ABSTRACT

Title: THE EFFECTS OF FRESHWATER FLOW AND GRAZING ON THE PLANKTON COMMUNITY STRUCTURE OF CHESAPEAKE BAY TRIBUTARIES

Matthew L. Reaugh, Master of Science, 2005

Directed By: Professor Michael R. Roman
Marine-Estuarine-Environmental Sciences

Changes in the plankton composition of estuarine systems are often driven by freshwater flow. These changes in species composition and abundance have the potential to affect trophic dynamics within the plankton community. In order to quantify the effects of freshwater flow in estuaries, the structure of the spring plankton community and copepod grazing were examined in an extreme dry (2002) and wet (2003) year in two tributaries of Chesapeake Bay. Increases in phytoplankton and copepod biomass in the wet year were large in comparison to the increase in microzooplankton biomass. Ample abundance of prey and high copepod community grazing potentials indicate that microzooplankton biomass was influenced by strong top-down control in the high flow year. While no evidence of a copepod-microzooplankton-phytoplankton trophic cascade was found, increased top-down control by grazers in combination with increased nutrient supply in wet years may be important in establishing spring phytoplankton blooms in Chesapeake Bay.

THE EFFECTS OF FRESHWATER FLOW AND GRAZING ON THE PLANKTON COMMUNITY STRUCTURE OF CHESAPEAKE BAY TRIBUTARIES

By

Matthew L. Reaugh

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2005

Advisory Committee:
Professor Michael R. Roman, Chair
Professor Diane K. Stoecker
Professor William C. Boicourt

Acknowledgements

I would like to thank my advisor, Dr. Michael Roman, for his guidance and assistance over these past 4 years. My thanks to him for his guidance and support span from my time as an REU intern through my graduate studies. Despite the many demands on his time as Director of Horn Point he has always had an "open-door." I also owe a great deal of thanks to my unofficial co-advisor Dr. Diane Stoecker, for her direction, guidance, and patience through countless revisions and corrections. Thank you also to Dr. William Boicourt for his insight into the physics of estuarine systems. I would like to thank Dr. David Kimmel for all his help with statistical analyses and his quick and insightful reviews of my thesis chapters; Dan Gustafson for all his help with the grazing experiments, carrying the plankton wheel, providing chl *a* and microzooplankton data, and his friendship and company on all the CBL excursions; and Tom Wazniak for providing the CTD data and all his help during field season. Dr. Bruce Haase has been an inspiration and a dear friend. His guidance and thirst for knowledge challenges me to strive for excellence, for this I am ever grateful. This research was funded by the U.S. EPA-Science To Acieve Results program (grant #: R82936601).

I also want to thank my parents for their love, support, prayers, and the provision of my education. The magnitude of which cannot be contained within the bounds of this thesis or degree. Finally, I would like to thank my beloved wife, Tiffany. Her faithful love and support have brought me through the many trials of graduate school. Of all the reasons for thankfulness and gratitude of support, guidance, friendship, and love I've expressed here, she is the ultimate fulfillment and example of them all. Thank you, and I love you.

Table of Contents

List of Tables

List of Figures

Introduction

The goal of my thesis was to examine the trophic interactions between zooplankton (primarily copepods) and microzooplankton in relation to spring blooms of small dinoflagellates in tributaries of Chesapeake Bay. I sought to determine if changes in plankton community structure and mesozooplankton grazing rates are able to create a window of opportunity for bloom development and propagation. This temporal and spatial window of opportunity would be a variation on the match/mismatch hypothesis as set forth by Cushing (1990), where an increase in top-down grazing pressure by mesozooplankton on heterotrophic protozoa may provide sufficient relief of top-down control of small phytoplankton to aid in bloom development. In order to accomplish this goal I examined changes in organism biomass and mesozooplankton grazing rates in response to changes in the physical environment, and the ability of *in situ* mesozooplankton populations to sufficiently reduce heterotrophic protozoa biomass via grazing. The data for my thesis were collected from March through May of 2002 and 2003. Sampling events and grazing experiments (as discussed in chapter 2) were conducted on a weekly basis in order to encompass pre-bloom, bloom, and post-bloom conditions.

Copepods may be the most numerous metazoan organisms on earth. Present in all aquatic systems, copepods are important components of aquatic food webs; where they act as both predator and prey, and nutrient regenerators. Many early studies of copepod feeding ecology classified copepods as strict herbivores, grazing on diatoms and other large phytoplankton species. However, within the last 30 years the studies have shown that copepods are rarely strict herbivores but ingest a wide variety of food types,

including detritus, protozoa, and other metazoans (Lonsdale et al. 1979, Roman 1984, Stoecker & Capuzzo 1990). In order to meet reproductive and metabolic needs, copepods require food sources with larger N:C ratios (Houde & Roman 1987), and ingestion of phytoplankton alone often does not meet this need (White & Roman 1992a). Since microzooplankton are rich in nitrogen containing compounds (Stoecker & Capuzzo 1990), they are able to fill the nutritional deficit created by feeding solely on phytoplankton (White & Roman 1992a). In addition to providing nitrogenous compounds, microzooplankton provide a source of superior food quality through a process known as trophic upgrading (Klein Breteler et al. 1999, Bec et al. 2003, Klein Breteler et al. 2004). Trophic upgrading involves the provision of essential lipids, not always available in algae, to copepods when they prey upon microzooplankton that are synthesizing these compounds as a result of grazing upon algae. Thus rather than just serving as trophic intermediary between algae and copepods, microzooplankton may upgrade the biochemical composition of phytoplankton for copepods.

Comparisons of copepod clearance and ingestion rates on various prey showed that copepods had higher rates on heterotrophic protozoans than on small phytoplankton (Dolan 1991, Gifford & Dagg 1991). Grazing rates on protozoans remained higher than the rates for small phytoplankton even when protozoans constituted a smaller portion of available prey biomass than the small phytoplankton species (Gifford & Dagg 1988, Rollwagen Bollens & Penry 2003). Rather, it is the microzooplankton that are the dominant grazers of small phytoplankton, and in some systems heterotrophic protozoans can consume 100% of daily phytoplankton production (Sherr & Sherr 1994). When compared with copepod grazing rates on similar phytoplankton species,

microzooplankton grazing rates were approximately 100 times greater (Sommer et al. 2002). Johnson et al. (2003) reported that microzooplankton in Chesapeake Bay exhibited grazing rates on the bloom forming dinoflagellates *Prorocentrum cordatum* and *Karlodinium micrum* that were greater than the maximum growth rates of those dinoflagellates. Thus microzooplankton could provide sufficient grazing pressure to prevent net growth, and therefore bloom formation, of small dinoflagellates in Chesapeake Bay. Microzooplankton also exhibit growth rates that are equal to, or greater than their phytoplankton prey (Sherr & Sherr 1994), so microzooplankton are able to increase community biomass at a rate which allows them to effectively graze rapidly forming phytoplankton blooms. Given this information and the large variability with which copepods graze harmful algal bloom forming species (Turner & Tester 1997), microzooplankton may have the greatest top-down impact on small bloom forming phytoplankton, and changes in microzooplankton biomass and grazing rates may prevent or aid in bloom formation. Copepods may then affect bloom dynamics indirectly through regulation of microzooplankton biomass, thus resulting in a trophic cascade that may or may not lead to windows of opportunity for algal bloom formation.

Copepods are the dominate mesozooplankton species in Chesapeake Bay, comprising > 65% of total zooplankton numbers (Brownlee & Jacobs 1987). The copepod species that dominate the Maryland portion of Chesapeake Bay are *Acartia tonsa*, *A. hudsonica*, and *Eurytemora affinis*. *Acartia* spp. are present throughout the year with *A. hudsonica* being the dominant species during the winter and spring and *A. tonsa* being the dominant *Acartia* and overall copepod species during the summer and fall and in salinities >18. Sullivan & McManus (1986) determined that the effect of temperature

on the reproductive success of each species was the reason for this seasonal succession. *E. affinis* is present in the oligo- and mesohaline portions of the Bay throughout the year and is the dominant copepod species in the winter and spring. In the mesohaline portion of the Bay *A. tonsa* succeeds *E. affinis* as the dominant copepod in the summer and then vice versa in the winter. The reason for this seasonal succession may be that *A. tonsa* is able to out compete *E. affinis* at higher temperatures and salinities due osmotic stress, as evidenced by changes in protein expression in *E. affinis* (Bradley 1991, Kimmel & Bradley 2001).

It is important to note at this point that in the data presented in chapters 1 & 2 I did not distinguish between *A. tonsa* and *A. hudsonica*. In order to process the samples more quickly I combined the two species under the general title of *Acartia* spp., and when I calculated biomass and the percent of body carbon ingested I used equations developed for *A. tonsa*. I was able to do this because both species have comparable percentages of body carbon in relation to their dry weight (Ambler 1985, Cataletto & Fonda Umani 1994). White & Roman (1992b) noted differences in ingestion rates between the two species, but this is probably the result of the difference in temperature at which the rates were measured as Gaudy et al. (2000) found no significant differences in ingestion between the two species at the same temperature, salinity, and food concentrations.

Chapter 1 examines the changes in plankton community biomass and composition that occur when transitioning from low to high freshwater input in mesohaline tributaries of Chesapeake Bay. Increased freshwater input delivers more nutrients to the Bay through increased runoff from nutrient rich soils. Favorable bottom-up controls, such as

increased nutrient levels, often aid the development of spring algal blooms in Chesapeake Bay. The goal of this chapter was to determine if significant changes in biomass occur within the different levels of the plankton community (specifically focusing on copepods) with increasing freshwater input that would indicate the presence of possible windows of opportunity for bloom development. The structure of the grazer community is important because if a bloom becomes established without a sufficient abundance of grazers, bloom species may be able to outgrow their predators even if these predators exhibit high grazing rates (Turner & Tester 1997).

In chapter 2 the potential for copepod control of microzooplankton populations, and if there exists a copepod – microzooplankton – phytoplankton trophic cascade that may aid spring bloom formation are examined. Based on estimated clearance and ingestion rates for microzooplankton (Stoecker & Capuzzo 1990), copepods have the potential to exert substantial top-down grazing pressure on microzooplankton communities. As noted above, this ability is also dependent on a ample abundance of copepods (Turner & Tester 1997). The goals of this chapter were to determine if the increased copepod biomass, noted in chapter 1 in the wet year, leads to significant increases in ingestion of microzooplankton and the ability to control microzooplankton biomass through grazing rates that are equal to or greater than microzooplankton growth rates. The ability of copepods to control microzooplankton is important in relation to spring bloom formation because of the potential to create spatial and temporal windows of low grazing pressure on small (<10 μm) phytoplankton in conjunction with a sufficient supply of nutrients. The effect of similar trophic cascades have been linked to changes in

phytoplankton concentrations in freshwater systems (McQueen et al. 1986, Carpenter et al. 1987).

References

Ambler JW (1985) Seasonal factors affecting egg production and viability of eggs of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est Coast Shelf Sci 20:743-760

Bec A, Desvilettes C, Véra A, Lemarchand C, Fontvieille D, Bourdier G (2003) Nutritional quality of a freshwater heterotrophic flagellate: tophic upgrading of its microalgal diet for *Daphnia hyalina*. Aquat Microb Ecol 32:203-207

Bradley BP (1991) Distribution of copepods in coastal zone waters: seasonal succession in Chesapeake Bay. Bull Plankton Soc Jpn Spec Vol 129-131

Brownlee DC, Jacobs F (1987) Mesozooplankton and microzooplankton in the Chesapeake Bay. In: Austin HM (ed) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 217-269

Carpenter SR, Kitchell JF, Hodgson JR, Cochran PA, Elser JJ, Elser MM, Lodge DM, Kretchmer D, He X, von Ende CN (1987) Regulation of lake primary productivity by food web structure. Ecology 68:1863-1876

Cataletto B, Fonda Umani S (1994) Seasonal variation in carbon and nitrogen content of Acartia clausi (Copepoda, Calanoida) in the Gulf of Trieste (Northern Adriatic Sea). Hydrobiologia 292/293:283-288

Cushing DH (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. In: Southward AJ (ed) Advances in Marine Biology, Vol 26. Academic Press, San Diego, p 249-293

Dolan JR (1991) Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods. Mar Biol 111:303-309

Gaudy R, Cervetto G, Pagano M (2000) Comparison of the metabolism of *Acartia clausi* and *A. tonsa*: influence of temperature and salinity. J Exp Mar Biol Ecol 247:51-65

Gifford DJ, Dagg MJ (1988) Feeding of the estuarine copepod Acartia tonsa Dana: carnivory vs. herbivory in natural microplankton assmeblages. Bull Mar Sci 43:458-468

Gifford DJ, Dagg MJ (1991) The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanuc plumchrus* Murukawa. Mar Microb Food Webs 5:161-177

Houde SEL, Roman MR (1987) Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. Mar Ecol Prog Ser 40:69-77

Johnson MD, Rome M, Stoecker DK (2003) Microzooplankton grazing on *Prorocentrum minimum* and *Karlodinium micrum* in Chesapeake Bay. Limnol Oceanogr 48:238-248

Kimmel DG, Bradley BP (2001) Specific protein responses in the calanoid copepod *Eurytemora affinis* (Poppe, 1880) to salinity and temperature variation. J Exp Mar Biol Ecol 266:135-149

Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. Mar Ecol Prog Ser 274:199-208

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135:191-198

Lonsdale DJ, Heinle DR, Siegfried C (1979) Carnivorous feeding behavior of the adult calanoid copepod *Acartia tonsa* Dana. J Exp Mar Biol Ecol 36:235-248

McQueen DJ, Post JR, Mills EL (1986) Trophic relationships in freshwater pelagic ecosystems. Can J Fish Aquat Sci 43:1571-1581

Rollwagen Bollens GC, Penry DL (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). Mar Ecol Prog Ser 257:139-158

Roman MR (1984) Utilization of detritus by the copepod, *Acartia tonsa*. Limnol Oceanogr 29:949-959

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223-235

Sommer U, Berninger UG, Böttger-Schnack R, Cornils A, Hagen W, Hansen T, Al-Najjar T, Post AF, Schnack-Schiel SB, Stibor H, Stübing D, Wickham S (2002) Grazing during early spring in the Gulf of Aqaba and the northern Red Sea. Mar Ecol Prog Ser 239:251-261

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12:891-908

Sullivan BK, McManus LT (1986) Factors controlling seasonal succession of the copepods *Acartia hudsonica* and *A. tonsa* in Narragansett Bay, Rhode Island: temperature and resting egg production. Mar Ecol Prog Ser 28:121-128

Turner JT, Tester PA (1997) Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol Oceanogr 42:1203-1214

White JR, Roman MR (1992a) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar Ecol Prog Ser 86:239-249

White JR, Roman MR (1992b) Seasonal study of grazing by metazoan zooplankton in the mesohaline Chesapeake Bay. Mar Ecol Prog Ser 86:251-261

Chapter 1

Changes in estuarine plankton community structure in response to freshwater flow

Abstract

Changes in plankton composition and species abundance in estuaries are often driven by freshwater flow. This chapter describes the effects of below and above average freshwater flow on the spring plankton community structure of two sub-estuarine tributaries of Chesapeake Bay. The biomass of phytoplankton, microzooplankton, copepods, and gelatinous zooplankton were measured during the spring of consecutive dry (below average flow) and wet (above average flow) years. Changes in biomass of each of these groups were related to the effects of changes in both bottom-up and top-down controls. Significant increases in phytoplankton biomass and copepod biomass were measured in the wet year as compared to the preceding dry year. Microzooplankton biomass also increased in the wet year, however the difference between years was not significant. The ctenophore *Mnemiposis leidyi*, which was present during the dry year, was absent during the sampling period of the wet year. The increases in phytoplankton and copepod biomass (733% and 4110% respectively) were much larger than the 53% increase in microzooplankton biomass. The results suggest increased control of microzooplankton populations by the enlarged copepod community in the wet year and that this may have released top-down control on phytoplankton. Reduced grazing, in conjunction with increased nutrient availability, allowed for the large increase in phytoplankton biomass. Thus increased freshwater flow has the potential to influence trophic cascades.

Introduction

The variability of the physical, chemical, and biological processes in estuarine systems are often driven by the amount of freshwater input entering these estuaries from contributing rivers (Cloern et al. 1983, Garcia-Soto et al. 1990, Mallin et al. 1993). In Chesapeake Bay the maximum freshwater input occurs during the late winter and early spring (Schubel & Pritchard 1987). This spring freshet results in increased nutrient loading into the Bay and therefore indirectly leads to the spring phytoplankton bloom (Harding 1994). Periods of high precipitation that result in above average spring freshwater input cause enhanced spring bloom conditions whereas periods of low precipitation and below average freshwater input often preclude the spring bloom (Harding & Perry 1997). Changes in freshwater flow also correlate with changes in the temporal and spatial abundances of the dominant copepod species in the Bay (Kimmel & Roman 2004).

The effect of increased freshwater flow on the ecology of estuaries seems to be highly variable, with different effects of flow on the abundances of similar organisms in different estuaries (Flint 1985, Mallin et al. 1993, Kimmerer 2002). Whether freshwater flow has positive or negative effects on an organism's abundance in the San Francisco Estuary appears to be primarily controlled by physical factors such as changes in light limitation, salinity and temperature induced stress, and the residence time of the system (Kimmerer 2002). However, Alpine & Cloern (1992) found that the biomass of chlorophyll *a* (chl *a*) in the same estuary was controlled jointly by physical (river flow) and biological (grazing by predators) controls. Results of freshwater input from other systems showed similar patterns to Chesapeake Bay, where phytoplankton biomass

increased with increasing freshwater input (Mallin et al. 1993, Livingston et al. 1997, Mozetic et al. 1998). In Corpus Christi Bay, Flint (1985) found that 84 % of the variability in primary production was explained by changes in freshwater input. Other ecological impacts of changes in freshwater input to river-dominated estuaries include changes in the trophic structure, whereby the trophic structure of Apalachicola Bay changed from being herbivore dominated to being dominated by succeeding higher order carnivores during extended periods of decreased freshwater flow (Livingston et al. 1997).

Most studies on freshwater flow effects in Chesapeake Bay have focused on the seasonal variation of phytoplankton biomass and zooplankton in the main stem of the Bay (Malone et al. 1988, Harding 1994, Purcell et al. 1994, Kimmel & Roman 2004). However, relatively few studies have focused on the sub-estuary tributaries. These tributaries play an important role in the ecology of Chesapeake Bay. The tidal freshwater and brackish portions of these tributaries serve as nursery and spawning grounds for many commercially and recreationally import species of estuarine, freshwater, and anadromous fish (Setzler-Hamilton 1987, Houde & Rutherford 1993). The tributaries are locations of significant point and non-point source nutrient inputs (Boynton et al. 1995, Jordan et al. 2003). In Chesapeake Bay, blooms of the harmful or potentially harmful dinoflagellate species *Pfiesteria piscicida*, *Prorocentrum cordatum*, and *Karlodinium micrum* (formerly *Gyrodinium galatheanum*) often have their origin in the tributaries (Lewitus et al. 1995, Li et al. 2000). In the case of *P. cordatum*, the blooms are aided by the convergence of down-river flowing nutrient-rich freshwater (surface water) with up-river flowing deep water containing the dinoflagellate (Tyler & Seliger 1978). This convergence of nutrient rich freshwater and higher salinity deep water can be caused by

either the decreased water depth in the middle to upper portions of the tributaries or by wind-driven upwelling over sills at the mouth of the tributary (Sanford & Boicourt 1990). Thus these sub-estuary tributaries can often be the primary location for the interaction between nutrient replete runoff and estuarine phytoplankton species.

The goals of this research are to describe (1) phytoplankton, microzooplankton, and mesozooplankton composition and biomass and (2) spatial and temporal patterns of plankton biomass; (3) relate these patterns to environmental conditions; (4) predict how environmental changes may contribute to spring bloom dynamics in these estuarine tributaries during low and high freshwater flow years. I hypothesize that increased freshwater input into estuarine tributaries of Chesapeake Bay will increase phytoplankton and zooplankton biomass. I predict that increased freshwater input will increase the importance of physical controls (such as temperature and salinity) over mesozooplankton abundance, whereas prey abundance and availability will be more important with decreased freshwater input. This prediction is based on the hypothesis that in low flow years phytoplankton biomass is reduced due to nutrient limitation thereby limiting secondary production, due to food limitation. Conversely, wet years will not experience nutrient limitation and physical factors such as temperature and salinity may have more of an effect on zooplankton distributions.

Materials and Methods

Study Site and Sampling Locations

Sampling was conducted from March through May in 2002 and 2003 in the Patuxent and Choptank Rivers, which are western and eastern shore tributaries of

Chesapeake Bay, USA (Figure 1-1, Table 1-1). These rivers were chosen for their logistical convenience, the existence of ongoing monitoring programs (EPA Chesapeake Bay Program; www.chesapeakebay.net/data/index.htm) with real-time capabilities (Coastal Intensive Site Network; www.cisnet-choptank.org), and because of their similar bathymetry. Three stations (termed lower, middle, and upper) were chosen for the Patuxent River and two stations (termed middle and upper) were chosen for the Choptank River (Figure 1-1). Micro- (20-200 μm) and mesozooplankton (> 200 μm) samples were collected and CTD (Sea-Bird Electronics) casts were conducted to measure conductivity, temperature, and fluorescence. These stations will henceforth be referred to as the biological stations. Additional CTD casts were conducted in each river at various intervals along the main channels, proceeding from the mouth to the upriver limit of the salt intrusion.

Collection Methods and Sample Preservation

Mesozooplankton were collected by towing a 0.5 m diameter 200 μm mesh size plankton net and an attached flow meter (General Oceanics Inc.) at the surface. These samples were preserved on location with 5% (v/v) buffered formalin for future analysis. If gelatinous zooplankton were present, the wet weight biomass was determined by measuring the displacement volume. This was done by measuring the volume of the sample before and after removing the gelatinous zooplankton with a colander (3 mm diameter openings). Microzooplankton (20-200 μm) samples were collected from the surface by collecting water in a bucket, preserving the samples in 10 % (v/v) acid Lugol's solution (Parsons et al. 1984a) for determination of *in situ* biomass. Whole water

samples were also collected from the surface of each biological station and stored in polypropylene bottles in a dark cooler for transportation to the laboratory. A portion of the water samples was filtered through a 10 μm membrane filter to obtain a sample of water containing only <10 μm organisms. The whole water samples and the <10 μm water samples where then filtered through GF/F filters and we determined the chl *a* concentration in the extracted samples.

Sample Analysis and Calculations

Microzooplankton were enumerated by using settling chambers and an inverted microscope (Utermöhl 1958). Ciliates and heterotrophic dinoflagellates were grouped according to size, shape, or genera; from which their volume was determined (Table 1-2). The dimensions of Tintinnids, and Spherical and Conical Ciliates (Table 1-2) were determined by taking the average dimensions of at least 10 organisms of each type measured using an ocular micrometer in samples from both years and both rivers. In order to calculate Tininnid biovolume, the lorica volume (LV) was calculated using the volume equations for a cylinder with a cone attached to one end (Hillebrand et al. 1999). The biovolume was then estimated to be 31% of the LV (Gilron & Lynn 1989) and converted the biovolume to biomass by using a conversion factor of 0.14 pg C μm^{-3} (Putt & Stoecker 1989). Oligotrichous ciliate volume (μm^3 $cell^{-1}$) was converted to carbon units (μg C ml^{-1}) by using a conversion factor of 0.22 pg C μm^{-3}, which was determined by correcting the conversion factor of 0.14 pg C μm^{-3} for a 59% underestimation of biomass caused cell shrinkage when using 10% acid Lugol's solution (Stoecker et al.

