ABSTRACT

Title of Document:	ZOOPLANKTON ECOLOGY IN THE CHESAPEAKE BAY ESTUARINE TURBIDITY MAXIMUM, WITH EMPHASIS ON THE CALANOID COPEPOD *EURYTEMORA AFFINIS*
	Scott S. Lloyd, Ph. D., 2006
Directed By:	Professor Michael R. Roman, Horn Point Laboratory, University of Maryland Center for Environmental Science

The estuarine turbidity maximum (ETM) region of Chesapeake Bay, located near the limit of saltwater intrusion, is characterized by high total suspended solid (TSS) concentrations, high light attenuation, and high densities of zooplankton. Due to high light attenuation, primary production is generally low in ETMs, yet the Chesapeake Bay ETM region is often considered a 'hot spot' of zooplankton abundance within the Bay. The omnivorous copepod *Eurytemora affinis* is especially prevalent in the ETMs of Chesapeake Bay and its tributaries and in ETM regions worldwide. In order to determine the factors influencing 1) zooplankton distribution and abundance in the Chesapeake Bay ETM, 2) *E. affinis* reproduction in the Chesapeake Bay and Choptank River ETMs, and 3) zooplankton position maintenance, cruises in the Chesapeake Bay and Choptank River ETMs were

conducted in 1996 and 2001-2003. Laboratory experiments examining the egg

production cycle of *E. affinis* were also performed. The cruise results show that

zooplankton taxa within the Chesapeake Bay ETM region tend to be distributed along

a salinity gradient from up-estuary to down-estuary, with cladocerans being most

common in low salinity/freshwater regions, *E. affinis* found in slightly higher

salinities than cladocerans, and mysids and the copepod *Acartia tonsa* found in more

mesohaline conditions. *Eurytemora affinis* appears to be contained in the ETM by

freshwater limiting its up-estuary extent and biological interactions with *A. tonsa* and

salinity tolerances limiting its down-estuary abundance. Grazing and egg production

results indicate that *E. affinis* production is not food-limited in the ETM region and

that this copepod's particle selection ability favors its success in the ETM over that of

A. tonsa. Laboratory egg production experiments also suggest that the most accurate

estimates of *E. affinis* egg production (and of all brooding copepods in general) are

achieved by incorporating both a temperature-based estimate of interclutch duration

(the time between successive clutches) with a temperature-based estimate of egg

hatching time.

ZOOPLANKTON ECOLOGY IN THE CHESAPEAKE BAY ESTUARINE TURBIDITY MAXIMUM, WITH EMPHASIS ON THE CALANOID COPEPOD *EURYTEMORA AFFINIS*

By

Scott Steven Lloyd

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Advisory Committee:
Professor Michael R. Roman, Chair
Professor H. Rodger Harvey
Professor Edward D. Houde
Professor Lawrence P. Sanford
Professor Diane K. Stoecker
Associate Professor Patrick Kangas, Dean's Representative

Dedication

To my beautiful, motivating wife

and three furry children.

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This research has truly been a collaborative effort and should include a chapter consisting only of names.

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CHAPTER 1: General Introduction

INTRODUCTION

The estuarine turbidity maximum (ETM) region of Chesapeake Bay, located near the limit of saltwater intrusion, is traditionally characterized by high total suspended solid (TSS) concentrations, high light attenuation, and high densities of zooplankton and anadromous fish larvae (North and Houde 1999, Roman *et al.* 2001). Phytoplankton productivity is generally low in the ETM region due to light limitation (Wofsy 1983, Kemp and Boynton 1984, Fisher *et al.* 1988). Therefore, food webs in ETMs tend to be based on allochthonous detrital inputs, with important microbial components (Irigoien and Castel 1997, Crump *et al.* 1998, North and Houde 2001).

Abundances of the calanoid copepod *E. affinis* peak in the Chesapeake Bay ETM region. *Eurytemora affinis* is omnivorous, feeding on phytoplankton (Vijverberg 1980, Gasparini and Castel 1997), detritus (Heinle *et al.* 1977), protozoa (Berk *et al.* 1978, Merrell and Stoecker 1998), and bacteria (Boak and Goulder 1983). It is usually considered to be an epibenthic copepod (Heinle and Flemer 1975, Simenstad 1990), and gravid females, which presumably are visible to visual predators, are often rare in the water column (Vuorinen 1987).

The high abundance of *E. affinis* and other zooplankton in the most turbid regions of estuaries leads to the question: what factors influence zooplankton abundance and distribution in ETM regions? One hypothesis is that the high turbidity provides a refuge from visual predators, especially fish. Therefore, high copepod abundances in turbid regions may simply be the result of lower mortality rates

(Kimmerer *et al.* 1998). The ETM may also provide the optimal temperature-salinity-food regime for growth and reproduction. North and Houde (2001) suggest that survival of white perch and striped bass larvae in the Chesapeake Bay ETM is determined by freshwater input into the system, which affects temperature, salinity, and zooplankton abundance in this region. Temperature by itself may explain over 90 % of all growth rate variability for marine copepods (Huntley and Lopez 1992) and is a primary factor in determining developmental and reproductive rates for estuarine copepods (Katona 1970, Nagaraj 1992, Ban 1994).

RESEARCH QUESTIONS

The following research chapters attempt to describe zooplankton ecology in the Chesapeake Bay and Choptank River estuarine turbidity maximum (ETM) regions. This research focuses on factors influencing the distribution, abundance, and reproductive rates of the calanoid copepod *Eurytemora affinis*. Specifically, I seek to 1) resolve relationships influencing zooplankton distributions, abundances, and diversity; 2) consider a more direct approach to estimating brooding (egg-carrying) copepod egg production; 3) determine whether or not *E. affinis* egg production is food-limited in the Chesapeake Bay and Choptank River ETMs and what other factors may influence egg production rates; and 4) determine if *E. affinis* vertically migrates to maintain its position within the Chesapeake Bay ETM region.

Chapter I: Zooplankton distribution, abundance, and diversity

The goal of Chapter 1 is to determine the primary factors influencing zooplankton location and abundance, particular those of *E. affinis*. The various zooplankton taxa exhibit abundance peaks in different regions throughout the ETM region, and these distributions are likely the result of biological and physical interactions which change temporally and spatially. Resolving these interactions may allow for predictions of zooplankton abundance and distribution to be made as the physical environment changes seasonally, between years, and over longer periods of time.

Chapter 2: Egg production and interclutch duration

Brooding copepods (those that carry their eggs until hatching) differ from broadcast spawning copepods (those that release their unhatched eggs) in several ways, one way being that a brooding female cannot produce a new clutch of eggs until after her present clutch hatches or the unhatched (usually dead) eggs are released (Runge and Roff 2000). This adds an important time component to egg production rate – hatching time (HT). Several studies have focused on temperature effects on HT (also known as egg development time or embryonic development time), which is often defined differently by different authors (Heinle and Flemer 1975, Vijverberg 1980, Ban and Minoda 1992, Andersen and Nielsen 1997). Only one study has measured the effects of salinity on embryonic development time, though, as stated above, the authors did not incorporate a temperature-salinity interaction effect (Ishikawa *et al.* 1999).

Another time period in brooding copepod egg production must also be considered – interclutch duration (ID). Interclutch duration is the period from the

hatching of the last egg of a clutch to the extrusion of the first egg of another clutch. Ban (1994) found a significant effect of temperature on *E. affinis* ID but a nonsignificant food concentration effect. There do not appear to be any studies on the effects of salinity on ID.

Traditional egg production measurements involve incubating copepods in bottles filled with filtered water, natural water, or water with a food amendment (Runge and Roff 2000) over a period of 24 hours to capture any diel periodicity in egg production (White and Roman 1992). Although spawning copepods appear to lay distinct clutches (Mauchline 1998), they lay them over shorter periods than do brooding copepods, often during a 24-hour period. Measured HTs for *E. affinis* range from over 14 days at temperatures below 5ºC to ~1 day at 25ºC (Andersen and Nielsen 1997). Such long HTs mean that traditional incubation techniques may not be applicable for brooders. One solution to this problem is to run incubations in large bottles (4 l) containing male and female copepods and periodically remove subsamples to enumerate nauplii and eggs female^{-1} (Burdloff *et al.* 1999). Counts for these subsamples are then compared to subsamples taken at the beginning of the incubations. The major assumption of this method is that each subsample contains the same ratio of gravid to nongravid females and is a representative sample of nauplius concentration (nauplii are uniformly distributed). Thus, there is the potential for much error with this method, and egg production rates seem low for this method compared to other methods [compare Burdloff *et al.* (1999) to Katona (1975) and Ban (1994)].

The "egg ratio method", developed by Edmondson *et al.* (1962), for estimating the egg production rates of rotifers has also been used to estimate copepod egg production (e.g.Edmondson *et al.* 1968, Sabatini and Kiørboe 1994). The egg ratio is egg abundance (E, per unit volume) to total number of females (A, per unit volume), giving units of eggs female^{-1}. The egg ratio alone does not estimate production rate, but if the HT can be determined based on environmental conditions (temperature, salinity, etc.) at the time of copepod capture, production rate can theoretically be determined (Runge and Roff 2000). According to Edmondson *et al.* (1962), egg production rate (EP) should equal the egg ratio divided by HT:

$$EP = \left(\frac{E}{A(HT)} \right).$$

Therefore, the only time component one needs to estimate EP using this method is HT. Theoretically, ID and HT could be used directly in EP measurements by dividing average sample clutch size by the sum of both time components:

$$EP = \left(\frac{CS}{HT + ID} \right).$$

The major goals of Chapter 2 are to determine the influence of temperature on measurements of *E. affinis* ID, use these ID values to estimate EP from field-collected *E. affinis* samples, compare these values to the EP values obtained through the egg-ratio method, and compare both types of EP estimates to models of copepod growth. An evaluation can then be made concerning which approach to measuring copepod egg production is most useful and/or valid.

Chapter 3: Food quantity, quality, and E. affinis *egg production*

Measurements of copepod egg production are often used to estimate reproductive rates as well as larval and juvenile biomass production, as both often depend on the same environmental factors (Berggreen *et al.* 1988, Runge and Roff 2000). In theory, adult females allocate carbon that would be used for somatic growth (adult copepod nonreproductive biomass is relatively constant) towards the production of eggs. There are conditions, such as during periods of food limitation, where somatic growth of immature stages and reproductive output by adults are not equivalent, and the above assumption does not hold (Hopcroft and Roff 1998).

The major factors known to affect egg production rates (and growth rates) for most calanoid copepod species are temperature, food concentration and food quality. Body size is also positively related to absolute reproductive output, so growth rates are usually expressed as weight-specific growth (day^{-1}). Temperature has long been recognized as a primary factor affecting the physiological rates of poikilotherms (Bělehrádek 1935, cited by McLaren *et al.* 1966). As stated above, Andersen and Nielsen (1997) analyzed *E. affinis* egg HTs versus temperature (T) from five studies and found that a power function explained 87% of the variability. Huntley and Lopez (1992) pooled data for 33 species of marine copepods corresponding to a temperature range of 32.4ºC, and described two functions: one power function describing generation time (days) in terms of temperature ($r^2 = 0.91$) and one describing weight-specific growth rate (day^{-1}) in terms of generation time ($r^2 = 0.96$). The relationship between temperature and a physiological rate, such as egg production, does not need to be modeled as a power function. Temperature may have a linear effect (Ambler

1985) or a parabolic effect (White and Roman 1992) on *Acartia tonsa* egg production. A parabolic effect of temperature on egg production is logical, as most physiological processes have an optimum temperature range above and below which the rates decline (Mauchline 1998). Such differences of temperature effects do not necessarily represent a physiological difference between populations but may reflect variability in the other factors affecting egg production (Ambler *et al.* 1986, Jónasdóttir 1994, Hirst and McKinnon 2001).

Food quantity and quality are also known to affect egg production rates (Mauchline 1998, Runge and Roff 2000). Differentiating between quantity and quality, though, is somewhat subjective, because one must define quantity in an ecologically relevant manner. Food availability has traditionally been measured in terms of carbon, chlorophyll, or both (Heinle and Flemer 1975, Runge and Roff 2000). Chlorophyll, however, is often not a good predictor of estuarine copepod egg production due to allochthonous carbon inputs to estuaries (Ambler 1986, White and Roman 1992, Burdloff *et al.* 1999). In turbid regions of estuaries, other food sources, such as microzooplankton and detritus, become important (Berk *et al.* 1978; Heinle *et al.* 1977). The ratios of both chlorophyll and the sum of protein, carbohydrate, and lipid concentrations to TSS are correlated to *E. affinis* egg production in the Gironde estuary (Burdloff *et al.* 1999). *Acartia* spp. egg production has been negatively correlated with seston C:N (Ambler 1986) and positively correlated with protein and specific fatty acid concentrations (Jónasdóttir 1994, Jónasdóttir *et al.* 1995) and microzooplankton abundance (White and Roman 1992).

The focus of Chapter 3 is to determine how biotic (food quantity and quality) and abiotic factors influence *E. affinis* clutch sizes (and thus egg production rates), and if any of these factors limit egg production in the Chesapeake Bay and Choptank River ETMs. Grazing experiments were also conducted to determine the diet of adult *E. affinis* both in the ETM and down-estuary from the ETM (where *A. tonsa* typically dominates the zooplankton community).

Chapter 4: Tidally-timed vertical migration

Several researchers have demonstrated that *E. affinis* uses tidally-timed vertical migration to maintain its position within an estuary (Hough and Naylor 1991, Simenstad *et al.* 1994, Morgan *et al.* 1997). In these studies, *E. affinis* was generally more abundant higher in the water column during a flood tide and deeper during ebb, thus being associated with the lowest velocity regions within the water column. Morgan *et al.* (1997) found that the principal lunar tidal component (M_2) described nearly half of all variability in *E. affinis* densities, further supporting the idea of a tidally-induced vertical migration. Vertical migrations based on the tidal cycle are common in the larvae of other, meroplanktonic organisms such as crabs (Cronin and Forward 1983, Tankersley and Forward 1994, Zeng *et al.* 1999), fish (Dauvin and Dodson 1990, Joyeux 1999), and molluscs (Roberts *et al.* 1989).

Other authors, however, have demonstrated that *E. affinis* populations may be sustained within estuaries by passive means. Castel and Viega (1990) determined that the swimming abilities of *E. affinis* are ineffective in retaining the population within the turbid Gironde estuary. This finding and the relationship between *E. affinis* abundance and suspended matter lead them to the conclusion that the copepods

behave as passive particles to maintain themselves within the Gironde estuary. Kimmerer *et al.* (1998) found that macrozooplankton (mysids and amphipods) exhibited tidally-induced vertical migrations that maintained their positions within San Francisco Bay (higher in the water column during flood, lower during ebb), but the copepods *E. affinis* and *Pseudodiaptomus forbesi* did not. *Eurytemora affinis'* position maintenance strategy apparently either differs with location / hydrodynamic properties of the estuary or is masked by certain hydrodynamic characteristics.

In Chapter 4, acoustically-determined zooplankton biovolume measurements are used to resolve the vertical distributions of zooplankton with respect to tidal stage and magnitude in the Chesapeake Bay ETM region. The major goal was to determine if *E. affinis* actively regulates its vertical position within the water column to enhance its retention in the ETM. The results are then compared to a model by Roman *et al.* (2001), which was developed using data from a year with markedly different hydrologic characteristics.

SUMMARY

Together, these four chapters should provide a better understanding of zooplankton ecology in the Chesapeake Bay ETM; the factors that influence *E. affinis* distribution and abundance; how *E. affinis* is retained in the Chesapeake Bay ETM; and, if production of *E. affinis* is food limited in the ETM.

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CHAPTER II: Zooplankton distribution, abundance, and diversity in the Chesapeake Bay estuarine turbidity maximum

ABSTRACT

Estuarine turbidity maximum (ETM) regions of many estuaries have been shown to contain high abundances of various zooplankton taxa. Previous research in the Chesapeake Bay ETM has been on a relatively large scale (10s of kilometers between stations) or has not focused on the different zooplankton taxa that comprise this community. I conducted high-resolution sampling in the Chesapeake Bay ETM in the spring, summer, and fall in 1996, 2001, and 2002. Freshwater input into the ETM region via the Susquehanna River was higher than normal in 1996 (a wet year), average in 2001 (a normal year), and low in 2002 (a dry year). The calanoid copepod *Eurytemora affinis,* cladocerans (*Bosmina longirostris* and *Daphnia* spp.), and amphipods (*Gammarus* spp.) were especially abundant during periods of high freshwater input. These zooplankton groups favor low salinity waters and were found further down-estuary during the wet year than in the dry or normal year. Their abundances, especially those of *E. affinis*, tended to stay above average for much of the year in 1996. Abundances of the copepod *Acartia tonsa* were highest in 2002, the dry year, and densities were elevated in regions where freshwater/low salinity species tend to dominate in the wet and normal years. Not only were zooplankton abundances and distributions related to environmental variables, but it was clear that biological interactions also influenced the spatial partitioning of the zooplankton community. Specifically, mysid predation is suggested, based on correlations with

both *A. tonsa* and *E. affinis*. Further, there appear to be interactions between *A. tonsa* and *E. affinis* themselves that may explain these species' distributions. Zooplankton taxa diversity tended to be lower during the fall when freshwater input was near or slightly below normal, and diversity in general was inversely correlated with salinity.

INTRODUCTION

The Susquehanna River discharges into the head of Chesapeake Bay and annually provides 50% of all freshwater input into the bay (Schubel and Pritchard 1986).The salt front created between this seaward flowing freshwater and landward moving tidal water is a site of enhanced suspended sediment concentrations, known as the estuarine turbidity maximum (ETM) region (Schubel 1968, Roman *et al.* 2001). The ETM region of Chesapeake Bay, located near the up-estuary limit of saltwater intrusion, is traditionally characterized by high total suspended solid (TSS) concentrations, high light attenuation, and high densities of zooplankton and anadromous fish larvae (North and Houde 2001, Roman *et al.* 2001).

Though primary production tends to be low in this region (Wofsy 1983, Kemp and Boynton 1984, Fisher *et al.* 1988), heterotrophs are abundant, possibly supported by senescent freshwater phytoplankton, protozoa, free and particle-attached heterotrophic bacteria, and detritus (Irigoien and Castel 1997, Crump *et al.* 1998, North and Houde 2001). The calanoid copepod *Eurytemora affinis* often dominates the ETM zooplankton community (Heinle and Flemer 1975, Castel and Viega 1990, Kimmerer *et al.*1998). *Eurytemora affinis* is omnivorous, feeding on phytoplankton (Vijverberg 1980, Gasparini and Castel 1997), detritus (Heinle *et al.* 1977), protozoa

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(Berk *et al.* 1978, Merrell and Stoecker 1998), and bacteria (Boak and Goulder 1983).

It is usually considered to be an epibenthic copepod (Heinle and Flemer 1975,

Simenstad 1990), and gravid females, which presumably are visible to visual

predators, are often rare in the water column (Vuorinen 1987).

Zooplankton distributions vary along the horizontal salinity gradient in ETM

regions (Roman *et al.* 2001, Simenstad 1994, Irigoien *et al.* 1993) and vertically

(Roman *et al.* 2001) in ETM regions and often move as the ETM itself moves with

changes in freshwater inputs, winds, and tidal cycles. Although zooplankton samples

have been collected monthly since 1984 by the Chesapeake Bay Monitoring Program

(http://www.chesapeakebay.net/data/index.htm) at selected stations in the main stem

of Chesapeake Bay (Jacobs *et al.* 1985), there have been few studies of species

composition and abundances on small spatial scales (on the order of kilometers) in

upper Chesapeake Bay. Such data can provide information on the effects of abiotic

factors affecting zooplankton distribution and abundance and on biological

interactions within the ETM region.

High zooplankton densities in the ETM suggest that there are benefits of

retention in this region, such as physiological optima (Hough and Naylor 1992,

Kimmerer *et al.* 1998), avoidance of visual predators, and/or a greater reproductive

output (Kimmerer *et al.* 1998, Roman *et al.* 2001). Hough and Naylor (1992) showed

that *E. affinis* collected from portions of the Conwy estuary displayed tidally induced

migratory behaviors to maintain themselves within a specific range of salinities.

These behaviors varied depending on the sampling location and tidal range (spring

versus neap). In tidal pools along the St. Lawrence estuary, *E. affinis* has been found

to migrate into areas of dense algal biomass along the shore during the day and back into the less turbid central regions during the night, presumably to avoid predation by sticklebacks (Castonguay and FitzGerald 1990). *Eurytemora hirudinoides*, a related species, performs diel vertical migration in the Archipelago Sea to escape predation (Vuorinen 1987). However, studies have also shown that some *E. affinis* populations do not actively select their location within estuaries, relying on passive behaviors (like a non-living particle) to remain in the ETM region (Kimmerer *et al.* 1998, Roman *et al.* 2001).

The purpose of this paper is to describe the variability in abundance and distribution of different zooplankton groups in relation to each other and to abiotic factors within the Chesapeake Bay ETM region. The goal was to determine the primary factor(s) influencing zooplankton location and abundance, particular those of *E. affinis*. Five major zooplankton groups were identified whose abundance peaks in the ETM region at least part of the year. Principal component analyses suggest that physical and biological interactions influence distribution and abundance of these taxa. Linear and quadratic regression analyses suggest that larger *E. affinis* individuals may be able to control their location within the ETM and have a significant effect on autotrophic biomass.

MATERIALS AND METHODS

Mesozooplankton samples were collected using a multi-net,1-m^2, 280-µm Tucker trawl in May (spring), July (summer), and October (autumn) of 1996, 2001, and 2002 in the Chesapeake Bay ETM region (Figs. 1 and 2) aboard the R/V Cape

Henlopen. Each net was opened at one of three depths (surface: ~0 to 4 m; middle: ~4 to 8 m; bottom: ~8 to 12 m) and deployed for 2 minutes at 5 to 7 stations along the channel of the ETM, for a total of 6 minutes spent sampling. The exceptions were the 1996 cruises, in which only two depth strata (surface and bottom) were sampled. The average volume filtered in a 2-minute deployment over all 9 cruises was 122 m^3. Once onboard, the plankton samples were preserved in ethyl alcohol for future species identificaiton and enumeration. Ctenophore (*Mnemiopsis leidyi*) and sea nettle (*Chrysaora quinquecirrha*) displacement biovolumes were also measured whenever collected. This sampling regime allowed for zooplankton composition and abundance to be quantified and compared spatially throughout the ETM region, between seasons, and between years with differing environmental conditions. Physical data were collected just prior to Tucker trawl deployment using a Seabird CTD equipped with a transmissiometer, fluorometer, and oxygen sensor.

Zooplankton samples were split using a plankton splitter, and organisms were identified and enumerated under a dissecting microscope. Three separate subsamples were counted and averaged for each sample. For most subsamples, at least 200 individuals of the most abundant taxon were counted to minimize errors common to small sample sizes. In samples with the lowest densities of zooplankton, at least 50 individuals of the most abundant taxon were counted. The taxa enumerated included *Eurytemora affinis* (life stage, sex, and female reproductive state), *Acartia tonsa* (life stage and sex), *Cyclops* spp., barnacles (nauplii and cyprids), ostracods, polochaetes larvae, harpacticoid copepods, *Podon* spp., *Bosmina* spp., *Daphnia* spp., *Leptodora kindtii*, amphipods, mysids, decapod larvae (including crab zoea), mollusks (veligers),

and mites. The biological groups included in statistical analyses include numerical abundances of *E. affinis*, *A. tonsa*, cladocerans (*Podon* spp. + *Bosmina* spp., + *Daphnia* spp. + *Leptodora kindtii*), mysids (*Neomysis* spp.), amphipods (*Gammarus* spp.), chlorophyll-*a* (chl-*a*) concentrations and biovolume of the ctenophore *Mnemiopsis leidyi* (for July cruises only). Temperature, salinity, and total suspended solids (TSS) served as the physical variables, and river km (down-estuary from Havre de Grace) and water depth were also included in statistical analyses.

Taxon diversity was calculated using the Shannon-Weiner index:

$$H = -\Sigma (P \times Log(P)),$$

where H = diversity index and P = proportion of each taxon. This diversity index was chosen because it is sensitive to rare species (Odum 1983), and the data were skewed so that the two most abundant taxa for each cruise represented 97 to 98% of all individuals. Shannon-Weiner indices can also be used in mean-comparison statistical methods, such as ANOVA.

Diversity is comprised of two components, richness (number of taxa per unit volume) and evenness (the apportionment of each individual among the taxa). Evenness (E) can be calculated from H using the formula:

$$E = H/log(S),$$

where S = the number of taxa. Log(S) represents the maximum possible diversity, and, assuming that the total number of taxa remains the same between treatments (cruises), E varies proportionately with changes in H (Odum 1983). Because of this relationship, results from analyses involving H can be inferred to correspond to E, and thus E is not considered further.

Because of the many variables measured, principal component analyses (PCA) was used to reduce the dimensionality of the data and to visually detect relationships between variables. Principal components analysis is a linear transformation approach to simplifying complex data sets. Principal components (PCs) are determined by plotting all data points (and their corresponding axes) in space (in this study, there are 11 axes) and running a new axis though the data 'cloud' so that this new axis accounts for the maximum amount of variance of the original data. This is the first principal component (PC 1). The second principal component (PC 2) is orthogonal (independent) to PC 1 and accounts for more variance than the third, PC, and so on. A biplot of PC 1 and PC 2 can visually help determine relationships between the original variables (Sokal and Rohlf 1994). The contribution of each variable to a PC (the eigenvectors) is known as the PC loadings, and these allow PCs to be characterized in terms of the original variables.

All statistical analyses were conducted using JMP v. 5.01 (SAS Institute), and contour plots were created using Surfer v. 8 software (Golden Software).

RESULTS

Total daily streamflow values for May, July, and October in the lower Susquehanna River show 1996 streamflow to be above average for all months, with most days at or above the 37-year mean (Fig. 2). In May of 2002, the week prior to the sampling cruise had above-average freshwater streamflow, while streamflow during the week prior the May, 2001 cruise is below average. July streamflow conditions are reversed from the May pattern, with 2002 having low-flow conditions

preceding the cruise and 2001 streamflow being near average. October streamflow

prior to the sampling cruises was below average during both 2001 and 2002. Physical

variables change predictably throughout the year in response to temperature and

changes in freshwater input (Table 1).

Mesozooplankton annual comparisons by season

May. *Eurytemora affinis* peak abundances in 1996 are five times greater than

in 2001. It is possible that the 1996 sampling stations did not include locations with

the highest densities of this copepod (Table I). Most zooplankton groups were

located 20 to 30 km further up-estuary in 2001 compared to 1996, presumably due to

decreased Susquehanna flow in 2001, and those groups favoring freshwater or low

salinity (cladocerans, amphipods, *E. affinis*) are generally much more abundant in

1996 than in 2001 (Fig. 3 and 4). *Eurytemora affinis* is very abundant in May 2002

($>200 \ l^{-1}$) and peak about 20 km above the salt front (Fig. 5). Springtime cladoceran

densities in 2002 are comparatively low and are divided between a *Bosmina* spp.

population above the salt front and a *Daphnia* spp. population in freshwater. *Podon*

spp. and *Leptodora kindtii* were found at very few stations and at densities never

exceeding 0.01 individuals l^{-1}, so are not considered separately in any analyses.

Acartia tonsa densities in 2002 are at their highest springtime values measured during

all three cruises ($2.5 \ l^{-1}$; Fig. 5).

July. Maximum densities of *E. affinis* in 1996 are $>20 \ l^{-1}$, their highest

summer values for all years (Table I, Fig. 6). These densities are comparable to

maximum abundances of *A. tonsa*; under conditions of normal freshwater input

(2001). *Eurytemora affinis* summer abundances decrease drastically from springtime

densities and are much lower than maximum *A. tonsa* values (Fig. 7). *E. affinis* abundances in 2001 are two orders of magnitude lower than 1996 values, while *A. tonsa* densities are at similar levels as 1996 and were greater down-estuary. Mysids and cladocerans are at lower concentrations in 2001, while amphipod densities increase from 1996 values (Fig. 7). Maximum densities of *A. tonsa* in 2001 are the highest measured out of all 9 cruises, peaking at values over 37 individuals l^{-1}. *Eurytemora affinis* peaks further upstream in 2002 than in 1996 or 2001, presumably in response to differences in salinity structures between these years. Measured cladoceran abundances are also lower in 2002 than in 1996, possibly because their peak densities are landward of the survey area, and *Daphnia* spp. and *Bosmina* spp. distributions always overlap. *Daphnia* spp. is an order-of-magnitude more abundant than *Bosmina* in 2001 and 2002, and both genera are at equivalent densities in 1996. Amphipod and mysid concentrations are low in 2002 (Fig. 8).

October. *Eurytemora affinis* in 1996 is at levels similar to those in July, likely due to high Susquehanna River streamflow in that year. Cladocerans and amphipods are also much more abundant in 1996 than in other years where they are often absent from October measurements (Fig. 9). In 1996, *Bosmina* spp. and *Daphnia* spp. distributions again overlap, but *Bosmina* spp. is generally an order-of-magnitude more abundant. Maximum *A. tonsa* densities in 1996 are between maximum values for 2001 and 2002 (Figs. 9, 10, and 11). It is possible that the peak in *A. tonsa* abundance during October 1996 occurred further down-estuary from the transect area due to the salt front being pushed further seaward compared to the other years. Low abundances were found for all zooplankton groups in 2001. The following October

(2002), *A tonsa* abundances were highest in October for all three years. *Eurytemora affinis* is conspicuously absent from the 2002 survey.

<u>Principal components analyses.</u>

Principal components (PC) analyses for each of the three months (for all three years) show varied relationships between organisms and their environment as the year progresses. In May, *E. affinis* is positively related to river km (distance down-estuary from Havre de Grace) and negatively related to chl-*a* concentration and TSS (Fig. 12). The orthogonal placement of *E. affinis* versus *A. tonsa,* mysids, salinity, and depth in the biplot suggest that there is no relationship between *E. affinis* and these variables. *Acartia tonsa* is positively related to salinity and negatively to dissolved oxygen (DO) concentration, which is likely due to this copepod species being found further down-estuary than *E. affinis* where DO concentrations tend to be lower. When *Daphnia* spp. and *Bosmina* spp. were considered separately in PC analyses, they were found to overlap with each other on all monthly biplots. They are therefore grouped as "Cladocerans" for all PC analyses. In May, cladocerans are positively related to DO and negatively related to salinity. Temperature appears to have a minimal influence on any biological variable (Fig. 12). The greatest loadings in the first PC (PC1) come from physical variables and *A. tonsa*. Total suspended solids, *E. affinis*, river km, chl-*a*, and DO are strongly loaded in PC2 (Fig. 12).

The PCA on July data again shows no relationship between copepod species (Fig. 13). *Acartia tonsa* abundance is unrelated to temperature, DO, and salinity, while *E. affinis* shows a positive relationship with temperature and DO and a negative relationship with salinity and ctenophore biovolume. Both *A. tonsa* and mysids have

negative relationships with chl-*a*. Cladocerans are negatively related to both river km and salinity. (Fig. 13). The loadings for PC1 are dominated by physical variables, and the biological variables (zooplankton and chl-*a*) are heavily loaded into PC2 (Fig. 13).

The October PCA suggests that *E. affinis*, *A. tonsa*, river km, and amphipods all strongly correlated with one another; cladocerans showed a weak correlation with these variables. All other variables were orthogonal to the above variables and thus not related. Whereas TSS and DO were strongly related to each other, they were negatively related to salinity, depth, chl-*a*, temperature, and mysids, which were positively correlated (Fig. 14). With the exception of cladocerans, PC1 is again primarily influenced by physical variables. Biological and physical variables both influence PC2, with *E. affinis* and amphipods being loaded most heavily.

Mesozooplankton seasonal comparisons by year.

1996. The spring Tucker trawl survey shows maximum densities of *E. affinis* around 70 l^{-1} that overlap with a population of *A. tonsa* with maximum densities an order of magnitude lower (Fig. 3). However, *A. tonsa* abundances generally peak further seaward in the mesohaline region of Chesapeake Bay. Amphipod abundance peaks further up-estuary. Cladoceran abundance peaks at the same distance down-estuary as amphipods, but the cladocerans occurred near the surface. Both amphipods and cladocerans are generally more abundant in the freshwater regions of Chesapeake Bay. All zooplankton populations shift 20 to 40 km upstream from spring to summer, presumably due to decreased freshwater input via the Susquehanna River (Fig. 6). *Acartia tonsa* abundance increases 10- to 100-fold in July; this copepod generally

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experiences its abundance peak in the summer. Densities of *E. affinis* remain at levels comparable to springtime values. Copepod, amphipod, and mysid abundance peaks tend to overlap, with mysid abundance also extending further down-estuary. Cladoceran densities are at a maximum up-estuary of the salt front and are dominated by *Bosmina* spp (Fig. 6). *Eurytemora affinis* remains at the July abundance levels into October and is spatially separated from *A. tonsa* with very little overlap (Fig. 9).

2001. Maximum springtime *E. affinis* densities are less than in 1996, though still at the same order of magnitude. Cladocerans, amphipods, and *E. affinis* are all located landward of the salt front and are spatially separated from both *A. tonsa* and mysids, which peak further down-estuary (Fig. 4). In July, both cladocerans and amphipods are again found primarily on the freshwater side of the salt front, with *E. affinis* being found both up- and down-estuary of the salt front. Mysids and *A. tonsa* are located seaward of the salt front. Their summer abundances increase greatly from springtime densities, but peak density values cannot be determined because the transect did not extend far enough down-estuary to completely sample both populations (Fig. 7). Abundances of all zooplankton are lower in October, with cladocerans and amphipods completely absent in the sampled region. Because the salt front is landward of our most up-estuary station it is possible these zooplankton groups and *E. affinis* are limited to the lower salinity/freshwater of the Susquehanna River. Maximum turbidity and *E. affinis* lags behind the salt front for most surveys but does appear to follow its movement up- and down-estuary (Fig. 10).

2002. Maximum springtime densities of *A. tonsa* and *E. affinis* in 2002 are the highest of all three years sampled, with abundances of *A. tonsa* exceeding those of *E.*

affinis. All zooplankton are clustered between 30 and 40 km down-estuary with the exception of mysids, which do not appear until around 50 km. The zooplankton and turbidity are below the salt front, which was moving down-estuary due to an ebb tide (Fig. 5). The salt front in July is further up-estuary in 2002 than in either of the other two summer transects. *Eurytemora affinis*, mysids, and *A. tonsa* are distinctly separated from up-estuary to down-estuary, and *A. tonsa* densities are the highest recorded during the three sampling years. Maximum amphipod concentrations overlap with maximum mysid concentrations and are the lowest measured for amphipods during the three summers. Cladocerans again appear to be shifted up-estuary into fresher waters (Fig. 8). Very few amphipods and no *E. affinis* or cladocerans are found in the sampling area in October. *Acartia tonsa* and mysids are found at relatively high levels (especially *A. tonsa*) with patchy, non-overlapping distributions (Fig. 11).

Diversity

Because it is designed to test significant differences between the means of >2 treatments under conditions of unequal sample size, the Tukey-Kramer test was used to examine differences in taxa diversity between months (May, July, and October) and years (1996, 2001, and 2002). Diversity during the October 2001 and 2002 cruises was significantly lower than in May or July of 2001 and 2002. No other diversity comparisons between cruises were significant (Table II). An inverse semi-log regression analysis showed diversity to be negatively related to salinity ($p <$ 0.0001, $r^2 = 0.48$; Fig. 15).

I was interested in how freshwater input into this region might affect the location and turbidity of the ETM and thus performed a regression between both turbidity (TSS) and distance down-estuary of maximum TSS values (ETM location) and Susquehanna River input (measured at the Conowingo dam). There is not a significant relationship between TSS or ETM location and streamflow for the same month as the cruises, but regressions between TSS or ETM location and Susquehanna River flow for the previous month were significant (Fig. 16a). *Acartia tonsa* and *E. affinis* peak abundance locations are related to freshwater input during the same month of study (Fig. 16b). An ANOVA comparing adult *E. affinis* female prosome length and the depth at which the females were collected shows that females nearest the bottom are significantly larger than those higher in the water column (Fig. 17). This analysis was based on data from the spring samples, when *E. affinis* was most abundant.

Using log-transformed values for *A. tonsa*, *E. affinis*, and chlorophyll-*a* (after adding 1 to abundance/concentration measurements), I grouped chlorophyll-*a* data by month (May, July, or October) for 2001 and 2002 and regressed it against the log-transformed values of abundance for both copepod species. During the May peak in *E. affinis* abundance in both 2001 and 2002, log-transformed chlorophyll-*a* concentrations are negatively related to the log of *E. affinis* abundance (Fig. 18). To determine the decrease in chl-*a* entering the ETM via the Susquehanna River that can be attributed to dilution, the mean of peak concentrations of chl-*a* at maximum ebb tides (corresponding to chl-*a* maxima) and maximum flood tides (corresponding to

chl-*a* minima) were calculated from a 25-hour survey in the ETM region in May, 2002. These values were then used to create a linear regression of chl-*a* versus salinity to compare to the actual relationship of chl-*a* versus salinity for all May cruises (Fig. 19). Also, all 2001 and 2002 log-transformed chlorophyll-*a* data were negatively related to the log abundance of *A. tonsa*, though to a lesser degree than to *E. affinis* abundance (Fig. 20).

