Abstract

A STUDY OF THE ABUNDANCE, DISTRIBUTION, AND GRAZING EFFECTS OF ZOOPLANKTON IN THE CHLOROPHYLL MAXIMUM (CMAX) OF THE NEUSE RIVER ESTUARY, NORTH CAROLINA

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Phytoplankton tend to accumulate in distinct zones referred to as chlorophyll maxima, or CMAX. A pronounced CMAX occurs in the Neuse River Estuary (NRE), North Carolina, where as much as over 60% of the estuary's phytoplankton biomass is located. A sampling study was initiated to determine the seasonal variability in mesozooplankton abundance and species composition in relation to the CMAX in the NRE. Sampling was conducted at four stations along a 40-km transect of the Neuse River from March to October, 2011. The stations were chosen to include areas both inside and outside of the CMAX and the timing of the study included sampling in spring, summer, and fall. Large zooplankton (collected in a 200μm net) were not found to be spatially coincident with the CMAX during our study. Smaller zooplankton (collected in a 60μm net) were present throughout the estuary and showed no spatial differences with respect to the CMAX. Abundances of larger mesozooplankton peaked in spring along with the peak in Chl *a*, however these peaks were spatially separated. This suggests a diminished grazing role for larger-sized mesozooplankton within the CMAX. Grazing experiments were conducted in June, 2011 in order to quantify the effect of mesozooplankton grazing on phytoplankton abundance both within the CMAX and outside the CMAX. Whole zooplankton community grazing on phytoplankton was highest upon large phytoplankton (>20 μm) upstream

of the CMAX. Grazing upon the >20 um fraction of chlorophyll within the CMAX was negative, suggesting no grazing occurred. Grazing by mesozooplankton was minimal in both locations and lowest on >20 um chlorophyll within the CMAX. Microzooplankton grazing rates were positive in all locations and were highest within the CMAX. Data from this study showed significant differences in grazing between the CMAX and upstream locations depending on both the size of zooplankton grazers and the size of the phytoplankton being grazed. When phytoplankton are larger, mesozooplankton will graze them directly. However, mesozooplankton appear unable to directly graze smaller-sized phytoplankton (< 20 um). As the majority of phytoplankton biomass is comprised of small cells (< 20 um) and mesozooplankton are spatially separated from the area of highest phytoplankton biomass (CMAX), grazing by large mesozooplankton on phytoplankton was found to be minimal. This result suggestions that the majority of mesozooplankton grazing occurs on microzooplankton, leading to increased importance of the microbial loop and less efficient energy transfer to higher trophic levels. This finding is consistent with other eutrophic systems that are typified by a decrease in phytoplankton size and increased importance of the microbial food web.

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CHAPTER 1

INTRODUCTION

Estuaries are highly productive systems that provide ecologically and economically valuable finfish and shellfish refuges, larval fish nurseries, and dynamic nutrient transformation zones at the interface between freshwater and marine environments (Nixon 1995). In estuarine and coastal systems worldwide, human activity puts considerable stress on ecosystem functioning. Nearly three-quarters of the world's population lives in coastal river basins (Vitousek et al. 1997), which has led to a massive increase in nutrient, sediment, and other pollutant loads to estuarine waters (Nixon 1995, Cloern 2001). Excess nutrient loading due to human activity, termed cultural eutrophication, creates a number of negative effects, including increasing phytoplankton blooms, hypoxia, and overall ecological decline (Nixon 1995). Cultural eutrophication has been well-studied (Cloern 2001); however, many aspects of cultural eutrophication are less easy to understand, consisting of complex, non-linear interactions (Paerl et al. 2004). It is the goal of this thesis to determine the distribution of mesozooplankton within a highly eutrophic system (Neuse River estuary) and investigate their grazing impact on phytoplankton stocks.

Phytoplankton dominate pelagic primary production in most estuarine and coastal waters and play a central role in carbon, nutrient, and oxygen cycling in estuaries (Nixon 1986, Paerl et al. 1998). They are highly sensitive indicators of physical forcing in estuarine systems such as vertical mixing, changes in flushing and residence times, and altered optical properties (Paerl et al. 2010). Because phytoplankton have fast growth rates (i.e., doubling times of a day or less) and can rapidly respond to a wide range of environmental perturbations, they represent a sensitive and important indicator for detecting ecological change in estuaries. Phytoplankton

biomass tends to accumulate in distinct zones where high nutrient loads coincide with optimal (for growth) light, salinity, mixing and residence time conditions (Kennedy 1984, Cloern 2001). Chlorophyll *a* (Chl *a*) is often used as a proxy for phytoplankton biomass and as such, the zone of phytoplankton accumulation is referred to as the chlorophyll maximum or "CMAX" (Kononen et al. 1998, Murty et al. 2000)

CMAX are common features of many estuaries (Stanley and Nixon 1992, Malone et al. 1996, Pennock et al. 1999, Harding et al. 2002), including those comprising North Carolina's Neuse River Estuary (Pinckney et al. 1999; Paerl et al. 2004). In the Neuse River Estuary (NRE), as much as over 60% of the estuary's phytoplankton biomass (Paerl et al. 1998) is found in the CMAX zone. From here the phytoplankton biomass is presumably transferred to higher trophic levels, mineralized in the water column or deposited to the sediments (Calbet and Landry 1999, Sipura et al. 2003). The standard perception of most ecologists is that Chl *a* peaks are common in many estuaries, and are simply the result of optimum light, nutrient and flushing (residence time) conditions. Cloern and Jassby (2008) reviewed phytoplankton biomass (chlorophyll *a*) time series from 114 estuaries, lagoons, inland seas, bays, and shallow coastal waters around the world, searching for seasonal patterns. Their analysis revealed no distinct seasonal patterns, with large variability across and within ecosystems, suggesting that phytoplankton dynamics may be uniquely driven in individual estuaries. Therefore, the mesozooplankton response to phytoplankton patterns is also suspected to vary in a similar fashion.

Zooplankton constitute an extremely important part of most marine systems by serving as a link between phytoplankton and higher trophic levels, such as larval fish. The larvae and juveniles of many commercially- and recreationally-important fish species utilize crustacean

zooplankton as their primary food source (Burbidge 1974, Kjelson et al. 1975, Stickney et al. 1975). It has been shown in numerous studies that zooplankton abundance and community structure can change seasonally (Mallin 1991, Lawrence et al 2004, Buskey 1993, Kimmel and Roman 2004), but there has been little research done on whether zooplankton abundance and community structure may change in relation to the CMAX in estuaries. There has also been no research done specifically on the abundance and community structure of zooplankton in the NRE since Mallin's 1991 study. I aimed to address the question of whether seasonal abundance, taxonomic composition, and location of zooplankton in the Neuse River Estuary are related to the location of the CMAX and to determine the impact of mesozooplankton grazing on the phytoplankton within and without the CMAX of the NRE.

The position, magnitude and composition of the CMAX are highly sensitive to nutrient and climatic disturbances in the NRE (Pinckney et al. 1999, Valdes-Weaver et al. 2006). Using data from the biweekly NRE Modeling and Monitoring Project (ModMon), the magnitude and location of the CMAX and its relationship to dissolved inorganic nutrient concentrations can be tracked. This work shows that the CMAX undergoes seasonal shifts in its location (Figure 1-1). The peak chlorophyll a location changes from spring into summer and a decrease in available nutrients coincides with the peaks in chlorophyll (Fig 1). The CMAX can contribute a significant proportion (up to two-thirds in the James River) of water column primary production (Bukaveckas et al. 2011). However, it is unclear if the CMAX is an important area of trophic transfer within the NRE as direct measurements of grazing within this region have not been reported. Following high levels of nutrient-rich freshwater input from hurricanes and tropical storms, phytoplankton biomass accumulates in the upper NRE (Wetz and Paerl 2008). Increased

freshwater input has been linked to increases in the abundances of phytoplankton and copepods in estuaries and coastal waters (Cloern et al. 1983, Kimmerer 2002, Kimmel and Roman 2004). Elevated levels of microzooplankton biomass also often occur simultaneously with these hydrologic events (Wetz and Paerl 2008). Microzooplankton (ciliates and heterotrophic dinoflagellates) can respond quickly to accumulations of phytoplanktonic prey due to their high growth rates, which can often approach those of many phytoplankton taxa (Strom and Morello 1998). Variability in microzooplankton grazing pressure may provide "windows of opportunity" for net growth of dinoflagellates and other phytoplankton in response to nutrient loading and a general lack of grazing pressure on phytoplankton stocks (Stoecker et al. 2008).

The Neuse River Estuary is part of North America's largest lagoonal estuarine ecosystem (Albermarle-Pamlico Estuare System), an important region for juvenile fish habitat and a variety of commercially exploited species (Paerl et al. 2007). There are two long-term monitoring programs in place (ModMon and FerryMon; both funded through at least 2011) that serve as databases for observational and experimental research and modeling (Paerl et al. 2004). The NRE drains some of North Carolina's most rapidly expanding agricultural and urban regions, and is impacted by human and climate-induced changes in nutrient loading (Paerl et al. 1998, 2006). Nutrient sources into the NRE are well characterized, with non-point source nutrient pollution contributing over 75% of external nitrogen and phosphorous inputs (NC-DENR 1998). Finally, and most importantly, the NRE exhibits relatively long residence times of 30-90 days (Huzzey and Nolan 2005, Wang et al. 2004); a residence time of this duration favors the buildup, release, and overall biogeochemical importance of "internal" regenerated nutrients which can support phytoplankton growth (Christian et al. 1991, Nixon et al. 1996). Long residence time also tends

to promotes higher abundances of omnivorous mesozooplankton than in well-flushed systems (Pace et al. 1992, Kibirige et al. 2006). Wetz et al. (2011) state that residence time may play an important role in structuring planktonic food webs and subsequent carbon flow in estuaries, as higher abundances of omnivorous mesozooplankton in these systems could allow for greater potential for top-down control of microzooplankton and cascading effects reaching the phytoplankton. Thus, the NRE is an ideal location to investigate the seasonal variability in mesozooplankton populations in relation to a CMAX and determine the mesozooplankton impact on this feature through grazing.

OBJECTIVE AND HYPOTHESES

The overall objective of this chapter is to determine the seasonal variability in mesozooplankton abundance and species composition in relation to the chlorophyll maximum in the Neuse River estuary.

Hypothesis 1: There will be no difference in either chlorophyll *a* concentration between station or season.

Hypothesis 2: There will be no difference in mesozooplankton abundance as sampled with a 200 µm net between station or season.

Hypothesis 3: There will be no difference in mesozooplankton abundance as sampled with a 60 µm net between station or season.

Approach: Fluorometric measurement of chlorophyll *a*, net sampling of mesozooplankton biweekly during March through October. Identification of species composition and abundance in

the laboratory. Two-way analysis of variance to determine differences in abundance between stations and season.

METHODS

Sampling location

Sampling was conducted at four stations (50, 70, 120, 160) located on a 40-km transect of the Neuse River, NC (Figure 1-2 and Table 1-1). These stations were chosen for sampling as the CMAX typically occurs between the area where the river begins to widen and where the river begins to bend towards the northeast (Paerl et al. 2007). These sampling stations should therefore give a reliable estimate of zooplankton abundances within, upstream from, and downstream from the CMAX. Depths at the stations ranged from between 6 and 7 meters at Station 160 to between 3 and 4 meters at station 50.

Sampling and analysis Procedure

Water quality parameters such as water temperature, salinity, dissolved oxygen (DO), conductivity, chlorophyll *a* fluorescence, turbidity, depth, and pH were measured with a YSI 6600 multiparameter water quality sonde coupled to a YSI 650 MDS logger as a part of the ModMon program's biweekly sampling trip. As an indicator of total phytoplankton community biomass, chlorophyll *a* (chl *a*) concentrations are also measured as a part of the ModMon sampling program. Near-surface and near-bottom grab samples were analyzed for chl *a* by filtering 100 mL of NRE water onto Whatman glass fiber filters (GFF, 0.7µm porosity). Filters were sonicated in 90% acetone, extracted overnight, and analyzed fluorometrically for chl *a* (Paerl et al. 1995)

Mesozooplankton samples were collected biweekly from 23 March 2011 to 24 October 2011. Samples were collected only once in the months of April, June, and September. Sampling dates were grouped into seasons to reflect the typical seasonal temperature changes in eastern North Carolina. Two separate plankton tows were conducted at each station beginning on 25 May, using each of two 0.5m diameter opening Sea-Gear plankton nets. One net was equipped with a 60µm-mesh net and cod end, while the other was equipped with a 200µm-mesh net and cod end. For the first three sampling dates only the 60µm-mesh net was used. It was later decided that the two net sizes would give a more accurate representation of the zooplankton present in the system. Tows lasted between 2 and 3 minutes each, and spanned the top 1 to 4 meters of the water column, depending on the station depth. Flowmeters (General Oceanics) were also attached to each net to determine the volume of water sampled. Organisms collected during each tow were rinsed into 100ml glass sample jars and field-preserved with 5% formalin.

In the laboratory, the mesozooplankton samples were counted using an Olympus SZX10 dissecting microscope and an 1810 Ward counting wheel. Zooplankton samples were re-suspended in water and diluted to a known volume (usually either 200 or 400ml). Extremely dense samples were split as needed using an acrylic splitting wheel (Wildco). From the resulting diluted volume, 10ml subsamples were taken with a Hensen-Stempel pipette. Sufficient aliquots were chosen so that at least approximately 300 zooplankton per sample were counted. In some samples having particularly low zooplankton densities or high detritus-to-zooplankton ratios, as few as 90 zooplankton were counted. Zooplankton were identified to species where possible, with some types such as *Oithona* and *Temora* identified only to genus. Copepod nauplii were all

grouped together as identification can be difficult during naupliar and copepodid stages (Johnson and Allen 2005).

Statistical Analysis

Two-way ANOVA will be used to test for significant differences in chlorophyll concentration and zooplankton abundance depending on sampling station and sampling season (Zar 1974). Tukey's test will be used for post-hoc statistical analysis (Zar 1974). Models were considered significant at the $\alpha = 0.05$ level.

