Mesopelagic zooplankton feeding ecology and effects on particle repackaging and carbon transport in the subtropical and subarctic North Pacific Ocean

Stephanie E. Wilson

College of William and Mary - Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/etd

Part of the Marine Biology Commons, and the Oceanography Commons

Recommended Citation


https://dx.doi.org/doi:10.25773/v5-8jmb-vw33

This Dissertation is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.
Mesopelagic zooplankton feeding ecology and effects on particle repackaging and carbon transport in the subtropical and subarctic North Pacific Ocean

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the requirements for the Degree of
Doctor of Philosophy

By
Stephanie Erin Wilson
2008
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy

Stephanie E. Wilson

Approved, by the Committee, November 2008

Deborah K. Steinberg, Ph.D.
Committee Chairman/Advisor

Elizabeth A. Canuel, Ph.D.

Fu-Lin E. Chu, Ph.D.

Hugh W. Ducklow, Ph.D.

Kam W. Tang, Ph.D.

Mary W. Silver, Ph.D.
University of California
Santa Cruz, California
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>viii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>7</td>
</tr>
<tr>
<td>CHAPTER 1 – 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</td>
<td>11</td>
</tr>
<tr>
<td>Abstract</td>
<td>12</td>
</tr>
<tr>
<td>Introduction</td>
<td>14</td>
</tr>
<tr>
<td>Methods</td>
<td>17</td>
</tr>
<tr>
<td>Results</td>
<td>21</td>
</tr>
<tr>
<td>Discussion</td>
<td>27</td>
</tr>
<tr>
<td>Summary and Conclusion</td>
<td>35</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>37</td>
</tr>
<tr>
<td>CHAPTER 2 – Feeding ecology of mesopelagic zooplankton of the subtropical and subarctic North Pacific Ocean determined using lipid biomarkers</td>
<td>62</td>
</tr>
<tr>
<td>Abstract</td>
<td>63</td>
</tr>
<tr>
<td>Introduction</td>
<td>65</td>
</tr>
<tr>
<td>Methods</td>
<td>68</td>
</tr>
<tr>
<td>Results</td>
<td>73</td>
</tr>
<tr>
<td>Discussion</td>
<td>80</td>
</tr>
<tr>
<td>Summary and Conclusion</td>
<td>90</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>92</td>
</tr>
<tr>
<td>CHAPTER 3 – Autotrophic picoplankton in mesozooplankton guts: Evidence of aggregate feeding in the mesopelagic zone and export of small phytoplankton</td>
<td>118</td>
</tr>
<tr>
<td>Abstract</td>
<td>119</td>
</tr>
<tr>
<td>Introduction</td>
<td>120</td>
</tr>
<tr>
<td>Methods</td>
<td>123</td>
</tr>
<tr>
<td>Results</td>
<td>127</td>
</tr>
<tr>
<td>Discussion</td>
<td>133</td>
</tr>
<tr>
<td>Summary and Conclusion</td>
<td>140</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>141</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I would like to thank my advisor, Debbie Steinberg for all of her support and encouragement. Her enthusiasm about all things zooplankton, the opportunities she gave me, and her awesome laugh were some of the many reasons I became her student. I am very lucky to have had her as an advisor. I am grateful to my committee: Liz Canuel, Fu-Lin Chu, Hugh Ducklow, Mary Silver, and Kam Tang, for their support, ideas and comments on my research, and use of their labs and equipment. Thanks to Ken Buesseler for his leadership in VERTIGO and for his help at sea. I am thankful to the captains and crews of the R/V Kilo Moana and R/V Roger Revelle for their assistance during the VERTIGO cruises. Also Carl Lamborg, Toru Kobari, Jim Bishop, Sue Coale, and the Café Thorium crew for their help with sample processing, use of samples and equipment, and comments on my past and future publications.

Thanks to my lab: Rob Condon, Joe Cope, Bethany Eden, Sarah Goldthwait, and Grace Saba. I especially wish to thank Joe for his help with everything from collecting samples and identifying animals to his statistical genius. I am grateful to Grace and Bethany for their advice and support, and for being wonderful officemates and good friends. Much gratitude towards Fu-Lin’s lab as well: Eric Lund, Paul Littreal, Kate Ruck, Vince Encomio, Bryce Brylowsky, and Jennifer Podbesek. Eric Lund was instrumental in educating me on all things lipid, and was always available to answer my questions. Kevin Kiley, Walker Smith, Jennifer Dreyer, Wayne Coats, and Paul Wassman were helpful with either use of equipment, methodology, species identification, or commenting on manuscripts.

My classmates and good friends Amy Chuiciolo, Meredith Fagan, Curt Freund, Danielle Johnston, Lindsey Kraatz, Beth Lerberg, Adriana Veloza, and Emily Yam were an indispensable source of fun times, nights out, and moral support. Exercise and sport was essential to keeping my sanity along the way and I am grateful to my work-out buddies, Jess Small, Marilyn Bowers, Judy and Pat Hurley, Mat Wright, Bob Ornelaz, Holly Northup, Sheena Booth, Erin Ferer, Keith McCaffrey and many others for keeping me in shape and on track. I’m also glad to have my best canine buddy Nick to come home to these past few years. He is a big fan of fecal pellets too, especially rolling in them.

Finally, thank you to my Family: mom and dad, Phyllis and Jack Wilson, my sister Allison Jonas and her husband John Jonas, my nephew Owen, and niece Ashley all in CA, as well as my family from up north for their everlasting support, encouragement, love and not thinking it is too crazy to spend an extra 8 years after college on more schooling.

iv
LIST OF TABLES

CHAPTER 1

Table 1. RGB values of selected fecal pellets .......................................................... 45
Table 2. Median carbon values per fecal pellet ...................................................... 46
Table 3: Fecal pellet characteristics of common taxa ........................................... 47

CHAPTER 2

Table 1. Common fatty acid biomarkers for zooplankton diet ............................. 98
Table 2. Major individual fatty acids for both zooplankton and > 51 µm particles .................................................................................................................. 99
Table 3. Major individual fatty acids for individually sorted zooplankton taxa ......................................................................................................................... 101

CHAPTER 3

Table 1. Target zooplankton species at both stations collected from 0–1000 m .................................................................................................................. 145
Table 2. Percentage of zooplankton gut contents containing other non-phytoplankton material ................................................................................ 146
LIST OF FIGURES

CHAPTER 1

Figure 1. Recognizable fecal pellet POC fluxes compared to the remainder of POC flux (non-pellets).................................................................48

Figure 2. Recognizable fecal pellet flux.............................................. 50

Figure 3. Fecal pellet carbon distribution from NBSTs.........................52

Figure 4. Example zooplankton fecal pellets from sediment trap samples indicating major types of pellets identified at each location.............. 54

Figure 5. Flux of fecal pellets categorized by shape and color.............. 56

Figure 6. Fecal pellet color/shape combinations and taxa..................... 58

Figure 7. Broken recognizable fecal pellets ..................................... 60

CHAPTER 2

Figure 1. Depth profiles of total fatty acid concentration in zooplankton and >51 µm particles.................................................................104

Figure 2. Herbivory biomarkers vs. depth.......................................... 106

Figure 3. Diatom and dinoflagellate biomarkers vs. depth................... 108

Figure 4. Carnivory/omnivory biomarkers vs. depth...........................110

Figure 5. Calanoid copepod biomarkers vs. depth...............................112

Figure 6. Particle-feeding biomarkers vs. depth..................................114

Figure 7. Scatter plot of bulk zooplankton vs. >51 µm particle fatty acids.............................................................................................................116
CHAPTER 3

Figure 1: Zooplankton gut contents as viewed under epifluorescence microscopy ................................................................. 151

Figure 2: Zooplankton at each depth interval with food in their gut at ALOHA ................................................................. 153

Figure 3: Zooplankton at each depth interval with food in their gut at K2 ................................................................. 155

Figure 4: Cyanobacteria density in zooplankton guts in each depth interval at ALOHA ................................................................. 157

Figure 5: Eukaryotic phytoplankton density in zooplankton guts in each depth interval at ALOHA ................................................................. 159

Figure 6: Cyanobacterial density in zooplankton guts in each depth interval at K2 ................................................................. 161

Figure 7: Eukaryotic phytoplankton density in zooplankton guts in each depth interval at K2 ................................................................. 163

CONCLUSION AND FUTURE DIRECTION

Figure 1: Conceptual diagram of the biological pump ......................... 170
ABSTRACT

Differences in zooplankton community structure and diet within the mesopelagic zone (base of euphotic zone to 1000 m) play a key role in affecting the efficiency by which organic matter is exported to depth, but how the structure of mesopelagic food webs change with depth or location is poorly known. I examined how mesopelagic zooplankton affect particle export in an oligotrophic (Hawaii Ocean Time-series site ALOHA) compared to a mesotrophic (Japanese time series site K2) open-ocean system. In the first part of the study, I investigated how fecal pellet characteristics change with depth in order to quantify the extent of particle repackaging by mesopelagic zooplankton. There was significant evidence of mesozooplankton repackaging of sinking particles in the mesopelagic zone, as indicated by presence of new fecal pellet types (as different size, color, or shape) occurring at different depths. Fecal pellets reflected the disparate zooplankton community structure at the two sites, and larger pellets at K2 likely increased transfer efficiency of POC there compared to ALOHA. In the second portion of this study, I analyzed fatty acids (FA) in zooplankton and particles to characterize zooplankton diet and large (>51 μm) particles in the mesopelagic zone of these two contrasting regions. Total FA concentration was higher in zooplankton tissue at K2, largely due to FA storage by ontogenetic vertical migrating species there. FA biomarkers that were indicative of particle feeding were also evident at both sites. Finally, I quantified cyanobacteria and small eukaryotic phytoplankton in the guts of mesopelagic zooplankton using light and epifluorescence microscopy to determine if mesopelagic zooplankton mediate the export of small phytoplankton to the deep sea. Cyanobacteria and small, eukaryotic phytoplankton occurred in the guts of nearly all target species sampled from the surface to 1000 m, indicating mesozooplankton grazing on aggregates is a pathway by which export of carbon and picoplankton can be enhanced. Guts of diel vertical migrators still contained picoplankton at their deep, daytime residence depths, indicating active export of these cells. In all three studies it was apparent that carnivory becomes an increasingly important component of mesopelagic zooplankton diet with depth. Evidence included the presence in sediment traps of red fecal pellets produced by carnivores, fatty acids in zooplankton tissue indicative of an increasingly carnivorous diet with depth, and the presence of chitin, gastropod shells, and microzooplankton in mesopelagic zooplankton gut contents. Changes in zooplankton feeding ecology from the surface through the mesopelagic zone, and between contrasting environments, have important consequences for the quality and quantity of organic material transported to the deep sea.
Mesopelagic zooplankton feeding ecology and effects on particle repackaging and carbon transport in the subtropical and subarctic North Pacific Ocean
INTRODUCTION

The biological pump and carbon flux through the mesopelagic zone

The ocean is a massive carbon reservoir due to its ability to draw down both anthropogenic and naturally-derived CO₂ from the atmosphere through the carbonate buffering system (Longhurst and Harrison, 1989). Through the biological pump, CO₂-derived carbon from the surface layer is vertically transported to the deeper layers of the ocean via passive sinking of particles, active transport via vertical migration of zooplankton, and physical mixing of dissolved organic carbon (DOC; Ducklow et al., 2001; Smayda, 1969; Steinberg et al., 2000; Zhang and Dam, 1997). In the upper ocean, atmospheric-derived CO₂ is taken up during photosynthesis to produce particulate organic carbon (POC) biomass (phytoplankton cells). Phytoplankton may sink directly as aggregates or are consumed by zooplankton in surface waters that produce sinking fecal pellets. Diel vertically migrating (DVM) zooplankton feed at night in the surface waters and migrate to depth during the day. The carbon ingested at the surface is metabolized at depth and thereby ‘actively’ transported into the deep-sea, a key mechanism by which carbon export is enhanced (Al-Mutairi and Landry, 2001; Longhurst et al., 1990; Schnetzer and Steinberg, 2002a; Steinberg et al., 2000; Zhang and Dam, 1997). Vertical advective supply of DOC from surface waters can also represent a substantial flux of C to the oceans’ interior and thus be an important component of the biological pump (e.g.,
Carlson et al., 1994; Hansell and Carlson, 2001). Dissolved organic carbon (DOC) excreted by zooplankton or CO\textsubscript{2} respired by organisms may remain in the upper or deep ocean where the DOC can be utilized by bacteria, or the CO\textsubscript{2} may re-enter the atmosphere (Ducklow et al., 2001). The carbon that originated in the atmosphere as CO\textsubscript{2} may thus become sequestered in the deep ocean for thousands of years (Mann and Lazier, 1996).

The flux of biologically-derived particles sinking from the surface to the deep sea attenuates rapidly in the mesopelagic zone (base of euphotic zone – 1000 m, Buesseler et al., 2008; Buesseler et al., 2007; Martin et al., 1993; Martin et al., 1987; Morales, 1999; Steinberg et al., 2008b; Wakeham and Canuel, 1988). This is due to biotic factors such as decomposition and remineralization by bacteria, and particle feeding, fragmentation, and active transport by zooplankton (Cho and Azam, 1988; Goldthwait et al., 2005; Koppelmann et al., 2004; Steinberg, 1995) and abiotic factors such as physical aggregation, disaggregation, and mineral ballasting (Armstrong et al., 2002; Jackson and Burd, 1998). 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 zone or “twilight zone”—depths below the euphotic zone to 1000 m (Fowler and Knauer, 1986; Lampitt, 1992; Sasaki et al., 1988). However, few studies have examined the processes that affect how ‘efficiently’ particulate organic matter is transported from where it is produced in surface waters to the deep sea. In particular, the role of the mesopelagic food webs in affecting carbon cycling at depth is poorly known, although some previous studies have indicated that the community structure and feeding activities of mesopelagic zooplankton can affect POC flux (Angel, 1989; Bishop et al.,
Sinking particles as a food source for mesopelagic zooplankton

Sinking aggregates, also referred to as detritus or marine snow, may contain uneaten phytoplankton, bacteria, cyanobacteria, protozoans, zooplankton fecal pellets, shells, carapace material, and mucous feeding webs (Alldredge and Silver, 1988; Bishop et al., 1977; Lampitt et al., 1993). These aggregates can be sticky, and as they sink, can form larger aggregates or break apart by various biological, chemical, and physical processes (Jackson and Burd, 1998; Michaels and Silver, 1988). Marine snow particles are microbial hotspots (Alldredge and Silver, 1988; Azam, 2003; Michaels and Silver, 1988) and a food source for mesopelagic zooplankton (e.g. Dilling et al., 1998; Schnetzer and Steinberg, 2002b; Steinberg, 1995), but detrital feeding can be difficult to recognize in zooplankton. Some species of zooplankton associated with marine snow also graze directly on attached phytoplankton, the detrital material itself, or on other associated picoplankton and microzooplankton (e.g. Schnetzer and Steinberg, 2002b; Steinberg, 1995). Feeding on aggregates is considered a food web ‘shortcut’, as most mesozooplankton cannot directly feed on free-living microorganisms (Dilling et al., 1998; Lampitt et al., 1993). Through zooplankton feeding on picoplankton in aggregates and their subsequent egestion of fecal pellets, non-sinking picoplankton may be exported to the deep sea (Richardson and Jackson, 2007; Silver et al., 1986).
This dissertation examines the role of zooplankton in mesopelagic food webs by focusing on the processing of particulate organic carbon (POC) by zooplankton. It is part of a multidisciplinary, international study on particle flux – VERtical Transport In The Global Ocean (VERTIGO). The major hypotheses of the VERTIGO project were that the efficiency of particle transport in the sea is dominantly controlled by 1) particle source characteristics, or 2) feeding or metabolic activities of mesopelagic zooplankton or bacteria (Buesseler et al., 2008; Buesseler et al., 2007). In VERTIGO we elucidated the multiple biological processes such as bacteria decomposition of POC, and zooplankton vertical migration, fecal pellet production, and particle feeding, that impact attenuation of POC flux though the mesopelagic zone (Buesseler et al., 2008; Buesseler et al., 2007; Steinberg et al., 2008a; Steinberg et al., 2008b).

The three chapters in this dissertation focus on aspects of particle consumption and repackaging by zooplankton within the poorly understood mesopelagic zone at two biogeochemically contrasting sampling locations in the North Pacific Ocean. These are the Hawaii Ocean Time-series station ALOHA (subtropical, oligotrophic), and the Japanese time-series station K2 (subarctic, mesotrophic; Honda et al., 2006; Karl and Lukas, 1996). In the first chapter I investigated the change in fecal pellet characteristics (e.g., size, shape, color) with depth to determine the extent of particle repackaging by zooplankton and the importance of fecal pellet flux to total POC export. In the second chapter I examined the role of zooplankton in mesopelagic food webs at these contrasting sites by determining the relative importance of carnivory, herbivory, and particle feeding by mesopelagic zooplankton with depth using specific fatty acid biomarkers and fatty acid ratios in both zooplankton tissue and large particles. The third and final chapter
focused on the role that mesopelagic zooplankton play in consumption and export of small phytoplankton by quantifying picoplankton (and other food items) in the guts of mesozooplankton. I analyzed the gut contents of zooplankton using epifluorescence microscopy to determine if cyanobacteria and eukaryotic phytoplankton too small to be ingested individually were present. Picoplankton, through aggregation and subsequent grazing by mesozooplankton, may allow for a greater contribution of small cells to export flux than previously thought (Richardson and Jackson, 2007; Richardson et al., 2004). The data obtained from this study will provide key information about the influence of zooplankton on POC flux to the deep ocean, provide better characterization of mesopelagic food webs in two contrasting environments, and test hypothesized mechanisms for the export of small cells to depth.
Literature Cited


CHAPTER 1

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

Stephanie E. Wilson¹, Deborah K. Steinberg¹, and Ken O. Buesseler²

¹Virginia Institute of Marine Science, 1208 Greate Rd., Gloucester Point, Virginia, 23062, USA. ²Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, 266 Woods Hole Road, Woods Hole, Massachusetts, 02543, USA.

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.
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.
Methods

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° N, 158° 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° N, 160° 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., 2008b). 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., 2008b).

**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.

**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 sp., 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.

Results

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).

**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, $\chi^2$ 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, $\chi^2$ 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: $p < 0.001$).

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 $\mu$m; 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* sp. (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 1.1 mm).

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* sp. 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).

*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,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 ontogenetic 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,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).
Discussion

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., 2008a).

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., 2008b), 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 (Paffenhofer 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).

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 (Paffenhofer 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 Kjørboe, 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., 2008a).

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 exoskeletons) 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., 2008a), 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.
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).

Summary and Conclusion

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; Paffenhofer 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. 2008b). 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.
Literature Cited


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©

<table>
<thead>
<tr>
<th>Color Classification</th>
<th>Red</th>
<th>Green</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>light brown</td>
<td>201 ± 23</td>
<td>195 ± 72</td>
<td>160 ± 39</td>
</tr>
<tr>
<td>dark brown</td>
<td>140 ± 36</td>
<td>123 ± 40</td>
<td>82 ± 38</td>
</tr>
<tr>
<td>transparent &amp; white</td>
<td>219 ± 31</td>
<td>219 ± 29</td>
<td>213 ± 40</td>
</tr>
<tr>
<td>red</td>
<td>182 ± 48</td>
<td>141 ± 65</td>
<td>117 ± 64</td>
</tr>
</tbody>
</table>
Table 2. Median carbon values (µg C) per fecal pellet. D1 = deployment 1; D2 = deployment 2; n = sample size.