DISCUSSSION

This study examined zooplankton abundances in upper Chesapeake Bay on a smaller spatial scale than in previous studies. Using 15 years of monitoring data collected at fixed sites throughout Chesapeake Bay, Kimmel and Roman (2004) determined that freshwater input into the oligohaline region of Chesapeake Bay (via the Susquehanna River) is the primary environmental force affecting zooplankton abundances. Many of the zooplankton species/groups (i.e., *E. affinis* and cladocerans) have relatively narrow salinity tolerances and tend to track along a specific salinity range. For example, in May 1996, a year of above-average freshwater input, I found an abundance of zooplankton groups that favor low salinities or freshwater, and the salt front was located further down-estuary than during 2001 or 2002. It has been suggested that *E. affinis* abundance and distribution is influenced by both temperature and salinity, with increases in temperature decreasing the maximum salinity tolerance of this species (Kimmel and Bradley 2001). The same may hold true for other estuarine zooplankton, especially those that dominate in the freshwater/saltwater transition area.

Because *E. affinis* is often considered a major food source for larval fish (i.e. striped bass and white perch) and mysids, especially in the spring when anadromous fish spawn in the ETM region (North and Houde 2001, Winkler and Greve 2004; Shoji et al. 2005), a positive relationship between TSS and *E. affinis* abundance might represent an adaptation to reduce predation by visual predators (Gilmurray and Daborn 1981, Castel and Viega 1990, Roman *et al.* 2001). Female *E. affinis* collected during spring cruises were larger at depths greater than 8 m than higher in the water column. *Eurytemora affinis* is a brooding copepod, thus gravid females are especially visible to predation and may actively seek or become passively entrained into areas of reduced visibility (Roman *et al.* 2001). Large and/or gravid individuals may also appear to accumulate in deeper or higher turbidity waters due to greater predation outside of these regions.

E. affinis abundance and turbidity were not positively correlated for any of the cruises. Morgan *et al.* (1997) also found that turbidity patterns in the Columbia River estuary did not match *E. affinis* distributions. If *E. affinis* actively migrates to maintain its position within the ETM region (Vuorinen 1987, Simenstad *et al.* 1994) or is more readily suspended by tidal currents than sediments (Roman *et al.* 2001), *E. affinis* abundance and turbidity would not be expected to coincide. The PCA for July shows a negative relationship between *E. affinis* abundance and salinity. As stated above, under high temperature conditions, *E. affinis* may modify its location to be in a general low salinity habitat rather than targeting high turbidity. To test this possibility, I categorized the mean salinity at each station, averaged over depth, as low (0-3), middle (3-6), or high (6+) and then used the nonparametric Wilcoxon

rank sums test to analyze *E. affinis* abundance each month grouped for all years

versus salinity label. The May data show no difference in *E. affinis* abundance versus

salinity label (χ^2 = 2.31, p = 0.31), while *E. affinis* abundance was significantly

higher in the "low" salinity group than in either of the other salinity groups for both

July (χ^2 = 22.27, p < 0.0001) and October (χ^2 = 17.97, p < 0.0001). Salinities

between 0 and 5 have been shown to be optimal for *E. affinis* with the May

temperatures ranges of the present study (12.8 to 19.6ºC; Roddie *et al.* 1984,

Soltanpou-Gargari and Wellershaus 1985). Nearly all of the study areas during the

May cruises would be considered to have 'low' or 'middle' salinities, suggesting that

most of this region is optimal habitat (in terms of temperature and salinity) for this

copepod. Therefore, a relationship between copepod abundance and salinity in May

would not be expected. These results suggest that *E. affinis* may display active

migrations to maintain itself in a particular salinity range. Because our data represent

snapshots of physical and biological variables in a dynamic environment, the lack of a

significant relationship between *E. affinis* and salinity in the correlation analyses does

not mean that salinity is unimportant in determining this copepod's location and

abundance in the ETM region (as suggested by the Wilcoxon ranked sums analyses).

The lack of a direct relationship between the May *E. affinis* with turbidity and

salinity, and the positive relationship with river km suggest that physical variables do

not greatly influence *E. affinis* abundance in the ETM region in spring. With higher

temperatures and reduced freshwater input in July the volume of habitat available to

E. affinis declines, as does *E. affinis* abundance (Hoffmeyer 2004, Kimmel and

Roman 2004). In October, temperatures decrease to spring values while freshwater input remains close to July levels, and *E. affinis* concentrations either remain near July values or decrease. The effects of freshwater input on the location of the ETM (the TSS peak) and *E. affinis* abundance peaks as well as the relationship between *E. affinis* and salinity in July (when water temperature are greatest) show the importance of freshwater input to sediment and zooplankton dynamics in the Chesapeake Bay ETM region.

The general distributional progression of zooplankton in the Chesapeake Bay ETM region from fresh- to saltwater (north to south) was the freshwater copepod *Cyclops* spp. (data not shown), amphipods, *Daphnia* spp., *Bosmina* spp., *E. affinis*, and *A. tonsa*, with mysids often covering a large salinity range. North and Houde (2003), working in the Chesapeake Bay ETM in 1998 and 1999, found a similar pattern between *Bosmina longirostris* and *E. affinis*. These distributional patterns and previous studies suggest that there are interactions between *E. affinis* and *A. tonsa* that help to define their distributions. *Acartia tonsa* has been shown to preferentially prey on the nauplii of other copepods species, including those of *E. affinis*, over its own nauplii (Londsdale *et al*. 1979). *Eurytemora affinis* may not be able to establish a population outside of the ETM due to such predation pressure. Live collections of *A. tonsa* from the field often show adults feeding on smaller copepodites, though it is not clear if this prey is of a different species (pers. obs.). *Acartia tonsa* has been shown to graze less efficiently on autotrophic prey than *E. affinis* in water collected from the ETM of the Choptank River, a Chesapeake Bay tributary (Lloyd, Chapter 3). A positive correlation between chlorophyll-*a* and turbidity in July, when *A. tonsa*

abundance peaks, may reflect a grazing rate decrease influenced by the concentration of suspended, inedible particles (Burdloff *et al.* 2002). *Eurytemora affinis*, however, appears be able to graze at similar rates on autotrophic prey in water from the ETM and water down-estuary (Lloyd, Chapter 3).

In general, peak abundances of *E. affinis*, and *A. tonsa* did not overlap along the ETM transect in summer and fall, and in 2001, the distributions appeared to be spatially separated all three cruises. *A. tonsa*'s salinity tolerances are broader than *E. affinis*, and *A. tonsa* has been shown to tolerate salinities ranging from 0 to over 70 (Cervetto *et al.* 1999). *Eurytemora affinis*' salinity tolerances generally range from freshwater to around 15, though they have been found at higher salinities in the later winter/early spring (Vaupel-Klein and Weber 1975, Roddie *et al.* 1984). As noted earlier, increasing temperatures tend to decrease the salinity range of *E. affinis* (Kimmel and Bradley 2001). *Acartia tonsa* ingestion rates are possibly too low in the ETM to sustain a population, and *E. affinis* is limited in its down-estuary distribution by predation pressure from *A. tonsa* and mysids. *Acartia tonsa*, in turn, may be limited to surface and middle depths in the summer due to hypoxic conditions (< 2 mg O_2 l^{-1}) in bottom waters beginning around river km 40 and continuing seaward .

The influence of predation on zooplankton abundance also may influence zooplankton abundance and distribution from summer to autumn as mysids and ctenophores increase in abundance in July and either remain at equivalent levels or decrease into October (i.e. Kimmel and Roman 2004). Whereas there is no significant correlation between *E. affinis* and mysids in July, there is a strong positive correlation in July between *A. tonsa* and mysid abundance that could represent the

two groups either coexisting without interaction or mysids actively seeking out

copepod prey. Among zooplankton, mysids are especially capable swimmers

(Kimmerer *et al.* 1998, Azeiteiro *et al.* 1999), are zooplanktivorous, and often select

for *E. affinis* before most other prey (Viherluoto and Viitasalo 2001, Chigbu 2004).

However, *E. affinis* has also been shown experimentally to have a greater escape

response than *Acartia* spp. regarding mysid predation and may actually interfere with

mysid predation on *Acartia* spp. (Viitasalo and Rautio 1998). October correlations

are similar to July, where mysids and *A. tonsa* share opposite relationships with the

same variables (though not all of these relationships are significant) and have a

significant positive relationship with each other.

The ctenophore *Mnemiopsis leidyi* is another predator that is often a large

source of mortality upon crustacean zooplankton (Purcell *et al.* 2001) and may release

microzooplankton (ciliates in particular) from copepod grazing mortality (Graneli and

Turner 2002). Abundances of *M. leidyi* usually peak in the late summer into the fall

at salinities > 4. *Eurytemora affinis* and other oligohaline zooplankton may be

protected from ctenophore and sea nettle (which usually has a negligible volume in

the study area compared to ctenophores) predation due to their salinity preferences

and tolerances, as peak abundances of *E. affinis* and cladocerans in the July and

October surveys occur at salinities below those where ctenophores are usually found.

Summer PCAs showed a nearly non-existent relationship between both *E. affinis* and

A. tonsa and ctenophores in the ETM region. This may not be the case in other areas

of Chesapeake Bay as Purcell and Decker (2005) concluded that ctenophore

abundance can significantly reduce *A. tonsa* abundance in years when populations of

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the sea nettle, *Chrysaora quinquecirrha*, a predator of ctenophores, are forced down-estuary due to high freshwater input.

Winkler *et al.* (2003) demonstrated a reduction of autotrophic biomass from freshwater into the low salinity zone of the St. Lawrence Estuary that was greater than a decrease caused by dilution and coincided with high *E. affinis* concentrations. This decrease in chlorophyll along the ETM occurred prior to any significant change in salinity or turbidity. *Eurytemora affinis* has been known to preferentially graze phytoplankton under conditions where autotrophic biomass is dwarfed by the mass of heterotrophs, detritus, and inorganic particles (Vijverberg 1980, Gasparini and Castel 1997). The inverse log-log relationship between *E. affinis* abundance and chlorophyll-*a* concentration in the May cruises of the present study (Figs. 19 and 20) is similar to results in the St. Lawrence Estuary. The logarithmic decline of chl-*a* concentrations versus *E. affinis* and the mismatch between chl-*a* concentrations and the dilution-based calculations suggest that the up- to down-estuary decrease in chl-*a* concentrations in May in northern Chesapeake Bay is possibly due to zooplankton grazing.

A plot of chlorophyll-*a* versus *A. tonsa* abundance suggests that *A. tonsa* may have impacted autotrophic biomass via grazing during the July and October 2001 and 2002 cruises (Fig. 18). Research in Chesapeake Bay has demonstrated variability on the effects of *A. tonsa* grazing on phytoplankton standing stock (White and Roman 1992, Cuker and Watson 2002). Abundances of *A. tonsa* and phytoplankton (using chl-*a* concentrations as a proxy) in the Chesapeake Bay ETM region often do not overlap, suggesting that copepod grazing may significantly influence the abundance

of autotrophs. However, this relationship is much weaker than the relationship between chl-*a* concentration and *E. affinis* density, and it could be the result of chl-*a* dilution from freshwater (the Susquehanna River) to the ETM region, phytoplankton senescence and corresponding chl-*a* degradation in the ETM, and/or reduced primary production due to light limitation.

Cruise comparisons of diversity show little change from May to July throughout the entire ETM region, but, under normal streamflow conditions, diversity does appear to decrease from July to October. The inverse log relationship between diversity and salinity suggests that, at salinities ranging from 0 to 14, diversity decreases from up-estuary to down-estuary. Zooplankton diversity in the St. Lawrence Estuary has been shown to be at a minimum at mesohaline locations (Laprise and Dodson 1994), and it is possible that zooplankton diversity increases from the middle bay to the bay mouth (Tafe 1990, Vieira *et al.* 2003). Salinities in the ETM region were higher during the October 2001 and 2002 cruises than during May or July for both of those years, and this hydrographic change may explain why diversity was lower during the October 2001 and 2002 cruises.

Zooplankton distributions and densities appear to be primarily influenced by freshwater input and to a lesser extent through biological interactions. *Eurytemora affinis* is a species found in freshwater and brackish habitats. The seaward limit of other zooplankton, such as cladocerans and amphipods, moves up-estuary in July, presumably in response to both reduced streamflow and increased temperatures. High temperatures and salinities in the summer likely increase energy expenditures due to osmoregulation and increased respiration rates and may contribute to the

general decline of zooplankton diversity with salinity (Kimmel and Bradley 2001).

Copepods, especially *E. affinis*, in the Chesapeake Bay ETM region appear to have a

grazing impact on autotrophic biomass, suggesting that the Chesapeake Bay ETM is

not necessarily a net heterotrophic system in the spring, even when turbidities are

highest (i.e. Winkler *et al.* 2003). The filtering efficiency of cladocerans (Gliwicz

1980, Uitto 1996), feeding selectivity of copepods (Bollens and Penry 2003, Tackx *et

al.* 2003), abundant detritus for amphipods (Zimmerman *et al.* 1979, Sanford *et al.*

2001), and zooplankton available to mysids creates a dynamic ecosystem in which a

change in freshwater input can broadly impact the location and densities of

autotrophic and heterotrophic plankton.

Table 2.1 (part 1 of 2). Median, (bold values), minimum (min), and maximum (max) values for all abiotic variables and the most abundant zooplankton groups. dO = dissolved oxygen (mg l^{-1}), Temp = Temperature (ºC), Salinity = PSU, Chl-*a* = chlorophyll-*a* (µg l^{-1}). All zooplankton abundances are in number m^{-3} except for Jelly Volume (the sum of ctenophore and sea nettle volume, ml m^{-3}). S = surface, B = bottom, M = medium.

Cruise	Depth	dO$_2$	min	max	Temp	min	max	Salinity	min	max	Chl-*a*	min	max	TSS	min	max
May, 1996	S	**9.3**	8.2	11.0	**15.8**	15.4	16.5	**1.8**	0.1	4.0	**2.4**	2.3	2.3	**25.2**	12.0	45.1
	B	**6.9**	5.6	10.8	**14.2**	12.8	16.1	**5.6**	0.2	8.0	**2.8**	1.5	5.0	**40.7**	29.3	63.7
July, 1996	S	**7.0**	6.4	8.9	**25.0**	24.6	25.7	**3.3**	0.3	5.8	**8.0**	8.0	8.0	**63.3**	46.4	251.8
	B	**4.7**	2.1	6.2	**24.1**	22.9	25.6	**8.3**	0.4	11.9	**8.0**	8.0	8.0	**22.3**	1.7	45.5
October, 1996	S	**10.3**	9.2	12.3	**14.1**	12.5	15.6	**0.3**	0.1	4.0	**1.9**	1.9	2.0	**32.4**	17.1	51.6
	B	**7.4**	6.8	11.9	**15.7**	12.8	16.5	**5.7**	0.2	9.1	**1.9**	1.8	1.9	**16.8**	10.9	32.7
May, 2001	S	**7.0**	4.2	14.2	**18.5**	17.5	19.5	**0.6**	0.2	1.9	**1.9**	1.6	4.5	**20.0**	15.2	27.2
	M	**7.0**	3.4	7.9	**18.2**	16.8	19.5	**1.1**	0.2	4.0	**2.1**	1.7	2.1	**19.6**	18.4	32.8
	B	**6.6**	5.3	7.3	**17.6**	15.0	19.6	**2.2**	0.2	7.8	**2.2**	1.8	2.9	**40.6**	26.4	52.4
July, 2001	S	**5.9**	5.8	6.0	**26.0**	25.7	26.4	**1.0**	0.2	3.1	**2.1**	1.9	2.5	**16.6**	15.1	17.4
	M	**5.9**	4.8	5.9	**26.0**	25.5	26.6	**1.2**	0.2	4.4	**2.5**	2.0	2.6	**16.7**	14.8	19.9
	B	**5.7**	2.1	5.9	**25.6**	24.2	26.7	**1.6**	0.2	9.7	**2.5**	2.2	2.7	**28.8**	20.8	51.7
October, 2001	S	**6.8**	6.9	7.4	**16.4**	20.2	20.7	**5.6**	4.4	10.9	**2.1**	1.7	2.8	**6.7**	4.1	10.2
	M	**6.8**	5.4	7.0	**16.4**	15.9	17.1	**4.6**	2.0	10.8	**2.2**	2.1	2.6	**6.5**	4.0	11.7
	B	**6.4**	5.4	6.7	**16.6**	16.2	17.1	**6.4**	2.4	10.8	**1.9**	1.7	2.7	**11.7**	7.6	19.4
May, 2002	S	**7.3**	6.7	9.9	**16.6**	16.1	17.0	**0.7**	0.1	4.2	**4.4**	3.3	8.5	**206.5**	200.0	224.0
	M	**6.7**	4.2	9.4	**16.5**	14.4	17.0	**1.3**	0.1	10.9	**5.0**	2.9	9.3	**172.4**	153.7	212.1
	B	**5.4**	3.3	7.2	**14.7**	13.5	16.9	**8.9**	0.1	14.8	**2.5**	2.3	10.3	**114.5**	65.3	126.5
July, 2002	S	**6.3**	6.0	6.9	**27.0**	25.9	27.1	**1.8**	0.3	5.4	**4.9**	2.4	5.8	**5.7**	4.1	7.7
	M	**5.9**	4.2	6.2	**26.3**	25.3	26.5	**4.7**	2.6	7.2	**5.1**	2.1	5.9	**6.0**	2.8	12.0
	B	**3.8**	0.4	4.1	**25.8**	23.0	26.0	**4.9**	4.7	15.0	**2.0**	1.5	2.5	**11.1**	10.3	31.6
October, 2002	S	**7.1**	6.9	7.4	**20.7**	20.2	20.7	**7.3**	4.4	10.9	**2.3**	1.7	2.8	**6.1**	4.1	10.2
	M	**7.0**	6.4	7.1	**20.9**	20.8	21.0	**8.0**	6.4	13.9	**2.6**	1.8	2.9	**8.9**	5.3	9.7
	B	**6.9**	6.2	7.1	**21.0**	20.9	21.2	**8.3**	6.6	14.9	**2.5**	1.8	2.7	**12.2**	8.3	21.1

Table 2.1 (part 1 of 2). Median, (bold values), minimum (min), and maximum (max) values for all abiotic variables and the most abundant zooplankton groups. dO = dissolved oxygen (mg l^{-1}), Temp = Temperature (ºC), Salinity = PSU, Chl-*a* = chlorophyll-*a* (µg l^{-1}). All zooplankton abundances are in number m^{-3} except for Jelly Volume (the sum of ctenophore and sea nettle volume, ml m^{-3}). S = surface, B = bottom, M = medium.

Cruise	Depth	Cladocerans	min	max	Amphipods	min	max	Mysids	min	max	Adult *E. affinis*	min	max	Adult *A. tonsa*	min	max
May, 1996	S	397.9	0.0	758.9	0.0	0.0	2.9	0.0	0.0	0.0	10587.4	1043.6	29053.8	0.0	0.0	890.1
	B	105.7	0.0	352.9	0.0	0.0	5.7	0.0	0.0	0.0	6231.4	1448.6	9146.5	40.3	0.0	64.4
July, 1996	S	0.0	0.0	302.1	0.0	0.0	20.1	0.0	0.0	17.9	0.0	0.0	5518.4	4263.1	232.0	9280.7
	B	0.0	0.0	470.7	0.0	0.0	156.9	21.2	0.0	172.2	21.2	0.0	22281.6	2217.7	56.7	34363.9
October, 1996	S	110.2	0.0	243.0	0.0	0.0	5.8	0.0	0.0	0.0	340.3	0.0	863.2	60.0	0.0	16522.3
	B	2.3	0.0	264.8	0.5	0.0	66.2	0.0	0.0	0.0	48.6	0.0	25286.4	720.5	0.0	9990.2
May, 2001	S	31.0	7.9	70.2	6.9	0.0	28.6	0.0	0.0	0.0	529.9	264.2	1347.9	40.8	0.0	114.3
	M	11.2	3.5	64.0	20.0	1.8	25.6	0.5	0.0	0.8	801.8	140.7	1992.4	10.7	0.0	409.7
	B	19.2	6.0	186.4	22.1	6.4	186.4	3.5	0.0	30.6	2307.9	156.7	16673.6	28.1	0.0	1436.6
July, 2001	S	0.0	0.0	37.0	9.5	0.0	64.7	0.0	0.0	43.1	178.6	0.0	267.6	2379.0	0.0	12523.4
	M	0.0	0.0	17.5	97.6	28.0	362.4	0.7	0.0	129.2	203.5	84.0	322.0	3996.5	0.0	37261.2
	B	0.0	0.0	83.5	32.6	0.0	183.7	0.0	0.0	47.0	75.6	0.0	225.4	75.0	0.0	20690.1
October, 2001	S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.8	0.0	0.0	0.0	862.1	36.9	1432.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	2.5	3.8	1.9	23.4	693.2	61.3	2694.7
	B	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	10.6	1.4	0.0	72.1	1662.3	779.8	4992.8
May, 2002	S	57.4	9.3	242.1	10.1	0.0	27.8	0.0	0.0	5.0	340.9	64.4	986.1	241.4	0.0	538.0
	M	81.9	0.0	378.0	17.8	0.8	37.3	0.0	0.0	0.9	598.2	25.8	1278.6	641.6	0.0	2492.4
	B	30.5	0.0	138.4	19.9	3.3	138.2	0.0	0.0	32.9	1090.0	35.4	2418.2	675.3	0.0	3610.0
July, 2002	S	23.6	0.0	472.8	0.0	0.0	0.0	0.0	0.0	0.1	47.3	0.0	55.6	1237.7	703.1	19824.5
	M	0.0	0.0	0.0	0.0	0.0	14.0	0.7	0.0	14.0	26.4	0.0	56.0	1575.5	1080.5	12545.7
	B	0.0	0.0	83.3	0.0	0.0	0.0	0.0	0.0	4.7	93.6	0.0	353.9	1593.3	993.5	2612.8
October, 2002	S	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	13.8	0.0	0.0	0.0	50.8	36.9	1432.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	10.5	2.5	31.1	0.0	0.0	0.0	591.0	188.4	815.6
	B	0.0	0.0	0.0	0.0	0.0	2.6	5.4	1.1	14.1	0.0	0.0	0.0	579.7	60.3	1319.5

Table 2.2. Shannon-Weiner diversity index means (H ± 1 SD). October 2001 and 2002 H values are significantly lower than May and July of the respective years and lower than October, 1996 (Tukey-Kramer means comparison, α = 0.05).

