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Deborah K. Steinberg
Virginia Institute of Marine Science

Benjamin A.S. Van Mooy

Ken O. Buesseler

Philip W. Boyd

Toru Kobari

See next page for additional authors

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

Deborah K. Steinberg[1]
Virginia Institute of Marine Science, 1208 Greate Rd., Gloucester Point, Virginia 23062

Benjamin A. S. Van Mooy and Ken O. Buesseler
Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, 266 Woods Hole Road, Woods Hole, Massachusetts 02543

Philip W. Boyd
NIWA Centre for Chemical and Physical Oceanography, Department of Chemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand

Toru Kobari
Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan

David M. Karl
Department of Oceanography, University of Hawaii, Honolulu, Hawaii 96822

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 (150–1,000 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.

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 to be 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 1,000 m) with the majority of the sinking POC lost between 100 m and 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 affect remineralization of sinking POC to carbon dioxide (CO_2) (Fig. 1).

Bacterial abundance also decreases with depth (Ducklow 1993; Nagata et al. 2000) (although the relative abundance

[1] Corresponding author (debbies@vims.edu).

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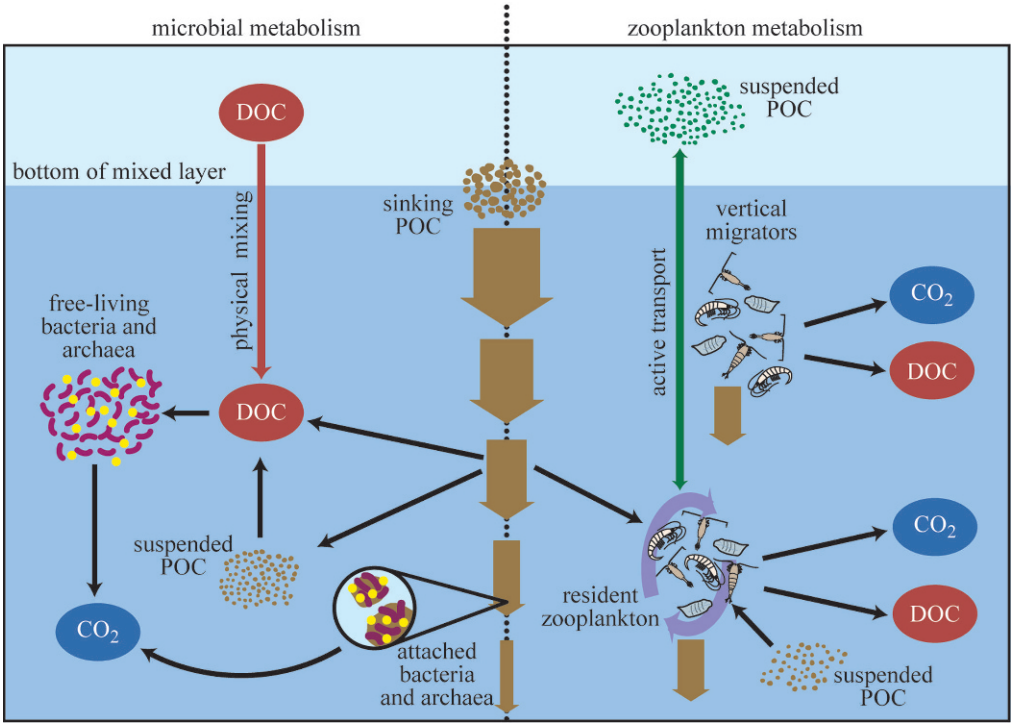

Fig. 1. Models of mesopelagic microbial (bacteria and archaea) and zooplankton metabolism. Mesopelagic microbes and zooplankton have fundamentally distinct nutritional modes, and thus affect attenuation of sinking POC with depth (shrinking brown arrows) differently. Particle-associated bacteria solubilize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

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 carbon (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 that sinking detritus, or 'marine snow,' is an important food source for both deep-sea nonmigrating (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 northwest subarctic

Pacific. At both sites we compared losses of sinking POC measured by neutrally buoyant sediment traps with metabolic requirements of bacteria and zooplankton 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 meet their nutritional and metabolic requirements.

