Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone

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Abstract

The downward flux of particulate organic carbon (POC) decreases significantly in the ocean's mesopelagic or 'twilight' zone due both to abiotic processes and metabolism by resident biota. Bacteria and zooplankton solubilize and consume POC to support their metabolism, but the relative importance of bacteria vs. zooplankton in the consumption of sinking particles in the twilight zone is unknown. We compared losses of sinking POC, using differences in export flux measured by neutrally buoyant sediment traps at a range of depths, with bacteria and zooplankton metabolic requirements at the Hawaii Ocean Time-series station ALOHA in the subtropical Pacific and the Japanese times-series site K2 in the subarctic Pacific. Integrated (150-1000 m) mesopelagic bacterial C demand exceeded that of zooplankton by up to 3-fold at ALOHA, while bacteria and zooplankton required relatively equal amounts of POC at K2. However, sinking POC flux was inadequate to meet metabolic demands at either site. Mesopelagic bacterial C demand was 3- to 4-fold (ALOHA), and 10-fold (K2) greater than the loss of sinking POC flux, while zooplankton C demand was 1- to 2-fold (ALOHA), and 3- to 9-fold (K2) greater (using our "middle" estimate conversion factors to calculate C demand). Assuming the particle flux estimates are accurate, we posit that this additional C demand must be met by diel vertical migration of zooplankton feeding at the surface, and by carnivory at depth–with both processes ultimately supplying organic C to mesopelagic bacteria. These pathways need to be incorporated into biogeochemical models that predict global C sequestration in the deep sea.

Introduction

Quantifying the processes that control transport of particulate organic carbon (POC) from the surface to the deep ocean is fundamental to understanding the global cycling of carbon and energy sources for deep-sea food webs. In the sunlit, surface ocean photosynthetic organisms convert inorganic carbon into organic carbon that is transferred from the surface to the deep sea via mixing of dissolved organic matter, active transport by animals, and sinking of particles–collectively known as the "biological pump". In particular, downward transport of biogenic particles is considered a key mechanism in sequestering C to the ocean's interior. The vertical POC flux attenuates rapidly with depth in the ocean's mesopelagic or "twilight" zone (depths immediately below the euphotic zone down to 1000 m) with the majority of the sinking POC lost between 100-500 m (Martin et al. 1987), due to both biotic (metabolism by resident biota) and abiotic (mineral dissolution) processes. Bacteria and zooplankton solubilize and consume sinking POC to support their metabolic demands. However little is known about their relative contributions to POC flux attenuation, whether these contributions vary with depth and locale, or how the fundamentally different mechanisms by which bacteria and zooplankton obtain C in the mesopelagic may effect remineralization of sinking POC to CO_2 (Fig. 1).

Bacterial abundance also decreases with depth (Ducklow 1993; Nagata et al. 2000) (although the relative abundance of archaea– a diverse group of prokaryotes, increases below the euphotic zone, Karner et al. 2001) and the concomitantly decreasing POC flux supports spatially heterogeneous bacterial populations in the mesopelagic (Hewson et al. 2006).

Bacterial activity on sinking particles appears insufficient to account for the attenuation of POC flux with depth (Karl et al. 1988), and bacterial production (BP) appears to be fueled by enzymatic hydrolysis of sinking particles to dissolved organic carbon (DOC), which then supplies the suspended, "free-living" bacterial pool that completes the remineralization of organic C to CO_2 (Cho and Azam 1988) (Fig. 1). Measurements of BP in the meso- and bathypelagic suggest that bacterial carbon demand (BCD) accounts for 14 to >100% of the loss of sinking POC with depth (Cho and Azam 1988; Nagata et al. 2000; Reinthaler et al. 2006).

Zooplankton in the mesopelagic zone include both full-time residents as well as diel (or seasonal) vertical migrators which feed on phytoplankton and other POC in the euphotic zone and mixed layer at night and return to mesopelagic depths during the day (Fig. 1). Evidence of a significant role for mesopelagic zooplankton in attenuation of sinking POC originates from dietary studies and calculation of zooplankton community metabolic requirements. Diet studies show sinking detritus, or 'marine snow,' is an important food source for both deep-sea non-migrating (Steinberg 1995) and migrating zooplankton species (Lampitt et al. 1993). Furthermore, zooplankton can fragment large sinking marine snow into smaller slower-sinking or suspended aggregates (Goldthwait et al. 2004), which also diminishes POC flux to depth. Zooplankton metabolic requirements have been calculated to account for 4 to 86% of the loss of sinking POC with depth (reviewed in Koppelmann et al. 2004, Table 6). While processing of sinking POC by bacteria and zooplankton has been investigated, their relative roles in this critical process have yet to be quantified simultaneously in the twilight zone.

As part of the VERTIGO (VERtical Transport In the Global Ocean) study we characterized the mesopelagic planktonic community at two contrasting oceanic sites: The Hawaii Ocean Time-series (HOT) station ALOHA in the oligotrophic subtropical Pacific gyre, and the Japanese times-series site (K2), located in a high nutrient, seasonally variable chlorophyll region of the NW subarctic Pacific. At both sites we compared losses of sinking POC measured by neutrally buoyant sediment traps with bacteria and zooplankton metabolic requirements at both sites to determine the relative role that bacteria and zooplankton play in the attenuation of POC with depth. Furthermore, we explore the options by which mesopelagic biota can met their nutritional and metabolic requirements.

Methods

Study sites

Samples were collected and experiments conducted aboard the *R/V Kilo Moana* at the Hawaii Ocean Time-series (HOT) station ALOHA (22° 45' N, 158° W) from 22 June- 09 July 2004, and aboard the *R/V Roger Revelle* at the Japanese times-series site (K2) (47° N 160° E) from 22 July-18 August 2005. An overview for each site of physical and particle properties, and primary production (PP) and particle flux is presented in Buesseler et al. (2007). During our study period ALOHA was characterized by warm waters (26°C at surface), a mixed layer depth of 49 m, mixed layer nutrients at nanomolar concentrations, PP 180-220 mg C m^{-2} d^{-1} (note- measured via shipboard deck incubations, and lower than the HOT in situ PP climatology), low Chl *a* (~0.1 mg m^{-3} at surface), and a phytoplankton assemblage consisting of small diatoms, coccolithophorids, and picoplankton. K2 was characterized by colder waters (10°C at surface), a mixed layer depth of 26 m, higher surface nutrients (12 μmol L^{-1} mixed