	May	July	October
1996	0.59 (0.39)	0.45 (0.31)	0.62 (0.26)
2001	0.76 (0.44)	0.63 (0.58)	0.06 (0.049)
2002	0.89 (0.13)	0.36 (0.41)	0.15 (0.046)
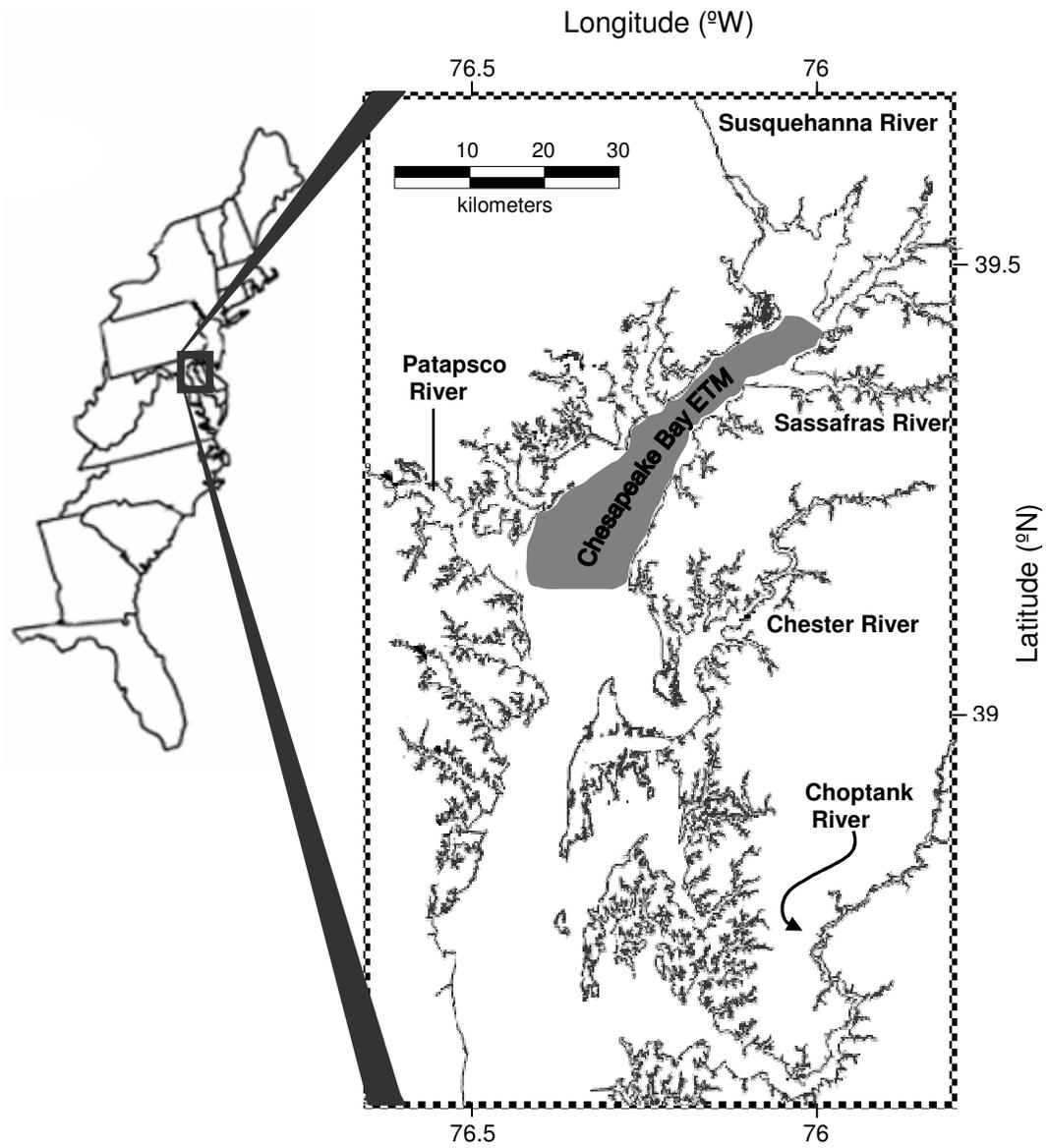
Figure 2.1. Map of upper Chesapeake Bay showing the ETM region (shaded).
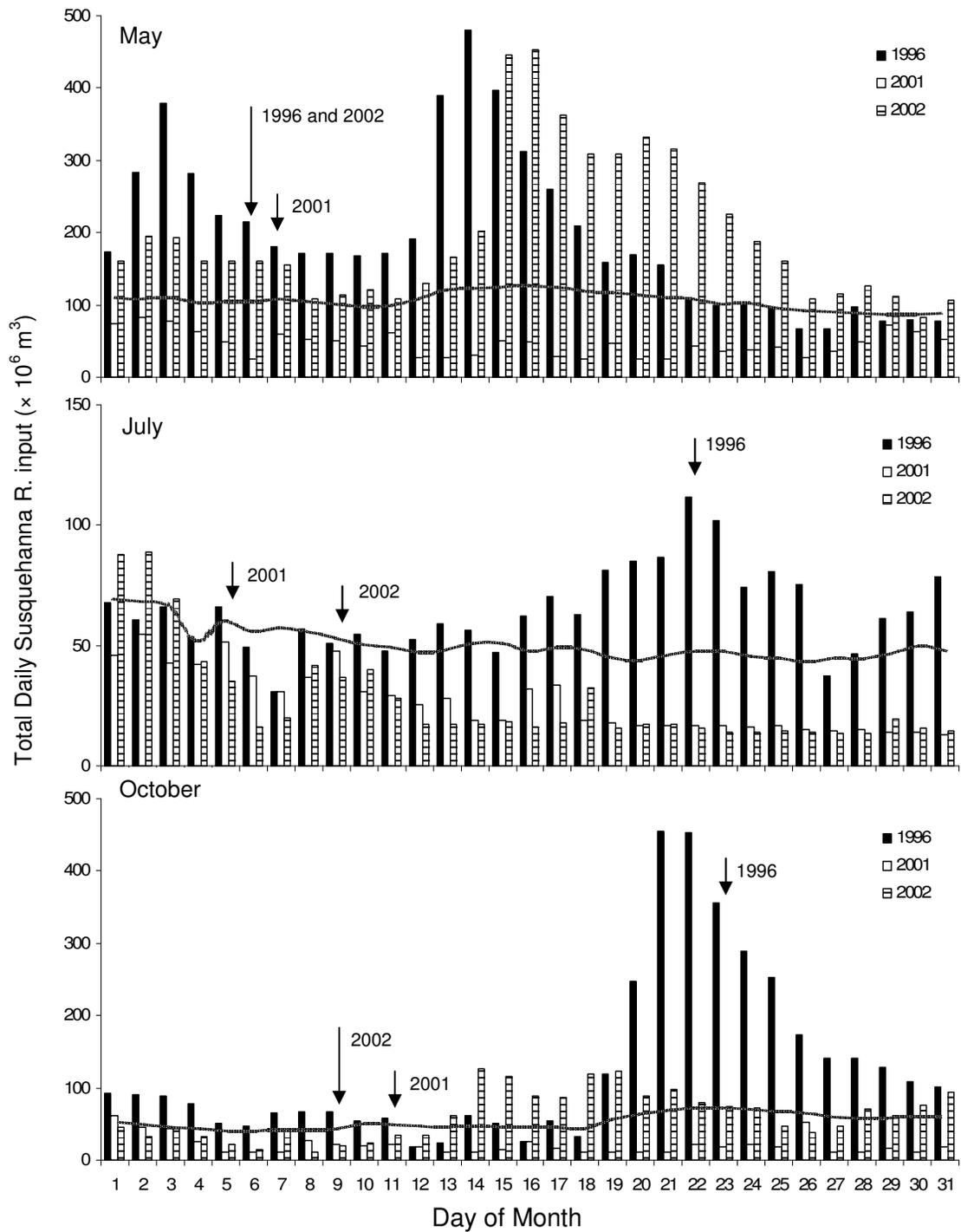
Figure 2.2. Total monthly Susquehanna River streamflow as measured at the Conowingo dam for the three study years. The line is the monthly mean from 1968 to 2003.
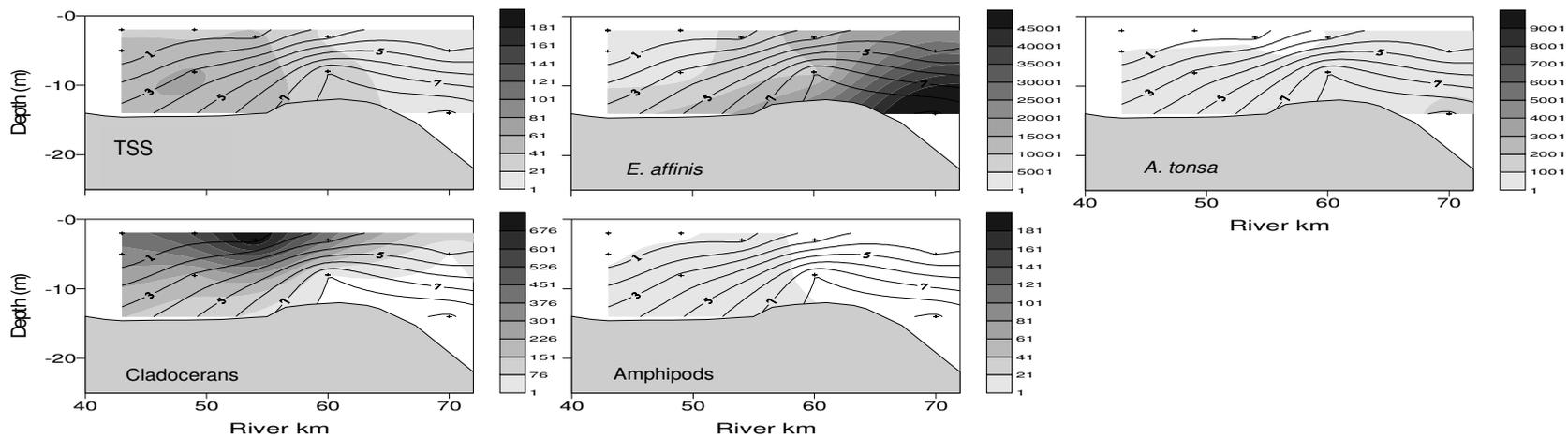
Figure 2.3. May 1996 axial transect through ETM region. The lines in each plot represent isohalines. Crosses represent the mean depths of Tucker trawl samples. River km is the distance down-estuary (south) from Havre de Grace. Zooplankton abundances are in number of individuals m^{-3}. TSS = total suspended solids (mg l^{-1}). All samples were taken at night.
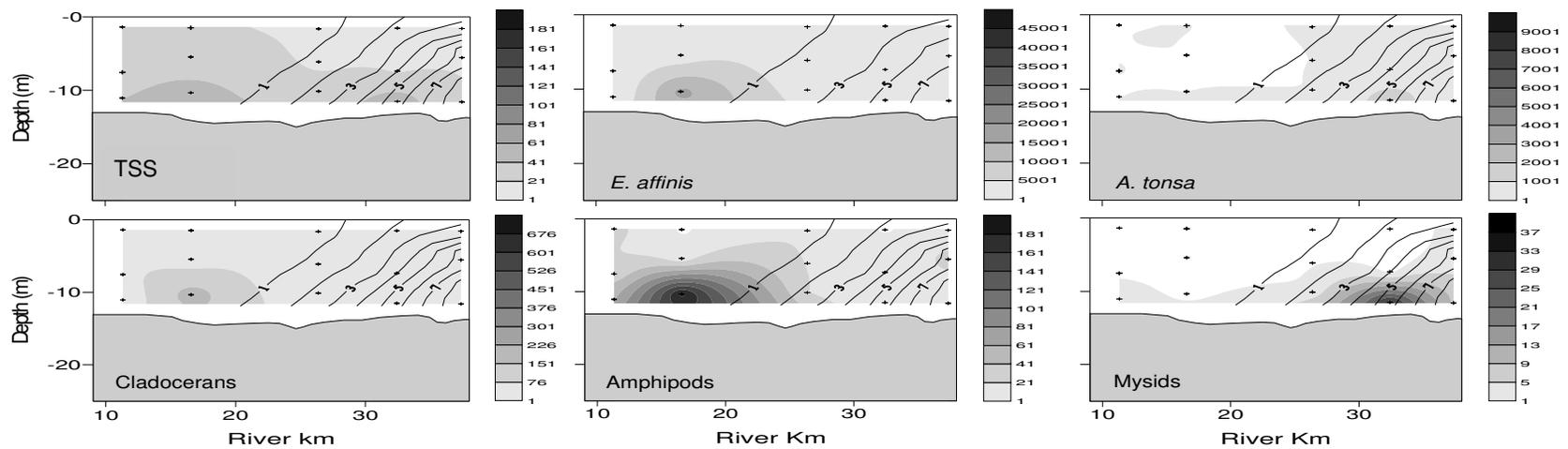
Figure 2.4. May 2001 axial transect through ETM region. All samples were taken at night. See Figure 3 caption for an explanation of the plots.
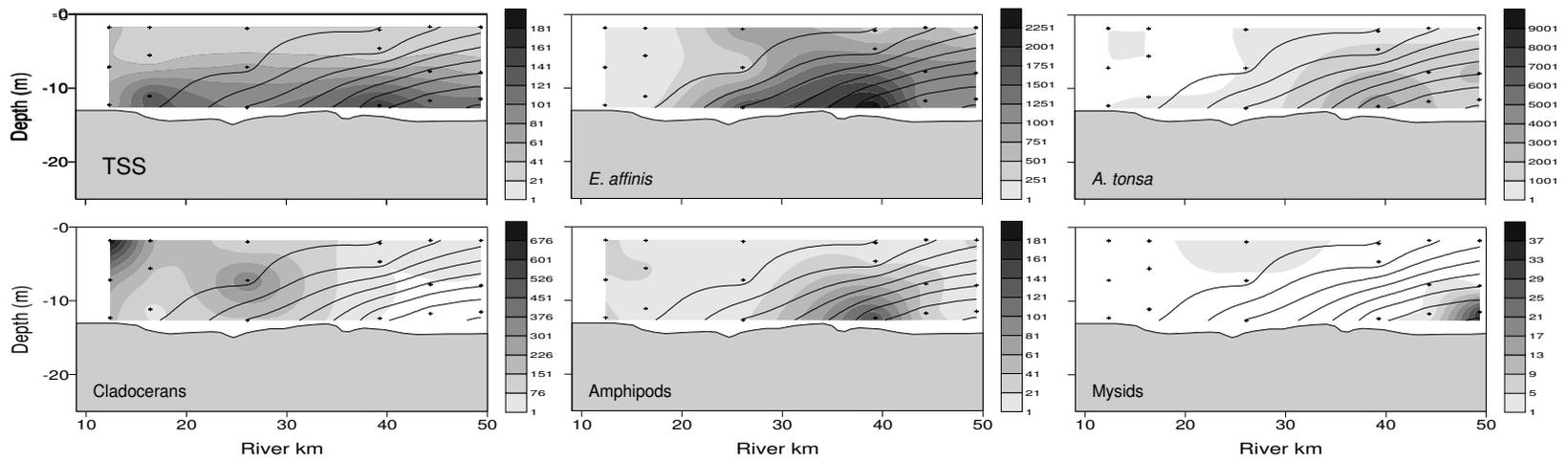
Figure 2.5. May 2002 axial transect through ETM region. All samples were taken at night. See Figure 3 caption for an explanation of the plots.
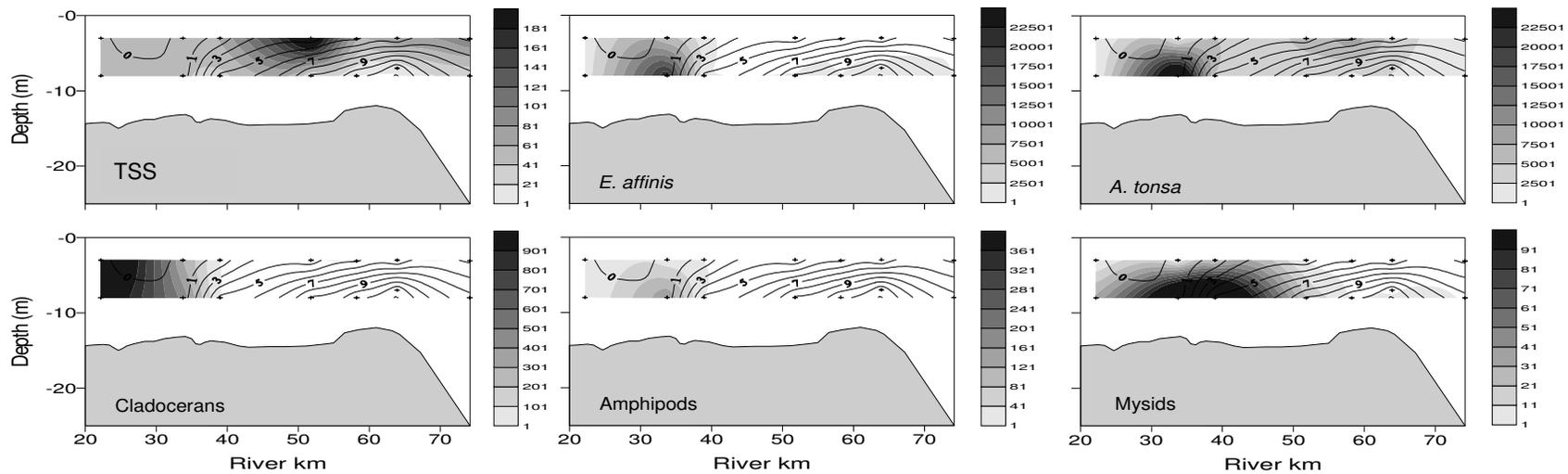
Figure 2.6. July 1996 axial transect through ETM region. All samples were taken at night. See Figure 3 caption for an explanation of the plots.
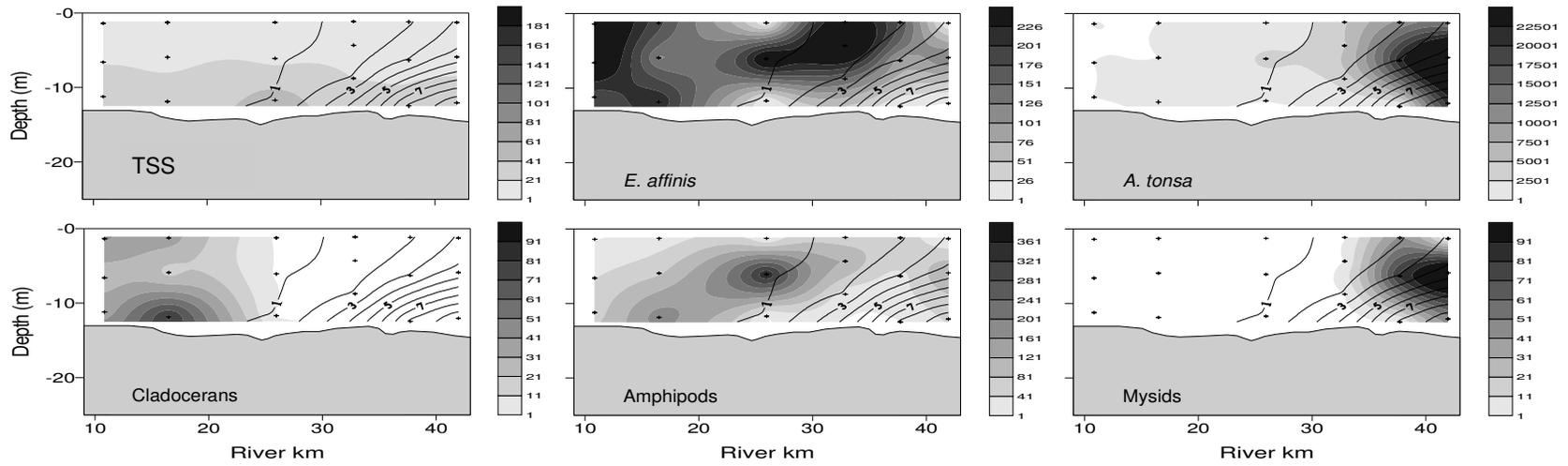
Figure 2.7. July 2001 axial transect through ETM region. All samples were taken at night. See
Figure 3 caption for an explanation of the plots.
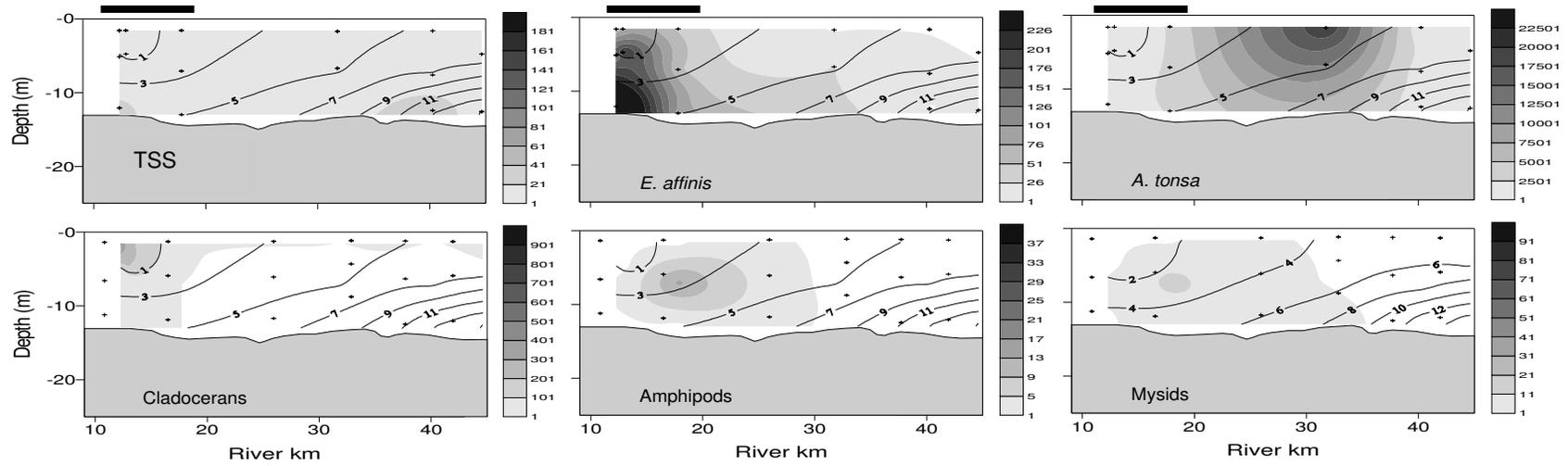
Figure 2.8. July 2002 axial transect through ETM region. All samples were taken at night. See Figure 3 caption for an explanation of the plots.
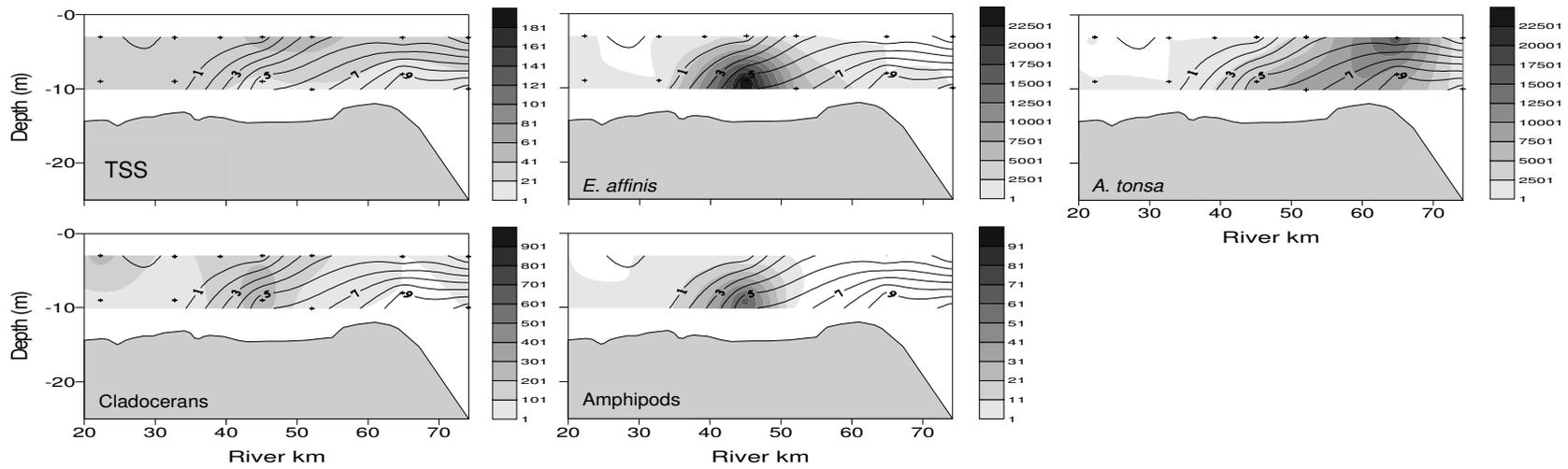
Figure 2.9. October 1996 axial transect through ETM region. All samples were taken at night. See Figure 3 caption for an explanation of the plots.
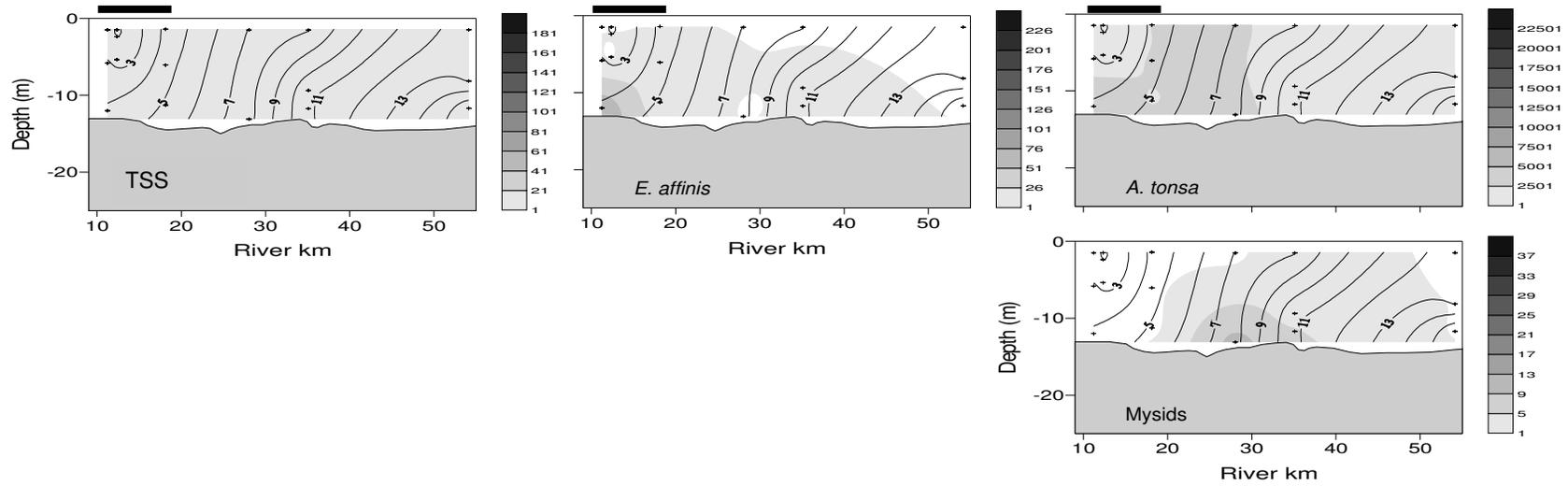
Figure 2.10. October 2001 axial transect through ETM region. All samples were taken at night. See Figure 3 caption for an explanation of the plots.
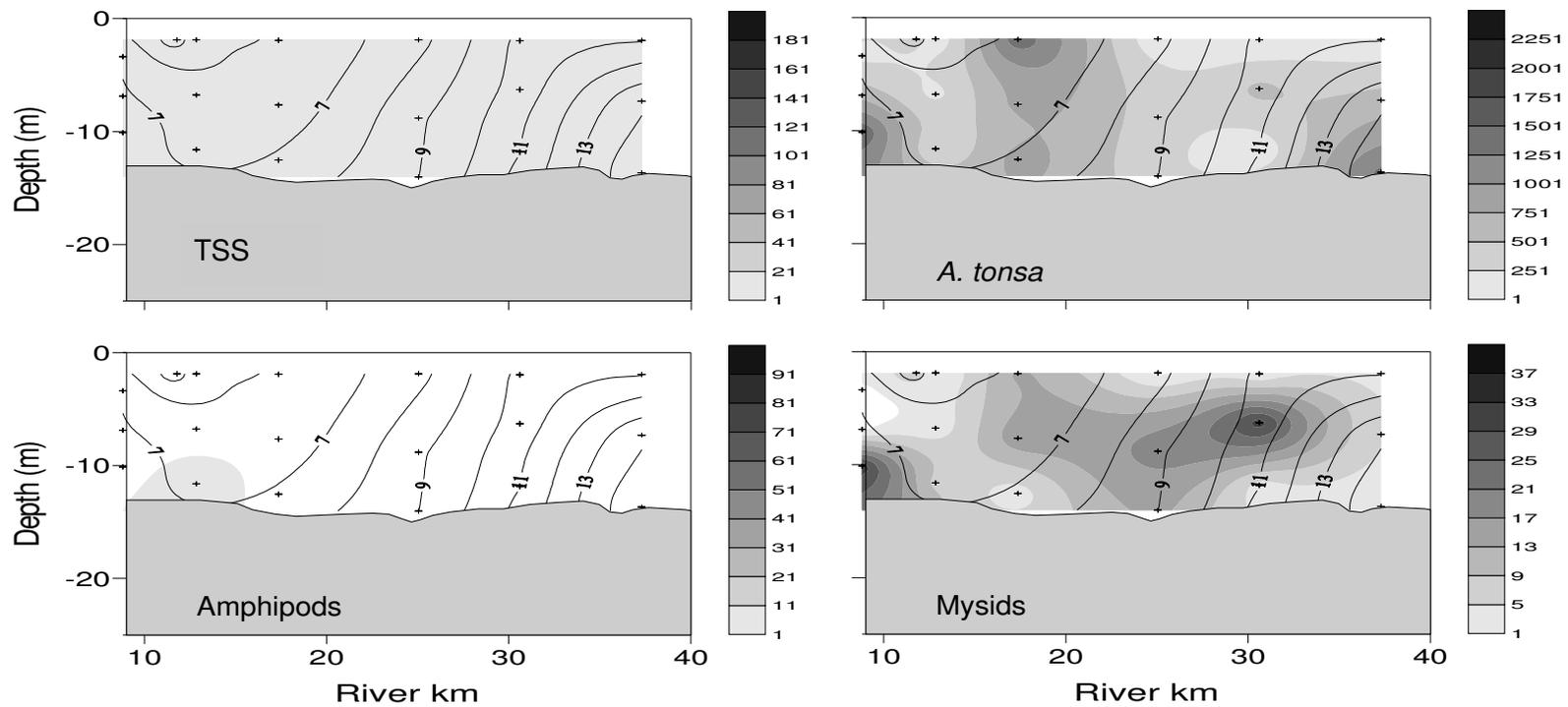
Figure 2.11. October 2002 axial transect through ETM region. All samples were taken at night. See
Figure 3 caption for an explanation of the plots.
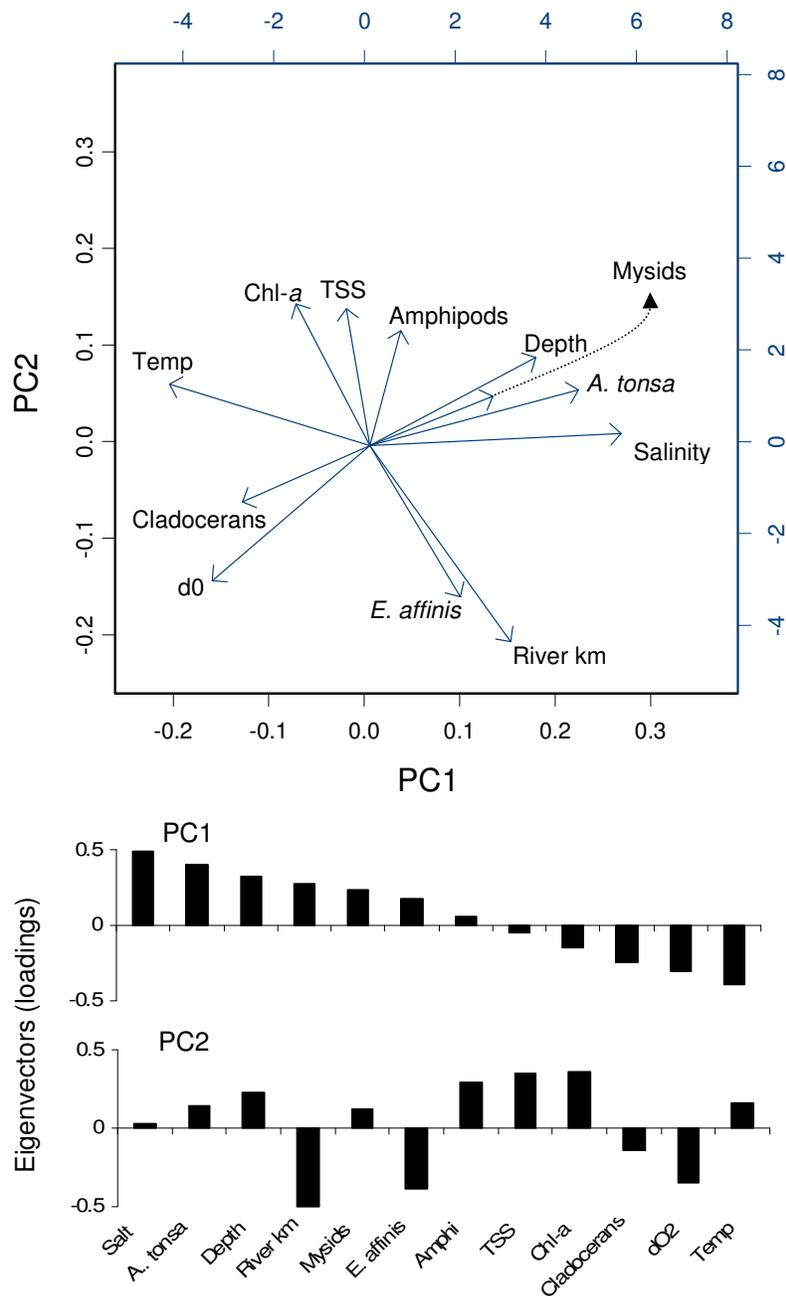
Figure 2.12. Principal components analysis (PCA) biplot of principal component 1 (PC 1) versus PC 2 for all May (1996, 2001, and 2002) data based on correlations. The relative lengths of arrows represent the amount of variation explained by the two principal components, and arrow direction represents the type of relationship (arrows in same direction mean variables are positively related, arrows at right angle mean variables are unrelated). PC loadings for PC1 and PC2 are shown below the biplot.
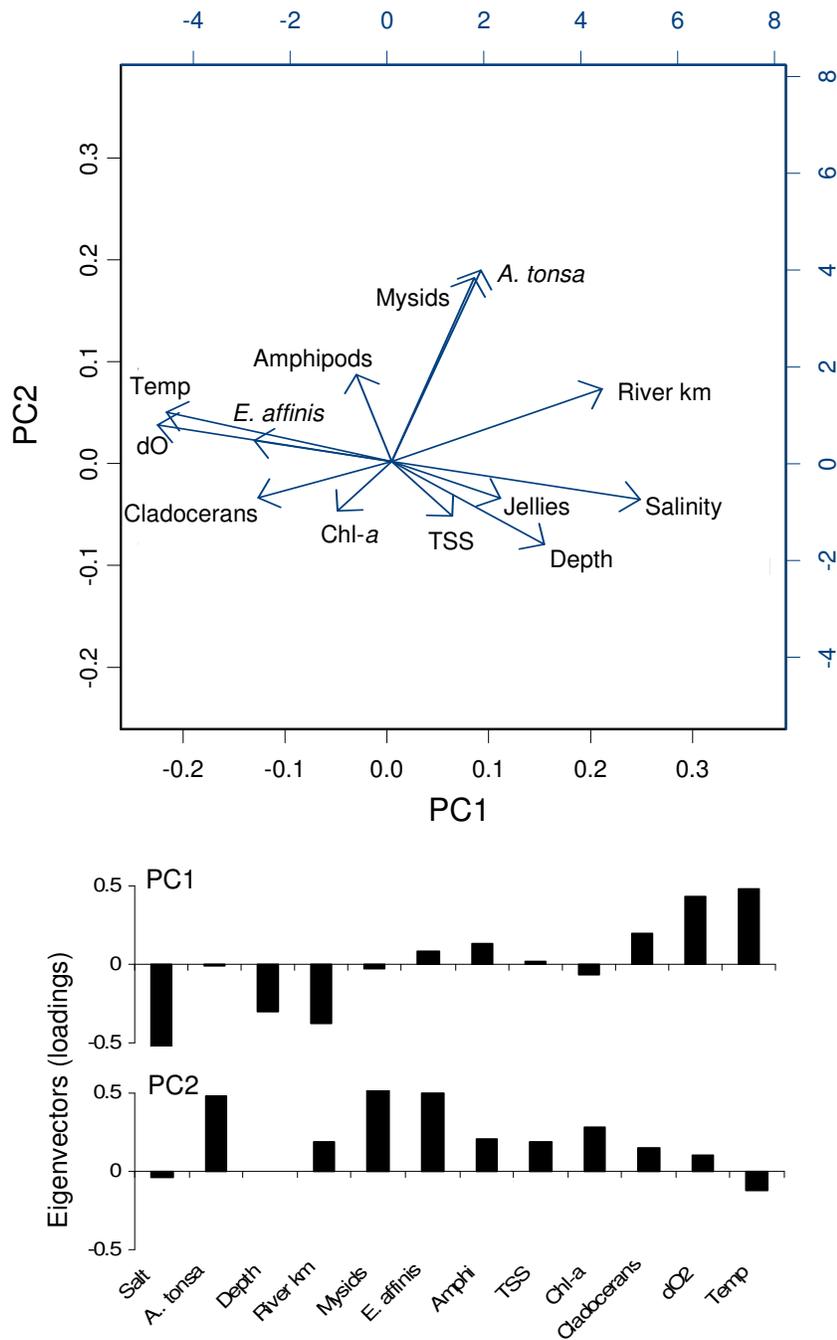
Figure 2.13. Principal components analysis (PCA) biplot of principal component 1 (PC 1) versus PC 2 for all July data based on correlations. The relative lengths of arrows represent the amount of variation explained by the two principal components, and arrow direction represents the type of relationship (arrows in same direction mean variables are positively related, arrows at right angle mean variables are unrelated). PC loadings for PC1 and PC2 are shown below the biplot.
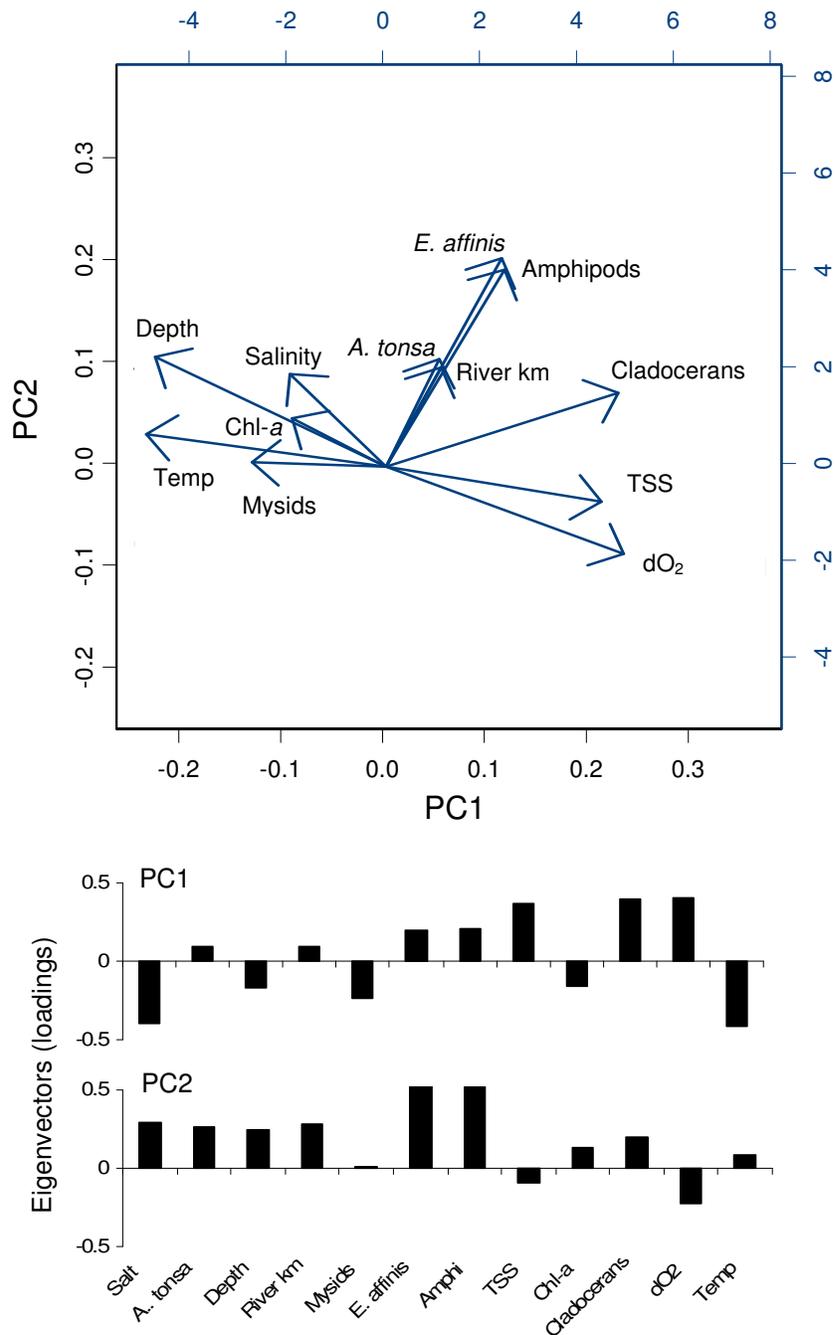
Figure 2.14. Principal components analysis (PCA) biplot of principal component 1 (PC 1) versus PC 2 for all October data based on correlations. The relative lengths of arrows represent the amount of variation explained by the two principal components, and arrow direction represents the type of relationship (arrows in same direction mean variables are positively related, arrows at right angle mean variables are unrelated). PC loadings for PC1 and PC2 are shown below the biplot.
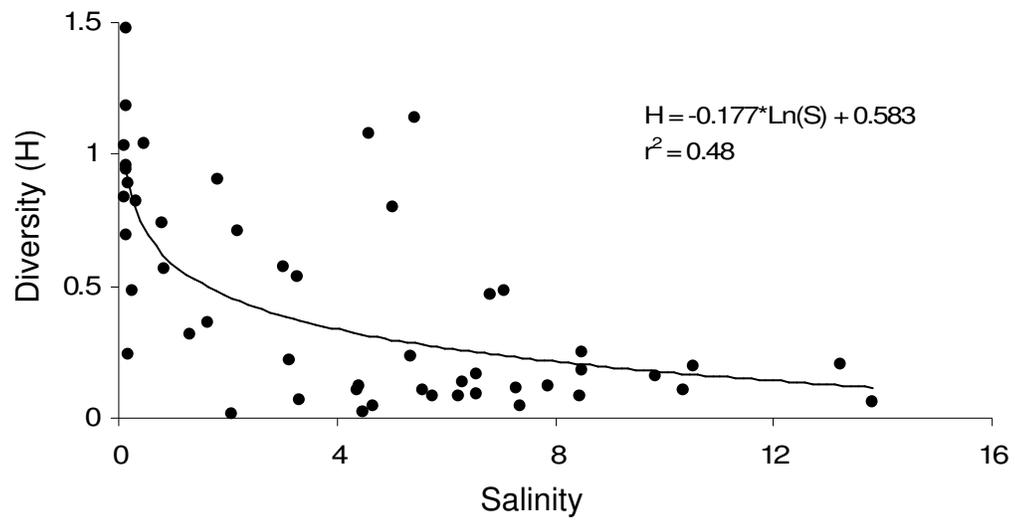
Figure 2.15. Relationship between Shannon-Weiner diversity index (H) and Salinity (S; psu). Diversity (H) was regressed on log salinity.
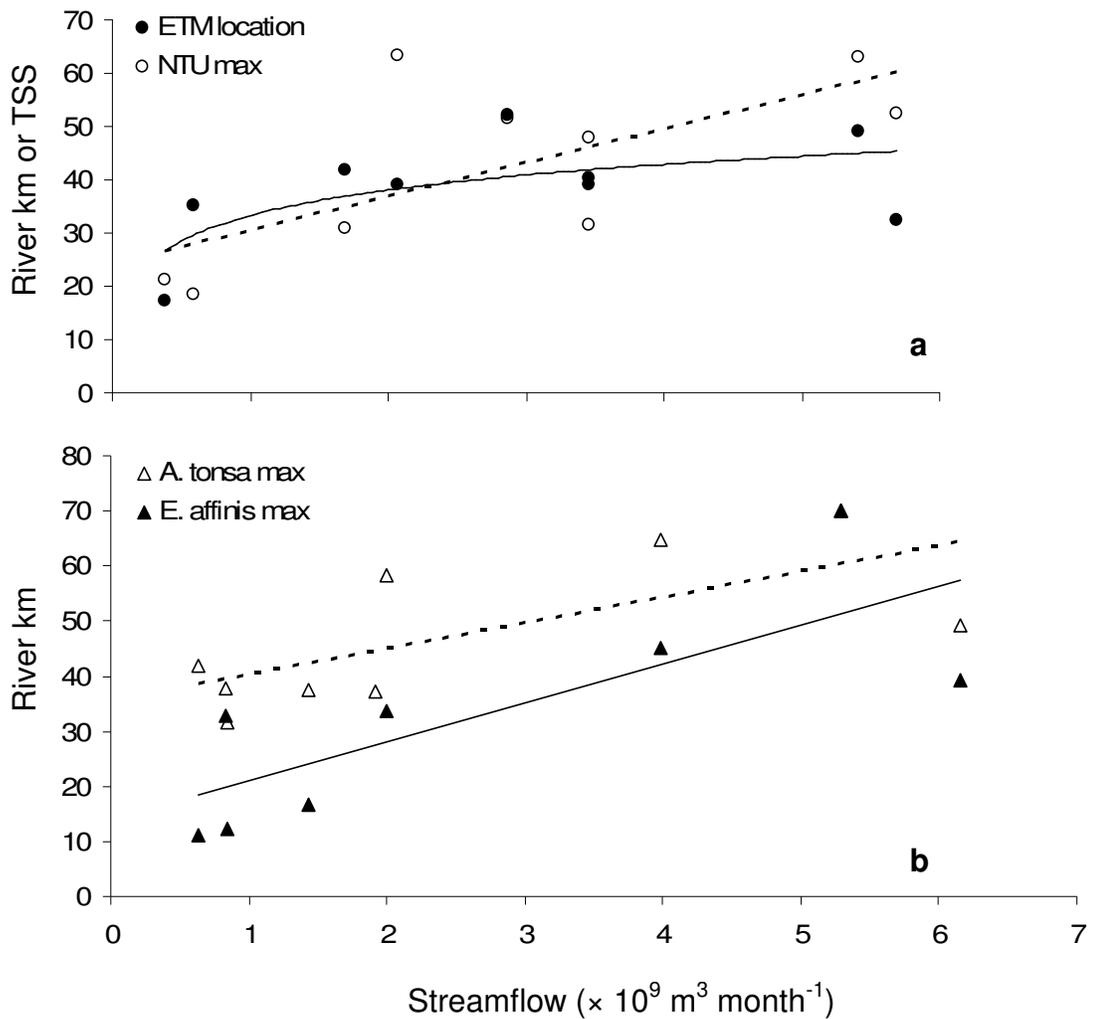
Figure 2.16. a) Regressions between both maximum TSS and River km (ETM location; distance down-estuary from Havre de Grace) versus total monthly Susquehanna River streamflow in the month prior to the measurements. The TSS versus streamflow regression is the solid linear regression line and has an r^2 of 0.49 (TSS = 6.0×10^{-9}(Streamflow) + 24.1); the River km versus streamflow regression was calculated using $\log_{10}$(streamflow) and has an r^2 of 0.42 (River km = 6.9 × Log_{10}(Streamflow) – 110.0). Both regressions are significant at the 0.05 alpha level. b) Regressions between the locations of *A. tonsa* and *E. affinis* peaks versus total monthly Susquehanna River streamflow in the same month as the cruises. The *A. tonsa* peak location versus streamflow regression is the stippled line and has an r^2 of 0.50 (*A. tonsa* = 5.0×10^{-9}(Streamflow) + 35.6), and the *E. tonsa* peak location versus streamflow regression has an r^2 of 0.61 (*E. affinis* = 7.0×10^{-9}(Streamflow) + 14.0). Both regressions are significant at the 0.05 alpha level.

