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Functional responses of aggregate-colonizing copepods

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Abstract

Zooplankton consumption of aggregates, such as marine snow, is an important factor in determining the efficiency of the biological carbon pump. However, the feeding rates of aggregate-associated small harpacticoid and poecilostomatoid copepods are largely unknown, as are the factors that influence these rates. We measured the functional responses of pellet production (PP) of aggregate-feeding copepods on appendicularian houses, algal and detrital aggregates, and on *Trichodesmium* spp. tufts. The PP rates of all copepods increased with increasing aggregate concentrations, but the response varied depending on the aggregate type. The pelagic harpacticoid copepod *Microsetella norvegica* had the highest PP rates on algal aggregates, while the poecilostomatoid copepod *Oncaea* spp. and the benthic harpacticoid copepod *Amonardia normanni* PP rates were highest on appendicularian houses. The ingestion rates of *M. norvegica* and *Oncaea* spp. were typically 0.04–0.13 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$, with the exception of ca. three times higher rates of *Oncaea* spp. on appendicularian houses and ca. 10 times higher rates of *M. norvegica* on algal aggregates. The ingestion rates of the larger species, *A. normanni*, were generally higher, 0.3–1.3 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ on algal aggregates, and even ca. 10-fold higher on appendicularian houses. Our results suggest that the aggregate degradation rates by copepods can vary many-fold depending on the quality (origin) of the aggregate and the copepod species. This can have large consequences for the attenuation of vertical carbon flux.

Degradation of sinking aggregates is one of the main processes influencing the efficiency of the biological pump, and therefore carbon sequestration in the ocean. It has been long acknowledged that zooplankton play an important role in this process through both fragmentation and direct consumption of aggregates (Dilling and Alldredge 2000; Steinberg et al. 2008). Feeding on aggregates by copepods is likely to be common in the ocean. This notion is supported by findings of high concentrations of metazoans on aggregates (Green and Dagg 1997; Kiørboe 2000), the demonstrated ability of some species to follow chemical trails to find aggregates (Lombard et al. 2013), and the observations indicating that some species are

specialized in feeding on aggregated food (Koski et al. unpubl.). Model results have suggested that zooplankton feeding can remove most aggregates before they leave the euphotic layer (Kiørboe 2000), and field observations combining the feeding rates on aggregates and abundances of particle-colonizing copepods suggest that these organisms are important for the carbon budgets of the surface ocean (Koski et al. 2020).

Many zooplankton species have been observed feeding on aggregates. These include amphipods (Lampitt et al. 1993b), Polychaeta larvae (Bochdansky and Herndl 1992), euphausiids, and large calanoid copepods (Dilling et al. 1998; Van der Jagt 2020) that can ingest whole aggregates similar to feeding on large phytoplankton cells or ciliates, as well as small non-calanoïd copepods, nematodes, meroplankton larvae, and copepod nauplii that colonize the aggregates and feed on their component parts (Shanks and Walters 1997; Kiørboe 2000). There is evidence that small harpacticoid and poecilostomatoid copepods might be particularly important degraders of aggregates (Alldredge 1972; Ohtsuka et al. 1993; Kiørboe 2000) as their concentrations in aggregates are often many-fold higher than their concentrations in the surrounding seawater (Kiørboe 2000). These latter species include the pelagic harpacticoid *M. norvegica* and oncaeid copepods that can be extremely abundant in widely varying environments, from

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Additional Supporting Information may be found in the online version of this article.

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Arctic fjords (Arendt et al. 2013; Svensen et al. 2018) to subtropical and tropical oceans (Böttger-Schnack 1995; Paffenhöfer and Mazzocchi 2003). These species appear to be more efficient in feeding on aggregated food than on suspended phytoplankton and can obtain high short-term feeding rates (Koski et al. 2005). In environments where particle-colonizing copepods dominate the zooplankton community, zooplankton degradation of sinking particles could be equally important to zooplankton-mediated production of sinking particles or active carbon transport through vertical migration, which are the zooplankton activities that have typically been considered important for the biological carbon pump (Brun et al. 2019).

Despite the potentially high impact of aggregate-colonizing copepods on the efficiency of the biological pump, we know little of the factors influencing their feeding rates. To be able to consume aggregates, copepods need to find and colonize the aggregates, and to stay on them for the time that it takes to fill their guts. While the encounter rates are influenced by the concentrations and sizes of the aggregates and the chemical trails that they may leave in their wakes (Kjørboe 2000; Lombard et al. 2013), the ability of copepods to extract nutrition from aggregates could depend on the chemical composition, and therefore, origin of the aggregates. The concentration, origin, and quality of aggregates can in turn vary greatly between geographic areas, seasons, and water depths. For instance, while diatoms are likely to be important constituents of aggregates during spring phytoplankton blooms in the North Atlantic (Daniels et al. 2015), *Trichodesmium* spp. filaments form an important surface area for multiple microorganisms in the subtropical and tropical oceans (Sheridan et al. 2002). Zooplankton-derived aggregates such as fecal pellets and appendicularian houses can dominate in many seasons and areas (Turner 2015). Consumption rates of copepods on aggregates might therefore vary depending both on their concentration and on their origin/quality.

We studied the interacting effects of aggregate type, concentration, and copepod species on the consumption of aggregates to reveal (1) whether different aggregate-colonizing copepod species have similar feeding rates and aggregate preferences and (2) whether the aggregate type influences the maximum ingestion rate. This is necessary information if we are to quantify the effect of these abundant copepods on flux attenuation, and therefore on the efficiency of the biological carbon pump.

Materials

We investigated the functional response of copepod feeding on diverse types of aggregates, using fecal pellet production (PP) as an indication of feeding rate. The copepod species included the pelagic harpacticoid *Microsetella norvegica*, the semi-benthic harpacticoid *Amonardia normanni*, and the poecilostomatoid copepod *Oncaea* spp. (Table 1). The aggregates included discarded houses of the appendicularian

Oikopleura dioica, laboratory-created algal aggregates from diatoms, cyanobacteria, and a diatom-dominated mixture of algae, field-collected tufts of the cyanobacteria *Trichodesmium* sp., and field-collected detritus and algal aggregates (Table 2).

Copepods and aggregates

The experiments were conducted during different cruises or laboratory campaigns between 2005 and 2014 (Table 1). All copepods were collected during spring or summer, using a WP2 net with a 90–100- μm mesh size and non-filtering cod end, with plankton tows that typically extended from below the chl-max to the surface. Copepods were individually sorted into air-tight closed culture flasks (*M. norvegica* and *Oncaea* spp.) or open containers (*A. normanni*), and fed with an excess ($> 300 \mu\text{g C L}^{-1}$) concentration of the diatom *Thalassiosira weissflogii* until their use in experiments. The experiments at sea or in field stations were conducted within 48 h from the capture of the copepods. The laboratory experiments with *M. norvegica* and *Oncaea* spp. were conducted within 1–3 months of the capture of the copepods, whereas *A. normanni* was grown in the laboratory for > 100 generations before the start of the experiments. The incubation temperature was close to the in situ temperature during the cruises, and typically 15°C in the laboratory experiments (Table 1), and all experiments were conducted in the dark. *T. weissflogii* that was fed to copepods prior to experiments was grown in semi-continuous batch cultures with 16 : 8 h light : dark cycle, at 18°C , using F/2 (+ Si) media (Guillard 1975). The cultures were diluted three times per week to keep the algae in an exponential growth stage.