130 layer DIN) and PP (365-530 mg C m^{-2} d^{-1}), variable but higher Chl a (~0.8 mg m^{-3} at surface),
131 and a phytoplankton assemblage consisting of picoplankton and large diatoms. Conditions
132 were relatively uniform during our ~3-week occupation of each site, with the exception of an
133 increase in particle flux over the study period at K2 (see Results).
134
135 *Particle flux*
136 We measured particle flux using neutrally buoyant sediment traps (NBSTs) during two
137 consecutive 3-5 day deployments at 150, 300, and 500 m at each site (Buesseler et al. 2007).
138 NBSTs were used to minimize potential hydrodynamic sampling biases due to fluid flow over
139 and within the trap (Buesseler et al. 2007). Replicate NBSTs were deployed (up to 3 at 150 m)
140 with good agreement between traps (*see* Results). Zooplankton swimmers were carefully
141 removed from all samples first via screening followed by hand picking under a dissecting
142 microscope (250-350x magnification). POC was obtained by difference between total C,
143 measured by CHN analysis, and particulate inorganic carbon (PIC). PIC was measured by
144 acidification of the sample with phosphoric acid and titration of CO_2 by a coulometric method.
145 POC flux at 1000 m was calculated by fitting a power function (Buesseler et al. 2007; Martin
146 et al. 1987) to mean trap POC fluxes at each depth. We then compared losses of sinking POC,
147 using differences in export flux measured by NBSTs at different depths, with metabolic C
148 requirements of bacteria and zooplankton for each of the two NBST deployments at each site.
149
150 *Bacteria respiration and carbon demand*
151 Depth integrated bacteria respiration (BR) and carbon demand (BCD) was based on our
152 measures of bacteria production (BP) at discrete depths throughout the water column, and

153 published bacterial growth efficiency (BGE). Bacterial production (BP) was determined using
154 30 mL [^{3}H]-thymidine incorporation incubations conducted shipboard at atmospheric pressure
155 and in situ temperatures (Fuhrman and Azam 1982) on water samples collected from the
156 surface to 1000 m. Thymidine incorporation was converted to carbon demand using the
157 commonly reported range of thymidine conversion factors (1.0 - 2.0 x 10^{18} cells mol^{-1})
158 (Ducklow 2000), applying a carbon conversion factor of 15 fg C $cell^{-1}$ (Ducklow 2000), and a
159 BGE range of 0.10 - 0.15 for open-ocean bacteria (Del Giorgio and Cole 2000; Reinthaler et al.
160 2006). This sensitivity analysis allowed us to account for uncertainties inherent in the
161 conversions and provided a middle (applying a thymidine conversion factor of 2.0 x 10^{18} cells
162 mol^{-1}, and a BGE of 0.15), lower (thymidine conversion factor of 1.0 x 10^{18} cells mol^{-1}, and a
163 BGE of 0.15), and upper (thymidine conversion factor of 2.0 x 10^{18} cells mol^{-1}, and a BGE of
164 0.1) estimate (Table 1). Bacteria were also enumerated at each site using DAPI (4',6-
165 diamidino-2-phenylindole) staining and epifluorescence microscopy.
166
167 While the influence of pressure on bacterial production conversion factors has not yet
168 been systematically examined, there is no a priori reason to expect that they should vary with
169 depth, and conversion factors for surface communities are commonly applied to the
170 mesopelagic (Nagata et al. 2000; Reinthaler et al. 2006). However, the thymidine
171 incorporation rates derived from incubations conducted at atmospheric pressure may
172 underestimate true rates (Bianchi et al. 1999), and, thus, the BP rates we present here are likely
173 to be conservative. The range of BGE values that we applied was lower than the value of 0.20
174 that Nagata et al. (2000) applied to the mesopelagic; similar to our range, their value was
175 derived from literature reports for the surface community. Recently very low BGE values

(~0.02) were reported by Reinthaler et al. (2006) for mesopelagic communities, but these were determined at atmospheric pressure, and there is evidence to suggest that decompression associated with bringing samples to the surface can result in BGE estimates that are artificially low (Tamburini et al. 2003). An average BGE of 0.09 for ALOHA mesopelagic bacteria that we estimated independently by electron transport system (ETS) activity was comparable to the range we applied. The rate of mineralization of organic carbon to CO_2 (BR) is given by the equation (Nagata et al. 2000):

$$BR\ (mg\ C\ m^{-2}\ d^{-1}) = (1-BGE)/BGE \cdot BP \quad (1)$$

Total organic carbon entering into bacteria (BCD) is given by the equation (Nagata et al. 2000):

$$BCD\ (mg\ C\ m^{-2}\ d^{-1}) = BP/\ BGE \quad (2)$$

Zooplankton respiration and carbon demand

Depth integrated zooplankton respiration (ZR) and carbon demand (ZCD) was based on our measures of size-fractionated zooplankton biomass and temperature, and published relationships of zooplankton body weight and respiration rate, and zooplankton assimilation efficiency. Zooplankton biomass and taxonomic composition was determined from net tows in 9 discrete depth intervals from 0-1000 m with a 1 m^2, 335 μm mesh MOCNESS or IONESS (Multiple Opening/Closing Net and Environmental Sampling System or Intelligent Operative Net Sampling System) during both day and night. The net tow samples were split: Half was

size-fractionated (5, 2, 1, 0.5, and 0.3 mm fractions) and frozen for biomass analyses (dried 24 h at 60°C and weighed), and half was preserved in sodium borate-buffered 4% formaldehyde for taxon analyses. Animals in each size fraction in each depth interval were counted and the mean dry weight animal^{-1} calculated. Gelatinous zooplankton, with the exception of large scyphozoan medusae, were included in counts and dry weight analyses. For K2, we subtracted the biomass contributed by several copepod species and stages in diapause (*Neocalanus cristatus* and *N. plumchrus* C5 and adult stages; *N. flemingeri* C4, C5, and adults; *Eucalanus bungii* C3, C4, C5, and adults; *Calanus jashnovi* and *C. pacificus* C5) as they do not feed while in diapause and thus would not be consuming sinking particles (Yamaguchi et al. 2002). Thus they are omitted from the calculation of ZR and ZCD below.

ZR was calculated using the empirical allometric relationships of Ikeda (1985) based on mean body mass for each size class and mean temperature for each depth interval, and converted to carbon equivalents following Al-Mutairi and Landry (2001). ZR for each depth interval (mg C m^{-2} d^{-1}) was calculated by multiplying ZR by the number of individuals m^{-3} in each size fraction times the depth interval (m), and summing all size fractions. ZR was converted to C consumption rates (ZCD) using the following equation:

$$\text{ZCD (mg C m}^{-2}\text{ d}^{-1}) = \text{ZR}/\text{R} \cdot \text{AE} \qquad (3)$$

where R is the fraction of assimilated C respired, and AE is the assimilation efficiency (fraction of C consumed that was assimilated) (Steinberg et al. 1997).