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Figure 2.17. Welch's ANOVA between *E. affinis* prosome length and water depth [surface (0-4 m), middle (4-8 m), and bottom (8+ m) waters]. Prosome length is significantly greater in deeper waters (p < 0.05). Diamonds represent the 95% confidence interval.
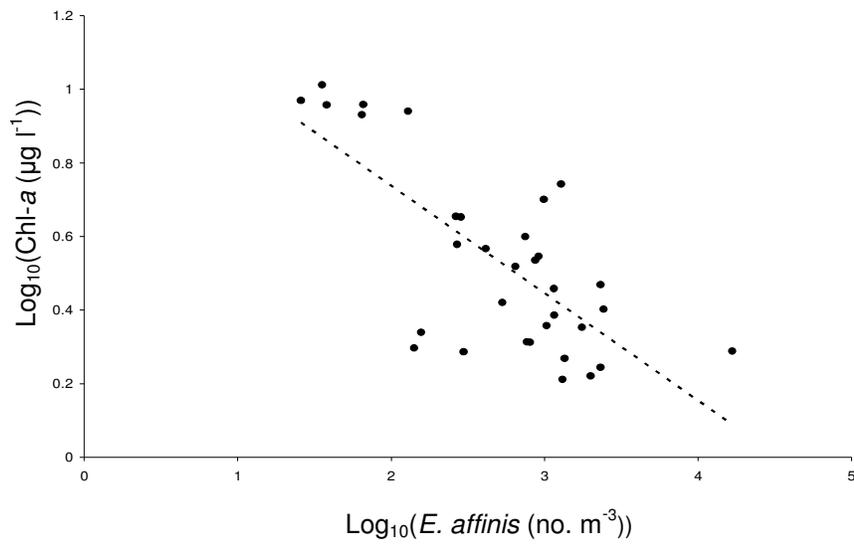
Figure 2.18. Regression between log-transformed chlorophyll-*a* concentration versus log-transformed *E. affinis* abundance for all 2001 and 2002 May data (p < 0.001).
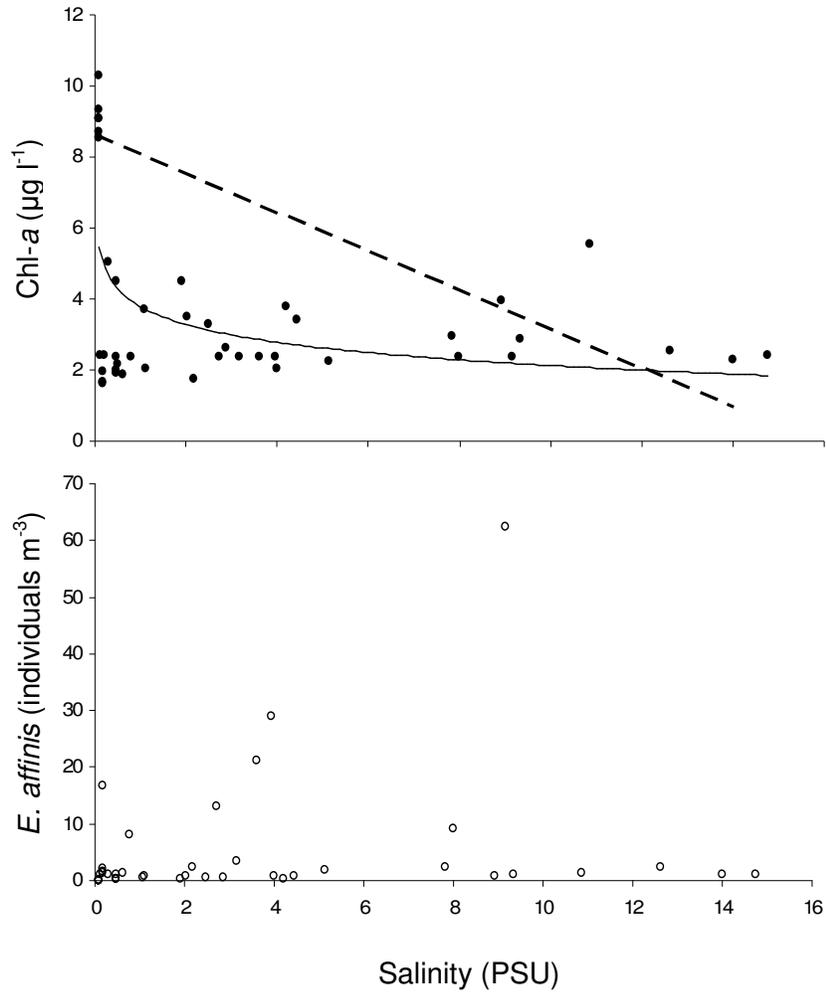
Figure 2.19. Chlorophyll-*a* (chl-*a*) and *Eurytemora affinis* (*E. affinis*) concentrations versus salinity. The dashed line represents the calculated chl-*a* concentration decrease by dilution alone; the solid line is the logarithmic fit to actual chl-*a* values versus salinity (p < 0.01).
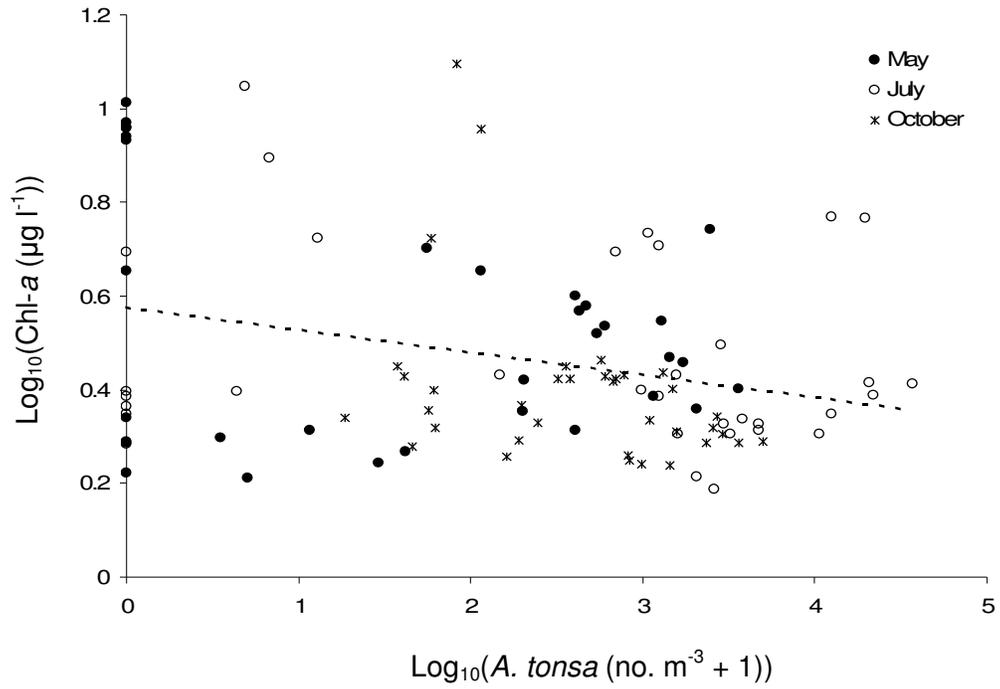
Figure 2.20. Regression between log-transformed chlorophyll-*a* concentration versus log-transformed *A. tonsa* abundance for all 2001 and 2002 data (p < 0.05).

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CHAPTER III: Incorporating interclutch duration into estimates of brooding copepod egg production

ABSTRACT

Egg production rates for copepods which brood their eggs are usually based on indirect methods which incorporate a temperature-dependent value for hatching time (= embryonic development) and the ratio of eggs to adult females. An alternative method for estimating brooding copepod egg production is described using the copepod *Eurytemora affinis* as an example. This method takes into account the life histories of brooding copepods and incorporates hatching time, interclutch duration, and mean clutch size into egg production estimates. Egg production estimated fromboth the clutch size method and the egg-ratio method were compared to estimates from three different empirical models of copepod production. The clutch size method consistently provided a better fit to predicted values from the models, with the egg-ratio method generally overestimating egg production compared to the clutch-size method. Although the clutch size method requires further research to be performed on the relationships between temperature and interclutch duration, it appears to provide more accurate estimates of egg production rates. The close fit between the somatic growth model outputs and egg clutch size-derived estimates suggests that egg production rates for *E. affinis* can be used to estimate juvenile growth under food-replete conditions.

INTRODUCTION

Brooding copepods (those that carry eggs until hatching) differ from broadcast-spawning copepods (those that release unhatched eggs) in several ways, one being that a brooding female cannot produce a new clutch of eggs until after her present clutch hatches or the unhatched (usually dead) eggs are released (Runge and Roff 2000). This adds an important time component to egg production rate – hatching time (HT). Temperature effects on HT (also known as egg development time or embryonic development time) have been the focus of several studies (e.g. Heinle and Flemer 1975, Vijverberg 1980, Ban and Minoda 1992, Andersen and Nielsen 1997). Essentially, HT is the time from extrusion of the first egg into an egg mass to hatching or release of the last egg in that mass. Hatching time values for *Eurytemora affinis* can be calculated from the equation:

$$HT = 36.8 \times T^{-1.04}$$

developed by Andersen and Neilsen (1997). This equation incorporates data from several studies and has a regression coefficient of 0.87.

Another time component in brooding copepod egg production that must also be considered is interclutch duration (ID; Chow-Fraser and Maly 1991). Interclutch duration, which has receivedless study, is the period from the hatching of the last egg of a clutch to the extrusion of the first egg of another clutch. Ban (1994) found a significant, negative power relationship between temperature and *E. affinis* ID, while Vijverberg (1980) found reduced *E. affinis* ID at temperatures >25ºC and reported ID values much lower than Ban (1994). Food limitation appeared to influence ID only at

near starvation levels (see Ban's Table III). The above time periods (HT and ID) comprise the egg production cycle for brooding copepods (Fig. 1).

Egg production measurements of broadcast-spawning copepod involve incubating animals in bottles filled with filtered water, natural water, or water with a food supplement (Runge and Roff 2000) over a period of 24 hours to capture any diel periodicity in egg production (White and Roman 1992). Measured HT for *E. affinis* ranged from over 14 days at temperatures below 5ºC to ~1 day at 25ºC (Andersen and Nielsen 1997). Such long hatching times at cooler temperatures mean that bottle incubation techniques may not be applicable for brooding copepods. One solution to this problem is to conduct incubations in large bottles (i.e. ≥ 4 l) containing male and female copepods and periodically remove subsamples to enumerate nauplii and eggs female^{-1} (Burdloff et al. 1999). Counts from these subsamples are then compared to subsamples taken at the beginning of the incubations. The major assumption of this method is that each subsample contains the same ratio of gravid to nongravid females and is a representative sample of nauplius concentration (nauplii are uniformly distributed). Thus, there is the potential for significant sample error with this method, and egg production rates appear low for this method compared to other methods [compare Burdloff et al. (1999) to Katona (1975) and Ban (1994)].

The "egg ratio method" developed by Edmondson (1968) to estimate the egg production rates of rotifers has been applied to brooding copepods (e.g. Edmondson et al. 1962, Sabatini and Kiørboe 1994). The egg ratio is egg abundance (E, per unit volume) divided by the total number of females with and without eggs (A, per unit volume), giving units of eggs female^{-1} (E/A). The egg ratio alone does not estimate

the production rate, but because HT can be determined from temperature at the time

of copepod capture, egg production rate can theoretically be determined (Runge and

Roff 2000). Assuming that all eggs are viable, egg production rate (EP, eggs female^{-1}

day^{-1}) should equal the egg ratio divided by hatching time:

$$EP = \left(\frac{E}{A(HT)} \right).$$

This definition assumes that ID is incorporated into E/A, because A includes all

gravid and nongravid adult females. Therefore, the only time component one needs

to estimate EP using this method is HT.

Interclutch duration and hatching time could be used directly in EP

measurements by dividing the mean clutch size (CS; of completely formed clutches)

by the sum of both time components:

$$EP = \left(\frac{\overline{CS}}{(ID + HT)} \right).$$

where $\overline{CS}$ = mean clutch size of fully-formed clutches. This method assumes that all

mature females are reproductively capable., The measured mean clutch size

represents the average potential egg output of all females, thus only gravid females

must be included in the calculation.

Traditional methods of zooplankton collection (nets, pumps) may

undersample bottom depths within the water column (Omori and Hamner 1982), and

because *E. affinis* is often considered an epibenthic copepod, with the proportion of

gravid females increasing with depth (Kimmerer et al. 1998) or light attenuation

(Castonguay and FitzGerald 1990), measured E/A may not represent the actual

population E/A. This would lead to inaccurate estimates of EP. Egg production

estimates based on the clutch size method are not dependant on ratios and are also

well-suited for conditions of low copepod abundances, where ratios may be especially misleading.

One purpose of this study is to provide a temperature-based model to estimate *E. affinis* interclutch duration (ID). These values can then be used to provide an EP estimate based directly on the complete reproductive cycle of *E. affinis* (CS) and can be compared to traditional E/A derived estimates of EP. Such comparisons suggest that *E. affinis* EP estimates based on the CS method provide a better fit to several empirical models of copepod growth rates than do estimates based on the E/A method. Further, the relationships between the model outputs and actual data suggest that EP can be used as proxy for somatic growth of *E. affinis* under food satiating conditions

MATERIALS AND METHODS

Interclutch duration experiments

Live *E. affinis* were collected by a horizontal net tow (mesh size = 200 μm) from the Horn Point Laboratory dock on the Choptank River (38.590 N/76.139 W, Cambridge, Maryland, USA), the catch was diluted with ambient water and the stored in a cooler. A 20-l carboy of 200-μm filtered water was also collected on site.

In the laboratory, four, 2-l aspirator bottles were each filled with 200-μm filtered water, and approximately 250 ml of water from beakers containing copepods. The four bottles were placed into one of four environmental chambers set at 5ºC, 13.6ºC, 22.6ºC, and 27.5ºC. The bottles were gently bubbled (~1 bubble s^{-1}) and left

undisturbed for 24 h under a 12:12 day/night cycle. The ambient water temperature at collection was 10.1ºC.

Following the 24 h temperature acclimation, 14 male and gravid female pairs from each temperature regime were collected using a wide-bore Pasteur pipette and each pair placed into 50 ml beakers containing 20 ml of 200-µm filtered water from the Choptank River. To ensure that the copepods in natural water (NW) were not food limited, the diatom *Thalassiosira weissflogii*(Tw) was added to four of the fourteen beakers in each of the temperature treatments to achieve a final concentration of $5*10^4$ cells ml^{-1} (NW+Tw). Diatom concentration was determined by a regression between fluorescence and cell number using a Coulter Counter ($r^2 =$ 0.98). An ANOVA was performed using JMP v. 5.0.1 software (SAS Institute) to test for differences in ID between NW and NW+Tw.

The incubation beakers were maintained in their respective environmental chambers under a screen to reduce light to ~20% incident levels. Once daily, 80% of the water in each beaker was replaced with 200-µm filtered Choptank River water, with care not to pour out the copepods, and *T. weissflogii* added to the appropriate beakers. Copepods in the 27.5ºC and 22.6ºC treatments were checked under a dissecting microscope at least every 5 hours for survival and the presence of eggs. Copepods in the 5ºC and 13.6ºC treatments were checked at least every 8 hours. When the last egg hatched from an egg mass was observed, a female was noted as being nongravid (in the ID phase) and was considered nongravid until the first egg of the next egg mass appeared. Any dead male or female copepods, were replaced using *E. affinis* cultures maintained at the same temperature and food rations.

Mortality was high at 27.5ºC, and egg development data was collected for only four

copepod pairs in the NW treatment and two pairs in the NW+Tw treatment.

Measured egg production versus model predictions

Eurytemora affinis were collected in 2002 (Chesapeake Bay) and 2003

(Choptank River) using a plankton net with a 200 μm mesh. Egg production rates

estimated by the E/A and CS methods were compared to three copepod growth

models. The Huntley and Lopez (1992) model assumes that the weight-specific

growth rate (G, d^{-1}) of copepods is solely dependant on temperature (T, in ºC) and

using published growth rates at different temperatures, they developed the model:

$$G=0.0445e^{(0.111T)}.$$

This is the simplest of the three models, as it is independent of copepod size and food

concentration.

Ikeda and Motoda (1978) developed a copepod growth model predicting G in

terms of habitat temperature (T) and individual copepod dry weight in mg (W) and in

μg carbon (W_c):

$$G=\frac{7.714\times10^{(0.02538T-0.125)}\times W^{(-0.01089T-0.818)}}{W_c}.$$

This growth equation is derived from an estimate of respiration that is

converted to carbon using a 0.8 respiratory quotient. This model assumes an

assimilation efficiency of 0.7 (Conover 1978) and a gross growth efficiency of 0.3

(Roman et al. 2000, Ikeda and Motoda 1978, Hirst and Sheader 1997).

Copepod W (mg) for the Ikeda-Motoda model was calculated from the

equation

$$Log(W)=2.11\times Log(PL)-5.29 \quad ,$$

where PL is prosome length in μm (Böttger and Schnack 1986). Carbon weight (in μg) was calculated using the equation

$$C=6.25\times(PL/1000)^{2.83} \quad ,$$

determined by Kankaala and Johansson (1986).

The third growth model is a multiple linear regression developed by Hirst and Sheader (1997):

$$\log_{10}(g)=0.0246T-(0.2962\log_{10}(W_c))-1.1355$$

with temperature (T) and copepod body carbon (W_c)

This model was developed with a meta-analysis approach using multiple copepod species growth data collected from the poles to the tropics. As copepod mass (W_c) increases this model predicts reduced growth rates.

These growth models were developed using primarily somatic growth data. Comparing them to growth data derived from egg production measurements allows for 1) a comparison between the E/A and CS methods for estimating egg production and 2) a verification of the use of EP as a proxy for somatic growth, which is a common use for EP estimates (Runge and Roff 2000).

RESULTS

The interclutch duration experiments indicated that ID has a strong negative power relationship with temperature that is similar to Ban's (1994) data (Fig. 2), and is described by the equation

$$ID=161.62\times T^{-1.523}.$$

Interclutch duration is apparently not food limited, as the NW+Tw treatments did not differ from NW treatments (Fig. 3). Therefore, NW+Tw treatment data with NW data were grouped in the analysis of ID versus temperature (Fig. 2). Summing HT (from Andersen and Nielsen 1997) and ID (from the present study) and plotting versus temperature gives the equation

$$TC=169.07\times T^{-1.3114},$$

where TC = total reproductive cycle length (Fig. 4).

The, 2002 weight-specific EP values for Chesapeake Bay fit each of the three growth models better than did the 2003 EP data from the Choptank River, and CS-based estimates have higher coefficients of determination than do E/A-based calculations when plotted versus model predictions (Table 1). The Huntley-Lopez (1992) temperature-based model demonstrates greater EP predictability for 2002 when the CS method is used compared to the E/A method; the 2003 regressions for both methods are not significant (Table 1). The Huntley-Lopez model is a slightly better EP predictor for these data than is the Ikeda-Motoda model, although the coefficients of determination for the two are similar. In 2002, the CS method greatly improves the model fit over the E/A approach for the Ikeda-Motoda model (Table 1). The Hirst-Sheader model shows the best fit for all data and is the only model that significantly fits any 2003 data (CS-derived EP; Table 1, Figure 5).

CS-derived EP was calculated using data from Ban (1994) and Crawford and Daborn (1986) and plotted these with 2002 and 2003 data (Figure 5). The Crawford and Daborn (1986) EP values were calculated from measurements of *E. herdmani*. The three data points from Ban (1994) were measured under satiating food conditions

and complement the 2002 data. With the Ban (1994) and 2002 data sets plotted together, the slope between actual EP and modeled EP is 0.90. A comparison of EP estimates derived from the E/A method versus the CS method suggests that as EP increases, the E/A method provides slightly higher EP estimates than the CS method (Figure 6).

DISCUSSION

Previous research suggests that temperature is the primary variable affecting the time components of the brooding copepod reproductive cycle. Hatching time is probably not affected by food limitation because the eggs are already formed and extruded at the beginning of the measurement. Ban (1994) demonstrated that ID remains relatively stable at a given temperature until food concentrations are nearly nonexistent. The addition of *T. weissflogii* had no effect on ID in the *E. affinis* used in our experiments, supporting the idea that ID was not generally food limited.

In field studies, *E. affinis* clutch sizes as well as female size tend to decrease with increasing temperature (Ban 1994, Hirche 1992). Thus, temperature effects are confounded by the strong relationship between female size and clutch size. It is possible that under satiating food conditions, *E. affinis* of a given size is 'programmed' to produce an egg clutch of particular number regardless of temperature. Therefore, laboratory estimates of ID may not reflect actual ID in the field if the copepods being incubated at the different temperatures are the same size, because larger clutches may take longer to produce. If this is the case, then ID values for this experiment may be overestimated for temperatures >10ºC and slightly

underestimated for temperatures <10ºC because all females used in the experiments were collected at ~10ºC, and their sizes (and thus clutch sizes) would be expected to represent the temperature of collection (mean female prosome ± SD = 935.1 ± 80.3 μm) instead of their incubation temperatures. Ban (1994) used *E. affinis* hatched and raised at the experimental temperatures, and his ID patterns are similar to those of this study (Figure 3). Any size effect on *E. affinis* clutch size appears to exert a minimal control on ID. Therefore, the ID-temperature relationship can be used for field estimates of EP when using the clutch size method.

The Ikeda-Motoda model (incorporating temperature and copepod weight) is the most complex of the three models, but predicts G (= EP) no better than the Huntley-Lopez model (incorporating temperature only). This is likely because the Ikeda-Motoda model is a more indirect estimate of EP, having a number of assumptions and calculations prior to calculating a growth value. The high predictability of the Huntley-Lopez model is due in part to the indirect incorporation of female size into the calculation. Generally, water temperature is similar during both an individual's somatic development and its reproductive period, and the temperature value in the equation accounts for both of these factors. Both models also rely on a temperature-based power function, which may also add to their similarity.

The Hirst-Sheader model is a multiple linear regression using log-transformed data to create linear relationships between predictors (temperature and copepod weight) and the regressor (G). This model provides the best fit for all data regardless of year or EP estimation method but is not better than the Huntley-Lopez model. The

W_c in this dataset covers a size range of 3.9 μg, while the Hirst-Sheader model covers a W_c size range of 511 μg. Thus, the W_c range in this study's *E. affinis* dataset may simply be too small to capture the effects of W_c on EP.

There were large differences in the model fits between 2002 (Chesapeake Bay) and 2003 (Choptank River; Figure 5). Preliminary analysis of available food in both of these regions suggests that these animals were not food limited (Chapter 3). 2002 was a warm, dry year compared to 2003, which had unseasonably cool temperatures and was one of the wettest years on record in this region (http://water.usgs.gov/). *Eurytemora affinis* flourishes in cool, wet conditions (Kimmel and Roman 2004), and abundances in both systems were notably higher in 2003 than in 2002. Enhanced predation on large, gravid *E. affinis* females in 2003 by greater number of anadromous fish larvae may increase the proportion of smaller females and/or smaller clutch sizes in a population, which would cause underestimates of mean clutch size, the egg-ratio, and EP. Whatever the cause of the year/system differences, all three models predict CS-derived EP estimates better than EP estimated by the traditional E/A approach. I propose that the CS method be included in any study measuring brooding copepod EP along with either E/A estimates or incubation measurements to allow for further comparisons of the various methods of EP estimation. Specifically, the equation for TC can be used to estimate *E. affinis* EP once a mean sample clutch size of fully-formed clutches is determined:

$$EP = \frac{\overline{CS}}{TC} \ .$$

The relationship between E/A-derived EP and CS-derived EP supports the idea that the E/A may provide overestimates of EP. The tendency for *E. affinis* to be found

near the bottom where they are often not sampled, suggests that gravid females would be undersampled and EP thus underestimated.

Not all *E. affinis* populations appear to have the same innate reproductive time components (Andersen and Nielsen 1997). Ban (1994) in Japan and Vijverberg (1980) in the Netherlands found different ID values versus temperature for *E. affinis*. Andersen and Nielsen (1997) point out that rate differences between populations of a species are often far fewer than between species. Therefore, extrapolating ID values from one population to another for a particular species can be justified. Ideally, estimates of ID versus temperature for a species will include data from many different sub-populations.

A large potential error in the clutch-size method of EP estimation is from the determination of mean CS. The standard deviations of *E. affinis* clutch size sample means from Chesapeake Bay represent from 25 to 50% of the means and are influenced by female prosome variability, whose standard deviations vary by up to 10% of the mean. If incomplete egg masses are used to estimate mean CS, EP will be underestimated. Under food-saturating conditions, the relationship between CS and prosome length (PL) in Chesapeake Bay is linear and has an r^2 of 0.85. A complete or nearly complete clutch can also be identified by its hemispheric appearance (Fig. 7). If food is limiting, clutch size would still be expected to be related to prosome length but would have a lower mean number of eggs.

Because these growth models were developed using primarily juvenile growth data, *E. affinis* EP (an easily measured rate) appears to provide a good estimate for *E. affinis* somatic growth (a more difficult rate to measure) in Chesapeake Bay. Hirst

and Sheader (1997) performed a meta-analysis using their multiple regression model and literature values that included copepod juvenile growth and egg production, obtaining an r^2 of 0.64 ($r^2 = 0.74$ for juvenile growth data only). The coefficient of determination using the same model with CS-derived *E. affinis* EP data was 0.88 for 2002 in Chesapeake Bay. All three growth models provide accurate estimates of *E. affinis* EP in 2002 and can be used to predict EP, with the Hirst-Sheader model providing the best fit. Copepods are not usually food limited in Chesapeake Bay (White and Roman 1992), and, under such conditions, reproductive rates should serve as reliable proxies for somatic growth (Hopcroft and Roff 1998). This research supports the use of egg production rates as a measure for juvenile growth for *E. affinis* in Chesapeake Bay.

Similar to hatching time, interclutch duration appears to be primarily affected by temperature. The addition of the diatom supplement did not have any effect on *E. affinis* interclutch duration, suggesting that this time component was not food-limited in this experiment. Clutch size-derived EP estimates provided a better fit to the copepod growth models than the egg-ratio estimates. The data also suggest that gravid females were slightly oversampled compared to the rest of the population during high production periods (usually late spring, early summer), i.e., the sample E/A is greater than population E/A (see Figure 6). As noted above, the vertical depth separation often observed between gravid and nongravid *E. affinis* does not support the use of the E/A method in estuarine systems (Kimmerer et al. 1998, Castonguay and FitzGerald 1990).

The better fit to the independent growth models as well as avoiding sampling errors associated with the E/A method, support the use of the clutch size method in addition to the egg-ratio method for estimating brooding copepod reproductive rates. Continued use of the egg-ratio method will allow future data to be compared to previous studies of copepod egg production. Caution should be taken when estimating EP with the clutch size method under low food conditions, as laboratory estimates of ID may not be representative of field ID. Further, it must be noted that because of the poor fit of the 2003 data to growth models, indirect methods of estimating copepod egg production, namely the egg-ratio method and clutch size method, may not always be appropriate, and incubations may provide better estimates.

Egg production rates have often been used to estimate somatic growth due to the general ease of determining EP (Berggreen et al. 1988). This approach assumes that any resources that would go into somatic growth for juveniles would be allocated to reproductive growth in adults. Our observations in the Chesapeake Bay suggest growth of *E.* affinis based on egg production can be used to estimate juvenile somatic growth.

Table 3.1. Results of linear regressions performed between model outputs of egg production (EP) versus EP estimates by the egg-ratio (E/A) and clutch size (CS) methods. m = regression slope; b = Y-intercept.

Year	Model	EP method	m	b	r^2
2002	Huntley and Lopez (1992)	E/A	1.44	0.014	0.54**
		CS	2.61	-0.16	0.82***
	Ikeda and Motoda (1978)	E/A	1.41	-0.045	0.52**
		CS	2.64	-0.18	0.78***
	Hirst and Sheader (1997)	E/A	0.742	-0.36	0.62**
		CS	0.99	-0.13	0.84***
2003	Huntley and Lopez (1992)	E/A	0.37	0.29	0.06
		CS	1.58	0.10	0.15
	Ikeda and Motoda (1978)	E/A	0.31	0.20	0.07
		CS	1.39	0.04	0.17
	Hirst and Sheader (1997)	E/A	0.20	-0.71	0.09
		CS	0.32	-0.59	0.32*

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Hatching Time
(<2 days at 25°C,
>14 days at 5°C)

First egg of new
clutch extruded

Last egg of clutch
hatches/falls off

Interclutch Duration
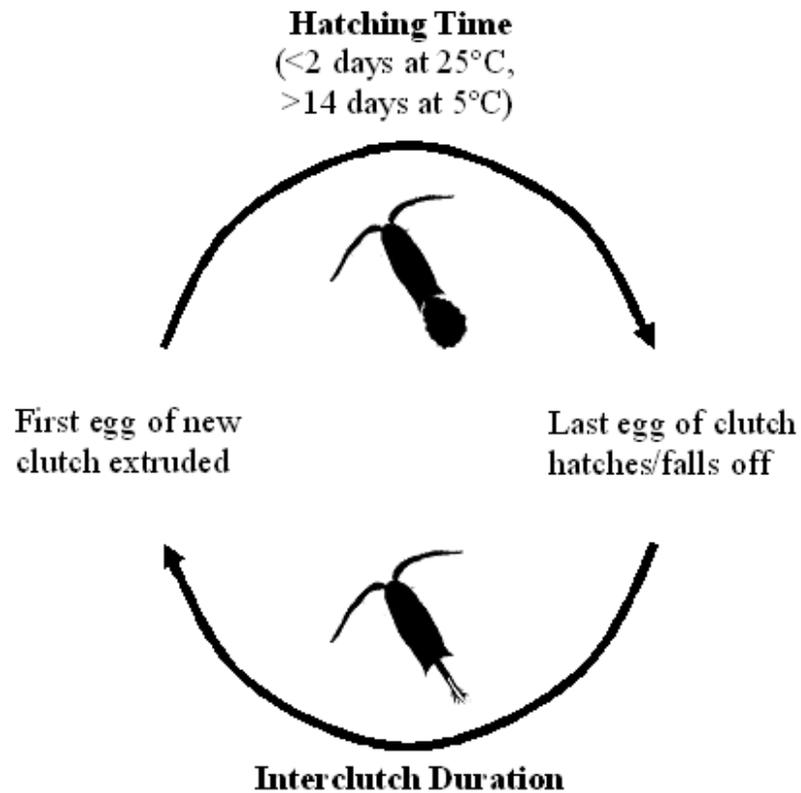
Figure 3.1. Conceptual diagram of reproductive cycle of *E. affinis* showing the two
major time components, hatching time and interclutch duration. Hatching time is
primarily influenced by temperature, and a similar relationship with temperature is
presumed for interclutch duration.
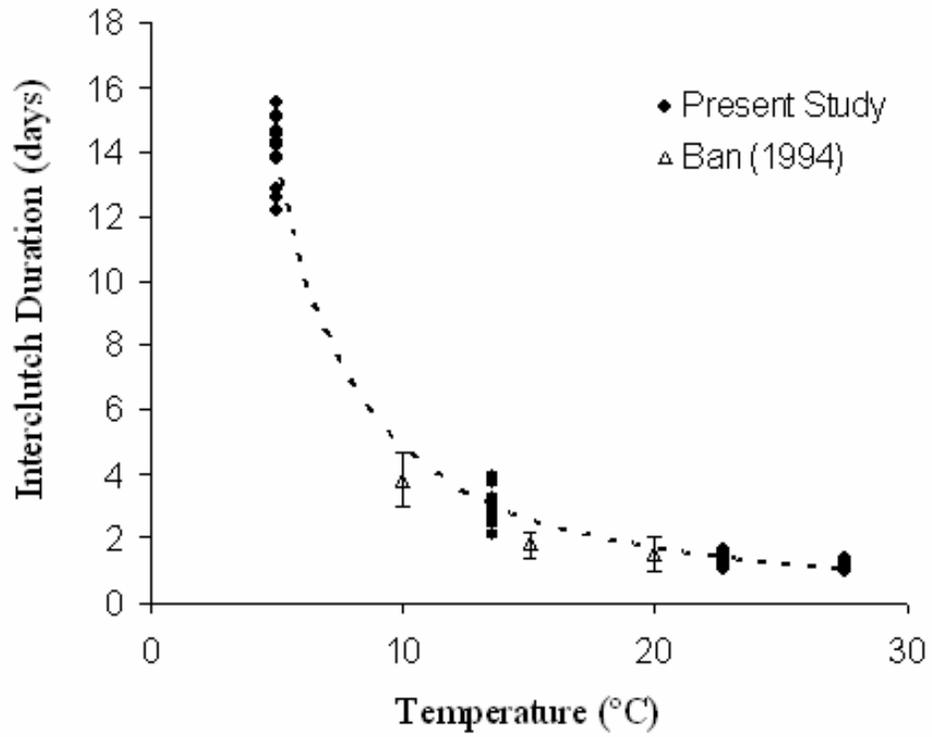
Figure 3.2. Relationship between interclutch duration (days) and temperature (°C). Ban (1994) data ± 1 SD. Fitted power function r^2=0.98 (does not include Ban (1994) data).
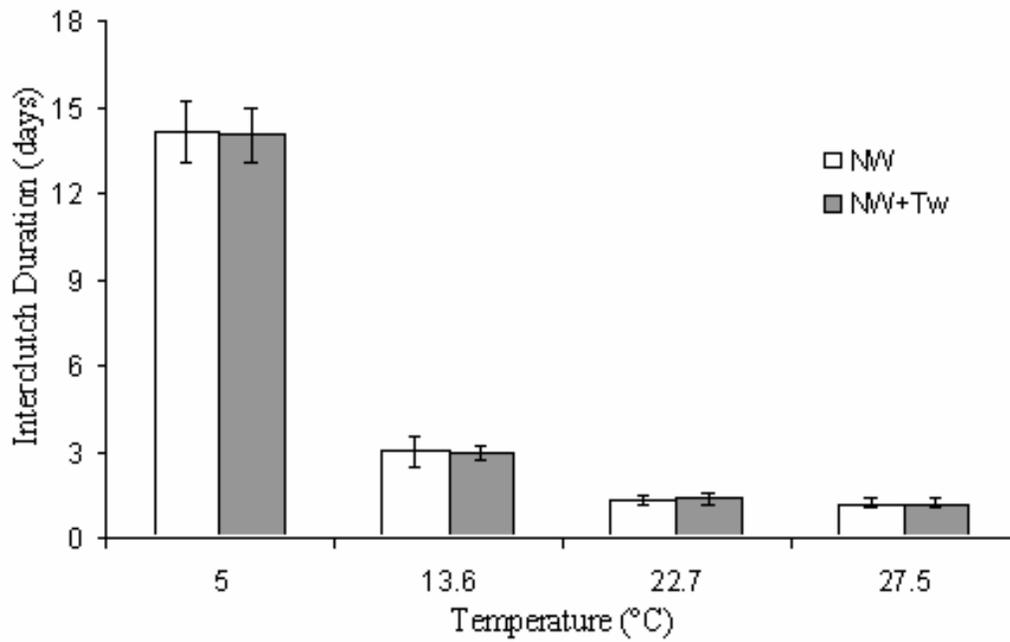
Figure 3.3. Comparison of Interclutch Durations for copepods fed 200-μm filtered Choptank River water (NW) and those fed Choptank River water plus a *Thalassiosira weissflogii* supplement (NW+Tw) at the four experimental temperatures.
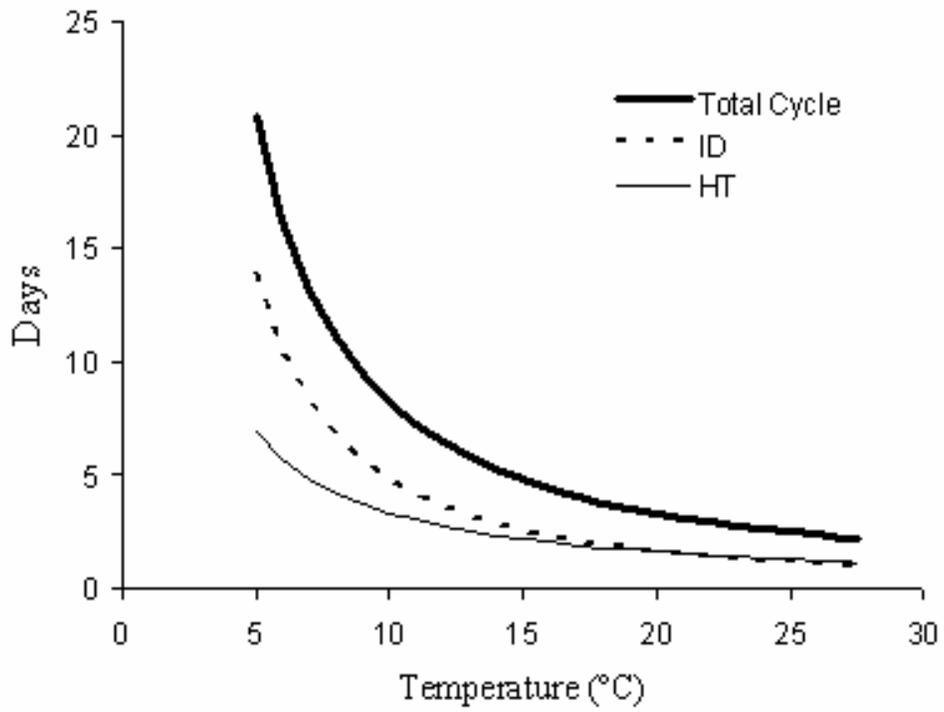
Figure 3.4. Graphical representation of the time components of *E. affinis*' reproductive cycle. HT = hatching time; ID = interclutch duration; TC = total cycle length (sum of HT and ID).
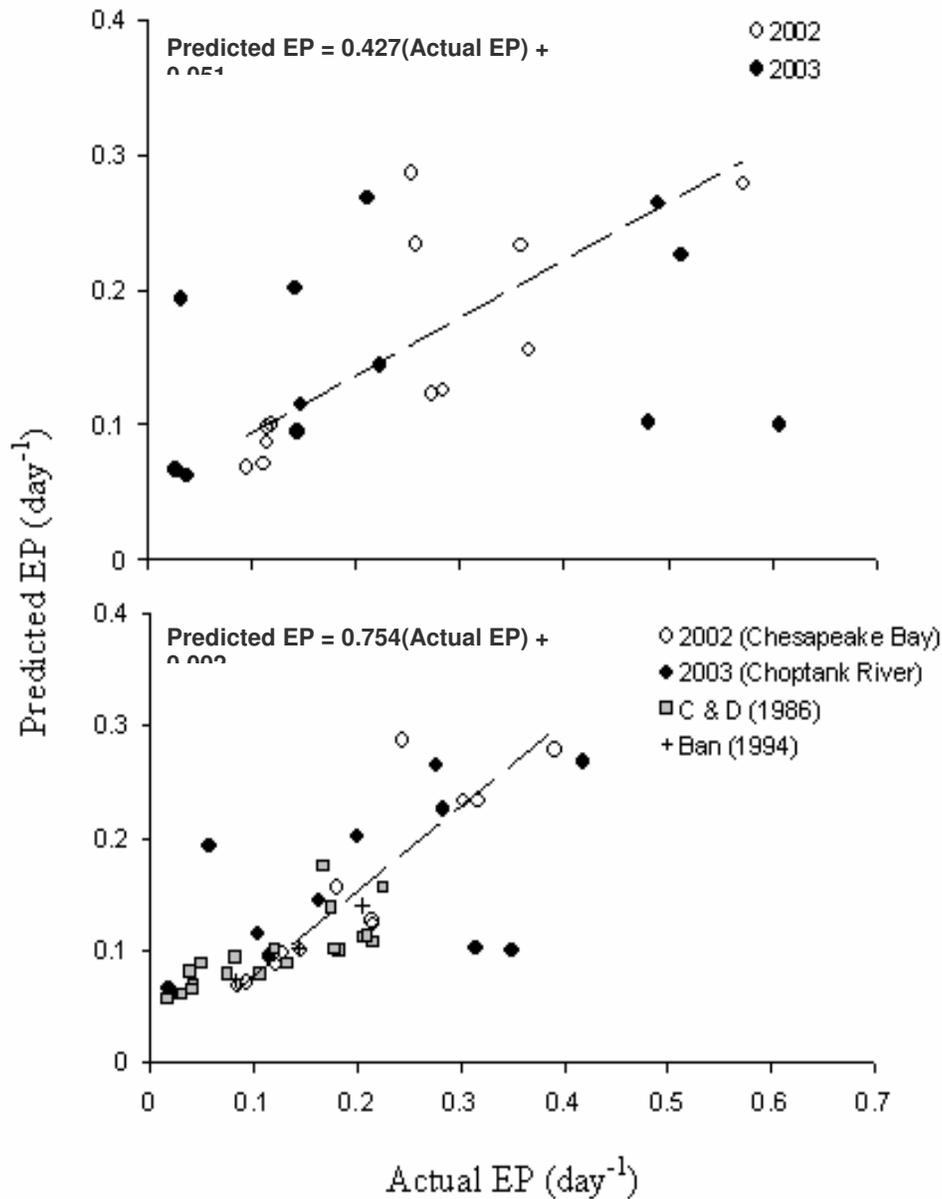
Figure 3.5. Hirst-Sheader (H-S) model weight-specific EP predictions versus actual weight-specific EP. (a) Actual EP values calculated by the egg-ratio (E/A) method. The dashed line is the linear fit to of H-S model output to 2002 data. 2003 data versus predicted values was not significant. (b) Actual EP values calculated by the mean clutch size (CS) method. The dashed line is the linear fit to of H-S model output to 2002 data. Linear fits to both 2002 and 2003 data were significant. 'C & D (1986)' = Crawford and Daborn (1986). See Table 1 for all linear regression statistics.

