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Copepod carcasses, mortality and population dynamics in the tributaries of the Lower Chesapeake Bay

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Copepod Carcasses, Mortality and Population Dynamics in the Tributaries of the Lower Chesapeake Bay

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

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

by
David Thomas Elliott
2010
APPROVAL SHEET

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Doctor of Philosophy

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ABSTRACT

Several studies have documented the occurrence of substantial numbers of zooplankton carcasses in marine field samples. However, the potential effect of carcasses on conclusions resting on zooplankton abundance estimates, and the reasons for carcass occurrence have been largely disregarded. Many field studies do not account for the presence of carcasses in their sampling methodology. Zooplankton carcasses *in situ* are significant for several reasons. As concentrated particles of organic matter in the water column, zooplankton carcasses can be important vehicles for organic matter transport and hotspots of microbial abundance and activity. If dead animals are treated alive, carcasses could bias the ecological conclusions of field studies. Finally, naturally occurring carcasses lacking injuries likely represent instances of non-predatory mortality, a poorly studied phenomenon in marine zooplankton ecology. The goal of my research was to resolve the importance of naturally occurring zooplankton carcasses with regard to the roles described above. A detailed evaluation was made of the neutral red vital staining method, to resolve method limitations for quantifying zooplankton carcasses *in situ*. The method gave reliable results for common copepods in the lower Chesapeake Bay, and artifact collection mortality was negligible. Thus, neutral red is a valuable method for quantifying naturally occurring copepod carcasses in the lower Chesapeake Bay. A two year study was then done to quantify carcasses in the lower Chesapeake Bay. Carcasses were a persistent feature in the water column throughout the study, with a repeating pattern of higher carcass abundance during the summer and fall in each year. The fate of carcass organic matter was then determined using a combined laboratory, field, and mathematical modeling approach to quantify removal by sinking, necrophagy (consumption of carcasses), and microbial decomposition. Carcass removal due to sinking was impeded by turbulent mixing in the estuary, and the rate of removal depended on the magnitude of ingestion by necrophages and the effects of water temperature on microbial decomposition. The resulting carcass abundances and removal rates were then used to determine errors resulting from counting carcasses as alive in ecological field studies, and also to determine the contribution of non-predatory factors to zooplankton mortality in the lower Chesapeake Bay. When carcasses were treated as alive, there were substantial errors in mortality rates derived from field abundances. This demonstrated the importance of identifying carcasses in zooplankton field samples. Non-predatory mortality accounted for 8% to 42% of total zooplankton mortality. The importance of non-predatory factors (*e.g.* disease, starvation, environmental stress) deserves more attention in ecological studies of marine zooplankton mortality and population dynamics.
AUTHOR'S NOTE

The primary research chapters (Chapters 2-5) of this dissertation were written in the format of the journal for which each is published, in review, or to be submitted. The citations for these chapters are as follows:

Chapter 2

Chapter 3

Chapter 4

Chapter 5
THE OCCURRENCE OF CARCASSES, MORTALITY AND POPULATION DYNAMICS OF COPEPODS IN THE LOWER CHESAPEAKE BAY
CHAPTER 1

Introduction to the dissertation
INTRODUCTION

In the pelagic marine environment, mesozooplankton are a vital link from phytoplankton to higher trophic level organisms. Copepods are the dominant mesozooplankton in many coastal seas, have the potential to consume substantial amounts of phytoplankton and other suspended particles (Richman et al. 1977; White and Roman 1992; Hoover et al. 2006), and represent an important food resource for many other marine animals including fish (Rilling and Houde 1999; North and Houde 2003). In coastal seas where copepod populations declined sharply (e.g. Black and Caspian Seas), the collapse of the areas' fisheries followed (Cloern 2001; Bilio and Niermann 2004). Because of their pivotal role in marine food webs, detailed studies of copepod population dynamics are fundamental to the study of marine ecology and ocean biogeochemistry.

The factors controlling spatial and temporal variation in copepod abundance include population growth and mortality, as well as advection and diffusion. Advection and diffusion are rather poorly understood, but with regard to copepods, may be considered negligible over large enough spatial and temporal scales. Population growth is a function of egg production, development through 13 distinct life-stages, and individual growth and biomass addition; all of which have been studied for decades by zooplankton ecologists. One of the most important, yet poorly constrained parameters in copepod population dynamics is mortality. Constraining mortality estimates in natural copepod populations is difficult, partly due to the scale over which measurements must be made. Individual processes such as egg production and stage-to-stage development can be observed in laboratory incubations. However, bottle incubations are less likely to give representative estimates of copepod mortality. To quote Ohman and Wood (1995):
while it is often feasible to incubate a zooplankter with a broad spectrum of natural prey items, it is rarely possible to include a spectrum of predators that may range from heterotrophic dinoflagellates, small poecilostomatoid copepods, and scyphostome medusae to mobile planktivorous fish and mammals.

Thus, mortality rates of natural copepod populations may be better estimated from field sampling, with additional insights provided by laboratory experiments. A number of methods exist to estimate mortality and other copepod population parameters from field census data (Aksnes et al. 1997).

Generally, zooplankton population census data are obtained by counting preserved field samples collected using plankton nets, pumps or other devices. In most cases all intact animals in the preserved samples are assumed to have been alive at the time of collection. The validity of this assumption is questionable, and a number of studies have reported carcasses of copepods and other zooplankton occurring in substantial numbers in marine plankton samples (see Ch. 2 for review). Zooplankton carcasses occurring in situ represent instances of natural mortality. After death, these carcasses will remain in the water column until removed by sinking, necrophagy, or microbial decomposition. However, copepod carcasses are rarely identified or quantified in field studies, due in part to the added workload and difficulty in identifying carcasses after sample preservation.

Failure to identify carcasses in field samples will lead to overestimation of the abundance and biomass of live zooplankton. How large could such an overestimation be? In samples collected in summer 2005 from the lower Chesapeake Bay, Tang et al. (2006) reported that, on average 29% of the copepods were dead. While this high value may
have been due to the unusually high water temperatures during summer 2005, a follow-up study throughout the entire bay in 2007 observed that an average of 18% of the collected copepods were carcasses (Tang et al. 2007). Thus, a substantial fraction of copepods in Chesapeake Bay may actually be dead in situ, necessitating careful consideration of live/dead composition when collecting copepod samples.

Copepod carcasses can be identified either based on signs of damage and internal decomposition (Wheeler 1967) or by using the vital stain neutral red (Tang et al. 2006). This second method is especially promising for widespread use in field studies. It is relatively simple to implement and less subjective than inspection for signs of injury or decomposition, since live animals stain red but dead animals do not. Use of neutral red to determine the live/dead composition of copepods in field samples would allow for more accurate assessment of live copepod abundance and biomass, and could allow direct and quantitative studies of the mortality processes resulting in carcasses in situ, such as partial predation (Genin et al. 1995; Haury et al. 1995) and non-predatory mortality, the latter being poorly studied in marine zooplankton populations (Gupta et al. 1994).

A more mechanistic understanding of mortality is imperative to studies of copepod population dynamics. In addition to predation, which is well established to be an important cause of copepod mortality (Carlotti et al. 2000), non-predatory mortality could be caused by factors such as disease, harmful algal blooms, hypoxia, chemical pollution, other environmental stressors, or starvation (Carpenter et al. 1974; Uye and Flemiger 1976; Byron et al. 1984; Vecchione 1989; Kimmerer and McKinnon 1990; Roman et al. 1993; Hall et al. 1995; Delgado and Alcaraz 1999; Calbet et al. 2003; Sopanen et al. 2007). In a global meta-analysis, Hirst and Kiørboe (2002) compared longevities of
copepods in the laboratory and in the field, estimating that predatory mortality accounted for 67-75% of total mortality of copepods in the sea. The remaining 25-33% represented non-predatory mortality, suggesting that non-predatory factors may be similar in magnitude to predation mortality in marine copepod population dynamics.

STRUCTURE OF DISSERTATION

The overall goal of my dissertation research was to make a detailed evaluation of the importance of copepod carcasses in studies of estuarine zooplankton ecology. This was done by quantifying the prevalence of carcasses and the processes resulting in removal of carcasses from the water column, and then determining the implications of these carcasses for zooplankton sampling methodology and non-predatory zooplankton mortality. This dissertation is divided into six chapters with Chapters 2 to 5 describing the results of laboratory experiments, a two-year field study of zooplankton in the lower Chesapeake Bay, and several modeling exercises.

Chapter 2 describes laboratory experiments testing the accuracy of live/dead information obtained using neutral red staining under a variety of environmental conditions. In addition, a detailed evaluation was made as to whether the live/dead information obtained in field samples reflects natural mortality in situ or a substantial artifact of copepods killed by sample collection and processing. The suitability of neutral red for live/dead determinations of zooplankton in field samples is discussed.

Chapter 3 describes a two-year field study of the dominant copepod species in the lower Chesapeake Bay, reporting the spatial and temporal variation in the abundances of live copepods and carcasses. The relationship between these abundances and measured
hydrographic variables was also explored. Results are discussed in context of the ecology of the copepod species studied.

Chapter 4 describes laboratory experiments, field observations, and modeling exercises examining the relative importance of sinking, necrophagy, and microbial decomposition for removal of copepod carcass organic matter from the estuarine water column. The resulting removal rates were used to predict the fate of carcass organic matter under different environmental conditions. The role of copepod carcasses in the lower Chesapeake Bay is discussed, including their fates, turnover times in the water column, and potential ecological and trophic importance.

Chapter 5 synthesizes the results of the field study (Chapter 3) and the carcass fate study (Chapter 4) to evaluate the implications of copepod carcasses for zooplankton population studies. Copepod mortality rates were estimated from abundance data (Chapter 3) both with and without live/dead information, to quantify errors occurring as a result of counting carcasses as alive in samples. Quantitative estimates of the predatory and non-predatory components of mortality were derived from data on carcass abundance (Chapter 3), and using estimated carcass turnover times from the carcass fate model (Chapter 4). Using a simple population dynamics model, I then evaluated the importance of these mortality terms for regulating copepod populations in the lower Chesapeake Bay.

Chapter 6 summarizes the broader conclusions resulting from this dissertation research. Based on these conclusions, I identified promising directions for future research in studies of marine zooplankton mortality and population dynamics.
REFERENCES


CHAPTER 2

A simple staining method for differentiating live and dead marine zooplankton in field samples
ABSTRACT

A method is described and evaluated for the use of neutral red staining to differentiate live and dead zooplankton in marine field samples. The protocol can be easily incorporated into shipboard zooplankton sampling. The use of neutral red in laboratory studies is common, but its application for quantifying natural live/dead zooplankton composition under field conditions has not been evaluated in detail. Here the accuracy and precision of the method were tested for a range of salinities and temperatures, and for common estuarine zooplankton groups. Detailed descriptions of staining intensities and patterns were provided. In addition, potential artifact mortality due to collection and sample handling was evaluated. The method produced accurate results under conditions tested, and artifact mortality was negligible using the recommended protocol. Neutral red staining is ideal for quantification of zooplankton carcasses in field samples, which will allow for more systematic study of in situ zooplankton mortality and related processes.
INTRODUCTION

A fundamental need in marine zooplankton ecological research is accurate assessment of population abundances. Traditionally researchers collect, preserve and enumerate zooplankton field samples without evaluating the vital state of the animals. Estimates of animal abundance from field samples are then used to extrapolate per capita ecological rates to population rates (e.g. grazing, metabolism, growth, and reproduction). The underlying assumption in this common practice is that all animals in field samples are live and active; any deviation from such an assumption could result in erroneous understanding of many fundamental processes in the pelagic ecosystem. A number of studies have shown that zooplankton carcasses are prevalent at times in the marine environment (Table 1). Carcasses with visible wounds could be results of partial predation (Genin et al. 1995; Haury et al. 1995), whereas carcasses showing signs of internal decomposition but otherwise intact may represent mortality from different causes, such as parasitism, harmful algal blooms, starvation, and environmental stress (Byron et al. 1984; Hall et al. 1995; Gomez-Gutierrez et al. 2003; Sopanen et al. 2007). Without carefully identifying zooplankton carcasses in samples, researchers may grossly overestimate the abundances of live individuals, especially in cases when a large percentage of the animals are dead in situ.

Inspection of preserved animals for visible signs of damage or decomposition can help to identify carcasses (e.g., Wheeler 1967; Weikert 1977). However, this method is time consuming and subjective, and does not easily distinguish recently dead animals from live animals. A simple method to quickly and reliably differentiate live and dead zooplankton in preserved field samples is therefore needed. Here we describe such a method using the vital stain neutral red. Dressel et al. (1972) first described the use of
neutral red staining to differentiate live and dead marine copepods. The method is promising for determining live/dead status of zooplankton in field samples for a number of reasons. It provides a clear color distinction between live and dead animals, making it less subjective and less time consuming than inspecting for signs of injury or decomposition. It also allows for identification of recently dead individuals that may have no visible signs of decomposition. The method is inexpensive, the stain is non-toxic, and the protocol for staining is simple, making it easy to incorporate into routine field sampling.

As with any method, there are likely inherent limitations with the application of neutral red staining to zooplankton in field samples. Dressel et al. (1972) described neutral red staining of *Acartia tonsa*, *Eurytemora affinis*, and several other crustacean zooplankton species, but provided no information on the accuracy of the method. Crippen and Perrier (1974) described a modified protocol for staining additional zooplankton groups, but did not report a thorough evaluation of the accuracy of the staining either. Further, the protocol that they proposed requires long staining time (1-6 hours), making it impractical for routine field application. Fleming and Coughlan (1978) further modified the method to increase sample storage times, but they also reported no data on the assessment of staining accuracy. Hence, there is still uncertainty concerning the accuracy and precision of neutral red staining for differentiating live and dead zooplankton. In addition, the published protocols require either long staining times, or excessive manipulation of zooplankton and hazardous chemical during staining and preservation, or both, making them less than ideal for routine shipboard application.
Although neutral red staining has commonly been used to determine the vital state of zooplankters in laboratory studies, use of the method for determining live/dead composition of zooplankton in field samples introduces many variables with untested effects on the staining results. This includes variations in temperature, salinity, zooplankton species compositions and abundances, and interference from other particles within samples (detritus and phytoplankton). There have been only five studies using neutral red on zooplankton field samples, based on a thorough review of the literature. Of these, two were for the specific purpose of assessing copepod mortality associated with power plant runoff (Carpenter et al. 1974; Hoffmeyer et al. 2005), another two did not describe nor cite the staining protocol that was used (Vinogradov et al. 1997, 1998), and none of these presented any information regarding the accuracy or precision of their protocols. Tang et al. (2006) were the first to examine the effects of different environmental variables on staining results. They measured the accuracy and precision of staining results, testing for effects of carcass age, the duration of the staining period, the killing method (exposure to various chemicals), and the possibility of artifact mortality due to sample handling. They found no significant effects of any of these factors on the accuracy of live/dead determinations for copepodites of *Acartia tonsa*.

Despite their limitations, these published studies provide a solid foundation for a broadly applicable neutral red staining method for field use. However, more tests are required to resolve the accuracy and precision of the method. It is necessary to develop standardized guidelines for interpreting staining patterns and color intensities, as stain uptake can vary among individuals (Fleming and Coughlan 1978), species and developmental stages (Omori and Ikeda 1984). Furthermore, the effects on staining
results of the variable conditions that occur in the field are unknown. Finally, the question
of artifact collection and handling mortality in field samples requires more study. The
first published study to address the possibility of artifact mortality was Tang et al. (2006).
However, their conclusions were based on a small sample size (8 replicate field
zooplankton tows), and they did not consider artifact mortality in the more fragile
naupliar stages of copepods. Limitations associated with neutral red staining of
zooplankton field samples can be resolved by testing the accuracy and precision of the
results of a single standardized protocol, and with regard to the factors mentioned above
(variable staining pattern/intensity, variable environmental condition, and artifact
mortality). This will ensure the accuracy of in situ live and dead composition data
obtained by neutral red staining. Here we describe a standardized protocol for collecting,
staining, and analyzing zooplankton field samples for in situ live and dead determinations
of animals. The protocol was tested across a broad range of environmental variability, its
applicability to a range of common estuarine zooplankton groups was determined, and
the issue of artifact mortality in naupliar and advanced stages of copepods was addressed
in detail. The result is a simple and reliable protocol for determination of the vital status
of common zooplankton groups in estuarine field samples.
MATERIALS AND PROCEDURES

Preparation of neutral red stock solution

Stock solution is prepared by adding 0.1 g of neutral red powder (Neutral Red high purity biological stain, Acros Organics) to 10 ml of deionized water, and slowly stirring the solution under dim light overnight to completely dissolve the powder. After preparation, the stock solution can be stored in the dark at room temperature in a sealed amber borosilicate glass vial. The exact shelf life of the stock solution was not tested, but we obtained good staining performance using a single stock for a month. It is therefore recommended that the stock solution be replaced monthly or after exposure to excessive heat or light.

Collecting zooplankton

The protocol for collection and staining of field zooplankton samples, and subsequent live/dead sorting is outlined in Figure 1. Sampling is done using a plankton net, and samples are stained prior to preservation. We used standard conical plankton nets in this study. The net should be towed at a slow speed (≤1 m s⁻¹) to avoid damaging the animals. Prior to every tow, the net should be rinsed out thoroughly to minimize accidental carryover of dead animals from earlier tows. Tow duration should be kept as short as possible while still collecting an adequate sample size. The concentration of animals can affect the staining process, and samples containing too many animals will result in live individuals being only weakly stained. We obtained good staining results when zooplankton concentration was <75,000 individuals l⁻¹ in the cod-end sample. Assuming a net with 0.5 m mouth diameter and 100 ml final cod-end volume, the recommended maximum tow distance can be calculated as:
Tow distance (m) = 38,200 / \textit{in situ} zooplankton concentration (ind. m$^{-3}$) \quad (1)

This is also equivalent to the tow duration (in seconds) at the recommended maximum tow speed (1 m s$^{-1}$). Upon net retrieval after each tow, the cod end contents should be carefully transferred into a staining jar (e.g. polyethylene or glass screw cap bottle) and neutral red stain added as described below. The net should not be hosed down prior to this transfer, as this may kill the animals and inflate the numbers of dead zooplankton.

\textit{Staining and sample storage}

Once a sample has been transferred to the staining jar, neutral red stock solution is added at a volume of 1.5 ml per 1000 ml of sample. For samples with an exceptionally high number of animals (or in samples with high concentrations of phytoplankton or detritus), additional neutral red stock may be added to increase stain uptake without causing harm to the animals. As a rough guideline, the water should appear bright red and not pink (too little stain) or brown (too much stain). After stain addition, samples are incubated for 15 min at \textit{in situ} temperature (a dark ambient water bath works well). Afterward, samples are concentrated onto fine nylon mesh disks and rinsed briefly with filtered seawater (near \textit{in situ} temperature and salinity) to remove excess stain. The mesh disks are then placed flat and sample side up in petri dishes, and stored on ice in the dark. Upon return to the laboratory, samples should be stored at −20°C in the dark until use. In an earlier test of the effect of preservation and storage on staining results, we took subsamples from a single copepod population using a Folsom Plankton Splitter. Triplicate subsamples were stained and counted immediately; additional triplicate subsamples were preserved by freezing at −20°C, or using 3.7% unbuffered formaldehyde followed by refrigeration at 4°C. Preserved samples were counted after 36
d of storage. There was no significant effect of preservation method on the staining results (Table 2). However, preservation with formaldehyde resulted in lower and more variable percentages of stained copepods when compared with samples that were counted immediately or preserved by freezing. We also observed that frozen samples retained the stain for over 2 months when stored properly; after 3 months samples began to degrade, making counting difficult. We therefore recommend that stained samples be stored at 
-20°C in the dark, and be processed within 2 months of collection.

**Microscopic analysis of stained samples**

Frozen samples can be thawed by resuspension in filtered seawater. Samples are then acidified to a pH of <7 to develop the stain’s color inside the animals. Acidification can be done using any acidic solution, and the addition of 1 ml of 1M HCl per 10 ml of sample works well in our experience. Samples are then viewed with a dissecting microscope. We used a Nikon SMZ1000 stereomicroscope with C_DSD diascopic stand. Microscopy lighting is an important factor, and excessive lighting may cause stained animals to appear pale, and unstained animals to appear pink. For adult copepods dark field lighting should be used. This same lighting in combination with a red overhead light aids stain visibility for copepod nauplii and small copepodites. Animals alive at the time of staining are stained bright red in parts or all of their tissues (mainly prosome tissue for copepods); animals dead before the staining will appear unstained, cloudy white, or light pink. Color will begin to fade in one hour following resuspension and acidification. This is particularly problematic for smaller animals such as copepod nauplii. The use of cold seawater for the thawing and resuspension reduces this problem when compared to using water at room temperature.
ASSESSMENT

Although a protocol for neutral red staining of zooplankton was described as early as Dressel et al. (1972), questions still remain as to the potential limitations of the method. Our goal is to describe and test a standardized protocol for staining and live/dead sorting that can be applied easily to field samples. In order for the method to be incorporated as a regular part of zooplankton field sampling, it is important to assess the accuracy and precision of staining results when applied to different zooplankton groups and across a range of environmental conditions. In coastal and estuarine environments, both salinity and temperature can vary greatly over space and time, and may influence the staining process. Temperature directly influences cellular activity, and could therefore influence stain uptake. We also observed that neutral red did not work well for freshwater zooplankton samples (see also Bickel et al. 2009). One possible explanation for this is the effect of pH. Neutral red forms a hydrophilic cation in acidic solution, but a lipophilic anion under alkaline conditions (Horobin and Kiernan 2002). Cells might take up more readily the lipophilic form that is present in slightly alkaline solutions such as seawater. Finally, for application in field studies, it is important that the staining results are representative of the natural live/dead compositions of the zooplankton. If artifact mortality occurs due to collection or handling of the samples, it must be quantified and live/dead composition data corrected accordingly.

**General staining patterns**

To test if the method would present false results (false positive or false negative staining), a large number (>600) of live and active *Acartia tonsa* copepodites were collected. Of these, some were killed by immersion in 0.2 μm filtered seawater at 50°C
for 5 min. Several other killing methods were also used, including the use of dilute HCl, NaN₃, and freezing. The heat method was verified on multiple occasions to quickly and effectively kill the zooplankton, whereas survival of some individuals was commonplace after freezing, sodium azide and acid exposure. One problem does occur when using heat to kill the zooplankton: animals killed by heat will initially stain quite brightly as described by Crippen and Perrier (1974). This is perhaps due to residual cellular and enzymatic activities. For this reason, heat killed animals were held in water at 20°C for 5 min prior to staining, after which no visible stain uptake was observed. Assemblages of live animals and heat killed animals were treated with neutral red separately. The results were unequivocal: 100% of the live and active animals were stained bright red, and 100% of heat killed individuals appeared unstained (Figure 2A). Live individuals were stained throughout part or all of their prosome, and often in the antennules and urosome as well. Over all, a bright red concentration of stain in prosome tissues indicated a live individual, regardless of whether the entire prosome was stained or staining was patchy.