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>ALOHA n</th>
<th>K2 (D1) n</th>
<th>K2 (D2) n</th>
<th>K2 Total n</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 m</td>
<td>0.036</td>
<td>0.236</td>
<td>0.136</td>
<td>0.170</td>
</tr>
<tr>
<td>300 m</td>
<td>0.048</td>
<td>0.156</td>
<td>0.202</td>
<td>0.179</td>
</tr>
<tr>
<td>500 m</td>
<td>0.043</td>
<td>0.079</td>
<td>0.084</td>
<td>0.081</td>
</tr>
</tbody>
</table>
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.

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

ALOHA

<table>
<thead>
<tr>
<th>Site</th>
<th>Pellet Source</th>
<th>n</th>
<th>Shape</th>
<th>Color</th>
<th>Volume (mm$^3$)</th>
<th>C content (mg C mm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 1.

(A) Station ALOHA
Flux (mgC/m²/d)

(B) Station K2
Flux (mgC/m²/d)
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 2.

(A) Pellet Proportion of POC Flux (%)

(B) Pellet POC Flux (mgC/m²/d)

Depth (m)

- ALOHA
- K2 (D1)
- K2 (D2)
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 3.

(A) 150m NBST

- ALOHA
- K2 (D1)
- K2 (D2)

(B) 300m NBST

- ALOHA
- K2 (D1)
- K2 (D2)

(C) 500m NBST

- ALOHA
- K2 (D1)
- K2 (D2)
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 5.

(A) ALOHA: Pellet Shape
- spherical
- ovoid
- cylindrical
- amorphous

(B) ALOHA: Pellet Color
- red
- white/transparent
- dark brown
- light brown

(C) K2: Pellet Shape (D1)
- spherical
- ovoid
- cylindrical
- amorphous

(D) K2: Pellet Color (D1)
- red
- white/transparent
- dark brown
- light brown

(E) K2: Pellet Shape (D2)
- spherical
- ovoid
- cylindrical
- amorphous

(F) K2: Pellet Color (D2)
- red
- white/transparent
- dark brown
- light brown

Flux (μg C/m²/d)
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 6.

(A) ALOHA: Pellet Type

(B) K2: Pellet Type D1

(C) K2: Pellet Type D2
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 7.

![Bar graph showing the percentage of broken pellets in traps at different depths. The graph compares two groups: ALOHA (filled squares) and K2 (open squares). The depth levels are 150, 300, and 500 meters. The percentage of broken pellets ranges from 0 to 100.](image)
CHAPTER 2

Feeding ecology of mesopelagic zooplankton of the subtropical and subarctic North Pacific Ocean determined using fatty acid biomarkers

S.E. Wilson¹, D.K. Steinberg¹, F.L. Chu¹, J.K.B. Bishop²,³

¹Virginia Institute of Marine Science, College of William and Mary, Route 1208 Great Rd. Gloucester Point, VA 23062

²Department of Earth and Planetary Science, University of California Berkeley, Berkeley, CA 94720

³EO Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720

To be submitted in Deep-Sea Research I
Abstract

Mesopelagic zooplankton may meet their nutritional and metabolic requirements in a number of ways including consumption of sinking particles, carnivory, and vertical migration. How these feeding modes change with depth or location, however, is poorly known. We analyzed fatty acid (FA) profiles to characterize zooplankton diet and large particle (> 51 μm) composition in the mesopelagic zone (base of euphotic zone -1000 m) at two contrasting time-series sites in the subarctic (station K2) and subtropical (station ALOHA) Pacific Ocean. Total FA concentration was 15.5 times higher in zooplankton tissue at K2, largely due to FA storage by seasonal vertical migrators such as Neocalanus and Eucalanus. FA markers specific to herbivory implied a higher plant-derived food source at mesotrophic K2 than at oligotrophic ALOHA. Zooplankton FA profiles indicated that diatoms, and to a lesser extent, dinoflagellates were important food sources at K2. At ALOHA, dinoflagellate FA were more prominent. Bacteria-specific FA biomarkers in zooplankton tissue were used as an indicator of particle feeding, and peaks were recorded at depths where known particle feeders were present at ALOHA (e.g., ostracods at 100 – 300 m). In contrast, depth profiles of bacterial FA were relatively consistent with depth at K2. Diatom, dinoflagellate, and bacterial markers were found in similar proportions in both zooplankton and particles with depth at both locations, providing additional evidence that mesopelagic zooplankton consume sinking particles. Carnivory indices were higher and increased significantly with depth at ALOHA, and exhibited distinct peaks at K2, representing an increase in dependence on other zooplankton for food in deep waters. These changes in zooplankton feeding ecology from
the surface through the mesopelagic zone, and between contrasting environments, have
important consequences for the quality and quantity of organic material transported to the
deep sea
Introduction

Zooplankton residing within the mesopelagic zone (base of the euphotic zone to ~1000 m) include a diverse assemblage of organisms that play a key role in the biological pump (Angel, 1989; Steinberg *et al.*, 2008a; Steinberg *et al.*, 2008b). The community structure and feeding activities of mesopelagic zooplankton are biotic factors that can affect the efficiency by which sinking POM is transported to the deep sea (Angel, 1989; Bishop *et al.*, 1987; Buessler *et al.*, 2008; Buessler *et al.*, 2007; Fowler and Knauer, 1986; Lampitt, 1992; Noji, 1991; Sasaki *et al.*, 1988). Mesopelagic zooplankton must obtain their food via carnivory and particle feeding (on aggregates of sinking or suspended detritus), or migrate to the epipelagic zone (0-150 m) to feed (Steinberg *et al.*, 2008b). However, there is limited information on the feeding ecology and nutrition of the zooplankton living within this zone.

Approaches to studying the feeding ecology of mesopelagic zooplankton have included microscopic analysis of gut contents or fecal pellets, and observations of changes in types of zooplankton fecal pellets with depth. Gut content and fecal pellet analysis is useful for detecting remains of food items such as phytoplankton tests, crustacean carapaces, and detritus in mesopelagic zooplankton (e.g., Kosobokova *et al.*, 2002; Lampitt *et al.*, 1993b; Schnetzer and Steinberg, 2002; Silver and Bruland, 1981; Steinberg, 1995; Uttal and Buck, 1996). Carnivory and particle feeding in the upper mesopelagic is evidenced by the emergence in lower mesopelagic sediment traps of new, distinctive fecal pellet types derived from crustacean or gelatinous carnivores and filter feeding larvaceans, respectively (Wilson *et al.*, 2008). These changes in fecal pellet type
with depth are generally consistent with observations of distinct peaks in both particle feeders (e.g., salps, ostracods, and poecilostomatoid copepods) and carnivores (e.g., chaetognaths, medusae, mysids, and ctenophores) within the mesopelagic zone in the subarctic and subtropical North Pacific Ocean (Steinberg et al., 2008a).

Lipid biomarkers provide a useful tool for investigating zooplankton nutrition and food-web interactions. Fatty acids \([x:y(n-z), \text{ where } x=\text{number of carbon atoms}, y=\text{number of double bonds/degree of unsaturation}, \text{ and if unsaturated, } n-z \text{ is used where } z=\text{the location of first double bond on the carbon chain after } CH_3] \) are the main constituents of lipids and can be linked to certain classes of zooplankton, algae, or bacteria based on structural features including number of double bonds, methyl group structure, and carbon chain length. These compounds (or biochemistries) are vital for normal growth and development of zooplankton and most fatty acids are incorporated into the animal without modification. As a result, fatty acids can be used to investigate diet history, nutrient upgrading, trophic transfer, and prey assimilation (reviewed by Dalsgaard et al., 2003).

Fatty acid biomarkers, with their respective biochemical structural features, for different classes of plankton are shown in Table 1. For example, a comparatively high ratio of (n-3) to (n-6) polyunsaturated fatty acids (PUFA) (or low (n-6)/(n-3)) is a general indicator of herbivory in zooplankton and bivalves (Desvilettes et al., 1997; Stevens et al., 2004a; Viso and Marty, 1993). Fatty acid markers such as docosahexanoic acid (DHA, 22:6(n-3)), arachidonic acid (AA, 20:4(n-6)) and 22 carbon-chain (C22) PUFA are markers for dinoflagellates, whereas eicosapentanoic acid (EPA, 20:5(n-3)), 16:1(n-7), 16:1/16:0, and 20 carbon-chain (C20) PUFA are markers for diatoms (Ackman et al.,
1968; Budge and Parrish, 1998; Dalsgaard et al., 2003; Reuss and Poulsen, 2002; Viso and Marty, 1993). Carnivores tend to accumulate oleic acid (OA, 18:1(n-9)) and have a high ratio of 18:1(n-9)/18:1(n-7) (Au et al., 2002; Cripps and Atkinson, 2000; Nelson et al., 2001; Phleger et al., 1998; Stevens et al., 2004b; Stevens et al., 2004c). Calanoid copepods are the only zooplankton taxa able to biosynthesize long chain monounsaturated fatty acids (MUFA) 20:1(n-9) and 22:1(n-11), which if measured in other non-calanoid zooplankton, could be an indication of carnivory as well (Dalsgaard et al., 2003).

Sinking particles are microbial hotspots (Alldredge and Silver, 1988; Azam and Long, 2001) and a food source for mesopelagic zooplankton, but detrital particles can be difficult to recognize in guts. As many larger, non filter-feeding zooplankton cannot directly feed on free-living microorganisms such as bacteria and picophytoplankton (Dilling et al., 1998), zooplankton feeding on marine snow is a food web ‘shortcut’ (Dilling et al., 1998; Lampitt et al., 1993a). Fatty acids within zooplankton tissue that are specific to bacteria would therefore provide evidence of particle feeding. Experiments in which fatty acid markers have been utilized specifically for indicating microbial/particle feeding are rare and have varied results based on location and interpretation (e.g., Desvilettes et al., 1997; Stevens et al., 2004c). Odd-numbered or branched FA (OBFA) such as 13:0, 15:0, and 17:0 have been used as biomarkers for heterotrophic bacteria (e.g., Budge and Parrish, 1998; Graeve et al., 1997; Kaneda, 1991; Meziane and Tsuchiya, 2000; Pranal et al., 1996; Stevens et al., 2004b; Wakeham, 1995). Increasing values of MUFA and combinations of particular MUFA have also been used as indicators for bacterial association/feeding (Desvilettes et al., 1997; Pranal et al., 1996).
In this study, the concentration and composition of FA associated with zooplankton and particles (a combination of both sinking and suspended, > 51 μm) throughout the mesopelagic zone were measured to investigate diets of the zooplankton community in the subarctic and subtropical North Pacific Ocean. The study was part of the VERTical Transport In the Global Ocean (VERTIGO) project, designed to investigate the controls on the efficiency of particle export to the deep sea (Buesseler et al., 2008; Buesseler et al., 2007). We examined the relative importance of carnivory, herbivory, and particle feeding by mesopelagic zooplankton with depth in these two contrasting regions using specific fatty acid biomarkers and fatty acid ratios previously established as indicators of zooplankton diet. Ultimately this information can be used to increase our understanding of how depth-related changes in zooplankton feeding ecology and particle composition can affect the biological pump.

Methods

Sample collection

Zooplankton were collected between 0–1000 m using a 1 m², 335 μm mesh, 10 net MOCNESS (Multiple Opening/Closing Net and Environmental Sensing System, Wiebe et al. 1985) and 7 net IONESS (Intelligent Operative Net Sampling System, similar to MOCNESS) at two contrasting sites in the North Pacific Ocean (Steinberg et al., 2008a). Collections were made at nine discrete depth intervals: 0-50, 50-100, 100-150, 150-200, 200-300, 300-400, 400-500, 500-750, and 750-1000 meters. Paired
day/night tows were conducted at the Hawaii Ocean Time series-HOT station ALOHA in the oligotrophic N. Pacific subtropical gyre (27.75°N, 158°W) June 22-July 9, 2004 aboard the R/V Kilo Moana, and at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) time-series site K2, in a high nutrient, variable chlorophyll region of the N. Pacific subarctic gyre (47°N, 160°E) July 22-August 11, 2005 aboard the R/V Roger Revelle. At ALOHA, the base of the euphotic zone was 125 m (0.1% surface irradiance), water column-integrated net primary production was 180-220 mgCm⁻²d⁻¹, new production was 18-38 mgCm⁻²d⁻¹, mixed layer nutrients were at nanomolar concentrations, and the phytoplankton assemblage consisted of small diatoms, coccolithophorids, dinoflagellates, picoplankton and cyanobacteria (Boyd et al., 2008; Buesseler et al., 2008; Buesseler et al., 2007; Elskens et al., 2008; Lamborg et al., 2008).

At K2, the base of the euphotic zone (50 m) was shallower than at ALOHA; net primary production (302-603 mgCm⁻²d⁻¹), new production (70-150 mgCm⁻²d⁻¹), and nutrients (12 μM mixed layer DIN) were all higher than at ALOHA, and the K2 phytoplankton assemblage consisted of picoplankton and large diatoms (Boyd et al., 2008; Buesseler et al., 2008; Buesseler et al., 2007; Lamborg et al., 2008).

A total of 4 day/night MOCNESS/IONESS tows were conducted at each location. Upon net recovery, the 9 cod-ends were removed, stored in buckets, and processed within ~6 hours. The two pairs of day/night tows at each site were used for bulk zooplankton lipid analyses, and the data presented herein are an average of day and night samples. These samples were divided using a Folsom plankton splitter and 1/8, 1/16, or 1/32 of the total biomass was rinsed with Milli-Q water to remove salts and stored at -80° C until analysis. Specific taxa of zooplankton (chaetognaths, euphausiids, and assorted calanoid
copepods: *Eucalanus bungii, Paraeuchaeta* sp., *Neocalanus cristatus*, and *N. plumchrus*) were sorted from a sub-sample of the remaining two IONESS paired tows at K2, and miscellaneous calanoid copepods and euphausiids were sorted from both the MOCNESS and additional net tows at station ALOHA for individual animal lipid analysis. These additional net tows were made using a 1-m diameter opening and closing conical net with a 335-μm mesh and non-filtering cod end. One set of multiple individuals was processed for each depth the taxa were present (n = 1 per depth). Individuals were collected from 2-7 different depth intervals. The remainder of the samples were used for analysis of zooplankton gut contents, size-fractionated biomass, and enumeration of taxa (Kobari *et al.*, 2008b; Steinberg *et al.*, 2008a; Wilson et al. in prep).

At both K2 and ALOHA, a combination of large (>51 μm) suspended and sinking particles were collected simultaneously from 3 to 12 depths within the upper 1000 m (ca. 10, 35, 60, 85, 135, 185, 235, 315, 465, 565, and 765 m) using a Multiple Unit Large Volume Filtration System (MULVFS), which consists of 12 specialized particulate matter pumps tethered to a 1000 m cable (Bishop *et al.*, 1985; Bishop and Wood, 2008). The MULVFS collected three size fractions of particles, <1μm, 1-51μm, and >51 μm. MULVFS protocols for VERTIGO are described in further detail by Bishop and Wood (2008). Particle samples acquired for this study were collected during two day-night deployments at each location, from approximately 1/8 of the large-fraction, 51 μm polyester filter on the MULVFS. Volumes of water filtered represented by these subsamples range from ~500 L in the euphotic zone to ~1500 L in deeper waters. The >51μm fraction was chosen for analysis as it includes large phytoplankton, aggregates, suspended and sinking particles available to the mesozooplankton. Visible zooplankton
were removed from filters, and particles were then rinsed onto pre-weighed GF/F filters, lightly rinsed again with Milli-Q water to remove salts, and frozen at-80°C until further analysis. The data presented from MULVFS particles also represent an average of day and night samples.

Sample analysis

Lipid analyses were conducted on zooplankton and sinking/suspended particles to determine the relative importance of particle/detritus feeding, herbivory, and carnivory between different mesopelagic taxa and between the two sites. Bulk zooplankton splits from tows were thawed and homogenized using an Ultraturrax T-25 homogenizer. Two 1.5 ml aliquots were placed in test tubes, one was used for lipid extraction and the second was placed in a drying oven overnight at 50°C to determine dry-weight for each aliquot. The sorted zooplankton species and the MULVFS particles on pre-weighed and combusted GF/F filters were freeze dried using a Virtis Alcatel 2008A freeze dryer. Dry weight of bulk zooplankton, sorted zooplankton species, and MULVFS particle samples was determined using a Sartorious BP211D microbalance. Lipids were extracted from both the second 1.5 ml bulk zooplankton aliquot and the freeze-dried samples using chloroform:methanol:water in the ratio 2:2:1 v/v/v (modified from Bligh and Dyer, 1959; Folch et al., 1957). Extracted samples were dried completely under nitrogen gas and weighed on the same microbalance to obtain mg of total lipid, then resuspended in 1 ml chloroform:methanol (1:1 v/v) and stored in a -20°C freezer.

A separate aliquot of the extracted lipid samples were utilized for fatty acid analysis. 20 μg of 23:0 FA internal standard was added to the lipid aliquot and
subsequently derivatized to form fatty acid methyl esters (FAMEs) using boron trifluoride (BF₃) (Metcalf and Schmitz, 1961) in a 100° C circulating water bath. The FAMEs were then extracted with carbon disulfide (CS₂) (Marty et al., 1992) and suspended in hexane for gas chromatography-flame ionization detection (GC-FID) analysis. FAMEs were analyzed on a Varian model 3800 GC-FID using a DB-WAX capillary column (25 m x 0.32 mm; 0.2 μm film thickness; J&W Scientific, Folsom, CA). FAMEs were identified by the comparison of retention times to those of commercially authenticated laboratory standards. Each fatty acid peak was quantified based on the 23:0 internal standard and expressed as both a percentage of the total fatty acid and as μg FA mg C⁻¹.

To determine total organic carbon content, duplicates of dried, pulverized bulk zooplankton samples were weighed (1-10 mg) in acetone-rinsed tin capsules and acidified using 10% HCl to remove inorganic carbon (Hedges and Stern 1984). Total organic carbon (TOC) was measured using a Fisions CHN analyzer (Model EA 1108). Particulate organic carbon (POC) from MULVFS particle samples were measured using a Carlo Erba elemental analyzer (Bishop and Wood 2008) using standard procedures described in Bishop et al. (1985). Along with the gravimetric measurements of total lipid, this information was used to calculate μg lipid mg C⁻¹.