As for BCD, we performed a sensitivity analysis for the calculation of ZCD, using an R of 50% and AE of 60% (middle), 70% (lower), and 50% (upper) for mesopelagic zooplankton consuming detritus (Steinberg et al. 1997) and which includes the AE (70%) commonly used in modeling studies. Note we did not perform sensitivity analysis on ZR rates, as they are based on an algorithm derived from hundreds of respiration measurements of epipelagic zooplankton (although including many vertically migrating species) from multiple phyla (Ikeda 1985) and in which differences in temperature and body weight (the two principle factors affecting zooplankton respiration) are already incorporated. We made no adjustment for possible depth-related changes in respiration rate. Previous studies of marine zooplankton indicate no decline in respiration rates with depth (Thuesen et al. 1998, and references therein). However, Ikeda et al. (2006, 2007) show respiration rates of mesopelagic copepods (adjusted for temperature differences) in the subarctic Pacific range from 90% (at 200 m) to 50% (at 1000 m) of their epipelagic (e.g., 100 m) counterparts (calculated from equation given in Fig. 2, Ikeda et al. 2006). Thus, zooplankton respiration in the lower mesopelagic may be overestimated for the copepod component of the community. However, at K2 the majority of the deep copepods were in diapause and not included in our respiration calculation anyway. At ALOHA, overestimation of deep copepod respiration may be more likely. However, it is difficult to assess how applicable depth-related changes in mesopelagic copepod respiration rates from one location in the subarctic Pacific (Ikeda et al. 2006, 2007) are to other locations with different fauna, such as ALOHA.

All zooplankton respiration and carbon demand calculations were made using a combination of day (13.5 h for ALOHA or 14.5 h for K2) + night (10.5 h for ALOHA or 9.5 h

for K2) biomass data (mean day and night length at each site during our study). This method thus includes C requirements of diel migrators residing at depth during the day, which may (Lampitt et al. 1993) or may not consume sinking particles. There was no significant difference in respiration or C demand for any depth interval using this method vs. only using night data in order to exclude C requirements of diel migrators (Student's *t*-test, $p > 0.05$). (Likely as some diel migrators only migrated as shallow as 150-250 m, and some came from below 1000 m into the mesopelagic zone at night, Fig. 3, Steinberg et al. in press).

Active flux of CO_2 and DOC by zooplankton vertical migrators

Downward active flux of CO_2 by migrant zooplankton (mg C m^{-2} d^{-1}) was calculated as in Al-Mutairi and Landry (2001) for the 0-150 m depth intervals, assuming migrants reside below the mixed layer 13.5 h and 14.5 h during the day at ALOHA and K2, respectively (see above), with the remainder of time spent in the surface waters at night, and applying the average temperature experienced by migrants at depth during the day at each site (Al-Mutairi and Landry 2001; Steinberg et al. 2000). Downward active flux of DOC by migrant zooplankton (mg C m^{-2} d^{-1}) was calculated as 31% of downward active flux of CO_2 (Steinberg et al. 2000).

Results

Plankton community structure

Both bacteria and zooplankton biomass were considerably higher at K2 than ALOHA. Bacterial abundances above 150m were up to 2-fold higher at K2 (range 2.1-10.5 x 10^5 cells

268 mL^{-1}) than ALOHA (range 1.8 - 5.5 x 10^5 cells mL^{-1}), and decreased exponentially with depth
269 at both sites, becoming up to 9-fold higher in the mesopelagic (≥150 m) at K2 (0.9 - 4.9 x 10^5
270 cells mL^{-1}) than reported at ALOHA (0.1 – 4.8 x 10^5 cells ml^{-1})(Karner et al. 2001) (Fig. 2).
271 Daytime mesopelagic zooplankton biomass (150-1000 m) was an order of magnitude higher at
272 K2 (mean ±1 SD = 6.9 ±0.7 g dry wt m^{-2}, *n*=4) than ALOHA (0.5±0.1 g dry wt m^{-2}, *n*=4),
273 partially due to high abundance of the large copepods *Neocalanus* spp. and *Eucalanus* sp. at
274 K2 (Fig. 3). Diel vertical migration was pronounced at both sites: Nighttime zooplankton
275 biomass was higher than daytime biomass in the upper 0-150 m by a factor of 1.7 ±0.5 at
276 ALOHA, as previously reported (Al-Mutairi and Landry 2001), and by a factor of 2.5 ±1.4 at
277 K2. Copepods constituted 74 ±0.5% and 70 ±4% of daytime mesopelagic zooplankton
278 abundance at ALOHA and K2, respectively (Steinberg et al. in press).
279
280 *Bacteria and zooplankton metabolic requirements*
281 At ALOHA bacteria were primarily responsible for metabolizing sinking POC, while at
282 K2 zooplankton and bacteria both contributed equally. At ALOHA the estimated bacterial
283 respiration (BR; remineralization of organic C to CO_2) significantly exceeded zooplankton
284 respiration (ZR) at nearly all depths for both deployments (Fig. 4), with integrated BR 2- to 10-
285 fold higher than ZR for both deployments (Table 1). Bacterial carbon demand (BCD) is
286 defined as the carbon required for respiration and growth, while zooplankton carbon demand
287 (ZCD) is carbon ingested and subsequently assimilated for use in respiration, excretion,
288 growth, and reproduction, plus unassimilated carbon egested as feces. ALOHA BCD also
289 exceeded ZCD at nearly all depths (Fig. 4). Integrated mesopelagic BCD ranged from slightly
290 lower than ZCD to 4-fold higher than ZCD (Table 1). The profiles of sinking particle flux at

291 ALOHA were nearly identical between the two deployments, with 75% of the 150 m POC flux
292 removed by 500 m (Fig. 4).
293
294 Carbon demand of mesopelagic bacteria and zooplankton was considerably higher at
295 K2 than at ALOHA (Fig. 5, note x-axis scale is double that of Fig. 4), despite the colder
296 temperatures at K2 (22ºC vs. 2ºC at 150 m, and 8ºC vs. 3ºC at 500 m, at ALOHA vs. K2,
297 respectively). This reflects the higher bacteria and zooplankton biomass at K2. Mesopelagic
298 integrated BR was up to 5-fold higher than ZR (Table 1). However, BCD and ZCD are
299 comparable to one another at depths below 200 m (and not statistically different at any depth,
300 Fig. 5), with integrated BCD less than a factor of 2 higher or lower than ZCD (Table 1).
301 Sinking particle flux was higher at K2 than ALOHA and decreased between deployments; but
302 on both deployments only ~ 25% of the 150 m POC flux at K2 was removed by 500 m (Fig. 5).
303 Vertical patterns in both BCD and ZCD were similar between deployments at each site.
304
305 *Comparison of metabolic requirements to attenuation of sinking POC*
306 Integrated BR and BCD accounted for 2-4 times the loss of sinking POC in the
307 mesopelagic zone at ALOHA, while ZR was approximately half, and ZCD twice the loss of
308 sinking POC flux (Table 1, using middle estimate conversion factors). At K2, BCD and ZCD
309 accounted for an even higher proportion of sinking POC loss with depth vs. at ALOHA, due
310 both to the considerably smaller decrease in sinking flux (Figs. 4 and 5), and the considerably
311 higher mesopelagic zooplankton biomass-derived ZCD at K2 (Fig. 3). Thus our results also
312 indicate that K2 BCD was 10-fold greater than the loss of sinking POC, while ZCD was 3- to
313 9-fold higher. In Fig. 6, we extract the integrated 150-1000 m BCD and ZCD data from Table

