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Spatial and Temporal Distributions of Live and Dead Copepods in the Lower Chesapeake Bay (Virginia, USA)

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Abstract Hydrography and copepod abundances (*Acartia tonsa*, *Eurytemora affinis*, and nauplii) were regularly monitored for 2 years in sub-estuaries of the lower Chesapeake Bay. Copepod vital status was determined using neutral red. Abundances of *A. tonsa* copepodites and nauplii peaked in late summer and were related to water temperature. *E. affinis* was present in early fall and winter–spring. Copepod carcasses were a persistent feature in the plankton from 2007 to 2009, with similar annual patterns of occurrence during both years. The relative abundance of carcasses varied among species and developmental stages, with means of 30% dead for stages NI–NIII copepod nauplii, 12–15% for stages NIV–NVI nauplii and *A. tonsa* copepodites, and 4–8% for *E. affinis* copepodites. Percent dead was also higher for adult male than female *A. tonsa*. No strong relationships were found between measured hydrographic variables and percent dead, but the higher percent dead in young nauplii and adult male *A. tonsa* may indicate greater susceptibility of these stages to death from environmental stressors.

Keywords Zooplankton sampling · Mortality · Vital staining · Copepod carcasses

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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 and Tang 2010). 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, hypox-

ia, 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 the collected *A. 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 for 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.

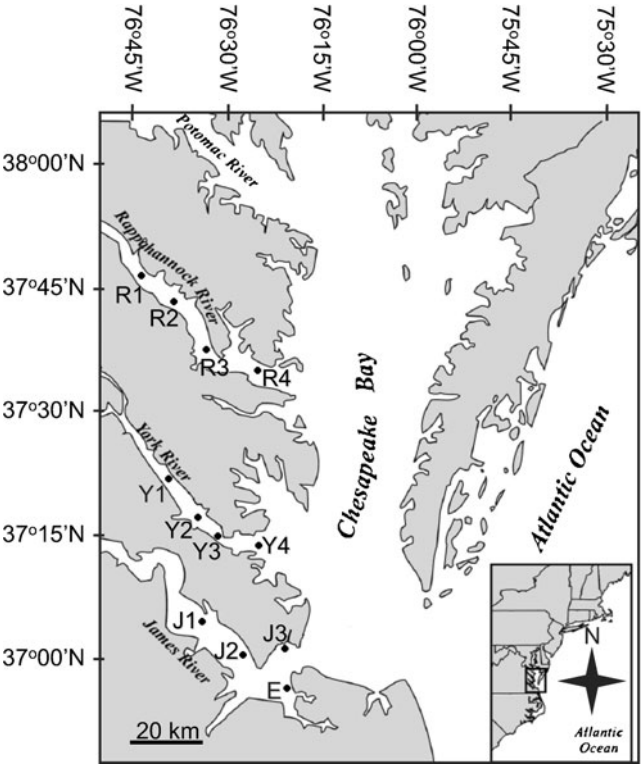

Fig. 1 Map of the lower Chesapeake Bay with sampling stations as circles (*J* = James, *E* = Elizabeth, *Y* = York, and *R* = Rappahannock River stations)

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), salinity, 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 ($\Delta\rho$) 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}. Previous study has shown 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 petri dishes, and stored at −40°C until enumeration (Elliott and Tang

2009). Samples were enumerated in the laboratory within 2 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* and *E. affinis* carcasses were quantified in samples taken during September 2009, a period when high percentages of dead copepodites occurred (see the "Results" section). 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 pacifica* preying on the copepod *Pseudocalanus* sp. (Ohman 1984).

Statistical Analyses

An average of 350 individuals was 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 the "Results" section), 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. *E. affinis* was rare or absent in many samples and the abundance data for this species 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 percent dead copepods among the three rivers during 2009 and among developmental stage groups for the entire study period. Two-way ANOVAs were used to test for differences in 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. A paired *t* test was also used to test for differences in the percent dead between sexes of adult *A. tonsa*. Principal components analysis was used to identify main gradients in hydrographic environmental parameters. The relationship between environmental parameters and copepod abundance and percent dead was explored by multiple linear regressions with stepwise selection of environmental variables.

Results

Hydrographic Environment

Dissolved oxygen data are not shown, but the water column water was usually well oxygenated and hypoxic conditions were observed only at Y1 on June 18, 2008 (1.79 mg L^{-1}) and at 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 (Fig. 2), and the annual temperature range within each tributary was smaller downstream (4.9–27.7°C at E, 5.3–27.6°C at Y4, and 2.7–26.8°C at R4) than upstream (4.5–28.6°C at J1, 5.1–28.2°C at Y1, and 2.4–28.2°C at R1). Salinity increased downstream within each tributary, rising on average 3.5 units from J1 to E, 2.3 units between Y1 and Y4, and 5.0 units between R1 and R4. Temporally, salinity 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 ($\Delta\rho$) observed during the 2-year study. Otherwise, the water column was most strongly stratified in late spring and summer (May–September). Chlorophyll-*a* concentration typically ranged from 3 to 20 μg L^{-1}. Mean chlorophyll was 8 μg L^{-1} in the York and James Rivers, and 12 μg L^{-1} in the Rappahannock River. Unusually high chlorophyll concentrations were observed at Y4 in May 2009 (depth average 31 μg L^{-1}) and at R1 in September 2009 (depth average 54 μg L^{-1}).