1994). Heterotrophic dinoflagellate volume was converted to carbon units using the following equation from Menden-Duer & Lessard (2000):

$$\mu g\ C = (0.760 * volume^{0.819}) * 10^{-6} \qquad \text{Eqn. 1}$$

where volume is expressed as $\mu m^3\ cell^{-1}$. The concentration of chl *a* was measured at all stations by extracting the chl *a* in 90% Acetone and using a Turner flurometer as described in Parsons et al. (1984a). For each biological station the amount of extracted chl *a* in the <10 μm fraction was subtracted from the amount in the whole water fraction to determine the amount of >10 μm chl *a*. The >10 μm chl *a* concentration ($\mu g\ L^{-1}$) fraction was then converted to carbon units ($\mu g\ C\ L^{-1}$), using a carbon to chl *a* ratio of 30:1 (Parsons et al. 1984b), so that it could be compared to microzooplankton biomass. Only the fraction of chl *a* > 10 μm was used to determine the amount of chl *a* available to as potential copepod food because the lower size limit for particle capture of the copepods *Acartia* spp. and *Eurytemora affinis* is 7-10 μm (Nival & Nival 1976, Berggreen et al. 1988). Mesozooplankton were counted and grouped according to class and copepods were further divided into genera. The abundance of copepods (number L^{-1}) was then calculated using the following equation:

$$\text{Abundance (number } L^{-1}) = [\text{Number of copepods / Tow volume } (m^3)] / 1000 \qquad \text{Eqn. 2}$$

where tow volume was obtained from the flow meter attached to the plankton net. The prosome lengths of the first 50 adult copepods counted were measured, and the average length was used to determine body carbon content using equation 3a for *Acartia* spp. (Berggreen et al. 1988) and 3b for *E. affinis* (Kankaala & Johansson 1986):

$$C = (1.11*10^{-8}) * PL^{2.92} \quad \text{Eqn. 3a}$$

$$C = (2.02*10^{-8}) * PL^{2.83} \quad \text{Eqn. 3b}$$

where C is carbon content (in µg C) and PL is the average prosome length (µm) for that species.

Due to the two-year sampling program of this study, the results contained in this paper were compared to a long-term (1985 – present) database from the Chesapeake Bay Program (CBP, http://www.chesapeakebay.net/data/index.htm). The CBP monitors various physical and biological parameters at locations in both the Patuxent (CBP station LE 1.1) and Choptank (CBP station ET 5.2) rivers that are near the upper biological stations of both rivers. The yearly average spring chl *a* concentration and copepod abundance data collected during the study presented in this paper were added to data that the CBP had collected starting in 1985 in the Patuxent River and 1988 in the Choptank River. The historical data on microzooplankton abundance was not included due to differences in enumeration and classification methods. The average spring stream flow data (obtained from USGS website as referenced in Figure 1-2) was also extended to include these additional years. Years of high conditions (stream flow, chl *a* concentration, and copepod abundance) were determined to be those with averages greater than the 3rd quartile, and years of low conditions are those with averages below the 1st quartile.

Statistical analyses were conducted using JMP Statistical Discovery Software 4.0.4 (SAS Institute Inc.). Student's t-tests were applied to the data to determine differences between the dry and wet years.

Results

Comparison of Physical Data

Stream flow for both the Patuxent and Choptank Rivers was much higher in 2003 (wet year) than 2002 (dry year) (Figure 1-2). In 2003 the mean flow during the sampling period was greater than the long term mean (Patuxent - 25 years, Choptank - 55 years) flow for March through May, but was less than the long term mean flow in 2002. Thus, 2003 (10.09 $m^3 s^{-1}$) was an above average (5.77 $m^3 s^{-1}$) wet year and 2002 (2.67 $m^3 s^{-1}$) was a below average dry year. Due to the large variability in daily measured stream flow, the data was grouped into monthly averages in order to determine if statistical differences in river flow existed between the 2 years. Average monthly stream flow was significantly greater in 2003 as compared to 2002 in both the Choptank (Student's t-test, $p < 0.05$) and the Patuxent (Student's t-test, $p < 0.05$) Rivers. Average temperature and salinity showed a general trend (Figure 1-3 & 1-4) of decreased values at each biological sample location in the wet year as compared to the dry year for both rivers. Student's t-test results indicated statistically significant decreases in average salinity in 2003 at all the biological sample stations.

Comparison of Biological Data

There was an increase in monthly averaged phytoplankton biomass in the wet year as compared to the dry year in both rivers and at all biological sample locations (Figure 1-5). There was also a greater degree of variability in the phytoplankton biomass in the wet year. The variance in biomass at the biological stations in both rivers ranged from 0.32 – 0.89 in the dry year as compared to a range of 0.66 – 1.25 in the wet year.

The variance was greater in the wet year (F test, $p < 0.01$) than the dry year at all biological stations of both rivers. The greatest phytoplankton biomass estimates in both rivers (Choptank = 99.17 µg L^{-1}, Patuxent = 225.28 µg L^{-1}) were recorded near the upper biological sample stations, which correspond to the general area of the estuarine turbidity maximum (ETM) of both rivers. Another effect of the increased flow in the wet year was the displacement of the average chl *a* maximum downriver from its position in the dry year. The position of the average chl *a* maximum in the dry year was at 47.71 km and 48.97 km (Patuxent and Choptank respectively) from the mouth of each river compared to 35.04 km and 31.60 km (Patuxent and Choptank respectively) in the wet year.

Average microzooplankton (composed of ciliates and heterotrophic dinoflagellates, Table 1-2) biomass (Figure 1-5) increased in the wet year as compared to the dry year at all biological sample stations in both rivers, except the upper station of the Patuxent River. The greatest average amount of biomass was measured in the Choptank River (0.50 µg C ml^{-1}), at the middle biological station. Variability in biomass was sufficient enough during the sampling period to negate any statistically significant differences between the wet and dry years, except for the middle Choptank station. Variance in the average biomass was greater in the wet year (ranging from 0.98 – 1.42) than the dry year (ranging from 0.39 – 1.33). The upper station of the Choptank River was the only station where variance (F test, $p = 0.78$) was greater in the dry year. While microzooplankton biomass increased in the wet year, its percentage in relation to total carbon available to mesozooplankton predators decreased (Table 1-3). The average (of all stations) percent of microzooplankton that comprised the total available <200 µm plankton carbon was 47% in the dry year as compared to 14% in the wet year.

Average total copepod biomass, consisting of *Acartia* spp. and *Eurytemora affinis*, was higher at all biological stations in both rivers in the wet year (Figure 1-6). This increase was significant at the middle station of the Choptank River and lower station of the Patuxent River ($p < 0.05$). As in the case of chl *a*, total copepod biomass was more variable in the wet year, with variance ranging from 0.08 – 1.68 in the wet year and 0.88 – 1.45 in the dry year. Variance was greater in the dry year at the lower and upper stations of the Patuxent River, but was only significantly greater at the upper station (F test, $p << 0.001$). This was due in part to the presence of monospecific *E. affinis* aggregations observed at the middle and upper stations of the Choptank River and the upper station of the Patuxent River in early to mid April of the wet year. During these aggregations, *E. affinis* abundance at the surface was recorded to be as high as 120 L^{-1} at the upper biological station of the Choptank River, whereas the average abundance of adult *E. affinis* at the same station in the dry and wet years were 1 and 21 L^{-1}, respectively. The recorded abundance of copepods present during the blooms may be underestimated because there were so many copepods that the plankton nets became clogged.

The presence of the monospecific aggregations of *E. affinis* also denotes another difference in the copepod community between the wet and dry year, wherein *E. affinis* comprised a greater proportion of the total copepod abundance in the wet year (Table 1-3). The abundance of *E. affinis* was greater at all biological stations in the wet year than in the dry year, and there were more *E. affinis* than *Acarita* spp. at the upper stations of both rivers. During the dry year the species *Centropages* sp. was also present, but never comprised more than 5% of the total copepod abundance.

The only gelatinous zooplankton species collected was the ctenophore *Mnemiopsis leidyi*. *M. leidyi* were present during the sampling period in the dry year in both rivers (Figure 1-6), and no gelatinous zooplankton species were collected during the sampling period of the wet year. The greatest average biovolume of *M. leidyi* (7.86 ml m^{-3}) was measured at the upper biological station of the Patuxent River (Figure 1-6).

Although there was an increase in the biomass of all 3 food web components mentioned above (copepods, microzooplankton, and phytoplankton) in the wet year, the magnitude of increase was not uniform among all 3 plankton groups (Figure 1-7). Rather, the percent increase in copepod biomass was the greatest at 4110%, followed phytoplankton biomass at 733%, and both were much greater than the increase in microzooplankton biomass (88%).

Comparison with Historical Data

The historical data from the CBP website was used to determine if there were other years which displayed the same trend as was noted during the dry year (2002; low flow, low chl *a* concentration, low copepod abundance) and the wet year (2003; high flow, high chl *a* concentration, high copepod abundance). In the Choptank River, 1994 had high flow and chl *a* concentrations that correlated with above average copepod abundance, and 1998 experienced high chl *a* concentration and copepod abundance with above average flow (Figure 1-8). In 1988 the pattern was consistent with 2002 where all 3 parameters were low, and in 1992 there was low flow and copepod abundance corresponding to below average chl *a* concentration, and 1999 had low flow and chl *a* concentration with below average copepod abundance (Figure 1-8). In the Patuxent

River the high trend in 2003 was supported by a similar results in 1998, and the low trend of 2002 was similar to 1999 and 1992 (Figure 1-9). The results of paired comparison tests showed strong positive correlations between flow and chl *a* concentration ($r^2 = 0.53$, $p < 0.05$) and copepod abundance ($r^2 = 0.63$, $p < 0.05$), and between copepod abundance and chl *a* concentration ($r^2 = 0.47$, $p = 0.07$) in the Choptank River. In the Patuxent River, river flow and copepod abundance showed a strong positive correlation ($r^2 = 0.46$, $p < 0.05$), whereas chl *a* was negatively correlated with copepod abundance ($r^2 = -0.61$, $p < 0.01$) and flow ($r^2 = -0.23$, $p = 0.35$). Thus there is historical support from both rivers for the influence of river flow on phytoplankton and copepod biomass as we observed for 2002 and 2003.

Discussion

In estuarine systems phytoplankton production is driven by nutrient inputs, circulation patterns, residence times, and vertical stability of the water column (Malone et al. 1988). All of these control factors are driven partially, if not completely, by freshwater input. Freshwater flow is strongly correlated with increased nutrient input (Harding & Perry 1997) and hence chl *a* concentration (Malone et al. 1988). Thus an increase in flow can be expected to produce an increase in phytoplankton biomass, as observed during the wet year of this study, where total phytoplankton biomass increased 733% in the wet year. Additionally, the biomass of >10 μm phytoplankton increased by an average of 956% in the wet year. Additionally, the biomass of >10 μm phytoplankton increased by an average of 956% in the wet year. Increased freshwater flow and nutrient inputs may favor large phytoplankton over <10 μm phytoplankton due to the potential for

increased large diatom concentrations as a result of environmental conditions, such as increased turbidity and vertical mixing, that are associated with greater flow and favor diatoms over smaller nanoplankton (Malone 1980 and references therein).

It is particularly interesting to note that this increase occurred congruently with a large increase in copepod biomass (see below), as large phytoplankton (>10 μm) are generally considered to be a major component of copepod's diets (Kleppel 1993, Stibor et al. 2004a). The increase in large phytoplankton biomass in conjunction with increasing copepod biomass may be the result of greater phytoplankton growth rates than copepod grazing rates, or of a poor spatial-temporal match between the large phytoplankton and copepod biomasses. However, the increase in large phytoplankton biomass, and total phytoplankton biomass in general, could also be a product of the comparatively small increase in microzooplankton biomass in the wet year (see below). It is possible that copepods predominantly grazed upon microzooplankton rather than phytoplankton during the wet year, thus allowing for a release of grazing pressure on both total and >10 μm chl *a*. Vadstein et al. (2004) described a situation where an increased presence of copepods correlated with increased phytoplankton biomass through the removal of herbivorous microzooplankton. While this effect was most evident in the nanoplankton biomass, increased total phytoplankton biomass also correlated with an increasing presence of copepods. In order to determine if copepods were predominately grazing on microzooplankton as opposed to large phytoplankton during the wet year, comparative grazing experiments need to be conducted, where the relative amounts of clearance and ingestion rates of these two prey types are compared.

The abundance of planktonic grazers (both herbivorous and omnivorous) is directly linked to river flow through bottom-up mechanisms such as the dispersion of grazer communities by increased river flow, and the abundance of prey (Livingston et al. 1997). Increased abundances of phytoplankton benefit microzooplankton communities, as herbivorous microzooplankton are the dominant predators of phytoplankton (Sherr & Sherr 1994). Thus it could be expected that increases in phytoplankton biomass would lead to increased protozoan grazer biomass. However, there was a < 2x increase in microzooplankton biomass in the wet year despite an approximately 8x increase in total phytoplankton biomass (Figure 1-7). This apparent mismatch could be the result of weak coupling between primary producers and herbivores, as was noted by Micheli (1999). However, that study focused on interactions between mesozooplankton and phytoplankton and did not examine correlations between microzooplankton and phytoplankton biomass. Filemann & Burkill (2001) found that while there was a weak relationship between phytoplankton and mesozooplankton, close coupling existed between microzooplankton and phytoplankton. Furthermore, there is evidence of strong trophic coupling between microzooplankton and phytoplankton in tributaries of Chesapeake Bay (Gallegos 1989). The large percentage of daily primary production that is consumed by microzooplankton also suggests close trophic coupling. Microzooplankton have been found to consume 25-100% of daily phytoplankton production, depending on their growth rate (Gifford & Dagg 1988, Verity et al. 1993, Stoecker et al. 2000). Thus it does not seem likely that the small increase in microzooplankton biomass in the wet year as compared to phytoplankton biomass is the result of weak coupling between the two trophic levels.

It is also possible that the small increase in microzooplankton was a result of top-down effects of mesozooplankton grazing. Copepods exhibit high clearance rates for microzooplankton, and it is estimated that they can remove > 100% if the microzooplankton from surface waters (Dolan 1991, Merrell & Stoecker 1998). High clearance rates and ingestion rates of 21 to 93% of microzooplankton production per day (Nejstgaard et al. 2001, Zeldis et al. 2002) suggest that copepods can potentially control microzooplankton populations. In the wet year of this study, copepod biomass increased by approximately 42x over the dry year biomass, while the increase in microzooplankton biomass was < 2x that of the dry year (Figure 1-7). Thus it seems most likely that the small increase in microzooplankton in the wet year was a result of top-down grazing pressure from an increased copepod population. However, to confirm this hypothesis results from copepod grazing experiments on microzooplankton need to be examined to determine if community-grazing rates removed a sufficient proportion of daily microzooplankton standing stock to limit growth.

The abundance and biomass of copepods are controlled by various physical and biological factors, which can produce or relieve physiological. Temperature and salinity directly effect copepod physiology, which is a contributing factor in determining the dominant species in a given location. For instance, *Acartia* spp. prefer higher salinities and temperatures than *E. affinis* (Jeffries 1962, Kimmel & Roman 2004), and are negatively correlated with flow in the upper Chesapeake Bay (Jeffries 1962, Kimmel & Roman 2004). *Acartia tonsa* and *A. hudsonica* (the 2 species included under the title *Acartia* spp.) experience maximum reproduction rates at 20 and 15 °C respectively (Sullivan & McManus 1986). The average water temperature at all stations in the

Patuxent River in both the dry and wet years were below 15 °C and were slightly above 15 °C during the only the wet year at both stations in the Choptank River (Figure 1-3 & 1-4).

We know that physical factors such as temperature control copepod abundance by influencing rates of feeding (via ingestion rates), respiration, and development and growth (White & Roman 1992b, Mauchline 1998). Salinity also controls copepod abundance through osmoregulatory stress. Euryhaline copepods are able to osmoregulate by adjusting the intracellular concentration of free amino acids in order to maintain cell volume (Goolish & Burton 1989, Burton 1991). However, this is energetically costly and thus decreases the available energy needed for feeding and reproduction. Food quantity and the presence of predators are biological factors that control copepod populations. Reproduction, as measured by egg production rates, is directly linked to food quantity and quality. Copepods in food-limited environments produce significantly fewer eggs than those in phytoplankton and protozoan enriched environments (Ambler 1986, Sullivan & Banzon 1990, White & Roman 1992a). Durbin et al. (1983) found that, in addition to increased egg production, greater food quantities led to significant increases in mean dry weight and cephalothorax length in *A. tonsa.* In addition they noted that egg production and the mean dry weight copepods tracked the seasonal trend of chl *a* in Narragansett Bay. Additionally, increased microzooplankton biomass (which was observed in the wet year, Figure 1-5) is nutritionally advantageous to copepods as microzooplankton prey can be a better source of protein and amino acids than phytoplankton (Houde & Roman 1987, Stoecker & Capuzzo 1990). Reproductive advantages for copepods, such as increased egg production rates, have also been linked to

an increased availability of microzooplankton (Stoecker & Egloff 1987, Tiselius 1989). While food limitation can limit copepod abundance through decreasing reproduction and growth, predation may be the greatest biological factor limiting copepod abundance in Chesapeake Bay (Purcell et al. 1994, Roman et al. 2005).

Increased copepod biomass in the wet year may also be the result of decreased top-down control due to the absence of ctenophores, as an increased presence of gelatinous zooplankton correlates with decreased copepod abundance (Granéli & Turner 2002, Purcell & Decker 2005). Granéli and Turner (2002) found that an increased presence of ctenophores led to increased ciliate abundance and a decreased phytoflagellate abundance through significant predation of copepods. Other studies have also found that phytoplankton abundance increased in the presence of gelatinous zooplankton, due to the gelatinous zooplankton exerting top-down control over copepods (Deason & Smayda 1982, Schneider & Behrends 1998). The ctenophore *Mnemiopsis leidyi*, which was the only gelatinous zooplankton collected during our sampling, is a voracious predator of copepods. Blooms of *M. leidyi* often occur in the spring and fall in Chesapeake Bay (Purcell 1988) and they are thought to be able to control copepod populations whenever not inhibited by predation from *Chrysaora quinquecirrha* medusa (Purcell & Decker 2005). In the Narragansett Bay, the amount of copepod abundance consumed by this ctenophore is estimated to be as much as 90% (Deason & Smayda 1982), and regional estimates from Chesapeake Bay indicate that it grazes 11-31% of daily copepod standing stock (Bishop 1967, Purcell et al. 1994).

The lack of *M. leidyi* during the wet year sampling period (Figure 1-6) is puzzling as its abundance has a strong positive correlation to freshwater input in Chesapeake Bay

(Kimmel & Roman 2004). So why were there no *M. leidyi* during the wet year? *M. leidyi* did appear in large numbers in the Chesapeake Bay in 2003, but not until mid summer, after our sampling ended (personal observation). Since temperature is the most important environmental variable affecting growth and development of *M. leidyi* (Kremer 1994), the development and migration of juvenile *M. leidyi* in the mesohaline region of Chesapeake Bay was delayed. Even though there was an increase in freshwater flow during the wet year, this advantage was negated by the below average temperatures in late winter to early spring.

In conclusion, it was found that increased freshwater flow into tributaries of Chesapeake Bay had different effects on each of the 3 plankton groups. Increased freshwater input led to large increases in phytoplankton and copepod biomass but a small and comparatively negligible increase in heterotrophic protozoan biomass. The results suggest the presence of strong grazing control over microzooplankton populations by metazoan predators, thus accounting for the small increase in microzooplankton biomass. The ability of mesozooplankton to control microzooplankton biomass has important implications for phytoplankton bloom development and persistence, as changes in the abundance of herbivorous microzooplankton communities and their predators create the potential for top-down control of blooms (Hansen et al. 1993, Granéli & Turner 2002). The results also suggest that increased freshwater input strengthens the trophic cascade between meso- and microzooplankton and leads to a partial disruption of the microbial food web. This disruption occurs when increased nutrient levels lead to increased primary production but a decrease in the transfer of this production up the food web as there is less grazing by microzooplankton.

Thus we find that changes in the abundances of planktonic grazers create a potential for top-down control of phytoplankton biomass in estuaries. Recent studies from other estuarine systems support this conclusion and emphasize the importance of changing food web structure in controlling phytoplankton biomass (Hansen et al. 1993, Kivi et al. 1996, Stibor et al. 2004a, Vadstein et al. 2004). In order to effectively determine if the changes in microzooplankton and phytoplankton biomass were a direct result of top-down control from the mesozooplankton, additional investigations are needed to determine if the mesozooplankton grazing rates during the wet year were sufficient to limit microzooplankton growth in conjunction with the increased phytoplankton biomass.

References

Alpine AE, Cloern JE (1992) Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol Oceanogr 37:946-955

Ambler JW (1986) Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est Coast Shelf Sci 23:183-196

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341-352

Bishop JW (1967) Feeding rates of the ctenophore *Mnemiopsis leidyi*. Chesapeake Sci 8:259-264

Boynton WR, Garber JH, Summers R, Kemp WM (1995) Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. Estuaries 18:285-314

Burton RS (1991) Refulation of proline synthesis in osmotic response: effects of protein synthesis inhibitors. J Exp Zool 259:272-277

Cloern JE, Alpine AE, Cole BE, Wong RLJ, Arthur JF, Ball MD (1983) River discharge controls phytoplankton dynamics in the northern San Francisco Bay estuary. Est Coast Shelf Sci 16:415-429

Deason EE, Smayda TJ (1982) Ctenophore-zooplankton-phytoplankton interactions in Narragansett Bay, Rhode Island, USA, during 1972-1977. J Plankton Res 4:203-217

Dolan JR (1991) Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods. Mar Biol 111:303-309

Durbin EG, Durbin AG, Smayda TJ, Verity PG (1983) Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnol Oceanogr 28:1199-1213

Fileman E, Burkill P (2001) The herbivorous inpact of microzooplankton during two short-term Lagrangian experiments off the NW coast of Galicia in summer 1998. Prog Oceanogr 51:361-383

Flint RW (1985) Long-term estuarine variability and associated biological response. Estuaries 8:158-169

Gallegos CL (1989) Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar Ecol Prog Ser 57:23-33

Garcia-Soto C, de Madariaga I, Villate F, Orive E (1990) Day-to-day variability in the plankton community of a coastal shallow embayment in response to changes in river runoff and water turbulence. Est Coast Shelf Sci 31:217-229

Gifford DJ, Dagg MJ (1988) Feeding of the estuarine copepod Acartia tonsa Dana: carnivory vs. herbivory in natural microplankton assemblages. Bull Mar Sci 43:458-468

Gilron GL, Lynn DH (1989) Assuming a 50% cell occupancy of the lorica overestimates tintinnine ciliate biomass. Mar Biol 103:413-416

Goolish EM, Burton RS (1989) Energetics of osmoregulation in an intertidal copepod: effects of anoxia and lipid reserves on the pattern of free amino acid accumulation. Funct Ecol 3:81-89

Granéli E, Turner JT (2002) Top-down regulation in ctenophore-copepod-ciliate-diatom-phytoflagellate communities in coastal waters: a mesocosm study. Mar Ecol Prog Ser 239:57-68

Hansen FC, Reckermann M, Klein Breteler WCM, Riegman R (1993) *Phaeocystis* blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. Mar Ecol Prog Ser 102:51-57

Harding LW (1994) Long-term trends in the distribution of phytoplankton in Chesapeake Bay: roles of light, nutrients, and streamflow. Mar Ecol Prog Ser 104:267-291

Harding LW, Perry ES (1997) Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. Mar Ecol Prog Ser 157:39-52

Hillebrand H, Dürselen C-D, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403-424

Houde ED, Rutherford ES (1993) Recent trends in estuarine fisheries - predictions of fish production and yield. Estuaries 16:161-176

Houde SEL, Roman MR (1987) Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. Mar Ecol Prog Ser 40:69-77

Jeffries HP (1962) Succession of two *Acartia* species in estuaries. Limnol Oceanogr 7:354-364

Jordan TE, Weller DE, Correll DL (2003) Sources of nutrient inputs to the Patuxent River estuary. Estuaries 26:226-243

Kankaala P, Johansson S (1986) The influence of individual variation on length-biomass regressions in three crustacean zooplankton species. J Plankton Res 8:1027-1038

Kimmel DG, Roman MR (2004) Long-term trends in mesozooplankton abundance in Chesapeake Bay, USA: influence of freshwater input. Mar Ecol Prog Ser 267:71-83

Kimmerer WJ (2002) Effects of freshwater flow on abundance of estuarine organisms: physical effects or trophic linkages? Mar Ecol Prog Ser 243:39-55

Kivi K, Kuosa H, Tanskanen S (1996) An experimental study on the role of crustacean and microprotozoan grazers in the planktonic food web. Mar Ecol Prog Ser 136:59-68