Methods

Study sites—Samples were collected and experiments conducted aboard the RV *Kilo Moana* at the HOT station ALOHA (22°45′N, 158°W) from 22 June 2004 to 09 July 2004 and aboard the RV *Roger Revelle* at K2 (47°N, 160°E) from 22 July 2005 to 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 $m^{-2}\,d^{-1}$ (PP was measured via shipboard deck incubations and was lower than the HOT in situ PP climatology.), low Chl *a* (~0.1 mg m^{-3} at the surface), and a phytoplankton assemblage consisting of small diatoms, coccolithophorids, and picoplankton. K2 was characterized by colder waters (10°C at the surface), a mixed layer depth of 26 m, higher surface nutrients (12 μmol L^{-1} mixed layer dissolved inorganic nitrogen [DIN]) and PP (365–530 mg C $m^{-2}\,d^{-1}$), variable but higher Chl a (~0.8 mg m^{-3} at the surface), and a phytoplankton assemblage consisting of picoplankton and large diatoms. Conditions were relatively uniform during our ~3-week occupation of each site, with the exception of an increase in particle flux over the study period at K2 (*see* Results).

Particle flux—We measured particle flux using neutrally buoyant sediment traps (NBSTs) during two consecutive 3–5 d deployments at 150 m, 300 m, and 500 m at each site (Buesseler et al. 2007). NBSTs were used to minimize potential hydrodynamic sampling biases due to fluid flow over and within the trap (Buesseler et al. 2007). Replicate NBSTs were deployed (up to n = 3 at 150 m) with good agreement between traps (*see* Results). Zooplankton swimmers were carefully removed from all samples first via screening followed by hand-picking under a dissecting microscope (250–350× magnification). POC was obtained by difference between total C, measured by carbon, hydrogen nitrogen (CHN) analysis, and particulate inorganic carbon (PIC). PIC was measured by acidification of the sample with phosphoric acid and titration of CO_2 by a coulometric method. POC flux at 1,000 m was calculated by fitting a power function (Buesseler et al. 2007; Martin et al. 1987) to mean trap POC fluxes at each depth. We then compared losses of sinking POC, using differences in export flux measured by NBSTs at different depths, with metabolic C requirements of bacteria and zooplankton for each of the two NBST deployments at each site.

Bacteria respiration and carbon demand—Depth integrated bacteria respiration (BR) and BCD was based on our measures of BP at discrete depths throughout the water column, and published bacterial growth efficiency (BGE). BP was determined using 30 mL [^{3}H]-thymidine incorporation incubations conducted shipboard at atmospheric pressure and in situ temperatures (Fuhrman and Azam 1982) on water samples collected from the surface to 1,000 m. Thymidine incorporation was converted to carbon demand using the commonly reported range of thymidine conversion factors (1.0–2.0 × 10^{18} cells mol^{-1}; Ducklow 2000), applying a carbon conversion factor of 15 fg C $cell^{-1}$ (Ducklow 2000), and a BGE range of 0.10–0.15 for open-ocean bacteria (Del Giorgio and Cole 2000; Reinthaler et al. 2006). This sensitivity analysis allowed us to account for uncertainties inherent in the conversions and provided a middle (applying a thymidine conversion factor of 2.0 × 10^{18} cells mol^{-1} and a BGE of 0.15), lower (thymidine conversion factor of 1.0 × 10^{18} cells mol^{-1} and a BGE of 0.15), and upper (thymidine conversion factor of 2.0 × 10^{18} cells mol^{-1} and a BGE of 0.1) estimate (Table 1). Bacteria were also enumerated at each site using DAPI (4',6-diamidino-2-phenylindole) staining and epifluorescence microscopy.

While the influence of pressure on bacterial production conversion factors has not yet been systematically examined, there is no a priori reason to expect that they should vary with depth, and conversion factors for surface communities are commonly applied to the mesopelagic (Nagata et al. 2000; Reinthaler et al. 2006). However, the thymidine incorporation rates derived from incubations conducted at atmospheric pressure may underestimate true rates (Bianchi et al. 1999), and, thus, the BP rates we present here are likely to be conservative. The range of BGE values that we applied was lower than the value of 0.20 that Nagata et al. (2000) applied to the mesopelagic; similar to our range, their value was 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):

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

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

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

Zooplankton respiration and carbon demand—Depth integrated zooplankton respiration (ZR) and zooplankton carbon demand (ZCD) were 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 nine discrete depth intervals from 0 m to 1,000 m with a 1-m^2, 335-μm mesh MOCNESS (Multiple Opening/Closing Net and Environmental Sampling System) or IONESS (Intelligent Operative Net Sampling System) during both day and night. The net tow samples were split: Half were size-fractionated (5-mm, 2-mm, 1-mm, 0.5-mm, and 0.35-mm fractions) and frozen for biomass analyses (dried 24 h at 60°C and then weighed), and half were 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) because 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:

