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The missing piece of the upper mesopelagic carbon budget? Biomass, vertical distribution and feeding of aggregate-associated copepods at the PAP site

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Key words: Marine snow, copepod, *Microsetella norvegica*, *Oncaea* spp., feeding, carbon demand

Abstract: Although zooplankton are recognized as important consumers of marine snow, our knowledge of the contribution of the small (< 1 mm) zooplankton to the degradation of sinking particles, and therefore on the efficiency of the biological pump, is limited. To estimate the marine snow consumption by small aggregate-feeding copepods, we measured the daily changes in biomass and vertical distribution of the harpacticoid *Microsetella norvegica* and the poecilostomatoid *Oncaea* spp. over a period of 10 days and combined these with estimates of their feeding and respiration rates. We estimated copepod feeding rates in multiple ways, using: gut chlorophyll content, maximum ingestion rates from functional responses on two types of aggregates, carbon demand based on the respiration measurements, and egg production rates. *Microsetella norvegica* and *Oncaea* spp. biomass levels varied > 2 -fold between sampling dates for geographically-close sampling stations. *Microsetella norvegica* resided mainly in the surface layer, at or immediately below the fluorescence peak, while *Oncaea* spp. was typically deeper. *Microsetella norvegica* fed actively on *Trichodesmium* filaments, with a maximum ingestion rate of ca. $0.11 \mu\text{g C ind.}^{-1} \text{d}^{-1}$, while their ingestion of detritus aggregates was low. Feeding on *Trichodesmium* was reflected in their gut chl-*a* content, which was high for *M. norvegica* at all but one sampling time. In contrast, *Oncaea* spp. had significantly lower gut chl-*a* content and may have been feeding on other types of aggregates. Respiration of both copepods was variable and as much as $0.08 \mu\text{l ind.}^{-1} \text{h}^{-1}$ for *M. norvegica* and $0.04 \mu\text{l ind.}^{-1} \text{h}^{-1}$ for *Oncaea* spp. Based on individual biomass, vertical distribution, and carbon demand, aggregate-associated copepods could degrade up to $79 \pm 33 \text{ mg C m}^{-2} \text{d}^{-1}$, a value similar to the surplus in the epipelagic carbon budget of the area. This large degradation rate demonstrates that zooplankton < 1 mm can have a large influence on the vertical flux and that the factors controlling their abundances and feeding rates should be high in the priority list of future zooplankton studies.

1. Introduction

Understanding the processes influencing the carbon sequestration in the ocean has been a major focus of biological and chemical studies during the last half century (Alldredge & Silver 1988, Longhurst & Harrison 1989, De La Rocha & Passow 2007, Buesseler et al. 2007). The biological carbon pump plays a large role in carbon sequestration, and the processes influencing its efficiency – particle production, sinking and degradation – need to be understood and accurately modelled, if we are to predict the carbon sequestration in the future ocean (Passow & Carlson 2012). While particle production, sinking, bacterial degradation and zooplankton fragmentation of particles have received much attention in past (Alldredge & Silver 1988, Ploug et al. 2008, Steinberg et al. 2008, Buesseler & Boyd 2009), degradation by small copepods has been rarely included in carbon flux studies, partly because small copepods are sampled poorly with traditional plankton nets of 200 μm (Turner 2004). Nevertheless, these copepods can potentially have a large effect on flux attenuation (Koski et al. 2005). Studying their effect on carbon flux at the Porcupine Abyssal Plain (PAP) - an area with a long history of observing carbon fluxes (Lampitt et al. 2010a, b) – provides a way to incorporate the effect of these understudied copepods on the flux attenuation, and thereby on the efficiency of the biological carbon pump.

During the last decades, research on the ocean's biological carbon pump has focused on particle production (Alldredge & Silver 1988, Jackson 1990, Kiørboe 1990), sinking (Shanks & Trent 1980, Iversen & Ploug 2010) and degradation rates (Simon et al. 1990, Kiørboe 2000, McDonnell et al. 2015). These and other studies have demonstrated the importance of coagulation (Jackson 1990, Kiørboe 1990), production of fecal pellets and feeding nets (Hansen et al. 1996, Wexels Riser et al. 2002, Wilson et al. 2013), active carbon transport by vertically-

migrating copepods (Steinberg et al. 2000) and the effect of particle density (Iversen & Ploug 2010) in enhancing the vertical flux. The studies that have concentrated on flux attenuation below the euphotic layer have investigated bacterial remineralization and solubilization (Herndl & Reinthaler 2013, McDonnell et al. 2015) and particle fragmentation by zooplankton (Dilling & Alldredge 2000). The conclusion has been that bacteria and zooplankton contribute approximately equally to the flux attenuation with depth (Steinberg et al. 2008). The export flux in the depth range of 50 to 100 m typically varies between 64 and 207 mg C m⁻² d⁻¹ (Lampitt et al. 2008, Thomalla et al. 2008, Riley et al. 2012), making up ca. 15% of the net primary production (Frigstad et al. 2015). However, only 0.6-4% of the primary production reaches the deep sea (Lampitt et al. 2010b, Frigstad et al. 2015). This semi-labile or refractory carbon forms a relatively stable food source for the benthic community that at the PAP site consists mainly of bacteria and suspension and deposit feeding megafauna (Durden et al. 2017).