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). PC-1 separated spring and summer samples from fall and winter and was most closely associated with degree of stratification ($\Delta\rho$) and dissolved oxygen concentration. PC-2 separated samples both temporally and spatially, was most closely associated with chlorophyll-*a* concentration and salinity, and likely represented a gradient in the degree of freshwater influence. PC-3 separated summer samples from all others based on temperature and chlorophyll-*a* concentration, likely representing the annual cycle of water temperature. PC-4 separated samples based primarily on salinity.

Copepod Abundances

Abundance of copepods ranged from <1,000 individuals m^{-3} to more than 200,000 nauplii m^{-3} and 20,000 copepodites m^{-3}. Abundances of nauplii and *A. tonsa* copepodites (Fig. 3) were lowest in winter (December–March) and had a bimodal annual pattern with peaks in spring (March–June) and late summer–early fall (July–October). *E. affinis* was

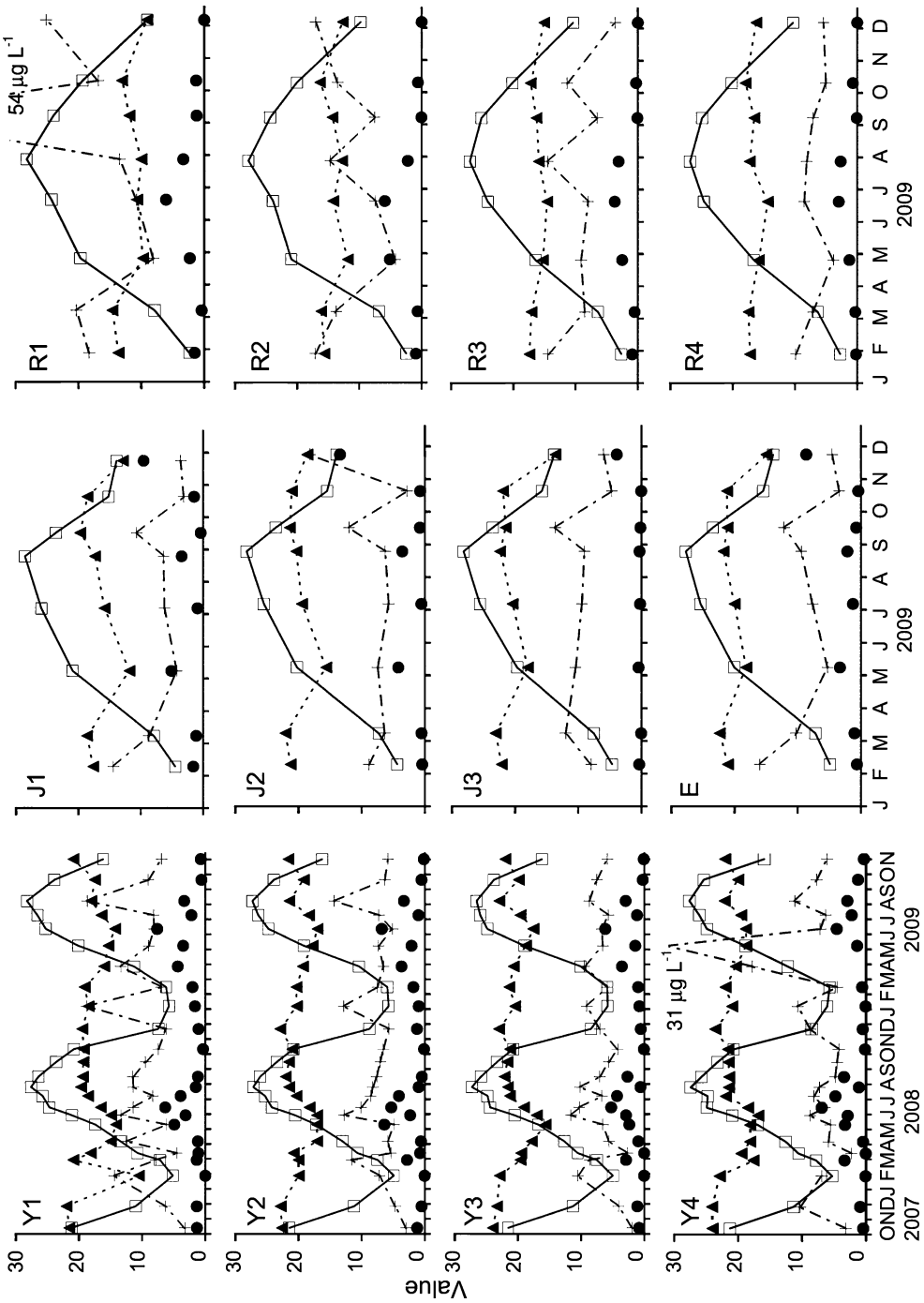

Fig. 2 Hydrographic conditions for each sampling date and station (station labels as in Fig. 1). Values shown are depth-averages for each vertical profile of temperature (°C; *box with solid line*), salinity (*filled triangle with dotted line*), and chlorophyll-*a* (µg L^{-1}; *plus sign with dashed–dotted line*). Values for the stratification index ($\Delta\rho$; *filled circle*) 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)