RESULTS

Water temperature in the estuary ranged from a low of 15.6°C at Station 160 on 23 March to a high of 30.4°C at Station 70 on 2 August (Table 1-2). Salinity increased linearly with movement downstream towards the ocean (Table 1-2) with a low of 0.58 occurring at Station 50 on 25 April and a high of 24.96 occurring at Station 160 on 15 August.

Chlorophyll *a* (Chl *a*) concentration ranged from a low of 5.02µg L^{-1} on 30 August at Station 50 to a high of 19.44 µg L^{-1} on 23 March at Station 70 (Table 1-2 and Figure 1-3). With the exception of the spring CMAX at Station 70, the highest average chlorophyll *a* concentrations tended to occur at Station 50 during most of the year (Figure 1-4). These averages declined with movement spatially downstream towards Station 160 and the ocean. A distinct CMAX occurred at Station 70 in the spring (Figure 1-4). The highest chlorophyll *a* levels in the summer and fall occur at Station 50, but there is high overlap with Station 70 (seen in the error bars of Figure 1-4). ANOVA revealed significant differences in mean chlorophyll *a* among the stations ($p<0.0001$, Table 1-3). Post-hoc analysis (Tukey's test) reveals that Chl *a* concentrations were significantly higher at Stations 50 and 70 than concentrations at Station 160. Further

ANOVA analysis revealed significant differences in mean chlorophyll *a* concentration by season ($p=0.0244$, Table 1-6), with chlorophyll *a* concentrations highest in spring (Figure 1-4).

The highest zooplankton abundance recorded using the 60µm-mesh nets was 50.05 individuals L^{-1} on 12 October at Station 160 (Table 1-4). The lowest abundance recorded using the 60µm-mesh nets was 0.13 individuals L^{-1} on 23 March at Station 70. Abundances recorded using the 200µm-mesh nets ranged from a low of 0.03 individuals L^{-1}on 24 October at Station 50 to a high of 26.68 individuals L^{-1} on 15 August at Station 160 (Table 1-4).

Average zooplankton abundances in 60µm-mesh samples were highest in fall and lowest in spring (Figure 1-5). Zooplankton abundances at Station 50 collected in the 60µm mesh nets increased rather dramatically from relatively low abundances in the spring and summer to much higher average abundances in the fall (Figure 1-6). Stations 50 and 70 did not exhibit a wide range of seasonal variation in the 200µm mesh nets (Figure 1-7), but Stations 120 and 160 both showed decreases in average abundance from spring to summer and from summer to fall (Figure 1-8).

ANOVA revealed significant differences in mean zooplankton abundance (as measured by 200 µm net) by station ($p=0.0077$, Table 1-5). Mesozooplankton abundances were significantly higher at Station 160 than at Stations 50 and 70 (Tukey's test, $p=0.0093$, $p=0.027$ respectively). ANOVA did not reveal any statistical difference in mean zooplankton abundance (as sampled with the 60 µm net) stations ($p>0.05$, Table 1-3). Further ANOVA analysis revealed significant differences in mean zooplankton (60 µm fraction and 200 µm fraction) by season ($p<0.0001$, $p=0.0128$ respectively, Tables 5 and 6). Post-hoc tests revealed that the 60 µm zooplankton abundance is significantly higher in fall than in summer ($p=0.0085$) or spring

($p<0.0001$). The 200 µm fraction of zooplankton abundance, on the other hand, is significantly higher in spring compared to fall (Tukey's test, $p=0.0286$).

The majority of the zooplankton samples collected using the 60µm-mesh nets consisted of *Acartia tonsa* (Dana 1849) and copepod nauplii (Figures 10 and 11). Polychaete larvae are only present most notably during the spring, and only at Station 50 in the summer. *Coullana canadensis* (Willey 1923), a harpacticoid copepod, follows a similar pattern, only appearing in the spring and at Station 50 in a very small percentage during the summer. *Oithona*, on the other hand, is not present in the spring and increases in abundance from summer to fall. *Podon polyphemoides* (Leuckert 1859), a cladoceran, is also most abundant in the spring. *A. tonsa* occurs at a higher percentage in the samples as you move from Station 50 downstream towards Station 160 (Figure 1-9). A general decrease can also be seen in the percentage of copepod nauplii composing samples moving from Station 50 downstream to Station 160. The samples collected using 200µm-mesh nets were dominated by *Acartia tonsa*, with all samples consisting of at least 80% *Acartia* (Figure 1-10). *Balanus* nauplii were present at Station 50 all year, and were present to a lesser extent at stations further downstream during the fall only. *Balanus* cyprids were most abundant during the spring at Station 50, and to a lesser extent in the summer and fall at the same station. *Podon polyphemoides* were abundant at all stations in the spring, with a steep decline as the year progressed into summer. *Pseudevadne tergestina* (Claus, 1877), another cladoceran, became abundant in the summer, especially at Station 160. They were absent in the spring and decreased in abundance as summer progressed into fall. Crab zoea were most abundant in the spring, decreasing in abundance with movement downstream from Station 50.

They remained present at Station 50 in the summer, but were not present in the summer at downstream stations and were nearly absent entirely in the fall.

DISCUSSION

My results showed a CMAX that occurs at or near Station 70 in the spring, and between Stations 50 and 70 during the summer and fall. Chlorophyll *a* concentration peaked in the spring, but remained at high levels throughout the year (Figure 1-4). Mesozooplankton (collected with 200µm nets) occur at a significantly higher abundance at Station 160 than at upstream Stations 50 and 70 and are therefore not spatially coincident with the CMAX (Figures 6 and 8). This result suggests that there is a spatial separation between >200 µm mesozooplankton and the CMAX. Therefore, grazing on phytoplankton within the CMAX by >200 µm mesozooplankton may be minimal (see Chapter 2). The abundance of smaller zooplankton (collected in 60µm nets) showed no significant difference with respect to station. This indicates that smaller mesozooplankton are present throughout the estuary and show no spatial differences with respect to the CMAX. There are significant differences in mesozooplankton abundance depending on the season, with mesozooplankton caught in the 60 µm net being highest in fall and mesozooplankton caught in the 200 µm net being highest in spring (Tables 4 and 5, Figures 6 and 8). These results suggest that the peak in chlorophyll *a* that occurs in the spring is spatially separate from the peak in >200 µm mesozooplankton, again suggesting a diminished grazing role for larger sized mesozooplankton within the CMAX.

A correlation between copepod abundance and water temperature has been observed in the Neuse River (Mallin 1991), the Pamlico River estuary (Peters 1968), and the Beaufort-area estuaries (Fulton 1984) of North Carolina. In our study, the highest average temperatures in the

estuary occurred in the summer. However, the highest average zooplankton abundances did not occur in the summer. The highest average abundances collected using the 60µm-mesh nets occurred in the fall and the highest average abundances collected using the 200µm-mesh nets occurred in the spring. Mallin's study in 1991 sampled at stations located most closely to our Station 160. Temperatures peaked at Station 160 in early August as at all stations in our study. Peak abundance of zooplankton collected in the 200um-mesh nets (consisiting mostly of *A. tonsa*, a copepod) at Station 160 occurred on August 15, the date of our second-highest measured water temperutures at that station.

The zooplankton abundances determined in this study were lower than those found in Mallin's 1991 study. The mean zooplankton abundance in 1989 was 31.224 individuals L^{-1}. The mean zooplankton abundances determined in this study for the entire study period were 6.348 individuals L^{-1} in the 60µm-mesh nets and 2.752 individuals L^{-1} in the 200µm-mesh nets. While lower than the abundances in Mallin's study, our results are on par with results from other, similar studies conducted in southeastern United States estuaries (Table 1-7). Also, if we include only samples from Station 160 in our study (which is in closest proximity to the stations in Mallin's study) the mean abundances increase to 12.685 individuals L^{-1} in the 60µm nets and 6.66 individuals L^{-1} in the 200um nets. If we consider only zooplankton collected at Station 160 during the summer, our mean abundances increase to 13.31 individuals L^{-1} and 8.73 individuals L^{-1} for zooplankton collected in 60µm and 200µm nets, respectively. The exact reason for the difference in our results and Mallin's results is difficult to determine, but predatory ctenophores could explain the reduced abundances determined in this study. Mallin does mention the presence of ctenophores during the summers in which he sampled, but he does not quantify their

numbers. We also did not include ctenophore abundances in our study, but their presence during summer sampling was significant. The numbers of ctenophores in the Neuse during the summer were so high that they would sometimes clog our nets and make preserving the zooplankton samples difficult. We attempted methods for excluding ctenophores from the sampling nets with limited success, including placing a "chicken-wire"-type mesh over the net opening. Still, the overwhelming concentration of ctenophores in the water during sampling may have played a role in the decreased zooplankton abundances of our study compared to Mallin's.

Zooplankton can be grouped into size classes, with species between 20 and 200µm considered microzooplankton and those larger than 200µm considered mesozooplankton (Sieburth et al. 1978). We can therefore predict that the 60µm-mesh nets used in our study will consist more exclusively of large species of microzooplankton and juvenile stages of larger mesozooplankotn (copepod nauplii) while the 200µm-mesh nets will give a more accurate depiction of the mesozooplankton (>200 µm) population. However, it is important to note that microzooplankton in the 20-60µm size class will not be collected in the 60µm nets. It must also be noted that zooplankton nets collect anything larger than their mesh size, so mesozooplankton could be (and were) collected in the 60µm nets. These size differentiations are evident in the taxonomic breakdown of our samples, as the 60µm-mesh nets collected a greater abundance of copepod nauplii while the 200µm-mesh net samples consisted of mostly *Acartia tonsa*. Smaller net mesh sizes often lead to considerably greater zooplankton density estimates (Turner 1982). Hence, an added benefit of using both net sizes was not only in their ability to sample different size classes of zooplankton, but also in support of Turner's assertions that smaller net sizes lead to greater density estimates. The 60µm-mesh nets yielded abundances, on average, nearly three

times higher than the 200um-mesh nets. The use of smaller nets, when possible, may yield larger densities but the use of multiple mesh sizes can provide interesting comparisons across size classes.

The results of this study could be showing top-down control on large microzooplankton by mesozooplankton in the NRE, which in turn leads to an increase in chlorophyll *a* as there are fewer microzooplankton available to feed on phytoplankton. Studies have documented intense microzooplankton grazing upon estuarine phytoplankton (Gallegos 1989, Strom et al 2001, Reaugh et al. 2007), at times capable of destroying blooms or altogether preventing bloom (CMAX) formation (Strom et al 2001). If the NRE microzooplankton are indeed being consumed by mesozooplankton, then we might expect subsequent increases or possible bloom events in the phytoplankton, which can be seen in our results. At Station 70 during the spring, mean chlorophyll *a* concentration was at its highest level of the year (17.066 ug L^{-1}), representing a well-defined CMAX. At this same station and during the same spring season, mean abundance of small zooplankton (collected in 60um nets) was lower (0.9395 individuals L^{-1}) than mean abundance of large (collected in 200um nets) (1.0533 individuals L^{-1}); however, these abundances were not significantly different. Spring was the only season during which average large zooplankton abundance was higher than average small zooplankton abundance at any station (also occurring at Stations 120 and 160). However, both abundances were low, indicating a possible "window" for phytoplankton blooms to occur due to limited grazing. During the rest of the year, average small zooplankton abundance was more than five times higher than mesozooplankton abundance in the NRE (10.07 individuals L^{-1} to 1.92 individuals L^{-1}, respectively). Thus, phytoplankton concentrations in the summer tend to decrease and this may

be due to the increase in the role of microzooplankton grazing. Concurrent with the decline in chlorophyll is a decline in larger mesozooplankton, possibly due to ctenophore predation (Deason and Smayda 1982, Purcell et al. 1994), thus indicating that phytoplankton may be experiencing significant grazing by microzooplankton. Indeed, this was observed in the NRE by Wetz et al. (2011) when they highlighted the importance of microzooplankton in controlling estuarine phytoplankton growth and phytoplankton size structure during warmer seasons. Microzooplankton grazing rates in this study exceeded *in situ* picophytoplankton and >3µm phytoplankton growth rates in nearly all summer experiments.

The results of my investigation illuminate several important areas for future research. One of these areas concerns the fate of carbon in the estuarine system. Given the high concentrations of chlorophyll *a* in the NRE due to eutrophication, what is the fate of this organic matter? My study suggests that much of the phytoplankton biomass in the spring appears to be unrelated to mesozooplankton grazers in terms of colocation in the estuary, a fact that may or may not indicate a lack of grazing (see Chapter 2). If meso- and microzooplankton are both consuming phytoplankton, are they doing so at the same or different rates? In their review of "exploitation ecosystems", Oksanen et al. (1981) hypothesize that changes in higher trophic levels should have a radical impact on lower ones. My results on the spatial and temporal variability in mesozooplankton populations suggest that mesozooplankton could be important players within a trophic cascade. This has also been suggested both within the NRE (Wetz et al. 2011) and other systems (Reaugh et al. 2007, Verity and Smetacek 1996). If the available carbon in the estuary is being consumed first by microzooplankton, then a reduced transfer efficiency of the system may occur as an extra trophic level is added to the food web and this

also indicates an active role for the microbial food web (Azam et al. 1983). The trophic pathway through microzooplankton results in a decrease in the export of primary production both in terms of sinking and/or consumption by higher trophic levels (Irigoien et al. 2005).

The fate of phytoplankton blooms and primary production in estuaries can no longer be explained by simple bottom-up or top-down factors. There is much more to these systems than the classical food chain of phytoplankton-zooplankton-fish (Fenchel 1988). Many copepods and their nauplii composed a sizeable proportion of the zooplankton abundance found in the NRE. Many copepods, such as *A. tonsa*, are omnivores (Bollens and Penry 2003) which have shown the ability to submit significant grazing pressure on both other smaller zooplankton (Merrell and Stoecker 1998, Bollens and Penry 2003) and larger phytoplankton (Mallin and Paerl 1994). There is a clear need to conduct further experimentation in order to gain a better understanding of the interactions between phytoplankton, zooplankton, and physical parameters in the NRE. Answering these questions will not be easy, since coastal aquatic systems will continue to be impacted by man through introduction of nutrient loads and removal of key trophic level individuals such as fish. Grazing experiments designed to determine the impact of multiple trophic levels on primary productivity in the CMAX could lead to sophisticated food web models which incorporate direct and indirect pathways in further understanding this ecologically important site of trophic activity, and this will be the focus of the next chapter.