Appendicularian houses were collected from a culture of *O. dioica*, kept in the laboratory at DTU (Lombard and Kjørboe 2010). The houses were collected shortly after they had been produced, using a wide-mouth pipette and with help of illumination from below the culture containers. The houses used in experiments were > 1 -h old (see Lombard and Kjørboe 2010). In situ houses were collected from a zip-on 3-L Plexiglass cod end after a surface tow with a 200- μm net, using a pointed light source and wide-mouth pipettes. Algae and detritus aggregates from the Iceland Sea and PAP site were collected from a marine snow catcher (Cavan et al. 2017), while *Trichodesmium* sp. filaments both at the PAP site and Sargasso Sea were collected from plankton tows. The algae aggregates from the Iceland Sea consisted mainly of a mixture of *Phaeocystis* sp. and diatoms. The algae aggregates originating from a mesocosm were produced by incubating the water from two different mesocosms in rotating containers (Moriceau et al. 2018). The plankton community in both mesocosms was dominated by diatoms and dinoflagellates, with smaller contributions from other algal groups. The aggregates were collected on day 17 of the mesocosm experiment, at the time when the chlorophyll *a* (Chl *a*) had stagnated and the algae, therefore, had likely reached a stationary growth phase (Moriceau et al. 2018). Diatom and cyanobacteria

Table 1. Experimental set up. Origin and density of copepods in experiments, type and concentration of aggregates (in aggregates L⁻¹ and in µg C L⁻¹), estimated reduction in aggregate concentration during the experiments (%), incubation temperature (T; °C), and number of replicate experiments (n). (a) Experiments with multiple aggregates; (b) experiments with single aggregates. Settled houses indicate the experiments where the appendicularian houses were allowed to settle on the bottom of the vials, all other incubations were conducted in rotating bottles (see Methods section). Algae refer to aggregates that were formed from natural phytoplankton, and thus consisted of several algal species or groups (see Table 2). (Houses) appendicularian houses, (Tricho) *Trichodesmium* sp., (TW) *T. weissflogii*, (TP) *Thalassiosira pseudonana*, (NOD) *Nodularia* sp., (APH) *Aphanizomenon flos-aquae*.

ID (#)	Species	Copepod			Aggregate			Reduction (%)	T	n	Time and place
		Origin	Density (Ind. L ⁻¹)	Type	Abundance (# L ⁻¹)	Concentration (µg C L ⁻¹)	(%)				
(a) Experiments with multiple aggregates											
1	<i>M. norvegica</i>	Godhåbsfjord (SW Greenland)	83	Settled houses	0, 17, 50, 83, 167	0, 32, 96, 160, 321	0–3	6	3	DTU; 2008	
2	<i>M. norvegica</i>	Godhåbsfjord	83	Houses	0, 10, 33, 50, 83	0, 19, 64, 96, 160	1–7	15	1–2	DTU; 2008	
3*	<i>M. norvegica</i>	North Sea	40–80	Houses	0, 20	0, 39	1–4	15	3–4	R/V Dana; Jul 2005	
4	<i>M. norvegica</i>	Iceland Sea	133	Algae	0, 13, 40, 67, 167, 220	0, 6, 18, 30, 76, 100	26–79	8	2–3	RV meteor; cruise #M87-1; 2012	
5	<i>M. norvegica</i>	Iceland Sea	133	Detritus	0, 28, 83, 139, 277	0, 28, 83, 139, 277	4–38	8	2–3	RV meteor	
6†	<i>M. norvegica</i>	Porcupine Abyssal Plain (PAP)	93	Detritus	0, 13, 40, 90, 157	0, 317, 951, 2140, 3720	0–2	12	2	RSS James cook; cruise #87; 2013	
7‡	<i>M. norvegica</i>	PAP site	107	Tricho	0, 14, 43, 71, 129	0, 1870, 5610, 9350, 18,710	<0.1	12	2–3	RSS James cook	
8	<i>Oncaea</i> spp.	Godhåbsfjord	107	Houses	0, 17, 33, 50, 83	0, 33, 66, 99, 165 (1.9–6.2)	2–6	15	2	DTU; 2008	
9	<i>Oncaea</i> spp.	Hopavågen marine lagoon (W Norway)	107	Algae	0, 27, 40, 67, 93, 133	0, 45, 67, 112, 156, 223	2–15	18	2	Sletvig Field Station NTNU; 2012	
10	<i>Oncaea</i> spp.	Hopavågen	107	Algae	27, 40, 67, 93, 133	45, 67, 112, 156, 223	3–20	18	2	Sletvig Field Station	
11	<i>Oncaea</i> spp.	Sargasso Sea	147	Tricho	0, 13, 27, 40, 67, 133	0, 4290, 8590, 12,880, 21,470, 42,940	<0.1	22	2–3	RV Dana; Mar 2014	
12	<i>A. normanni</i>	Culture‡	67	Houses	0, 10, 17, 33, 50, 83	0, 20, 33, 66, 99, 165	2–78	15	6–9	DTU; 2009	
13	<i>A. normanni</i>	Culture	67	Settled houses	17, 33, 83	33, 66, 165	8–36	15	2–3	DTU; 2009	
14	<i>A. normanni</i>	Culture	67	TW	0, 17, 33, 50, 83, 167, 250	0, 4.3, 8.6, 13, 22, 43, 65	5–58	15	3	DTU; 2013	
15	<i>A. normanni</i>	Culture	67	TP	0, 17, 33, 50, 83, 167, 250	0, 6.2, 12, 19, 31, 62, 93	5–44	15	2–3	DTU; 2013	
16	<i>A. normanni</i>	Culture	67	NOD	0, 17, 33, 50, 67, 83, 133, 167, 250	0, 6.7, 13, 20, 27, 34, 54, 67, 101	1–20	15	2–3	DTU; 2013	
(b) Experiments with single aggregates											
17	<i>A. normanni</i>	Culture	67	NOD	1	1.4–101	7–100	15	19	DTU; 2013	
18	<i>A. normanni</i>	Culture	67	TW	1	1.3–72	7–100	15	21	DTU; 2013	
19	<i>A. normanni</i>	Culture	67	APH	1	9.1–17.7	6–100	15	7	DTU; 2013	
20	<i>A. normanni</i>	Culture	67	Houses	1	9.5–48.0	1–30	15	26	DTU; 2013	

*Koski et al. (2007).

†Koski et al. (2020).

‡Copepods were originally collected in Gullmarsfjord, Western Sweden, but were cultured in the laboratory for > 100 generations.

Table 2. Aggregate characteristics. Type, origin, volume (mm^3 ; mean \pm SD), Chl *a* content ($\mu\text{g aggregate}^{-1}$), and carbon content ($\mu\text{g C aggregate}^{-1}$) of the different types of aggregates used in experiments. The carbon content is either calculated from the volume according to Allredge (1998), or from the Chl *a* content using the Chl *a* to carbon ratio from Roman (1978). The volumes of detritus aggregates from the PAP site and *Trichodesmium* tufts (printed in italics) were estimated based on the calculated carbon concentrations, using Allredge (1998). The numbers of measured aggregates are indicated in parenthesis. ID refers to the numbers of experiments as listed in Table 1.