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183-195

Kremer P (1994) Patterns of abundance for *Mnemiopsis* in US coastal waters: a comparative overview. ICES J Mar Sci 51:347-354

Lewitus AJ, Jensien RV, Kana TM, Burkholder JM, Glasgow HB, May E (1995) Discovery of the "phantom" dinoflagellate in Chesapeake Bay. Estuaries 18:373-376

Li A, Stoecker DK, Coats DW (2000) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. J Plankton Res 22:2105-2124

Livingston RJ, Niu X, Lewis FG, Woodsum GC (1997) Freshwater input to a gulf estuary: long-term control of trophic organization. Ecol Appl 7:277-299

Mallin MA, Paerl HW, Rudek J, Bates PW (1993) Regulation of estuarine primary production by watershed rainfall and river flow. Mar Ecol Prog Ser 93:199-203

Malone TC (1980) Algal size. In: Morris I (ed) The physiological ecology of phytoplankton, Vol 7. Blackwell Scientific Publications, Los Angeles, CA, p 433-463

Malone TC, Crocker LH, Pike SE, Wendler BW (1988) Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. Mar Ecol Prog Ser 48:235-249

Mauchline J (1998) The biology of calanoid copepods. Academic Press, San Diego, CA

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569-579

Merrell JR, Stoecker DK (1998) Differential grazing on protozoan microplankton by developmental stages of the calaniod copepod *Eurytemora affinis* Poppe. J Plankton Res 20:289-304

Micheli F (1999) Eutrophication, fisheries, and consumer-resource dynamics in marine pelagic ecosystems. Science 285:1396-1398

Mozetic P, Umani Fonda S, Cataletto B, Malej A (1998) Seasonal and inter-annual plankton variability in the Gulf of Trieste (nothern Adriatic). ICES J Mar Sci 55:711-722

Nejstgaard JC, Naustvoll L-J, Sazhin A (2001) Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. Mar Ecol Prog Ser 221:59-75

Nival P, Nival S (1976) Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. Limnol Oceanogr 21:24-38

Parsons TR, Maita Y, Lalli CM (1984a) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK

Parsons TR, Takahashi M, Hargrave B (1984b) Biological oceanographic processes, 3rd edn. Pergamon Press, Oxford, UK

Purcell, JE (1988) Quantification of *Mnemiopsis leidyi* (Ctenophora, Lobata) from formalin-preserved plankton samples. Mar Ecol Prog Ser 45:197-200

Purcell JE, Decker MB (2005) Effects of climate on relative predation by scyphomedusae and ctenophores on copepods in Chesapeake Bay during 1987-2000. Limnol Oceanogr 50:376-387

Purcell JE, White JR, Roman MR (1994) Predation by gelationous zooplankton and resource limitation as potential controls of *Acartia tonsa* copepod populations in Chesapeake Bay. Limnol Oceanogr 39:263-278

Putt M, Stoecker DK (1989) An experimentally determined carbon : volume ratio fo marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34:1097-1103

Roman M, Zhang X, McGilliard C, Boicourt W (2005) Seasonal and annual variability in the spatial patterns of plankton biomass in Chesapeake Bay. Limnol Oceanogr 50:480-492

Sanford LP, Boicourt WC (1990) Wind-forced salt intrusion into a tridutary estuary. J Geophys Res 95:13357-13371

Schneider G, Behrends G (1998) Top-down control in a neritic plankton system by *Aurelia aurita* medusae - a summary. Ophelia 48:71-82

Schubel JR, Pritchard DW (1987) A brief physical description of Chesapeake Bay. In: Majumdar SK, Hall LW, Austin HM (eds) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 1-32

Setzler-Hamilton EM (1987) Utilization of Chesapeake Bay by early life history stages of fishes. In: Majumdar SK, Hall LW, Austin HM (eds) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 63-93

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223-235

Stibor H, Vadstein O, Diehl S, Gelzleichter A, Hansen T, Hantzsche F, Katechalis A, Løseth K, Peters C, Roederer W, Sandow M, Sundt-Hansen L, Olsen Y (2004) Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. Ecol Lett 7:321-328

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12:891-908

Stoecker DK, Egloff DA (1987) Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. J Exp Mar Biol Ecol 110:293-299

Stoecker DK, Gifford DJ, Putt M (1994) Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. Mar Ecol Prog Ser 110:293-299

Stoecker DK, Stevens K, Gustafson DE (2000) Grazing on *Pfiesteria piscicida* by microzooplankton. Aquat Microb Ecol 22:261-270

Strüder-Kypke MC, Kypke ER, Agatha S, Warwick J, Montagnes DJS (2002) The user-friendly guide to coastal planktonic ciliates. The University of Liverpool

Sullivan BK, Banzon PV (1990) Food limitation and benthic regulation of populations of the copepod *Acartia hudsonica* Pinhey in nutrient-limited and nutrient-enriched systems. Limnol Oceanogr 35:1618-1631

Sullivan BK, McManus LT (1986) Factors controlling seasonal succession of the copepods *Acartia hudsonica* and *A. tonsa* in Narragansett Bay, Rhode Island: temperature and resting egg production. Mar Ecol Prog Ser 28:121-128

Tiselius P (1989) Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. Mar Ecol Prog Ser 56:49-56

Tyler MA, Seliger HH (1978) Annual subsurface transport of a red tide dinoflagellate to its bloom area: water circulation patterns and organism distribution in the Chesapeake Bay. Limnol Oceanogr 23:227-246

Utermöhl, H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitt Int Ver Limnol 9:1-38

Vadstein O, Stibor H, Lippert B, Løseth K, Roederer W, Sundt-Hansen L, Olsen Y (2004) Moderate increase in the biomass of omnivorous copepods may ease grazing control of planktonic algae. Mar Ecol Prog Ser 270:199-207

Verity PG, Stoecker DK, Sieracki ME, Nelson JR (1993) Grazing, growth and mortality of microzooplankton during the 1989 North Altantic spring bloom at 47°N, 18°W. Deep-Sea Res I 40:1793-1814

White JR, Roman MR (1992a) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar Ecol Prog Ser 86:239-249

White JR, Roman MR (1992b) Seasonal study of grazing by metazoan zooplankton in the mesohaline Chesapeake Bay. Mar Ecol Prog Ser 86:251-261

Zeldis J, James MR, Grieve J, Richards L (2002) Omnivory by copepods in the New Zealand subtropical frontal zone. J Plankton Res 24:9-23

Table 1-1. Dates of sampling for the Choptank and Patuxent Rivers and stations where biological samples were collected. Surface temperature is the temperature at a depth of 1m. C = Choptank River, P = Patuxent River, L = Lower, M = Middle, U = Upper

Sampling Date	River	Experimental Stations (Surface Temperature [^{0}C])			Year	Sampling Date	River	Experimental Stations (Surface Temperature [^{0}C])		
11-Mar	C		M(8)	U(9)	**2003 (wet)**	19-Mar	C		M(6)	U(7)
14-Mar	P	L(8)	M(9)	U(10)		27-Mar	P	L(3)	M(5)	U(6)
21-Mar	C		M(10)	U(11)		1-Apr	C		M(9)	U(10)
26-Mar	C		M(9)	U(9)		3-Apr	P	L(9)	M(11)	U(12)
28-Mar	P	L(10)	M(9)	U(11)		14-Apr	C		M(11)	U(11)
3-Apr	C		M(13)	U(14)		16-Apr	P	L(10)	M(11)	U(12)
8-Apr	C		M(11)	U(12)		25-Apr	C		M(13)	U(14)
11-Apr	P	L(12)	M(13)	U(15)		28-Apr	C		M(14)	U(15)
19-Apr	C		M(15)	U(16)		30-Apr	P	L(14)	M(16)	U(18)
24-Apr	C		M(16)	U(17)		7-May	C		M(16)	U(17)
1-May	P	L(16)	M(17)	U(18)		14-May	C		M(17)	U(17)
8-May	C		M(19)	U(19)		20-May	C		M(16)	U(17)
16-May	C		M(18)	U(19)		22-May	P	L(16)	M(17)	U(17)
23-May	C		M(18)	U(19)		28-May	C		M(17)	U(17)
30-May	C		M(23)	U(23)						

Table 1-2. Microzooplankton classification (class, shape, or genus), size category, dimensions, lorica volume (LV), and biovolume (μm^3 $cell^{-1}$) used for microscopic counts and biomass calculations. Ciliate size categories are based on anterior diameter. Dimensions of non-spherical organisms are listed as diameter * length.

Microzooplankton Class, Shape, or Genus	Size Categories (μm)	Dimensions (μm)	LV (μm^3)	Biovolume (μm^3 $cell^{-1}$)
Tintinnid	<20	15 * 105	$1.86 * 10^4$	$5.75 * 10^3$
	>20	25 * 72	$3.24 * 10^4$	$1.00 * 10^4$
Spherical Ciliate	<20	17	-	$2.57 * 10^3$
	>20	34	-	$2.06 * 10^4$
Conical Ciliate	<20	17 * 42	-	$3.18 * 10^3$
	>20	34 * 56	-	$1.69 * 10^4$
Myrionecta rubra	-	17 * 20	-	$3.03 * 10^3$ [a]
Didinium gargantua	-	50 * 70	-	$8.50 * 10^4$ [b]
***Dinophysis* sp.**	-	-	-	$3.28 * 10^3$ [c]
***Protoperidinium* spp.**	-	-	-	$5.75 * 10^4$ [c]
***Gyrodinium* spp.**	-	-	-	$3.10 * 10^3$ [c]

a) Merrell & Stoecker 1998

b) Strüder-Kypke et al. 2002 (http://www.liv.ac.uk/ciliate/)

c) Phytoplankton Guide to the Chesapeake Bay and Other Regions (http://www.serc.si.edu/labs/phytoplankton/guide/index.jsp)

Table 1-3. The percent of the total prey carbon available to copepods and the percent of total copepod abundance (no. L^{-1}) that is comprised of *Acartia* spp. and *Eurytemora affinis*.

River	Station	Year	Percentage of Total Available Carbon		Percentage of Total Copepod Abundance	
			> 10 µm Chl *a*	Micro-zooplankton	*Acartia* spp.	*Eurytemora affinis*
Choptank	Middle	Dry	48	52	97	1
		Wet	75	25	72	28
	Upper	Dry	36	64	92	8
		Wet	75	25	46	54
Patuxent	Lower	Dry	55	45	95	0
		Wet	92	8	96	4
	Middle	Dry	71	29	96	0
		Wet	91	9	98	2
	Upper	Dry	53	47	100	0
		Wet	97	3	48	52

Figure Legends

Figure 1-1. Diagram of Chesapeake Bay. The Choptank and Patuxent River watersheds are shown enlarged in the inserts. White circles denote the biological sampling stations and are titled "Lower", "Middle", and "Upper." White triangles note the location of stations were only CTD casts were conducted.

Figure 1-2. Mean stream flow (m^3 s^{-1}) from March 1 through May 30 as recorded at US Geological Survey stream gauges, where a) is the Patuxent River and b) is the Choptank River. The long-term mean for the Patuxent River encompasses 25 years of data and 55 years of data for the Choptank River. The data was obtained from the USGS at: a) http://waterdata.usgs.gov/md/nwis/uv?01594440 (station located near Bowie, Maryland) and b) http://waterdata.usgs.gov/md/nwis/uv?01491000 (station located near Greensboro, Maryland).

Figure 1-3. Graphs of temperature during the dry and wet sampling years at each biological sampling station. The dashed line denotes the dry year, and the solid line denotes the wet year.

Figure 1-4. Graphs of salinity during the dry and wet sampling years at each biological sampling station. The dashed line denotes the dry year, and the solid line denotes the wet year.

Figure 1-5. Average microzooplankton (20-200 μm) and total phytoplankton biomass (μg C ml^{-1}) at all biological sampling stations in the dry year and wet year. The black bars represent the standard error of the means. Statistical differences between the dry and wet years are noted as follows: * ($p < 0.05$) and ** ($p < 0.005$).

Figure 1-6. Average copepod biomass (μg C L^{-1}) and average ctenophore biomass (liters of ctenophore per liter of sample) from March – May of the dry and wet years. The following copepod species are represented in the average copepod biomass: *Acartia tonsa*, *Acartia hudsonica*, and *Eurytemora affinis*. No ct. = no ctenophores were present in the samples during the wet year. The black bars represent the standard error of the means. Statistical differences between the dry and wet years are noted as follows: * ($p < 0.05$) and ** ($p < 0.005$).

Figure 1-7. Diagram of the difference in copepod, microzooplankton, and phytoplankton biomass in the dry and wet years. The volumes of the boxes are related to the biomass of each category (where 1 cm^2 = 20 μg C L^{-1}). The bar graph at the bottom represents the percent increase in biomass of the 3 categories in the wet year. The biomass values for a given category were determined by averaging all the biomass values at each station and from both rivers in each year.

Figure 1-8. Average yearly spring (March – May) a) stream flow (data obtained from the same source as noted in Fig. 2), b) chl a concentration, and c) copepod abundance from the Choptank River. The open bars represent the data from this study, the shaded bars

represent data from the Chesapeake Bay Program website. The solid horizontal lines represent the average value from 1988-2003. The dashed horizontal lines indicate the 1st and 3rd quartiles of the data set. Years average values below the 1st quartile are considered low value years, and those with average values above the 3rd quartile are considered high value years.

Figure 1-9. Average yearly spring (March – May) a) stream flow (data obtained from the same source as noted in Fig. 2), b) chl a concentration, and c) copepod abundance from the Patuxent River. The open bars represent the data from this study, the shaded bars represent data from the Chesapeake Bay Program website. The solid horizontal lines represent the average value from 1985-2003. The dashed horizontal lines indicate the 1st and 3rd quartiles of the data set. Years average values below the 1st quartile are considered low value years, and those with average values above the 3rd quartile are considered high value years. The copepod species included in the copepod abundance values are *Acartia hudsonica*, *Acartia tonsa*, and *Eurytemora affinis*.

Figure 1-1.

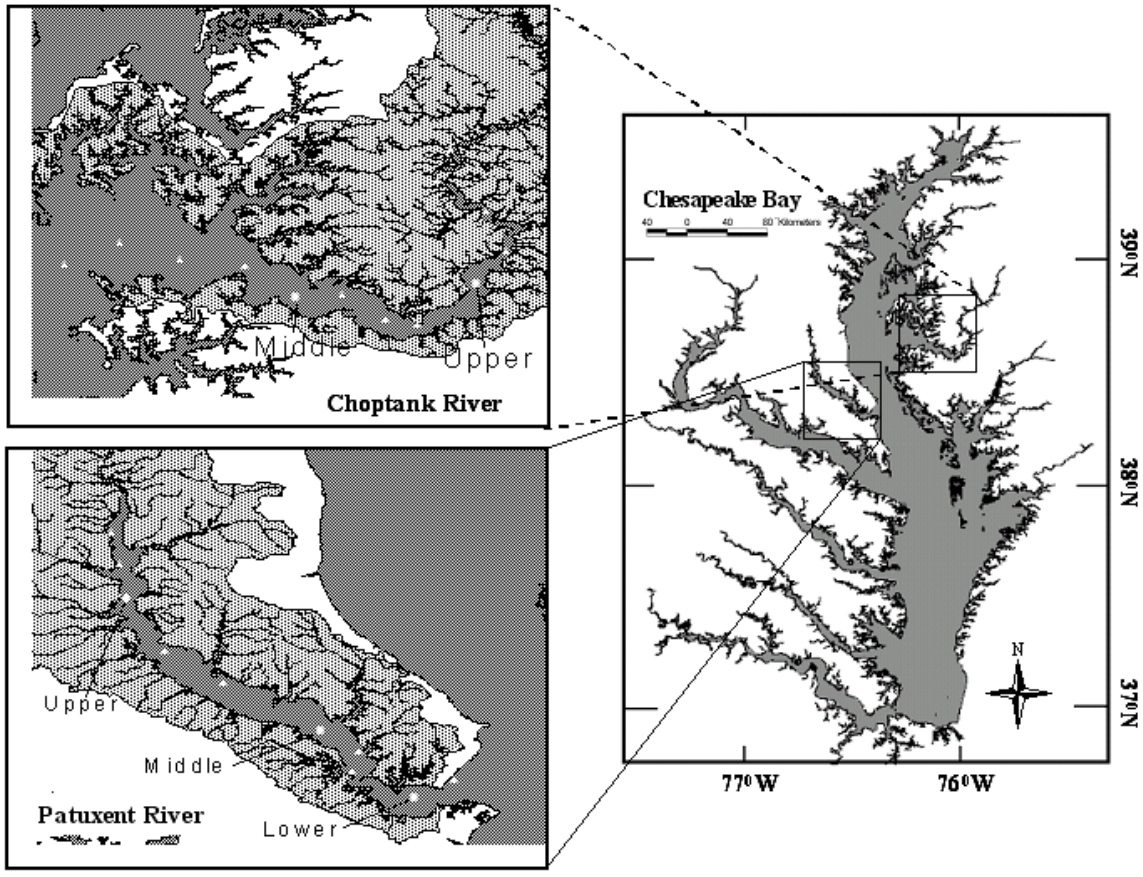

Chesapeake Bay
40 0 40 80 Kilometers
39°N
38°N
37°N
77°W
76°W
N
Middle
Upper
Choptank River
Upper
Middle
Lower
Patuxent River

Figure 1-2.

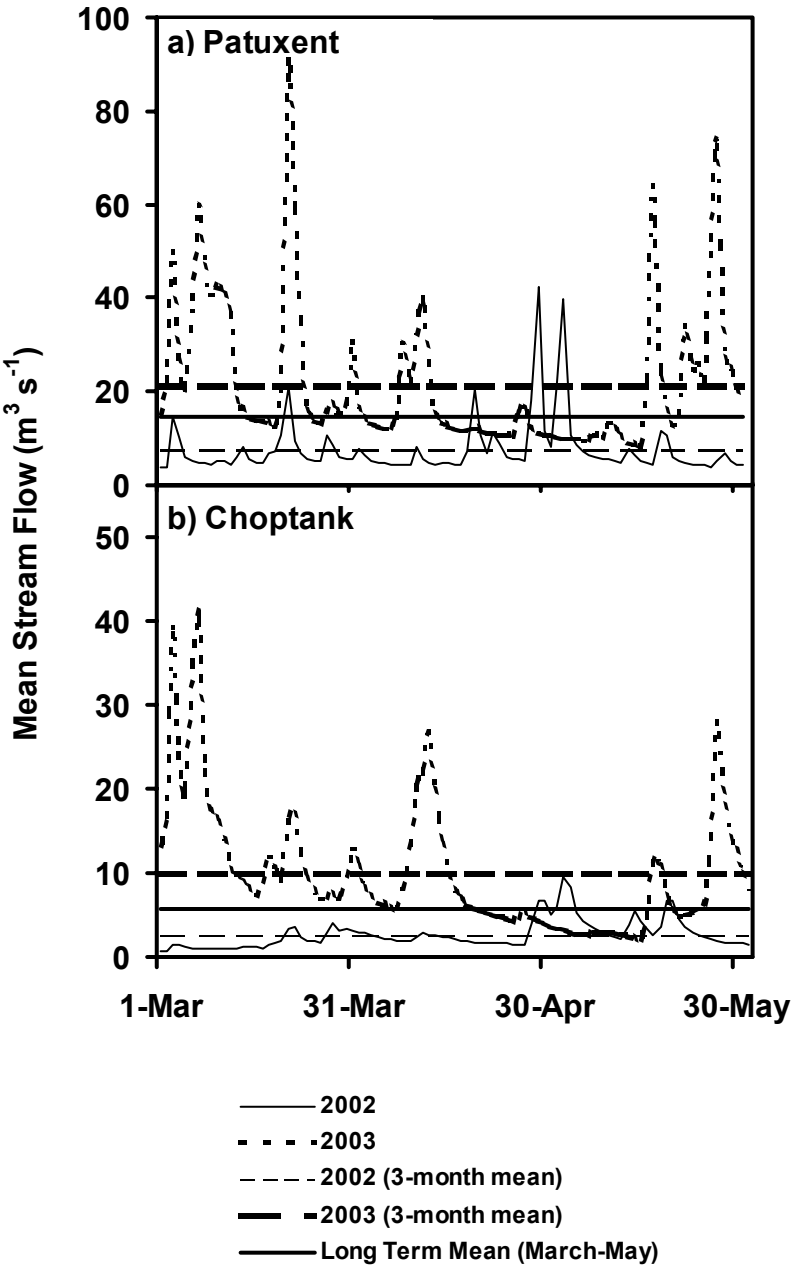

a) Patuxent
b) Choptank
Mean Stream Flow ($m^3 s^{-1}$)
100
80
60
40
20
0
50
40
30
20
10
0
1-Mar
31-Mar
30-Apr
30-May
2002
2003
2002 (3-month mean)
2003 (3-month mean)
Long Term Mean (March-May)

Figure 1-3.

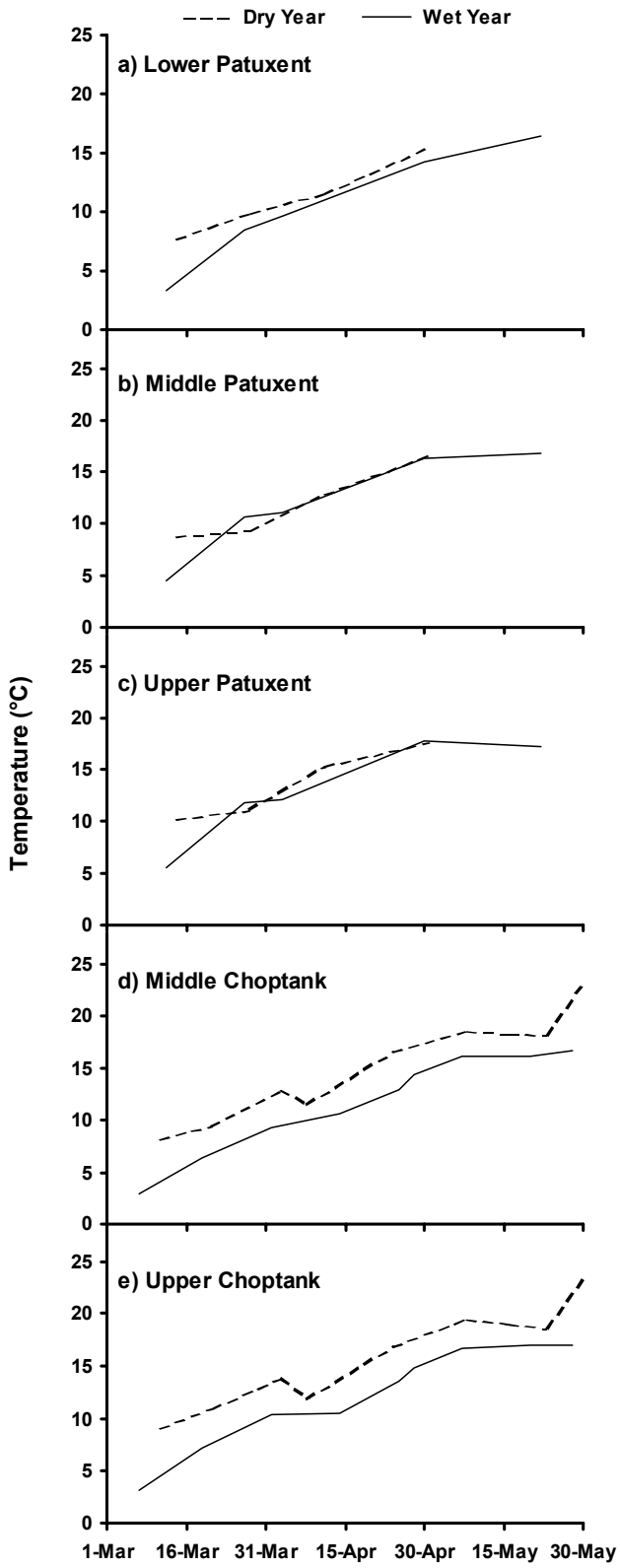

Dry Year
Wet Year
a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
Temperature (°C)
0
5
10
15
20
25
1-Mar
16-Mar
31-Mar
15-Apr
30-Apr
15-May
30-May

Figure 1-4.