Vertical flux and its attenuation have been intensively studied at the PAP (Lampitt et al. 2010a, b), and many of the new insights result from these studies. For instance, the attenuation coefficient, $-b$, of Martin et al. (1987) has been estimated in several studies at the PAP, yielding values ranging from 0.5 to 0.7 (Marsay et al. 2015, Belcher et al. 2016, Cavan et al. 2017), and implying a high flux attenuation in the area (Frigstad et al. 2015). The focus of many of these studies has been to balance the carbon budget of the epipelagic and mesopelagic zones (Burd et al. 2010, Giering et al. 2014), which typically show an oversupply of carbon at the surface layer, but undersupply in the mesopelagic (Giering et al. 2014, Belcher et al. 2016). Explanations for the mismatch in the carbon budget include the effect of temperature (Marsay et al. 2015), difficulties in connecting the source and sink (Belcher et al. 2016, Frigstad et al. 2015), solubilization, transformation of the large particles into smaller ones (Giering et al. 2014,

Belcher et al. 2016) and too-coarse resolution of the measurements immediately below the euphotic layer (Jackson et al. 2015). However, it has generally proven difficult to match the variation in the flux at the deep sea to the upper ocean processes (Lampitt et al. 2010b).

We argue that part of the mismatch in carbon budgets results from ignoring the small size fraction of zooplankton. In general, studies on particle consumption and breakup include bacterial metabolism and activities of larger zooplankton of > 1 mm in size, but ignore the potential effect of small copepods that are not efficiently captured with conventional nets (Svensen et al. 2011). These smaller copepods include abundant and widely distributed species, such as *Oithona* spp., *Oncaea* spp. and *Microsetella norvegica*, which can have large effects on both food web dynamics and biogeochemical cycles (Turner 2004). For example, *Oncaea* spp. and *M. norvegica* have been observed in marine snow particles (Green & Dagg 1997) and are known to feed on these (Alldredge 1972), potentially at high rates (Kiørboe 2000, Koski et al. 2005). In fact, some of these pelagic species, such as *M. norvegica*, depend on marine snow particles for their nutrition (Koski et al. 2005, Koski et al. 2017). Estimates suggest that particle degradation by these and other zooplankton could consume most of the marine snow before it leaves the euphotic layer (Kiørboe 2000). However, as the distribution of the species is patchy, and their biology and physiological rates (including diet and feeding) poorly known, their overall importance is difficult to estimate.

Jackson (1993) introduced the term “flux feeding,” suggesting that a zooplankton ‘gate-keeping’ community residing just below the particle maximum could consume a large proportion of sinking particles at rates depending on their fluxes rather than concentrations (Jackson & Checkley 2011). We suggest that aggregate-associated copepods at the PAP site could be acting as ‘gate-keepers’ and that feeding by these copepods might account for the surplus carbon at the

euphotic layer. Understanding the diet and feeding rates of these species will help to balance the carbon budgets in both the epipelagic and mesopelagic zones. Therefore, we investigated the biomass, vertical distribution and feeding of small aggregate-associated copepods *Microsetella norvegica* and *Oncaea* spp., in order to 1) investigate their diets and feeding rates, 2) estimate their carbon requirements, and 3) estimate their potential contributions to the attenuation of vertical flux.

2. Methods

2.1 Sampling and sample preservation

This study was conducted onboard RRS James Cook (National Oceanography Centre, Southampton), during the cruise number 87 to the PAP site from 31 May to 18 June 2013. Vertical distribution of zooplankton abundance was measured eight times during the cruise: six times at the PAP site and twice at nearby sites (Table 1). Zooplankton were sampled in five depth layers, 0-50, 50-100, 100-300, 300-500 and 500-1000 m. Sampling was conducted with Multinet Midi (Hydrobios) 50 μm mesh size nets equipped with a flowmeter. The nets were hauled vertically from the deepest sampling depth to the surface at the maximum speed of ca. 0.5 m s^{-1} . Multinet samples were preserved immediately with 2% formalin. A CTD was typically deployed in the upper 200 m of the water column less than 6 h before or after the multinet sampling and used to obtain vertical profiles of temperature, salinity and fluorescence.

Microsetella norvegica and *Oncaea* spp. for gut chlorophyll analyses and incubations were sampled using a modified WP2 net with a 90 μm mesh size and non-filtering cod end. The WP2 net was towed vertically from a depth of ca. 200 m to the surface, at the speed of ca. 1 m s^{-1} .

¹. Upon recovery, the contents of the cod end were diluted into ca. 20 L of surface seawater. We immediately moved 43-70 adults or late copepodites of *M. norvegica* and 31-60 adults or late copepodites of *Oncaea* spp. from the cod end samples onto GF/F filters for the analysis of their gut chl-*a* contents. We made filter blanks by filtering an equal amount of the seawater used to transport copepods, but without copepods, at each sampling time. The filters were placed into glass vials with added acetone, and refrigerated for 24 h before analysis. For functional response and respiration incubations, *M. norvegica* and *Oncaea* spp. were individually taken from the samples and placed into <0.7 µm filtered seawater at ca. 12 °C for a minimum of 12 h before being used in experiments. The seawater for incubations originated from the CTD or a marine snow catcher (Cavan et al. 2017), and was filtered through a GF/F filter before use.