**General methodology**

To assess the performance of the neutral red method, laboratory tests were done with mixtures of known numbers of live and dead zooplankton. For each test, a large number (approximately 300 or more) of live and active animals were collected from a single station in the York River estuary, USA (37.24°N, 76.45°W). Of these, approximately half were killed by heat as described earlier. Following the preparation of dead zooplankton, known numbers of live and dead animals were mixed and treated with the neutral red method. For all the tests described in this study, samples were stained, preserved and analyzed according to the protocol described in the Materials and
Procedures section, with storage time ranging from several hours to less than two days. Staining efficiency is defined as \( 100 - \% E \), where \( \% E \) is the difference between the known percent live animals (expected) and the counted percent live animals (observed). A staining efficiency of 100% represents perfect staining. A value >100% indicates that a lower percentage of dead individuals were observed relative to the expected value (dead animals incorrectly stained or were preferentially lost). A value <100% indicates a higher percentage of dead individuals observed relative to the expected value (live animals failed to stain or were preferentially lost).

**Application to other zooplankton groups**

To date, neutral red has been applied mainly to calanoid copepods (Dressel et al. 1972; Tang et al. 2006), whereas other zooplankton groups reportedly require staining time longer than what is convenient for field applications (Crippen and Perrier 1974). As part of the method development, we assessed the applicability of our protocol to several common estuarine zooplankton groups: copepod nauplii, copepodites of *Acartia tonsa* (Calanoida) and *Oithona* sp. (Cyclopoida), barnacle nauplii, and planktonic polychaete larvae. For each zooplankton group, replicates of known numbers of live and dead individuals (approximately 100 or more individuals per replicate) were mixed, stained, and counted. The appearances and staining patterns of the groups tested are shown in Figure 2 (B and C). The results show that the neutral red method worked for all tested groups (Table 3), with a mean staining efficiency of 99.1% (s.d. 1.5%). However, the usefulness of the method depends not only on the initial staining efficiency, but also on the ease of visibility of absorbed stain and retention of stain after uptake. Live polychaete larvae initially stained bright red. However, the stain faded to barely visible levels in 10
min after thawing and acidification. Live barnacle nauplii also took up the stain, but the staining was confined to weak pink coloration at joints. Copepod nauplii and small copepodites both stained efficiently. Due to the small size of these individuals, the staining result was more difficult to see than for larger copepodites. Also, the stain faded noticeably as soon as 0.5 h after thawing and acidification. Large calanoid copepods stained the most clearly, and retained the stain for a long time after thawing. In conclusion, the use of neutral red staining for some zooplankton groups requires special attention. Samples of polychaete larvae need to be analyzed quickly after thawing; and barnacle nauplii need to be inspected closely for stain uptake. Other groups, such as copepod nauplii and copepodites, can be confidently determined as live or dead with relative ease. For copepod nauplii and small copepodites, samples can be carefully counted on high magnification promptly after acidification, and with the aid of a red overhead light.

**Effects of environmental conditions**

To evaluate the effects of environmental conditions on staining efficiency, tests were conducted on *Acartia tonsa* (copepodite stage IV through adult) across a range of salinities and temperatures analogous to field conditions. *A. tonsa* is commonly found in meso- and polyhaline environments (salinity 5-30), and can tolerate salinities between 0 and 52 (Cervetto et al. 1999). The geographic range of this species is mainly restricted to the temperate zone (approximately 5-25°C), although it can tolerate temperatures between –1 and 32°C (Gonzalez 1974). Field collected *A. tonsa* were acclimated to laboratory conditions similar to the *in situ* conditions when they were caught (10°C; salinity 20). Groups of animals were then transferred to water of the desired conditions,
adjusting by 5°C temperature or 5 salinity increments, and with 24 h acclimation periods between adjustments. Temperature treatments were set up by adjusting the incubation temperature and allowing the water to equilibrate naturally. Salinity was adjusted by adding deionized water or brine (made with Instant Ocean®) to the containers until the desired salinity was achieved. DI water or brine solution was added very slowly and carefully with constant mixing to achieve uniform salinity within the containers. After 24 h of acclimation, live and active copepods were selected from each treatment (salinity 10-30 maintained at 10°C; temperature 5-30°C maintained at 20 salinity). Approximately half were killed by heat exposure, and known numbers of live and dead individuals were mixed, stained with neutral red, and counted in triplicate (approximately 100 or more individuals per replicate). There was no significant effect of salinity on staining efficiency (Table 4), with a mean staining efficiency of 100.2% (s.d. 1.5%). The effect of temperature on staining efficiency was significant (Table 4). Post-hoc pairwise comparisons showed that staining efficiency was significantly lower for the lowest temperature (mean 98.5 %) than for higher temperatures (99.6-100.8%). 5°C is at the lower end of the temperature range that A. tonsa experiences in the Chesapeake Bay mainstem, and it is likely that cellular and enzymatic activities, hence stain uptake, are reduced at this low temperature. However, the error even at 5°C was less than 2%.

Additional tests were done to determine if salinity fluctuations might affect neutral red staining results. A “salinity shock” was administered to simulate the effect of passing the animals through a strong halocline, or rinsing them with water of salinity very different than the in situ salinity. For this test, field collected Acartia tonsa copepodites were acclimated to laboratory conditions as described above. Duplicate groups of live
animals acclimated to a salinity of 20 were quickly transferred to 25 and stained immediately. Additionally, duplicate groups acclimated to a salinity of 15 were transferred to 10 and stained immediately. These treatments would not cause immediate death of the copepods, as *A. tonsa* has been observed to survive salinity shocks of similar or greater magnitudes, at least over short (hours) time periods (Cervetto et al. 1999). Neither an upward nor downward salinity shock resulted in strong bias in staining efficiency. We obtained good staining efficiency with the upward shocked treatment (mean 100.0%, s.d. 1.3%), which was not significantly different from 100% according to one-sample *t*-test (*t*=−0.03, df=1, *p*=0.98). The downward shocked treatment yielded a somewhat lower staining efficiency (mean 97.5%, s.d. 2.5%), but this was not significantly different from 100% (*t*=1.42, df=1, *p*=0.39). A slightly lower staining efficiency in downward salinity shock treatment is expected, as animals could be removing solutes from their cells in order to maintain osmotic equilibrium, which could work against the uptake of charged neutral red molecules.

**Artifact mortality in the field**

Another potential problem with neutral red staining of field zooplankton samples is artifact mortality associated with animal collection and handling. Zooplankton net tows likely impose high mechanical stresses on the captured animals. The turbulent and barrier ridden environment inside the net, and the subsequent confinement to the cod end may cause mortality to the animals. Handling of the samples prior to staining may also cause stress and mortality to the animals. While steps should be taken to minimize these stresses, it is still possible that artifact mortality of zooplankton could occur during capture (cod-end mortality) and handling (handling mortality).
To quantify cod-end mortality, we conducted field tests that consisted of a series of successive net tows of different durations at a single location in the field. If cod-end mortality occurred, longer tow duration would result in an increase in the percent dead zooplankton. Two of these field experiments were done at a single location in the York River estuary, one for copepodites (with 200 μm mesh net) and one for copepod nauplii (with 63 μm mesh net). Linear least squares regression was used to test for a relationship between tow duration and % dead copepods. In neither of these experiments was the slope of the regression line significantly different from zero (Figure 3). In addition, we compared tows with filtering cod-end and tows with non-filtering cod-end. Non-filtering cod-end is designed to reduce the stress experienced by the trapped animals. If cod-end mortality occurred, for two analogous tows, the one using a non-filtering cod-end would have lower % dead copepods compared to the one using a filtering cod-end. The resulting means were 8.3% dead for filtering cod-end, and 12.9% dead for solid cod-end. There was no significant difference between cod-end types in % dead copepods according to 2-sample t-test (arcsine transformed data, t=-1.92, df=22, p=0.07).

Additional laboratory experiments were conducted to test whether artifact mortality could occur during collection and handling. Large numbers (100-460) of live and active *Acartia tonsa* copepodites and copepod nauplii were collected from the York River estuary and placed in ambient water. These animals were then siphoned through a tubing (12 mm internal diameter) into submerged miniature reproductions of plankton nets (For copepodites: 200 μm mesh, 2.27 cm² mesh surface area, 0.9 cm mouth diameter, 5.5 ml cod-end volume; for nauplii: 63 μm mesh, 2.54 cm² mesh surface area, 0.5 cm mouth diameter, 3 ml cod-end volume). The siphoning lasted for 1.5-2 min at a
flow rate of 1.25-2.5 l min⁻¹. Based on the surface area of the collection nets and the speed of the flow, these laboratory conditions were equivalent to towing a 0.5 m diameter plankton net for the same duration (1.5-2 min) at a speed of ≥1 m s⁻¹. The final concentrations of animals in the collection containers were 2.3-8.4 × 10⁷ ind. m⁻³, comparable to the typical cod-end concentration of zooplankton in our field tows. After siphoning, the collected animals were diluted to ensure good staining efficiency, and stained with neutral red. During the entire process the animals were transferred, sieved, and pipetted multiple times, with the total time and amount of handling far exceeding what our protocol recommends. In all cases, less than 2% of the animals appeared unstained following this process (copepodites: n=3, mean=1.6% dead, s.d.=0.5%; nauplii: n=3, mean=1.2% dead, s.d.=0.6%). These results suggest that artifact mortality associated with collection and handling of copepods per our protocol is negligible. This is perhaps not surprising, given that neutral red stain is taken up intracellularly. Even copepods damaged during collection and handling should continue to take up stain until activity at the cellular level has ceased. Indeed, one important observation of the siphoning tests was the occurrence of several stained copepods with severe wounds. The small amount of tissue remaining inside the carapace was stained bright red, indicating that animals torn apart by the rough handling were still active enough at the cellular level to take up the stain. Conversely, the few animals that appeared unstained (dead) during the siphoning tests were completely intact, and may have represented either false negative staining, or an accidental carryover of dead animals between replicates. Regardless, artifact mortality associated with collection and handling of field samples can be avoided if our protocol is followed properly.
DISCUSSION

The lack of information on the vital state of the animals represents a major oversight and limitation in traditional zooplankton sampling. Neutral red vital staining is a promising method that provides this missing information. In this paper we described an improved protocol for neutral red staining of marine zooplankton in field samples, and provided detailed guidelines for visual examination of staining results. We verified that the method was reliable for determining live/dead zooplankton compositions across a range of environmental conditions and for different zooplankton groups. We also showed that artifact mortality associated with sample collection and handling was negligible when the protocol was followed properly.

With the neutral red method researchers can now easily and reliably quantify live/dead zooplankton compositions in situ as part of routine field sampling. This information will improve estimates of live zooplankton population abundance, as well as estimates of ecological rates at the population level. Ability to quantify carcasses in field samples will also make it more feasible to study natural zooplankton mortality and its causes. In addition to improvement of future studies, insights into recurring patterns of carcass abundance and distribution within a system will allow for re-examination of past studies of the same system where live/dead zooplankton composition was not considered. Such a retrospective effort could change some of our long-held understandings in marine zooplankton ecology.
COMMENTS AND RECOMMENDATIONS

In this study, the neutral red method was evaluated for its applicability under coastal and estuarine conditions and for common estuarine zooplankton groups. It is particularly well suited for copepods, the most abundant zooplankton in many marine environments (Humes 1994). However, application of the method to untested zooplankton taxa (including untested copepod species) should be attempted only after testing to ensure that the targeted species take up the stain and retain it for an adequate period of time during preservation and microscopic analysis. This is because stain uptake varies with different taxa (Omori and Ikeda 1984). For those taxa that begin to lose stain shortly after acidification (e.g. polychaete larvae), it is suggested that color photographs be taken for later detailed analysis. Use of the method in environments differing greatly from estuarine settings requires additional considerations. For example, long tow duration may be needed to collect sufficient sample in the open ocean, and the effects of long tow duration (>5 min) on artifact mortality need to be evaluated carefully. Also, rigorous washing of the net down into the cod end is a common practice in open ocean net tows, but should be avoided for samples intended for use in live/dead determination. While this may influence the accuracy of abundance estimates in these samples, duplicate tows can be collected, one for live/dead determinations and another for abundance estimation. Another untested factor is the complications associated with bringing zooplankton up from great depths. Drastic changes in temperature and pressure may be experienced by zooplankton brought up from deep water, and the consequences on artifact mortality and staining efficiency are unknown. Finally, the neutral red method was found to significantly underestimate the number of live Acartia tonsa individuals near the lower
temperature limit for this species. It is also possible that very high temperatures could inflate the number of live individuals, as implied by the observed uptake of stain after heat killing of zooplankton. Overall, extreme temperatures appear to affect stain uptake and this should be accounted for if the method is to be used in such environments.
REFERENCES


Table 1. Literature reports of the percentage of marine zooplankton identified as dead in field samples. Visual discrimination was based on microscopic inspection of individual animals for signs of tissue decomposition or injuries.

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>Carcass identification method</th>
<th>% dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheeler 1967</td>
<td>Atlantic off American Coast (20°-38° N)</td>
<td>Visual discrimination</td>
<td>50-70%</td>
</tr>
<tr>
<td>Weikert 1977</td>
<td>Atlantic off African Coast (10°-20° N)</td>
<td>Visual discrimination</td>
<td>16-28%</td>
</tr>
<tr>
<td>Roe 1988</td>
<td>N. Atlantic (31.3° N; 25.4° W)</td>
<td>Visual discrimination</td>
<td>25-50%</td>
</tr>
<tr>
<td>Terazaki &amp; Wada 1988</td>
<td>Sea of Japan (38°-42° N; 132°-140° E)</td>
<td>Visual discrimination</td>
<td>16-28%</td>
</tr>
<tr>
<td>Böttger Schnack 1990</td>
<td>Red Sea (21.4° N; 38° E)</td>
<td>Visual discrimination</td>
<td>1-50%</td>
</tr>
<tr>
<td>Geptner et al. 1990</td>
<td>S.W. Indian Ocean off African Coast</td>
<td>Visual discrimination</td>
<td>10-90%</td>
</tr>
<tr>
<td></td>
<td>Throughout Red Sea</td>
<td>Visual discrimination</td>
<td>&lt;10-29%</td>
</tr>
<tr>
<td>Böttger Schnack 1995</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genin et al. 1995</td>
<td>Gulf of Eilat (ca. 29.5° N; 35° E)</td>
<td>Visual discrimination</td>
<td>10-60%</td>
</tr>
<tr>
<td>Haury et al. 1995</td>
<td>Pacific near California Bight (30°-33° N)</td>
<td>Visual discrimination</td>
<td>10-60%</td>
</tr>
<tr>
<td>Böttger Schnack 1996</td>
<td>Arabian Sea</td>
<td>Visual discrimination</td>
<td>5-70%</td>
</tr>
<tr>
<td>Yamaguchi and Ikeda 2001</td>
<td>North Pacific (42° N; 145.5° E)</td>
<td>Visual discrimination</td>
<td>0-75%</td>
</tr>
<tr>
<td>Yamaguchi et al. 2002</td>
<td>Across North Pacific (40°-50° N)</td>
<td>Visual discrimination</td>
<td>10-90%</td>
</tr>
<tr>
<td>Yahel et al. 2005</td>
<td>Gulf of Aqaba (29° N; 34.5°-35° E)</td>
<td>Visual discrimination</td>
<td>10-20%</td>
</tr>
<tr>
<td>Tang et al. 2006</td>
<td>Lower Chesapeake Bay and tributaries</td>
<td>Neutral red staining</td>
<td>13-37%</td>
</tr>
</tbody>
</table>
Table 2. Comparison of preservation methods on the % stained *Acartia tonsa* copepodites. Copepodites were counted immediately after staining, or preserved by freezing or with formaldehyde for 36 d prior to counting. There was no significant difference among treatments (ANOVA of arcsine transformed data; $F_{2,6} = 3.92, p = 0.08$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean ± 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate count</td>
<td>3</td>
<td>74.0 ± 7.0</td>
</tr>
<tr>
<td>Freezing (-20°C)</td>
<td>3</td>
<td>72.4 ± 11.9</td>
</tr>
<tr>
<td>3.7% formaldehyde</td>
<td>3</td>
<td>61.9 ± 20.6</td>
</tr>
</tbody>
</table>
Table 3. Results of staining efficiency tests for the various zooplankton groups. (n = the number of animals added vs. recovered in each replicate, % dead expected = the % dead animals initially present in the sample, % dead observed = the % dead animals determined by the neutral red method)

<table>
<thead>
<tr>
<th>Group</th>
<th>n (added, recovered)</th>
<th>% dead expected</th>
<th>% dead observed</th>
<th>Staining efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copepod nauplii</td>
<td>(136,121)</td>
<td>61.8</td>
<td>62.8</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>(100,89)</td>
<td>81.0</td>
<td>82.0</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>(94,87)</td>
<td>43.6</td>
<td>43.7</td>
<td>99.9</td>
</tr>
<tr>
<td>Acartia tonsa</td>
<td>(181,178)</td>
<td>47.5</td>
<td>47.2</td>
<td>100.3</td>
</tr>
<tr>
<td></td>
<td>(176,176)</td>
<td>43.2</td>
<td>46.0</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>(173,173)</td>
<td>49.7</td>
<td>51.4</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>(159,159)</td>
<td>57.2</td>
<td>57.1</td>
<td>100.2</td>
</tr>
<tr>
<td></td>
<td>(173,173)</td>
<td>61.3</td>
<td>63.6</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>(213,213)</td>
<td>39.9</td>
<td>40.2</td>
<td>99.7</td>
</tr>
<tr>
<td>Oithona sp.</td>
<td>(98,91)</td>
<td>76.5</td>
<td>78.0</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>(92,92)</td>
<td>46.7</td>
<td>44.1</td>
<td>102.7</td>
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<tr>
<td></td>
<td>(96,96)</td>
<td>65.6</td>
<td>64.6</td>
<td>101.0</td>
</tr>
<tr>
<td>Barnacle nauplii</td>
<td>(90,89)</td>
<td>50.0</td>
<td>49.4</td>
<td>100.6</td>
</tr>
<tr>
<td></td>
<td>(96,96)</td>
<td>49.0</td>
<td>50.0</td>
<td>99.0</td>
</tr>
<tr>
<td></td>
<td>(96,95)</td>
<td>52.1</td>
<td>53.7</td>
<td>98.4</td>
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<td></td>
<td>(262,255)</td>
<td>64.9</td>
<td>66.3</td>
<td>98.6</td>
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<tr>
<td></td>
<td>(362,358)</td>
<td>50.0</td>
<td>50.6</td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td>(173,171)</td>
<td>58.4</td>
<td>60.2</td>
<td>98.1</td>
</tr>
<tr>
<td>Polychaete larvae</td>
<td>(40,38)</td>
<td>22.5</td>
<td>26.3</td>
<td>96.2</td>
</tr>
</tbody>
</table>
Table 4. Staining efficiencies for salinities and temperatures tested (Acartia tonsa CIV-CVI). Column labels are the same as for Table 3. For the salinity treatments, there was no significant effect of salinity on staining efficiency according to ANOVA ($F_{4,12}=1.12$, $p=0.41$). For the temperature treatments, there was a significant effect of temperature on staining efficiency according to ANOVA ($F_{5,15}=4.51$, $p=0.02$), with significantly lower staining efficiency at 5°C (according to post-hoc pairwise Bonferroni comparisons).

<table>
<thead>
<tr>
<th>Salinity treatments at 10°C</th>
<th>n (added, recovered)</th>
<th>% dead expected</th>
<th>% dead observed</th>
<th>Staining efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>(110,110)</td>
<td>52.7</td>
<td>53.6</td>
<td>99.1</td>
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<tr>
<td></td>
<td>(132,128)</td>
<td>55.3</td>
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<td></td>
<td>(191,189)</td>
<td>71.2</td>
<td>67.7</td>
<td>103.5</td>
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<tr>
<td>15</td>
<td>(130,120)</td>
<td>73.8</td>
<td>73.3</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>(96,96)</td>
<td>68.8</td>
<td>68.8</td>
<td>100.0</td>
</tr>
<tr>
<td>20</td>
<td>(101,99)</td>
<td>48.5</td>
<td>50.5</td>
<td>98.0</td>
</tr>
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Figure 1. Flow diagram of the recommended protocol of the neutral red method for collection, staining, and live/dead sorting of zooplankton samples
Collection:
Thoroughly rinse collection gear to reduce carryover of dead animals

Collect plankton sample (<1 m s\(^{-1}\) tow speed & minimal tow duration)

Carefully concentrate tow contents and transfer to staining jar

Staining and preservation:
Add 1.5 ml neutral red stock (10 g l\(^{-1}\) conc.) per 1 l concentrated sample (final concentration approximately 1:67,000)

Stain for 15 min at in situ temperature (water bath/dark)
Concentrate stained sample onto nylon mesh
Rinse briefly with filtered seawater (in situ T & S) to remove excess stain
Preserve and store sample immediately (-20\(^{\circ}\)C, <2 months)

Analysis:
Thaw frozen mesh in cold filtered seawater (ca. in situ salinity)
Acidify sample to pH<7 (1:10 final concentration 1M HCl works well)
Count sample under dark field microscopy (patchy or uniform red stained tissue = live; pink/unstained=dead; red overhead light facilitates contrast in copepod nauplii and other small individuals)
Figure 2. Appearance of neutral red treated zooplankton under a stereomicroscope (Nikon SMZ1000) and recommended lighting. Shown are 100% dead and 100% live *Acartia tonsa* (A), patchily stained and pink individuals (B), and the live and dead individuals of various developmental stages and groups tested (C). Pictures were taken with a Nikon Coolpix 4300 digital camera.
Figure 3. Results of tow duration field experiments to assess potential cod-end mortality in field sampling. Percentages of dead animals were determined by neutral red staining. Linear least square regression function was fitted to the data for (A) copepodites ($F_{1,31} = 0.56, p = 0.46$) and (B) nauplii ($F_{1,12} = 0.59, p = 0.46$).
(A) $y = 0.007x + 10.12$

$r^2 = 0.018$

$p = 0.46$

(B) $y = 0.023x + 30.23$

$r^2 = 0.047$

$p = 0.46$
CHAPTER 3

Spatial and temporal distributions of live and dead copepods in the lower Chesapeake Bay (Virginia, USA)
ABSTRACT

Hydrographic parameters and abundances of *Acartia tonsa*, *Eurytemora affinis*, and copepod nauplii were monitored *ca.* monthly or seasonally for up to two years in tributaries of the lower Chesapeake Bay. The vital status of copepods was determined using neutral red staining. Abundances of *A. tonsa* and nauplii were highest in summer and early fall, and increased with water temperature. *E. affinis* was present in early fall and winter through spring. The percent of copepods that were dead varied between species, among developmental stages, and in a recurring annual pattern. Percent dead was highest in summer and fall samples. Mean percent dead was highest for young nauplii (NI-NIII) at 30%. Means of 12-15% of older nauplii (NIV-NVI) and *A. tonsa* copepodites and 4-8% of *E. affinis* were dead. Percent dead was higher for adults male than female *A. tonsa*, with study means of 40% and 9% dead, respectively. Percent dead was not directly related to measured environmental variables. Copepod carcasses were a persistent feature in the plankton from 2007-2009, reflecting unknown causes.
INTRODUCTION

Protocols for field sampling of zooplankton often assume that all collected and preserved animals were alive in situ. The resulting abundance data are then frequently used to extrapolate individual rate measurements to population rates, such as ingestion or egg production (e.g. Uye 1986; Hansen and van Boekel 1991; Morales et al. 1993). However, a number of studies have reported the occurrence of substantial numbers of zooplankton carcasses in field samples (reviewed by Elliott and Tang 2009). Consequently, flawed ecological conclusions could result when high numbers of carcasses occur in samples but are not accounted for.