Data Analysis

Results are presented as depth profiles using the median depth for each MOCNESS net sampling interval. Average water column depths were also computed: Since the sampling depth interval of the MOCNESS nets varied, depth-weighted averages
were calculated for the ten most abundant fatty acids (expressed as percent total fatty acid) as well as the ratios and the sum of all saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). This was done using the equation:

\[ \text{Zooplankton depth-weighted average (\%, ratio, or \( \mu \)g lipid / mg C)} = \sum (z_2 - z_1) \times x / z_{\text{tot}} \]

where \( z_2 - z_1 \) is the depth interval of each net (m), \( x \) is the fatty acid value (\%, ratio, or \( \mu \)g FA mg C\(^{-1}\)) and \( z_{\text{tot}} \) is the total depth interval (m) of interest. In order to compare the net tow zooplankton—collected in depth intervals, with the MULVFS particles—collected at single depths, a trapezoidal calculation was used to calculate depth-weighted averages using the equation:

\[ \text{Particle depth-weighted average (\%, ratio, or \( \mu \)g lipid / mg C)} = \sum [(x_1 - x_2)/2] \times (z_2 - z_1) / z_{\text{tot}} \]

where \( x_1 - x_2 \) is the difference in particle fatty acid values (\%, ratio, or \( \mu \)g FA mg C\(^{-1}\)) between depth intervals \( z_2 - z_1 \) (m) and \( z_{\text{tot}} \) is the total depth interval (m) of interest.

**Results**

*Comparison of bulk zooplankton and particle fatty acids with depth and between sites*
Total fatty acid (FA) concentration per unit carbon (µg FA mg C⁻¹) was considerably higher at K2 than ALOHA. On average, zooplankton FA concentrations were 7 (epipelagic zone; 0-150 m) to 18 (mesopelagic zone; >150-1000 m) times higher at K2, and 15.5 times larger at K2 for the entire 0-1000 m water column (Table 2). Peaks in total FA concentration were present in both the epipelagic and mesopelagic zones at K2 (~350 m, upper-mesopelagic) and ALOHA (450-650 m, mid-mesopelagic) (Fig. 1A,B). For particle samples collected from 10-850 m depth, total FA concentration per unit C was on average 7x (K2) and 2x (ALOHA) lower than for zooplankton (Table 2). Total FA per unit C generally decreased with depth for particles at ALOHA with some variability above 350m and lower average concentrations at depth (below 400 m; Fig. 1A). Total FA concentrations for particles tracked the concentrations for zooplankton at K2 (although with much lower concentrations; Fig. 1B). Particles from 10 - 850 m contained on average 5x more FA per unit C at K2 than at ALOHA (Table 2).

Total FA per unit volume (µg FA m⁻³ seawater) in the epipelagic was 33x higher for zooplankton collected at K2 than at ALOHA, and was 126x higher at K2 in the mesopelagic (Table 2). At ALOHA, FA concentrations (µg FA m⁻³ seawater) for particles were 2x (epipelagic) to 3x (mesopelagic) higher than for zooplankton. In contrast, FA concentrations (µg FA m⁻³ seawater) for particles from K2 were 10x lower than for zooplankton (Table 2). At both locations, FA concentrations per unit volume for both particles and zooplankton were generally highest in the surface waters and decreased with depth, with the exception of a large peak (3800 µg FA / m³) between 200 to 400 m at K2 (Fig. 1D). Since concentrations per unit carbon and volume of all individual major fatty acids were much higher at K2 than ALOHA, the remaining results are expressed as a
proportion (%) of the total FA at each station. This presentation allows a better comparison of the differences in FA composition between the two sites.

The integrated average concentrations of the major FA for both zooplankton and particles are presented in Table 2 for the epipelagic zone (0 – 150 m, which spans the different euphotic zone depths at K2 and ALOHA), mesopelagic zone (150 – 1000 m), and the 0-1000 m water column. For all depths, there were significant differences (paired t-test, p<0.05) in the contribution of a number of these fatty acids to the total FA (% total FA) between sites and between zooplankton and particles at each site. For both the epipelagic and mesopelagic zones, zooplankton at K2 had significantly higher proportions of 14:0, 20:1(n-9), 22:1(n-11), EPA, MUFA, C20 PUFA, 16:1/16:0, and (n-3)/(n-6) than ALOHA (paired t-test, p<0.05). Zooplankton at ALOHA had significantly higher 16:0, 18:0, OA, AA, DHA, SFA, PUFA (similar in epipelagic), OBFA, C22 PUFA, and the ratios DHA/EPA, and 18:1(n-9)/18:1(n-7) (paired t-test, p<0.05). These between-station trends were generally similar for particle samples collected over the entire depth range 0-1000 m. Particles collected from K2 had higher 16:0, 18:0, 16:1(n-7), 18:1(n-9), 18:1(n-7), SFA, OBFA, and DHA/EPA than K2 zooplankton while particles collected from ALOHA had higher 14:0, 18:0, and SFA than zooplankton.

**Major fatty acids in individual zooplankton taxa**

The percent composition of the major FAs was also calculated for individual zooplankton groups and was highly variable between species (Table 3). Statistically significant differences between taxa were observed for many FAs (one-way ANOVA, p>0.05). Of the taxa analyzed, *Neocalanus cristatus*, *Neocalanus plumchrus*, and
Eucalanus bungii are considered to be omnivorous, with diets changing seasonally from phytoplankton to microzooplankton plus sinking particles (Kobari et al., 2003 and references therein). Both Neocalanus spp. were high in (n-3)/(n-6), EPA, and C20 PUFA, which are consistent with diatom sources. However, *N. cristatus* had significantly higher 20:1(n-9) and 22:1(n-11) than any of the other zooplankton groups analyzed. *E. bungii* also had similarly high C20 PUFA and EPA, however ratios of (n-3)/(n-6) were lower and 16:0 and 16:1(n-7) were higher than *Neocalanus* spp., suggesting some ingestion of diatoms. Omnivorous euphausiids were collected from both stations but % OBFA, DHA, DHA/EPA, and C22 PUFA were generally higher at ALOHA, suggesting higher consumption of bacteria (from sinking and suspended particles) and dinoflagellates than at K2. Euphausiids at K2 had significantly higher EPA and 22:1(n-11) than ALOHA (indicating both diatoms and calanoid copepods in their diet). Euphausiids at both sites had moderate (n-3)/(n-6), 18:1(n-9), and C20 & C22 PUFA, providing additional evidence of their omnivorous diet. Carnivorous chaetognath FAs were generally similar to the FA content of their presumed prey (*Neocalanus* spp.). However, chaetognaths had higher %18:1(n-9) than *Neocalanus* spp., as is typical of carnivores. *Paraeuchaeta* sp. are generally considered carnivorous copepods. They had a high % MUFA, 18:1(n-9), 20:1(n-9), 22:1(n-11), 18:1(n-9)/18:1(n-7), and low ratio of (n-3)/(n-6), all indicative of carnivorous feeding. The miscellaneous large copepods collected at ALOHA had high % 18:1(n-9) and lower ratios of (n-3)/(n-6), which may reflect an omnivorous diet or a mixture of copepods ranging from herbivorous to carnivorous.
Depth profiles of fatty acid indicators of herbivory

A high ratio of (n-3) to (n-6) PUFA is a general indicator for herbivory. This ratio was higher at K2 than ALOHA for bulk/mixed zooplankton (Fig. 2A). At ALOHA, the ratio was relatively constant throughout the water column (5.6 ± 0.6) and decreased slightly (to 2.0 ± 0.7) in the deepest sample. In contrast the (n-3)/(n-6) ratio was constant in the euphotic zone at K2 and decreased in the mid and lower mesopelagic (Fig. 2A). An increase in the (n-3)/(n-6) ratio from 11.5 ± 0.04 at 200-300 m to 14.6 ± 1.5 at 750-1000 m depth at K2 may be an indication of elevated feeding on sinking/suspended particles which can include aggregates of senescent phytoplankton cells. The (n-3)/(n-6) ratio for individual calanoid copepods at both sites is shown in Fig. 2B for comparison. *Neocalanus* species at K2 had the highest ratios (16.2 ± 1.1, slightly higher than the bulk K2 profile of 13.9 ± 1.5) and did not change considerably in the mesopelagic zone, suggesting these species may also be feeding on sinking/suspended particles. The (n-3)/(n-6) ratio also decreased with depth for *Paraeuchaeta* sp. at ALOHA, possibly indicating a switch in feeding mode from omnivory to carnivory with depth. *E. bungii* at K2 was intermediate between *Neocalanus* spp. and *Paraeuchaeta* sp. Miscellaneous large calanoid copepods from ALOHA had low (n-3)/(n-6) ratios (5.5 ± 0.7) suggesting carnivory as well.

Although there was a diverse assemblage of phytoplankton groups at ALOHA and K2 (ref), we used several diatom and dinoflagellate biomarkers as examples to explore zooplankton herbivory in more detail. The % C20 PUFA and 16:1/16:0 ratio were used as an index for diatom sources (Fig. 3A,B) and % C22 PUFA and DHA/EPA ratio were used as an index for dinoflagellates (Fig. 3C,D). Both % C20 PUFA and 16:1/16:0
were higher at K2 (ANOVA, p<0.05), consistent with the greater importance of diatoms at K2 vs. ALOHA (Fig. 3A,B). The DHA/EPA ratio was close to 1 and the 16:1/16:0 was below 1 at K2, suggesting a dinoflagellate source of nutrition in the zooplankton at K2 as well (Fig. 3B,D). In contrast, % C18 (which also occurs in haptophytes), C22 PUFA, and DHA/EPA were higher at ALOHA than at K2 (ANOVA, p<0.05), consistent with a dinoflagellate source for the zooplankton residing in both the epipelagic and mesopelagic zones at ALOHA compared to K2 (Table 2, Fig. 3). Values for both dinoflagellate indices were relatively constant with depth (Fig. 3C,D).

Depth profiles of fatty acid indicators of carnivory/omnivory

OA (18:1(n-9)), which is found in larger proportions in carnivorous zooplankton, increased with depth in mixed bulk zooplankton at ALOHA, and peaked at both 100-200 m and 400-500 m at K2 (Fig. 4A), which corresponds to above and below the midwater peak in K2 zooplankton biomass (Steinberg et al., 2008a). Carnivorous copepods (e.g., Paraeuchaeta sp. at K2, large copepods at ALOHA) are significantly higher in OA than the Neocalanus spp. at K2 (ANOVA, p<0.05, Fig. 4B). In the middle range are E. bungii at K2 (Fig. 4B). The relative percentages of OA in Paraeuchaeta sp. also increased with depth, indicating an increase in carnivory deeper in the water column. The miscellaneous large copepods collected from ALOHA contained similar proportions of OA as Paraeuchaeta sp. below 300 m. Like OA, the omnivory ratio 18:1(n-9)/18:1(n-7) was also higher at ALOHA than at K2 (Table 2). The long chain MUFAs, 20:1(n-9) and 22:1(n-11), indicative of calanoid copepods, were both higher at K2 than ALOHA in the bulk zooplankton and increased through the mesopelagic (Fig. 5A). Chaetognaths, as well
as the calanoids *N. cristatus*, *N. plumchrus*, and *Paraeuchaeta* sp., were also elevated in these MUFAs (Fig. 5B). OA and 20:1(n-9) + 22:1(n-11) in particle samples at both locations increased with depth throughout the mesopelagic, indicating an increase in zooplankton-derived material (e.g., fecal pellets) (Fig. 4C, 5C).

**Depth profiles of fatty acid indicators of particle feeding**

Although minor components of the total fatty acid concentration, bacterial fatty acids in zooplankton may provide a useful index for particle feeding. Bacterial fatty acid biomarkers examined in zooplankton and particles include OBFA, 18:1(n-7), and %MUFA (although MUFA are not exclusive to bacteria). OBFA and 18:1(n-7) made-up a small percentage of the total FA in all samples (Table 2), and there was no difference in the 18:1(n-7) between stations in both bulk zooplankton and particles. However, OBFA was significantly higher (t-test, p<0.05) in zooplankton tissue throughout the mesopelagic and epipelagic zone at ALOHA vs. K2, and similar at ALOHA between the particles and zooplankton (Table 2). For the individual taxa, *E. bungii* at K2 and copepods at ALOHA were enriched in OBFA relative to the other species sampled (ANOVA, p<0.05). *E. bungii* at K2 and euphausiids at both sites were also higher in 18:1(n-7) than other species measured (Table 3). %MUFA in zooplankton was higher at K2 than ALOHA through 1000 m (Table 2) and increased significantly (ANOVA, p<0.05) with depth at both sites (Fig. 6A,B). %MUFA in both particles and zooplankton were similar with depth at ALOHA until 750-1000 m, where MUFA was slightly higher in zooplankton than in particles (Fig. 6A). % MUFA was similar in zooplankton and particles throughout the water column at K2 with the exception of the epipelagic zone where %MUFA was
higher in zooplankton (Table 2, Fig. 5B). MUFA was similar in individual taxa analyzed at both sites with the exception of *Paraeuchaeta* spp (due to higher OA, which is also a MUFA).

**Discussion**

*Bulk zooplankton total FA*

The higher fatty acid concentration (per unit carbon and per volume) for the bulk zooplankton at K2 vs. ALOHA is a reflection of the higher zooplankton biomass, larger body sizes, and lipid storage function in zooplankton at K2. Changing zooplankton FA concentration by volume (μg/ m³) with depth was correlated with zooplankton biomass which was an order of magnitude higher at K2 than ALOHA (Steinberg *et al.*, 2008a). The majority of the zooplankton biomass was < 2mm in size at station ALOHA and > 2mm at station K2, due to the high numbers of large *Neocalanus* spp. copepods at K2 present at all depths (Kobari *et al.*, 2008b; Steinberg *et al.*, 2008a). *Neocalanus* spp. and *E. bungii* at K2 were beginning their seasonal ontogenetic vertical migrations to depth during our study (Kobari *et al.*, 2008b; Steinberg *et al.*, 2008a; Tsuda *et al.*, 1999) and are well known to store lipids for their winter diapause and spawning (Tsuda *et al.*, 2001). Large lipid globules obtained by summer feeding were clearly visible in these species at K2 and contributed to their higher total lipid and total FA content, and thus the high lipid and FA content of bulk zooplankton. Lipid globules in copepods were not clearly visible at ALOHA, however particle lipid concentration per unit volume in seawater was much
higher than for zooplankton at ALOHA vs. K2. Higher concentrations of FA in particles at ALOHA could give mesopelagic zooplankton a steady supply of essential nutrition from a food source that is less seasonal than at K2, as suggested by Conte et al. (1998) for sinking particles in the Sargasso Sea.

FA concentration per unit C was variable with depth at both locations, peaking at two distinct depths at K2 (epipelagic and 200-400 m) and ALOHA (euphotic/upper epipelagic and 400-750 m). The peaks in FA concentration per unit C at K2 mirrored the abundance of several individual zooplankton taxa; calanoid copepods (mostly *Neocalanus* spp.), ostracods, chaetognaths, and hydrozoan medusae had bimodal distributions at K2 at similar depths (Steinberg *et al.*, 2008a). Other taxa at K2 such as radiolaria, pteropods, salps, and doliolids also peaked in abundance at 200-500 m. Similar to FA concentration per unit C at ALOHA, most individual species abundances at ALOHA decreased with depth and a few (including cyclopoid and harpacticoid copepods, gammarid amphipods, and mysids) increased slightly in abundance at 500-750 m (Steinberg *et al.*, 2008a). At K2, these FA peaks likely come from calanoid copepods (with large oil sacs such as *Neocalanus* and *Eucalanus* spp.) that were abundant at these depths and beginning their ontogenetic vertical migrations to depth (Dagg, 1993; Kobari and Ikeda, 1999, 2001; Shoden *et al.*, 2005). At ALOHA, although these peaks are small, they could be indicative of vertical migrators coming from both the mesopelagic (into the euphotic/epipelagic zone) and > 1000 m (into the lower mesopelagic, Steinberg *et al.*, 2008a). These deeper-residing taxa would likely have a greater stored lipid concentration (in the form of wax esters) than shallower-residing zooplankton (Lee and Hirota, 1973; Lee *et al.*, 1971). Total FA concentrations (both by
carbon and volume) from the bulk zooplankton samples collected at night in the epipelagic at ALOHA were indeed higher than those collected during the day (data not shown).

Modes of feeding – Herbivory

The contrasting community composition of the primary producers between the two sites was reflected in the diets of the mesozooplankton. At the times of our study, ALOHA was dominated by dinoflagellates and cyanobacteria (Prochlorococcus spp. and Synechococcus spp.) and K2 was dominated by both cyanobacteria (Synechococcus spp.) and larger diatoms (Chaetoceros spp. and Coscinodiscus spp.), and to a lesser extent dinoflagellates (Boyd et al., 2008; Buesseler et al., 2008; Zhang et al., 2008). There was a higher herbivory ratio \((n-3)/(n-6) \text{ PUPA}\) at K2 vs. ALOHA, likely due to higher phytoplankton productivity and biomass in the form of large diatoms at K2 (Boyd et al., 2008). Higher herbivory ratios in calanoid copepods have been reported in areas of high diatom production in the Arctic (Stevens et al., 2004a). A comparatively large proportion of \((n-6) \text{ PUFA}\) in grazers can be due to the consumption of both bacteria and ciliates (Desvilettes et al., 1997). Therefore the lower \((n-3)/(n-6)\) ratio at ALOHA may be due to a greater influence of either bacteria or ciliates in zooplankton diets there. Many studies do not use the general \((n-3)/(n-6)\) ratio as they instead relate the more specific biomarkers such as EPA, DHA, and \(C_{18}\text{PUFA-C_{22}PUFA}\) to infer dinoflagellate vs. diatom diets. However Viso and Marty (1993) proposed the use of \((n-6)/(n-3)\) as an index of nutritional value in bivalves in conjunction with the other more specific markers such as EPA and DHA.
Group-specific phytoplankton trophic markers were utilized to determine if
dinoflagellate- or diatom-based nutrition (directly or from feeding on aggregates)
dominated at each location. This approach has been verified by Graeve et al. (1994) who
used feeding experiments to show herbivorous calanoid copepods in the Arctic fed either
diatoms or dinoflagellates were capable of completely replacing dinoflagellate
biomarkers with diatom markers, and visa versa, within 24-42 days. EPA and DHA
made-up the highest proportions of individual fatty acids in bulk zooplankton tissue at K2
and ALOHA indicating diatoms and dinoflagellates were an important component of
zooplankton nutrition at both sites and at all depths. Although the ratio DHA/EPA in
zooplankton tissue indicated dinoflagellate feeding was important at ALOHA and diatom
feeding was important at K2, dinoflagellates were also a substantial dietary component at
K2. Diatom-derived C_{20} PUFA and 16:1/16:0 ratio, both higher in the bulk zooplankton
tissue at K2 than ALOHA, also provide evidence of a diatom-origin diet at K2. At depth,
these PUFA were likely obtained via vertical migration or feeding on sinking aggregates
(see below).

Although cyanobacteria were abundant in the water column (Zhang et al., 2008),
and consistently found in zooplankton guts at both locations (Wilson et al., in prep.), they
may be a poor food source for zooplankton (Müller-Navarra et al., 2000; Müller-Navarra
et al., 2004). Cyanobacteria contain less PUFA and EFA and therefore are less nutritious
than diatoms or dinoflagellates (Desvilettes et al., 1997; Viso and Marty, 1993). In
feeding experiments, the freshwater cladoceran Daphnia was fed a diet of Synechococcus
and other autotrophic picoplankton to determine if these crustaceans could get their
essential fatty acids from these small cells (Bec et al., 2006; Desvilettes et al., 1997). The
consumption of these species resulted in poor growth and fecundity without the addition of other phytoplankton (DeMott and Müller-Navarra, 1997; Desvilettes et al., 1997) or via trophic upgrading through an intermediate grazer (Bec et al., 2003; Bec et al., 2006). There is also speculation that cyanobacteria are resistant to digestion by zooplankton and pass through their guts still viable (Lampitt et al., 1993b; Silver and Alldredge, 1981).