absent from many samples, but measurable abundances occurred in August and September and in winter (December–March). Even during these times, *E. affinis* abundances were <1,000 individuals m^{-3}, with the exception of the Elizabeth River station in September of 2009 when >14,000 individuals m^{-3} were observed. *A. tonsa* copepodites were significantly more abundant in vertical than in horizontal tows (Fig. 4a), indicating higher abundance deeper in the water column than near the surface. No such vertical differences were observed for copepod nauplii. Several environmental variables were significantly related to copepod abundances (Table 2): Abundances of all groups were positively related to water temperature, and abundances of copepod nauplii were positively related to salinity.

Copepod Vital Status

Visible injuries occurred on an average of 1.7% (standard deviation 2.3%) of *A. tonsa* and *E. affinis* copepodite

Table 1 Results of principal components analysis on collected environmental data

	PC-1	PC-2	PC-3	PC-4
Eigenvalue	1.96	1.30	0.78	0.53
% of environmental variability explained	39.3%	26.0%	15.7%	10.6%
Correlation with				
Temp	0.43	0.35	0.67	0.39
Sal	−0.41	0.55	0.23	−0.64
DO	−0.57	−0.28	−0.01	0.40
Chl	0.10	−0.71	0.54	−0.44
$\Delta\rho$	0.57	−0.04	−0.45	−0.28

Temp temperature, *Sal* salinity, *DO* dissolved oxygen, *Chl* chlorophyll-*a*, $\Delta\rho$ stratification index

carcasses collected 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 both CI–CV and CVI *A. tonsa* copepodites (Fig. 4b), and 4–8% for *E. affinis* copepodites when this species was present (CI–CV and CVI). 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, and a significantly higher percentage of males than females were dead within each plankton tow sample (paired *t* test, $p<0.0005$). In the York River, annual variation in percent dead was similar during both years (Fig. 5). Highest percent dead copepod nauplii occurred from mid-spring through summer during