Zooplankton carcasses represent concentrated sources of labile organic matter, and a diversion of secondary production to the microbial loop (Tang et al. 2006b; 2009; Bickel & Tang in press). Carcasses lacking wounds are also evidence of mortality due to causes other than predation, such as starvation, parasitism, disease, environmental stress, or old age (e.g. Kimmerer and McKinnon 1990; Hall et al. 1995; Gomez-Gutierrez et al. 2003). However, direct measurements of such non-predatory zooplankton mortality in situ are rare in the literature. Quantifying zooplankton carcasses in preserved samples could be difficult because carcasses are similar in appearance to live animals for hours to days after death, depending on decomposition rate (Tang et al. 2006a). Recently, vital staining with neutral red has been used to differentiate live and dead copepods in zooplankton samples from Chesapeake Bay (Tang et al. 2006a), and rigorous testing of this method confirmed its reliability in generating live and dead information for various common estuarine zooplankton taxa (Elliott and Tang 2009).
Chesapeake Bay is the largest estuary in the USA, and supports a number of economically important activities including fisheries and aquaculture. Eutrophication, hypoxia, and other changes to the ecology and water quality of Chesapeake Bay are well documented (Kemp et al. 2005). The dominant copepods in Chesapeake Bay are *Acartia tonsa* and *Eurytemora affinis*, and they represent an important link in the pelagic food chain. Eutrophication could affect these copepod populations through altered trophic interactions or reduced survival associated with hypoxia (Kemp et al. 2005). Using the neutral red staining method Tang et al. (2006a) found that an average of 29% of collected *Acartia tonsa* copepodites were dead during summer 2005 in the York and Hampton Rivers, lower Chesapeake Bay. Such a high percentage of dead copepods suggests that mortality due to factors other than predation may be important in Chesapeake Bay zooplankton populations. The observations of Tang et al. 2006a did not extend to the naupliar stages of copepods, and were restricted to one summer when the surface water temperature was at a record high (average 27.5°C; maximum 33.4°C). Hence, it remains questionable if the observed high abundance of carcasses was a singular phenomenon, or a common feature of Chesapeake Bay.

In this study we sampled *A. tonsa*, *E. affinis*, and copepod nauplii regularly between 2007 and 2009 in the lower Chesapeake Bay. We described variations in copepod abundances and live and dead compositions among different tributaries, along the salinity gradient within each tributary, with depth, through time, and in relation to measured environmental conditions. This new information is then discussed in terms of mortality and population dynamics of Chesapeake Bay copepods.
METHODS

Sampling locations

Samples were collected at 12 stations in the lower Chesapeake Bay (Fig. 1), four along the salinity gradients of the York and Rappahannock Rivers, and three in the James River with a fourth at the mouth of the Elizabeth River (collectively referred to as James River herein). The depth at each station was 7-20 m except at J3 (3 m depth). Sampling of the York River stations occurred approximately monthly between October 2007 and December 2009 (Fig. 1, Y stations). Other stations were sampled twice each season throughout 2009.

Sample collection

Hydrographic data were collected using a hand-held YSI 6600 sonde measuring pressure (depth), conductivity, temperature, dissolved oxygen concentration, and chlorophyll-a concentration (as in situ fluorescence). Vertical profiles of these variables were recorded at each station, with measurements at 0.5 m intervals from the surface to ~1.5 m above the bottom. Water density was calculated from temperature and salinity, and the density difference between surface and bottom measurements (Δρ) was used as an indication of vertical stratification in each profile. Plankton sampling consisted of four plankton tows at each station, two with a 63 μm mesh net for copepod nauplii, and two with a 200 μm mesh net for copepodites. For each mesh-size net one tow was taken vertically from ~1.5 m above bottom to surface, and the other was taken horizontally just below the surface for ~60 s at a speed of ≤1 m s⁻¹. A previous study determined that this sampling procedure did not result in any significant artifact mortality (Elliott and Tang 2009). Sampled volumes for vertical tows were calculated as towed depth multiplied by
net mouth area, and volumes for horizontal tows were calculated based on readings of a
flowmeter attached to the net mouth. Between consecutive tows both the net and cod end
were rinsed thoroughly to avoid carryover of carcasses. To determine the vital status of
collected zooplankton, cod-end samples were first transferred to containers and stained
with neutral red for 15 min (1:67,000 final stain:water concentration), then concentrated
onto nylon mesh disks, sealed in perti dishes, and stored at -40°C until enumeration
(Elliott and Tang 2009). Samples were enumerated in the laboratory within two months
of collection. Frozen samples were thawed back into artificial seawater (20 salinity) and
split when necessary to obtain a manageable number of animals for counting. Samples
were then acidified with HCl to a pH of <7 to develop the neutral red stain color, and
viewed under a dissecting microscope with dark field illumination (Elliott and Tang
2009). Counts were made for live and dead copepod nauplii grouped into stages NI-NIII
and NIV-NVI, and copepodites of A. tonsa and E. affinis, each grouped into stages CI-
CV and CVI. To account for carcasses that could have resulted from partial predation,
injuries to A. tonsa carcasses were quantified in samples taken during September 2009, a
period when high percentages of dead copepodites occurred (see Results). A total of 851
carcasses were inspected for injury in antennule, urosome or prosome segments, which
may indicate partial predation as observed in laboratory experiments with Euphausia
pacificana preying on the copepod Pseudocalanus sp. (Ohman 1984).

Statistical analyses

An average of 350 individuals were enumerated in each tow sample, and samples
containing <50 individuals in total were excluded from further analysis. Copepodite
abundance was consistently higher in vertical than in horizontal tows (see Results),
suggesting a patchy distribution of animals with depth. Therefore, unless otherwise specified, copepodite abundance data from depth integrated vertical tows were used in the analysis, since these data were more representative of the total copepodite abundance at each sampling station. Because our sampling stations did not extend into the oligohaline portions of the estuaries, *E. affinis* was rare or absent in many samples. Therefore, data on its abundance were not analyzed statistically. Copepod abundances were log-transformed and percent dead data were arcsine-square root transformed prior to statistical analyses. One-way ANOVAs were used to test for differences in abundance and percent dead among the three rivers during 2009, and to test for differences in percent dead among developmental stage groups. Two-way ANOVAs were used to test for differences in abundance and percent dead among sampling events (dates) and stations within each river. As an indication of variation with depth, abundance and percent dead were compared between horizontal and vertical tows using paired *t*-tests, with pairs of horizontal and vertical tows taken on each date and at each station. Principal components analysis (PCA) was performed on the hydrographic data to identify the main underlying environmental gradients. These gradients (PCA axes) were then used as explanatory variables in multiple linear regressions to describe copepod abundance and percent dead in relation to the environment (principal components regression; Graham 2003). Stepwise multiple linear regressions were also performed to select the best set of individual hydrographic variables explaining copepod abundance and percent dead. However, the results were very similar to those of the principal components regressions, and are not included in the results.
RESULTS

Hydrographic environment

Water temperature, salinity, chlorophyll-α concentration, and stratification index (Δρ) are shown in Fig. 2. Dissolved oxygen data are not shown, but hypoxic conditions were observed on only two occasions at Y1 on June 18, 2008 (1.79 mg L⁻¹) and R2 on June 22, 2009 (1.92 mg L⁻¹). Water temperature was highest in late July-August and lowest in January-early February of both years, and the temperature range was smaller downstream within each tributary. Salinity increased downstream within each tributary, and was highest in late fall and early winter. An abrupt drop in surface salinity was observed throughout the James River in late November 2009, following several weeks of heavy rainfall. This freshwater lens was responsible for the strongest density stratification (Δρ) observed during the two-year study. Otherwise, the water column was most strongly stratified in late spring and summer (May–September). Chlorophyll-α concentration typically ranged from 3-20 µg L⁻¹ and increased upstream in all tributaries. Mean chlorophyll was 8 µg L⁻¹ in the York and James Rivers, and 12 µg L⁻¹ in the Rappahannock River. An unusually high chlorophyll concentration (depth-average 31 µg L⁻¹) was observed at the mouth of the York River in May 2009.

The first four axes derived from principal components analysis (PC-1, PC-2, PC-3, and PC-4) explained between 39% and 11% of the variability in environmental data (Table 1). Each PCA axis was most closely related to one or two environmental variables (Table 1). PC-1 separated spring and summer samples from fall and winter, and was most closely associated with degree of stratification (Δρ) and dissolved oxygen concentration. PC-2 separated samples both temporally and spatially, and was most closely associated
with chlorophyll-\(a\) concentration and salinity. PC-3 separated summer samples from all others based on temperature and chlorophyll-\(a\) concentration. PC-4 separated samples based primarily on salinity, describing an underlying gradient in the degree of freshwater influence.

**Copepod abundances**

Copepod abundance ranged from \(<1000\) individuals m\(^{-3}\) to more than \(3 \times 10^5\) nauplii m\(^{-3}\) and \(2 \times 10^4\) copepodites m\(^{-3}\) (Figs. 3, 4). Abundances of nauplii and \(A.\) *tonsa* copepodites were lowest in winter (December-March), increased in spring (March-June), and were highest in late summer and early fall (July-October). *E. affinis* was absent from many samples, but measurable abundances occurred in early fall and in winter and spring. With the exception of NI-NIII nauplii, abundances were not significantly different among rivers (Table 2). Within each river, abundances were significantly different among dates, but not among stations except for *A. tonsa* copepodites, which were significantly less abundant at station J3, and significantly less abundant at R3 and R4 than at R1 (both \(p<0.05\), Tukey pairwise comparisons). *A. tonsa* copepodites were significantly more abundant in vertical than in horizontal tows (Fig. 5a), indicating higher abundance deeper in the water column than near the surface. No such vertical differences were observed for copepod nauplii, suggesting a well mixed vertical distribution.

Several environmental gradients (PCA axes) consistently explained a substantial fraction of variation in copepod abundances (Table 3): Abundances of copepod nauplii and CI-CV *A. tonsa* were related to PC-1, with lower abundances associated with higher dissolved oxygen and weaker stratification present in fall and winter samples. Abundances of all groups were related to PC-3, with higher abundance associated with
higher water temperature. Abundances of copepod nauplii were related to PC-2, with higher abundance associated with lower chlorophyll-\(a\) concentration.

**Copepod vital status**

A mean of 1.7% (standard deviation 2.3%) of all copepod carcasses had visible injuries during September 2009. The most common injury was broken antennules, present in 1.1% of the carcasses, followed by prosome and urosome injuries, each accounting for 0.3% of the carcasses. The mean percentages dead were 30% for NI-NIII nauplii, 12-15% for NIV-NVI nauplii and *A. tonsa* copepodites (CI-CV and CVI), and 4-8% for *E. affinis* copepodites (CI-CV and CVI; Fig. 5b). Percent dead was significantly different among developmental stage groups according to ANOVA \((p<0.001)\), with higher percent dead for NI-NIII than for all other groups \((p<0.05\), Tukey pairwise comparisons\). Among adult *A. tonsa* a mean of 9% of females and 40% of males were dead. Temporal patterns in percent dead were similar during both years in the York River (Fig. 6). Highest percent dead copepod nauplii occurred from mid-spring through summer during both years (April–August; Fig. 6), coincident with highest naupliar abundances (Fig. 3). Percent dead *A. tonsa* copepodites were highest from mid-summer through early fall in both years (July–November; Fig. 6), during and immediately after peak abundances (Fig. 3). Percent dead in the other rivers followed a similar trend (Fig. 7), though sampling was limited to one year and had somewhat lower temporal resolution than for York sampling.

Percent dead was significantly different among rivers for NIV-NVI nauplii and CI-CV *A. tonsa* (Table 4), and pairwise comparisons showed that percent dead nauplii in the York River was significantly lower than in the Rappahannock River \((p=0.002\), Tukey
pairwise comparisons). Within each river, percent dead was significantly different among sampling dates for all developmental stage groups, but only significantly different among sampling stations for nauplii in the York River (Table 4). There were no significant differences in percent dead between horizontal and vertical tows (Fig. 5b). No environmental factors (PCA axes) explained more than a few percentage points of variability in percent dead (Table 3).
DISCUSSION

Hydrographic environment

The temperature and salinity ranges recorded during this study (Fig. 2) were characteristic of the meso- and polyhaline regions of a temperate estuary, and chlorophyll-\(a\) concentrations were typical for the eutrophic Chesapeake Bay (Harding and Perry 1997). The primary axis of environmental variability (PC-1; Table 1) reflected seasonal changes related to both stratification and dissolved oxygen concentration, reflecting the stratified conditions and deepwater oxygen depletion that occur in Chesapeake Bay and its tributaries in spring and summer (Taft 1980; Kemp 2005). True hypoxic conditions were rarely observed at our sampling stations. Severe and extensive hypoxia is more common in the deeper (>20 m) parts of the bay (Hagy et al. 2004), and our sampling design may have missed localized patches of hypoxia within the tributaries.

Copepod abundance

Observed spatial and temporal patterns in copepod abundance were comparable to previous reports for the mesohaline section of the Chesapeake Bay (Brownlee and Jacobs 1987; Roman et al. 1993; Kimmel and Roman 2004; Purcell and Decker 2005). The higher abundance of \(A.\ tonsa\) copepodites below the surface layer (\textit{i.e.} in vertical tows; Fig. 5a) was consistent with several other Chesapeake Bay studies that have reported higher abundance of larger copepodites deeper in the water column (Roman et al. 1993; Roman et al. 2001; Cuker and Watson 2002).

The positive association observed between copepod abundance and both temperature and chlorophyll was expected (PC-3; Table 3). Many zooplankton processes are temperature dependent, and in the absence of food limitation gross copepod
production increases with temperature within a species' temperature range (Heinle 1966; Huntley and Lopez 1992). The negative association of naupliar abundance with chlorophyll-\(a\) concentration in PC-2 was not expected (Table 3). However, PC-2 represented a gradient of lower chlorophyll in combination with higher salinity, conditions that occurred in fall (Fig. 2) when copepod abundances were still quite high (Figs. 3, 4), and this likely explained the observed relationship. Abundances of nauplii and juvenile copepodites were also related to the temporal gradient in \(\Delta p\) and dissolved oxygen (PC-1, Table 3). Although hypoxia can cause mortality and decrease copepod abundances, such a pattern was not seen in our study. Rather, lower abundances were associated with higher dissolved oxygen, and vice versa (Table 3). Thus, the relationship between PC-1 and copepod abundance probably reflected seasonal co-variation of these variables rather than causal relationships; with a less stratified (Fig. 2) and more oxygenated water column (data not shown) in winter when copepod abundances were lowest (Figs. 3, 4).

In the York River, springtime abundances of \(A.\ tonsa\) and nauplii increased earlier in 2008 than in 2009 (Fig. 8), possibly a result of the significantly warmer water temperature in winter 2007-2008 (mean 7.7\(^{\circ}\)C) than in 2008-09 (mean 5.9\(^{\circ}\)C; \(t\)-test, \(p=0.021\)). Higher water temperature could cause zooplankton abundance to increase earlier in the year and this has been hypothesized to suppress formation of the spring phytoplankton bloom in some New England estuaries (Keller et al. 1999; Oviatt 2004). Consistent with this hypothesis, measured York River chlorophyll-\(a\) concentrations were lower in January-May of 2008 (mean 7.10 \(\mu \text{g L}^{-1}\)) than the same period in 2009 (mean 10.64 \(\mu \text{g L}^{-1}\)), although this difference was not statistically significant (\(t\)-test, \(p=0.076\)).
**Copepod vital status**

Significantly higher percentages of copepod nauplii (NIV-NVI) were dead in spring 2008 than in 2009 (t-test, p<0.001; 16% dead 2008 and 9% dead in 2009), potentially explaining the lower maximum copepod abundance in spring 2008 (Fig. 8). Higher percent dead nauplii implied higher non-predatory mortality in 2008. This could have resulted from the lower chlorophyll-α in spring 2008 compared to 2009 (see previous section), since the development and survival of the early stages of marine copepod nauplii are particularly sensitive to starvation (Lopez 1996; Calbet and Alcaraz 1997).

We expected that the amount of dead copepods should be highest during and shortly after peaks in abundance, when mortality balanced or exceeded growth. This expectation was substantiated by our York River observations for A. tonsa and copepod nauplii in summer and early fall (Figs. 3, 6). Injuries in prosome, urosome or antennules were found in only 1.7% of the copepod carcasses in September 2009. Even if we increase this value to 2.6% to account for other possible injuries (e.g., swimming legs and mouthparts; Ohman 1984), partial predation and mechanical damages due to sample handling would still have accounted for a very small percentage of all carcasses during the period of high carcass abundance (September 2009), with the remaining >97% of carcasses likely representing instances of in situ non-predatory mortality.

The occurrence of copepod carcasses was not directly related to measured hydrographic variables (Table 3). Overall, the ambient environmental conditions in our study area were unlikely to directly result in A. tonsa mortality. For example, no spikes in percent dead were observed even in the James River in November 2009 (Fig. 7), when
stratification was strong enough that the copepods would have experienced a salinity shift of as much as 14 across the pycnocline in our vertical tows (Fig. 2, J2). This is not surprising, as *A. tonsa* has been shown to be quite tolerant to decreasing salinity, and transfer to a salinity of as low as 1 had no effect on its survival over 3 days in the laboratory (Cervetto et al. 1999), much longer than the time required to collect, stain and preserve our samples in the field.

One interesting observation was the higher percentage of dead adult *A. tonsa* males (40% mean) than females (9% mean). Given the similar size and morphology of adult male and female *A. tonsa*, there is no reason to expect that the rate of carcass turnover due to necrophagy, decomposition, and sinking would be substantially different between the two sexes (Elliott et al. 2010). Therefore, our field data suggested that non-predatory mortality was >4 times higher among *A. tonsa* males than females in the lower Chesapeake Bay. Male copepods have been shown to suffer higher predation risk (Kiørboe 2006; 2007); in addition, they are more susceptible to algal toxins and have a shorter maximum life span than female copepods (Avery et al. 2008; Rodríguez-Graña et al. 2010). The higher percent dead male *A. tonsa* that we observed may indicate that the environmental conditions were more detrimental to male copepods than female copepods in the lower Chesapeake Bay. Regardless of the specific cause(s), a higher mortality among male *A. tonsa* could limit mating success (Kiørboe 2007) and population growth of the species (Kiørboe 2006).

We found a mean of 14% *A. tonsa* copepodites dead in Chesapeake Bay from October 2007 through December 2009. This was lower than the 29% dead previously reported for this region (Tang et al. 2006a). However, the surface water temperature in
Chesapeake Bay during summer 2005 was at a record high (average 27.5°C; maximum 33.4°C), which may have caused anomalously high non-predatory mortality and carcass abundance (Tang et al. 2006a). Compared to *A. tonsa* copepodites and NIV-NVI copepod nauplii (12-15% dead; Fig. 5b), the percent dead *E. affinis* was low (mean 4% of CVI and 8% of CI-CV dead), and that of NI-NIII nauplii was high (mean 30% dead). Given the similar sizes and likely similar turnover times of *A. tonsa* and *E. affinis* copepodites, the lower percent dead for *E. affinis* implied that this species suffered lower non-predatory mortality than *A. tonsa*. Lower mortality would be expected for a *k*-selected species such as *E. affinis* (Hirche 1992), whereas an *r*-strategist such as *A. tonsa* would have a higher potential reproductive output (Mauchline 1998) and a broader environmental range. The higher percent dead NI-NIII nauplii represents a potentially large source of error in estimates of the abundance of live copepod nauplii. This higher percent dead nauplii could be a result of higher retention of the carcasses in the water column by turbulence relative to the larger carcasses (Elliott et al. 2010), or an indication of higher non-predatory mortality among the younger stages. Young stages of crustacean zooplankton are prone to mortality from environmental stressors, such as starvation (Threlkeld 1976; Lopez 1996; Calbet and Alcaraz 1997), salinity stress (Cervetto et al. 1999), and UV radiation (Leech and Williamson 2000). Susceptibility to environmental stressors and lower survival in these young stages, in combination with high egg mortality (Ohman and Wood 1995; Tang et al. 1998), may constitute a bottleneck to copepod population recruitment.

The present study was the first to describe the occurrence of copepod carcasses in the Chesapeake Bay throughout the entire year and for naupliar stages. We found that
carcasses consistently represented a substantial fraction of collected copepods throughout the lower Chesapeake Bay (Figs. 6, 7), suggesting that these carcasses are a persistent feature of the area. The percent dead varied in a repeatable seasonal manner between 2008 and 2009, and also varied among developmental stages and between species. These observations raise the question: Are zooplankton carcasses common in most shallow estuarine systems where sufficient turbulent energy exists to retain them in the water column (Elliott et al. 2010), or do these and earlier findings (Tang et al. 2006a; Tang et al. 2007) signal a degradation of environmental quality in Chesapeake Bay? Future studies should carefully consider the presence of carcasses when estimating abundances of zooplankton in Chesapeake Bay and other marine environments, particularly if these abundance data are to be used to estimate population rates. Identification of carcasses would also allow for assessment of mortality due to non-predatory factors as well as the importance of carcasses as microbial hotspots (Tang et al. 2009) and vehicles for transport of organic matter in the oceans (Bickel and Tang in press).
REFERENCES


*Marine Biology* 92:35–43.
**Table 1.** Results of principal components analysis on collected environmental data. Temp = temperature, Sal = salinity, DO = dissolved oxygen, Chl = chlorophyll-a, Δρ = stratification.

