Changes in fecal pellet characteristics with depth as indicators of zooplankton repackaging of particles in the mesopelagic zone of the subtropical and subarctic North Pacific Ocean

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Abstract

We investigated how fecal pellet characteristics change with depth in order to quantify the extent of particle repackaging by mesopelagic zooplankton in two contrasting open-ocean systems. Material from neutrally buoyant sediment traps deployed in the summer of 2004 and 2005 at 150, 300, and 500 m was analyzed from both a mesotrophic (Japanese time-series station K2) and an oligotrophic (Hawaii Ocean Time series-HOT station ALOHA) environment in the Pacific Ocean as part of the VERtical Transport In the Global Ocean (VERTIGO) project. We quantified changes in the flux, size, shape, and color of particles recognizable as zooplankton fecal pellets to determine how these parameters varied with depth and location. Flux of K2 fecal pellet particulate organic carbon (POC) at 150 and 300 m was 4-5 times higher than at ALOHA, and at all depths, fecal pellets were 2-5 times larger at K2, reflective of the disparate zooplankton community structure at the two sites. At K2, the proportion of POC flux that consisted of fecal pellets generally decreased with depth from 20% at 150 m to 5% at 500 m, whereas at ALOHA this proportion increased with depth (and was more variable) from 14% to 35%. This difference in the fecal fraction of POC with increasing depth is hypothesized to be due to differences in the extent of zooplankton-mediated fragmentation (coprohexy) and in zooplankton community structure between the two locations. Both regions provided indications of sinking particle repackaging and zooplankton carnivory in the mesopelagic. At ALOHA this was reflected in a significant increase in the mean flux of larvacean fecal pellets from 150 to 500 m of 3 to 46 μg C m^{-2} d^{-1}, respectively, and at K2 a large peak in larvacean mean pellet flux at 300 m of 3.1 mg C m^{-2} d^{-1}. Peaks in red pellets produced by carnivores occurred at 300 m at K2, and a variety of other fecal pellet classes showed significant changes in their distribution with depth. There was also evidence of substantially higher pellet fragmentation at K2 with nearly double the ratio of

broken:intact pellets at 150 and 300 m (mean of 67% and 64%, respectively) than at ALOHA where the proportion of broken pellets remained constant with depth (mean 35%). Variations in zooplankton size and community structure within the mesopelagic zone can thus differentially alter the transfer efficiency of sinking POC.

Key Words: fecal pellets, mesopelagic, particle flux, zooplankton

1. Introduction

Fecal pellets produced by zooplankton can significantly contribute to vertical flux of particulate organic carbon (POC) and are thus a key component of the biological pump (Bishop et al., 1977; Urrère and Knauer, 1981; Lampitt et al., 1990; Silver and Gowing, 1991; Carroll et al., 1998; Turner, 2002). The contribution of fecal pellets to total sinking POC flux is highly variable and is affected by multiple factors (Taguchi and Saino, 1998; González et al., 2000; Wassmann et al., 2000; Turner, 2002). Zooplankton community composition, vertical migration behavior, and mode of nutrition can all determine fecal pellet abundance and composition and thus the delivery of POC to the deep sea (Noji, 1991; Noji et al., 1991; Steinberg et al., 2000).

Zooplankton feeding activity can affect the rate at which particles reach the deep ocean, with much of this modification of sinking POC flux occurring within the mesopelagic "twilight zone," or depths below the euphotic zone to 1000 m (Fowler and Knauer, 1986; Sasaki et al., 1988; Lampitt, 1992). In a resource-limited environment such as the mesopelagic, zooplankton must obtain their nutrition via vertical migration and surface feeding, or carnivory and particle feeding within the mesopelagic (Angel, 1989; Steinberg, 1995; Uttal and Buck, 1996; Schnetzer and Steinberg, 2002). Little is known about the extent of particle feeding by zooplankton within the mesopelagic, or in general about food web processes affecting carbon cycling within this region (Angel, 1989; Dagg, 1993; Kosobokova et al., 2002; Schnetzer and Steinberg, 2002). Mesopelagic zooplankton consume smaller sinking or suspended particles and 'repackage' them as dense, quickly-sinking fecal pellets. While sinking, fecal pellets can become fragmented into slower or non-sinking particles (coprohexy) via sloppy feeding or swimming activity, re-ingested (coprophagy) by other zooplankton, or stick to other particles to form aggregates of marine snow

(Alldredge and Silver, 1988; Lampitt et al., 1990; Noji, 1991; González et al., 1994; Dilling and Alldredge, 2000; Goldthwait et al., 2004). Fecal pellets can contain large amounts of undigested or partially-digested material that is utilized by bacteria and microzooplankton (Pomeroy et al., 1984), and alteration of pellets by zooplankton and bacteria can slow the export of carbon (Longhurst and Harrison, 1989; Lampitt et al., 1990).

Changes in fecal pellet type with depth can be used as an indication of zooplankton repackaging of particles in the mesopelagic zone (Carroll et al., 1998). Fecal pellets are produced in a variety of sizes, shapes, and, colors dependant upon the species and their diet. For example, euphausiids produce long (> 1 mm) cylindrical pellets (Fowler and Small, 1972; González, 1992); salps produce large (> 1 mm), tabular-shaped, fragile pellets that sink rapidly (Bruland and Silver, 1981; Anderson, 1998; Yoon et al., 2001; Madin et al., 2006); and larvaceans produce dense, ellipsoid pellets that also sink rapidly (Gorsky and Fenaux, 1998; Taguchi and Saino, 1998). Carnivores such as chaetognaths (Dilling and Alldredge, 1993) and heteropods (personal observation) produce irregularly-shaped semi-transparent fecal pellets. Small, spherical "mini pellets" (< 60 μm) are produced by zooplankton nauplii and microzooplankton (Gowing and Silver, 1985; Gowing et al., 2001; Turner, 2002). Copepods vary in shape and size and their pellets are also variable and can be small, ellipsoid or ovoid in shape, or large and cylindrical with rounded or pointed ends (Martens, 1978; Yoon et al., 2001).

Pellet color can be a general indicator of zooplankton diet. Although pellet color can fade with increased bacterial decomposition (Hansen et al., 1996) and with the addition of formaldehyde in sample preservation, white and lighter pellets (including some cylindrical transparent pellets, personal observation) may indicate feeding on detritus, fecal pellets, or transparent flagellates (Urrère and Knauer, 1981; Noji *et al.*, 1991). Green and darker brown

colors may indicate feeding on phytoplankton; lighter brown pellets may indicate feeding on a mixture of diatoms, protists, and marine snow (Honjo, 1978; Hansen et al., 1996; Urban-Rich et al., 1998). Red, orange, and most other transparent pellets reflect carnivorous feeding on mid-water prey species (Dilling and Alldredge, 1993; Urban-Rich et al., 1998).

We quantified the extent of particle repackaging by mesopelagic zooplankton as part of a study investigating particle flux and transformations in the mesopelagic zone (VERtical Transport In the Global Ocean -VERTIGO). We analyzed fecal pellets from sediment traps deployed at 150, 300, and 500 m in the subtropical and the subarctic North Pacific Ocean to investigate the change in fecal pellet characteristics (e.g., size, shape, color) with depth and determined the importance of fecal pellet flux to POC export. Due to the differences in zooplankton and phytoplankton community structure between the two contrasting sites, a comparison of particle repackaging by zooplankton communities at these sites will help elucidate how plankton community structure may affect the biological pump.

2. Methods

2. 1 Sediment trap collections

Neutrally buoyant sediment traps (NBSTs), were deployed at two contrasting sites in the North Pacific Ocean twice for 3-4 days at 150, 300, and 500 m at each site. These traps are designed to reduce horizontal flow across the mouth of the trap and are mounted on a neutral-density float (Buesseler et al., 2000; Stanley et al., 2004; Buesseler et al., 2007). The six baffled collection cylinders on each trap were partially filled with a brine and formaldehyde solution and once traps were recovered, contents were preserved in 4% buffered formaldehyde solution

(Buesseler et al., 2007). To avoid pellet breakage during processing, trap samples used for our analysis were not screened – settled samples were gently poured whole into sample jars for analysis.

The first trap collections were made June 22-July 9, 2004 at the Hawaii Ocean Time series-HOT station ALOHA in the oligotrophic subtropical gyre (27.75^{o} N, 158^{o} W) aboard the R/V *Kilo Moana*. The second collections were made July 22-August 11, 2005 at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) time-series site K2, in a high nutrient, variable chlorophyll region of the subarctic gyre (47^{o} N, 160^{o} E) aboard the R/V *Roger Revelle*. At ALOHA, primary production was 180-220 mg C m^{-2} d^{-1}, new production was 18-38 mg C m^{-2} d^{-1}, mixed layer nutrients were at nanomolar concentrations, and the phytoplankton assemblage consisted of small diatoms, coccolithophorids, picoplankton, and cyanobacteria (Buesseler et al., 2007; Buesseler et al., 2008; Lamborg et al., 2008). At K2, primary production (365-530 mg C m^{-2} d^{-1}), new production (70-150 mg C m^{-2} d^{-1}), and nutrients (12 μM mixed layer DIN) were all higher than ALOHA, and the K2 phytoplankton assemblage consisted of picoplankton and large diatoms (Buesseler et al., 2007, Buesseler et al. 2008; Lamborg et al., 2008). Zooplankton biomass in the surface 150 m was an order-of-magnitude higher at K2 than ALOHA (Steinberg et al., 2008 b). The majority of the zooplankton biomass was < 2 mm in size at station ALOHA and > 2 mm at station K2, due to the high numbers of large *Neocalanus* spp. calanoid copepods at K2 (Kobari et al., 2008; Steinberg et al., 2008 b).