ID (#)	Type	Origin	Volume (mm^3)	Chl <i>a</i> (μg)	Carbon ($\mu\text{g C}$)
(a) Experiments with multiple aggregates					
1–3, 8, 12–13	Houses	DTU; laboratory	4.3 \pm 3.2(30)		1.9
4	Algae (<i>Phaeocystis</i> spp. and diatoms)	Iceland Sea	0.36 \pm 0.85(48)		0.42
9, 10	Algae (diatoms and dinoflagellates)	Mesocosm	2.4 \pm 4.3(30)		1.5
5	Detritus	Iceland Sea	5.4 \pm 5.5(55)		2.1
6	Detritus	PAP site	396	0.17(1)	22.2
7	<i>Tricho</i>	PAP site	12,025	0.97(1)	131
11	<i>Tricho</i>	Sargasso Sea	3680	0.42 \pm 0.12(5)	70.8
14	TW	DTU; laboratory	0.08 \pm 0.07(19)		0.26
15	TP	DTU; laboratory	0.15 \pm 0.29(19)		0.37
16	NOD	DTU; laboratory	0.18 \pm 0.41(10)		0.40
(b) Experiments with single aggregates					
17	NOD	DTU; laboratory	0.01–32.4(16)		0.08–6.0
18	TW	DTU; laboratory	0.01–17.0(20)		0.08–6.0
19	APH	DTU; laboratory	0.3–1.6(7)		0.5–1.3
20	House	DTU; laboratory	0.2–25.8(24)		0.5–5.4

APH, *Aphanizomenon flos-aquae*; Houses, appendicularian houses; NOD, *Nodularia* sp.; TP, *Thalassiosira pseudonana*; *Tricho*, *Trichodesmium* sp.; TW, *Thalassiosira weissflogii*.

aggregates were produced from senescent cultures of *T. weissflogii*, *Thalassiosira pseudonana*, *Nodularia* sp., and *Aphanizomenon flos-aquae*, by slowly rotating (at 0.5 RPM) a high concentration of algae ($> 1000 \mu\text{g C L}^{-1}$) in air-tight closed bottles in a rolling table. Aggregates of different sizes started forming after 1–2 d of rotation. The algae were grown in batch cultures with 16 : 8 h light : dark cycle, at 18°C. *T. weissflogii* and *T. pseudonana* were grown in F/2 (+ Si) media, at the salinity of ca. 30 ppm. *Nodularia* sp. and *A. flos-aquae* were grown under brackish (ca. 5 ppm) salinity, using a modified Z8 media (Lehtimäki et al. 1994). *Nodularia* sp. is known to produce the toxin nodularin, while *A. flos-aqua* strain was non-toxic. The aggregates thus differed in quality and included both aggregates that consisted mainly of a single algae (experiments 7, 11, 14–19) and a mixture of algae (experiments 4, 9, 10), as well as appendicularian houses (experiments 1–3, 8, 12–13), and detritus aggregates (experiments 5–6) that likely contained both algal remains and a microbial community (Table 1).

Aggregate volumes were estimated by measuring the main dimensions of the aggregates, using a stereo microscope with a precision of 19.6 μm , and an appropriate geometric formula. Seven to fifty-five aggregates of diverse types were measured in each experiment. The aggregates were grouped roughly into three size classes, whereas most algal aggregates were small (0.1–0.4 mm^3), appendicularian houses, algal aggregates originating

from a mesocosm, and detritus aggregates from the Iceland Sea were ca. 10 times larger (2.4–5.4 mm^3) and *Trichodesmium* tufts and detritus aggregates from PAP site were ca. 100 times larger ($> 400 \text{mm}^3$; Table 2). Aggregate volumes were converted to carbon according to Allredge (1998). *Trichodesmium* spp. tufts and detritus aggregates from PAP site were not measured, but their carbon content was estimated based on Chl *a*. For this, groups of ca. 40 aggregates were pipetted onto GF/F filters (after rinsing them carefully in filtered seawater) and extracted in acetone for 24 h. The Chl *a* was converted to carbon using the chl : carbon ratio of Roman (1978).

Set up

Incubations were conducted either with increasing numbers of aggregates (experiments 1–16) or with increasing size of a single aggregate (experiments 17–20), with typically 2–3 replicate experiments for each aggregate type and density (Table 1). The aggregate numbers ranged from zero to 280 L^{-1} , which in most experiments corresponded to carbon concentrations ranging from zero to 320 $\mu\text{g C L}^{-1}$ or volume concentrations from zero to 700 ppm, with the exception of *Trichodesmium* spp. tufts where the large size of the tufts resulted in peak carbon concentrations $> 18 \text{mg C L}^{-1}$ (Table 1). The lower half of these concentrations are within the range of the peak aggregate abundances and concentrations that have been measured in, e.g., Baltic Sea (28 aggregates L^{-1} ;

Möller et al. 2012), North Sea (130 ppm; Tiselius and Kuylenstierna 1996), and northeast Atlantic (50–100 ppm or 12 aggregates L⁻¹; Lampitt et al. 1993a). Most experiments were conducted in a rotating plankton wheel turning at a speed of 1 RPM, which kept most of the aggregates suspended in the water. However, in experiments 1 and 13, the bottles were not rotated and the aggregates were allowed to settle to the bottom of the container.

The set up was similar in all experiments. Adult females or late copepodites (CIV-V) were collected from the culture flasks, and starved in <0.2 μm filtered seawater for a minimum of 12 h before the start of the incubations. After the starvation period, 5–15 females or late copepodites were individually sorted out, placed into 70- or 100-mL flat tissue culture flasks containing filtered seawater and the target concentration of aggregates, and the flasks were closed air-tight and placed into the plankton wheel. The aggregates were added to the bottles individually using a wide-mouthed pipette: aggregates with roughly similar sizes were selected for experiments 1–16, whereas as many aggregate sizes as possible were used for experiments 17–20. After 24 h, the contents of the bottles were carefully filtered onto a 15-μm net and flushed into Petri dishes (*A. normanni*) or poured directly into Petri dishes (*M. norvegica* and *Oncaea* spp.). Copepods were counted and their condition (dead/alive) was checked, and their prosome (*A. normanni* and *Oncaea* spp.) or total (*M. norvegica*) lengths were measured using a stereo microscope with 18-μm precision. The numbers of pellets were counted using a stereo microscope. In most experiments, the food concentration was not likely to decrease below ca. 40% of the original concentration (estimated based on the ingestion rates and densities of copepods), and aggregates or their remains were visible in the bottles after the incubations. However, in incubations where copepod ingestion rates were highest (see Results section), > 50% of the original food source was consumed (Table 1), and the ingestion rates should therefore be considered as conservative estimates.

The possible occurrence of coprophagy was tested by plotting the saturated PP (in numbers of pellets ind.⁻¹ d⁻¹) as a function of copepod density in incubations (copepods L⁻¹), assuming that if coprophagy was important, the PP would be density-dependent and decrease at higher copepod densities. There was no relationship between the PP and copepod density in incubations with *A. normanni* and *Oncaea* spp. (linear regression; $p > 0.05$), whereas the PP of *M. norvegica* tended to increase with increasing copepod density (linear regression; $R^2 = 0.39$; $p < 0.01$; data not shown). Therefore, coprophagy was assumed not to be important in the current experiments.