2.2 Incubations

Trichodesmium spp. filaments were collected from the plankton tows, while detritus aggregates were collected using a marine snow catcher deployed at the time of the net tows at depths of ca. 100 m below the mixed layer depth (Cavan et al. 2017). Chl-*a* contents of these aggregates were used as indications of their sizes: replicate samples containing ca. 35 *Trichodesmium* filaments or 43 detritus aggregates were collected on GF/F filters and analyzed in a manner similar for gut chl samples. *Microsetella norvegica* fecal pellet production on two types of aggregates, *Trichodesmium* spp. filaments and detritus aggregates, was measured at concentrations of 0, 1, 3, 5 and 10 aggregates per 70 mL sample bottle. At the start of the incubations, starved *M. norvegica* were poured carefully into a Petri dish, from which 7 actively-swimming individuals were sampled using a glass pipette and transferred into 2-3 replicate vials containing filtered seawater and target aggregate concentrations. The bottles were closed air-tight

and incubated at 12 °C in the dark for 24 h. During the incubation period, the bottles were manually turned around 2-3 times. After 24 h, the contents of the bottles were carefully poured into Petri dishes, the numbers and conditions (dead / alive) of copepods were checked and their fecal pellets were counted.

To measure copepod respiration rates, 5-15 starved *Microsetella norvegica* or 9-27 starved *Oncaea* spp. were placed into 2 mL measurement chambers containing GF/F filtered seawater and immersed into a temperature-controlled water bath. After ca. 5 min, the O₂ electrode was injected through the chamber lid and the measurements began. Respiration was measured using Unisense microrespirometer system (www.unisense.dk), which consists of a Clarke-type oxygen microsensor, an amplifier and a stirring system. The O₂ concentration was measured at 2 s intervals and the data was logged using MicOx software. The measurements continued for 15 min. At each station, a background measurement was conducted with filtered seawater to account for potential microbial respiration or oxygen consumption of the electrode. After the incubations, contents of the respiration chambers were poured onto a Petri dish, the conditions of the copepods were recorded and their prosome lengths were measured with a precision of 20 µm.

2.3 Analysis and calculations

All Multinet samples were processed at the NMFRI Plankton Sorting and Identification Center in Szczecin, Poland. Zooplankton were identified to species or genus and life-stage, with at least 300 individuals per sample counted, using a stereo microscope. Ten individuals of each stage and species were measured with a precision of 8 µm and the number of eggs in egg sacs counted. The carbon weights of individuals were estimated from their stage-specific body

lengths and using published carbon to length relationships of Uye et al. (2002) for *Microsetella norvegica* and Satapoomin (1999) for *Oncaea* spp. The weighted mean depth of the population was calculated by multiplying the abundances in each depth layer by the mean depth of the sampled layer, and dividing the sum of this by the total abundance.

Average reproductive rate was estimated by multiplying the numbers of egg sacs per female in the Multinet samples by the average clutch size in the same samples and then dividing by the total number of females (taking into account that *Oncaea* spp. females carry two egg sacs) and the egg development period. The development time of eggs was calculated using the temperature at the weighted mean depth of females from the relationships of Uye et al. (2002) for *M. norvegica*, and the average of those from McLaren et al. (1969) and Nielsen et al. (1991) for *Oncaea* spp.

Copepod ingestion and carbon demand were estimated several ways from the above estimates:

1) Gut chl was converted to feeding rate by multiplying with the gut clearance coefficient (k). The coefficient k was calculated using temperature (T) at the weighted mean depth of the population, using the equation of Dam & Peterson (1988):

$$k = 0.0117 + 0.001794T \quad (1)$$

which resulted in k varying between 0.03 and 0.04 min⁻¹. For *Microsetella norvegica*, we also estimated k based on the functional response of fecal pellet production on *Trichodesmium* filaments, according to Harris et al. (2000), and assuming that no more than one fecal pellet can be in the gut at a time. The value of k estimated from fecal pellet production was considerably lower at 0.006 ± 0.003 min⁻¹. Ingestion of Chl-*a* was then converted to carbon ingestion rate assuming that most of the chl-*a* in the gut originated from feeding on *Trichodesmium* spp. and by

using a carbon to chl-*a* conversion of Roman (1978) for *Trichodesmium* sp. *Microsetella norvegica*, and probably also *Oncaea* spp. rely on aggregates for their food rather than consuming phytoplankton (Koski et al. 2005, Koski et al. 2017), and were thus not expected to feed on suspended particles.

2) Fecal pellet production was converted to carbon ingestion by using the relationship between fecal pellet production and ingestion by *M. norvegica* at 12 °C when feeding on the diatom *Thalassiosira weissflogii* ($R^2 = 0.5$; Koski et al. unpubl.). Aggregate carbon was calculated from chlorophyll concentration using the carbon to chlorophyll ratio from Roman (1978). The average chl-*a* content of the *Trichodesmium* filaments in our study was 0.97 µg Chl-*a*, which corresponded to the carbon content of 135 µg C filament⁻¹. The chl-*a* content of detritus aggregates was 0.17 µg Chl-*a*, which, assuming the same carbon to chlorophyll conversion than for *Trichodesmium*, corresponded to 22.2 µg C aggregate⁻¹.

3) Respiration rate was calculated from the rate of oxygen decrease in µmol min⁻¹ L⁻¹ and converted to µL h⁻¹ ind.⁻¹. The oxygen consumption rate in the background measurement (filtered seawater only) was subtracted from the respiration in chambers containing copepods to isolate the copepod rates. The respiration rate was converted to carbon by using the respiratory quotient of 0.97 (Ikeda et al. 2000).