<table>
<thead>
<tr>
<th></th>
<th>PC-1</th>
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<th>PC-3</th>
<th>PC-4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eigenvalue</strong></td>
<td>1.96</td>
<td>1.30</td>
<td>0.78</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>% of environmental variability explained:</strong></td>
<td>39.3%</td>
<td>26.0%</td>
<td>15.7%</td>
<td>10.6%</td>
</tr>
<tr>
<td><strong>Correlation with:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp</td>
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<td>0.35</td>
<td>0.67</td>
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<td>Sal</td>
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<td>DO</td>
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<td>-0.28</td>
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<td>Chl</td>
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<td>-0.45</td>
<td>-0.28</td>
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Table 2. Results of ANOVAs testing for differences in copepod abundance (A) among rivers and (B) within each river among sampling stations and dates. James River data include one station in the Elizabeth River. Statistics shown are p-values (degrees of freedom) for the effect of given factors on abundances of each copepod developmental stage group. Developmental stage group abbreviations as in Fig. 3.

<table>
<thead>
<tr>
<th>(A)</th>
<th>River</th>
<th>River</th>
<th>River</th>
<th>River</th>
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</thead>
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<tr>
<td>NI-NIII</td>
<td>0.012 (2)</td>
<td>NIV-NVI</td>
<td>0.172 (2)</td>
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</table>

<table>
<thead>
<tr>
<th>(B)</th>
<th>River</th>
<th>Station</th>
<th>Date</th>
<th>Station</th>
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<th>Station</th>
<th>Date</th>
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<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>York</td>
<td>0.889 (3)</td>
<td>&lt;0.001 (22)</td>
<td>0.622 (3)</td>
<td>&lt;0.001 (22)</td>
<td>0.925 (3)</td>
<td>&lt;0.001 (23)</td>
<td>0.703 (3)</td>
<td>&lt;0.001 (23)</td>
<td></td>
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<tr>
<td>Rappahannock</td>
<td>0.470 (3)</td>
<td>&lt;0.001 (7)</td>
<td>0.443 (3)</td>
<td>&lt;0.001 (7)</td>
<td>0.001 (3)</td>
<td>&lt;0.001 (7)</td>
<td>0.010 (3)</td>
<td>&lt;0.001 (7)</td>
<td></td>
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<tr>
<td>James</td>
<td>0.289 (3)</td>
<td>&lt;0.001 (7)</td>
<td>0.414 (3)</td>
<td>0.001 (7)</td>
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Table 3. Results of principal components regression using the environmental PCA axes (Table 1) as explanatory variables of (A) copepod abundance and (B) percent dead copepods. Statistics shown are the percentage of variability in copepod abundance and percent dead explained by each PCA axis. Developmental stage group abbreviations as in Fig. 3.

<table>
<thead>
<tr>
<th></th>
<th>PC-1</th>
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<th>PC-3</th>
<th>PC-4</th>
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</thead>
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<td><strong>(A) Abundance:</strong></td>
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<td></td>
</tr>
<tr>
<td>NI-NIII</td>
<td>12.4%</td>
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<td>11.7%</td>
<td>0.3%</td>
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<td>16.0%</td>
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</tr>
<tr>
<td>ACI-ACV</td>
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<td>3.7%</td>
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<td>1.5%</td>
</tr>
<tr>
<td>ACVI</td>
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<td>1.5%</td>
<td>8.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td><strong>(B) Percent Dead:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NI-NIII</td>
<td>2.2%</td>
<td>1.9%</td>
<td>2.2%</td>
<td>2.8%</td>
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<tr>
<td>NIV-NVI</td>
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<td>3.2%</td>
<td>0.1%</td>
<td>0.6%</td>
</tr>
<tr>
<td>ACI-ACV</td>
<td>0.1%</td>
<td>0.0%</td>
<td>0.1%</td>
<td>0.0%</td>
</tr>
<tr>
<td>ACVI</td>
<td>0.3%</td>
<td>0.5%</td>
<td>4.0%</td>
<td>1.9%</td>
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Table 4. Results of ANOVAs testing for differences in the percent dead copepods (A) among rivers and (B) within each river among sampling stations and dates. James River data include one station in the Elizabeth River. Statistics shown are $p$-values (degrees of freedom) for the effect of given factors on percent dead of each copepod developmental stage group. Developmental stage group abbreviations as in Fig. 3.

<table>
<thead>
<tr>
<th></th>
<th>NI-NIII</th>
<th>NIV-NVI</th>
<th>ACI-ACV</th>
<th>ACVI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) River</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.126 (2)</td>
<td>0.003 (2)</td>
<td>0.034 (2)</td>
<td>0.105 (2)</td>
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<tr>
<td><strong>(B) River</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>York</td>
<td>0.014 (3)</td>
<td>&lt;0.001 (22)</td>
<td>0.080 (3)</td>
<td>0.729 (3)</td>
</tr>
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<td>Rappahannock</td>
<td>0.113 (3)</td>
<td>0.002 (7)</td>
<td>0.136 (3)</td>
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</tr>
<tr>
<td>James</td>
<td>0.398 (3)</td>
<td>&lt;0.001 (7)</td>
<td>0.017 (3)</td>
<td>&lt;0.001 (7)</td>
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Fig. 1. Map of the lower Chesapeake Bay with sampling stations as circles (J=James, E=Elizabeth, Y=York, and R=Rappahannock River stations).
**Fig. 2.** Hydrographic conditions for each sampling date and station (station labels as in Fig. 1). Values shown are the depth-averaged values for each vertical profile of temperature (°C; □, solid line), salinity (▲, dotted line), and chlorophyll-a (μg L⁻¹; +, dashed-dotted line). Values for the stratification index (Δρ; ●) are the difference between surface and bottom density within each vertical profile. Note that two off-scale chlorophyll-a measurements are labeled with their values (Y4 May 2009 and R1 September 2009).
Fig. 3. Copepod abundances during the study period (x-axes) and along the salinity gradient of the York River (y-axes; station labels as in Fig. 1). Each panel represents a separate species and developmental stage group (NI-NIII = naupliar stage one to three; NIV-NVI = naupliar stage four to six; ACI-ACV = *Acartia tonsa* copepodid stage one to five; ACVI = *A. tonsa* adults; ECI-ECV = *Eurytemora affinis* copepodid stage one to five; ECVI = *E. affinis* adults). Dashed lines indicate contours of 50, 500, and 5000 individuals m$^{-3}$, and >50,000 individuals m$^{-3}$ is solid black. Contours were generated by triangulation data interpolation among samples taken at each of four stations in a tributary and on each sampling date (indicated by ▼ at the top).
**Fig. 4.** Copepod abundances during the study period (x-axes) and along the salinity gradients of the James and Rappahannock Rivers (y-axes; station labels as in Fig. 1). Each panel represents a separate species and developmental stage group (abbreviations as in Fig. 3). Shading and legend value are the same as in Figure 3. Dashed lines indicate contours of 50, 500, and 5000 individuals m$^{-3}$, and >50,000 individuals m$^{-3}$ is solid black. Contours were generated by triangulation data interpolation among samples taken at each of four stations in a tributary and on each sampling date (indicated by ▼ at the top).
Fig. 5. Study mean (a) abundance and (b) percent dead of nauplii and *Acartia tonsa* copepodites in horizontal and vertical plankton tows (developmental stage group abbreviations as in Fig. 3). Error bars are +/- 95% confidence intervals. Note the log scale of y-axis in (A). Abundances were significantly higher in vertical than in horizontal tows for *A. tonsa* CI-CV and CVII (paired t-tests: *p*<0.0005 for both), but not for nauplii (paired t-tests: *p*>0.05). There were no significant differences in percent dead between vertical and horizontal tows (paired t-tests, *p*>0.05 in all tests).
Fig. 6. Percent of collected copepods that were dead during the study period (x-axes) and along the salinity gradient of the York River (y-axes; station labels as in Fig. 1). Each panel represents a separate species and developmental stage group (abbreviations as in Fig. 3). Dashed lines indicate contours of 15, 30, and 45% dead. Contours were generated by triangulation data interpolation among samples taken at each of the four stations in a tributary and on each sampling date (indicated by ▼ at the top).
**Fig. 7.** Percent of collected copepods that were dead during the study period (x-axes) and along the salinity gradients of the James and Rappahannock Rivers (y-axes; station labels as in Fig. 1). Each panel represents a separate species and developmental stage group (abbreviations as in Fig. 3). Shading and legend value are the same as in Figure 6. Dashed lines indicate contours of 15, 30, and 45% dead. Contours were generated by triangulation data interpolation among samples taken at each of the four stations in a tributary and on each sampling date (indicated by ▼ at the top).
Fig. 8. Mean monthly abundances of copepod nauplii plus *A. tonsa* copepodites in the York River during spring 2008 and 2009. Error bars are + 95% confidence intervals.
CHAPTER 4

Dead in the water: The fate of copepod carcasses in the York River estuary, Virginia
ABSTRACT

Using laboratory and field experiments we investigated three fates of copepod carcass organic matter in the York River estuary, Virginia: ingestion by planktivores (necrophagy), microbial decomposition, and removal by gravitational settling in the presence of turbulence (sinking). The ctenophore *Mnemiopsis leidyi* ingested live copepods and carcasses indiscriminately in feeding experiments. Microbial decomposition led to ca. 50% of carcass dry weight loss within 8 h after death. Carcass settling velocities in still water were ca. 0.1 cm s$^{-1}$, implying short residence time (hours) in the shallow estuary. However, turbulent mixing kept carcasses in suspension much of the time, reducing sinking losses. Rates of carcass organic matter removal were combined in a simple mathematical model predicting the fate of estuarine copepod carcasses. When sinking was considered, it removed a large fraction of carcass organic matter (≥58% for copepodites, ≥35% for nauplii), with most of the remainder being removed by microbial decomposition. In the absence of sinking losses, necrophagy became proportionally more important in removing carcass organic matter (≥49% except in summer).
INTRODUCTION

Studies of marine zooplankton population abundance rarely consider the vital status of the animals collected. However, zooplankton carcasses can at times comprise 10% or more of the total individuals in field samples (reviewed by Elliott and Tang 2009). These carcasses likely result from non-predatory mortality, defined here as mortality where individuals are not immediately ingested, and having causes such as injuries, resource limitation, disease, parasitism, harmful algal blooms, environmental stresses, or old age (Kimmerer and McKinnon 1990; Hall et al. 1995; Gomez-Gutierrez et al. 2003). Non-predatory mortality appears to be common among freshwater crustacean zooplankton (McKee et al. 1997; Gries and Glide 1999; Dubovskaya et al. 2003), but its importance in marine systems is poorly known because of the difficulty in measuring it. Nevertheless, a global analysis suggests that non-predatory factors account for 1/4 to 1/3 of the total mortality in marine planktonic copepods (Hirst and Kiørboe 2002), and the resultant carcasses represent a concentrated pool of labile organic substrates for microorganisms (Tang et al. 2006b, 2009), a potential food source for other organisms (Zajaczkowski and Legeżyńska 2001), and a vehicle for transporting essential biomolecules, toxins and pollutants (Lee and Fisher 1994; Frangoulis et al. 2005; Bickel and Tang in press). Several recent studies suggest that copepod carcasses can at times represent a large fraction (~1/4-1/2) of total vertical downward flux of particulate organic carbon to the deep sea (Sampei et al. 2009; Frangoulis et al. 2010). Hence, quantification of non-predatory mortality and the fate of the resulting carcasses are critical for
understanding zooplankton population dynamics, ocean trophodynamics and biogeochemistry.

Available data suggest that copepod carcasses may be common in marine and estuarine environments. Using neutral red stain to identify carcasses, Tang et al. (2006a) observed that on average 29% of collected copepods were dead during the summer of 2005 in the lower Chesapeake Bay, Virginia. Between 2007 and 2008, an average of 13-28% of collected copepods were found to be dead in the York River sub-estuary of Chesapeake Bay, and the percentage was higher among younger developmental stages (D. T. Elliott unpubl.). Non-predatory mortality rate for estuarine and oceanic copepods has been estimated to be ca. 0.1 d⁻¹ (Mauchline 1998; Tang et al. 2006a), indicating that on average 10% of copepod production could be diverted to carcass-based trophic pathways.

The goal of the present study was to determine the fate(s) of copepod carcasses and associated organic matter in the York River estuary. A combined experimental, observational and modeling approach was used to quantify rates of removal of carcass organic matter from the water column through ingestion by planktivores (necrophagy), microbial decomposition, and gravitational settling in the presence of turbulence (sinking losses).
METHODS

Our study included three components: 1) Carcass removal: Laboratory experiments were conducted to determine the potential rates of removal of copepod carcasses via necrophagy, microbial decomposition, and gravitational settling. 2) Effects of turbulence: The likelihood that resuspension and turbulent diffusion prolong residence time of copepod carcasses in the water column was investigated by an additional laboratory experiment and field sampling. 3) Fate of carcass organic matter: Results of these experiments and observations were combined with literature information and applied to a mathematical model that predicted the fate of copepod carcass organic matter under various environmental conditions in the York River estuary.

Study area

The York River is a partially mixed microtidal estuary, with depths ranging from 6-20 m along the main channel, maximum tidal currents nearing 1 m s\(^{-1}\), a tidal range of 0.7-0.85 m, and a typical bed stress of around 1.10 cm s\(^{-1}\) (Kim et al. 2000; Friedrichs 2009). Water temperatures in the York River range from 2-31°C on an annual cycle (Reay and Moore 2009). Phytoplankton abundance and chlorophyll-a concentration peak in the spring and summer, and primary production is high (60-70 mg C m\(^{-3}\) h\(^{-1}\)) from March through August (Marshall 2009). The dominant mesozooplankton taxa are copepods (Steinberg and Condon 2009). The calanoid copepod *Acartia tonsa* is dominant in the summer and is replaced by *A. hudsonica* in the winter, while *Eurytemora affinis* is more abundant in the spring and in the mesohaline portion of the estuary (Steinberg and Condon 2009). The ctenophore
Mnemiopsis leidyi is present persistently throughout much of the York River, becoming particularly abundant at times (Steinberg and Condon 2009). *M. leidyi* and other gelatinous planktivores are the major predators of mesozooplankton in Chesapeake Bay, particularly during the summer (Baird and Ulanowicz 1989; Steinberg and Condon 2009).

**Source of carcasses**

Copepod carcasses for laboratory experiments were produced from live and active *Acartia tonsa* collected from the York River estuary. Animals were held in 20 salinity Instant Ocean artificial seawater (ASW) in polycarbonate bottles. Water temperature was slowly raised to 50°C and maintained for 5 min, after which the animals were collected onto a sieve and returned to ASW at room temperature. This procedure killed almost all copepods while keeping the carcasses intact. Intact carcasses were sorted by age class into nauplii, copepodite stages I to III (CI-CIII), and copepodite stages IV through adult (CIV-CVI).

**Carcass removal: Ctenophore selectivity**

The rate at which planktivores consume copepod carcasses was quantified using the ctenophore *Mnemiopsis leidyi* from the York River estuary as a model organism, and testing for ctenophore selectivity between live copepods and carcasses. Incubations were performed using cydippid larvae (<10 mm diameter) and adult *M. leidyi* (ca. 40 mm diameter). For acclimation, 20-30 similarly sized ctenophores were incubated in the dark for 24 h in ASW at 20°C containing similar numbers of live copepods and carcasses. Experiments with cydippid larvae consisted of triplicate 4 L polycarbonate bottles of ASW at 20°C, each with three cydippid larvae and either live
or dead *Acartia tonsa* (CIV-CVI; 100 copepods per bottle). Triplicate predator-free controls were also set up to account for copepod and carcass loss during processing. Adult ctenophore incubations were modified to include one ctenophore with both live copepods and carcasses in equal amounts (50 + 50 total per bottle) in each 4 L bottle. To distinguish between live copepods and carcasses in these mixed treatments, live copepods were stained with neutral red and mixed with unstained carcasses in three replicate bottles, and stained carcasses were mixed with unstained live copepods in another three replicate bottles. The separate staining of live copepods and carcasses was to control for possible preferential ingestion or rejection of individuals due to the stain. Live copepods were stained with a 1:670,000 stain concentration (10% of concentration recommended by Elliott and Tang 2009) for 3 min; afterward, the copepods remained alive, active, and visibly stained for the duration of the incubations (2-4 h). Stained carcasses were prepared by immersing live copepods in a 1:33,500 stain concentration (200% concentration recommended by Elliott and Tang 2009) for 15 min, after which animals were heat killed while still in the staining solution. The resulting carcasses were visibly stained for ~6 h. To begin feeding experiments, acclimated ctenophores were introduced into bottles with known amounts of copepods and carcasses, and incubated in the dark on a plankton wheel (2 revolutions per min, rpm) for 2-4 h. At the end of the incubations ctenophores were removed and remaining copepods and carcasses were enumerated. An average of 83% (cydippid larvae incubations) and 41% (adult incubations) of the initial prey remained at end of incubation. Ctenophore clearance rates were calculated from the change in copepod or carcass abundance after correcting for losses of prey in
predator-free controls (average of 14% live copepods and 18% carcasses lost in controls).

**Carcass removal: Microbial decomposition**

The decomposition of *Acartia tonsa* carcasses was observed through time at different temperatures and dissolved oxygen concentrations. To create carcasses at different stages of decomposition, fresh carcasses (CIV-CVI) were placed in petri dishes (50 carcasses per dish) with 50 mL of 5 μm filtered York River water, and incubated at 5°C, 15°C, or 25°C on an orbital shaker table (30 rpm). Incubation containers were covered but not sealed, so that normoxic treatments consisted of water at atmospheric equilibrium. To account for the influence of seasonal hypoxia in Chesapeake Bay (Diaz 2001), additional hypoxic and anoxic treatments were incubated at 25°C in a sealed incubation chamber (VWR model A-143) filled with specific gas mixtures (hypoxic gas: 3.8% O_2, 0.1% CO_2, balance N_2; anoxic gas: 2.5% H_2, balance CO_2). The water for the hypoxic and anoxic treatments was first bubbled for 10 min with the incubation gas; treated water was then measured into petri dishes and copepod carcasses added, taking care not to disturb and re-oxygenate the water. Additional incubations were done for carcasses of naupliar and CI-CIII age classes at 15°C under normoxic condition. Incubation lasted 30-120 h depending on incubation conditions, and triplicate petri dishes from each treatment were removed at discrete times to measure carcass dry weight, abundance of carcass associated bacteria, and carcass settling velocity (see next section) at different stages of decomposition.
Microbial decomposition of copepod carcasses was quantified as change in carcass dry weight and separately as change in carcass associated bacterial abundance in the incubations described above. For dry weight measurements, carcasses (25-50 for copepodites; 75-150 for nauplii) were collected onto combusted pre-weighed GF/C filters, dried at 60°C for 24 h and then weighed on a microbalance (Sartorius CPA2P-F; resolution 1 μg). For bacterial abundances, measurements were taken only for adult female *A. tonsa* carcasses decomposing at 15°C normoxic conditions. These carcasses were rinsed briefly with sterile seawater and homogenized with a tissue grinder. The homogenates were filtered onto 0.2 μm black Nuclepore filters and stained with SYBR Gold™ (Chen et al. 2001), and stained bacteria were counted by epifluorescence microscopy (Bickel and Tang in press).

**Carcass removal: Hydrodynamic settling properties**

Laboratory measurements were designed to provide estimates of carcass settling velocity, density, and equivalent spherical diameter (ESD). These hydrodynamic properties were estimated for carcasses at varying stages of decomposition from each age class of copepods (nauplii, CI-CIII, and CIV-CVI) as follows.

Carcass incubations for measurement of settling velocities were set up identically to those for dry weight and bacterial abundance measurements. Carcass settling velocities were measured in a transparent glass column (height 21 cm, diameter 2 cm) filled with ASW at 20°C. Individual carcasses at different stages of decomposition were gently released just beneath the water surface, and observed by eye (for copepodites) or using a video stereomicroscope (AMG model AMS-MV2;
for naupliii). The carcasses were allowed to sink ~4 cm below the release point, and then timed as they passed five consecutive 1-cm intervals. The settling velocities of nine carcasses were observed for each discrete stage of decomposition and age class. Water-soluble red dye was used to check for thermal convection in the settling column. The dye was added at the column surface; it immediately sank to the bottom, and slowly and uniformly diffused upward. Once the dye had spread throughout the bottom half of the water, the column was emptied and refilled with new ASW and dye. In some cases we observed rapid upward transport of dye or a non-uniform upward plume of dye. This was taken to indicate a convective current and we discarded data from these trials. On several occasions carcasses were observed to slow their descent as they approached the glass column walls; therefore data were also discarded if sinking carcasses came within several body lengths of a wall.

Fresh carcass density was measured using a variation of the density gradient method (Køgeler et al. 1987): Small flasks (50 mL) were filled with ten fresh CIV-CVI carcasses and brine of various salinities (40-70 salinity) and temperatures (0-20°C). The flasks were mixed gently by inversion, and allowed to sit for 10 min undisturbed. After 10 min, the number of carcasses that had sunk to the bottom was noted, as well as those in suspension or floating near the surface. Carcasses that were neutrally buoyant (i.e., not sinking or floating) were considered to be of equal density to the solution containing them. The density of fresh nauplii and CI-CIII carcasses was assumed to be the same as measured for CIV-CVI carcasses.

The gravitational settling velocity of a copepod carcass in water depends on the density and size (ESD) of the carcass, as well as the density and viscosity of the
water. Carcass settling velocity measurements and fresh carcass density were used to estimate carcass ESD using a modified Stokes’ Law for irregularly shaped particles (Ferguson and Church 2004):

\[ \omega_s = \frac{RgD^2}{C_1 \nu + (0.75C_2 RgD^3)^{0.5}} \]  

where \( \omega_s \) is particle (carcass) settling velocity, \( R \) is submerged specific gravity \(((\rho_{\text{particle}} - \rho_{\text{fluid}})/\rho_{\text{fluid}}) \), where \( \rho \) is density), \( g \) is gravitational acceleration, \( D \) is particle ESD, \( \nu \) is fluid kinematic viscosity, and \( C_1 \) and \( C_2 \) are constants (24 and 1.2, respectively; as recommended for angular grains by Ferguson and Church 2004).

