^{-1}$ for ctenophores (Fig. 4A, B; Fig. 5A, B). In contrast, there was no significant difference in weight specific excretion rates between both species (NH$_4^+$: $p = 0.096$, PO$_4^{3-}$: $p = 0.125$, Table II). For *M. leidyi* ctenophores, both NH$_4^+$ and PO$_4^{3-}$ excretion rates significantly increased with dry body weight (g) and temperature (°C) (Table II), but there was no difference between weight-specific excretion of inorganic nutrients at temperatures ≥ 20°C (Fig. 4). *Chrysaora* medusae NH$_4^+$ and PO$_4^{3-}$ excretion also increased with dry body weight and temperature (Fig. 5, Table II). The slopes of log-transformed regressions relating *M. leidyi* body mass to inorganic N and P excretion rates were similar (NH$_4^+$: $b = 0.79$, PO$_4^{3-}$: $b = 0.83$, Table II), suggesting a decrease in weight-specific excretion rate with increase in body size. The same analysis for *C. quinquecirrha* medusae indicates the slopes were slightly higher (NH$_4^+$: $b = 0.91$, PO$_4^{3-}$: $b = 0.94$, Table II) compared to ctenophores.

Daily turnover of NH$_4^+$ and PO$_4^{3-}$ by *M. leidyi* ctenophores was greater than for *C. quinquecirrha* medusae (NH$_4^+$: $p < 0.001$; PO$_4^{3-}$: $p < 0.001$). Inorganic nutrient turnover by ctenophores was high with similar turnover rates for NH$_4^+$ and PO$_4^{3-}$ ($p = 0.47$, Table III). For *C. quinquecirrha*, inorganic N turnover was comparable to DON turnover, but significantly lower than inorganic P turnover ($p < 0.001$, Table III). Multiple regressions
of dry weight and temperature showed that NH$_4^+$ and PO$_4^{3-}$ turnover by *M. leidyi* were negatively correlated with body mass but positively correlated to temperature (Table III). In contrast, NH$_4^+$ and PO$_4^{3-}$ turnover by medusae were positively correlated to both body size and temperature (Table III).

**C, N, and P stoichiometry of DOM and inorganic nutrients**

DOC:DON ratios of DOM released by *M. leidyi* averaged 29:1, higher than Redfield (6.6C:1N), but were highly variable, ranging 0.62 – 472:1 (Fig. 6A). In comparison, *Chrysaora* medusae DOC:DON ratios were closer to Redfield C:N, averaging 8.1 (Fig. 6A). DOC:TDN ratios (mean = 2.8:1) were significantly lower than DOC:DON for *M. leidyi* ctenophores (*p* < 0.001) but were similar for medusae (mean = 5.6; Fig. 6B). In addition, DOC:TDN release ratios by *M. leidyi* were negatively correlated to both increasing body size and increasing temperature (*p* < 0.05). For *M. leidyi*, DOC:DOP release ratios were variable but on average simultaneous release of DOC and DOP (118:1, Fig. 6C) was similar to C:P Redfield stoichiometry (106:1). Multiple regressions of DOC:DOP decreased with ctenophore dry weight (*p* < 0.05) and increased with temperature (*p* < 0.05), although correlations were weak (*r^2 = 0.43*). Mean release ratios of DON and DOP (6:1) were below Redfield N:P ratio (16:1) for *M. leidyi* ctenophores and *C. quinquecirrha* medusae (9:1, Fig. 6D). Linear regressions relating DOC:DON and DON:DOP release rates with body size and temperature were non significant for both gelatinous species.
In general, NH$_4^+$: PO$_4^{3-}$ excretion ratios were similar or slightly below Redfield stoichiometry of 16N:1P for *M. leidy* ctenophores (mean = 13.1) and *C. quinquecirrha* medusae (mean = 9.3), although data exhibited high variability (Fig. 6E). NH$_4^+$: PO$_4^{3-}$ excretion ratios significantly increased with temperature for both gelatinous species ($p < 0.001$), but were not related to body weight.

**York River bulk DOC and inorganic nutrient concentrations**

Bulk DOC concentrations were typically between 200 – 300 μM and were higher upriver compared to downriver (log-transformed, $F = 2.93$, $p < 0.001$, df = 191; Fig. 6A, B). In general, York River DOC (μM) followed a seasonal pattern at both locations with lowest DOC concentrations observed during summer (May – July), followed by an increase during autumn (August – December) before reaching a maximum during late winter and spring (January – April) (Fig. 6A, B); spring 2005 (March – April) was an exception in that low DOC concentrations were observed.

Inorganic N and P concentrations varied greatly across seasons (Fig. 6C-F). Within sample dates, there was no significant difference in concentrations of NH$_4^+$ (Fig. 7C, D) and NO$_x$ (NO$_2^-$ + NO$_3^-$) between upriver and downriver locations, however, there were significantly higher PO$_4^{3-}$ concentrations upriver compared to downriver ($p < 0.05$; Fig. 7E, F). DIN concentrations were high during late winter-spring (January-April), but both NH$_4^+$ (Fig. 7C, D) and NO$_x$ were low and often below detection during summer months (May-August). In contrast, PO$_4^{3-}$ concentrations were low and often below analytical
detection limits during spring (January-April), and significantly higher during summer months (July - Sept; Fig. 7E, F). DIN:DIP on bulk inorganic N (NO\(_x^–\) + NH\(_4^+\)) and P pools were high during spring (> 100N:P), resulting in phytoplankton P-limitation throughout the York River. During summer, DIN:DIP ratios were significantly lower upriver (5.8:1) compared to downriver (13.7:1, p = 0.04).

**Gelatinous zooplankton biomass and contributions to DOC, DIN, and DIP pools**

Biomass of gelatinous zooplankton in the York River was dominated by *Mnemiopsis* ctenophores. High biomass of *M. leidyi* ctenophores (g DW m\(^{-3}\)) was observed during summer and winter months, with significantly higher ctenophore biomass occurring upriver (p < 0.001). During summer, peak ctenophore biomass consistently occurred during May (average range 0.4 – 1.2 g DW m\(^{-3}\)) and June (average range 0.3 – 1.5 g DW m\(^{-3}\)), although high biomass occurred at other times. Winter biomass peaks usually occurred during January and February and were comparable to summer peaks (average range 0.02 – 1.2 g DW m\(^{-3}\)). *Chrysaora* medusae were present in the York River during July and August and primarily occurred at the upriver station. *Chrysaora* biomass was about an order of magnitude lower than ctenophores, and ranged 0.01 – 0.23 g DW m\(^{-3}\) during July and 0.002 – 0.14 g DW m\(^{-3}\) during August.

Contributions by gelatinous zooplankton to DOC and dissolved inorganic N and P pools were greatest during *M. leidyi* blooms (Fig. 7A,B). In general, release of DOC by *Mnemiopsis* populations was higher upriver compared to downriver, with high release of
DOC occurring during summer (May-July) and minor contributions during winter and spring (February-April) (Fig. 7A,B). Daily contributions by ctenophore populations to bulk DOC pools were low (<1%); however, when compared to labile DOC pools contributions were higher with maximum daily contributions ranging 18 – 29% (Fig. 7A). Daily contributions by *C. quinquecirrha* populations to bulk and labile DOC pools were low (<1%).

Maximum excretion of NH$_4^+$ by gelatinous zooplankton populations was highest upriver during summer (May-July) when annual bulk DIN concentrations were lowest (Fig. 7C,D). At these times, mean daily NH$_4^+$ production by *M. leidyi* populations represented 2 – 50% of York River NH$_4^+$ concentrations, and individual estimates often exceeded 100% (Fig. 7C, D). Net PO$_4^{3-}$ excretion by gelatinous zooplankton was high upriver during late summer (July-August, Fig. 7E, F); however during March-April low release of PO$_4^{3-}$ by ctenophore populations still represented a major daily source of DIP to bulk pools (57 – 119% of PO$_4^{3-}$; Fig. 7E, F).

**DISCUSSION**

**Comparison of excretion rates with previous studies**

The general trends of higher NH$_4^+$ and PO$_4^{3-}$ excretion rates with increased body size and temperature, and decreased (b < 1.0) or stable (b = 1.0) weight-specific inorganic excretion in larger animals are consistent with previous studies on gelatinous zooplankton
excretion (Table IV). However, in our study weight-specific release rates of DOM by both gelatinous species, and NH$_4^+$ excretion by *M. leidyi* ctenophores, are higher than in previous studies (Table IV). High inorganic excretion and nutrient turnover has been reported for gelatinous zooplankton fed on high prey concentrations (Kremer et al. 1986), in short incubations (3 hr), and at higher temperatures (> 25°C) (Malej 1989). In addition, measurements based on long incubations (> 12 hr) without correction for bacterial utilization of metabolites may underestimate DOM release rates (Hansson & Norrman 1995, Condon et al. In prep). In this study, experimental incubations were short (4-12 hr), bacterial uptake of DOM was low, and release rates were measured on recently fed animals. Moreover, inorganic N and P excretion rates by *Chrysaora* medusae and PO$_4^{3-}$ excretion rates by ctenophores were similar to previous studies. We suggest that higher weight-specific excretion rates of inorganic N by ctenophores were primarily due to release of excess assimilated N as evidenced by high daily turnover rates of body N as NH$_4^+$. 

**Factors controlling DOM excretion by gelatinous zooplankton**

Both *M. leidy* ctenophores and *C. quinquecirrha* medusae release high quantities of DOC. Released DON and DOP also comprise a sizable fraction of the total N and P released. Comparisons of DOP released on a per individual and dry weight basis were similar for ctenophores and medusae. However, daily turnover rates of DOC and DON were higher in ctenophores than in the medusae, and the ratios in which these organic compounds were released differed between the species. Ultimately, the turnover of
assimilated C, N and P elements is linked to both the metabolic conditions (e.g., temperature), and the elemental stoichiometric requirements of the animal (Sterner & Elser 2002). We suggest that differences in the release of DOC and DON observed between the two species were related to the production of mucus in ctenophores and retention of organic C and N for body structural components and nematocysts in scyphomedusae.

*Mnemiopsis* ctenophores released DOM with high organic C content as indicated by significantly higher release of DOC compared to DON. High release of DOC by gelatinous zooplankton has been attributed to their “leaky” nature (Kremer 1977), and to mucus production (Hansson & Norrman 1995, Steinberg et al. 2000). Lobate ctenophores, such as *M. leidyi*, primarily use mucous lined lobes to capture and digest prey (Costello et al. 1999). As a result, mucus production is key for maintaining daily elemental body requirements via assimilation of prey. The biochemical composition of *M. leidyi* mucus is unknown, but colloids released by corals and scyphomedusae are comprised of glycoproteins that are C-rich relative to N due to their high carbohydrate content (Ducklow & Mitchell 1979, Hansson & Norrman 1995, Cohen & Forward 2003). If mucus release is the primary pathway for DOC and DON production, then organic C and N release rates would be independent of the physical controls of metabolism, and related more to biometric parameters involved in ctenophore feeding (e.g., surface area on lobes) because the elemental composition and production of mucus is independent of intracellular physiology (Heeger & Moller 1987). Our results indicate that DOC and DON release by ctenophores were significantly positively correlated with body size,
however weight-specific DOC and DON release was either weakly or not correlated with temperature and remained the same with body size. Furthermore, DOC:DON release ratios were not related to body size and temperature, suggesting that ctenophores primarily release DOC and DON compounds of similar elemental proportions independent of intracellular metabolism. Collectively, these results support the hypothesis that mucus production is the principal mechanism of DOC and DON release in lobate ctenophores.

In contrast, turnover of C and N, as released DOC and DON, by individual C. quinquecirrha scyphomedusae were low and on average <3% of body C and N per day. This is consistent with a prior study showing that DOC turnover in Aurelia medusae was a minor component of the C budget and equivalent to C allocated to reproduction (Hansson & Norrman 1995). Although weight-specific release rates between medusae and ctenophores were similar for DOC but higher for DON in medusae, individual medusae have a higher dry weight (> 1 gram) and a greater amount of organic C and N compared to individual ctenophores. Thus, similarities in organic release rates between individual ctenophores and medusae further emphasize the relatively low release rates of DOC and DON by C. quinquecirrha medusae.

In contrast to ctenophores, there was no relationship between DOC and DON release and body size in medusae. And similar to ctenophores, there was no difference in weight-specific DOC release with a 10°C increase in temperature, suggesting that organic C and N release was not linked to C or N metabolism. Scyphomedusae are tentaculate predators
that slough mucus and nematocysts as a defense strategy (Shanks & Graham 1988), and DOC:DON release ratios were not indicative of C-rich mucus production as observed for *M. leidyi* ctenophores. Rather, medusae DOC:DON release ratios were closer to the C:N elemental ratios of *Chrysaora* organic body content of about 4C:1N by atom (Nemazie et al. 1993). *Chrysaora* scyphomedusae are more robust than *M. leidyi* ctenophores due to higher amounts of N-rich collagen fibers in the mesoglea per individual medusae (Arai 1997). In addition, nematocyst capsules used in prey capture are made of C- and N-based chitin molecules (Hessinger & Lenhoff 1988). Thus, compared to ctenophores, medusae have high organic C and N requirements related to body structure that potentially increase as the medusa grows. This is supported by medusae daily DON turnover rates that significantly decreased with increased body size, suggesting retention of organic N in larger animals. We suggest that low turnover of DOC and DON for *C. quinquecirrha* scyphomedusae is due to preferential retention of these compounds for use in medusae structural components and that the release of DOC and DON by medusae is associated with the turnover of these structural components, rather than due to feeding or C and N metabolism.

**Importance of gelatinous zooplankton for DOC cycling in the York River**

The highest contributions by gelatinous zooplankton to DOC pools occurred during summer, with the majority of DOC contribution associated with *M. leidyi* ctenophore blooms. While ctenophore populations contributed <1% to bulk DOC pools, they contributed up to 18% and 28% d⁻¹ to labile DOC pools in upriver and downriver
locations. This ctenophore production of labile DOC could support bacterial production comparable to that supported by phytoplankton production of DOC (del Giorgio & Cole 1998, Condon et al. In prep). In the spring, DOC contribution by *M. leidyi* blooms was minor; this is a time when DOC exudates released by spring phytoplankton blooms potentially contribute the majority of labile DOC (Raymond & Bauer 2000). Therefore, the importance of *M. leidyi* blooms as a major source to labile DOC pools is likely restricted to summer months when ctenophore DOC release rates are high and allochthonous sources of DOC to the York River are low (Raymond & Bauer 2000). At these times, ctenophore populations may further impact labile DOM pools because the high release of C-rich DOM would shift the stoichiometric balance toward organic ratios biased for DOC relative to DON and DOP. This is in contrast to phytoplankton-dominated systems which control the ratio of organic C, N and P in seawater close to canonical Redfield stoichiometry - 106C:16N:1P by atoms (Redfield et al. 1963, Steinberg et al. 2000, Sterner & Elser 2002).

The high contribution of labile DOC by *M. leidyi* blooms has implications for bacterioplankton communities in the York River estuary, which have high C metabolic demands and are utilizing DOC from large pools that are primarily refractory (Raymond & Bauer 2000, Schultz Jr. & Ducklow 2000). This process may be accentuated in the York River estuary because temporal shifts have occurred in *M. leidyi* blooms that have increased both C residence times and potential release of DOC by gelatinous zooplankton populations (Condon & Steinberg In review). This potential utilization of jellyfish-derived DOC by bacteria may represent a primary C pathway in gelatinous zooplankton-
dominated systems (Riemann et al. 2006), whereby C (as well as N and P) can be assimilated by bacteria and reincorporated into planktonic food webs (Riemann et al. 2006, Condon et al. In prep).

In contrast, C. quinquecirrha populations contributed minor amounts (< 1%) to labile DOC pools and thus direct release of DOC by medusa is likely not important in DOC cycling in the York River. Sloppy feeding or leaching of fecal material was not addressed in this study because medusae were not fed during experiments. Leaking of organic material via feeding may be a more important mechanism by which medusae contribute to labile DOC pools (Hansson & Norrman 1995).

**Importance of DON and DOP metabolites released by gelatinous zooplankton**

To our knowledge there are no previously published rates of DOP release for gelatinous zooplankton. However, high daily turnover of DOP, increased release of organic N and P with body size, and high population biomass of M. leidyi compared to C. quinquecirrha, suggest that ctenophore populations might be an important source of DON and DOP in the York River estuary. Using an average biomass of 1.5 g DW m$^{-3}$ and daily weight-specific release rates of 48.0 μmol-DON g DW$^{-1}$ d$^{-1}$ and 14.4 μmol-DOP g DW$^{-1}$ d$^{-1}$, we estimate that during summer M. leidyi populations release 72.0 μmol-DON m$^{-3}$ d$^{-1}$ and 21.6 μmol-DOP m$^{-3}$ d$^{-1}$. During summer, organic N and P concentrations in the York River typically range 10-15 μM for DON (R. Condon, unpublished data), and 0.2-0.6 μM for DOP, assuming similar concentrations to lower Chesapeake Bay (Conley et al. 1995).
Comparison of population release rates with bulk DON and DOP concentrations suggest that *M. leidyi* populations contribute more to DOP (3.6-11%) than DON pools (< 1%). The release of DOP compounds by gelatinous zooplankton may thus have important implications for bacterioplankton and P cycling in coastal and estuarine systems (Karl & Bjorkman 2002), particularly as DOP is preferentially remineralized by bacteria (Loh & Bauer 2000).

