

LETTER

Deep zooplankton rely on small particles when particle fluxes are low

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Scientific Significance Statement

Deep-sea communities rely on organic matter (OM) produced almost exclusively in surface waters. Pulses of fast sinking material are their main food source, but here we show that when the quantity of that material is low, small particles can sustain deep zooplankton communities. Small particles are often overlooked as a source of OM to deep food webs, yet they are found in higher concentrations than larger, faster sinking particles, and their relative contribution to the total pool is expected to increase in the future. Our results highlight the importance of small particles as a food source sustaining deep food webs, which may become more widespread given the predicted changes in overlying surface waters.

Abstract

The fate of organic matter (OM) in the deep ocean remains enigmatic, with little understood regarding the flux and its utilization by deep food webs. We used compound-specific nitrogen stable isotope ratios of source amino acids measured in particle size classes and deep zooplankton (700–1500 m) to determine the contribution of small (0.7–53 μm) vs. large particles ($> 53 \mu\text{m}$) to their diet at four sites in the North Pacific. Our results show that small particles constitute between 9% and 98% of zooplankton diets, being the contribution higher at sites with lower flux regimes. The contribution of small particles to the diet of deep zooplankton was also higher when biomass of vertical migrators, and therefore actively transported OM, was lower. Climate-driven changes in primary production and export are expected to shift particle fluxes to smaller size classes, and thus their importance in midwater food webs may become more widespread.

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Deep marine ecosystems depend on the downward flux of detrital organic matter (OM) from the euphotic zone. Only 5–25% of net primary production is exported below the euphotic layer (Siegel et al. 2014), and mass balance calculations argue that this fixed carbon must be adequate to meet the respiratory demands of deep-sea food webs. However, the estimated metabolic carbon demand of deep-sea fauna frequently exceed carbon supplied from overlying waters (Burd et al. 2010). These carbon budget calculations mainly take into account larger particles ($> 53 \mu\text{m}$; Lam et al. 2011), which are more efficiently collected by sediment traps (Burd et al. 2010) and reach the deep ocean by rapidly sinking through the water column. However, small particles ($< 53 \mu\text{m}$; Lam et al. 2011) exist in higher concentrations relative to larger aggregates (Alonso-González et al. 2010), and their concentrations are not attenuated as rapidly with increasing depth (Herndl and Reinthaler 2013). Although smaller particles are regarded as suspended in the water column, they too may sink, albeit at a slower rate. Indeed recent work suggests that small particles may also contribute significantly to the organic particle flux to the deep ocean (e.g., Richardson and Jackson 2007; Alonso-González et al. 2010). Moreover, several studies further argue that surface production in mid and low latitudes will decrease in the future due to increasing stratification (e.g., Riebesell et al. 2009). The resulting transition to a phytoplankton community dominated by smaller cells (Bopp et al. 2005) may thus favor increasing contributions of small particles to the total organic carbon flux.

Small particles are often overlooked as a food source to deep ecosystems; however, they are known to enter the food web through zooplankton (Hannides et al. 2013) with pathways leading to micronekton in the oligotrophic North Pacific Subtropical Gyre (NPSG; Choy et al. 2015; Gloeckler et al. 2018). The extent to which this process occurs in other deep ecosystems and under what conditions small particles are an important food source remain unknown. It has been suggested that the dependency on small particles is higher when overall particle fluxes are lower (Hannides et al. 2020), but this hypothesis has never been rigorously tested.

Smaller particles remain suspended longer in the midwater column than larger particles, consequently particle size classes have different organic compound compositions (Abramson et al. 2010) and are associated with different microbial communities (Duret et al. 2019). This also leads to distinct $\delta^{15}\text{N}$ values of small and large particles, so that below the euphotic zone small particles typically have higher $\delta^{15}\text{N}$ values than large particles (e.g., Altabet et al. 1991). During microbial degradation of particles, ^{14}N -containing bonds are preferentially cleaved in the extracellular enzymatic hydrolysis of proteins (Hannides et al. 2013; Ohkouchi et al. 2017), leaving small particles, which are exposed to longer periods of microbial alteration, enriched in ^{15}N relative to living biomass or unaltered material.