Measured values of fresh carcass density \( (\rho_{\text{particle}}) \) and settling velocity \( (\omega_s) \) were applied in Eq. 1 to calculate carcass fresh carcass ESD. Because the shape of the chitin carapace of a copepod carcass remains relatively intact for several days after death (Tang et al. 2006a, b), we assumed that carcass ESD was constant during decomposition. Therefore, ESD derived for fresh carcasses was applied to all other stages of decomposition, and measured carcass settling velocities were then used to estimate densities \( (\rho_{\text{particle}}) \) of the partially decomposed carcasses based on Eq. 1.

**Effects of turbulence: Laboratory experiment**

Average vertical current flow in the York River estuary is negligible compared to along channel current velocity, as is true in most shallow systems (Pond and Pickard 1983). However, turbulent velocity fluctuations vertically mix the water column such that particles, including copepod carcasses, which have congregated at or near the benthos could be resuspended and mixed by upward turbulent diffusion. The Rouse model (Orton and Kineke 2001) approximates the vertical distribution of
suspended particle concentration by assuming turbulent diffusion and gravitational settling dominate transport, and that the turbulent eddy viscosity varies as a parabola with depth. If suspension follows the Rouse model, then the concentration of particles \( C_z \) at any height \( z \) above the bottom is given by:

\[
C_z = C_a \left( \frac{z(h - z_a)}{z_a (h - z)} \right)^{-Pr}
\]

(2)

where \( C_a \) is particle concentration at a reference height \( z_a \) above the bottom, \( h \) is total water column depth, and \( Pr \) is the Rouse parameter:

\[
Pr = \frac{\omega_s}{\kappa u*}
\]

(3)

where \( \omega_s \) is particle settling velocity, \( \kappa \) is von Kármán’s constant (0.408), and \( u* \) is shear velocity. The Rouse parameter \( (Pr) \) indicates the relative importance of settling and turbulent fluxes, and values <1 indicate that particle suspension is occurring throughout most of the water column (van Rijn 1993). Application of Eq. 2 to copepod carcasses in an estuary, however, requires extra caution because 1) settling velocity \( (\omega_s) \) is not constant for carcass at different stages of decomposition, 2) the assumption of parabolic eddy viscosity may be invalid when the water column is stratified, and 3) the vertical settling and diffusion terms may not be in balance as is assumed in the Rouse model, due to the production of new carcasses within the water column or horizontal advection.

A laboratory experiment was conducted to test whether Eq. 2 adequately described the vertical distribution of copepod carcasses in a closed and homogenous system. A transparent 13 L container (30 cm high, 24 cm diameter) was filled to 24 cm height with ASW at 20°C, and continuously mixed by two 8 cm long Teflon stir...
bars attached to an impellor shaft. Stir bars were fixed at 2 cm and 15.5 cm off the bottom, and protruded 3.5 cm from either side of the impellor, which rotated at 7 rpm, reversing direction every minute (Fig. 1). This setup was based on that of Petersen et al. (1998) to simulate intermediate turbulent mixing conditions in the Choptank River, Chesapeake Bay. To begin the experiment 1000 fresh A. tonsa (CIV-CVI) carcasses (~100 individuals L⁻¹) were added to the container. A video-stereomicroscope (AMG model AMS-MV2) attached to a motorized track slowly traversed the height of the water column 3 times every hour for 19 h, each time recording a profile of copepod carcass vertical distribution (56 profiles recorded). Each recorded profile was analyzed by enumerating the carcasses within the microscope’s field of view at 10 evenly spaced depths from 1-22.6 cm off the bottom. Eq. 2 was fit to these observations using non-linear least squares regression. C₂ was set to the number of carcasses observed in the video-microscope field of view at each of the 10 depths, Z_a was set to 2.18 cm off the bottom, and C_a was the mean number of carcasses observed at Z_a. The Rouse parameter (Pr) was estimated by fitting Eq. 2 to these data by the regression. For comparison, a simpler linear model was also fit to the vertical carcass distribution data.

Effects of turbulence: Field observations

A field study was conducted to evaluate the role that resuspension may play in determining the residence times of copepod carcasses in the water column, and to test whether the Rouse model (Eq. 2) reasonably described the vertical distribution of copepod carcasses in the York River estuary. Copepod carcasses were sampled with a custom-built plankton pump at discrete depths at a single station in the York River
(37.24°N, 76.50°W). This station was 9.1 m deep (±0.4 m tidal range), and adjacent to a data buoy equipped with ADCP for current velocity measurements (http://chsd.vims.edu/realtime/). The pump was housed in a watertight enclosure, and the water intake was fitted with a 63 μm mesh collection canister. Water was drawn directly into the canister such that the animals did not come into contact with the pump apparatus. The low flow rate of the pump (0.032 m³ min⁻¹) was unlikely to cause mortality of copepods (Elliott and Tang 2009), but would likely under-sample live copepods with escape jump behavior. However, our purpose was to sample carcasses, hence potential under-sampling of live copepods was not a concern.

Samples were collected on 13 May 2009 from 08:00 to 17:00 h Eastern Standard Time, spanning the majority of a tidal cycle (low tide ca. 06:55 h, high tide ca. 13:00 h, and subsequent low tide ca. 18:50 h). The pump was run for 5 min at a time followed by closure of the intake via a messenger. Samples were collected at shallow (2-3 m), intermediate (3-5 m), and deep (5-8 m) depths. A Conductivity-Temperature-Depth (CTD) profiler attached to the pump recorded the exact depth of each sample along with continuous temperature and salinity measurements. A total of 18 discrete depth samples were collected. To identify carcasses, collected samples were immediately stained with neutral red solution prior to preservation (Elliott and Tang 2009). Within each sample carcasses of copepod nauplii and *A. tonsa* copepodite stages CI-CIII and CIV-CVI were enumerated, and all samples were processed within 10 d of collection. Each sample was counted in its entirety, and counts were converted to *in situ* carcass abundances. Samples containing a total of less than 5 carcasses were excluded from further analysis, since abundance estimates have very
low precision when such low numbers are counted (Harris et al. 2000). Resulting data on vertical carcass distribution were fit with Eq. 2 by non-linear least squares regression, and the Rouse parameter (Pr, indicating the importance of resuspension) was estimated from the regressions.

**Fate of carcass organic matter: Application to the York River estuary**

To estimate the fate of copepod carcass organic matter in the York River estuary, all three removal mechanisms- necrophagy, sinking, and microbial decomposition- were combined in a simple mathematical model. This model represented the loss of carcass organic matter through time, beginning with 100 units of mass of fresh copepod carcass organic matter at mid-depth within the water column, and proceeding until only the equivalent dry weight of the chitin carapace remained (assuming 8.35% of initial dry weight was chitin; Cauchie et al. 1997).

Table 1 shows the formulations and parameterization used in the mathematical model. Our experiments showed no statistically significant difference in ctenophore clearance rates on live copepods vs. carcasses (see Results). We therefore estimated ctenophore population clearance rates on copepod carcasses from size-dependent individual *M. leidyi* clearance rate on live copepods (Purcell et al. 2001) and *M. leidyi* abundances and size distributions from the York River (Condon and Steinberg 2008). To account for the interplay between sinking and turbulent upward diffusion, two extreme scenarios for sinking losses were considered in the model. At one extreme carcasses were assumed to remain suspended indefinitely (no sinking loss), and at the other extreme carcasses were assumed to be removed immediately upon reaching the bottom (maximum sinking loss). The model was run separately for CIV-CVI, CI-CIII
and nauplii, for each season, for an additional anoxic summer scenario, and for both sinking loss scenarios.
RESULTS

Carcass removal: Ctenophore selectivity

The presence or absence of stain had no significant effect on the ingestion of live copepods and carcasses by adult *Mnemiopsis leidyi*, regardless of which was stained (2-sample t-test, t=0.81, df=3, p=0.477). Since ctenophores were not selective based on stain, live stained and dead stained treatments were combined for further analysis. The mean clearance rates by cydippid larvae on live copepods and carcasses were 0.03 and 0.07 L individual$^{-1}$ h$^{-1}$, respectively. For adult ctenophores, the mean clearance rates were 0.61 and 0.64 L individual$^{-1}$ h$^{-1}$ on live copepods and carcasses, respectively. There were no significant differences in clearance rates for live copepods vs. carcasses (2-sample t-tests: for larvae, t=-1.31, df=3, p=0.281; for adults, t=-0.16, df=9, p=0.875).

Carcass removal: Microbial decomposition

Loss of carcass dry weight occurred most rapidly in the 25°C normoxic treatment, where dry weight dropped below detection limit within 30 h (Table 2). CIV-CVI carcass dry weight decreased significantly and linearly with the logarithm of time since death ($R^2=0.547$; t-test, t=-9.93, df=80, p<0.0005). The dry weight of CI-CIII and naupliar carcasses at 15°C also decreased significantly and linearly with the logarithm of time since death (for CI-CIII $R^2=0.396$; t-test, t=-2.63, df=9, p=0.03; for nauplii $R^2=0.242$; t-test, t=-2.20, df=12, p=0.05). The rate of decrease of carcass dry weight was positively temperature dependent; that is, the slope of the linear regression line describing CIV-CVI carcass dry weight through time was significantly steeper for incubation at 25°C than at 5°C (t-test, t=2.98, df=35, p<0.05). Though line
slopes were not significantly different between normoxic and anoxic incubations (t-test, t=0.07, df=35, p>0.05), dry weight did decrease more slowly in anoxic incubations (Table 2). Therefore, we included both dissolved oxygen and temperature as explanatory variables in a regression model describing carcass dry weight (dry wt) as a function of time t since death:

\[
dry wt_t = dry wt_i + (\ln(t) + 1.39) \times \left[ K_1 \times (1 - e^{k \times T}) + (K_2 \times DO) \right]
\]

where dry wt is carcass dry weight (µg carcass\(^{-1}\)) at time t after death (from 0.25 h onward), dry wt\(_i\) is initial carcass dry weight, \(T\) is water temperature (°C), \(DO\) is an indicator variable (0 = normoxic; 1 = hypoxic or anoxic), and \(K_1\), \(K_2\), and \(k\) are constants that were estimated by the non-linear regression using an iterative approach. Eq. 4 predicts dry wt\(_t\) to decrease linearly with \(\ln(t)\), with the rate of decrease (slope) determined as a linear function of dissolved oxygen condition and an exponential saturation function of temperature. The parameter estimates that resulted in the best fit of Eq. 4 to CIV-CVI carcass dry weight data were dry wt\(_i\)=4.178 µg carcass\(^{-1}\), \(K_1=-4.166\), \(K_2=0.046\), and \(k=-0.008°C^{-1}\). The resulting model fit the data with \(R^2=0.631\).

The mean initial bacterial abundance associated with adult female carcasses was \(3.78 \times 10^4\) carcass\(^{-1}\). Changes in the abundance of carcass-associated bacteria did not coincide with changes in carcass dry weight, as indicated by the lack of a significant correlation between the averages of these two metrics (Pearson Correlation, \(r=-0.577, t=-1.41, df=4, p=0.231\)). For example, maximum dry weight loss occurred in the first 8 h after death, during which time bacterial abundance remained nearly constant (Table 2).
Carcass removal: Hydrodynamic settling properties

Copepod carcass settling velocity decreased rapidly in the first several hours after death, and more slowly thereafter (Table 3). Mean fresh carcass density was 1049 kg m\(^{-3}\) (± 0.3 kg m\(^{-3}\); 95% confidence interval), and was combined with fresh carcass settling velocity (mean of Table 3 initial values; \(a_0 = 0.125, 0.08, \text{ and } 0.03 \text{ cm s}^{-1}\) for CIV-CVI, CI-CIII, and nauplii, respectively) to calculate ESD using Eq. 1 (\(\rho_{\text{fluid}} = 1013 \text{ kg m}^{-3}\) and \(\nu = 1.0 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}\)). The resulting ESDs were 333 \(\mu\text{m}\) for CIV-CVI, 243 \(\mu\text{m}\) for CI-CIII, and 141 \(\mu\text{m}\) for nauplii. Using these ESDs and measured carcass settling velocities (Table 3) in Eq. 1, carcass densities were calculated at different stages of decomposition. Resulting densities of carcasses of both copepodite groups decreased significantly and linearly with the natural logarithm of time (Fig. 2 for CIV-CVI \(R^2 = 0.378; t\)-test, \(t = -23.87, df = 936, p < 0.0005\); for CI-CIII \(R^2 = 0.099; t\)-test, \(t = -3.87, df = 128, p < 0.0005\)), but not significantly for nauplii (\(R^2 = 0.007; t\)-test, \(t = 1.13, df = 40, p = 0.264\)). As was the case with dry weight, the rate of decrease of carcass density was positively temperature dependent, with a linear regression line describing decrease in CIV-CVI carcass density through time that was significantly steeper at 25\(^\circ\)C than at 5\(^\circ\)C (2-sample \(t\)-test, \(t = 2.1, df = 413, p < 0.05\)). Although line slopes did not significantly differ between normoxic and anoxic incubations (2-sample \(t\)-test, \(t = 0.02, df = 389, p > 0.05\)), carcass density did decrease more slowly in anoxic incubations (Fig. 2 for CIV-CVI). Therefore, we included both temperature and oxygen as explanatory variables in a regression model describing carcass density \((\rho)\) as a function of time \(t\) since death:

\[
\rho_t = \rho_i + (\ln(t) + 1.39) \times [K_1 \times (1 - e^{k \times T}) + (K_2 \times DO)]
\]  

\(5\)
Eq. 5 is analogous to Eq. 4, where \( \rho_t \) is predicted carcass density (kg m\(^{-3}\)) at time \( t \) after death (from 0.25 h onward). Parameter estimates for CIV-CVI carcasses from non-linear regression using an iterative approach were \( \rho_i \) (initial carcass density) = 1045 kg m\(^{-3}\), \( K_1 = -3.78 \), \( K_2 = 0.731 \), and \( k = -0.329^\circ C^{-1} \). The resulting model fit the data with \( R^2 = 0.398 \). Model predictions of carcass density from Eq. 5 are shown as lines in Fig. 2.

Settling velocities predicted from Eqs. 1 and 5 were on average 23% higher than our empirical measurements (Table 3), suggesting that carcass ESD may have decreased slightly during decomposition. To account for this, predicted settling velocities were adjusted by a factor of 1/1.23. The adjusted predictions agreed closely with observation for copepodites and nauplii (Fig. 3; \( R^2 = 0.733 \); \( t \)-test of regression slope, \( t = 10.0 \), df=36, \( p < 0.0005 \)), and this adjustment was subsequently made to predicted settling velocities used in the Rouse model (Eq. 2) and in the mathematical model predicting the fate of carcass organic matter (Table 1).

**Effects of turbulence: Laboratory experiment**

Copepod carcass abundance increased with depth in all 56 recorded profiles (Fig. 4). The linear regression model described vertical carcass distribution quite well (\( t \)-test, \( t = 21.28 \); df=513; \( p < 0.0005 \); \( R^2 = 0.42 \)), but the Rouse model (Eq. 2) provided a slightly better fit to the data (\( R^2 = 0.48 \)). The estimated 95% confidence interval of the Rouse parameter (Pr) was 0.36-0.42, corresponding to a shear velocity (\( u_* \)) of \(~0.5\) cm s\(^{-1}\) based on an intermediate \( \omega_b \) of 0.085 cm s\(^{-1}\) chosen to be representative of the 19 h experimental period.
Effects of turbulence: Field observations

Copepodite carcass abundance increased with depth in the York River (Fig. 5A, B). The Rouse model (Eq. 2) was fit to these abundances, resulting in characteristic profiles over the sampling period (Fig. 5 ‘Rouse prediction’). The regression was not attempted for naupliar carcasses, whose abundance did not vary with depth (Fig. 5C). Reference height \((z_a)\) in these regressions was set as 6.1 m above bottom (3 m depth), where we had the most replicate samples and likely highest accuracy in measured concentrations. Reference concentration \((C_a)\) was set to the mean carcass abundance at \(z_a\). There was a strong relationship between observed copepodite carcass abundance and Eq. 2 predictions \((R^2=0.725 \text{ for CIV-CVI}; R^2=0.809 \text{ for CI-CIII})\). The estimated 95% confidence intervals for Rouse parameters \((Pr)\) were 0.29-0.52 for CIV-CVI and 0.44-0.68 for CI-CIII, indicating that resuspension of carcasses was important as well as gravitational settling \((Pr<1)\).

Assuming settling velocities of intermediate aged carcasses (18 h, Table 3, \(\omega_s=0.06\) cm s\(^{-1}\) for both CIV-CVI and CI-CIII) these Rouse parameters corresponded to a shear velocity of ~0.2-0.5 cm s\(^{-1}\) (95% confidence intervals: 0.28-0.51 cm s\(^{-1}\) for CIV-CVI, and 0.22-0.33 cm s\(^{-1}\) for CI-CIII).

Fate of carcass organic matter: Application to the York River estuary

Model estimates of carcass fate were expressed as percentages of carcass organic matter removed by the three different mechanisms (Table 4). The model was run under two different assumptions of sinking losses to consider the implications of turbulence. The first assumed no resuspension (maximum sinking losses) and the
second that resuspension retained carcasses in the water column indefinitely (no sinking losses).

When maximum sinking loss was considered, the time required for removal of all copepod carcass organic matter was 3-4 h for CIV-CVI, 5-7 h for CI-CIII, and 11-20 h for nauplii (Table 4). Sinking was the dominant removal mechanism, although it was relatively less important for nauplii because of their lower settling velocities. Microbial decomposition removed much of the remainder of carcass organic matter (7-30% for CIV-CVI, 9-39% for CI-CIII, and 12-57% for nauplii), and its importance varied seasonally based on water temperature and competition with necrophagy. Compared to decomposition and sinking, necrophagy by ctenophores removed a small amount of carcass organic matter (2-6% for copepodites and 6-14% for nauplii) except in the spring when ctenophore population clearance rate was highest (Table 1). Seasonal anoxia caused only a slight decrease in losses to microbial decomposition and a slight increase in sinking losses (2-5%), but no change in losses to necrophagy (Table 4).

At the other extreme, when the model neglected sinking losses, it took 19-111 h for carcass organic matter to be removed (Table 4). These results applied to all developmental stage groups since the same rates of dry weight loss and ctenophore clearance were applied to all stages. In the absence of sinking, the importance of ctenophore ingestion (necrophagy) became disproportionately larger, and was comparable to microbial decomposition in removing copepod carcasses (Table 4).
DISCUSSION

In this section we summarize our findings on carcass removal via necrophagy, microbial decomposition, and gravitational settling in the presence of turbulence; and compare our result to those of other studies. Then we synthesize our findings into a general representation of the fate of copepod carcass organic matter in the York River estuary. Finally, we discuss the ecological implications in estuaries like the York River, and in the larger marine environment.

Carcass removal: Ctenophore selectivity

We found that ctenophores did not strongly select for live copepods or carcasses, consuming each in similar proportion to their abundance in the water. Several studies have shown particle selectivity by ctenophores and other gelatinous planktivores (Purcell et al. 1991; Costello et al. 1999; Waggett and Costello 1999). However, in our bottle incubations selectivity between live copepods and carcasses was not detected. Ctenophores are not the only York River planktivore that may ingest copepods and their carcasses. However, *M. leidyi* is both abundant and persistent in the York River (Steinberg and Condon 2009), and represents the dominant predator on mesozooplankton in Chesapeake Bay (Baird and Ulanowicz 1989). Therefore, as a first approximation we assumed that both live copepods and carcasses were cleared at the same rate in the field, and that this clearance rate was set by *M. leidyi* abundance and size distribution.

Carcass removal: Microbial decomposition

Following death, the dry weight (and density) of copepod carcasses decreased rapidly at first, and more slowly as time progressed. This log-linear type of trajectory
of decomposition has been found in other studies of zooplankton carcass decomposition (Seiwell and Seiwell 1938; Lee and Fisher 1994; Bickel and Tang in press), and also specifically for the decomposition of detritus (Jenny et al. 1949; Olson 1963; Wider and Lang 1982). Overall, the regression models (Eqs. 4 and 5) performed well at describing carcass decomposition (dry weight and density losses) through time and for different temperature and dissolved oxygen conditions ($R^2$ describing 40-63% of variation). Some of the unexplained variation may have been due to grouping of multiple developmental stages. Size difference can be large among developmental stages of *A. tonsa*, and carcass settling velocity and dry weight will vary accordingly. For example, the dry weight of CIV *A. tonsa* is approximately half that of CVI, and length of CIV is approximately 2/3 that of CVI (Heinle 1966).

Although individual copepods were selected randomly for experiments, variation around the mean of measured dry weight and settling velocity could have been magnified by a disproportionately large number of individuals of a single developmental stage within specific replicates.

The abundance of bacteria associated with copepod carcasses (Table 2) was comparable to reports for naturally occurring Chesapeake Bay calanoid copepods ($0.3-9.6 \times 10^5$ cells carcass$^{-1}$; Heidelberg et al. 2002) and laboratory *A. tonsa* cultures ($10^3-10^5$ cells carcass$^{-1}$; Tang 2005). Maximum dry weight loss occurred in the first 8 h after death, but bacterial abundance remained nearly constant until 16 h after death. This initial 8-16 h period may represent a lag phase in the growth of bacteria decomposing the carcasses, during which time the bacteria actively produced exoenzymes to break down copepod tissues, but bacterial population growth was still
slow (Bickel and Tang in press). Assuming a bacterial cell diameter of 1.0 μm, a
volume-to-carbon conversion of 0.09 × volume⁰.⁶ (Norland 1993), and a carbon
content of 50% of dry weight (Norland 1993), the observed maximum carcass
associated bacterial abundance (104,700 cells carcass⁻¹) corresponded to a dry weight
of <0.13 μg, or <7% of total carcass dry weight. Hence, carcass associated bacteria
contributed a negligible fraction to the total carcass dry weight.