4) Ingestion based on egg production was calculated by multiplying the numbers of eggs by the egg carbon content. Egg carbon content for *M. norvegica* was taken from Uye et al. (2002). To calculate the egg carbon content for *Oncaea* spp., we multiplied the ratio of egg carbon to female carbon from Böttger-Schnack & Schnack (2005) with the size of *Oncaea* spp. females in our samples. The weight-specific egg production was converted to ingestion by

assuming a gross growth efficiency (GGE) of 0.2, the lower range of the average GGE of copepods in Straile (1997).

Finally, carbon demands of *M. norvegica* and *Oncaea* spp. were calculated by multiplying their biomass values with the average weight-specific ingestion / carbon demand estimated from the gut chl-*a*, functional responses and respiration. Ingestion based on the egg production was not included, as many zero rates suggested that the egg production was controlled by factors other than ingestion rate.

3. Results

3.1 Hydrography

The water column at the PAP site (Sta. 1-6, 10 and 11) was weakly stratified, with the temperature decreasing from 13.6 °C at the surface to 11.6 °C at 200 m. Salinity in the upper 200 m was 35.5-35.6 ppm. Fluorescence peaked at 30-33 m, thereafter decreasing to low values around 100 m (Fig. 1). Most of the chl-*a* was composed of nanoplankton (< 10 µm), which on average contributed to 82% of the total chl-*a* (Gašparović et al., 2018). Stations outside the PAP site (7-9) had slightly higher surface temperature (14.3 °C) and fluorescence, with a fluorescence peak closer to the surface (data not shown). Also Sta. 5, with a deeper distribution of copepods (see below), had a higher temperature and a greater peak fluorescence. The hydrography at the PAP site during the cruise has been described in more detail in Cavan et al. (2017) and Gašparović et al. (2017).

3.2 Biomass, vertical distribution and egg production

Total biomass of particle-colonizing copepods varied between 55 and 280 mg C m⁻², corresponding to 0.27-1.32 x 10⁶ ind. m⁻² over the 1000 m water column (Fig. 2a and b). *Microsetella norvegica* and *Oncaea* spp. contributed approximately equally to the total biomass. Juveniles (nauplii + copepodites) of *M. norvegica* at the PAP site typically made up ca. 50 % of the total biomass, with the exception of Sta. 1 when most of the population consisted of adults (Fig. 2a). Juveniles of *Oncaea* spp. made up between 25 and 50% of the total biomass (Fig. 2b). The stations away from PAP had a lower total biomass, and no nauplii of either species were observed.

Egg production of both species ranged between zero and ca. 1.5 eggs female⁻¹ d⁻¹ (Fig. 2c and d). The proportion of females with eggs was mostly low ($\leq 10\%$ of the population) for both species, although Sta. 3 (22 and 50% for *M. norvegica* and *Oncaea* spp., respectively) and Sta. 11 (35% for *M. norvegica*) had substantially more. For females bearing eggs, clutch size was 8 ± 1 eggs clutch⁻¹ for *M. norvegica* and 12 ± 5 eggs clutch⁻¹ for *Oncaea* spp. There was no correlation between the numbers of eggs and numbers of first nauplius stages of either species. The average female: male ratio (F: M) of *Microsetella norvegica* of all samples was 1.5 ± 1 , although Sta. 2 had ten times more females than males (Fig. 2c). The average F:M ratio for *Oncaea* spp. was relatively constant at 0.9 ± 0.5 (Fig. 2d). No connection between sex ratio and numbers of eggs or nauplii in the water column was found for either of the species (Spearman rank correlation analysis; $p > 0.05$).

Most of the *Microsetella norvegica* at all stations resided in the upper 100 m of the water column (Fig. 2e, g). For only one profile (Sta. 5) was the weighted mean depth of that population deeper than 100 m. In contrast, the weighted mean depth of *Oncaea* spp. was always 100 m or deeper, with the two deepest population distributions at stations outside the PAP site. Station 5

also had the deepest distribution of *Oncaea* spp. (Fig. 2f). Nauplii, copepodites and females of *M. norvegica* were typically in the near surface (0-50 m) water layer, while the weighted mean depth of males was slightly deeper at 50-100 m (data not shown), with little variation between stations (Fig. 2e, g). The vertical distributions of different life stages of *Oncaea* spp. were much more variable (Fig. 2f, h), with the weighted mean depth of nauplii, copepodites, females and males varying between 30-300 m, 50-400 m, 90-300 m and 90-250 m, respectively (data not shown). *Microsetella norvegica* was observed predominantly at or just below the fluorescence maximum at the temperatures between 12.5 and 14 °C, while *Oncaea* spp. usually resided below the thermocline. The deepest distributions of both species, Sta. 5 in PAP site, and Sta. 7 and 8 outside PAP, coincided with warmer surface temperatures and greater fluorescence maxima (cf. Fig. 1 and 2).

3.3 Feeding

Microsetella norvegica had measurable amounts of chl-*a* in its gut (Fig. 3) for all stations but Sta. 11, indicating feeding on phytoplankton or on the *Trichodesmium* filaments that were abundant at the PAP site during the cruise (F. Norrbin, University of Tromsø, pers.com.). The gut chl-*a* content of *M. norvegica* varied between 0.03 and 0.2 ng chl-*a* ind.⁻¹ with an average of 0.09 ± 0.08 ng chl-*a* ind.⁻¹. In contrast, gut chl-*a* values of *Oncaea* spp. were significantly lower than those of *Microsetella* in the same samples (2-way ANOVA; $F_{1,31} = 6.0$; $p < 0.05$), varying between non detectable levels and 0.15 ng chl-*a* ind.⁻¹. Similar to biomass, gut chl-*a* of both species varied many-fold between stations, with the highest gut chl-*a* being observed at the station outside the PAP site (11.6.; $F_{5,31} = 6$; $p < 0.001$; Tukey HSD; $p < 0.05$). The average

feeding rates calculated using the gut chl-*a* relationships were 0.43 ± 0.35 and 0.18 ± 0.26 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ for *M. norvegica* and *Oncaea* spp., respectively (Table 3).