2.2 Fecal pellet analysis

Preserved subsamples of the NBST sediment trap material from the two sites at all three depths (see Fig. 1 caption for replication at each site and depth) were analyzed using an Olympus

SZX12 stereo dissecting microscope and digital camera under dark- and light-field illumination. Digital images were analyzed using ImagePro© and Adobe Photoshop© software. The type of particles caught in sediment traps was recorded (e.g. fecal pellets, fecal 'fluff', mucous feeding webs, and phytodetritus). Changes in fecal pellet size, shape, color, condition (intact vs. broken), and flux with depth, station, and deployment were used as an indication of the amount of zooplankton processing and repackaging at depth. Separate one-way ANOVAs were used to test for differences between sites and among depths in all parameters unless otherwise noted in the text.

Particles recognizable as fecal pellets were counted, measured and categorized by shape and color. Pellets were placed into four shape categories: ovoid, cylindrical, spherical, and amorphous. Fecal pellets were categorized as intact (i.e., peritrophic membrane present, smooth edges) or broken/degraded yet still recognizable as a pellet (i.e., peritrophic membrane partially absent, frayed edges, fragmented). Particles that were unrecognizable as fecal pellets yet may have been fecal in origin (e.g. fecal 'fluff,' with peritrophic membrane completely absent) were not counted or measured in this study but were recorded when present. Pellet color, a factor that is dependent on the food available, was analyzed both by eye and using standard RGB (red, green, and blue) values from the software ImagePro© and Adobe Photoshop© (Table 1) to provide an additional, more objective, reference for color. Sections of pellets were quantitatively analyzed for average RGB values using the images that were photographed under constant light conditions and camera settings. Pellets were categorized into four color classes (with corresponding diet): dark brown (herbivory); light brown (omnivory, detritivory); red (carnivory); and white, transparent, or multi-colored (omnivory, detritivory) (Table 1). Dominant shape/color pairs characteristic of pellets from major zooplankton taxa were also analyzed. These

included larvaceans (ellipsoid/light brown), large copepods (cylindrical/beige or light brown), decapods and euphausiids (cylindrical/white or transparent), and small herbivorous copepods and nauplii (ovoid/light or dark brown, spherical/light or dark brown).

Fecal pellets were converted to carbon to determine the contribution of fecal pellet carbon flux to total trap carbon flux and compared between the two sites and depths. Pellet volume was calculated based on length and width measurements (using an ocular micrometer or ImagePro© software) and applying the formula for a sphere, cylinder, or ovoid shape that most closely resembled the pellet shape. Fecal pellet volume was converted to carbon using a conversion factor of 0.08 mg C mm^{-3}, a mid-range estimate based on a range of conversion factors (0.01 to 0.15 mg C mm^{-3}) from the literature (Silver and Gowing, 1991; Lundsgaard and Olesen, 1997; Carroll et al., 1998; Taguchi and Saino, 1998; Urban-Rich et al., 1998; Roy et al., 2000; Wassmann et al., 2000; Gowing et al., 2001; Wexels Riser et al., 2001; Suzuki et al., 2003; Huskin et al., 2004; Olesen et al., 2005; Reigstadt et al., 2005) and from our own measurements at station K2 (see below and results). Extremely large pellets of the heteropod *Carinaria spp.* (see results) were only found at station ALOHA at 150 m (in all replicates). We excluded heteropod pellet POC contribution in our analyses as we have no reliable estimate of their carbon-to-volume content.

2.3 Live animal fecal pellet collection

Sediment traps contain an array of different types of zooplankton fecal pellets, many of which are from an unknown source (Martens, 1978; Urrère and Knauer, 1981; Carroll et al., 1998). We performed incubations with live zooplankton to help identify the source of fecal pellet types found in the sediment traps. Live animals were collected at multiple depths at both stations

using a 1 m-diameter, 333 μm mesh, opening/closing net equipped with a non-filtering cod end. Species that were abundant and in good condition were placed into 1 L jars (in groups of 4 to 100 per jar) of 0.2 μm filtered seawater fitted with either a 300 or 500 μm (depending on animal size) nitex mesh "trap" to separate fecal pellets from the live animals (to avoid coprophagy). Fecal pellets were collected after 12-24 hr and photographed. Fecal pellet shape, color, and size were measured under a dissecting microscope and used to help identify and categorize pellets in sediment traps. Pellets collected from live animals at station ALOHA included calanoid copepods, the heteropod *Carinaria* spp., euphausiids, and ostracods, and at K2 included calanoid copepods *Paraeuchaeta* spp., *Neocalanus* spp., and *Eucalanus bungii*; chaetognaths; euphausiids; and ostracods. Carbon content of 78 larvacean pellets selected from the sediment trap samples at K2 (no replicates), and two to three replicates of 7-140 fresh fecal pellets from a variety of the K2 zooplankton mentioned above were measured with a high temperature combustion technique on a Thermo Electron Flash EA 1112 C/N analyzer. This analysis was performed on samples filtered onto silver membrane filters (Sterlitech; nominal pore size 1.2 μm). There was insufficient material available from the ALOHA incubations to measure fecal pellet carbon.

3. Results

3.1 Fecal Pellet Carbon Flux

At station ALOHA, trap total POC flux with depth was not significantly different between deployments (Buesseler et al., 2007; Lamborg et al., 2008), therefore fecal pellet results were combined for deployments 1 and 2. As the trap total POC flux at K2 decreased three-fold

between deployments (D1 and D2) (Fig. 1, Buesseler et al. 2007), fecal pellet results were separated by deployment. The total pellet carbon flux was up to 3.2 times higher at station K2 than station ALOHA at 150 m, and up to 5.4 times higher at 300 m. Total pellet carbon flux was also higher at all three depths during deployment 1 vs. 2 at K2 (Fig. 1). The proportion of the total POC flux that was recognizable fecal pellets ranged from 14.2-35% at ALOHA and 2.8-28.5% at K2 (Fig. 1, Fig. 2a). This proportion increased slightly but not significantly with depth at ALOHA (Fig. 2a, mean % ± 1 s.d.: 150 m = 14.2 ± 9.6, 300 m = 22.1 ± 23.1, 500 m =35.0 ± 23.4; p = 0.47), decreased significantly with depth in the second deployment at K2 (Fig. 2a, mean % ± s.d: 150 m = 28.5 ± 3.6, 300 m = 20.0 ± 3.0, 500 m = 5.6 ± 4.8; p = 0.01) and from 300 to 500 m in the first deployment at K2 (Fig. 2a, mean % ± s.d: 150 m = 12.1 ± 0.6, 300 m = 14.7 ± 0.5, 500 m = 2.8; p = 0.01).

Although total trap POC flux decreased with depth at station ALOHA (Fig. 1a), fecal pellet carbon flux (mean pellet carbon flux for all three depth intervals ± s.d.: 1.7 ± 1.2 mg C m^{-2} d^{-1}) was not significantly different between depth intervals (Fig. 2b, p = 0.49). At station K2 however, the fecal pellet carbon flux did decrease significantly with depth in both deployments (Fig. 2b, mean pellet carbon flux, mg C m^{-2} d^{-1} ± 1 s.d. D1: 150 m = 7.6 ± 0.4; 300 m =6.9 ± 0.8; 500 m = 0.8; p = 0.02. D2: 150 m = 6.7 ± 1.4; 300 m = 3.2 ± 0.01; 500 m = 0.7 ± 0.6; p=0.003). Differences in total pellet carbon flux between deployments at K2 were observed at 150 m where the proportion of pellet POC increased two-fold (p = 0.045) and pellet POC decreased two-fold at 300 m, (p = 0.02) from D1 to D2 (Fig. 2).