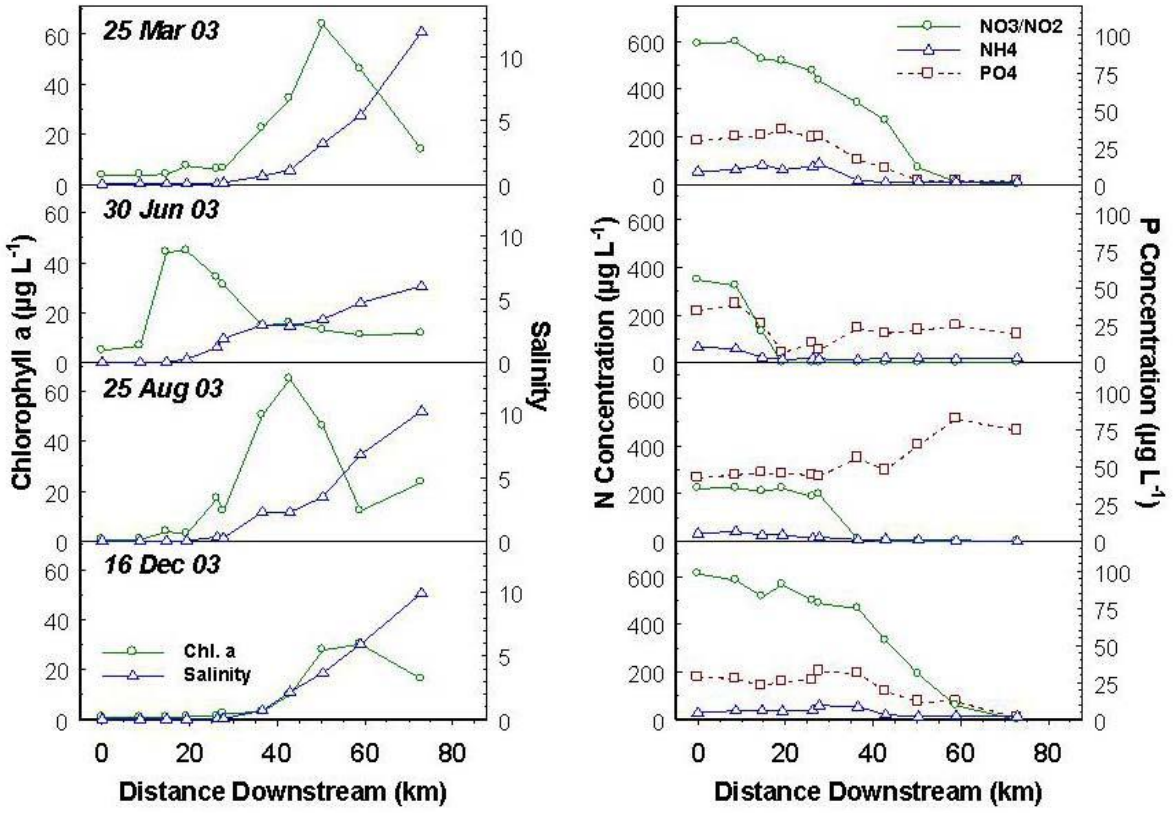

Figure 1-1. Seasonal locations (shown by date) of the CMAX in response to freshwater discharge (as salinity) and nutrient availability in the NRE during a representative year, 2003. (Hans W. Paerl, personal communication, 2012)

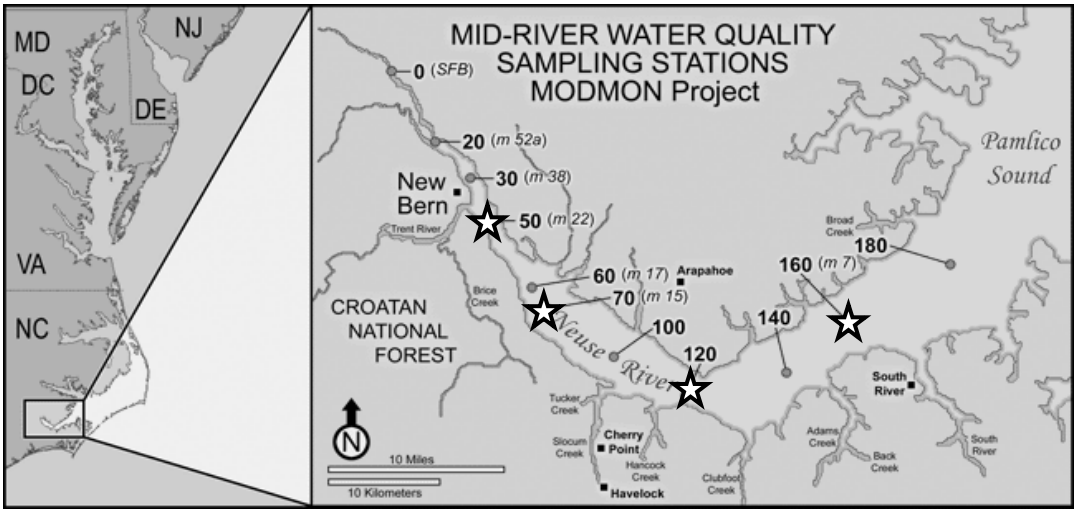

Figure 1-2. Map of NRE showing ModMon sampling site. CMAX typically resides between stations 30 and 120. Stars represent sampling sites for grazing experiment.

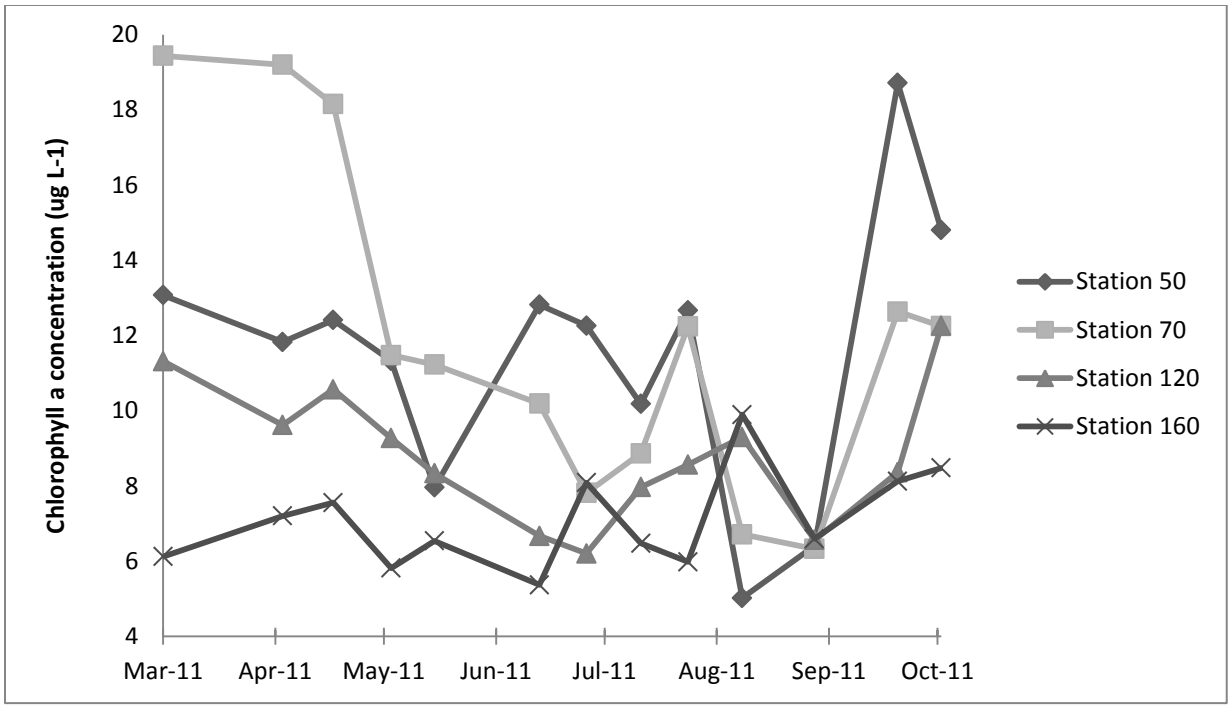

Figure 1-3. Chlorophyll *a* concentration at each date by station from March to October 2011.

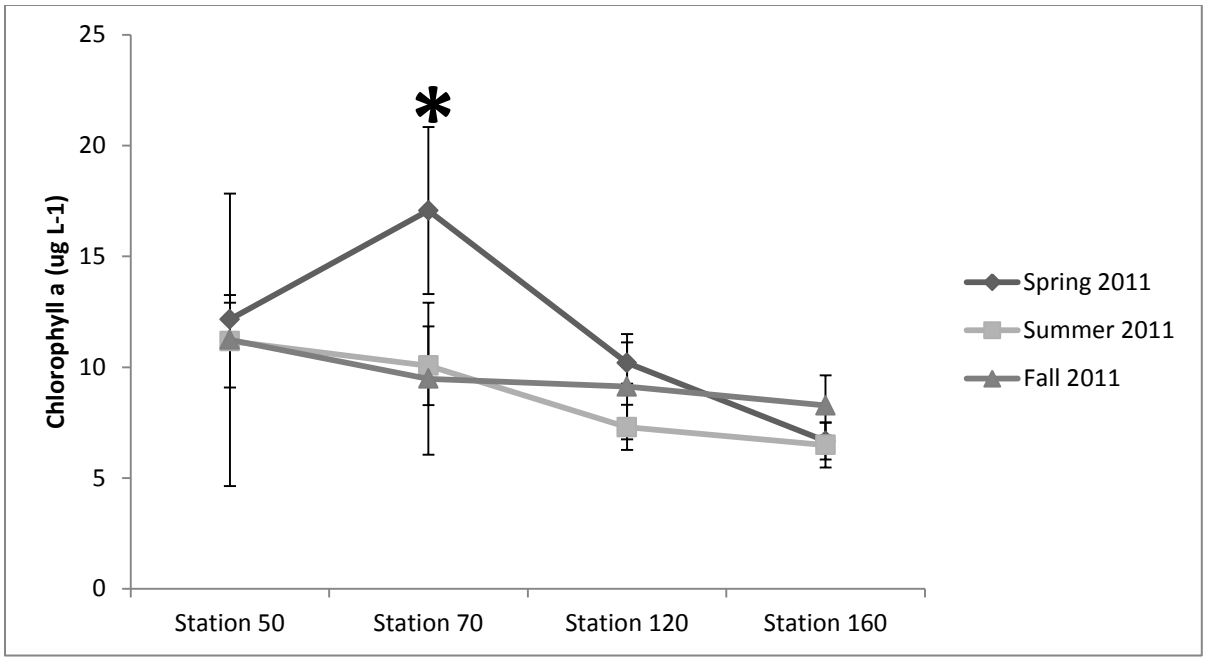

Figure 1-4. Average seasonal chlorophyll *a* concentration at each station. Error bars represent standard deviation. The asterisk represents the statistically significant spring CMAX at Station 70. Spring dates include 23 March, 25 April, 9 May, 25 May; summer dates include 6 June, 5 July, 18 July, 2 August, 15 August; fall dates include 30 August, 19 September, 12 October, 24 October.

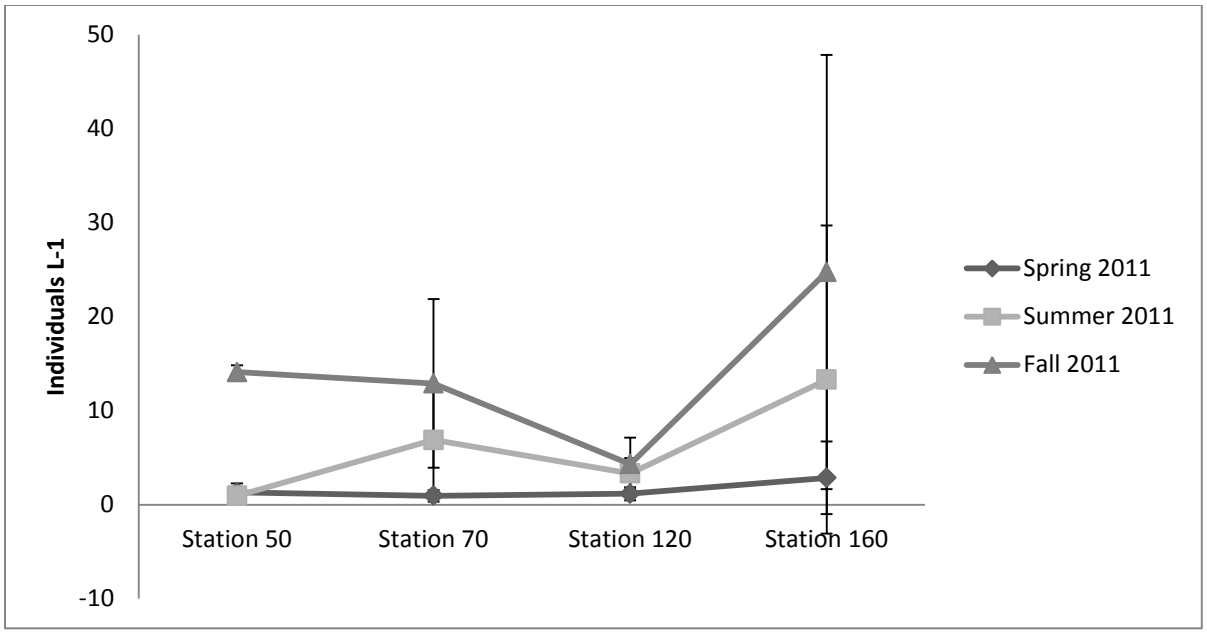

Figure 1-5. Average seasonal zooplankton abundance at each station from samples collected using 60µm-mesh nets. Error bars represent standard deviation. Spring dates include 23 March, 25 April, 9 May, 25 May; summer dates include 6 June, 5 July, 18 July, 2 August, 15 August; fall dates include 30 August, 19 September, 12 October, 24 October.

