

Mesopelagic zooplankton metabolic demand in the North Pacific Subtropical Gyre

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Abstract

We assess the metabolic demand of mesopelagic zooplankton for carbon, nitrogen, and phosphorus in the North Pacific Subtropical Gyre (NPSG). We compare zooplankton metabolic demand with the attenuation of sinking particle fluxes, and find the average metabolic demand for resident midwater zooplankton can account for 1.3× the loss of sinking particulate organic carbon and particulate nitrogen fluxes, and 2.6× the loss of particulate phosphorus fluxes. Zooplankton metabolic demand for carbon remains significant (0.4–1.9×) relative to the loss in sinking particulate fluxes, even when new depth-specific dry weight conversion factors and recent global-bathymetric models of zooplankton metabolism are applied. These new models reduce zooplankton carbon demands to reasonably match particle flux attenuation in the mesopelagic zone. Zooplankton metabolic demand for phosphorus is particularly large in comparison to particle flux attenuation (1.5–9×), and when temporal change in the molar carbon : phosphorus ratio of the attenuation in particulate fluxes are considered, mesopelagic zooplankton in the NPSG may episodically become phosphorus-limited. Midwater zooplankton have the potential to be important mediators of carbon flux to the deep ocean in the NPSG.

Zooplankton in the mesopelagic zone (between about 150 m to 1000 m water depth) must survive in a challenging environment characterized by reduced light levels, increased hydrostatic pressure, cold temperatures, and a low and intermittent food supply. A recent study evaluating midwater zooplankton stable isotope composition (Hannides et al. 2013) indicates that a major food source for these populations is particulate material that sinks from surface waters through the mesopelagic zone. However, evaluation of zooplankton metabolism at depth indicates that their carbon (C) demand can be greater than the attenuation (or midwater removal) of sinking particulate organic C (POC) fluxes by 1–9 fold (Steinberg et al. 2008b). If the metabolic demand of microbial populations is also considered (Steinberg et al. 2008b), midwater C budgets become even more imbalanced. Alternate food sources at depth could help reduce these discrepancies; however, a major source of uncertainty in these calculations is the allometric relationships used to calculate zooplankton metabolism (Burd et al. 2010). While these relationships have been largely based on epipelagic zooplankton populations (Ikeda 1985), previous studies indicate that animal metabolism can decrease significantly with depth (Seibel and Drazen 2007). Recent experiments involving mesopela-

gic and bathypelagic zooplankton supports depth-dependence in their metabolic rates (Ikeda et al. 2007a; Ikeda and Takahashi 2012; Ikeda 2013a). Assimilation efficiencies and dry weight (dry wt) conversion factors (e.g., dry wt individual (ind.)⁻¹) used to estimate metabolic demand are also poorly constrained at depth.

The primary goal of this research is to evaluate midwater resident zooplankton C, and for the first time nitrogen (N) and phosphorus (P), metabolic demand, and compare the stoichiometry of this demand with the attenuation of sinking particle fluxes in the mesopelagic zone of the North Pacific Subtropical Gyre (NPSG). We further apply new dry wt conversion factors and recently described global-bathymetric models of zooplankton respiration (Ikeda et al. 2007a) to assess the potential of these relationships for reducing the imbalance in midwater C budgets found in previous studies at this site (Steinberg et al. 2008b).

Methods

Zooplankton were collected in late August 2011 at a station west of Oahu, Sta. Kahe (21.3°N, 158.3°W), and at the NPSG Hawaii Ocean Time-series (HOT) station, Sta. ALOHA (22.45°N, 158°W). In early September 2013, zooplankton were collected at a station north of Oahu, Sta. Kaena Point

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(21.6°N, 158.2°W). All zooplankton collections were conducted using a Multiple Opening-Closing Net and Environmental Sensing System (MOCNESS; B.E.S.S., Falmouth, Massachusetts) fitted with 1 m² 200 μm mesh plankton nets. Plankton were collected at Sta. ALOHA during the daytime (10:00-14:00 h) and at all sites during the nighttime (22:00 h–02:00 h) by oblique tows at the following depth intervals between the surface and 1000 m: 0-50 m, 50-100 m, 100-150 m, 150-200 m, 200-250 m, 250-300 m, 300-500 m, 500-700 m, and 700-1000 m (the last interval was sampled in 2011 only). Onboard, zooplankton were rapidly wet-sieved in filtered sea water using 0.2 mm, 0.5 mm, 1.0 mm, 2.0 mm, and 5.0 mm mesh sieves into different size fractions and frozen at –20°C. In the laboratory, zooplankton were defrosted and weighed to determine wet weight (wet wt) biomass, sample was removed for counts of total zooplankton abundance (2013 only); then, all remaining sample was lyophilized and weighed again to determine dry weight (dry wt) biomass. Numbers of individuals (ind.) per filter were calculated from microscopic analysis of samples, and new dry wt conversion factors (dry wt ind.⁻¹) determined for each depth and size fraction.