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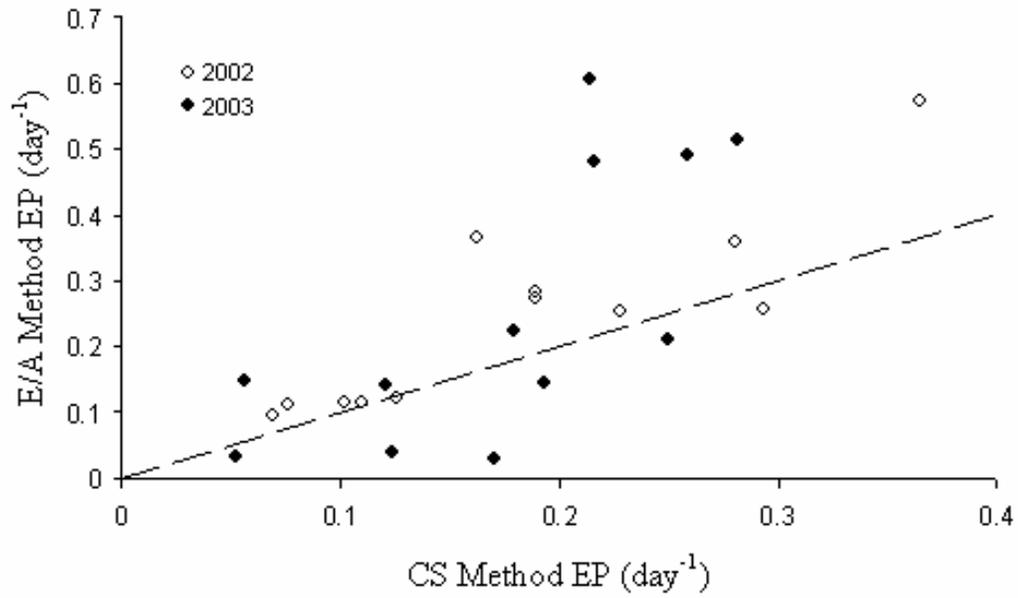
Figure 3.6. Comparison of EP rates estimated by the egg-ration method (E/A) and the mean clutch size method (CS). Dashed line is the 1:1 relationship.

Figure 3.7. Dorso-lateral view of gravid *E. affinis* showing hemispheric nature of the egg mass. The two darker regions of the egg mass (em) are separated by the cleft (light-colored region) below the attached spermatophores (sp). The urosome (ur) is lifted dorsally in this photograph but would normally rest in the cleft region.

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CHAPTER IV: Egg production and grazing by the copepod Eurytemora affinis in the Chesapeake Bay estuarine turbidity maximum

ABSTRACT

The calanoid copepod *Eurytemora affinis* tends to exhibit maximum abundance in the estuarine turbidity maxima (ETMs) of Chesapeake Bayand in estuaries around the world. Autotrophic production is generally low in these turbid regions due to light limitation, yet ETMs usually contain high abundances of zooplankton. In this study, egg clutch size and grazing measurements of *E. affinis* were conducted to help understand why this copepod species reaches maximum abundance within ETM regions. The comparison of clutch size and numerous biotic and abiotic variables revealed that *E. affinis* egg production does not appear to be food limited in the Chesapeake Bay or Choptank River ETMs because prosome length and not any measure of food quantity or quality was the best predictor for egg clutch size. Regression tree analysis using residuals from the prosome versus clutch size regression suggested that salinity had a slight positive influence on clutch size in 2002 (Chesapeake Bay; a dry year), while food quality may have had a greater influence on clutch size in 2003 (Choptank River; a wet year). Grazing measurements indicated that *E. affinis* ingests autotrophs (<20 μm equivalent spherical diameter) at higher rates inside the ETM than outside, but grazes on >20 ESD protists at higher rates outside the ETM. Neither of these sources, however, sufficiently accounted for total grazing by *E. affinis*. The copepod, *E. affinis,* likely obtains a majority of its ingested carbon from additional sources which were not quantified, such as free-living nanoplankton and detrital communities. Benefits of

living in the ETM for *E. affinis* likely include decreased interspecific interactions (competitive and predator-prey) and abundant food resources (due to *E. affinis*' particle selection ability).

INTRODUCTION

The estuarine turbidity maximum (ETM) regions of the main stem of Chesapeake Bay and the Choptank River (Fig. 1), located near the limit of saltwater intrusion, are traditionally characterized by high total suspended solid (TSS) concentrations, high light attenuation, and high densities of zooplankton and anadromous fish larvae (North and Houde 1999, Roman *et al.* 2001). Phytoplankton productivity is generally low in ETM regions due to light limitation (Wofsy 1983, Kemp and Boynton 1984, Fisher *et al.* 1988). Therefore, food webs in ETMs tend to be based on allochthonous detrital inputs, with important microbial components (Irigoien and Castel 1997, Crump *et al.* 1998, North and Houde 2001). Abundances of the epibenthic copepod *Eurytemora affinis* peak in the Chesapeake Bay ETM region and in ETMs worldwide (Fig. 2; Heinle and Flemer 1975, Hough and Naylor 1992, Gasparini *et al.* 1999, Kimmel and Roman 2004). *Eurytemora affinis* is omnivorous, feeding on phytoplankton (Vijverberg 1980, Gasparini and Castel 1997), detritus (Heinle *et al.* 1977), protozoa (Berk *et al.* 1978, Merrell and Stoecker 1998), and bacteria (Boak and Goulder 1983).

Assuming that *E. affinis* is retained within the ETM by either passive or active means, greater egg production rates (EPr) within the ETM versus up- and downstream of the ETM will increase population size. Higher EPr within the ETM

would suggest that the ETM is a suitable nutritional environment for copepods. Previous research has demonstrated that copepod egg production rates respond positively to increases in food quality. For example, in the Gironde estuary, *E. affinis* EPr increases with a higher nutritional quotient (NQ; carbohydrates+proteins+lipids:TSS) and with the ratio of chl-*a* to TSS (Burdloff *et al.* 2000). Gasparini *et al.* (1999) demonstrated that high TSS values in the Gironde estuary decreased ingestion of chl-*a* by *E. affinis* and suggested that this likely has an adverse effect on its EPr. In *Acartia* spp., egg production has been negatively correlated to seston C:N (Ambler 1986) and positively correlated with the protein and specific fatty acid concentrations of seston (Jónasdóttir 1994, Jónasdóttir *et al.* 1995), microzooplankton abundance (White and Roman 1992), and chl-*a*:TSS values (Burdloff *et al.* 2002).

This study compared *E. affinis* egg clutch sizes throughout the Chesapeake Bay (2002) and Choptank River (2003) ETM regions with biotic and abiotic variables to determine 1) if EPr are consistently higher in regions of high turbidity compared to up- or down-estuary of these areas, and 2) what factors, if any, might limit egg production. I also conducted grazing experiment to determine the diets of *E. affinis* in the ETM and down-estuary from the ETM.

MATERIALS AND METHODS

Egg production

Field work. I conducted five cruises in 2002 and six in 2003 into the ETM regions of Chesapeake Bay (2002) and the Choptank River (2003), a tributary of

Chesapeake Bay (Fig. 1). I selected stations downstream, within, and upstream of the ETM in each location based on salinity and turbidity profiles measured by a Seabird CTD (Chesapeake Bay) or a YSI Salinometer with a weighted sensor (Choptank River). Water samples were collected at mid-depth at each station using either a Niskin bottle or a submersible pump for analysis of chlorophyll-*a* (chl-*a*), phaeophytin, total suspended solids (TSS), carbon (C), nitrogen (N), total carbohydrates (CH_2O), total protein, and total lipids. *Eurytemora affinis* was collected by replicate plankton tows from the bottom to the surface with a 64-μm mesh size plankton net, 0.5 m in diameter, equipped with a General Oceans flowmeter. Zooplankton samples were immediately fixed in a 5% formalin solution for later processing.

Seston analysis. Water samples collected for seston analyses were first sieved through a 64-μm sieve and the filtrate collected. The resulting maximum particle size in the filtrate (< 64 μm) was expected to correspond to published values of the maximum particle size available for *E. affinis* grazing (Richman *et al.* 1977). Filtrate from each station was filtered onto either uncombusted (chl-*a,* phaeophytin, and TSS) Whatman GF/F filters or GF/F filters that had been combusted at 450ºC for 4 hours (C, N, CH_2O, protein, and lipids). Chl-*a* and phaeophytin values were determined fluorometrically using the methods of Parsons and Strickland (1963). I calculated TSS by drying a filter in a 60ºC oven to a constant weight and subtracting the initial weight of the filter. Filters for organic C and N analysis were dried at 60ºC and analyzed on an Exeter Analytical Inc. CE-440 Elemental Analyzer. Carbohydrates were determined spectrophotometrically using an Hitachi U-3110 spectrophotometer

following the protocol of Dubois *et al.* (1956). Proteins were determined using bicinchoninic acid protein assay reagent (Smith *et al.* 1985) following the addition of sodium deoxycholate, which aids in protein release from filters (Nguyen and Harvey, 1994). Protein concentrations were then estimated on a Hitachi U-3110 spectrophotometer using BSA as a standard at an absorbance of 560 nm. Lipids were determined gravimetrically by extraction in a 1 CH_2CL_2:1 MeOH (vol/vol) solution, immersing the capped extraction tube in an ice-cold sonicating bath for 10 min., then following the Bligh and Dyer (1959) protocol.

     Zooplankton processing. Mesozooplankton samples were subsampled using a plankton splitter and 2 to 3 subsamples counted using a dissecting microscope. All *E. affinis* eggs, females (gravid and nongravid), and males were counted from each subsample (usually totaling ~100 copepods/subsample), and all gravid female prosome lengths were measured with an eyepiece micrometer. Female *E. affinis* C was calculated based on an equation by Kankaala and Johansson (1986) relating prosome length to C content:

$$\text{Female C} = 6.25 \times \text{Pro}^{2.83},$$

where Pro is prosome length (in mm). Egg numbers were multiplied by 0.048 to give egg C (Heinle and Flemer 1975, Hirche 1992). Clutch sizes were converted to egg production using the method of Lloyd and Roman (see Chapter 1). Briefly, the mean sizes of complete clutches were calculated for each station and divided by the sum of hatching time (HT) and interclutch duration (ID). Both of these time components are temperature dependant, with

$$HT = 36.8 \times T^{-1.04}, \text{ and}$$

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$$ID = 161.62 \times T^{-1.523}.$$

Statistical analyses. I used JMP v. 5.0.1a (SAS Institute, Inc.) statistical software to perform linear regression and regression trees analyses on the biotic and abiotic factors affecting *E. affinis* clutch size. Regression trees split data at terminal nodes (inflection points between the dependent and a predictor variable) based on a single predictor variable, creating two groups that are both homogenous as possible with a minimum sum of squares (De'ath and Fabricius 2000). The data can be split as many times as there are predictor variables, but, as the number of splits increases, the less likely it is that a relationship exits between the predictor and dependent variable.

I chose to use regression tree analysis over multiple regression because the independent variables were often related, which violates an assumption of multiple regression analysis. Further, regression trees are a more exploratory method for determining relationships of multiple factors on a single regressor and are often easier to interpret due to their graphical nature (Dzeroski and Drumm 2003). Due to limited output, regression trees can be limiting in their ability to provide a predictive model. They also are not capable of incorporating strong linear relationships (De'ath and Fabricius 2000), so I used the residuals of a linear regression between clutch size (dependent variable) and female prosome length (independent variable) in the analyses.

Food concentration and growth calculations. Using equations derived by Huntley and Boyd (1984), I calculated both maintenance food concentrations (C_m; copepod carbon assimilation equals respiratory demands) and the critical food

concentration (C_c), above which copepod growth is assumed not to be food limited. These values are based solely on carbon concentrations and therefore must be analyzed cautiously, given that 1) all particulate carbon in the seston may not be available for copepod consumption and/or assimilation (Huntley and Boyd 1984), and 2) copepod reproductive and somatic growth often depends on food quality and not just the gross amount of carbon (Jónasdóttir *et al.* 1995, Burdloff *et al.* 2000). These calculations assume that carbon (food) requirements for copepods are based on dry body mass (W, in mg) and temperature, which is incorporated into several variables (b, k, m, and n), and that copepods have an assimilation efficiency (mass of carbon passing through the gut wall into the body / total mass of ingested carbon) of 0.7 (Conover 1966).

The equation for maintenance carbon concentration is:

$$C_m = \frac{kW^{(m-n)}}{ab},$$

where k is the respiratory coefficient, W is the individual dry weight in mg, m is the respiratory exponent, n is the clearance rate exponent, a is the assimilation efficiency (0.7), and b is the clearance rate coefficient. The derivations of these variables are explained in Huntley and Boyd (1984). The food concentration at which growth, and presumably reproduction (Hopcroft and Roff 1998), is not limiting is explained by the equation:

$$C_c = \frac{G_{hour}}{abW^{(n-1)}},$$

where the variables are as described above (Huntley and Boyd 1984). G_{hour} is a variable that represents the maximum hourly weight- or carbon-specific growth rate, assuming that food is replete.

I also compared our measured egg production rates with the values obtained from the Huntley and Boyd (1984) equation for egg production under food-replete conditions, with temperature (T) as the only variable:

$$G_{daily} = 0.0542e^{(0.110T)}.$$

Both of these approaches (calculating carbon requirements and growth under food-replete conditions) allow us to examine egg production rates in terms of food limitation.

Grazing experiments

Experimental design. I performed the grazing experiment in August, 2003, following the grazing experiment protocol as described by Gifford (1993). *Eurytemora affinis* used in grazing experiments were collected from the ETM of the Choptank River (Fig. 1; 0.1 salinity, 21.8°C), by plankton tows with a 200 μm plankton net. The contents of the cod end were gently poured into a carboy containing unfiltered water from the same site. Water from the surface mixed layer was collected from both the ETM station and a site several kilometers downstream from the ETM. The water from both of these sites was poured through a 200 μm sieve to be used in the grazing experiments.

Grazing experiments were performed by incubating *E. affinis* in 250 ml bottles containing 200-μm-filtered seawater from either the ETM site or the non-ETM site. In cases where copepods were incubated in the water of a different site from

which they were collected, the copepods were acclimated at an intermediate salinity for approximately 1 hour. Active, adult females were hand picked with a Pasteur pipette using a dissecting microscope and placed into their respective experimental bottles, with each bottle containing a total of ten individuals. There were three replicate bottles for each treatment combination, giving a total of 12 bottles (3 bottles containing *E. affinis* in ETM water, 3 bottles containing *E. affinis* in non-ETM water, 3 bottles containing *A. tonsa* in ETM water, 3 bottles containing *A. tonsa* in non-ETM water). Additionally, there were four sets of control bottles which included no copepods (i.e. ETM t_0 control, ETM t_{24} control, non-ETM t_0 control, non-ETM t_{24} control) with 3 replicates each.

The sealed bottles were transferred to an environmental chamber at 20ºC and loaded onto a plankton wheel set for 2 RPM for 24 hours. The control t_0 bottles were harvested after 1 h following the start of the experiment to allow for the dissipation of any microplankton killed or damaged prior to the beginning of the experiment, while all other bottles (control and treatment) were collected after 24 hours. The contents from the treatment bottles were gently poured separately through a 64 um sieve to recover the copepods. The contents of both sets of control bottles were treated in the same manner. Water samples from all control and experimental bottles were preserved in 10% (v/v) acid Lugol's solution for later enumeration (Parsons et al. 1984). All preserved samples were refrigerated in the dark until analysis.

Copepod gut fluorescence was measured according to Bamstedt *et. al* (2000) after the 24 h grazing period. The copepods were filtered onto Whatman GF/F filters which were then transferred into centrifuge tubes containing 5 ml of 90% acetone.

The tubes were kept dark and were placed in a freezer. After 24 h, the tubes were shaken, vortexed, and centrifuged. Fluorescence was measured with a Turner fluorometer and converted to chlorophyll-*a* concentration using a chlorophyll-*a* versus fluorescence regression.

Microscopic enumeration. The water samples preserved in Lugol's solution were concentrated by settling 10 ml subsamples in Utermöhl chambers. All samples were enumerated with an inverted microscope at 200x magnification. Microplankton between 10 µm and 200 µm were counted for subsamples from all bottles, including the t_0 controls and t_{24} controls. Pico- and nanoplankton were not evaluated. Cells were grouped according to size (10-20 µm or >20 µm) into the following prey categories: Aloricate ciliates, dinoflagellates, diatoms, loricate ciliates, or other autotrophs (e.g. cryptophytes, colonial algae, desmids).

Calculations and statistics. Prey carbon content was calculated from cell volumes and converted to carbon using the equations of Montagnes *et al.* (1994) and Menden-Deuer and Lessard (2000). Carbon-specific gut fluorescence was measured following the protocol of Barquero *et al.* (1998), and chlorophyll-*a* concentrations were converted to carbon using a 25:1 carbon to chlorophyll-*a* ratio (Steele and Baird 1961, Steele and Baird 1962, Landry *et al.* 1995).

Microscope cell counts of samples from the control bottles and bottles with copepods allowed carbon-specific prey ingestion rates (I, day^{-1}) to be determined by using the equations of Frost (1972). One-way ANOVA tests and multiple range tests (Tukey method) using SAS 8e 2000 software were performed to detect any significant ($p < 0.05$) differences between the treatment combinations for mean gut

content, carbon-specific ingestion rates, and percent abundance changes in the control bottles. Specific prey ingestion rates were analyzed with JMP 4 software using Student's t-test comparisons for each species by water type and each water type by species.

RESULTS

Seston and microplankton

In 2002 (Chesapeake Bay), POC and chl-*a* concentrations and the chl-*a*/TSS ratio were greatest during the early spring and decreased throughout the year (Table I). The nutritional quotient (NQ), however, was relatively stable during the sampling period and never fell below 0.02, twice as much as the 0.01 value determined to limit *E. affinis* egg production (Burdloff *et al.* 2000). The POC values in 2003 (Choptank River) showed a similar seasonal trend as in Chesapeake Bay, but the C:N ratio remained relatively constant over the year (Table I). The chl-*a*/TSS ratio in the Choptank River in 2003 followed the same trend as in Chesapeake Bay in 2002 (high in spring, generally decreasing throughout year).

Microplankton measurements were performed for the 2003 sampling year (Choptank River; Table II). Ciliate biomass peaked in the late spring, with large tintinnids dominating. With the exception of June, ciliate biomass remained at relatively high levels through mid-autumn. A *Prorocentrum minimum* bloom was evident in April and May. Besides a number of unidentified dinoflagellates, the only other dinoflagellate species of particular interest (in terms of biomass) were *Gyrodinium* spp. in July and *Heterocapsa* spp. in early spring and mid-autumn.

Diatom biomass peaked in early spring and dominated the microplankton community. In general, microplankton biomass was dominated by autotrophs (diatoms, dinoflagellates, and other autotrophic flagellates) in early spring, while ciliate biomass increased in late spring/early summer and remained high throughout the remainder of the year.

Egg production

A linear regression between clutch size (CS) and female prosome length (PL) explained 85% and 88% of clutch size variability for 2002 (Chesapeake Bay) and 2003 (Choptank River), respectively (Fig. 3). The 2002 regression tree using the residuals of CS versus PL as the regressor variable suggests that salinity was the major factor affecting CS residuals in the Chesapeake Bay ETM region that year, with all residual CS values being split at a salinity of 1.75 (Fig. 4). Although two nutritional variables are used in the second-level split of the data, chl-*a*/TSS (a measure of food quality) and N concentration, the mean values allotted to these nodes decrease with increasing chl-*a*/TSS and N values, suggesting that these variables do not affect CS. Therefore, I did not split the data further.

Although salinity is also the first split of the 2003 CS residuals, the node created at salinity < 0.3 contains about 4% of the data (Fig. 5). The second split is based on a C:N of 8.64, with higher C:N values having lower mean. The third level of splits is based on a calculated nutritional quotient (NQ), which is the sum of all particulate carbohydrates, proteins, and lipids (in μg), divided by TSS (in mg). Both nodes formed by the split of the "salinity ≥ 0.3" node show a positive relationship

between NQ and the CS residuals, as one would expect if there is a true effect of NQ on the residuals.

A regression tree for all 2002-2003 data shows a salinity value of 1.75 as the major variable describing CS residuals (Fig. 6). The "salinity< 1.75" node, is then split in terms of C:N, with C:N values > 8.2 having a lower CS residual mean then values < 8.2. The split of the "salinity > 1.75" node suggests for stations with an NQ ≥ 0.037, residual CS values are lower than when NQ < 0.037. Similar to the second split of the 2002 regression, this is likely a function of working with such a small variance, as one would not expect CS residuals to decrease with an increasing NQ.

Prosome length was significantly related to water temperature two weeks prior to the prosome measurement (Fig. 7). Weight-specific egg production rates for *E. affinis* generally increased with temperature in the Chesapeake Bay ETM region (2002 but showed no relationship with *in-situ* temperature in the Choptank River (2003;Fig 8). Hirche (1992) developed a curve representing *E. affinis* egg production rates at saturating food concentrations versus temperature. The Chesapeake Bay (2002) data tended to fall along this line in the spring and fall, but in the summer the model overpredicts egg production rates, suggesting food limitation. The 2003 data is more variable compared to 2002 and was egg production was also overpredicted by the model at warmer temperatures (Fig 9).

Burdloff et al. (2000) determined that the NQ limiting copepod egg production is around 0.010. Our minimum NQ was 0.016, suggesting that food quality should not limit egg production rates in the Chesapeake Bay or Choptank River ETMs.

Particulate organic carbon concentrations and model comparison

Particulate organic carbon (POC) concentrations were usually an order of magnitude greater than those concentrations calculated to be the threshold between food-limited and non-food-limited growth (Fig. 10). *Eurytemora affinis* growth, and presumably reproduction, should not be limited by food quantity in the Chesapeake Bay and Choptank Rive ETM regions.

Measured reproductive rates compared to Huntley and Boyd (1984) model outputs assuming food-replete conditions suggests that the measured rates (egg production) are 2.5 times greater than modeled rates (Figure 11). When only the 2002 data is used, the model explains 82% of the variance but under-estimates actual values; with both 2002 and 2003 data used, the r^2 is nearly cut in half to 0.43 (Figure 11).

Grazing experiment

Eurytemora affinis ingestion rates for the <20 μm size fraction are lower in non-ETM water than in the ETM water (Table III). The only positive ingestion rate in this size class is for the autotroph group in ETM water, which includes colonial algae, desmids, cryptophytes, and other autotrophic flagellates, and this ingestion rate in ETM water is significantly greater than in the non-ETM (Student's t-test, $p < 0.05$). This group is clearly an important food item for *E. affinis*, as the autotroph ingestion rates are greater than protozoa in either size class. In the >20 μm protozoan group, *E. affinis* fed more in the less turbid, non-ETM water type. Specifically, aloricate ciliates appeared to be the targeted prey type, with *E. affinis* individuals feeding on 24.11 cells (4.48 ng C) day^{-1} in the non-ETM water versus a negative grazing rate in

the ETM water. The only group in ETM water experiencing ingestion rates greater than in the non-ETM water were loricate ciliates, with 0.13 cells (0.40 ng C) consumed copepod^{-1} day^{-1}.

The copepod gut fluorescence measurements following the grazing experiments suggest that the grazing experiments can strongly underestimate carbon intake (Fig. 12). Further, there was no significant difference in gut fluorescence between the ETM and non-ETM treatments. Total C-specific grazing rates calculated from microscope counts are 5 and 3 orders-of-magnitude lower than the grazing rates determined by gut fluorescence in the ETM and non-ETM treatments, respectively (Fig. 12, Table III).

DISCUSSION

The strong relationship between clutch size and prosome length suggests that *E. affinis* egg production in both the Chesapeake Bay (r^2 = 0.85) and Choptank River (r^2=0.88) ETM regions is not food limited. Regressions using the residuals of these analyses versus salinity and TSS show no relationship (data not shown), meaning that copepod size is not related to either of these variables. The dynamic nature of the Chesapeake Bay and Choptank River ETM regions suggest that copepods likely experience constantly changing salinity and TSS concentrations throughout their lifetimes. Their ability to thrive in such variable environments may preclude *E. affinis* locating itself in a specific salinity or TSS range.

A salinity of 1.75 was the first node of the 2002 regression tree. Devreker *et al.* (2004), working with copepods collected from the Seine Estuary, found that first-

and second-stage *E. affinis* nauplii showed lowest survival in freshwater compared to other salinities up to 35. The point at which 50% of the nauplii in their experiments survived was < 12 hours in freshwater but close to 3 days at a salinity of 2.5. In a dynamic environment like the ETM, gravid females find themselves exposed to variable salinities throughout a reproductive cycle. If a female produces an egg clutch while in higher salinities and then is transported into freshwater, there should be no effect of low salinity on clutch size. Any extended time in low salinities may elicit a reduced reproductive output either through adaptation or increased energy consumption due to osmoregulatory energy demands.

Eurytemora affinis' tolerances to low salinities may depend heavily on their natural environment, with freshwater populations reproductively outperforming estuarine populations in freshwater, even after salinity acclimation. Katona (1970), using specimens collected in brackish water, determined that *E. affinis* can produce viable eggs at salinities ranging from 1.5 to 20 and temperatures ranging from 2 to 23°C. Ban (1994), using *E. affinis* from a freshwater lake, measured some of the highest egg production rates for this species in the literature (see his Table III). Ishikawa et al. (1999) performed salinity tolerance experiments again using *E. affinis* from a freshwater lake and found that hatching times were not significantly different from 0 to 10 ‰ and slowly increased as salinity increased. Over salinities of 25, hatching success was near 0 %. Salinities of up to 15 appeared to result in the greatest hatching success and survival; this is also the salinity range that *E. affinis* tends to be found in Chesapeake Bay and its tributaries. The maximum salinity tolerances of these freshwater populations seem to be consistent with their estuarine

counterparts, but estuarine copepods salinity tolerances seem to decline rapidly below 2 (also see Roddie *et al.* 1984).

Regression tree analysis of the Choptank River clutch size residuals suggests that food quality may have played a minor role in *E. affinis* clutch size in 2003. The nutritional quotient (NQ) provides the first major split in the residual data, with a value of 0.038. This value is much greater than the value of 0.01 suggested by Burdloff et al. (2000) that should limit *E. affinis* egg production rates, and the mean NQ for the < 0.038 group is 0.024. Given that less than 5% of the residual data is included in the < 0.038 group, NQ did not appear to significantly affect clutch size in the Choptank River ETM in 2003. The next split of the > 0.038 group is based on a CH_2O concentration of 0.49 mg l^{-1}, a measurement of food quantity. The < 0.49 mg l^{-1} CH_2O group is split by the a chl-*a*:TSS ratio of 7.8×10^{-5}, a measure of food availability, with 70% of the data at or above the node value. The ≥ 0.49 mg l^{-1} CH_2O group is split based on the concentration of spherical ciliates < 20 μm in diameter, with 62% of data falling below the node of 2.03 ciliates ml^{-1} and 38% equal to or above the node. The inclusion of ciliates in the regression tree should not be unexpected, as *E. affinis* may actually selectively graze heterotrophic microzooplankton, especially ciliates, over chl-*a* (Merrell and Stoecker 1998).

Compared to the 2002 regression tree analysis, the 2003 regression tree nodes are based primarily on food quantity or quality related variables. The high flow of 2003 likely increased available habitat for *E. affinis* in the upper Choptank River estuary compared to the low flow years (Kimmel and Roman 2004). Osmoregulatory energy demands are lower under such conditions (Kimmel and Bradley 2001) and are

unlikely to affect clutch sizes or egg production rates. This is supported by food limitation variables (in terms of quality and quality) providing the major splits in the 2003 regression tree analyses. In 2002, salinity described most of the differences in the clutch size residuals, with food-related variables being secondary. However, analyses of available food and food requirements suggest that food was not limiting either year. Because prosome length was shown to be the major factor affecting clutch size, the overall influence of salinity and food on clutch size is minimal for both years.

Eurytemora affinis egg production does not appear to be food limited in Chesapeake Bay or the Choptank River ETM regions. Though food quality and quantity appear to play a larger role in the Choptank River in 2003 than in Chesapeake Bay in 2002, both regression trees were created using the residuals of linear regressions that explained a large majority of the variability surrounding clutch size. Therefore, only a small amount of added variability is explained by using this statistical approach. Salinity and NQ may explain a small amount of clutch size variability for 2002 and 2003, respectively, but prosome length is the major forcing on clutch size in this study and in others (Crawford and Daborn 1986, Hirche 1992, Ban 1994). It is difficult to determine the effects of temperature and prosome length on clutch size because of the strong relationship between prosome and temperature. Chow-Fraser and Maly (1991) determined that clutch size is temperature-related for *Diaptomus minutus and D. oregonensis*, while Williamson and Butler (1987) found no significant effect of temperature on *D. pallidus* clutch size.

Several issues exist which may confound conclusions drawn from the clutch size analysis. There may be differences between the seston measured and that which *E. affinis* actually consumes. Great care has been taken to 1) sample water from the location (including depth) at which *E. affinis* was sampled and 2) to include only particles in the appropriate size range. Other studies have taken a similar approach and have found significant results between the nutritional environment and copepod egg production (Jónassdóttir et al. 1995, Burdloff et al. 2000). Jónassdóttir et al. (1995) found that specific unsaturated fatty acids were predictors in describing *A. hudsonica* and *Temora longicornis* egg production rates, and this type of analysis might be necessary in conditions where C is plentiful, and micronutrients may play an important role in egg production. However, because egg production is based on copepod feeding days and sometimes weeks prior to clutch extrusion, temporal changes in seston composition could also compromise this sort of analysis. This is especially true in warmer months, when organic material turnover is expected to be greater. Female age has also been implicated in differences in clutch sizes and egg production rates (Runge and Plourde 1996). Ianora and Poulet (1993) found that egg production and hatching success decrease for *A. hudsonica*, and it is reasonable to assume that other estuarine copepods, like *E. affinis*, might experience the same reduction in reproductive output with age.

The Huntley and Boyd (1984) model for growth and carbon requirements also suggest that copepods are not food limited in the Chesapeake Bay and Choptank River ETMs. During the years sampled, measured POC values were much higher than those required by *E. affinis* based on weight and temperature by the Huntley and

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Boyd (1984) model. In terms of the food quantity available, these copepods should not have limited growth or reproductive output. The growth model fit the 2002 egg production data, but the 2003 data again proved to be more complicated. When both years' egg production rates were compared to the growth model outputs, the r^2 dropped from 0.82 (for 2002 data only) to 0.43 (2002 and 2003 data together).

Berggreen et al. (1988) suggested that under conditions where copepods were food satiated, egg production rates should be reliable proxies for somatic growth, assuming that adult females allocate carbon that would go towards somatic growth to reproduction. Coastal and estuarine environments generally contain enough food resources so that copepods are rarely, if ever, food limited (McLaren 1978, Huntley and Boyd 1984). Assuming a C-specific ingestion rate of 1.25 day^{-1} (= 125% body C day^{-1}) from Figure 12 and a gross growth efficiency of 0.3 (Mauchline 1998), total C available to growth (or reproduction) is ~42% of body C day^{-1} (0.42 day^{-1}). This value is well within the bounds shown for egg production values in Figure 11, further suggesting that E. affinis egg production is not food limited in the Chesapeake Bay or Choptank River ETM regions. The estimated ingestion rates used in this calculation are within the limits for all literature values of E. affinis ingestion (Mauchline 1998).

Eurytemora affinis abundance often increases during years of high freshwater input (Fig. 13), but such an increase does not imply a change in food availability. Gaston and Lawton (1990) reported that population abundance generally increases with increasing habitat size. Christie et al. (2003) found that macrofaunal abundances in a kelp bed were significantly related to habitat availability and not influenced by food resources. In Chesapeake Bay, increases in freshwater input via the

Susquehanna River tend to lower salinities in the oligo- and mesohaline portions of the bay, thus extending the habitat available to *E. affinis* further down-estuary (Kimmel and Roman 2004). Increased habitat volume could account for the increase in *E. affinis* abundance during high-flow years.

The several approaches used to measure copepod egg production in reference to available food suggest that *E. affinis* egg production (and juvenile somatic growth) is not food limited. Therefore, one can assume that the copepods are feeding enough to meet their respiratory and growth needs. Our grazing experiments suggest that *E. affinis* grazes primarily on organisms <20 μm in size. Thus, *E. affinis* may get its carbon from autotrophic flagellates, colonial algae, and desmids in mostly the <20 μm size range, with ciliates >20 μm also supplementing their diet. Given the low grazing rates for these organisms, it appears likely that *E. affinis* in the field is obtaining carbon and other nutritional requirements from other sources. It is also possible that there were bottle effects during the grazing experiments that reduced ingestion rates below *in situ* values.

Eurytemora affinis is known to be an omnivore, feeding on detritus, bacteria, and both autotrophic and heterotrophic Protozoa. It is possible that *E. affinis* gains most of its carbon from sources not measured in the experiments, namely detritus, particle-attached bacteria and protists, and 5-10 μm nanoflagellates. Heinle *et al.* (1977) found that *E. affinis* consumed detritus that had been sterilized of all microbiota (protozoa and bacteria) and that which contained a microbiotic community. Survival and egg production was much higher in the non-sterilized treatment; the sterilized treatment exhibited no egg production. Copepod grazing

may be high on particle attached bacteria and protozoans (Crump *et al.* 1998), but these organisms were not counted in our grazing experiments.

The grazing experiments results suggest that *E. affinis* can obtain enough food outside of the ETM to survive and possibly thrive. Why *E. affinis* remains primarily in the ETM region may have to do with physical tolerances; the elevated turbidity providing a refuge from visual predators; or possibly interactions with other zooplankton species. Mysids (Winkler and Greve 2004) and fish larvae (North and Houde 2001), both abundant in the ETM are known predators of *E. affinis* .The copepod *Acartia tonsa*, usually peaks in abundance seaward of the ETM, and thus may limit *E. affinis* distributions through competition and predation (see Lonsdale 1979). *Eurytemora affinis* may be trapped like a particle in the ETM due to convergent flow patterns (Castel and Viega 1990; Roman et al. 2001) or it may actively maintain itself in the ETM area through tidally-based vertical migrations (Hough and Naylor 1991, Simenstad *et al.* 1994, Morgan *et al.* 1997). I do not know if *E. affinis* is able to exploit specific food resources of the ETM better than other zooplankton species or if other organisms prevent *E. affinis* from establishing a population outside of the ETM region. Further studies on these biological and physical interactions should help elicit why *E. affinis* dominates ETM regions.

Table 4.1. Mean monthly values for nutritional and abiotic variables (minus microplankton) measured in the Chesapeake Bay (2002) and Choptank River (2003) ETM regions. C = carbon; N = nitrogen; Phaeo = phaeophytin; TSS = total suspended solids; CH_2O = carbohydrates; Prot = proteins; NQ = nutritional quotient (sum of CH_2O, Prot, and Lipids divided by TSS).

Year	Month	C (mg/l)	N (mg/l)	Molar C:N	Chl-*a* (µg/l)	Phaeo (µg/l)	TSS (mg/l)	Temp (ºC)	Sal (PSU)	CH_2O (mg/l)	Prot (mg/l)	Lipid (mg/l)	NQ (mg/mg)	Chl-*a*:TSS (mg/mg)
2002	March	4.17	0.65	7.37	51.79	46.77	39.02	7.62	8.39	0.97	0.04	0.44	0.045	1.99E-03
	April	3.94	0.46	9.76	31.42	125.93	113.04	11.41	4.01	1.22	0.27	0.60	0.020	4.42E-04
	May	2.05	0.22	10.62	4.87	23.63	42.33	15.51	7.61	0.40	1.28	0.17	0.052	1.83E-04
	June	2.86	0.29	11.47	2.69	23.76	48.17	24.36	0.11	0.76	0.37	0.30	0.030	5.75E-05
	July	0.94	0.17	8.58	2.51	17.45	12.27	26.08	3.46	0.19	0.16	0.15	0.042	2.08E-04
2003	March	4.44	0.70	7.88	34.12	92.92	22.38	12.65	4.18	0.73	0.07	0.23	0.044	1.52E-03
	May	2.66	0.34	9.24	4.59	13.51	28.33	17.00	3.10	0.43	0.87	0.17	0.052	1.61E-04
	June	1.85	0.25	8.75	2.33	6.38	35.09	22.45	0.90	0.41	1.14	0.32	0.053	6.53E-05
	July	1.46	0.21	8.06	3.43	4.59	23.62	27.20	3.40	0.36	0.67	0.18	0.051	1.46E-04
	Sept	1.56	0.23	7.97	10.81	52.14	23.54	23.60	0.80	0.49	0.65	0.18	0.056	4.59E-04
	Oct	1.20	0.16	8.64	3.37	15.06	15.65	14.20	2.40	0.23	0.50	0.11	0.054	2.15E-04

Table 4.2. Mean monthly microplankton biomass (pg C ml^{-1}) as measured in the Choptank River in 2003. Tin = tintinnids; Con = conical ciliates; Sph = spherical ciliates; Hetero = *Heterocapsa* spp.; P. min = *Prorocentrum minimum*; Karlo = *Karlodinium* spp.; Oxy = *Oxyrrhis* spp.; Gyro = *Gyrodinium* spp.; Gymno = *Gymnodinium* spp.; Pperi = *Protoperidinium* spp.; UnID Dino = unidentified dinoflagellates; Diat = diatoms; Myr/Meso = *Myrionecta* spp. or *Mesodinium* spp.; AFlag = autotrophic flagellates (includes cryptophytes).