Overall, small and large particles constitute two potential food sources supporting deep food webs with distinctly

different $\delta^{15}\text{N}$ values that can be used to estimate the size of particles consumed by mesopelagic metazoans (Hannides et al. 2013). However, the interpretative power of this approach is limited because variations in bulk $\delta^{15}\text{N}$ values of consumers can result from a change in trophic position as well as from differences in $\delta^{15}\text{N}$ values at the base of the food web. This limitation can be resolved using $\delta^{15}\text{N}$ values of individual amino acids, which comprise the most abundant compounds within particulate organic matter (POM; Sheridan et al. 2002). In metazoans, “source” amino acids (e.g., phenylalanine, serine, lysine, or glycine; Popp et al. 2007; Chikaraishi et al. 2009) change little with trophic transfer such that their $\delta^{15}\text{N}$ values ($\delta^{15}\text{N}_{\text{Src-AA}}$) largely resemble the isotopic composition of phytoplankton or detrital particles at the base of the food web. Gloeckler et al. (2018) showed that $\delta^{15}\text{N}_{\text{Src-AA}}$ values in small particles are higher than in large particles at depths of ~ 250 – 1200 m in the NPSG. Hence, the difference in $\delta^{15}\text{N}_{\text{Src-AA}}$ values between particle size classes can be traced into higher trophic levels in the mesopelagic to determine the relative contribution of large and small particles as food sources.

We analyzed $\delta^{15}\text{N}_{\text{Src-AA}}$ values in particle size classes and deep zooplankton (700–1500 m) to estimate the contribution of small particles to zooplankton diet. We compared two sites in the Equatorial Pacific (5°N and 8°N , 155°W) with results from Sta. ALOHA situated in the NPSG (22°N , 158°W) during summer when particle export flux is high and winter, when export flux is low. The Equatorial Pacific is generally productive, especially at 5°N , which is supported by the equatorial upwelling, although the intensity of upwelling was curtailed by strong El Niño conditions when we sampled this site (Umhau et al. 2019). This allowed us to examine four sites of contrasting biogeochemistry and flux attenuation of sinking particles. We show that lower export flux as determined using $^{234}\text{Th} : ^{238}\text{U}$ disequilibria, and lower active flux of OM as determined from biomass of migrant zooplankton, lead to a higher dependency of deep zooplankton on small particles.

Results and discussion

We found that deep-sea zooplankton communities can be sustained on small particles, with large POM appearing as a minor food source. Small particles were a food source (directly or indirectly through the food web) for all deep (700–1500 m) zooplankton (1–2 mm) with the relative contribution varying largely among sites. Small particles represented between $98.4\% \pm 1.9\%$ of zooplankton diet at 5°N and $9.3\% \pm 9.3\%$ at Sta. ALOHA during summer (Table 1). Small particles have been previously reported as a food source for zooplankton in the NPSG (Sta. ALOHA; Hannides et al. 2013; Hannides et al. 2020). Here, the comparison among contrasting ecosystems allowed us to further investigate the underlying mechanisms that facilitate the reliance on small particles in deep communities.

Table 1. Average $\delta^{15}\text{N}_{\text{Sr-AA}}$ values for SP and LP between 600 and 1250 m used as end-members for the calculation of the relative contribution of small particles (f_{SP}) to the diet of zooplankton (1–2 mm) collected between 700 and 1500 m depth. $\Delta\delta^{15}\text{N}_{\text{Sr}}$ is the increase in $\delta^{15}\text{N}_{\text{Sr-AA}}$ values between surface waters (0–150 m) and deep waters (600–1250 m) for SP and LP.

Station	$\delta^{15}\text{N}_{\text{Sr-SP}}$	$\delta^{15}\text{N}_{\text{Sr-LP}}$	f_{SP} (%)	$\Delta\delta^{15}\text{N}_{\text{Sr-SP}}$	$\Delta\delta^{15}\text{N}_{\text{Sr-LP}}$
5°N	10.9 ± 0.8	6.4 ± 0.3	98.4 ± 1.9	4.3	0.8
8°N	10.4 ± 1.0	8.5 ± 0.5	66.7 ± 25.1	6.2	2.7
ALOHA summer	6.9 ± 0.2	2.7 ± 0.1	9.3 ± 9.3	5.2	1.4
ALOHA winter	6.0 ± 0.6	2.4 ± 0.5	48.2 ± 18.8	6.8	2

