

Microbial decomposition of proteins and lipids in copepod versus rotifer carcasses

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Abstract Zooplankton carcasses are common within aquatic systems and potentially serve as organic-rich substrates for bacteria. We compared the microbial decomposition of representative crustacean (copepod) and non-crustacean (rotifer) zooplankton carcasses and monitored changes in carcass protein and lipid contents. Our results showed that carcass decomposition was mainly driven by bacteria colonizing from the surrounding water. Carcass-associated bacteria displayed higher protease and lipase activities than free-living bacteria. Protein content of copepod carcasses decreased by 70% within the first 8 h and shifted from larger to smaller sized proteins, while protein loss in rotifer carcasses was insignificant. Carcass lipid content did not change significantly over 24 h in either zooplankton type, although polar branched fatty acids increased on copepod carcasses indicating an increase in viable microbial biomass. Our results suggest differential turnover of protein versus lipid within a zooplankton carcass and that carcasses from different zooplankton groups would affect water column microbial processes differently.

Introduction

Organic aggregates play an important role in shaping aquatic microbial food web dynamics and transporting

surface-water-derived particulate organic matter (POM) to depth. The formation, prevalence and decomposition of organic aggregates, as well as the associated microbial processes, have been studied extensively in freshwater and marine systems (reviewed in Simon et al. 2002). One source of POM that has been largely overlooked is zooplankton carcasses. Quantitative information on zooplankton carcass abundances is limited due to the difficulty in identifying live/dead individuals in field samples; nevertheless, the available literature data show that carcasses can comprise up to 90% of the sampled populations with a median of 35% (reviewed by Elliott and Tang 2009). If we assume there is 10^{14} g of zooplankton carbon biomass in the world's ocean (Libes 1992), approximately 3.5×10^{13} g C could be available in the form of zooplankton carcasses for microbial decomposition and/or export to depth. In comparison, approximately 3×10^{16} g C is currently present in the world's oceans as detritus (Libes 1992). While zooplankton carcasses contribute only 0.1% to the global detrital carbon pool, processes associated with zooplankton carcasses could be significant on a local scale. In a recent sediment trap study in Arctic waters, Sampei et al. (2009) found that carcasses represented less than 5% of all copepods within traps yet contributed 36% of the annual particulate organic carbon flux. Consequently, ignoring the presence of zooplankton carcass could lead to gross underestimation of the local vertical carbon flux. Organic aggregate carbon-to-nitrogen ratio ranges from 5 to 20, and particulate combined amino acids account for 8–51% of aggregate bound POM (Simon et al. 2002). By comparison, calanoid copepods, the most abundant marine mesozooplankton taxa, have an average C:N ratio close to 3, and proteins commonly account for 30–70% of their dry weight (Båmstedt 1986). This suggests that although

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few in number, zooplankton carcasses provide higher quality POM than detrital aggregates. Also, unlike phytoaggregates, which rarely become anoxic (Ploug 2001), decomposing zooplankton carcasses can quickly become hypoxic or anoxic and support anaerobic microbial processes that otherwise could not occur in the water column (Glud et al. unpubl. data).

During the decomposition of estuarine crustacean zooplankton carcasses, there was visible internal tissue loss and accumulation of bacteria inside the carapace (Tang et al. 2006a). A shift in the carcass-associated microbial community composition and a fourfold to sixfold increase in protease activity associated with carcasses were also observed during the decomposition of freshwater copepods and cladocerans (Tang et al. 2006b). Follow-up experiments showed enhanced carcass-associated bacterial production and enzyme activity relative to free-living bacteria and reduction in carcass C and N contents over time (Tang et al. 2009); however, no measurements of biomolecules (i.e., proteins or lipids) were made. It remains unknown how the biochemical composition of carcasses changes during the decomposition process, thus influencing their effectiveness as transport mechanisms for high quality organic materials to depth.

Most previous studies of zooplankton carcass decomposition have focused on crustacean species. Non-crustacean zooplankton are common in freshwater and marine systems and at times can dominate the zooplankton community (Loeb et al. 1997; Holst et al. 1998; Chiba et al. 1998), but decomposition of their carcasses has rarely been studied (Riemann et al. 2006; Titelman et al. 2006). The exoskeleton of crustacean zooplankton is composed primarily of chitin, which is relatively resistant to bacterial degradation (Kirchner 1995) and may hinder bacterial colonization and decomposition of the carcasses. In comparison, non-crustacean zooplankton lack a chitinous exoskeleton, and earlier qualitative observations suggested that their carcasses may decompose faster, although quantitative measurements were not available (Tang et al. 2006a).

In the present study, we compared the decomposition of crustacean and non-crustacean zooplankton carcasses. Incubation experiments were conducted with or without natural bacterial communities to determine the relative importance of free-living bacteria, attached bacteria and zooplankton tissue autolysis in the decomposition process. Additionally, we monitored microbial activity and changes in carcass protein and lipid contents to determine the change in quantity and quality of carcass-derived POM during the decomposition process.