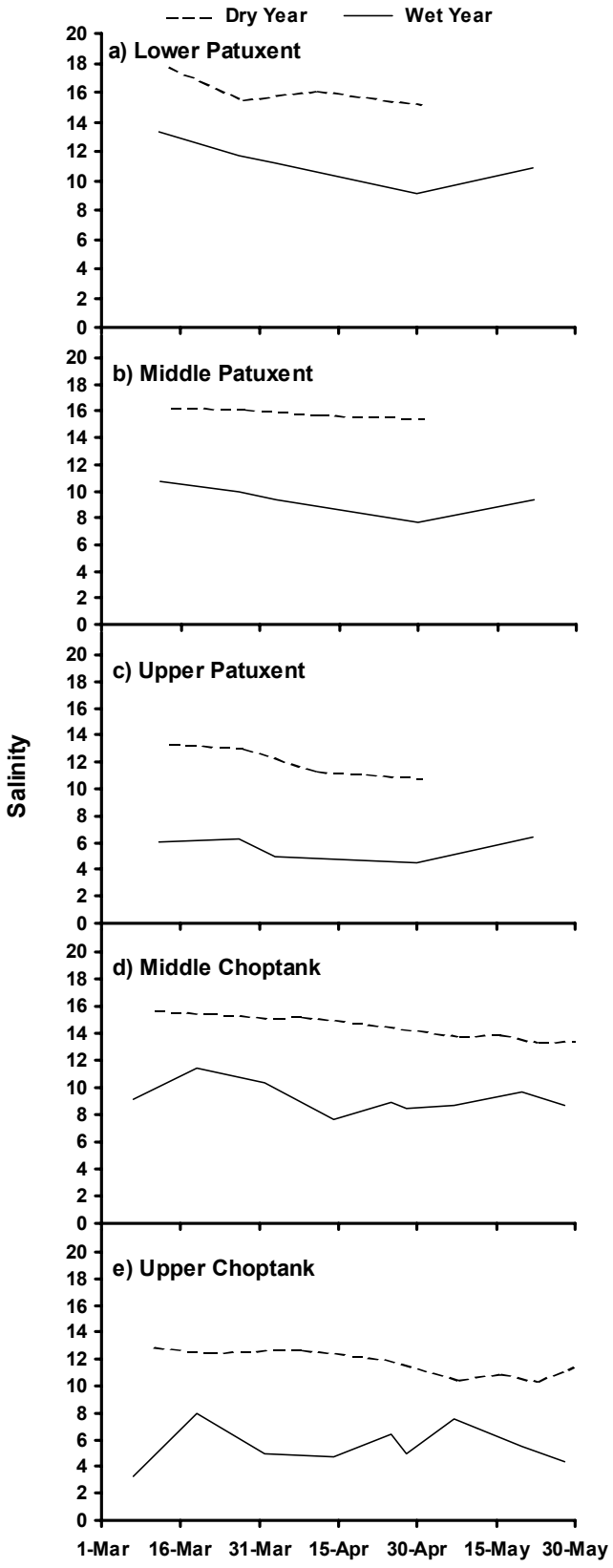

Dry Year
Wet Year
a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
Salinity
0
2
4
6
8
10
12
14
16
18
20
1-Mar
16-Mar
31-Mar
15-Apr
30-Apr
15-May
30-May

Figure 1-5.

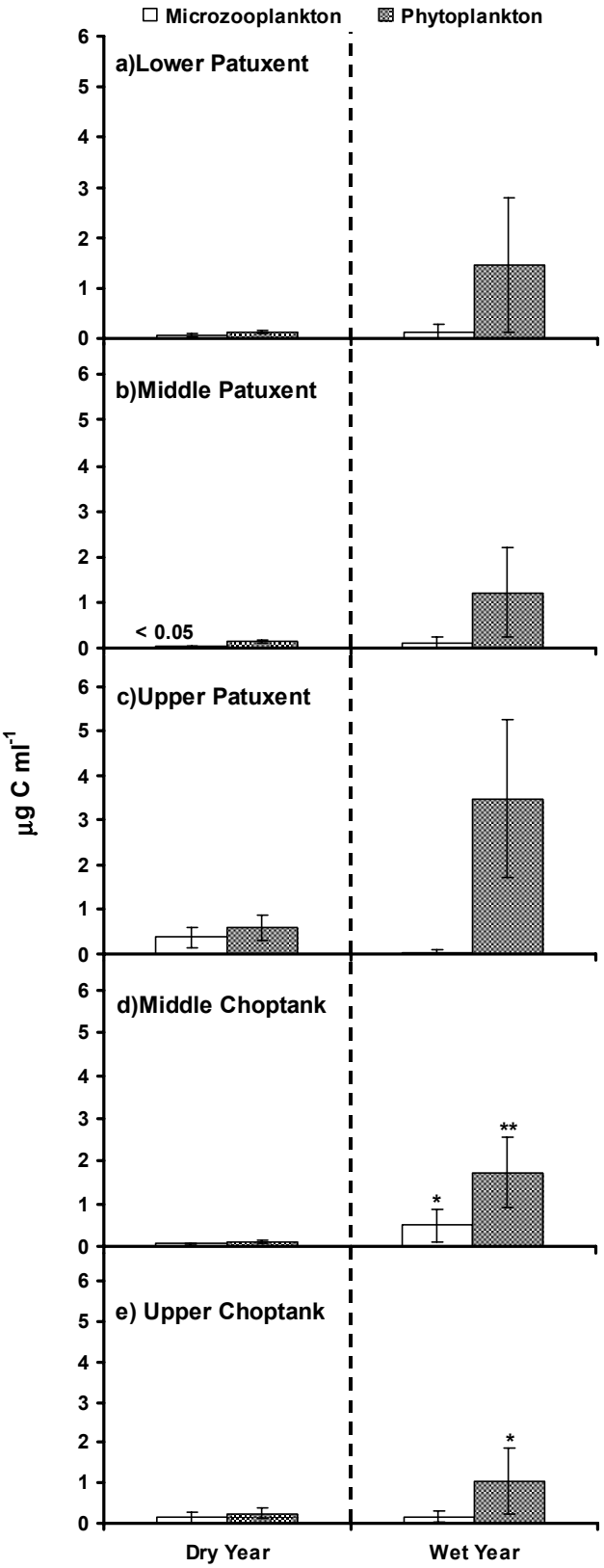

Microzooplankton
Phytoplankton
a)Lower Patuxent
b)Middle Patuxent
< 0.05
c)Upper Patuxent
d)Middle Choptank
e) Upper Choptank
µg C ml-1
Dry Year
Wet Year

Figure 1-6.

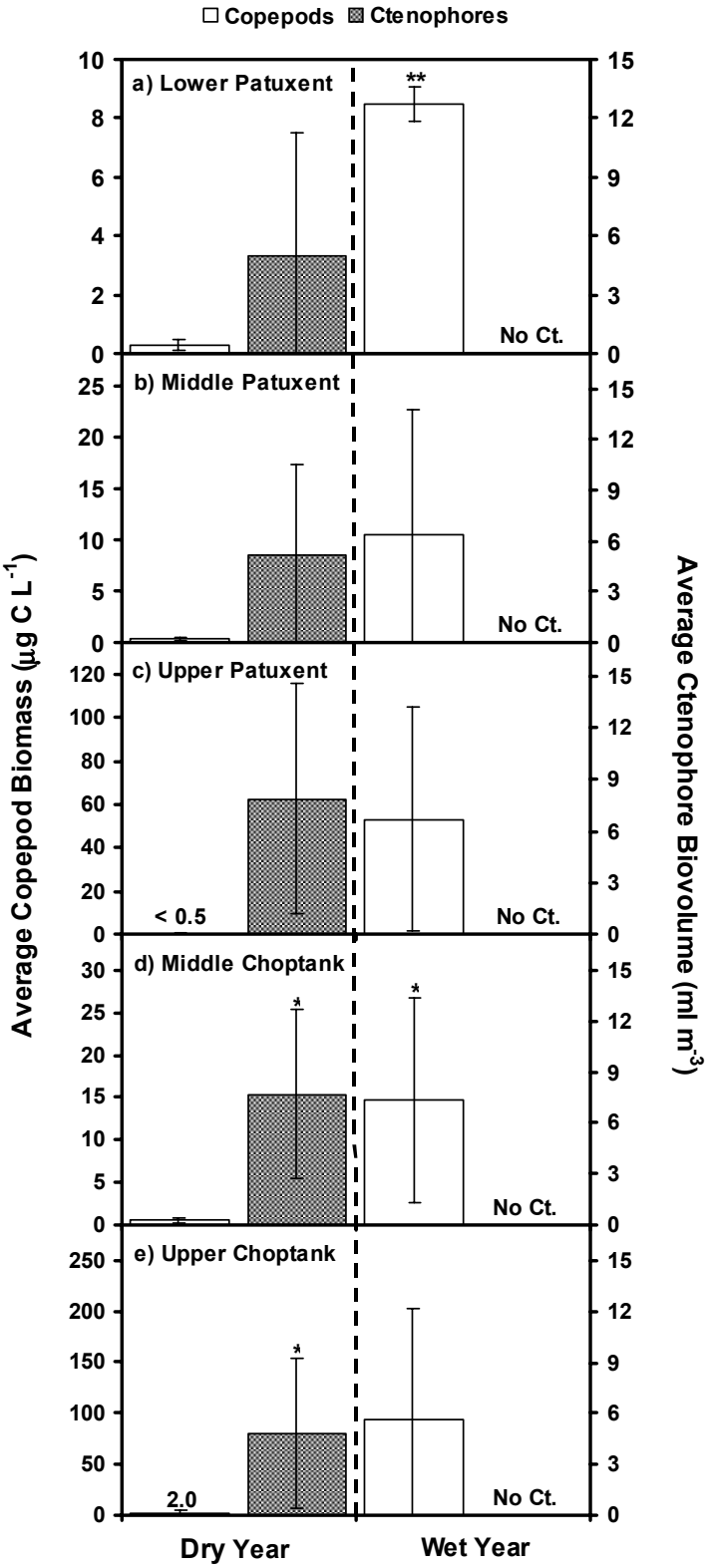

Copepods
Ctenophores
a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
**
*
No Ct.
< 0.5
2.0
Average Copepod Biomass (µg C L-1)
Average Ctenophore Biovolume (ml m-3)
Dry Year
Wet Year

Figure 1-7.

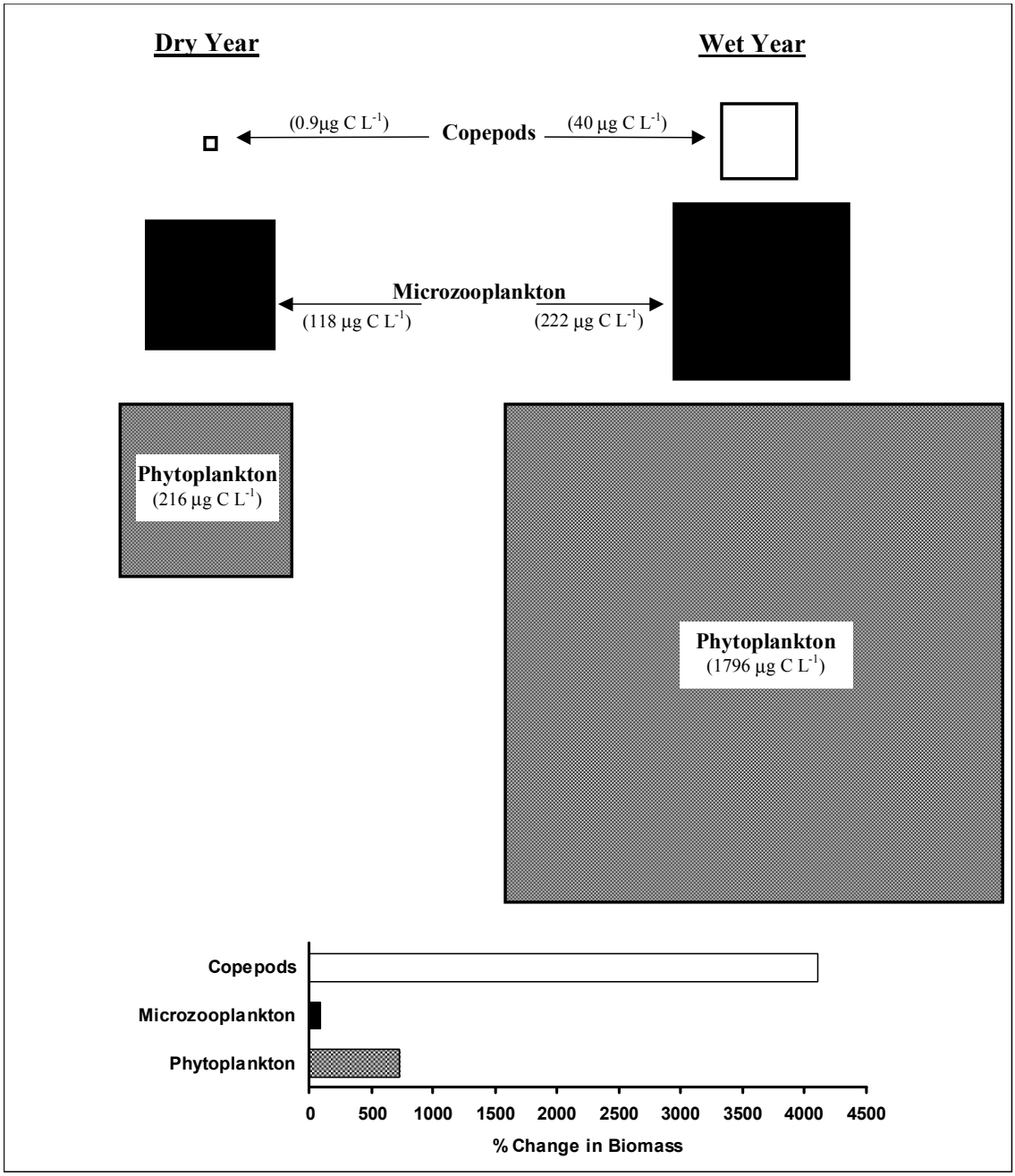

Dry Year
Wet Year
(0.9µg C L-1)
Copepods
(40 µg C L-1)
Microzooplankton
(118 µg C L-1)
(222 µg C L-1)
Phytoplankton
(216 µg C L-1)
Phytoplankton
(1796 µg C L-1)
Copepods
Microzooplankton
Phytoplankton
0
500
1000
1500
2000
2500
3000
3500
4000
4500
% Change in Biomass

Figure 1-8.

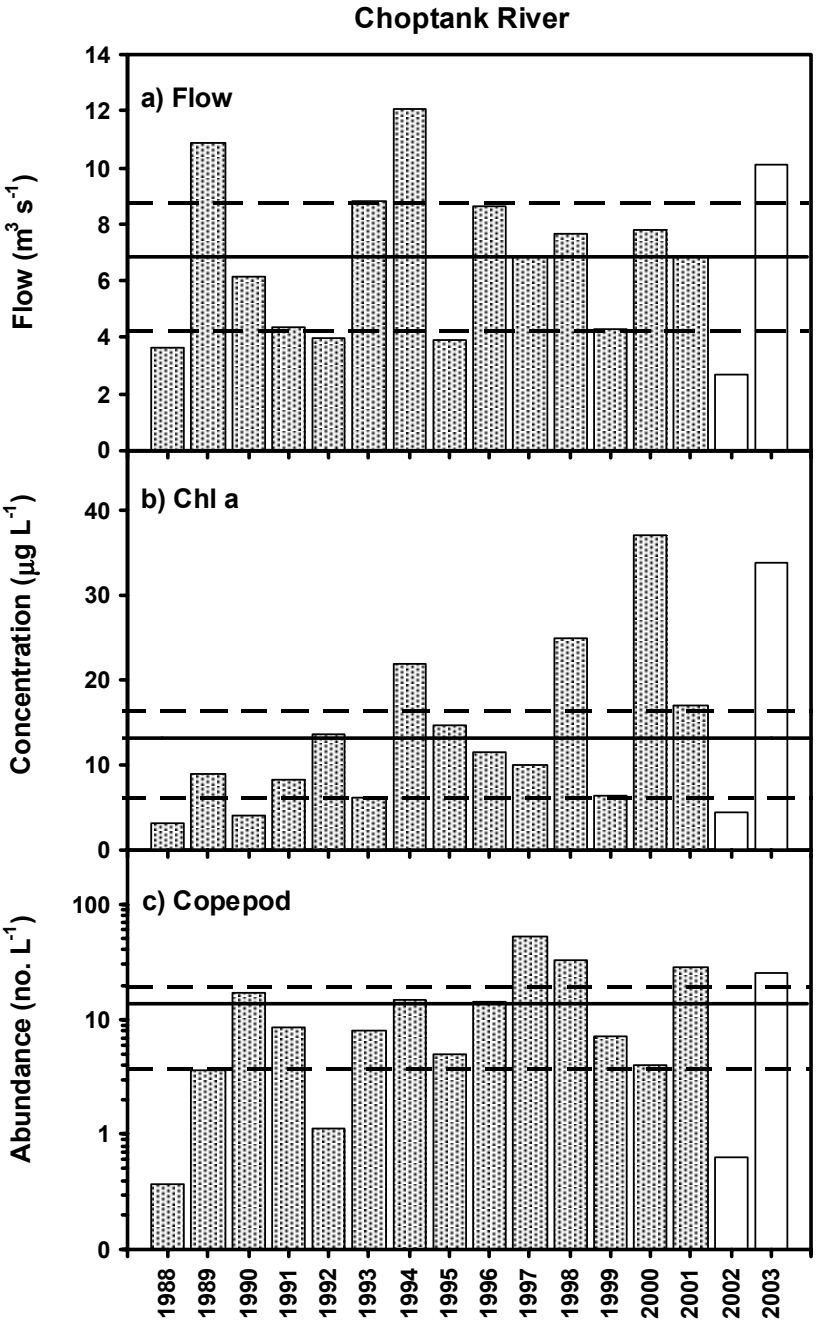
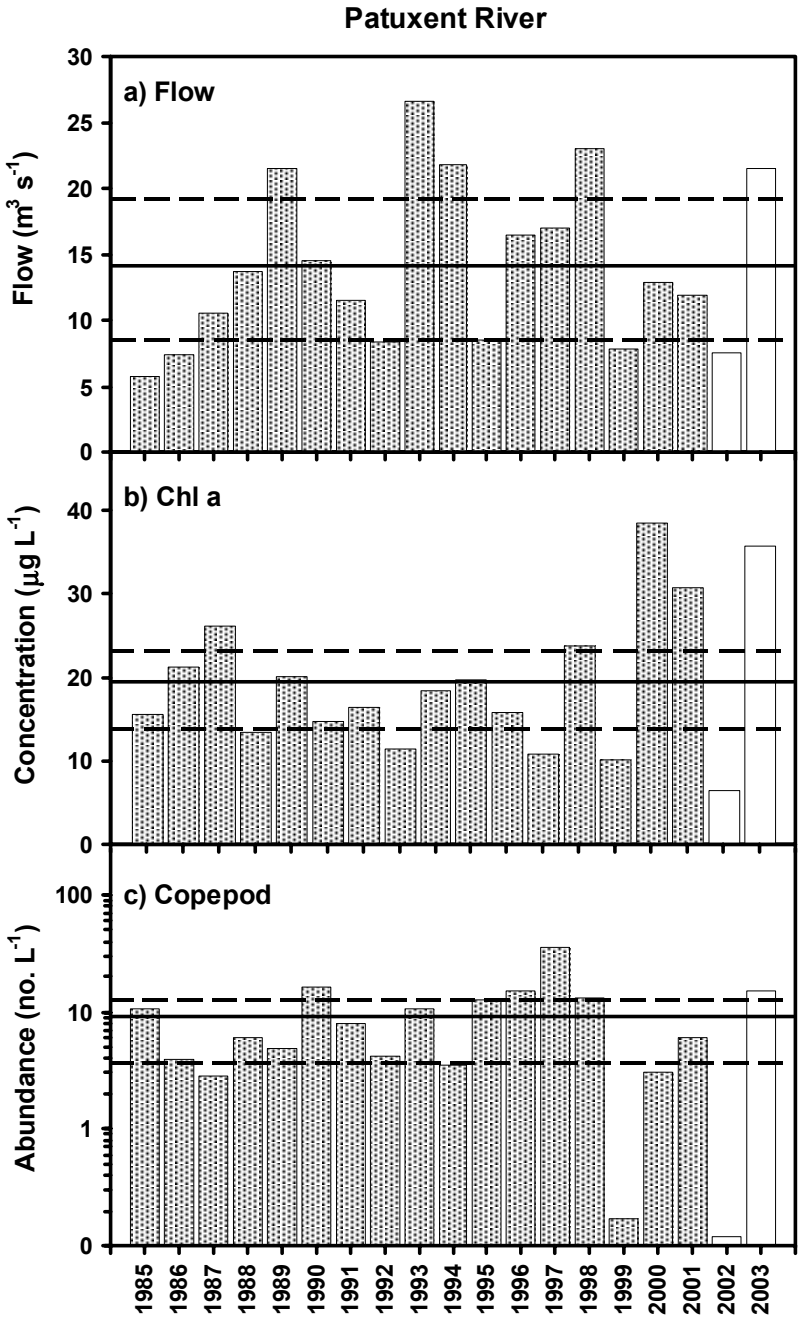

Choptank River
a) Flow
Flow (m3 s-1)
b) Chl a
Concentration (µg L-1)
c) Copepod
Abundance (no. L-1)
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003

Figure 1-9.


Patuxent River
a) Flow
Flow ($m^3 s^{-1}$)
b) Chl a
Concentration ($\mu g L^{-1}$)
c) Copepod
Abundance (no. L^{-1})
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003

Chapter 2

The role of copepod abundance in regulating top-down control of estuarine microzooplankton

Abstract

This chapter examines the hypothesis that high copepod abundance results in top-down control of microzooplankton and release of <10 μm phytoplankton from grazing pressure (i.e. a trophic cascade). Copepod grazing on phytoplankton and microzooplankton were compared during the springs of an extreme dry (2002) and wet (2003) year in 2 tributaries of Chesapeake Bay. The potential for copepod control over microzooplankton biomass was evaluated by comparing the potential copepod community grazing rate on microzooplankton (d^{-1}) to microzooplankton growth rate (d^{-1}). Multiple regression analysis was used to determine if copepod biomass was inversely related to microzooplankton biomass but directly related <10 μm phytoplankton biomass, which would indicate a copepod – microzooplankton – small phytoplankton trophic cascade. Grazing pressure on microzooplankton was greatest in the wet year, with copepods ingesting 0.21-1.58 μg of microzooplankton C copepod^{-1} d^{-1} and removing up to 100% of the daily standing stock. In the dry year copepods ingested 0.14 – 0.71 μg C copepod^{-1} d^{-1}, and a maximum of 3% of daily microzooplankton standing stock. Potential copepod grazing pressure was equivalent to microzooplankton growth in the wet year, implying strong top-down control. However, no inverse relationships were found between *in situ* measurements of copepod and microzooplankton biomass, nor did these biomass relationships support the presence of a trophic cascade.

Introduction

Calanoid copepods are the most abundant mesozooplankton species in Chesapeake Bay, comprising >65 % of total zooplankton numbers (Brownlee & Jacobs 1987). Once thought to be strictly herbivorous, copepods are now known to ingest a wide variety of food types (Lonsdale et al. 1979, Roman 1984, Stoecker & Capuzzo 1990), and many species display the ability to feed actively by selecting or rejecting different food types (Donaghay & Small 1979, Strickler 1982). As a result of their ability to selectively choose food, copepods can exhibit food-switching behaviors between herbivory and carnivory, often feeding disproportionately on the most abundant prey available (Landry 1981, Kleppel et al. 1988, Kleppel 1993). Recent work suggests that microzooplankton are the preferred food source for copepods, as they clear microzooplankton at higher rates than phytoplankton even in cases where microzooplankton comprise a smaller portion of the total available prey biomass (Gifford & Dagg 1988, Merrell & Stoecker 1998, Rollwagen Bollens & Penry 2003). In addition, copepods have greater clearance rates and greater prey concentration specific ingestion rates for protozoans than other food sources (Stoecker & Sanders 1985, Gifford & Dagg 1988, 1991, Roman & Gauzens 1997, Levinsen et al. 2000). The ingestion of protozoan microplankton also benefits copepod populations by increasing egg production rates and egg hatching success (Stoecker & Egloff 1987, Tiselius 1989, Tang et al. 2001). Given the prevalence of copepods in Chesapeake Bay and the ability to actively ingest microzooplankton, copepods have the potential to significantly influence the structure of estuarine planktonic food webs (e.g. Stibor et al. 2004a).