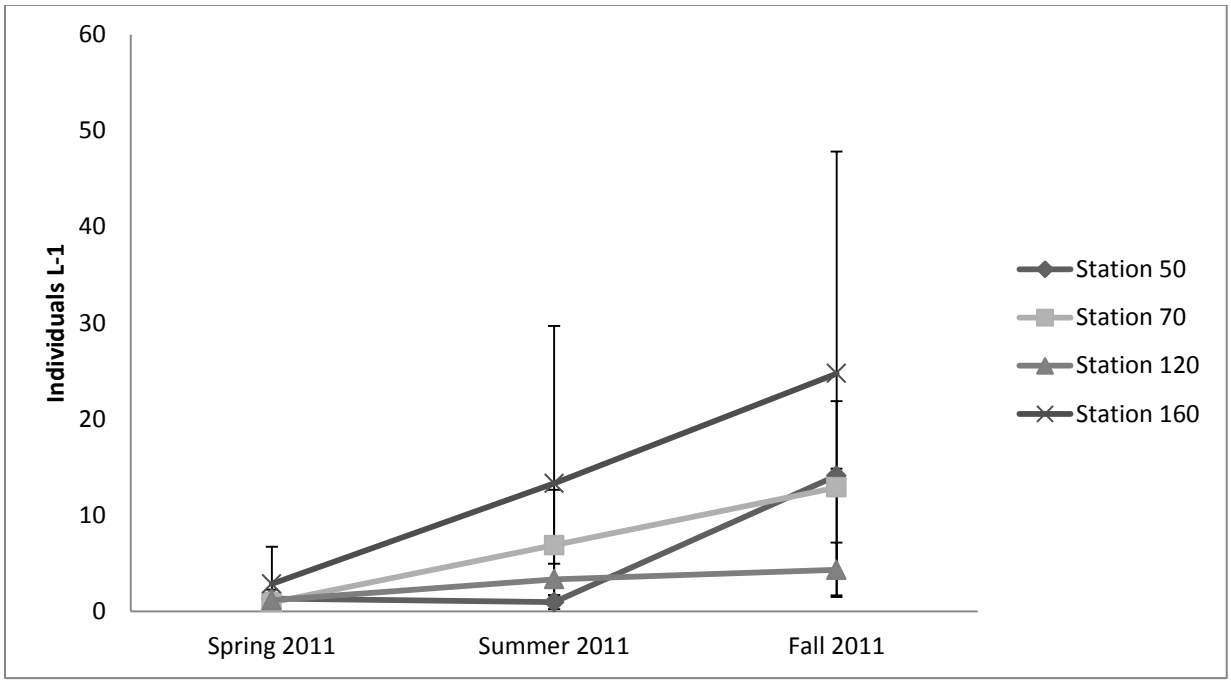

Figure 1-6. Average zooplankton abundance during each season by station from samples collected using 60um-mesh nets. Error bars represent standard deviation. Spring dates include 23 March, 25 April, 9 May, 25 May; summer dates include 6 June, 5 July, 18 July, 2 August, 15 August; fall dates include 30 August, 19 September, 12 October, 24 October.

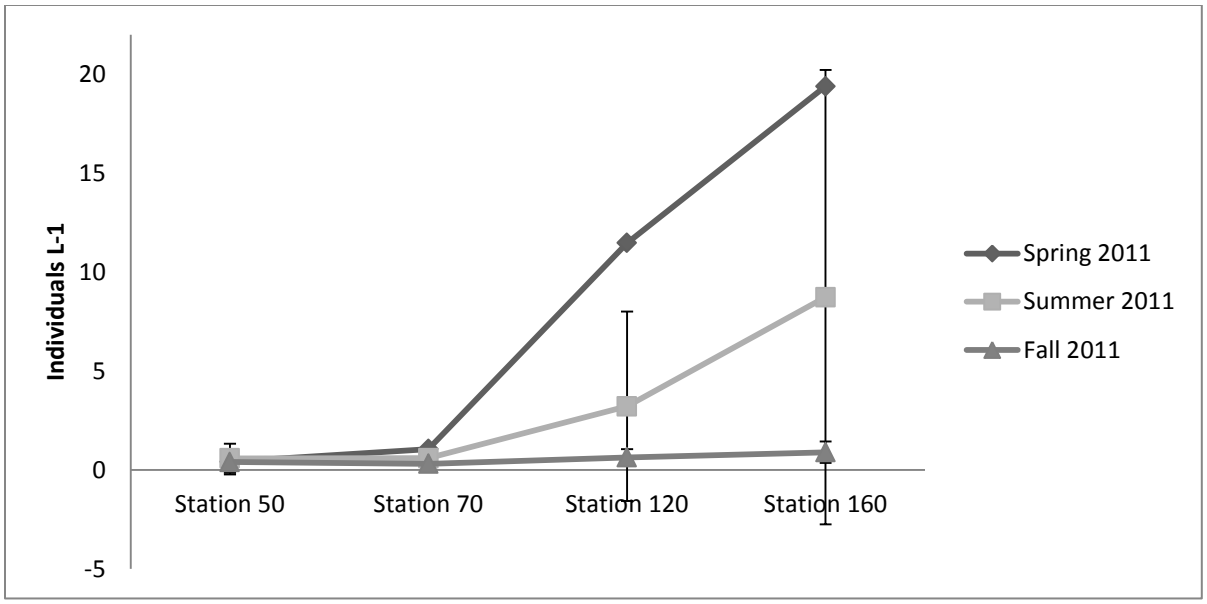

Figure 1-7. Average seasonal zooplankton abundance at each station from samples collected using 200µm-mesh nets. Error bars represent standard deviation. Spring dates include 23 March, 25 April, 9 May, 25 May; summer dates include 6 June, 5 July, 18 July, 2 August, 15 August; fall dates include 30 August, 19 September, 12 October, 24 October.

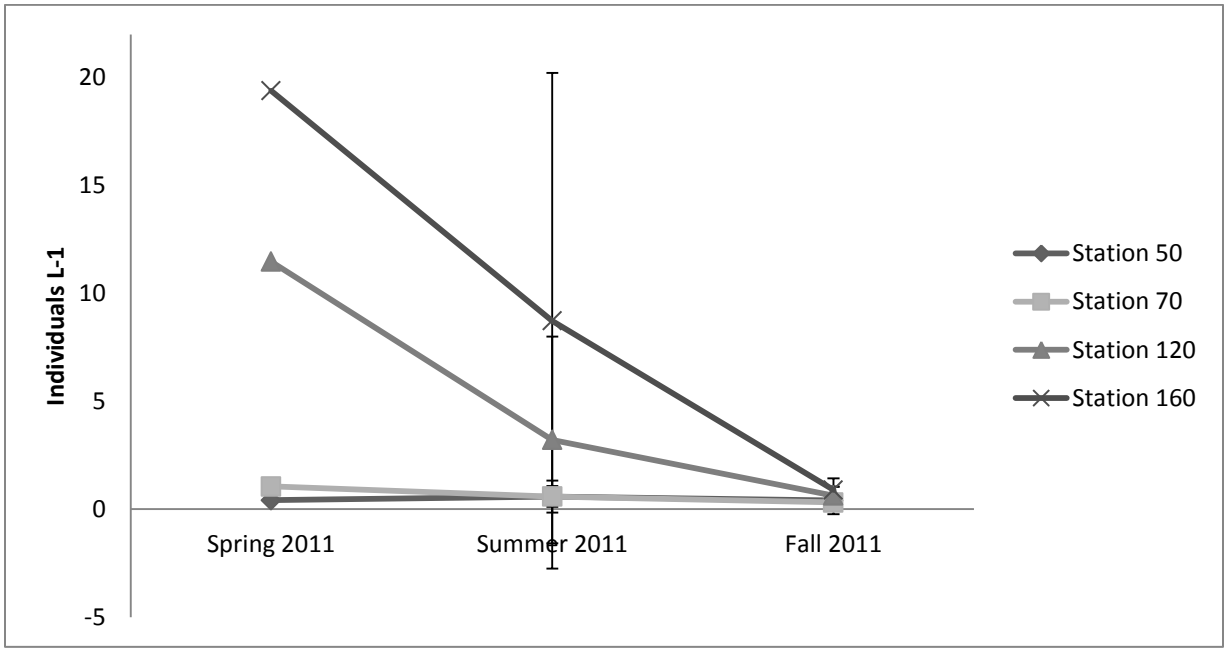

Figure 1-8. Average zooplankton abundance during each season by station from samples collected using 200µm-mesh nets. Error bars represent standard deviation. Spring dates include 23 March, 25 April, 9 May, 25 May; summer dates include 6 June, 5 July, 18 July, 2 August, 15 August; fall dates include 30 August, 19 September, 12 October, 24 October.

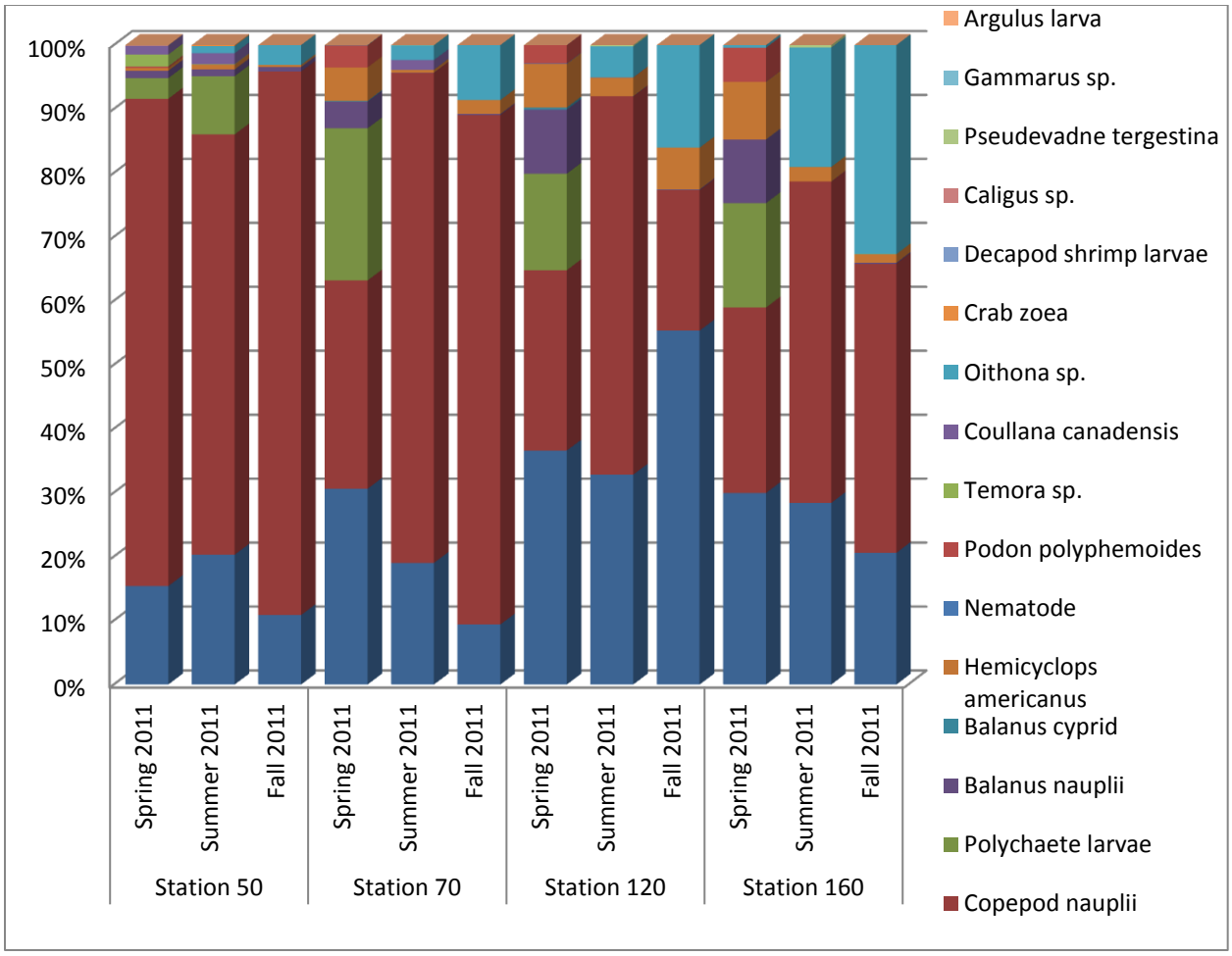

Figure 1-9. Percentage of samples collected in each season composed of observed taxonomic groups in 60µm-mesh nets, by station.