Materials and methods

Zooplankton carcass production

The calanoid copepod *Acartia tonsa* Dana was used as the model crustacean zooplankton while the rotifer *Brachionus plicatilis* Mueller served as the model non-crustacean zooplankton. *A. tonsa* is dominant among the mesozooplankton within Chesapeake Bay in Virginia, USA, and can be found year-round, reaching peak abundances during the summer months (Kimmel and Roman 2004). Likewise, *B. plicatilis* is common in Chesapeake Bay during the spring and summer and plays an integral trophic role in the system (Dolan and Gallegos 1992). For this study, *A. tonsa* were collected from the mesohaline portion of the York River estuary, a sub-estuary within Chesapeake Bay. Upon return to the lab, live zooplankton were gently concentrated onto a 200- μm mesh sieve and then killed by brief exposure to a weak acid (approximately 10 s in <3% HCl). The carcasses were rinsed extensively with 0.2- μm filtered artificial seawater (FASW, 20 psu) to remove the acid and then back-rinsed into a petri dish. Adult *A. tonsa* were identified under a dissecting microscope and transferred into a second petri dish containing 0.2 μm FASW. No differentiation between male and female copepods was made. Laboratory cultures of *B. plicatilis* were hatched from resting eggs (Florida Aqua Farms Inc. USA, Dade City, FL), maintained in 0.2- μm filtered York River water (FYRW) at 25°C and fed a diet of the cryptophyte *Rhodomonas salina*. For the experiments, rotifer cultures were concentrated onto an acid-washed 35- μm mesh sieve, back-rinsed with 0.2 μm FASW into an acid-washed beaker and brought to a known volume with 0.2 μm FASW. The beaker was gently mixed, and three aliquots of 5 ml were removed and preserved with Lugol's stain to determine rotifer abundance via direct counts. To produce carcasses for the enzyme and protein decomposition experiments, aliquots of the rotifer concentrate were transferred to sterile, polystyrene centrifuge tubes and placed in a 55°C water bath for 10 min. Heat was used instead of acid because the latter was found to be ineffective at killing rotifers. The same procedures were followed to produce copepod and rotifer carcasses for lipid decomposition experiments with the exception that pre-combusted borosilicate glass culture tubes were used in place of plastic tubes during heat-killing of rotifers.

Laboratory incubation experiments

To examine microbial response to zooplankton carcasses and subsequent changes in carcass biochemical composition, a

series of laboratory incubation experiments was conducted. Incubation water was collected from the York River estuary 1–2 days prior to each experiment, gravity filtered through a series of decreasing pore size sieves and stored at 15°C. All incubation experiments, except those for lipid decomposition, were performed with two different water filtrates: a 5- μm filtrate with the majority of larger organisms removed but ambient bacteria retained and a 0.2- μm filtrate where most free-living bacteria were removed.

To monitor bacterial abundance and exoenzyme activities, 25 similarly sized *A. tonsa* (approximate length: 1,000 μm) or 250 *B. plicatilis* (approximate length: 200 μm) carcasses were transferred into a sterile polystyrene petri dish filled with 25 ml of 5 μm or 0.2 μm FYRW. To measure carcass protein, freshly prepared carcasses (200 copepods or approximately 10,000 rotifers) were incubated in acid-washed and sterilized borosilicate glass bottles with 200 or 1,000 ml of 5 μm or 0.2 μm FYRW, respectively. To determine the change in carcass lipid content during decomposition, 700 freshly prepared copepod carcasses or approximately 20,000 rotifer carcasses were incubated in acid-washed and pre-combusted borosilicate glass bottles with GF/D (nominal pore size 2.7 μm) filtered YRW at a final concentration of 1 copepod carcass or 10 rotifer carcasses per ml. In all experiments, triplicate incubations containing carcasses and corresponding controls of FYRW (no carcasses added) were established for each time point. All containers were incubated at 15°C on orbital shaker tables rotating at low speeds to provide gentle mixing. Exoenzyme activities and bacterial abundances were measured for up to 4 days. Protein content of copepod and rotifer carcasses was measured at 0, 8, 16 and 24 h; carcass lipid content was measured at 0 and 24 h.

Exoenzymatic activities

To measure enzyme activities attributed to free-living bacteria, 3-ml aliquots of incubation water were removed from each replicate treatment and control and transferred to separate cuvettes. Two aliquots were taken from each replicate: one for protease measurement and the other for lipase. Two additional 3-ml aliquots were taken from one of the replicates, and 300 μl of 35% formaldehyde was added to stop all exoenzymatic activities to correct for background fluorescence.

To measure enzyme activities of copepod carcass-associated bacteria, five carcasses were removed from the incubation vessel and transferred to a 1-ml glass tissue grinder. It was unavoidable that a small amount of incubation water was also transferred with the carcasses; however, our calculations indicated that the bacterial abundance within this water was negligible compared to

the number of bacteria associated with the carcass itself. The carcasses were ground vigorously to break up zooplankton tissues and release bacteria from within the carcasses. Carcass homogenate was then transferred to a cuvette; 0.2 μm FASW was used to rinse the tissue grinder three times into the same cuvette and bring the final volume to 3 ml. Two additional samples of 5 copepod carcasses were taken from one of the replicates, ground as previously described, and 300 μl of 35% formaldehyde was added to stop all exoenzymatic activities to correct for background fluorescence. Due to the smaller size of rotifers, 50 carcasses were used for each carcass-associated measurement to ensure detectable signals. Rotifer carcasses were processed in the same manner as copepods.

The fluorogenic substrate analogs 4-methylumbelliferyl heptanoate (MUH) (Williams and Jochem 2006) and L-leucine 7-amido-4-methylcoumarin (Leu-MCA) (Hoppe 1983) were used to measure lipase and protease activities, respectively. Concentrated stocks (5 mM) of the analogs were prepared by dissolving MUH or Leu-MCA powder into 2-methoxyethanol or Milli-Q water, respectively. A 60- μl aliquot of 5 mM MUH or Leu-MCA stock was added to each sample cuvette for a final concentration of 0.1 mM. All cuvettes were incubated in the dark for 1 h at 15°C. Fluorescence intensity was measured immediately after the 1-h incubation on a Shimadzu RF-1501 spectrofluorophotometer (Shimadzu Corp., Japan) at $\lambda_{\text{ex}} = 380 \text{ nm}$ and $\lambda_{\text{em}} = 440 \text{ nm}$ for protease, $\lambda_{\text{ex}} = 365 \text{ nm}$ and $\lambda_{\text{em}} = 445 \text{ nm}$ for lipase. Fluorescence intensities were converted into substrate cleavage rates based on calibration curves prepared with 7-amido-4-methylcoumarin (AMC) for protease and 4-methylumbelliferone (MUF) for lipase. Fluorescence measurements were normalized to DAPI or SYBR-Gold total direct counts (see *Bacterial enumeration*) from the same replicate to determine cell-specific enzyme activities.