The importance of top-down control of microzooplankton protozoans in marine food webs remains inconclusive. The results of some studies support bottom-up control of microzooplankton through food limitation (Verity 1986, Nielsen & Kiørboe 1994), while others show possible top-down control from mesozooplankton grazing (Dolan 1991, Kivi et al. 1996). Despite these inconsistencies, large changes in copepod biomass have the potential to impact microzooplankton biomass given their estimated clearance and ingestion rates (Stoecker & Sanders 1985, reviewed by Stoecker & Cappuzzo 1990).

Increasing freshwater input has been linked to increases in the abundances of phytoplankton and mesozooplankton in Chesapeake Bay (Kimmel & Roman 2004). The typical bottom-up control of phytoplankton biomass hypothesis suggests that increased nutrient inputs during periods of increased freshwater input are responsible for creating phytoplankton blooms (Malone et al. 1988, Harding 1994). However, given the strong trophic coupling between microzooplankton and phytoplankton (Gallegos 1989, Fileman & Burkill 2001), it is also possible phytoplankton blooms are created (in part) by mesozooplankton removal of microzooplankton. Similar trophic cascades have been documented in freshwater lakes, where the effects of removing or adding piscivorous and planktivorous fish effect changes in phytoplankton abundance through a trophic cascade (McQueen et al. 1986, Carpenter et al. 1987). Verity & Smetacek (1996) report that while some evidence supports such trophic cascades in the marine literature, the complex hydrodynamics of marine environments and the resultant spatial and temporal variability in plankton communities, has made it difficult to investigate these relationships in marine systems. This is especially true for estuaries, which have a variety of physical processes that can aggregate or disperse different plankton groups depending on their relative size,

generation times, and behavior (Roman et al. 2005). Nonetheless, changes in freshwater flow may enhance trophic cascades and top-down control within estuarine plankton communities via changes in the abundances of mesozooplankton, microzooplankton, and primary producers.

The effects of different nutrient inputs and mesozooplankton abundances on microzooplankton populations were studied in 2 tributaries of Chesapeake Bay. The research objective was to evaluate the potential for copepods to control the microzooplankton community through grazing and to examine if the effects of such control would cascade down the food web to impact small (<10 μm) phytoplankton biomass.

Materials and Methods

Samples were collected from the Choptank and Patuxent Rivers, two mesohaline sub-estuarine tributaries of Chesapeake Bay (Figure 2-1). Two locations in the Choptank River (termed middle and upper) and 3 locations in the Patuxent River (termed lower, middle, and upper) were chosen based on their positions within 3 distinct hydrographical regions of the rivers: (1) wind-driven circulation in the lower estuary, (2) 2-layer gravitational circulation in the middle estuary, and (3) the up-river limit of salt intrusion in the upper estuary. Samples were collected during the spring (March through May) of 2002 and 2003. As reported in chapter 1, the freshwater flow in each of the rivers was below the long-term average in 2002 and above the long-term average in 2003. As a result, 2002 will henceforth be referred to as the "dry" year and 2003 as the "wet" year.

Collection Methods

Mesozooplankton samples were collected by conducting horizontal tows at the surface with a 0.5 m diameter, 202 µm mesh size plankton net, equipped with a General Oceanics flowmeter. The samples were preserved with 5 % (v/v) buffered formalin for future analysis. Separate mesozooplankton tows with a 0.5 m diameter, 202 µm mesh size, closed cod end net were used to collect live copepods for use in grazing experiments. The contents of these tows were stored in 4 L polycarbonate bottles and kept in flow-through seawater bins in order to keep the samples at ambient temperature. Natural microzooplankton (20 – 200 µm) samples were collected by preserving a sample of the surface water in 10 % (v/v) acid Lugol's solution (Parsons et al. 1984a, Gifford 1988, Sherr & Sherr 1993), and stored in tinted glass bottles. Additional whole water samples were collected from the surface of each station and stored in polypropylene bottles in the dark until the chlorophyll *a* (chl *a*) concentration could be determined at the laboratory. Approximately 10 L of surface water was filtered through a 202 µm mesh screen to remove mesozooplankton predators before being stored in 10 L clear polycarbonate bottles and placed in the flow-through seawater bins for use in the grazing experiments.

Grazing Experiments

Grazing experiments were conducted following the protocol described by Gifford (1993a). For each station, nine 500 ml clear polycarbonate bottles were filled with the <202 µm surface water. Six of the bottles for each station were designated as controls (no copepods present) and 3 were designated as experimental bottles (copepods added).

Ten adult female copepods were added to each of the experimental bottles. The species of copepod used was either *Acartia* spp. (*tonsa* and *hudsonica*) or *Eurytemora affinis*, depending on which species was the dominant copepod at a given time and sample location (Table 2-1). The bottles were placed on a rotating plankton wheel contained within a transparent UV resistant acrylic box with a screen that reduced light within the box by approximately 60% of surface irradiance. Temperature within the box was maintained at ambient levels by flowing river water through the box (Figure 2-2). The box was located outside in order to maintain seasonal light intensities and durations.

After one hour, 3 control bottles for each station were removed in order to determine the initial concentrations of phytoplankton and microzooplankton. These samples are referred to as the t_0 controls. Water to be used in chl *a* analysis was divided between total and <10 μm size fractions. The total chl *a* samples were filtered onto GF/F filters and frozen in the dark until analysis. The <10 μm size fraction chl *a* was obtained by filtering whole water through a 10 μm polycarbonate membrane filter and collecting the <10 μm fraction on GF/F filters. The filters were frozen and kept in the dark until analysis. Microzooplankton samples were preserved in 10 % acid Lugol's solution and kept in dark polycarbonate bottles. Additional microzooplankton samples were preserved in 1 % (v/v) gluteraldehyde for use with epifluorescent microscopy. After 24 hours, the other 3 control (termed t_{24} controls) and the 3 experimental bottles were removed from the plankton wheel in order to determine prey growth rates and copepod clearance and ingestion rates for both phytoplankton and microzooplankton. Phytoplankton and microzooplankton samples were preserved as above.

Sample Analysis

The mesozooplankton from the preserved field samples were counted and grouped according to class, and copepods were further divided into genera. In order to estimate the biomass of copepods, the prosome length of 50 adult female copepods from each sample were measured and these measurements were used to calculate carbon content based on equations 3a & b in chapter 1. Microzooplankton were grouped into the categories shown in Table 2-2, and biomass (both *in situ* and from the grazing experiments) was calculated by the methods specified in chapter 1. Since the water used in the grazing experiments was filtered through a 202 µm mesh screen upon collection, the abundances of organisms in the *in situ* microzooplankton samples were compared to the grazing experiment t_0 microzooplankton samples to determine if the there were significant reductions microzooplankton abundance that would effect the results of the grazing experiments. Total and <10 µm chl *a* concentration was determined using a Turner flurometer as described in Parsons et al. (1984a). The >10 µm chl *a* concentration as calculated by subtracting the <10 µm fraction from the total chl *a* concentration. Chl *a* concentration (µg L^{-1}) was converted to biomass (µg C L^{-1}) using a carbon to chl *a* ratio of 30:1 (Parsons et al. 1984b). Only the concentration of >10 µm phytoplankton was considered to be available food for the copepods, as the lower size limit of particle capture for *Acartia* spp. and *E. affinis* is 7 – 10 µm (Nival & Nival 1976, Berggreen et al. 1988).

Calculations and Statistical Analyses

Clearance (ml copepod^{-1} d^{-1}) and ingestion (µg C copepod^{-1} d^{-1}) rates of copepods on microzooplankton and >10 µm phytoplankton were calculated based on the modified Frost (1972) equations given in Gifford (1993a). Since 2 species of copepods were used at different times in the grazing experiments (Table 2-1), the clearance rates for microzooplankton and total phytoplankton of both species were compared in order to determine if they were comparable so that we could combine the rates into one estimate. The clearance rate for both microzooplankton and phytoplankton were not significantly different (Student's t-test) between *Acartia* spp. or *E. affinis*. Thus the calculated potential grazing rates were independent of the species of copepod used in the grazing experiments.

In order to examine the impact of copepod grazing on prey populations, the percent of prey standing stock removed (d^{-1}) was calculated. This was calculated as the product of copepod abundance (copepods L^{-1}) and the clearance rates (L copepod^{-1} d^{-1}) for >10 µm phytoplankton and each microzooplankton category at each biological station, and then expressed as a percentage. A potential grazing rate (d^{-1}) to prey growth rate (d^{-1}) analysis was used to determine the ability of copepod grazing to control microzooplankton biomass. The potential grazing rate of copepods was calculated in the same manner as standing stock removed, except it is not expressed as a percentage. Microzooplankton growth rates (µ) were calculated using the following equation from Gifford (1993a):

$$\mu = \ln (C_2 / C_1) / t$$

where C_2 is the microzooplankton concentration in the t_{24} control samples, C_1 is the microzooplankton concentration in the t_0 control samples, and t is the total time of incubation.

All statistical analyses were conducted using JMP Statistical Discovery Software 4.0.4 (SAS Insitiute Inc.). Student's t-tests were used to determine statistical differences between dry and wet year copepod biomass estimates and $\log_{10}$ transformed initial prey concentrations, and between dry and wet year percentages of standing stock removed. The nonparametric Wilcoxon test was used to determine differences in clearance rates, ingestion rates, potential grazing rates, and microzooplankton growth between the dry and wet years. Linear correlations were tested for significant differences using analysis of variance (ANOVA), and the nonparametric Spearman rank test was used to test relationships within multiple correlation analyses.

Results

Comparison of Microzooplankton Abundances in Environmental Samples and in Grazing Experiments

It is usually assumed that the t_0 samples from microzooplankton grazing experiments reflect the ambient microzooplankton community. Although the water used in the grazing experiments presented here was collected simultaneously with the preservation of natural samples from each station, the t_0 abundances in the grazing experiments were less than in the field samples. The greatest decreases in abundance occurred in the large heterotrophic dinoflagellate and large ciliate categories, such as *Protoperidinium* spp. and *Didinium gargantua* (Table 2-3). The average percentage of

decrease in the t_0 abundances from the field abundances was 51%. Percentages of decrease in t_0 abundance ranged from 7 – 98% decrease from field samples (Table 2-3). Pre-filtering collected seawater through 202 µm mesh, storage for up to 5 hours in a carboy, pouring the water into the experimental grazing chambers, and mixing the contents by end-over-end rotation for 1 h (when the t_0 sample was collected) resulted in large decreases in microzooplankton abundance (Table 2-3). These discrepancies are most pronounced when large heterotrophic dinoflagellates or large ciliates are important members of the assemblage, such as those mentioned above. Some implications of this problem are discussed below.

Grazing Experiment Data

Analysis of chl *a* data showed significantly higher biomass of >10 µm phytoplankton at the beginning of the wet year grazing experiments as compared to the dry year experiments at both stations in the Choptank River (Figure 2-2). Increased biomass was also measured in the Patuxent River, but statistical differences between the 2 years were not detected probably due to the small sample size. While the initial biomass of microzooplankton appears to have been greater in the wet year experiments, there were no significant differences between the wet and dry years (Figure 2-2). Phytoplankton biomass (>10 µm) available to copepods during the grazing experiments ranged from 0.04 – 0.19 µg C ml^{-1} in the dry year, and 0.31 – 0.87 µg C ml^{-1} in the wet year. The available microzooplankton biomass in the dry year ranged from 0.014 – 0.074 µg C ml^{-1}, and from 0.013 – 0.111 µg C ml^{-1} in the wet year. Thus there was approximately a 7-fold increase in the average available phytoplankton biomass as

compared to a <2-fold increase in average microzooplankton biomass in the grazing experiments during the wet year.

Examination of clearance rates of copepods on >10 μm phytoplankton revealed no specific trends between the dry and wet years, as instances of significantly greater clearance rates were found in both the dry and wet years (Table 2-4). This was also true of clearance rates of copepods for microzooplankton, except that there was only one instance of a significantly greater clearance rate, which occurred at the upper station of the Patuxent River in the dry year (Table 2-4). Negative clearance rates were calculated for the >10 μm phytoplankton prey category, and occurred in both the dry and wet years. Negative rates denote situations in which the presence of copepods resulted in increased net prey growth compared to the controls without added copepods. All negative clearance rates were assumed to be zero clearance in calculating the averages in Table 2-4. Significant increases in the ingestion of >10 μm phytoplankton in the wet year were calculated at 2 of the 5 biological stations and at 3 of the 5 stations for ingestion of microzooplankton in the wet year (Table 2-4).

Despite the lack of difference in individual copepod clearance rates for microzooplankton between the dry and wet years, large increases in copepod abundances (Figure 2-3) in the wet year led to an increased depletion of microzooplankton standing stock d^{-1} (Figure 2-4). The percentage of microzooplankton standing stock removed d^{-1} was significantly greater at all stations in the wet year, with the exception of the upper Patuxent station. Percentages of microzooplankton standing stock removed ranged from < 1 – 3% in the dry year and 9 – 60 % in the wet year. The removal of >10 μm phytoplankton standing stock significantly increased in the wet year at both stations of

the Patuxent River, but there was no difference in removal at any of the Choptank River stations (Figure 2-4).

In order to estimate whether the increased copepod ingestion of microzooplankton biomass in the wet year was great enough to limit microzooplankton growth, the potential copepod community grazing rates were compared to the microzooplankton growth rates obtained from the experimental controls (Table 2-5). The potential grazing rate of copepods on microzooplankton significantly increased at all but the middle Patuxent River station in the wet year. However, it is important to note that there were no significant increases in microzooplankton growth rates in the wet year over those of the dry year (Table 2-5). At all biological stations with a positive microzooplankton growth rate, the ratio of potential copepod grazing rate to microzooplankton growth rate increased in the wet year (Table 2-4). The values of this ratio ranged from 0.05 – 0.13 in the dry year, and from 0.39 – 2.10 in the wet year. In the dry year, the microzooplankton growth rate was significantly greater than the potential copepod grazing rate at the upper Choptank and middle Patuxent River stations. In comparison, there were no significant differences between microzooplankton growth and potential grazing rate in the wet year (Table 2-5). Thus, there was a shift from significantly greater growth rates than grazing rates in the dry year (which would allow for net population growth) to no significant differences between growth and grazing in the wet year (Table 2-5).

Response of *In Situ* Biomass

Measurements of the *in situ* biomass of copepods, microzooplankton, and phytoplankton were reported in chapter 1. Increases in copepod biomass (4110%) and

phytoplankton biomass (733%) were much greater than microzooplankton (88%) in the wet year as compared to the dry year (Figure 1-7). Microzooplankton biomass showed no dependence upon copepod biomass in either the dry (ANOVA, $p = 0.26$) or wet year (ANOVA, $p = 0.83$), or both years combined (ANOVA, $p = 0.98$). The results of multiple regression analysis (Table 2-6) showed that copepod biomass and microzooplankton biomass had no effect on small (<10 μm) phytoplankton biomass when the dry and wet years were analyzed separately, but copepod biomass had a significant positive effect on <10 μm phytoplankton biomass when both years were combined. A non-parametric correlation analysis revealed a significant positive correlation (Spearman's Rho = 0.41, $p < 0.005$) between the potential copepod grazing rate for microzooplankton, as measured in the grazing experiments, and *in situ* <10 μm phytoplankton biomass from the combined years.

Discussion

Impact of the Experimental Method

Protozoan microplankton, particularly aloricate ciliates, are fragile organisms that can lyse as a result turbulence and contact with netting (Gifford 1993a). Gifford (1985) reports that up to 37% of ciliate biomass can be lost due to filtering through a 202 μm mesh screen. This problem is demonstrated in this paper by the decrease in microzooplankton abundances in the t_0 samples from the grazing experiments as compared to samples preserved on station (Table 2-3). The water containing microzooplankton used in the grazing experiments was poured through a 202 μm mesh screen upon collection and turbulence during handling and transfer into the experimental

bottles both may have caused cells to lyse. This may have caused considerable differences in microzooplankton abundances between the experimental samples and *in situ* samples, particularly among the larger protozoans (Table 2-3). The decreased abundance of protozoan microplankton in the grazing experiments, compared to nature, may have resulted in an underestimation of ingestion and an over estimation of clearance, and hence the potential community grazing rate of copepods on microzooplankton. Assuming that copepods select for the largest microzooplankton (Berggreen et al. 1988), clearance rates could be underestimated by as much as 59%.

Using an analysis of prey removal in bottle incubation experiments that contain several trophic levels to estimate copepod grazing potentials creates the potential for underestimation of copepod clearance and ingestion rates on prey types that are consumed by more than one level of grazer (Nejstgaard et al. 2001). For instance, in the grazing experiments described in this paper, phytoplankton may be consumed by both microzooplankton and copepods in the experimental bottles, but only by microzooplankton in the control bottles. Therefore, significant grazing pressure on microzooplankton as exerted by copepods has the potential to relieve grazing pressure on phytoplankton and allow for net phytoplankton growth in the grazing experiment bottles. However, microzooplankton grazing may limit net phytoplankton growth in control bottles, thus creating a situation where the final phytoplankton concentrations are greater in the control bottles than the experimental bottles. The calculation of negative clearance rates of copepods for phytoplankton, as mentioned above, is an outworking of this problem. The calculation of negative grazing rates is common when using this method of estimating copepod grazing rates (e.g. Hansen et al. 1993, Nejstgaard et al. 1997,

Nejstgaard et al. 2001). Copepods undoubtedly do ingest phytoplankton during the experiments, but the ability to measure this signal is drown-out by a significant release of phytoplankton mortality due to decreased grazing from the microzooplankton community. Thus, negative clearance and ingestion rates cause the actual grazing pressure of copepods on phytoplankton to be underestimated and an overestimation of the contribution of microzooplankton to the copepods diet (Nejstgaard et al. 2001). These negative rates are however indicative of the presence of trophic cascades with in the experimental bottles, and are quantitatively related to trophic cascades in nature, as they are dependent upon the number of copepods in the bottles.

Microzooplankton Biomass

Plankton biomass is controlled by both bottom-up and top-down mechanisms. In the case of heterotrophs, bottom-up control is typically attributed to food limitation, and top-down control is attributed to predation (Kiørboe 1998). In chapter 1 it was reported that the small increase in the biomass of microzooplankton in the wet year was negligible compared to the large increases of phytoplankton and copepod biomass during the same period. The lack of a significant increase in microzooplankton biomass in response to the large increase in phytoplankton biomass is puzzling given the strong trophic coupling between microzooplankton and phytoplankton (Gallegos 1989, Fileman & Burkill 2001). Assuming a gross growth efficiency of 30 – 40%, a phytoplankton growth rate of 0.97 d^{-1}, a phytoplankton grazing mortality rate of 0.53 d^{-1}, and that microzooplankton ingest 59.7% of primary production in estuarine habitats (Landry & Calbet 2004); and using the wet year phytoplankton biomass estimate in chapter 1, it is estimated that

microzooplankton biomass should have been on the order of 338 – 450 µg C L^{-1} in the wet year. However, the average microzooplankton biomass was 222 µg C L^{-1}, which is 34 – 51% less than the calculated potential biomass. Ranges in the average total and <10 µm phytoplankton biomass in the wet year grazing bottles were 610 – 1850 µg C L^{-1} and 120 – 1540 µg C L^{-1}, respectively. Both of these ranges are greater than the reported threshold of 50 µg C L^{-1} required for sustaining microzooplankton growth (Verity 1991), also see (Strom & Morello 1998). In light of the evidence against food limitation, the results of the grazing experiments and the copepod population estimates presented in this paper imply grazer control of microzooplankton biomass by copepods.

Copepod clearance rates of microzooplankton did not significantly change between the dry and wet years (Table 2-4), thus the increased control of the microzooplankton community in the wet year was a result of the increase in copepod abundance (Figure 2-3). The increase in copepod abundance resulted in increased copepod community grazing potential of the microzooplankton population in the wet year (Table 2-5), and created potential grazing rates that were equivalent to microzooplankton growth rates. This was in contrast to the dry year when microzooplankton growth rates were larger than the potential grazing rate, thus enabling the microzooplankton to out-grow the copepod grazing pressure (Table 2-5). The effect of increased copepod abundance on the ability to control microzooplankton community biomass is further demonstrated by the increased removal of microzooplankton standing stock removed d^{-1} in the wet year, when as much as 60% of the microzooplankton community was cleared d^{-1} (Figure 2-4).

Rates of copepod ingestion of microzooplankton typically decrease as the concentration of phytoplankton increase (Stoecker & Sanders 1985, Stoecker & Egloff 1987). The opposite effect was observed during this study, as copepod ingestion of microzooplankton increased in the wet year, when phytoplankton was abundant (Table 2-4). The average ratio of ingested microzooplankton (μg C copepod^{-1} d^{-1}) to copepod weight (μg C copepod^{-1}) increased from 0.09 d^{-1} in the dry year to 0.23 d^{-1} in the wet year. Thus the ingestion rates increased because each copepod was eating more microzooplankton carbon in proportion to its body weight. The increased ingestion rates together with increased selection for microzooplankton (Table 2-7) demonstrate that microzooplankton are an important food source for estuarine copepods, even in eutrophic conditions.

There exist numerous studies supporting mesozooplankton control of microzooplankton biomass in freshwater environments (Carrick et al. 1991, Burns & Schallenberg 1998, Adrian & Schneider-Olt 1999, Burns & Schallenberg 2001). These studies have focused on the effect of zooplankton grazing on protozoan net growth rates through *in situ* grazing experiments. They report that protozoan net growth rates are inversely related to zooplankton biomass and that there is tight trophic coupling between the 2 levels, where zooplankton biomass accounts for as much as 84 % of protozoan variability (Carrick et al. 1991, Adrian & Schneider-Olt 1999). As the importance of protozoans to the diets of marine zooplankton has gained greater understanding in marine environments, studies have recognized the potential for copepods to control microzooplankton distributions and biomass (Stoecker & Sanders 1985, Stoecker & Capuzzo 1990, Nielsen & Kiørboe 1994, Kivi et al. 1996, Vadstein et al. 2004).

Copepods have the ability to clear ciliates from 35 – 200 % of the surface water column per day (Dolan 1991, Kivi et al. 1996). Vadstein et al. (2004) report that copepods exert control over ciliates not only by the direct effects of grazing, but also by greater consumption of mutual food resources.

Despite the large decrease in top-down grazing pressure from copepods (Table 2-5, Figure 2-4), microzooplankton biomass in the dry year was less than the biomass of the wet year. Small phytoplankton biomass in the dry year grazing experiments generally seemed to be sufficient (ranging from 30 – 180 μg C L^{-1}) to sustain maximum protozoan growth (Verity 1991, Strom & Morello 1998).

There was not a greater concentration of microzooplankton in the dry as compared to the wet year when there was significantly less top-down control by copepods and there seemed to be ample prey concentrations to support microzooplankton growth. One possible explanation is that only adult copepods were used in the grazing experiments. In the natural environment there are many more possible predators of microzooplankton. In this study, adult *Acartia* spp. and *E. affinis* only comprised an average of 16% (Choptank River) and 34% (Patuxent River) of the total mesozooplankton community in the dry year, and 28% (Choptank River) and 42% (Patuxent River) in the wet year. Grazing experiments using copepod nauplii have found that they contribute to much as 56% of the total copepod community grazing on microzooplankton, and including naupliar ingestion rates adds an additional grazing impact on ciliates of 9 – 84% d^{-1} (White & Roman 1992b, Merrell & Stoecker 1998). During the dry year there were additional zooplankton species present, such as the calanoid species *Centropages hamatus* and the cladoceran *Podon polyphemoides*, that are

not usually present during the spring in the mesohaline portion of Chesapeake Bay (Brownlee & Jacobs 1987). Some evidence exists to suggest that *P. polyphemoides* may have greater ingestion rates for microflagellates (>10 μm) than the copepod species used in this experiments (Turner & Granéli 1992). There were also many larval stages of organisms that typically inhabit the more southern, higher salinity portions of the Bay, but that were able to move into the mesohaline region due to increased salinities in the dry year (Figure 1-4). Therefore, by not using these organisms in the grazing experiments, the top-down grazing pressure of zooplankton on the microzooplankton community was potentially underestimated.