The relationship between fecal pellet production by *Microsetella norvegica* and concentrations of *Trichodesmium* spp. filaments demonstrated feeding on cyanobacteria (Fig. 4a). *Microsetella norvegica* fecal pellet production increased from 4 to 10 fecal pellets $\text{ind.}^{-1} \text{d}^{-1}$ as *Trichodesmium* spp. concentrations increased, with a response that can be described as a functional response type II (Holling 1965):

$$I = \frac{\beta C_{\text{prey}}}{1 + \beta C_{\text{prey}} \tau} \quad (2)$$

where I is the ingestion rate (or fecal pellet production rate), β is the encounter rate kernel (search volume rate), C_{prey} is the food concentration and τ is the handling time. We used the ingestion rate (in carbon) derived from fecal pellet production and food concentration converted to carbon (see Methods) and fitted the equation of hyperbola:

$$y = \frac{ax}{b + x} \quad (3)$$

This gave parameter values of $a = 0.11$ and $b = 3737$ ($R^2 = 0.53$). We then used $\frac{a}{b}$ and $\frac{1}{a}$ to calculate β and τ , respectively. This relationship implied that *M. norvegica* was searching for *Trichodesmium* aggregates at the low rate of $0.03 \text{ ml ind.}^{-1} \text{d}^{-1}$, and had a handling time of $8.8 \text{ days aggregate}^{-1}$. The maximum ingestion rate of *Trichodesmium* aggregates could then be calculated as an inverse of handling time, and was $0.11 \mu\text{g C ind.}^{-1} \text{d}^{-1}$. The fecal pellet production on detrital aggregates was always < 5 fecal pellets $\text{ind.}^{-1} \text{d}^{-1}$, and with no discernable functional response (Fig. 4b). The maximum feeding rate on detritus aggregates was calculated from the average pellet production rate of 4.6 ± 1.1 pellets $\text{ind.}^{-1} \text{d}^{-1}$ to be $0.05 \mu\text{g C ind.}^{-1} \text{d}^{-1}$, which indicated a handling time of $21 \text{ days aggregate}^{-1}$ (Table 3).

Most of the measured respiration rates of *Microsetella norvegica* were between 0.005 and 0.02 $\mu\text{L ind.}^{-1} \text{h}^{-1}$, with a few greater rates at each station (0.04-0.08 $\mu\text{L ind.}^{-1} \text{h}^{-1}$) (Table 2). The average respiration rates of both *M. norvegica* and *Oncaea* sp. were similar, 0.02 ± 0.03 and 0.03 ± 0.02 $\mu\text{L ind.}^{-1} \text{h}^{-1}$, respectively. The respiration rates indicated carbon demands ranging from 0.17 to 0.31 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ for *M. norvegica* between the stations (average of 0.24 ± 0.33 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$) and 0.32 ± 0.25 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ for *Oncaea* spp. (Table 3).

3.4 Potential carbon consumption of *Microsetella norvegica* and *Oncaea* spp.

The total average carbon demands of *Microsetella norvegica* and *Oncaea* spp. were 79 ± 33 mg C m^{-2} (Fig. 5), ranging from 16.5 to 95 mg C m^{-2} for *M. norvegica* and from 12.3 to 75 mg C m^{-2} for *Oncaea* spp. between the days. In most stations, the two species consumed approximately equally amounts of carbon, with the exception of a few stations where *M. norvegica* dominated. Most carbon was consumed in the 0-50 m surface layer, with *M. norvegica*'s consumption decreasing rapidly with depth. Carbon consumption of *Oncaea* spp. was more evenly distributed with depth and exceeded the carbon consumption of *M. norvegica* at depths ≥ 100 m (Fig. 5).

4. Discussion

4.1 The contribution of aggregate-associated copepods to the carbon budget

The estimated total carbon consumption of *Microsetella norvegica* and *Oncaea* spp. varied among sampling sites, but was always sufficiently high to account for a considerable part of the export flux. Average primary production during the sampling period was ca. 1000 mg C

$\text{m}^{-2} \text{d}^{-1}$, of which $50 \text{ mg C m}^{-2} \text{d}^{-1}$ was exported from the euphotic zone and $25 \text{ mg C m}^{-2} \text{d}^{-1}$ reached the deep sea (Cavan et al. 2017). The consumption of particles was thus high in the euphotic layer (average mixed layer depth of 30 m; Frigstad et al. 2015), but relatively low in the twilight zone (Cavan et al. 2017), similar to other studies from the PAP site (Belcher et al. 2016). Belcher et al. (2016) estimated an excess POC supply of $172 \text{ mg C m}^{-2} \text{d}^{-1}$ in the upper mesopelagic, which could not be explained by respiration of either larger zooplankton collected with a $200 \mu\text{m}$ net or bacteria. This imbalance has been suggested to be due to an increase in bacterial and protozoan degradation of sinking particles, enhanced by their fragmentation by zooplankton activities (Giering et al. 2014, Belcher et al. 2016, Cavan et al. 2017). Our results indicate that feeding by small aggregate-associated copepods also could account for the oversupply of carbon in the epipelagic. This is in agreement with model calculations that suggest that zooplankton consumption of sinking particles could degrade most of the POC flux before it leaves the euphotic zone (Kjørboe 2000).