Bacterial enumeration

For each filtrate experiment, a 1-ml aliquot of water was taken from each replicate treatment and control, stained with 4',6-diamidino-2-phenylindole (DAPI) and filtered onto a 0.2- μm black polycarbonate filter to enumerate free-living bacteria (Porter and Feig 1980). To enumerate carcass-associated bacteria, 5 copepod or 50 rotifer carcasses were ground as described previously. The carcass homogenates were filtered onto 0.2- μm black polycarbonate filters and stained with SYBR-Gold (Chen et al. 2001). Staining with SYBR-Gold provided greater contrast between bacteria and carcass detritus than DAPI and was therefore more preferable for counting carcass-associated bacteria.

Carcass protein content and molecular weight distribution

The entire content of each incubation bottle was gravity filtered onto a separate 25 mm (for copepods) or 47 mm (for rotifers) diameter, 5- μ m-pore size polycarbonate filter. Afterward, the actual number of copepod carcasses on the filter was determined under a dissecting microscope. Visual verification was not done for rotifers due to the small size and large number of carcasses used. The total number of rotifer carcasses used for protein measurement was therefore estimated from the concentration of rotifer stock and aliquot volume. Each filter was cut into small pieces with a surgical scalpel, placed into a separate microcentrifuge tubes and stored at -40°C until processing.

To prepare the samples for total protein measurement, 600 μ l of 0.2 μ m FASW was added to each microcentrifuge tube, which was then sonicated on ice with a microprobe for 60 s (output power 20 W). The homogenate was then centrifuged for 15 min at 14,000g at -9°C to pellet out the filter. Total protein of the supernatant was determined colorimetrically using the Bradford method (Bradford 1976) and quantified against a standard curve of bovine serum albumen. Changes in the size composition of carcass proteins were monitored by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE). To denature the proteins, samples from different stages of decomposition were vortexed with β -mercaptoethanol, centrifuged for 15 s at 14,000g, heated at 95°C for 4 min and again centrifuged at 14,000g for 15 s. All samples were run on a 12% acrylamide resolving gel with a 4% acrylamide stack for 45 min at 200 V (Bankey et al. 1995; Van Veld and Westbrook 1995). Within each gel, one lane was loaded with protein standard of different molecular sizes (EZ-RunTM Rec Protein Ladder, Fisher BioReagents). Each gel was dual stained with coomassie blue and silver stain to visualize the protein bands. Gels were photographed using Alpha Innotech software (San Leandro, CA).

Carcass lipid content

The content of each incubation bottle was gravity filtered onto a pre-combusted, 47 mm diameter, GF/D filter. Filters were stored in foil packets at -40°C until analysis. All tools were thoroughly rinsed with Milli-Q water and hexane between samples. Frozen filters were thawed, cut into small pieces and mixed with hydromatrix (Varian Inc., Palo Alto, CA) to remove excess water. Neutral and polar lipid fractions were extracted sequentially from each filtered sample using accelerated solvent extraction (Poerschmann and Carlson 2006). Neutral and polar extracts were concentrated down to 1 ml with a Turbo-Vap (Zymark, Germany), blown dry under nitrogen gas and

brought to 100 μ l final volume with hexane (for neutral lipids) or chloroform (for polar lipids). Total extracted neutral and polar lipids were determined gravimetrically. Afterward, both polar and neutral fractions were dried under nitrogen gas and saponified with 1 N KOH in methanol for 2 h at 110°C (Poerschmann and Carlson 2006). To analyze fatty acid composition, acids were methylated to fatty acid methyl esters (FAMES) with 3% boron trifluoride (BF₃) in methanol at 85°C for 1 h (Poerschmann and Carlson 2006). FAMES, alcohols and sterols were analyzed with gas chromatography and were quantified by comparison to internal standards (Supelco Inc., St. Louis, MO; Poerschmann and Carlson 2006).

Statistical analyses

A one-way ANOVA was performed on bacterial abundances, cell-specific enzyme activities and carcass protein content to detect temporal changes for each of the parameters. A post-hoc Tukey test of 95% confidence intervals was performed to compare individual time points. To assess differences in the abundances and enzymatic activities among control, free-living and carcass-associated bacterial populations, two-way nested ANOVAs were performed to eliminate bias of time within the measurements. A one-sample *t* test was performed to assess changes in total extractable lipid content over the incubation period, and a 2-sample *t* test was performed to detect shifts in neutral-to-polar lipid ratio. No statistical analyses could be performed on the fatty acid composition data as only one sample from each time point was analyzed. Consequently, only general observations on the trends of fatty acid composition were made. All statistical analyses were performed using Minitab statistical software (State College, PA).

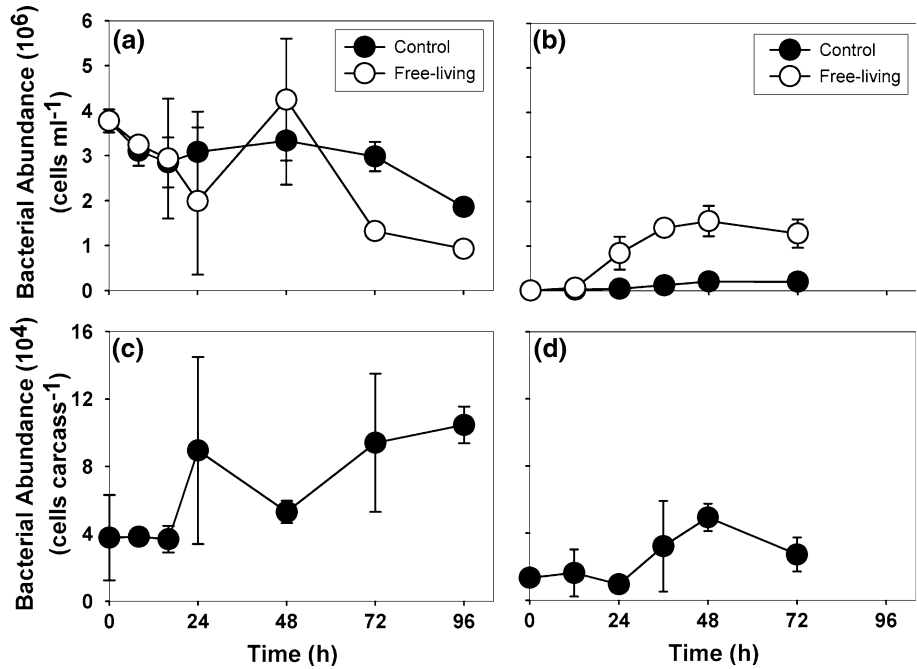
Results

Bacterial abundance

From here on, “carcass-associated” refers to bacteria found inside or attached to the external surface of a carcass; the term “free-living” is used in reference to bacteria in ambient water when carcasses were present, and “control” refers to bacteria in ambient water when no carcasses were present.