Analysis

Quantifying the feeding rates of small copepods that will only ingest part of an aggregate is complicated, since their activity does not necessarily result in a measurable reduction of aggregate concentration or volume in experiments. We, therefore, used the PP of copepods as an indirect measure of

ingestion, and existing empirical relationships between ingestion and fecal PP on diatom diets to convert PP to ingestion. PP was converted to ingestion using the linear regression between the PP (in pellets ind.⁻¹ d⁻¹) and carbon ingestion (I ; μg C ind.⁻¹ d⁻¹; Supporting Information Fig. S1). The regression relationship for *A. normanni* ($R^2 = 0.63$; $p < 0.0001$; $n = 139$) was based on data obtained at 15°C with the same laboratory culture as used in the present experiments, whereas the regression for *M. norvegica* was obtained for copepods collected from a north Norwegian fjord Balsfjord ($R^2 = 0.51$; $p < 0.0001$; $n = 52$; Koski et al. unpubl.). The diatom *T. weisslogii* was used as food in both experiments. The regression relationship of *M. norvegica* was also used for *Oncaea* spp. The ingestion rates corresponding to the maximum PP rates obtained through these regressions were compared to maximum ingestion rates estimated assuming that weight-specific PP would account for 20–40% of the weight-specific ingestion as estimated for diverse copepod species and diets by Debs (1984), Dutz et al. (2008), and Franco-Santos et al. (2018). However, this percentage depends on food concentration and quality, with diatoms inducing proportionally higher PP than, e.g., flagellates (Besiktepe and Dam 2002), which could result in an overestimation of feeding on diatom-dominated aggregates, although the ingestion rates estimated by different methods agreed in most cases reasonably well (Supporting Information Table S1).

To estimate the weight-specific PP, the pellet volumes of *M. norvegica* and *Oncaea* spp. were estimated based on the linear regression between copepod body size and pellet size from a data set including both *M. norvegica* and *Oncaea* spp. (Uye and Kaname 1994). The pellet volume of *A. normanni* was estimated based on the measured lengths and widths of 53–211 pellets and assuming a cylindrical shape. The pellet volume was 247,300 μm³ when feeding on appendicularian houses, 301,700 μm³ when feeding on diatoms, and 193,900 μm³ when feeding on cyanobacteria aggregates (Supporting Information Table S1). Pellet volume was converted to carbon by averaging the carbon to volume ratios of Ploug et al. (2008) and Svensen et al. (2014). Body sizes of *M. norvegica* and *Oncaea* spp. were obtained from measurements conducted in the study area at the time of the copepod collection or from measurements in nearby areas (Supporting Information Table S1) and converted to carbon using the length-weight regressions of Uye et al. (2002) and Satapoomin (1999), respectively. The body size of *A. normanni* in earlier experiments was measured to be 2.2 μg C ind.⁻¹ (Koski et al. 2005).

Ingestion rates obtained through regressions were plotted against the aggregate concentration (in carbon), and the Holling II (Holling 1965) equation was used to estimate the search volume rate (β), handling time (τ), and maximum ingestion rate (i)

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

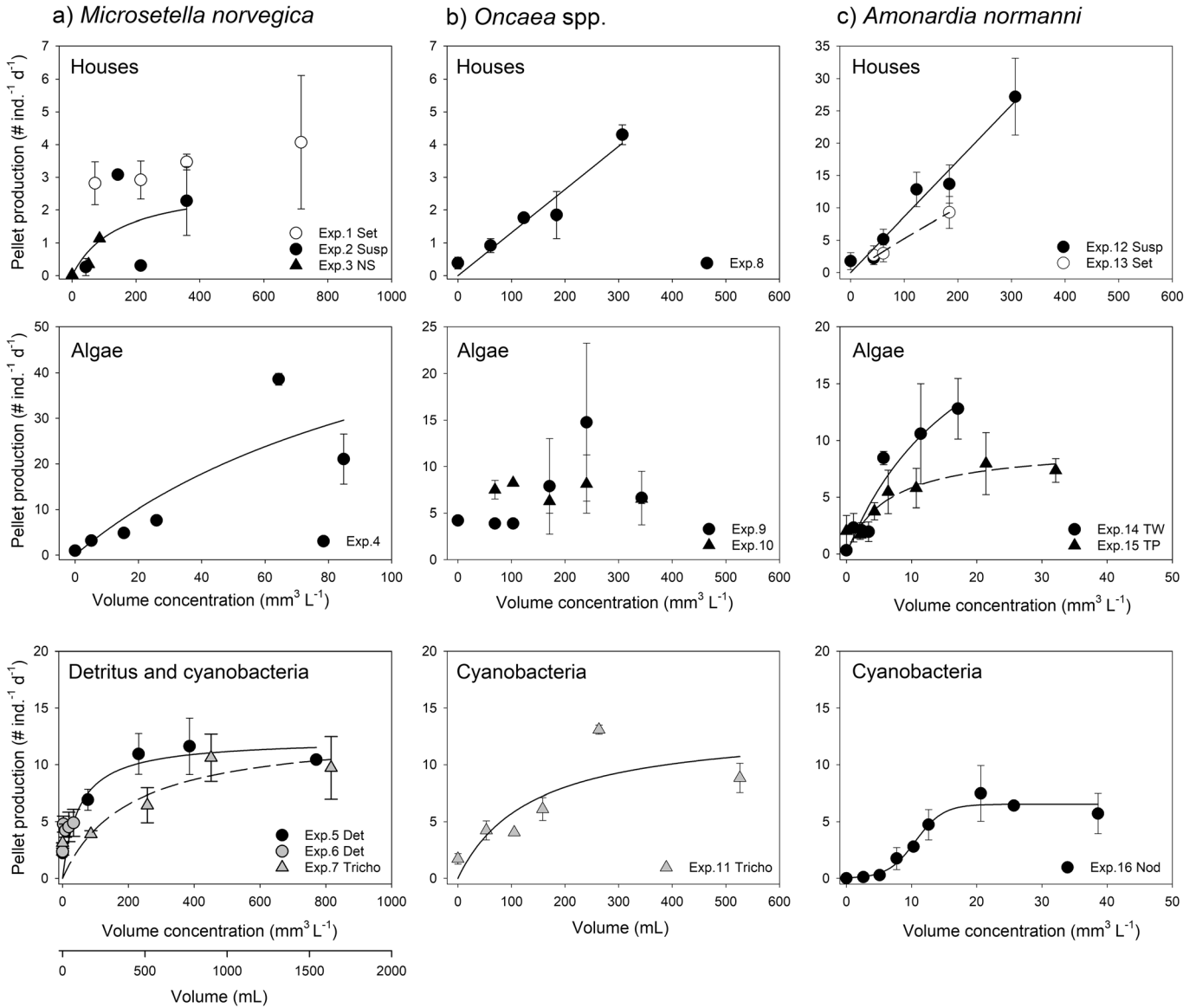


Fig. 1. Functional response of (a) *M. norvegica*, (b) *Oncaea* spp., and (c) *A. normanni* pellet production (pellets ind.⁻¹ d.⁻¹; mean ± SE) on increasing aggregate volume (mm³ L⁻¹). Different symbols represent different experiments (cf. Table 1). (Susp) aggregates that were suspended in the water, (Set) aggregates that were allowed to settle on the bottom of the incubation flasks, (NS) aggregates collected from the North Sea, (Tricho) *Trichodesmium* sp. aggregates, (Det) detritus aggregates, (TW) aggregates made from *T. weissflogii*, (TP) aggregates made from *Thalassiosira pseudonana*, and (Nod) aggregates made from *Nodularia spumigena*. The gray symbols indicate the experiments where the aggregate volume is presented in the secondary x-axis (experiments 6 and 7). The lines indicate the significant Holling II or III (*A. normanni* on *Nodularia* sp. aggregates) types of functional responses that were fitted to the observations. Note that the figure presents the pellet production and aggregate volume and not the rates converted to carbon, which were used to calculate the parameters in Table 3. Note different scales of the axis.