The smaller zooplankton species such as *Microsetella norvegica* and *Oncaea* spp. have a strong impact on vertical flux, a conclusion supported by several lines of evidence. First, small zooplankton species, including *Oncaea* spp. and harpacticoids, have been observed associated with marine snow particles in numerous studies from both the Atlantic and Pacific Oceans, sometimes with concentrations that are several orders of magnitude higher than in the surrounding water (Alldredge 1972, Bochdansky & Herndl 1992, Ohtsuka et al. 1993, Green & Dagg 1997, Shanks & Walters 1997). Second, it has been long known that these species feed on the surface of marine snow (Alldredge 1972, Metz 1998), and that their individual feeding rates can be as high as the feeding rates of calanoid copepods on suspended phytoplankton (Koski et al. 2005, 2007, 2017). Neither *M. norvegica* nor *Oncaea* spp. are efficient in feeding on

suspended particles, but are dependent on a surface, such as an aggregate, to obtain food (Koski et al. 2005, 2017). Third, mechanistic understanding of the processes of finding and colonizing marine snow particles has started to emerge, with direct observations having shown that copepods follow chemical trails to marine snow particles (Kiørboe 2001, Lombard et al. 2013). Fourth, both observations based on particle-size spectra (Jackson 1993, Jackson & Checkley 2011) and model calculations (Kiørboe 2000, Koski et al. 2005) suggest that realistic concentrations of aggregate-associated copepods could clear the water column of most of the sinking marine snow particles. Our observation of an average carbon consumption of *M. norvegica* and *Oncaea* spp. of ca. $80 \text{ mg C m}^{-2} \text{ d}^{-1}$ - approximately half of the unaccounted POC in Belcher et al. (2016) – is thus well in line with previous studies. It strongly suggests that consumption of marine snow by zooplankton $< 1 \text{ mm}$ should be taken into account in epipelagic and mesopelagic carbon budgets.

4.2 Factors controlling the distribution and abundance of aggregate-associated copepods

Although it is clear that small zooplankton are important for flux attenuation, we do not know what controls their abundance or distribution. For instance, *Microsetella norvegica* can dominate the zooplankton community, with abundances of 10^4 - 10^5 ind. m^{-3} reported from Scotian Shelf (Dugas & Koslow 1984), Sea of Japan (Uye et al. 2002) and sub-arctic fjords in Norway and Greenland (respectively, Svensen et al. 2018, Arendt et al. 2013). In other areas, *M. norvegica* appears to be a regular but not dominant member of the zooplankton community, with concentrations that are many-folds lower than the peak concentrations (Paffenhöfer & Mazzocchi 2003, Beşiktepe et al. 2015, Thor et al. 2005). As demonstrated by our sampling, the

biomass levels of *M. norvegica* and *Oncaea* spp. were also patchy within a restricted geographic area, with 2-fold differences in biomass between the stations.

Microsetella norvegica distribution can be controlled by marine snow abundance and turbulence intensity (Maar et al. 2006). However, *M. norvegica* does not appear to be dependent on marine snow in productive areas (Uye et al. 2002), and turbulence can have both beneficial (Uye et al. 2002) and negative (Maar et al. 2006) effects on its distribution. Although *M. norvegica* appears to be associated with warmer water masses at the surface (Uye et al. 2002, Svensen et al. 2018), its distribution spans from sub-tropical and tropical ocean (Paffenhöfer & Mazzocchi 2003) to sub-arctic and arctic fjords (Arend et al. 2013, Svensen et al. 2018). Temperatures over this region vary from a few degrees above 0° C to more than 25 °C, a range that makes it difficult to conclude whether temperature controls *M. norvegica* distribution. The factors controlling the distribution of *Oncaea* spp. are even more elusive, complicated by the fact that *Oncaea* spp. in an area can include > 20 species, each with its specific vertical and geographic niche (see Böttger-Schnack 1995 for *Oncaea* spp. in the Mediterranean). Both the drivers of patchiness and their scales for *M. norvegica* and *Oncaea* spp. are, therefore, unknown. Our results suggest that advection could also be an important factor determining the biomass at the PAP site, a possibility suggested by the simultaneous drop in the abundance and weighted mean depth of both species at Sta. 5, whose hydrographic profile also differed from those of the other stations.