1 to illustrate the "best" and "worst" case scenarios by comparing the middle, minimum, and maximum estimated C demand (from our sensitivity analysis) to POC flux attenuation. It is evident that even in the "best case" scenario (lower range limit of error bar), BCD and ZCD are higher than POC flux attenuation for all deployments. As a "worst case" (higher range extremes least favorable to the model), community C demand far exceeds sinking POC flux attenuation– with BCD up to 16 times, and ZCD up to 11 times the sinking POC flux attenuation (Table 1, Fig. 6).

Discussion

Excess metabolic C demand in the mesopelagic

It is evident that sinking particles alone can not adequately satisfy the metabolic requirements of mesopelagic biota at ALOHA and K2. Previous studies have noted that sinking POC flux as measured by sediment traps was insufficient to fuel mesopelagic C demand in the subarctic Pacific (Boyd et al. 1999; Simon et al. 1992) and the Arabian Sea (Ducklow 1993). Our study, however, is the first to systematically examine the C demand by both bacteria and zooplankton in the mesopelagic, which together considerably exceeded the delivery of organic C by sinking particles.

Other sources of C for mesopelagic biota

This excess metabolic C demand suggests a source of organic C to the mesopelagic other than sinking POC (Fig. 1). Vertical advective supply of DOC from surface waters (Carlson et al. 1994; Emerson et al. 1997) could support a portion of either the BCD when taken up directly, or ZCD via the microbial loop (VERTIGO did not address the contributions

337 of protozoan grazers, which are an important link in the microbial loop between bacteria and
338 zooplankton but undoubtedly contribute an additional C demand in the mesopelagic, Gowing et
339 al. 2003). However, the average daily rate of downward DOC export to the mesopelagic at
340 ALOHA (30 mg C m^{-2} d^{-1} below 100 m) (Emerson et al. 1997) is insufficient to support even
341 the observed BCD above 200 m. Furthermore, we sampled at ALOHA during summer
342 stratification when vertical mixing is minimal. At K2 it is possible that vertical mixing was
343 more significant, but DOC export would need to exceed POC export by an order of magnitude
344 to balance the mesopelagic C demand; to our knowledge this has never been observed in the
345 open ocean. The ambient DOC in bathypelagic waters is 4000-6000 years old (Bauer et al.
346 1992) and thought to be relatively unavailable to bacteria; global distributions of DOC and
347 BCD support this assertion (Nagata et al. 2000). DOC use also accounts for only ~10-20% of
348 the apparent oxygen utilization in the mesopelagic global ocean, suggesting an alternate C
349 source (Arístegui et al. 2005).
350
351 Furthermore, suspended POC concentrations at depth are inadequate to support
352 sustained metabolic demand. For example, at K2 suspended POC below 150 m was ~6 mg C
353 m^{-3}, and with a combined metabolic C demand at K2 of 0.4-0.6 mg C m^{-3} d^{-1} for both
354 zooplankton and bacteria (Table 1), POC stocks would be depleted in just 10-15 days. Thus a
355 new supply of POC (other than from sinking particles) would be required to keep up with the
356 demand, for which there is no evidence (e.g., no significant advection). Thus, while a
357 complete C budget is beyond the scope of our study, even our most conservative estimates
358 indicate neither sinking POC, suspended POC, nor imported DOC can meet the significant
359 excess C demand in the mesopelagic during our occupation of the two sites.

We posit that zooplankton diel vertical migration and carnivory sustain much of the excess C demand we observed (Fig. 1). By feeding in surface waters at night and metabolizing their food below the mixed layer during the day, zooplankton diel migrators can actively transport dissolved organic and inorganic C (via excretion and respiration, respectively) to depth (Al-Mutairi and Landry 2001; Steinberg et al. 2000). To test this hypothesis, we compared metabolism of zooplankton migrators with community C requirements at depth. This spatial uncoupling of ingestion and metabolism, while still only a few percent of surface layer photosynthetic production (Buesseler et al. 2007), could support 15-88% of our observed zooplankton respiratory C requirements (Table 2). Although variable– active transport of CO_2 by migrating zooplankton averaged (for both stations and all deployments combined) 47% of 150-1000 m zooplankton respiration, with a 95% confidence interval of 10-85%, respiration by migrators was not significantly different from integrated (150-1000 m) zooplankton community respiration (*t*-test, $p>0.05$). Thus, we conclude mesopelagic zooplankton could sustain a significant amount of their C demand by diel vertical migration. Excretion by zooplankton (migratory or non-migratory) may also provide a source of labile DOC that could fuel mesopelagic BCD (Steinberg et al. 2000), with migratory zooplankton excretion supporting up to 7% of BCD in our study (Table 2). Although not measured in our study, vertically migrating micronekton (e.g., decapods and fishes) may also actively transport C to depth (Hidaka et al. 2001) (as well as contribute further to C demand). Mortality of diel vertically migrating copepods during the day also can supply POC to the mesopelagic. Using metabolic C requirements of the non-migrating, mesopelagic micronekton predator community at ALOHA to estimate prey mortality, Al-Mutairi and Landry (2001) calculated mortality of