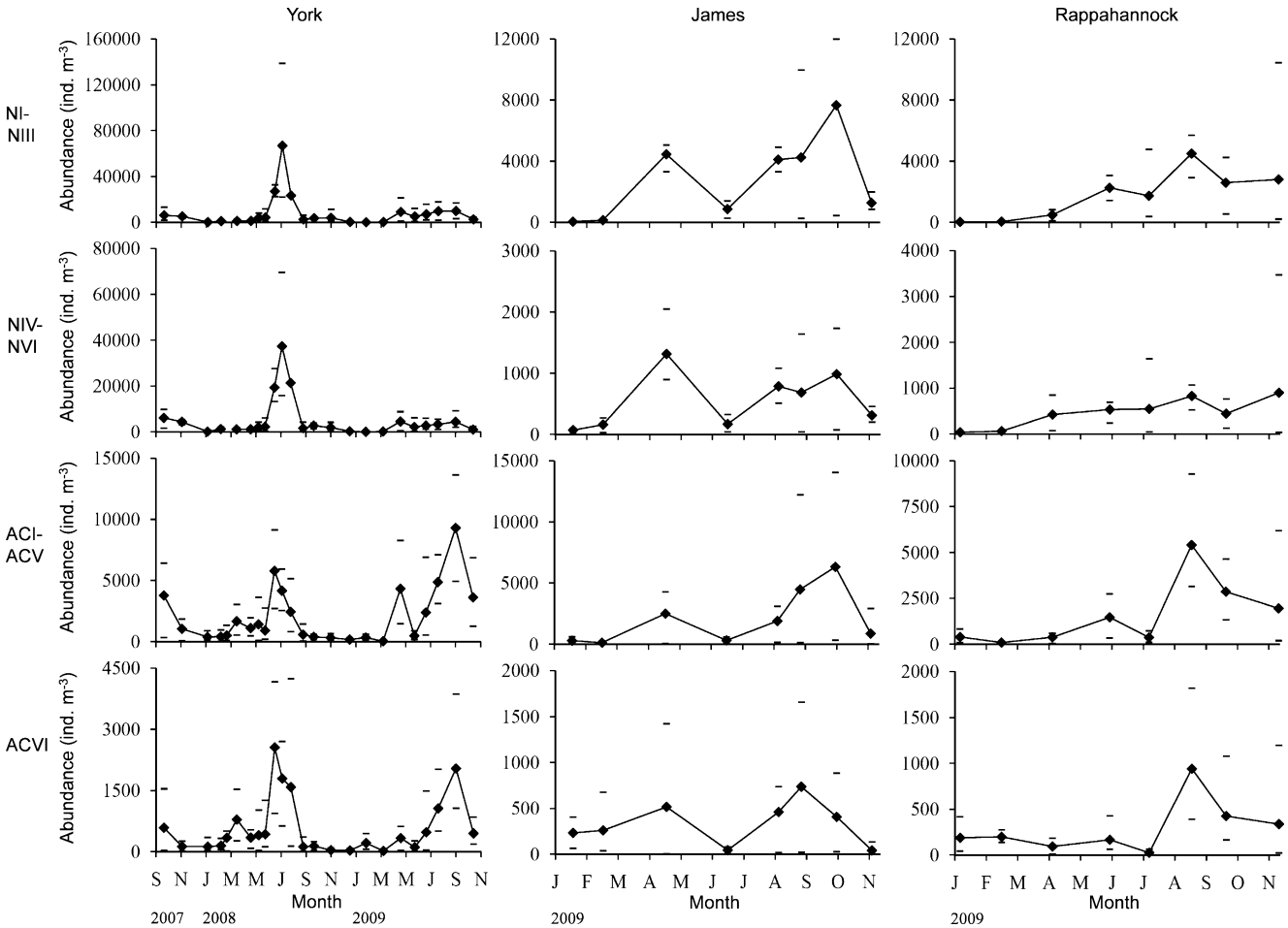

Fig. 3 Mean (*filled diamond with solid line*) and range (*dash*) of copepod abundance (individuals m^{-3}) within a river on each sampling date. *Columns of panels* represent different rivers and *rows* represent different copepod developmental stage groups (NI–NIII = naupliar stages one to three; NIV–NVI = naupliar stages four to six; ACI–ACV = *Acartia tonsa* copepodite stages one to five; ACVI = *A. tonsa* adults). Due to the scarcity of samples containing *Eurytemora affinis*, abundance data for this species are not shown, but are discussed in the text

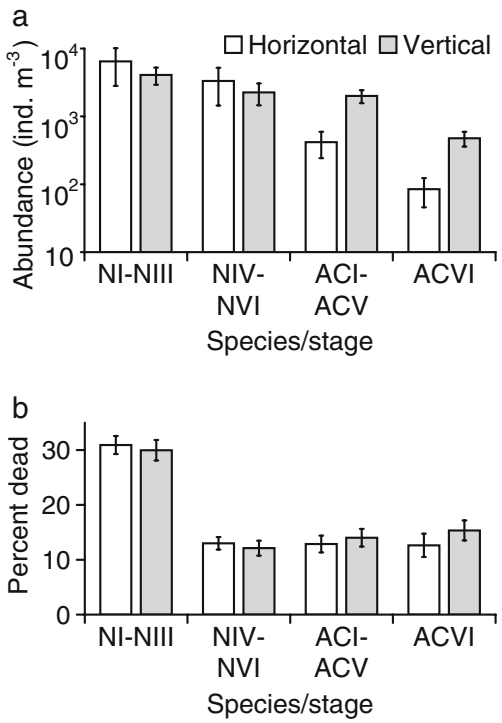

Fig. 4 Mean **a** abundance (individuals m^{-3}) and **b** percent dead of nauplii and *Acartia tonsa* copepodites in horizontal and vertical plankton tows (developmental stage group abbreviations as in Fig. 3). *Error bars* are ±95% confidence intervals. Note the log scale of *y*-axis in **a**. Abundances were significantly higher in vertical than in horizontal tows for *A. tonsa* CI–CV and CVI (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)

both years (April–August; Fig. 5), coincident with highest naupliar abundances (Fig. 3). Percent dead *A. tonsa* copepodites was highest during mid-summer through early fall in both years (July–November), concurrent with and immediately after the peak annual abundances. Percent dead in the other rivers was of a similar magnitude to that in the York River, although samples were taken with somewhat lower temporal resolution and only for 1 year.

Percent dead was significantly different among rivers for NIV–NVI nauplii and CI–CV *A. tonsa* (Table 3), 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. 4b), indicating similar abundances of carcasses relative to total copepods near the surface and throughout the water column. According to multiple linear regressions (Table 2), percent dead of NI–NIII nauplii and *A. tonsa* adults were positively related to temperature, percent dead of both naupliar groups were negatively related to salinity and positively to dissolved oxygen concentration, and percent dead of *A. tonsa* adults was negatively related to degree of stratification. However, these relationships were generally weak, and each multiple regression model explained <10% of the variability in percent dead.