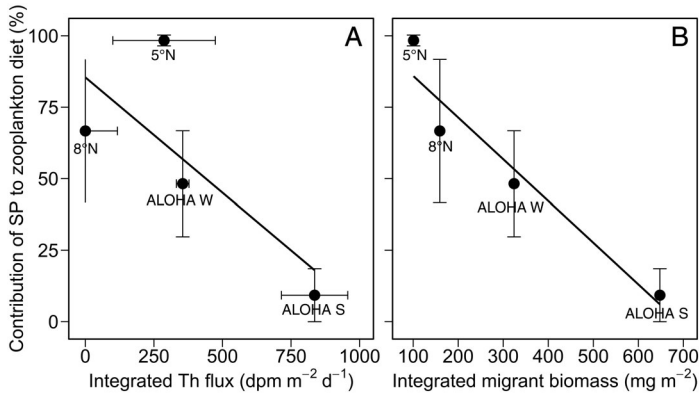


Fig. 1. (A) Relationship between the contribution of small particles in the diet of deep zooplankton (f_{SP}) as percentage and integrated flux of thorium in the upper 200 m ($R^2 = 0.57$; $p = 0.24$). (B) Relationship between the contribution of small particles in the diet of deep zooplankton (f_{SP}) as percentage and total integrated biomass of zooplankton migrating below 200 m depth ($R^2 = 0.93$; $p < 0.05$).

We used $^{234}\text{Th} : ^{238}\text{U}$ disequilibria and derived ^{234}Th export fluxes integrated over the upper 200 m of the water column as an indicator of the net particle flux below the euphotic zone (Umhau et al. 2019). We hypothesized that a lower export flux will lead to a higher consumption of smaller particles by deep zooplankton because they are available in a higher proportion in mesopelagic waters (Lam et al. 2015). Our results showed a trend supporting our hypothesis except for at 5°N (Fig. 1A), where the contribution of small particles as a food source to deep zooplankton (fraction small particles, f_{SP} ; see “Methods” section) was higher than expected. This suggests the dominance of smaller particles in the flux at this site, which agrees with Umhau et al. (2019). Also, at 5°N a higher proportion of carbonates within the small particle pool was measured (Supporting Information Table S1), similar to previous results from the Equatorial Pacific during El Niño (Hernes et al. 2001), probably due to the decline in abundance of diatoms and the dominance of nanoeucaryotes like coccolithophorids that are captured in the small size fraction. The presence of carbonates increases the settling velocity of particles (Armstrong et al. 2001), which might enhance the availability of small particles deeper in the water column.

Despite variations in export flux, particles underwent similar processes of degradation with depth at all sites, as determined by their $\delta^{15}\text{N}_{\text{Sr-AA}}$ values (Fig. 2). These values increased mainly throughout the upper 250 m, especially in small particles due to microbial degradation (Hannides et al. 2013). Interestingly, the increase in $\delta^{15}\text{N}_{\text{Sr-AA}}$ values was lower at 5°N than other sites for both particle size classes (Table 1). This indicates lower microbial alteration of particles and is consistent with a higher settling velocity due to their high carbonate content. Below 250 m, the $\delta^{15}\text{N}_{\text{Sr-AA}}$ values remained fairly constant and distinct for both particle size classes (Fig. 2). This supports findings of the limited exchange between large and small particle pools below the euphotic layer (Abramson et al. 2010; Duret et al. 2019), and suggests that small particles are not primarily formed from disaggregation of large particles in the mesopelagic zone.

The export flux obtained from $^{234}\text{Th} : ^{238}\text{U}$ disequilibria accounts for passively sinking particles, but also for particles removed by zooplankton in the surface and released at depth as fecal pellets. Diel vertical migration (DVM)-mediated transport of OM can make a significant contribution to the downward flux of sinking particles, which has been calculated to represent on average 14% of the global export flux (Archibald et al. 2019). Vertical migrators typically descend during the day to avoid predators after feeding closer to the surface at night. As a result, in this study, daytime zooplankton biomass in the upper mesopelagic zone (300–700 m) was always higher than nighttime biomass (Supporting Information Fig. S1). When migrant zooplankton biomass (i.e., the difference between daytime and nighttime biomass) is higher, the export flux of larger sinking particles ($> 53 \mu\text{m}$) below the euphotic zone should also increase due to the production of fecal pellets at depth during the daytime. The contribution of DVM-mediated transport of OM to the total flux is generally higher in central gyres than in equatorial regions (Archibald et al. 2019) and this was consistent with our results of a higher vertical migrator biomass at Sta. ALOHA relative to the Equatorial Pacific (Supporting Information Fig. S1). Moreover during El Niño when production is lower, the contribution of actively transported OM by DVM to total export flux decreases (Zhang and Dam 1997), favoring the observed higher dependence on small particles by deep zooplankton at 5°N. At sites