**Impacts of DIN and DIP excretion on inorganic nutrient cycling**

Large seasonal variations in inorganic nutrients occurred throughout the York River, consistent with previous reports on nutrient cycling in this region (Sin et al. 1999, Schultz Jr. et al. 2003, Kemp et al. 2005). In spring, concentrations of DIN relative to DIP were higher than Redfield (16N:1P by atoms) resulting in P-limited conditions for phytoplankton and bacterioplankton. In contrast, during summer DIN:DIP ratios were typically below Redfield ratios resulting in N-limitation, particularly in upriver locations where DIN was commonly below detection in surface waters.

*M. leidyi* ctenophores and *C. quinquecirrha* medusae excreted higher or similar amounts of NH$_4^+$ and PO$_4^{3-}$ relative to DON and DOP, as indicated by high NH$_4^+$:DON and PO$_4^{3-}$:DOP release ratios, and our calculations suggest that gelatinous zooplankton are a major source of recycled nutrients to DIN and DIP pools in the York River estuary. The highest contributions of inorganic N and P occurred during summer in upriver locations that supported high gelatinous zooplankton biomass. For the York River which sustains high
phytoplankton biomass during summer (Sin et al. 1999, Condon & Steinberg In review), the release of nutrients by gelatinous zooplankton may favor phytoplankton growth because their excretion of NH$_4^+$ and PO$_4^{3-}$ occurs in ratios similar to Redfield N:P stoichiometry (Kemp et al. 2005).

During summer, daily primary production rates in lower Chesapeake Bay are approximately 83.3 mmol C m$^{-2}$ d$^{-1}$ (Chesapeake Bay Remote Sensing Program: http://www.cbrsp.org/). If we assume similar phytoplankton production rates for the York River and Redfield nutrient uptake kinetics, daily N and P production rates by phytoplankton are 12.6 mmol N m$^{-2}$ d$^{-1}$ and 0.78 mmol P m$^{-2}$ d$^{-1}$. Comparison of these N and P production rates with daily inorganic N and P released by gelatinous zooplankton (rates taken from Fig. 7) indicate that recycled nutrients by ctenophores and medusae combined supports < 4% of daily primary production. This is similar to results from the mesohaline Chesapeake Bay, where ctenophores and medusae support up to 3% of primary production (Nemazie et al. 1993). Gelatinous zooplankton excretion thus supports a small fraction of primary production in Chesapeake Bay, and phytoplankton must largely utilize other N and P sources for production. For the York River, additional sources might include the flux of NH$_4^+$ and PO$_4^{3-}$ from sediments during hypoxia (Kemp et al. 2005), river runoff, desorption of PO$_4^{3-}$ from particles (Sin et al. 1999), and regeneration of NH$_4^+$ by non-gelatinous zooplankton (Park & Carpenter 1987, Miller & Glibert 1998, Kirchman 2000).
These results also imply an alternative sink to phytoplankton for rapid utilization of gelatinous zooplankton inorganic excreta because high inorganic excretion occurred at times when DIN and DIP pools were low or devoid of nutrients. Advection may be responsible for the removal of some of these nutrients, but low river flow, summer water residence times greater than inorganic production rates, and strong vertical stratification gradients would limit flushing and ensure retention of nutrients in surface waters (Hayward et al. 1982, Shen & Haas 2004; L. Haas, pers. comm). We hypothesize that the release of inorganic N and P by ctenophores and medusae favors growth of bacterial communities because there maybe a stoichiometric imbalance in labile organic pools created by the high release of C-rich DOM by M. leidyi populations that drives supplemental uptake of inorganic nutrients in order to satisfy relatively high bacterial N and P demands (Kirchman 2000). Here, bacteria would have a competitive advantage over phytoplankton for inorganic resources because of their higher surface area:volume ratios. Low DOC:TDN and DOC:TDP release ratios by gelatinous zooplankton that are similar to bacterial C:N and C:P elemental ratios and nutrient stoichiometric requirements (Goldman et al. 1987, Kirchman 2000) would also favor uptake of inorganic N and P released by bacteria over phytoplankton. Therefore, understanding the nature of interactions between gelatinous zooplankton with bacteria are important if we are to fully understand the role gelatinous zooplankton play in DOM and inorganic nutrient cycling in regions where gelatinous zooplankton proliferate.
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Condon RH, Steinberg DK, del Giorgio PA, Bouvier TC (In prep) The 'jelly pump': consequences of jellyfish blooms for increased carbon metabolism within the microbial loop. Science


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Fig. 1. Release rates of dissolved organic matter by *Mnemiopsis leidyi* ctenophores. (a) Dissolved organic carbon (DOC), (b) nitrogen (DON) and (c) phosphorus (DOP) release rates (μmol ind⁻¹ h⁻¹). Ctenophore weight-specific release (μmol g DW⁻¹ h⁻¹) across temperatures of (d) DOC, (e) DON, and (f) DOP. Error bars are ±1 standard deviation. Sample size (n) for each temperature given in Table 1. DW = dry weight. * = p < 0.05.
Fig. 1

![Graphs showing DOC, DON, and DOP concentrations](image-url)
Fig. 2. Release rates of dissolved organic matter by *Chrysaora quinquecirrha* medusae. (a) Dissolved organic carbon (DOC), (b) nitrogen (DON) and (c) phosphorus (DOP) release rates (μmol ind⁻¹ h⁻¹) by individual seyphomedusae. Medusae weight-specific release (μmol g DW⁻¹ h⁻¹) across temperatures of (d) DOC, (e) DON, and (f) DOP. Error bars are ± 1 standard deviation. Sample size (n) for each temperature given in Table 1. DW = dry weight. nd = no data.
Fig. 2

DOC

DON

DOP

A

B

C

D

E

F

μmol l⁻¹ h⁻¹

μmol g (DW)⁻¹ h⁻¹

Temperature (°C)

0.1 1 10

0.1 1 10

0.1 1 10

0.1 1 10

0.01

1 10

1 10

1 10

1 10

nd

nd
Fig. 3. Comparison of inorganic vs. organic nitrogen (N) and phosphorus (P) release by ctenophores and medusae. (a) Percent total dissolved N released as dissolved organic N (DON) and ammonium (NH$_4^+$), and (b) percent total dissolved P released as dissolved organic P (DOP) and phosphate (PO$_4^{3-}$), by Mnemiopsis leidyi ctenophores across all experimental temperatures and Chrysaora quinquecirrha scyphomedusae at 27°C. Error bars are ± 1 standard error.
Fig. 3

(a) % N released as DON or NH$_4^+$

(b) % P released as DOP and PO$_4^{3-}$

Mnemiopsis

Chrysaora

n = 43

n = 9

n = 35

n = 6

DON

NH$_4^+$

DOP

PO$_4^{3-}$
Fig. 4. Excretion rates of (a) ammonium (NH$_4^+$) and (b) phosphate (PO$_4^{3-}$) by *Mnemiopsis leidyi* ctenophores in μmol ind$^{-1}$ h$^{-1}$. Ctenophore weight-specific excretion (μmol g DW$^{-1}$ h$^{-1}$) of (c) NH$_4^+$ and (d) PO$_4^{3-}$ across experimental temperatures. Sample size (n) for each temperature given in Table 1. Error bars are ±1 standard deviation. DW = dry weight. * = p < 0.05.
Fig. 5. Excretion rates of (a) ammonium (NH$_4^+$) and (b) phosphate (PO$_4^{3-}$) by Chrysaora quinquecirrha medusae in µmol ind$^{-1}$ h$^{-1}$. Medusae weight-specific excretion (µmol g DW$^{-1}$ h$^{-1}$) of (c) NH$_4^+$ and (d) PO$_4^{3-}$ across experimental temperatures. Sample size (n) for each temperature given in Table 1. Error bars are ± 1 standard deviation. DW = dry weight. nd = no data. * = p < 0.05. Circled data indicates outliers removed from statistical analyses (Table II).
Fig. 6. Box plots comparing organic and inorganic release ratios by individual *Mnemiopsis leidyi* ctenophores and *Chrysaora quinquecirrha* medusae. (a) Dissolved organic carbon (DOC) to nitrogen (DON), (b) DOC to total dissolved nitrogen (TDN), (c) DOC to dissolved organic phosphorus (DOP), (d) DON to DOP, and (e) ammonium ($\text{NH}_4^+$) to phosphate ($\text{PO}_4^{3-}$) release ratios. Box and whiskers represent 10th, 25th, median, 75th and 90th percentiles. Outliers not shown. Out of range value in parenthesis. Vertical dashed lines = average release ratio.
Fig. 6
Fig. 7. Upriver and downriver concentrations of (lines) (a, b) dissolved organic carbon (DOC), (c, d) ammonium (NH$_4^+$) and (e, f) phosphate (PO$_4^{3-}$) ($\mu$mol L$^{-1}$), and contributions made by gelatinous zooplankton populations (bars) ($\mu$mol L$^{-1}$ d$^{-1}$) to DOC, NH$_4^+$ and PO$_4^{3-}$ pools in the York River. Upriver and downriver regions are located at stations 1 (37° 20.046'N, 076° 36.052'W) and 2 (37° 14.273'N, 076° 30.274'W), and stations 3 (37° 14.233'N, 076° 14.232'W) and 4 (37° 14.535'N, 076° 20.633'W), respectively, according to Condon and Steinberg (In review). BDOC = bulk DOC. LDOC = labile DOC. LDOC defined as 2.8% of BDOC following Raymond and Bauer (2000). Error bars are ± 1 standard deviation.
Table I. Physical conditions and incubation times for laboratory experiments. *Mnem* = *Mnemiopsis leidyi* ctenophores. *Chry* = *Chrysaora quinquecirrha* medusae. na = not applicable.

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<th>Salinity (psu)</th>
<th>Incubation (h)</th>
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<td>5 May 2007</td>
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Table II. Linear and multiple linear regressions of dissolved organic matter (DOM) and inorganic nutrient release rates by *Mnemiopsis* ctenophores and *Chrysaora* medusae. Error bars are ± 1 standard deviation.

<table>
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<th>WS Rel. (μmol g DW⁻¹ h⁻¹)</th>
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<th>log Y = a₀ + a₁ log W + a₂ T</th>
<th>a₀</th>
<th>a₁</th>
<th>a₂</th>
<th>r²</th>
<th>p value</th>
<th>a₀</th>
<th>a₁</th>
<th>a₂</th>
<th>r²</th>
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<td>DOC</td>
<td>5 - 27</td>
<td>59</td>
<td>12.0 ± 15.0</td>
<td>2.54</td>
<td>0.63</td>
<td>0.18</td>
<td>**</td>
<td>-0.09</td>
<td>0.53</td>
<td>0.023</td>
<td>0.29</td>
<td>**</td>
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<tr>
<td></td>
<td>5 - 27</td>
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<td>1.00</td>
<td>0.39</td>
<td>**</td>
<td>-0.53</td>
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<td>0.40</td>
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<tr>
<td></td>
<td>14 - 27</td>
<td>35</td>
<td>0.2 ± 0.2</td>
<td>0.29</td>
<td>1.28</td>
<td>0.62</td>
<td>**</td>
<td>-0.09</td>
<td>1.24</td>
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<td></td>
<td>NH₄⁺</td>
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<td>4.9 ± 5.5</td>
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<td>0.23</td>
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<td>5 - 27</td>
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<td>0.3 ± 0.3</td>
<td>0.18</td>
<td>0.83</td>
<td>0.48</td>
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<td>-1.22</td>
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<td>DOC</td>
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ML, *Mnemiopsis leidyi*; CQ, *Chrysaora quinquecirrha*; Y, gelatinous zooplankton release rate (μmol ind⁻¹ h⁻¹); DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; NH₄⁺, ammonium; PO₄³⁻, phosphate; W, dry weight (g); T, temperature (°C); WS Rel., mean weight-specific release rate of DOM or inorganic nutrients; a, constants; b, slope of the regression lines relating organic or inorganic release to body mass; var., organic or inorganic variable; c, outliers removed from analyses (see Fig. 3); n, sample size; r², correlation coefficient; *, p < 0.05; **, p < 0.001; NS, non significant; nd, no data.
Table III. Linear and multiple regressions of daily dissolved organic matter and inorganic nutrient turnover rates by *Mnemiopsis* ctenophores and *Chrysaora* medusae. Error bars are ± 1 standard deviation.

<table>
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<th>Var.</th>
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<th>DT (% released d⁻¹)</th>
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<td>59</td>
<td>25.2 ± 30.4</td>
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<td>NS</td>
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<tr>
<td>DON</td>
<td>5 - 27</td>
<td>45</td>
<td>18.3 ± 19.2</td>
<td>14.1 0.09</td>
<td>0.01</td>
<td>NS</td>
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<td>0.05</td>
<td>0.006</td>
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<tr>
<td>DOP</td>
<td>14 - 27</td>
<td>35</td>
<td>26.1 ± 18.5</td>
<td>36.3 0.28</td>
<td>0.07</td>
<td>NS</td>
<td>2.00</td>
<td>0.24</td>
<td>-0.023</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>5 - 27</td>
<td>49</td>
<td>53.0 ± 42.7</td>
<td>31.6 -0.07</td>
<td>0.004</td>
<td>NS</td>
<td>0.71</td>
<td>-0.31</td>
<td>0.035</td>
<td>0.54</td>
<td>**</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>5 - 27</td>
<td>58</td>
<td>42.6 ± 31.6</td>
<td>21.9 -0.17</td>
<td>0.04</td>
<td>NS</td>
<td>0.87</td>
<td>-0.27</td>
<td>0.023</td>
<td>0.36</td>
<td>**</td>
</tr>
</tbody>
</table>

| CQ   | DOC       | 14 - 27 | 13 | 2.9 ± 3.3 | 1.99 -0.37 | 0.06 | NS | -0.15 | -0.46 | 0.021 | 0.16 | NS     |
| DON  | 27       | 9      | 2.4 ± 2.4 | 2.39 -1.19 | 0.62 | *  | nd  |       |       |       |       |        |
| DOP  | 27       | 6      | 14.0 ± 9.9 | 16.2 -1.08 | 0.44 | NS | nd  |       |       |       |       |        |
| NH₄⁺ | 14 - 27  | 14     | 3.0 ± 3.0 | 1.13 0.91  | 0.16 | NS | -1.84 | 0.42  | 0.087 | 0.87 | **    |
| PO₄³⁻| 14 - 27  | 14     | 11.4 ± 5.5 | 9.64 0.21  | 0.08 | NS | 0.47  | 0.08  | 0.024 | 0.54 | *     |

*DT*, daily turnover (% released d⁻¹); for remainder of abbreviations see Table II.
Table IV. Comparison of weight-specific release rates of dissolved organic and inorganic nutrients for ctenophore and scyphomedusae species (adapted from Nemazie et al. 1993).

<table>
<thead>
<tr>
<th>Species</th>
<th>Temp (°C)</th>
<th>NH$_4^+$</th>
<th>PO$_4^{3-}$</th>
<th>DOC</th>
<th>DON</th>
<th>DOP</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WS Ex. (μmol g DW$^{-1}$ h$^{-1}$)</td>
<td>b</td>
<td>WS Ex. (μmol g DW$^{-1}$ h$^{-1}$)</td>
<td>b</td>
<td>WS Rel. (μmol g DW$^{-1}$ h$^{-1}$)</td>
<td>b</td>
<td>WS Rel. (μmol g DW$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>Ctenophores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mnemiopsis leidyi</td>
<td>5 - 27</td>
<td>0.2 - 23.2</td>
<td>0.79</td>
<td>0.06 - 1.0</td>
<td>0.83</td>
<td>0.4 - 61.6</td>
<td>0.63</td>
</tr>
<tr>
<td>M. leidyi</td>
<td>18 - 27</td>
<td>3.0 - 4.8</td>
<td>0.74</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>M. leidyi</td>
<td>10 - 24</td>
<td>0.4 - 1.5</td>
<td>0.89</td>
<td>0.08 - 0.20</td>
<td>0.53</td>
<td>0.18 - 0.86</td>
<td>nd</td>
</tr>
<tr>
<td>M. mccradyi</td>
<td>17 - 24</td>
<td>0.06 - 0.11</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ocyropsis sp.</td>
<td>22</td>
<td>0.4 - 1.8</td>
<td>0.94</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Bolinopsis vitrea</td>
<td>25</td>
<td>0.7 - 0.9</td>
<td>0.76</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Beroe ovata</td>
<td>25</td>
<td>0.25 - 1.1</td>
<td>1.08</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Eurhamphaea</td>
<td>25</td>
<td>2.3</td>
<td>0.93</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>vexilegera</td>
<td></td>
<td>0.5</td>
<td>0.93</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Bathocyroe fosteri</td>
<td>9 - 13</td>
<td>0.1</td>
<td>1.20</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Scyphomedusae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysaora</td>
<td>14 - 27</td>
<td>0.1 - 7.8</td>
<td>0.91</td>
<td>0.12 - 0.44</td>
<td>0.89</td>
<td>1.3 - 46.4</td>
<td>0.64</td>
</tr>
<tr>
<td>quinquecirrha</td>
<td>18 - 28</td>
<td>3.5 - 5.0</td>
<td>1.00</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C. quinquecirrha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aurelia aurita</td>
<td>15</td>
<td>1.2 - 3.9</td>
<td>0.93</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>A. aurita</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelagia noctiluca</td>
<td>21</td>
<td>1.9 - 4.1</td>
<td>0.90</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>P. noctiluca</td>
<td>12 - 23</td>
<td>0.3 - 7.2</td>
<td>0.65</td>
<td>0.8 - 1.5</td>
<td>1.06</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