Of the specific taxa analyzed, Neocalanus cristatus, N. plumchrus, and Eucalanus bungii are considered omnivorous with diets changing seasonally from phytoplankton to microzooplankton and sinking particles (Dagg, 1993; Kobari and Ikeda, 1999, 2001; Kobari et al., 2008a; Kobari et al., 2003; Shoden et al., 2005). The high (n-3)/(n-6) ratios in Neocalanus spp. are consistent with a more herbivorous diet in the late summer at K2 that included diatoms (e.g., Kobari and Ikeda 1999, 2001), as indicated by their high EPA and C20 PUFA content. A high % EPA and C20 PUFA in Neocalanus spp. was generally consistent throughout the 1000 m depth profile. Mesopelagic Neocalanus spp. are likely obtaining these diatom biomarkers through particle feeding as mentioned above. E. bungii, however, contained lower (n-3)/(n-6) ratios, higher oleic acid, as well as higher 16:1(n-7), EPA, and C20 PUFAs, indicating an omnivorous diet with a large diatom influence, consistent with feeding on sinking particles (e.g., Shoden et al. 2005).

**Modes of feeding – Carnivory/Omnivory**

Carnivores are an abundant component of the mesopelagic food web and carnivory is essential (along with diel vertical migration) for satisfying the metabolic demands of deeper fauna (Steinberg et al., 2008b). Carnivores and omnivores tend to accumulate particular MUFA in their tissue therefore the high relative proportions of OA
18:1(n-9), 18:1(n-9)/18:1(n-7), as well as 20:1(n-9) and 22:1(n-11) (in non-calanoid zooplankton) is consistent with the increasing importance of carnivory. Mayzaud et al. (1999) determined that the arctic euphausiid Meganyctiphanes norvegica was a carnivore from its comparatively high percentages of OA, 20:1(n-9), and 22:1(n-11). Mayzaud et al. (2007) further demonstrated carnivory in select zooplankton species analyzed in a subtropical station compared to a subantarctic one using these same fatty acid markers. In the subtropical station, an increase in the importance of carnivory was related to a lack of algal food (Mayzaud et al., 2007). Similarly, OA and 18:1(n-9)/18:1(n-7) indices were both higher at ALOHA than at K2, likely due to a greater reliance on carnivory (also likely due to lower primary productivity) at our subtropical site. The increase in OA and/or 20:1(n-9) + 22:1(n-11) biomarkers with depth at both locations also signifies the increasing importance of carnivory with depth. This increasing dependence on carnivory with depth has been shown in the Arctic, where the carnivorous amphipod Themisto abyssicorum had a higher 18:1(n-9)/18:1(n-7) than its congener Themisto libellula (also carnivorous). T. abyssicorum is distributed deeper and preys upon other carnivorous zooplankton whereas the surface dwelling T. libellula were preying upon diatom-feeding copepods (Auel et al., 2002). The lower proportions of 20:1(n-9) and 22:1(n-11) at ALOHA likely result from a lack of exclusively herbivorous feeding calanoid copepods in this region; however, like K2, the proportion of these FAs do increase by ~40% from 25 m to 1000 m, supporting the hypothesis of an increase in carnivory with depth.

At K2, there were distinct peaks in the abundance of carnivores (e.g., chaetognaths at 0-50 and 150-200 m, medusae at 200-300 m, and ctenophores at 50-150 m) within the mesopelagic zone (Steinberg et al., 2008a; Steinberg et al., 2008b). These
depths correspond to peaks observed in OA and 20:1(n-9) + 22:1(n-11) in this study. Within the tissue of the carnivorous taxa analyzed at K2 (chaetognaths and *Paraeuchaeta* sp.), 20:1(n-9) and 22:1(n-11) were in similar proportions to their prey, *N. plumchrus*. Similarly, Auel et al. (2002) found higher amounts of 20:1(n-9) and 22:1(n-11) in carnivorous Arctic amphipods as did Graeve et al. (2008) in Arctic ctenophores, both suspected of consuming *Calanus* spp.

Mesopelagic carnivores are also important for supplying 'repackaged' carbon at depth by the production of fecal pellets (Wilson *et al.*, 2008). Mayzaud *et al.* (2007) showed that the fecal pellets of carnivores can be important vectors of essential fatty acids to deeper waters, with PUFA, DHA+EPA, and 20:1(n-9) + 22:1(n-11) content all highest in the fecal pellets of the most carnivorous copepods and euphausiids sampled. The increase in OA and 20:1(n-9) + 22:1(n-11) in particles with depth at both K2 and ALOHA supports this assertion.

**Modes of feeding – Sinking/suspended particles**

Fatty acids associated with particulate organic matter generally reflect their origins which could be from a combination of phytoplankton, zooplankton, and bacterial sources – which makes finding appropriate biomarkers for detrital feeding difficult (Wakeham, 1995). We used the proportions of OBFA and 18:1(n-7) in zooplankton tissue as indicators of feeding on bacteria. Relative abundances of these FA tend to be small, causing their importance to be overshadowed by the larger contribution of other fatty acids from zooplankton and phytoplankton (Wakeham and Canuel, 1988). In our study, %OBFA was useful for indicating particle feeding between sites and with depth but not
to other FAs. Stevens et al. (2004c) also used an OBFA index to determine the importance of microbial feeding in Arctic zooplankton around a polynya in Baffin Bay and concluded that particle feeding by copepods occurred but varied by region. A strong linear correlation between OBFA in marine seston and the calanoid copepod, Calanus glacialis was also found (Stevens et al., 2004a). At both ALOHA and K2, there were distinct peaks in abundance of known particle feeders (e.g., salps above 150 m at ALOHA and between 200-400 m at K2, ostracods 100-300 m at ALOHA and 150 - 400 m at K2, and poecilostomatoid copepods above 200 m at ALOHA and below 200 m at K2 (Steinberg et al., 2008a). Similar to salps, poecilostomatoid copepod, and ostracod distribution at ALOHA, the % OBFA was highest between 0-300 m. The % OBFA at K2 however, remained constant throughout the mesopelagic zone. OBFA in particles and zooplankton was significantly higher at ALOHA than K2 although the %18:1(n-7) was similar at both sites. %18:1(n-7) can also be present in copepod tissue in more productive regions due to the elongation of 16:1(n-7) into 18:1(n-7) (Stevens et al., 2004c). As a result, this biomarker may not be useful as an index for bacteria at K2, as illustrated by the somewhat higher %18:1(n-7) in the epipelagic zone where diatoms dominated, than the mesopelagic (Table 2). In contrast, %18:1(n-7) was higher in bulk zooplankton collected from the mesopelagic vs. those collected from the epipelagic zone at ALOHA (Table 2). However 18:1(n-7) is also produced in cyanobacteria, coccolithophores, and flagellates; all present at ALOHA (Boyd et al., 2008). Thus the 18:1(n-7) measured in zooplankton tissue at ALOHA was likely obtained via feeding on sinking particles which can aggregate phytoplankton taxa (see below).
EPA and DHA, as well as other (n-3) and (n-6) PUFAs can be indicators of particle feeding at depths below the epipelagic. These compounds are considered essential fatty acids (EFAs). Zooplankton cannot synthesize EFAs de novo so these FA must be obtained from food sources for normal growth and development (Dalsgaard et al., 2003). In order to obtain EFAs, mesopelagic zooplankton would have to vertically migrate to surface waters to feed, feed on sinking particles, or ingest other zooplankton. Vertical migration was significant at both K2 and ALOHA with both the strength and amplitude varying between regions (Steinberg et al., 2008a; Steinberg et al., 2008b).

Sinking particles however, include fecal pellets, viable or dead/dying phytoplankton cells as well as bacteria which would also carry nutritious PUFAs and EFAs to the deep sea (e.g., Falk-Peterson et al., 1999; Fowler and Knauer, 1986; Wakeham and Canuel, 1988). EPA and DHA however, are also assumed to be an indicator of “freshness” of sinking particles as the half lives of these and other highly labile PUFAs are on the order of days (Canuel and Martens, 1996; Conte et al., 2003; Conte et al., 1995). The percentage of total PUFA in particles at ALOHA and K2 decreases slightly from the epipelagic to the mesopelagic but remained at high levels in total FA throughout. The availability of PUFA in the mesopelagic zone is undoubtedly an important nutritional option for zooplankton residing there.

The correlation between compounds found in both zooplankton and particles may also be a general indicator of particle feeding. Alternatively, this relationship could mean that zooplankton ingest particles in constant proportion. Many of the FA depth profiles of sinking particles mirrored the FA depth profiles of the zooplankton at both ALOHA and K2. For example, the proportion of MUFA in bulk zooplankton and particles at ALOHA
and K2 both increase and are nearly identical with depth (Fig. 6). Increasing values of total MUFA (or combinations of specific MUFA) have also been used as indicators of bacterial association/feeding (e.g., Desvilettes et al., 1997; Pranal et al., 1996). The percentages of (n-3) EFA (including EPA and DHA), OBFA (at ALOHA, not shown due to scale), PUFA (at K2 only), as well as MUFA in bulk zooplankton all were significantly correlated with the percentages measured in the particles (Fig. 7, Pearson’s r, p<0.05). These linear correlations provide additional evidence that zooplankton can obtain essential FA from sinking and suspended particles in the mesopelagic zone.

Although a poor nutritional supplement, the presence of cyanobacteria in zooplankton tissue can also demonstrate particle feeding. Like bacteria, cyanobacteria are too small to be eaten by non-filter feeding zooplankton. The increase in 16:1/16:0 in zooplankton tissue at ALOHA and K2 with depth may be due to the fact that 16:1(n-7) is found in bacteria and cyanobacteria as well as diatoms (e.g., Desvilettes et al., 1997; Wakeham, 1995). Wakeham (1995) found increasing 16:1(n-7) in POC of the deeper 1000m waters in the central north pacific was well as in the anoxic region of the black sea, indicative of bacterial decomposition of POC.

Zooplankton fecal pellets sinking through the mesopelagic can also provide essential nutrition to zooplankton residing there. The increase with depth in both 18:1(n-9) and 20:1(n-9) + 22:1(n-11) MUFA in the particles is indicative of an increase in zooplankton-derived material, likely fecal pellets, within the mesopelagic zone. As mentioned above, FA in subantarctic and subtropical zooplankton fecal pellets contained significant amounts of EFA, including DHA and EPA (Mayzaud et al., 2007). Sheridan et al. (2002) and Conte et al. (1998) both conclude that the production and consumption
of fecal pellets produced by mesopelagic zooplankton contributes to or has an effect on the nutritional composition of particles reaching the deep sea. At ALOHA and K2 the percentage of the total carbon flux that consisted of intact fecal pellets was as much as 35% and 29%, respectively (Wilson et al., 2008). A larger proportion of sinking material may also be of fecal origin but in the form of broken-up "fecal fluff" which was present in large amounts at K2 but not quantifiable (Wilson et al., 2008).

Summary and Conclusion

Our results illustrate the changing feeding ecology of zooplankton from the surface through the mesopelagic zone, and between locations, both of which have implications for the transport of organic material to the deep sea. Biomarkers for herbivory remained more pronounced in zooplankton at K2 during late summer than ALOHA where, although at the end of a diatom bloom, primary production was still higher. On an annual basis however, K2 is more seasonal than ALOHA, making lipid storage in seasonal migrators at K2 essential. FA composition at K2 was consistent with zooplankton feeding on diatom and diatom-derived aggregate material throughout the mesopelagic. In contrast, dinoflagellate markers were proportionately more abundant in zooplankton at ALOHA, which like diatoms at K2, can also supply essential fatty acids to zooplankton at depth. At ALOHA, where production is lower and particulate organic carbon attenuated more rapidly than at K2, carnivory was more prominent at depth. Carnivory may help mesopelagic zooplankton compensate for the lower export efficiency.
of POC at ALOHA vs. K2. Particle feeding by zooplankton was evident at both locations, and fecal pellets are likely to be a major source of essential fatty acids for mesopelagic zooplankton. Food-web transfer and alteration of fatty acids through the mesopelagic zone thus affects the efficiency of the biological pump as differences in zooplankton feeding ecology can alter the quality and quantity of organic material reaching the deep sea.
Literature Cited


Stevens, C.J., Deibel, D., Parrish, C.C., 2004b. Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment


Table 1. Common fatty acid biomarkers for zooplankton diet used in this study. n/a = no common name for ratio or group of compounds. FA = fatty acids. PUFA = polyunsaturated fatty acids.

<table>
<thead>
<tr>
<th>Food Source</th>
<th>Biomarker</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>general herbivory</td>
<td>(n-3)/(n-6)</td>
<td>n/a</td>
</tr>
<tr>
<td>diatoms</td>
<td>16:1(n-7)</td>
<td>palmitoleic acid</td>
</tr>
<tr>
<td></td>
<td>20:5(n-3)</td>
<td>eicosapentanoic acid (EPA)</td>
</tr>
<tr>
<td></td>
<td>C₂₀ PUFA</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>16:1/16:0</td>
<td>n/a</td>
</tr>
<tr>
<td>dinoflagellates</td>
<td>DHA/EPA</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>20:4(n-6)</td>
<td>arachidonic acid (AA)</td>
</tr>
<tr>
<td></td>
<td>22:6(n-3)</td>
<td>docosahexanoic acid (DHA)</td>
</tr>
<tr>
<td></td>
<td>C₂₂ PUFA</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>C₁₈ PUFA</td>
<td>n/a</td>
</tr>
<tr>
<td>heterotrophic bacteria</td>
<td>OBFA</td>
<td>odd or branched FA</td>
</tr>
<tr>
<td></td>
<td>18:1(n-7)</td>
<td>vaccenic acid</td>
</tr>
<tr>
<td>general carnivory</td>
<td>18:1(n-9)</td>
<td>oleic acid (OA)</td>
</tr>
<tr>
<td></td>
<td>18:1(n-9)/18:1(n-7)</td>
<td>n/a</td>
</tr>
<tr>
<td>calanoid copepods</td>
<td>20:1(n-9)</td>
<td>eicosenoic acid</td>
</tr>
<tr>
<td></td>
<td>22:1(n-11)</td>
<td>docosenoic acid</td>
</tr>
</tbody>
</table>
Table 2. Major individual fatty acids (FA) as a proportion of total FA, FA ratios, and the total FA concentration for both zooplankton and > 51 um particles at ALOHA and K2. Data are presented for the epipelagic zone (0 – 150m), mesopelagic zone (150 – 1000m) and the 0-1000 m water column. For zooplankton, depth-weighted averages (± standard error) are presented. Values for particles are trapezoidal integrated averages (± standard error). SFA = saturated fatty acids. MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. OBFA = iso/anteiso 13:0, 15:0, and 17:0 SFA. C20,22 = 20,22 carbons in fatty acid. n = 4 for both K2 and ALOHA zooplankton and particles.
Table 2.

![Table 2. Total Fatty Acid Ratios](image-url)

**Zooplankton**

<table>
<thead>
<tr>
<th>Fatty Acids (%)</th>
<th>ALOHA</th>
<th>K2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0</td>
<td>5.1 ± 0.3</td>
<td>7.9 ± 0.6</td>
</tr>
<tr>
<td>16:0</td>
<td>22.4 ± 1.3</td>
<td>19.8 ± 1.1</td>
</tr>
<tr>
<td>18:0</td>
<td>7.1 ± 0.8</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>4.1 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>8.2 ± 0.7</td>
<td>17.9 ± 1.2</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>1.8 ± 0.2</td>
<td>2.2 ± 0.07</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.57 ± 0.06</td>
<td>1.04 ± 0.3</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>1.6 ± 0.06</td>
<td>1.6 ± 0.09</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>7.9 ± 0.6</td>
<td>6.2 ± 0.15</td>
</tr>
<tr>
<td>22:1(n-11)</td>
<td>0.5 ± 0.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>21.7 ± 1.7</td>
<td>18.1 ± 0.5</td>
</tr>
<tr>
<td>SFA</td>
<td>36.9 ± 2.5</td>
<td>31.4 ± 1.6</td>
</tr>
<tr>
<td>MUFA</td>
<td>19.0 ± 0.8</td>
<td>32.1 ± 2.2</td>
</tr>
<tr>
<td>PUFA</td>
<td>40.5 ± 2.3</td>
<td>35.6 ± 0.6</td>
</tr>
<tr>
<td>OBA</td>
<td>3.4 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>C20 PUFA</td>
<td>11.0 ± 0.4</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>C22 PUFA</td>
<td>24.3 ± 1.7</td>
<td>22.4 ± 0.6</td>
</tr>
<tr>
<td>% SFA</td>
<td>25.1 ± 1.2</td>
<td>21.2 ± 1.6</td>
</tr>
<tr>
<td>% MUFA</td>
<td>39.0 ± 0.8</td>
<td>39.6 ± 2.8</td>
</tr>
<tr>
<td>% PUFA</td>
<td>39.4 ± 0.9</td>
<td>35.6 ± 0.6</td>
</tr>
<tr>
<td>% OBA</td>
<td>2.6 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>% C20 PUFA</td>
<td>9.9 ± 0.2</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>% C22 PUFA</td>
<td>25.0 ± 0.8</td>
<td>22.4 ± 0.6</td>
</tr>
</tbody>
</table>

**Fatty Acid Ratios**

<table>
<thead>
<tr>
<th>DHA/EPA</th>
<th>18:1(n-9)/18:1(n-7)</th>
<th>16:1/16:0</th>
<th>Total Fatty Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.1 ± 2.5</td>
<td>8.1 ± 1.2</td>
<td>8.4 ± 1.3</td>
<td>43.5 ± 11.7</td>
</tr>
<tr>
<td>11.9 ± 1.1</td>
<td>10.4 ± 0.4</td>
<td>10.1 ± 0.3</td>
<td>39.3 ± 7.6</td>
</tr>
<tr>
<td>12.7 ± 0.9</td>
<td>14.1 ± 0.0</td>
<td>12.7 ± 0.9</td>
<td>40.0 ± 6.9</td>
</tr>
<tr>
<td>18.9 ± 1.2</td>
<td>10.6 ± 0.4</td>
<td>11.9 ± 0.5</td>
<td>11.9 ± 0.5</td>
</tr>
<tr>
<td>13.7 ± 1.1</td>
<td>13.5 ± 1.1</td>
<td>12.6 ± 0.9</td>
<td>11.0 ± 0.3</td>
</tr>
<tr>
<td>14.6 ± 1.3</td>
<td>15.1 ± 0.9</td>
<td>13.5 ± 0.7</td>
<td>10.9 ± 0.4</td>
</tr>
<tr>
<td>15.1 ± 0.9</td>
<td>14.6 ± 1.3</td>
<td>13.5 ± 0.7</td>
<td>10.9 ± 0.4</td>
</tr>
<tr>
<td>16.2 ± 1.1</td>
<td>16.2 ± 1.1</td>
<td>13.1 ± 0.2</td>
<td>11.1 ± 0.2</td>
</tr>
<tr>
<td>16.6 ± 1.1</td>
<td>16.6 ± 1.1</td>
<td>11.8 ± 1.1</td>
<td>10.5 ± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Fatty Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(μg/mgC)</td>
</tr>
<tr>
<td>(μg/m³)</td>
</tr>
</tbody>
</table>