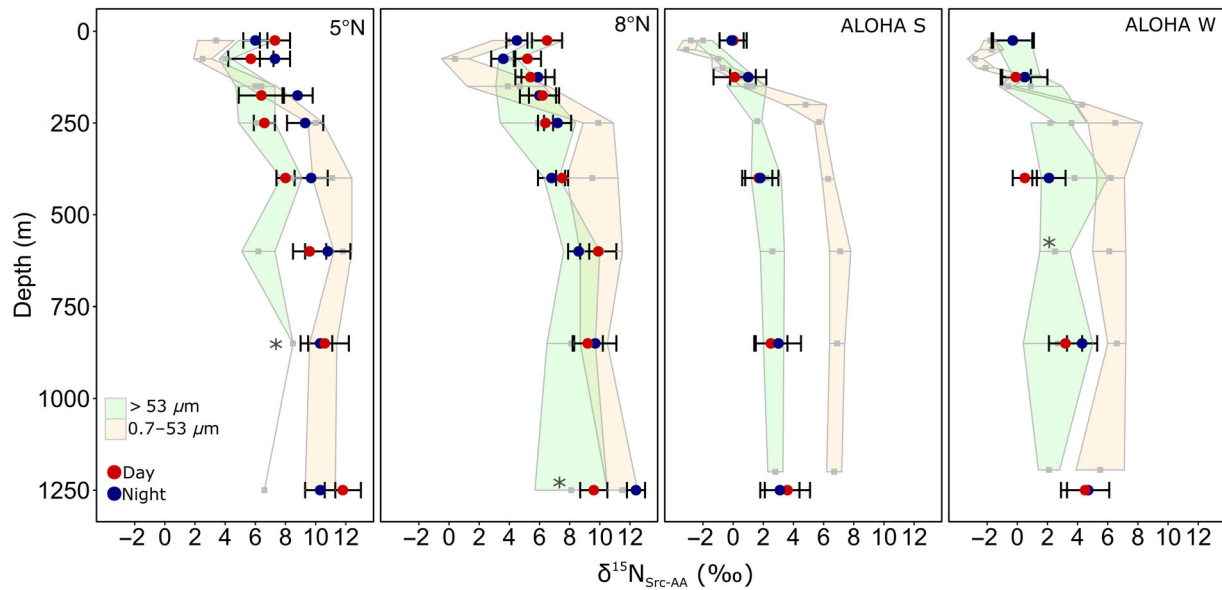


Fig. 2. Average source amino acid $\delta^{15}\text{N}_{\text{Src-AA}}$ (mean $\pm$ propagation error) for zooplankton (1–2 mm) collected during the day (red circles) and at nighttime (blue circles), and for large ($> 53 \mu\text{m}$; green shading) and small particles ($0.7\text{--}53 \mu\text{m}$; orange shading; mean $\pm$ propagation error) at 5°N , 8°N , and Sta. ALOHA during summer and winter seasons. Asterisks indicate depths at which $\delta^{15}\text{N}$ values of large particles had one amino acid missing so the resulting average $\delta^{15}\text{N}_{\text{Src-AA}}$ was not comparable to other depths, and were excluded in the calculations of end-members (Table 1).

where DVM biomass was lower, deep zooplankton showed a significantly higher f_{SP} ($R^2 = 0.93$; $p < 0.05$; Fig. 1B). This indicates that active transport of OM is a key determinant of the importance of small particles for zooplankton nutrition in the lower mesopelagic zone.