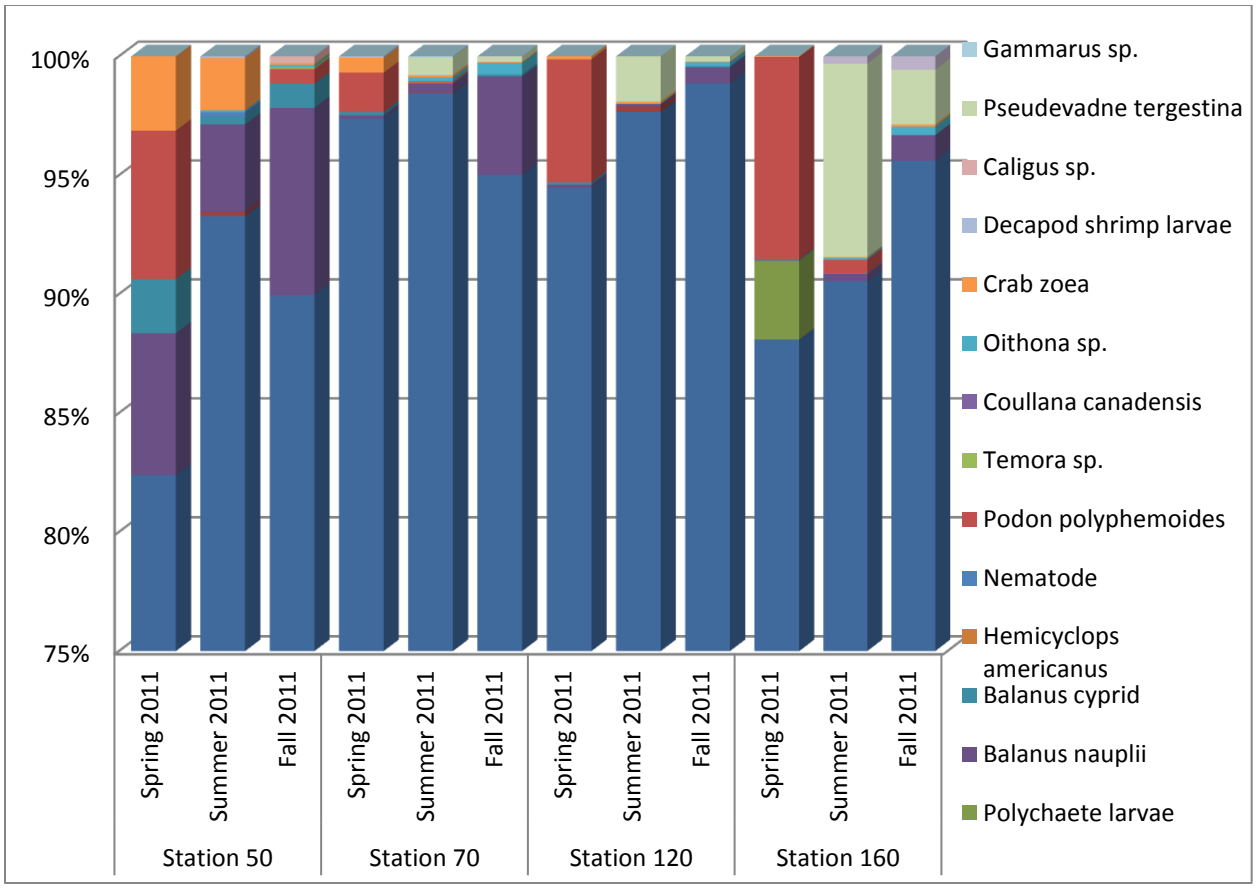

Figure 1-10. Percentage of samples composed of observed taxonomic groups in 200µm-mesh nets, by station. The y-axis begins at 75% to more clearly show differences in less numerous taxa.

Table 1-1. ModMon project water quality sampling station information. Highlighted stations were used in this study. *Depth of stations can fluctuate by up to 1m or more throughout the year.

Station #	Latitude (deg)	Longitude (deg)	Distance Downstream (km)	Approximate Station Depth (m)*
0	35.21023	77.12267	0	5.0
20	35.15330	77.07648	8.67	3.5
30	35.11375	77.03525	14.70	3.5
50	35.07952	77.00640	19.32	4.0
60	35.02465	76.96925	26.29	3.8
70	35.01472	76.95943	27.71	3.9
100	34.97660	76.87550	36.56	4.8
120	34.94888	76.81515	42.88	6.5
140	34.96610	76.73740	50.38	5.7
160	35.01440	76.66407	58.99	7.8
180	35.06413	76.52602	72.93	7.2

Table 1-2. Average temperature, salinity, and chlorophyll *a* concentration at each sampling station on each sampling date.

		23-Mar-11	25-Apr-11	9-May-11	25-May-11	6-Jun-11	5-Jul-11	18-Jul-11
Average Temp (°C)	Station 50	17.43	22.67	21.75	24.17	26.36	29.47	28.38
	Station 70	17.20	21.85	22.01	25.44	26.36	28.47	28.05
	Station 120	16.01	20.98	21.45	23.94	26.42	28.52	28.17
	Station 160	15.55	20.72	21.33	23.43	26.02	28.03	27.86
		2-Aug-11	15-Aug-11	30-Aug-11	19-Sep-11	12-Oct-11	24-Oct-11	
	Station 50	30.04	28.94	25.20	23.07	21.07	19.42	
	Station 70	30.38	28.63	25.47	22.53	20.76	18.50	
	Station 120	30.11	29.00	26.04	22.74	20.61	18.81	
	Station 160	29.63	29.13	26.27	23.01	20.44	18.67	
		23-Mar-11	25-Apr-11	9-May-11	25-May-11	6-Jun-11	5-Jul-11	18-Jul-11
Average Salinity	Station 50	2.62	0.58	6.22	5.53	12.34	9.17	10.64
	Station 70	5.85	4.80	6.79	10.51	12.84	15.88	14.28
	Station 120	9.34	11.31	11.10	18.11	16.63	19.21	19.65
	Station 160	13.16	14.15	14.84	20.91	19.20	21.42	22.48
		2-Aug-11	15-Aug-11	30-Aug-11	19-Sep-11	12-Oct-11	24-Oct-11	
	Station 50	17.91	13.63	10.54	13.88	10.95	10.39	
	Station 70	18.07	18.04	9.17	14.87	12.36	9.81	
	Station 120	21.62	22.26	16.26	16.95	15.83	15.76	
	Station 160	24.19	24.96	22.42	20.19	18.37	18.42	
		23-Mar-11	25-Apr-11	9-May-11	25-May-11	6-Jun-11	5-Jul-11	18-Jul-11
Average [Chl *a*] (µg L^{-1})	Station 50	13.07	11.83	12.41	11.32	7.96	12.82	12.26
	Station 70	19.44	19.20	18.15	11.48	11.23	10.19	7.81
	Station 120	11.32	9.62	10.56	9.27	8.33	6.67	6.20
	Station 160	6.12	7.20	7.55	5.81	6.54	5.37	8.09
		2-Aug-11	15-Aug-11	30-Aug-11	19-Sep-11	12-Oct-11	24-Oct-11	
	Station 50	10.18	12.67	5.02	6.41	18.71	14.80	
	Station 70	8.86	12.24	6.71	6.32	12.63	12.24	
	Station 120	7.97	8.56	9.30	6.56	8.36	12.25	
	Station 160	6.47	5.98	9.89	6.59	8.13	8.48	

Table 1-3. ANOVA for log of 60µm zooplankton abundance by Station and Season. * indicates significant results.

Source	DF	Sum of Squares	F ratio	Prob > F
Station	3	7.596220	1.9797	0.1350
Season	2	33.117581	12.9466	<0.0001*
Station x Season	6	10.874547	1.4171	0.2359
Model	11	56.83	4.04	0.0008*
Error	35	44.77	-	-

Table 1-4. Zooplankton abundance (individuals L^{-1}) measured at each sampling station on each sampling date, using either 60 or 200 µm-mesh nets as indicated . Dashes represent samples that were not taken or were lost.

		23-Mar-11	25-Apr-11	9-May-11	25-May-11	6-Jun-11	5-Jul-11	18-Jul-11
60 µm nets	Station 50	0.2133191	1.365351	2.479153	1.2370396	1.68312	1.54359	1.154129
	Station 70	0.1328943	1.048687	1.614732	0.961646	13.4686	11.0008	1.782557
	Station 120	0.5781569	1.082435	0.830703	2.1631801	3.72963	3.90901	4.949805
	Station 160	0.5369223	0.744468	1.516615	8.6275104	41.5278	3.72251	13.87727
		2-Aug-11	**15-Aug-11**	**30-Aug-11**	**19-Sep-11**	**12-Oct-11**	**24-Oct-11**	
	Station 50	0.248232	0.1283639	-	14.634099	13.27038	14.41091	
	Station 70	0.160341	7.9855265	-	3.751549	13.26907	21.67474	
	Station 120	3.482564	0.6096523	-	2.3125104	-	6.314463	
	Station 160	2.645687	4.7765212	-	19.382006	50.04519	4.81838	
		23-Mar-11	**25-Apr-11**	**9-May-11**	**25-May-11**	**6-Jun-11**	**5-Jul-11**	**18-Jul-11**
200 µm nets	Station 50	-	-	-	0.417069	0.5149	0.275	1.881782
	Station 70	-	-	-	1.0532853	1.18331	0.66378	0.902897
	Station 120	-	-	-	11.468026	0.07755	0.49801	0.78221
	Station 160	-	-	-	19.386724	1.06699	0.72978	1.273407
		2-Aug-11	**15-Aug-11**	**30-Aug-11**	**19-Sep-11**	**12-Oct-11**	**24-Oct-11**	
	Station 50	0.110396	0.1600666	1.3537168	0.168173	0.052786	0.032295	
	Station 70	0.085252	0.1213521	0.8151609	0.0556036	0.238176	0.135238	
	Station 120	11.49805	3.2084917	0.9717803	0.1331801	0.439233	0.984652	
	Station 160	13.88983	26.683673	0.9175562	0.2716468	1.581809	0.803257	

Table 1-5. ANOVA for log of 200µm zooplankton abundance by Station and Season. * indicates significant results.

Source	DF	Sum of Squares	F ratio	Prob > F
Station	3	27.298915	4.8497	0.0077*
Season	2	19.167986	5.1079	0.0128*
Station x Season	6	3.585703	0.3185	0.9218
Model	11	53.37	2.56	0.021*
Error	28	52.54	-	-

Table 1-6. ANOVA for log of mean chlorophyll *a* abundance by Station and Season. * indicates significant results.

Source	DF	Sum of Squares	F ratio	Prob > F
Station	3	1.9092250	9.5569	<0.0001*
Season	2	0.5431394	4.0781	0.0244*
Station x Season	6	0.8131105	2.0351	0.0833
Model	11	3.31	4.52	0.0002*
Error	40	2.66	-	-

Table 1-7. Comparison of mean zooplankton abundance for selected estuaries in the southeastern United States. [a] Post-naupliar copepod data only presented. (Adapted from Table 3 of Mallin 1991).

Study	Study Site	Net mesh (um)	Individuals L^{-1}
Birkhead et al. (1979)	Cape Fear River, NC	156	6.7 (1971-1973) 8.2 (1974-1976)
Fulton (1984)[a]	Beaufort, NC	76	21.9 (30 months)
Lonsdale and Coull (1977)	North Inlet, SC	156	9.257 (20 months)
Mallin (1991)	Neuse River Estuary, NC	76	31.224 (1989) 34.530 (20 months)
Thayer et al. (1974)	Newport River, NC	156	4.000 (1970) 8.400 (1971)
McGlaughon (2012)	Neuse River Estuary, NC	60 200	6.348 (2011) 2.752 (2011)
McGlaughon (2012) (Station 160 only)	Neuse River Estuary, NC	60 200	12.685 (2011) 6.66 (2011)

CHAPTER 2

INTRODUCTION

Carpenter et al (1985) adopted the term "trophic cascade" for limnetic plankton ecology to describe a downward transmission of top-down effects from fish to lake phytoplankton. They defined the consequence of a trophic cascade as a negative correlation between the biomass of adjacent trophic levels and a positive one between trophic levels two links apart. The opposite, or "bottom-up", effect would show a positive correlation between all trophic levels where biomass of a trophic level is determined by its resources. Such cascades have been studied in North American estuaries (Reaugh et al. 2007, Smayda 2008, Stoecker et al. 2008), though evidence for trophic cascades in estuaries is far outnumbered by examples from lakes (Carpenter et al 2001, Tessier and Woodruff 2002). A simple example of a trophic cascade in an estuarine food web consisting of planktivorous larval fish, zooplankton, and phytoplankton might be seen if an increase in larval fish biomass leads to a decrease in zooplankton biomass, which leads to a subsequent increase in phytoplankton biomass. Bottom-up effects might be seen if a significant increase in available nutrients led to increases at each ascending level of the food web. It is widely believed that pelagic food webs are largely forced by nutrient dynamics and a combination of bottom-up forcing and top-down control (Gliwicz 2002, Howarth et al. 1999).

The importance of top-down vs. bottom-up control in marine food webs remains inconclusive. In a cross-ecosystem analysis of the strengths of trophic cascades, Shurin et al. (2002) found that top-down control of plant biomass was stronger in water than on land. However, the differences found among the aquatic food webs were as great as those between aquatic and terrestrial systems. Microzooplankton grazing limits phytoplankton biomass accumulation under otherwise favorable environmental conditions in marine systems (Barber and

Hiscock 2006). Some copepods, such as *Acartia tonsa*, that occur in the Neuse River Estuary, NC are omnivores that feed on both microzooplankton and phytoplankton (Sipura et al 2003). They can therefore affect the estuarine food web in a number of ways. Reaugh et al (2007) found that in Chesapeake Bay, top-down control of microzooplankton by copepods may be an important factor in phytoplankton bloom formation. This study also demonstrated that microzooplankton are an important food source for estuarine copepods, even when phytoplankton are abundant. However, Wetz et al. (2011) found that phytoplankton biomass was negatively correlated with copepod abundances in grazing experiments conducted in the Neuse River Estuary, pointing to a potential direct grazing impact by copepods as well. Short-term microcosm grazing experiments conducted by Sipura et al. (2003) showed that copepods significantly decreased both microzooplankton and large phytoplankton (between 14 and 70µm in size) populations, and blooms of ciliates and diatoms were apparent in copepod removal treatments.

These differences in the grazing impacts of different types and sizes of zooplankton can have important effects in estuarine systems. In regions where phytoplankton is unable to be grazed directly by mesozooplankton, phytoplankton production is made available to the larger zooplankton through trophic intermediates represented by microzooplankton which signify an important carbon source to larger zooplankton (Gifford and Dagg 1988, Stoecker and Capuzzo 1990, Gifford 1993). Zooplankton community structure, production, and phytoplankton abundance can be influenced by the coupling of different trophic levels by trophic cascading (Pace et al. 1998, Calbet and Landry 1999). Trophic cascades in the oligotrophic temperate Kariega estuary in South Africa were consistent with the expectation of predator-prey cascades

(Froneman 2002). This study found microzooplankton as the primary consumers of Chl *a*, and mesozooplankton had little impact on Chl *a*. Mesozooplankton had a negative impact on net growth rates of microzooplankton which resulted in a decrease in the feeding impact of these organisms on the Chl *a* and bacteria. The data from this study are consistent with the model proposed by Menge and Sutherland (1976) which argues that the prevalence of omnivory in food webs should lead to increasing control by predation and decreasing control by resource limitation for populations at lower trophic levels. However, several authors have argued that systems with high levels of omnivory rarely exhibit community-level trophic cascades (Strong 1992, Polis et al. 2000).