3.2 Fecal Pellet Size

The median size (μg C pellet^{-1}) of individual fecal pellets were two to five times larger at K2 than at ALOHA at all depths (Table 2, Mann-Whitney two sample test: $p < 0.001$). The majority of the fecal pellets were small at ALOHA although there were some very large pellets (the largest pellets at ALOHA were from *Carinaria* spp. and were not included here, see methods). Fecal pellet carbon frequency distributions (normalized to 1000 pellets for each depth using: [number of pellets in a size class/total number of pellets]*1000) were also significantly different between ALOHA and K2 at all depths, showing clearly the higher abundance of larger size classes of pellets at K2 (Fig. 3, χ^2 test: $p < 0.001$ for all depths). Pellets were similar in size between all three depths at ALOHA (Table 2, Mann-Whitney two sample test: 150 m, $p = 0.13$; 300 m, $p = 0.78$; 500 m, $p = 0.12$) and different at K2 (Table 2, Mann-Whitney two sample test: $p < 0.001$ for all depths and deployments). The frequency distribution between the deployments at station K2 were different for all depths (Fig. 3, χ^2 test: $p < 0.001$), however at 150 m, the median fecal pellet POC in D1 was nearly double that of D2 (Table 2, Mann-Whitney two sample test: $p < 0.001$).

3.3 Fecal pellet characteristics- live animal fecal pellet collections

As fecal pellet color and shape can be taxon-specific, and change with food type and region, fecal pellets from live incubations were used to help identify fecal pellets in sediment traps (Table 3; some examples of which can be seen in Fig. 4). The heteropod *Carinaria* spp. produced large (mean length of 1.6 mm) distinct pellets at ALOHA (A, Fig. 4). These were fragile, transparent, and filled with pieces of copepods, other zooplankton species, and unidentified spines. Euphausiids at ALOHA produced cylindrical pellets during the incubations which averaged 1.6mm in length, and were red, light brown or transparent in color. These pellets

were also usually broken around the edges (B, Fig. 4). At K2, larger copepods such as *Neocalanus* spp. produced cylindrical light brown pellets (mean length of 465 μm; E, Fig. 4), however only *Neocalanus* spp. collected from 300 m or above produced pellets in the incubations (indicating they cleared their guts during capture, or were in dormancy and not feeding). *Eucalanus bungii*, a common particle feeder at K2 did not produce pellets despite multiple incubation attempts. Chaetognaths produced amorphous (mean length of 2.2 mm) red pellets containing orange lipid globules and copepod parts; many of their pellets floated to the surface of the incubation chamber. *Paraeuchaeta* spp. (a carnivorous copepod) produced cylindrical (mean length of 474 μm), transparent pellets with a pointed end that also contained lipid globules. Euphausiids at K2 produced long (mean length of 1.1 mm), thin white or light brown cylindrical pellets (e.g., F, Fig. 4) and ostracods produced multi-colored crescent-shaped pellets (mean length of 1.1mm).

Pellet carbon measurements (mg C mm^{-3}) for incubated zooplankton at K2 were as follows (mean ± 1 s.d.): chaetognaths, 0.03 ± 0.01; euphausiids, 0.08 ± 0.01; larvaceans, 0.085 (one replicate); *Neocalanus* spp. copepods, 0.13 ± 0.04, and *Paraeuchaeta* spp. copepods, 0.15 ± 0.03. Mean fecal pellet carbon of all pellets and taxa analyzed was 0.11 ± 0.04 mg C mm^{-3} (not normalized to the actual contribution of these various taxa to pellets in the traps and also excludes chaetognaths, as their pellets were not observed in the traps).

3.4 Changing fecal pellet types with depth

General differences in fecal pellet size as discussed above, and type (see below) between stations and with depth can be seen in Figure 4. The transparent pellets of the heteropod, *Carinaria spp*. were the largest of all the pellets in the traps (A, Fig. 4), and were only found at

station ALOHA at 150 m. The large cylindrical pellets of euphausiids, decapods, and large calanoid copepods were common in the traps and observed at all depths (B, Fig. 4). Smaller copepods (C, Fig. 4) and larvacean pellets (D, Fig. 4), were also observed in the traps at ALOHA. At station K2, the large cylindrical pellets from the calanoid copepod *Neocalanus* spp. were extremely common (E, Fig. 4) as were the longer, thinner euphausiid pellets (F, Fig. 4). Red pellets produced by carnivores (G, Fig. 4), and larvacean pellets (D, Fig. 4) emerged at deeper depths at K2. Broken pellets and fecal 'fluff' (significantly degraded and therefore unrecognizable remnants of pellets) were present in traps at both locations and all depths. Fecal 'fluff' (H, Fig. 4), was more apparent at K2, as were broken pellets (I, Fig. 4). Chaetognath pellets were not found in any of the sediment trap samples analyzed and ostracod pellets were rare.

The flux of pellets of different colors and shape changed with depth at station ALOHA, although the data were highly variable and not statistically significant when tested for differences with depth (Fig. 5a and b; $p > 0.05$). Cylindrical pellets contributed the most to fecal pellet carbon flux at all three depths (Fig. 5a). Flux of dark brown pellets (indicative of herbivory) was highest at 150 m, while flux of red pellets (indicative of carnivory) increased with depth (Fig. 5b). White and specific transparent pellets (indicative of detrital particle feeding) comprised the largest proportion of the fecal pellet flux at 150 and 300 m (69.1% and 57.7% respectively). Flux of light brown pellets was highest at 500 m (46.7% of total pellet POC flux) (Fig. 5b).

Sediment traps in both deployments at K2 contained similar fecal pellet shape and color distribution with some significant differences between depth and deployment (Fig. 5c-f). Cylindrical pellets contributed the most to pellet POC flux in both deployments at 150 m (78.8%) and significantly decreased with depth (D1, $p = 0.004$; D2, $p = 0.05$). *Neocalanus* spp.

and *E. bungii* are ontogentic vertical migrators and during this late summer period have begun their dormancy at depth during which time they do not feed (Dagg, 1993; Kobari and Ikeda, 2001; Kobari et al., 2008). Thus these copepod species collected from > 300 m did not produce pellets in our incubation experiments. This cessation of feeding and defecation may also partially account for this significant decrease in cylindrical fecal pellet flux in the traps with depth. In both K2 deployments, the flux of white & transparent pellets decreased significantly below 150 m (D1, $p = 0.02$; D2, $p = 0.009$). A red pellet flux maximum at 300 m was observed in D2 which was significantly higher than at other depths ($p = 0.026$). Cylindrical and spherical pellets decreased significantly from the first to the second deployment at 300 m ($p = 0.035$, $p = 0.015$ respectively) as did light brown and white and transparent pellets ($p = 0.040$, $p = 0.003$ respectively).

Depth distributions of fecal pellet color and shape combinations (characteristic of various taxa and feeding modes) that were common at both locations are shown in Figure 6. Larvacean fecal pellet POC flux increased significantly with depth at station ALOHA (Fig. 6a, $p = 0.011$) and were also significantly more abundant at 300 m vs. other depths in both deployments at station K2 (Fig. 6b and c; D1, $p = 0.01$; D2, $p = 0.004$). The pellet color/shape combinations (cylindrical beige or light brown) indicative of the dominant large copepods such as *Neocalanus* spp. were abundant at 150 m and decreased significantly in the deeper samples at K2 D2 ($p = 0.026$). Cylindrical and white or transparent pellets, made by a combination of decapods, euphausiids, *Neocalanus* spp. and other large calanoid copepods, were significantly higher at 150 m and decreased with depth (D1, $p = 0.017$; D2, $p = 0.008$). Ovoid/red pellets, produced by carnivores, were present in the traps at most depth levels although they were marginally highest at 300 m D2 ($p = 0.066$).

4. Discussion

4.1 Contribution of fecal pellets to sediment trap POC flux

The contribution of fecal pellets to POC flux at depth can be highly variable, with factors such as zooplankton community structure and behavior, as well as sampling location and season, playing an important role (Karl and Knauer, 1984; Wexels Riser et al., 2001; Wexels Riser et al., 2002; Huskin et al., 2004). At ALOHA and K2 mesozooplankton fecal pellets contributed from 14- 35%, and 3-39%, respectively, of the downward flux of POC through the mesopelagic zone. Seasonally-productive regions such as the Southern Ocean exhibit variations in fecal pellet contribution to POC flux at 100 m ranging from a low value of 2-7% in the summer to 22-63% in the spring (Dagg et al., 2003), while fecal pellet contribution from more oligotrophic regions such as the North Atlantic subtropical gyre was on average 30% (ranging from 2-82%) of the total POC flux at 200 m (Huskin et al., 2004) and in the Mediterranean ranged seasonally from 8%-24% at 200-2000 m (Carroll et al., 1998). Indeed some of this variability between studies may be due to differences in sampling depths, methodology and pellet carbon estimation.

The proportion of total trap POC flux that were fecal pellets at station ALOHA (although highly variable) increased slightly with depth, indicating fecal pellets may be a more important contribution to POC flux deeper. Fecal pellets as a proportion of POC flux increased significantly with depth to 200 m in the North Atlantic subtropical gyre (Huskin et al., 2004) and the importance of pellets as a component of POC flux can increase with depth via strong vertical migration of mesozooplankton due to feeding in surface waters and egestion at depth (Karl and Knauer, 1984), particle repackaging at depth, and other *in situ* processes such as the production

of new pellets at depth from carnivorous zooplankton species (Turner and Ferrante, 1979; Wassmann et al., 2000; Huskin et al., 2004). The flux of sinking POC able to support deeper biomass in oligotrophic regions such as ALOHA would be largely through the fecal pellet production of the predominantly small (< 2 mm) zooplankton residing there (Paffenhöfer and Knowles, 1979; Small et al., 1987; Steinberg et al., 2008 a).