Water temperature (°C), salinity, and oxygen (O₂) content (μmol kg⁻¹) were determined using a Sea-Bird SBE 9/11 Conductivity–Temperature–Depth recorder and a Sea-Bird SBE 43 dissolved oxygen sensor on seven casts during the August 2011 cruise. Average temperature, salinity, and O₂ content for each zooplankton depth interval (*see* earlier) were determined, and these values were then averaged across the seven casts. O₂ content was converted to a fraction of O₂ saturation by applying the equations of Garcia and Gordon (1992) to determine O₂ solubility in seawater.

Zooplankton C, N, and P demand at depth was initially determined following the methods Steinberg et al. (2008b) used for C. Briefly, rates of O₂ respiration, ammonium excretion, and phosphate excretion were determined by applying the empirical allometric relationships of Ikeda (1985) to resident zooplankton dry wt biomass at Sta. ALOHA (*i.e.*, the biomass of zooplankton residing at depths of 150-1000 m at night, when vertical migrants were feeding in surface waters). Depth-specific dry wt conversion factors for each size fraction (dry wt ind.⁻¹) and average water temperatures evaluated at each depth interval were used in these calculations. O₂ consumption rates were converted to respiratory C equivalents assuming a respiratory quotient of 0.8, that is, a protein-based diet. All hourly rates were converted to daily rates assuming that resident zooplankton metabolize 24 h a day at depth, resulting in the calculation of daily zooplankton respiration (ZR: g C m⁻² d⁻¹), N excretion (Z_N: g N m⁻² d⁻¹), and P excretion (Z_P: g P m⁻² d⁻¹) in the mesopelagic zone. Finally, rates of zooplankton respiration and excretion were converted to total zooplankton C, N, and P metabolic demand following the equation Steinberg et al. (1997) used for C, that is:

Table 1. The fraction of assimilated C that is respired (R_C) and the assimilation efficiencies of C (AE_C). When necessary, R_C was calculated from net growth efficiencies. “Upper,” “Middle,” and “Lower” refer to AE_C and R_C used to calculate “upper,” “middle,” and “lower” values of zooplankton C demand in the mesopelagic zone.

	R_C	Reference	AE_C	Reference
	0.43	Mauchline (1998)	0.44	Mauchline (1998)
	0.51	Mauchline (1998)	0.51	Paffenhöfer and Koster (2005)
	0.54	Mauchline (1998)	0.65	Almeda et al. (2011)
	0.68	Almeda et al. (2011)	0.68	Mauchline (1998)
	0.76	Pagano and Saint-Jean (1994)	0.69	Landry et al. (1984)
	0.77	Almeda et al. (2011)	0.69	Pagano and Saint-Jean (1994)
	0.78	Mauchline (1998)	0.73	Paffenhöfer and Koster (2005)
	0.79	Pagano and Saint-Jean (1994)	0.79	Landry et al. (1984)
	0.88	Mauchline (1998)	0.81	Landry et al. (1984)
	0.89	Mauchline (1998)	0.84	Mauchline (1998)
	0.91	Mauchline (1998)	0.85	Landry et al. (1984)
	0.91	Pagano and Saint-Jean (1994)	0.86	Mauchline (1998)
	0.91	Mauchline (1998)	0.86	Almeda et al. (2011)
	0.93	Mauchline (1998)	0.89	Mauchline (1998)
	0.93	Pagano and Saint-Jean (1994)	0.92	Paffenhöfer and Koster (2005)
			0.93	Paffenhöfer and Koster (2005)
Upper	0.4		0.4	
Middle	0.7		0.7	
Lower	0.9		0.9	

$$ZCD \text{ (mg C m}^{-2}\text{d}^{-1}\text{)} = ZR/(R_C \times AE_C) \quad (1)$$

$$ZND \text{ (mg N m}^{-2}\text{d}^{-1}\text{)} = Z_{E_N}/(E_N \times AE_N) \quad (2)$$

$$ZPD \text{ (mg P m}^{-2}\text{d}^{-1}\text{)} = Z_{E_P}/(E_P \times AE_P) \quad (3)$$

R_C , E_N , and E_P are the fraction of assimilated C, N, or P that is respired (R_C) or excreted (E_N and E_P), and AE_C , AE_N , and AE_P are the assimilation efficiencies for C, N, and P (fraction of C, N, or P that is consumed which was assimilated). To assess the possible range in zooplankton metabolic demand at depth, we calculated “lower,” “middle,” and “upper” values of ZCD, ZND, and ZPD based on literature R_C , E_N , E_P , AE_C , AE_N , and AE_P values (Tables 1-3). For example, upper ZPD values were determined using an E_P of 0.6 and an AE_P of 0.2 while lower ZPD values were determined using an E_P of 0.9 and an AE_P of 0.8 (Table 3).