383 zooplankton diel migrators was equal to 32% of the diel migrant respiratory flux. Using the
384 approach of Zhang and Dam (1997) to estimate weight-specific mortality of diel migrators in
385 our study yields a mean diel mortality flux at ALOHA that is equal to, and at K2 is 1.3-fold,
386 the diel respiratory flux at each site (Table 2). Similarly, mortality loss of ontogenetic vertical
387 migrators in the mesopelagic zone during winter can also supply a significant amount of POC
388 annually. This is particularly important in the subarctic Pacific, where mortality loss of
389 ontogenetic migrators is equal to 92% of annual POC flux measured by sediment traps at 1000
390 m (Kobari et al. 2003).
391
392 Furthermore, the proportion of zooplankton biomass that is carnivorous increases with
393 depth (Vinogradov and Tseitlin 1983), thus mesopelagic zooplankton must meet a significant
394 fraction of their energy requirements via carnivory. In the northwest subarctic Pacific
395 carnivorous zooplankton comprised ~ 25% of the zooplankton biomass between 200-500 m
396 and > 50% of the biomass between 500-1000 m (Vinogradov and Tseitlin 1983). At K2 we
397 measured increases in carnivore abundance at depth, forming distinct layers in the
398 mesopelagic. Chaetognath density, for example, increased up to 30-fold between 150-300 m
399 compared to the upper 150 m (Steinberg et al. in press). Processes associated with carnivory,
400 such as DOM release from "sloppy feeding" could also fuel BCD. However, we emphasize
401 that ultimately many mesopelagic carnivores get their energy from sinking particles, because
402 the carnivores feed on animals that they themselves were feeding on sinking particles. Thus,
403 carnivory doesn't help solve the excess C demand problem, unless the animals the carnivores
404 consume come from outside the system (e.g., via advection, or diel vertically migrating
405 carnivores feeding on animals in the euphotic zone), or if the carnivory occurs on a different

406 time scale than our study- such as the fall and winter supply of ontogenetic migrators. Further
407 studies of taxonomic community structure and food web dynamics of the mesopelagic zone are
408 needed to determine the C demand that can be met by consumption of other animals.
409
410 Mesopelagic zooplankton (full-time residents and migrators) also produce fecal pellets
411 at depth that are consumed by detritivores (Sasaki et al. 1988; Yamaguchi et al. 2002), as
412 evidenced by the appearance of new classes of fecal pellets in our deeper 300 m and 500 m
413 NBST's compared to the 150 m traps (Wilson et al. in press). Migrators also actively transport
414 POC as fecal pellets produced at depth as a result of their surface feeding (Schnetzer and
415 Steinberg 2002), which can be consumed by zooplankton or solubilized by bacteria. This
416 consumption of animals and re-processing of sinking particles adds further complexity to
417 developing C budgets for the mesopelagic and in modeling the relative roles of heterotrophic
418 bacteria and zooplankton in the understudied deep ocean.
419
420 Both bacterial and zooplankton communities are important remineralizers and
421 consumers of sinking POC in the ocean's twilight zone, but sinking POC supplies only a
422 portion of the C they require. Certainly, episodic production of particles in the upper ocean
423 and their subsequent export could lead to a temporal offset in any direct comparison of
424 contemporaneous processes (Karl et al. 2003). However, we argue that a significant fraction of
425 the zooplankton C demand in the mesopelagic must be met by spatially uncoupled organic C
426 consumption and production by migrating zooplankton, as well as by carnivory. The result is
427 an active microbial loop in the dark waters of the mesopelagic that is ultimately supported by
428 phytoplankton but proximately by zooplankton. These pathways, and their linkages between

429 the microbial and zooplankton communities, need to be further explored and incorporated into
430 biogeochemical models that predict global C sequestration in the deep sea.
431

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549

550 Table 1. Metabolic carbon requirements of bacteria and zooplankton in the twilight zone as
551 compared to loss of (Δ) sinking particulate organic carbon flux in the same depth interval. All
552 units are mg C m^{-2} d^{-1}, with the exception of metabolic C requirements as loss of POC flux
553 (%). nd=not determined. Mean values are reported (using middle estimate conversion factors)
554 with the range given in parentheses (using lower - upper estimate conversion factors) from our
555 sensitivity analysis (*see* Materials and methods for details). No range is given for zooplankton
556 respiration (*see* Methods). Replication is as reported in Figs. 4 and 5. The ΔPOC flux was
557 calculated from measurements in Figs. 4 and 5, with 1000 m flux calculated from fitting a
558 power function (Buesseler et al. 2007; Martin et al. 1987) to mean trap organic carbon fluxes
559 measured at 150, 300, and 500 m.

	Metabolic C requirements				Metabolic C requirements as % loss of POC flux			
	ALOHA		K2		ALOHA		K2	
	1st Dep.	2nd Dep.	1st Dep.	2nd Dep.	1st Dep.	2nd Dep.	1st Dep.	2nd Dep.
Bacteria respiration								
150-500 m	31.8 (15.9 – 50.5)	34.7 (17.4 – 55.2)	nd	92.4 (46.2 – 146.7)	234% (117 – 372)	246% (123 – 391)	nd	892% (446 – 1416)
150-1000 m	45.0 (22.5 - 71.5)	55.1 (27.6 - 87.5)	nd	132.1 (66.1 – 209.8)	280% (140 – 444)	331% (166 – 526)	nd	932% (466 – 1481)
Bacteria C demand								
150-500 m	37.4 (18.7 – 56.2)	40.9 (20.4 – 61.3)	nd	108.7 (54.3 – 163.0)	276% (138 – 413)	290% (145 – 435)	nd	1049% (524 – 1573)
150-1000 m	53.0 (26.5 – 79.5)	64.8 (32.4 – 97.2)	nd	155.4 (77.7 – 233.1)	329% (165 – 494)	390% (195 – 585)	nd	1097% (548 – 1645)
Zooplankton respiration								
150-500 m	6.6	6.2	27.8	27.6	49%	44%	84%	267%
150-1000 m	10.0	9.1	39.7	40.0	62%	54%	103%	282%
Zooplankton C demand								
150-500 m	22.0 (18.9 – 26.4)	20.6 (17.7 – 24.8)	92.7 (79.6 – 113.3)	92.1 (79.1 – 110.6)	162% (139 – 194)	146% (126 – 176)	280% (240 – 336)	889% (763 – 1068)
150-1000 m	33.1 (28.5 – 39.8)	30.2 (25.9 – 36.2)	132.2 (113.6 – 158.9)	133.1 (114.3 – 159.9)	206% (177 – 247)	181% (156 – 218)	341% (293 – 410)	939% (807 – 1128)
Δ POC flux								
150-500 m	13.6	14.1	33.1	10.4				
150-1000 m	16.1	16.6	38.7	14.2				

Table 2. Active transport of CO_2 and DOC by zooplankton vertical migration at ALOHA (22° 45' N, 158° W) and K2 (47° N 160° E). All migratory fluxes are calculated across 150 m (*see* Methods). Active transport of CO_2 and DOC is compared to zooplankton respiration (ZR) and bacterial carbon demand (BCD), respectively, in the mesopelagic zone at each site (from Table 1). *n*=2 day and night pairs for each deployment. nd=not determined.