In contrast to ALOHA, fecal pellet POC flux (both absolute flux and as a proportion of total trap POC flux) decreased with depth at station K2, as appears to be typical of regions with higher zooplankton biomass (Roy et al., 2000; Suzuki et al., 2003). Andreassen et al. (1990) and Suzuki et al (2003) both found a decrease in fecal pellet flux with depth related to the appearance of smaller sinking particles which they hypothesized were the remnants of fecal pellets (fecal 'fluff' in the present study– see below) and attributed to alteration by zooplankton (Lampitt et al., 1990; Noji et al., 1991). Zooplankton at K2 were predominately large (> 2 mm), and an order-of-magnitude higher in biomass than ALOHA (Steinberg et al., 2008 b), and produced ~1 mm long cylindrical fecal pellets which broke apart easily (observed in live incubations). Zooplankton-mediated processes such as coprohexy and coprophagy as well as the fragile nature of the pellets likely reduce the number of fecal pellets that make it through the mesopelagic intact at K2.

Our estimate of the contribution of fecal pellet POC to total POC flux is partially dependent upon the fecal pellet carbon-to-volume conversion. We applied a fecal pellet carbon-to-volume conversion of 0.08 mg C mm^{-3}, a mid-range value from the literature and close to our measured fecal pellet carbon content of several key species of 0.11 mg C mm^{-3}. A low-range carbon-to-volume conversion of 0.03 (Urrère and Knauer, 1981; Wassmann et al., 2000) decrease estimates of fecal pellet contribution threefold to 5-13% (ALOHA) and 2-8% (K2) of

the total POC flux. A higher range estimate of 0.11 measured here at K2 and in Carroll et al., (1998) would increase estimates of fecal pellet contribution by a factor of 1.4 to 19-48% (ALOHA) and 7-29% (K2) of the total POC flux. Further investigation of the C content of large, rare heteropod pellets, which may substantially increase fecal pellet contribution to POC flux at station ALOHA, is needed.

Fecal pellet size distribution also differed greatly between the two locations and likely influenced the contribution of fecal pellets to POC flux as well as the amount of the attenuation of vertical POC flux through the mesopelagic (Buesseler et al., 2007). Zooplankton fecal pellet size is correlated to zooplankton body size (Uye and Kaname, 1994), with larger zooplankton producing larger, faster-sinking pellets and smaller zooplankton producing smaller, slower-sinking pellets that are recycled quickly (Paffenhöfer and Knowles, 1979; Poulsen and Kiørboe, 2006), which could account for some of the difference in POC flux attenuation (higher at ALOHA) between the 2 sites (Buesseler et al., 2007, Lamborg et al., 2008).

4.2 Particle repackaging and carnivory

To meet their nutritional requirements, zooplankton in the mesopelagic zone may intercept and consume sinking particles (e.g. fecal pellets, marine snow), filter feed on small suspended particles, vertically migrate to feed on surface particles, or consume other zooplankton. Detrital particles are colonized by bacteria and microzooplankton (Alldredge and Silver, 1988; Azam and Long, 2001) and once ingested, are subsequently repackaged into fast sinking fecal pellets, exporting POC to the deep ocean (Turner and Ferrante, 1979 and references therein; Turner, 2002). Particle repackaging and carnivory in the mesopelagic were evident in our study due to changes in the presence of distinct fecal pellet types with depth.

Many ubiquitous zooplankton species in both oligotrophic and mesotrophic regimes are commonly recognized as sinking detrital particle repackagers. At ALOHA small poecilostomatoid and cyclopoid copepods were common sinking particle feeders (e.g. *Oncea* spp. and *Oithona* spp. (González and Smetacek, 1994). Svensen and Nejstgaard (2003) showed that when the abundance of *Oithona spp*. is high, fecal pellet flux is low, and hypothesize that the inverse relationship between the magnitude of POC export and the presence of *Oithona* may be common. Indeed in our study, both *Oncea spp.* and *Oithona spp*. copepods decreased in biomass below 150 m while fecal pellet flux slightly increased with depth at ALOHA. This pattern may be more apparent in oligotrophic regions such as ALOHA where the system is dominated by smaller zooplankton species where smaller pellets with slower sinking speeds are available for capture (Paffenhöfer and Knowles, 1979; Uye and Kaname, 1994; Wassmann *et al.*, 2000). There was also a low coprophagy rate of larger pellets by *Oithona* reported in several Sub-Arctic and Arctic studies (Sampei et al., 2004; Reigstad et al., 2005; Poulsen and Kiørboe, 2006). At K2, poecilostomatoid and cyclopoid copepods increased in biomass below 150 m as did fecal pellet flux, however these species constitute a smaller proportion of the zooplankton biomass at K2 compared to ALOHA. The high biomass at K2 of larger particle feeders such as *Eucalanus bungii*, *Neocalanus* spp., and ostracods may be more influential in particle feeding and fragmentation there (Uye and Kaname, 1994; Yamaguchi et al., 2002; Sampei et al., 2004).

The presence of larvacean fecal pellets in sediment traps at all sampled depths at ALOHA and K2 indicates repackaging of suspended POC in the mesopelagic. Larvaceans filter suspended particles from the water column using a mucous feeding web, or "house" with particles as small as 5-0.1 μm retained by their inner filter mesh (Alldredge and Madin, 1982; Deibel, 1998), therefore larvaceans can bypass the classical microbial loop by transforming small, suspended

particles into fast sinking fecal pellets (Michaels and Silver, 1988; Urban et al., 1992; Gorsky et al., 1999). At ALOHA larvacean pellet flux increased with depth and nearly all pellets were intact, except at 500 m where some pellets were partially decomposed or fragmented. At K2, the highest larvacean pellet flux occurred at 300 m. There was also an increase in the number of pellets in the larger size classes (1-2.5 mm) at 300 m, which may be attributed to this increase in larvacean fecal pellets. Nearly 90% of these larvacean pellets at 300 m were partially fragmented, which was higher than at the other two sampling depths at K2 (50% at 150 m, and 29% at 500 m), and may indicate stratified populations of larvaceans through the mesopelagic. Stratified mesozooplankton net sampling did not reveal a mesopelagic peak in larvacean abundance, however, these delicate animals are damaged easily and thus not sampled well by these nets (Steinberg et al., 2008 a).

Zooplankton that feed on other animals either living within the mesopelagic region or migrating through it can also contribute substantially to fecal pellet flux with depth (Small and Ellis 1992). Carnivorous zooplankton generally increase in abundance with depth and can produce new fecal pellets at depth that contribute to the sinking flux and that can be consumed by detritivores (Vinogradov and Tseitlin, 1983; Small and Ellis, 1992; Yamaguchi et al., 2002). Evidence of carnivorous feeding at both ALOHA and K2 include changes in the depth distribution of both red fecal pellets (the color deriving from crustacean prey with red or orange chitinous exoskeltons) and white/transparent pellets (deriving from prey with white or clear chitinous exoskeletons, transparent gelatinous zooplankton, or microzooplankton) at depth. At K2, flux of red, oval pellets was highest at 300 m (G, Fig. 4) as a result of carnivorous feeding between 150 and 300 m depth. The carnivorous zooplankter that produced these fecal pellets is unknown. Carnivorous chaetognaths were numerous in the zooplankton tows at both locations,

with abundance peaks in the mesopelagic (Steinberg et al., 2008 a), yet their distinctive pellets were rare in the traps. Chaetognath pellets collected from the incubation experiments at K2 were rich in lipid globules from their copepod prey which would make them a nutritious and labile food source. Dilling and Alldredge (1993) measured mesopelagic chaetognath pellet sinking rates off California and indicated that the pellets sank slower than other herbivorous zooplankton pellets of comparative size with some also remaining positively or neutrally buoyant. A small number of floating chaetognath pellets were also observed in the K2 incubation experiments. These slow sinking or floating chaetognath fecal pellets may be easily accessible as food to other zooplankton taxa. Thus we propose chaetognath fecal pellets may be consumed quickly while sinking and could supply the mesopelagic with an abundant and highly labile food source.

4.3 – Pellet fragmentation via swimming action and coprohexy

Zooplankton can efficiently fragment fecal pellets while swimming and feeding in a process known as coprohexy (Lampitt et al., 1990). Coprohexy has the potential to significantly increase the retention time of fecal pellet carbon in the water column by producing smaller, slower sinking particles vulnerable to zooplankton repackaging and microbial remineralization, and (as observed at station K2) decrease the number of intact pellets making it to deeper waters (Noji et al., 1991; Andreassen et al., 1996; Suzuki et al., 2003). Fecal pellets and other particles can fragment through abiotic processes such as turbulence in the mixed layer (Karl *et al.*, 1988), zooplankton swimming action (Dilling and Alldredge, 2000; Goldthwait et al., 2004), and sloppy feeding (Lampitt et al., 1990). The degree of fragmentation can also vary with season (Wassman et al. 1999). Many copepod species create a feeding current to obtain phytoplankton for ingestion

and any fecal material captured may be broken apart but not necessarily consumed (Poulsen and Kiørboe, 2005). Some copepod species will preferentially consume the peritrophic membrane of a fecal pellet and discard the rest (Lampitt et al., 1990; Noji et al., 1991; Small and Ellis, 1992; Alldredge et al., 1993), leaving the pellet more vulnerable to fragmentation.