When copepod carcasses were incubated in 5 μ m FYRW, free-living bacterial abundance was highly variable but significantly decreased over the incubation period (Fig. 1a; ANOVA, $P = 0.006$; Tukey test $P = 0.009$). In contrast, free-living bacterial abundance increased over the incubation period with rotifer carcasses under the same

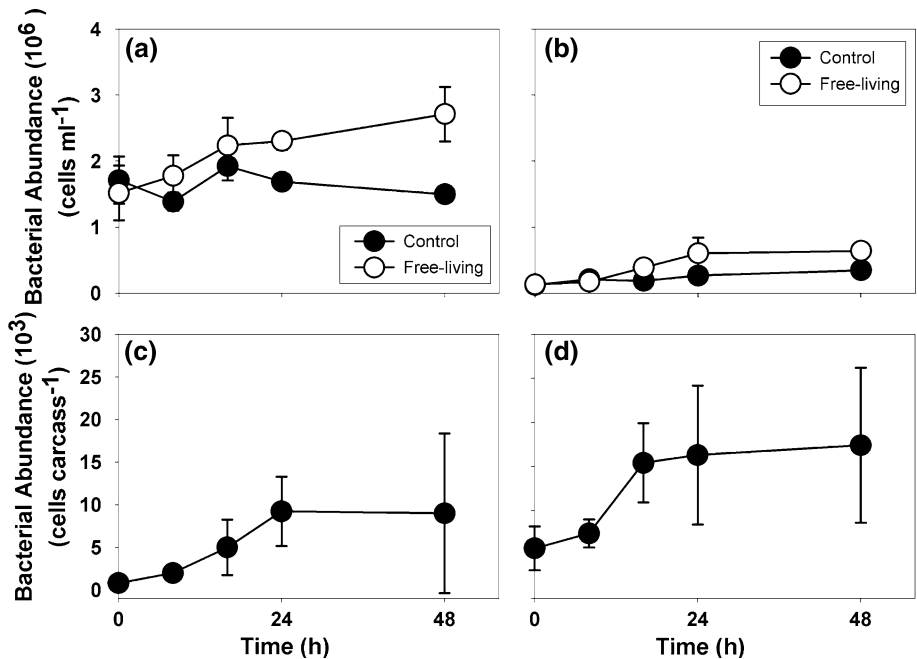
Fig. 1 Abundances of free-living and control bacteria (a, b) and carcass-associated bacteria (c, d) during the decomposition of copepod carcasses in 5 μm FYRW (a, c) or 0.2 μm FYRW (b, d) (mean ± S.D., n = 3)



conditions (Fig. 2a; ANOVA, $P = 0.016$; Tukey test, $P = 0.003$). Carcass-associated bacterial abundance increased significantly on copepods incubated in 5 μm FYRW (Fig. 1c; ANOVA, $P = 0.029$), whereas it did not change significantly with rotifers (Fig. 2c). When incubated in 0.2 μm FYRW, free-living bacterial abundances in both copepod (Fig. 1b) and rotifer (Fig. 2b) treatments increased by 24 h (ANOVA, $P < 0.001$; Tukey test, $P < 0.001$ for both copepods and rotifers) but remained lower than those in the 5-μm incubations. Carcass-

associated bacteria in the 0.2 μm FYRW incubations increased on copepods (Fig. 1d; ANOVA, $P = 0.035$) but did not change significantly on rotifer carcasses (Fig. 2d). Bacterial abundance per copepod carcass was on average an order of magnitude higher than abundance per rotifer carcass. When initial carcass-associated bacterial abundances (5 μm FYRW incubation) were normalized to literature C-content values (4.6 μg C copepod⁻¹, Tang et al. 1999; 170 ng C rotifer⁻¹, Øie et al. 1997), copepod carcasses also contained more bacteria than rotifer carcasses:

Fig. 2 Abundances of free-living and control bacteria (a, b) and carcass-associated bacteria (c, d) during the decomposition of rotifer carcasses in 5 μm FYRW (a, c) or 0.2 μm FYRW (b, d) (mean ± S.D., n = 3)



8206 ± 5506 cells $\mu\text{g C}^{-1}$ versus 2914 ± 990 cells $\mu\text{g C}^{-1}$ (mean ± SD).

Exoenzymatic activities

The potential cell-specific protein and lipid analog hydrolysis activities were calculated for each of the bacterial groups (control, free-living and carcass-associated). These enzymatic measurements will henceforth be referred to as protease and lipase activities. Among the 5 μm FYRW incubations, cell-specific protease activity of free-living bacteria in the copepod treatment reached a maximum within 24 h (Fig. 3a; ANOVA, $P = 0.009$); no change was detected in the rotifer treatment (Fig. 3c). Copepod

carcass-associated bacteria in 5 μm FYRW incubations showed no change in cell-specific protease activity through time, whereas their cell-specific lipase activity peaked by 8 h (ANOVA, Tukey test, $P < 0.001$; Fig. 3a, b). Rotifer carcass-associated bacteria demonstrated high initial cell-specific protease and lipase activities, which sharply declined by 8 h (ANOVA, Tukey test, $P < 0.001$ for both enzymes; Fig. 3c, d). In general, copepod and rotifer carcass-associated bacteria exhibited much higher cell-specific protease and lipase activities than their free-living counterparts when incubated in 5 μm FYRW.