We conclude that small, slowly sinking particles are a main food source for deep zooplankton when large particle fluxes are low. Conversely, we hypothesize that when export flux is high, large sinking particles will entirely sustain the deep food web. In fact, resident zooplankton in the upper mesopelagic (300–500 m), where flux attenuation is lower, showed a dependence on small particles only at 5°N ($f_{\text{SP}} = 46.6\%$). To test this hypothesis, studies in more productive systems are needed. We showed here that using amino acid compound-specific isotope analysis (CSIA-AA) is remarkably useful for characterizing food sources, elucidating their fate into deep food webs, and comparing ecosystems of contrasting biogeochemistry. In this study, export fluxes were low in all the investigated sites relative to upwelling regions under normal conditions. However if areas with a similar or lower flux become widespread in the future, then small particles will be an important food source supporting deep food webs globally. The dependency on small particles by deep zooplankton was close to 50% at Sta. ALOHA in winter, where the average export flux is $\sim 30 \text{ mg C m}^{-2} \text{ d}^{-1}$ at 150 m depth (Church et al. 2013). Hence, a deep food web with an overlying export flux lower or similar is hypothesized to depend on small particles for more than 50% of its nutrition. Using a multimodel mean of global export flux (Supporting Information; Jones

et al. 2014), we estimated that regions with an export flux lower than $30 \text{ mg C m}^{-2} \text{ d}^{-1}$ at 150 m depth, and therefore with deep zooplankton food webs mainly supported by small particles, occupy $\sim 37\%$ of the ocean surface area today.

The fact that small particles are a food source to deep food webs has significant implications for understanding the biogeochemical cycle of carbon. Consumption of slow sinking particles in the lower mesopelagic can enhance the sequestration flux, defined as the transport of carbon below 1000 m depth (Passow and Carlson 2012). It is generally assumed that carbon sequestration is only possible for large rapidly sinking particles because small particles tend to be more easily remineralized in the water column (Passow and Carlson 2012). However, if zooplankton feed on the small pool close to the sequestration depth, they can enhance the transport of carbon to depth by producing fecal pellets that sink more quickly. Moreover, the expected climate-driven changes in ocean biogeochemistry include an expansion of oligotrophic waters (Polovina et al. 2008), reduction in primary producer size, and an increase in the proportion of small size particles within particle flux (Bopp et al. 2005). In fact, in the Equatorial Pacific it is expected that temperature increases will drive changes similar to those occurring during El Niño. Given predictions of global export flux by Jones et al. (2014), by 2100 the area of the ocean with deep zooplankton food webs mainly supported by small particles will increase from the current 37% to $\sim 46\%$. Thus, the importance of small particles to the nutrition of deep mesopelagic zooplankton is likely to become more widespread, potentially enhancing deep

midwater ecosystems to adjust to the predicted decreases in primary production and large particle export from overlying surface waters.

Methods

Particles and zooplankton collection

Samples of particles and zooplankton were collected at 5°N and 8°N (155°W) between August and September 2015. Particles were collected using in situ McLane pumps equipped with mini-MULVFS (Bishop et al. 2012) two-tiered filter holders. Particle collection captured sequentially large ($> 53 \mu\text{m}$) particulates on acid-cleaned Nitex mesh filters and small particles ($< 53 \mu\text{m}$) on precombusted GF/F glass microfiber filters ($0.7 \mu\text{m}$) or QMA quartz filters ($1 \mu\text{m}$) at discrete depths for CSIA-AA (25, 75, 150, 250, 400, 600, 850, and 1250 m) and for particulate carbon (10, 25, 50, 75, 100, 125, 150, and 200 m). This method is designed to exclude motile metazoans but include all other ambient, nonswimming particulate matter (see Bishop et al. 2012). Immediately after collection, large particles were rinsed off of the Nitex screens and onto precombusted 25-mm QMA filters using $0.2 \mu\text{m}$ filtered seawater. All filters were frozen at -20°C or -80°C as soon as possible after collection. Zooplankton were collected using a 1 m^2 MOCNESS net during the day ($\sim 09:00\text{--}15:00 \text{ h}$) and at night ($\sim 20:00\text{--}03:00 \text{ h}$) at depths intervals of 0–50, 50–100, 100–150, 150–200, 200–300, 300–500, 500–700, 700–1000, and 1000–1500 m. Night and day tows were repeated to obtain replicate samples for biomass and isotopic analyses. Upon collection, each sample was size-fractionated, filtered onto preweighed 47 mm filters of 0.2 mm Nitex mesh, and stored frozen at -80°C . Similarly, particles and zooplankton samples were collected at Sta. ALOHA (22°N , 158°W) in February (winter) and August–September (summer) of 2014 as described in Gloeckler et al. (2018) and Hannides et al. (2020).