4.3 Feeding rates

As with their distribution and abundance, we do not know what controls the feeding rates of *Microsetella norvegica* and *Oncaea* spp., how variable their feeding rates are in the natural

environment or in fact, what they are feeding on. The presence of chlorophyll in the gut of *M. norvegica* and its absence in the gut of *Oncaea* spp. implies that these copepods have different diets, although might both have been feeding on aggregates (including *Trichodesmium* spp.). In addition, the vertical distribution of *M. norvegica* in the surface layer and *Oncaea* spp. below the euphotic layer is consistent with their having different diets, as is the difference between the respiration-based feeding rates compared to those from gut chlorophyll content. While the feeding rate of *Oncaea* spp. based on respiration was approximately twice that of the feeding rate estimated using gut chlorophyll, the feeding rate of *M. norvegica* based on the chlorophyll was higher than that based on respiration. This difference suggests that feeding on autotrophic organisms was sufficient to fill the carbon requirements of *M. norvegica* but not of *Oncaea* spp. *Oncaea* spp. have been shown to feed on aggregates of diatoms and *Phaeocystis* sp. (Metz 1998), on appendicularian houses (Ohtsuka et al. 1993), and on diatoms, detritus and other zooplankton (Turner 1986, Wu et al. 2004). *Microsetella norvegica* has been shown to feed on diatoms (Koski et al. 2005) and appendicularian houses (Ohtsuka et al. 1993, Koski et al. 2007). Both species appear to be omnivorous, with diets influenced by encounter rates with different types of aggregates within their distribution depths and aggregate properties, including concentration and sinking rate. In the past, the typical aggregate at the PAP site included phytodetritus (Belcher et al. 2016), which was also a dominant particle type during this study (Cavan et al. 2017). However, turfs of *Trichodesmium* sp. were also abundant, being observed regularly in video plankton recorder profiles (F. Norrbin, pers.com.). Although *Trichodesmium* sp. is typically assumed to be degraded at the surface (Mulholland 2007), it has also been found in deep sediment traps at the North Atlantic (Pabortsava et al. 2017), and could thus contribute to the diet of copepods at all depths.

Our estimates of feeding rates fit well with the previously reported feeding rates of *Oncaea* spp. at 0.2-0.4 $\mu\text{g C ind.}^{-1} \text{ d}^{-1}$ (Metz 1998) and *Microsetella norvegica* at 0.14 $\mu\text{g C ind.}^{-1} \text{ d}^{-1}$ (Koski et al. 2007). Similarly, the measured respiration rates were of the same magnitude as the rates reported for *Oncaea* spp. at corresponding temperatures (Paffenhöfer 2006). The estimated feeding rates based on reproduction were considerably lower than the estimated rates based on other methods. Reproduction could have been limited by other factors than food concentration, such as by the effect of temperature (Bunker & Hirst 2004), fertilization (Ceballos et al. 2014) or food quality (Koski et al. 1998), and therefore not related to ingestion. The relatively even sex ratio and moderate clutch size, but low proportion of females carrying eggs, could suggest that temperature rather than food was limiting reproduction, since the spawning frequency is typically thought to be regulated by temperature (Rhyne et al. 2009). Functional response of fecal pellet production indicated feeding rates that were lower than feeding rates based on gut chlorophyll or respiration, and suggested that the aggregate type influences the shape of the response and the maximum feeding rate. The calculated search volume rate on *Trichodesmium* spp. aggregates at $<< 1 \text{ mL d}^{-1}$ was much lower than what has been estimated based on the swimming velocity (Koski et al. 2005), and might have been due to the high biomass of *Trichodesmium* aggregates in the experiments. Nevertheless, the incubations confirmed feeding on *Trichodesmium* spp. filaments and, to a lesser degree, on detritus aggregates, although more experiments are needed to assess the effect of aggregate type on encounter rate or handling time.

4.4 Conclusions

The fate of the primary production – food web transfer, microbial loop or sinking – essentially determines the important ecosystem services of fisheries production and carbon sequestration by the biological pump. Whether primary production is consumed by large zooplankton at the surface ocean, remineralised by microbial activity, exported and sequestered in the deep ocean, or exported and remineralised in the mesopelagic layer, depends, for instance, on the size structure and physiological state of the phytoplankton community, community composition and ecophysiology of zooplankton, and abundance and activity of bacteria and microplankton. The abundance and feeding of aggregate-associated copepods contribute to the degradation of sinking matter both in the epipelagic and below, and will therefore be important in determining the fate of the primary production, although the geographic or seasonal variation of this activity is unknown.

Increasing sea surface temperature will induce diverse changes in zooplankton communities, including distributional shifts, changes in phenology, and decrease of mean size (Richardson 2008, Garzke et al. 2014). For instance, it is conceivable that small copepods might be better in coping with increasing stratification and decreasing biomass of large phytoplankton than large calanoids, and might thus become proportionally more abundant in the future ocean. Although our knowledge on the biology of aggregate-associated species is too limited to predict their future abundance or distribution, we do know that these species can have a large influence on the efficiency of the biological pump, and that changes in the species composition of zooplankton, particularly between vertically migrating calanoids and aggregate-associated species, might have a large effect on the fate of the primary production (Sanders et al. 2014, Koski et al. 2017). Factors controlling abundances and feeding rates of aggregate-associated copepods should therefore feature high in the priority list of the future zooplankton studies.

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Figure legends:

Fig. 1. Hydrography at the sampling site. Vertical distribution of temperature ($^{\circ}\text{C}$), salinity (ppm) and fluorescence (uncalibrated units) at the upper 200m of the water column at the PAP site (average of all stations $\pm$ SD). The inserted figure shows temperature, salinity and fluorescence on 9.6. when the weighted mean depth of *Microsetella norvegica* was substantially lower than in other sampling times (see Fig. 2e).

Fig. 2. Biomass, reproduction and vertical distribution of *Microsetella norvegica* and *Oncaea* spp. Daily variation in the biomass (mg m^{-2}) of a) *M. norvegica* and b) *Oncaea* spp., reproduction and sex ratio (eggs $\text{f}^{-1} \text{d}^{-1}$; grey columns and F:M; solid circles, respectively) of c) *M. norvegica* and d) *Oncaea* spp., weighted mean depth (m) of e) *M. norvegica* and f) *Oncaea* spp. and vertical distribution (% from the total population per each depth layer) of g) *M. norvegica* and h) *Oncaea* spp. during the cruise. (N) Nauplii, (C) copepodites, (CVI) adults. The arrows indicate the stations which were outside the PAP site (see Table 1).