C_{prey} is the aggregate concentration. If the regression was not significant, the ingestion rate was estimated as the average of all ingestion rates in that experiment (excluding filtered seawater), and the handling time was estimated as an inverse of that rate. We also fitted all data with the Holling III type of functional response according to

$$i = \alpha \beta e^{1 - \frac{\alpha}{C_{prey}}} \quad (2)$$

where α is the threshold food concentration for feeding. For most of the copepod-aggregate combinations, no obvious feeding threshold was detected and Holling 2 resulted in higher or similar R^2 as Holling 3, although both functions gave roughly similar maximum ingestion rates. Holling 2 was therefore used to estimate the search volume rates and handling times for most copepod-aggregate combinations. The exception to this was *A. normanni* feeding on *Nodularia*

Table 3. Parameters from the functional responses of copepod feeding. Search volume rates (β ; mL ind.⁻¹ d⁻¹), handling times (τ ; days) and maximum ingestion rates (I_{\max} ; $\mu\text{g C ind.}^{-1} \text{d}^{-1}$) of the three copepod species on different types of aggregates. Adjusted R^2 , level of significance and numbers of observations (in parenthesis) from the Holling 2 or Holling 3 (only *Amonardia* on *Nodularia* sp.) types of functional responses relating ingestion rates ($\mu\text{g C ind.}^{-1} \text{d}^{-1}$) to aggregate concentrations ($\mu\text{g L}^{-1}$) are indicated in the table. (°), (*), (**), and (***) significant at the level of $p < 0.1$, < 0.05 , < 0.01 , and < 0.001 , respectively. (NS) not significant ($p > 0.1$). The maximum ingestion rates in experiments where the equation was not significant represent the mean (\pm SD) of all measured rates in the presence of aggregates, and the handling time is calculated as the inverse of the maximum ingestion rate. The value in parenthesis after β (*Amonardia* on *Nodularia* sp.) indicates the threshold food concentration for feeding (in $\mu\text{g L}^{-1}$).

Copepod	Aggregate	β (mL ind. ⁻¹ d ⁻¹)	τ (d)	I_{\max} ($\mu\text{g C ind.}^{-1} \text{d}^{-1}$)	R^2
<i>M. norvegica</i>	Houses	0.74	32.6	0.04	0.37* (9)
	Settled houses			0.04 \pm 0.02	NS (12)
	Algae	4.2	1.2	0.85	0.73*** (16)
	<i>Tricho</i>	0.03	8.2	0.13	0.40* (12)
	Detritus (Iceland Sea)	0.01	8.4	0.12	0.65*** (12)
	Detritus (PAP)		23.2	0.04 \pm 0.03	NS (10)
<i>Oncaea</i> sp.	Houses	0.3	3.2	0.32	0.78*** (10)
	Algae		13.7	0.07 \pm 0.06	NS (12)
	Algae		14.0	0.07 \pm 0.02	NS (10)
	<i>Tricho</i>	0.02	8.6	0.12	0.58** (12)
<i>A. normanni</i>	Houses	5.5	0.08	13.2	0.44*** (46)
	Settled houses	2.2	0.76	1.3	0.68* (7)
	TW	9.9	0.78	1.3	0.64*** (21)
	TP	11.7	3.2	0.31	0.44** (20)
	NOD	3.1 (33.9)	9.5	0.10	0.65*** (26)

APH, *Aphanizomenon flos-aquae*; Houses, appendicularian houses; NOD, *Nodularia* sp.; TP, *Thalassiosira pseudonana*; *Tricho*, *Trichodesmium* sp.; TW, *Thalassiosira weissflogii*.

sp. aggregates, where Holling 3 resulted in a better fit. The maximum ingestion rate for *A. normanni* on *Nodularia* sp. was calculated as $\alpha\beta e^1$ and handling time was assumed to be an inverse of this rate (Almeda et al. 2018). In most cases, the maximum ingestion rates estimated by the model corresponded well to the maximum rates measured in experiments. The exceptions to this were experiments where feeding did not saturate, including *M. norvegica* on algal aggregates, and *Oncaea* spp. and *A. normanni* on appendicularian houses (Fig. 1; Supporting Information Table S1).

PP was tested for differences between copepod species, aggregate types, and aggregate numbers using a three-way ANOVA, and between aggregate types and numbers using a two-way ANOVA, separately for each species. The aggregate numbers for the analysis were pooled into four groups (10–20, 30–50, 80–120, and > 120 aggregates L⁻¹). However, since all copepod-aggregate type-aggregate number combinations were not represented, the degrees of freedom in the analysis varied (Supporting Information Table S2). All data were tested for normality and equal variance, and if the conditions were not met, square-root transformed. A link with access to all data along with associated metadata will be available from <https://ecotip-geo.iopan.pl/geonetwork/>.

Results

Differences between species, aggregate types, and aggregate numbers

The three copepod species differed in their response to aggregate types, although the PP of all species increased with increasing aggregate numbers (three-way ANOVA, $F_{2,149} = 19.1$; $p < 0.001$; Fig. 1; Supporting Information Table S2). *M. norvegica* had significantly different PP rates both between different types of aggregates and between different aggregate numbers, with a significant interaction between the two (two-way ANOVA, $F_{12,47} = 4.7$; $p < 0.001$; Supporting Information Table S2). Thus, different aggregate types induced a different shape of the functional response (Fig. 1a). Typically, PP on appendicularian houses was lower than on other aggregate types, while the PP on algal aggregates was higher (Tukey HSD; $p < 0.05$; Fig. 1a).