Small Phytoplankton Biomass

Microzooplankton have greater ingestion rates for small (typically defined as <10 or <20 μm) phytoplankton and phytoflagellates than do mesozooplankton, and are generally more efficient at grazing small phytoplankton because they have comparable growth rates that allow microzooplankton populations to rapidly respond to increases in phytoplankton biomass (Kiørboe 1993, Ingrid et al. 1996). As a result, small phytoplankton biomass is often kept in check by microzooplankton grazing (Kiørboe 1998). If microzooplankton growth is balanced by mortality due to mesozooplankton grazing, then there exists the possibility for windows of reduced grazing pressure on small phytoplankton. If these windows occur in conjunction with ample nutrient supplies, then phytoplankton may be able to exploit the decrease in grazing pressure and out-grow their protozoan predators. Such instances of the release of phytoplankton by zooplankton grazing on protozoans have been observed in other aquatic systems

(Vadstein et al. 2004). Hansen et al. (1993) described a situation in which copepod grazing on protozoans released *Phaeocystis* from top-down control and aided in bloom formation. In mesocosm experiments Stibor et al. (2004a) found that the presence of copepods had a positive effect on small algae biomass, and the presence of gelatinous zooplankton had negative effects on small algae biomass. The removal of other phytoplankton-consuming organisms, such as the appendicularian *Oikopleura dioica*, by copepods has also been found to have strong positive correlations with small phytoplankton biomass (Stibor et al. 2004b). The presence of negative clearance rates in the grazing experiment results could represent situations where the >10 μm phytoplankton were partly released from the top-down control of microzooplankton due to copepod ingestion of microzooplankton (Nejstgaard et al. 1997, Nejstgaard et al. 2001). It addition less top-down control, copepod grazing of microzooplankton may also increase nutrient supplies to phytoplankton by removing the predators of nitrogen regenerators (Miller et al. 1995).

In situ small phytoplankton biomass did show a significant positive relationship with copepod biomass (Table 2-6), but only when the data from both the dry and wet years were combined, otherwise there was no significant trend in each individual year. Additionally, one would expect to find negative relationships between microzooplankton biomass and small phytoplankton biomass, but this was also not observed in either the dry or wet year, nor with both years combined (Table 2-6). This lack of correlation may be due to sampling intervals that are not of the necessary temporal resolution to detect biomass changes that would occur due to grazing, and a the wrong spatial scale to account for communities being moved down-river before the next sampling date.

The results of the grazing experiments indicated strong top-down control of microzooplankton biomass but *in situ* biomass measurements did not support this hypothesis. If copepods were able to control microzooplankton biomass then one would expect to find an inverse relationship between *in situ* copepod biomass and microzooplankton biomass. However, there was no significant correlation between the natural abundances of copepods and microzooplankton in the dry year (Spearman's Rho = 0.04, $p = 0.26$), the wet year (Spearman's Rho < 0.01, $p = 0.83$), or both years combined (Spearman's Rho << 0.001, $p = 0.98$). A possible explanation for this observation is that phytoplankton biomass, particularly in the wet year, was great enough to saturate copepod grazing and therefore release microzooplankton from top-down control (Nielsen & Kiørboe 1994, Levinsen & Nielsen 2002). However, in the experiments presented here, clearance rates of copepods for microzooplankton were not depressed in the wet year. Furthermore, it is not believed that copepod ingestion was saturated by the phytoplankton biomass in the wet year because there was a greater use of microzooplankton carbon by copepods in the wet year (Table 2-7). Congruently, the ratio of copepod prey ingested to phytoplankton biomass stayed relatively the same between the two years (Table 2-7). Thus, copepod ingestion of microzooplankton was not impacted by high phytoplankton concentrations, but rather increased in the wet year.

There are several possible explanations for the lack of correlation between the results of the grazing experiments and the *in situ* biomass measurements. First, this lack of correlation may be due to an overestimation of clearance rates for microzooplankton in the grazing experiments because of decreased microzooplankton concentrations in the experimental bottles as compared to the field samples, as clearance rates decrease as a

function of increasing prey concentration (Frost 1972). This would result in artificially high potential copepod community grazing rates in the experiments as compared to actual *in situ* rates. Another possible explanation for the lack of correlation between copepods and <10 µm phytoplankton is the effects of patchy plankton distributions estuaries. Not only are planktonic organisms not homogenous in their horizontal distributions, but due to mixing and the ability to vertically migrate, there is also patchiness in vertical distributions (Mauchline 1998). The fact that sampling was only conducted during the day, and within the top one meter of the water column, may serve to accentuate the effect of patchiness on our results. A third possibility is that turbulence created from the rotation of the plankton wheel may have disrupted microzooplankton patches and increased encounter rates between copepods and their prey, thus increasing copepod ingestion rates (Saiz et al. 1992) in the grazing experiments as compared to actual *in situ* rates. Davis et al. (1991) found that moderate to high levels of turbulence can both disrupt planktonic patches and increase encounter raters for copepods through creating homogeneity of prey.

Summary

Copepods had substantially greater ingestion and potential community grazing rates of microzooplankton in the wet year as compared to the dry year. As a result of this increase in ingestion, as well as the higher copepod biomass in the wet year, it is estimated that copepod assemblages had the potential to control microzooplankton biomass in the wet year. The presence of top-down control of the microzooplankton community is a possible explanation for the relatively small increase in

microzooplankton biomass, relative to phytoplankton and copepod biomass, in the wet year as reported in chapter 1. The presence of strong top-down control of microzooplankton community biomass may create a window of low grazing pressure by which small (<10 μm) phytoplankton may be able to out-grow microzooplankton predators. This window of low grazing pressure created by mesozooplankton removal of microzooplankton may be an important factor in small phytoflagellate bloom formation.

To my knowledge, the results of this study are unique in that this is the first estuarine study to compare the grazing potential of copepods to microzooplankton growth rates, where both the grazing potential and the growth rates were obtained from the same experimental data set. Other studies (Gifford 1993b, Nielsen & Kiørboe 1994, Levinsen & Nielsen 2002) have compared potential grazing rates of copepods to microzooplankton growth rates, but they used estimated clearance rates or microzooplankton growth rates that were not obtained from the same experimental data set as the potential grazing rates. While the experimental methods of this study were not extended as far as freshwater studies have done, conducting the experiments *in situ*, this study has advanced the understanding of estuarine zooplankton control of microzooplankton by combining potential grazing rate and growth rate analysis. It is believed that using this method of analysis in future studies will further enhance the study of plankton dynamics by creating the ability to easily compare different systems and the effects of different zooplankton species through the use of data with equivalent units of measure.

References

Adrian R, Schneider-Olt B (1999) Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. J Plankton Res 21:2175-2190

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341-352

Brownlee DC, Jacobs F (1987) Mesozooplankton and microzooplankton in the Chesapeake Bay. In: Majumdar SK, Hall LW, Austin HM (eds) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 217-269

Burns CW, Schallenberg M (1998) Impacts of nutrients and zooplankton on the microbial food web of an ultra-oligotrophic lake. J Plankton Res 20:1501-1525

Burns CW, Schallenberg M (2001) Calanoid copepods versus cladocerans: consumer effects on protozoa in lakes of different trophic status. Limnol Oceanogr 46:1558-1565

Carpenter SR, Kitchell JF, Hodgson JR, Cochran PA, Elser JJ, Elser MM, Lodge DM, Kretchmer D, He X, von Ende CN (1987) Regulation of lake primary productivity by food web structure. Ecology 68:1863-1876

Carrick HJ, Fahnenstiel GL, Stoermer EF, R.G. W (1991) The importance of zooplankton-protozoan trophic couplings in Lake Michigan. Limnol Oceanogr 36:1335-1345

Davis CS, Flierl GR, Wiebe PH, Franks PJS (1991) Micropatchiness, turbulence and recruitment in plankton. J Mar Res 49:109-151

Dolan JR (1991) Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods. Mar Biol 111:303-309

Donaghay PL, Small LF (1979) Food selection capabilities of the estuarine copepod *Acartia clausi*. Mar Biol 52:137-146

Fileman E, Burkill P (2001) The herbivorous inpact of microzooplankton during two short-term Lagrangian experiments off the NW coast of Galicia in summer 1998. Prog Oceanogr 51:361-383

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805-815

Gallegos CL (1989) Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar Ecol Prog Ser 57:23-33

Gifford DJ (1985) Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). Mar Ecol Prog Ser 23:257-267

Gifford DJ (1988) Impact of grazing by microzooplankton in the Northwest arm of Halifax Harbour, Nova Scotia. Mar Ecol Prog Ser 47:249-258

Gifford DJ (1993a) Consumption of protozoa by copepods feeding on natural microplankton assemblages. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, p 723-729

Gifford DJ (1993b) Protozoa in the diets of *Neocalanus* spp. in the oceanic subarctic Pacific Ocean. Prog Oceanogr 32:223-237

Gifford DJ, Dagg MJ (1988) Feeding of the estuarine copepod Acartia tonsa Dana: carnivory vs. herbivory in natural microplankton assemblages. Bull Mar Sci 43:458-468

Gifford DJ, Dagg MJ (1991) The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchrus* Murukawa. Mar Microb Food Webs 5:161-177

Hansen FC, Reckermann M, Klein Breteler WCM, Riegman R (1993) *Phaeocystis* blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. Mar Ecol Prog Ser 102:51-57

Harding LW (1994) Long-term trends in the distribution of phytoplankton in Chesapeake Bay: roles of light, nutrients, and streamflow. Mar Ecol Prog Ser 104:267-291

Ingrid G, Andersen T, Vadstein O (1996) Pelagic food webs and eutrophication of coastal waters: impact of grazers on algal communities. Marine Pollut Bull 33:22-35

Kimmel DG, Roman MR (2004) Long-term trends in mesozooplankton abundance in Chesapeake Bay, USA: influence of freshwater input. Mar Ecol Prog Ser 267:71-83

Kiørboe T (1993) Turbulence, phytoplankton cell size, and the structure of pelagic food webs. In: Blaxter JHS, Southward AJ (eds) Advances in Marine Biology, Vol 29. Academic Press, LTD., London, p 1-72

Kiørboe T (1998) Population regulation and role of mesozooplankton in shaping marine pelagic food webs. Hydrobiologia 363:13-27

Kivi K, Kuosa H, Tanskanen S (1996) An experimental study on the role of crustacean and microprotozoan grazers in the planktonic food web. Mar Ecol Prog Ser 136:59-68

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183-195

Kleppel GS, Frazel D, Peiper RE, Holliday DV (1988) Natural diets of zooplankton off southern California. Mar Ecol Prog Ser 49:231-241

Landry MR (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. Mar Biol 65:77-82

Landry MR, Calbet A (2004) Microzooplankton production in the oceans. ICES J Mar Sci 61:501-507

Levinsen H, Nielsen TG (2002) The trophic role of marine pelagic ciliates and heterotrophic dinoflagellates in arctic and temperate coastal ecosystems: a cross-latitude comparison. Limnol Oceanogr 47:427-439

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in artic marine ecosystems. Mar Ecol Prog Ser 204:65-77

Lonsdale DJ, Heinle DR, Siegfried C (1979) Carnivorous feeding behavior of the adult calanoid copepod *Acartia tonsa* Dana. J Exp Mar Biol Ecol 36:235-248

Malone TC, Crocker LH, Pike SE, Wendler BW (1988) Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. Mar Ecol Prog Ser 48:235-249

Mauchline J (1998) The biology of calanoid copepods. Academic Press, San Diego, CA

McQueen DJ, Post JR, Mills EL (1986) Trophic relationships in freshwater pelagic ecosystems. Can J Fish Aquat Sci 43:1571-1581

Merrell JR, Stoecker DK (1998) Differential grazing on protozoan microplankton by developmental stages of the calaniod copepod *Eurytemora affinis* Poppe. J Plankton Res 20:289-304

Miller CA, Penry DL, Glibert PM (1995) The impact of trophic interaction on rates of nitrogen regeneration and grazing in Chesapeake Bay. Limnol Oceanogr 40:1005-1011

Nejstgaard JC, Gismervik I, Solberg PT (1997) Feeding and reproduction by *Calanus finmarchicus*, and microzooplankton grazing during mesocosm blooms of diatoms and the coccolithophore *Emiliania huxleyi*. Mar Ecol Prog Ser 147:197-217

Nejstgaard JC, Naustvoll L-J, Sazhin A (2001) Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. Mar Ecol Prog Ser 221:59-75

Nielsen TG, Kiørboe T (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnol Oceanogr 39:508-519

Nival P, Nival S (1976) Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. Limnol Oceanogr 21:24-38

Parsons TR, Maita Y, Lalli CM (1984a) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK

Parsons TR, Takahashi M, Hargrave B (1984b) Biological oceanographic processes, 3rd edn. Pergamon Press, Oxford, UK

Rollwagen Bollens GC, Penry DL (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). Mar Ecol Prog Ser 257:139-158

Roman MR (1984) Utilization of detritus by the copepod, *Acartia tonsa*. Limnol Oceanogr 29:949-959

Roman MR, Gauzens AL (1997) Copepod grazing in the equatorial Pacific. Limnol Oceanogr 42:623-634

Roman M, Zhang X, McGilliard C, Boicourt W (2005) Seasonal and annual variability in the spatial patterns of plankton biomass in Chesapeake Bay. Limnol Oceanogr 50:480-492

Saiz E, Alcaraz M, Paffenhöfer G-A (1992) Effects of small-scale trubulence on feeding rate and gross-growth efficiency of three *Acartia* species (Copepoda: Calanoida). J Plankton Res 14:1085-1097

Sherr EB, Sherr BF (1993) Preservation and storage of samples for enumeration of heterotrophic protists. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, p 207-212

Stibor H, Vadstein O, Diehl S, Gelzleichter A, Hansen T, Hantzsche F, Katechalis A, Løseth K, Peters C, Roederer W, Sandow M, Sundt-Hansen L, Olsen Y (2004a) Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. Ecol Lett 7:321-328

Stibor H, Vadstein O, Lippert B, Roederer W, Olsen Y (2004b) Calanoid copepods and nutrient enrichment determine population dynamics of the appendicularian *Oikopleura dioica*: a mesocosm experiment. Mar Ecol Prog Ser 270:209-215

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12:891-908

Stoecker DK, Egloff DA (1987) Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. J Exp Mar Biol Ecol 110:293-299

Stoecker DK, Sanders NK (1985) Differential grazing by *Acartia tonsa* on a dinoflagellate and a tinitinnid. J Plankton Res 8:85-100

Strickler JR (1982) Calanoid copepods, feeding currents, and the role of gravity. Science 218:158-160

Strom SL, Morello TA (1998) Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. J Plankton Res 20:571-584

Strüder-Kypke MC, Kypke ER, Agatha S, Warwick J, Montagnes DJS (2002) The user-friendly guide to coastal planktonic ciliates. The University of Liverpool

Tang KW, Jakobsen HH, Visser AW (2001) *Phaeocystis globosa* (Prymnesiophyceae) and the planktonic food web: feeding, growth, and trophic interactions among grazers. Limnol Oceanogr 46:1860-1870

Tiselius P (1989) Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. Mar Ecol Prog Ser 56:49-56

Turner JT, Granéli E (1992) Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms form the west coast of Sweden. J Exp Mar Biol Ecol 157:19-31

Vadstein O, Stibor H, Lippert B, Løseth K, Roederer W, Sundt-Hansen L, Olsen Y (2004) Moderate increase in the biomass of omnivorous copepods may ease grazing control of planktonic algae. Mar Ecol Prog Ser 270:199-207

Verity PG (1986) Growth rates of natural tintinnid populations in Narragansett Bay. Mar Ecol Prog Ser 29:117-126

Verity PG (1991) Measurements and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol Oceanogr 36:729-750

Verity PG, Smetacek V (1996) Organism life cycles, predation, and the structure of marine pelagic ecosystems. Mar Ecol Prog Ser 130:277-293

White JR, Roman MR (1992) Seasonal study of grazing by metazoan zooplankton in the mesohaline Chesapeake Bay. Mar Ecol Prog Ser 86:251-261

Table 2-1. Dates on which the grazing experiments were conducted, and the copepod species that was used at each station.

Date	Choptank River	
	Middle	Upper
11-Mar-02	*Acartia* spp.	*Acartia* spp.
21-Mar-02	*Acartia* spp.	*Acartia* spp.
26-Mar-02	*Acartia* spp.	*Acartia* spp.
3-Apr-02	*Acartia* spp.	*Acartia* spp.
8-Apr-02	*Acartia* spp.	*Acartia* spp.
19-Apr-02	*Acartia* spp.	*Acartia* spp.
24-Apr-02	*Acartia* spp.	*Acartia* spp.
8-May-02	*Acartia* spp.	*Acartia* spp.
16-May-02	*Acartia* spp.	*Acartia* spp.
23-May-02	*Acartia* spp.	*Acartia* spp.
30-May-02	*Acartia* spp.	*Acartia* spp.
19-Mar-03	*E. affinis*	*E. affinis*
1-Apr-03	*E. affinis*	*E. affinis*
14-Apr-03	*E. affinis*	*E. affinis*
25-Apr-03	*Acartia* spp.	*E. affinis*
28-Apr-03	*Acartia* spp.	*E. affinis*
7-May-03	*Acartia* spp.	*Acartia* spp.
14-May-03	*Acartia* spp.	*Acartia* spp.
20-May-03	*Acartia* spp.	*Acartia* spp.

Date	Patuxent River		
	Lower	Middle	Upper
14-Mar-02	*Acartia* spp.	*Acartia* spp	*Acartia* spp
28-Mar-02	*Acartia* spp	ne	*Acartia* spp
11-Apr-02	*Acartia* spp	*Acartia* spp	*Acartia* spp
1-May-02	*Acartia* spp	*Acartia* spp	*Acartia* spp
27-Mar-03	*Acartia* spp	*Acartia* spp	*E. affinis*
3-Apr-03	*Acartia* spp	*Acartia* spp	*E. affinis*
16-Apr-03	*Acartia* spp	*Acartia* spp	*E. affinis*
30-Apr-03	*Acartia* spp	*Acartia* spp	*Acartia* spp
22-May-03	*Acartia* spp	*Acartia* spp	*Acartia* spp

Table 2-2. Microzooplankton classification (class, shape, or genus), size category, dimensions, lorica volume (LV), and biovolume (μm^3 $cell^{-1}$) used for microscopic counts and biomass calculations. Ciliate size categories are based on anterior diameter. Dimensions of non-spherical organisms are listed as diameter * length. Table is from Chapter 1.

Microzooplankton Class, Shape, or Genus	Size Categories (μm)	Dimensions (μm)	LV (μm^3)	Biovolume (μm^3 $cell^{-1}$)
Tintinnid	<20	15 * 105	$1.86 * 10^4$	$5.75 * 10^3$
	>20	25 * 72	$3.24 * 10^4$	$1.00 * 10^4$
Spherical Ciliate	<20	17	-	$2.57 * 10^3$
	>20	34	-	$2.06 * 10^4$
Conical Ciliate	<20	17 * 42	-	$3.18 * 10^3$
	>20	34 * 56	-	$1.69 * 10^4$
Myrionecta rubra	-	17 * 20	-	$3.03 * 10^3$ [a]
Didinium gargantua	-	50 * 70	-	$8.50 * 10^4$ [b]
***Dinophysis* sp.**	-	-	-	$3.28 * 10^3$ [c]
***Protoperidinium* spp.**	-	-	-	$5.75 * 10^4$ [c]
***Gyrodinium* spp.**	-	-	-	$3.10 * 10^3$ [c]

a) Merrell & Stoecker 1998

b) Strüder-Kypke et al. 2002 (http://www.liv.ac.uk/ciliate/)

c) Phytoplankton Guide to the Chesapeake Bay and Other Regions (http://www.serc.si.edu/labs/phytoplankton/guide/index.jsp)

Table 2-3. Average estimated abundance of microzooplankton in field samples and in corresponding t_0 samples from copepod grazing experiments. The decrease in abundance in t_0 samples compared to field samples is expressed as the % decrease in abundance in the t_0 samples. The field samples were preserved at each sample station immediately after collection. Abundance values listed are the average abundances of all the samples from both rivers. Field abundances that are significantly greater than the t_0 abundance are denoted by * (Student's t-test, $p < 0.05$).

Microzooplankton Class, Shape, or Genus	**Field Abundance (number ml^{-1})**	**t_0 Abundance (number ml^{-1})**	**% Decrease in t_0 Abundance**
Tintinnids (<20µm)	4.17	2.18	48
Tintinnids (>20µm)	3.26	1.38	58
Conical Ciliate (<20µm)	6.89	3.41	51
Conical Ciliate (>20µm)	1.66	1.54	7
Spherical Ciliate (<20µm)	10.32	7.59	26
Spherical Ciliate (>20µm)	1.87	1.18	37
Myrionecta rubra	49.14	39.19	20
Didinium gargantua	1.70	0.06	96
Dinophysis spp.	1.35	0.40	70
Protoperidinium spp.	18.75*	0.33	98
Gyrodinium spp.	46.07	22.34	52

Table 2-4. Average clearance (F; ml copepod^{-1} d^{-1}) and ingestion (I; µg C copepod^{-1} d^{-1}) rates of copepods on >10 µm phytoplankton and microzooplankton. The numbers in parentheses are the standard error of the mean. If a negative clearance or ingestion rate was calculated for any given experiment, that rate was considered to be zero clearance or ingestion when the averages were calculated. Averages that are statistically different (Wilcoxon test) in the wet year compared to the dry year are denoted with * ($p < 0.05$) and ** ($p < 0.005$), and clearance and ingestion rates that are significantly greater on one prey type than the other are denoted by † ($p < 0.05$) and ‡ ($p < 0.005$).

			Phytoplankton		Microzooplankton	
River	**Station**	**Year**	**F**	**I**	**F**	**I**
Choptank	Middle	Dry	7.51(2.18)	0.48(0.12)†	79.13(7.23)‡	0.18(0.02)
		Wet	15.30(2.57)*	7.76(1.46)**‡	72.61(11.23)‡	0.46(0.10)*
	Upper	Dry	4.59(1.28)	0.25(0.07)	77.80(8.24)‡	0.73(0.19)†
		Wet	13.61(3.68)	1.77(0.55)*	53.43(6.31)‡	0.93(0.20)
Patuxent	Lower	Dry	22.99(6.52)	0.80(0.17)‡	114.43(10.73)‡	0.25(0.03)
		Wet	5.07(2.03)*	1.83(0.70)†	103.00(8.76)‡	0.21(0.03)
	Middle	Dry	15.95(6.11)	0.42(0.15)	63.95(14.24)†	0.10(0.02)
		Wet	12.84(4.43)	3.26(1.18)†	66.68(6.22)‡	0.24(0.05)*
	Upper	Dry	5.00(2.03)	0.96(0.37)	59.43(8.14)‡	0.30(0.07)
		Wet	4.29(2.28)	2.37(1.13)	29.88(7.15)*†	2.64(0.98)*
Average		Dry	9.18 (1.41)	0.49 (0.07)	79.24 (4.25)*‡	0.38 (0.07)
		Wet	11.21 (1.52)	3.57 (0.50)*‡	64.72 (4.11)‡	0.86 (0.17)

Table 2-5. Average microzooplankton growth rate (μ, d^{-1}), potential grazing rate of copepods on microzooplankton (d^{-1}), and the ratio of copepod grazing to microzooplankton growth. The entry entitled "Average" represents the average growth rate and potential grazing rate during of all biological stations for the dry and wet years. The potential grazing rate of copepods on microzooplankton is the product of copepod abundance (no. L^{-1}) and copepod clearance rates of microzooplankton. Significant differences (Wilcoxon test) in averages between the dry and wet years are denoted by * ($p < 0.05$) and ** ($p < 0.005$), growth rates that are significantly ($p < 0.05$) greater than the potential grazing rate are denoted with the † symbol. nr = negative ratio

River	Station	Year	μ (d^{-1})	Potential grazing rate (d^{-1})	Potential grazing / μ
Choptank	Middle	Dry	0.23	0.02	0.09
		Wet	0.17	0.23*	1.35
	Upper	Dry	0.35†	0.04	0.11
		Wet	0.41	0.86*	2.10
Patuxent	Lower	Dry	0.08	0.01	0.13
		Wet	0.39	0.22*	0.56
	Middle	Dry	0.20†	0.01	0.05
		Wet	0.41	0.16	0.39
	Upper	Dry	-0.21	0.02	nr
		Wet	0.25	0.49**	1.96
Average		Dry	0.19†	0.02	0.11
		Wet	0.32	0.41**	1.28

Table 2-6. Results of a multiple linear regression of <10 μm phytoplankton biomass (μg C ml^{-1}) as a function of copepod and microzooplankton biomass (μg C ml^{-1}). All categories were log_{10} transformed to obtain normal distributions (Shaprio-Wilk test, $p > 0.05$). Dry year $r^2 = 0.13$, wet year $r^2 = 0.04$, combined $r^2 = 0.26$.