WS Ex., weight-specific excretion rate; WS Rel., weight-specific release rate; NH$_4^+$, ammonium; PO$_4^{3-}$, phosphate; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; b, slope of the regression lines relating organic or inorganic release to body mass; nd, no data; Ref, reference; 1, this study; 2, Nemazie et al. (1993); 3, Kremer (1977); 4, Park and Carpenter (1987); 5, Kremer (1982); 6, Kremer et al. (1986); 7, Youngbluth et al. (1988); 8, Schneider (1989); 9, Hansson and Norrman (1995); 10, Morand et al. (1987); 11, Malej (1989).
CHAPTER 4

Phylogenetic Changes to Natural Bacterioplankton Communities Caused by Uptake of Zooplankton-Derived Dissolved Organic Matter
Abstract

Chesapeake Bay is a highly productive estuarine ecosystem that sustains large populations of copepods, lobate ctenophores (*Mnemiopsis leidyi*), and scyphomedusae (*Chrysaora quinquecirrha*) in its mainstem and tributaries. At times of high biomass, these zooplankton may provide bacterial communities with substantial quantities of organic substrate for growth via release of dissolved organic matter (DOM) in their metabolites. We conducted two laboratory experiments during spring and summer, 2005, to determine the response of natural, free-living bacterioplankton assemblages to zooplankton-derived DOM, measured in terms of bacterial cell growth, production, dissolved organic carbon (DOC) and nitrogen (DON) uptake, and phylogenetic community composition. In spring, highest bacterial growth and production occurred in the copepod treatment, but low growth occurred in ctenophore metabolites. In contrast, bacterial biomass and production rapidly increased (within 6-12 h) in *Mnemiopsis* ctenophore and *Chrysaora* scyphomedusae treatments during summer. Counts of bacterial phylogenetic groups using molecular probes (fluorescence *in situ* hybridization, FISH) showed that DOM released by crustacean and gelatinous zooplankton stimulated growth of different, resource-specific phylotypes that were not dominant in the initial inoculum assemblage. Large increases in *Bacteroidetes* were observed during spring (50%) and summer (64%) in copepod treatments due to high uptake of N-rich high-molecular weight compounds released in copepod metabolites. In contrast, both ctenophore and medusae metabolites supported high growth of *gammaproteobacteria* during spring (22%) and summer (50 - 61%). High uptake of DOC relative to DON in
gelatinous zooplankton metabolites, and the emergence of \textit{gammaproteobacteria} in the glucose addition control, suggests that this phylotype prefers poor-quality substrate for growth. In light of worldwide increases of gelatinous zooplankton blooms, these results may have significant implications for carbon transfer in estuarine and coastal food webs, as there is greater potential for more carbon to be metabolized inefficiently by ‘opportunistic’ phylogenetic groups.

\textbf{Introduction}

Dissolved organic matter (DOM) in marine systems represents one of the largest reservoirs of carbon (C), nitrogen (N) and phosphorus (P) on earth (Hansell and Carlson, 2002; Carlson et al., 2007). As major consumers of DOM in aquatic systems, bacterioplankton play a key role in C transfer through uptake of large quantities of C that can be potentially reincorporated into planktonic food webs or returned to the inorganic C reservoirs (i.e., CO$_2$) (del Giorgio and Cole, 1998; Kirchman, 2000). Traditionally, studies examining the processing of C within the ‘microbial loop’ have treated bacterial communities as one unit (Yokokawa et al., 2004); however, recent studies have demonstrated that bacterial communities are comprised of a diverse array of functionally independent phylotypes that are specialized in the uptake of specific organic substrates (Bouvier and del Giorgio, 2002; del Giorgio and Bouvier, 2002; Cottrell and Kirchman, 2004; Alonso-Sáez and Gasol, 2007). Furthermore, the importance of considering individual phylogenetic components is exemplified by metabolism of rare phylotypes,
which is often disproportionate to the abundance of these phylotypes within the total bacterial assemblage (Cottrell and Kirchman, 2000; Yokokawa et al., 2004).

DOM generated from primary production is generally considered the major source of organic substrate sustaining bacterial communities in aquatic systems, but rates of primary and bacterial production are only weakly correlated, and highly variable (del Giorgio and Cole, 1998; Carlson et al., 2007). Recent studies have also demonstrated that both crustacean and gelatinous zooplankton can provide bacterial communities with substantial quantities of organic substrate for growth via release of DOM in their excretia (Hansson and Norrman, 1995; Nelson et al., 2004; Condon and Steinberg, In Prep). For gelatinous zooplankton, this process may be accentuated through mucus production (Shanks and Graham, 1988; Hansson and Norrman, 1995; Condon and Steinberg, In Prep), although increased microbial respiration on gelatinous zooplankton-derived DOM suggests it is of poor quality for microbial growth (Condon et al., In prep). In addition, zooplankton may contribute to DOM pools via sloppy feeding and leaching from fecal material (Møller et al., 2003; Møller, 2007), but these processes are not considered in this paper. Determining how zooplankton impact the structure of bacterial phylogenetic assemblages is integral to our understanding of how C is processed within the ‘microbial loop’, particularly in light of large temporal and spatial increases in gelatinous zooplankton in estuarine and coastal systems (Mills, 2001; Sullivan et al., 2001; Purcell et al., 2007; Condon and Steinberg, In review).
Chesapeake Bay is a highly dynamic and productive estuarine ecosystem that sustains large biomass of copepods and gelatinous zooplankton in its mainstem and tributaries (Kimmel and Roman, 2004; Purcell and Decker, 2005; Breitburg and Fulford, 2006; Condon and Steinberg, In review). For calanoid copepods, blooms of *Eurytemora affinis* occur during spring and are replaced by *Acartia tonsa* in summer months (Roman et al., 2001; Kemp et al., 2005). Massive blooms of lobate ctenophores, *Mnemiopsis leidyi*, occur during both seasons and their high predation restricts copepod populations to low densities (Condon and Steinberg, In review). *Chrysaora quinquecirrha* scyphomedusae also appear in mid summer and drastically deplete *M. leidyi* blooms (Purcell and Cowan, 1995; Condon and Steinberg, In review). This study examines the impact of these zooplankton species on estuarine bacterioplankton community structure using dilution experiments conducted during spring and summer, which measured changes in bacterial growth, phylogenetic diversity, and DOM and inorganic nutrient uptake of a natural bacterial inoculum exposed to various zooplankton metabolites. We employed the fluorescent *in situ* hybridization (FISH) technique using rRNA oligonucleotide probes to identify broad bacterial groups and changes to phylogenetic community structure caused by differential uptake of metabolites released by crustacean and gelatinous zooplankton.

**Results**

*Patterns of bacterial growth and production between experiments*
Markedly different patterns of bacterial abundance (BA; Fig. 1a & b), bulk production (BP, Fig. 1c & d), and single-cell production (BP_{sc}; Fig. 1e & f) were observed in the gelatinous zooplankton compared to copepod metabolite treatments for both experiments (spring and summer). In spring, at 10°C, significantly higher BA, BP, and BP_{sc} occurred in the copepod excretia treatment with initial responses within the first 6-12 h (Fig. 1a,c,e). High BA and BP in copepod excretia continued for the remainder of the experiment at rates greater than three times that of natural water controls. In contrast, there was moderate growth and BP in the natural water control, but significantly low growth and BP in the ctenophore metabolite treatment except for a small spike in BP and BP_{sc} at 18 h (Fig. 1c,e). High BA, BP and BP_{sc} was also observed in the glucose addition (labile DOC control) but there was a lag in the bacterial response of approximately 24 h. There was no increase in cells and negligible BP in the low nutrient controls (data not shown) relative to the Mnemiopsis metabolite treatment and natural water control. In general, similar patterns occurred within treatments and controls in comparisons of BA (cells x 10^{6} mL^{-1}) and BP (μg C L^{-1} h^{-1}) uncorrected for differences in DOC concentrations. The exception was a significantly higher BP in the natural water control compared to the Mnemiopsis metabolites treatment (but with no difference in BA).

In contrast, during the summer experiment conducted at 25°C, significantly higher BA, BP, and BP_{sc} occurred in the gelatinous zooplankton treatments (both M. leidyi ctenophores and C. quinquecirrha scyphomedusae) (Fig. 1). This dramatic response of bacteria was reflected in the two-fold increase in BA within the first 12 h of the experiment, after which BA declined. Similar, but lower in magnitude, initial increases
followed by declines in BA also occurred in copepod excretia and glucose addition treatments. DOC normalized BP and BP_{sc} within the first 12 hours was two to three times higher in gelatinous zooplankton metabolites, but production rates dropped to levels observed in the copepod treatment and labile DOC control after 12 h. Decreases in BA, BP, and BP_{sc} occurred in the natural water control throughout the experiment.

Comparison of BA and BP results uncorrected for differences in DOC concentrations (data not shown) were similar as described above except that within the first 12 h in the glucose addition, BA was higher than all zooplankton treatments and BP was similar to gelatinous zooplankton metabolite treatments.

*Comparison of live gelatinous zooplankton and metabolite treatments*

Increases in BA were significantly higher in live *Mnemiopsis* and *Chrysaora* treatments compared to their metabolite treatments (Fig. 2a). However, there were more bacterial cells in the original inoculum for live treatments and comparison of percent increases between the initial and 12 h timepoints for live and metabolite treatments were the same (ANOVA, p = 0.12) (Fig. 2a). Comparison of live gelatinous zooplankton with respective metabolite treatments yielded a similar response of increased production in the first 12 h. However, declines in BP following removal of ctenophores and medusae after 12 h in the live containers were gradual compared to metabolite containers (Fig. 2b).

*Response of phylogenetic groups to zooplankton excretia*
Changes in phylogenetic community structure are determined by comparing FISH results from initial and 12 and 48 h time points for summer and spring, respectively, as these time points corresponded to the largest differences in BA and BP in treatment vs. control containers (Fig. 1, 2). Comparisons between cells hybridized with the total bacteria (EUB338) probe and cells stained with DAPI indicated hybridization efficiencies were higher in summer ranging 57% to >100%, although efficiencies were >55% in all zooplankton treatments and glucose addition controls in spring. In addition, the ability to detect changes in the phylogenetic community structure within the domain bacteria was higher in summer with our FISH probe set accounting for 74-105% of total EUB counts as compared to BRGI that ranged 21-90%.

Consistent changes in phylogenetic community assemblage (Fig. 3 and 4) and specific growth rates (Table 2) were measured within zooplankton treatments and controls in both experiments. In spring, the phylogenetic composition of the initial inoculum assemblage was dominated by betaproteobacteria (65%) with equal proportions (15%) of gammaproteobacteria and Bacteroidetes, with the Planctomycetales being rare (<5%) (Fig. 3a). Consistent with these observations, growth of betaproteobacteria was highest in the natural water control (ANOVA, p < 0.001, Fig. 3a, Table 2), although these bacteria also moderately increased in Mnemiopsis and copepod metabolite treatments (Fig. 3a). Comparison of zooplankton treatments demonstrated growth of different and rare phylogenetic bacterial groups on the different zooplankton metabolites. In particular, significant increases in abundance and high specific growth rates of Bacteroidetes occurred in copepod excretia (ANOVA, p < 0.001), and moderate increases
and high specific growth rates of *gammaproteobacteria* occurred in *Mnemiopsis* metabolites (ANOVA, p < 0.001) (Fig. 3a, Table 3). The majority of increased cell abundance in the glucose addition control in spring was due to growth of *gammaproteobacteria* (ANOVA, p < 0.05; Table 2; Fig. 3a). There was no growth of *Planctomycetales* in any treatment or the control (Fig. 3a).

In summer, the inoculum was comprised primarily by *alphaproteobacteria* (51%) with low proportions of beta- and gamma-proteobacteria and Bacteroidetes (~15% each), and *Planctomycetales* (< 2%) also present (Fig. 3b). Similar to spring, copepod excretia supported the highest increase in *Bacteroidetes* (ANOVA, p < 0.05), and *gammaproteobacteria* predominated in the glucose addition control (ANOVA, p < 0.001). In addition, large increases and high specific growth rates of *gammaproteobacteria* occurred in the gelatinous zooplankton metabolite treatments for both species (*Mnemiopsis* vs. *Chrysaora*, Tukey’s HSD test, t = 1.4, p = 0.63), but there was no increase in *gammaproteobacteria* in copepod excretia (Fig. 3b, Table 2). In addition, some growth of *Bacteroidetes* occurred in ctenophore and medusae metabolites (12-25% increase) at specific growth rates similar to that in copepod excretia (Table 2). Similarly, moderate increases in abundance and high specific growth rates of *betaproteobacteria* occurred in the medusae and copepod treatments (ANOVA, F = 11.9, p < 0.05), but not in the ctenophore treatments and glucose addition control. All zooplankton treatments supported similar increases of *alphaproteobacteria* (ANOVA, F = 2.65, p = 0.13) but the percent changes in cells were lower than for *gammaproteobacteria* and *Bacteroidetes*. Small increases in *Planctomycetales* and
archaea occurred in the zooplankton treatments but were relatively minor in comparison to overall changes in community structure. There was no difference in proportional changes to phylogenetic community structure between the live ctenophore and ctenophore metabolite treatments (ANOVA, F = 0.28, p = 0.61; Fig 3b), although specific growth rates of alphaproteobacteria and Bacteroidetes were higher in the live Mnemiopsis treatment (Table 2). There was no increase in any bacterial group in the natural water control (ANOVA, p < 0.001) (Table 2).

Patterns of nutrient uptake by bacteria

In spring, cell-specific uptake of DOC and DON after 48 hours was significantly higher in the natural water controls compared to the glucose addition control and zooplankton treatments (DOC: F = 56.0, p < 0.001; DON: F = 87.1, p < 0.001; Fig. 4a). Furthermore, DOC uptake by bacteria was significantly higher in the glucose addition control compared to zooplankton treatments, however, DON uptake per bacteria was slightly and significantly higher in the copepod excretia treatment (Tukey’s post hoc pairwise comparisons, Fig. 4a). With the exception of the copepod treatment, cell-specific NH4⁺ uptake was the same between treatments and controls but PO₄³⁻ uptake was significantly higher in the glucose control (p < 0.001) (Fig. 4b). The highest DOC uptake, relative to DON, occurred in the glucose addition and Mnemiopsis metabolite treatments (F = 116.8, p < 0.001) (Fig. 4c). High DOC uptake relative to total dissolved N (TDN) was also observed for bacteria in the ctenophore treatment (F = 11.6, p < 0.05), but DOC:TDN uptake ratios were low in the other treatments (Fig. 4c). Similarly, high DOC: PO₄³⁻
bacterial uptake ratios were observed in the natural water control (F = 33.1, p < 0.001).

After 48 h, there were no detectable changes in DOP concentrations in any of the treatments (data not shown).

For summer, there was significantly higher cell-specific DOC and DON uptake in the live ctenophore treatment vs. controls and other treatments after 12 h (Fig. 4d), and no difference between other treatments and controls, although negative uptake was observed in both gelatinous zooplankton metabolite treatments (Fig. 4d). While significantly higher bacterial uptake of \( \text{NH}_4^+ \) (F = 3.0, p < 0.05) and \( \text{PO}_4^{3-} \) (F = 14.6, p < 0.001) occurred in the medusae treatment, DIN:DIP uptake rates (5.9:1 ± 5.0) were below Redfield (16:1) indicating a preference for \( \text{PO}_4^{3-} \) uptake by bacteria (Fig. 4d). Bacteria in the glucose, ctenophore, and medusae treatments utilized significantly higher amounts of DOC compared to DON (p < 0.001) relative to Redfield ratios (6.6C:IN), but DOC:DON uptake ratios were the same between the live ctenophore and the copepod treatments. In contrast, bacterial uptake of DOC relative to TDN in the labile DOC and ctenophore containers was significantly higher than copepod and \textit{Chrysaora} treatments (F = 6.3, p < 0.05; Fig. 4f).

\textit{Principal components analysis}

We used principal components analyses (PCA) as a descriptive tool to determine consistencies in phylotype changes between spring and summer and to link changes in phylogenetic community assemblages and specific growth rates with DOM and nutrient
uptake rates between treatments and controls. PCA using correlation matrices separated bacterial, FISH, and nutrient uptake results into two and three linear variables for spring and summer, respectively, that combined represented a large proportion of the total variability in the original data (75-78% of total variability, Fig. 5). For spring, growth variables associated with *alphaproteobacteria* and *Planctomycetales* were removed from analyses, as there was little or no change in these phylotypes. The first principal component (PC1; 42% of total variability) was negatively correlated to *gammaproteobacteria* growth, high DOC:DON and DOC:TDN relative to Redfield ratios, and total PO$_4^{3-}$ uptake in the glucose addition and *Mnemiopsis* treatments, and positively correlated to growth and proportional increases in *betaproteobacteria*, high DOC:PO$_4^{3-}$, and DOC and DON uptake in copepod excretia and the natural water control (Fig. 5a). PC2 (33% of variation) was negatively correlated to *Bacteroidetes* growth in the copepod treatment and positively correlated to inorganic N uptake and high NH$_4^+$:PO$_4^{3-}$ uptake ratios (Fig. 5b).