	Mean (±1 SD) (mg C m^{-2} d^{-1})				% ZR 150-1000 m			
	ALOHA		K2		ALOHA		K2	
	1st Dep.	2nd Dep.	1st Dep.	2nd Dep.	1st Dep.	2nd Dep.	1st Dep.	2nd Dep.
Migratory CO_2 flux	5.9 (1.4)	1.4 (0.4)	11.9 (16.9)	35.1 (32.9)	59%	15%	30%	88%
					% BCD 150-1000 m			
Migratory DOC flux	1.8 (0.4)	0.4 (0.1)	3.7 (5.2)	10.9 (10.2)	3%	1%	nd	7%

560 **Figure captions**

561

562 **Figure 1.** Models of mesopelagic microbial (bacteria and archaea) and zooplankton
563 metabolism. Mesopelagic microbes and zooplankton have fundamentally distinct nutritional
564 modes, and thus affect attenuation of sinking POC with depth (shrinking brown arrows)
565 differently. Particle-associated bacteria solubilize sinking POC into DOC, which is either
566 taken up directly and respired, or respired by suspended, "free-living" bacteria. Physical
567 mixing (red arrow) is another source of DOC to bacteria. Full-time resident zooplankton
568 consume sinking or suspended particles and convert POC to CO_2 through their respiration,
569 excrete DOC which could fuel microbial metabolism, and egest fecal pellets which augment
570 sinking POC flux (brown arrows). Vertical migrators, however, can fuel their C requirement
571 by ingesting POC in the surface mixed layer, and then subsequently metabolizing it at depth
572 (active transport, green arrow), or by directly consuming sinking POC. A proportion of the
573 resident and migrant zooplankton in the mesopelagic are carnivorous and feed on each other
574 (purple arrows). Both bacteria and zooplankton also fragment sinking particles into smaller,
575 non-sinking POC, diminishing POC flux.

576

577 **Figure 2.** Bacteria (plus archaea) abundance at stations ALOHA and K2. (a) Station ALOHA
578 (22° 45' N, 158° W) and (b) station K2 (47° N 160° E) bacteria (plus archaea) abundance
579 profiles. Presented in (a) are a compilation of Hawaii Ocean Time-series (HOT) core data
580 from immediately before (16 June 2004) and after (17 August 2004) the VERTIGO cruise, all
581 available HOT core data from depths ≥ 200 m, and mesopelagic (≥ 100 m) total
582 bacteria+archaea counts from Karner et al. 2001 ("all DAPI-stained cells" in supplementary

583 material) (Karner et al. 2001). Presented in (b) are counts from DAPI-stained samples
584 collected on the VERTIGO K2 cruise. Error bars (for Karner et al. 2001 data) are 1 SD.
585
586 **Figure 3.** Zooplankton biomass at stations ALOHA and K2. (a) Station ALOHA (22° 45' N,
587 158° W) and (b) station K2 (47° N 160° E) day and night size fractionated zooplankton
588 biomass. Values are mean (plotted at the midpoint of each of 9 depth intervals: 0-50, 50-100,
589 100-150, 150-200, 200-300, 300-400, 400-500, 500-750, and 750-1000 meters) of n=2
590 MOCNESS or IONESS (Multiple Opening/Closing Net and Environmental Sensing System or
591 Intelligent Operative Net Sampling System) casts during each sediment trap deployment.
592
593 **Figure 4.** Bacteria and zooplankton metabolic carbon requirements and POC flux at station
594 ALOHA in the N Pacific subtropical gyre. (a-d) Station ALOHA twilight zone bacteria and
595 zooplankton respiration (remineralization to CO_2) and total metabolic C demand (for bacteria=
596 C for respiration + growth, for zooplankton= C ingestion). For bacteria, n=1 cast taken during
597 each of two sediment trap deployments (values based on n=3 replicate incubations depth^{-1}
598 through the water column –integrated into depth bins). For zooplankton, values are mean (± 1
599 SE) of n=2 casts taken during each of two sediment trap deployments. A 3-way ANOVA (site
600 x depth interval x taxa) was performed on transformed respiration values ($1/x^2$) and on ranked
601 C demand values (for deployments 1 and 2 combined) after data were tested for homogeneity
602 and normality. Significant differences (ANOVA, $p< 0.05$) between bacteria and zooplankton
603 respiration were seen at all depth intervals with the exception of 400-500 m and 500-750 m. A
604 significant difference ($p<0.05$) between bacteria and zooplankton C demand was only observed
605 between 750-1000 m. (e) Sediment trap POC flux with power curve fit (note- power curve fit

606 overlaps for deployments 1 and 2). (Deployment 1– rate of flux attenuation "b"= -1.29,
607 r^2=0.89; deployment 2– "b"= -1.38, r^2=0.89; *see* Martin et al. 1987 for equation). Note:
608 Bacteria respiration and carbon demand, and zooplankton C demand, shown is calculated using
609 middle estimate conversion factors from our sensitivity analysis (*see* Methods).
610
611 **Figure 5.** Bacteria and zooplankton metabolic carbon requirements and POC flux at station
612 K2 in the NW subarctic Pacific. (a-d) Station K2 twilight zone bacteria and zooplankton
613 respiratory (remineralization to CO_2) and total metabolic C demand (for bacteria= C for
614 respiration + growth, for zooplankton= C ingestion). For bacteria in deployment 2, values are
615 mean (± 1 SE) of n=2 casts taken during the sediment trap deployment (for each cast, values
616 based on n=3 replicate incubations depth^{-1} through the water column –integrated into depth
617 bins). (Bacteria C requirements were not measured in deployment 1.) For zooplankton, values
618 are mean (± 1 SE) of n=2 casts taken during each of two sediment trap deployments. A 3-way
619 ANOVA (site x depth interval x taxa) was performed on transformed respiration values ($1/x^2$)
620 and on ranked C demand values (for deployments 1 and 2 combined) after data were tested for
621 homogeneity and normality. A significant difference (ANOVA, $p< 0.05$) between bacteria and
622 zooplankton respiration was only seen in the 150-200 m depth interval. No significant
623 differences between bacteria and zooplankton C demand were seen at any depth interval ($p<$
624 0.05). (e) Sediment trap POC flux with power curve fit. (Deployment 1– rate of flux
625 attenuation "b"= -0.52, r^2=0.88; deployment 2– "b"= -0.50, r^2=0.92; see Martin et al. 1987 for
626 equation). Note: Bacteria respiration and carbon demand, and zooplankton C demand, shown is
627 calculated using middle estimate conversion factors from our sensitivity analysis (*see*
628 Methods).

Figure 6. Integrated (150-1000 m) bacteria and zooplankton metabolic carbon demand compared to loss of sinking particulate organic carbon flux (ΔPOC) in the same depth interval. Values are from Table 1, with bars representing bacteria and zooplankton carbon demand using middle estimate conversion factors, with the range shown as error bars (with low and high range values determined using lower and upper estimate conversion factors, respectively, from our sensitivity analysis, *see* Materials and methods for details). Loss of POC flux represents mean values from Table 1.