Amino acid compound-specific stable isotope analysis

For CSIA-AA, particles and zooplankton were freeze-dried and analyzed following the methods of Hannides et al. (2013). $\delta^{15}\text{N}_{\text{Src-AA}}$ values for both particles and zooplankton were calculated as the average $\delta^{15}\text{N}$ value of serine, phenylalanine, lysine, and glycine (Hannides et al. 2020).

Contribution of small particles to zooplankton diet

We used an isotope mass balance mixing model to calculate the relative contribution, as percentage, of small particles to the diet of zooplankton (f_{SP}) for each site:

$$\delta^{15}\text{N}_{\text{Src-Zoo}} = f_{\text{SP}}\delta^{15}\text{N}_{\text{Src-SP}} + f_{\text{LP}}\delta^{15}\text{N}_{\text{Src-LP}},$$

with $f_{\text{SP}} + f_{\text{LP}} = 1$ and where $\delta^{15}\text{N}_{\text{Src-SP}}$ and $\delta^{15}\text{N}_{\text{Src-LP}}$ are the end-members for small (SP) and large (LP) particles, respectively. The end-members are the average $\delta^{15}\text{N}_{\text{Src-AA}}$ values for particles collected between 600 and 1250 m depth. Only $\delta^{15}\text{N}_{\text{Src-AA}}$ values resulting from the average of $\delta^{15}\text{N}$ values of

the four source amino acids (serine, phenylalanine, lysine, and glycine) were included in the calculations of end-members (Fig. 2). The $\delta^{15}\text{N}_{\text{Src-Zoo}}$ values are the averaged $\delta^{15}\text{N}_{\text{Src-AA}}$ values of zooplankton (1–2 mm fraction) collected at night and during the day between 700 and 1000 m, and between 1000 and 1250 m depth. We analyzed zooplankton in these deep waters because shallower populations will likely have a contribution of surface material in their diets through vertical migration. For a few samples, the $\delta^{15}\text{N}_{\text{Src-Zoo}}$ values were higher than the $\delta^{15}\text{N}_{\text{Src-AA}}$ values of small particles, likely because only a subset of highly microbially altered small particles was used as a food source (Hannides et al. 2020). In these cases, we assumed a f_{SP} of 1.

Thorium flux

Total ^{234}Th was measured in samples of seawater collected at discrete depths using Niskin bottles as described in Umhau et al. (2019). These samples were collected during the same period as particles and zooplankton at each site. The ^{234}Th flux ($\text{dpm m}^{-2} \text{ d}^{-1}$) at each depth was calculated from the $^{234}\text{Th} : ^{238}\text{U}$ disequilibrium and assuming a steady state (Umhau et al. 2019). The ^{234}Th flux at 200 m was obtained by integrating the flux for the overlying depths.

Migrant zooplankton biomass

Freeze-dried zooplankton filters for all size fractions were weighed to calculate zooplankton biomass (mg in dry weight/ m^3) at each depth during the day and at nighttime. Deep migrator biomass was obtained from the difference in total biomass (i.e., all size-fractions) between zooplankton collected during the day and at nighttime at a certain depth and integrated for depths below 200 m (Supporting Information Fig. S1). We used deep migrator biomass because migrator biomass at the upper 200 m was not equal to deep migrator biomass below 200 m for all the sites, pointing to an effect of net avoidance when zooplankton was collected during the day close to the surface.

References

- Abramson, L., C. Lee, Z. Liu, S. G. Wakeham, and J. Szlosek. 2010. Exchange between suspended and sinking particles in the northwest Mediterranean as inferred from the organic composition of in situ pump and sediment trap samples. *Limnol. Oceanogr.* **55**: 725–739. doi:10.4319/lo.2009.55.2.0725
- Alonso-González, I. J., and others. 2010. Role of slowly settling particles in the ocean carbon cycle. *Geophys. Res. Lett.* **37**: 1–5. doi:10.1029/2010GL043827
- Altabet, M. A., W. G. Deuser, S. Honjo, and C. Stienen. 1991. Seasonal and depth-related changes in the source of sinking particles in the North Atlantic. *Nature* **354**: 136–139. doi:10.1038/354136a0
- Archibald, K. M., D. A. Siegel, and S. C. Doney. 2019. Modeling the impact of zooplankton diel vertical migration on the