Month	Tin <20 µm	Tin >20 µm	Con <20	Con >20	Sph <20	Sph >20	Hetero	P. min	Karlo	Oxy	Gyro	Gymn	Pperi	UnID Dinos	Diat	Myr/ Meso	AFlag	Total Carbon
March	0.00	0.00	0.00	0.00	0.00	25.02	19.51	0.00	0.00	0.00	0.00	0.00	0.00	49.29	221.99	6.62	31.94	354.37
April	0.00	174301.71	3062.48	1.79	1.79	1.79	0.00	3957.65	0.00	0.00	0.00	0.00	0.00	0.00	14.40	20.52	7.10	181381.60
May	1645.22	176445.77	628.55	0.00	0.27	0.27	0.00	30.99	4.50	0.00	0.00	0.00	0.00	71.40	26.50	1.61	14.81	178869.90
June	0.00	0.00	1257.09	0.67	0.27	4.02	0.00	1.33	0.00	0.28	0.00	0.00	0.00	52.70	29.47	1.95	6.88	1354.68
July	4113.10	89448.09	785.68	5.18	2.02	3.52	0.00	2.11	0.00	0.00	102.95	4.13	1.58	69.92	25.43	2.87	23.46	94590.05
Sept	3290.45	83484.91	1257.09	0.27	2.69	5.76	4.43	3.44	0.00	0.00	0.28	0.00	0.28	43.99	31.56	2.94	58.99	88194.04
Oct	8226.21	71558.48	628.55	0.54	1.07	1.22	196.03	0.00	0.00	0.00	2.39	0.00	0.00	261.96	67.94	0.31	21.10	80965.80

Table 4.3. Mean carbon-specific ingestion rates, I, [µg C ingested (µg copepod body C)$^{-1}$] for *E. affinis* in the Estuarine Turbidity Maximum (ETM) and downstream from the ETM for each prey type, listed by size class: 10-20 µm, >20 µm; N=3. Autoflag = Autotrophic flagellates. Standard deviations are in parentheses. Total C ingested shows the sum of positive ingestion rates only.

Size Class	Prey Category	In ETM		Downstream of ETM	
10–20 µm	Loricate cil	0		-2.51×10^{-3}	(3.95×10^{-5})
	Aloricate cil	-9.63×10^{-5}	(5.77×10^{-4})	-7.58×10^{-4}	(7.89×10^{-4})
	Unidentified cil	-1.24×10^{-5}	(9.53×10^{-5})	-2.02×10^{-4}	(1.75×10^{-4})
	Diatoms	-1.96×10^{-5}	(2.06×10^{-5})	0	
	DF	0		-1.41×10^{5}	(1.64×10^{-6})
	Autoflag	3.78×10^{-5}	(1.23×10^{-4})	-5.16×10^{-4}	(2.63×10^{-4})
	Total C ingested	**3.78×10^{-5}**		**0**	
>20 µm	Loricate cil	1.94×10^{-4}	(6.08×10^{-4})	-2.22×10^{-3}	(5.28×10^{-3})
	Aloricate cil	-2.20×10^{-4}	(8.0×10^{-4})	2.16×10^{-2}	(1.72×10^{-3})
	Unidentified cil	2.04×10^{-5}	(1.71×10^{-5})	5.11×10^{-4}	(5.12×10^{-4})
	Diatoms	-7.33×10^{-5}	(5.58×10^{-5})	5.19×10^{-5}	(2.66×10^{-4})
	DF	0		2.01×10^{-4}	(5.95×10^{-4})
	Autoflag	0		0	
	Total C ingested	**2.14×10^{-4}**		**2.23×10^{-2}**	
	Total C ingested for both size classes	**2.52×10^{-4}**		**2.23×10^{-2}**	
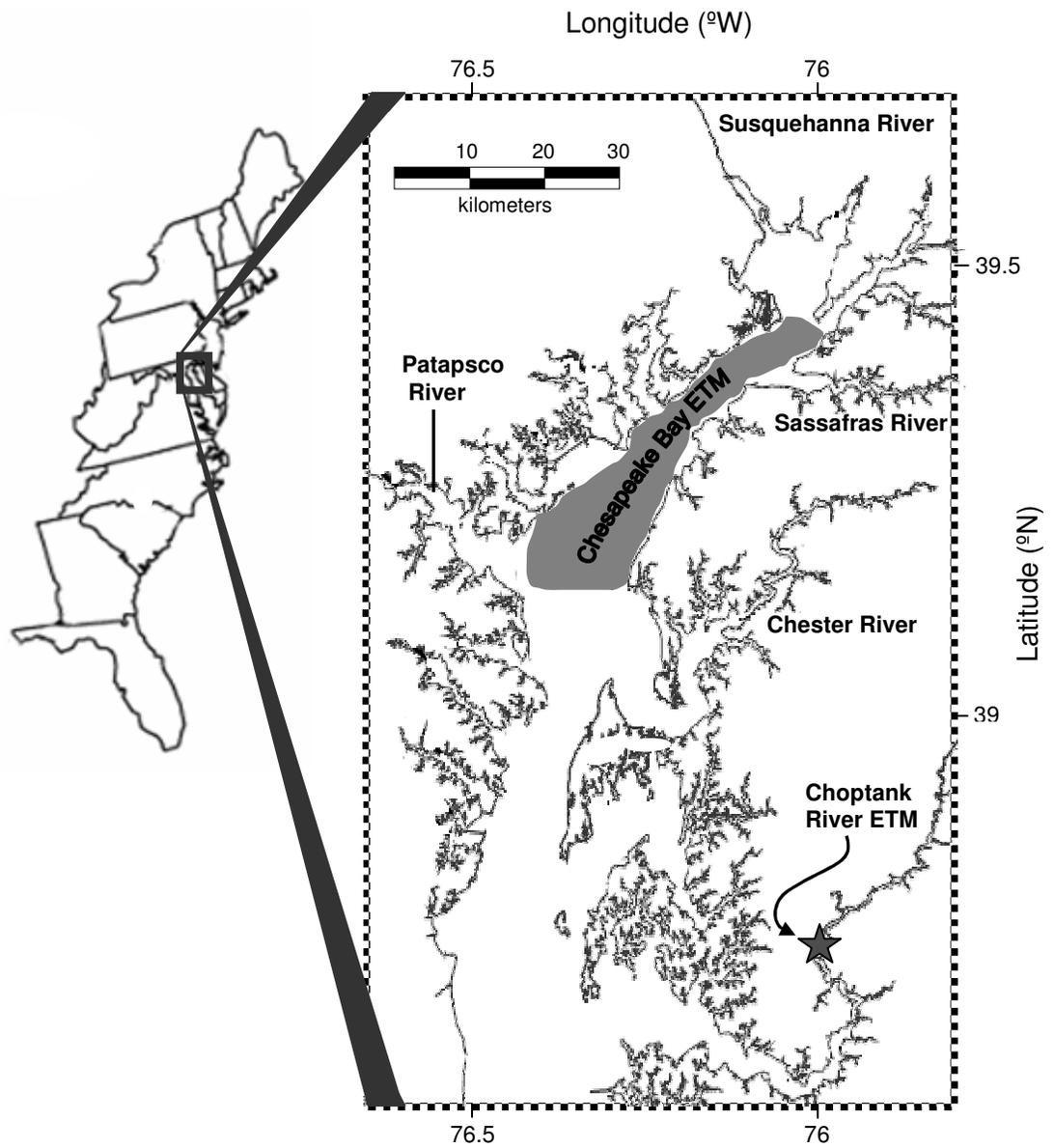
Figure 4.1. A map of the USA east coast, with Chesapeake Bay and its tributaries enlarged. The estuarine turbidity maximum (ETM) region of the Choptank River are shown using filled stars.
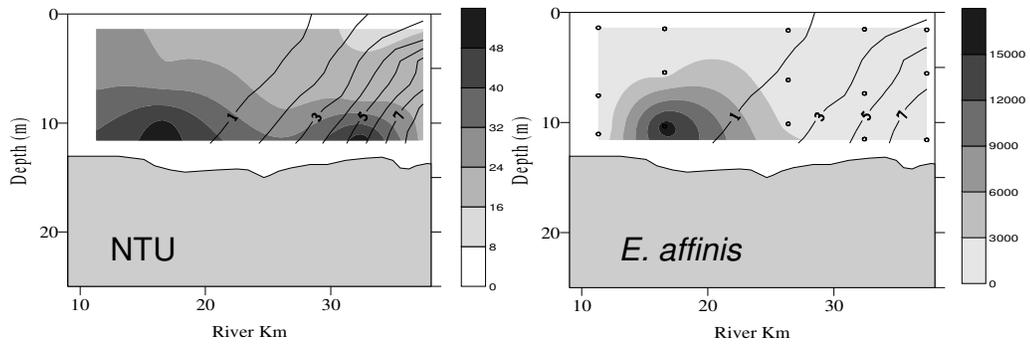
Figure 4.2. May, 2001 contour maps of turbidity (in nephalometric turbidity units, NTU) and *E. affinis* abundance (# m^{-3}) in northern Chesapeake Bay. Note the relationship between turbidity (NTU) and copepod abundance. River km is measured as the distance downstream from Havre de Grace.
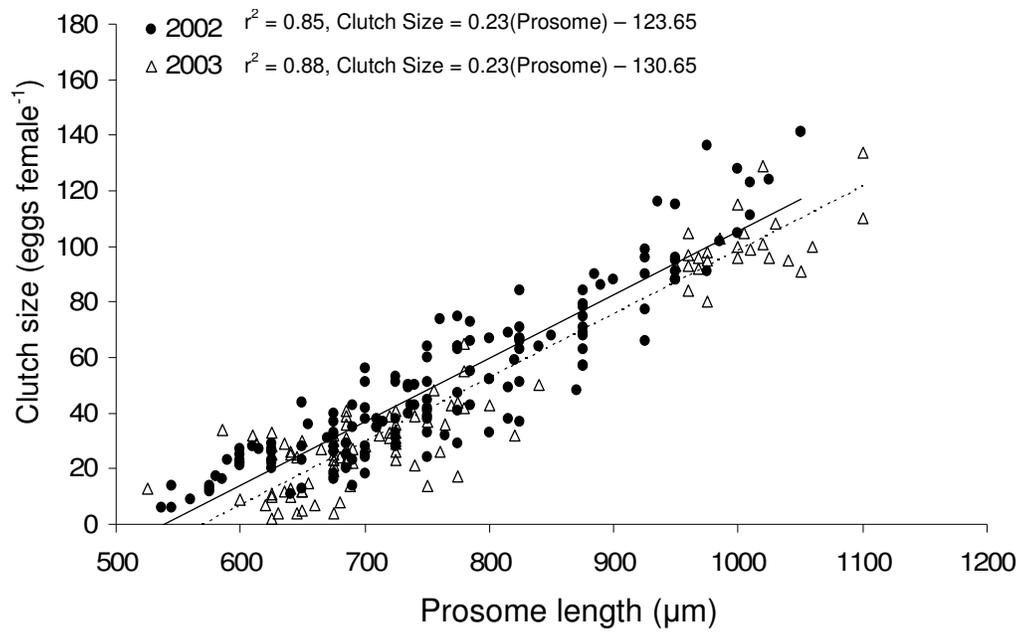
Figure 4.3. Linear regressions of clutch size versus female prosome length for 2002 (Chesapeake Bay) and 2003 (Choptank River). Only completely formed clutches were used in this analysis.
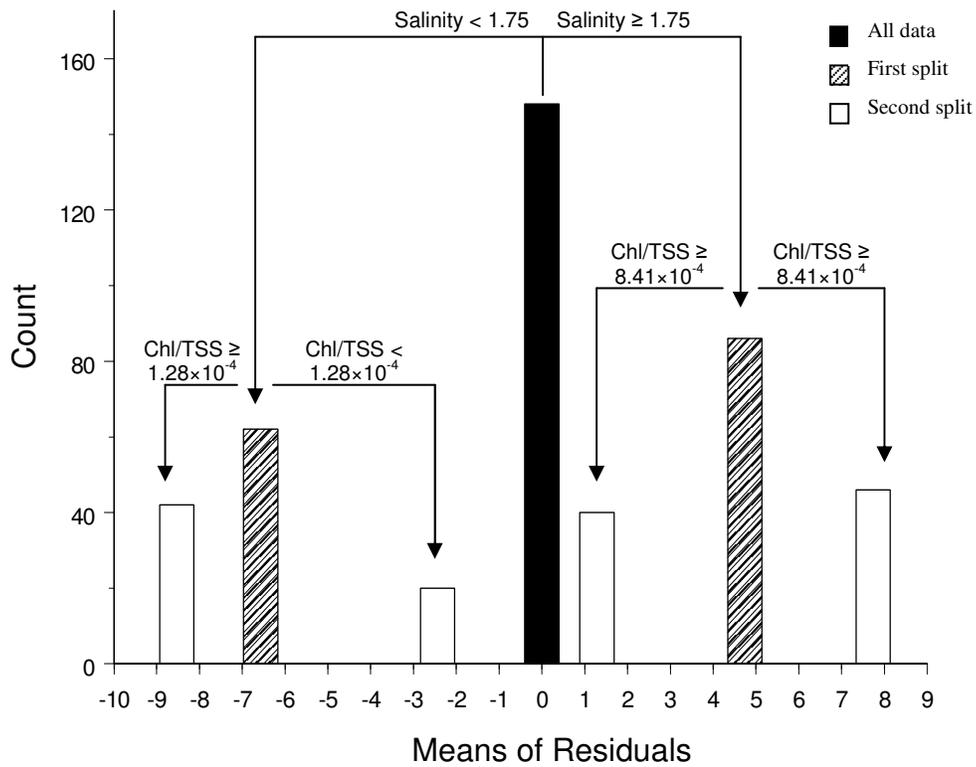
Figure 4.4. Regression tree analysis using the residuals from the 2002 (Chesapeake Bay) clutch size versus prosome length linear regression against the environmental variables measured (see Table I for a full list). 'Count' refers to the number of data points falling at that mean value. See Table I caption for abbreviation information.
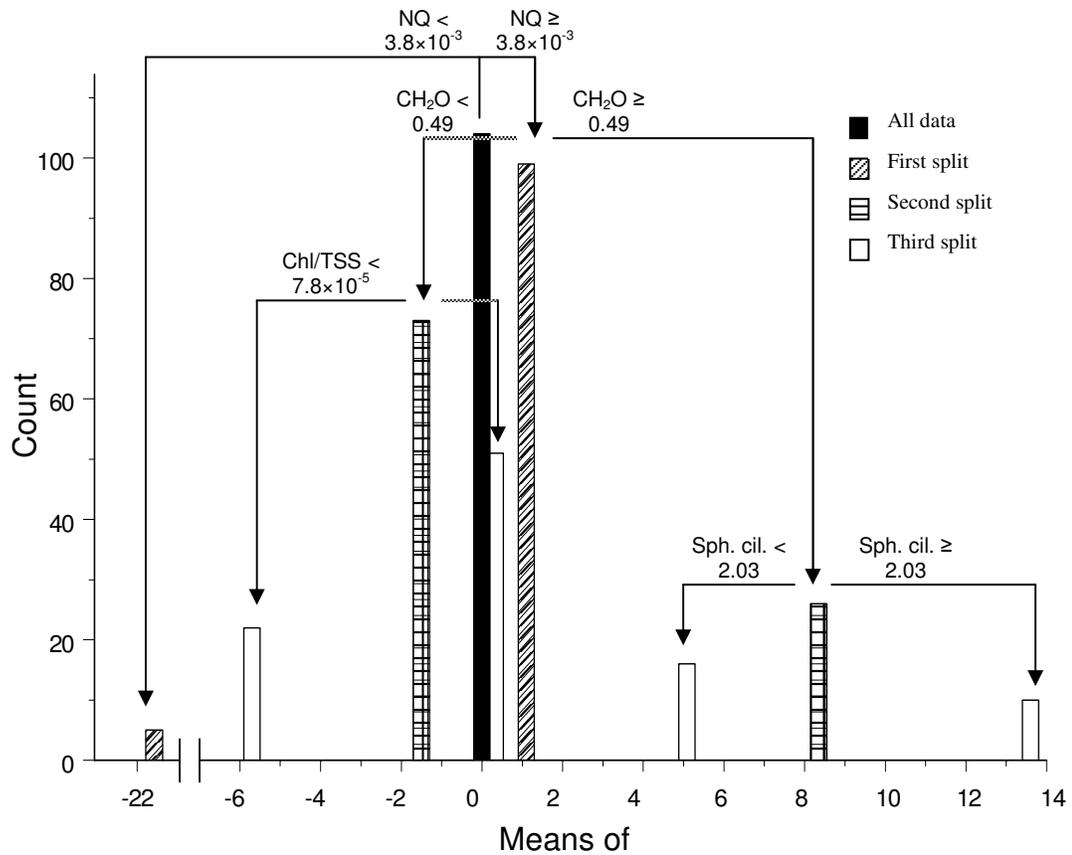
Figure 4.5. Regression tree analysis using the residuals from the 2003 (Choptank River) clutch size versus prosome length linear regression against the environmental variables measured (see Table I for a full list). 'Count' refers to the number of data points falling at that mean value. See the captions for Tables I and II for abbreviation information.
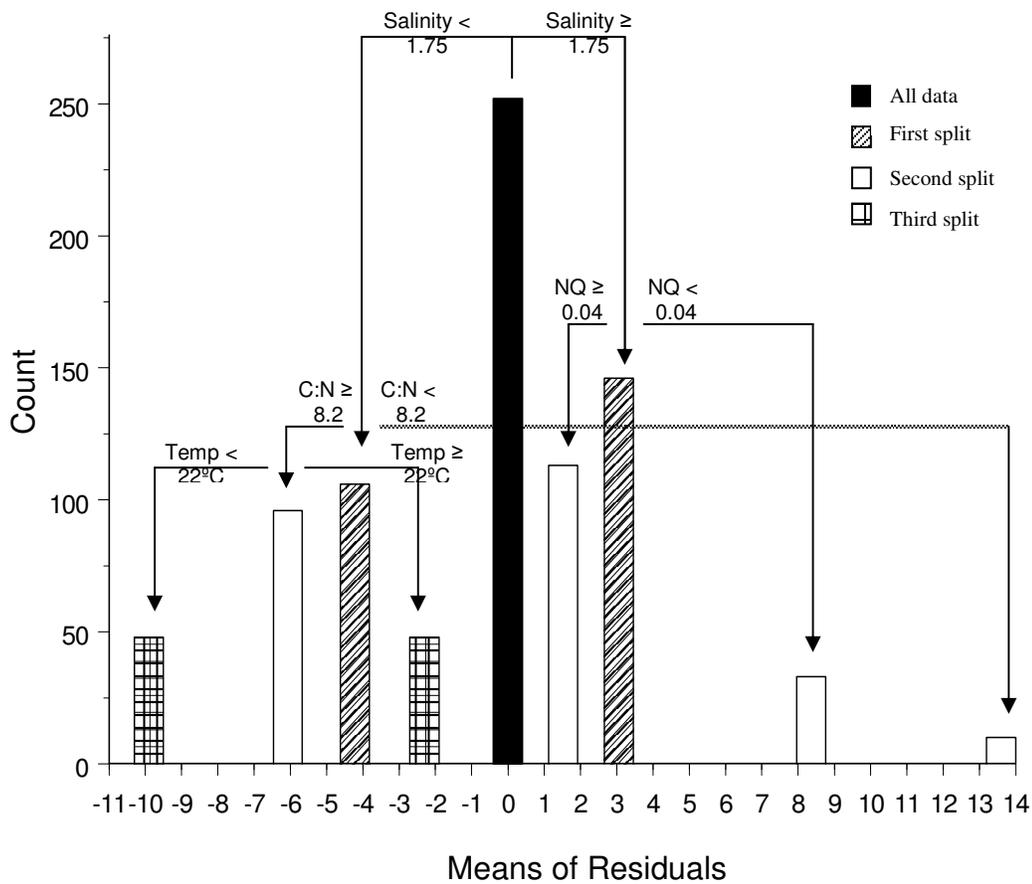
Figure 4.6. Regression tree analysis using the residuals from the 2002 (Chesapeake Bay) and 2003 (Choptank River) clutch size versus prosome length linear regression against the environmental variables measured (see Table I for a full list). 'Count' refers to the number of data points falling at that mean value. See the captions for Tables I and II for abbreviation information.
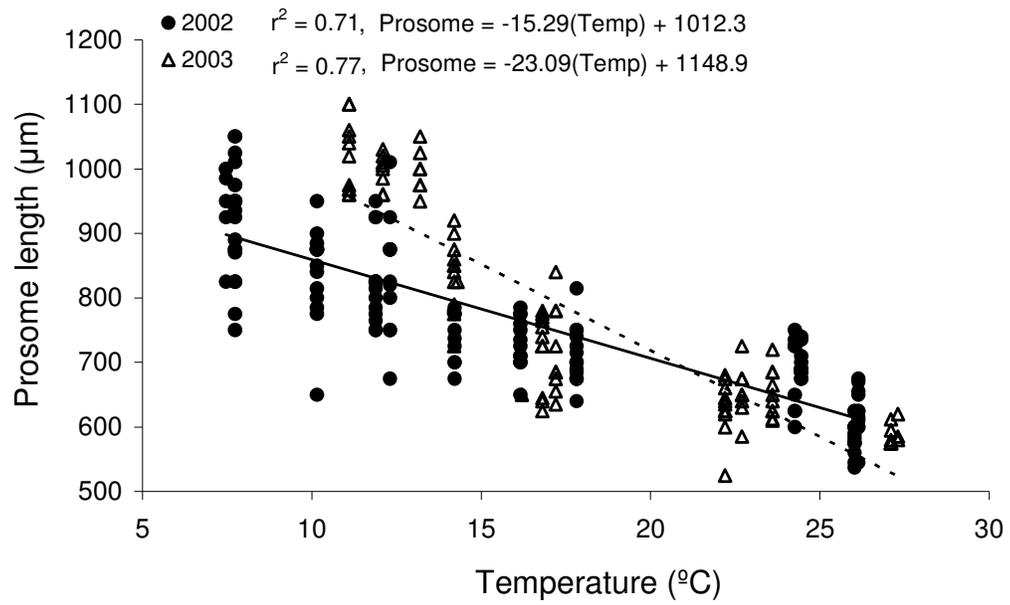
Figure 4.7. Linear regressions of female prosome length and estimated mean temperature during development for 2002 (Chesapeake Bay) and 2003 (Choptank River).
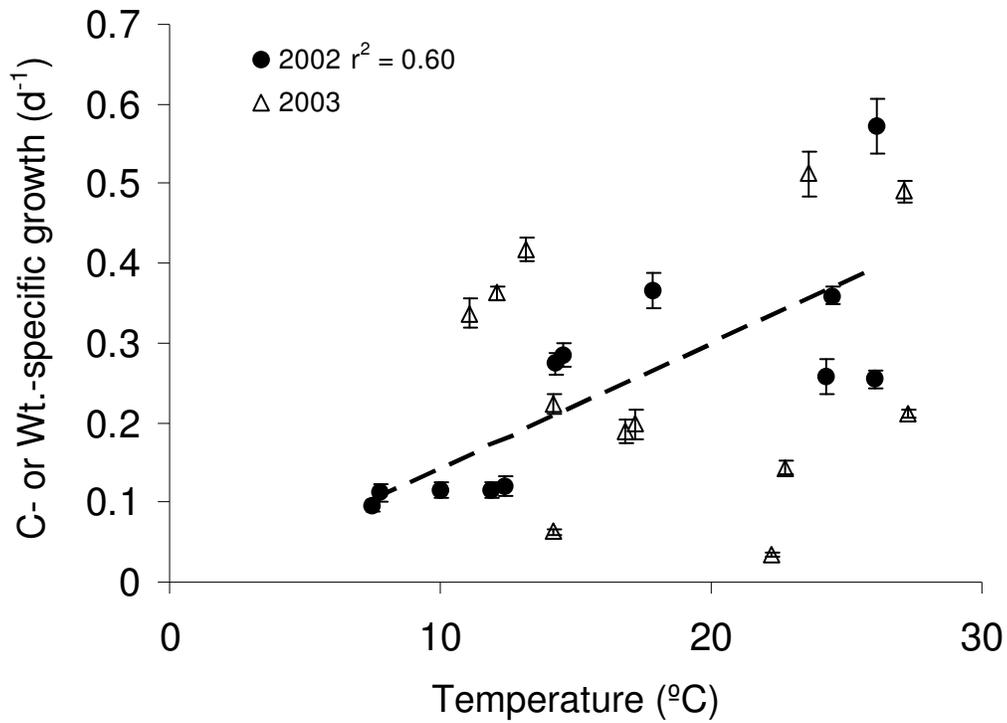
Figure 4.8. Carbon-specific egg production rates plotted versus temperature. Linear regression is for 2002 (Chesapeake Bay) data and is significant at $p < 0.01$.
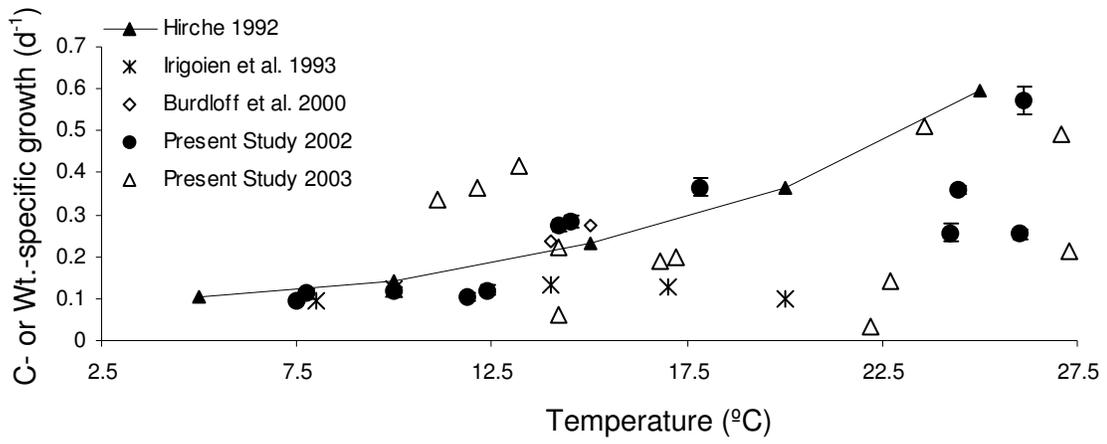
Figure 4.9. Comparisons of C-specific egg production rates from this and other studies to the Hirche (1992) model of *E. affinis* egg production under food replete conditions.
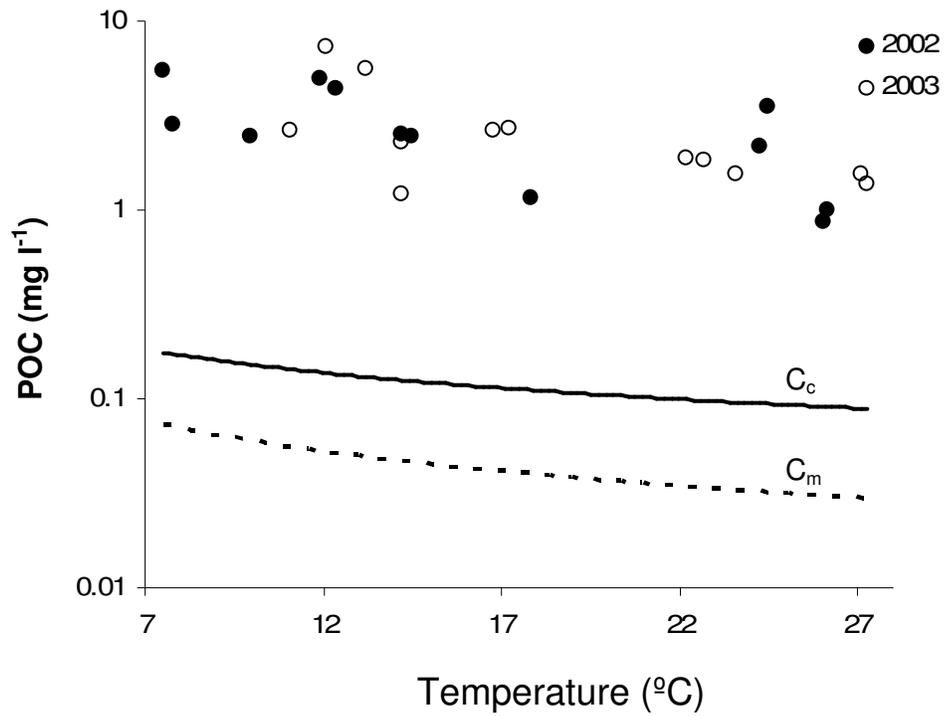
Figure 4.10. Calculations for particulate carbon concentrations (POC) concentrations required for copepod maintenance (C_m; copepod carbon assimilation = respiratory demands, stippled line) and non-food limited copepod growth (C_c, solid line). Actual POC concentrations for 2002 and 2003 are also plotted.
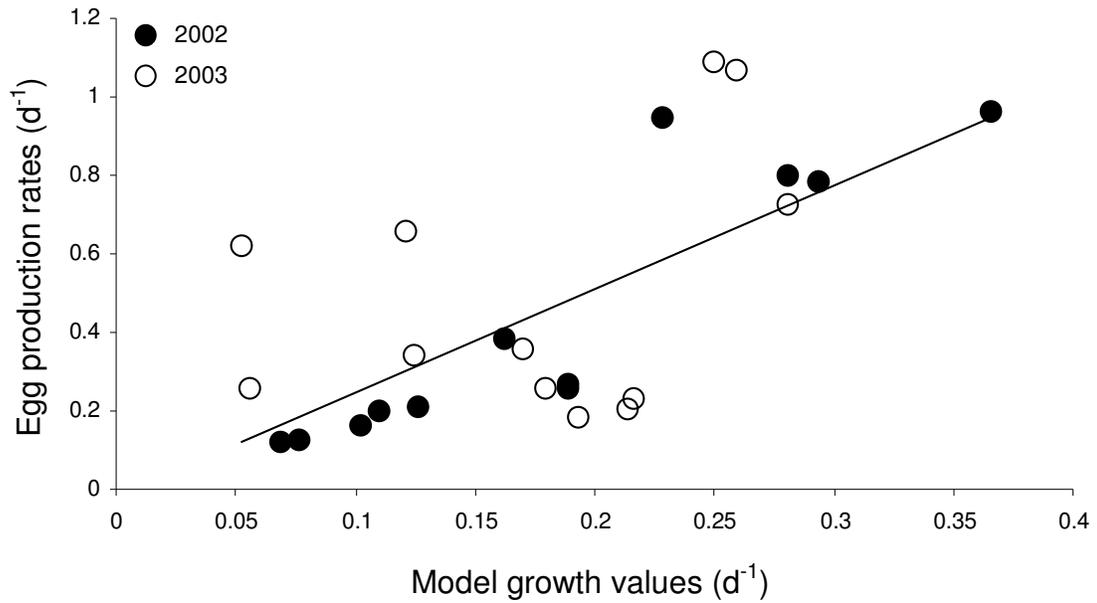
Figure 4.11. *Eurytemora affinis* egg production rates versus modeled rates from Huntley and Boyd (1984). The modeled rates are based on temperature and copepod weight and assume no food limitation. The r^2 for the linear regression is 0.43 (p < 0.05).
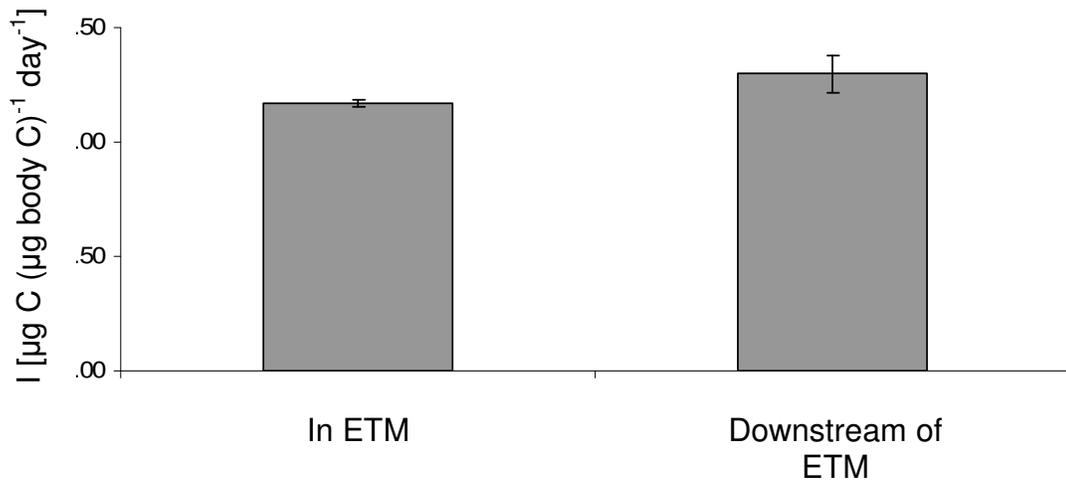
Figure 4.12. Results of gut fluorescence measurements following grazing experiment. Chlorophyll-*a* has been converted to carbon units in order to C-specific ingestion on autotrophic prey.
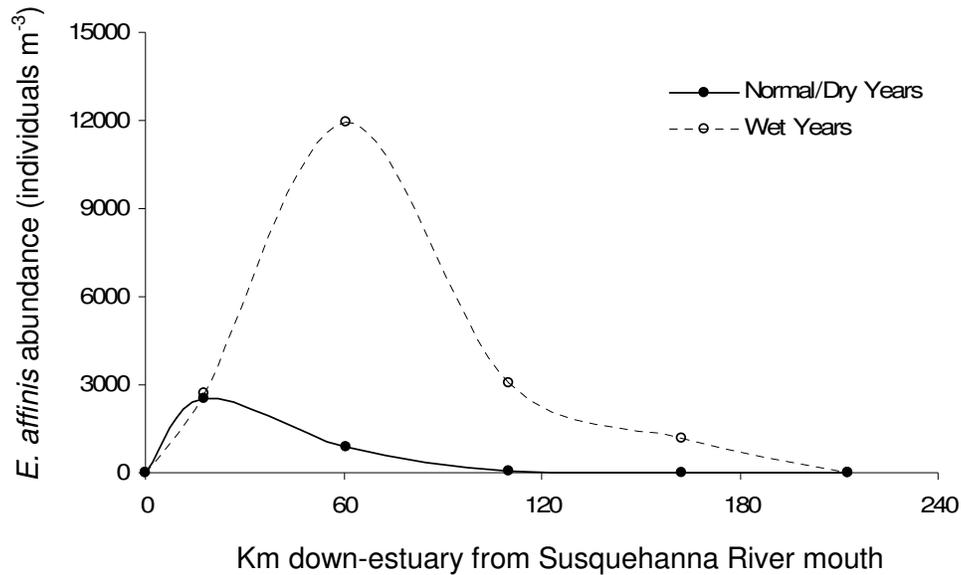
Figure 4.13. Means of *E. affinis* abundance at Chesapeake Bay Program sampling stations from 1986-1995. 1990 and 1993 are considered years of enhanced Susquehanna River freshwater input ("wet" years). 1986, 1987, 1988, 1989, and 1992 are considered "normal" and "dry" years. The years following wet years (1991, 1994, and 1995) were not included in this analysis because *E. affinis* abundance those years was highly influenced by resting eggs laid the previous year (Kimmel and Roman 2004).

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CHAPTER V: Tidally-timed vertical migration of zooplankton in the Chesapeake Bay estuarine turbidity maximum

ABSTRACT

Estuarine zooplankton often use some behavioral means of position maintenance, such as tidally-timed vertical migration, to maintain themselves in distinct salinity ranges. To determine if zooplankton, especially the copepod *Eurytemora affinis*, vertically migrate to maintain their position within the Chesapeake Bay estuarine turbidity maximum (ETM) region, I conducted time-series measurements of salinity, temperature, fluorescence, total suspended solids (TSS), current velocities and acoustically determined zooplankton biovolume (BV). Zooplankton BV was significantly related to TSS near the bottom and middle depths, but there was no relationship higher into the water column. Whereas TSS concentrations in the water column appeared to be primarily the result of tidal current scouring along the bottom, the lack of concordance between zooplankton BV and TSS higher in the water column suggests active vertical movement by zooplankton. It is possible that zooplankton in the Chesapeake Bay ETM vertically migrate in response to tides, but the data are ambiguous. The data do suggest that zooplankton in the up-estuary regions of the ETM might vertically migrate upward during ebb tides. In the down-estuary region, zooplankton are concentrated near the bottom during flood tides. Such movements could position zooplankton at depths with the greatest horizontal movement, creating a conveyor belt of zooplankton movement through the ETM region. Additionally, the Susquehanna River may provide a

freshwater source to sweep zooplankton into the ETM. Such a migration pattern would retain zooplankton in their optimal salinity range while possibly allowing them to be maintained in, or find food-rich regions. It is clear that, if zooplankton migrate, they do not migrate solely in response to tidal phase (based on BV and TSS versus current direction relationships), and that any migration patterns do not minimize horizontal displacement.