When copepod carcasses were incubated in 0.2 μm FYRW, the cell-specific protease and lipase activities of free-living bacteria dropped after the initial measurements

Fig. 3 Cell-specific protease (a, c) and lipase (b, d) activities for control, free-living and carcass-associated bacteria during the decomposition of copepod (a, b) and rotifer (c, d) carcasses in 5 μm FYRW (mean ± S.D., $n = 3$)

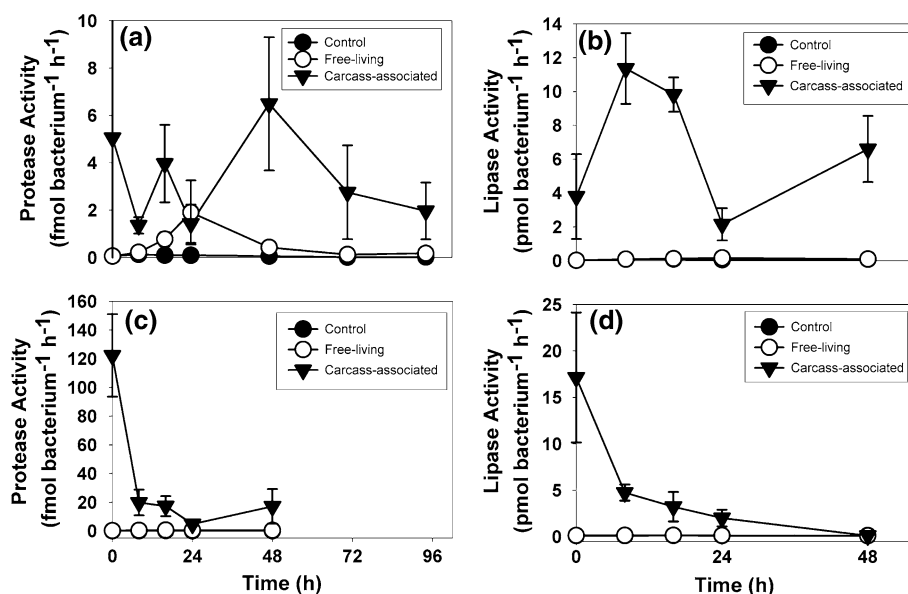
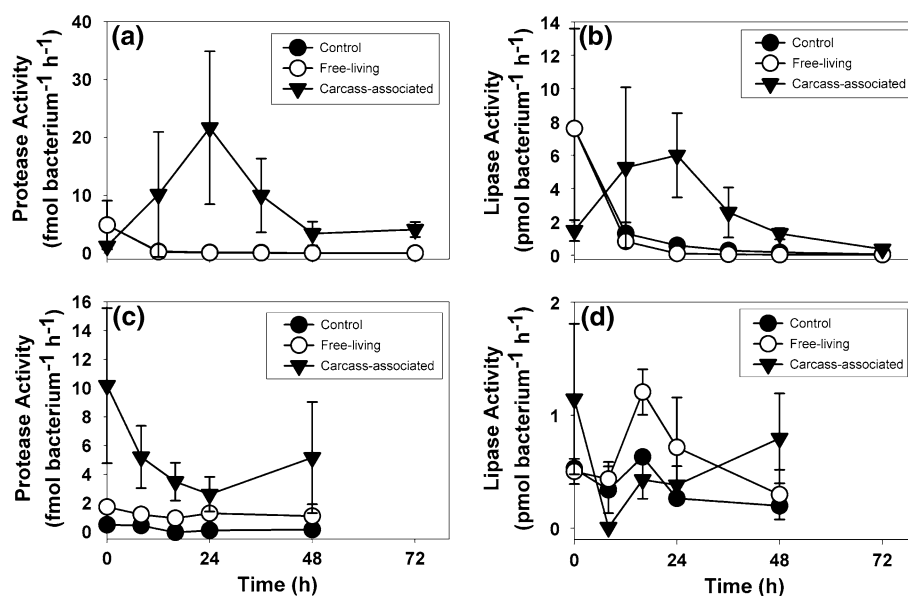


Fig. 4 Cell-specific protease (a, c) and lipase (b, d) activities for control, free-living and carcass-associated bacteria during the decomposition of copepod (a, b) and rotifer (c, d) carcasses in 0.2 μm FYRW (mean ± S.D., $n = 3$)



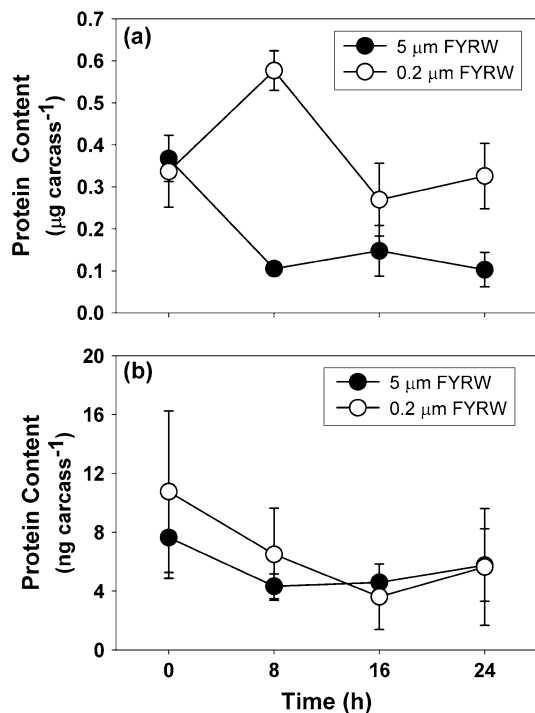


Fig. 5 Total protein content of copepod (a) and rotifer (b) carcasses incubated in two water filtrates (mean \pm S.D., $n = 3$)

and remained low for the duration of the incubation (Fig. 4a, b). In the similar experiment with rotifer carcasses, free-living bacteria showed no change in cell-specific protease activity throughout the experiment, while their cell-specific lipase activity increased to a maximum at 16 h before decreasing (Fig. 4c, d). The cell-specific protease and lipase activity of both copepod and rotifer carcass-associated bacteria exhibited general temporal patterns similar to those observed in the 5 μ m FYRW treatments. None of the temporal changes were significant, however, due to the reduced scale of the measurements and variation among the replicates (Fig. 4). While carcass-associated bacterial exoenzymatic activities were still elevated over free-living bacterial activities, the elevation was either delayed (copepods) or occurred on a much smaller scale (rotifers) than in the 5 μ m FYRW treatments.