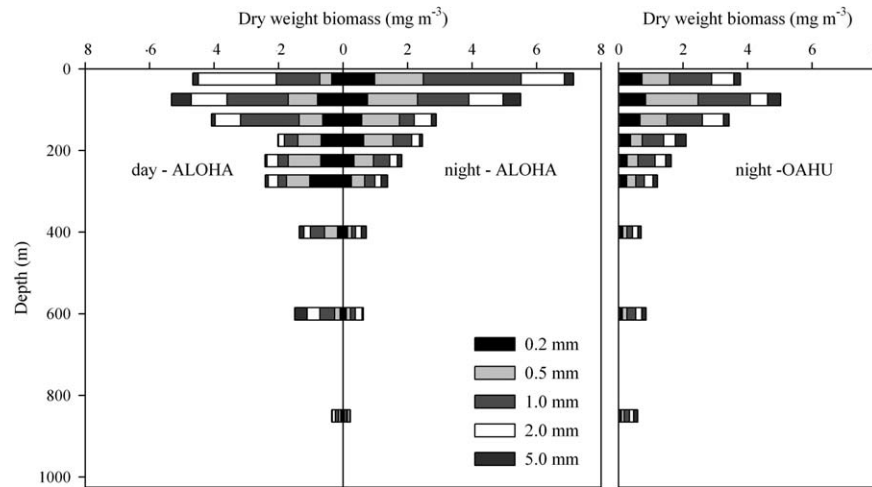


Fig. 1. Day and night size-fractionated zooplankton biomass at Sta. ALOHA (each: $n = 1$) and mean night size-fractionated zooplankton biomass at stations (Sta. Kahe and Kaena Point) off the island of Oahu ($n = 2$). Zooplankton biomass is centered within each depth interval of collection (0-50 m, 50-100 m, 100-150 m, 150-200 m, 200-250 m, 250-300 m, 300-500 m, 500-700 m, and 700-1000 m) and is calculated using improved MOCNESS conversion factors relative to Hannides et al. (2013).

A more conservative estimate of ZCD at depth was further calculated using the empirical global-bathymetric model of Ikeda et al. (2007a). Rates of O_2 consumption were determined based on the Ikeda et al. (2007a) allometric relationships, mean depth of capture, resident zooplankton dry wt biomass in the mesopelagic zone at Sta. ALOHA, measured depth-specific dry wt conversion factors (dry wt ind.⁻¹), average water temperatures, and O_2 saturation at each depth interval. These rates were converted to respiratory C equivalents as above, and a ZR ($mg\ C\ m^{-2}\ d^{-1}$) determined. Finally, total ZCD was calculated based on the equations given above and literature values for R_C and AE_C (Table 1).

Sinking particle fluxes were measured at Sta. ALOHA in the NPSG in July 2011 during the HOT cruise immediately before our August 2011 zooplankton collections. Particle fluxes were sampled using MultiPIT traps deployed at 150 m for approximately 60 h and analyzed for particulate C (PC), particulate inorganic C (PIC), N (PN), and P (PP) following the methods outlined in Karl et al. (1996) and in the HOT Data Organization and Graphical System (HOT-DOGS) website: <http://hahana.soest.hawaii.edu/hot/hot-dogs/index.html>). POC fluxes at 150 m were determined by subtracting PIC fluxes from total PC fluxes. To evaluate POC, PN, and PP fluxes at 500 m and 1000 m depth, and thus, calculate the loss of POC, PN, and PP over these depth intervals, flux attenuation was modeled following Martin et al. (1987), as:

$$F_z/F_0 = (z/z_0)^{-b} \quad (4)$$

with F_0 the flux of POC, PN, or PP at depth z_0 (here 150 m) and the attenuation coefficient ($b \pm$ standard error (SE_b)) determined to be on average 1.31 ± 0.17 for POC, 1.48 ± 0.26 for PN, and 1.03 ± 0.41 for PP at Sta. ALOHA in

summer during VERTIGO (VERTical Transport In the Global Ocean; Lamborg et al. 2008).

Results

Zooplankton biomass at Sta. ALOHA ranged from $0.22\ mg\ m^{-3}$ to $7.2\ mg\ m^{-3}$, and mean zooplankton biomass at stations closer to the island of Oahu ranged from $0.59\ mg\ m^{-3}$ to $5.0\ mg\ m^{-3}$ (Fig. 1), in all cases decreasing exponentially with depth as has been observed in many other regions of the world's oceans. In surface waters ($< 150\ m$), zooplankton biomass varied from $0.70\ g\ dry\ wt\ m^{-2}$ to $0.78\ g\ dry\ wt\ m^{-2}$, which is within the range determined by the HOT program at Sta. ALOHA (0.25 - $2.4\ g\ dry\ wt\ m^{-2}$ from 1996 to 2011; HOT-DOGS dataset). Our results use improved MOCNESS conversion factors and supplant the biomass values presented by Hannides et al. (2013). Steinberg et al. (2008b) document slightly higher biomass values for surface zooplankton but similar biomass values for midwater zooplankton collected with a $1\ m^2\ 335\ \mu m$ mesh MOCNESS system at Sta. ALOHA in July 2004. We focus on zooplankton biomass and metabolic demand at Sta. ALOHA to allow comparison with sinking particulate fluxes and with the results of Steinberg et al. (2008b).