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

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 an 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 because 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 1,000 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, $p > 0.05$). (This is likely because some diel migrators only migrated as shallow as 150–250 m and some came from below 1,000 m into the mesopelagic zone at night; *see* fig. 3 in 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 150 m were up to 2-fold higher at K2 (range 2.1–10.5 $\times$ 10^5 cells mL^{-1}) than ALOHA (range 1.8–5.5 $\times$ 10^5 cells mL^{-1}), and decreased exponentially with depth at both sites, becoming up to 9-fold higher in the mesopelagic ($\geq$150 m) at K2 (0.9–4.9 $\times$ 10^5 cells mL^{-1}) than reported at ALOHA (0.1–4.8 $\times$ 10^5 cells mL^{-1}) (Karner et al. 2001) (Fig. 2). Daytime mesopelagic zooplankton biomass (150–1,000 m) was an order of magnitude higher at K2 (mean $\pm$1 SD = 6.9 $\pm$ 0.7 g dry wt m^{-2}, $n = 4$) than ALOHA (0.5 $\pm$ 0.1 g dry wt m^{-2}, $n = 4$), partially due to high abundance of the large

copepods *Neocalanus* spp. and *Eucalanus* sp. at K2 (Fig. 3). Diel vertical migration was pronounced at both sites: Nighttime zooplankton biomass was higher than daytime biomass in the upper 0–150 m by a factor of 1.7 ± 0.5 at ALOHA, as previously reported (Al-Mutairi and Landry 2001), and by a factor of 2.5 ± 1.4 at K2. Copepods constituted 74 ± 0.5% and 70 ± 4% of daytime mesopelagic zooplankton abundance at ALOHA and K2, respectively (Steinberg et al. in press).

Bacteria and zooplankton metabolic requirements—At ALOHA bacteria were primarily responsible for metabolizing sinking POC, while at K2 zooplankton and bacteria both contributed equally. At ALOHA the estimated BR (remineralization of organic C to CO_2) significantly exceeded ZR at nearly all depths for both deployments (Fig. 4), with integrated BR 2- to 10-fold higher than ZR for both deployments (Table 1). BCD is the carbon required for respiration and growth, while ZCD is carbon ingested and subsequently assimilated for use in respiration, excretion, growth, and reproduction, plus unassimilated carbon egested as feces. ALOHA BCD also exceeded ZCD at nearly all depths (Fig. 4). Integrated mesopelagic BCD ranged from slightly lower than ZCD to 4-fold higher than ZCD (Table 1). The profiles of sinking particle flux at ALOHA were nearly identical between the two deployments, with 75% of the 150 m POC flux removed by 500 m (Fig. 4).

Carbon demand of mesopelagic bacteria and zooplankton was considerably higher at K2 than at ALOHA (Fig. 5, note *x*-axis scale is double that of Fig. 4), despite the colder temperatures at K2 (22°C vs. 2°C at 150 m, and 8°C vs. 3°C at 500 m, at ALOHA and K2, respectively). This reflects the higher bacteria and zooplankton biomass at K2. Mesopelagic integrated BR was up to 5-fold higher than ZR (Table 1). However, BCD and ZCD are comparable to one another at depths below 200 m (and not statistically different at any depth, Fig. 5), with integrated BCD less than a factor of two higher or lower than ZCD (Table 1). Sinking particle flux was higher at K2 than ALOHA and decreased between deployments; but on both deployments only ~25% of the 150 m POC flux at K2 was removed by 500 m (Fig. 5). Vertical patterns in both BCD and ZCD were similar between deployments at each site.

Comparison of metabolic requirements to attenuation of sinking POC—Integrated BR and BCD accounted for two to four times the loss of sinking POC in the mesopelagic zone at ALOHA, while ZR was approximately half, and ZCD accounted for twice the loss of sinking POC flux (Table 1, using middle estimate conversion factors). At K2, BCD and ZCD accounted for an even higher proportion of sinking POC loss with depth vs. at ALOHA, due both to the considerably smaller decrease in sinking flux (Figs. 4 and 5), and the considerably higher mesopelagic zooplankton biomass-derived ZCD at K2 (Fig. 3). Thus our results also indicate that K2 BCD was 10-fold greater than the loss of sinking POC, while ZCD was 3- to 9-fold higher. In Fig. 6, we extract the integrated 150–1,000 m BCD and ZCD data from Table 1 to illustrate