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) was related to both stratification and dissolved oxygen concentration, reflecting the stratified conditions and deepwater oxygen

Table 2 Multiple linear regression results, including regression coefficents (*p* values), and coefficients of determination (R^2) for the relationships between environmental variables and copepod (A) abundance and (B) percent dead

		NI–NIII	NIV–NVI	ACI–ACV	ACVI
(A)	Temp	0.163 (<0.0005)	0.116 (<0.0005)	0.098 (<0.0005)	0.062 (<0.0005)
	Sal	0.11 (<0.0005)	0.145 (<0.0005)	NS	NS
	DO	NS	NS	NS	NS
	Chl	NS	NS	NS	NS
	$\Delta\rho$	NS	NS	NS	NS
	Model R^2	43.03%	28.01%	23.53%	8.13%
(B)	Temp	0.248 (<0.0005)	NS	NS	0.279 (<0.0005)
	Sal	−0.45 (<0.0005)	−0.29 (0.009)	NS	NS
	DO	0.3 (0.005)	0.23 (0.008)	NS	NS
	Chl	NS	NS	NS	NS
	$\Delta\rho$	NS	NS	NS	−0.71 (0.017)
	Model R^2	9.51%	3.55%	0.00%	6.36%

Environmental variable abbreviations are as in Table 1 and developmental stage group abbreviations as in Fig. 3

NS environmental variable not selected

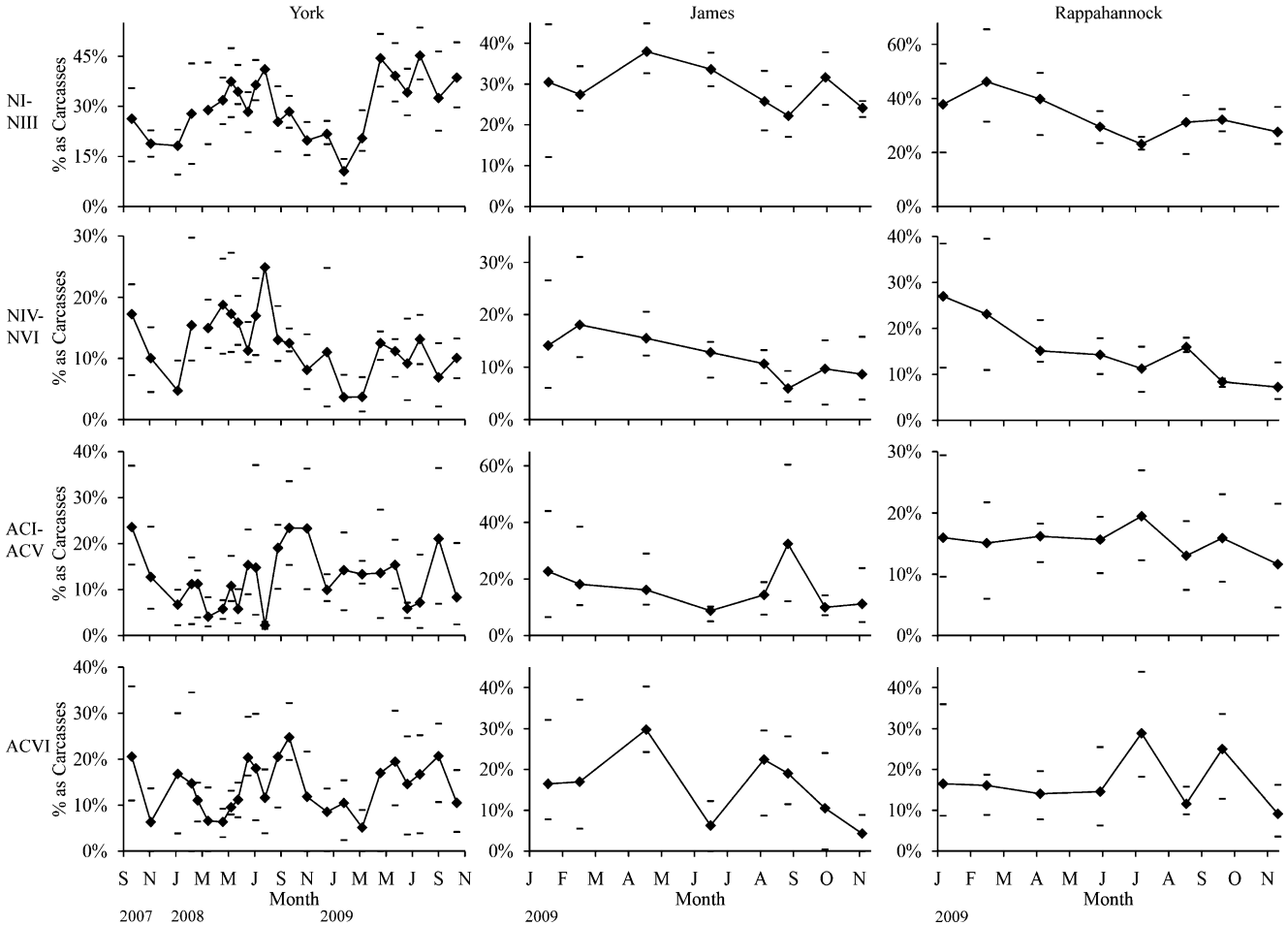

Fig. 5 Mean (*filled diamond with solid line*) and range (*dash*) of percent dead of copepods collected within a river on each sampling date. *Columns of panels* represent different rivers and *rows* represent different copepod developmental stage groups (developmental stage group abbreviations as in Fig. 3). Due to the scarcity of samples containing *Eurytemora affinis*, percent dead data for this species are not shown, but are discussed in the text

depletion that occur in Chesapeake Bay in spring and summer (Taft et al. 1980; Kemp et al. 2005). However, true hypoxic conditions were rare at our sampling stations, and severe oxygen depletion is more common further north and deeper (>20 m) in the main stem of the bay (Hagy et al. 2004).