Application of the standard allometric relationships of Ikeda (1985) to assess midwater zooplankton metabolic demand results in a middle range ZCD of $28.2\ mg\ C\ m^{-2}\ d^{-1}$, a ZND of $5.6\ mg\ N\ m^{-2}\ d^{-1}$, and a ZPD of $0.91\ mg\ P\ m^{-2}\ d^{-1}$ for zooplankton from 150 m to 1000 m at Sta. ALOHA (Table 4). Comparing these metabolic demands with the loss of particulate fluxes through 1000 m indicates that ZCD ranges from $0.6\times$ to $3\times$ (middle: $1.3\times$) POC flux losses, ZND ranges from $0.6\times$ to $4\times$ (middle: $1.3\times$) PN flux losses,

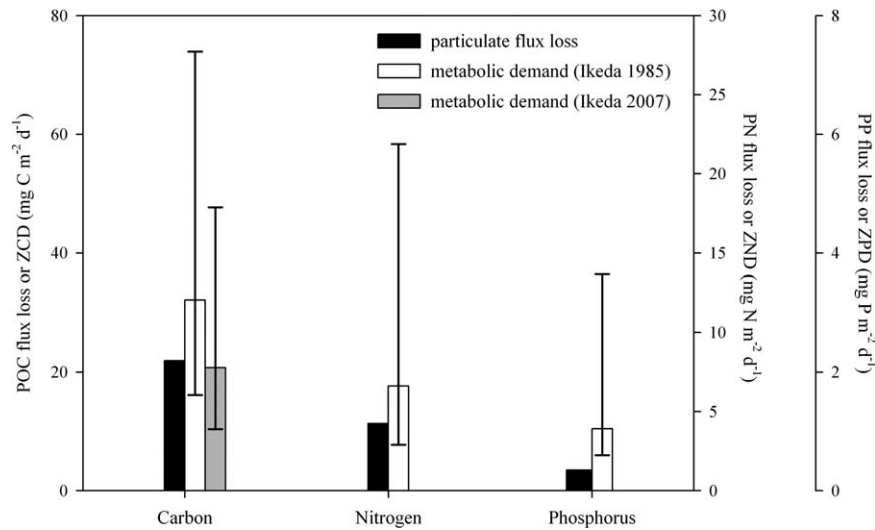


Fig. 2. Zooplankton metabolic demand or the loss of particulate fluxes from 150 m to 1000 m water depth for carbon, nitrogen, and phosphorus at Sta. ALOHA. Zooplankton metabolic demand (ZCD, ZND, or ZPD) was calculated using allometric relationships from Ikeda (1985) or Ikeda et al. (2007a; ZCD only). Error bars represent “upper” (positive error bars) or “lower” (negative error bars) range values for ZCD, ZND, or ZPD calculated assuming different assimilation efficiencies and net growth efficiencies (Tables 1-3).

and ZPD ranges from 1.5× to 9× (middle: 2.6×) PP flux losses (Table 4; Fig. 2). We further apply the Ikeda et al. (2007a) global-bathymetric model and find a more conserva-

tive, yet still appreciable ZCD of 17.9 mg C m⁻² d⁻¹ (middle range), which is 0.4-1.9× (middle: 0.8×) the loss of POC fluxes from 150 m to 1000 m (Table 4). Overall the C : N : P

Table 4. Total midwater resident zooplankton C, N, and P demand and metabolic demand as a percentage of the loss (Δ) of POC, PN, and PP fluxes through the mesopelagic zone (150-1000 m) at Sta. ALOHA in the North Pacific Subtropical Gyre. All metabolic demands were estimated with empirical relationships from Ikeda (1985) using zooplankton dry wt biomass, and ZCD was also estimated using Ikeda et al. (2007a). Molar C : N : P ratios for zooplankton metabolic demand [from “middle” estimates of ZCD, ZND, and ZPD using Ikeda (1985)] and for the potential food supply (i.e., the loss of particulate fluxes between 150 m and 1000 m; Δ POC, Δ PN, and Δ PP fluxes) are also shown. Error in Δ POC, Δ PN, and Δ PP flux is derived from propagation of the standard error of the attenuation coefficient (SE_b).

Resident metabolic demand		Metabolic demand as % loss of particulate flux
ZCD (mg C m ⁻² d ⁻¹)—Ikeda (1985)		% Δ POC flux
150-500 m	22.4 (11.2–51.5)	118%±6% (59±3–272±14)
150-1000 m	28.2 (14.1–64.9)	129%±4% (65±2–296±9)
ZCD (mg C m ⁻² d ⁻¹)—Ikeda et al. (2007)		% Δ POC flux
150-500 m	15.6 (7.8–35.9)	82%±4% (41±2–190±10)
150-1000 m	17.9 (9.0–41.2)	82%±2% (41±1–188±6)
ZND (mg N m ⁻² d ⁻¹)		% Δ PN flux
150-500 m	4.5 (2.0–14.8)	120%±8% (53±3–396±25)
150-1000 m	5.6 (2.5–18.6)	132%±4% (58±2–437±14)
ZPD (mg P m ⁻² d ⁻¹)		% Δ PP flux
150-500 m	0.70 (0.40–2.43)	243%±49% (139±28–849±172)
150-1000 m	0.91 (0.52–3.19)	264%±34% (151±20–921±120)
Metabolic demand C:N:P		Loss of particulate flux C:N:P
150-500 m	83:14:1	171±36:29±6:1
150-1000 m	80:14:1	163±22:27±4:1

Table 2. The fraction of assimilated N that is excreted (E_N) and the assimilation efficiencies of N (AE_N). When necessary, E_N was calculated from net growth efficiencies. "Upper," "Middle," and "Lower" defined as in Table 1 but for N.