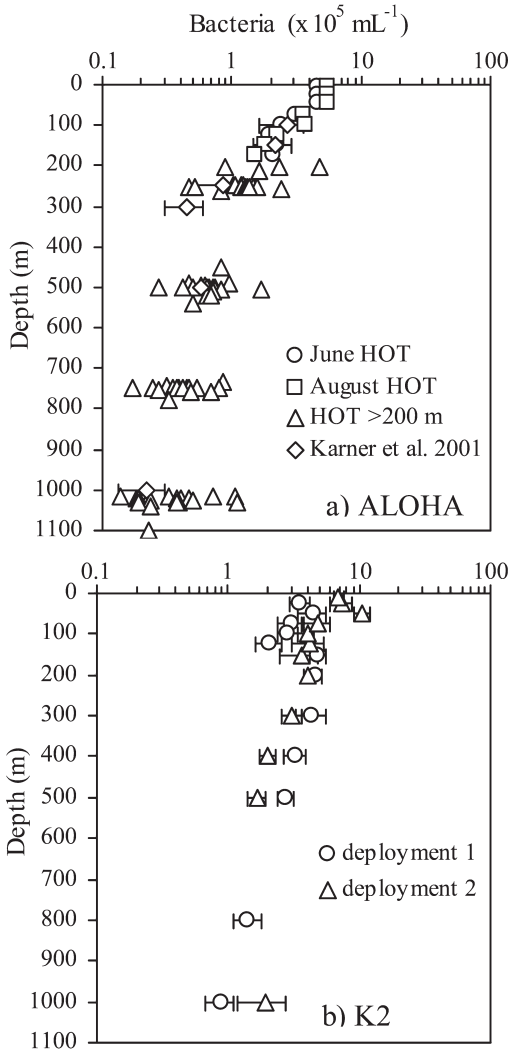

Fig. 2. Bacteria (plus archaea) abundance at stations ALOHA and K2. (a) Station ALOHA (22°45′N, 158°W) and (b) station K2 (47°N, 160°E) bacteria (plus archaea) abundance profiles. Presented in (a) are a compilation of Hawaii Ocean Time-series (HOT) core data from immediately before (16 June 2004) and after (17 August 2004) the VERTIGO cruise, all available HOT core data from depths ≥200 m, and mesopelagic (≥100 m) total bacteria+archaea counts from Karner et al. 2001 (''all DAPI-stained cells'' in supplementary material) (Karner et al. 2001). Presented in (b) are counts from DAPI-stained samples collected on the VERTIGO K2 cruise. Error bars (for Karner et al. 2001 and K2 data) are 1 SD.

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).