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 (i.e., in vertical tows; Fig. 4a) 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 temperature was expected (Table 2). 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 positive association between naupliar abundance and salinity may reflect a larger population of the dominant copepod *A. tonsa* in higher salinity regions. The optimal salinity range for *A. tonsa* is around 15–22 (Cervetto et al. 1999), and decreased salinity can be accompanied by a decrease in egg production rate (Peck and Holste 2006, salinity < ca. 14), egg hatching success (Holste and Peck 2006, salinity < ca. 15), and naupliar survivorship (Chinnery and Williams 2004, salinity < full seawater). Our data showed that lower salinity was associated with lower naupliar abundances and also higher percent dead nauplii (Table 2), possibly reflecting higher naupliar mortality in the low salinity environment.

Table 3 Results of ANOVAs testing for differences in the percent dead copepods among rivers

	NI–NIII	NIV–NVI	ACI–ACV	ACVI
River	0.126 (2)	0.003 (2)	0.034 (2)	0.105 (2)

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 are as in Fig. 3

Copepod Vital Status

We expected that the amount of dead copepods should be highest during and shortly after peaks in abundance, when the abundance began to level off and decline as mortality balanced or exceeded population growth. This expectation was substantiated by our York River observations for *A. tonsa* copepodites and copepod nauplii in summer and early fall (Figs. 3 and 5). 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 resulting from in situ non-predatory mortality. On the other hand, visible injuries do not necessarily indicate partial predation as the cause of death if the wounds are inflicted post-mortem.

No measured hydrographic variables were significantly related to the occurrence of copepod carcasses across all developmental stage groups (Table 2). The positive relationship between temperature and percent dead NI–NIII nauplii and adult *A. tonsa* was reflective of the higher percent dead in the summer (Fig. 5). As noted above, the negative relationship between salinity and percent dead nauplii (Table 2) may reflect decreased survival of *A. tonsa* nauplii in an environment below their optimal salinity range. Higher percent dead nauplii and *A. tonsa* adults were also associated with higher dissolved oxygen or weaker stratification (Table 2), suggesting that the degree of vertical mixing may influence observed percent dead. However, the inclusion of these parameters in the multiple regression models increased the R^2 value by only 2% or less, and no combinations of environmental variables explained a large amount of variability in percent dead. Overall, the environmental conditions that we observed were unlikely to cause immediate death of *A. tonsa* copepodites. For example, no spikes in percent dead were observed even in the James River in November 2009 (Fig. 5), 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). *A. tonsa* copepodites have been shown to be quite tolerant to decreasing salinity, and transfer to a salinity of as low as 1 had no effect on their 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 significantly 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, or sinking would be substantially different between the two sexes (Elliott et al. 2010). Therefore, our field data implied that non-predatory mortality could be >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).

Table 4 Results of ANOVAs testing for differences in the percent dead copepods among stations and dates within each river

River	NI–NIII		NIV–NVI		ACI–ACV		ACVI	
	Station	Date	Station	Date	Station	Date	Station	Date
York	0.014 (3)	<0.001 (22)	0.080 (3)	<0.001 (22)	0.729 (3)	<0.001 (23)	0.886 (3)	0.019 (23)
Rappahannock	0.113 (3)	0.002 (7)	0.136 (3)	<0.001 (7)	0.091 (3)	0.548 (7)	0.210 (3)	0.005 (7)
James	0.398 (3)	<0.001 (7)	0.017 (3)	<0.001 (7)	0.328 (3)	0.045 (7)	0.570 (3)	0.034 (7)

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 are as in Fig. 3

We found a mean of 14% *A. tonsa* copepodites dead in Chesapeake Bay from October 2007 through December 2009. This was lower than the 18–29% dead previously reported (Tang et al. 2006a; Tang et al. 2007), reflecting the fact that these previous studies were confined to the summer when higher percent dead copepods was expected. Compared to *A. tonsa* copepodites and NIV–NVI copepod nauplii (12–15% dead; Fig. 4b), the percent dead *Eurytemora 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* copepodite carcasses, 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 higher sustainable mortality rate.