**> 51 μm Particles**

<table>
<thead>
<tr>
<th>Fatty Acids (%)</th>
<th>ALOHA</th>
<th>K2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0</td>
<td>9.9 ± 0.3</td>
<td>8.6 ± 1.0</td>
</tr>
<tr>
<td>16:0</td>
<td>25.1 ± 1.2</td>
<td>21.2 ± 1.6</td>
</tr>
<tr>
<td>18:0</td>
<td>6.9 ± 0.8</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>5.9 ± 0.2</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>7.4 ± 0.4</td>
<td>13.9 ± 1.2</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>2.3 ± 0.4</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.14 ± 0.10</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>1.8 ± 0.06</td>
<td>0.94 ± 0.14</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>6.9 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>22:1(n-11)</td>
<td>0.27 ± 0.19</td>
<td>1.4 ± 1.1</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>16.8 ± 1.1</td>
<td>10.5 ± 0.8</td>
</tr>
<tr>
<td>SFA</td>
<td>45.5 ± 0.3</td>
<td>39.9 ± 1.7</td>
</tr>
<tr>
<td>MUFA</td>
<td>17.6 ± 0.3</td>
<td>28.6 ± 2.9</td>
</tr>
<tr>
<td>PUFA</td>
<td>36.5 ± 0.02</td>
<td>25.7 ± 1.3</td>
</tr>
<tr>
<td>OBA</td>
<td>3.3 ± 0.2</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>C20 PUFA</td>
<td>9.5 ± 0.2</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>C22 PUFA</td>
<td>20.3 ± 0.05</td>
<td>12.7 ± 1.0</td>
</tr>
<tr>
<td>% SFA</td>
<td>25.6 ± 1.6</td>
<td>21.5 ± 1.6</td>
</tr>
<tr>
<td>% MUFA</td>
<td>31.7 ± 0.7</td>
<td>39.3 ± 1.7</td>
</tr>
<tr>
<td>% PUFA</td>
<td>44.1 ± 1.2</td>
<td>28.1 ± 3.0</td>
</tr>
<tr>
<td>% OBA</td>
<td>4.1 ± 0.9</td>
<td>26.3 ± 1.6</td>
</tr>
<tr>
<td>% C20 PUFA</td>
<td>8.6 ± 1.0</td>
<td>34.0 ± 1.2</td>
</tr>
<tr>
<td>% C22 PUFA</td>
<td>10.5 ± 0.3</td>
<td>32.7 ± 1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fatty Acid Ratios</th>
<th>DHA/EPA</th>
<th>18:1(n-9)/18:1(n-7)</th>
<th>16:1/16:0</th>
<th>Total Fatty Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(μg/mgC)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>(μg/m³)</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>
Table 3. Major individual fatty acids (FA) as a proportion of total FA, as FA ratios, and the total FA concentration for individually sorted zooplankton taxa from K2 and ALOHA. Values are depth-weighted averages (± standard error) for zooplankton collected between 0-1000 m. n = number of replicates for each taxa. SFA = saturated fatty acids. MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. OBFA = iso/anteiso 13:0, 15:0, and 17:0 SFA. C20,22 = 20,22 carbons in fatty acid.
Table 3.

<table>
<thead>
<tr>
<th>Fatty Acids (%)</th>
<th>chaetognaths (n=7)</th>
<th>Paraenococcus sp. (n=5)</th>
<th>euphausiids (n=2)</th>
<th>N. plumchrus (n=7)</th>
<th>N. cristatus (n=6)</th>
<th>E. bungii (n=3)</th>
<th>ALOHA lg. copepods (n=4)</th>
<th>euphausiids (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>6.7 ± 2.4</td>
<td>1.2 ± 0.3</td>
<td>4.2 ± 1.0</td>
<td>13.6 ± 0.7</td>
<td>7.8 ± 0.2</td>
<td>6.5 ± 0.2</td>
<td>4.1 ± 1.3</td>
<td>5.0 ± 0.5</td>
</tr>
<tr>
<td>16:0</td>
<td>12.8 ± 0.9</td>
<td>14.0 ± 1.1</td>
<td>22.5 ± 2.2</td>
<td>9.0 ± 0.6</td>
<td>6.8 ± 0.14</td>
<td>24.7 ± 0.5</td>
<td>19.2 ± 5.2</td>
<td>20.7 ± 0.3</td>
</tr>
<tr>
<td>18:0</td>
<td>3.6 ± 0.9</td>
<td>9.3 ± 2.2</td>
<td>1.6 ± 0.09</td>
<td>4.6 ± 0.15</td>
<td>3.7 ± 0.4</td>
<td>2.0 ± 0.2</td>
<td>9.2 ± 4.1</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>7.8 ± 0.7</td>
<td>13.4 ± 1.4</td>
<td>4.8 ± 0.9</td>
<td>2.7 ± 0.2</td>
<td>2.2 ± 0.09</td>
<td>16.6 ± 0.4</td>
<td>4.7 ± 0.6</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>7.5 ± 1.5</td>
<td>23.6 ± 1.1</td>
<td>9.9 ± 0.8</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.04</td>
<td>6.2 ± 0.07</td>
<td>18.7 ± 4.0</td>
<td>9.5 ± 0.2</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>1.9 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.03</td>
<td>6.5 ± 0.3</td>
<td>2.1 ± 0.13</td>
<td>3.5 ± 0.12</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>2.2 ± 0.3</td>
<td>1.9 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>0.93 ± 0.11</td>
<td>3.4 ± 0.2</td>
<td>0.47 ± 0.02</td>
<td>1.0 ± 0.4</td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.57 ± 0.05</td>
<td>0.23 ± 0.01</td>
<td>1.3 ± 0.4</td>
<td>0.52 ± 0.03</td>
<td>0.60 ± 0.06</td>
<td>0.95 ± 0.04</td>
<td>1.5 ± 0.14</td>
<td>4.0 ± 0.15</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>12.7 ± 1.6</td>
<td>4.2 ± 1.2</td>
<td>20.6 ± 1.6</td>
<td>13.3 ± 0.9</td>
<td>16.5 ± 0.8</td>
<td>21.4 ± 0.3</td>
<td>7.4 ± 0.9</td>
<td>13.3 ± 0.2</td>
</tr>
<tr>
<td>22:1(n-11)</td>
<td>4.5 ± 1.1</td>
<td>5.1 ± 1.3</td>
<td>1.3 ± 0.3</td>
<td>4.2 ± 0.5</td>
<td>10.4 ± 0.9</td>
<td>0.3 ± 0.01</td>
<td>0.7 ± 0.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>12.7 ± 1.9</td>
<td>5.9 ± 1.5</td>
<td>11.4 ± 4.6</td>
<td>6.5 ± 1.5</td>
<td>6.2 ± 0.3</td>
<td>4.7 ± 1.0</td>
<td>15.1 ± 3.3</td>
<td>23.4 ± 0.6</td>
</tr>
<tr>
<td>SFA</td>
<td>24.0 ± 2.9</td>
<td>25.2 ± 2.9</td>
<td>29.2 ± 3.3</td>
<td>28.2 ± 0.5</td>
<td>19.4 ± 0.6</td>
<td>33.6 ± 0.9</td>
<td>35.2 ± 3.1</td>
<td>32.0 ± 0.1</td>
</tr>
<tr>
<td>MUFA</td>
<td>33.1 ± 3.3</td>
<td>54.3 ± 5.2</td>
<td>26.8 ± 3.7</td>
<td>16.0 ± 0.5</td>
<td>25.0 ± 1.1</td>
<td>32.9 ± 0.1</td>
<td>30.9 ± 5.1</td>
<td>18.5 ± 0.8</td>
</tr>
<tr>
<td>PUFA</td>
<td>32.8 ± 3.2</td>
<td>15.9 ± 3.0</td>
<td>41.3 ± 7.9</td>
<td>34.7 ± 2.4</td>
<td>37.9 ± 1.3</td>
<td>32.7 ± 1.0</td>
<td>31.5 ± 3.5</td>
<td>48.6 ± 0.5</td>
</tr>
<tr>
<td>OBFA</td>
<td>1.2 ± 0.1</td>
<td>0.69 ± 0.1</td>
<td>0.69 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.25 ± 0.04</td>
<td>2.7 ± 0.5</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>C20 PUFA</td>
<td>14.1 ± 1.7</td>
<td>5.0 ± 1.3</td>
<td>23.1 ± 2.1</td>
<td>10.9 ± 0.9</td>
<td>20.1 ± 0.9</td>
<td>23.5 ± 0.3</td>
<td>9.7 ± 1.3</td>
<td>17.8 ± 0.03</td>
</tr>
<tr>
<td>C22 PUFA</td>
<td>13.9 ± 1.8</td>
<td>6.2 ± 1.6</td>
<td>12.4 ± 4.7</td>
<td>7.3 ± 1.5</td>
<td>7.0 ± 0.3</td>
<td>5.6 ± 0.9</td>
<td>16.7 ± 3.4</td>
<td>25.6 ± 0.6</td>
</tr>
</tbody>
</table>

Fatty Acid Ratios

<table>
<thead>
<tr>
<th>DHA/EPA (n=4)</th>
<th>1.0 ± 0.1</th>
<th>1.4 ± 0.1</th>
<th>0.5 ± 0.2</th>
<th>0.47 ± 0.07</th>
<th>0.38 ± 0.02</th>
<th>0.22 ± 0.04</th>
<th>2.1 ± 0.3</th>
<th>1.8 ± 0.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:1(n-9)/18:1(n-7)</td>
<td>4.0 ± 0.9</td>
<td>16.8 ± 1.0</td>
<td>1.8 ± 0.2</td>
<td>1.1 ± 0.07</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.03</td>
<td>9.3 ± 2.3</td>
<td>2.7 ± 0.04</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>0.78 ± 0.08</td>
<td>1.1 ± 0.1</td>
<td>0.27 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>0.48 ± 0.02</td>
<td>0.72 ± 0.01</td>
<td>0.5 ± 0.3</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>(n-3)(n-6)</td>
<td>15.4 ± 1.3</td>
<td>8.4 ± 2.3</td>
<td>8.8 ± 0.6</td>
<td>15.9 ± 1.5</td>
<td>16.5 ± 0.4</td>
<td>9.0 ± 0.3</td>
<td>5.5 ± 0.6</td>
<td>5.0 ± 0.1</td>
</tr>
</tbody>
</table>

Total Fatty Acid

<table>
<thead>
<tr>
<th>µg/mg dry weight</th>
<th>75.2 ± 9.1</th>
<th>159.4 ± 17.7</th>
<th>107.9 ± 45.6</th>
<th>174.9 ± 28.4</th>
<th>159.4 ± 6.0</th>
<th>142.1 ± 49.6</th>
<th>85.4 ± 25.2</th>
<th>24.9 ± 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/mg individual</td>
<td>n/d</td>
<td>278.1 ± 60.1</td>
<td>n/d</td>
<td>158.6 ± 51.1</td>
<td>630.5 ± 103</td>
<td>71.3 ± 26.5</td>
<td>94.6 ± 62.3</td>
<td>29.3 ± 0.8</td>
</tr>
</tbody>
</table>
Figure 1. Depth profiles of total fatty acid (FA) concentration in zooplankton and >51 μm particles (μg / mg C) at (A) ALOHA and (B) K2, and (μg / m³) at (C) ALOHA and (D) K2. For zooplankton, values are plotted at the median depth of each net sampling interval. For particles, values are plotted at actual depth of sampling of the MULVFS. sw = seawater. Error bars = 1 standard error. n = 4 for both zooplankton and particles.
Figure 1.

(A) ALOHA Total FA (µg/mgC)

(B) K2 Total FA (µg/mgC)

(C) ALOHA Total FA (µg/m^3 sw)

(D) K2 Total FA (µg/m^3 sw)
Figure 2. Herbivory biomarkers vs. depth. Depth profiles of (n-6)/(n-3) polyunsaturated fatty acids (PUFA) herbivory index for (A) bulk zooplankton (error bars = 1 standard error, n = 4), and (B) calanoid copepods at ALOHA and K2 (n = 1). Values are plotted at the median depth of each net sampling interval. For (B), dashed lines indicate carnivorous taxa, solid lines indicate omnivorous taxa. N, Neocalanus. ALOHA = lg. calanoids. K2 = N. plumchrus, N. cristatus, E. bungii. and Paraeuchaeta sp.
Figure 2.

(A) Bulk Zooplankton (n-3)/(n-6) vs. Depth (m)

(B) Calanoid Copepods (n-3)/(n-6) vs. Depth (m)

- N. plumchrus
- N. cristatus
- E. bungii
- ALOHA
- K2
- Paraeuchaeta spp.
- lg. calanoids
Figure 3. Diatom and dinoflagellate biomarkers vs. depth. (A) Bulk zooplankton mean % C20 polyunsaturated fatty acid (PUFA) (diatom biomarker) of total fatty acid, (B) bulk zooplankton 16:1/16:0 ratio (diatom biomarker), (C) bulk zooplankton mean % C22 PUFA (dinoflagellate biomarker) of total fatty acid, and (D) docosahexanoic acid / eicosapentanoic acid (DHA/EPA) ratio (dinoflagellate biomarker). Values are plotted at the median depth of each net sampling interval. Error bars = 1 standard error. n=4
Figure 3.

(A) % C20 PUFA

(B) 16:1/16:0

(C) % C22 PUFA

(D) DHA/EPA
Figure 4. Carnivory/omnivory biomarkers vs. depth. (A) bulk zooplankton, (B) calanoid copepod, and (C) particle mean carnivory/omnivory biomarker % oleic acid 18:1(n-9) of total fatty acid versus depth. Values are plotted at the median depth of each net sampling interval (error bars = 1 standard error. n = 4). For (B), dashed lines indicate carnivorous taxa, solid lines indicate omnivorous taxa. N, Neocalanus. ALOHA = lg. calanoids. K2 = N. plumchrus, N. cristatus, Eucalanus sp. and Paraeuchaeta sp. n = 1.
Figure 4.

(A) Bulk Zooplankton % 18:1(n-9)

(B) Calanoid Copepods % 18:1(n-9)

(C) Particles % 18:1(n-9)

- ALOHA
- K2
- N. plumchrus
- N. cristatus
- E. bungii
- Paraeuchaeta spp.
- lg. calanoids

Depth (m)
Figure 5. Calanoid copepod biomarkers vs. depth. (A) bulk zooplankton, (B) K2 assorted zooplankton, and (C) particle mean % 20:1(n-9)+22:1(n-11) of total fatty acid versus depth. For (A) zooplankton, values are plotted at the median depth of each net sampling interval (error bars = 1 standard error. n = 4.). For (B), dashed lines indicate carnivorous taxa, solid lines indicate omnivorous taxa. N, *Neocalanus*. n=1. For particles (C), values are plotted at actual depth of sampling of the MULVFS. For ALOHA, n=1 to 3, For K2, n=4.
Figure 6. Particle-feeding biomarkers vs. depth. (A) ALOHA and (B) K2 bulk zooplankton and >51 um particle mean % monounsaturated fatty acids (MUFA). White plots, dashed line = particles. Black plots, solid line = bulk zooplankton. Values are % of total fatty acid. Error bars = 1 standard error. For zooplankton, values are plotted at the median depth of each net sampling interval. For particles, values are plotted at actual depth of sampling of the MULVFS. For ALOHA, n=1 to 3, For K2, n=4.
Figure 6.

(A) ALOHA % MUFA

(B) K2 % MUFA

Depth (m)

- ■ Zooplankton
- ▲ Particles

Zooplankton

Particles
Figure 7. Scatter plot of (A) ALOHA and (B) K2 bulk zooplankton vs. >51 um particle fatty acids. PUFA = polyunsaturated fatty acids, MUFA = monounsaturated fatty acids, EFA = essential fatty acids. Pearson’s $r$ correlation coefficient: For ALOHA, PUFA $r = 0.61$, MUFA $r = 0.865$, (n-3)EFA $r = 0.694$. For K2, PUFA $r = 0.763$, MUFA $r = 0.932$, (n-3) EFA $r = 0.757$. $p < 0.05$ for all data shown.
Figure 7.

(A) ALOHA

(B) K2
CHAPTER 3

Autotrophic picoplankton in mesozooplankton guts: Evidence of aggregate feeding in the mesopelagic zone and export of small phytoplankton

S.E. Wilson, D.K. Steinberg

Virginia Institute of Marine Science, College of William and Mary

To be submitted in Marine Biology
Abstract

Zooplankton play a key role in affecting the efficiency by which organic matter is exported to depth. Mesozooplankton consumption of detrital aggregates has been hypothesized as a mechanism to enhance the export of picoplankton from surface layers. We analyzed the guts of mesopelagic zooplankton using light and epifluorescence microscopy to determine if cyanobacteria and eukaryotic phytoplankton too small to be ingested individually were present. Hind-guts were dissected from multiple zooplankton species collected in discrete depth intervals between 0 to 1000 m during day and night at contrasting sites in the subtropical (Hawaii Ocean Time-series site ALOHA) and subarctic (Japanese Time-series site K2) Pacific Ocean. Autofluorescing cyanobacteria and small, eukaryotic phytoplankton were found in the guts of nearly all species sampled from the surface to 1000 m, indicating consumption of aggregates. Foraminiferans, tintinnid lorica, chitin, and gastropod shells were also common in guts of many species and throughout the range of sampling depths. Tintinnid lorica and gastropod shells were higher in abundance in zooplankton guts at K2. At both sites most species' guts contained higher concentrations of cyanobacteria and small phytoplankton at night than during the day. Ostracod guts at ALOHA contained higher densities of picoplankton than at K2, reflecting the predominance of smaller cells at ALOHA. Guts of diel vertical migrators still contained picoplankton at their deep, daytime residence depths, indicating active export of these cells. Our results indicate mesozooplankton grazing on aggregates is a pathway by which flux of picoplankton can be enhanced.
Introduction

The trophic ecology of the mesopelagic zooplankton community plays an important role in affecting the flux of particulate organic matter to the deep sea (Jackson, 1993; Lampitt, 1992; Noji, 1991; Sarnelle, 1999; Steinberg et al., 2000). Zooplankton residing in the mesopelagic zone (base of the euphotic zone to 1000 m) must obtain their nutrition via feeding on sinking aggregates (including fecal pellets and detrital material), vertical migration to the surface, or carnivory. While all of these processes help to determine the efficiency of the biological pump (Angel, 1989; Fowler and Knauer, 1986; Michaels and Silver, 1988; Schnetzer and Steinberg, 2002a; Steinberg et al., 2008b), studies of the feeding ecology of midwater organisms are limited. In particular, feeding on sinking aggregates of detritus ('marine snow') is difficult to quantify, but is assumed to be widespread (Dagg, 1993; Dilling and Brzezinski, 2004; Dilling et al., 1998; Lampitt et al., 1993; Schnetzer and Steinberg, 2002b; Steinberg, 1995).