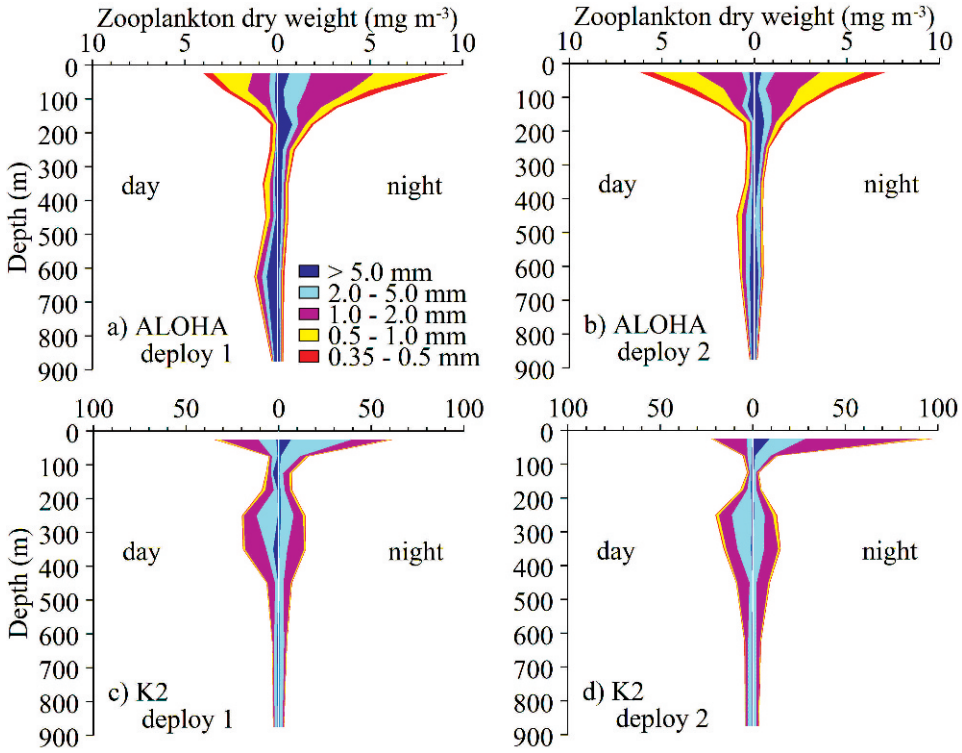

Fig. 3. Zooplankton biomass at stations ALOHA and K2. (a) Station ALOHA (22°45'N, 158°W) and (b) station K2 (47°N, 160°E) day and night size fractionated zooplankton biomass. Values are mean (plotted at the midpoint of each of nine depth intervals: 0–50, 50–100, 100–150, 150–200, 200–300, 300–400, 400–500, 500–750, and 750–1,000 meters) of $n = 2$ MOCNESS or IONESS (Multiple Opening/Closing Net and Environmental Sensing System or Intelligent Operative Net Sampling System) casts during each sediment trap deployment.

Discussion

Excess metabolic C demand in the mesopelagic—It is evident that sinking particles alone cannot 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 of protozoan grazers, which are an important link in the microbial loop between bacteria and zooplankton but undoubtedly contribute an additional C demand in the mesopelagic, Gowing et al. 2003). However, the average daily rate of downward DOC export to the mesopelagic at ALOHA (30 mg C m^{-2} d^{-1} below 100 m; Emerson et al. 1997) is insufficient to support even the observed BCD above 200 m. Furthermore, we sampled at ALOHA during summer stratification when vertical mixing is minimal. At K2 it is possible that vertical mixing was more significant, but DOC export would need to exceed POC export by an order of magnitude to balance the mesopelagic C demand; to our knowledge this has never been observed in the open ocean. The ambient DOC in bathypelagic waters is 4,000–6,000 years old (Bauer et al. 1992) and thought to be relatively unavailable to bacteria; global distributions of DOC and BCD support this assertion (Nagata et al. 2000). DOC use also accounts for only ~10–20% of the apparent oxygen utilization in the mesopelagic global ocean, suggesting an alternate C source (Arístegui et al. 2005).

Furthermore, suspended POC concentrations at depth are inadequate to support sustained metabolic demand. For example, at K2 suspended POC below 150 m was ~6 mg C m^{-3}, and with a combined metabolic C demand at K2 of 0.4–0.6 mg C m^{-3} d^{-1} for both zooplankton and bacteria (Table 1), POC stocks would be depleted in just 10–15 d. Thus a new supply of POC (other than from sinking particles) would be required to keep up with the demand, for which there is no evidence (e.g., no significant advection). Thus, while a complete C budget is beyond the scope of our study, even our most conservative estimates

Table 1. Metabolic carbon requirements of bacteria and zooplankton in the twilight zone as compared to loss of (Δ) sinking particulate organic carbon flux in the same depth interval. All units are mg C m^{-2} d^{-1}, with the exception of metabolic C requirements as loss of particulate organic carbon (POC) flux (%). Mean values are reported (using middle estimate conversion factors) with the range given in parentheses (using lower–upper estimate conversion factors) from our sensitivity analysis (*see* Methods for details). No range is given for zooplankton respiration (*see* Methods). Replication is as reported in Figs. 4 and 5. The ΔPOC flux was calculated from measurements in Figs. 4 and 5, with 1,000-m flux calculated from fitting a power function (Buesseler et al. 2007; Martin et al. 1987) to mean trap organic carbon fluxes measured at 150, 300, and 500 m.

	Metabolic C requirements				% Metabolic C requirements 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% (446–1,416)
150–1,000 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% (466–1,481)
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	1,049% (524–1,573)
150–1,000 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	1,097% (548–1,645)
Zooplankton respiration								
150–500 m	6.6	6.2	27.8	27.6	49%	44%	84%	267%
150–1,000 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% (763–1,068)
150–1,000 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% (807–1,128)
Δ POC flux								
150–500 m	13.6	14.1	33.1	10.4				
150–1,000 m	16.1	16.6	38.7	14.2				

* nd, not determined.