INTRODUCTION

Position maintenance within particular regions of an estuary can require behavioral mechanisms that reduce advection (Kimmerer *et al.* 1998; Forward and Tankersley 2001). Zooplankton vertical migration patterns are often attributed to the diel cycle of solar irradiance (e.g. Cushing 1951, Ohman 1990). During light-induced diel vertical migration, zooplankton migrate to the surface at night to feed and back to depth as sunrise approaches to avoid predation (i.e, Huntley and Brooks 1982, Frost 1988, Verheye and Field 1992). This phenomenon is especially prevalent in oceans and lakes, where potential advection from a particular habitat is less than it is in estuaries (Ohman 1990; Ringelberg 1995). However, due to changes in tidal current velocities and direction with depth and over time, vertical migrations cued to tidal cycles are common in the estuarine larvae of meroplanktonic organisms such as crabs (Cronin and Forward 1983, Tankersley and Forward 1994, Zeng *et al.* 1999), fish (Dauvin and Dodson 1990, Joyeux 1999), and molluscs (Roberts *et al.* 1989) and in certain holoplankton such as the calanoid copepod *Eurytemora affinis* (Hough and Naylor 1991).

The estuarine turbidity maximum (ETM) region of most partially-mixed estuaries is located near the landward limit of salt water intrusion and contains, by definition, a higher concentration of suspended solids than waters up- or down-estuary. Such high turbidity may reduce diel migration patterns by increasing light attenuation (Kimmerer *et al.* 1998; Roman *et al.* 2001). *Eurytemora affinis* is an epibenthic copepod whose abundance usually peaks in low-salinity ETM regions worldwide (Simenstad *et al.* 1990; Hough and Naylor 1992; Sautour and Castel 1995; Roman *et al.* 2001). In order for a population maximum of *E. affinis* to occur in an ETM region with a net flow out of that region (seaward) there must be 1) active movement by individuals to transport themselves into areas that will promote their retention, 2) physical processes that resuspend and settle organisms in a way that promotes retention, and/or 3) higher reproductive output within the region than up-or down-estuary (Kimmerer *et al.* 1998).

Eurytemora affinis has been found to utilize tidally-timed vertical migration to maintain its position within the ETM in some estuaries (Hough and Naylor 1991, Simenstad *et al.* 1994, Morgan *et al.* 1997). In these studies, *E. affinis* was generally dominant higher in the water column during flood tide and deeper during ebb, thus being associated with the lowest velocity regions within the water column. Morgan *et al.* (1997) found that the principal lunar tidal component (M_2) described nearly half of all variability in *E. affinis* abundance, further supporting the idea of a tidally-timed vertical migration.

Other studies have demonstrated that *E. affinis* populations may be retained within estuaries by passive means. Castel and Viega (1990) determined that the

swimming abilities of *E. affinis* are ineffective in retaining the population within the turbid Gironde estuary. This finding and the positive relationship between *E. affinis* abundance and suspended matter concentration led them to conclude that the copepods utilize hydrodynamic processes to maintain themselves within the Gironde estuary; that is, they behave as passive particles. Kimmerer *et al.* (1998) found that macrozooplankton (mysids and amphipods) exhibited tidally-induced vertical migrations that maintained their positions within San Francisco Bay (higher in the water column during flood, lower during ebb), but the copepods *E. affinis* and *Pseudodiaptomus forbesi* did not. However, both copepod species were found higher in the water column than expected, probably due to the high turbidity of northern San Francisco Bay which would provide a predation refuge. *Pseudodiaptomus forbesi*, a brooding copepod like *E. affinis*, has been known to use setae on its antennules to attach itself to detrital particles, which 1) could reduce mortality by visual predators and 2) allow the copepod to utilize the particle trapping dynamics of the ETM to maintain its position within the ETM. *Eurytemora affinis* and other planktonic ETM fauna may adopt a similar strategy.

Eurytemora affinis may also exercise plasticity in its migratory behaviors. Roman *et al.* (2001) used acoustical methods to measure zooplankton biovolume (BV) and concluded that, under conditions of high freshwater input, zooplankton in the Chesapeake Bay ETM are aggregated and resuspended by the same physical processes that affect sediments. They proposed a conceptual model in which zooplankton and sediments are resuspended into the water column landward of the salt front during an ebb tide. During flood conditions, zooplankton and sediments

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below the salt front are also readily resuspended but are limited in their vertical suspension by the pycnocline. Resuspension in this model is based on the spatial location where maximum bottom currents scour the seafloor (i.e., above the salt front during ebb flow and below the salt front during a flood).

In this study, I examined zooplankton biovolume in the Chesapeake Bay ETM using a larger dataset and longer time series under conditions of freshwater input that were much reduced compared to those observed by Roman *et al.* (2001). Our goals were to determine if zooplankton in the Chesapeake Bay ETM region (dominated by *E. affinis*) actively regulate their positions via tidally-timed vertical migrations and to compare the results with the Roman *et al.* (2001) model.

METHODS

Field Sampling

I conducted two surveys in May, 2002, in the channel of the Chesapeake Bay ETM region (Fig. 1). Tucker trawl samples during this period show that *E. affinis* was the most abundant zooplankton species in the ETM region (Chapter 1). Our surveys consisted of sampling 3 to 7 fixed stations sequentially over a 22 to 27 hour time period following a hydrographic survey to locate the ETM. The first May survey (May 8-9) included thirteen axial (north to south) transects through the ETM region; the second survey (May 14-15) included eleven. At each station, a vertical profile was taken using a Sea Bird CTD rosette equipped with sensors to measure salinity, temperature, fluorescence, light transmission, and oxygen concentration. In addition, a shipboard Acoustic Doppler Current Profiler (ADCP) was used to measure

current velocities at 0.5 m vertical bins. All data was later averaged into 1 m vertical bins.

Fluorescence measurements were converted to chlorophyll-*a* (chl-*a*; μg l⁻¹) concentration by collecting water samples, extracting chl-*a* in a 90% acetone solution, and then performing a regression between chl-*a* and fluorescence. Total suspended solid (TSS; mg l⁻¹) concentration was determined by filtering water samples onto pre-weighed Whatman GF/F filters, drying to constant weight, and subtracting the initial filter weight. These TSS measurements were then regressed against transmissiometer readings, allowing all transmissiometer readings to be converted to TSS.

Zooplankton biovolume (BV) was quantified using a Tracor Acoustic Profiling System (TAPS). This device is capable of taking fine-scale vertical (< 1 m) measurements of zooplankton BV concentration (mm³ m⁻³) by measuring acoustical backscattering of frequencies ranging from 300 kHz to 3 MHz. Several BV measurements are made each meter and are later averaged into 1 m bins. Details on TAPS theories, operation, and data analysis can be found in Holliday and Pieper (1995) and Roman *et al.* (2001). TAPS measurements of zooplankton biomass and abundance are highly correlated with traditional net and pump estimates (Fig. 2) (Barans *et al.* 1997; Pieper *et al.* 2001; Roman *et al.* 2001). The minimum linear size of detection is around 200 μm, making TAPS ideal for measuring zooplankton ranging from nauplii to mysids while excluding most sediments. Even flocculated material that may exceed 200 μm equivalent spherical diameter is not usually detected by acoustical methods, which instead detect flocs as their constituent grains and not as single particles (Fugate and Friedrichs 2002).

<u>Data Analyses</u>

Visual exploratory analyses of temporal and spatial patterns were performed using the data contouring program Surfer (Golden Software, v. 8). Single-station time series were plotted for stations centered on river km (distance from Havre de Grace, Maryland) 30, where zooplankton counts from Tucker trawl surveys collected just prior to the present surveys indicated that *E. affinis* was the most abundant zooplankton species (Chapter 1). Contour plots of each transect of the 25-hour surveys were also created.

Statistical analyses were chosen based on the assumption that zooplankton tidally-timed migration is indicated by 1) the depth of zooplankton BV maxima with respect to tidal direction and velocity and 2) relationships between BV and TSS. Because zooplankton may respond to current velocity (magnitude and direction) based on their location within the ETM region, BV and TSS values for stations up-estuary from river km 28 (located at approximately river km 26, 22, 18, and 16), which was near the center point of all transects, were averaged in 1 m depth bins for each transect. The same process was repeated for down-estuary stations (located at approximately river km 30, 34, and 38). The resulting dataset was composed of depth-specific, transect-averaged BV and TSS values for both landward (up-estuary) and seaward (down-estuary) regions. Depths of 0-4 m were grouped as 'surface' depths, 4-8 m were grouped as 'middle' depths, and 8-12 m were grouped as 'bottom' depths. Linear regressions between both BV and TSS versus current velocity (by depth) were then performed.

Pairwise correlation analyses were also used to determine relationships between BV and TSS in surface, middle, and bottom waters.

RESULTS

Zooplankton BV aggregations are centered in the upper and lower water column, with significantly less BV being measured at mid-depths (Table I). Surface BV is greater than the other depths and both surface and bottom BV values are significantly higher than middle BV measurements.

Zooplankton BV is positively related to TSS concentrations in middle and bottom waters for both cruises (Table II). There are no significant relationships between the zooplankton BV depth maxima and time-of-day, indicating no diel vertical migration (data not shown).

At river km 30, both BV and TSS become elevated in bottom waters during flood tides in the first survey, with TSS resuspension occurring after zooplankton resuspension (Fig. 3). Increases in zooplankton biomass correspond to a peak in flood currents. Peaks in surface ebb current flow match surface maxima in zooplankton biomass (Fig. 3). In contrast, TSS and BV are significantly greater during flood currents at the bottom and surface for the second cruise (Fig 4).

Displacement calculations for the May and July cruises show that following complete tidal cycles, water in the upper water column is displaced down-estuary and water near the bottom was displaced up-estuary (Table III). The average displacement calculated over 3 to 4 complete tidal cycles was -3.38 km (negative denotes a down-estuary direction) in surface waters, -0.36 km in middle waters, and

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+1.32 km in bottom waters. These displacements are consistent with the estuarine circulation expected at a location downstream of the salt front.

The relationships between both zooplankton BV and TSS concentrations versus current direction are variable in contour plots of the multiple fixed-station surveys. In general, zooplankton BV during the first May survey tend to be concentrated near the surface in transects e through j (Fig. 5), which covered both flood and ebb tides (Fig. 3). In the other 5 surveys conducted during both flood and ebb, zooplankton are concentrated equally near the surface and the bottom. Most TSS resuspension occurred during flood tides and was isolated to bottom and middle depths. TSS maxima were also limited to the lower water column during the second May survey (Fig. 6). Zooplankton BV maxima do not appear to follow a trend in water column location versus tidal stage during this survey. In general, BV and TSS maxima are more intense during flood tides, especially along the bottom (Fig. 6, frames a, j, and k). It should be noted that contour plots of the second survey only extend from river km 22 to 40, while some transects extend to river km 18 and 16.

Regression analyses of BV and TSS versus tidal velocity suggest that zooplankton BV location within the water column is related to current velocity in different ways with regard to location within the estuary (up-estuary or down-estuary) (Figs. 7 and 8). In the up-estuary region of the ETM, ebb currents (seaward) are related to higher BV concentrations in middle and surface depths. Bottom BV concentrations increase as both ebb and flood currents increase. Although some regressions show a positive, parabolic relationship between TSS and current velocity, TSS concentrations tend to increase at all depths as current velocity in the flood

direction increases (Figs. 7). Seaward of the ETM, BV increases as flood tide

velocities increase in both bottom and middle depths. Concentrations of TSS increase

with flood velocities at all depths (Fig. 8). There is no significant relationship

between BV and current velocity in surface waters seaward of the ETM.

DISCUSSION

Freshwater input into Chesapeake Bay in 2001 was below the 50-year

average (http://md.water.usgs.gov/monthly/bay.html) and has been shown to

positively influence *E. affinis* location and abundance in Chesapeake Bay (Kimmel

and Roman 2004) and other estuaries (Peitsch *et al.* 2000; Kimmerer 2002). Further,

spring *E. affinis* abundance in northern Chesapeake Bay is affected by freshwater

input the previous autumn, with greater Susquehanna River input in the autumn

leading to a greater abundance of *E. affinis* the following spring (Kimmel and Roman

2004). Below-average freshwater input may explain the relatively low copepod

abundances found during our ETM sampling program (Chapter 1) compared to other

years (Roman *et al.* 2001; Kimmel and Roman 2004). Roman *et al* (2001) conducted

their ETM field work in 1996, which was a year of exceptionally high Susquehanna

River flow, and *E. affinis* was abundant throughout the year (Kimmel and Roman

2004). Freshwater input was less in 2002 than in 1996, producing a seasonal shift in

zooplankton abundance in the ETM, with the copepods *Acartia tonsa* and *E. affinis*

exhibiting similar abundances at down-estuary stations (maximum values of 200-300

ind l^{-1}, determined by May Tucker trawl surveys). May acoustic surveys were

analyzed for evidence of zooplankton vertical migration under the presumption that

data primarily represented *E. affinis*, which was the dominant zooplankton species at this time. In spite of this dominance by *E. affinis*, *A. tonsa* BV is possibly incorporated in the down-estuary measurements of total zooplankton BV.

There is also the possibility that cladoceran BV is included in the BV measurements. North and Houde (2003), working in the Chesapeake Bay ETM in May 1998 and 1999, reported abundances of the cladoceran *Bosmina longirostris* peaking in the ETM region and up-estuary of the ETM. Lloyd (Chapter 1) also suggests that maximum abundances of cladocerans, *Daphnia* spp. and especially *B. longirostris*, occur up-estuary of the ETM, usually in freshwater. While *E. affinis* and *B. longirostris* distributions overlap to some extent in the Chesapeake Bay ETM, abundance maxima of *E. affinis* (adults and copepodites) are often an order-of-magnitude greater than *B. longirostris* density (Fig. 9; North and Houde 2003). Contour plots and calculations from a Tucker trawl survey conducted just days apart from both 25-hour surveys show that average and maximum cladoceran (*B. longirostris* and *Daphnia* spp.) densities in the ETM in May, 2002, are 10% and 32% of average and maximum *E. affinis* densities, respectively. Assuming 1) an average cladoceran size of 750 μm (the largest individuals of the more abundant *Bosmina longirostris* are ~500 μm (Zaret and Kerfoot 1975), and the largest *Daphnia* spp. individuals collected in the ETM are slightly > 1000 μm), 2) the average size of an *E. affinis* individual is ~1000 μm, and 3) a given length-to-BV ratio, the average and maximum calculated BV of cladocerans in the ETM are 8% and 14% of *E. affinis* BV, respectively. Therefore, while cladoceran BV is likely incorporated into

estimates of zooplankton BV, the cladoceran signal is likely small relative to copepod BV.

The single-station contour plots (Figs. 3 and 4) show a general trend of zooplankton BV and TSS resuspension in bottom waters during a flood tide that is either timed with, or precedes, a surface BV peak. Roman *et al.* (2001) noted that zooplankton BV resuspension tends to lead TSS as they both track with tidal currents. This same phenomenon appears to exist in the current study. The phasing between current speed and the resuspension of sediments and zooplankton may be due to the cohesive nature (i.e. stickiness) of sediments which must be disrupted by current energy before sediments can be eroded (Sanford *et al.* 2001). Thus, increasing tidal currents would first resuspend zooplankton, which are aggregated near the bottom, followed by sediment resuspension (Roman *et al.* 2001).

The two time series centered on the station near river km 30 (Figs. 3 and 4) demonstrate noticeable differences in water column zooplankton BV and TSS concentrations relative to current direction. Zooplankton concentrations along the bottom and at the surface increase with flood tides, and TSS increases in the lower water column with flood tides. Ebb currents appear to create zooplankton surface maxima but generally lack enhanced near-bottom zooplankton or TSS values. Zooplankton biomass maxima along the bottom coincide with TSS peaks, but surface BV peaks do not correspond with surface turbidity peaks (see Figs. 3 and 4). If zooplankton were simply responding passively to the same forces acting on sediments, TSS and BV concentrations should overlap at all depths. Suspended sediment concentrations, however, rarely increase above 30 mg l^{-1} above a depth of

6m for both May surveys, which is near the background TSS concentration for

Chesapeake Bay (Sanford *et al.* 2001). It appears that both zooplankton and TSS are

resuspended by current-induced scouring along the bottom, with resuspension of

zooplankton preceding the resuspension of sediments. However with decreased

current speed, zooplankton remain suspended, perhaps by actively swimming, in the

water column longer than sediments, which apparently settle.

Sanford *et al.* (2001), working in the Chesapeake Bay ETM in 1996, showed

that the average settling speed for the smallest, most abundant particles (in

concentrations over 40 mg l^{-1}) in the ETM was less than 0.02 mm s^{-1}. The largest size

class of particles (at concentrations less than 3 mg l^{-1}) settled at approximately 1.3

mm s^{-1}, and the mean settling speed for all particle size classes was around 0.3 mm s^{-1}. Morgan *et al.* (1997) determined the average sinking speed of *E. affinis* to be 3.3

mm s^{-1}. Based on these results, it seems unlikely that, once suspended, sediments

would sink faster than *E. affinis* if this copepod behaves as a passive particle (i.e., is

not swimming).

It is also known that sediment flocculation can change the density, mass and

size characteristics of particles in the Chesapeake Bay ETM and other water bodies.

Manning and Dyer (1999), using a laboratory flume, developed a power function

predicting settling velocity (W_s) versus floc diameter (D_x) which had an r^2 of 0.85:

$$W_s = 0.0116 \, (D_x)^{0.8446}.$$

Flocs sizes may range up to 300 μm in the Chesapeake Bay ETM (L. Sanford, pers.

comm.), giving a calculated sinking speed of 1.43 mm s^{-1}, still below that calculated

for *E. affinis*. Therefore, after factoring in flocculation processes, it still seems unlikely that sediment settling rate would be greater than *E. affinis* sinking velocities.

A fixed, single-station analysis is suitable for analyzing changes in TSS and BV at a point of interest but does not provide details about the spatial complexity of sediments and zooplankton in the larger ETM region. It is possible that the separation between bottom and surface TSS and zooplankton BV peaks only occurs due to the time between sampling events – the two peaks may be part of the same anomaly. Both time series have 'arms' of lower BV concentrations at mid-depths that seemingly connect well-defined surface and bottom peaks. Behavioral mechanisms, such as swimming and sinking, would serve to maintain these maxima. Assuming a sinking rate of 4 m for every 20 min (Morgan *et al.* 1997), a copepod could passively sink the equivalent depth of the Chesapeake Bay ETM in just over 1 hour.

The above description of the time series at river km 30 assumes that the ETM channel is a closed system. Another possible explanation for the disjoined TSS and BV peaks is that tidal currents scouring adjacent shoals cause copepods to be swept into the surface waters of the channel. The synchronous timing of the surface and bottom peaks would correspond to the same process (tides) acting on different regions of the ETM (channel bed and shoal bed). A shoal effect such as this would also be expected to result in surface TSS peaks, which are not present.

Unlike the Roman *et al.* (2001) results in 1996, a year of exceptionally high Susquehanna River input, the 2002 single fixed-station surveys did not reveal large resuspensions of TSS and zooplankton BV high into the water column during ebb tides. I did observe surface zooplankton BV increases during both flood and ebb

flows that may correspond to a resuspension of zooplankton from below. However, unlike the 1996 survey, there were no TSS maxima extending into the upper water column during either flood or ebb tides. Because of the differences in freshwater input between 1996 and 2002, comparisons of the physical conditions between those years should be undertaken with caution.

The conclusions drawn from single-station analyses are limited because data from these stations could represent the vertical movements of either a single zooplankton population or the movements of multiple populations from down- or up-estuary through the station. The 25-hour multiple fixed-station axial surveys (Figs. 5 and 6) allow for greater spatial analysis of BV and TSS movements. However, as stated above, a pattern of zooplankton abundance by depth with respect to tidal stage is not apparent in contour plots of the multiple fixed-station surveys. Again, the general trend for the first survey is that zooplankton abundance is greatest near the surface while TSS peaks in bottom waters. During the second survey, TSS is also highest near the bottom, especially during flood tides, and BV peaks throughout the water column. Quite often, TSS and BV maxima in one transect seem to be horizontally transported to a new river km in plots of the next transect. It is likely that BV variations during the surveys were related to zooplankton being tidally transported, i.e., displaced horizontally with water movements, rather than to only vertical movements.

The contour plots of the 25-hour surveys suggest that a different pattern of zooplankton resuspension and vertical transport was present in May surveys of 2002 and 1996, but the 2002 pattern is ambiguous based on the present data. Although

Tucker trawl data collected during the same time period as the acoustic surveys suggest that *E. affinis* is the most abundant zooplankton species in the ETM in 2002, it is possible that cladocerans in the up-estuary region and *A. tonsa* in the down-estuary region contaminate the acoustic survey data. Cladocerans are primarily found in greatest abundance near the surface in freshwater (North and Houde 2003; Lloyd, Chapter 1). High BV measurements at the surface during ebb tides may indicate cladocerans being transported down-estuary into the study area. Down-estuary of the ETM, variable BV measurements could indicate the presence of *A. tonsa*, which is usually found in the mesohaline regions of Chesapeake Bay. Migration behaviors of this copepod are primarily diel in nature and therefore may mask tidally-timed migration patterns of other taxa, namely *E. affinis*.

The apparent inconsistency in zooplankton location relative to tidal phase between the two 2002 surveys as suggested by the multiple-station contour plots (Figs. 5 and 6) probably results from 1) incomplete sampling of the water column and 2) low tidal displacements relative to the size of the ETM region. The CTD and its associated instruments, including TAPS, incompletely sample the top 1-2 m of surface waters and bottom 1-2 m along the seabed. *Eurytemora affinis* is an epibenthic copepod, and incomplete bottom sampling may therefore miss a large proportion of its population(s) within the ETM, while partial surface sampling would create an incomplete dataset with regard to vertical migration patterns. The average axial tidal displacements for each depth class (surface, middle, and bottom) were a maximum of a few kilometers, while habitat in the 0-5 salinity range usually extended more than 10 km along the bay axis (depending on depth and tidal phase). Kimmerer

et al. (1998) suggested that salinity cues may be involved in vertical migration

behaviors. Thus, any migration pattern may be masked by the fact that, over a single

tidal cycle, ETM zooplankton may not be transported out of the optimal salinity range

and will therefore not display a vertical migration pattern. Separating the ETM into

up- and down-estuary regions allowed zooplankton within particular salinity ranges to

be isolated, making migration patterns easier to statistically clarify.

Assuming that most BV measured during these surveys belongs to *E. affinis*,

one possible conclusion from the contour plots and regression analyses is that

zooplankton in the ETM possess the ability to regulate their vertical positions in the

water column and do so in response to tidal and salinity cues. The general pattern

appears to be tidal suspension and active vertical migration toward the surface during

ebb tides at up-estuary (freshwater/low salinity) locations and active migration and/or

passive sinking toward the seafloor during flood tides in down-estuary (more saline)

regions. In 2002, TSS and BV do not coincide in surface waters, and there is a

different response of TSS and BV versus current magnitude relative to estuarine

location (Figs. 7 and 8). The general relationship between tidal currents and TSS and

zooplankton BV derived from regression analyses suggest that, in the up-estuary

region of the ETM, zooplankton along the bottom are resuspended regardless of

current direction. The parabolic relationship between both BV and TSS versus

current direction suggests that zooplankton are passively resuspended as current

velocity increases. Zooplankton BV concentrations in middle and surface waters

increase with increasing ebb (seaward) tidal currents, especially at surface depths.

This pattern would position copepods in locations with the greatest down-estuary

displacement. In the more saline, down-estuary area of the ETM, a near-opposite

pattern exists. Zooplankton BV concentrations in the bottom and middle water

column increase with increasing current velocities in the flood direction, placing them

at depths with the greatest landward displacement. Based on this pattern,

zooplankton are primarily found at depths where the likelihood of advective transport

is maximized.

Previous studies of tidally-timed vertical migration have concluded that

vertical movement in which zooplankton are higher in the water column during flood

and deeper during ebb should serve to minimize seaward flux and enhance position

maintenance (Hough and Naylor 1992; Morgan *et al.* 1997; Kimmerer *et al.* 1998).

The pattern described for the 2002 BV data would place zooplankton at depths where

horizontal transport is most likely, moving zooplankton along a 'conveyor belt' of

water movement – up-estuary transport occurs along the bottom, and down-estuary

transport occurs near the surface (Fig. 10). Therefore, zooplankton position would be

maintained within the ETM over a larger area than if zooplankton vertically migrated

to depths with the least horizontal transport.

Roddie *et al.* (1984) showed that *E. affinis* survival was greatest in salinities

between 0 and 5 at May water temperatures (15-19ºC). During both May cruises,

zooplankton BV was significantly more concentrated in lower salinities and appeared

to be especially dense between salinities of 0 and 5 (Fig. 11). Although the vertical

migration pattern described for the May cruises probably results in greater advection

than the pattern described by Morgan *et al* (1997) and Kimmerer *et al.* (1998), it

would appear to keep zooplankton in the low salinity ETM region. This 'conveyor

belt' migration pattern may also serve to keep *E. affinis* in areas of high food (phytoplankton, protists, and detritus with its associated biota) concentrations or may enhance its search for food.

A variation on the 'conveyor belt' hypothesis described above is that the up-estuary region is a source of zooplankton that are tidally transported into the ETM. As they are transported down-estuary into more saline waters, the zooplankton may passively sink and/or actively migrate downward. Ensuing flood currents resuspend zooplankton from the bottom and transport them back up-estuary. Here, they may be resuspended, especially by ebb tides, and again transported down-estuary along with zooplankton recently advected from up-estuary.

The 2002 survey contour plots appear to show a different pattern of zooplankton and TSS resuspension than plots of the 1996 data. As stated above, 1996 was one of the wettest years on record in the Chesapeake Bay watershed, resulting in high Susquehanna River freshwater input into Chesapeake Bay. In October of 1996, bottom ebb current magnitudes (driven by freshwater input) were greater than flood, while the "average" October is usually marked by a minimum in freshwater input (see Fig. 9 in Roman *et al.* 2001). High Susquehanna River input pushes the ETM region further down-estuary (possibly beyond the survey area) and increases turbidity, presumably by increasing TSS resuspension (North and Houde 2001; Sanford *et al.* 2001). Enhanced ebb tides due to high freshwater input may have caused the high water column concentrations of TSS and zooplankton during ebb flow. This phenomenon was not present during the 2002 surveys. In 2002, zooplankton BV was unrelated to TSS concentrations in surface waters. The possibility of zooplankton

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advection to and from adjacent shoals is improbable because surface TSS values do not increase or decrease with BV fluxes.

Due to the ambiguity of these data, it is unclear as to whether or not zooplankton in the Chesapeake Bay ETM actively regulate their vertical position in the water column and do so in response to tidal and salinity cues. If they do, the general pattern appears to be vertical migration toward the surface during ebb tides at up-estuary locations and toward the seafloor during flood tides in down-estuary regions, with the Susquehanna River possibly supplying zooplankton to surface waters up-estuary of the ETM. Vertical migration behaviors might be more apparent during periods of reduced freshwater input via the Susquehanna River (i.e., 2002), presumably because of reduced habitat and a greater tidal influence in the upper Chesapeake Bay (Kimmel and Roman 2004). Enhanced freshwater inputs like those in 1996 create a larger-than-average ETM region that is located further down-estuary than the 2002 ETM (compare isohaline and maximum TSS locations between Roman *et al.* (2001) Figs. 2, 3, and 4 versus Lloyd *et al.* (Chapter 1) Figs. 5, 8, and 11, respectively), making comparisons between these two years difficult.

The possible differences between the 1996 and 2002 ETM surveys should encourage future research on tidally-timed migration in the Chesapeake Bay ETM, which should ideally incorporate both acoustic and traditional (net, pump) sampling methods. While the 2002 data are inconclusive regarding a tidal migration pattern zooplankton, the parabolic relationship between near-bottom BV and current velocity up-estuary of the ETM suggests that if zooplankton do migrate, they do not migrate solely in response to tidal phase (Fig. 7, 'Bottom' frames). Further, if there is active,

tidally-timed migration in the Chesapeake Bay ETM, it does not serve to minimize

advection, unlike migration patterns observed in other estuaries.

Table 5.1. Median, minimum, and maximum values for Total Suspended Solids (TSS, mg l^{-1}) and Zooplankton Biovolume (BV, mm^3 m^{-3}) grouped for both surveys. Surface = 3-6m, Middle = 6-9m, Bottom = 9-12m.

	Total Suspended Solids			**Zooplankton BV**		
Depth	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**
Surface	10.4	6.4	25.0	920	12	88948
Middle	14.0	6.6	61.7	281	9	3379
Bottom	33.8	9.3	177.9	1096	107	25863

Table 5.2. Correlation coefficients by depth of pairwise correlation analyses between TSS and BV for both cruises. Surface = 0-4 m; Middle = 4-8 m, Bottom = 8-12 m. Values with an asterisk are statistically significant (p < 0.001).

	May 8-9	May 14-15
Surface	-0.10	0
Middle	0.48*	0.33*
Bottom	0.61*	0.57*

Table 5.3. Calculated water displacements (km) for both surveys at three depths (3.5m, 7.5m, and 11.5m). Total displacement is the net average displacement over two tidal cycles except for 7.5 and 11.5m depths for the May 14-15 survey, which are calculated over one tidal cycle. Negative values indicate displacement down-estuary (ebb direction), positive values indicate displacement up-estuary (flood direction).