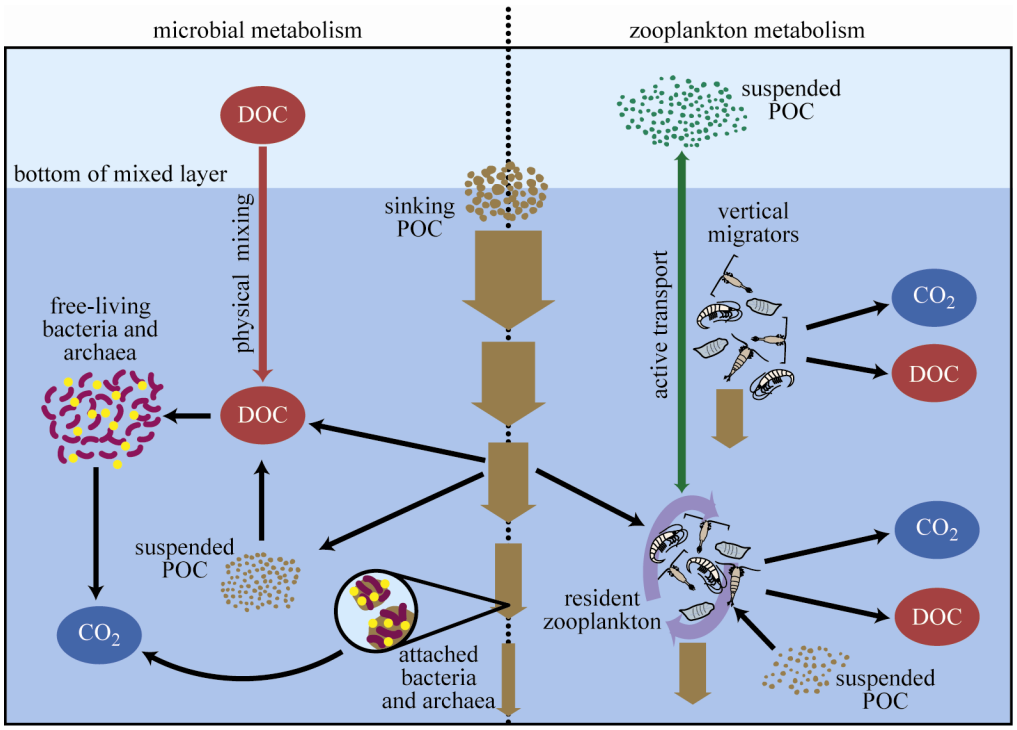
microbial metabolism
zooplankton metabolism
DOC
bottom of mixed layer
physical mixing
free-living
bacteria and
archaea
DOC
suspended
POC
CO2
sinking
POC
attached
bacteria
and archaea
suspended
POC
active transport
vertical
migrators
CO2
DOC
resident
zooplankton
CO2
DOC
suspended
POC

Figure 1

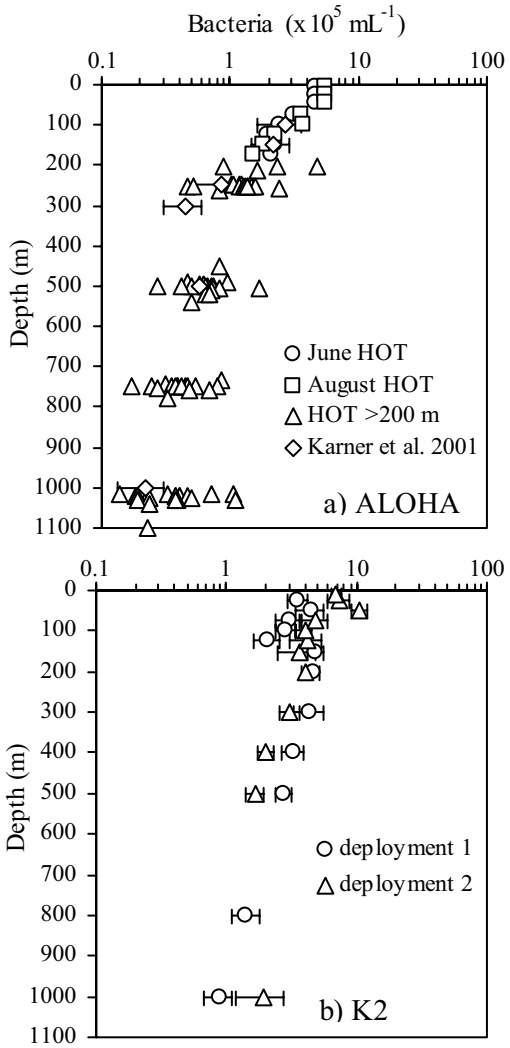
Bacteria (x 10^5 mL^-1)
0.1
1
10
100
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
June HOT
August HOT
HOT >200 m
Karner et al. 2001
a) ALOHA
deployment 1
deployment 2
b) K2

Figure 2

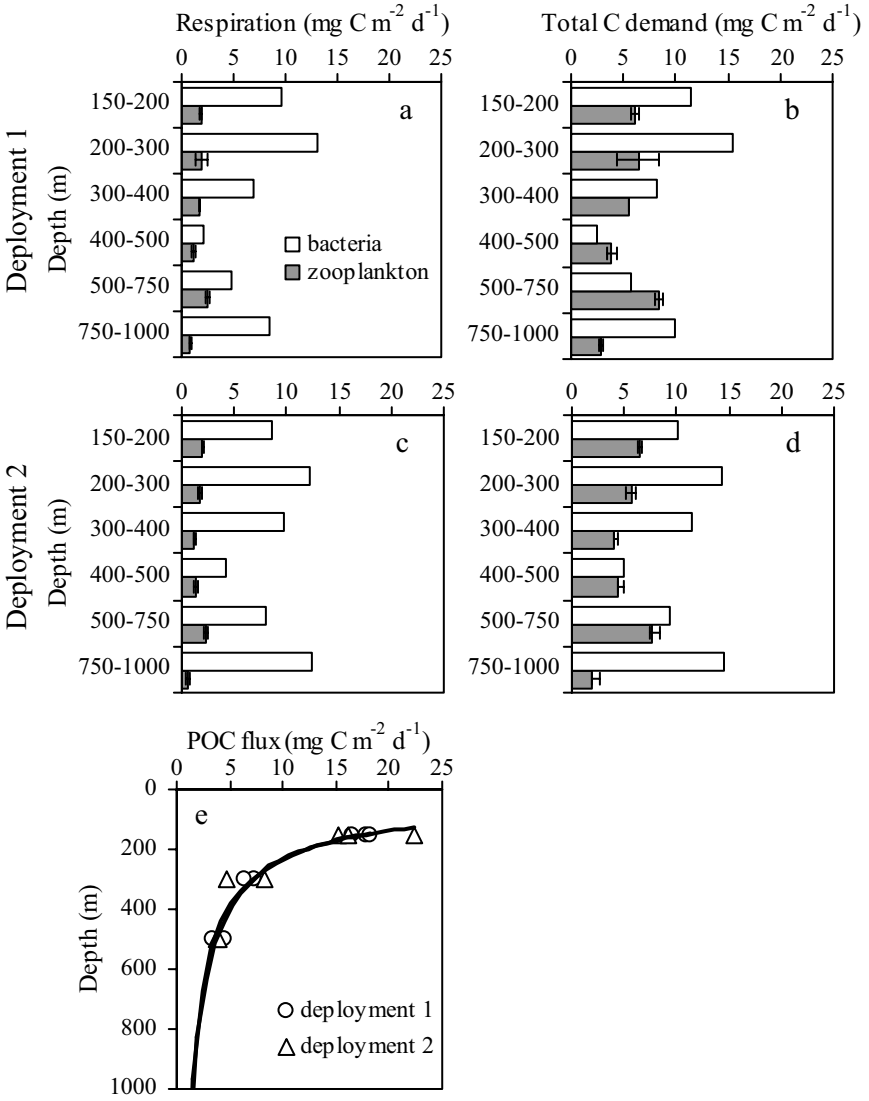
Respiration (mg C m-2 d-1)
Total C demand (mg C m-2 d-1)
0 5 10 15 20 25
Deployment 1
Deployment 2
Depth (m)
150-200
200-300
300-400
400-500
500-750
750-1000
a
b
c
d
bacteria
zooplankton
POC flux (mg C m-2 d-1)
0 200 400 600 800 1000
e
deployment 1
deployment 2