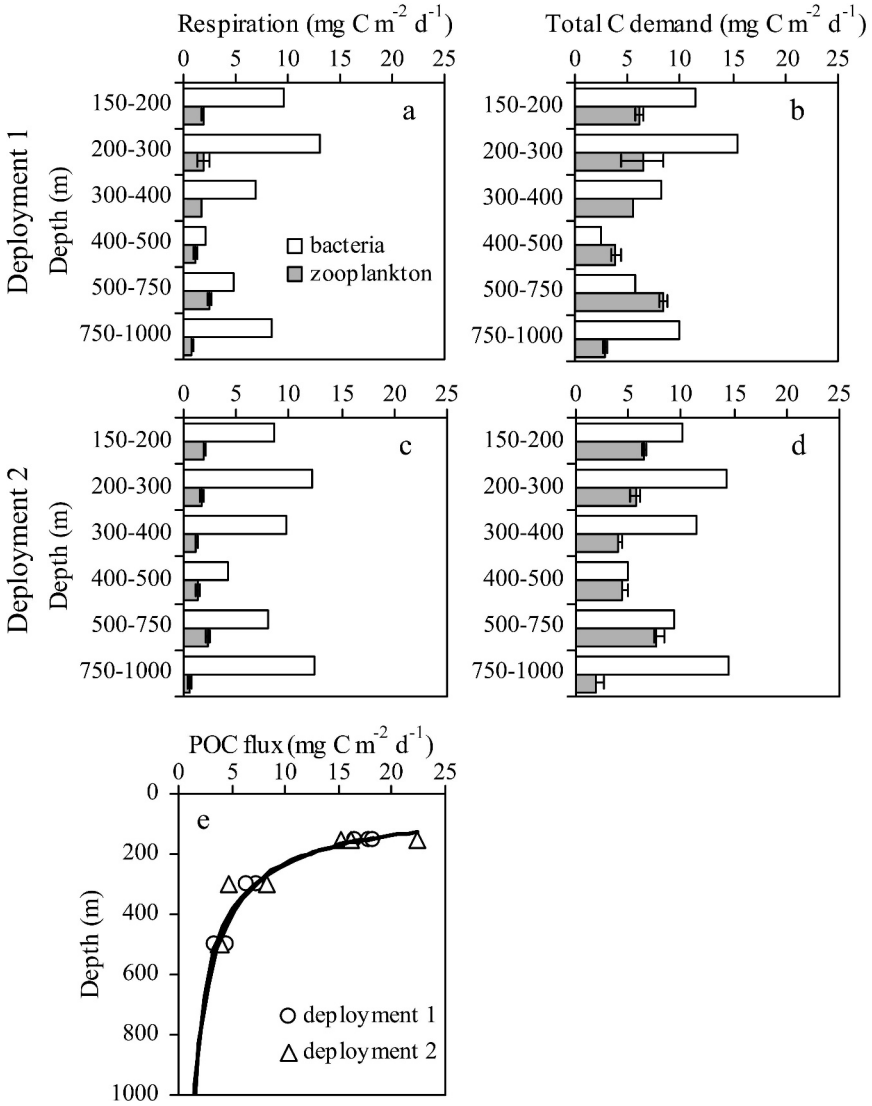

Fig. 4. Bacteria and zooplankton metabolic carbon requirements and POC flux at station ALOHA in the N Pacific subtropical gyre. (a–d) Station ALOHA twilight zone bacteria and zooplankton respiration (remineralization to CO_2) and total metabolic C demand (for bacteria = C for respiration + growth, for zooplankton = C ingestion). For bacteria, $n = 1$ cast taken during each of two sediment trap deployments (values based on $n = 3$ replicate incubations depth^{-1} through the water column –integrated into depth bins). For zooplankton, values are mean (± 1 SE) of $n = 2$ casts taken during each of two sediment trap deployments. A 3-way ANOVA (site $\times$ depth interval $\times$ taxa) was performed on transformed respiration values ($1/x^2$) and on ranked C demand values (for deployments 1 and 2 combined) after data were tested for homogeneity and normality. Significant differences (ANOVA, $p < 0.05$) between bacteria and zooplankton respiration were seen at all depth intervals with the exception of 400–500 m and 500–750 m. A significant difference ($p < 0.05$) between bacteria and zooplankton C demand was only observed between 750–1,000 m. (e) Sediment trap POC flux with power curve fit (note- power curve fit overlaps for deployments 1 and 2). (Deployment 1– rate of flux attenuation "b" = -1.29, $r^2 = 0.89$; deployment 2– "b" = -1.38, $r^2 = 0.89$; *see* Martin et al. 1987 for equation). Note: Bacteria respiration and carbon demand, and zooplankton C demand, shown is calculated using middle estimate conversion factors from our sensitivity analysis (*see* Methods).

indicate neither sinking POC, suspended POC, nor imported DOC can meet the significant 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