Dry Year				
	Coefficient	**SE**	**t**	**p**
Copepod Biomass	0.006	0.055	0.11	0.91
Microzooplankton Biomass	0.238	0.120	1.98	0.06
Wet Year				
	Coefficient	**SE**	**t**	**p**
Copepod Biomass	0.109	0.147	0.74	0.47
Microzooplankton Biomass	0.109	0.143	0.76	0.45
Combined				
	Coefficient	**SE**	**t**	**p**
Copepod Biomass	0.177	0.046	3.85	<0.001
Microzooplankton Biomass	0.172	0.101	1.71	0.09

Table 2-7. Ratio of the concentration of prey ingested by copepods during the grazing experiments (µg C) to the concentration of prey available in the grazing experiment bottles at the start of the experiments (µg C). The values denote the concentration of prey ingested relative to the concentration available.

River	Station	>10 µm Phytoplankton		Microzooplankton	
		Dry Year	Wet Year	Dry Year	Wet Year
Choptank	Middle	0.16	0.37	0.21	0.34
	Upper	0.08	0.11	0.20	0.25
Patuxent	Lower	0.29	0.04	0.29	0.32
	Middle	0.20	0.13	0.14	0.28
	Upper	0.10	0.08	0.11	0.48

Figure Legends

Figure 2-1. Map of Chesapeake Bay. The Choptank and Patuxent River watersheds are shown enlarged in the inserts. White circles denote the biological sampling stations and are titled "Lower", "Middle", and "Upper."

Figure 2-2. Comparisons of the salinity (in the experimental bottles) and incubation temperature of each grazing experiment in the dry and wet years. All the grazing experiments for a given date were incubated at the same temperature, thus graphs f) and g) apply to all stations of that particular river.

Figure 2-3. The average biomass of microzooplankton and large (>10μm) phytoplankton available to copepods at the beginning of the grazing experiments in the wet and dry years. The averages were obtained from the t_0 control samples. Bars represent the standard error of the mean. Statistical differences in the biomass of a given prey type between the dry and wet years are denoted by * ($p < 0.05$) and ** ($p < 0.005$).

Figure 2-4. Average copepod abundance estimates as obtained from *in situ* samples in the dry and wet years. Bars represent the standard error of the mean. Statistical differences in the biomass between the dry and wet years are denoted by * ($p < 0.05$) and ** ($p < 0.005$).

Figure 2-5. Average percentage of microzooplankton and large (>10 μm) phytoplankton standing stock removed d^{-1} by copepod grazing. The percentages are the product of

copepod abundance and average clearance rates. Bars represent the standard error of the mean. Statistical differences in the percentage of standing stock removed of a given prey type between the dry and wet years are denoted by * ($p < 0.05$) and ** ($p < 0.005$).

Figure 2-1.

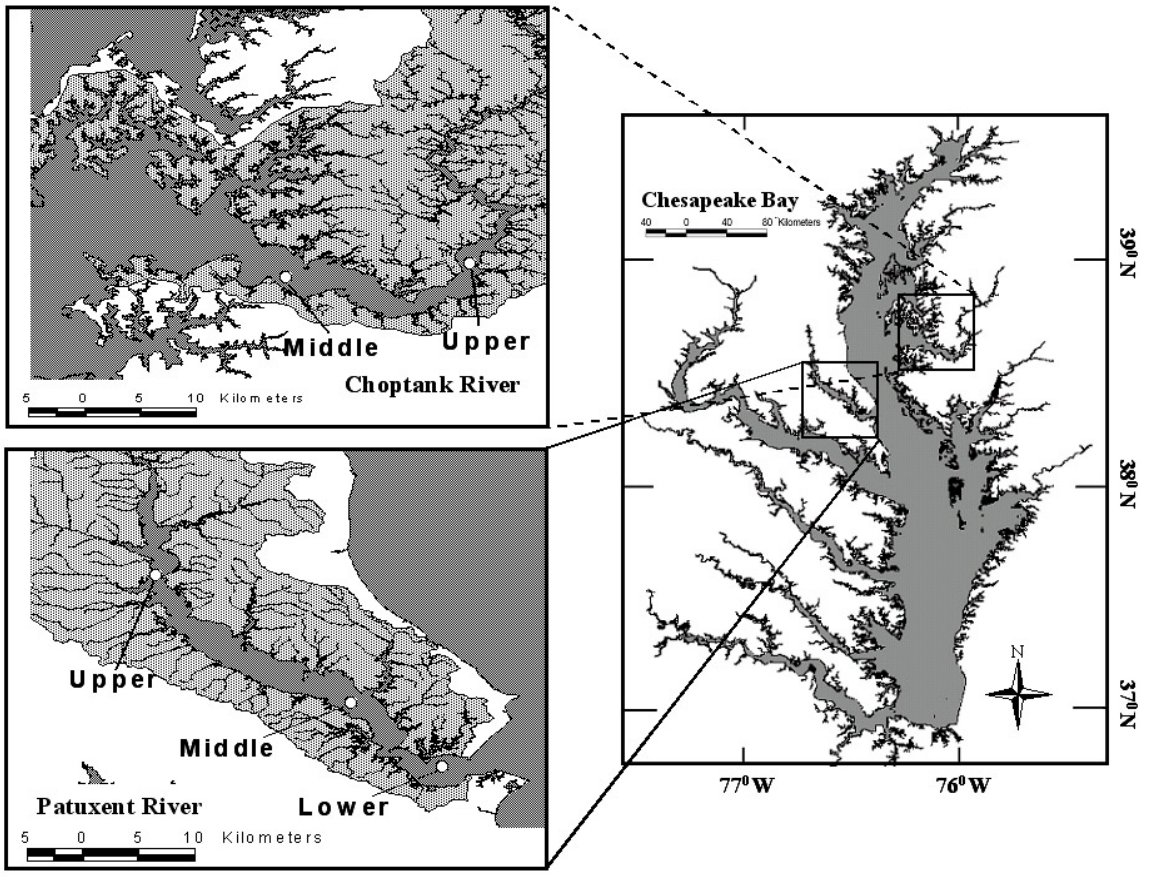

Middle
Upper
Choptank River
5 0 5 10 Kilometers
Upper
Middle
Patuxent River
Lower
5 0 5 10 Kilometers
Chesapeake Bay
40 0 40 80 Kilometers
N
39⁰ N
38⁰ N
37⁰ N
77⁰ W
76⁰ W

Figure 2-2.

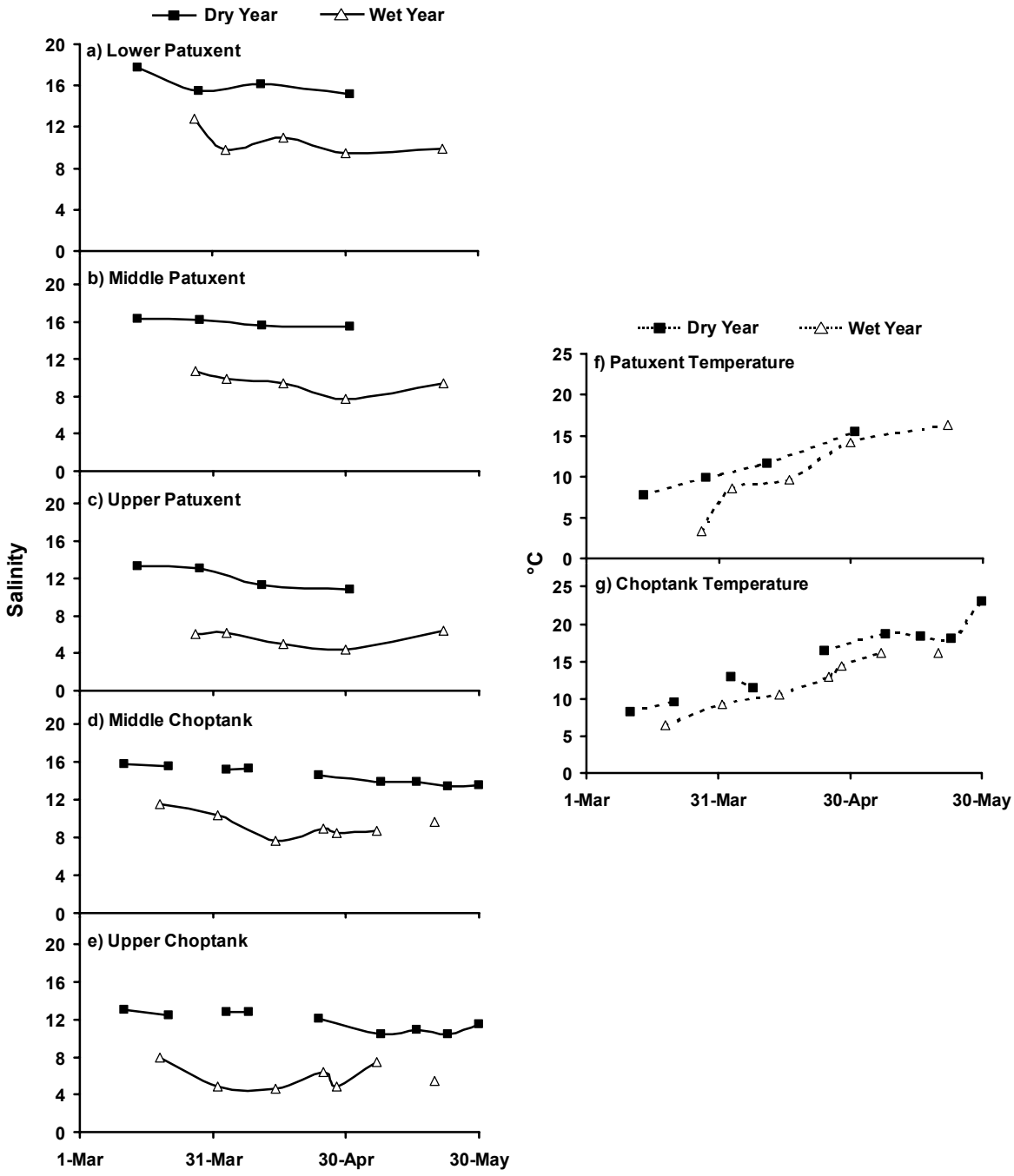

Dry Year
Wet Year
a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
Salinity
1-Mar
31-Mar
30-Apr
30-May
f) Patuxent Temperature
g) Choptank Temperature
°C

Figure 2-3.

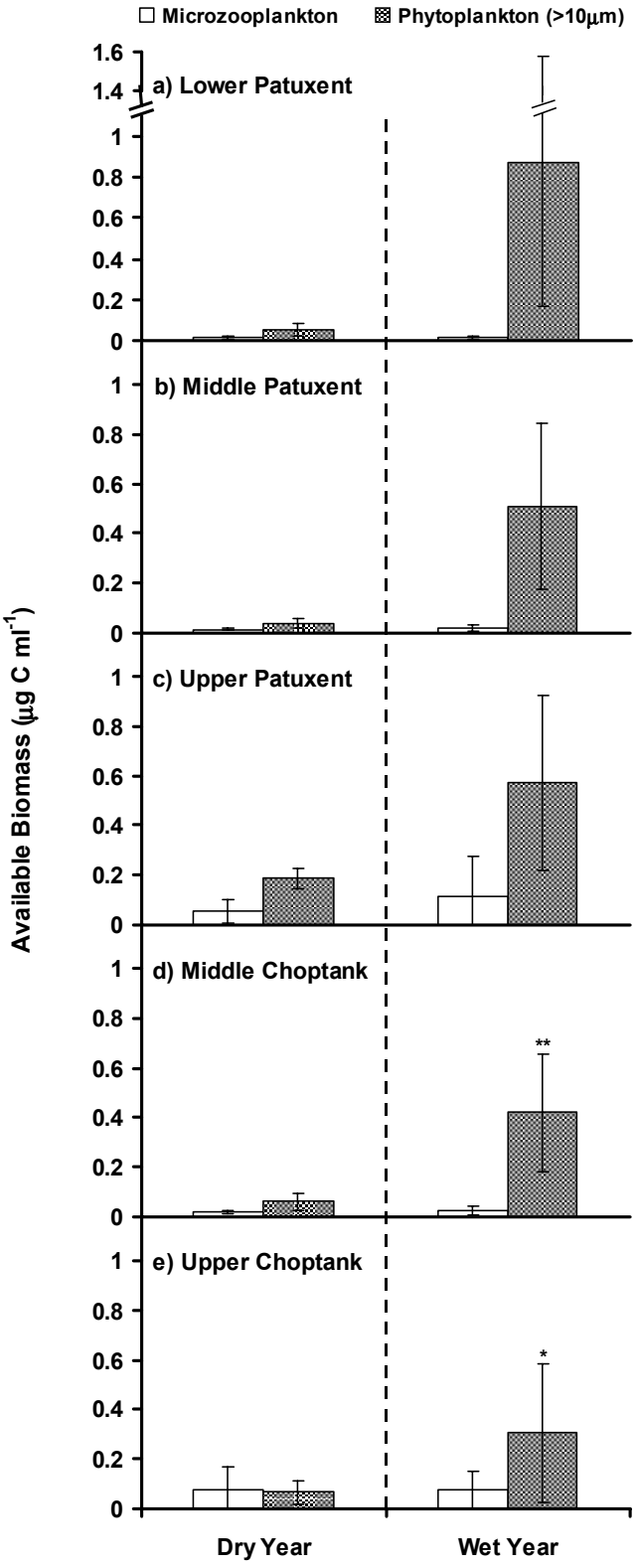

Microzooplankton
Phytoplankton (>10μm)
a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
Available Biomass (μg C ml-1)
Dry Year
Wet Year
**
*

Figure 2-4.

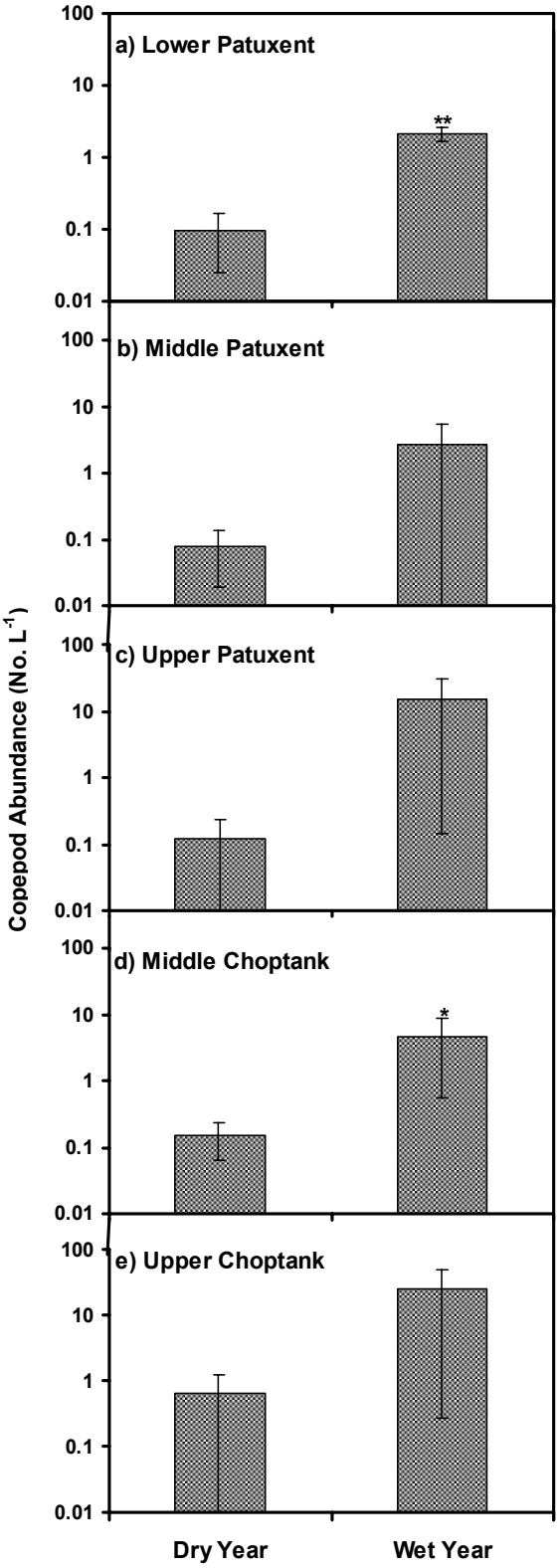
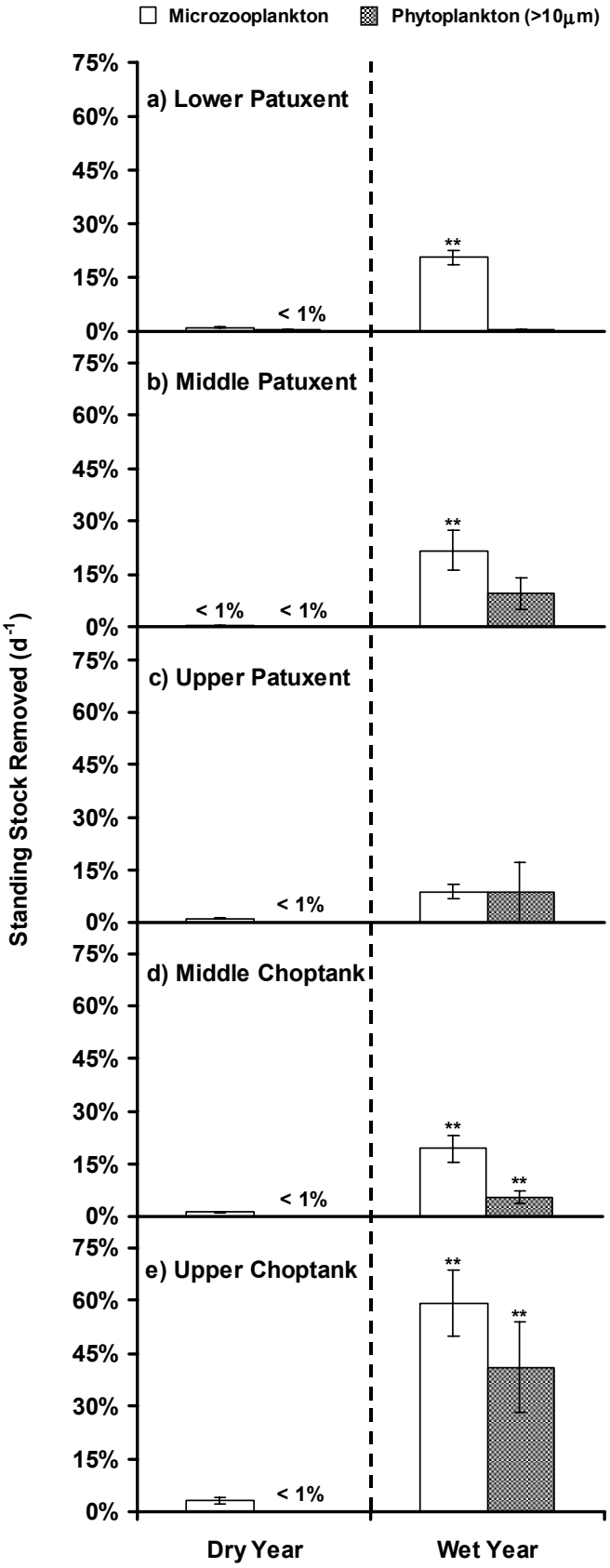

a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
**
*
100
10
1
0.1
0.01
Copepod Abundance (No. L-1)
Dry Year
Wet Year

Figure 2-5.


Microzooplankton
Phytoplankton (>10μm)
a) Lower Patuxent
b) Middle Patuxent
c) Upper Patuxent
d) Middle Choptank
e) Upper Choptank
Standing Stock Removed (d-1)
75%
60%
45%
30%
15%
0%
**
< 1%
Dry Year
Wet Year

Summary

The results of chapter 1 show that increasing freshwater input is correlated with increases in the biomass of planktonic organisms. However, these increases in biomass are not uniform across all plankton groups, as large increases were noted in phytoplankton and copepod biomass but a small and comparatively negligible increase was observed in microzooplankton biomass. Given the strong trophic coupling between phytoplankton and microzooplankton (Gallegos 1989, Fileman & Burkill 2001) it is possible that the large (733%) increase in phytoplankton was due impart to the small (88%) increase in microzooplankton biomass. The strong trophic coupling between microzooplankton and phytoplankton raises a question as to why the microzooplankton biomass wasn't substantially greater in the wet year given the abundance of prey. The answer to this question seems to be top-down control from the large increase (4110%) in copepod biomass observed in the wet year. The increase in copepod biomass was in turn related to the absence of ctenophores in the wet year. An increase in food availability in the wet year was not likely to be the cause of the large increase in biomass over the dry year, as copepods are rarely (if ever) food limited in estuarine environments (Huntley & Boyd 1984, Huntley & Lopez 1992). Thus the structure of the plankton community in the wet year seems to suggest the presence of strong top-down controls and a possible trophic cascade caused by copepod predation on microzooplankton.

In order to test the hypotheses raised by the results of chapter 1, the results of copepod grazing experiments were analyzed, as discussed in chapter 2. Analysis showed that copepods had increased ingestion rates and community grazing potentials of microzooplankton in the wet year compared to the dry year. The increase in copepod

community grazing potential was the result of increased copepod biomass, as clearance rates showed no significant change between the two years. These results demonstrate that copepods were able to control microzooplankton biomass in the wet year, as potential grazing rates were equivalent to microzooplankton growth rates. However, *in situ* biomass estimates did not display significant negative relationships between copepod and microzooplankton biomass, as would be expected if copepod grazing was controlling microzooplankton biomass. That increased copepod ingestion rates of microzooplankton occurred during the same period of significantly increased phytoplankton biomass demonstrates that microzooplankton are an important food source for estuarine copepods, particularly in eutrophic conditions. While no experimental evidence existed for the presence of a trophic cascade, combining *in situ* biomass estimates from both years resulted in a significant positive dependence of small (<10 μm) phytoplankton biomass on copepod biomass. However, when each year was examined independently, there was no relationship.

While there were no significant relationships indicating the presence of a trophic cascade in the wet year, there was one instance where grazing by microzooplankton may have prevented a bloom of *Karlodinium micrum* in the Choptank River. I thought that it would be interesting to include a brief examination of this instance here, as it provides some evidence in support of the existence of estuarine trophic cascades. In late April to early May of the wet year *K. micrum* abundance began to rapidly increase (Figure 3-1). In conjunction with the initiation of this increase there was a decrease in potential microzooplankton grazing rates on *K. micrum* and an increase in potential copepod grazing rates on microzooplankton, indicated by the first arrows in all 3 panels of Figure

3-1. The start of the rapid increase in *K. micrum* abundance just before this date may have been initiated by the decrease in microzooplankton grazing pressure due to the increased copepod community grazing potential. The second arrow in each panel indicate a period of decreased potential grazing by copepods, increased microzooplankton potential grazing, and a large decrease in the rate of *K. micrum* population growth (Figure 3-1). It is possible that the slowed rate of increase and leveling off of the *K. micrum* population growth was the result of a greater ability of microzooplankton to graze the dinoflagellate due to decreased top-down control from copepods.