The presence of fecal-fragment-derived marine snow (fecal 'fluff') in the trap samples could not be quantified as it was difficult to discern from other non-fecal-derived marine snow particles (Shanks and Trent, 1980; Sasaki et al., 1988). Sediment traps deployed in the Kerguelen Ocean and plateau (Southern Ocean) containing polyacrylamide gels, which capture particles relatively intact and in the form they sink (Lundsgaard, 1995; Waite et al., 2000), revealed that the majority of the sinking aggregates were fecal in origin and thus flux in the study area was hypothesized to be controlled by zooplankton grazers (Ebersbach et al., 2006). Fecal 'fluff' was considerably more apparent at all depths and in all traps at K2 than at ALOHA, and a significantly higher number of recognizable fecal pellets that were broken or partially fragmented were present at K2 at 150 and 300 m than at ALOHA, whereas at 500 m they were similar (Fig. 7). Thus, microbial processes and coprohexy, that transform pellets into fecal 'fluff' and render some pellets unrecognizable in our trap samples, may have resulted in an increase in fecal 'fluff' at K2, and an underestimation of the contribution of fecal pellets to total flux.

The most common pellets in the traps at K2 were produced by *Neocalanus cristatus*, *Neocalanus flemingeri*, and *Neocalanus plumchrus*. These copepods, along with *Eucalanus bungii* comprise the majority of the zooplankton biomass at K2 and are recognized as opportunistic herbivorous/omnivorous and particle feeders (Dagg, 1993; Shoden et al., 2005). *Neocalanus* spp. produced large, cylindrical pellets which were generally in large fragments in the sediment trap samples and accounted for 10 to 22% of the total POC flux across 150 m. This

range is considerably lower than an estimated 141-223 % for *Neocalanus* spp. pellets as a proportion of the total POC flux at 150 m at K2, based on copepod metabolic requirements (Kobari et al., 2008); this difference is likely due to coprophagy, coprohexy and microbial processes.

The dominance of the larger size and biomass of the mesozooplankton at K2 also likely enhanced fragmentation in the water column due to their faster swimming speeds and higher magnitude of vertical migration (Goldthwait et al., 2004) compared to ALOHA. Finally, sampling artifacts of breakage of pellets during handling of the material likely leads to additional error and further underestimation of fecal pellet flux in our study. Comparative studies with polyacrylimide-based gel traps will be useful to quantitatively determine both the prevalence of fecal-derived marine snow and the extent of pellet breakage from sinking and handling of samples (Lundsgaard, 1995; Waite et al., 2000).

5. Conclusions

This study provides evidence of both detrital particle repackaging and carnivory within the mesopelagic zone of the subtropical and subarctic North Pacific Ocean that can influence both the magnitude and character of sinking POC. Mesozooplankton community structure is important in determining the flux of fecal pellet carbon through the mesopelagic zone at both sites, with changes in fecal pellet types with depth indicating considerable repackaging of particles by a variety of different taxa. Recycling of fecal pellets by small zooplankton may play a large role in affecting POC export to depth in oligotrophic regions such as ALOHA (Small et al., 1987; Paffenhöfer and Knowles, 1979). In more mesotrophic regions such as K2, the larger

size and biomass of zooplankton and their fecal material promote high POC flux and increased transport efficiency of POC to depth (Buesseler et al., 2007, Steinberg et al. 2008). As the ocean surface continues to warm, the plankton biomass and community structure will be affected (Karl et al., 1996; Karl et al., 2001). By comparing mesopelagic food webs in contrasting environments, and how particles are made and modified by animals in the ocean's interior, we can gain some insight onto how predicted changes in the plankton community will affect the flux of carbon to the deep ocean.

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Yamaguchi, A., Watanabe, Y., Ishida, H., Harimoto, T., Fursawa, K., Suzuki, S., Ishizaka, J., Ikeda, T., Takahashi, M., 2002. Community and trophic structures of pelagic copepods down to greater depths in the western subarctic Pacific (WEST-COSMIC). Deep-Sea Research I 49, 1007-1025.

Yoon, W.D., Kim, S.K., Han, K.N., 2001. Morphology and sinking velocities of fecal pellets of copepod, molluscan, euphausiid, and salp taxa in the northeastern tropical Atlantic. Marine Biology 139, 923-928.

Figure 1. Recognizable fecal pellet POC fluxes at stations a) ALOHA and b) K2 compared to the remainder of POC flux (non-pellets). The value of stacked bars indicates total POC flux. Data for the two trap deployments were combined for ALOHA, as there was no significant difference in total POC flux between deployments (see results), and was kept separate for K2. Values are mean ± 1 s.d. Error bar is standard deviation of fecal pellet carbon flux. For ALOHA, n = 3 for all samples. For K2, n = 2 for 150, and 300 m for both deployments; n = 1 (D1) and n = 3 (D2) at 500 m. D1, deployment 1; D2, deployment 2.

Figure 2. Recognizable fecal pellet flux at ALOHA and K2 shown as a) fecal pellets as a proportion of total POC flux , and b) fecal pellet POC flux. Values are mean ± 1 s.d. For ALOHA, n = 3 for all samples. For K2, n = 2 for 150 m, and 300 m for both deployments; n = 1 (D1) and n = 3 (D2) at 500 m. D1, deployment 1; D2, deployment 2.

Figure 3. Fecal pellet carbon distribution at ALOHA and K2 from NBSTs deployed at a) 150 m, b) 300 m and c) 500 m. For ALOHA: 150 m, n = 421; 300 m n = 473; 500 m n = 580. For K2 (D1): n = 1201; 300 m n = 1175; 500 m n = 174: For K2 (D2): 150 m, n = 1867; 300 m n = 519; 500 m n = 463. Sample size normalized to 1000 pellets for each location, deployment, and, depth (see results). See also Table 2 for median values for each location, deployment, and depth. D1, deployment 1; D2, deployment 2.

Figure 4. Example zooplankton fecal pellets from sediment trap samples indicating major types of pellets identified at each location. Scale bar is 500 μm. A) heteropod *Carinaria* spp. B) large

copepod or euphausiid, C) small copepod, D) larvacean, E) *Neocalanus* spp., F) Euphausiid, G) unknown carnivorous zooplankton, H) fecal 'fluff,' I) broken pellet.

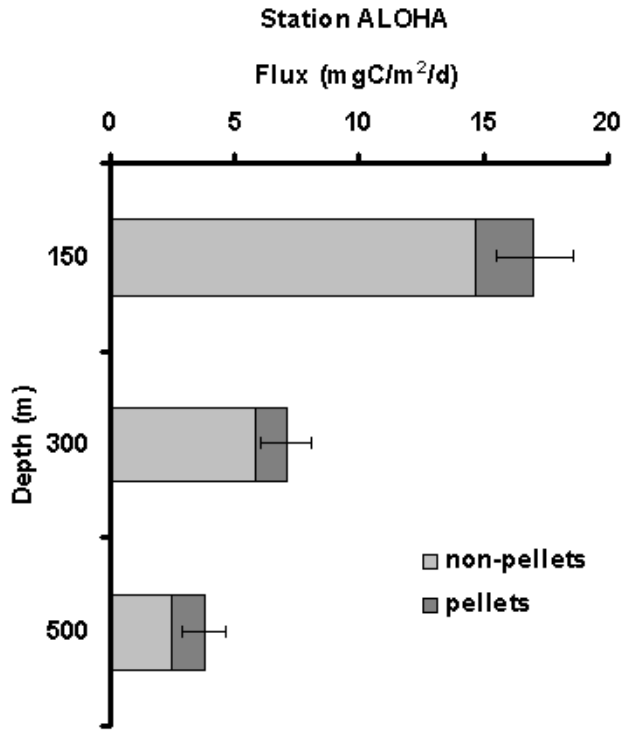
Figure 5. Flux of fecal pellets at ALOHA and K2 categorized by shape and color. Values are mean ± 1 s.d. A * indicates significant differences (ANOVA, $p < 0.05$). D1, deployment 1; D2, deployment 2.

Figure 6. Fecal pellet color/shape combinations and taxa for ALOHA (a) and K2 (b,c). Ovoid or spherical brown pellets (ovo/sph brown) may be attributed to small copepods and herbivores. Ovoid red pellets are attributed to carnivores. Cylindrical pellets that are beige or light brown (cyl beige & light) are attributed to omnivorous large copepods. Cylindrical pellets that are white or transparent (cyl white & trans) are attributed to particle feeders (i.e. large copepods and euphausiids). Values are mean ± 1 s.d. A * indicates significant differences (ANOVA, $p < 0.05$). D1, deployment 1; D2, deployment 2.