A better understanding of mesopelagic food webs is thus needed to determine how zooplankton affect particle flux and carbon transport. There are only a handful of studies of the diets of mesopelagic zooplankton, and microscopy has been used to identify phytoplankton tests, crustacean carapaces, and detritus in their guts (e.g., Dagg, 1993; Kosobokova et al., 2002; Nishida et al., 1991; Schnetzer and Steinberg, 2002b; Steinberg, 1995; Uttal and Buck, 1996). Previous studies indicate a large proportion of zooplankton gut content is detrital in origin (Kosobokova et al., 2002; Schnetzer and Steinberg, 2002b; Steinberg, 1995). For example, gut contents of the Antarctic mesopelagic copepod, *Spinocalanus antarcticus*, were almost entirely comprised of
detritus balls (Kosobokova et al., 2002). Mesopelagic copepods associated with larvacean mucous feeding webs ('houses') had fragments of diatoms, microzooplankton, fecal pellets, and pieces of the mucous web in their gut (Steinberg, 1995). Guts of diel vertically migrating zooplankton in the Sargasso Sea near Bermuda also contained items indicative of feeding on marine snow (Schnetzer and Steinberg, 2002b).

Marine snow aggregates contain phytoplankton, bacteria, and cyanobacteria (Alldredge and Silver, 1988; Azam and Long, 2001; Lampitt et al., 1993; Lochte and Turley, 1988; Michaels and Silver, 1988) and concentration of picoplankton in aggregates may be orders of magnitude higher than in surrounding seawater (Azam and Long, 2001; Lampitt et al., 1993; Waite et al., 2000; Zhang et al., 2008). Free-living picoplankton are too small to be individually captured by mesozooplankton (Lampitt et al., 1993; Nival and Nival, 1976; Silver and Bruland, 1981) other than filter and mucous web feeders (e.g., Deibel and Lee, 1992; e.g., Pfankuche and Lochte, 1993). Thus high densities of cyanobacteria such as *Synechococcus* spp. occurring in the gut and fecal pellets of mesozooplankton is a good indicator of detritivory (Lampitt et al., 1993; Nishida et al., 1991; Pfankuche and Lochte, 1993; Schnetzer and Steinberg, 2002b).

Recent studies have suggested that autotrophic picoplankton, through aggregation and subsequent grazing by mesozooplankton, may make a larger contribution to export flux than previously thought (Richardson and Jackson, 2007; Richardson et al., 2004). In the equatorial Pacific, up to 76% of picoplankton net primary production is estimated to contribute to POC flux via mesozooplankton out of the euphotic zone (Richardson et al., 2004). The hypothesized mechanisms for export of these small cells is grazing of aggregates and picoplankton-fed microzooplankton, (Richardson et al., 2004). Support
for these assertions includes the discovery of *Synechococcus*-like cyanobacteria in abundance in bathypelagic sediment traps, fecal pellets, and in abyssal sea-floor sediments (Lampitt *et al.*, 1993; Lochte and Turley, 1988; Pfankuche and Lochte, 1993; Silver *et al.*, 1986).

Furthermore, N$_2$-fixing (diazotrophic) colonial cyanobacteria such as *Trichodesmium* spp., endosymbiotic *Richelia* spp., and newly discovered picoplanktonic nitrogen fixers (3-10 µm, (Montoya *et al.*, 2004; Zehr *et al.*, 2001) have recently been determined to be food sources for zooplankton through δ$^{15}$N measurements (Montoya *et al.*, 2002). The ingestion of diazotrophs by zooplankton is an important but little studied pathway by which fixed nitrogen is incorporated into higher trophic levels (Carpenter and Capone, 2008), and of interest to the present study as a mechanisms by which new nitrogen may be transported to depth.

In this study we investigated the role that mesopelagic zooplankton play in consumption and export of small phytoplankton by quantifying picoplankton (and other visible food items) in the guts of mesozooplankton at depths ranging from the surface to 1000 m. This study was part of a larger project investigating the controls on particle flux through the mesopelagic zone in the subtropical (Hawaii Ocean Time-series site ALOHA) and subarctic (Japanese time-series site K2) North Pacific Ocean (VERtical Transport In the Global Ocean- VERTIGO, Buesseler *et al.*, 2007; Steinberg *et al.*, 2008b). These data not only characterize mesopelagic food webs in two contrasting environments, but allow us to test hypothesized mechanisms for the export of small cells to depth and how picoplankton flux may contribute to the biological pump.
Methods

Sample collection

A 1 m², 335 μm mesh MOCNESS (Multiple Opening/Closing Net and Environmental Sensing System) (Wiebe et al. 1985) and IONESS (Intelligent Operative Net Sampling System) were used to collect zooplankton between 0-1000 m at ALOHA and K2 (Buesseler et al., 2007; Steinberg et al., 2008a). Paired day/night tows were conducted in nine discrete depth intervals (0-50, 50-100, 100-150, 150-200, 200-300, 300-400, 400-500, 500-750, and 750-1000 meters). The samples were collected at the Hawaii Ocean Time series-HOT station ALOHA in the oligotrophic N. Pacific subtropical gyre (27.75°N, 158°W) on June 22-July 9, 2004 aboard the R/V Kilo Moana, and at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) time-series site K2, in a high nutrient, variable chlorophyll region of the N. Pacific subarctic gyre (47°N, 160°E) on July 22-August 11, 2005 aboard the R/V Roger Revelle.

Environmental parameters at the two sites differed including water column-integrated net primary production (ALOHA – 180-220 mgCm⁻²d⁻¹, K2 – 302-603 mgCm⁻²d⁻¹), new production (ALOHA – 18-38 mgCm⁻²d⁻¹, K2 – 70-150 mgCm⁻²d⁻¹) and mixed layer nutrients (ALOHA – nanomolar concentrations, K2 – 12 μM DIN) (Boyd et al., 2008; Buesseler et al., 2008; Elskens et al., 2008; Lamborg et al., 2008; Zhang et al., 2008). The phytoplankton assemblage consisted of small diatoms, dinoflagellates, coccolithophorids, picoplankton and cyanobacteria at ALOHA and picoplankton and large diatoms at K2 (Boyd et al., 2008; Lamborg et al., 2008; Zhang et al., 2008).
A total of four paired day/night MOCNESS/IONESS tows were conducted at each location and one set of paired tows from ALOHA and K2 were split to collect zooplankton for this study. At ALOHA, where net primary production was uniform during our sampling period (Boyd et al., 2008), the third day/night cast (3-4, July 2004) was randomly selected for this study. At K2 net primary production decreased during our sampling period (Boyd et al., 2008; Buesseler et al., 2008) and the first set of day/night tows (31, July 2005 - 1, August 2005), taken before the bloom diminished, was selected to provide maximum contrast between the two sites. Upon net recovery (tows were ~3.25 to 4.5 hr duration), the cod-ends were removed and stored in buckets (1 to ~4 hrs.) until processing. To minimize net/bucket feeding artifacts, animals selected for gut content analysis were large species with long gut passage times (1.5 to >3 hr.; (Dagg and Wyman, 1983; Schnetzer and Steinberg, 2002a and references therein), and their foreguts were removed (see below). Furthermore, many animals from deeper nets were no longer alive by the time of net recovery. Samples from each depth/cod end were divided using a Folsom plankton splitter and splits (1/8 to 1/16 of whole sample) were stored at -80°C until analysis. Remaining sample aliquots were used for analysis of size fractionated biomass, lipid biomarkers, and enumeration of taxa (Kobari et al., 2008; Steinberg et al., 2008a; Wilson et al., in prep).

Sample analysis

Frozen samples were thawed the day of their analysis and immediately sorted for target zooplankton species while kept cold and dark. Several calanoid copepod genera and miscellaneous ostracods were selected from each sample depth at which they were
present at both sites. Target species were selected based on hypothesized diet, vertical migration patterns, and large size. At ALOHA, copepod taxa analyzed were: *Pleuromamma xiphias*, *P. piseki/gracilis* (grouped together), *P. abdominalis*, *Neocalanus robustior*, *Paraeucalanus attenuatus*, *Lucicutia* sp., and miscellaneous large calanoid copepods (>3mm). At K2 target copepod species were: *Neocalanus cristatus*, *N. plumchrus* and *Eucalanus bungii* (which are abundant, ontogenetic vertical migrators (Kobari et al., 2008), *Paraeuchaeta* spp., *Metridia pacifica*, and *Pleuromamma quadrangulata*. Ostracods were not individually identified to species, but both stations contained members of the diverse genera *Conchoecia* and *Porroecia* (Steinberg et al., 2008a). At both sites, usually 10 or more replicates of each taxa per depth interval were examined for visible gut contents, although some depths at both sites contained fewer than 10 individuals of a given taxa (Steinberg et al., 2008a).

Replicates of each species present at each location, depth interval, and time (day and night) were sorted into a multi-welled petri dish placed on ice and filled with filtered seawater. Before dissection, animals were photographed using an Olympus SZX12 stereo dissecting microscope and Olympus DP71 digital camera, and the presence or absence of gut contents was noted. Up to five animals (if available) from each taxa with visible gut contents were then dissected on a microscope slide. Gut contents of *Neocalanus* spp., *E. bungii*, and *Paraeuchaeta* spp. at K2 were only analyzed from day samples; for the remaining target species both day and night samples were analyzed. Upper gut contents were not included in the study due to the possibility of bucket and net feeding artifacts. To obtain the mid- and lower gut, the upper cephalosome of the copepods was removed leaving the rest of the body and the lower 2/3 of the gut. The remaining gut contents were
photographed and measured on the dissection microscope and the volume of gut material was calculated using the formula for the volume of a cylinder for copepods, and an ovoid for ostracods. Samples were smeared on the slide with a probe, and analyzed using a Nikon Labophot-2 epifluorescence microscope under blue (510-560 nm) and green (450-490 nm) excitation filters, and under normal light. Red (Chl a- containing eukaryotic phytoplankton) and yellow (phycoerythrin- and phycocyanin containing cyanobacteria) autofluorescing cells were enumerated under blue or green light. Other recognizable gut contents such as shells, microzooplankton, and crustacean parts were also enumerated.

All samples were photographed with the same digital camera system described above, and ImagePro and Adobe Photoshop software was used to enumerate and measure phytoplankton cells and other items in the guts. Data for cyanobacteria and eukaryotic phytoplankton are reported as the number of cells per cubic millimeter (cells mm$^{-3}$) of gut material. For guts containing high densities or large clusters of cyanobacteria cells (Fig. 1A-B), cell numbers were estimated using the ImagePro “count and measure” function or by counting the cells within a smaller area and extrapolating to total area of gut material. Yellow-fluorescing cyanobacteria cells were categorized as small, 1-5 $\mu$m and large >5 $\mu$m in diameter size classes (although the yellow-orange fluorescing algal cryptophytes are also included in the latter category). The 1-5 $\mu$m cells likely include Synechococcus spp., Richelia spp. (at ALOHA), and small unicellular diazotrophs (Silver pers. comm., Lampitt et al., 1993; Zehr et al., 2007). The >5 $\mu$m category likely includes single cells from Trichodesmium spp. trichomes (at ALOHA), unicellular diazotrophs, and cryptophytes (Silver pers. comm., Zehr et al., 2007). Only intact red fluorescing cells >3 $\mu$m were enumerated. The predominant red cells were pennate and centric diatoms and
dinoflagellates, however amorphous red-pigmented material was common in ostracods and in zooplankton that had consumed foraminiferans (e.g. symbionts that were visible inside mixotrophic foraminifera) and were not included in the red cell counts. Tiny (<1μm) red-fluorescing cells, presumably Prochlorococcus spp. were not enumerated in this study.

Results

Proportion of zooplankton with gut contents

The proportion of individuals with visible gut contents varied by taxa at both ALOHA and K2 (Table 1). At ALOHA, the proportion of zooplankton with guts that contained food ranged from 34% (epipelagic species Euchaeta rimana) to 90% (ostracods) (Table 1). At K2, the proportion of target species with food in their guts ranged from 17% (Neocalanus plumchrus) to 89% (Pleuromamma quadrangulata) (Table 1). Identifiable gut material from zooplankton at both locations included cyanobacteria, eukaryotic phytoplankton (e.g. centric and pennate diatoms, dinoflagellates) foraminiferans, radiolarians, gastropod shells, tintinnid lorica, and crustacean carapace fragments (Fig. 1A-E).

For zooplankton residing primarily in the upper 150 m at ALOHA (N. robustior, Paraecalanus. attenuatus, and E. rimana), the proportion of guts with food was significantly higher at night (mean 45% ± 13% standard error, range 18-100%, 0-150 m) than day (mean 28% ± 11%, range 0-50%) (Mann-Whitney test, p<0.05; Fig. 2A). The
percentage of diel vertically migrating (DVM) copepods at ALOHA (e.g. *P. piseki/gracilis*, *P. abdominalis*, and *P. xiphias*) containing food in their guts was also higher at night while in the epipelagic zone (mean 83% ± 10%, range 56-100%, 0-150 m) than during the day while in the mesopelagic zone (mean 23% ± 8%, range 0-41%, 150-1000 m, Mann-Whitney test, p<0.05; Fig. 2B). Nearly all ostracods (most of which are DVM) examined contained food in their guts, with no significant difference between night and day (Mann-Whitney test, p>0.05; Fig. 2C). *Lucicutia* sp. was only present at ALOHA during the day at 150-200 m and 36% of the individuals analyzed contained food (Fig. 2C). Most of the miscellaneous large calanoid copepods at ALOHA were only present at night in the lower mesopelagic and most of them had food in their guts (Fig. 2C). The proportion of *N. robustior*, *P. attenuatus*, *P. abdominalis*, *P. xiphias*, and misc. ostracods with visible gut content generally decreased with depth, especially at night (Fig. 2A-C).

At K2, ontogenetic vertically migrating (OVM) copepods *N. plumchrus*, *N. cristatus* and *E. bungii* were the dominant mesozooplankton and at the time of our study had begun their descent to enter into their diapause phase below 1000m (Kobari *et al.*, 2008). The proportion of these individuals with food in their guts decreased with depth down to 300 m, and below 300 m these species contained no visible food in their guts (with one exception – a single *N. plumchrus* at 750-1000 m containing food; Fig. 3A). The other taxa analyzed at K2, *M. pacifica* and *P. quadrangulata* (both DVM), ostracods, and *Paraeuchaeta* spp., all had food in their guts at all depths at which they were abundant (Fig. 3B-C). Nearly all of the DVM zooplankton caught while they were feeding in the epipelagic zone at night had food in their guts, however many still
contained food in their guts while at their daytime mesopelagic resident depths as well (Fig. 3B-C).

Cyanobacteria and eukaryotic phytoplankton

At both ALOHA and K2, a large proportion, >80%, of the 1-5 individuals of each target species with gut content at every depth contained 1-5 μm cyanobacteria and >60% included small eukaryotic phytoplankton (Table 1). Considerably more zooplankton at ALOHA contained larger, >5μm cyanobacteria in their guts (82–100%) than at K2 (36% to 86%) (Table 1).

Copepod species E. rimana and N. robustior collected from the epipelagic zone at ALOHA had significantly higher mean concentrations of cyanobacteria in their guts at night (6.8 x 10^5 ± 5.7 x 10^5 and 3.2 x 10^5 ± 3.8 x 10^5 cells/mm^3 respectively) vs. day (3.4 x 10^4 ± 2.6 x 10^4 and 2.6 x 10^4 ± 1.3 x 10^4 cells/mm^3 respectively, Mann-Whitney test, p<0.05; Fig. 4A), and E. rimana also contained higher concentrations of eukaryotic phytoplankton at night above 150m (mean 1.3 x 10^4 ± 7.0 x 10^3 cells/mm^3, Mann-Whitney test, p<0.05; Fig. 5A). Diel vertically migrating P. piseki/gracilis, P. abdominalis, and P. xiphias at ALOHA had significantly higher concentrations of cyanobacteria in their guts at night in the lower epipelagic zone than during the day in the mesopelagic zone (Mann-Whitney test, p<0.05; Fig. 4B). There was one individual P. abdominalis at 300-400 m (night) with high cyanobacterial density as well (Fig. 4B). There was no significant difference between night vs. day for small eukaryotic phytoplankton in DVM species’ guts throughout their depth range, however, maximum values were highest in the mesopelagic during the day and epipelagic at night (Fig 4B).
Cyanobacteria density in guts of misc. ostracods, which included migrating species, was at its maximum in the mesopelagic during the day (mean $1.9 \times 10^5 \pm 1.1 \times 10^5$ cells/mm$^3$) and epipelagic at night (mean $2.1 \times 10^5 \pm 1.1 \times 10^5$ cells/mm$^3$, Fig. 4C). The density of eukaryotic phytoplankton in ostracod guts was variable with depth although generally higher above 300m at night (Fig 5C). The deeper residing species *Lucicutia* sp. was only present during the day above 200m. Densities of cyanobacteria and phytoplankton in the guts of *Lucicutia* sp. were comparable to those of ostracods (Fig. 4C; Fig. 6C).

Miscellaneous large calanoids, generally more carnivorous than the other species (see below), had low densities of cyanobacteria and phytoplankton in their guts (Fig. 4C; Fig. 6C). *P. xiphias* (at 500-750 m) had the highest mean density of eukaryotic phytoplankton of all the zooplankton sampled at ALOHA ($4.4 \times 10^4 \pm 3.5 \times 10^4$ cells mm$^{-3}$) followed by *N. robustior* at 100-150 m and *Lucicutia* sp. at 150-200m (Fig. 5A-C).

At K2, densities of cyanobacteria and eukaryotic phytoplankton in the guts of OVM copepods *Neocalanus* spp. and *E. bungii* (only analyzed during the day) were highest in the upper epipelagic and upper mesopelagic zones, with the exception of a single *N. plumchrus* at 750-1000m that contained high gut densities of cyanobacteria and eukaryotic phytoplankton in its gut (Fig. 6A, Fig. 7A). Gut densities of cyanobacteria and eukaryotic phytoplankton in *Paraeuchaeta* spp. decreased with depth in the mesopelagic zone (Fig. 6A, Fig. 7A). At K2, diel vertically migrating *M. pacifica* had significantly higher concentrations of cyanobacteria in their guts at night in the epipelagic zone ($4.8 \times 10^5 \pm 1.4 \times 10^5$ cells mm$^{-3}$) than during the day at their mesopelagic residence depths ($3.8 \times 10^4 \pm 1.5 \times 10^4$ cells mm$^{-3}$, Mann-Whitney test, p<0.05; Fig. 6B). *M. pacifica* gut eukaryotic phytoplankton density depth maxima was similar to that of the cyanobacteria,
with the exception of a single individual at 500-750m with a high eukaryotic phytoplankton density in its gut (Fig. 7B). The diel vertical migrator *P. quadrangulata* concentrated between 300 and 500 m during the day and was distributed between 50 and 1000 m at night. However, densities of cyanobacteria and eukaryotic phytoplankton in the guts of *P. quadrangulata* were similar during day and night, with no density maximum in the epipelagic zone as seen for *M. Pacifica* (Fig. 6B; Fig. 7B). At K2 the maximum density of cyanobacteria in ostracod guts was above 200 m at night and below 300 m during the day (Fig. 6B), and gut densities of eukaryotic phytoplankton were maximum at 50-100 m at night and 400-500 m during the day (Fig. 7B). *M. pacifica* had the highest day and night gut concentrations of eukaryotic phytoplankton, although All DVM species at K2 had similar concentrations of eukaryotic phytoplankton in their guts throughout their depth of occurrence (Fig. 7B).