For summer, PC1 (33% of total variability) was positively correlated with increases in *betaproteobacteria* as well as specific growth rates of *Planctomycetales* and archaea, and negatively correlated to *gammaproteobacteria* growth and higher DOC compared to DON and TDN uptake in the glucose addition control and *Mnemiopsis* metabolite treatment (Fig. 5b). PC2 (25% of variation) was positively correlated to *alphaproteobacteria* growth, *Bacteroides* growth, and to DOC and DON uptake in the copepod and live ctenophore treatments (Fig. 5b). Increases in *Bacteroidetes*, *Planctomycetales* and *Archaea* abundances were positively correlated, and inorganic
nutrient uptake was negatively correlated with PC3 and explained 21% of the total variance (data not shown).

Discussion

Evaluation of FISH technique and experimental design

We have experimentally demonstrated that in the absence of bacterivory, bacterial uptake of various zooplankton metabolites had significant ‘bottom-up’ effects on microbial community structure at the class and subclass level, by stimulating growth and production of specific and rare bacterial phylotypes from within the natural assemblage. The interpretation of our results is dependent on being able to detect and characterize changes in bacterial phylotypes using oligonucleotide probes that are representative of the majority of phylotypes found in natural microbial communities. Comparison of total bacteria cells hybridized with the EUB338 probe with total counts for each specific probe suggests that only a small fraction of the bacterial community was uncharacterized by our FISH probe set, and that this uncharacterized fraction was generally associated with low nutrient and natural water controls that experienced low bacteria cell increases and production rates. Furthermore, hybridization efficiencies were low in the spring and high in the summer experiment. As temperature was a major difference between experiments, it is likely that relatively lower growth, ribosomal content, and intracellular metabolism in the spring effected hybridization of cells (Bouvier and del Giorgio, 2003; Yokokawa et al., 2004). Irrespective of these disparities, the major trends and emergence of substrate-
specific phylotypes within treatments was reproducible in both experiments, despite obvious differences in the phylogenetic structure of the inoculum water between seasons. These patterns suggest that FISH was an effective technique in detecting changes in microbial community structure.

A common criticism of the design of dilution experiments is that the response of bacterial communities might not be related to the quality of material in metabolites but rather to exposure to high organic and nutrient concentrations (Bouvier and del Giorgio, 2007). The inclusion of live ctenophore and medusae treatments in our experimental design allows us to assess this potential methodological artifact because DOM and inorganic metabolites are released gradually over time. Comparisons between live and metabolite treatments for ctenophores and medusae yielded the same results in terms of bulk bacterial production, inorganic nutrient uptake, and relative increases in bacterial abundance, however, bacterial uptake of DOC and DON was higher in the live ctenophore treatment. This higher uptake of DOM suggests that filtration of metabolites removed some organic substrate in colloidal form, or that bacteria utilized some of the DOM during metabolite isolation. However, this had no effect on the response of bacterial communities because increases in the same specific bacterial phylotypes occurred in both live ctenophore and ctenophore metabolite treatments. In addition, large increases in *gammaproteobacteria* have been demonstrated with incubations of high inorganic nutrient concentrations (Fuchs et al., 2000; Bouvier and del Giorgio, 2002). While this phylotype solely increased in glucose addition controls, a mixture of dominant and rare bacterial phylotypes emerged in the gelatinous zooplankton metabolites and
there were low growth or decreases in **gammaproteobacteria** in copepod excretia treatments. Collectively, these comparisons suggest that bacterial communities were responding primarily to the quality rather than the quantity of organic substrate in the experiments.

We evaluated the impact of zooplankton on bacterial communities by determining changes in phylotypes between two time points – the initial and a time point with highest bacterial growth and production. It is conceivable that there was bacteria community turnover prior to our final time point measurements. This potential effect on our community characterization would be most likely in spring because compositional changes were based on time points two days apart. However, specific growth rates for each phylotype were lower in spring and we observed the same bacterial response within a short time frame during summer, suggesting that results from both experiments are reflective of changes due to zooplankton metabolites and not indicative of bacterial community turnover.

Minor increases in the domain **archaea** and **Planctomycetales** occurred in some of the zooplankton treatments. The **Planctomycetales** have been observed attached to outer surfaces of particles and phytoplankton, and some increase in this bacterial group could have been due to detachment from zooplankton. This result is consistent with previous studies demonstrating that these prokaryotes are genetically rare components of marine microbial assemblages (Glöckner et al., 1999). The domain **archaea** are traditionally considered ‘extremophiles’ predominating under anoxic conditions such as in sediments
and summertime bottom waters of Chesapeake Bay (Bouvier and del Giorgio, 2002), inside marine aggregates (Ploug et al., 1997), or in the guts of animals (DeLong and Pace, 2001). The low proportion of *archaea* in our samples further supports the notion that zooplankton-derived DOM originated via the release of assimilated biomass and not from other confounding sources such as fecal material. In general, *archaea* and *Planctomycetales* were absent from FISH counts so the release of zooplankton metabolites likely has little effect on these rare phylotypes.

*‘Bottom-up’ controls by zooplankton on bacterial community structure*

The release of metabolites by copepods and gelatinous zooplankton had pronounced but dissimilar ‘bottom-up’ effects on bacterial growth dynamics by stimulating increases in specific and rare bacterial phylotypes from within the natural assemblage. Prior studies have demonstrated that various phylotypes are specialized for the uptake of specific organic substrates (Cottrell and Kirchman, 2000, 2004; Alonso-Sáez and Gasol, 2007), and due to higher specific growth rates, rare groups can out compete dominant bacterial groups when specific organic resources become available (Yokokawa et al., 2004). Similarly, we suggest that the significant treatment-specific shifts in bacterial community composition in our experiments were caused by high affinities of specific phylotypes for different biochemical compounds released as metabolites by crustacean and gelatinous zooplankton (Condon and Steinberg, In Prep; Condon et al., In prep). In this study, the major changes in microbial assemblages involved the emergence of the rare bacterial
phylogenotypes, namely *gammaproteobacteria, Bacteroidetes*, and in summer, *betaproteobacteria*.

During both seasons, *gammaproteobacteria* responded favorably to metabolites released by both gelatinous zooplankton species, particularly during summer when large and rapid increases in bacterial abundance and production were accompanied by similar increases and high specific growth rates of this phylotype. Even at low levels of bacterial growth and production during spring, a relatively large proportion of total bacteria growth was by *gammaproteobacteria* in ctenophore metabolites. The *gammaproteobacteria* have been considered an ‘opportunistic’ bacterial group because they can respond rapidly and sustain high but inefficient growth on poor-quality organic substrates (Fuchs et al., 2000; Carlson et al., 2007). This was exemplified in the glucose addition control where extremely high bacterial growth was supported by *gammaproteobacteria*. *Mnemiopsis* ctenophores and *Chrysaora* medusae are known to release high quantities of DOC in their metabolites (Hansson and Norrman, 1995; Condon and Steinberg, In Prep), and while the biochemical composition is unknown, Condon et al. (In prep) considered gelatinous zooplankton DOC poor quality because bacteria utilized this material inefficiently for growth. Furthermore, the release of poor organic substrate may be accentuated in gelatinous zooplankton through mucus production (Ducklow and Mitchell, 1979; Shanks and Graham, 1988; Hansson and Norrman, 1995). To this end, we suggest that the release of high amounts of poor-quality, C-rich substrate by gelatinous zooplankton stimulates rapid growth of *gammaproteobacteria* observed in our experiments and suggests the biochemical nature of their released DOC is similar to
glucose (Condon et al., In prep). This hypothesis is supported by high uptake of DOC, relative to DON, by bacteria in glucose addition and gelatinous zooplankton metabolite containers and PCA correlations linking high DOC:DON and DOC:TDN uptake ratios with increased biomass and specific growth rates of *gammaproteobacteria* in glucose addition and ctenophore metabolite containers.

Changes in the phylogenetic composition in copepod metabolites was dominated by high growth of *Bacteroidetes* but also moderate growth of *betaproteobacteria*, however, there was little or no growth from *gammaproteobacteria*. For the *Bacteroidetes*, these changes were caused by higher cell-specific growth relative to total bacterial growth rates and high uptake of DON reflected in low DOC:DON uptake ratios. In addition, the close link of *Bacteroidetes* and *betaproteobacteria* were confirmed with PCA that correlated growth parameters for both phylotypes to copepod treatments or to uptake of DON. A previous study demonstrates that copepods can support growth of *Bacteroidetes* when this phylotype is a dominant component of the bacterial assemblage (Zubkov and López-Urrutia, 2003), and our results clearly show a high affinity of *Bacteroidetes* for copepod-derived compounds because this phylotype was initially rare in the inoculum. The *Bacteroidetes* and *betaproteobacteria* are known to utilize N-rich, high molecular weight organic compounds including chitin and *N*-acetylglucosamine, a monomeric unit of chitin (Cottrell and Kirchman, 2000). The uptake of chitin-based compounds by these phylotypes is relevant in our experiments because these compounds form the chemical structure of the copepod exoskeleton. In contrast to gelatinous zooplankton (Condon and Steinberg, In Prep), copepods excrete high quantities of DON (Miller and Glibert, 1998)
and can release high molecular weight (HMW), N-rich compounds (Urban-Rich et al., 2006), thus contributions by copepods to DOM pools that are qualitatively relevant to *Bacteroidetes* and *betaproteobacteria* communities was likely high and the probable cause for increases in these bacterial groups in the copepod excretia treatments.

Interestingly, in addition to *gammaproteobacteria*, small to moderate increases in *Bacteroidetes* also occurred in the gelatinous zooplankton treatments during summer. Furthermore, increases in *Bacteroidetes* were accompanied by similar increases in *betaproteobacteria* cells in medusa metabolites but not in any of the ctenophore treatments. These disparities may be explained by differences in feeding mechanisms and the turnover of structural components between lobate ctenophores and semaeostome scyphomedusae (Condon and Steinberg, In Prep). In particular, *Chrysaora* scyphomedusae capture prey using tentacles laden with nematocysts composed of chitin (Hessinger and Lenhoff, 1988), whereas *Mnemiopsis* ctenophores trap prey in feeding lobes lined with mucus, that are formed by glycoprotein colloids (Ducklow and Mitchell, 1979). In this regard, *Chrysaora* medusae are biochemically similar to copepods because both have high chitin requirements, but are dissimilar to *Mnemiopsis* ctenophores which are essentially devoid of chitin molecules. This hypothesis is further supported by PCA for summer that correlated both copepod and *Chrysaora* metabolite treatments with increases and specific growth rates of *betaproteobacteria*. Moreover, potentially higher production and turnover of mucous compounds in *M. leidyi* compared to *C. quinquecirrha* at higher temperatures (Condon and Steinberg, In Prep) would result in greater concentrations of proteins, but not chitin, in ctenophore treatments. As
Betaproteobacteria utilize low molecular weight amino acids, preferential growth of Bacteroidetes would be expected in ctenophore metabolites because they can also utilize HMW proteins (Cottrell and Kirchman, 2000).

Opposite trends were observed in the spring experiments with high growth of betaproteobacteria but no growth of Bacteroidetes in the ctenophore treatment. Release of DON (and DOP) in M. leidyi metabolites was low during spring (Condon and Steinberg, In Prep), so increases in betaproteobacteria biomass in this experiment must be due to an alternative organic source. Betaproteobacteria are dominant members of the microbial assemblage in low salinity systems (Cottrell and Kirchman, 2000; Bouvier and del Giorgio, 2002; Yokokawa et al., 2004), but growth may also be driven by bulk DOC pools in freshwater systems (Bouvier and del Giorgio, 2002). It is thus interesting to note that the highest increases and specific growth rates of betaproteobacteria occurred in untreated, natural water in which bacterial assemblages dominated by this phylotype were exposed to DOM pools that were consistent with field conditions. Furthermore, high growth of betaproteobacteria in the natural water control was complemented by high DON uptake and low DOC:DON and DOC:TDN uptake ratios by bacteria. While zooplankton metabolites were isolated in low nutrient water, a portion (20% by volume) of the natural water was included in diluted amounts in each container as part of the initial bacterial inoculum. As the response of bacteria is the result of DOM quality and betaproteobacteria increased in all zooplankton and natural water containers, we suggest that growth of this phylotype in spring could be due to utilization of material in the bulk DOM pool. The exception was in the glucose addition control, but high growth rates
exhibited by *gammaproteobacteria* coupled with high DOC uptake dynamics may have masked the response of *betaproteobacteria* in these containers. It is unclear what biochemical component of the DOM pool is utilized by *betaproteobacteria*, but high affiliation for HMW N compounds (Cottrell and Kirchman, 2000) would be advantageous in Chesapeake Bay tributaries that typically contain high concentrations of humic material (See and Bronk, 2005).

*Alphaproteobacteria* are common components of the microbial assemblage in marine systems (Rappé et al., 1997; Hagström et al., 2002; Selje et al., 2004) and in the summer experiment small to moderate increases were observed in all zooplankton treatments. *Alphaproteobacteria* can take up substantial amounts of glucose and amino acids (Alonso-Sáez and Gasol, 2007), and in addition to glucose, amino acids are reportedly released by gelatinous zooplankton (Webb and Johannes, 1967; Kremer, 1977). We suggest that this is not the case in our experiments because *alphaproteobacteria* did not increase under added glucose conditions, and aminoletic *betaproteobacteria* did not increase in *Mnemiopsis* metabolite treatments. As an alternative, we suggest that in zooplankton treatments increases in *alphaproteobacteria* were attributed to their competitive ability to prosper in nutrient-deprived conditions, because starting DOP and PO$_4^{3-}$ concentrations were low or individual containers shifted to P-limitation during experimentation via release of C-rich DOM. This conclusion is supported by field data indicating a negative correlations between *alphaproteobacteria* biomass and PO$_4^{3-}$ concentrations (Bouvier and del Giorgio, 2002).
Ecological implications for carbon cycling of zooplankton-mediated changes in bacteria phylogenetic assemblages

Exploring the processes that explain patterns in diversity and community structure is a challenging research area in community ecology. Theory predicts that in high productivity system, such as Chesapeake Bay, predation is the major influence on diversity patterns, and organisms that are successful at avoiding predation are expected to be dominant (Bohannan and Lenski, 2000). The corollary of this prediction is that the non-dominant species are successful at competing for resources, because of the existence of a trade-off between “exploitation ability” and “predator resistance” (Bouvier and del Giorgio, 2007). Our observations were consistent with such predictions. We observed that the in situ low abundance phylogenetic groups were better at exploiting DOM resources and that this resource may largely proceed from copepod and gelatinous zooplankton metabolites during their seasonal blooming in estuarine and marine systems (Condon and Steinberg In Prep). This has important implications for C cycling and bacterial metabolism at the community level, because high release of DOM by zooplankton combined with high affinities for these substrates would result in higher quantities of C, N, and P being processed by rare phylotypes. The greatest impacts of zooplankton on microbial communities would occur during spring and summer when blooms of copepods and gelatinous zooplankton occur in Chesapeake Bay and its tributaries (Kimmel and Roman, 2004; Kemp et al., 2005; Purcell and Decker, 2005; Condon and Steinberg, In review).
Our results suggest that copepod populations would mostly influence *Bacteroidetes* communities. Utilization of copepod metabolites by these bacterial groups can have significant consequences for C cycling because labile, preformed, organic molecules are utilized more efficiently for bacterial production (Alonso-Sáez and Gasol, 2007). In turn, this creates an important trophic pathway whereby large amounts of C, N, and P can be incorporated into bacterial biomass and potentially reincorporated into planktonic food webs (Condon et al., In prep). This process would be accentuated in regions where *Bacteroidetes* are dominant members of the phylogenetic community assemblage. *Bacteroidetes* are commonly attached to particles, and are dominant phylotypes in estuarine turbidity maxima (ETM) that have high particle loads (Crump et al., 1999; Bouvier and del Giorgio, 2002), and entrap high copepod biomass in the ‘salt wedge’ (Roman et al., 2001). The potential high turnover of copepod biomass could provide a valuable source of labile DOM for *Bacteroidetes* in the ETM, because cells have high intracellular maintenance requirements under conditions of high environmental stress and detachment from particles reduces a source of organic substrate and surface for growth (Bouvier and del Giorgio, 2002; del Giorgio and Bouvier, 2002).

Gelatinous zooplankton blooms can have direct and indirect effects on the structure of microbial communities and C cycling through both the direct release of DOM in their metabolites and their high predation rates on copepods (Condon and Steinberg, In Prep, In review). In particular, Condon et al. (In prep) suggested that high assimilation of copepods by gelatinous zooplankton converts large amounts of primary and secondary production into gelatinous biomass and subsequent release of ‘junk DOC’ in metabolites
favors growth of opportunistic bacterial groups. Our results suggest that predation on copepods would partially impact *Bacteroidetes* communities by reducing a major source of labile DOM, but in turn would stimulate *gammaproteobacteria* growth that can rapidly utilize the poor quality DOM in gelatinous zooplankton metabolites. This has deleterious implications for C cycling because opportunistic bacterial groups, such as *gammaproteobacteria*, grow inefficiently on this substrate and increased respiration would result in high amounts of C returning to dissolved inorganic pools rather than being reincorporated into the planktonic food web (Condon et al., In prep).