The higher percent dead NI–NIII nauplii represent a potentially large source of error in estimates of the abundance of live copepod nauplii. In addition, the observed higher percent dead NI–NIII nauplii could imply higher non-predatory mortality among these younger stages, provided that water column retention times do not differ substantially between naupliar and copepodite carcasses. Elliott et al. (2010) found that turbulent energy would be strong enough to resuspend even large *A. tonsa* carcasses in the York River water column. This suggests that carcass removal by sinking to the seabed is of minor importance in shallow tidal environments. Carcasses could also be removed by ingestion or microbial decomposition, but neither of these should discrepantly affect observed percent dead of various developmental stages. A dominant planktivore in the lower Chesapeake Bay is the ctenophore *Mnemiopsis leidyi* (Steinberg and Condon 2009). *M. leidyi* can feed on a wide range of motile and non-motile prey the sizes of adult copepods and nauplii (Waggett and Costello 1999), do not strongly select for either live copepods or carcasses (Elliott et al. 2010), and would therefore not strongly alter percent dead copepods by their feeding activities. Finally, Elliott et al. (2010) found that both *A. tonsa* copepodites and nauplii decomposed at similar rates in laboratory incubations (their Table 2). Thus, the consistently higher percent dead in stage NI–NIII nauplii in situ likely reflect higher non-predatory mortality in these 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; Chinnery and Williams 2004), and UV radiation (Leech and Williamson 2000). Susceptibility to environmental stressors and lower survival in these young stages may contribute to limitation of population recruitment, similar to the bottleneck created by high egg mortality (Ohman and Wood 1995; Tang et al. 1998).

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 (Fig. 5), suggesting that these carcasses are a persistent feature of the area. The percent dead varied in a similar annual pattern during both 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, 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 2010).

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References

Avery, D.E., K.J.K. Altland, and H.G. Dam. 2008. Sex-related differential mortality of a marine copepod exposed to a toxic dinoflagellate. *Limnology and Oceanography* 53: 2627–2635.

Bickel, S.L., and K.W. Tang. 2010. Microbial decomposition of proteins and lipids in copepod versus rotifer carcasses. *Marine Biology* 157: 1613–1624.

Brownlee, D.C., and F. Jacobs. 1987. Mesozooplankton and microzooplankton in the Chesapeake Bay. In *Contaminant problems and management of living Chesapeake Bay resources*, ed. S.K. Malumadar, L.W. Hall, and H.M. Austin, 217–267. Philadelphia: Pennsylvania Academy of Sciences.

Calbet, A., and M. Alcaraz. 1997. Growth and survival rates of early developmental stages of *Acartia grani* (Copepoda, Calanoida) in relation to food concentration and fluctuations in food supply. *Marine Ecology Progress Series* 147: 181–186.

Cervetto, G., R. Gaudy, and M. Pagano. 1999. Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). *Journal of Experimental Marine Biology and Ecology* 239: 33–45.

Chinnery, F.E., and J.A. Williams. 2004. The influence of temperature and salinity on *Acartia* (Copepoda: Calanoida) nauplii survival. *Marine Biology* 145: 733–738.

Cuker, B.E., and M.A. Watson. 2002. Diel vertical migration of zooplankton in contrasting habitats of the Chesapeake Bay. *Estuaries* 25: 296–307.

Elliott, D.T., and K.W. Tang. 2009. Simple staining method for differentiating live and dead marine zooplankton in field samples. *Limnology and Oceanography: Methods* 7: 585–594.

Elliott, D.T., C.K. Harris, and K.W. Tang. 2010. Dead in the water: The fate of copepod carcasses in the York River estuary, Virginia. *Limnology and Oceanography* 55: 1821–1834.

Gomez-Gutierrez, J., W.T. Peterson, A. De Robertis, and R.D. Brodeur. 2003. Mass mortality of krill caused by parasitoid ciliates. *Science* 301: 339.

Hagy, J.D., W.R. Boynton, C.W. Wood, and K.V. Wood. 2004. Hypoxia in Chesapeake Bay, 1950–2001: Long-term changes in relation to nutrient loading and river flow. *Estuaries* 27: 634–658.

Hall, L.W., M.C. Ziegenfuss, R.D. Anderson, and W.D. Killen. 1995. Use of estuarine water column tests for detecting toxic conditions in ambient areas of the Chesapeake Bay watershed. *Environmental Toxicology and Chemistry* 14: 267–278.

Hansen, B.W., and W.H.M. van Boekel. 1991. Grazing pressure of the calanoid copepod *Temora longicornis* on a *Phaeocystis* dominated spring bloom in a Dutch tidal inlet. *Marine Ecology Progress Series* 78: 123–129.

Harding, L.W., and E.S. Perry. 1997. Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950–1994. *Marine Ecology Progress Series* 157: 39–52.

Heinle, D.R. 1966. Temperature and zooplankton. *Chesapeake Science* 10: 186–209.

Hirche, H.J. 1992. Egg production of *Eurytemora affinis*—Effect of k-strategy. *Estuarine, Coastal and Shelf Science* 35: 395–407.

Holste, L., and M.A. Peck. 2006. The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation. *Marine Biology* 148: 1061–1070.

Huntley, M.E., and M.G.D. Lopez. 1992. Temperature dependent production of marine copepods: A global synthesis. *The American Naturalist* 140: 201–242.

Kemp, W.M., W.R. Boyton, J.E. Adolf, D.F. Boesch, W.C. Boicourt, G. Brush, J.C. Cornwell, T.R. Fisher, P.M. Glibert, J.D. Hagy, L.W. Harding, E.D. Houde, D.G. Kimmel, W.D. Miller, R.I.E. Newell, M. R. Roman, E.M. Smith, and J.C. Stevenson. 2005. Eutrophication of Chesapeake Bay: Historical trends and ecological interactions. *Marine Ecology Progress Series* 303: 1–29.