Physical (light, residence time) and chemical (nutrients) forcing features have long been emphasized as main drivers of the location and timing of the Neuse River Estuary (NRE) chlorophyll maximum (CMAX) development, its composition, and ultimately the fate of its production (Paerl et al. 2007, Paerl et al. 2010). Recently it has become evident that the estuarine CMAX may be dominated by small cells such as pico- and nanophytoplankton (Phlips et al. 1999, Murrell and Lores 2004, Gaulke et al. 2010), which has brought attention to the role of top-down controls, particularly by microzooplankton which can rapidly consume small cells but also provide nutrients through excretion and sloppy feeding. Studies have documented intense microzooplankton grazing upon estuarine phytoplankton (Gallegos 1989, Strom et al 2001), at times capable of destroying blooms or altogether preventing bloom formation (Strom et al 2001). Grazing may not always be the dominant pathway for CMAX production, even when small phytoplankton dominate. For instance, in the Neuse River Estuary, microzooplankton grazing was not sufficient to prevent small phytoplankton from exhibiting net population growth

(beginning in spring), reach bloom proportions and sustain high biomass (during summer) (Wetz et al. 2011). The high mesozooplankton abundances associated with low microzooplankton abundances is consistent with the hypothesis that top-down controls on the microzooplankton allow for the small phytoplankton to bloom and sustain high biomass during summer. Depending on the timing, location and composition of estuarine blooms, top-down controls and trophic cascades may play a key role in determining the spatial-temporal distribution of the CMAX, its composition (through selective feeding), productivity (through nutrient recycling) and fate (through presence or absence of grazing pressure). The purpose of this chapter is to examine the role of mesozooplankton grazing within the CMAX region and upstream of the CMAX region of the NRE with a series of grazing experiments. My goal is to quantify the effect of mesozooplankton grazing on phytoplankton abundance in each location. Quantification of mesozooplankton grazing rates will shed further light onto the trophic dynamics of this eutrophic system.

HYPOTHESES

Hypothesis 1: There will be no difference in the chlorophyll *a* (whole phytoplankton community) ingestion rate of unfiltered water, water filtered through a 153 µm filter and water filtered through a 20 µm filter, between the CMAX and an area upstream of the CMAX.

Hypothesis 2: There will be no difference in the chlorophyll *a* (<20 µm sized phytoplankton community) ingestion rate of unfiltered water, water filtered through a 153 µm filter and water filtered through a 20 µm filter, between the CMAX and an area upstream of the CMAX.

Hypothesis 3: There will be no difference in the chlorophyll *a* (> 20 µm sized phytoplankton community) ingestion rate of unfiltered water, water filtered through a 153 µm filter and water filtered through a 20 µm filter, between the CMAX and an area upstream of the CMAX.

Approach: A 2-factor experiment where location (CMAX and upstream) and grazer size (Whole, <153 µm and <20 µm) were manipulated. The experiment was analyzed by 2-factor ANOVA, with Tukey's post-hoc tests for comparison (Zar, 1974). Note that the three different sized phytoplankton communities (whole, < 20 µm, >20 µm) were separated via filtration of each experimental carboy after the conclusion of the experiment.

METHODS

Collection

Experiments were conducted at the University of North Carolina-Chapel Hill's Institute of Marine Sciences (IMS) in Morehead City, North Carolina in June 2011. For each sampling event, surface water samples were collected mid-day in clean 20 L carboys from the CMAX (located with a flow-through chlorophyll *a* sensor aboard the sampling vessel) and from a few kilometers upstream of the CMAX, and stored under black tarps for transport back to IMS.

Experimental Procedure

Upon returning to IMS, water from each of the two sites was divided among several treatments. Triplicate transparent 4L-cubitainers were used for each of the 12 treatments listed in Table 2-1. For the grazing manipulation, water was first filtered through either a 20 µm mesh (to remove micro- and mesozooplankton), a 153 µm mesh (to remove mesozooplankton), or was left unfiltered (leaving the zooplankton community intact). Ten µmol L^{-1} urea or nitrate was

added to select treatments (as indicated in Table 2-1). These nutrient addition manipulations were used to offset phytoplankton death due to nutrient limitation. For the duration of the experiment (48 hours), cubitainers were incubated in an experimental pond at IMS which was continuously flushed with water from Bogue Sound, so as to mimic ambient temperature and light levels. Subsamples were taken from each cubitainer at 0 hrs, 24 hrs, and 48 hrs. Parameters measured from each subsample included inorganic nutrients, size-fractionated (< 20 µm, > 20 µm) phytoplankton pigments (using high-performance liquid chromatography, or HPLC) and phytoplankton/zooplankton abundance.

Grazing Rate Calculations

Growth constants (*K*) were calculated at the end of the experiment (T_{48}) for Total, <20µm, and >20µm Chl *a* using the following equation:

$$K = \left(\frac{(\ln C_1 - \ln C_0)}{(t_1 - t_0)} \right)$$

where *t* = time in days, and *C* = Chl *a* concentration (see Figure 2-1). Grazing coefficients were then calculated using the following equation (also see Figure 2-1):

$$g = K - \left(\frac{(\ln C_1^* - \ln C_0^*)}{(t_1 - t_0)} \right)$$

The calculated grazing coefficients were then used to calculate clearance rate (F) and ingestion rate (I) using the following equations:

$$F = gV$$

$$I = [C]F$$

where *V* is the cubitainer volume.

Statistical Analysis

Two-way ANOVA will be used to test for significant differences in ingestion rates depending on location inside or outside the CMAX and depending on the size of phytoplankton being grazed. Type-III Sum of Squares was used where necessary since in certain instances the ANOVA was unbalanced, and Type-III effect estimates are not a function of the frequency of observations in any group (Clark 2011). For the purposes of this discussion we labeled 20-153µm-filtered grazers as microzooplankton and >153µm-filtered grazers as mesozooplankton (see Table 2-2), even though these values do not match entirely with the ranges of Sieburth, et al. 1978.

RESULTS

At the start of our experiments, mean total chlorophyll *a* concentration from CMAX water was 16.958 µg L^{-1} with 92.7% of chlorophyll *a* composed of small phytoplankton (<20µm) (Figure 2-2). After 48 hours, mean total Chl *a* concentration from the CMAX was reduced to 5.403 µg L^{-1}, with 89.0% of this composed of small phytoplankton. This represents a 68.1% reduction in total chlorophyll from the beginning of the experiment to the end. In upstream samples, starting mean total Chl *a* concentration was 16.336 µg L^{-1}, with 88.5% being composed of small phytoplankton. Starting chlorophyll concentration did not differ significantly ($p>0.05$, ANOVA) between CMAX and Upstream samples (Table 2-3). After 48 hours, mean total Chl *a* concentration from upstream samples was 6.317 µg L^{-1}, with 81.8% composed of small phytoplankton. This represents a 61.3% reduction in chlorophyll concentration from the start of the experiment to then end.

The zooplankton community and its constituents will be defined using the definitions described in Table 2-2. Whole zooplankton community grazing on phytoplankton was highest upon large phytoplankton (>20 µm) upstream of the CMAX (Figures 2-3 and 2-4). Grazing upon the >20 um fraction of chlorophyll within the CMAX was negative, suggesting no grazing occurred. Grazing by mesozooplankton was minimal in both locations and lowest on >20um chlorophyll within the CMAX. Microzooplankton-specific grazing rates were positive in all locations and were highest within the CMAX.

ANOVA revealed significantly higher ingestion rates inside the CMAX by the zooplankton community as a whole upon small (<20µm) phytoplankton ($p=0.0125$, Figure 2-4 and Table 2-4). Ingestion rates by the whole zooplankton community upon large phytoplankton (>20µm) were significantly higher upstream from the CMAX ($p=0.0143$). Microzooplankton-specific ingestion rates upon small and total phytoplankton were significantly higher within the CMAX than upstream from the CMAX ($p=0.0006$ and 0.0322, respectively). Mesozooplankton-specific ingestion rates did not differ significantly on any phytoplankton size within or outside the CMAX.

ANOVA also revealed significant differences in ingestion rates of total phytoplankton and small phytoplankton (<20µm) at T_{48} between location in the CMAX vs. Upstream samples ($p=0.009$ and $p<0.0001$, respectively, Table 2-5), with ingestion of total and small Chl *a* being significantly higher in the CMAX than in Upstream treatments. Ingestion rates of large phytoplankton (>20µm) were significantly higher in Upstream treatments than CMAX treatments ($p=0.0056$, Table 2-5). ANOVA also revealed significant differences in ingestion rates on total Chl *a* depending on size of the zooplankton ($p=0.0023$, Table 2-5). Post-hoc

student's *t* testing (α=0.05) revealed significantly higher ingestion rates of total Chl *a* by microzooplankton than by mesozooplankton. Small phytoplankton were grazed differently depending on the size of the grazer (p=0.0004, Table 2-5), with microzooplankton exhibiting significantly higher ingestion rates on small phytoplankton than mesozooplankton. ANOVA revealed a significant interaction (p=0.0166, Table 2-5) between location (CMAX vs. Upstream) and zooplankton size on the ingestion rate of small phytoplankton. This signifies a non-linear relationship between small phytoplankton and grazers in the estuary, with grazing of small phytoplankton decreasing significantly from CMAX to Upstream samples across all zooplankton sizes. There were no significant differences in the ingestion rates of large phytoplankton depending on the size of zooplankton.

DISCUSSION

Data from this study showed significant differences in grazing between the CMAX and upstream locations depending on both the size of zooplankton grazers and the size of the phytoplankton being grazed. Chlorophyll *a* concentration at the start of the experiment did not differ significantly between the CMAX and non-CMAX samples. This can be explained by the lack of a distinct CMAX in the summer during which these experiments were conducted. While the CMAX samples were taken from an area of the estuary with increased chlorophyll levels, further grazing experiments conducted when there is a more distinct CMAX (such as the spring CMAX seen in Chapter 1) may be useful in further understanding differences in CMAX vs. non-CMAX grazing. Small phytoplankton (< 20 um) dominated the phytoplankton community regardless of location and were ingested at significantly higher rates inside the CMAX than upstream from the CMAX. The primary grazers of these small phytoplankton cells were

microzooplankton and not mesozooplankton (Figure 2-5, Tables 2-4 and 2-5). My results indicate that mesozooplankton play a limited role in direct phytoplankton grazing and that the majority of phytoplankton grazing occurs within the CMAX region by microzooplankton.

Seasonally high water temperatures promote regenerated nutrient release into the water column, especially in eutrophic, long-residence time systems such as the NRE (Cowan and Boynton 1996, Rizzo and Christian 1996). The combined effects of high temperatures and regenerated nutrients are known to favor small phytoplankton growth in estuaries (Agawin et al. 2000). Thus, the buildup of available energy in the NRE CMAX should exist predominantly in the form of small phytoplankton, which are consumed by microzooplankton at a very high rate. A study by Froneman (2002) in the Kariega estuary in South Africa yielded comparable results to those found in our study of the NRE. Microzooplankton were identified as the primary consumers of Chl *a*, and mesozooplankton had very little impact on Chl *a*. The phytoplankton in the estuary were too small to be grazed efficiently by larger zooplankton, leading to increased consumption of nano- and microzooplankton. This subsequent negative impact on microzooplankton resulted in a decreased feeding impact on small chlorophyll *a*.

Zooplankton can graze phytoplankton differently based on the size of phytoplankton and thereby alter algal assemblages through grazing (Bergquist et al. 1985, Lehman and Sandgren 1985, Liu and Dagg 2003, Stoecker et al. 2008), and it would appear that our study also supports these findings. In their study of trophic interactions in the plume of the Mississippi River, Liu and Dagg (2003) found that mesozooplankton grazing rates were highest where large phytoplankton (>20μm) were abundant. As large phytoplankton growth became nutrient limited and subsequently declined, microzooplankton grazing rates increased, causing declines in

phytoplankton biomass. While the differences were not significant, mesozooplankton in our experiment did ingest large phytoplankton at a higher rate upstream from the CMAX than within the CMAX (Figure 2-3). A paired *t*-test showed that concentrations of large phytoplankton (>20µm) were significantly higher at the start of our experiment (T_0) in samples from upstream water than in samples from CMAX water (t_2=3.084, p=0.0455). Thus, mesozooplankton grazing was higher in areas where large phytoplankton were more readily available. As smaller phytoplankton come to dominate the system, especially inside the CMAX, the availability of large phytoplankton decreases and the grazing pressure of mesozooplankton subsequently drops.

Another reason for the difference in grazing rate could be attributed to different species of mesozooplankton present in the CMAX and non-CMAX regions of the NRE. Copepods counted at the end of these experiments consisted of mostly *Acartia tonsa* and an unknown harpacticoid copepod, likely *Coullana canadensis* (Table 2-6). In my zooplankton sampling conducted during the same year and in the same part of the NRE (see Chapter 1), all mesozooplankton samples collected during the summer of 2011 (when these grazing experiments were conducted), regardless of sampling station (an area that included the CMAX and Upstream samples collected for these grazing experiments), were composed of at least 90% *Acartia tonsa*. *Acartia tonsa* are omnivores which feed on both microzooplankton and phytoplankton (Sipura et al 2003). *C. canadensis* is a benthic detritivore that has been found to be positively correlated with turbidity and more densely populated in bottom waters (Morgan et al 1997). Because of their affinity for turbid, bottom-water regions of estuaries, these harpacticoids would likely not be playing a major role in CMAX grazing. Being that *A. tonsa* was present as the dominant

mesozooplankton species at all locations in the NRE, there presumably should not have been any changes in grazing based solely on the type of mesozooplankton grazer present in the system.

Other studies on estuarine mesozooplankton grazing effects have returned varying results regarding ingestion upon phytoplankton by mesozooplankton. Lionard et al. (2005) found no significant grazing impact of mesozooplankton in the upper Schelde estuary, Belgium, despite experimental mesozooplankton densities that were much higher than those found in the field. Mesozooplankton studied in these experiments included the calanoid copepod *Eurytemora affinis*, the cyclopoid copepods *Acanthocyclops robustus* and *Cyclops vicinus*, and several cladoceran species. Experiments conducted in Florida Bay (Goleski et al. 2010) to elucidate the trophic impact of mesozooplankton on the microbial food web revealed ingestion rates of Chl *a* between -0.12 and 0.14 ug Chl *a* individual^{-1}day^{-1}, depending on the presence or absence of cyanobacteria blooms.