	E_N	Reference	AE_N	Reference
	0.38	Mauchline (1998)	0.36	Mauchline (1998)
	0.35	Pagano and Saint-Jean (1994)	0.34	Paffenhöfer and Koster (2005)
	0.38	Mauchline (1998)	0.54	Paffenhöfer and Koster (2005)
	0.39	Pagano and Saint-Jean (1994)	0.60	Mauchline (1998)
	0.46	Pagano and Saint-Jean (1994)	0.69	Pagano and Saint-Jean (1994)
	0.47	Mauchline (1998)	0.74	Landry et al. (1984)
	0.50	Mauchline (1998)	0.81	Landry et al. (1984)
	0.50	Pagano and Saint-Jean (1994)	0.81	Paffenhöfer and Koster (2005)
	0.55	Pagano and Saint-Jean (1994)	0.84	Landry et al. (1984)
	0.57	Mauchline (1998)	0.89	Mauchline (1998)
	0.60	Pagano and Saint-Jean (1994)	0.93	Landry et al. (1984)
	0.61	Mauchline (1998)	0.93	Paffenhöfer and Koster (2005)
	0.73	Mauchline (1998)	0.95	Paffenhöfer and Koster (2005)
	0.73	Pagano and Saint-Jean (1994)		
	0.95	Mauchline (1998)		
Upper	0.4		0.3	
Middle	0.5		0.7	
Lower	0.9		1.0	

stoichiometry of zooplankton metabolic demand calculated using standard allometric relationships (Ikeda 1985) is 80 : 14 : 1 while the stoichiometry of the loss of sinking particulate fluxes through 1000 m is 163 : 27 : 1 (Table 4).

To estimate zooplankton metabolic demand, we applied new dry wt conversion factors (dry wt ind.⁻¹) measured for each depth and size fraction to our allometric relationships. We find that average (\pm standard deviation) surface water (< 150 m) dry wt conversion factors for almost all size fractions (0.2 mm: 0.017 ± 0.004 , 0.5 mm: 0.040 ± 0.014 , 1.0 mm: 0.16 ± 0.10 , and 2.0 mm: 1.50 ± 0.28 mg dry wt ind.⁻¹) are significantly smaller than average midwater (> 150 m) conversion factors (0.2 mm: 0.030 ± 0.010 , 0.5 mm:

0.083 ± 0.012 , 1.0 mm: 0.34 ± 0.10 , and 2.0 mm: 2.80 ± 0.51 mg dry wt ind.⁻¹; Mann-Whitney $U = 0.0-1.0$, $df = 3, 6$, $p \leq 0.048$). For the 5 mm size fraction, surface (10.5 ± 1.2 mg dry wt ind.⁻¹) and midwater conversion factors (13.9 ± 8.8 mg dry wt ind.⁻¹) are not significantly different (Mann-Whitney U test, $p > 0.05$).

Discussion

Our analysis of midwater resident zooplankton metabolism at Sta. ALOHA indicates that zooplankton metabolic demand can be equal to or greater than the attenuation of particulate organic matter fluxes through the mesopelagic

Table 3. The fraction of assimilated P that is excreted (E_P) and the assimilation efficiencies of P (AE_P). When necessary, E_P was calculated from net growth efficiencies. "Upper," "Middle," and "Lower" defined as in Table 1 but for P.

	E_P	Reference	AE_P	Reference
	0.59	Mauchline (1998)	0.19	Liu et al. (2006)
	0.59	Pagano and Saint-Jean (1994)	0.38	Mauchline (1998)
	0.63	Pagano and Saint-Jean (1994)	0.69	Pagano and Saint-Jean (1994)
	0.66	Mauchline (1998)	0.72	Reinfelder and Fisher (1991)
	0.74	Mauchline (1998)	0.78	Liu et al. (2006)
	0.78	Mauchline (1998)		
	0.87	Pagano and Saint-Jean (1994)		
	0.89	Pagano and Saint-Jean (1994)		
Upper	0.6		0.2	
Middle	0.7		0.6	
Lower	0.9		0.8	

zone in the NPSG. Our ZCD calculations for zooplankton at Sta. ALOHA are similar to those Steinberg et al. (2008b) calculated at the same site in summer 2004. This indicates that, within a season, midwater resident zooplankton populations may exert a relatively consistent metabolic demand in the NPSG. However, while particulate fluxes are relatively high and potentially labile during the mid- to late summer at Sta. ALOHA, fluxes are lower the rest of the year (Karl et al. 2012). Mesopelagic flux attenuation has been shown to respond dynamically to changes in the export of material from surface waters (Lomas et al. 2010), and thus, midwater zooplankton populations should be evaluated during low-flux periods (i.e., winter) to further our knowledge of temporal change in the NPSG. Intraseasonal variability in metabolic demand is also not well constrained by our treatment, as data from only one night tow at Sta. ALOHA was available. Future studies should evaluate both intraseasonal and interseasonal variability in plankton metabolism and particle flux attenuation to gain a holistic perspective on the balance between C sources and sinks in the NPSG.