Cruise	Tidal phase	3.5m	7.5m	11.5m
May 8-9	Ebb	-13.87	-9.47	-4.25
	Flood	6.02	7.24	7.89
	Ebb	-10.97	-6.28	-4.73
	Flood	9.74	10.04	6.65
	Total	-4.54	0.77	2.78
May 14-15	**Tidal phase**			
	Ebb	-4.06	-3.44	-3.04
	Flood	1.85	3.33	2.97
	Ebb	-13.34	-4.80	-3.11
	Flood	11.09		
	Total	-2.23	-1.48	-0.13
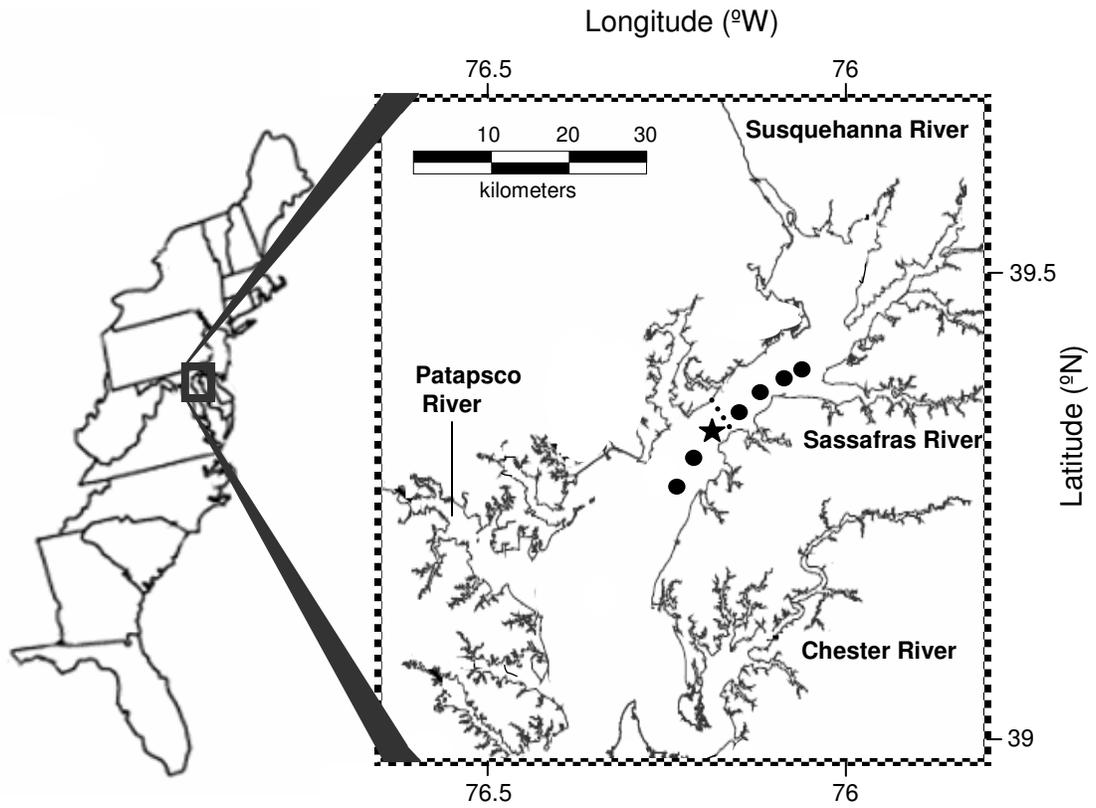
Figure 5.1. Map of the United States east coast with the Chesapeake Bay ETM region magnified. Solid circles and the star represent the approximate locations of all stations of the 25-hour surveys. The star represents the location of the station at river km 30. The dotted line perpendicular to the bay axis represents the dividing line between up-estuary and down-estuary stations and is at approximately river km 28.
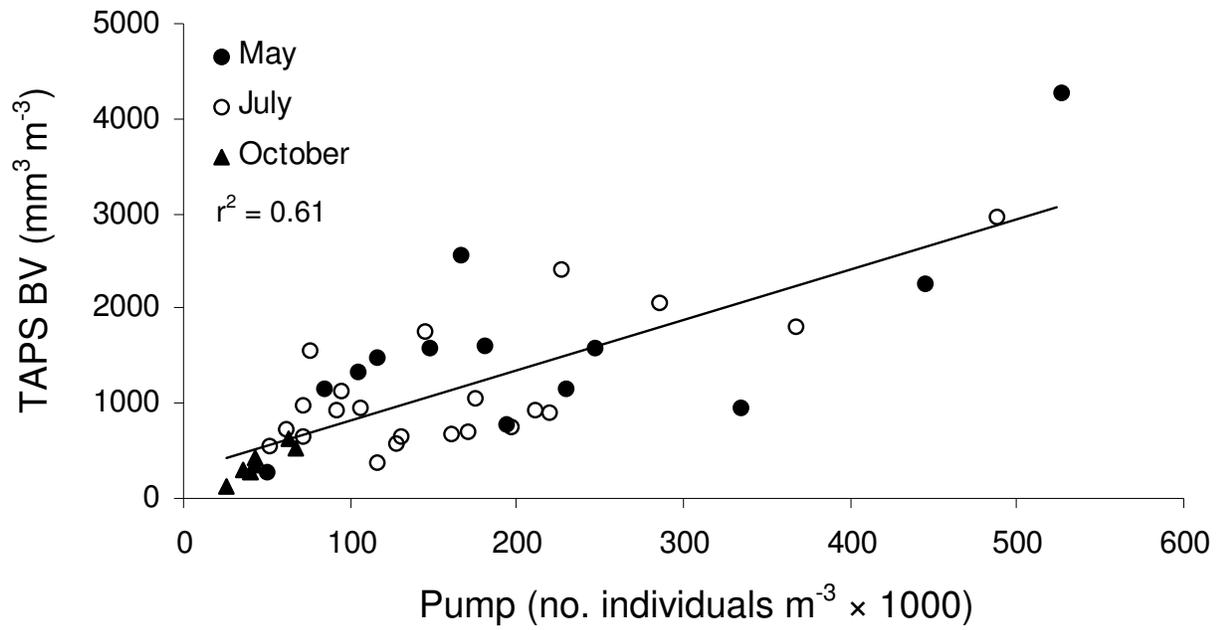
Figure 5.2. Comparison of TAPS Biovolume (BV) measurements with zooplankton counts from pump samples.
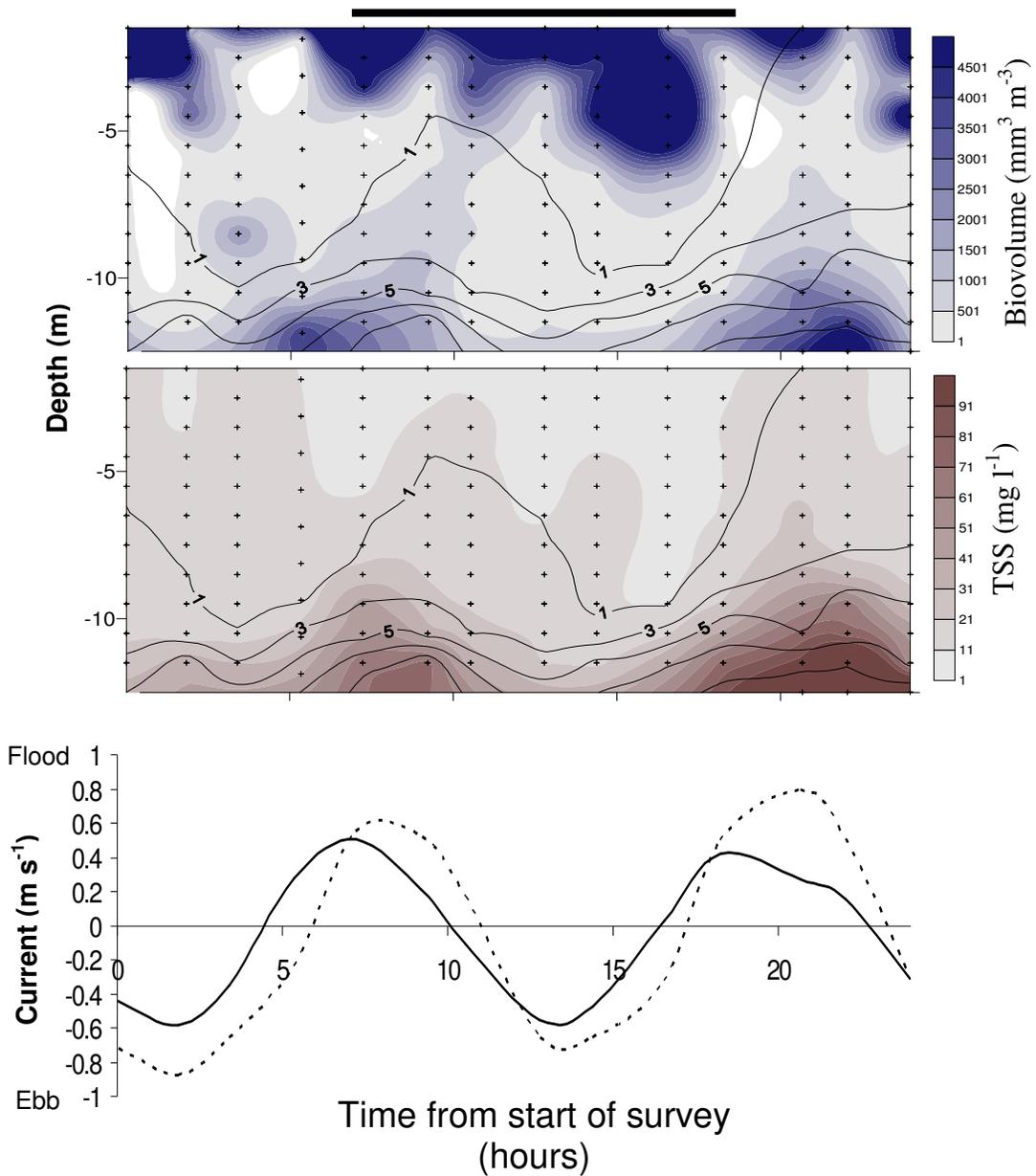
Figure 5.3. Salinity (line contours; PSU), zooplankton biovolume (top contour plot; mm^3 m^{-3}), total suspended solids (middle contour plot; mg l^{-1}), and current velocity (bottom plot; positive values indicate flood currents, negative values indicate ebb currents) for the first survey (May 8-9). The solid line in the current plot represents currents at 11m depth, the stippled line represents currents at 3 m depth. The black bar above the top frame marks stations that were sampled at night.
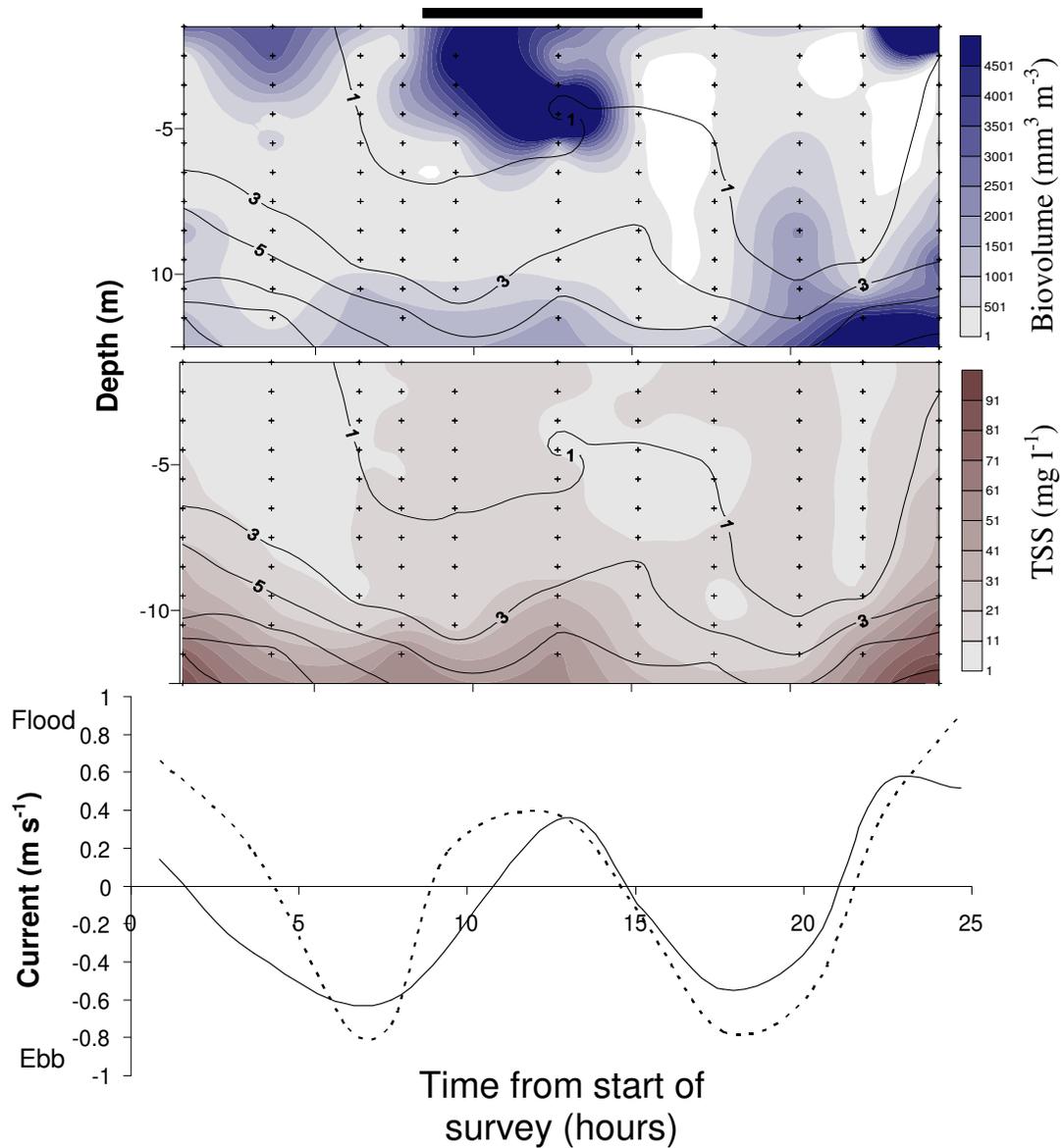
Figure 5.4. Salinity (line contours; PSU), zooplankton biovolume (top contour plot; mm^3 m^{-3}), total suspended solids (middle contour plot; mg l^{-1}), and current velocity (bottom plot; positive values indicate flood currents, negative values indicate ebb currents) for the second survey (May 14-15). The solid line in the current plot represents currents at 11m depth, the stippled line represents currents at 3 m depth. The black bar above the top frame marks stations that were sampled at night.

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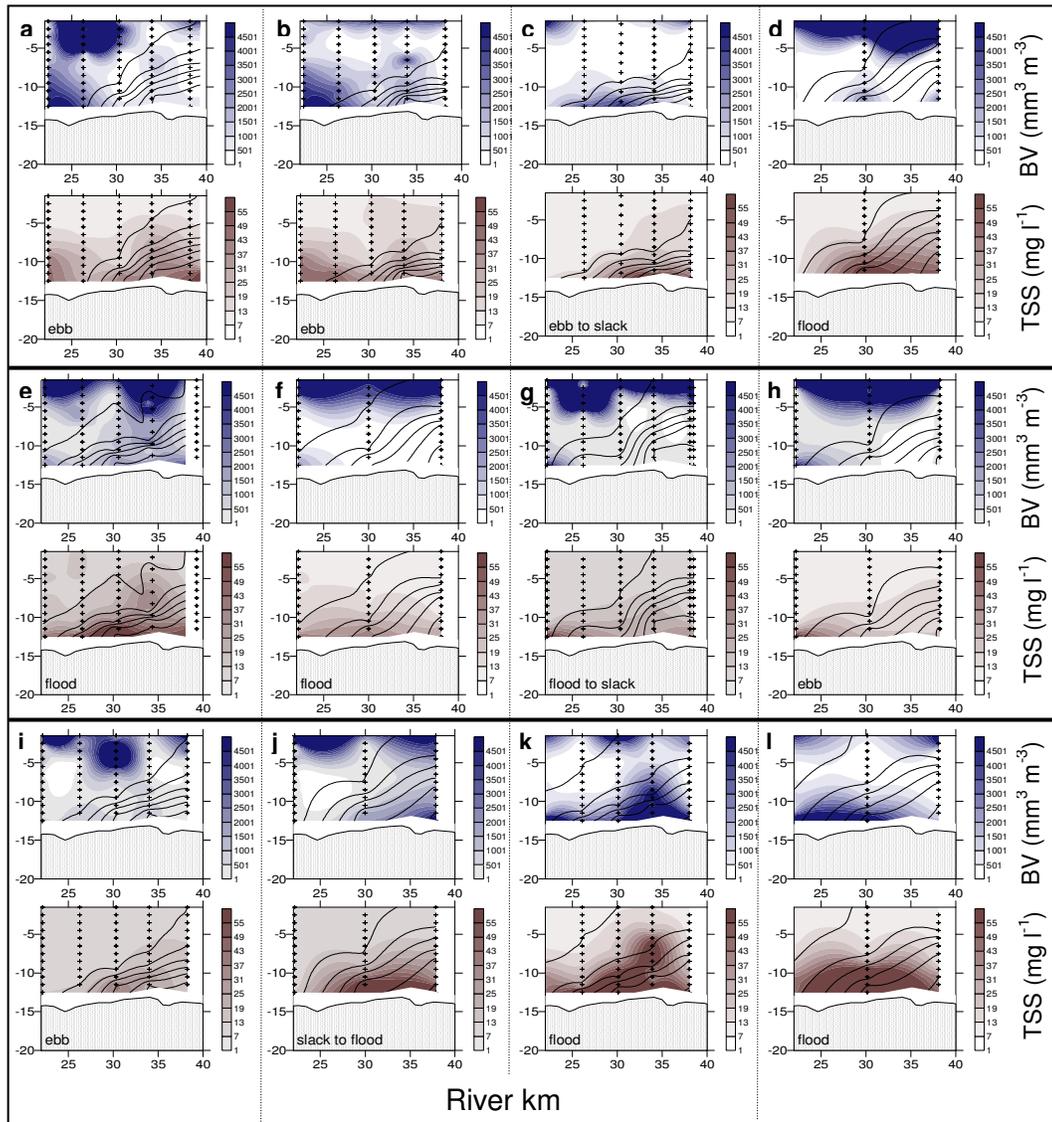
Figure 5.5. Multiple fixed-station contour plots of salinity (line contours; PSU), zooplankton biovolume (top contour plot; mm^3 m^{-3}), total suspended solids (middle contour plot; mg l^{-1}) versus river km (distance from Havre de Grace, Maryland) for the first survey (May 8-9). Transects were surveyed chronologically by letter (**a-l**). Tidal stage is given for each transect. The bold line represents the 1 PSU isohaline, each isohaline to the right increases by 2 PSU.
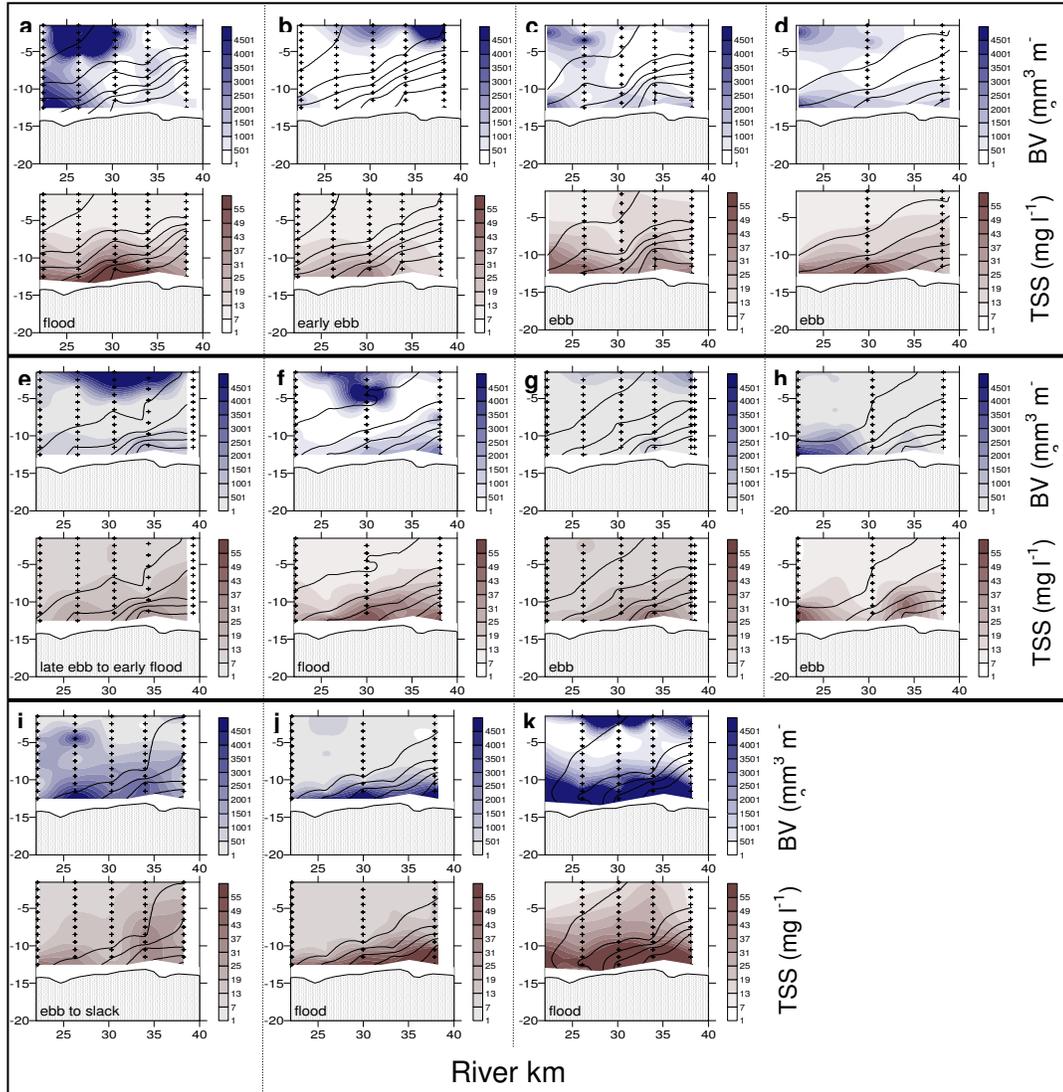
Figure 5.6. Multiple fixed-station contour plots of salinity (line contours; PSU), zooplankton biovolume (top contour plot; mm^3 m^{-3}), total suspended solids (middle contour plot; mg l^{-1}) versus river km (distance from Havre de Grace, Maryland) for the second survey (May 14-15). Transects were surveyed chronologically by letter (**a-k**). Tidal stage is given for each transect. The bold line represents the 1 PSU isohaline, each isohaline to the right increases by 2 PSU.
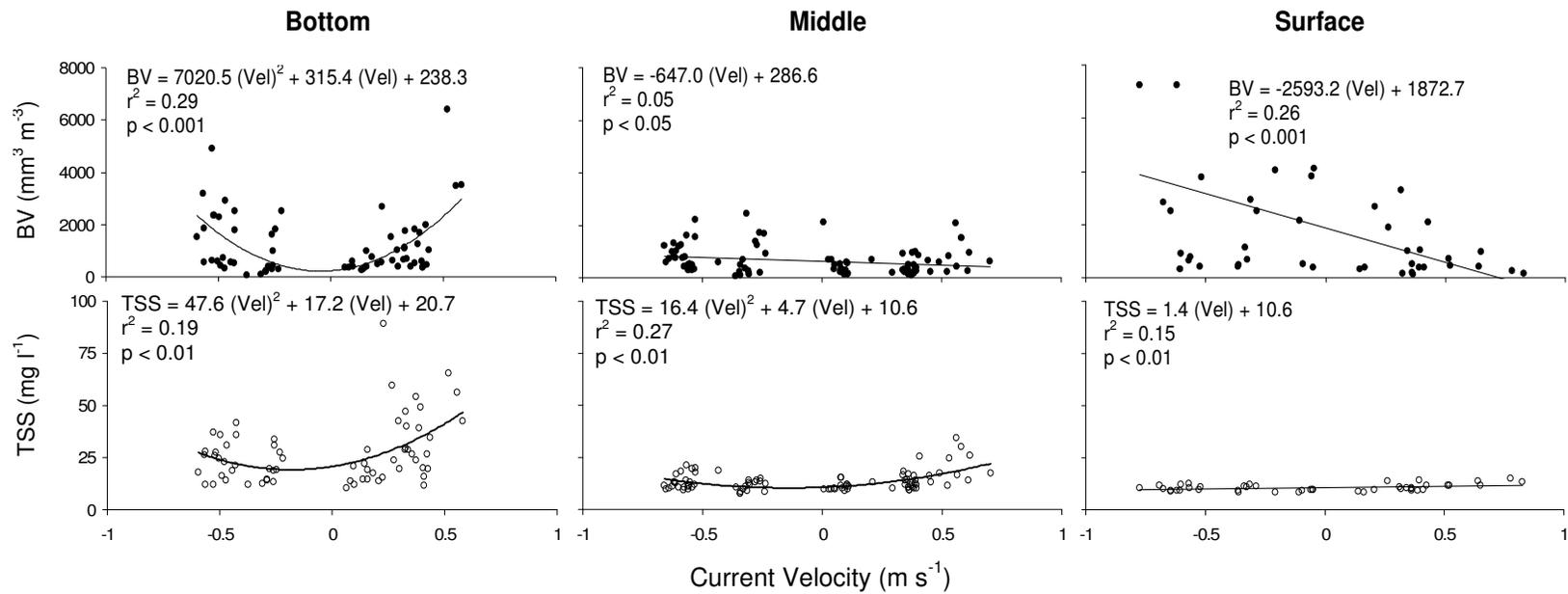
Figure 5.7. Regression analyses between both zooplankton biovolume (BV) and total suspended solids (TSS) versus current velocity (Vel) for transect stations up-estuary from river km 28. Bottom = 8-12 m depth; Middle = 4-8 m depth; Surface = 0-4 m depth.
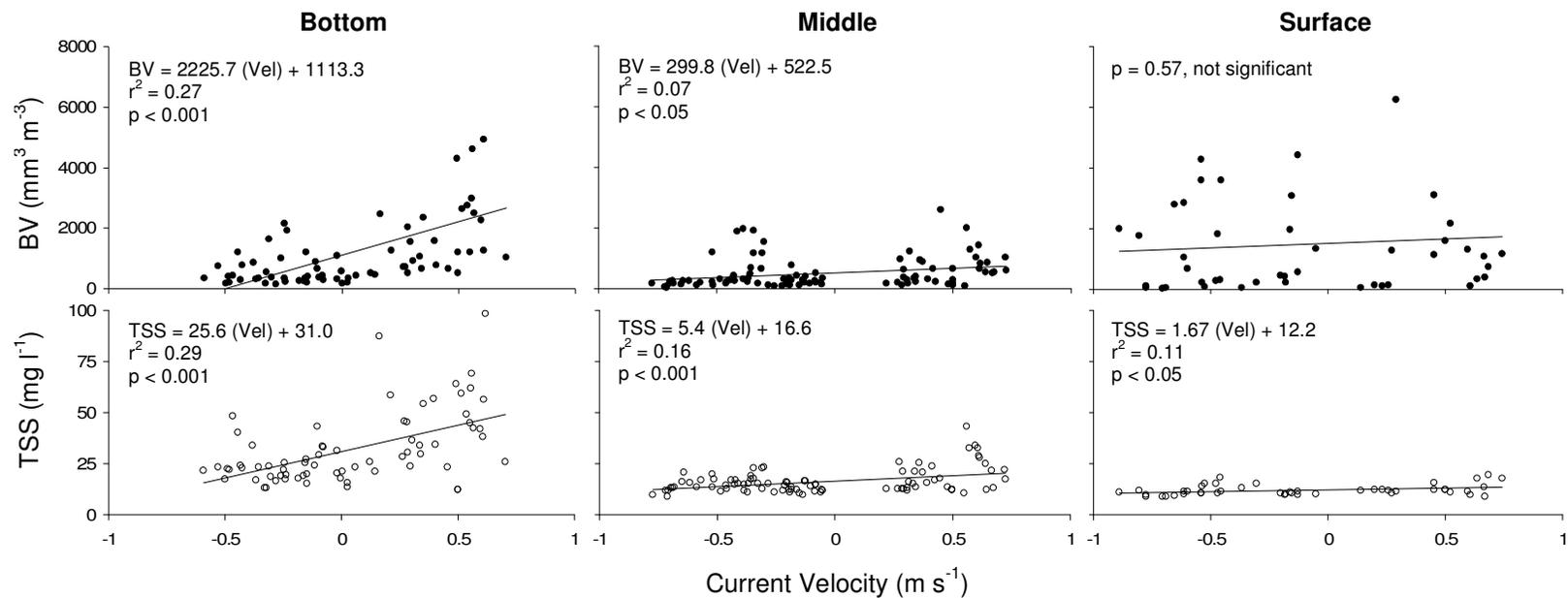
Figure 5.8.   Regression analyses between both zooplankton biovolume (BV) and total suspended solids (TSS) versus current velocity (Vel) for transect stations down-estuary from river km 28. Bottom = 8-12 m depth; Middle = 4-8 m depth; Surface = 0-4 m depth.
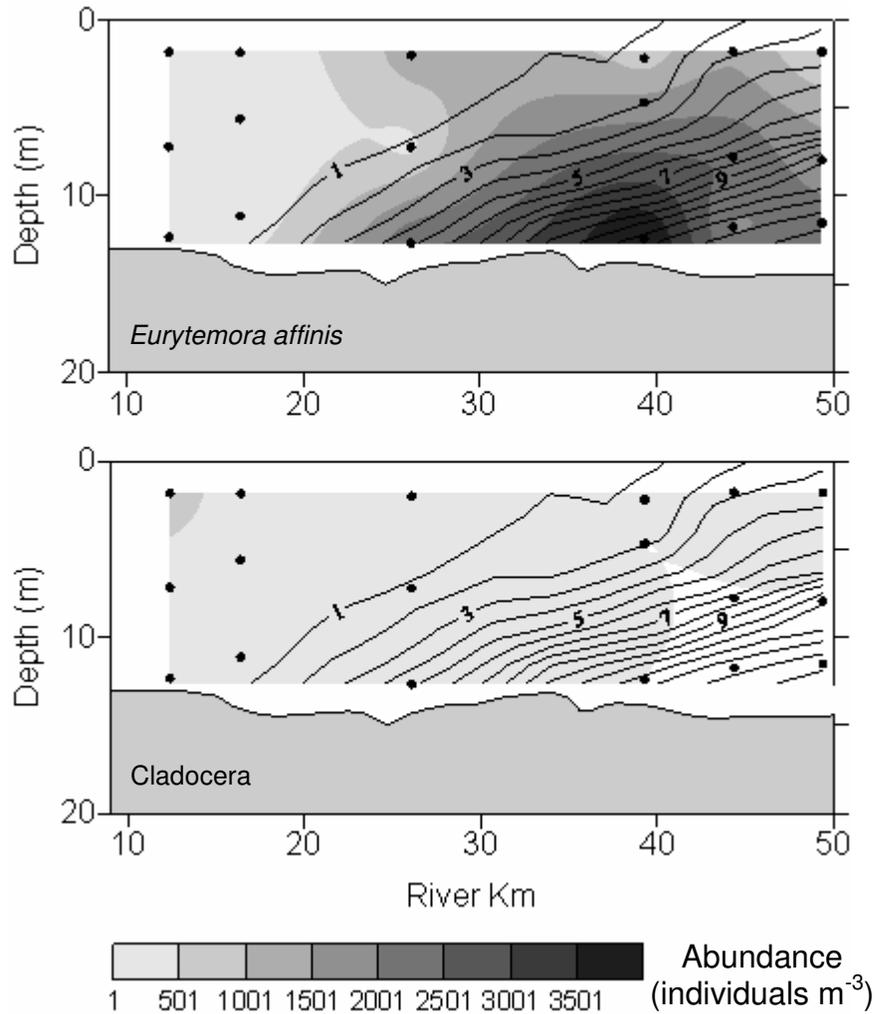
Figure 5.9. Contour plots of Tucker trawl data from Chesapeake Bay ETM region, May, 2002. Line contours – salinity (PSU); Color contours – *Eurytemora affinis* and cladoceran (*Bosmina longirostris* and *Daphnia* spp.) abundance (individuals m^{-3}); X-axis is distance down-estuary from Havre de Grace, Maryland.
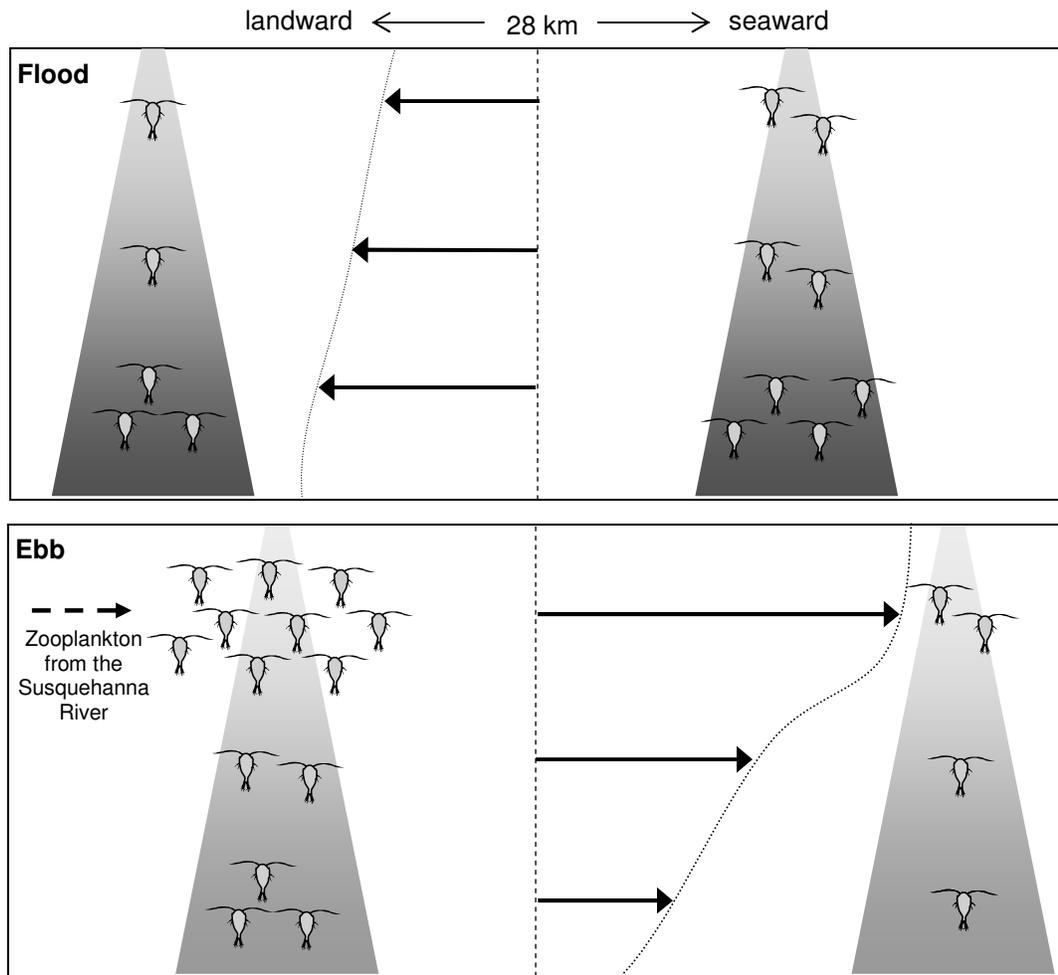
Figure 5.10. A hypothetical snapshot of zooplankton biovolume (BV; each   represents approx. 500 mm^{-3} of BV) and total suspended solid (TSS; relative intensity indicated by shading) concentrations during maximum flood and ebb conditions in the Chesapeake Bay ETM region in May, 2002. The bold arrows in both frames represent relative current velocities in surface (1-4 m depth), middle (4-8 m depth) and bottom (8-12 m depth) portions of the water column, and the stippled line shows the mean isohaline structure. On the seaward side of the mid-point between all transects (28 km from Havre de Grace), BV concentrations in the bottom and middle portions of the water column are significantly greater during flood than ebb. On the landward side, BV concentrations are significantly greater in surface and middle waters during ebb than flood. During flood, bottom scouring resuspends both TSS and epibenthic zooplankton in both landward and seaward regions, especially in bottom waters. During the slack before ebb, TSS concentrations decrease as sediments settle out of the water column. Zooplankton swimming behaviors and zooplankton resuspension during the following ebb may result in significantly greater BV in surface and middle waters than during flood. Additionally, zooplankton swept down-stream from the Susquehanna River might add to zooplankton BV in surface waters, especially during ebb tides. The locations of BV maxima during the tidal cycle suggest that zooplankton tend to be at the depths of maximum current velocities, resulting in a 'conveyor belt' of zooplankton movement. TSS concentrations are generally greater in both landward and seaward regions during flood than ebb at all depths, probably due to greater bottom stress during flood.

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Figure 5.11. Linear regression of zooplankton biovolume (BV) versus salinity of all May data.

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CHAPTER VI: General Conclusions

SUMMARY AND CONCLUSIONS

Although primary production is low in the Chesapeake Bay ETM (Fisher *et al.* 1988), this region is a dynamic, often flourishing, ecosystem. Phytoplankton and detritus washed into this region from upstream, the bacteria and protozoa organisms associated with them, and free-living microzooplankton all likely combine to form the base of the food web in the ETM. The calanoid copepod *Eurytemora affinis* is especially suited to occupy such a habitat given its 1) preference for low salinity and 2) ability to select particles based on nutritional value, which is enough to create a chlorophyll-*a* (chl-*a*) "hole" in this region. This copepod appears to feed more efficiently outside of the particle milieu of the ETM but is limited to the ETM region due to greater predation pressures down-estuary.

<u>Chapter 1</u>

The constraints (physical versus biological) on *E. affinis* distribution appear to be primarily based on the strength of Susquehanna River input into Chesapeake Bay. During periods of low flow (below the annual mean), the size of the ETM is reduced, and its location is pushed up-estuary (Kimmel and Roman 2004). Such conditions make biological interactions more discernible. For example, Susquehanna River flow generally peaks in the springtime (March, April, May) and is at a minimum around August; July and October tend to have similar values. Early-to-mid autumn flow may also increase if tropical cyclones pass over the Chesapeake Bay region.   In 2001 and 2002, years of average or slightly below average freshwater input, Tucker trawl data

show that *E. affinis* and *Acartia tonsa* distributions often overlapped in the spring but were spatially partitioned in the summer and fall. Laboratory experiments demonstrate that *E. affinis* is able to survive and reproduce at salinities and temperatures above those in which it was found at these times, suggesting that interactions with *A. tonsa* and/or mysids limited its down-stream abundance. Mysids and *A. tonsa* may also limit downstream *E. affinis* abundance during high flow periods, but, because such conditions increase available habitat, interactions between *E. affinis* and mesohaline zooplankton would affect a smaller proportion of the population.

More research concerning interactions between *E. affinis* and *A. tonsa*, mysids, ctenophores, and andadromous fish larvae should be performed for several reasons. Identifying the effects of these groups on *E. affinis'* abundance and reproductive rates will help to further elucidate why *E. affinis* is constrained primarily to the ETM. As these groups all directly interact, it would also be helpful to know, for example, what fraction of mysid prey consists of *E. affinis* and the proportions in which fish larvae prey on these groups. Because the ETM appears to be such a unique ecosystem, tracking carbon through the food web is interesting both academically and economically (i.e. fisheries).

Chapter 2

Egg production rates have often been used to estimate somatic growth due to the general ease of determining EP. This approach assumes that any resources that would go into somatic growth for juveniles would be allocated to reproductive growth in adults. The fact that 2002 EP estimates by the clutch size method fit all three

growth models, especially the Hirst-Sheader model, supports the use of EP estimates

to estimate juvenile somatic growth under food-replete conditions. Caution should be

taken when estimating EP with the clutch size method under low food conditions, as

laboratory estimates of ID may not be representative of field ID. Further, it must be

noted that because of the poor fit of the 2003 data to growth models, indirect methods

of estimating copepod egg production, namely the egg-ratio method and clutch size

method, may not always be appropriate, and incubations may provide better

estimates.

Two issues with the use of the clutch size method for estimate copepod egg

production rates are that 1) the experiments to determine ID are time intensive, and 2)

ID/temperature relationships for one copepod species cannot be extrapolated to

another. Further complicating such indirect methods of estimating egg production

rates, *E. affinis* may actually represent a species complex (Lee 1999) where particular

rates or tolerances appear to differ between populations. This is suggested by

differences in *E. affinis* ID/temperature relationships between Vijverberg (1980) and

Lloyd (Chapter 2). Acclimating *E. affinis* captured in low salinity waters to

freshwater conditions has proven difficult (pers. obs.), though many *E. affinis*

populations are found in the freshwater regions of river and in lakes (Ban 1994; Lee

1999).

There is also the possibility that copepod ID is dependent on the size of the

female. *Eurytemora affinis* females used in the ID experiments were collected from

waters around 10ºC and incubated at temperatures ranging from 5 to 27.5ºC. While

these females were about the same size, the same incubation temperatures in the field

would be associated with adult copepods of different sizes due to the effect of temperature on development rate (lower temperatures = larger adults, higher temperatures = smaller adults). While temperature is negatively related to female size, female size is positively related to clutch size, and a larger clutch (from a larger female) may take longer to create than a smaller clutch (from a smaller female). Therefore, if the goal of ID duration experiments is to estimate egg production rates of copepods in the field, the experiments should be conducted on females whose size is representative of the temperature at which they are incubated. It would be interesting to compare the results of such an experiment to the results of Chapter 2.

Chapter 3

Increases in freshwater input cause an increase in particulate input (Sanford *et al.* 2001) and, as stated above, an increase in the nutritional quality of particulates. Research during 2002 and 2003 in the Chesapeake Bay and Choptank River ETMs on food limitation suggests that, even during dry years, *E. affinis* is not food limited in terms of food quantity. There may be a slight increase food quality during wet years, but the increase in available habitat seems to be the greatest benefit to copepods.

Optimally, the research for this chapter would involve measuring *E. affinis* egg production in the same system (either Chesapeake Bay or the Choptank River) over multiple years. Also, microplankton (64-200 μm) counts for both years would have helped to better characterize the Chesapeake Bay and Choptank River ETMs.

Although the effects of freshwater input on the size and intensity of the ETMs in both systems seems to be the same, there is no guarantee that both *E. affinis* populations respond the same way to particular variables (see the Chapter 2 summary

above). However, the conclusion that *E. affinis* is not food limited in the ETM is supported by both the clutch size/prosome regression and the results of the grazing experiment.

Chapter 4

Freshwater input also influences *E. affinis* vertical distributions. Dry periods (months or years) allow for copepod responses to tidal current/salinity changes to affect their position within the ETM. Specifically, zooplankton migrate toward the surface during flood tides and downward during ebb, thus staying at depths with the lowest horizontal current velocities. When freshwater input is high, zooplankton may be swept out of the upper portion of the ETM due the enhanced seaward flow, thus masking any vertical migration patterns. Because the ETM usually extends further down-estuary during these conditions, zooplankton that are transported seaward may not necessarily be transported out of the ETM habitat.

If I were to carry out the vertical migration project again, I would collect data in April, optimally during a period of average freshwater input. Under such conditions, *E. affinis* abundance tends to be high while *Acartia tonsa* abundance is low and is greatest down-estuary of the ETM. The sampling protocol – 25-hour, multiple-station transects – is ideal for analyzing vertical movements that also have a tidal (horizontal) component, and the use of TAPS to collect BV data allowed for rapid data collection not possible through traditional net or pump sampling. The data might have been improved by binning different zooplankton size ranges, specifically to differentiate between *E. affinis* nauplii and copepodite stages. Also, tidal displacement could have been directly measured using an inert tracer.

The Chesapeake Bay and Choptank River turbidity maxima are unique, dynamic ecosystems. *Eurytemora affinis*, the most abundant zooplankton species in these regions for much of the year, is well adapted to ETM salinity, current, and food conditions. It is possible that competitive and predatory interactions with other zooplankton (*A. tonsa*, mysids, larval fish) exclude *E. affinis* from down-estuary regions that it may otherwise successfully colonize. Its ability to utilize the resources in highly turbid regions, however, allow for population abundances that are often greater than other species' abundances further up- or down-estuary of ETMs.

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