Figure 7. Broken recognizable fecal pellets at ALOHA and K2 shown as percent (%) of total number of pellets counted. These do not include any particles unrecognizable as fecal pellets or fecal 'fluff.' Values are mean ± 1 s.d. For ALOHA, n = 3. For K2, n = 4. Deployments at K2 were combined. ANOVA: 150 m, $p = 0.015$, 300 m, $p = 0.007$, 500 m, not significant.

Figure 1.

a


Station ALOHA
Flux (mgC/m^2/d)
0
5
10
15
20
Depth (m)
150
300
500
non-pellets
pellets

b

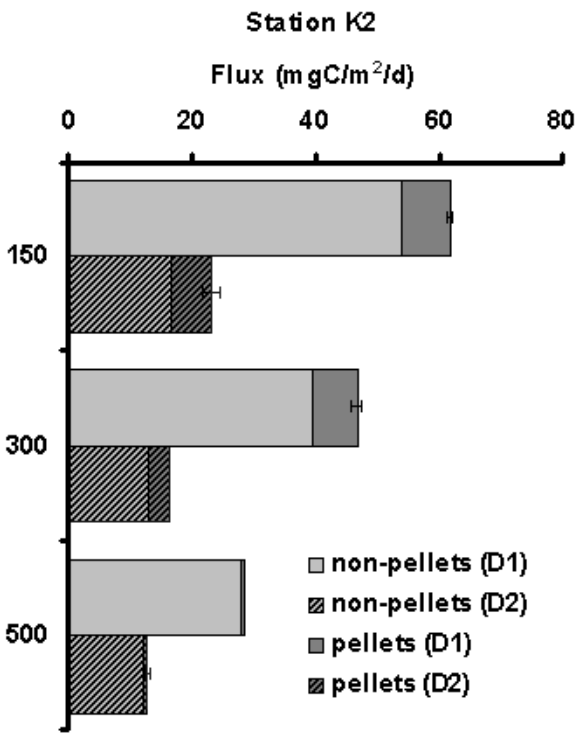
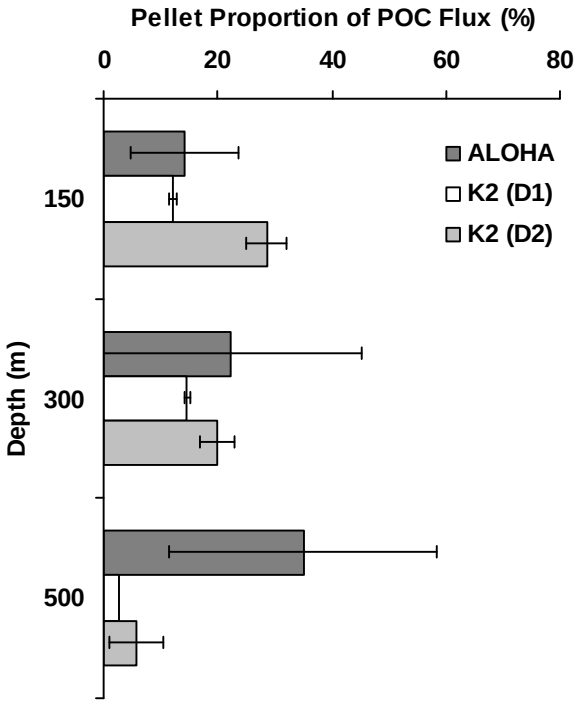

Station K2
Flux (mgC/m^2/d)
0
20
40
60
80
150
300
500
non-pellets (D1)
non-pellets (D2)
pellets (D1)
pellets (D2)

Figure 2.

a


Pellet Proportion of POC Flux (%)
0
20
40
60
80
ALOHA
K2 (D1)
K2 (D2)
150
300
500
Depth (m)

b

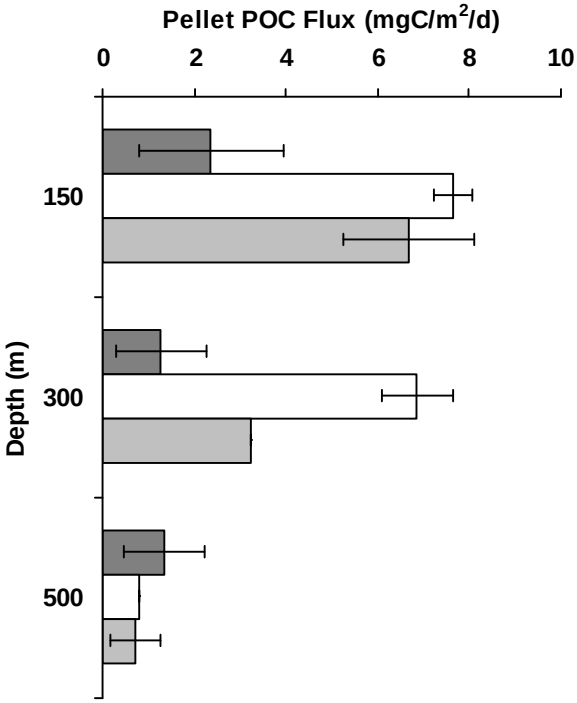
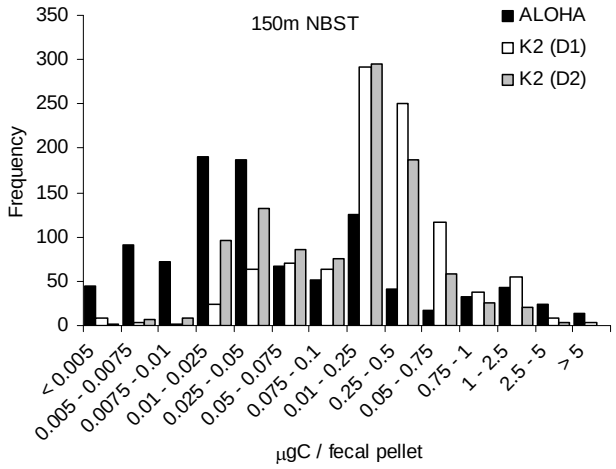
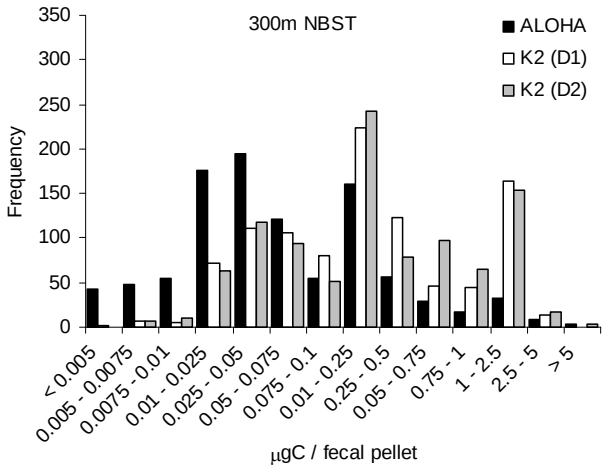

Pellet POC Flux (mgC/m2/d)
0
2
4
6
8
10
150
300
500
Depth (m)

Figure 3.

a


150m NBST
ALOHA
K2 (D1)
K2 (D2)
Frequency
350
300
250
200
150
100
50
0
< 0.005
0.005 - 0.0075
0.0075 - 0.01
0.01 - 0.025
0.025 - 0.05
0.05 - 0.075
0.075 - 0.1
0.01 - 0.25
0.25 - 0.5
0.05 - 0.75
0.75 - 1
1 - 2.5
2.5 - 5
> 5
μgC / fecal pellet

b


300m NBST
ALOHA
K2 (D1)
K2 (D2)
Frequency
350
300
250
200
150
100
50
0
< 0.005
0.005 - 0.0075
0.0075 - 0.01
0.01 - 0.025
0.025 - 0.05
0.05 - 0.075
0.075 - 0.1
0.01 - 0.25
0.25 - 0.5
0.05 - 0.75
0.75 - 1
1 - 2.5
2.5 - 5
> 5
μgC / fecal pellet

c

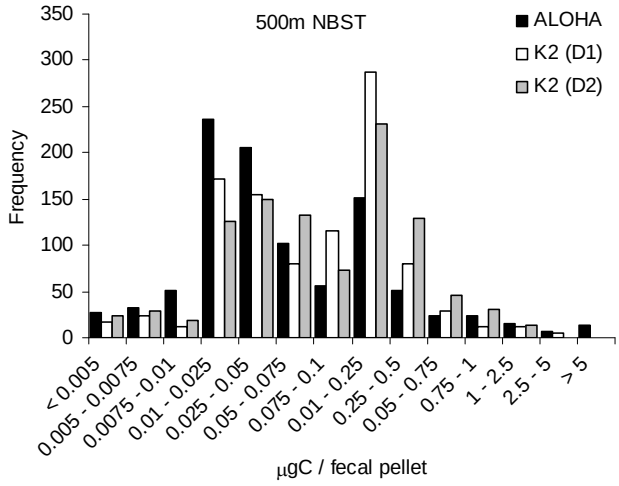

500m NBST
ALOHA
K2 (D1)
K2 (D2)
Frequency
350
300
250
200
150
100
50
0
< 0.005
0.005 - 0.0075
0.0075 - 0.01
0.01 - 0.025
0.025 - 0.05
0.05 - 0.075
0.075 - 0.1
0.01 - 0.25
0.25 - 0.5
0.05 - 0.75
0.75 - 1
1 - 2.5
2.5 - 5
> 5
μgC / fecal pellet

Figure 4.