Our further application of a global-bathymetric model of zooplankton respiration (Ikeda et al. 2007a) indicates that even with this more conservative approach, ZCD accounts for a significant fraction (0.4-1.9 \times) of POC loss through the mesopelagic zone. There are several caveats to this observation. First, the allometric relationships we use (Ikeda et al. 2007a) are based on the respiration rates of epipelagic, mesopelagic, bathypelagic, and abyssopelagic copepods. We focus on these empirical equations because copepods dominate zooplankton populations in surface and midwaters of the NPSG (Landry et al. 2001; Steinberg et al. 2008a), and thus, can contribute significantly to total ZCD at depth. We note that Ikeda and Takahashi (2012), Ikeda (2013a, b) report separate global-bathymetric models for chaetognath, euphausiid, and mysid respiration rates. However, comparison of rates standardized to the same body mass, habitat temperature, and O₂ saturation at 1000 m depth indicate that copepod respiration rates are between that of euphausiids and mysids (higher) and chaetognaths (lower; Ikeda 2013a). Thus, our ZCD derived using Ikeda et al. (2007a) should be regarded as a representative value for zooplankton metabolic demand at depth, but an appropriately conservative one when considering that copepods dominate midwater populations. Another caveat is that the deep-water copepod respiration rates that inform the Ikeda et al. (2007a) model are based primarily on experiments conducted in the subpolar North Pacific Ocean. However, Ikeda and Takahashi (2012) and Ikeda (2013a, b) use data from several deep-water sites and indicate a worldwide decline in plankton metabolism with depth for mesopelagic and bathypelagic populations. Thus, we posit that the Ikeda et al. (2007a) model can be applied to yield more conservative estimates of midwater zooplankton metabolic demand at Sta. ALOHA, but strongly suggest that further experiments should be conducted to

examine deep-water plankton metabolism in tropical, subtropical, and polar ocean biomes.

The allometric relationships used to estimate metabolic demand rely on dry wt conversion factors (dry wt ind.⁻¹) that are poorly constrained for zooplankton at depth. We find that these conversion factors are larger for midwater zooplankton as compared to those for the surface community. Accordingly, our microscope observations indicated larger sized zooplankton within each size fraction at depth, and dry wt is logarithmically related to prosome length in copepods (Mauchline 1998). A shift from primarily suspension feeders in surface layers to more detritivorous and carnivorous zooplankton at depth could also contribute to this trend, as Ikeda et al. (2006) have found higher water content in suspension feeders relative to detritivores and carnivores. Ultimately, the consequence of our findings is that applying a "blanket" surface ocean-derived dry wt conversion factor will result in erroneously large mesopelagic zooplankton metabolic demands. For example, if conversion factors derived from Landry et al. (2001; i.e., Table 2) are used, our estimated ZCD, ZND, and ZPD increase by roughly 30%. In summary, more conservative estimates of zooplankton metabolic demand in midwaters are achieved with depth- and size fraction-specific dry wt conversion factors, such as those we have found for the NPSG.

Understanding the potential effect of zooplankton populations on mesopelagic particulate fluxes relies on reasonable estimates of total zooplankton metabolic demand. While the allometric relationships and dry wt conversion factors used to model ZR, ZEN, and ZEP will obviously influence the resulting calculation of total metabolic demand (Burd et al. 2010), zooplankton assimilation efficiencies (AEC, AEN, and AEP) and net growth efficiencies (or resulting RC, EN, and EP) also exert a significant effect on final ZCD, ZND, and ZPD values. Assimilation and net growth efficiencies are poorly known for midwater populations, and thus, our approach is to apply a range of values based on the literature. Our most conservative estimates of zooplankton metabolic demand are achieved at high assimilation efficiencies and low net growth efficiencies, that is, assuming that midwater zooplankton assimilate a high proportion of the food they ingest (AEC, AEN, and AEP: 0.78-0.95; Tables 1-3) and shunt a high proportion of the assimilated food into metabolism (RC, EN, and EP: 0.89-0.95; Tables 1-3). At these ratios, little of the assimilated energy is used for growth. The very few metabolic studies that have been conducted on deep-sea copepods support high assimilation efficiencies. For example, investigations of deep-water carnivorous copepods in Norwegian fjords indicate an assimilation efficiency of 0.91-0.98 (by weight) for *Aetideopsis armatus* and an assimilation efficiency of 0.91-0.94 (by weight) for *Paraeuchaeta norvegica*. Conversely, there is no a priori reason to expect that deep-sea species should have greater assimilation efficiencies compared to shallow living species as energetic economy is selected for in all