In summary, I am led to the following conclusions; (1) years (or seasons) of significantly increased freshwater input correlate with increased biomass of estuarine plankton through a combination of bottom-up and top-down controls. Bottom-up controls primarily affect autotrophs, through nutrient supply. On the other hand, heterotrophic organisms appear to be primarily top-down controlled, as they can experience heavy grazing pressure from predators and are rarely food limited in estuarine and coastal environments. (2) There are several possible explanations for why the experimental results supported the top-down control hypothesis while the *in situ* samples neither supported this hypothesis or the idea of a planktonic trophic cascade in estuarine systems. The first of these explanations revolves around a long-standing difficulty for plankton ecologists, the issue of patchiness. Patchy distributions caused by the physics of estuarine systems and behavior factors make it difficult to properly sample plankton populations, and may cause 10 – 30% variation in measurements (Valiela 1995). In chapter 2, the use of horizontal tows to estimate copepod biomass reduced the effect of

patchiness, while the method used for collecting microzooplankton samples was highly dependent upon patchiness as it only a small sample of surface water from a single location. Thus a greater biomass of copepods could be attributed to the location of the sampled microzooplankton biomass than was actually the case. A second explanation for the contradiction between the experimental and *in situ* results may be the potential overestimation of copepod clearance rates of microzooplankton because of decreased microzooplankton concentrations in the experimental bottles as compared to the field samples. Finally, the turbulence and bottle effects associated with the grazing experiments may increase contact rates between prey and predators, thus creating artificially high potential grazing rates compared to actual *in situ* rates. (3) The collection and handling of microzooplankton samples used in the grazing experiments can potential cause ingestion and clearance rates of copepods for microzooplankton to be greatly underestimated, by as much as 59% if copepods select for the largest microzooplankton.

References

Fileman E, Burkill P (2001) The herbivorous inpact of microzooplankton during two short-term Lagrangian experiments off the NW coast of Galicia in summer 1998. Prog Oceanogr 51:361-383

Gallegos CL (1989) Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar Ecol Prog Ser 57:23-33

Huntley M, Boyd C (1984) Food-limited growth of marine zooplankton. Am Nat 124:455-478

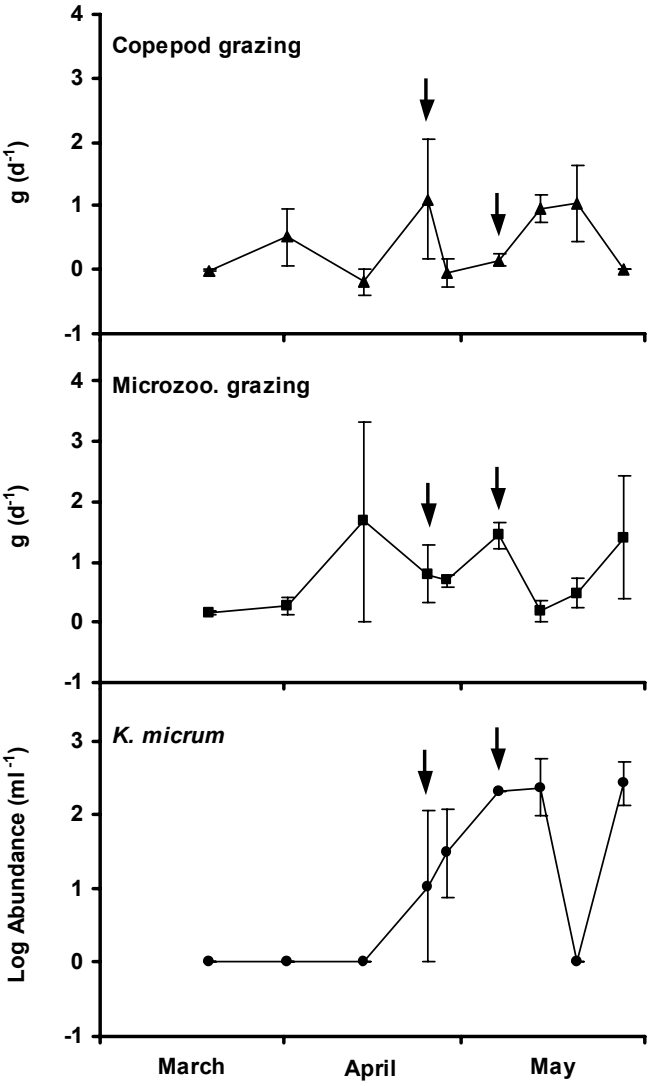
Huntley M, Lopez MDG (1992) Temperature-dependent production of marine copepods: a global synthesis. Am Nat 140:201-242

Valiela I (1995) Marine ecological processes, 2nd edn. Springer, New York, NY

Figure Legend

Figure 3-1. Figure of copepod community grazing potential on microzooplankton (d^{-1}), microzooplankton community grazing estimates on *K. micrum* (d^{-1}), and *K. micrum* abundance (log cells ml^{-1}) as a function of time. Arrows are defined in the text.

Figure 3-1.


Copepod grazing
g (d-1)
Microzoo. grazing
g (d-1)
K. micrum
Log Abundance (ml-1)
4
3
2
1
0
-1
March
April
May

Complete Reference List

Adrian R, Schneider-Olt B (1999) Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. J Plankton Res 21:2175-2190

Alpine AE, Cloern JE (1992) Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. Limnol Oceanogr 37:946-955

Ambler JW (1985) Seasonal factors affecting egg production and viability of eggs of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est Coast Shelf Sci 20:743-760

Ambler JW (1986) Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est Coast Shelf Sci 23:183-196

Bec A, Desvilettes C, Véra A, Lemarchand C, Fontvieille D, Bourdier G (2003) Nutritional quality of a freshwater heterotrophic flagellate: tophic upgrading of its microalgal diet for *Daphnia hyalina*. Aquat Microb Ecol 32:203-207

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99:341-352

Bishop JW (1967) Feeding rates of the ctenophore *Mnemiopsis leidyi*. Chesapeake Sci 8:259-264

Boynton WR, Garber JH, Summers R, Kemp WM (1995) Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. Estuaries 18:285-314

Bradley BP (1991) Distribution of copepods in coastal zone waters: seasonal succession in Chesapeake Bay. Bull Plankton Soc Jpn Spec Vol 129-131

Brownlee DC, Jacobs F (1987) Mesozooplankton and microzooplankton in the Chesapeake Bay. In: Majumdar SK, Hall LW, Austin HM (eds) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 217-269

Burns CW, Schallenberg M (1998) Impacts of nutrients and zooplankton on the microbial food web of an ultra-oligotrophic lake. J Plankton Res 20:1501-1525

Burns CW, Schallenberg M (2001) Calanoid copepods versus cladocerans: consumer effects on protozoa in lakes of different trophic status. Limnol Oceanogr 46:1558-1565

Burton RS (1991) Refulation of proline synthesis in osmotic response: effects of protein synthesis inhibitors. J Exp Zool 259:272-277

Carpenter SR, Kitchell JF, Hodgson JR, Cochran PA, Elser JJ, Elser MM, Lodge DM, Kretchmer D, He X, von Ende CN (1987) Regulation of lake primary productivity by food web structure. Ecology 68:1863-1876

Carrick HJ, Fahnenstiel GL, Stoermer EF, R.G. W (1991) The importance of zooplankton-protozoan trophic couplings in Lake Michigan. Limnol Oceanogr 36:1335-1345

Cataletto B, Fonda Umani S (1994) Seasonal variation in carbon and nitrogen content of Acartia clausi (Copepoda, Calanoida) in the Gulf of Trieste (Northern Adriatic Sea). Hydrobiologia 292/293:283-288

Cloern JE, Alpine AE, Cole BE, Wong RLJ, Arthur JF, Ball MD (1983) River discharge controls phytoplankton dynamics in the northern San Francisco Bay estuary. Est Coast Shelf Sci 16:415-429

Cushing DH (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. In: Southward AJ (ed) Advances in Marine Biology Vol 26, Vol 26. Academic Press, San Diego, p 249-293

Davis CS, Flierl GR, Wiebe PH, Franks PJS (1991) Micropatchiness, turbulence and recruitment in plankton. J Mar Res 49:109-151

Deason EE, Smayda TJ (1982) Ctenophore-zooplankton-phytoplankton interactions in Narragansett Bay, Rhode Island, USA, during 1972-1977. J Plankton Res 4:203-217

Dolan JR (1991) Microphagous ciliates in mesohaline Chesapeake Bay waters: estimates of growth rates and consumption by copepods. Mar Biol 111:303-309

Donaghay PL, Small LF (1979) Food selection capabilities of the estuarine copepod *Acartia clausi*. Mar Biol 52:137-146

Durbin EG, Durbin AG, Smayda TJ, Verity PG (1983) Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnol Oceanogr 28:1199-1213

Fileman E, Burkill P (2001) The herbivorous inpact of microzooplankton during two short-term Lagrangian experiments off the NW coast of Galicia in summer 1998. Prog Oceanogr 51:361-383

Flint RW (1985) Long-term estuarine variability and associated biological response. Estuaries 8:158-169

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805-815

Gallegos CL (1989) Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Mar Ecol Prog Ser 57:23-33

Garcia-Soto C, de Madariaga I, Villate F, Orive E (1990) Day-to-day variability in the plankton community of a coastal shallow embayment in response to changes in river runoff and water turbulence. Est Coast Shelf Sci 31:217-229

Gaudy R, Cervetto G, Pagano M (2000) Comparison of the metabolism of *Acartia clausi* and *A. tonsa*: influence of temperature and salinity. J Exp Mar Biol Ecol 247:51-65

Gifford DJ (1985) Laboratory culture of marine planktonic oligotrichs (Ciliophora, Oligotrichida). Mar Ecol Prog Ser 23:257-267

Gifford DJ (1988) Impact of grazing by microzooplankton in the Northwest arm of Halifax Harbour, Nova Scotia. Mar Ecol Prog Ser 47:249-258

Gifford DJ (1993a) Consumption of protozoa by copepods feeding on natural microplankton assemblages. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, p 723-729

Gifford DJ (1993b) Protozoa in the diets of *Neocalanus* spp. in the oceanic subarctic Pacific Ocean. Prog Oceanogr 32:223-237

Gifford DJ, Dagg MJ (1988) Feeding of the estuarine copepod Acartia tonsa Dana: carnivory vs. herbivory in natural microplankton assemblages. Bull Mar Sci 43:458-468

Gifford DJ, Dagg MJ (1991) The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanuc plumchrus* Murukawa. Mar Microb Food Webs 5:161-177

Gilron GL, Lynn DH (1989) Assuming a 50% cell occupancy of the lorica overestimates tintinnine ciliate biomass. Mar Biol 103:413-416

Goolish EM, Burton RS (1989) Energetics of osmoregulation in an intertidal copepod: effects of anoxia and lipid reserves on the pattern of free amino acid accumulation. Funct Ecol 3:81-89

Granéli E, Turner JT (2002) Top-down regulation in ctenophore-copepod-ciliate-diatom-phytoflagellate communities in coastal waters: a mesocosm study. Mar Ecol Prog Ser 239:57-68

Hansen FC, Reckermann M, Klein Breteler WCM, Riegman R (1993) *Phaeocystis* blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. Mar Ecol Prog Ser 102:51-57

Harding LW (1994) Long-term trends in the distribution of phytoplankton in Chesapeake Bay: roles of light, nutrients, and streamflow. Mar Ecol Prog Ser 104:267-291

Harding LW, Perry ES (1997) Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950-1994. Mar Ecol Prog Ser 157:39-52

Hillebrand H, Dürselen C-D, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403-424

Houde ED, Rutherford ES (1993) Recent trends in estuarine fisheries - predictions of fish production and yield. Estuaries 16:161-176

Houde SEL, Roman MR (1987) Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. Mar Ecol Prog Ser 40:69-77

Huntley M, Boyd C (1984) Food-limited growth of marine zooplankton. Am Nat 124:455-478

Huntley M, Lopez MDG (1992) Temperature-dependent production of marine copepods: a global synthesis. Am Nat 140:201-242

Ingrid G, Andersen T, Vadstein O (1996) Pelagic food webs and eutrophication of coastal waters: impact of grazers on algal communities. Marine Pollut Bull 33:22-35

Jeffries HP (1962) Succession of two *Acartia* species in estuaries. Limnol Oceanogr 7:354-364

Johnson MD, Rome M, Stoecker DK (2003) Microzooplankton grazing on *Prorocentrum minimum* and *Karlodinium micrum* in Chesapeake Bay. Limnol Oceanogr 48:238-248

Jordan TE, Weller DE, Correll DL (2003) Sources of nutrient inputs to the Patuxent River estuary. Estuaries 26:226-243

Kankaala P, Johansson S (1986) The influence of individual variation on length-biomass regressions in three crustacean zooplankton species. J Plankton Res 8:1027-1038

Kimmel DG, Bradley BP (2001) Specific protein responses in the calanoid copepod *Eurytemora affinis* (Poppe, 1880) to salinity and temperature variation. J Exp Mar Biol Ecol 266:135-149

Kimmel DG, Roman MR (2004) Long-term trends in mesozooplankton abundance in Chesapeake Bay, USA: influence of freshwater input. Mar Ecol Prog Ser 267:71-83

Kimmerer WJ (2002) Effects of freshwater flow on abundance of estuarine organisms: physical effects or trophic linkages? Mar Ecol Prog Ser 243:39-55

Kiørboe T (1993) Turbulence, phytoplankton cell size, and the structure of pelagic food webs. In: Blaxter JHS, Southward AJ (eds) Advances in Marine Biology, Vol 29. Academic Press, LTD., London, p 1-72

Kiørboe T (1998) Population regulation and role of mesozooplankton in shaping marine pelagic food webs. Hydrobiologia 363:13-27

Kivi K, Kuosa H, Tanskanen S (1996) An experimental study on the role of crustacean and microprotozoan grazers in the planktonic food web. Mar Ecol Prog Ser 136:59-68

Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. Mar Ecol Prog Ser 274:199-208

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135:191-198

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183-195

Kleppel GS, Frazel D, Peiper RE, Holliday DV (1988) Natural diets of zooplankton off southern California. Mar Ecol Prog Ser 49:231-241

Kremer P (1994) Patterns of abundance for *Mnemiopsis* in US coastal waters: a comparative overview. ICES J Mar Sci 51:347-354

Landry MR (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. Mar Biol 65:77-82

Landry MR, Calbet A (2004) Microzooplankton production in the oceans. ICES J Mar Sci 61:501-507

Levinsen H, Nielsen TG (2002) The trophic role of marine pelagic ciliates and heterotrophic dinoflagellates in arctic and temperate coastal ecosystems: a cross-latitude comparison. Limnol Oceanogr 47:427-439

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in artic marine ecosystems. Mar Ecol Prog Ser 204:65-77

Lewitus AJ, Jensien RV, Kana TM, Burkholder JM, Glasgow HB, May E (1995) Discovery of the "phantom" dinoflagellate in Chesapeake Bay. Estuaries 18:373-376

Li A, Stoecker DK, Coats DW (2000) Spatial and temporal aspects of *Gyrodinium galatheanum* in Chesapeake Bay: distribution and mixotrophy. J Plankton Res 22:2105-2124

Livingston RJ, Niu X, Lewis FG, Woodsum GC (1997) Freshwater input to a gulf estuary: long-term control of trophic organization. Ecol Appl 7:277-299

Lonsdale DJ, Heinle DR, Siegfried C (1979) Carnivorous feeding behavior of the adult calanoid copepod *Acartia tonsa* Dana. J Exp Mar Biol Ecol 36:235-248

Mallin MA, Paerl HW, Rudek J, Bates PW (1993) Regulation of estuarine primary production by watershed rainfall and river flow. Mar Ecol Prog Ser 93:199-203

Malone TC (1980) Algal size. In: Morris I (ed) The physiological ecology of phytoplankton, Vol 7. Blackwell Scientific Publications, Los Angeles, CA, p 433-463

Malone TC, Crocker LH, Pike SE, Wendler BW (1988) Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. Mar Ecol Prog Ser 48:235-249

Mauchline J (1998) The biology of calanoid copepods. Academic Press, San Diego, CA

McQueen DJ, Post JR, Mills EL (1986) Trophic relationships in freshwater pelagic ecosystems. Can J Fish Aquat Sci 43:1571-1581

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569-579

Merrell JR, Stoecker DK (1998) Differential grazing on protozoan microplankton by developmental stages of the calaniod copepod *Eurytemora affinis* Poppe. J Plankton Res 20:289-304

Micheli F (1999) Eutrophication, fisheries, and consumer-resource dynamics in marine pelagic ecosystems. Science 285:1396-1398

Miller CA, Penry DL, Glibert PM (1995) The impact of trophic interaction on rates of nitrogen regeneration and grazing in Chesapeake Bay. Limnol Oceanogr 40:1005-1011

Mozetic P, Umani Fonda S, Cataletto B, Malej A (1998) Seasonal and inter-annual plankton variability in the Gulf of Trieste (nothern Adriatic). ICES J Mar Sci 55:711-722

Nejstgaard JC, Gismervik I, Solberg PT (1997) Feeding and reproduction by *Calanus finmarchicus*, and microzooplankton grazing during mesocosm blooms of diatoms and the coccolithophore *Emiliania huxleyi*. Mar Ecol Prog Ser 147:197-217

Nejstgaard JC, Naustvoll L-J, Sazhin A (2001) Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. Mar Ecol Prog Ser 221:59-75

Nielsen TG, Kiørboe T (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnol Oceanogr 39:508-519

Nival P, Nival S (1976) Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. Limnol Oceanogr 21:24-38

Parsons TR, Maita Y, Lalli CM (1984a) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, UK

Parsons TR, Takahashi M, Hargrave B (1984b) Biological oceanographic processes, 3rd edn. Pergamon Press, Oxford, UK

Purcell, JE (1988) Quantification of *Mnemiopsis leidyi* (Ctenophora, Lobata) from formalin-preserved plankton samples. Mar Ecol Prog Ser 45:197-200

Purcell JE, Decker MB (2005) Effects of climate on relative predation by scyphomedusae and ctenophores on copepods in Chesapeake Bay during 1987-2000. Limnol Oceanogr 50:376-387

Purcell JE, White JR, Roman MR (1994) Predation by gelationous zooplankton and resource limitation as potential controls of *Acartia tonsa* copepod populations in Chesapeake Bay. Limnol Oceanogr 39:263-278

Putt M, Stoecker DK (1989) An experimentally determined carbon : volume ratio fo marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34:1097-1103

Rollwagen Bollens GC, Penry DL (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). Mar Ecol Prog Ser 257:139-158

Roman M, Zhang X, McGilliard C, Boicourt W (2005) Seasonal and annual variability in the spatial patterns of plankton biomass in Chesapeake Bay. Limnol Oceanogr 50:480-492

Roman MR (1984) Utilization of detritus by the copepod, *Acartia tonsa*. Limnol Oceanogr 29:949-959

Roman MR, Gauzens AL (1997) Copepod grazing in the equatorial Pacific. Limnol Oceanogr 42:623-634

Saiz E, Alcaraz M, Paffenhöfer G-A (1992) Effects of small-scale trubulence on feeding rate and gross-growth efficiency of three *Acartia* species (Copepoda: Calanoida). J Plankton Res 14:1085-1097

Sanford LP, Boicourt WC (1990) Wind-forced salt intrusion into a tridutary estuary. J Geophys Res 95:13357-13371

Schneider G, Behrends G (1998) Top-down control in a neritic plankton system by *Aurelia aurita* medusae - a summary. Ophelia 48:71-82

Schubel JR, Pritchard DW (1987) A brief physical description of Chesapeake Bay. In: Majumdar SK, Hall LW, Austin HM (eds) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 1-32

Setzler-Hamilton EM (1987) Utilization of Chesapeake Bay by early life history stages of fishes. In: Majumdar SK, Hall LW, Austin HM (eds) Contaminant problems and management of living Chesapeake Bay resources. The Pennsylvania Academy of Science, Phillipsburg, NJ, p 63-93

Sherr EB, Sherr BF (1993) Preservation and storage of samples for enumeration of heterotrophic protists. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, p 207-212

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223-235

Sommer U, Berninger UG, Böttger-Schnack R, Cornils A, Hagen W, Hansen T, Al-Najjar T, Post AF, Schnack-Schiel SB, Stibor H, Stübing D, Wickham S (2002) Grazing during early spring in the Gulf of Aqaba and the northern Red Sea. Mar Ecol Prog Ser 239:251-261

Stibor H, Vadstein O, Diehl S, Gelzleichter A, Hansen T, Hantzsche F, Katechalis A, Løseth K, Peters C, Roederer W, Sandow M, Sundt-Hansen L, Olsen Y (2004a) Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. Ecol Lett 7:321-328

Stibor H, Vadstein O, Lippert B, Roederer W, Olsen Y (2004b) Calanoid copepods and nutrient enrichment determine population dynamics of the appendicularian *Oikopleura dioica*: a mesocosm experiment. Mar Ecol Prog Ser 270:209-215

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12:891-908

Stoecker DK, Egloff DA (1987) Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. J Exp Mar Biol Ecol 110:293-299

Stoecker DK, Gifford DJ, Putt M (1994) Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. Mar Ecol Prog Ser 110:293-299

Stoecker DK, Sanders NK (1985) Differential grazing by *Acartia tonsa* on a dinoflagellate and a tinitinnid. J Plankton Res 8:85-100

Stoecker DK, Stevens K, Gustafson DE (2000) Grazing on *Pfiesteria piscicida* by microzooplankton. Aquat Microb Ecol 22:261-270

Strickler JR (1982) Calanoid copepods, feeding currents, and the role of gravity. Science 218:158-160

Strom SL, Morello TA (1998) Comparative growth rates and yields of ciliates and heterotrophic dinoflagellates. J Plankton Res 20:571-584

Strüder-Kypke MC, Kypke ER, Agatha S, Warwick J, Montagnes DJS (2002) The user-friendly guide to coastal planktonic ciliates. The University of Liverpool

Sullivan BK, Banzon PV (1990) Food limitation and benthic regulation of populations of the copepod *Acartia hudsonica* Pinhey in nutrient-limited and nutrient-enriched systems. Limnol Oceanogr 35:1618-1631

Sullivan BK, McManus LT (1986) Factors controlling seasonal succession of the copepods *Acartia hudsonica* and *A. tonsa* in Narragansett Bay, Rhode Island: temperature and resting egg production. Mar Ecol Prog Ser 28:121-128

Tang KW, Jakobsen HH, Visser AW (2001) *Phaeocystis globosa* (Prymnesiophyceae) and the planktonic food web: feeding, growth, and trophic interactions among grazers. Limnol Oceanogr 46:1860-1870

Tiselius P (1989) Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. Mar Ecol Prog Ser 56:49-56

Turner JT, Granéli E (1992) Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms form the west coast of Sweden. J Exp Mar Biol Ecol 157:19-31

Turner JT, Tester PA (1997) Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol Oceanogr 42:1203-1214

Tyler MA, Seliger HH (1978) Annual subsurface transport of a red tide dinoflagellate to its bloom area: water circulation patterns and organism distribution in the Chesapeake Bay. Limnol Oceanogr 23:227-246

Utermöhl, H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitt Int Ver Limnol 9:1-38

Vadstein O, Stibor H, Lippert B, Løseth K, Roederer W, Sundt-Hansen L, Olsen Y (2004) Moderate increase in the biomass of omnivorous copepods may ease grazing control of planktonic algae. Mar Ecol Prog Ser 270:199-207

Valiela I (1995) Marine ecological processes, 2nd edn. Springer, New York, NY

Verity PG (1986) Growth rates of natural tintinnid populations in Narragansett Bay. Mar Ecol Prog Ser 29:117-126

Verity PG (1991) Measurements and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. Limnol Oceanogr 36:729-750

Verity PG, Smetacek V (1996) Organism life cycles, predation, and the structure of marine pelagic ecosystems. Mar Ecol Prog Ser 130:277-293

Verity PG, Stoecker DK, Sieracki ME, Nelson JR (1993) Grazing, growth and mortality of microzooplankton during the 1989 North Altantic spring bloom at 47°N, 18°W. Deep-Sea Res I 40:1793-1814

White JR, Roman MR (1992a) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar Ecol Prog Ser 86:239-249

White JR, Roman MR (1992b) Seasonal study of grazing by metazoan zooplankton in the mesohaline Chesapeake Bay. Mar Ecol Prog Ser 86:251-261

Zeldis J, James MR, Grieve J, Richards L (2002) Omnivory by copepods in the New Zealand subtropical frontal zone. J Plankton Res 24:9-23