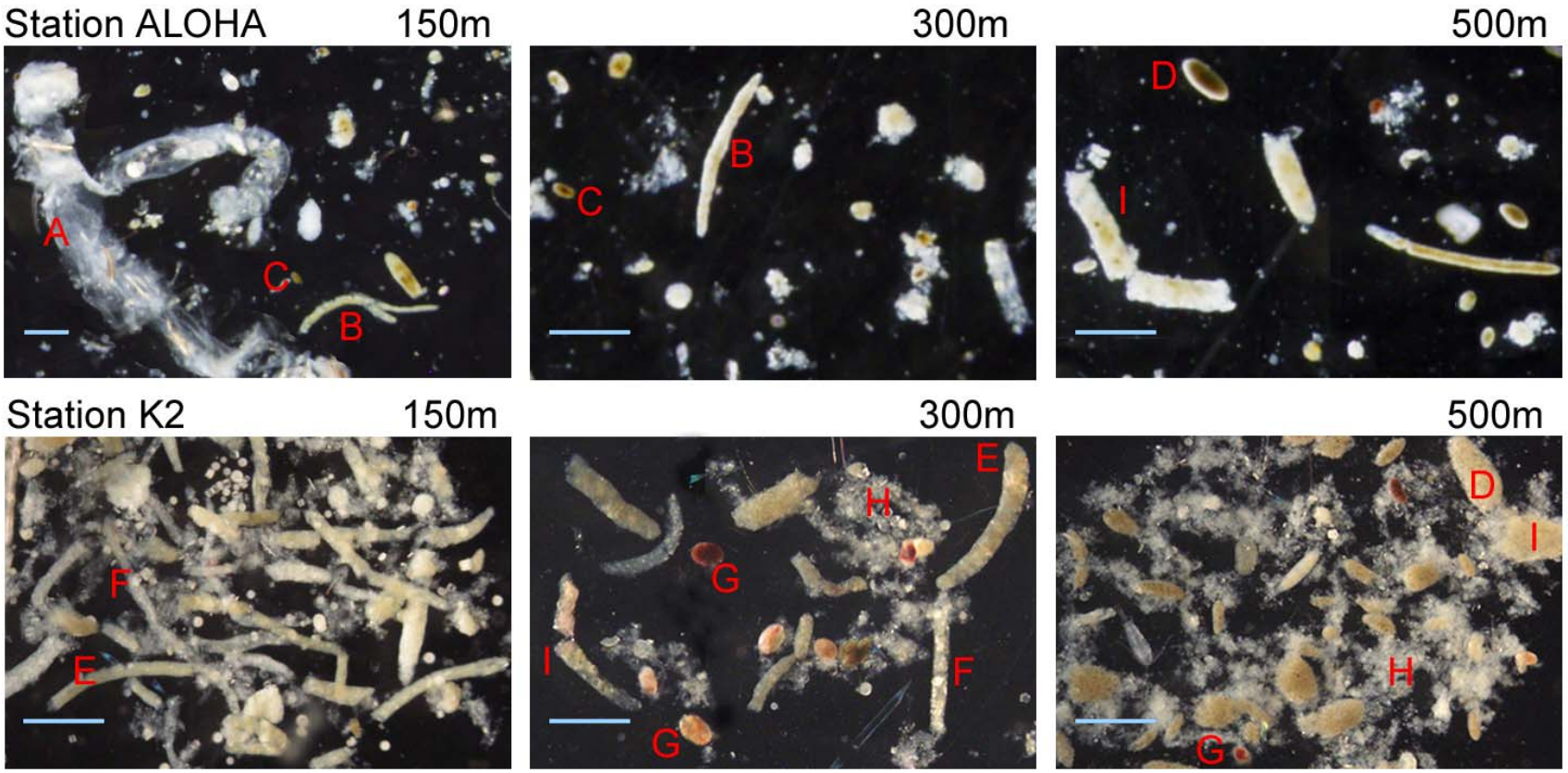

Station ALOHA
150m
300m
500m
A
C
B
B
C
D
I
Station K2
150m
300m
500m
F
E
E
H
G
I
F
G
D
I
H
G

Figure 5.

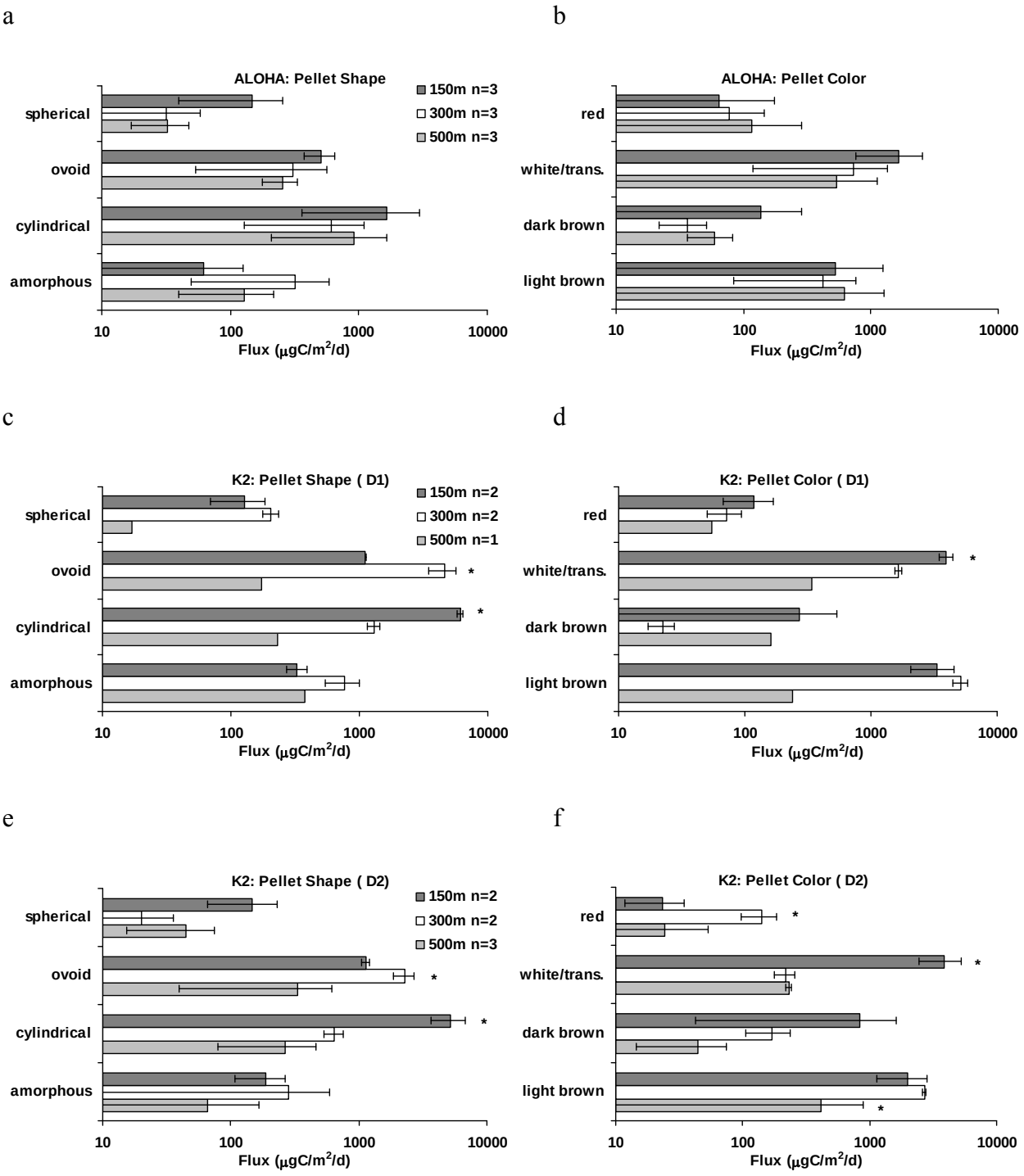
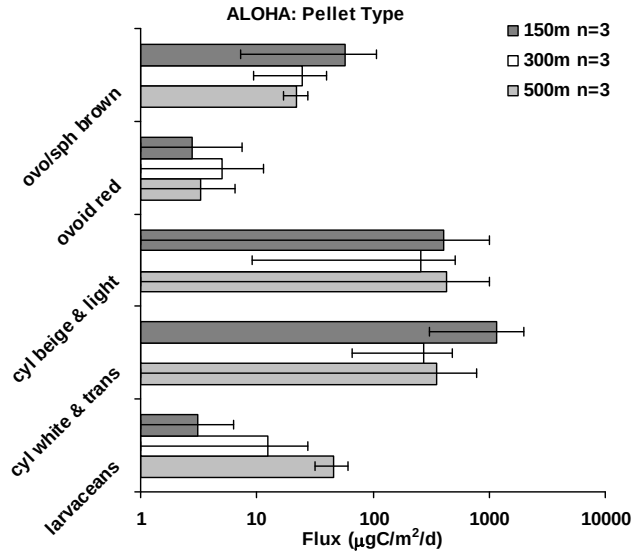
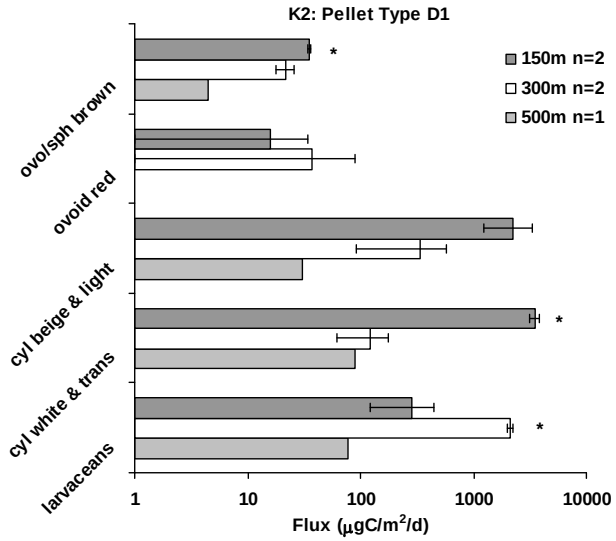