**Carcass removal: Hydrodynamic settling properties**

The gravitational settling velocity of a copepod carcass in water depends on
the density and size (ESD) of the carcass, and the density and viscosity of the water,
as described in Eq. 1. Measured settling velocity, density, and ESD of carcasses
agreed well with the literature. Our measurements of copepodite carcass settling
velocities (ca. 0.1 cm s⁻¹, Table 3) were comparable to reports for passively sinking
live *A. tonsa* (0.08 ± 0.021 cm s⁻¹; Jonsson and Tiselius 1990). The density of fresh *A.
tonsa* carcasses (1049 kg m⁻³ ± 0.3, 95% confidence interval) was slightly higher than
reported for *Calanus finmarchicus* (1027-1045 kg m⁻³; Knutsen et al. 2001) and
*Acartia clausii* (1045 kg m⁻³; Greenlaw 1977). ESDs estimated from settling
velocities and density (333 μm, 243 μm, and 141 μm for CIV-CVI, CI-CIII, and
nauplii, respectively) compared well with independent estimates based on *A. tonsa*
body volume (from Mauchline 1998) and assuming cylindrical (copepodites) and
spherical (nauplii) shapes (345 μm, 199 μm, and 125 μm for CIV-CVI, CI-CIII, and
nauplii, respectively). In our ESD calculations we assumed that the densities of CI-
CIII and copepod nauplii were identical to that measured for CIV-CVI. Although the
assumption is reasonable among copepodite stages, the density of nauplii may be
slightly higher, since lipids tend to contribute slightly less to total naupliar dry weight (van der Meeren et al. 2008). However, this difference is likely unimportant given the already low settling velocity of nauplii. For example, if instead we assume a density of 1071 kg m\(^{-3}\) (maximum reported \textit{Acartia} spp. density; Mauchline 1998), the difference in settling velocity predicted by Eq. 1 is small (<0.017 cm s\(^{-1}\)), and is within the range of variability of naupliar settling velocity measurements (Table 3).

\textit{Effects of turbulence}

A goal of the turbulence experiments was to verify that carcasses behaved as passive sinking particles throughout decomposition, which was substantiated by the observation that carcass concentration increased significantly with depth throughout decomposition in the laboratory (Fig. 4). A second purpose of these experiments was to evaluate the ability of the Rouse model (Eq. 2) to accurately describe the vertical distribution of copepod carcasses, and thereby provide a way to quantify the relative importance of vertical mixing and gravitational settling to the fate of copepod carcasses. The Rouse model described the vertical distribution of carcasses fairly well in the laboratory (Fig. 4) and in the field (Fig. 5A, B). The Rouse parameter \(Pr\) (Eq. 3) was useful for capturing the curvilinear shape of carcass distribution with depth (Figs. 4; 5A, B), which could not be done with the linear model (Fig. 4). The linear model also had the undesirable potential of predicting negative carcass abundances at shallow depths (negative y-intercept possible). Finally, the linear model required estimation of both line slope and intercept, while the Rouse model required estimation only of the Rouse parameter (\(Pr\)). We concluded that the Rouse model (Eq.
2) described carcass profiles better than the linear model, supporting its use to
describe the vertical distribution of copepod carcasses.

As a means of further assessment, estimates of $u^*$ from the regressions of the
Rouse model to carcass abundances in the field (Fig. 5A, B) were compared to
independent field derived $u^*$ estimates from the quadratic stress law (Orton and
Kineke 2001) and using ADCP current speed data (1.12 m above the bottom). A
typical drag coefficient of 0.0015 for the central portion of the York River was
assumed (Scully and Friedrichs 2003). Estimates of $u^*$ from the quadratic stress law
ranged from 0.06 to 2.04 cm s$^{-1}$, with a time-average of 0.82 cm s$^{-1}$, slightly higher
than estimates based on vertical carcass distribution measurements in the field (~0.2-
0.5 cm s$^{-1}$). This discrepancy was not surprising, given the tendency for thermohaline
and sediment induced stratification to reduce turbulent stresses and apparent shear
velocity in mid-water column of the York River (Friedrichs et al. 2000). As is typical
of this estuary, strong thermohaline stratification was present during our field
sampling. The change in water density from 2.5-8 m depth (the range of sample
collection) was 0.9 kg m$^{-3}$, and this may explain the lower shear velocities estimated
from observed carcass distribution.

Fate of carcass organic matter in the York River estuary

The predicted fate of carcass organic matter depended heavily on the
magnitude of sinking losses (Table 4). Assuming a settling velocity of ~0.1 cm s$^{-1}$
(Table 3) and an average channel depth of ~10 m (Dellapenna et al. 1998), copepodite
carcasses could sink out of the York River water column in under three hours.
However, several lines of evidence suggest that most carcasses remain in the water
column for much longer as a result of turbulent mixing: 1) Tang et al. (2006a) observed an average 29% of collected copepods were carcasses in the York River during summer 2005. In the absence of resuspension and upward turbulent diffusion, the high carcass abundance and high settling velocity translate to an extremely high post-hatch mortality rate of ca. 2.32 d⁻¹. This far exceeds the estimated population growth rate (≤0.73 d⁻¹) for the summer when copepod abundance is increasing (D.T. Elliott unpubl.). Thus, a more reasonable explanation is that vertical mixing helps retain carcasses in the water column. 2) Our laboratory experiment showed that copepod carcasses could be suspended throughout the water column by turbulence, and that concentrations increased with depth (Fig. 4). 3) Our field data confirmed that copepod carcasses were found throughout the water column, with abundances increasing with depth (Fig. 5A, B). The Rouse parameter (Pr) estimated from field abundances of copepodites was well below 1.0 (Fig. 5A, B), indicating that turbulent resuspension was occurring throughout much of the water column (van Rijn 1993). 4) Turbulent resuspension will be important for particles having a Rouse parameter of <1.0 (van Rijn 1993). The time-averaged shear velocity from ADCP data (u*=0.82 cm s⁻¹) corresponds a Rouse parameter below 1.0 for particles with settling velocities <0.3 cm s⁻¹. Our empirically derived settling velocities were all lower than this threshold (Table 3).

Thus, carcasses within the York River do sink but may congregate near the bottom where turbulent mixing is most intense. Some of the carcasses will be deposited to the bed, especially during periods of low turbulence such as at slack tide. These, however, may be subsequently resuspended and mixed into the overlying
water column especially when tidal currents and turbulent mixing increase. The true budget for the fates of copepod carcasses lies somewhere between the two extreme scenarios of zero and maximum sinking losses.

The influence of horizontal advection on carcass distribution should be analogous to that of suspended sediments of a similar settling velocity. Suspended sediments in the York River estuary often converge within one of two turbidity maxima, both located >20 km upstream of the estuary’s mouth (Friedrichs 2009). At a maximum carcass residence time of 111 h (Table 4) and a 7 cm s\(^{-1}\) net landward flow as a result of estuarine circulation (Friedrichs 2009), a carcass could be transported 28 km upstream, approximately half the length of the estuary. Hence, the overall effect of horizontal advection would be to retain carcasses within the estuary, and possibly concentrate them in the estuarine turbidity maxima. This would be especially important when carcass residence time is long such as in the winter (Table 4). Elevated copepod abundances have been observed in estuarine turbidity maximum regions (Morgan et al. 1997; Roman et al. 2001), and our results suggest that this phenomenon could partially result from passive concentration of carcasses there. In this case, one would expect to find high relative abundances of carcasses in zooplankton samples taken from turbidity maximum regions.

**Broader ecological implications**

Based on this and other studies it is clear that microbial decomposition of copepod carcasses represents an alternative trophic pathway for zooplankton secondary production. The importance of carcass organic matter to microbial production in the York River estuary can be estimated as follows: Bacterial carbon
demand (BCD) for the York River is estimated to be 0.8-4.2 mg C m⁻³ h⁻¹ (Schultz 1999 for a 10 m deep water column). Assuming a copepod production of 0.24 mg C m⁻³ h⁻¹ (Purcell et al. 1994, mesohaline portion of the Chesapeake Bay), and an instantaneous non-predatory mortality rate of ~0.1 d⁻¹ for copepods (Mauchline 1998; Tang et al. 2006a), we estimate a rate of 0.024 mg C m⁻³ h⁻¹ for the conversion of copepod production to carcasses. If 58% of this is directly utilized by bacteria (average of no sinking scenario; Table 4), then carcass decomposition will be equivalent to consumption of 0.014 mg C m⁻³ h⁻¹ by microbes, or 0.3-1.8% of York River BCD. This estimate is substantially higher than that by Tang et al. (2006a) for the summer of 2005, likely because it considers all copepod developmental stages rather than just adults, and includes springtime copepod production can be higher than in the summer.

We can make a similar calculation of the potential contribution of carcasses to water column particulate organic carbon (POC) and POC flux to the benthos. Total POC in York River surface water is on the order of 1-2 mg C L⁻¹ (Countway et al. 2003). Assuming a maximum carcass residence time of 111 h in the water column (Table 4), the estimated average production of copepod carcass organic matter (0.024 mg C m⁻³ h⁻¹, see above) corresponds to a carcass carbon concentration of 2.7 μg C L⁻¹. This suggests that copepod carcasses represent <0.3% of York River water column POC. Flux of POC to the benthos has been estimated as 20.6-42.5 mg C m⁻² h⁻¹ annually in the mesohaline portion of Chesapeake Bay (Roden et al. 1995). Assuming 0.24 mg C m⁻² h⁻¹ carcass organic matter production rate (0.024 mg C m⁻³ h⁻¹ for a 10 m deep water column, see above) and 63% sinking losses (average of sinking
scenarios; Table 4), copepod carcasses could account for 0.4-0.7% of organic matter deposited to the York River bottom.

These estimates suggest that copepod carcasses likely contribute only small fractions to the total York River BCD, water column POC, and POC flux to the benthos. Nevertheless, carcasses provide important localized hot spots of microbial growth and activity that may select for specific bacterial phylotypes and hence affect microbial diversity in the water column (Tang et al. 2006b, 2009). Carcasses are also labile particulate organic matters that could be a nutritious food source for benthic fauna (Zajączkowski and Legeżyńska 2001).

Our results also have implications for the fate of copepod carcasses in other environments. Considering an oceanic copepod species with initial carcass density and rate of density reduction that are identical to those measured for *A. tonsa*, a carcass decomposing in the relatively warm surface ocean (at 15-25°C) would approach a density of 1028 kg m⁻³ after 24–48 h (Fig. 2A). At this time it would be approximately neutrally buoyant with the water below the permanent thermocline (ς ≈ 28). Using Eqs. 1 and 5 we predicted sinking trajectories through time of a copepod carcass (prosome length 2000 μm, ESD 1028 μm) in freshwater (0 salinity), mesohaline water (18 salinity) and seawater (35 salinity), at temperatures of 5°C, 15°C, and 25°C, and in the absence of net vertical water movement. The effect of temperature on predicted trajectory was moderate. Carcass density decreased more slowly in cold water (5°C), but the carcass also sank more slowly since cold water is denser. Conversely, in warm water (25°C) carcass density decreased more rapidly, but the carcass also sank faster. The effect of salinity was more pronounced. Relative to
freshwater, predicted settling velocity decreased markedly in mesohaline water as the carcass decomposed and became less dense. However, the effect of decreasing carcass density was most notable in seawater, where the carcass was predicted to achieve neutral buoyancy between 40 and 77 h after death, during which time it would have sunk approximately 150-300 m. Assuming instead a water temperature of 0°C at the depth of neutral buoyancy, carcasses at this depth would decompose slowly enough to remain relatively intact for weeks. This may explain the congregations of zooplankton carcasses observed at or below the permanent thermocline in several oceanic studies (Terazaki and Wada 1988; Geptner et al. 1990; Böttger-Schnack 1996).

Using a combined experimental, observational, and modeling approach, this study quantified the relative importance of necrophagy, microbial decomposition, and sinking to removal of copepod carcass organic matter. We found that turbulent mixing in shallow tidal systems can keep carcasses in suspension much longer than gravitational settling alone would imply. Therefore, a large portion of copepod carcass organic matter may fuel microbial and necrophage production in the water column, as predicted in the model scenario that excluded sinking losses (Table 4). This may also occur in the open ocean, due to the large distances that a carcass must sink and the potential for a carcass to achieve neutral buoyancy.

The importance of removal of carcass that are near the bed by ingestion by benthic fauna in shallow systems is not presently clear, nor is the importance of carcass incorporation into sinking aggregates or burial in bed sediments. Further work on carcass sinking losses should focus on resolving the balance between gravitational
settling and upward turbulent diffusion, perhaps using a hydrodynamic model coupled with observations of carcass abundance close to and on the sea bed. The results of this study highlight the importance of considering live and dead composition in zooplankton sampling and pelagic food web models, given that carcasses can remain in the water column for extended times.
REFERENCES


doi: 10.1093/plankt/fbq010


Table 1. Formulation, parameterization, and sources of data used in the model to predict the fate of copepod carcass organic matter in the York River estuary.

<table>
<thead>
<tr>
<th>description</th>
<th>value(s)</th>
<th>units</th>
<th>Source (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$dCS/dt$ change in organic matter through time</td>
<td>$= -CS \times (a + b + c)$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS carcass dry weight pool</td>
<td>100</td>
<td>units of mass</td>
<td>assigned</td>
</tr>
<tr>
<td>$a$ ctenophore population clearance rate</td>
<td>winter = 0.020; spring = 0.089; summer = 0.015; fall = 0.026</td>
<td>h$^{-1}$</td>
<td>(Purcell et al. 2001; Condon and Steinberg 2008)</td>
</tr>
<tr>
<td>$b$ rate of transfer from CS to microbial decomposition</td>
<td>$(1 - \text{dry wt}<em>t/\text{dry wt}</em>{t,0})/\Delta t$</td>
<td>h$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>$c$ rate of transfer from CS to benthos</td>
<td>$= a_{k0}/(0.5 \times \text{depth})$</td>
<td>h$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>dry wt$_t$ carcass dry weight at time $t$ after death</td>
<td>variable</td>
<td>g copepod$^{-1}$</td>
<td>from Eq. 4</td>
</tr>
<tr>
<td>$T$ water temperature</td>
<td>winter = 5.5; spring = 18.0; summer = 26.3; fall = 15.4</td>
<td>°C</td>
<td><a href="http://www.chesapeakebay.net/">www.chesapeakebay.net/</a></td>
</tr>
<tr>
<td>DO dissolved oxygen condition</td>
<td>all seasons = 0; anoxic summer scenario = 1</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>$\Delta t$ time increment used</td>
<td>0.03125</td>
<td>h</td>
<td>assigned</td>
</tr>
<tr>
<td>$a_{k0}$ carcass settling velocity at time $t$ after death</td>
<td>variable</td>
<td>m h$^{-1}$</td>
<td>from Eqs. 1 and 5</td>
</tr>
<tr>
<td>depth channel depth of the York River estuary</td>
<td>10</td>
<td>m</td>
<td>(Dellapenna et al. 1998)</td>
</tr>
</tbody>
</table>
Table 2. Time series of copepod carcass dry weight (μg carcass\(^{-1}\); mean with standard deviation in parentheses) through decomposition for different developmental stages, temperatures, and dissolved oxygen conditions; and carcass associated bacterial abundance (bacterial cells carcass\(^{-1}\)) for CVI females decomposing at 15°C normoxic conditions (n=3 except in cases denoted by * where n=2 because insufficient carcasses were recovered for a third measurement).

<table>
<thead>
<tr>
<th>Approximate time since death (h)</th>
<th>Carcass dry weight</th>
<th>Bacterial abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5°C Normoxic</td>
<td>15°C Normoxic</td>
</tr>
<tr>
<td>0.25</td>
<td>3.6 (1.3)</td>
<td>5 (1.1)</td>
</tr>
<tr>
<td>2</td>
<td>3.2 (0.7)</td>
<td>2.4 (0.9)</td>
</tr>
<tr>
<td>4</td>
<td>1.4 (0.9)</td>
<td>1.3 (0.1)</td>
</tr>
<tr>
<td>6</td>
<td>2 (0.1)</td>
<td>2.6 (0.6)</td>
</tr>
<tr>
<td>8</td>
<td>3.1* (0.4)</td>
<td>3.3 (1.7)</td>
</tr>
<tr>
<td>10</td>
<td>3.5 (0.5)</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td>18</td>
<td>2.4 (0.8)</td>
<td>0.1* (0.1)</td>
</tr>
<tr>
<td>24</td>
<td>2.5* (1.1)</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>30</td>
<td>1.9 (0.3)</td>
<td>0.4 (0.2)</td>
</tr>
<tr>
<td>48</td>
<td>1.8 (0.4)</td>
<td>1.8 (0.4)</td>
</tr>
<tr>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>116</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

131
Table 3. Time series of copepod carcass settling velocity (cm s\(^{-1}\); mean with standard deviation in parentheses) through decomposition for different developmental stages, temperatures, and dissolved oxygen conditions (n=24-40 for copepodites and 7-9 for nauplii).

<table>
<thead>
<tr>
<th>Approximate time since death (h)</th>
<th>5°C Normoxic</th>
<th>15°C Normoxic</th>
<th>25°C Normoxic</th>
<th>25°C Hypoxic</th>
<th>25°C Anoxic</th>
<th>15°C Normoxic</th>
<th>15°C Nonnoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.120 (0.032)</td>
<td>0.124 (0.023)</td>
<td>0.120 (0.032)</td>
<td>0.129 (0.039)</td>
<td>0.078 (0.043)</td>
<td>0.027 (0.010)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>.</td>
<td>0.066 (0.015)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.078 (0.026)</td>
<td>0.076 (0.020)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>6</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>0.099 (0.058)</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>8</td>
<td>0.089 (0.021)</td>
<td>0.070 (0.025)</td>
<td>0.097 (0.025)</td>
<td>.</td>
<td>.</td>
<td>0.081 (0.024)</td>
<td>0.044 (0.021)</td>
</tr>
<tr>
<td>10</td>
<td>.</td>
<td>.</td>
<td>0.069 (0.025)</td>
<td>.</td>
<td>0.085 (0.038)</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>18</td>
<td>0.057 (0.021)</td>
<td>0.061 (0.022)</td>
<td>0.066 (0.022)</td>
<td>0.070 (0.032)</td>
<td>.</td>
<td>0.063 (0.032)</td>
<td>0.025 (0.007)</td>
</tr>
<tr>
<td>24</td>
<td>.</td>
<td>0.066 (0.021)</td>
<td>.</td>
<td>.</td>
<td>0.081 (0.044)</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>30</td>
<td>0.075 (0.022)</td>
<td>.</td>
<td>0.060 (0.029)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>0.030 (0.009)</td>
</tr>
<tr>
<td>48</td>
<td>0.085 (0.027)</td>
<td>0.047 (0.017)</td>
<td>.</td>
<td>0.057 (0.012)</td>
<td>0.050 (0.022)</td>
<td>0.041 (0.018)</td>
<td>0.049 (0.033)</td>
</tr>
<tr>
<td>72</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>0.056 (0.028)</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>116</td>
<td>0.060 (0.024)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
Table 4. Predictions resulting from the carcass fate model (Table 1), including % of total organic matter removed by each mechanism and model run time (time for all carcass organic matter except chitin to be removed). For the maximum sinking loss scenario, separate predictions are shown for each developmental stage group and season (ANX = anoxic). Predictions for the no sinking loss scenario apply to all developmental stages.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Season scenario</th>
<th>Microbial decomposition</th>
<th>Necrophagy</th>
<th>Sinking</th>
<th>Model run time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIV-CV: winter</td>
<td></td>
<td>7%</td>
<td>3%</td>
<td>90%</td>
<td>4</td>
</tr>
<tr>
<td>Sinking</td>
<td>spring</td>
<td>20%</td>
<td>10%</td>
<td>70%</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>30%</td>
<td>2%</td>
<td>68%</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>summer ANX</td>
<td>28%</td>
<td>2%</td>
<td>70%</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>fall</td>
<td>18%</td>
<td>4%</td>
<td>78%</td>
<td>4</td>
</tr>
<tr>
<td>CI-CIII: winter</td>
<td></td>
<td>9%</td>
<td>5%</td>
<td>86%</td>
<td>7</td>
</tr>
<tr>
<td>Sinking</td>
<td>spring</td>
<td>26%</td>
<td>16%</td>
<td>58%</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>39%</td>
<td>3%</td>
<td>58%</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>summer ANX</td>
<td>36%</td>
<td>3%</td>
<td>61%</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>fall</td>
<td>24%</td>
<td>6%</td>
<td>70%</td>
<td>7</td>
</tr>
<tr>
<td>Nauplii: winter</td>
<td></td>
<td>12%</td>
<td>14%</td>
<td>74%</td>
<td>20</td>
</tr>
<tr>
<td>Sinking</td>
<td>spring</td>
<td>34%</td>
<td>31%</td>
<td>35%</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>57%</td>
<td>6%</td>
<td>37%</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>summer ANX</td>
<td>52%</td>
<td>6%</td>
<td>42%</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>fall</td>
<td>34%</td>
<td>14%</td>
<td>52%</td>
<td>16</td>
</tr>
<tr>
<td>All stages: winter</td>
<td></td>
<td>20%</td>
<td>80%</td>
<td>NA</td>
<td>111</td>
</tr>
<tr>
<td>No sinking</td>
<td>spring</td>
<td>44%</td>
<td>56%</td>
<td>NA</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>summer</td>
<td>88%</td>
<td>12%</td>
<td>NA</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>summer ANX</td>
<td>86%</td>
<td>14%</td>
<td>NA</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>fall</td>
<td>51%</td>
<td>49%</td>
<td>NA</td>
<td>58</td>
</tr>
</tbody>
</table>
Figure 1. Experimental setup for laboratory observation of vertical copepod carcass distribution throughout decomposition and under sustained turbulence.
motorized track vertically traversing water column 3 times every h

impellor turning at 7 rpm and reversing direction every 1 min

video-scope

$\times 1000$

15.5 cm

8 cm

2 cm

24 cm

30 cm

24 cm
Figure 2. Mean density (symbols; derived from measured settling velocity) and predicted density (lines; derived from Eq. 5) for CIV-CVI copepod carcasses decomposing under different (A) temperatures and (B) dissolved oxygen conditions. Error bars are 95% confidence intervals. X-axes log-scale.
Carcass density (kg m$^{-3}$) vs. time since death (h)

A) Temperature effects:
- $25^\circ$C
- $15^\circ$C
- $5^\circ$C

B) Oxygenation conditions:
- Normoxic
- Hypoxic
- Anoxic
Figure 3. Relationship between measured copepod carcass settling velocities and those predicted for the same carcasses using Eqs. 1, 5, and the adjustment factor (0.81).
Observed settling velocity (cm s⁻¹)