- carbon export flux of the biological pump. *Global Biogeochem. Cycles* **33**: 181–199. doi:[10.1029/2018GB005983](https://doi.org/10.1029/2018GB005983)
- Armstrong, R. A., C. Lee, J. I. Hedges, S. Honjo, and S. G. Wakeham. 2001. A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **49**: 219–236. doi:[10.1016/S0967-0645\(01\)00101-1](https://doi.org/10.1016/S0967-0645(01)00101-1)
- Bishop, J. K. B., P. J. Lam, and T. J. Wood. 2012. Getting good particles: Accurate sampling of particles by large volume in-situ filtration. *Limnol. Oceanogr.: Methods* **10**: 681–710. doi:[10.4319/lom.2012.10.681](https://doi.org/10.4319/lom.2012.10.681)
- Bopp, L., O. Aumont, P. Cadule, S. Alvain, and M. Gehlen. 2005. Response of diatoms distribution to global warming and potential implications: A global model study. *Geophys. Res. Lett.* **32**: 1–4. doi:[10.1029/2005GL023653](https://doi.org/10.1029/2005GL023653)
- Burd, A. B., and others. 2010. Assessing the apparent imbalance between geochemical and biochemical indicators of meso- and bathypelagic biological activity: What the @\$#! is wrong with present calculations of carbon budgets? *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **57**: 1557–1571. doi:[10.1016/j.dsr2.2010.02.022](https://doi.org/10.1016/j.dsr2.2010.02.022)
- Chikaraishi, Y., and others. 2009. Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnol. Oceanogr.: Methods* **7**: 740–750. doi:[10.4319/lom.2009.7.740](https://doi.org/10.4319/lom.2009.7.740)
- Choy, C. A., B. N. Popp, C. C. S. Hannides, and J. C. Drzen. 2015. Trophic structure and food resources of epipelagic and mesopelagic fishes in the North Pacific Subtropical Gyre ecosystem inferred from nitrogen isotopic compositions. *Limnol. Oceanogr.* **60**: 1156–1171. doi:[10.1002/lno.10085](https://doi.org/10.1002/lno.10085)
- Church, M. J., M. W. Lomas, and F. Muller-Karger. 2013. Sea change: Charting the course for biogeochemical ocean time-series research in a new millennium. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **93**: 2–15. doi:[10.1016/j.dsr2.2013.01.035](https://doi.org/10.1016/j.dsr2.2013.01.035)
- Duret, M. T., R. S. Lampitt, and P. Lam. 2019. Prokaryotic niche partitioning between suspended and sinking marine particles. *Environ. Microbiol. Rep.* **11**: 386–400. doi:[10.1111/1758-2229.12692](https://doi.org/10.1111/1758-2229.12692)
- Gloeckler, K., C. A. Choy, C. C. S. Hannides, H. G. Close, E. Goetze, B. N. Popp, and J. C. Drzen. 2018. Stable isotope analysis of micronekton around Hawaii reveals suspended particles are an important nutritional source in the lower mesopelagic and upper bathypelagic zones. *Limnol. Oceanogr.* **63**: 1168–1180. doi:[10.1002/lno.10762](https://doi.org/10.1002/lno.10762)
- Hannides, C. C. S., B. N. Popp, C. Anela Choy, and J. C. Drzen. 2013. Midwater zooplankton and suspended particle dynamics in the North Pacific Subtropical Gyre: A stable isotope perspective. *Limnol. Oceanogr.* **58**: 1931–1936. doi:[10.4319/lo.2013.58.6.1931](https://doi.org/10.4319/lo.2013.58.6.1931)
- Hannides, C. C. S., and others. 2020. Seasonal dynamics of midwater zooplankton and relation to particle cycling in the North Pacific Subtropical Gyre. *Prog. Oceanogr.* **182**: 102266. doi:[10.1016/j.pocean.2020.102266](https://doi.org/10.1016/j.pocean.2020.102266)
- Herndl, G. J., and T. Reinthaler. 2013. Microbial control of the dark end of the biological pump. *Nat. Geosci.* **6**: 718–724. doi:[10.1038/ngeo1921](https://doi.org/10.1038/ngeo1921)
- Hernes, P. J., M. L. Peterson, J. W. Murray, S. G. Wakeham, C. Lee, and J. I. Hedges. 2001. Particulate carbon and nitrogen fluxes and compositions in the central equatorial Pacific. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* **48**: 1999–2023. doi:[10.1016/S0967-0637\(00\)00115-1](https://doi.org/10.1016/S0967-0637(00)00115-1)