Cross comparisons of total gut cyanobacteria and eukaryotic phytoplankton densities for zooplankton collected at ALOHA and K2 resulted in few significant differences between groups (e.g. DVM calanoid copepods, ostracods) and depth ranges (e.g. mesopelagic vs. epipelagic) likely due to the large variation in gut cell densities at both sites. Ostracod day/night combined cyanobacteria and eukaryotic phytoplankton gut densities were both significantly higher at ALOHA ($1.4 \times 10^5 \pm 9.5 \times 10^4$ and $5.1 \times 10^3 \pm 5.7 \times 10^3$ cells/mm$^3$ respectively) than K2 ($1.2 \times 10^5 \pm 1.5 \times 10^5$ and $1.2 \times 10^3 \pm 1.3 \times 10^3$ cells/mm$^3$ respectively) throughout their depth range (Mann-Whitney test, $p<0.05$). Also, there were marginally higher densities of eukaryotic phytoplankton in the guts of zooplankton at mesopelagic depths during the day and all DVM calanoid copepods at ALOHA, than at K2.
Other food items present in guts

All zooplankton gut contents from both K2 and ALOHA had items other than cyanobacteria and eukaryotic phytoplankton, with the majority being unidentifiable detrital material. The most abundant identifiable non-autotrophic items included foraminiferans, tintinnid lorica, pieces of carapace from crustaceans, and gastropod shells (Table 2). Other less frequently observed identifiable items included broken diatom tests, foraminiferan and radiolarian spines, chaetognath spines, whole ostracods, pieces of ostracod carapace, cnidarian nematocysts, crustacean nauplii, and zooplankton fecal pellets.

Foraminiferans were found most often in both ALOHA and K2 zooplankton guts within the epipelagic (both night and day) and upper mesopelagic during the day. All gut material from *P. attenuatus, N. cristatus*, and *P. quadrangulata* collected in the epipelagic zone (either day or night) contained one or more foraminiferans, many of which were still intact (Table 2). Many of the intact foraminiferans contained visible photosynthetic symbionts (Fig. 1C). Tintinnid loricae were found consistently and in nearly all target species at K2, vs. in only a few ostracods and *Pleuromamma* spp. at ALOHA (Table 2). The proportion of zooplankton at ALOHA with crustacean carapace material in their guts was highest in ostracods (nearly all ostracods, all depth zones), *P. xiphias* (500-1000m), and misc. lg. calanoid copepods (500-1000m) (Table 2). At K2, the highest proportion of zooplankton with carapace material in their guts was in ostracods (all depth categories), *P. quadrangulata* (all depth categories), and *Paraeuchaeta* spp. (150-500m and 500-1000m, (Table 2). Gastropod shells were found in the guts of *P. xiphias* and misc. lg. copepods at ALOHA and *P. quadrangulata* at K2 (Table 2).
Discussion

Zooplankton-mediated picoplankton export

Cyanobacteria and small eukaryotic phytoplankton were present in the guts of all species examined. Picoplankton-sized cyanobacteria (e.g. *Synechococcus* sp.) are generally too small to be ingested individually by these large zooplankton and were likely ingested as part of marine snow particles or within the guts of other organisms (Lampitt *et al.*, 1993; Nival and Nival, 1976; Silver and Bruland, 1981). For example, the preferred food particle size for *Neocalanus cristatus* is >20 μm (Liu *et al.*, 2005), considerably larger than the majority of phytoplankton cells we found in *Neocalanus* guts. Water column cyanobacteria cell abundance for *Synechococcus* spp. in the euphotic zone at K2 (0-50m) averaged $1.39 \times 10^4$ cells ml$^{-1}$, and just west of ALOHA in the north Pacific gyre (0-200m) averaged $1.7 \times 10^3$ cells ml$^{-1}$ (Zhang *et al.*, 2008). Within marine snow, the concentrations of picoplankton are considerably higher, and this plus their incorporation in aggregates makes cyanobacteria more accessible to grazers than in the surrounding water column (Lampitt *et al.*, 1993; Silver *et al.*, 1986; Waite *et al.*, 2000). For example, in order for *Pleuromamma* spp. or *Neocalanus* spp. to ingest the mean number of cyanobacteria measured in its gut directly from individual cells in the water column (given the above *Synechococcus* spp. abundance), they would need to filter 1018 ml and 1176 ml of seawater, respectively. For *Neocalanus* spp., with clearance rates of ~17 to ~124 ml copepod$^{-1}$ hr$^{-1}$ [high range(Dagg and Wyman, 1983), at chlorophyll levels comparable to those at K2 (Boyd *et al.*, 2008)], and gut passage times of ~1.8 hours (Dagg and Walser Jr., 1987), obtaining this concentration of gut cyanobacteria via
feeding on free-living cells (which, regardless, are not in the size range preferred by these larger copepods) would be unfeasible.

Ingestion of picoplankton-containing marine snow and its subsequent egestion as fast-sinking fecal pellets will enhance vertical export of small cells from the euphotic zone (Lampitt et al., 1993; Richardson and Jackson, 2007; Silver et al., 1986; Waite et al., 2000). The sedimentation of zooplankton fecal pellets from the upper ocean has been documented to enhance the flux of picoplankton to the deep-sea (Lampitt et al., 1993; Pfankuche and Lochte, 1993; Richardson and Jackson, 2007; Silver et al., 1986; Waite et al., 2000). Also, Lampitt et al. (1993) and (Silver and Alldredge, 1981) speculated that picoplanktonic cyanobacteria may be resistant to digestion by invertebrates, as viable cells are found in fecal pellets and deep-sea sediments.

Three of the target species at K2, *Eucalanus bungii*, *Neocalanus plumchrus*, and *N. cristatus* are the most abundant calanoid copepod species in the subarctic Pacific Ocean (Kobari et al., 2008; Mackas et al., 2007; Steinberg et al., 2008a). At the time of our sampling these copepods were beginning their seasonal ontogenetic vertical migration to their bathypelagic overwintering depths. These copepods are hypothesized to consume eukaryotic phytoplankton, microzooplankton, and cyanobacteria via feeding on marine snow (Dagg, 1993; Kobari et al., 2008). Data from our study supports this assertion, making OVM copepods important contributors to active export of picoplankton. OVM species produced large cylindrical fecal pellets at K2 which made up to ~79% of the intact fecal pellet C flux at 150 m, ~20% at 300 m and ~38 % at 500m (Wilson et al., 2008), and OVM fecal matter-derived flux is hypothesized to account for ~15% of the total K2 sinking C flux at 1000m (Kobari et al., 2003). Only one OVM
individual contained food in its guts below 300 m, confirming that generally these species do not feed when going into diapause (Kobari and Ikeda, 1999). Therefore a portion of the OVM zooplankton pellets produced above 300 m are passively sinking to the lower mesopelagic which are bringing along a considerable number of cyanobacteria. The two target epipelagic/upper mesopelagic species at ALOHA (N. robustior, P. attenuatus) are abundant above 200 m (Landry et al., 2001) and nearly all gut contents of these species contained cyanobacteria. There is little in the literature regarding the feeding ecology of these two species however Sheridan et al. (in press) conducted a Compound-Specific nitrogen Isotope Analysis (CSIA, which estimates trophic positions) in N. robustior and hypothesized that its low trophic position may be reflective of feeding on recycled material (e.g. fecal pellets). This study revealed that densities of cyanobacteria and eukaryotic phytoplankton in the guts of N. robustior and P. attenuatus during daytime were similar in densities to those of the OVM species at K2 above 200 m. Thus there is likely a substantial input of cyanobacteria to depth at ALOHA as well.

Active transport of C via diel vertical migration is a key mechanism by which carbon export to the deep-sea is enhanced (Al-Mutairi and Landry, 2001; Longhurst et al., 1990; Schnetzer and Steinberg, 2002a; Steinberg et al., 2000; Zhang and Dam, 1997), and is a mechanism by which small phytoplankton cells are actively exported out of the euphotic zone (Lampitt et al., 1993; Richardson and Jackson, 2007, this study). The guts of diel vertically migrating copepods (Pleuromamma piseki/gracilis, P. abdominalis and P. xiphias at ALOHA, and P. quadrangulata and M. pacifica at K2) and miscellaneous ostracods contained high concentrations of cyanobacteria and small eukaryotic phytoplankton while feeding at night in the epipelagic, as well as at their mesopelagic
depths during the day. Gut passage times of diel vertically migrating zooplankton are longer than their surface-living counterparts, allowing food consumed in the epipelagic to be egested at depth (e.g., Dagg et al., 2003; Nishida et al., 1991; Schnetzer and Steinberg, 2002a). These “fresh” fecal pellets produced at depth by migrators will be less decomposed compared to pellets produced in the epipelagic zone, and may be a valuable source of nutrition for mesopelagic biota (Schnetzer and Steinberg, 2002b).

The copepod Lucicutia sp. occurred at the base of the epipelagic zone at ALOHA during the day, but at night disappeared from our samples completely, inferring that it may have migrated to depths below 1000m at night – however this assumption is inconclusive. The mesopelagic copepod Lucicutia grandis is not known to vertically migrate and predominately resides in oceanic oxygen minimum zones (Gowing and Wishner, 1998; Koppelmann and Weikert, 2005; Wishner et al., 2008; Wishner et al., 2000). Other species of epipelagic Luiciutia spp. have been known to exhibit an asynchronous vertical migration in which they reside in deep layers during the day and either spread out throughout the water column or remain at depth at night (Castro et al., 2007; Paffenhöfer and Mazzocchi, 2003). L. grandis is however, also known to consume sinking detritus as supported by occurrence of heterotrophic bacteria and cyanobacteria cells in their guts, which is converted into compact sinking fecal pellets, enhancing vertical flux of both bacteria and cyanobacteria to depth (Gowing and Wishner, 1998).

Consumption of diazotrophs and implications for transfer in marine food webs and export of new Nitrogen
There is recent evidence of zooplankton ingestion of N₂ fixing cyanobacteria and the importance of small N₂ fixers to nitrogen budgets in oligotrophic waters (Carpenter and Capone, 2008; Montoya et al., 2002). In this study we have provided further evidence that *Trichodesmium* spp. and picoplanktonic cyanobacteria are being ingested by zooplankton. Cyanobacterial cells in zooplankton guts at K2 and ALOHA varied in size with higher proportions of cells >5 μm found at ALOHA. The higher percentages of >5μm cyanobacterial cells in zooplankton guts at ALOHA are likely individual *Trichodesmium* spp. trichomes and *Richelia* spp. (endosymbiotes in diatoms). In addition, newly discovered small, unicellular diazotrophs may comprise some of the 3-5 μm cells in guts (Silver, pers. com., Montoya et al., 2004; Zehr et al., 2001). Although not likely directly grazing on *Trichodesmium* spp. colonies at ALOHA (i.e. few zooplankton are known to consume *Trichodesmium* spp., which is thought to be noxious or harmful, O'Neil and Roman, 1994). The zooplankton in this study may be consuming trichomes or pieces of *Trichodesmium* spp. within aggregates, as the density of >5 μm cyanobacteria in their guts was relatively low. There was a smaller proportion of >5μm cyanobacteria in the guts of zooplankton at K2 than at ALOHA, presumably due to the comparatively lower abundance of larger colonial cyanobacteria at K2, but also the high density of small (<5 μm) cyanobacteria in zooplankton guts at K2, as the small diazotrophs are now assumed to be broadly distributed across most oceanic environments (Montoya et al., 2004; Montoya et al., 2007; Zehr et al., 2007). The indirect grazing on these diazotrophs by particle feeding zooplankton could be an important pathway by which new nitrogen is exported to depth.
Carnivory/omnivory in mesopelagic zooplankton

There was considerable evidence for carnivorous/omnivorous feeding by copepods and ostracods at both K2 and ALOHA. As previously mentioned, it is possible that some of the cyanobacteria and other small cells ingested originated from inside the guts, or as symbionts, of microzooplankton which were in turn consumed by the larger species investigated in this study. For example, foraminiferan shells with endosymbiotic eukaryotes within and dense clusters of cyanobacteria, which may have originated from microzooplankton guts or fecal pellets, were found in many zooplankton guts at both stations (e.g. Figure 1A-C). Many K2 zooplankton guts contained numerous protozoan tintinnid lorica, which may have been consumed on marine snow. Ciliated protozoans such as tintinnids and oligotrichs are known to colonize marine snow particles and “ride” within them to depth (Silver et al., 1984). Lampitt et al (1993) considered the presence of tintinnid lorica along with cyanobacteria in fecal pellets of the amphipod *Themisto compressa* as a signature of marine snow consumption. Radiolarian spines were also observed in the guts of zooplankton at both stations. Steinberg et al. (2008) found high densities of the particle feeding phaeodarian radiolarians throughout the mesopelagic at K2, which are known to feed on detritus (Gowing and Coale, 1989; Gowing and Wishner, 1986).

Ostracods in our study appear to be opportunistic feeders, consuming phytoplankton, detritus, or other animals, as have been found in previous studies (Angel, 1970; Lampitt et al., 1993; Lochhead, 1968; Vannier et al., 1998). Gut contents of the mesopelagic ostracod *Conchoecia pseudodiscophora* from the Japan Sea contained detritus, phytoplankton, microzooplankton, and crustacean appendages (Ikeda, 1990).
Ikeda (1990) hypothesized that *C. pseudodiscophora* ate mostly dead or nearly dead material because they did not feed much on active *Artemia salina* in culture. It is thus possible that much of the carapace material in the guts of mesopelagic ostracods at K2 and ALOHA is a result of consumption of sinking carcasses and detritus.

In this study, only a few ostracods and large calanoid copepods had gut content consisting solely of animal material (e.g. crustacean carapace fragments). However, at K2 many guts of zooplankton that were empty of recognizable food particles contained red-pigmented oil, evidence of consumption of oil-rich subarctic copepods (Kobari and Ikeda, 1999, 2001). Oil droplets were found in the guts of a deep water copepod, *Cephalophanes* spp, which feeds on sinking crustacean carcasses, but may have also come from its own intra-cellular oil droplets (Nishida et al., 2002). Sheridan et al. (in press) determined that *E. rimana* at ALOHA was carnivorous using CSIA-based estimates of trophic position. The presence of crustacean carapace, tintinnid lorica, and foraminiferans in the guts of *E. rimana* in this study concurs with their results (however small cyanobacteria were also present in *E. rimana* in the present study).

Evidence of increasing carnivory with depth was apparent in this and other VERTIGO studies (e.g., Steinberg et al., 2008a; Wilson et al., 2008; Wilson et al. in prep). In this study, large misc. calanoid copepods at ALOHA were present at night below 400m with guts containing whole ostracods as well as carapace fragments. At K2, *Paraeuchaeta* spp. also had guts containing crustacean carapace at mesopelagic depths. The percentage of ostracod guts containing crustacean carapace also generally increased with depth at both ALOHA and K2. Wilson et al. (2008) determined there was an increase in the presence of carnivores in the mesopelagic with the emergence of “red”
fecal pellets produced by carnivores. An increase in the monounsaturated fatty acid oleic acid, with depth at both K2 and ALOHA is also linked to an increase in the importance of carnivory or more likely, omnivory through the mesopelagic zone (Wilson et al. in prep).

**Summary and Conclusion**

This study provides further evidence that mesozooplankton grazing on aggregates is a pathway by which flux of picoplankton can be enhanced. Picoplanktonic cyanobacteria were present in nearly all gut contents analyzed indicating that consumption of detritus is an important means by which both epipelagic and mesopelagic zooplankton at K2 and ALOHA obtain their nutrition, and that subsequent egestion of this material as fecal pellets enhances passive vertical export of small cells (some of which are likely diazotrophs). Diel or ontogenetic vertically migrating zooplankton are also important contributors to the active flux of picoplankton out of the epipelagic at K2 and ALOHA. Microzooplankton and other animal material were also common in zooplankton guts, evidence of the importance of carnivory for zooplankton nutrition in the mesopelagic zone. While ALOHA and K2 are contrasting environments with different phytoplankton and zooplankton community structure, mesopelagic zooplankton at both sites increase the transfer efficiency of picoplankton carbon to the deep sea.


Nival, P., Nival, S., 1976. Particle retention efficiencies of an herbivorous copepod, 
Acartia clausi (adult and copepodite stages): Effects on grazing. Limnology and 
Oceanography 21 (1), 24-38.

Noji, T.T., 1991. The influence of macrozooplankton on vertical particle flux. Sarsia 76, 
1-9.

by pelagic harpacticoid copepods, Macrosetella, Miracia and Oculosetella. 
Hydrobiologia 292/293, 235-240.


Pfankuche, O., Lochte, K., 1993. Open ocean pelago-benthic coupling: cyanobacteria as 

Richardson, T.L., Jackson, G.A., 2007. Small phytoplankton and carbon export from the 

through food webs of the eastern equatorial Pacific: an inverse approach. Deep-Sea 
Research I 51, 1245-1274.

Sarnelle, O., 1999. Zooplankton effects on vertical particulate flux: Testable models and 
experimental results. Limnology and Oceanography 44 (2), 357-370.


Schnetzer, A., Steinberg, D.K., 2002b. Natural diets of vertically migrating zooplankton 


Silver, M.W., Bruland, K.W., 1981. Differential feeding and fecal pellet composition of 
salps and pteropods, and the possible origin of the deep-water flora and olive-green 


Table 1: Target zooplankton species at K2 and ALOHA collected from 0–1000 m. Data are presented for percentage (%) of the total number of species from all depths containing: any food in their guts, cyanobacteria (cyano) in two different size classes, and eukaryotic phytoplankton (phyto) > 2μm. Values for carapace length (l) are mean ± standard error and are from tip of rostrum to the end of metasome (prosome length) for copepods and dorsal valve length for ostracods. For ALOHA, n=11 to 137 total individuals examined for visible gut contents, and n=8 to 67 for guts that contained material. For K2, n=31 to 146 individuals examined for visible gut contents, and n=7 to 61 for guts that contained material.