Predicted settling velocity (cm s⁻¹)

\[ y = 1.00x + 0.006 \]
Figure 4. Observed profile of the vertical distribution of CIV-CVI copepod carcasses from the laboratory turbulence experiment. Values are means (averaged across all 56 profiles) of the fraction of the observed carcasses that occurred at each depth interval in the experimental container. Error bars are 95% confidence intervals. Also shown are estimates made using the Rouse and linear regression models fit to these data.
Figure 5. Vertical copepod carcass distribution in the York River estuary for (A) CIV-VI, (B) CI-CIII, and (C) nauplii. Black circles are observed carcass abundances (from pump samples); lines are best fits of Eq. 2 (Rouse model). No regression solution was attempted for nauplii. Pr is the estimated 95% confidence interval of the Rouse parameter derived from the regressions, and $z_a$ is the reference depth used in Eq. 2. Note different scales for x-axes.
CHAPTER 5

From beyond the grave: Incorporating copepod carcasses into mortality rate calculations, and the implications for population dynamics
ABSTRACT

Using two years of field data on the abundance and live/dead composition of copepod nauplii and Acartia tonsa copepodites in the lower Chesapeake Bay, we evaluated the potential for the presence of copepod carcasses to distort or create bias in our ecological understanding of the system. Copepod population mortality rates were estimated from the field data both with and without live/dead information. Instantaneous mortality rates varied from near $0.0 \text{ d}^{-1}$ in winter to summer maximums of around $0.6 \text{ d}^{-1}$ for nauplii and $0.8 \text{ d}^{-1}$ for A. tonsa copepodites. Resulting mortality rates differed, often substantially, depending on whether live/dead information was taken into account. A simple model of A. tonsa population dynamics was used to evaluate the effect of both mortality estimates on predicted copepod abundances. Model predictions more closely matched observations when mortality rates that accounted for carcasses were used, indicating the importance of live/dead composition for field studies in zooplankton ecology. We used the same field dataset to estimate the predatory and non-predatory components of mortality rates. Non-predatory mortality comprised between 8 and 42% of the total mortality. Predatory and non-predatory components of mortality were used separately in the population model, to evaluate the implications of each component for A. tonsa abundances. Predatory mortality alone was insufficient to keep A. tonsa population growth in check during the summer growing season (June-October), demonstrating the importance of non-predatory mortality copepod population dynamics in the lower Chesapeake Bay.
INTRODUCTION

Mortality is a poorly constrained parameter in zooplankton population dynamics. Uncertainty in mortality rates stems from the inherent difficulties in measuring \textit{in situ} mortality (Ohman and Wood 1995). However, variation of zooplankton mortality rate can drastically affect the behavior of both zooplankton population dynamics and nutrient-phytoplankton-zooplankton models (Steele and Henderson 1992; Edwards and Yool 2000; Plourde et al. 2008; Carlotti and Poggiale 2010), and \textit{a posteriori} adjustments of mortality rates are often required to match model predictions to observed data (Runge et al. 2004). Thus, suitable formulation of the mortality parameter in plankton models is a major difficulty facing plankton ecologists. To quote Runge et al. (2004):

\begin{quote}
\textit{Accurate depiction of mortality schedules is one of the greatest challenges in the modeling of marine population dynamics. Good demographic studies of mortality in the sea are rare and in many models, mortality rates are crudely imposed as values from limited observations.}
\end{quote}

Copepods are the most abundant multicellular zooplankton in the ocean. There are several methods to estimate copepod mortality rates from field census data, including horizontal and vertical life table approaches and inverse methods. The horizontal life table approach estimates mortality by following the development of a cohort of copepods, with the primary assumption that physical processes do not disrupt the cohort (Aksnes et al. 1997). The vertical life table approach analyzes the distribution of copepod developmental stages in discrete samples, assuming that recruitment is relatively constant for the duration of the stages (Aksnes et al. 1997).
Inverse methods apply known values of parameters other than mortality to population models; mortality is then estimated by fitting model predictions to observed data, by varying mortality manually (tuning) or estimating it by regression (Aksnes et al. 1997). These methods, however, require accurate measurements of all model parameters, which is often not possible. Consequently, the mortality term will contain error from all other parameters in the model, and may be quite different from the true value of mortality in situ.

Field estimates have demonstrated that copepod mortality rates vary spatially and temporally, interspecifically, and among developmental stages (~0.0-1.0 d⁻¹; Eiane and Ohman 2004; Thor et al. 2008; Plourde et al. 2009). The causes of these variations are less well known (Twombly et al. 2007; Plourde et al. 2008), although predatory mortality is related to predator abundance (Ohman et al. 2008), adult copepod density if cannibalism occurs on young stages (Landry 1978; Ohman and Hirche 2001), differences in prey vulnerability due to behavior, morphology, or distribution (Ohman and Wood 1995), or the affect of water clarity on visual predator success (Giske et al. 1994; Aksnes et al. 2004).

Studies of copepod population dynamics have often focused on predatory mortality, to the exclusion of non-predatory factors. A few population dynamics studies have considered non-predatory mortality due diet and starvation (Carlotti et al. 2000; Mazzocchi et al. 2006). In addition, non-predatory mortality could result from disease and parasitism, environmental stressors, or ageing (Carpenter et al. 1974; Kimmerer and McKinnon 1990; Rodríguez-Graña et al. 2010). There is no a priori reason to consider natural non-predatory mortality of copepods as negligible. Indeed,
a meta-analysis of longevity data from field and laboratory studies suggested that non-predatory factors account for 25-33% of total mortality among adult copepods in the sea (Hirst and Kiørboe 2002).

Non-predatory mortality leaves behind an intact carcass that may remain in the water column indefinitely (e.g. Wheeler 1967; Weikert 1977; Terazaki and Wada 1988; Tang et al. 2006), and failure to identify carcasses in samples could skew estimates of live copepod abundance and associated ecological parameters. For example, consider the copepod species *Acartia tonsa*, which may have nine generations in a year (Mauchline 1998), and is a dominant copepod species in the Chesapeake Bay, USA. Studies over multiple years found that approximately 10-30% of *A. tonsa* were carcasses in the lower Chesapeake Bay (Tang et al. 2006; Elliott and Tang submitted). Assuming, conservatively, that at any time 15% of *A. tonsa* were actually carcasses for each of nine generations (i.e., only 85% are alive and producing), the annual production predicted from abundances would be overestimated by a factor of \((1/0.85)^9 = 4.32\) if carcasses were counted as live copepods.

Identifying carcasses in plankton samples can also permit direct quantification of non-predatory mortality *in situ*. Copepod carcasses with injuries could be due to partial predation (Genin et al. 1995; Haury et al. 1995), but intact carcasses likely originate from non-predatory mortality (Weikert 1977; Tang et al. 2006). In a recent study, Elliott and Tang (submitted) described the abundances of live copepods and carcasses in the lower Chesapeake Bay over two years. Carcasses were identified using neutral red vital staining, which has been rigorously tested for live/dead determinations of estuarine copepods *in situ* (Elliott and Tang 2009). Elliott and Tang
(submitted) found that copepod carcasses were prevalent throughout the study area, and visibly injured carcasses were rare; therefore, the majority of the carcasses appeared to have resulted from non-predatory mortality.

In the present study we used the field data from Elliott and Tang (submitted) to estimate mortality rates of copepod nauplii and A. tonsa copepodites both with and without including live/dead (vital status) information of the collected animals. Our purpose was to examine the effect of considering vital status on mortality rate estimates. We also estimated the contribution of predatory and non-predatory factors to mortality based on abundances of intact carcass. Finally, we used a simple A. tonsa population dynamics model to evaluate both the importance of considering vital status and the implications of predatory and non-predatory factors for population dynamics.
METHODS

Data for mortality estimates

Data on the abundance and vital status of *A. tonsa* and copepod nauplii were taken from Elliott and Tang (*submitted*), who explain the data collection methods in detail. Samples were collected at 12 stations in the James, Elizabeth, Rappahannock and York Rivers. The first three tributaries were sampled eight times during 2009 (twice per season), and York River stations were sampled *ca.* monthly from October 2007 through November 2009. Depth profiles of water temperature, salinity, dissolved oxygen, and chlorophyll-*a* were measured with a YSI 6600 sonde. Copepod nauplii were collected by towing a 63 μm mesh conical plankton net twice at each station, once horizontally near the surface for 1 min, and once vertically from ~1.5 m above bottom to the surface. Copepodites were collected by a vertical tow at each station with a 200 μm mesh net. Plankton samples were stained with neutral red and frozen for later abundance and vital status determinations (Elliott and Tang 2009). Counts were made for live and dead copepod nauplii (stages NI-NIII and NIV-NVI) and *A. tonsa* copepodites (stages CI-CV and CVI). In mortality calculations and formulation of a population dynamics model (*see below*), the 12 post-hatch developmental stages of the copepods were each assigned an Arabic numeral (*see symbol* *i* in Table 1). The age group containing NI-NIII nauplii is denoted *i*=1-3, and NIV-NVI is *i*=4-6, the *A. tonsa* age group containing CI-CV copepodites is *i*=7-11, and CVI is *i*=12.
Estimating total mortality with and without live/dead information

Using the vertical life table (VLT) approach, we estimated total mortality from abundance data both with and without including vital status information. VLT estimates of mortality rely on the ratio of abundances in two consecutive copepod age groups. Recruitment to the younger group is assumed constant, and the expected abundance of the older group is calculated from observed abundance of the younger group and the stage durations of each group. The difference between expected and observed abundances in the older group is then attributed to mortality (Aksnes and Ohman 1996). Because dead individuals in the younger group can never develop into the older group. Therefore, failure to account for carcasses in the younger group would inflate the mortality estimates. Conversely, failure to distinguish carcasses from live individuals in the older group would underestimate mortality. Hence, inclusion of live/dead information for both age groups will improve VLT mortality estimates.

Using the VLT approach, we estimated total mortality rates for copepod nauplii (i=1-6) and A. tonsa copepodites (i=7-12) based on two different abundance estimates. In the first attempt, abundances consisted of all intact animals, including both live copepods (stained) and intact carcasses (unstained). This represents the conventional practice of ignoring vital status of the copepods (i.e. counting intact carcasses as live individuals). In the second attempt, abundances included only live (stained) copepods. Differences between the two approaches will indicate the bias in mortality estimates when the vital status of collected copepods is ignored, and we refer to estimates from the second attempt as ‘corrected total mortality’.
Naupliar mortality rates in each sample were calculated according to Aksnes et al. (1997) as:

\[
\frac{A_{1:3}}{A_{4:6}} = \frac{e^{m_n \times s_{1:3}} - 1}{1 - e^{-m_n \times s_{4:6}}}
\]

(1)

where \(A_{1:3}\) is NI-NIII abundance, \(A_{4:6}\) is NIV-NVI abundance, \(m_n\) is the shared mortality rate for all naupliar stages, and \(s_{1:3}\) and \(s_{4:6}\) are the cumulative stage durations of NI-NIII and NIV-NVI, respectively; calculated as the number of stages (three in each group) times the temperature-dependent individual stage duration (Table 1; equation 2). Although nauplii were not identified to the species level, \(A.\) tonsa was the numerically dominant adult copepod in >95% of the samples and was at least 10 times more abundant than the next most abundant species (\(Eurytemora affinis\)) in 87% of the samples (Elliott and Tang submitted). Therefore, we estimated the stage duration of nauplii from a published Bělehrádek function describing \(A.\) tonsa development time (Leandro et al. 2006). Isochronal development of \(A.\) tonsa was assumed (Miller et al. 1977), appropriate because we grouped multiple developmental stages and our calculations were therefore less sensitive to possible variation in the duration of different stages (Landry 1983). Equation 1 was then solved iteratively for mortality.

The equation to calculate copepodite mortality in each sample was:

\[
m_c = \frac{\ln \left( \frac{A_{7:11}}{A_{12}} + 1 \right)}{s_{7:11}}
\]

(3)

where \(m_c\) is the shared mortality rate for \(A.\) tonsa copepodites (CI-CVI), \(A_{7:11}\) is CI-CV abundance, \(A_{12}\) is CVI abundance, and \(s_{7:11}\) is the cumulative stage duration of
CI-CV; calculated as the number of stages (five) times the temperature-dependent individual stage duration (Table 1; equation 2). VLT mortality estimates are only reliable with sufficient replication (Aksnes and Ohman 1996). Thus, mortality rates presented here are means of all samples in a tributary on a given date (4-8 replicates).

**Estimating non-predatory mortality from VLT**

We also estimated the predatory and non-predatory components of mortality using the VLT approach with abundances of live copepods and carcasses. Mortality rates were calculated using Equations 1 and 3, with abundances of the younger groups (A1:3 and A7:11) consisting only of live copepods, and abundances of the older groups (A4:6 and A12) consisting of all intact copepods (live plus dead). This estimation represents mortality due to removal of copepods from the water column (e.g. consumption); thus, representing predatory mortality. Next we repeated the calculation using only live abundances for both age groups to obtain corrected total mortality (see above). The difference between corrected total mortality and predatory mortality gave non-predatory mortality.

**Estimating non-predatory mortality from carcass turnover times**

Non-predatory mortality was also independently estimated by a second approach, using the turnover time of carcasses in the water column (CTT approach). By assuming that the relative abundance of carcasses to live copepods was constant for the turnover time of carcasses in the water column (a period of several days; Elliott et al. 2010), we estimated non-predatory mortality rate as:

\[
d_i = \frac{C_i}{A_i \times t}
\]  

(4)
where $d_i$ is non-predatory mortality rate of age group $i$, $C_i$ and $A_i$ are the abundances of carcasses and live animals, respectively, in age group $i$, and $t$ is carcass turnover time.

To use this approach, carcass turnover time ($t$) was estimated by considering the potential for carcass removal by sinking, necrophagy, and microbial decomposition. Elliott et al. (2010) found that turbulence under normal tidally driven flows was sufficient to retain carcasses in the York River water column for an extended time such that sinking losses could be neglected. Elliott et al. (2010) also found that the dominant Chesapeake Bay planktivore *Mnemiopsis leidyi* did not select for live copepods or carcasses in feeding experiments. Such non-selective feeding would not alter the ratio of carcasses to live copepods, hence would not affect the mortality estimates. Thus, $t$ was estimated as the time required for microbial decomposition of a fresh copepod carcass down to an empty chitin carapace (8.35% of initial dry weight; Cauchie et al. 1997), using an equation relating the temporal change in *A. tonsa* carcass dry weight to water temperature and dissolved oxygen concentration (Elliott et al. 2010, their equation 4), and solving for $t$:

$$
t = e^{\left(\frac{3.83}{4.166 \left(1 - e^{-0.0087T} \right) + 0.046DO} - 1.39\right)}
$$

(5)

where $t$ is the hours from death to an empty chitin carapace, $T$ is the ambient water temperature, and $DO$ is an indicator variable that is 0 unless dissolved oxygen concentration is $<2$ mg L$^{-1}$, when it is 1.
**Predictive mortality functions**

The relationships between mortality rates and environmental variables were explored by multiple linear least squares regressions with stepwise selection of independent variables. Independent variables included depth-averaged water temperature, salinity, and chlorophyll-α, depth minimum dissolved oxygen, and stratification as the difference in potential density between surface and bottom waters. Only water temperature was significant in all of these analyses, always accounting for >98% of the explained variability ($R^2$) in mortality rates. Therefore, simple linear regressions of mortality rates versus water temperature were used to parameterize mortality in a population dynamics model (see next section). We also tested for density dependent mortality by using linear least squares regressions of corrected total mortality rates versus adult *A. tonsa* abundance.

**Population dynamics model**

A model describing *A. tonsa* population dynamics was used to evaluate the implications of: (1) the two mortality rate estimates that included or neglected live/dead information, and (2) the predatory and non-predatory components of mortality. The model (Table 1) consisted of 13 coupled differential equations describing temporal change in *A. tonsa* abundances for eggs ($A_6$) through adults ($A_{12}$). Model output was cumulative copepodite abundance ($A_{7:12}$), and was compared with archived data to evaluate model performance. Monthly mean abundances of *Acartia* spp. copepodites were calculated using archived Chesapeake Bay Program (CBP) mesozooplankton monitoring project data (http://www.chesapeakebay.net/data_plankton.aspx) from January 2000 to November 2002.
(York River stations RET4.3 and WE4.2). Data before 2000 were omitted due to underestimation of mesozooplankton abundances prior to this time (Chesapeake Bay Program 2000), and the project was terminated after 2002.

The initial abundance values in the model (day 0, Table 1) were approximated from CBP abundances for January of 2000-2002. However, it should be noted that model behavior was insensitive to these initial abundances. Changing initial abundances influenced the overall magnitude of predicted abundances, but not the annual pattern or timing of increases or decreases in abundance. Modeled rates of change in abundance of each developmental stage ($dA/dt$; Table 1, equations 6-8) were based on development rates ($D$) and mortality rates ($m$). Stage specific development rates for eggs through CV ($D_i$; Table 1, equation 9) were the reciprocal of individual stage durations ($s_i$; Table 1, equations 10 and 2). These stage durations came from reported Bélehrádek functions describing $A. tonsa$ egg and post-hatch development times relative to temperature, and assuming isochronal development of post-hatch stages. The equivalent of development rate for adults ($D_{12}$) was not used to predict their abundance (Table 1, equation 8), since adults remain as CVI until removed by mortality. Instead, $D_{12}$ was the adult female egg production rate, reported as a unimodal function of temperature (Table 1, equation 11), and used to predict gains to the egg stage (Table 1, equation 6). For simplicity, half of adults were specified as female to estimate population egg production (Table 1, equation 6).

The model was run for 365 days, with temperature determined each day from a cosine function (Table 1, equation 12). This function was fit by non-linear regression to York River CBP temperature data for 2000-2002, estimating parameters
iteratively ($A=16.133$, $B=-11.132$, and $C=-28.076$). Naupliar and copepodite mortalities ($m_{1:12}$) were parameterized in the model using the regression equations describing mortality rates versus temperature. At very low temperatures these regressions predicted negative mortalities, in which case model mortality rate was set to zero. Because data on egg abundances were not available, model egg mortality ($m_e$) was predicted using a published regression of egg mortality versus temperature for broadcast spawning copepods (Table 1; equation 13).
RESULTS

Total mortality

Total mortality rates varied in a cyclic manner annually, peaking in the summer (Figure 1). Corrected total mortality for nauplii varied from near 0 d\(^{-1}\) in the fall and winter to a maximum of 0.27 d\(^{-1}\) in June of 2008 and 0.55 d\(^{-1}\) in August of 2009 (Figure 1a). The occurrence of several negative mortality rates is indicative of violation of VLT assumption(s) on those dates, such as the assumption of representative sampling of each developmental stage. Corrected total mortality for *A. tonsa* copepodites was near 0 d\(^{-1}\) in the winter, and around 0.6 d\(^{-1}\) in summer 2008 and 0.8 d\(^{-1}\) in summer 2009, with a maximum of 1.29 d\(^{-1}\) in July of 2009 (Figure 1b).

Bias to mortality rate estimates due to the presence of carcasses was obvious for nauplii, where estimates ignoring vital status were consistently higher than corrected total mortality (Figure 1a). Mortality rate estimates for *A. tonsa* copepodites were only minimally influenced by the occurrence of carcasses (Figure 1b), due to the fact that a similar percent dead was found in both copepodite stage groups (Elliott and Tang submitted).

In the stepwise multiple regressions, water temperature predicted the vast majority of explained variation in mortality rates, being responsible for >98% of each model \(R^2\). Based on simple linear regression, mortality rates estimated with and without live/dead information both increased significantly with increasing water temperature (Figure 2). Density dependent mortality was not observed, since mortality rates were not significantly related to *A. tonsa* adult abundances (for nauplii \(R^2 < 0.0005, p=0.663\); for copepodites \(R^2 < 0.0005, p=0.401\)).
The equations describing mortality versus temperature (Figure 2) were used to parameterize mortality in the population model (Table 1). For comparative purposes, the model was run with a fixed mortality rate throughout time, calculated as the average of corrected total mortalities for 2007-2009 (Figure 1). Fixed mortality resulted in low abundances until late August, when the population quickly grew above realistic abundances before decreasing again in December (Figure 3). Predictions based on total mortality rates estimated without live/dead information showed population growth until March, when the population decreased and approached extinction by June (Figure 3). Even if we varied the temperature-mortality regression slope by up to 200% to account for the scatter (Figure 2), the predicted abundances still never reached 1000 ind. m⁻³ in summer-fall (data not shown).

Predictions based on corrected total mortality rates most closely matched the CBP observed abundances, and the population displayed a typical annual cycle rather than going extinct or growing to unrealistic abundances (Figure 3). It is worth-noting that a small difference between corrected and uncorrected mortality rates (Figure 1) led to substantially different population development, especially later in the year. This difference occurred likely because the higher uncorrected naupliar mortality rates resulted in lower adult abundances and this effect reverberated through multiple generations in our model run. Hence, model predictions that relied on only live copepod abundances differed substantially from, and were more realistic than predictions made without live/dead information.
Predatory and non-predatory components of mortality

Non-predatory mortality rates estimated from VLT were significantly correlated with those from CTT (Pearson correlation for nauplii $r=0.877$, $p<0.0005$; for copepodites $r=0.832$, $p<0.0005$), but the latter were frequently higher than the former by a factor of ~3 for nauplii and ~1.5 for copepodites (Figure 4a and b). Such a discrepancy could result from underestimation of carcass residence times (see Equation 4). In situ carcass decomposition rate could be lower than the laboratory measurements of Elliott et al. (2010) indicated, due to loss of carcass associated bacteria to advection and predation (Tang et al. 2009). This would lead to overestimation of non-predatory mortality rates by the CTT approach. Thus, we used the more conservative VLT estimates in subsequent analyses of the importance of non-predatory mortality.

VLT non-predatory mortality rates varied in a cyclic annual manner, peaking in the summer (Figure 5). Naupliar non-predatory mortality ranged from near 0 to 0.15 $d^{-1}$ throughout the study period, accounting for a median 20% of corrected total mortality. Copepodite non-predatory mortality ranged from 0 to 0.19 $d^{-1}$, accounting for a median 7.5% of corrected total mortality. Both predatory and non-predatory components of mortality were significantly and positively related to water temperature (Figure 6). Furthermore, the sum of predicted predatory and non-predatory mortality rates was equal to the predicted corrected total mortality rate.