The results of this study stress the importance of including specific phylotypic components of the bacterial community assemblages in models of biogeochemical cycling and bacterial metabolism. In addition, we did not consider the potential effects of bacterivory by heterotrophic nanoflagellates or viral lysis which also play a part in controlling bacterial diversity within zooplankton dominated systems (Weinbauer and Rassoulzadegan, 2004; Bouvier and del Giorgio, 2007; Zhang et al., 2007). These biological components should also be considered if we are to fully comprehend the effects of zooplankton derived-DOM on microbial metabolic processes.
Experimental Procedures

Bacterial dilution experiments

We conducted a bacterial dilution experiment comparing exponential growth of free-living natural bacterial assemblages exposed to zooplankton metabolites (treatments), natural *in situ* water (natural water control), and labile DOM substrate (labile DOC control) during spring (10°C, BRG1) and summer (25°C, BRG2), 2005. Free-living bacteria were isolated from York River surface waters (0-2 m) via filtration through 1.2 μm Millipore™ AP15 glass fiber (GF) filters using a peristaltic pump (del Giorgio et al., 2006). Acid-cleaned polycarbonate carboys filled with 6 L of grazer-free treatment or control water were inoculated with 1.5 L of filtrate containing natural bacterial assemblages (Nelson et al., 2004), and prokaryote growth monitored over 3 days. Samples for bacterial abundance (BA), bacterial production (BP), DOM (DOC, DON, DOP), dissolved inorganic N (NO₂⁻, NO₃⁻, NH₄⁺) and P (PO₄³⁻) were taken every 12 h from each of three replicate treatment and control containers. BP and BA normalized to nutrient concentrations were used as a proxy for bacterial growth.

The starting physical and biological conditions are summarized in Table 3. Treatment and control media were prepared as follows. Natural control water was collected from surface waters (0-2 m), and sequentially filtered through 142 mm, 1.2μm AP15 GF and then 0.2 μm polycarbonate filters. Gelatinous zooplankton metabolite media was prepared by incubating eight *M. leidyi* (*Mnemiopsis* treatment) or one *C. quinquecirrha*...
(Chrysaora treatment) in separate 6 L, acid-cleaned, polycarbonate containers filled with low nutrient, Nanopure™-diluted, 0.2 μm-filtered Sargasso seawater. After 12 h, gelatinous zooplankton were removed for biometric and chemical analysis. For copepod excretia treatments, copepods were concentrated in 0.2 μm-filtered York River water using back filtration (Harris et al., 2000), and transferred to 5 L, acid-washed polycarbonate containers filled with 0.2 μm-filtered Sargasso Sea water diluted with Nanopure water to in situ salinity. After 12 h, copepods were collected in 200 μm acid-washed sieves and the copepod excretia filtrate was retained for experiments. Copepod samples were fixed in 4% buffered formaldehyde and enumerated under the light microscope. Water containing gelatinous zooplankton or copepod-derived DOM was screened through acid-rinsed, 100 μm nitex mesh and gravity filtered through autoclaved Whatman™ Polygap 0.2 μm membrane filtration cartridges (Nelson et al., 2004). Cartridges were acid rinsed and flushed thoroughly with 0.45 μm-filtered Nanopure™ water prior to filtration. The low amount of particulates retained on the mesh screen indicated egestion was minimal during incubations and the potential contribution to zooplankton-derived DOM pools via sloppy feeding and leaching of fecal material was low.

As incubation water was non axenic and post incubation filtration removed colloidal organic substrate (e.g., mucus), a portion of DOM released by jellyfish was potentially utilized by prokaryotes before the experiment. To evaluate this effect, 'live jellyfish' treatments were included during BRG2, whereby equal numbers of M. leidyi (live Mnemiopsis) or C. quinquecirrha (live Chrysaora) were incubated in bacteria-inoculated,
York River control water for the first 12 h of the experiment (i.e., same gelatinous zooplankton biomass and time period as for metabolite incubations). Because the water was not refiltered following removal of jellyfish, carboys were inspected and particulates removed using sterile pipette tips.

To evaluate the lability of DOM released by jellyfish, we compared metabolite and live treatments to a nutrient enrichment (labile DOC) control. Nutrient lability controls consisted of Nanopure water-diluted Sargasso Sea water with added glucose (C₆H₁₂O₆, 30 μM final conc.), NH₄⁺ (1.5 μM), and PO₄³⁻ (0.75 μM). Glucose was chosen as a labile DOC control because it is the most abundant neutral sugar in the ocean, and it is preferentially utilized by bacteria relative to other monosaccharides (Rich et al., 1996).

**Microbiological analyses**

Methodology for determining BA, BP, and community composition are described in detail elsewhere (Smith and Azam, 1992; Kana et al., 1994; del Giorgio et al., 1996; Bouvier and del Giorgio, 2002). Briefly, 10 mL BA samples were fixed in 0.2 µm filtered molecular grade formaldehyde (2% final conc.) buffered with sodium borate and stored in a -80°C freezer. For bacterial counts, fixed cells were stained using SYTO-13 (Invitrogen Molecular Probes, S7575) green fluorescence nucleic acid stain (del Giorgio et al., 1996; Gasol et al., 1999) and enumerated using a Coulter Epics Altra or a Becton Dickinson FACScalibur flow cytometer, both equipped with 488 nm argon lasers. Prior to counting, calibrated 1.1 µm microsphere bead stock (Molecular Probes™, Cat. No.
F8888) was added to each of 3 sub-sample vials and total bead counts used to determine
BA (no. bacteria ml\(^{-1}\)). BP (\(\mu\text{g C L}^{-1} \text{ h}^{-1}\)) was measured in triplicate 1.5 ml sub samples
using the \(^3\text{H}-\text{leucine incorporation technique (Smith and Azam, 1992), modified}
according to Pace et al. (2004) and assuming a C conversion factor of 3.1 kg C mol leu\(^{-1}\)
Kirchman, 1993). To compare relative changes in bacterial metabolism across treatments
it was necessary to normalize BP rates on a single-cell basis (\(\mu\text{g C cell}^{-1} \text{ h}^{-1}\)) by dividing
each parameter by BA (cells L\(^{-1}\)).

**Phylogenetic diversity**

To analyze microbial community structure and responses of various prokaryote
phylogenotypes to gelatinous zooplankton DOM we employed the fluorescent in situ
hybridization (FISH) technique using oligonucleotide probes conjugated with CY3
(Bouvier and del Giorgio, 2007). The FISH probes targeted specifically the domain
*bacteria* (EUB338), including five of its main phylogenetic groups: the *alpha-* (ALF1b),
*beta-* (BET42a), *gamma-proteobacteria* (GAM42a), the *Bacteroidetes* (CF319a), and the
*Planctomycetales* (PLA886) lineage, as well as the domain *archaea* (ARCH915) (Table
1). The hybridization methodology we adopted in this study is detailed in Bouvier and del
Giorgio (Bouvier and del Giorgio, 2002, 2007) with the following changes: samples
were generated from BA samples that were stored in a \(-80^\circ\text{C}\) freezer before FISH sample
preparation, filters were hybridized for five hours, concentrated stock of DAPI stain was
added to the Citifluor mounting solution (Ted Pella Inc., London, UK), and hybridized
slides were kept in the dark at \(-20^\circ\text{C}\) for 1-2 days prior to counting. FISH counts were
made on a Zeiss Axiophot epifluorescence microscope with an external Hg light source under green light excitation. To correct for autofluorescing and non-target cells, we subtracted counts from the control probe (NON338) (Table 1) from all prokaryote probe counts. With the exception of the live Chrysaora treatment (BRG2, data excluded from analyses), percentages of fluorescing cells using the control probe was low, averaging < 0.5% of the cells counted with the EUB338 probe. To check for hybridization efficiency (i.e., ratio of counted hybridized cells and actual bacterial cells), EUB338 counts were compared to total bacteria counts made on the same filter with DAPI staining under UV light. Efficiencies ranged 24 (low nutrient control, bacteria experiment 1) to >100% (copepod excretia treatment, BRG1) which is within the range of literature values (Bouvier and del Giorgio, 2003), but efficiencies were typically > 60%.

Specific growth rates of the different phylogenetic groups in incubations were calculated on the basis of the changes in the number of cells of each phylotype using the equation
\[ \mu = \frac{\ln(A_t/A_0)}{T}, \]
where \( A_0 \) is the initial bacterial abundance, \( A_t \) the bacterial abundance at time \( t \), \( T \) the unit time interval and \( \mu \) the specific growth rate (Kirchman, 2002). Specific growth rates were compared to total bacterial growth rates as calculated above but with cell changes with the EUB338 probe.

**Chemical analyses**

Water was filtered through pre-combusted (500°C) Whatman\textsuperscript{TM} GF/F filters, and dissolved nutrients determined from the filtrate. DOC concentrations were measured via
high temperature combustion on a Shimadzu™ 5000A Total Organic Carbon (TOC) analyzer using potassium hydrogen phthalate (C$_8$H$_5$O$_4$K) standard (Peltzer et al., 1996). Prior to combustion, 6N HCl was added to 5 mL sample (pH < 3) and sparged for two minutes with C ultra free air to ensure removal of dissolved inorganic C. DOC concentrations were based on the best three of a maximum five column injections within an analytical detection error set to a peak area standard deviation ±120 or coefficient of variance of 0.8%. Samples with ±1.5 μM error were reanalyzed. In addition, data precision, instrument accuracy, and platinum catalyst efficiency were quality checked with low C (1-2 μM DOC) and deep Sargasso Sea water (44-46 μM DOC) reference standards provided by the carbon reference material program (Sharp, 2002).

Total dissolved N (TDN) and P (TDP) by persulfate oxidation (Bronk et al., 2000; Sharp, 2002), nitrate (NO$_3^-$) by spongy cadmium (Cd) method, and nitrite and phosphate (Koroleff, 1983) were measured on a Lachat™ QuikChem 8500 nutrient autoanalyzer. During analysis, the conversion of NO$_3^-$ to NO$_2^-$ by Cd catalyst was monitored and columns regenerated if reduction efficiency was < 96%. The efficiency of the persulfate reagent to convert organic N and P to respective inorganic constituents was evaluated using 10 μM urea (CH$_4$N$_2$O) and 10 μM glucose-6-phosphate (C$_6$H$_{12}$O$_9$PNa) standards for DON and DOP, respectively. NH$_4^+$ was measured on a Shimadzu™ UV-1601 spectrophotometer by the manual hypochlorite method (Koroleff, 1983) using standard curves corrected for sample salinity. DON and DOP were determined by calculating the difference between total dissolved and inorganic fractions for N (TDN - Σ [NO$_3^-$ + NO$_2^-$ + NH$_4^+$]) and P (TDP - Σ [PO$_4^{3-}$]), respectively (Sharp, 2002).
Statistical analyses

Normalized and raw data describing changes in bacterial community composition using FISH, bacterial abundance and production, specific growth rates of phylotypes, and uptake of DOM and inorganic nutrients were analyzed using analysis of variance (ANOVA) (level of significance of $\alpha = 0.05$). If ANOVA’s were significant, post hoc pairwise comparison of means using Tukey’s HSD tests were performed (Quinn and Keough, 2002). Prior to analyses, data were checked for normality and homogeneity of variance using Kolmogorov-Smirnov tests, box plots and histograms of data and residuals, and non-conforming data were improved through log_{10} or fourth-root transformations (Quinn and Keough, 2002). In addition, principal components analyses were conducted to describe underlying patterns of changes in phylotypes observed within treatments and between experiments. PCAs were based on raw data on percent changes and specific growth rates of phylotypes, and DOM and nutrient uptake rates in a correlation matrix because the variables had different variances.

References


Fig. 1. Patterns of bacterial growth and production. A and B. Bacterial abundance (BA) within control and treatment containers for the spring (A) and summer (B) dilution experiments. C and D. Bacterial production within control and treatment containers for spring (C) and summer (D). E and F. Single-cell bacterial production (BP_{sc}) within control and treatments for spring (E) and summer (F). Data are expressed as mean values of n=3 replicate containers normalized to DOC concentrations (A-D) and BA (E and F). Error bars are ± 1 standard error. Natural water control (NWC), glucose addition control (+Glu), copepod excretia treatment (Cope), *Mnemiopsis* ctenophore metabolite treatment (Mnem), and *Chrysaora* scyphomedusae metabolite treatment (Chry). Value in parentheses is an out of range value for the +Glu treatment. There was no *Chrysaora* scyphomedusae excretia treatment for BRG1.
Fig. 1

Spring

Summer

Bacterial Abundance
(10^6 cells x DOC)

0 15 30 45 60 75 90

Bacterial Production
(μg C mg^{-1} DOC h^{-1})

0 15 30 45 60 75 90

BP_{sc}
-lg C cell^{-1} h^{-1})

0 15 30 45 60 75 90

Time (h)

- NWC
- +Glu
- Cope
- Mnem
- Chry

(410 ± 17)
Fig. 2. Comparison of mean bacterial abundance (A), and net bacterial production (B) between live gelatinous zooplankton (white symbols) and metabolite (black symbols) treatments in the summer dilution experiment (see Experimental Procedures section). Insert in Fig 2A shows the percent increase in total bacterial cells after 12 h between live animal and metabolite treatments. Values are mean ± 1 standard error of n = 3 replicates. Note: data are not normalized to DOC concentration. Animals in live treatments were removed after 12 h (bolded R) vs. no animals present at all in metabolite treatments.
Fig. 2

(a) Bacterial Abundance (Cells * 10^6 ml^-1)

(b) Bacterial Production (ugC l^-1 h^-1)

Time (h)
Fig. 3. Phylogenetic composition in the inoculum water and changes in abundance for each phylotype after 48 h and 12 h in the spring (A) and summer (B) experiments, respectively. Data are presented as percent changes in cells relative to the composition of the original inoculum (see Results section). Error bars are ± 1 standard deviation. Inoculum water (Inoc), natural water control (NWC), copepod excretia (Cope), glucose addition control (+Glu), *Mnemiopsis* metabolite treatment (Mnem), live *Mnemiopsis* treatment (Live Mn), *Chrysaora* metabolite treatment (Chry). *Alphaproteobacteria* (α), *betaproteobacteria* (β), *gammaproteobacteria* (γ), *Bacteroidetes* (Bact), *Planctomycetales* (PLA), *Archaea* (Arch). There are no *Archaea* data for spring. Note that data for NWC are not presented for summer as there was no increase in any phylotype.
**Fig. 4.** Patterns of nutrient uptake by bacteria. Cell-specific uptake of DOC (black bars) and DON (grey bars) for spring (A) and summer (B). C and D. Cell-specific uptake of NH$_4^+$ (black bars) and PO$_4^{3-}$ (grey bars) for spring (C) and summer (D). E and F. Uptake ratios of DOC:DON (black bars) and DOC:TDN (grey bars) for spring (E) and summer (F). Data are mean ± 1 standard deviation of total DOM or inorganic nutrient uptake per cell within treatments after 48 h for spring and 12 h for summer. Treatment and controls connected by the same letter (black bars) and numbers (grey bars) are not significantly different using Tukey’s *post hoc* analyses ($p < 0.05$) where $A > B > C$ and $1 > 2 > 3$ (see Results section). Parentheses represent out of range values.
Fig. 4

Spring

[Graph showing nmol cell\(^{-1}\) vs. DOC: DON across different treatments labeled NWC, +Glu, Mnem, and Cope.]

Summer

[Graph showing nmol cell\(^{-1}\) vs. DOC: DON across different treatments labeled +Glu, Mnem, Chry, and Cope.]

Treatment
Fig. 5. Principal components analysis (PCA) score plots of first (PC1) and second (PC2) principal components for spring (A) and summer (B). Loadings (correlations) represented by stars. Natural water control (NWC), copepod excretia treatments (Cope), glucose addition control (+Glu), *Mnemiopsis* metabolite treatment (Mnem), live *Mnemiopsis* treatment (Live Mn), *Chrysaora* metabolite (Chry). Specific growth rates (u) and per cent increase in phylotypes (%) of *alphaproteobacteria* (A), *betaproteobacteria* (Be), *gammaproteobacteria* (G), *Bacteroidetes* (Ba), *Planctomycetales* (P), *Archaea* (Ar). Uptake rates for dissolved organic carbon (DOC), nitrogen (DON), and uptake ratios for DOC and DON (DOC:DON) and total dissolved N (DOC:TDN). Circles represent positive and negative correlations between loadings and treatment and control scores for PC1 and PC2 (see Results section).
Fig. 5

(a) PC1 (42%) vs. PC2 (33%)

(b) PC1 (33%) vs. PC2 (25%)

Legend:
- Chry
- Cope
- + Glu
- Live Mn
- Mnem

Data points represent various substances and conditions, with different symbols indicating different samples or treatments.
Tables and table captions

**Table 1.** Oligonucleotide probes used in this study. rRNA position based on *Escherichia coli* numbering (Bouvier and del Giorgio, 2007). %FA = Percentage of formamide in hybridization buffer. IS: ionic strength (NaCl, M).

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<th>Target taxa</th>
<th>Probe sequence (5’-3’)</th>
<th>rRNA position</th>
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Table 2. Growth rate of specific bacterial groups (day⁻¹) in zooplankton treatments and controls for the spring and summer dilution experiments. NC, the growth rate could not be calculated because of a decrease in cell abundance. ND, no data. *Alpha*, *alphaproteobacteria*. *Beta*, *betaproteobacteria*. *Gamma*, *gammaproteobacteria*. PLA, *Planctomycetales*.

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(*) Growth rate calculations based on cells undetected at the initial timepoint.
Table 3. Starting physical and biological conditions for spring and summer bacterial dilution experiments. BMD = below minimum detection. NA = not applicable. Errors are ± 1 standard deviation.

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<td>16 July to 20 July, 2005</td>
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<td>20.4</td>
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<td>Mnemiopsis metabolites</td>
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CHAPTER 5

The 'Jelly Pump': Consequences of Jellyfish Blooms for Increased Carbon Metabolism Within the Microbial Loop
One-sentence summary: Jellyfish release high quantities of carbon-rich dissolved organic matter that rapidly stimulates growth and metabolism of specific bacterial phylotypes, thereby providing an alternative carbon pathway within the planktonic food web that shunts carbon away from higher trophic levels and increases the microbial respiratory C sink.