Kimmel, D.G., and M.R. Roman. 2004. Long-term trends in mesozooplankton abundance in Chesapeake Bay USA: Influence of freshwater input. *Marine Ecology Progress Series* 267: 71–83.

Kimmerer, W.J., and A.D. McKinnon. 1990. High mortality in a copepod population caused by a parasitic dinoflagellate. *Marine Biology* 107: 449–452.

Kiørboe, T. 2006. Sex, sex-ratios, and the dynamics of pelagic copepod populations. *Oecologia* 148: 40–50.

Kiørboe, T. 2007. Mate finding, mating, and population dynamics in a planktonic copepod *Oithona davisae*: There are too few males. *Limnology and Oceanography* 52: 1511–1522.

Leech, D.M., and C.E. Williamson. 2000. Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? *Ecological Applications* 10: 1530–1540.

Lopez, M.G.D. 1996. Effect of starvation on development and survivorship of naupliar *Calanus pacificus* (Brodsky). *Journal of Experimental Marine Biology and Ecology* 203: 133–146.

Mauchline, J. 1998. The biology of calanoid copepods. In *Advances in marine biology*, eds. J.H. Blaxter, A. Southward, and P.A. Tyler, 1–13. NY: Academic710.

Morales, C.E., R.P. Harris, R.N. Head, and P.R.G. Tranter. 1993. Copepod grazing in the oceanic northeast Atlantic during a six week drifting station: the contribution of size classes and vertical migrants. *Journal of Plankton Research* 15: 185–211.

Ohman, M.D. 1984. Omnivory by *Euphausia pacifica*: The role of copepod prey. *Marine Ecology Progress Series* 19: 125–131.

Ohman, M.D., and S.N. Wood. 1995. The inevitability of mortality. *ICES Journal of Marine Science* 52: 517–522.

Peck, M.A., and L. Holste. 2006. Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures. *Aquaculture* 255: 341–350.

Purcell, J.E., and M.B. Decker. 2005. Effects of climate on relative predation by scyphomedusae and ctenophores on copepods in Chesapeake Bay during 1987–2000. *Limnology and Oceanography* 50: 376–387.

Rodríguez-Graña, L., D. Calliari, P. Tiselius, B.W. Hansen, and H. N. Sköld. 2010. Gender-specific ageing and non-Mendelian inheritance of oxidative damage in marine copepods. *Marine Ecology Progress Series* 401: 1–13.

Roman, M.R., A.L. Gauzens, W.K. Rhinehart, and J.R. White. 1993. Effects of low oxygen waters on Chesapeake Bay zooplankton. *Limnology and Oceanography* 38: 1603–1614.

Roman, M.R., D.V. Holliday, and L.P. Sanford. 2001. Temporal and spatial patterns of zooplankton in the Chesapeake Bay turbidity maximum. *Marine Ecology Progress Series* 213: 215–227.

Steinberg, D.K., and R.H. Condon. 2009. Zooplankton of the York River. *Journal of Coastal Research* 57: 66–79.

Taft, J.L., W.R. Taylor, E.O. Hartwig, and R. Loftus. 1980. Seasonal oxygen depletion in Chesapeake Bay. *Estuaries* 3: 242–247.

Tang, K.W., H.G. Dam, and L.R. Feinberg. 1998. The relative importance of egg production rate, hatching success, hatching duration and egg sinking in population recruitment of two species of marine copepods. *Journal of Plankton Research* 20: 1971–1987.

Tang, K.W., C.S. Freund, and C.L. Schweitzer. 2006a. Occurrence of copepod carcasses in the lower Chesapeake Bay and their decomposition by ambient microbes. *Estuarine, Coastal and Shelf Science* 68: 499–508.

Tang, K.W., K.M.L. Hutalle, and H.P. Grossart. 2006b. Microbial abundance, composition and enzymatic activity during decomposition of copepod carcasses. *Aquatic Microbial Ecology* 45: 219–227.

Tang, K.W., C.S. Freund, A.N. Parrish, and S.L. Bickel. 2007. A simple staining method for differentiating live and dead copepods in natural samples. Estuarine Research Federation Biennial Conference. Providence, RI

Tang, K.W., S.L. Bickel, C. Dziallas, and H.P. Grossart. 2009. Microbial activities accompanying decomposition of cladoceran and copepod carcasses under different environmental conditions. *Aquatic Microbial Ecology* 57: 89–100.

Threlkeld, S.T. 1976. Starvation and the size structure of zooplankton communities. *Freshwater Biology* 6: 489–496.

Uye, S. 1986. Impact of a copepod grazing on the red-tide flagellate *Chatonella antiqua*. *Marine Biology* 92: 35–43.

Waggett, R., and J.H. Costello. 1999. Capture mechanisms used by the lobate ctenophore, *Mnemiopsis leidyi*, preying on the copepod *Acartia tonsa*. *Journal of Plankton Research* 21: 2037–2052.