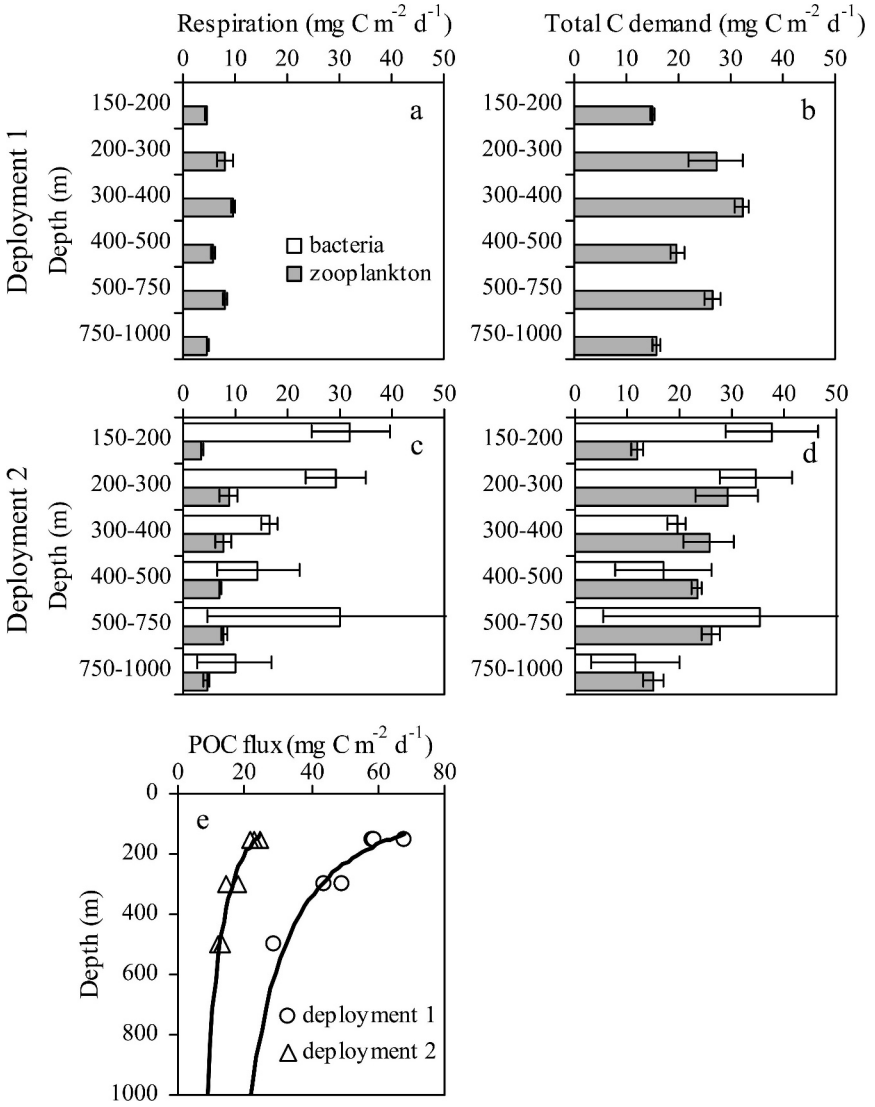

Fig. 5. Bacteria and zooplankton metabolic carbon requirements and POC flux at station K2 in the NW subarctic Pacific. (a–d) Station K2 twilight zone bacteria and zooplankton respiratory (remineralization to CO_2) and total metabolic C demand (for bacteria = C for respiration + growth, for zooplankton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

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 150–1,000 m zooplankton respi-

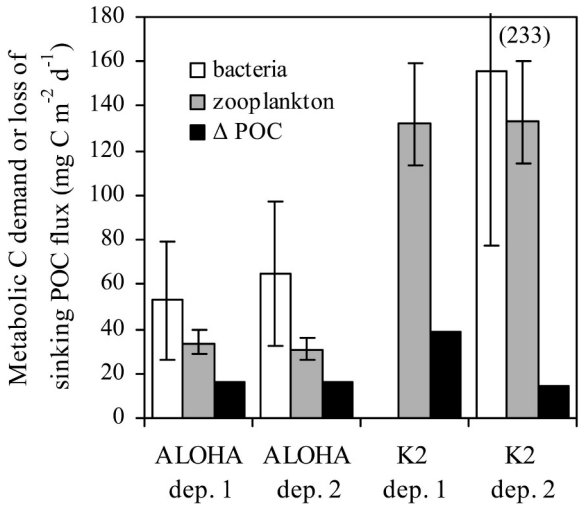

Fig. 6. Integrated (150–1,000 m) bacteria and zooplankton metabolic carbon demand compared to loss of sinking particulate organic carbon flux (ΔPOC) in the same depth interval. Values are from Table 1, with bars representing bacteria and zooplankton carbon demand using middle estimate conversion factors, 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* Methods for details). Loss of POC flux represents mean values from Table 1.