To evaluate the importance of predatory and non-predatory mortality for $A. tonsa$ population dynamics, the regression equations in Figure 6 were used to parameterize mortality in the population model (Table 1). Considering only non-
predatory mortality, predicted abundance increased rapidly in the spring when egg
production and development rates increased with higher temperatures (Figure 7).
Considering only predatory mortality, model prediction agreed fairly well with
archived CBP observations in winter and early spring, after which the predicted
abundances increased unrealistically before they stabilized in late fall (Figure 7).
Only when both non-predatory and predatory mortality rates were considered together
did the model closely replicate the CBP observations (Figure 7). Thus, while non-
predatory mortality alone does not explain observed *A. tonsa* population behavior, it
was critical to the overall population dynamics, particularly in the summer months
when predatory mortality did not keep population growth in check.
DISCUSSION

Quantification of perceived live/dead composition of zooplankton in field samples has rarely been done because of the difficulty of identifying carcasses and the extra sample processing time needed. Nevertheless, one may ask: Is it necessary to distinguish between live and dead zooplankton in field samples? Previous studies have shown that copepod carcasses can be prevalent in marine environments including Chesapeake Bay, often accounting for 10% to 50% of collected copepods (see Elliott and Tang 2009 for review). In the present study, we directly demonstrated that failure to identify copepod carcasses can lead to errors when estimating population parameters such as mortality rates (Figure 1), which could then create bias in understanding of copepod population dynamics (Figure 3). It is therefore clear that quantifying the live/dead composition of field samples is an important consideration in zooplankton studies. Moreover, the use of the simple and inexpensive neutral red staining method should allow researchers to accomplish that goal with relative ease (Elliott and Tang 2009).

In our study, mortality rates of copepod nauplii and _A. tonsa_ copepodites were more closely related to temperature than to any other measured variables including adult population density. Life history theory suggests that mortality risk increases at higher temperatures, which is offset by the faster development to reproductive stage (Myers and Runge 1983; Kiørboe and Hirst 2008). Previous studies have found that temperature described variation in mortality rates better than predator abundance or density dependence alone (Hirst and Kiørboe 2002; Hirst et al. 2007; Plourde et al. 2009). Predator abundance and predation rate are likely to be temperature dependent.
(Myers and Runge 1983), and higher temperature could also increase disease and parasitic infection in natural populations (Harvell et al. 2002). Among poikilotherms, such as copepods, temperature can directly affect metabolic processes, with higher temperatures resulting in increased starvation risk (Tsuda 1994) and decreased maximum lifespan (Gophen 1976; Hirst and Kiørboe 2002).

In Atlantic estuaries including Chesapeake Bay, the growth season for *A. tonsa* occurs when water temperature approaches 15°C and above (Jeffries 1962; Elliott and Tang submitted). At these temperatures our regression models (Figure 2, Figure 6) predicted that non-predatory mortality represented 8-9% and 30-42% of corrected total mortality for *A. tonsa* copepodites and copepod nauplii, respectively. These results were consistent with those of Hirst and Kiørboe (2002), who concluded that non-predatory mortality accounted for ca. 1/4-1/3 of copepod mortality globally.

Our population dynamics model indicated that in June-July the increase in *A. tonsa* population growth exceeded the increase in predation mortality, and predatory mortality alone could not keep abundances in check (Figure 7). During spring and summer, the three dominant planktivores in Chesapeake Bay are ctenophores (especially *Mnemiopsis leidyi*), the sea nettle *Chrysaora quinquecirrha*, and the bay anchovy *Anchoa mitchilli* (Baird and Ulanowicz 1989). Although sea nettles prey on copepods, they can also exert top-down control on ctenophore populations, and the net effect of high sea nettle abundances *ca.* June may be decreased predation pressure on *A. tonsa* (Purcell and Decker 2005; Condon and Steinberg 2008). On the other hand, the magnitude of planktivory by bay anchovy in Chesapeake Bay does not increase substantially until later in the summer, *ca.* August (Wang and Houde 1995).
Therefore, between June and July non-predatory mortality likely supplements predatory mortality in a manner that is critical for *A. tonsa* population dynamics.

As discussed above, several non-predatory mortality factors might increase with temperature, including disease and parasite infections, starvation risk, and ageing. In addition, anoxic conditions can cause *A. tonsa* mortality (Roman et al. 1993; Stalder and Marcus 1997). Low oxygen conditions begin in Chesapeake Bay around June and persist throughout the summer (Hagy et al. 2004), corresponding to the period when non-predatory mortality became most important (Figure 7). Although Elliott and Tang (*submitted*) rarely observed hypoxia in their field study, they did not focus on areas known to be seasonally hypoxic, and their sampling protocol may have overlooked localized patches of hypoxic deepwater. Mortality rates of a comparable magnitude to ours were reported for the summer *A. tonsa* populations in Mariager Fjord, Denmark, which also experiences severe oxygen depletion (Tiselius et al. 2008). Our mortality rates estimates can also be compared with those of Heinle (1966) for *A. tonsa* in the Patuxent River estuary, Chesapeake Bay. In May through October of 1963 and 1964, Heinle (1966) found instantaneous mortality rates of ca. 0.74-0.89 d\(^{-1}\) for *A. tonsa* nauplii and 0.11-0.95 d\(^{-1}\) for *A. tonsa* copepodids.

Interestingly, while our copepodite mortality rates agree quite closely with the Patuxent’s range for copepodids (Figure 1b), our naupliar mortality rates are substantially lower than those of Heinle, particularly after correcting for the occurrence of carcasses (Figure 1a).

This study demonstrated the importance of live/dead information for proper understanding of zooplankton population dynamics and related ecological processes.
We estimated predatory and non-predatory mortalities of copepods in Chesapeake Bay based directly on field observations of live/dead copepod composition, and these agreed well with the previous indirect estimates of Hirst and Kjørboe (2002). Although non-predatory mortality generally accounted for less than half of total mortality, it was crucial to obtaining realistic abundance predictions from the population dynamics model. Future studies should evaluate the importance of non-predatory mortality for other zooplankton populations and other locations, especially areas that experience hypoxia. Research is also needed to identify the main causes of non-predatory mortality. In combination with an improved understanding of what regulates predatory mortality, research on non-predatory mortality will contribute to a mechanistic and predictive representation of the mortality term that is so critical to understanding how copepod populations vary in nature.
REFERENCES


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Hirst, A. G., D. Bonnet, and R. P. Harris. 2007. Seasonal dynamics and mortality rates of *Calanus helgolandicus* over two years at a station in the English Channel. Marine Ecology Progress Series 340:189-205.


Table 1. Formulations, parameterizations, and sources of data used in the *Acartia tonsa* population dynamics model.

<table>
<thead>
<tr>
<th>Model organization:</th>
<th>Symbol</th>
<th>Value</th>
<th>Units</th>
<th>Equation</th>
<th>Source</th>
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<tbody>
<tr>
<td>Day in model</td>
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<td>d</td>
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<td></td>
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<tr>
<td>Temperature</td>
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<td>= A + B * cos(2π(day-C)/365)</td>
<td>°C</td>
<td>12</td>
<td></td>
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<tr>
<td>Developmental stage</td>
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<td>= egg (e); nauplii (1-6); copepodid (7-11); adult (12)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 0 abundances</td>
<td>A&lt;sub&gt;i&lt;/sub&gt;</td>
<td>= 500 nauplii and 200 copepodites per stage</td>
<td>ind m&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>CBP database</td>
<td></td>
</tr>
</tbody>
</table>

**Governing equations:**

| Rate of change (i = e) | dA<sub>i</sub>/dt | = 0.5 * A<sub>12</sub>(D<sub>12</sub>) - A<sub>e</sub>(D<sub>e</sub>) - A<sub>e</sub>(m<sub>e</sub>) | ind m<sup>-3</sup>d<sup>-1</sup> | 6 |
| Rate of change (i = 1-11) | dA<sub>i</sub>/dt | = A<sub>i</sub>-(D<sub>i</sub>) - A<sub>i</sub>(m<sub>i</sub>) | ind m<sup>-3</sup>d<sup>-1</sup> | 7 |
| Rate of change (i = 12) | dA<sub>i</sub>/dt | = A<sub>11</sub>(D<sub>11</sub>) - A<sub>12</sub>(m<sub>12</sub>) | ind m<sup>-3</sup>d<sup>-1</sup> | 8 |

**Specific formulations:**

| Stage duration (i = e) | s<sub>i</sub> | = 489(T - 1.8)<sup>2.05</sup> | d | 10 | McLaren et al. 1969 |
| Stage duration (i = 1-11) | s<sub>i</sub> | = 458(T + 0.96)<sup>2.05</sup> | d | 2 | Leandro et al. 2006 |
| Development rate (i = e-11) | D<sub>i</sub> | = 1/s<sub>i</sub> | d<sup>-1</sup> | 9 |
| Adult egg production rate | D<sub>12</sub> | = 50.9 * ((34-T)/9.22)<sup>3.95</sup> * e<sup>[3.95*(T-24.78)/9.22]</sup> | d<sup>-1</sup> | 11 | Holste and Peck 2006 |
| Mortality rate (i = e) | m<sub>i</sub> | e<sup>[0.0725T-1.112]</sup> | d<sup>-1</sup> | 13 | Hirst and Kørboe 2002 |
| Mortality rate (i = 1-12) | m<sub>i</sub> | specific to model run | d<sup>-1</sup> | this study |        |
Figure 1. Time series (month and year on x-axes) of total mortality rates for (a) copepod nauplii and (b) *Acartia tonsa* copepodites without live/dead information (gray circles and lines) and after correcting for carcasses (black circles and lines). Error bars are 95% confidence intervals in a tributary on a specific date.
Figure 2. Results of regression analysis of temperature versus total copepod mortality rates for (a) copepod nauplii without live/dead information, (b) copepod nauplii after correcting for carcasses, (c) *Acartia tonsa* copepodites without live/dead information, and (d) *Acartia tonsa* copepodites after correcting for carcasses. Statistics for each regression are shown on the graphs.
1.2 y = 0.0237x - 0.199
\[ R^2 = 0.469 \]
\[ p < 0.0005 \]

\[ y = 0.0155x - 0.145 \]
\[ R^2 = 0.275 \]
\[ p < 0.0005 \]

\[ y = 0.0331x - 0.189 \]
\[ R^2 = 0.711 \]
\[ p < 0.0005 \]

\[ y = 0.0343x - 0.203 \]
\[ R^2 = 0.696 \]
\[ p < 0.0005 \]
Figure 3. Predicted abundances of *Acartia tonsa* copepodites from the population dynamics model using different mortality formulations. Solid gray line = total mortality without live/dead information (regression equations from Figure 2a and c); solid black line = corrected total mortality (regression equations from Figure 2b and d); dashed line = temporally constant mortality; asterisks = monthly mean abundances of *Acartia* sp. copepodites from Chesapeake Bay Program.
Figure 4. Comparison of non-predatory mortality rates based on vertical life table and carcass turnover time for (a) copepod nauplii and (b) *Acartia tonsa* copepodites. 1:1 lines (dashed) are shown for reference.
Vertical life table non-pred. mortality (d⁻¹)

Turnover non-pred. mortality (d⁻¹)

(a)

(b)
Figure 5. Time series (month and year on x-axes) of vertical life table estimates of non-predatory mortality rates for (a) copepod nauplii and (b) *Acartia tonsa* copepodites. Error bars are 95% confidence intervals in a tributary on a specific date.
Figure 6. Results of regression analysis of temperature versus (a) predatory and (b) non-predatory mortality rates for nauplii, and (c) predatory and (d) non-predatory mortality rates for *Acartia tonsa* copepodites (all derived from vertical life table approach). Statistics for each regression are shown on the graphs.
(a) $y = 0.0114x - 0.121$
$R^2 = 0.159$
$p < 0.0005$

(b) $y = 0.00407x - 0.0247$
$R^2 = 0.531$
$p < 0.0005$

(c) $y = 0.0309x - 0.176$
$R^2 = 0.705$
$p < 0.0005$

(d) $y = 0.00343x - 0.0257$
$R^2 = 0.328$
$p < 0.0005$
Figure 7. Predicted abundances of *Acartia tonsa* copepodites from the population dynamics model using different components of mortality. Diamonds = corrected total mortality (as in Figure 3); solid black line = predatory mortality only (regression equations from Figure 6a and c); solid gray line = non-predatory mortality only (regression equations from Figure 6b and d); asterisks = monthly mean abundances of *Acartia* sp. copepodites from Chesapeake Bay Program.
CHAPTER 6

Conclusions
Zooplankton Carcasses in the Marine Environment

A number of studies have demonstrated that zooplankton carcasses can be common in marine environments (see review in Chapter 2). The importance of copepod carcasses in marine systems is threefold. First, they are concentrated particles of organic matter that can be important hotspots of microbial activity (Tang et al. 2009), potential food for consumers (Zajączkowski and Legeżyńska 2001), or a means of vertical transport of essential biomolecules, toxins and pollutants (Lee and Fisher 1994; Frangoulis et al. 2005; Bickel and Tang in press). In addition to their direct role in ocean trophodynamics and biogeochemistry, quantification of copepod carcasses is important to improve studies of marine plankton ecology. Substantial numbers of carcasses in samples could produce errors in ecological studies that assume all collected animals to be alive in situ. Finally, abundances of intact carcasses are valuable data for evaluating in situ non-predatory mortality.

Mortality is one of the most critical, yet poorly constrained vital rates describing copepod population dynamics (Ohman and Wood 1995), and the mechanisms controlling spatial and temporal variation in copepod mortality rates are largely unknown. A global meta-analysis suggested that non-predatory factors likely account for an important fraction of total mortality (Hirst and Kiorboe 2002); hence, separation of mortality into predatory and non-predatory components is an important step toward constraining mortality rates in marine zooplankton populations. In addition, studying mortality from direct in situ evidence of death (a carcass) represents a promising approach for identifying the mechanisms causing non-predatory mortality in nature.
In this dissertation, I have completed a detailed evaluation of the importance of copepod carcasses for studies in estuarine zooplankton ecology. My main findings were:

(1) Neutral red staining gave accurate results for many common Chesapeake Bay zooplankton species and for different copepod developmental stages. Collection induced mortality was negligible when the recommended collection and handling procedures were followed. Accordingly, neutral red staining represents a simple and reliable way to quantify estuarine copepod carcasses in situ. (Chapter 2)

(2) In tributaries of the lower Chesapeake Bay between fall 2007 and 2009, the fraction of collected copepods that were dead varied between copepod species, among developmental stages, and with a recurring annual pattern in each of the two years. Furthermore, carcasses rarely had visible injuries, suggesting negligible copepod mortality due to partial predation and damage during collection and handling (cf. Harding 1973; Ohman 1984; Haury 1995). Copepod carcasses were a persistent feature in the lower Chesapeake Bay tributaries and were likely the result of natural non-predatory mortality. (Chapter 3)

(3) The rate of removal of copepod carcass organic matter depended mainly on the effects of water temperature on the rate of microbial decomposition and on necrophage abundance. Removal of copepod carcasses by sinking was much less important in the York River estuary than the settling velocities of fresh carcasses would imply, due to turbulence and decreases in carcass body density during decomposition. (Chapter 4)
(4) Copepod mortality rates often differed substantially, particularly for nauplii, depending on whether estimates were made with or without considering the *in situ* live/dead composition of the copepods collected. Mortality rates derived without live/dead information resulted in spurious predictions from a simple population dynamics model, and predictions were substantially improved when live/dead information was taken into consideration. (Chapter 5)

(5) In the tributaries of the lower Chesapeake Bay, non-predatory mortality represented 8-9% of total population mortality for *A. tonsa* copepodites, and 30-42% of mortality for copepod nauplii. Although less than half of total mortality, this non-predatory component was essential to producing realistic model predictions of *A. tonsa* abundance throughout the annual cycle, since predatory mortality alone was insufficient to control potential population growth in the summer months. (Chapter 5)

**Conclusions and Future Research Directions**

Overall, my research has underscored both the necessity and the benefits associated with identifying zooplankton carcasses in field samples. The neutral red method provides a relatively simple way to quantify carcasses. In the tributaries of the lower Chesapeake Bay, copepod carcasses were a persistent feature in plankton samples from 2007-2009. This could represent a common estuarine phenomenon or it could indicate a degradation of local water quality. Future studies could resolve this question by quantifying copepod carcasses in other estuarine and coastal systems. Considering the potential fates of copepod carcasses, it is apparent that carcass turnover times of at least several days are feasible, since carcass sinking losses may be diminished by turbulent mixing in a typical estuary and by decomposition to
neutral density with seawater in the open ocean. These mechanisms of carcass retention provide a reasonable explanation for the high abundances of zooplankton carcasses reported in previous studies. My research provided, as far as I know, the first estimates of non-predatory mortality of marine copepods to be based on field abundances of carcass. My results agreed quite well with the previous indirect estimates (~25-33% of total mortality) of Hirst and Kiørboe (2002).

Temperature was a useful proxy for describing variations in $A. tonsa$ mortality rates in Chesapeake Bay. However, a more mechanistic understanding of mortality is needed. Obviously, the relationship between predatory mortality and temperature was not one of direct cause and effect, and predatory mortality will depend on predator abundance and predation rate rather than directly on temperature. Natural non-predatory mortality may result directly from higher temperatures, due to either physiological temperature stress (Carpenter 1974; Vecchione 1989) or decreased maximum life span at high temperatures (Gophen 1976; Hirst and Kiørboe 2002). Still, these factors are only two of the many potential causes of non-predatory mortality, and other important factors could deviate strongly from temperature dependence. For example, ‘outbreaks’ of disease or parasite infection could transcend temperature dependence, and periods of starvation mortality would depend on food availability as well as temperature. Such events could rapidly and drastically alter zooplankton populations, causing variation in zooplankton abundance at scales over which predatory mortality may be relatively constant; e.g. daily or interannual scales. Such non-predatory mortality events could go unnoticed in zooplankton populations if carcasses are sampled and counted as alive, and the resulting population
fluctuations could be mistakenly attributed to resource limitation, stochastic/chaotic behavior, or even sampling variability.

My research suggested that non-predatory mortality is an important mechanism that regulates *A. tonsa* populations. The results of the population dynamics model demonstrated the ability of *A. tonsa* populations to grow extremely rapidly in the absence of a balancing mortality term, going from low abundances to high abundances that are not observed *in situ*, all in a period of only several days to weeks (see Ch. 5 Figs. 3 and 7). The rate of growth of predator populations is generally lower than that of their prey (McCauley et al. 1993), and it is difficult to imagine that predator populations and therefore predatory mortality would always keep pace with the rapid growth of *A. tonsa* populations. On the other hand, factors such as disease and parasite infection, and food depletion and starvation could 'respond' more rapidly to increasing *A. tonsa* abundances. Indeed, *A. tonsa* is a *r*-strategist among copepod species (Hirsche 1992; Ch. 3), and *r*-strategist populations are often regulated by non-predatory factors such as disease and starvation (Southwood and Comins 1976).

The scarcity of existing research on natural non-predatory mortality of marine copepods precludes the development of mechanistic relationships between non-predatory mortality and environmental conditions. The importance of non-predatory factors in population dynamics has been recognized and modeled for many taxa, including freshwater crustacean zooplankton, arthropods, and various marine taxa (Table 1). Apart from starvation, such formulations are lacking in marine zooplankton population models (Carlotti et al. 2000). This lack of attention to non-predatory
zooplankton mortality may stem from the same bias that has caused a focus on
growth processes to the exclusion of mortality (Ohman and Wood 1995); specifically,
the motivating interest of predicting fishery yield from studies of plankton
production, and the appeal of the often overly simple trophic-dynamic view of aquatic
ecosystems in which zooplankton consume phytoplankton and are then consumed by
fish. From such a viewpoint, it is tempting to view zooplankton mortality exclusively
from the standpoint of direct consumption and transfer of energy up the food chain.
However, quoting Wetzel (1995) about aquatic ecosystems:

\[
\text{The common idea that the living net production in organisms, particularly} \\
\text{algae and microbes but also metazoans, dies largely by ingestive predation is} \\
\text{not only unsubstantiated but intuitively unreasonable.}
\]

\[
\text{Many if not most organisms, particularly bacteria and fungi but also algae} \\
\text{and higher organisms simply mature physiologically, senesce, and die, and} \\
\text{then enter the combined particulate and dissolved detrital pool for microbial} \\
\text{heterotrophic utilization.}
\]

\[
\text{Just because we cannot measure non-predatory mortality well does not mean} \\
\text{it does not exist or even dominate at most times of the year.}
\]

The results of my dissertation research provide strong support for these bold claims.
Future work on non-predatory mortality of marine zooplankton is essential, given the
central role that these mesozooplankton play in ocean trophodynamics and biogeochemistry.
REFERENCES


Table 1. Literature examples of population dynamics models incorporating at least one non-predatory mortality factor into population predictions.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Taxon</th>
<th>Mortality factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crustacean zooplankton</td>
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<tr>
<td></td>
<td><em>Daphnia</em> sp.</td>
<td>starvation</td>
<td>McCauley et al. (1996)</td>
</tr>
<tr>
<td></td>
<td><em>Daphnia</em> sp.</td>
<td>parasitism</td>
<td>Ebert et al. (2000)</td>
</tr>
<tr>
<td></td>
<td><em>Daphnia</em> sp.</td>
<td>starvation and ageing</td>
<td>Rinke and Vijverberg (2005)</td>
</tr>
<tr>
<td>Arthropods</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><em>Zeiraphera diniana</em> (larch bud moth) and other forest insects</td>
<td>pathogenic infections</td>
<td>Anderson and May (1980)</td>
</tr>
<tr>
<td></td>
<td><em>Aonidiella auranti</em> (red scale, insect)</td>
<td>parasitism</td>
<td>Murdoch (1994)</td>
</tr>
<tr>
<td></td>
<td><em>Brachycentrus americanus</em> (stream caddis-fly)</td>
<td>parasitism</td>
<td>Kohler and Holland (2001)</td>
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<td></td>
<td><em>Apis mellifera</em> (honey bee)</td>
<td>viral diseases</td>
<td>Martin (2001)</td>
</tr>
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<td></td>
<td><em>Corophium volutator</em> (benthic amphipod)</td>
<td>parasitism</td>
<td>Poulin and Mouritsen (2006)</td>
</tr>
<tr>
<td>Marine taxa</td>
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<tr>
<td></td>
<td>Fisheries species</td>
<td>parasitism</td>
<td>Dobson and May (1987)</td>
</tr>
<tr>
<td></td>
<td>Phytoplankton</td>
<td>viral disease</td>
<td>Beltrami and Carroll (1994)</td>
</tr>
<tr>
<td></td>
<td><em>Clupea harengus</em> (North Sea herring)</td>
<td>parasitism</td>
<td>Paterson (1996)</td>
</tr>
</tbody>
</table>
VITA

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