Carcass protein content

Approximately 70% of the initial total copepod protein was lost within the first 8 h of incubation in 5 μ m FYRW (Fig. 5a), after which there was no significant change in total protein (ANOVA, Tukey test, $P = 0.013$). This overall decrease in total protein as well as changes in protein composition was noted in the SDS-PAGE results (Fig. 6a). The compositional changes included: (1) higher molecular weight proteins (e.g., 150 and 100 kD) present at the beginning disappeared as decomposition progressed;

(2) intermediate-sized proteins (approximately 60 kD) appeared after 16 h and then disappeared by 24 h; (3) lower molecular weight proteins (e.g., 45 kD) persisted throughout the decomposition process. Copepod carcasses incubated in 0.2 μ m FYRW showed an inexplicable increase in protein content after 8 h, but overall there was no significant decrease in the amount of particulate protein over 24 h (Fig. 5a); SDS-PAGE confirmed that proteins of all sizes, including the large sizes, persisted throughout the experiment (Fig. 6b). Rotifer carcasses incubated in 5 μ m FYRW exhibited a non-significant decrease in total protein content during the first 8 h (Fig. 5b), and the majority of the protein bands persisted throughout the incubation (Fig. 6c). Likewise, rotifer carcasses incubated in 0.2 μ m FYRW showed non-significant decrease in the total particulate protein content (Fig. 5b), and no major change was noted in the protein size distribution (Fig. 6d).

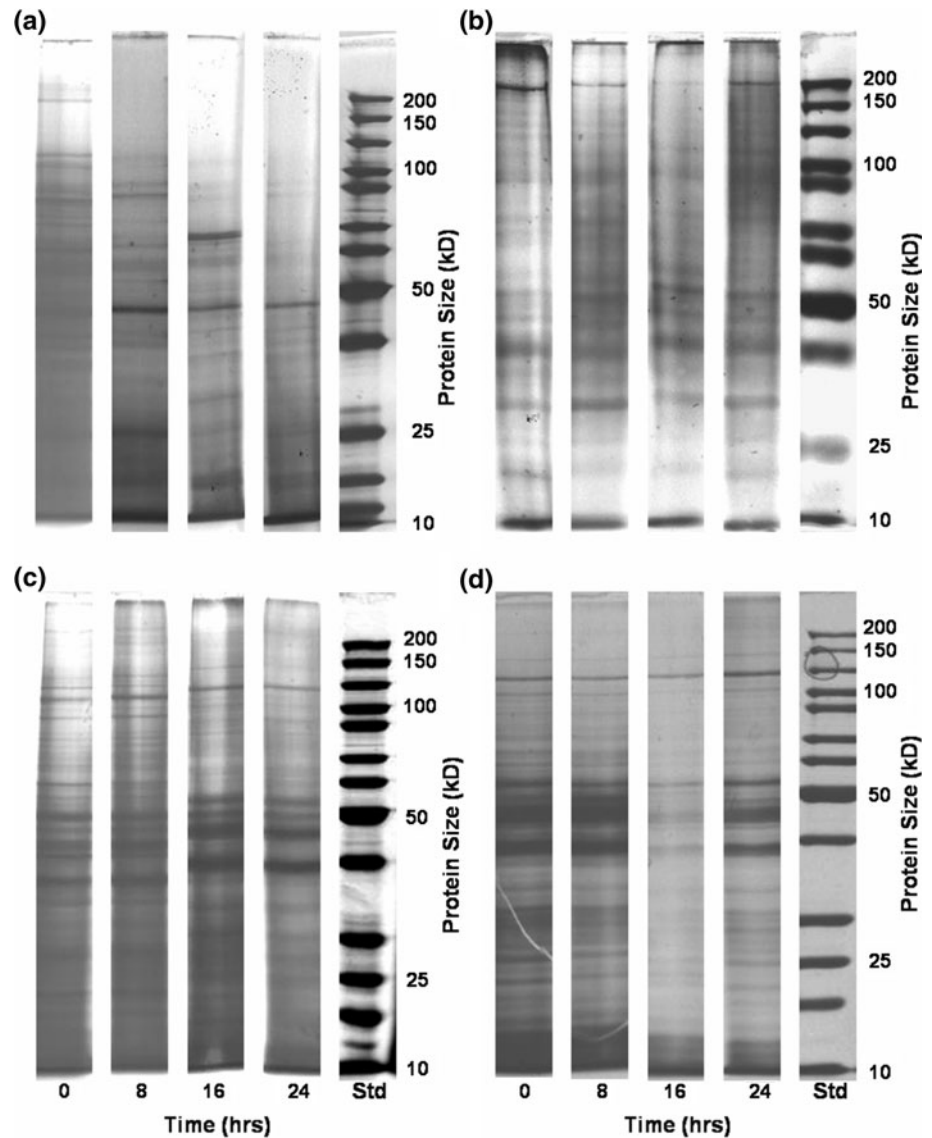
Carcass lipid content

On average, the gravimetrically determined total neutral and polar lipid fractions declined within copepod and rotifer carcasses over 24 h when incubated in GF/D FYRW, although the decrease was not significant (data not shown). No significant shift in the neutral-to-polar lipid ratio per carcass was noticed over 24 h of incubation for either copepod or rotifer carcasses. GC analysis of selected samples revealed somewhat different trends in lipid content than were observed from the gravimetric measurements. A slight increase in the total polar fatty acid content of both copepods (0.78 ng individual⁻¹) and rotifers (0.60 ng individual⁻¹) was noted over the 24-h incubation (Fig. 7a). In the copepod experiments, this increase was driven by increases in monounsaturated and branched polar fatty acids, both of which comprised a larger fraction of the total fatty acid content at 24 h (Fig. 8). The increase in polar fatty acids in the rotifer experiment was due to increases in monounsaturated and polyunsaturated polar fatty acids (Fig. 7a). Copepod carcasses demonstrated a slight increase in all classes of neutral fatty acids (4.73 ng individual⁻¹), while rotifer carcasses showed a slight decrease (-2.40 ng individual⁻¹; Fig. 7b).