The maximum ingestion rates of *M. norvegica* on appendicularian houses, detritus, and *Trichodesmium* aggregates were ca. 0.04–0.13 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$, and the maximum ingestion rate on algal aggregates was ca. 0.85 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ (Table 3). This corresponded to weight-specific ingestion rates of 0.2–0.7 $\mu\text{g C} (\mu\text{g C})^{-1} \text{d}^{-1}$ on houses, detritus, and *Trichodesmium* and 4.6 $\mu\text{g C} (\mu\text{g C})^{-1} \text{d}^{-1}$ on algal aggregates (Supporting Information Table S1). The ingestion rates were

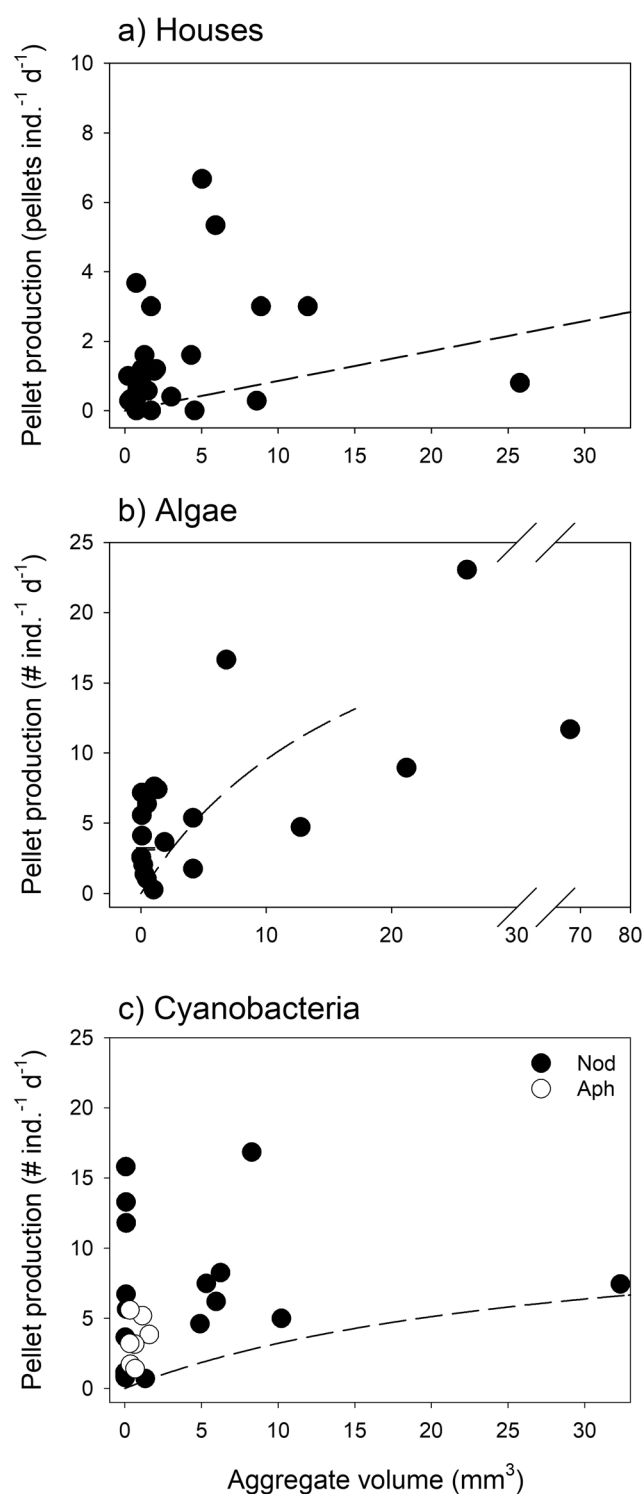


Fig. 2. Pellet production (pellets ind.⁻¹ d⁻¹; mean \pm SE) of *A. normanni* as a function of aggregate volume (mm³) in incubations with single (a) appendicularian house, (b) cyanobacteria aggregate, or (c) diatom aggregate, compared to the functional response of the pellet production in incubations with increasing aggregate numbers (cf. Fig. 1; dashed line). (Open symbols; APH) *A. flos-aquae*, (closed symbols; NOD) *Nodularia* sp. Note different scales of the axis.

estimated based on the regression between PP and ingestion, and the ingestion rates were estimated assuming that weight-specific PP corresponded to 20–40% of the weight-specific ingestion, deviated by ca. 30% (Supporting Information Table S1). The search volume rate (β) was low irrespective of the aggregate type, ranging from 0.01 to 4.2 mL ind.⁻¹ d⁻¹, and the handling times of 1–23 d suggested that one *M. norvegica* individual would be unlikely to consume a whole aggregate of a size used in these experiments (> 0.4 mm³). The low search volume rate with *Trichodesmium* spp. aggregates was likely an artifact due to the large size and correspondingly high-carbon content in the incubations with these aggregates.

Also the PP of *Oncaea* spp. differed between the aggregate types and aggregate numbers (two-way ANOVA; $F_{3,31} = 12.2$; $p < 0.001$), but there was no significant interaction between the two (Supporting Information Table S2). The PP rates of *Oncaea* spp. on appendicularian houses and *Trichodesmium* spp. tufts were higher than the PP on the two types of algae aggregates (Tukey HSD; $p < 0.001$; Fig. 1b). The maximum ingestion rate varied from 0.07 to 0.32 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ (0.06–0.24 $\mu\text{g C} (\mu\text{g C})^{-1} \text{d}^{-1}$) with a similarly low search volume rate and long handling time as with *M. norvegica* (Tables 3, S1). Similar to the experiments with *M. norvegica*, the extremely low search volume rate with *Trichodesmium* spp. aggregates was likely due to the high-carbon concentrations of aggregates in the incubations. The ingestion rates estimated by the two methods deviated by 20–40% (Supporting Information Table S1).

A. normanni had the highest PP rate on appendicularian houses, which at 27 ± 18 pellets ind.⁻¹ d⁻¹ was > 2 times higher than the peak PP on other types of aggregates (Fig. 1c). These high PP rates were only obtained when the aggregates were suspended in the water column, while the PP rates of the aggregates that were allowed to settle on the bottom of the experimental flasks were ca. half of the rates of the suspended aggregates. There was no significant interaction between the aggregate type and aggregate number (Supporting Information Table S2), suggesting that *A. normanni* PP responded to increasing amounts of aggregates in a similar way with all aggregate types. The high PP on suspended houses was mainly due to efficient feeding and the absence of saturation in the investigated concentration range (Fig. 1c; Table 3), rather than due to the differences in search volume rate, which ranged from ca. 4 to ca. 12 mL ind.⁻¹ d⁻¹ among all suspended aggregates. The non-saturated feeding also resulted in a high maximum ingestion rate of 13.2 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ (6.0 $\mu\text{g C} (\mu\text{g C})^{-1} \text{d}^{-1}$), whereas the maximum ingestion rate on other types of aggregates ranged from 0.1 to 1.3 $\mu\text{g C ind.}^{-1} \text{d}^{-1}$ (0.05–0.6 $\mu\text{g C} (\mu\text{g C})^{-1} \text{d}^{-1}$; Tables 3, S1). In contrast to all other copepod-aggregate combinations, the functional response of *A. normanni* PP on cyanobacteria aggregates followed Holling-III-type sigmoidal response, with a feeding threshold that was estimated to be ca. 34 $\mu\text{g C L}^{-1}$ (Fig. 1c).

Table 4. Maximum ingestion (I; $\mu\text{g C ind.}^{-1} \text{d}^{-1}$) and weight-specific ingestion (W-spec I; $\mu\text{g C } (\mu\text{g C})^{-1} \text{d}^{-1}$) rates of (a) *M. norvegica*, (b) *Oncaea* spp., and (c) *A. normanni* estimated using different methods and compared to previous estimates. The body weight ($\mu\text{g C ind.}^{-1}$) that was used to estimate the weight-specific ingestion rate is indicated in parenthesis. All reported rates are for late copepodites (CIV-V) or adults. If not reported in the original article, the respiration rate was converted to carbon ingestion by using the respiratory quotient of 0.97 (Ikeda et al. 2001). To estimate the ingestion based on reproduction, we used the maximum reproduction rate reported in the article, assuming a gross growth efficiency of 0.3 (Straile 1997).