ration, with a 95% confidence interval of 10–85%, respiration by migrators was not significantly different from integrated (150–1,000 m) zooplankton community respiration (*t*-test, $p > 0.05$). Thus, we conclude that mesopelagic zooplankton could sustain a significant amount of their C demand by diel vertical migration. Excretion by zooplankton (migratory or nonmigratory) 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 nonmigrating, mesopelagic micronekton predator community at ALOHA to estimate prey mortality, Al-Mutairi and Landry (2001) calculated that mortality of zooplankton diel migrators was equal to 32% of the diel migrant respiratory flux. Using the approach of Zhang and Dam (1997) to estimate weight-specific mortality of diel migrators in our study yields a mean diel mortality flux at ALOHA that is equal to, and at K2 is 1.3-fold, the diel respiratory flux at each site (Table 2). Similarly, mortality loss of ontogenetic vertical migrators in the mesopelagic zone during winter can also supply a significant amount of POC annually. This is particularly important in the subarctic Pacific, where mortality loss of ontogenetic migrators is equal to 92% of annual POC flux measured by sediment traps at 1,000 m (Kobari et al. 2003).

Furthermore, the proportion of zooplankton biomass that is carnivorous increases with depth (Vinogradov and Tseitlin 1983), thus mesopelagic zooplankton must meet a significant fraction of their energy requirements via carnivory. In the northwest subarctic Pacific carnivorous zooplankton comprised ~25% of the zooplankton biomass between 200 m and 500 m and >50% of the biomass between 500 m and 1,000 m (Vinogradov and Tseitlin 1983). At K2 we measured increases in carnivore abundance at depth, forming distinct layers in the mesopelagic. Chaetognath density, for example, increased up to 30-fold between 150 m and 300 m compared to the upper 150 m (Steinberg et al. in press). Processes associated with carnivory, such as dissolved organic matter (DOM) release from "sloppy feeding," could also fuel BCD. However, we emphasize that ultimately many mesopelagic carnivores get their energy from sinking particles, because the carnivores feed on animals that were themselves feeding on sinking particles. Thus, carnivory doesn't help solve the excess C demand problem unless the animals the carnivores consume come from outside the system (e.g., via advection or diel vertically migrating carnivores feeding on animals in the euphotic zone), or if the carnivory occurs on a different time scale than our study—such as the fall and winter supply of ontogenetic migrators. Further studies of taxonomic community structure and food web dynamics

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 dissolved organic carbon (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.

	Mean (±1 SD) (mg C m^{-2} d^{-1})				% ZR 150–1,000 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%
					%z BCD 150–1,000 m			
Migratory DOC flux	1.8 (0.4)	0.4 (0.1)	3.7 (5.2)	10.9 (10.2)	3%	1%	nd*	7%

* nd, not determined.

of the mesopelagic zone are needed to determine the C demand that can be met by consumption of other animals.

Mesopelagic zooplankton (full-time residents and migrators) also produce fecal pellets at depth that are consumed by detritivores (Sasaki et al. 1988; Yamaguchi et al. 2002), as evidenced by the appearance of new classes of fecal pellets in our deeper 300 m and 500 m NBST's compared to the 150 m traps (Wilson et al. in press). Migrators also actively transport POC as fecal pellets produced at depth as a result of their surface feeding (Schnetzer and Steinberg 2002), which can be consumed by zooplankton or solubilized by bacteria. This consumption of animals and reprocessing of sinking particles adds further complexity to developing C budgets for the mesopelagic and in modeling the relative roles of heterotrophic bacteria and zooplankton in the understudied deep ocean.

Both bacterial and zooplankton communities are important remineralizers and consumers of sinking POC in the ocean's twilight zone, but sinking POC supplies only a portion of the C they require. Certainly, episodic production of particles in the upper ocean and their subsequent export could lead to a temporal offset in any direct comparison of contemporaneous processes (Karl et al. 2003). However, we argue that a significant fraction of the zooplankton C demand in the mesopelagic must be met by spatially uncoupled organic C consumption and production by migrating zooplankton, as well as by carnivory. The result is an active microbial loop in the dark waters of the mesopelagic that is ultimately supported by phytoplankton but proximately supported by zooplankton. These pathways, and their linkages between the microbial and zooplankton communities, need to be further explored and incorporated into biogeochemical models that predict global C sequestration in the deep sea.

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