<table>
<thead>
<tr>
<th>ALOHA Target Groups</th>
<th>carapace l (mm)</th>
<th>% w/ food</th>
<th>% w/ cyano (1-5μm)</th>
<th>% w/ cyano (&gt;5μm)</th>
<th>% w/ phyto (&gt;2μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euchaeta rimana</td>
<td>2.0 ± 0.3</td>
<td>34.3</td>
<td>86.4</td>
<td>81.8</td>
<td>77.3</td>
</tr>
<tr>
<td>Lucicutia sp.</td>
<td>0.9 ± 0.0</td>
<td>36.4</td>
<td>50.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Neocalanus robustior</td>
<td>2.2 ± 0.2</td>
<td>37.1</td>
<td>83.3</td>
<td>88.9</td>
<td>66.7</td>
</tr>
<tr>
<td>Paraueucalanus attenuatus</td>
<td>2.8 ± 0.4</td>
<td>38.5</td>
<td>92.9</td>
<td>92.9</td>
<td>71.4</td>
</tr>
<tr>
<td>Pleuromamma abdominalis</td>
<td>2.2 ± 0.1</td>
<td>50.0</td>
<td>100.0</td>
<td>92.9</td>
<td>85.7</td>
</tr>
<tr>
<td>Pleuromamma piseki/gracilis</td>
<td>1.3 ± 0.1</td>
<td>39.3</td>
<td>100.0</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>Pleuromamma xiphias</td>
<td>2.5 ± 0.4</td>
<td>79.5</td>
<td>100.0</td>
<td>95.5</td>
<td>81.8</td>
</tr>
<tr>
<td>misc. lg. calanoids</td>
<td>3.1 ± 0.8</td>
<td>81.8</td>
<td>88.9</td>
<td>100.0</td>
<td>88.9</td>
</tr>
<tr>
<td>misc. ostracods</td>
<td>1.2 ± 0.2</td>
<td>90.5</td>
<td>98.5</td>
<td>87.9</td>
<td>83.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>K2 Target Groups</th>
<th>carapace l (mm)</th>
<th>% w/ food</th>
<th>% w/ cyano (1-5μm)</th>
<th>% w/ cyano (&gt;5μm)</th>
<th>% w/ phyto (&gt;2μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalanus bungii</td>
<td>5.1 ± 0.7</td>
<td>33.04</td>
<td>100.0</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Metridia pacifica</td>
<td>2.5 ± 0.2</td>
<td>56.96</td>
<td>100.0</td>
<td>60.0</td>
<td>83.3</td>
</tr>
<tr>
<td>Neocalanus cristatus</td>
<td>7.2 ± 0.2</td>
<td>47.29</td>
<td>100.0</td>
<td>85.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Neocalanus plumchrus</td>
<td>4.1 ± 0.2</td>
<td>17.04</td>
<td>100.0</td>
<td>70.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Paraueuchaeta spp.</td>
<td>3.7 ± 0.7</td>
<td>50.53</td>
<td>92.9</td>
<td>35.7</td>
<td>42.9</td>
</tr>
<tr>
<td>Pleuromamma quadrangulata</td>
<td>2.6 ± 0.1</td>
<td>88.89</td>
<td>100.0</td>
<td>70.7</td>
<td>78.0</td>
</tr>
<tr>
<td>misc. ostracods</td>
<td>1.2 ± 0.1</td>
<td>75.38</td>
<td>98.4</td>
<td>49.2</td>
<td>60.7</td>
</tr>
</tbody>
</table>

148
Table 2: Percentage of zooplankton gut contents containing other non-phytoplankton material (foraminifera, tintinnid lorica, crustacean carapace material, and gastropod shells) for target species at ALOHA and K2. Data are pooled and presented for the epipelagic (0 – 150m), upper mesopelagic (150 – 500m), and lower mesopelagic (500 – 1000m) zones. 0 = animals with food in guts, but zero of the listed items were present. - = animals were present at that depth interval but had empty guts. Blank = no animals present at that depth interval. n=1-19, * indicates n < 3.
Table 2.

ALOHA

<table>
<thead>
<tr>
<th></th>
<th>E. rimana</th>
<th>Lucicutia sp.</th>
<th>N. robustior</th>
<th>misc. ostracods</th>
<th>P. attenuatus</th>
<th>P. abdominalis</th>
<th>P. piseki/graclis</th>
<th>P. xiphias</th>
<th>Ig. copepods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day</td>
<td>night</td>
<td>day</td>
<td>night</td>
<td>day</td>
<td>night</td>
<td>day</td>
<td>night</td>
<td>day</td>
</tr>
<tr>
<td>0-150m</td>
<td>0</td>
<td>15</td>
<td>38</td>
<td>30</td>
<td>0</td>
<td>13</td>
<td>100</td>
<td>22</td>
<td>33</td>
</tr>
<tr>
<td>forams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tintinnids</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>44</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>chitin</td>
<td>50</td>
<td>38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>shells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>150-500m</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>6</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>forams</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tintinnids</td>
<td>0*</td>
<td>0*</td>
<td>5</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>chitin</td>
<td>100*</td>
<td>75</td>
<td>89</td>
<td>78</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>shells</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>500-1000m</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>forams</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0*</td>
</tr>
<tr>
<td>tintinnids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0*</td>
<td>-</td>
<td>0*</td>
</tr>
<tr>
<td>chitin</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>33</td>
<td>-</td>
<td>-</td>
<td>100*</td>
<td>0*</td>
<td>100*</td>
</tr>
<tr>
<td>shells</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0*</td>
<td>-</td>
<td>0*</td>
</tr>
</tbody>
</table>

K2

<table>
<thead>
<tr>
<th></th>
<th>E. bungii</th>
<th>M. pacifica</th>
<th>misc. ostracods</th>
<th>N. cristanus</th>
<th>N. plumichus</th>
<th>Peraeochaeta</th>
<th>P. quadrangulata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day</td>
<td>night</td>
<td>day</td>
<td>night</td>
<td>day</td>
<td>night</td>
<td>day</td>
</tr>
<tr>
<td>0-150m</td>
<td>38</td>
<td>-</td>
<td>30</td>
<td>10</td>
<td>100</td>
<td>n/d</td>
<td>14</td>
</tr>
<tr>
<td>forams</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tintinnids</td>
<td>25</td>
<td>-</td>
<td>70</td>
<td>10</td>
<td>100</td>
<td>n/d</td>
<td>14</td>
</tr>
<tr>
<td>chitin</td>
<td>25</td>
<td>-</td>
<td>0</td>
<td>100</td>
<td>20</td>
<td>n/d</td>
<td>14</td>
</tr>
<tr>
<td>shells</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>n/d</td>
<td>n/d</td>
<td></td>
</tr>
<tr>
<td>150-500m</td>
<td>-</td>
<td>n/d</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>forams</td>
<td>-</td>
<td>n/d</td>
<td>54</td>
<td>50</td>
<td>26</td>
<td>0</td>
<td>0*</td>
</tr>
<tr>
<td>tintinnids</td>
<td>-</td>
<td>n/d</td>
<td>46</td>
<td>33</td>
<td>100</td>
<td>95</td>
<td>50</td>
</tr>
<tr>
<td>chitin</td>
<td>-</td>
<td>n/d</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>shells</td>
<td>-</td>
<td>n/d</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>500-1000m</td>
<td>-</td>
<td>n/d</td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>100*</td>
</tr>
<tr>
<td>forams</td>
<td>-</td>
<td>n/d</td>
<td>-</td>
<td>-</td>
<td>100*</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>tintinnids</td>
<td>-</td>
<td>n/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100*</td>
<td>-</td>
</tr>
<tr>
<td>chitin</td>
<td>-</td>
<td>n/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>shells</td>
<td>-</td>
<td>n/d</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

n/d - not determined
Figure 1: Zooplankton gut contents as viewed under epifluorescence microscopy. A, Individual cyanobacteria (cy), cyanobacterial clusters (cc), pennate diatoms (p) and dinoflagellate (di) in ostracod gut from 150-200 m at ALOHA. B, high density of cyanobacteria in *Metridia pacifica* gut from 0-50 m at K2. C, foraminiferan (f) with red autofluorescing symbionts in *Pleuromamma quadrangulata* gut from 300-400 m at K2. D, presumed individual cells from *Trichodesmium* spp. trichome (t) in miscellaneous calanoid copepod gut from 400-500 m at ALOHA. E, tintinnid lorica (lo) and crustacean carapace fragments (cf) in *P. quadrangulata* gut from 150-200 m at K2. (scale bars = 20 μm)
Figure 1.
Figure 2: Zooplankton at each depth interval with food in their gut at ALOHA. (A) *Neocalanus robustior*, *Paraeucalanus attenuatus*, and *Euchaeta rimana*. (B) *Pleuromamma piskei/gracilis*, *Pleuromamma abdominalis*, and *Pleuromamma xiphias*. (C) *Lucicutia* sp., miscellaneous (misc.) ostracods, and misc. large copepods. Open bars = total number of each species analyzed. Filled bars = number of each species analyzed containing food in gut.
Figure 3: Zooplankton at each depth interval with food in their gut at K2. (A) *Eucalanus bungii*, *Neocalanus cristatus*, and *Neocalanus plumchrus*. (B) *Metridia pacifica* and *Pleuromamma quadrangulata*. (C) miscellaneous (misc.) ostracods, and *Paraeuchaeta* spp. Open bars = total number of each species analyzed. Filled bars = number of each species analyzed containing food in gut.
Figure 3.

(A) K2 zooplankton with food in gut (g)  
Day  Night  20  15  10  5  0  5  10  15  20  25  30
0-50  50-100  100-150  150-200  200-300  300-400  400-500  500-750  750-1000
Depth (m)

(B) K2 zooplankton with food in gut (g)  
Day  Night  20  15  10  5  0  5  10  15  20  25  30
0-50  50-100  100-150  150-200  200-300  300-400  400-500  500-750  750-1000
Depth (m)

(C) K2 zooplankton with food in gut (g)  
Day  Night  15  10  5  0  5  10  15
0-50  50-100  100-150  150-200  200-300  300-400  400-500  500-750  750-1000
Depth (m)

- E. bungii
- M. edulis
- M. plumchrus
- M. pacifica
- P. quadrangula
- P. antarctica
- P. antarctica
- P. antarctica
- P. antarctica
- P. antarctica
- P. antarctica
- P. antarctica
- P. antarctica
- P. antarctica
Figure 4: Cyanobacteria density in zooplankton guts in each depth interval at ALOHA (x 10^3 cells mm^-3). Cyanobacteria density includes both 1-5 μm cells and >5 μm cells. (A) *Neocalanus robustior, Paraeucalanus attenuatus, and Euchaeta rimana*. (B) *Pleuromamma piskei/gracilis, Pleuromamma abdominalis, and Pleuromamma xiphias*. (C) *Lucicutia* sp., miscellaneous (misc.) ostracods, and misc. large copepods. Values are mean ± 1 standard error. of n=1-5 guts per species per depth.
Figure 4.

(A) ALOHA gut total cyanobacteria density (x10^9 cells mm^-3)

Day Night

0-50 50-100 100-150 150-200 200-300 300-400 400-500 500-750 750-1000

Depth (m)

- R. rubescens
- P. attenuata
- E. reniforme

(B) ALOHA gut total cyanobacteria density (x10^9 cells mm^-3)

Day Night

500 400 300 200 100 0 100 200 300 400 500 900

Depth (m)

- P. pisekigracila
- P. stolomnells
- P. uphiales

(C) ALOHA gut total cyanobacteria density (x10^9 cells mm^-3)

Day Night

500 400 300 200 100 0 100 200 300 400 500

Depth (m)

- Lucicola sp.
- misc. cianobacteria
- misc. large cianobacteria

158
Figure 5: Eukaryotic phytoplankton density in zooplankton guts in each depth interval at ALOHA (x $10^3$ cells mm$^{-3}$). (A) *Neocalanus robustior*, *Paraeucalanus attenuatus*, and *Euchaeta rimana*. (B) *Pleuromamma piskei/gracilis*, *Pleuromamma abdominalis*, and *Pleuromamma xiphias*. (C) *Lucicutia* sp., miscellaneous (misc.) ostracods, and misc. large copepods. Values are mean ± 1 standard error of n=1-5 guts per species per depth.
Figure 5.
Figure 6: Cyanobacterial density in zooplankton guts in each depth interval at K2 (x 10^3 cells mm^3). Cyanobacteria density includes both 1-5μm cells and >5 μm cells. (A) *Eucalanus bungii, Neocalanus cristatus, Neocalanus plumchrus* and *Paraeuchaeta spp.* (B) *Metridia pacifica, Pleuromamma quadrangulata, and miscellaneous (misc.)* ostracods. Values are mean ± 1 standard error. of n=1-5 guts per species per depth. No data (N/D) for (A) night.
Figure 6.

(A) K2 gut total cyanobacteria density (x10^9 cells.mm^-3)

(B) K2 gut total cyanobacteria density (x10^9 cells.mm^-3)
Figure 7: Eukaryotic phytoplankton density in zooplankton guts in each depth interval at K2 (x 10^3 cells mm^-3). (A) *Eucalanus bungii*, *Neocalanus cristatus*, *Neocalanus plumchrus* and *Paraeuchaeta* spp. (B) *Metridia pacifica*, *Pleuromamma quadrangulata*, and miscellaneous (misc.) ostracods. Values are mean ± 1 standard error of n=1-5 guts per species per depth. No data (N/D) for (A) night.
Figure 7.

(A) K2 gut eukaryotic phytoplankton density (x10^6 cells mm^{-3})

Day Night Day Night
-10 -8 -6 -4 -2 0 2 4 6 8

-10 -8 -6 -4 -2 0 2 4 6 8

Depth (m)

0-50
50-100
100-150
150-200
200-300
300-400
400-500
500-750
750-1000

N/D

Species:
- E. bangi
- K. creatius
- K. plumchrus
- Paraeuchaeta spp

(B) K2 gut eukaryotic phytoplankton density (x10^6 cells mm^{-3})

Day Night Day Night
-10 -8 -6 -4 -2 0 2 4 6 8 54

-10 -8 -6 -4 -2 0 2 4 6 8 54

Depth (m)

0-50
50-100
100-150
150-200
200-300
300-400
400-500
500-750
750-1000

Species:
- K. pecifica
- P. quadrangulae
- misc. ostracods
CONCLUSION AND FUTURE DIRECTION

The VERTIGO study provided a multidisciplinary approach to understanding the efficiency of the flux of particulate organic carbon (POC) through the mesopelagic and into the deep sea, and how POC transport efficiency varies between contrasting environments in a changing ocean (Buesseler et al., 2008 and references therein). My study provided significant evidence that mesopelagic zooplankton play a key role in affecting POC export, and that elucidating trophic transfers through the mesopelagic food web is important for understanding POC export and carbon sequestration in the deep ocean (Fig. 1). It is also one of few studies that combine analyses of both zooplankton and particles (from sediment traps and large volume filtration systems) to enhance our understanding of zooplankton food webs in the mesopelagic zone.

I found through the examination of zooplankton fecal pellets in sediment traps within the upper mesopelagic zone evidence at both sites for repackaging of detritus and carnivory at depth. The appearance of new fecal pellet classes at depth such as from filter-feeding larvaceans at both sites is indicative of an increase in importance of mesopelagic particle feeding. The emergence of red and transparent pellets with depth indicates carnivores also play a role in affecting particle flux. Zooplankton body size and diet was a key factor influencing both the magnitude and character of sinking POC. In more mesotrophic regions such as K2, the larger size and biomass of zooplankton and
their fecal material promotes increased transport efficiency of POC to depth. Recycling of fecal pellets by small zooplankton in oligotrophic regions such as ALOHA may affect POC export to depth, as evidenced by increases in broken pellets with depth at ALOHA. Thus variations in mesopelagic zooplankton community structure, size, and trophic ecology can differentially alter the characteristics and transfer efficiency of sinking POC.

There were also marked differences in zooplankton feeding ecology with depth and between sites as indicated by fatty acid biomarkers for food sources for mesopelagic zooplankton. The seasonality of the subarctic Pacific makes it necessary for zooplankton at K2 to store lipid reserves in their large bodies for overwintering and diapause (Kobari et al., 2008), and as a result K2 zooplankton had considerably higher total lipid concentration than ALOHA zooplankton. Fatty acid biomarkers indicated that zooplankton at K2 were generally feeding on diatom or diatom-derived aggregates, vs. dinoflagellate-derived food at ALOHA both of which likely providing a source of essential fatty acids for even deeper food webs. At ALOHA, where primary production was lower and particulate organic carbon attenuated more rapidly than at K2 (Buesseler et al., 2007), biomarkers for carnivory were more prominent in zooplankton. An increase in carnivory-derived fatty acids with depth may help compensate mesopelagic zooplankton at ALOHA for the lower export efficiency of POC at ALOHA vs. K2. These results further demonstrate how changing feeding ecology of zooplankton from the surface through the mesopelagic zone has implications for the quality of organic material to the deep sea.

Recent studies have asserted that photosynthetic picoplankton (e.g., cyanobacteria, small eukaryotic phytoplankton) contribute more substantially to export of
POC than previously thought, and that grazing by mesozooplankton is likely an important pathway by which picoplankton are exported to depth (Montoya et al., 2004; Richardson and Jackson, 2007; Zehr et al., 2001). At both K2 and ALOHA, picoplanktonic cyanobacteria were present in guts of nearly all zooplankton analyzed, indicating that consumption of detritus is an important means by which both epipelagic and mesopelagic zooplankton obtain their nutrition, and that subsequent egestion of this material as fecal pellets enhances passive vertical export of small cells (some of which are likely diazotrophs). Diel or ontogenetic vertically migrating zooplankton are also important contributors to the active flux of picoplankton out of the epipelagic at K2 and ALOHA. Microzooplankton and other animal material were also common in zooplankton guts, providing evidence for the importance of carnivory as a source of zooplankton nutrition in the mesopelagic zone. While ALOHA and K2 are contrasting environments with different phytoplankton and zooplankton community structure, mesopelagic zooplankton at both sites enhance the export of picoplankton carbon to the deep sea.

As the ocean surface continues to warm, plankton biomass and community structure will be affected (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 a better understanding of how predicted changes in plankton community will affect the flux of carbon to the deep ocean. Future studies on how changes occurring in the ocean surface will affect processes in the mesopelagic zone will be essential to accomplish this, which may require long-term studies of the mesopelagic zone at biogeochemical time-series sites such as K2 or ALOHA, or the Bermuda Atlantic Time-series Study (BATS) site. There is a documented long-term increase in epipelagic
zooplankton biomass at both ALOHA and BATS (Sheridan and Landry 2004, Steinberg et al. 2008), but nothing is known of changes in the zooplankton community that are most certainly occurring deeper as well. In order to predict how C cycling in the mesopelagic zone may be changing over time, biogeochemical studies will need to move towards longer-term studies of the deep ocean.

The VERTIGO data presented in this dissertation as well as additional data not presented for chapters 1 and 2 are available online at the Ocean Carbon and Biogeochemistry Data Management Office website at:

http://ocb.whoi.edu/vertigo.html
Literature Cited


Figure 1: Conceptual diagram of the biological pump and the role of zooplankton in affecting transport of particulate organic matter (POM) to depth (modified from Buesseler et al. 2007), as investigated in this dissertation. Sinking POM can include fecal pellets and aggregates of detritus (A). These sinking particles can change in shape, color, size, and fatty acid composition with depth as material is repackaged by zooplankton that also vary in their community composition with depth. Zooplankton gut material contains picoplankton such as cyanobacteria (B) which is acquired via feeding on sinking POM, which enhances picoplankton export via egestion of sinking fecal pellets. Carnivory (C) is also an important mesopelagic feeding mode, as indicated by the presence of red fecal pellets, crustacean carapace material in gut contents, and an increase in carnivory fatty acid biomarkers in zooplankton tissue with depth.
Figure 1.
VITA

Stephanie Erin Wilson

Born in Los Angeles, California, on July 27, 1974. Graduated from North Hollywood High School in 1992. Attended the University of New South Wales in Sydney, Australia as an exchange student in 1995. Received a bachelor’s degree from the University of California at Santa Cruz in 1996. Earned a Master’s of Science in Marine Environmental Science at the State University of New York, Stony Brook in 2001. Thesis investigated the escape responses of three calanoid copepod species from a juvenile fish predator. Advisor: Dr. Jeannette Yen. Employed as a laboratory research assistant for Dr. Deborah Steinberg from 2001 to 2003. Worked as a contract zooplankton ecologist on the United States Antarctic Marine Living Resources program in the Antarctic peninsula in 2003 and 2005. Entered the Ph.D. program at the Virginia Institute of Marine Science, College of William and Mary in 2003 under graduate advisor Dr. Deborah Steinberg.