I	W-spec. I (weight)	Method	T	Food	Reference
(a) <i>M. norvegica</i>					
0.04–0.85	0.19–4.6(0.18–0.21)	Pellet production	6–17	Aggregates	This study
0.08	0.46(0.18)	Size-based I (model)	15	Diverse	Kjørboe and Hirst (2014)
0.05	0.30(0.18)	Size and T-based R (model)	15	Diverse	Ikeda et al. (2001)
0.6	1.5(0.4)	Particle disappearance	15	<i>T. weissflogii</i>	Koski et al. (2005)
0.43	2.4(0.18)	Gut Chl	12	In situ	Koski et al. (2020)
0.24	1.3(0.18)	Respiration	12	FW	Koski et al. (2020)
0.26	0.26(1.0)	Reproduction	20	In situ	Uye et al. (2002)
0.31	1.53(0.20)	Reproduction	4	In situ	Koski et al. (2021)
0.48	0.20(0.42)	Reproduction	8	In situ	Barth-jensen et al. (2020)
(b) <i>Oncaea</i> spp.					
0.07–0.32	0.06–0.24(0.5–2.0)	Pellet production	15–22	Aggregates	This study
0.63	0.63(1.0)	Size-based I (model)	15	Diverse	Kjørboe and Hirst (2014)
0.19	0.19(1.0)	Size and T-based R (model)	15	Diverse	Ikeda et al. (2001)
3.2	0.87(2.8)	Particle disappearance	20	<i>Gymnodinium</i>	Paffenhöfer (1993)
4.66	3.01(1.55)	Chl disappearance	0	<i>Phaeocystis</i>	Metz (1998)
0.18	0.25(0.71)	Gut chl	12	In situ	Koski et al. (2020)
0.32	0.45(0.71)	Respiration	12	FW	Koski et al. (2020)
0.012	0.015(0.8)	Respiration	3	In situ	Nishibe and Ikeda (2008)
1.33	2.90(0.46)	Carbon demand	18	Aggregates	Koski et al. (2017)
0.04	0.24(0.16)	Reproduction	23	In situ	Böttger-Schnack and Schnack (2005)
0.07–0.09	0.10–0.13(0.70)	Reproduction	>21	In situ	De Melo et al. (2021)
(c) <i>A. normanni</i>					
0.1–13.2	0.05–6(2.2)	Pellet production	15	Aggregates	This study
2.0	0.91(2.2)	Particle disappearance	15	<i>T. weissflogii</i>	Koski et al. (2005)
0.88–1.2	0.90(0.84)	Particle disappearance, gut chl, pellet production	20	<i>Nitzschia</i> sp.	Souza-Santos and Castel (2013)

R, Respiration; T, temperature.

Effect of aggregate size and aggregate number

The PP rates of *A. normanni* were linearly related to aggregate size when the copepods were feeding on single diatom aggregates, although aggregate volume only explained 26% of the variation in PP ($[\text{pellets ind.}^{-1} \text{d}^{-1}] = 5.08 + 0.18 V [\text{mm}^3]$; $R^2 = 0.26$, $n = 20$, $p < 0.05$; Fig. 2). No relationship between PP and aggregate size was evident when copepods were feeding on appendicularian houses or cyanobacteria aggregates ($p > 0.05$). In most cases, and particularly on cyanobacteria aggregates, *A. normanni* appeared to have higher PP rates when feeding on a single aggregate (Fig. 2), compared to the same aggregate volume divided between several aggregates (Fig. 1), suggesting that *A. normanni* remained in the aggregate that it found first. The response of *A. normanni* was not influenced by the cyanobacteria species, although *Nodularia* sp. was toxic and *A. flos-aquae* was not. No relationship was

observed between the encounter rate, handling time, or maximum ingestion rate of any of the copepods and the average aggregate volume (Spearman rank correlation; $p > 0.05$; data not shown).

Discussion

Species-specific feeding rates and aggregate preferences

Our results demonstrated that all types of aggregates could be ingested by copepods, but that the ingestion rates varied over two orders magnitude, from $< 0.1 \mu\text{g C ind.}^{-1} \text{d}^{-1}$ to $> 10 \mu\text{g C ind.}^{-1} \text{d}^{-1}$, depending on the aggregate type and copepod species. Pelagic *M. norvegica* appeared best fit to feed on algal aggregates, whereas semi-benthic *A. normanni* preferred appendicularian houses, and *Oncaea* spp. appeared to have a broad diet.

The maximum ingestion rates of *M. norvegica* on houses, detritus, and *Trichodesmium* aggregates were relatively similar to rates that can be estimated based on size-specific ingestion according to Kiørboe and Hirst (2014) or temperature- and size-specific respiration according to Ikeda et al. (2001; Table 4). In contrast, the maximum ingestion rate on algae aggregates was substantially higher than what would be expected based on allometric relations, and more similar to the ingestion rates estimated based on reproduction rate, gut chlorophyll content, or particle disappearance (Table 4). Although the model estimates of maximum ingestion rates on algal aggregates were likely overestimated, with weight-specific rates that exceeded the body size of *M. norvegica* several times, it was clear that *M. norvegica* had substantially higher ingestion rates on algal aggregates than on other types of aggregates. The observations of high *M. norvegica* abundances in areas with high algal biomass (Ohtsuka et al. 1993; Uye et al. 2002), reproductive peak during and right after arctic spring bloom (Svensen et al. 2018), and a high gut Chl *a* content (Koski et al. 2020) suggest that *M. norvegica* may be more dependent on phytoplankton than the two other species. In contrast, appendicularian houses seemed to present a challenge for *M. norvegica*, whose feeding might have been hampered by the mucus glue of the houses.

The ingestion rates of *Oncaea* spp. fell within the range of most previously measured values (Table 4), although the studies that have measured ingestion directly using algal cultures as food tended to result in higher rates (Paffenhöfer 1993; Metz 1998). Nevertheless, *Oncaea* spp. appeared to be able to obtain nutrition from aggregates at a similar rate than estimated for diverse other (in situ) food sources (e.g., Böttger-Schnack and Schnack 2005; De Melo et al. 2021), and appeared less dependent on phytoplankton and better able to feed on a gelatinous matrix of the appendicularian houses than *M. norvegica*. *Oncaea* spp. diet includes gelatinous colonies of *Phaeocystis* spp. (Metz 1998), diatoms (Wu et al. 2004), and other crustaceans (Turner 1986; Kattner et al. 2003), indicating that its feeding appendages are suitable for a broad selection of food items. *Oncaea* species in current experiments were not identified, and the species-specific feeding habits could therefore have caused the observed differences in ingestion rates between, e.g., appendicularian houses and *Trichodesmium* tufts. The species used in experiments with houses was likely *Triconia borealis*, which is the dominant oncaeid in the area where copepods for these experiments were collected (Arendt et al. 2013), whereas *O. media* and *O. conifera* were the dominant species during the collection for the experiments with *Trichodesmium* tufts (Koski et al. 2007). Although the feeding behavior of different *Oncaea* spp. on aggregates is not known, the existing studies that have included several *Oncaea* species do not suggest large species-specific differences in the diet (Kattner et al. 2003; Wu et al. 2004). Our experiments indicate that the broad diet of *Oncaea* spp. is likely to include several types of aggregates.