Fig. 3. Gut chl-*a* of *Microsetella norvegica* and *Oncaea* spp. Gut chlorophyll of *M. norvegica* (solid symbols) and *Oncaea* spp. (open symbols) at different sampling stations ($\mu\text{g ind.}^{-1}$). The arrows indicate the stations which were outside the PAP site (see Table 1).

Fig. 4. Functional response of *Microsetella norvegica* feeding on aggregates. Functional response of *M. norvegica* fecal pellet production (fecal pellets $\text{ind.}^{-1} \text{d}^{-1}$) on two types of

aggregates, a) *Trichodesmium* sp. filaments and b) detritus (mean $\pm$ SD). The line indicates the Holling type II functional response (hyperbola).

Fig. 5. Carbon requirements of aggregate-associated copepods. Average depth-specific carbon requirements of *Microsetella norvegica* (black columns) and *Oncaea* spp. (grey columns) at the PAP site and surrounding areas (mg C m^{-3} ; mean $\pm$ SE). The total carbon consumption of both species is indicated in the figure (mg C m^{-2} ; mean $\pm$ SD).

Table 1. Sampling station, date, time and location, sampling depth, measured variables and incubations set up with *Microsetella norvegica*. (R) Respiration, (F) functional response of feeding. Numbers of replicate incubations are indicated in parenthesis. The locations in bold indicate the stations outside the PAP site.

Station	Date	Location	Sampling depths	Measurements	Incubations
1	5.6. (17:23)	48°38.91 016°08.57	Multinet: 0-50, 50-100, 100-300, 300-500, 500-1000 WP2: 0-100	Abundance, biomass, vertical distribution	
2	6.6. (10:53)	48°38.92 016°08.56			
3	7.6. (13:35)	48°38.91 016°08.58			R (3)
4	8.6. (11:31)	48°29.98 016°29.31	WP2: 0-100	Gut chl	R (7), F (2-3)
5	9.6. (10:21)	48°38.92 016°08.56	Multinet: 0-50, 50-100, 100-300, 300-500, 500-1000 WP2: 0-100	Abundance, biomass, vertical distribution, gut chl	
6	10.6. (12:34)	48°38.92 016°08.57			
7	10.6. (16:55)	48°40.47 016°32.68			
8	11.6. (15:20)	48°46.408 016°41.302			R (8)
9	12.6. (07:00)	48°47.41 017°02.14	WP2: 0-100	Gut chl	F (2)
10	13.6. (12:33)	48°38.917 016°08.574	Multinet: 0-50, 50-100, 100-300, 300-500, 500-1000 WP2: 0-100	Abundance, biomass, vertical distribution, gut chl	
11	14.6. (09:40)	48°38.91 016°08.58			R (3; <i>Oncaea</i> spp.)

Table 2. Respiration rate of *Microsetella norvegica* and *Oncaea* spp. Respiration rate of *M. norvegica* and *Oncaea* spp. at four sampling stations ($\mu\text{L ind.}^{-1} \text{ h}^{-1}$; mean $\pm$ SD). The station outside the PAP site is indicated in bold (see Table 1). The number of replicate measurements is indicated in parenthesis.

Species	Date	Respiration (n)
<i>M. norvegica</i>	7.6	0.025 ± 0.020 (3)
	8.6	0.015 ± 0.013 (6)
	11.6.	0.022 ± 0.038 (8)
	Average	0.020 ± 0.027 (17)
<i>Oncaea</i> spp.	14.6.	0.026 ± 0.020 (3)

Table 3. Average ingestion rates of *Microsetella norvegica* and *Oncaea* spp. estimated based on the gut chlorophyll, functional responses on two types of aggregates, respiration and reproduction rates ($\mu\text{g C ind.}^{-1} \text{ d}^{-1}$; mean $\pm$ SD). The rate in parenthesis indicates ingestion if the fecal pellet production on *Trichodesmium* sp. aggregates is used to calculate the gut clearance rate (k). The ingestion rate based on reproduction is the mean ($\pm$ SD) of the eight sampling times (including the dates where no egg-carrying females were observed). (Blank) No data.

	<i>M. norvegica</i>	<i>Oncaea</i> spp.
Gut chl	0.43 ± 0.35 (0.07 ± 0.06)	0.18 ± 0.26
Functional response (<i>Trichodesmium</i> sp.)	0.11	
Functional response (detritus)	0.05	
Respiration	0.24 ± 0.33	0.32 ± 0.25
Reproduction	0.06 ± 0.09	0.01 ± 0.02

Fig. 1.

Fig. 2

Fig. 3

Functional response

Fig. 4

Fig. 5

The missing piece of the upper mesopelagic carbon budget? Biomass, vertical distribution and feeding of aggregate-associated copepods at the PAP site

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Highlights:

- Aggregate-associated copepods can have a large influence on the attenuation of the vertical flux
- Particle degradation by these copepods can equal the surplus in the epipelagic carbon
- Biomass of *M. norvegica* and *Oncaea* spp. is variable both in time and in space
- Understanding their distribution is needed to predict the changes in the biological pump

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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