Large jellyfish blooms of lobate ctenophores (*Mnemiopsis leidyi*) and scyphomedusae (*Chrysaora quinquecirrha*) occur in many coastal areas, including large summer blooms in Chesapeake Bay. High jellyfish biomass coincides with peaks in microbial activity and biomass, but few studies have investigated the potential link between jellyfish blooms and microbial functioning in coastal ecosystems. We measured dissolved organic matter (DOM) production by jellyfish, and the response of free-living bacterioplankton to this C input, in terms of bacterial cell growth, metabolism, and phylogenetic community composition. Both species of jellyfish released large amounts of carbon (C)-rich DOM, and bacterioplankton quickly responded with large increases in metabolic activity. Enumeration of prokaryotic phylogenetic groups using fluorescence in situ hybridization (FISH) showed specific bacterial groups were responsible for increased metabolism resulting from jellyfish-generated DOM. Furthermore, decreases in bacterial growth efficiency suggest a shunt of C consumed towards bacterial respiration. In the context of worldwide increases in jellyfish, our results suggest the possibility of major shifts in marine microbial structure and function, and a potential for a large
bacterial C sink away from higher trophic levels, including commercially important fish species.

Massive increases of ctenophores and medusae (hereafter referred to as ‘jellyfish’) have occurred in many estuarine and marine ecosystems worldwide over the past decade. The reasons for observed increases in jellyfish biomass are not fully understood, but likely include eutrophication, over-fishing, global climate change, and direct introduction into exotic regions (1, 2). Given current and projected global increases in ocean temperature, combined with anthropogenic influences, these trends are likely to continue into the near future (3) with unknown consequences.

Voracious predation by jellyfish converts large quantities of C (as well as nitrogen [N] and phosphorus [P]) fixed by primary producers and consumed by secondary producers (e.g., copepods) into gelatinous biomass, which is not readily consumed by higher trophic level predators (2). In this regard, jellyfish can have a negative impact on C transfer by limiting C bioavailability to higher trophic levels (e.g., planktivorous fish) via direct predation, thereby representing a trophic ‘dead-end’ for C transfer (3). However, live jellyfish may play important roles in C transfer through enhanced release of plankton-derived dissolved organic matter (DOM) (2, 4), which fuels bacterioplankton growth (2, 4, 5). The relevance of this trophic link between jellyfish and the microbial loop is poorly understood, but has consequences for C cycling because relatively large amounts of C can be repackaged by microbes and reincorporated into the planktonic food web, rather than being ‘locked up’ in jellyfish biomass. Little is known about the large-scale
effects of DOM release by jellyfish blooms on the production and respiration of C by bacterioplankton (6).

The objective of this study was to determine the impact of jellyfish blooms on planktonic C cycling, focusing on the fates of jellyfish C during these blooms and potential alterations to C pathways in jellyfish-dominated systems. Our conclusions are based on laboratory experiments of jellyfish and bacteria metabolism and C cycling combined with field surveys conducted in the York River estuary, a southern Chesapeake Bay tributary. Chesapeake Bay is a well-studied estuarine system that sustains high jellyfish biomass (Fig. SI). Blooms of two native species, *M. leidyi* ctenophores and *C. quinquecirrha* scyphomedusae, dominate throughout the estuary during late spring (May) and summer (June-August), and are the focus of this study (2).

We measured the simultaneous release of DOM and inorganic constituents by *M. leidyi* ctenophores and *C. quinquecirrha* medusae during short laboratory incubations. Jellyfish directly release assimilated material as DOM and inorganic nutrients via excretion and mucus production. Since organic and inorganic constituents are produced via different processes but originate from assimilated material, we use the term ‘metabolites’ to describe material directly released by jellyfish. DOM may also be released indirectly through leaching of fecal material and ‘sloppy feeding’ but these processes are not considered here. Metabolite release rates were normalized to jellyfish dry weight to allow comparison between jellyfish species. Compared to organic N and P release, both jellyfish species released high amounts of DOC in their metabolites, particularly *M. leidyi*
ctenophores, which produced C-rich DOM as reflected in the high molar DOC: DON: DOP ratios of their metabolites (60.3: 5.0: 1) compared to the canonical Redfield ratio (106C:16N:1P) (Fig. 1). The biochemical nature of the DOC released by jellyfish is unknown, but C-rich compounds are typically energy-rich (7-9). This autochthonous production of DOC by jellyfish blooms has implications for microbial dynamics in estuarine and coastal systems through their potential high contribution of utilizable C into bulk DOC pools (10, 11).

Whether bacteria utilize jellyfish DOC for their growth will depend upon supplemental uptake of N and P for synthesis of essential intracellular compounds (12). Our results show that jellyfish also release high amounts of inorganic N and P relative to DON and DOP (Fig 2), which are energetically favorable and may be utilized as an alternative to support bacterial growth. Thus, while jellyfish DOM metabolites are C-rich, additional dissolved organic and inorganic N and P metabolites improve the potential for bacterial growth.

How efficiently microbes utilize jellyfish metabolites is central to our understanding of how C is cycled through the microbial loop in jellyfish-dominated systems. This is reflected in changes in bacterial growth efficiency (BGE), the ratio of biomass produced (bacterial production, BP) to substrate assimilated (BP + bacterial respiration, BR) (13). An increase in BGE would suggest improved functioning of the microbial loop with a higher proportion of jellyfish-derived C incorporated into microbial biomass rather than being respired. In contrast, a decrease in BGE suggests inefficient functioning of the
microbial loop resulting from higher respiration of jellyfish-derived C (7). To quantify
the influence of jellyfish on BGE, we measured bacterial production and respiration on
natural, free-living microbial assemblages in closed incubations with and without
jellyfish metabolites. Results were compared to control incubations with natural water or
with natural water plus glucose as an added source of C-rich DOC.

Compared to the natural seawater control, the utilization of jellyfish metabolites by the
free-living microbial community had pronounced effects on microbial metabolism, with
significantly larger increases in single-cell respiration relative to single-cell production
observed within relatively short time periods (6-8 h) (Fig. 2). This pattern was stronger at
higher temperatures (20 and 25°C) where BR increased 82-159% compared to natural
water controls, and was 92-128% higher than BP within jellyfish treatments. At lower
temperatures (14°C), increases in BP and BR were the same because DOC release rates in
*M. leidyi* ctenophores is linked to metabolic activity such that release of DOC is reduced
at lower temperatures (14). As a result of these metabolic disparities, decreases of 10-
15% in BGE at the two higher temperatures were observed in the jellyfish treatments
(Fig. 2). These results not only suggest that zooplankton metabolites are rapidly utilized
for bacterial metabolism, but that metabolites have short residence times in the water
column – on the order of minutes to hours. Interestingly, similar trends between BP, BR,
and BGE were observed in the glucose addition control, suggesting that the quality of
jellyfish DOC is similar to glucose. Bacteria grown in energy and substrate-rich media
(e.g., glucose) often display an uncoupling of catabolism (respiration) from anabolism
(growth), resulting in non-growth energy dissipation (i.e, overflow metabolism) and
decreased BGE (7). This implies decreased efficiency of the microbial loop during jellyfish blooms because a higher proportion of jellyfish metabolites are used for respiration.

We further analyzed how jellyfish impact bacterial metabolism by examining prokaryotic community structure from a summer bacterial dilution experiment using the FISH technique (Table S2). Bacterial phylogenetic structure is important because some bacterial phylotypes are specialized in the uptake of specific organic substrates (15, 16), which ultimately determines bacterial metabolism at the community level (17, 18). Differences in prokaryote assemblages were characterized by comparing community compositional changes after 12 hr between the initial inoculum, a glucose addition (C-rich DOM), and jellyfish and copepod metabolite treatments, to assess the quality of jellyfish DOM (Fig 3). We observed consistent patterns within all treatments that promoted growth of resource-specific phylotypes from within the natural microbial assemblage. In particular, we observed high growth of \(\gamma\)-proteobacteria in the jellyfish and glucose treatments and high growth of Bacteroidetes in the copepod excretia treatment (Fig. 3).

The emergence of these phylotypes from within the natural microbial assemblage is indicative of differences in substrate quality combined with the capacity of specific phylogenetic groups to utilize various DOM molecules (9, 15, 16). Bacteroidetes bacteria have a high affinity for high molecular weight compounds (16); we suggest growth of these bacteria was due to efficient processing of high-quality DON compounds.
in the copepod excretia (19). In contrast, \(\gamma\)-proteobacteria specialize in uptake of poor-quality, low-molecular weight compounds (e.g., glucose) (9, 16). These results indicate jellyfish can greatly influence microbial community structure, forcing changes in metabolism and C flows within the microbial loop. As jellyfish consume high copepod biomass, a major source of high quality substrate (copepod excretia) for efficient bacterial growth will be reduced during summer jellyfish blooms (2). Instead, jellyfish repackage and release copepod biomass as low quality DOM that favors growth of opportunistic bacterial groups, such as \(\gamma\)-proteobacteria. We suggest that high growth rates of \(\gamma\)-proteobacteria relative to other dominant phylotypes (e.g., \(\alpha\)-proteobacteria) (20) combined with uptake of energy-rich jellyfish DOM stimulates rapid increases in bacterial respiration that leads to overall decreases in bacterial growth efficiencies in jellyfish-dominated systems (7).

Lastly, we evaluated the impact of jellyfish blooms on bacterial metabolism in situ by calculating daily amounts of Mnemiopsis- and Chrysaora-derived DOC respired or incorporated into biomass (BP) by bacterial communities in the York River estuary (Table 1). Our estimates of increased bacterial C respiration and production due to utilization of jellyfish DOC indicate that jellyfish blooms in the York River can significantly influence C transfer within the microbial food web. The potential for jellyfish blooms to fuel BR was high during peaks in jellyfish biomass, with substantial increases in bacterial respiration for \(M.\) leidy during June-July (up to 31 mg C m\(^{-3}\) day\(^{-1}\)) and for \(C.\) quinquecirrha during July-August (up to 60 mg C m\(^{-3}\) day\(^{-1}\)) (Table 1). In contrast, smaller increases in BP were estimated during blooms of both jellyfish species
Comparisons of increases in BR with total *in situ* rates of BP indicate up to 32-45% of *M. leidy* and 34-73% of *C. quinquecirrha* DOC released during blooms is respired by microbes as CO$_2$. This high proportion of respired material has implications for C transfer within jellyfish-dominated food webs and implies that jellyfish metabolites are 'junk DOM', because it results in large quantities of C being shunted into CO$_2$, rather then being potentially reincorporated into food webs (Fig. 4).

The jellyfish-induced changes in BGE were not solely caused by increases in BR but rather due to higher bacterial C consumption by microbial communities (i.e., higher BR relative to BP), thus there is evidence that jellyfish blooms play important roles in sustaining BP in the York River estuary. The current paradigm is that primary producer (phytoplankton) exudates support the bulk of bacterial growth in estuarine and marine systems; however, studies comparing primary production with net BP exhibit high variability (7, 13, 21). Likewise, a weak but significant correlation between phytoplankton biomass (Chl-a) and BP exists in the York River suggesting alternative DOM sources must fuel microbial growth (Fig. S2). Our calculations of increases in BP for the York River indicate that high jellyfish biomass can support a relatively large proportion of BP during summer (*Table 1*). Specifically, the release of DOM by *M. leidy* and *C. quinquecirrha* populations were estimated to support up to 20% (43 mg C m$^{-3}$ day$^{-1}$) and 33% (27 mg C m$^{-3}$ day$^{-1}$) of BP during times of high biomass in the York River. Thus DOC production by jellyfish, coupled with simultaneous release of inorganic N and P, could be an important source of C for bacteria in the summertime
Chesapeake Bay, when there is high metabolic C demand, due to increased temperatures (22), and C is being drawn from a DOC pool that is predominantly refractory (10).

Our results demonstrate that the jellyfish-bacteria link is an important alternative C pathway for bacterioplankton production within jellyfish-dominated systems. The potential for this jellyfish-derived bacterial C to be reincorporated into the planktonic food web will initially be a function of the response of viruses and grazing by heterotrophic nanoflagellates (Fig. 4) (23, 24). A major impact of viruses would be to mediate return of jellyfish-derived C to bulk DOC pools via lysis of bacterial cells (25). This process may be beneficial for bacteria during jellyfish blooms by increasing labile DOM pools of preformed compounds that support higher growth efficiencies (6, 15). In contrast, bacterivory by flagellates would be the primary mechanism to reintroduce jellyfish C into the planktonic food web (12). Increased flagellate biomass may have a 'bottom-up' effect with increases in flagellate predators (e.g., ciliates), and their predators (copepods). As jellyfish also consume ciliates (26, 27) and maintain copepod populations at low levels during summer (2), flagellate C would potentially be re-assimilated into gelatinous biomass. This creates a positive feedback 'jelly loop' that is controlled centrally by jellyfish predation and the pumping of 'junk DOM' through the microbial loop (Fig. 4). Assimilation of new prey by jellyfish continually expands the size of C pools trapped within the 'jelly loop'. In terms of C flows, the importance of microbial respiration in this cycle and strong links between jellyfish and bacterial metabolism are further emphasized, because BR acts as a valve that releases C from the loop in the form of CO₂ that prevents direct re-sequestration by jellyfish and heterotrophic microbes.
Ultimately, these jellyfish-mediated processes will further impact fisheries production by shunting C flows away from higher trophic levels. With anticipated further increases in jellyfish populations in the coming decades, there are important environmental, societal, and economic implications of this jellyfish-bacteria link for ecosystem functioning and fisheries production that need to be urgently addressed.
References


Fig 1. Weight-specific release rates of dissolved organic carbon (DOC), nitrogen (DON), phosphorus (DOP), and inorganic N (ammonium, NH$_4^+$) and P (phosphate, PO$_4^{3-}$) by (left) *Mnemiopsis leidyi* ctenophores at 20°C and 25°C, and (right) *Chrysaora quinquecirrha* medusae at 27°C. * = significantly higher values, ANOVA, $p < 0.05$. Error bars are ± 1 standard error.
**Mnemiopsis**

![Bar chart showing DOC or N excretion for Mnemiopsis at different temperatures (20°C and 25°C).](chart)

**Chrysaora**

![Bar chart showing P excretion for Chrysaora at 27°C.](chart)
Fig 2. Results from 6-hour closed bacterial incubations conducted at 14°C, 20°C and 25°C measuring changes in cell-specific bacterial production (BP), respiration (BR) and bacterial growth efficiencies (BGE) of free-living communities in treatments with added glucose (+Glu) and added metabolites from *Mnemiopsis leidyi* (Mnem) and *Chrysaora quinquecirrha* (Chry) relative to natural water controls (NWC). Mnem and Chry treatments were prepared by incubating *M. leidyi* ctenophores or *C. quinquecirrha* medusae with microbial communities prior to incubations for six hours. Results from treatments were compared to controls using ANOVAs. Vertical bars in left panels represent increases in BP and BR after 6-hours in +Glu, Mnem and Chry compared to relative changes in NWC containers. Vertical bars in right panels are mean BGE values.

\[ \text{BGE (\%)} = \frac{\text{BP}}{\text{BP} + \text{BR}} \times 100 \] following del Giorgio & Cole (13). Treatment and controls connected by the same letter are not significantly different \((p < 0.05)\), where \(A > B\). Error bars are ± 1 standard deviation. \text{nd} = \text{not determined.}
Fig 3. Bacterial phylotypes and the domain archaea in the natural bacterial inoculum (Inoc) and proportional changes in these phylotypes after 12 hours between the live *Mnemiopsis* (Live Mnem) and incubations with metabolites from *Mnemiopsis* ctenophores (Mnem), *Chrysaora* medusae (Chry) and copepods (Cope), and a glucose addition control (+Glu) in the bacterial dilution experiment (25°C). Phylotypes were detected with the fluorescent *in situ* hybridization (FISH) technique using oligonucleotide probes labeled with CY3 (see Table S2). FISH results from natural water controls are not included because there was a decrease in bacterial cells in these containers. Treatment and controls connected by the same letter are not significantly different ($p < 0.05$) where $A > B > C$ and $D > E > F$ with respect to increases in cell counts of $\gamma$-proteobacteria and *Bacteroidetes*.
Treatment

% Change in bacterial phylotypes after 12 hours

- Inoc
- +Glu
- Live Mnem
- Mnem
- Chry
- Cope

Legend:
- α-proteobacteria
- β-proteobacteria
- γ-proteobacteria
- Bacteroidetes
- Planctomycetales
- Archaea
**Fig. 4.** Suggested changes in carbon (C) pathways within the planktonic food web (a) before and (b) after (present time) increases in jellyfish blooms. Green arrows indicate flows reincorporating C into the planktonic food web and potential transfer to higher trophic levels. Red arrows signify C pathways impacted by jellyfish. Size of arrow indicates relative magnitude of C flow. The direct link and increased influence of jellyfish and microbial pathways are emphasized by (1) the shunting of C away from fish production, (2) the conversion of C into jellyfish biomass and subsequent release in metabolites as ‘junk’ dissolved organic matter (DOM), and (3) utilization of jellyfish material for bacterial metabolism, especially respiration. The ‘jelly loop’ involves the cycling of C between jellyfish, bacteria, heterotrophic nanoflagellates (HNF) and ciliates.
a  Before

Phytoplankton → Copepods → Jellyfish → Fish

Ciliates → Bacteria

MICROBIAL LOOP

HNF

Lysis
DOM

Viruses

b  Present

Phytoplankton → Copepods → Jellyfish

Ciliates → Bacteria

Junk DOM

MICROBIAL LOOP LINKED TO 'JELLY LOOP'

HNF

Lysis
DOM

Viruses

Respiration
Table 1. Physical and biological properties of the York River and *Mnemiopsis leidyi* and *Chrysaora quinquecirrha* blooms. Temp is temperature, Chl-\(a\) is chlorophyll-\(a\), BP\(_b\) is bulk bacterial production of unfiltered water, DOC is dissolved organic carbon, POC is particulate organic carbon and, BR and BP are increases in respiration and production by bacterial populations due to uptake of jellyfish metabolites. DOC production by jellyfish blooms was determined by multiplying daily weight specific DOC release rates (see Fig. 1) by biomass of *Mnemiopsis leidyi* and *Chrysaora quinquecirrha* populations (see Fig. S1). Increased BR and BP due to utilization of jellyfish DOC were based on differences in cell-specific BR and BP rates between natural water controls and both jellyfish treatments normalized to DOC released by jellyfish over the experiment (Fig. 2). Final calculations of BR and BP by bacterial populations were determined by multiplying DOC normalized single-cell metabolism by *in situ* bacterial abundances (Cells m\(^{-3}\)) and then by total daily amounts of DOC released by *M. leidyi* and *C. quinquecirrha* populations.