Figure 4

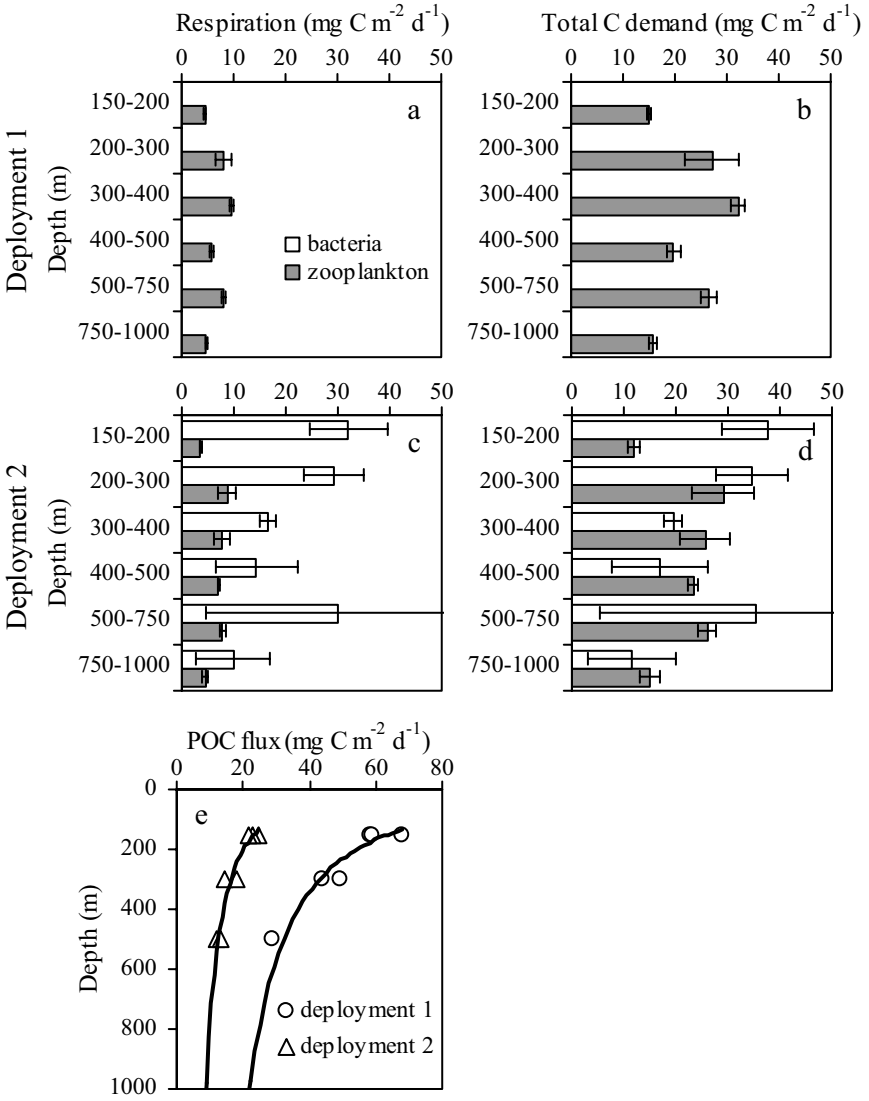
Respiration (mg C m-2 d-1)
Total C demand (mg C m-2 d-1)
0 10 20 30 40 50
150-200
200-300
300-400
400-500
500-750
750-1000
Deployment 1
Deployment 2
Depth (m)
a
b
c
d
bacteria
zooplankton
POC flux (mg C m-2 d-1)
0 20 40 60 80
0
200
400
600
800
1000
e
deployment 1
deployment 2

Figure 5

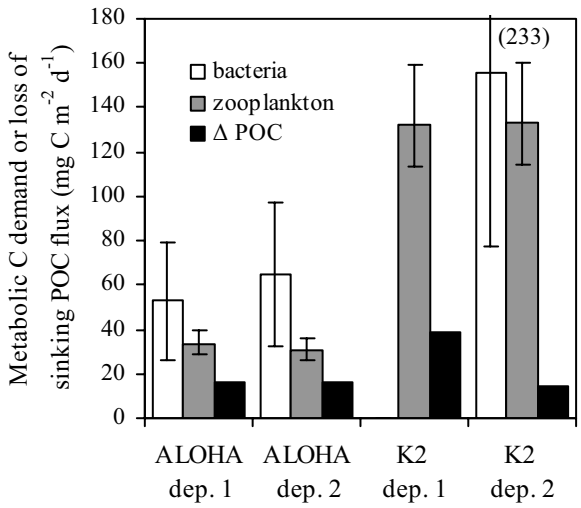
Metabolic C demand or loss of sinking POC flux (mg C m-2 d-1)
180
160
140
120
100
80
60
40
20
0
bacteria
zooplankton
Δ POC
(233)
ALOHA dep. 1
ALOHA dep. 2
K2 dep. 1
K2 dep. 2

Figure 6