Discussion

When carcasses were incubated with natural bacteria (5 μ m FYRW), carcass-associated bacteria exhibited enhanced protease and lipase activities over their free-living counterparts in both copepod and rotifer treatments (Fig. 3), which is consistent with previous observations using freshwater crustacean zooplankton carcasses (Tang et al. 2006b, 2009). However, cell-specific lipase activity

Fig. 6 Protein size composition of copepod (**a, b**) and rotifer (**c, d**) carcasses incubated in 5 μm FYRW (**a, c**) or 0.2 μm FYRW (**b, d**) based on SDS-PAGE



was three orders of magnitude higher in this study. As the production of specific exoenzymes is highly dependent upon microbial community structure (Arnosti 2004), this difference may be a consequence of differences in the microbial communities between the two studies. There were also differences in the decomposition process between copepod and rotifer carcasses. Bacterial abundance on copepod carcasses increased as decomposition progressed (Fig. 1), while there was no change in bacterial abundance on rotifer carcasses (Fig. 2). Initial protease and lipase activities on rotifer carcasses were respectively 22 and 4 times higher than the corresponding enzyme measurements on copepod carcasses (Fig. 2). The high enzyme activity on rotifers immediately after death may be partially attributed to residual digestive enzymes produced by the rotifers (de Araujo et al. 2000; Štrojsová and Vrba 2005). Enzyme production by rotifers and their epizootic

bacteria can be highly localized (Štrojsová and Ahlrichs 2009), and the fluorogenic substrate method is not able to differentiate between the two enzyme sources.

Preferential decomposition of proteins and nitrogen-rich substrates has been observed among other organic aggregates but at a much lower rate than what was measured in this study, likely reflecting differences in the biochemical quality of the organic materials. For example, Grossart and Ploug (2001) reported 50% loss of particulate organic nitrogen from diatom aggregates over 5.4 days. In contrast, we observed a rapid loss (70% over the first 8 h; Fig. 5) of total copepod carcass protein when incubated with the natural microbial community. This is comparable to the observation by Elliott et al. (2010) that 50% of copepod carcass dry weight was lost within the first 8 h due to microbial decomposition. This protein loss was corroborated by elevated carcass-associated protease activity

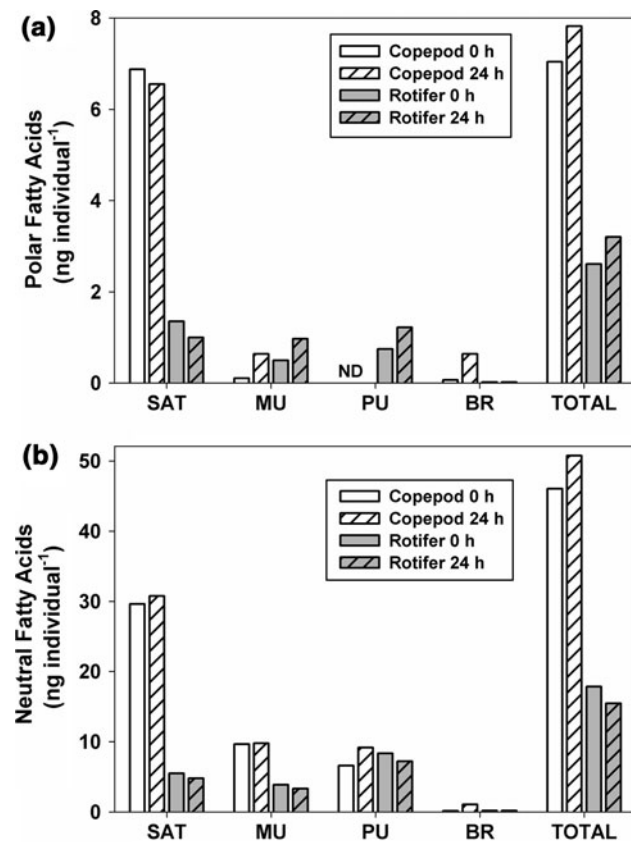


Fig. 7 Polar (a) and neutral (b) fatty acid contents per copepod or rotifer carcass at 0 and 24 h in GF/D filtered York River Water. *ND* not detected, *SAT* saturated fatty acids, *MU* monounsaturated fatty acids, *PU* polyunsaturated fatty acids, *BR* branched fatty acids, *TOTAL* sum of all classes of fatty acids

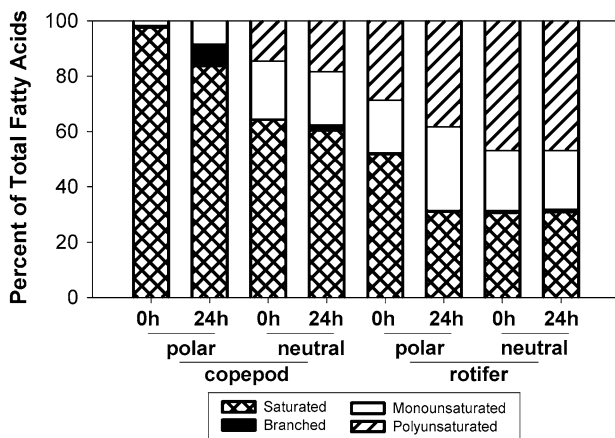


Fig. 8 Relative percentages of fatty acid classes present in polar and neutral lipids of copepod and rotifer carcasses

(Fig. 3a), while the disappearance of large protein molecules and ephemeral appearance of intermediate sized protein molecules indicate rapid hydrolysis of larger protein molecules into smaller forms through bacterial

exoenzymatic activity (Fig. 6a). In comparison, rotifer carcasses lost insignificant amounts of protein when exposed to bacteria (Figs. 5, 6c). Some proteins lost from the carcasses may have been retained within bacterial biomass. While it was not possible to separate bacterial and carcass proteins in our experiments, a back-of-the-envelope calculation using measured carcass-associated bacterial abundances and an average value of 34.4 fg protein per bacterial cell (Simon and Azam 1989) suggests that bacterial contributions to total protein measurements at any given time would be negligible (no more than 3%).