Bacterial metabolism data presented as monthly mean values ± 1 standard deviation. Maximum values are in parentheses. % BP\(_b\) are comparisons between increases in BR and BP with *in situ* BP\(_b\) measurements and calculated as follows: % BP\(_b\) = (BR or BP / BP\(_b\)) * 100.
Table 1.

<table>
<thead>
<tr>
<th>Month</th>
<th>Temp. (°C)</th>
<th>Salinity</th>
<th>Chl-a (µg l⁻¹)</th>
<th>BP₃ (mg C m⁻³ day⁻¹)</th>
<th>Bulk DOC (g C m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>16.4 - 25.1</td>
<td>13 - 20</td>
<td>3.2 - 8.5</td>
<td>7.1 - 40.8</td>
<td>2.8 - 3.4</td>
</tr>
<tr>
<td>June</td>
<td>20.2 - 29.1</td>
<td>9 - 21</td>
<td>4.9 - 24.3</td>
<td>40.2 - 319</td>
<td>2.1 - 3.6</td>
</tr>
<tr>
<td>July</td>
<td>27.2 - 29.7</td>
<td>15 - 26</td>
<td>4.1 - 24.3</td>
<td>81.7 - 231</td>
<td>2.5 - 3.5</td>
</tr>
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<td>August</td>
<td>26.5 - 31.2</td>
<td>14 - 22</td>
<td>8.3 - 29.9</td>
<td>10.4 - 281</td>
<td>2.6 - 5.5</td>
</tr>
<tr>
<td>Sept</td>
<td>26.7 - 27.2</td>
<td>19 - 21</td>
<td>5.1 - 22.7</td>
<td>60.7 - 160</td>
<td>2.9 - 6.0</td>
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**Physical and biological properties during jellyfish blooms**

<table>
<thead>
<tr>
<th>Month</th>
<th>Mnemiopsis blooms</th>
<th>Chrysaora blooms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POC Biomass (mg m⁻³)</td>
<td>DOC production (mg C m⁻³ day⁻¹)</td>
</tr>
<tr>
<td>May</td>
<td>18.7 ± 9.8 (43.6)</td>
<td>5.2 ± 11.1 (32.3)</td>
</tr>
<tr>
<td>June</td>
<td>19.2 ± 18.2 (62.1)</td>
<td>4.6 ± 5.2 (20.7)</td>
</tr>
<tr>
<td>July</td>
<td>5.2 ± 4.7 (16.4)</td>
<td>1.2 ± 2.7 (8.4)</td>
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<tr>
<td>August</td>
<td>3.5 ± 6.6 (99.8)</td>
<td>1.4 ± 5.3 (42.0)</td>
</tr>
<tr>
<td>Sept</td>
<td>0.2 ± 0.4 (0.8)</td>
<td>0.01 ± 0.02 (0.1)</td>
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</tbody>
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**Bacterial metabolism increases associated with jellyfish DOC production**

<table>
<thead>
<tr>
<th>Month</th>
<th>BR</th>
<th>% BR of BP₃</th>
<th>BR</th>
<th>% BR of BP₃</th>
</tr>
</thead>
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<tr>
<td></td>
<td>(mg C m⁻³ day⁻¹)</td>
<td>(mg C m⁻³ day⁻¹)</td>
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<td>(mg C m⁻³ day⁻¹)</td>
</tr>
<tr>
<td>May</td>
<td>1.2 ± 1.3 (3.0)</td>
<td>9.2 ± 12.5 (32.1)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>June</td>
<td>13.0 ± 21.2 (96.3)</td>
<td>12.7 ± 14.1 (45.2)</td>
<td>0.1 ± 0.3 (1.2)</td>
<td>0.1 ± 0.5 (2.4)</td>
</tr>
<tr>
<td>July</td>
<td>4.9 ± 9.5 (30.7)</td>
<td>3.7 ± 6.7 (18.7)</td>
<td>10.1 ± 19.1 (60.1)</td>
<td>7.2 ± 13.1 (34.2)</td>
</tr>
<tr>
<td>August</td>
<td>0.8 ± 1.8 (7.1)</td>
<td>0.5 ± 0.7 (2.7)</td>
<td>7.3 ± 15.1 (54.6)</td>
<td>9.4 ± 19.8 (73.1)</td>
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<tr>
<td>Sept</td>
<td>0.1 ± 0.1 (0.3)</td>
<td>0.1 ± 0.1 (0.2)</td>
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</table>

<table>
<thead>
<tr>
<th>Month</th>
<th>BR</th>
<th>% BR of BP₃</th>
<th>BR</th>
<th>% BR of BP₃</th>
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<tbody>
<tr>
<td></td>
<td>(mg C m⁻³ day⁻¹)</td>
<td>(mg C m⁻³ day⁻¹)</td>
<td></td>
<td>(mg C m⁻³ day⁻¹)</td>
</tr>
<tr>
<td>May</td>
<td>0.6 ± 0.6 (1.4)</td>
<td>4.2 ± 5.6 (14.5)</td>
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<td>0</td>
</tr>
<tr>
<td>June</td>
<td>5.9 ± 9.6 (43.4)</td>
<td>5.7 ± 6.4 (20.4)</td>
<td>0.1 ± 0.1 (0.5)</td>
<td>0.1 ± 0.2 (1.1)</td>
</tr>
<tr>
<td>July</td>
<td>2.2 ± 4.3 (13.9)</td>
<td>1.7 ± 3.0 (8.4)</td>
<td>4.6 ± 8.7 (27.4)</td>
<td>3.3 ± 6.0 (15.6)</td>
</tr>
<tr>
<td>August</td>
<td>0.3 ± 0.8 (3.2)</td>
<td>0.2 ± 0.3 (1.2)</td>
<td>3.3 ± 6.9 (24.9)</td>
<td>4.3 ± 9.0 (33.4)</td>
</tr>
<tr>
<td>Sept</td>
<td>0.1 ± 0.0 (0.3)</td>
<td>0.1 ± 0.1 (0.2)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Supporting Online Material

Materials and methods

Collection and preparation of zooplankton for experiments

*Chrysaora quinquecirrha* medusae were collected by dipnet or in 20 L buckets (for larger animals) from surface waters. *Mnemiopsis* ctenophores were collected during 30 second, gentle plankton tows using 200 μm mesh net and a non-filtering cod end. Upon collection, jellyfish were immediately transported to the laboratory and incubated with field-collected copepod prey at *in situ* temperature for 30 minutes. Prior to experimentation, animals were gently individually transferred to 20 L buckets filled with 0.2 μm filtered York River water for 15 minutes. This step rinsed the animals and provided them time to clear their guts, reducing potential confounding effects of sloppy feeding and leaching of dissolved organic matter (DOM) from fecal material during the experiments.

Jellyfish DOM and inorganic release experiments

We conducted five laboratory experiments between May and August 2003-2007 to determine the simultaneous release rates of DOM and inorganic nutrients by *M. leidyi* ctenophores and *C. quinquecirrha* scyphomedusae in the York River estuary. For each experiment, individual animals were incubated in the dark at 20 or 25°C for 4-12 h in 1.2
L (for *M. leidyi*) or 4 L (for *C. quinquecirrha*) acid-cleaned polycarbonate containers filled with 0.2 μm filtered (Nucleopore® polycarbonate) low-nutrient Sargasso seawater diluted with Nanopure Diamond (Barnstead®) water to *in situ* York River salinity (20-22 psu). The low DOM content (e.g., 40-50 μM DOC) in the experimental media reduced methodological error and improved precision of DOM measurements. At the start of the experiment, one jellyfish was randomly added to each experimental container (treatment) and the release of DOM and inorganic N and P determined by measuring changes in DOC, DON, DOP and inorganic constituents (nitrite [NO$_2^-$], nitrate [NO$_3^-$], NH$_4^+$ and PO$_4^{3-}$) in the water after 3-4 hours. Experimental controls consisted of chambers absent of jellyfish, although the addition of jellyfish was mimicked. These data were used to correct for the small addition of nutrients associated with transferring jellyfish into each chamber. At the completion of the experiment, jellyfish were removed and their wet and dry weights, and elemental composition determined according to Condon & Steinberg (1).

DOM and inorganic release rates were expressed as a function of body mass according to the allometric equation:

\[ Y = a_1 W^b \]  

where \( Y \) is the release rate of organic or inorganic metabolite (μmol ind$^{-1}$ h$^{-1}$), \( W \) is the dry weight (g DW), \( b \) is the exponent relating excretion to body mass, and \( a \) are constants (2, 3). Release rates were further characterized by comparing C, N, and P ratios of
released DOM and inorganic nutrients between the two gelatinous species and to the canonical Redfield ratio (106C:16N:1P). DOM and inorganic excretion rates were normalized to jellyfish dry weight (μmol g DW$^{-1}$ h$^{-1}$) allowing comparison between jellyfish species.

The possible influence of bacterial uptake of jellyfish DOM metabolites on measured excretion rates were investigated by measuring bacterial production (BP) in a subset of excretion chambers. Using a bacterial growth efficiency of (BGE) of 30% (Fig. 2), these measurements suggest that our DOM release rates were slightly underestimated with bacteria potentially utilizing between 1-13% of DOC released by jellyfish during incubations, and thus we do not correct for bacterial uptake.

**Objectives for bacteria experiments**

The goal of these experiments was to assess the lability (defined here as utilization by bacteria within hours) of jellyfish exudates and to determine whether the production and subsequent release of DOM by jellyfish blooms significantly impacts microbial metabolic processes, specifically bacterial production and respiration. To this degree, we were also interested in analyzing whether jellyfish control bacterial community assemblages because some bacterial phylotypes are specialized in the uptake of specific organic substrates, and changes in phylogenetic composition may impact metabolism at the community level (4-6). To achieve these objectives we conducted two types of laboratory experiments: bacterial dilution and bacterial growth efficiency.
Bacterial dilution experiments

We conducted a bacterial dilution experiment comparing exponential growth of free-living natural bacterial assemblages in exposed to zooplankton metabolites (treatments), natural *in situ* water (York River control), and labile DOM substrate (labile DOC control) during summer (25°C), 2005 (*Table S1*). Free-living bacteria were isolated from York River surface waters (0-2 m) via filtration through 1.2 μm Millipore AP15 glass fiber (GF) filters using a peristaltic pump (7). 1.5 L of filtrate was inoculated to acid cleaned polycarbonate carboys containing 6 L of grazer-free treatment or control water (8), and prokaryote growth monitored over 3 days. Every 12 h, samples for bacterial abundance (BA), BP, DOM (DOC, DON, DOP), dissolved inorganic N (NO₂⁻, NO₃⁻, NH₄⁺) and P (PO₄³⁻) were taken from each of three replicate treatment and control containers. BP and BA normalized to nutrient concentrations were used as a proxy for bacterial growth.

Treatment and control media were prepared as follows. York River control water was collected from surface waters (0-2 m), and filtered through 142 mm 1.2μm GF and 0.2 μm polycarbonate filters, respectively. Jellyfish metabolite media was prepared by incubating eight *M. leidy* (*Mnemiopsis treatment*) or one *C. quinquecirrha* (*Chrysaora treatment, summer only*) in separate 61 acid cleaned, polycarbonate containers filled with low nutrient Nanopure diluted 0.2 μm filtered Sargasso seawater. After 12 h, jellyfish were removed for biometric and chemical analysis. For copepod excretia treatments, copepods were concentrated in 0.2 μm filtered York River water using back filtration (9),
and transferred to 5 l acid-washed polycarbonate containers filled with 0.2 μm filtered Sargasso Sea water diluted with Nanopure water to in situ salinity. After 12 h, copepods were collected in 200 μm acid-washed sieves and copepod excretia filtrate retained for experiments. Copepod samples were fixed in 4% buffered formaldehyde and enumerated under the light microscope. Water containing jellyfish or copepod derived DOM was screened through acid rinsed 100 μm nitex mesh and gravity filtered through autoclaved Whatman Polygap 0.2 μm membrane filtration cartridges (8). Cartridges were acid rinsed and flushed thoroughly with 0.45 μm filtered Nanopure water prior to filtration. Low amount of particulates retained on the mesh screen indicated egestion was minimal during incubations and the potential contribution to zooplankton derived DOM pools via sloppy feeding and leaching of fecal material was low.

As incubation water was non-axenic and post incubation filtration removed colloidal organic substrate (e.g., mucus), a portion of DOM released by jellyfish was potentially utilized by prokaryotes before the experiment. To evaluate this effect ‘live jellyfish’ treatments were included, whereby same numbers of M. leidyi (live Mnemiopsis) or C. quinquecirrha (live Chrysaora) were incubated in bacteria inoculated York River control water for the first 12 h of the experiment (i.e., same jellyfish biomass and time period for metabolite incubations). Because the water was not refiltered following removal of jellyfish, carboys were inspected and particulates removed using sterile pipette tips.

To evaluate the lability of DOM released by jellyfish, we compared metabolite and live treatments to a nutrient enrichment (labile DOC) control. Nutrient lability controls
consisted of Nanopure water diluted Sargasso Sea water with added glucose ($C_6H_{12}O_6$, 30 µM final conc.), $NH_4^+$ (1.5 µM) and $PO_4^{3-}$ (0.75 µM). Glucose was chosen as a labile DOC control because it is the most abundant neutral sugar in the ocean, and it is preferentially utilized by bacteria relative to other monosaccharides (10).

**Bacterial growth efficiency experiments**

To further analyze direct linkages between jellyfish and bacterial communities, we designed experiments to examine how the production of jellyfish DOM influences BP (anabolism), bacterial respiration (BR, catabolism) and bacterial growth efficiencies (BGE), and C pathways through the microbial loop. Experiments consisted of measuring metabolic differences in free-living bacterial assemblages exposed to jellyfish metabolites (treatment), natural conditions (York River control), or added glucose (labile DOC control) during summer 2006 (July, 20°C and 25°C) and spring 2007 (May, 14°C and 20°C).

Preparation of treatments and controls occurred as follows. York River surface water (0-2 m) was filtered through 1.2 µm AP15 GF filters using peristaltic pumps and acid-washed tubing and 10 l of filtrate (York River control) was added to individual acid-washed buckets. Jellyfish treatments were prepared by incubating either 10 *M. leidyi* ctenophores (*Mnemiopsis* treatment) or one *C. quinquecirrha* medusa (*Chrysaora* treatment, summer only) in control water. For labile DOC controls, York River control water was enriched with the same concentrations of glucose and inorganic N and P as in the BRG
experiments. During treatment and control preparation, all buckets were maintained in
dark environmental chambers for 6 h and aerated to ensure aerobic conditions. After
preparation, jellyfish were removed from treatments, and experimental water was
screened through acid-washed 100 μm nitex sieves before being added to three replicate
experimental incubation setups. A description of the incubation setups are detailed in del
Giorgio and Bouvier (11). Briefly, experimental water was used to fill two 4 l acid-
washed Erlenmeyer flasks. One flask (reservoir) was placed on a stand and was
connected to the other sample flask below by acid-washed, conditioned Teflon tubing
that enabled flow between the sampling vessels. The sample flask was sealed airtight
with an acid-washed rubber stopper fitted with two glass tubes: one linked to the
reservoir via Teflon tubing, the other represented a sample port connected also by a
length of Teflon tubing. A pinch-valve with an adjustable screw at the end of the sample
port controlled the flow of water from the sample flask. Samples for BA, BP, BR, DOM
and inorganic nutrients were taken at the start of treatment preparation, and during
incubations at the initial (t0), mid- (t3, bacteria only) and final (t6) timepoints.

Field surveys

We combined data from laboratory experiments with field surveys to evaluate the
contributions by jellyfish blooms on C cycling within the microbial loop. Field surveys
were conducted during 2004-2006 along a salinity gradient in the lower York River to
collect the relevant biological, chemical and physical parameters from the field, including
species composition and biomass of jellyfish and mesozooplankton, bacterial community
dynamics (BA, BP), DOM (C, N, and P) and inorganic nutrients, chlorophyll-\(\alpha\) (Chl-\(\alpha\)) and physical properties (e.g., temperature). Descriptions of collection and analytical protocols employed for jellyfish, mesozooplankton and chl-\(\alpha\), and station descriptions are detailed in Condon and Steinberg (1). Samples for bacterial and nutrient parameters were determined from surface water (0-2 m) collected in 2 L dark, acid-washed polycarbonate bottles.