environments, for example, as has been shown for fishes (Drazen et al. 2007). Moreover, stable isotope analysis indicates that marine snow becomes a more important component of zooplankton diets with depth in the mesopelagic zone (Hannides et al. 2013). In general, animals with non-carnivorous or refractory diets have lower assimilation efficiencies [e.g., AE_C values of 0.64-0.83 for the vertical migrator *Euphausia pacifica* feeding on marine snow (Dilling et al. 1998)]. Net growth efficiencies for mesopelagic zooplankton have been the subject of few, if any, studies. Ikeda et al. (2007b) show that the ribonucleic acid : deoxyribonucleic acid ratio of copepods, an indicator of protein synthetic activity, decreases with depth from epipelagic to mesopelagic and bathypelagic waters. Thus, zooplankton activity, including growth, may be slower with depth in the water column (Ikeda et al. 2007b). Metabolism (Drazen and Seibel 2007) and growth (Drazen and Haedrich 2012) of fishes also declines with depth, although these studies were not able to evaluate growth efficiency due to a lack of data. In summary, the few available studies for mid- and deep-water zooplankton indicate assimilation efficiencies could be near the “lower” bounds we have established based on literature values. However, even at these high efficiencies ZCD, ZND, and ZPD still represent a considerable proportion (0.4-1.5 \times) of particle flux attenuation from 150 m to 1000 m at Sta. ALOHA.

Our use of recently published global-bathymetric metabolism models, new dry wt conversion factors, and a range of assimilation and net growth efficiencies significantly reduce the imbalance in midwater C, N, and P budgets in the NPSG. While this approach is based on a relatively straightforward comparison of metabolic demand and particle flux attenuation, we acknowledge more complex dynamics likely affect these processes. For example, migrant zooplankton that do not migrate to surface waters at night are included in our calculation of “resident” metabolic demand, and thus, we assume a constant fraction of the migrator community remains at depth each day. Migrating zooplankton that transit to the surface to feed at night may additionally feed while residing at midwater depths during the day, which would augment our estimate of zooplankton metabolic demand at depth. Finally, migrators may affect particulate fluxes through fecal pellet production in midwaters. While it is difficult to quantify these complexities given uncertainties surrounding their parameterization, they should be considered in future studies.

A recent strict focus on the balance of C flow into and out of the mesopelagic food web has resulted in several new approaches which can be applied to our system at Sta. ALOHA. For example, one can evaluate C budgets solely on the basis of zooplankton and microbial respiration (Giering et al. 2014), effectively defining an ultimate metabolic sink for C in midwaters. Using this approach, we find that midwater resident zooplankton respire 7.8 mg C m⁻² d⁻¹ (Ikeda

et al. 2007a)–12.3 mg C m⁻² d⁻¹ (Ikeda 1985). These zooplankton respiratory demands are still 0.4-0.6 \times the loss of POC in the mesopelagic zone or essentially ZCD at our “lower” bound of assimilation and net growth efficiencies. We can apply the same logic to midwater N and P budgets, and find that zooplankton excrete 2.2 mg N m⁻² d⁻¹ and 0.4 mg P m⁻² d⁻¹. Again, these excretory demands are significant (0.5 \times and 1.0 \times , respectively) relative to PN and PP attenuation in the mesopelagic zone and similar to ZND and ZPD at our “lower” assimilation and net growth efficiencies.

Recent studies have also used a dynamic upper boundary when evaluating particulate flux to the mesopelagic zone (Buesseler and Boyd 2009). For example, the C budget defined by Giering et al. (2014) is based on particulate fluxes measured from the depth of the mixed layer. This approach significantly contributed to the C balance found at their study site because C supply was larger than sinks between the mixed layer depth and 150 m. It is difficult to evaluate a dynamic upper boundary in our case as particulate fluxes were only measured at 150 m and other flux-relevant data (e.g., ²³⁴Th activity) are not available. However, we note that the depth of the euphotic zone (1% light level) was approximately 120 m at Sta. ALOHA during our study period (HOT-DOGS dataset). Using the simple model of Buesseler and Boyd (2009), particulate fluxes at this depth would likely be $\sim 1.06\times$ those measured in the sediment traps, indicating a loss of 1.53 mg C m⁻² d⁻¹, 0.29 mg N m⁻² d⁻¹, and 0.03 mg P m⁻² d⁻¹ from the base of the euphotic zone to 150 m. Zooplankton biomass is appreciable over this interval and the resulting estimates for zooplankton respiration and excretion are 2.49 mg C m⁻² d⁻¹, 0.46 mg N m⁻² d⁻¹, and 0.06 mg P m⁻² d⁻¹ (ZCD, ZND, and ZPD: 5.72 mg C m⁻² d⁻¹, 1.17 mg N m⁻² d⁻¹, and 0.16 mg P m⁻² d⁻¹). Thus, based on this simple model, we do not find C, N, and P sources to be greater than sinks in the upper mesopelagic zone when considering the euphotic zone as an upper boundary. We note that this balance could change, however, with seasonal variation in euphotic zone depth at Sta. ALOHA. In summary, a strict focus on balancing elemental budgets in the mesopelagic zone should focus on C, N, and P sources (the attenuation of particulate fluxes between a dynamic upper boundary and the base of the mesopelagic zone) and ultimate sinks (e.g., zooplankton respiration and excretion). However, even with these new approaches, we find zooplankton metabolism represents a considerable fraction (at least 0.4-1.0 \times) of the loss of PC, PN, and PP through the mesopelagic zone at Sta. ALOHA.