- Jones, D. O. B., A. Yool, C. L. Wei, S. A. Henson, H. A. Ruhl, R. A. Watson, and M. Gehlen. 2014. Global reductions in seafloor biomass in response to climate change. *Glob. Chang. Biol.* **20**: 1861–1872. doi:[10.1111/gcb.12480](https://doi.org/10.1111/gcb.12480)
- Lam, P. J., S. C. Doney, and J. K. B. Bishop. 2011. The dynamic ocean biological pump: Insights from a global compilation of particulate organic carbon, CaCO₃, and opal concentration profiles from the mesopelagic. *Global Biogeochem. Cycles* **25**: 1–14. doi:[10.1029/2010GB003868](https://doi.org/10.1029/2010GB003868)
- Lam, P. J., D. C. Ohnemus, and M. E. Auro. 2015. Size-fractionated major particle composition and concentrations from the US GEOTRACES North Atlantic Zonal Transect. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **116**: 303–320. doi:[10.1016/j.dsr2.2014.11.020](https://doi.org/10.1016/j.dsr2.2014.11.020)
- Ohkouchi, N., and others. 2017. Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. *Org. Geochem.* **113**: 150–174. doi:[10.1016/j.orggeochem.2017.07.009](https://doi.org/10.1016/j.orggeochem.2017.07.009)
- Passow, U., and C. A. Carlson. 2012. The biological pump in a high CO₂ world. *Mar. Ecol. Prog. Ser.* **470**: 249–271. doi:[10.3354/meps09985](https://doi.org/10.3354/meps09985)
- Polovina, J. J., E. A. Howell, and M. Abecassis. 2008. Ocean's least productive waters are expanding. *Geophys. Res. Lett.* **35**: 2–6. doi:[10.1029/2007GL031745](https://doi.org/10.1029/2007GL031745)
- Popp, B. N., B. S. Graham, R. J. Olson, C. C. S. Hannides, M. J. Lott, G. A. López-Ibarra, F. Galván-Magaña, and B. Fry. 2007. Stable isotopes as indicators of ecological change. *Terr. Ecol.* **1**: 173–190. doi:[10.1016/S1936-7961\(07\)01012-3](https://doi.org/10.1016/S1936-7961(07)01012-3)
- Richardson, T. L., and G. A. Jackson. 2007. Small phytoplankton and carbon export from the surface ocean. *Science* **315**: 838–840. doi:[10.1126/science.1133471](https://doi.org/10.1126/science.1133471)
- Riebesell, U., A. K. Rtzinger, and A. Oschlies. 2009. Sensitivities of marine carbon fluxes to ocean change. *Proc. Natl. Acad. Sci. USA* **106**: 20602–20609. doi:[10.1073/pnas.0813291106](https://doi.org/10.1073/pnas.0813291106)
- Sheridan, C. C., C. Lee, S. G. Wakeham, and J. K. B. Bishop. 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific Ocean. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* **49**: 1983–2008. doi:[10.1016/S0967-0637\(02\)00118-8](https://doi.org/10.1016/S0967-0637(02)00118-8)
- Siegel, D. A., K. O. Buesseler, S. C. Doney, S. F. Sailley, M. J. Behrenfeld, and P. W. Boyd. 2014. Global assessment of ocean carbon export by combining satellite observations and food-web models. *Global Biogeochem. Cycles* **28**: 181–196. doi:[10.1002/2014GB004832](https://doi.org/10.1002/2014GB004832)

- Umhau, B. P., C. R. Benitez-Nelson, H. G. Close, C. C. S. Hannides, L. Motta, B. N. Popp, J. D. Blum, and J. C. Drazen. 2019. Seasonal and spatial changes in carbon and nitrogen fluxes estimated using ^{234}Th : ^{238}U disequilibria in the North Pacific tropical and subtropical gyre. *Mar. Chem.* **217**: 103705. doi:[10.1016/j.marchem.2019.103705](https://doi.org/10.1016/j.marchem.2019.103705)
- Zhang, X., and H. G. Dam. 1997. Downward export of carbon by diel migrant mesozooplankton in the central equatorial Pacific. *Deep-Sea Res. Part II Top. Stud. Oceanogr.* **44**: 2191–2202. doi:[10.1016/S0967-0645\(97\)00060-X](https://doi.org/10.1016/S0967-0645(97)00060-X)

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Conflict of Interest

None declared.

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