**Microbiological analyses**

Methodology for determining BA, BP, BR, and community composition are described in detail elsewhere (5, 12-14). Briefly, 10 ml BA samples were fixed in 0.2 \(\mu\)m filtered molecular grade formaldehyde (2% final conc.) buffered with sodium borate and stored in a -80°C freezer. For bacterial counts, fixed cells were stained using SYTO-13 (Invitrogen Molecular Probes, S7575) green fluorescence nucleic acid stain (13, 15) and enumerated using a Coulter Epics Altra or a Becton Dickinson FACScalibur flow cytometer, both equipped with 488 nm argon lasers. Prior to counting, calibrated 1.1 \(\mu\)m microsphere bead stock (Molecular Probes, Cat. No. F8888) was added to each of 3 sub-sample vials and total bead counts used to determine BA (no. bacteria mL\(^{-1}\)). BP (\(\mu\)g C L\(^{-1}\) h\(^{-1}\)) was measured in triplicate 1.5 ml sub-samples using the \(^{3}\)H-leucine incorporation technique (12), integrating modifications in Pace *et al.* (16) and assuming a C conversion factor of 3.1 kg C mol leu\(^{-1}\) (17). For field samples, BA and BP were determined on whole (bulk) and filtered (< 1.2 \(\mu\)m) bacterial fractions. BR (\(\mu\)g C L\(^{-1}\) h\(^{-1}\)) was determined by quantifying the decline in oxygen (O\(_2\)) over the course of closed
incubations (18). O$_2$ samples were collected in airtight 7 ml glass stoppered vials (Chemglass), fixed with 2 µl 0.12M HgCl$_2$, and concentrations (µg O$_2$ L$^{-1}$ h$^{-1}$) determined using membrane-inlet mass spectrometry (14). O$_2$ uptake was transformed to C respired assuming an atomic weight C: O$_2$ conversion factor of 0.375. Total bacterial carbon demand (BCD) was determined through the addition of BP and BR measurements. Bacterial growth efficiency (BGE) was determined as the ratio of BP and BCD (BGE = BP/(BP + BR)) (19). To compare relative changes in bacterial metabolism across treatments it was necessary to normalize bulk BP and BR rates to a single-cell basis (µg C cell$^{-1}$ h$^{-1}$) by dividing each parameter by BA (cells l$^{-1}$).

**Phylogenetic diversity**

To analyze microbial community structure and responses of various prokaryote phylotypes to jellyfish DOM we employed the fluorescent *in situ* hybridization (FISH) technique using oligonucleotide probes conjugated with CY3 (20). The FISH probes targeted specifically the domain *bacteria* (EUB338), including five of its main phylogenetic groups (the alpha- (ALF1b), beta- (BET42a), gamma-proteobacteria (GAM42a), *Bacteroidetes* (CF319a), and the *Planctomycetales* (PLA886) lineage), as well as the domain *archaea* (ARCH915) (*Table S3*). The hybridization methodology we adopted in this study is detailed in Bouvier and del Giorgio (5, 20), except that samples were generated from BA samples that were stored in a -80°C freezer before FISH sample preparation, filters were hybridized for five hours, concentrated stock of DAPI stain was added to the Citifluor mounting solution (Ted Pella Inc., London, UK), and hybridized
slides were kept in the dark at -20°C for 1-2 days prior to counting. FISH counts were made on a Zeiss Axiophot epifluorescence microscope with an external Hg light source under green light excitation. To correct for autofluorescing and non-target cells, we subtracted counts from the control probe (NON338) (Table S3) from all prokaryote probe counts. With the exception of the live Chrysaora treatment (bacteria experiment #2, data excluded from analyses), percentages of fluorescing cells using the control probe was low, averaging < 0.5% of the cells counted with the EUB338 probe. To check for hybridization efficiency (i.e., ratio of counted hybridized cells and actual bacterial cells), EUB338 counts were compared to total bacteria counts made on the same filter with DAPI staining under UV light. Efficiencies ranged 24 (low nutrient control, bacteria experiment 1) -117% (copepod excretia treatment, bacteria experiment 1) which is within the range of literature values (21), but percentages were typically > 60%.

**Chemical analyses**

Water subsampled from incubation bottles was filtered through pre-combusted (500°C) Whatman GF/F filters, and dissolved nutrients determined from the filtrate. DOC concentrations were measured via high temperature combustion on a Shimadzu 5000A Total Organic Carbon (TOC) analyzer using potassium hydrogen phthalate (C₈H₅O₄K) standard (22). Prior to combustion, 6N HCl was added to 5 mL sample (pH < 3) and sparged for two minutes with ultra free air to ensure removal of dissolved inorganic C. DOC concentrations were based on the best three of a maximum of five column injections within an analytical detection error set to a peak area standard deviation ±120
or coefficient of variance of 0.8%. Samples with ±1.5 μM error were reanalyzed. In addition, data precision, instrument accuracy and platinum catalyst efficiency were quality checked with low C (1-2 μM DOC) and deep Sargasso Sea water (44-46 μM DOC) reference standards provided by the carbon reference material program, University of Miami (http://www.rsmas.miami.edu/groups/biogeochem/CRM.html) (23).

Total dissolved N (TDN) and P (TDP) were measured by persulfate oxidation (23, 24), NO$_2^-$ and NO$_3^-$ were measured by the spongy cadmium (Cd) method, and PO$_4^{3-}$ was measured by the molybdate (25) method on a Lachat QuikChem 8500 nutrient autoanalyzer. During analysis, the conversion of NO$_3^-$ to NO$_2^-$ by Cd catalyst was monitored and columns regenerated if reduction efficiency was < 97%. The efficiency of the persulfate reagent to convert organic N and P to respective inorganic constituents was evaluated using 10 μM urea (CH$_4$N$_2$O) and 10 μM glucose-6-phosphate (C$_6$H$_{12}$O$_9$PNa) standards for DON and DOP, respectively. NH$_4^+$ was measured on a Shimadzu UV-1601 spectrophotometer by manual hypochlorite method (25) using standards curves corrected for sample salinity. DON and DOP were determined by calculating the difference between total dissolved and inorganic fractions for N (TDN - Σ [NO$_3^-$ + NO$_2^-$ + NH$_4^+$]) and P (TDP - Σ [PO$_4^{3-}$]), respectively (23).

Particulate organic C content of pre-weighed sub samples of ground, dried jellyfish was measured on a Carlo Erbra EA-1108 CHN Elemental Analyzer (I).
Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and two-sample t-tests using Minitab statistical software (level of significance of $\alpha < 0.05$). If ANOVA’s were significant, post hoc pairwise comparison of means using Tukey’s HSD tests were performed (26). Prior to analysis, data were checked for normality and homogeneity of variance using Kolmogorov-Smirnov tests, box plots and histograms of data and residuals, and non-conforming data were improved through $\log_{10}$, or square- or fourth root transformations (26).
References


Fig. S1. Biomass of (blue) *Mnemiopsis leidyi* ctenophores and (red) *Chrysaora quinquecirrha* scyphomedusae in the lower York River estuary, USA, between 2003-2006. Values presented as mean values of replicate double-oblique plankton tows in surface waters (0-2 m). Error bars are ± 1 standard deviation.
**Fig. S2.** Linear regression of chlorophyll-α biomass and rates of bacterial production in surface waters (0-2 m) the York River. Data obtained from field surveys conducted between 2003-2006. Regression statistics are based on log-transformed data.
$BP_b = 1.20 \text{Chl} - 0.88$

$r^2 = 0.32, p < 0.001$
Tables

**Table S1.** Starting physical and biological conditions for bacterial dilution experiments.

BMD = below minimum detection. Error bars represent ± 1 standard deviation.

<table>
<thead>
<tr>
<th>Summer dilution experiment</th>
<th>DOC</th>
<th>DON</th>
<th>DOP</th>
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<tr>
<td><strong>Experiment dates</strong></td>
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</tr>
<tr>
<td></td>
<td>16 July to 20 July, 2005</td>
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<tr>
<td>Temperature (°C)</td>
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</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>Salinity</td>
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<td>BA inoculum (Cells x 10⁶ ml⁻¹)</td>
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<tr>
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<th>DOC</th>
<th>DON</th>
<th>DOP</th>
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<td>York River control</td>
<td>234.7 ± 1.2</td>
<td>15.8 ± 0.1</td>
<td>0.21 ± 0.18</td>
</tr>
<tr>
<td>Labile DOC control</td>
<td>465.5 ± 6.1</td>
<td>BMD</td>
<td>0.28 ± 0.20</td>
</tr>
<tr>
<td>Low nutrient</td>
<td>86.3 ± 2.6</td>
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<td>0.14 ± 0.06</td>
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<td><em>Mnemiopsis</em></td>
<td>104.8 ± 1.0</td>
<td>3.9 ± 3.1</td>
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<td>metabolites</td>
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<tr>
<td>Copepod excretia</td>
<td>131.6 ± 7.5</td>
<td>6.4 ± 0.9</td>
<td>0.18 ± 0.03</td>
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<tr>
<td><em>Chrysaora</em></td>
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<tr>
<td>Live <em>Mnemiopsis</em></td>
<td>256.5 ± 7.9</td>
<td>20.8 ± 3.4</td>
<td>BMD</td>
</tr>
<tr>
<td>Live <em>Chrysaora</em></td>
<td>245.9 ± 6.9</td>
<td>14.4 ± 1.1</td>
<td>BMD</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>NOₓ</td>
<td>NH₄⁺</td>
<td>PO₄³⁻</td>
</tr>
<tr>
<td>York River control</td>
<td>16.7 ± 0.5</td>
<td>3.2 ± 1.0</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>Labile DOC control</td>
<td>16.2 ± 0.1</td>
<td>3.8 ± 0.9</td>
<td>1.61 ± 0.04</td>
</tr>
<tr>
<td>Low nutrient</td>
<td>0.4 ± 0.1</td>
<td>2.4 ± 0.0</td>
<td>BMD</td>
</tr>
<tr>
<td><em>Mnemiopsis</em></td>
<td>0.9 ± 0.0</td>
<td>11.6 ± 0.5</td>
<td>0.33 ± 0.02</td>
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<td>metabolites</td>
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<tr>
<td>Copepod excretia</td>
<td>1.0 ± 0.4</td>
<td>3.7 ± 1.2</td>
<td>BMD</td>
</tr>
<tr>
<td><em>Chrysaora</em></td>
<td>1.7 ± 0.2</td>
<td>40.4 ± 1.5</td>
<td>1.90 ± 0.13</td>
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<tr>
<td>Live <em>Mnemiopsis</em></td>
<td>15.2 ± 1.4</td>
<td>20.1 ± 1.9</td>
<td>1.73 ± 0.11</td>
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<td>11.9 ± 0.2</td>
<td>39.8 ± 8.0</td>
<td>2.66 ± 1.10</td>
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Table S2. Oligonucleotide probes used in this study. rRNA position based on *Escherichia coli* numbering (20). %FA = Percentage of formamide in hybridization buffer. IS: ionic strength (NaCl, M).

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<tr>
<th>Probe</th>
<th>Target taxa</th>
<th>Probe sequence (5'-3')</th>
<th>rRNA position</th>
<th>%FA</th>
<th>IS</th>
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<tr>
<td>EUB338</td>
<td>Bacteria</td>
<td>GCTGCCTCCCCGTAGGAGT</td>
<td>16S (338-355)</td>
<td>30</td>
<td>102</td>
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<td>NON338</td>
<td>Non-prokaryotes, autofluorescing cells</td>
<td>ACTCCTACGGGAGGCAGC</td>
<td>16S (338-355)</td>
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<td>ALF1b</td>
<td>α-proteobacteria</td>
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<td>BET42a</td>
<td>β-proteobacteria</td>
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<td>23S (1027-1043)</td>
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<td>102</td>
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<td>CF319a</td>
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<td>16S (886-904)</td>
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</table>
CHAPTER 6

Conclusions
Conclusions

Dramatic temporal and spatial increases in gelatinous zooplankton biomass ('blooms') continue to occur in estuarine, coastal, and open ocean environments (Purcell et al. 2007, Condon & Steinberg In review) but little is known about how and why these blooms form or what impact blooms have on carbon (C) cycling. My research demonstrated that *Mnemiopsis leidyi* blooms form when mature ctenophores are released from temperature-limitation in late spring but decline rapidly in early-mid summer because of predation by *Chrysaora quinquecirrha* scyphomedusae (Ch. 2). Examination of long-term records of *M. leidyi* blooms suggest that biomass peaks now appear one month earlier compared to 40 years ago, possibly due to increased surface water temperature in the York River (Ch. 2). The implication of this temporal shift for C cycling is an increased residence time for C assimilated into gelatinous biomass in planktonic food webs because ctenophores are voracious predators on copepods, and represent a trophic 'dead-end' because there are no major predators on gelatinous zooplankton.

Recent studies have suggested that gelatinous zooplankton play important roles in C transfer by shunting C to the microbial loop (Hansson & Norrman 1995, Jackson et al. 2001, Riemann et al. 2006). In particular, Condon & Steinberg (In Review) suggested the enhanced release of dissolved organic matter (DOM) in gelatinous zooplankton metabolites that could fuel bacterial metabolism (Hansson & Norrman 1995, Condon et al. In prep) and provide a mechanism for the reincorporation of C into planktonic food webs (Condon et al. In prep). My laboratory experiments demonstrated that both
*Mnemiopsis* and *Chrysaora* release high amounts of DOC in their metabolites compared to organic N and P (Ch. 3; Ch. 5). In particular, *M. leidyi* ctenophores produced C-rich DOM as reflected in the high molar DOC: DON: DOP ratios of their metabolites (60.3: 5.0: 1) relative to the canonical Redfield ratio (106C:16N:1P) (Ch. 3). The biochemical nature of the DOC released by gelatinous zooplankton is unknown, but C-rich compounds are typically energy rich (Rich et al. 1996, Malmstrom et al. 2005, Carlson et al. 2007). This autochthonous production of DOC by gelatinous zooplankton blooms has implications for microbial dynamics in estuarine and coastal systems through their potential high contribution of utilizable C into bulk DOC pools (Raymond & Bauer 2000, Hansell & Carlson 2002).

The efficiency of bacterioplankton utilization of assimilated jellyfish metabolites is central to understanding how C is cycled through the microbial loop (del Giorgio & Cole 1998). This will depend on numerous factors including (1) how bacteria partition jellyfish metabolites for respiration and production, and (2) the structure of the natural bacterial community assemblage. My dissertation indicates a 10–15% decreases in bacterial growth efficiency (i.e., bacterial production relative to DOM uptake) on jellyfish metabolites during summer compared to filtered estuarine water, indicating a higher proportion of gelatinous zooplankton products are used for bacterial respiration relative to production (Ch. 5). These results clearly demonstrate that the release of DOM by gelatinous zooplankton can have pronounced impacts on C cycling in estuarine systems by mediating C pathways within the ‘microbial loop’.

Determining how jellyfish shape bacterial phylogenetic assemblages is integral to this understanding because some bacterial phylotypes are specialized in the uptake of
specific organic substrates (e.g., Cottrell & Kirchman 2000, Alonso-Sáez & Gasol 2007),
and changes in phylogenetic composition may impact metabolism at the community level
(Bouvier & del Giorgio 2002, del Giorgio & Bouvier 2002). Results from Chapter 4
suggest that jellyfish may cause dramatic shifts in bacterial community composition by
promoting the dominance of ‘opportunistic’ bacterial groups (e.g., *gammaproteobacteria*)
that have high affinities for the DOC released by gelatinous zooplankton and have
potentially higher respiration rates relative to dominant phylotypes. These factors
combined indicate jellyfish blooms may have the ability to reshape the microbial
community structure and the overall functioning of the microbial loop.

The results of this dissertation stress the importance of including gelatinous
zooplankton and specific phylotypic components of the bacterial community assemblages
in models of biogeochemical cycling and bacterial metabolism. In addition, we did not
consider the potential effects of bacterivory by heterotrophic nanoflagellates or viral
lysis, which also play a part in controlling whether gelatinous zooplankton-derived C is
reincorporated into planktonic food webs (Ch. 5, Fig. 4; Weinbauer & Rassoulzadegan
2004, Bouvier & del Giorgio 2007, Zhang et al. 2007). These biological components
should also be considered if we are to fully comprehend the effects of gelatinous
zooplankton blooms on C cycling in planktonic food webs.

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VITA

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Born in Melbourne, Victoria, Australia on May 27, 1974. Graduated from Haileybury College, Australia in 1991. Received a B.Sc. (Hons.) in Marine Zoology and Botany from the University of Melbourne, Vic., Australia in 1997. Worked as a research assistant at the Museum of Victoria and the University of Melbourne for Dr. Mark Norman. Conducted field and laboratory experiments in the cephalopod program. Worked as a senior laboratory technician between 1999 and 2002 at Horn Point Laboratory, University of Maryland Center for Environmental Science for Dr. Jennifer Purcell in the gelatinous zooplankton ecology lab and Dr. Paul del Giorgio in the microbial ecology lab. Entered the Masters program at the Virginia Institute of Marine Science, College of William and Mary in 2002 under graduate advisor Dr. Deborah K. Steinberg and bypassed into the Ph.D. program in 2004.