Further data from a second set of experiments done in August 2011 and planned future grazing experiments in March 2012 and beyond, will help to facilitate a broader understanding of the trophic interactions occurring in the NRE CMAX. Initial results do show that microzooplankton appear to be playing a very significant role in the grazing of phytoplankton inside the CMAX, while mesozooplankton do not appear to be grazing directly upon phytoplankton at a similarly high rate. This further supports the important role of estuarine microzooplankton as a link between available carbon in the form of chlorophyll *a* and higher trophic levels such as larval fish, especially in highly productive regions such as the chlorophyll maximum. While mesozooplankton serve as the direct energy source for higher estuarine trophic

levels, the microzooplankton are key in transferring available organic carbon up from the bottom of the food web.

My research has shown that size is an important driver of who is eating whom in the NRE. When phytoplankton are larger, mesozooplankton will graze them directly. However, mesozooplankton cannot easily graze smaller-sized phytoplankton. Thus, there are situations inside the NRE in which mesozooplankton are not grazing phytoplankton directly. This leads to a less efficient transfer of energy to higher trophic levels, and may be an effect of eutrophication which can drive phytoplankton sizes down (Paerl et al. 1998, 2004). All of this suggests the potential for a trophic cascade in the NRE where mesozooplankton are eating microzooplankton, which is reducing overall grazing on phytoplankton. However, grazing rates remain high because of the extremely fast growth rate of microzooplankton (Landry and Calbet 2004). The presence of these trophic cascades in the NRE suggest that there is still much research to be done before we fully understand the implications upon system activity in highly productive zones such as the CMAX. Further experimentation in the NRE with the goal of defining the individual roles of varying size classes of phytoplankton and zooplankton grazers will assist in further understanding this complex system.

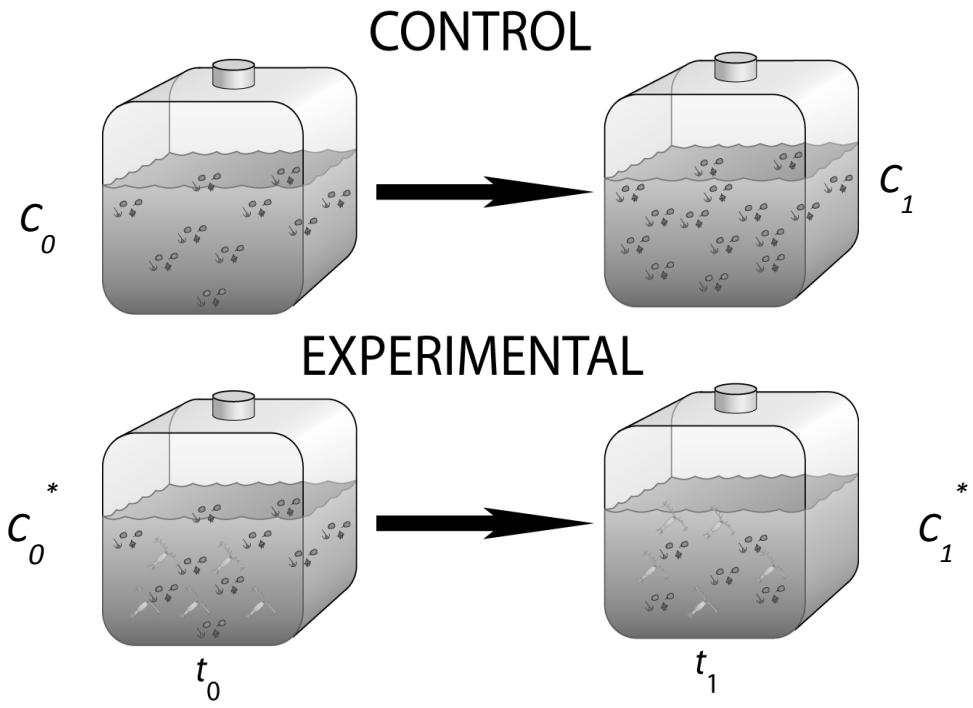

Figure 2-1. Sample experimental design for grazing experiment. *C* = chlorophyll *a* concentration in the control, *C** = chlorophyll *a* concentration in the experimental treatment, and *t* = time.

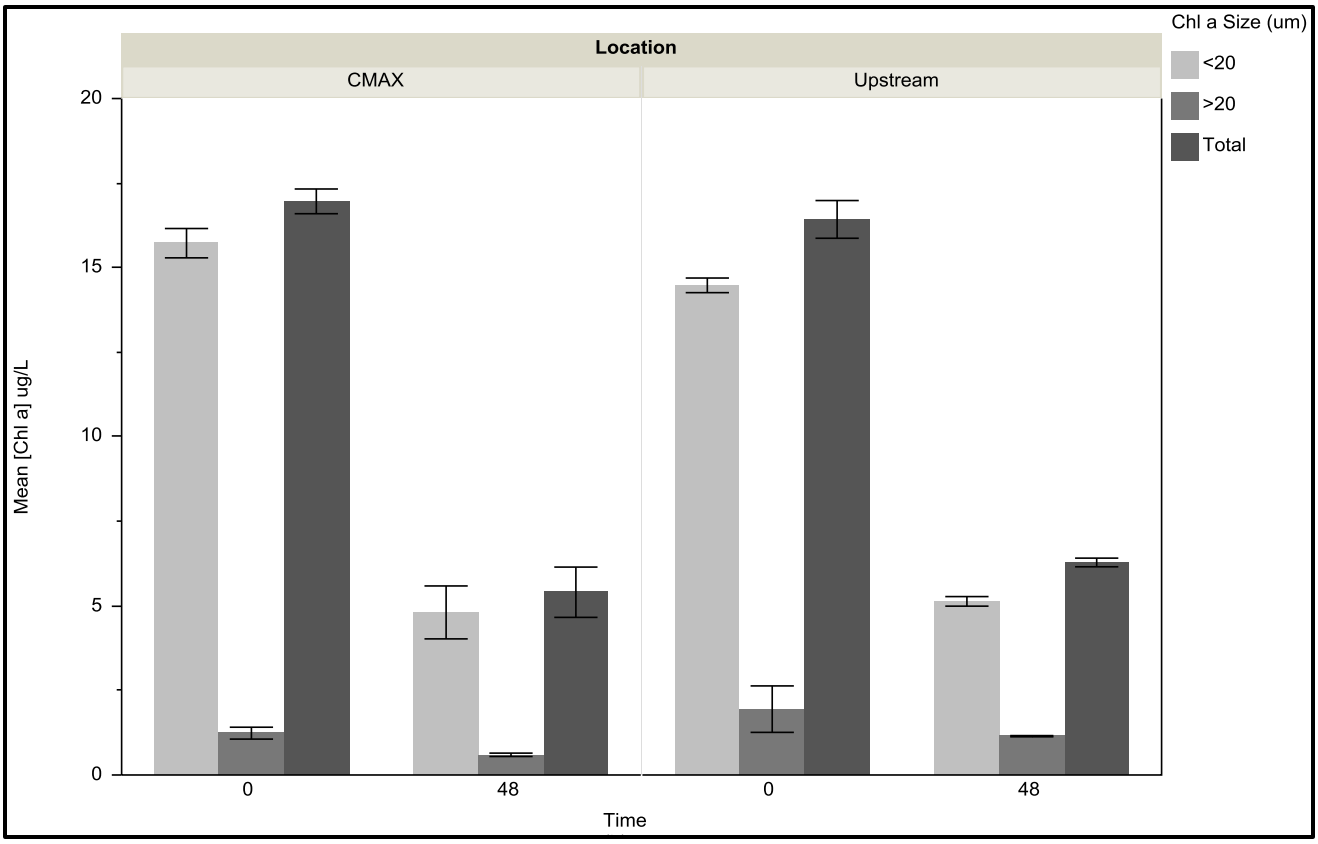

Figure 2-2. Starting (T_{0}) and ending (T_{48}) mean chlorophyll *a* concentrations for total, <20um, and >20um phytoplankton in CMAX and Upstream samples across all filtration treatments. Error bars constructed using one standard error from the mean.

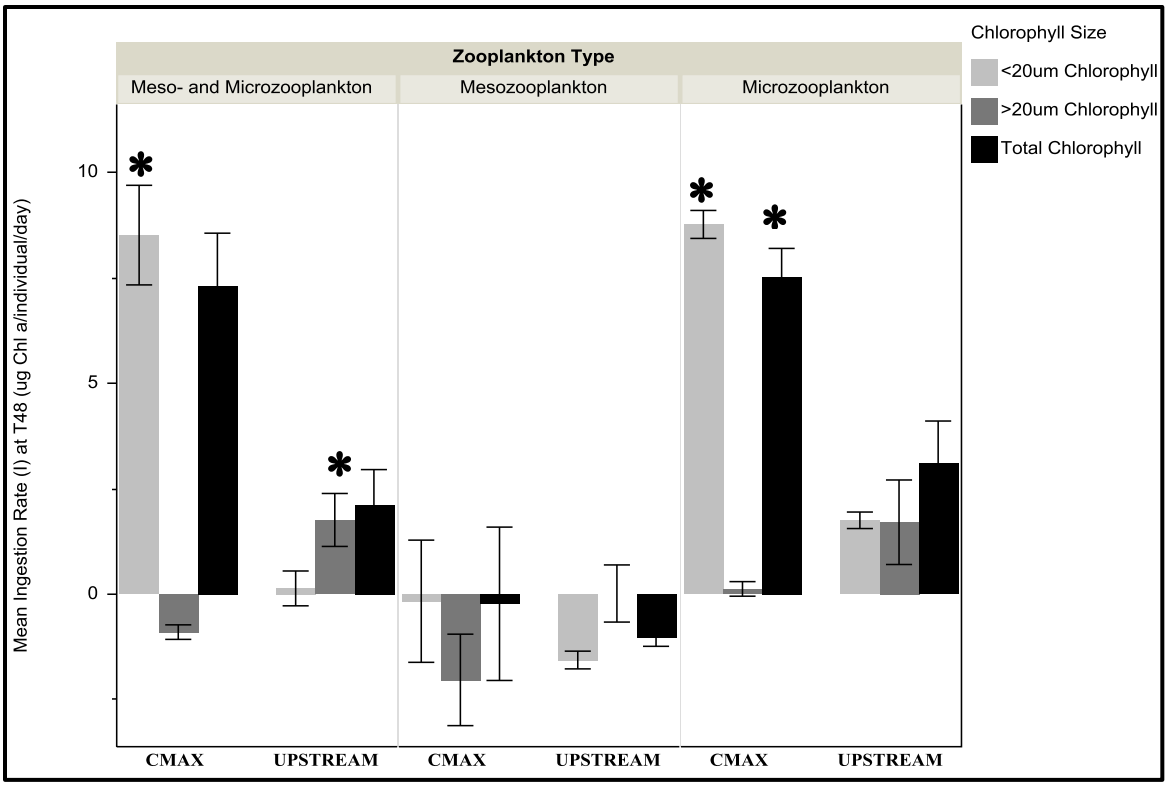

Figure 2-3. Mean ingestion rate of phytoplankton by mesozooplankton, microzooplankton, and the whole phytoplankton community at T_{48} within the CMAX and upstream from the CMAX. Error bars constructed using one standard error from the mean. Asterisks represent significantly higher values between location (CMAX vs. Upstream, $p<0.05$).

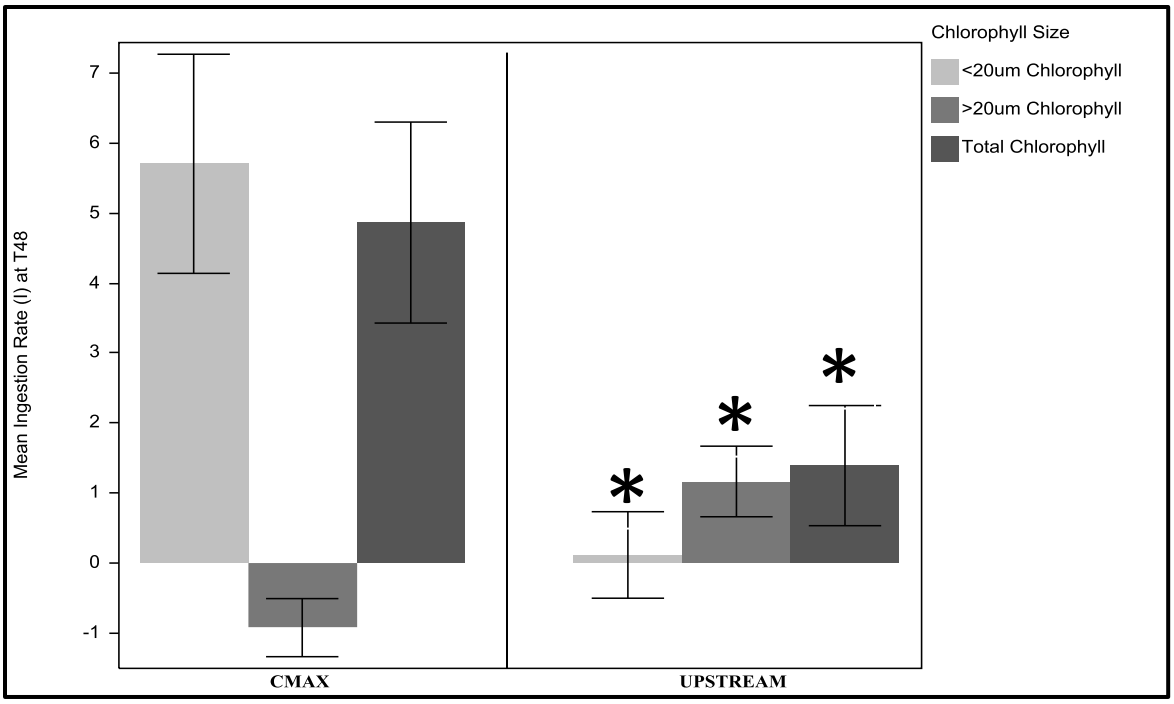

Figure 2-4. Mean ingestion rate of chlorophyll at T_{48} across all treatments within the CMAX and upstream from the CMAX. Error bars constructed using one standard deviation from the mean. Asterisks indicate Upstream values that are significantly different from CMAX values.