a
ALOHA: Pellet Shape
150m n=3
300m n=3
500m n=3
spherical
ovoid
cylindrical
amorphous
10
100
1000
10000
Flux (µgC/m²/d)
b
ALOHA: Pellet Color
red
white/trans.
dark brown
light brown
10
100
1000
10000
Flux (µgC/m²/d)
c
K2: Pellet Shape (D1)
150m n=2
300m n=2
500m n=1
spherical
ovoid
cylindrical
amorphous
*
*
10
100
1000
10000
Flux (µgC/m²/d)
d
K2: Pellet Color (D1)
red
white/trans.
dark brown
light brown
*
10
100
1000
10000
Flux (µgC/m²/d)
e
K2: Pellet Shape (D2)
150m n=2
300m n=2
500m n=3
spherical
ovoid
cylindrical
amorphous
*
*
10
100
1000
10000
Flux (µgC/m²/d)
f
K2: Pellet Color (D2)
red
white/trans.
dark brown
light brown
*
*
*
10
100
1000
10000
Flux (µgC/m²/d)

Figure 6.

a


ALOHA: Pellet Type
150m n=3
300m n=3
500m n=3
ovo/sph brown
ovoid red
cyl beige & light
cyl white & trans
larvaceans
1
10
100
1000
10000
Flux (µgC/m2/d)

b


K2: Pellet Type D1
150m n=2
300m n=2
500m n=1
ovo/sph brown
ovoid red
cyl beige & light
cyl white & trans
larvaceans
*
*
*
1
10
100
1000
10000
Flux (µgC/m2/d)

c

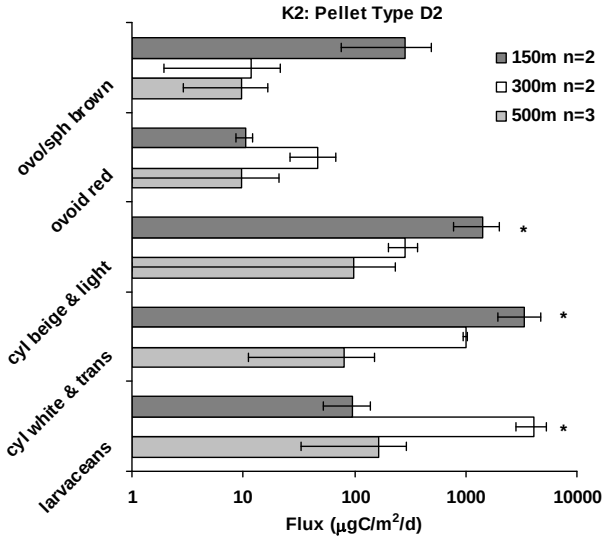

K2: Pellet Type D2
150m n=2
300m n=2
500m n=3
ovo/sph brown
ovoid red
cyl beige & light
cyl white & trans
larvaceans
*
*
*
1
10
100
1000
10000
Flux (µgC/m2/d)

Figure 7.

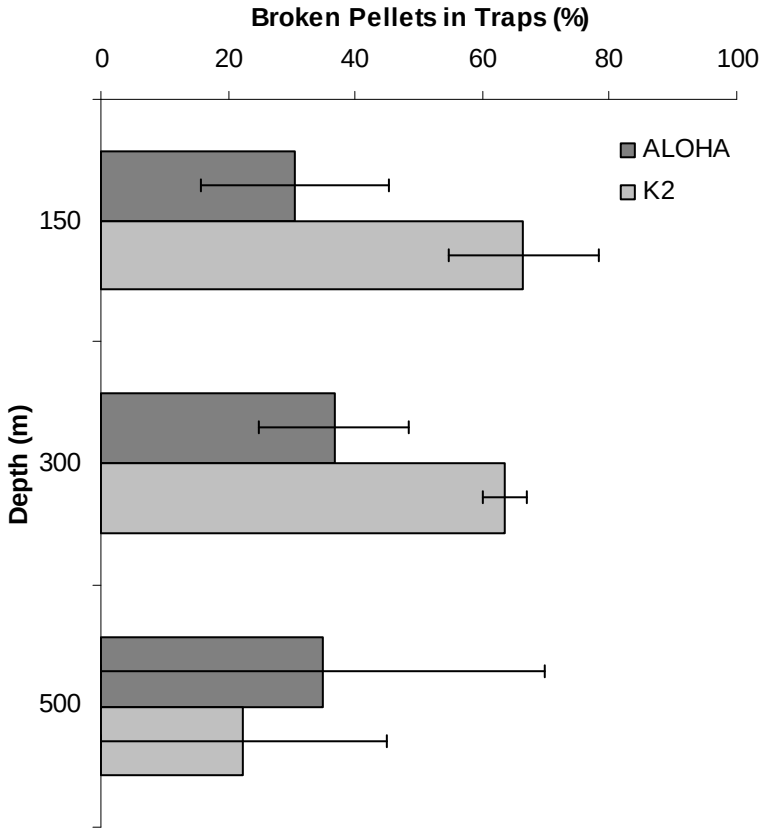

Broken Pellets in Traps (%)
0
20
40
60
80
100
ALOHA
K2
Depth (m)
150
300
500

Table 1. RGB values (mean ± 1 s.d.) of selected fecal pellets for classification purposes (RGB max = 255, n = 1758 pellets). RGB values are taken from sections of pellets and analyzed in ImagePro© and/or Adobe Photoshop©

Color Classification	Red	Green	Blue
light brown	201 ± 23	195 ± 72	160 ± 39
dark brown	140 ± 36	123 ± 40	82 ± 38
transparent & white	219 ± 31	219 ± 29	213 ± 40
red	182 ± 48	141 ± 65	117 ± 64

Table 2. Median carbon values (µg C) per fecal pellet. D1 = deployment 1; D2 = deployment 2; n = sample size.

	ALOHA	n	K2 (D1)	n	K2 (D2)	n	K2 Total	n
150 m	0.036	421	0.236	1201	0.136	1867	0.170	3068
300 m	0.048	473	0.156	1175	0.202	519	0.179	1694
500 m	0.043	580	0.079	174	0.084	463	0.081	637

Table 3: Fecal pellet characteristics of common taxa determined from live incubations. Volume and C values are mean ± 1 s.d. n = sample size; Misc., miscellaneous; n/d = not determined.

Site	Pellet Source	n	Shape	Color	Volume (mm^3)	C content (mg C mm^{-3})
K2						
	Neocalanus spp.	34	cylindrical	light brown	4.7 ± 2.7	0.13 ± 0.04
	Paraeuchaeta spp.	32	cylindrical	transparent	6.4 ± 3.3	0.15 ± 0.03
	misc. Euphausiids	39	cylindrical	light brown, transparent	9.2 ± 9.2	0.08 ± 0.01
	misc. Chaetognaths	14	amorphous	red	600.5 ± 151.0	0.03 ± 0.01
	misc. ostracods	2	crescent	multi	192.3 ± 5.9	n/d
ALOHA						
	Carinaria spp.	7	amorphous	transparent	110.1 ± 121.3	n/d
	misc. Euphausiids	5	cylindrical	light brown, white	10.8 ± 5.2	n/d