Whether considering ZPD or excretion, zooplankton metabolic demands for P in the mesopelagic zone are particularly large relative to the particulate fluxes purported to support them. Moreover, molar C : N : P ratios of zooplankton metabolic demand at depth are, based on middle ZCD, ZND, and ZPD values, 80 : 14 : 1, and may reach 70 : 10 : 1 if lower range values are used. In contrast, the C : N : P ratio of the

loss of particulate flux between 150 m and 1000 m (i.e., the supply of particles which potentially fuels zooplankton in midwaters) is 163 : 27 : 1. Thus, clearly zooplankton metabolic demand C : P and N : P ratios are lower than those observed for their food source. This imbalance raises the question of whether midwater zooplankton are P-limited. Hannides et al. (2009) previously investigated zooplankton stoichiometry in surface waters at Sta. ALOHA and found a zooplankton “threshold elemental ratio” (Andersen and Hessen 1995) for P (TER_P) of 191. That is, the growth of zooplankton consuming food with a C : P less than 191 will not be P-limited, and P is rather released with excretion and egestion. Assuming that this TER_P holds for midwaters, we do not find evidence for zooplankton P-limitation during the time period of our study. However, one caveat is that the calculation of TER_P depends in part on zooplankton body C : P ratios, and it is not known if these ratios change with depth. Calanoid copepod C : N ratios decrease significantly with depth, likely due to the loss of proteinaceous structures (i.e., muscle; Ikeda et al. 2006) and increase in lipid stores in deep waters. Depth-dependent change in zooplankton C : P ratios, thus, may be possible, and these values and their effect on zooplankton TER_P at depth should be investigated in future studies of mesopelagic zooplankton metabolism.

Another major caveat is that we have only investigated flux attenuation at one point in time (July 2011–August 2011), when particle fluxes are dominated by fast-sinking, relatively labile material (Karl et al. 2012). However, significant temporal variability in sinking particle stoichiometry has been observed, particularly deeper in the water column (Karl et al. 1996). If we apply the flux attenuation coefficients of Lamborg et al. (2008; see earlier) to POC and PP fluxes measured at 150 m from 2001 to 2011 at Sta. ALOHA, the resulting molar C : P ratios for the loss of POC compared to the loss of PP range from 94 to 585 and average 281 ± 97 (HOT-DOGS dataset). Thus, if zooplankton metabolic demand is relatively steady [as suggested by the close agreement of our ZCD with that determined by Steinberg et al. (2008b)], it is likely that the fluctuating food supply from surface waters occasionally drives midwater zooplankton to P-limitation. Clearly, more research is needed to ascertain zooplankton P content, P demand, and potential P limitation in the mesopelagic zone.

While ZCD, ZND, and ZPD are clearly significant relative to the loss of particulate fluxes from 150 m to 1000 m, microbial metabolic demand is likely large at these depths. For example, Steinberg et al. (2008b) found microbial metabolic C demand to be 10× the attenuation in POC fluxes at Sta. ALOHA. A sensitivity analysis by Burd et al. (2010) further found the ratio of POC flux attenuation to microbial C demand to depend heavily on bacterial growth efficiencies and assumptions regarding cellular C contents but most often to fall in the range of 0.1–1 at Sta. ALOHA. To gain a more holistic perspective on particle loss processes, future

studies should focus on both zooplankton and microbial metabolic demands at depth. For example, when microbial P metabolic demand is included in conjunction with our observed large ZPD, midwater P budgets are likely to become even more imbalanced.

In conclusion, we find the metabolic demand of mesopelagic zooplankton to be significant in comparison with the attenuation of particle fluxes in the NPSG. Alternate methods of calculating zooplankton metabolism, including using the recently described global-bathymetric models, new dry wt conversion ratios, and a sole focus on respiration, reduce ZCD to reasonably match particle flux attenuation in the mesopelagic zone. However, particularly when considering the additional metabolic demands of the microbial community at depth, it is likely that midwater zooplankton engage in a number of different feeding mechanisms to supplement the fuel needed for their metabolism. One potential alternate mechanism is carnivorous feeding on vertical migrants. Carnivory has been previously observed as an important feeding mode for midwater zooplankton, and feeding on vertical migrants could account in part for the relatively ^{15}N -depleted isotopic composition of zooplankton in the mesopelagic zone at Sta. ALOHA (Hannides et al. 2013). However, zooplankton typically limit their migrations to the upper- to mid-mesopelagic zone, and previous isotope analyses indicate that deep-water zooplankton (inhabiting 700–1000 m water depths) continue to be strongly influenced by surface water food sources. This “surface” link is most likely maintained through the consumption of sinking particles, and thus, zooplankton in the lower mesopelagic zone may have a particularly strong effect on particle attenuation at these depths. Overall, our results support the inclusion of zooplankton metabolism at depth in future studies of particle flux attenuation and the mechanisms influencing deep-water C sequestration.

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