The selective decomposition of some protein sizes and retention of others suggest that there were at least two protein pools within copepod carcasses. The first pool was likely highly labile and rapidly consumed by bacteria, while the second pool was more refractory and likely to be transported to depth. Reinfelder et al. (1993) also noted two separate protein fractions released from carcasses of the copepod *Anomalocera patersoni*: approximately 64% of the total protein was comprised of a rapidly exchanging pool with a half-life of 1.1 d, and the remainder of the protein belonged to a slowly exchanging pool with a half-life of 5.5 d.

Contrary to our initial expectations, proteins contained within crustacean zooplankton were decomposed at a higher rate than those of non-crustacean zooplankton. This indicates that the presence of a chitinous exoskeleton did not necessarily impede the decomposition process relative to non-crustaceans. Rather, the decomposition rate might be regulated by the overall quality of the protein. The SDS-PAGE results (Fig. 6) showed that copepods initially contained multiple protein bands larger than 70 kD and only three prominent protein bands in the size range of 40–60 kD. In contrast, rotifers had fewer larger sized proteins and 5 prominent protein bands in the 40–60 kD range. In both copepods and rotifers, larger sized proteins tended to disappear quickly as decomposition progressed, while the smaller sized proteins persisted. These observations suggest that the labile protein pool was primarily comprised of larger sized proteins and the refractory pool was dominated by smaller sized proteins. The dominance of small sized and presumably refractory proteins in rotifers might explain the insignificant loss of carcass protein during the experiment. Rotifer integument contains a layer of tightly packed keratin, which is likely responsible for maintaining rotifer body shape (Kleinow and Wratil 1995). A portion of the protein bands retained throughout our experiments could be keratin proteins, which typically have a size range of 40–70 kD (e.g., Lee et al. 1979; Kleinow 1993) and are highly resistant to decomposition (Kleinow 1993).

Polar fatty acids detected via gas chromatography are representative of membrane lipids, and branched polar fatty acids are commonly used to represent the viable microbial

community (Gillan and Johns 1986). The increase in absolute abundance (Fig. 7a) and relative proportion (Fig. 8) of branched polar fatty acids per copepod carcass over 24 h suggests an increase in carcass-associated bacteria, which is corroborated by microscopic counts (Fig. 1c). The lesser increase in branched fatty acids associated with rotifer carcasses (Fig. 7a) is also consistent with the highly variable but non-significant increase in carcass-associated bacteria (Fig. 2c). Contrary to expectations, the neutral lipid component, which is indicative of storage lipids, changed only slightly over time in both types of carcasses (Fig. 7b). Despite the commonality of lipid-hydrolyzing bacteria within aquatic systems (Mudryk and Skórczewski 2006), decomposition of lipids tends to occur over a longer time scale than that of proteins. In laboratory flow-through systems Harvey et al. (1995) noted a rapid decrease in the protein content of diatom detritus after 10 days while lipid content did not decrease until 20 days and was still detectable after 77 days. Similarly, 2–3 weeks were necessary to observe decomposition of algal fatty acids within sediments (Ding and Sun 2005). Even though we observed high lipase activities, it is possible that neutral storage lipids within copepod and rotifer carcasses would be decomposed over a longer time, similar to phytodetrital lipids. Our observations also suggest a loose coupling between lipase activity and lipid turnover such that enzymatic activity alone may not be a reliable indicator of the carcass decomposition process.

When most of the ambient free-living bacteria were removed (0.2 μm FYRW incubations), both copepod and rotifer carcasses showed insignificant loss of proteins (Figs. 5, 6) and a small or delayed enhancement of carcass-associated enzyme activity (Fig. 4). This suggests that both autolysis of zooplankton tissue and the activity of enteric or previously attached bacteria were minimal. A comparison of the 5 μm and 0.2 μm FYRW experiments indicates that carcass decomposition was largely initiated by colonizing, free-living bacteria, which is consistent with the findings of Harding (1973) and similar to what has been observed for the decomposition of zooplankton fecal pellets (e.g., Honjo and Roman 1978; Jacobsen and Azam 1984; Hansen and Bech 1996).

Our results showed that rotifer carcasses decomposed at a relatively low rate. In estuaries, the distribution of rotifers can be sporadic, and their abundances can increase or decrease by orders of magnitude within days (Dolan and Gallegos 1991; Park and Marshall 2000). While the ability to reproduce parthenogenically can explain rapid increases in rotifer abundance, the cause of population decline is less clear. If rotifers suffer from massive mortality due to non-predatory causes, slow decomposition may allow their carcasses to accumulate in the benthos in shallow parts of the estuaries thus

contributing to benthic food webs. Copepod carcasses are found throughout the Chesapeake Bay (Tang et al. 2006a, 2007). The different decomposition rates of protein and lipid fractions of copepod carcasses observed in this study suggest that the fate of these carcasses would largely depend on the biochemical composition of the copepod species within the system. Elliott et al. (2010) noted that copepod carcass sinking rates were positively correlated with dry weight, suggesting that as decomposition progresses, carcass dry weight and sinking rate decrease, allowing a larger fraction of a protein-rich copepod to be remineralized within the water column. In contrast, based on our results, the change in dry weight of a lipid-rich copepod carcass should be minimal, and sedimentation of carcasses would be more likely. With a maximum sinking rate of 0.12 cm s^{-1} (Elliott et al. 2010), fresh *Acartia* carcasses have the potential to sink approximately 34 m within 8 h, reaching the bottom in the majority of the Chesapeake Bay. If similar decomposition patterns are applicable to a copepod carcass in a deeper coastal system (e.g., >34 m), carcass proteins would likely be remineralized within the water column, while carcass lipids would still be transported to depth. Knowledge of carcass decomposition patterns, zooplankton community structure and biochemical composition of the dominant species could be coupled with an assessment of the prevalence of carcasses within the populations to allow for an estimation of the contribution of zooplankton carcasses to biogeochemical cycling in the system.

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