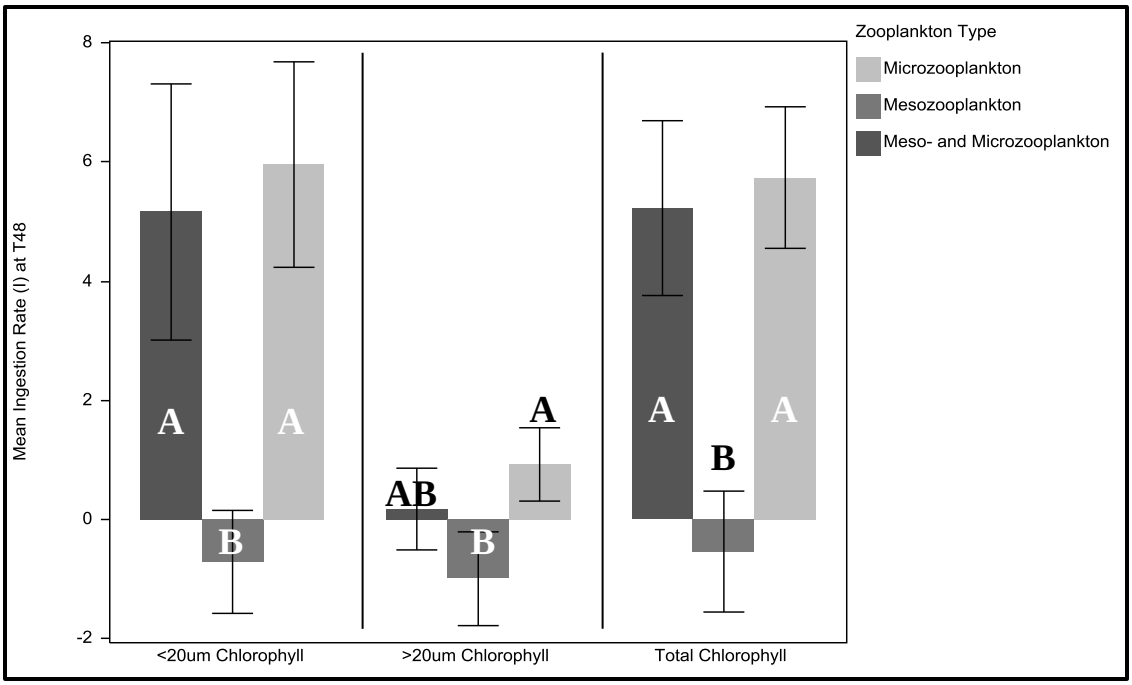

Figure 2-5. Mean ingestion rate of chlorophyll at T_{48} by microzooplankton, mesozooplankton, and total zooplankton. Error bars constructed using one standard error from the mean. Levels not connected by the same letter are significantly different according to student's *t* testing (α=0.05), for each phytoplankton size class.

Table 2-1. Sampling locations, nitrogen manipulations, and grazing manipulations for June, 2011 grazing experiments.

Sampling Location	Nitrogen manipulation	Grazing manipulation
CMAX	Urea	Whole Water
		<153 µm
		<20 µm
	Nitrate	Whole Water
		<153 µm
		<20 µm
	No addition	Whole Water
		<153 µm
		<20 µm
Upstream	No addition	Whole Water
		<153 µm
		<20 µm

Table 2-2. Filtration treatments used to calculate microzooplankton, mesozooplankton, and total community grazing rates upon phytoplankton. Grazing coefficient (*g)* calculated by comparing growth constants (*K*) in different filtration treatments.

Filtration	Name
None	Whole water (total zooplankton community)
<153µm	Microzooplankton (20-200µm*) only
None - <153µm	Mesozooplankton (>200µm*) only
<20µm	No grazers

***size ranges taken from Sieburth et al. 1978.**

Table 2-3. ANOVA results for starting (T_0) chlorophyll concentration by location inside the CMAX or upstream from the CMAX.

	DF	Sum of Squares	F ratio	Prob > F
Location	**1**	**2.1915**	**0.0446**	**0.8336**
Error	**49**	**2406.8920**	**-**	**-**

Table 2-4. ANOVA table for ingestion rate (I) by Location (CMAX vs. Upstream) upon small (<20µm), large (>20µm), and total phytoplankton. Asterisks indicate significant results ($p<0.05$).

Whole zooplankton Community	**Phytoplankton <20µm**				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	84.19	29.02	0.013*
	Error	3	8.70	-	-
	Phytoplankton >20µm				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	8.49	26.34	0.014*
	Error	3	0.32	-	-
	Total phytoplankton				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	32.44	8.99	0.58
	Error	3	10.82	-	-
Microzooplankton only	**Phytoplankton <20µm**				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	59.05	240.84	0.0006*
	Error	3	0.74	-	-
	Phytoplankton >20µm				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	2.50	2.42	0.26
	Error	2	2.07	-	-
	Total phytoplankton				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	23.47	14.38	0.03*
	Error	3	4.90	-	-
Mesozooplankton only	**Phytoplankton <20µm**				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	2.33	0.55	0.51
	Error	3	12.68	-	-
	Phytoplankton >20µm				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	4.20	2.57	0.25
	Error	2	3.27	-	-
	Total phytoplankton				
		DF	**Sum of Squares**	**F ratio**	**Prob > F**
	Location	1	0.78	0.12	0.75
	Error	3	19.91	-	-

Table 2-5. Type III Sum of Squares ANOVA for ingestion rate (I) by Location and zooplankton size. Asterisks indicate significant results ($p<0.05$).

Phytoplankton <20µm				
	DF	**Sum of Squares**	**F ratio**	**Prob > F**
Location	1	112.69	45.85	<0.0001*
Zooplankton Size	2	104.47	21.25	0.0004*
Location x Zooplankton Size	2	32.88	6.69	0.0166*
Phytoplankton >20µm				
	DF	**Sum of Squares**	**F ratio**	**Prob > F**
Location	1	13.96	15.50	0.0056*
Zooplankton Size	2	8.08	4.49	0.056
Location x Zooplankton Size	2	0.64	0.36	0.71
Total phytoplankton				
	DF	**Sum of Squares**	**F ratio**	**Prob > F**
Location	1	43.51	10.99	0.009*
Zooplankton Size	2	102.47	12.94	0.0023*
Location x Zooplankton Size	2	13.18	1.67	0.24

Table 2-6. Species and number of mesozooplankton present at end of grazing experiments in whole water treatments.

	CMAX Whole A	**CMAX Whole B**	**CMAX Whole C**	**Upstream Whole A**	**Upstream Whole B**
Acartia tonsa	145	166	250	9	13
Harpacticoid copepod	22	15	90	10	6
Copepod nauplius	0	1	0	0	0
Oithona sp.	0	0	1	0	0
Total	**167**	**182**	**341**	**19**	**19**

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APPENDIX

Table A-1. Data from CMAX grazing experiments. T_0 results taken at start of experiment, T_{24} results taken after 24 hours, T_{48} results taken after 48 hours.

		[Chl a] (T_0)	[Chl a] (T_{48})
Total Chl a	CMAX Whole A	16.790	4.995
	CMAX Whole B	17.332	4.746
	CMAX Whole C	17.510	4.273
	CMAX < 153 A	17.730	4.490
	CMAX < 153 B	18.471	4.587
	CMAX < 153 C	16.085	4.868
	CMAX < 20 A	16.218	6.941
	CMAX < 20 B	16.072	6.492
	CMAX < 20 C	16.422	7.234
	Upstream Whole A	17.683	6.577
	Upstream Whole B	15.564	5.929
	Upstream Whole C	17.149	-
	Upstream < 153 A	16.509	5.839
	Upstream < 153 B	17.787	6.312
	Upstream < 20 A	15.490	6.231
	Upstream < 20 B	15.599	7.129
	Upstream < 20 C	14.907	6.203
		[Chl a] (T_0)	**[Chl a] (T_{48})**
< 20 Chl	CMAX Whole A	15.279	4.328
	CMAX Whole B	16.882	4.207
	CMAX Whole C	16.797	3.745
	CMAX < 153 A	15.788	3.978
	CMAX < 153 B	15.814	3.557
	CMAX < 153 C	16.304	4.335
	CMAX < 20 A	14.886	6.603
	CMAX < 20 B	15.252	6.056
	CMAX < 20 C	14.487	6.454
	Upstream Whole A	13.292	5.111
	Upstream Whole B	14.322	5.159
	Upstream Whole C	14.527	-
	Upstream < 153 A	14.171	4.812
	Upstream < 153 B	15.099	4.972
	Upstream < 20 A	14.262	5.382
	Upstream < 20 B	15.131	5.646
	Upstream < 20 C	14.867	5.101
		[Chl a] (T_0)	**[Chl a] (T_{48})**
> 20 Chl	CMAX Whole A	1.511	0.667
	CMAX Whole B	0.450	0.539
	CMAX Whole C	0.712	0.528
	CMAX < 153 A	1.942	0.512
	CMAX < 153 B	2.657	1.031
	CMAX < 153 C	-0.219	0.533
	CMAX < 20 A	1.332	0.338
	CMAX < 20 B	0.820	0.436
	CMAX < 20 C	1.935	0.780
	Upstream Whole A	4.391	1.465
	Upstream Whole B	1.242	0.771
	Upstream Whole C	2.622	-
	Upstream < 153 A	2.338	1.028
	Upstream < 153 B	2.688	1.340
	Upstream < 20 A	1.228	0.848
	Upstream < 20 B	0.467	1.483
	Upstream < 20 C	0.039	1.102

Table A-2. Data from CMAX Grazing Experiments. Comparison represents treatment filtrations used to determine grazing rates for zooplankton types (g=grazing coefficient, F=clearance rate, I=ingestion rate).

Chl Size	Location	Comparison	Replicate	$g(T_{48})$	$F(T_{48})$	$I(T_{48})$	Zooplankton Type
Total	CMAX	153-Whole	A	-0.08	-0.24	-2.49	Meso only
Total	CMAX	153-Whole	B	-0.05	-0.15	-1.56	Meso only
Total	CMAX	153-Whole	C	0.11	0.32	3.37	Meso only
Total	CMAX	20-Whole	A	0.18	0.55	5.95	Meso and micro
Total	CMAX	20-Whole	B	0.19	0.58	6.17	Meso and micro
Total	CMAX	20-Whole	C	0.30	0.89	9.81	Meso and micro
Total	CMAX	20-153	A	0.26	0.79	8.59	Micro only
Total	CMAX	20-153	B	0.24	0.73	7.72	Micro only
Total	CMAX	20-153	C	0.19	0.56	6.23	Micro only
Total	UPSTREAM	153-Whole	A	-0.03	-0.08	-0.83	Meso only
Total	UPSTREAM	153-Whole	B	-0.04	-0.11	-1.24	Meso only
Total	UPSTREAM	153-Whole	C	-	-	-	Meso only
Total	UPSTREAM	20-Whole	A	0.04	0.12	1.26	Meso and micro
Total	UPSTREAM	20-Whole	B	0.09	0.27	2.95	Meso and micro
Total	UPSTREAM	20-Whole	C	-	-	-	Meso and micro
Total	UPSTREAM	20-153	A	0.06	0.19	2.07	Micro only
Total	UPSTREAM	20-153	B	0.13	0.38	4.11	Micro only
Total	UPSTREAM	20-153	C	-	-	-	Micro only
<20	CMAX	153-Whole	A	-0.06	-0.18	-1.74	Meso only
<20	CMAX	153-Whole	B	-0.05	-0.15	-1.49	Meso only
<20	CMAX	153-Whole	C	0.09	0.26	2.73	Meso only
<20	CMAX	20-Whole	A	0.22	0.67	7.23	Meso and micro
<20	CMAX	20-Whole	B	0.23	0.70	7.44	Meso and micro
<20	CMAX	20-Whole	C	0.35	1.04	10.87	Meso and micro
<20	CMAX	20-153	A	0.28	0.85	9.11	Micro only
<20	CMAX	20-153	B	0.28	0.85	9.08	Micro only
<20	CMAX	20-153	C	0.26	0.77	8.10	Micro only
<20	UPSTREAM	153-Whole	A	-0.06	-0.19	-1.77	Meso only
<20	UPSTREAM	153-Whole	B	-0.04	-0.13	-1.35	Meso only
<20	UPSTREAM	153-Whole	C	-	-	-	Meso only
<20	UPSTREAM	20-Whole	A	-0.01	-0.03	-0.28	Meso and micro
<20	UPSTREAM	20-Whole	B	0.02	0.05	0.55	Meso and micro
<20	UPSTREAM	20-Whole	C	-	-	-	Meso and micro
<20	UPSTREAM	20-153	A	0.05	0.16	1.56	Micro only
<20	UPSTREAM	20-153	B	0.06	0.19	1.95	Micro only
<20	UPSTREAM	20-153	C	-	-	-	Micro only
>20	CMAX	153-Whole	A	-0.26	-0.77	-0.95	Meso only
>20	CMAX	153-Whole	B	-0.56	-1.69	-3.12	Meso only
>20	CMAX	153-Whole	C	-	-	-	Meso only
>20	CMAX	20-Whole	A	-0.28	-0.83	-0.69	Meso and micro
>20	CMAX	20-Whole	B	-0.41	-1.22	-0.76	Meso and micro
>20	CMAX	20-Whole	C	-0.30	-0.91	-1.24	Meso and micro
>20	CMAX	20-153	A	-0.02	-0.06	-0.05	Micro only
>20	CMAX	20-153	B	0.16	0.47	0.30	Micro only
>20	CMAX	20-153	C	-	-	-	Micro only
>20	UPSTREAM	153-Whole	A	0.14	0.41	0.69	Meso only
>20	UPSTREAM	153-Whole	B	-0.11	-0.33	-0.66	Meso only
>20	UPSTREAM	153-Whole	C	-	-	-	Meso only
>20	UPSTREAM	20-Whole	A	0.36	1.09	1.13	Meso and micro
>20	UPSTREAM	20-Whole	B	0.82	2.45	2.39	Meso and micro
>20	UPSTREAM	20-Whole	C	-	-	-	Meso and micro
>20	UPSTREAM	20-153	A	0.23	0.68	0.70	Micro only
>20	UPSTREAM	20-153	B	0.93	2.78	2.71	Micro only
>20	UPSTREAM	20-153	C	-	-	-	Micro only