The ingestion rates of *A. normanni* on diatom aggregates were similar to the few earlier measurements of ingestion rates on different diatom species (Sousa-Santoz and Castel 2013; Table 4), whereas the ingestion rates on cyanobacteria aggregates were substantially lower and ingestion rates on appendicularian houses substantially higher. The mouthparts of *A. normanni* could be better adjusted to feed on the gelatinous matrix of appendicularian houses or on the phytoplankton and bacteria trapped in the houses than the mouthparts of the pelagic species. *A. normanni* nauplii seem to be able to feed on bacteria by scratching them from the surface of diatom cells (Decho and Fleeger 1988), and microbial food sources appear in general to be common for benthic harpacticoids (Couch 1989). It could be that benthic or semi-benthic copepods profit from a larger size of an aggregate, and that the feeding rates therefore were higher on appendicularian houses that were > 10 times larger than the phytoplankton aggregates offered to *A. normanni*. *A. normanni* is a substantially slower swimmer than, e.g., *M. norvegica* (Koski et al. 2005), and could therefore prefer to stay in an aggregate once it has found it, as also indicated by the difference in PP rates between the experiments with single aggregates compared to the experiments with multiple aggregates. This is a common behavior for benthic organisms, which might use aggregates as vehicles to return to the sea bed (Shanks and Edmondson 1990).

Zooplankton species that create a feeding current or species that search for their prey, can be characterized as “active feeders” and tend to have a type 3 functional response. In contrast ambush feeders, forms that wait passively for their prey that is captured by fast attacks, can be characterized as “passive feeders” and tend to have a type 2 functional response (Kiørboe 2016; Almeda et al. 2018). For suspension-feeding copepods that feed on motile or non-motile prey that is smaller than themselves, prey density, motility, and predation risk determine which feeding strategy is most efficient, since active feeding is more efficient on non-motile prey and passive feeding is less risky in terms of predation (Almeda et al. 2018). Copepods that feed on aggregates could be considered active feeders, due to their demonstrated ability to search and follow chemical trails to aggregates (Kiørboe 2000; Lombard et al. 2013), but could also follow a passive ambush feeding mode, assuming that aggregates have sufficiently high sinking rates to be encountered efficiently. The observed low search volume rates of 0.1–12 mL ind.⁻¹ d⁻¹ would be realistic if the copepods remain passive in the water column waiting for aggregates, whereas active searching should result in higher search volume rates known from previous studies (Koski et al. 2005). If we estimate the search volume rates that would result in the observed concentrations of harpacticoids in aggregated particles (0.1–1 ind. aggregate⁻¹; Kiørboe 2000) and assume a copepod abundance of 50 ind. L⁻¹, which is the average summer abundance of *M. norvegica* in the surface layer in a subarctic fjord (Svensen et al. 2018), the expected search

volume rates would range from 2 to 20 mL ind.⁻¹ d⁻¹. However, the abundances of aggregate-colonizing copepods are variable, and an abundance of 1–10 ind. L⁻¹ as measured, e.g., in the North Sea (Dugas and Koslow 1984) would suggest much higher search volume rates. The role of the species-specific feeding behavior in copepod-aggregate interactions clearly needs more attention in the future.

Effect of aggregate quality on copepod ingestion rates

The effect of aggregate type seemed generally to override the effect of aggregate size. Besides the differences in copepod behavior or morphology of the feeding appendages, the differences in feeding rates between the aggregate types could have been due to the different nutritional contents of the aggregates. In general, detritus can be expected to be of a poorer nutritional quality than, e.g., living phytoplankton (Roman 1984). Aggregates that have different origin sustain different primary production rates and heterotrophic activity, with both rates being typically higher on diatom aggregates than on appendicularian houses or fecal pellet aggregates (Alldredge and Gotschalk 1989). Also, algal aggregates tend to have lower C : N ratios than appendicularian houses or fecal pellets, although the ratios both vary and overlap (Alldredge 1976; Dilling et al. 1998). The aggregates formed from living phytoplankton cultures or collected from the North Atlantic during the spring bloom were likely to have a high Chl *a* content, ongoing photosynthesis, and a C : N ratio of 5–10 (Brzezinski 1985), whereas the appendicularian houses probably included the algae that the appendicularians had been feeding on (the cryptophyte *Rhodomonas salina*) and bacteria that were trapped in the feeding structures (Alldredge and Gotschalk 1989) and likely had a C : N ratio ranging from 7 to 16 (Alldredge 1976). *Trichodesmium* tufts probably harbored a rich microbial community of bacteria, diatoms, and protozoans (Sheridan et al. 2002) and as N-fixing organisms had a low C : N ratio of 3–5 (Roman 1978). These qualitative differences, however, did not seem to determine the ingestion rates of copepods, which were perhaps more connected to their ability to extract nutrition from different types of aggregates (structure of the mouth parts) or to their swimming behavior, although a low quality of some of the aggregates could also have induced compensatory feeding, as demonstrated by Roman (1984) for detritus.

Effect of aggregate-associated copepods on flux attenuation

Although the effect of fecal PP and vertical migration of large copepods on the efficiency of the biological pump can be estimated (Brun et al. 2019), the effect of small non-calanoid copepods on flux attenuation through their feeding on aggregates has so far not been included in regional or global models of any type. This is clearly a problem considering the potentially high maximum ingestion rates demonstrated in this and previous studies (Table 4). The biomass of,

e.g., *M. norvegica* frequently exceeds 50 mg C m⁻³ (Uye et al. 2002; Arendt et al. 2013) and if we assume its average weight-specific ingestion rate to be 1.3 μg C (μg C)⁻¹ d⁻¹ (the average of present experiments), the population consumption rates of *M. norvegica* alone can reach 64 mg C m⁻³, and a similar rate could be expected for *Oncaea* spp. This exceeds the net primary production in the upper mixed layer in the Western North Atlantic (up to 21 mg C m⁻³; Fox et al. 2020), and, assuming an even distribution of aggregate-colonizing copepods in the upper mixed layer, also exceeds the spring bloom export flux measured at 100 m in the North Atlantic (400–600 mg C m⁻² d⁻¹; Martin et al. 2011). Aggregate-feeding copepods could thus qualify as the “gate keepers” of the vertical flux (Jackson and Checkley 2011).

The feeding rates of aggregate-associated copepods can vary many-fold, depending not only on the species and aggregate type, but also on the environmental conditions or the method that is used to estimate these rates (Table 4). This presents a challenge for modeling, and emphasizes the need for a better understanding of the species-specific feeding behavior of aggregate-colonizing copepods. For instance, the preference of different types of aggregates by different copepod species might modify both the quality and attenuation of vertical flux. Obviously, particle degradation by aggregate-colonizing copepods cannot be ignored if we are to understand the processes influencing the efficiency of the biological pump and predict the future carbon sequestration in the ocean.

References

- Allredge, A. L. 1972. Abandoned larvacean houses: A unique food source in the pelagic environment. *Science* **177**: 885–887. doi:10.1126/science.177.4052.885
- Allredge, A. L. 1976. Discarded appendicularian houses as sources of food, surface habitats, and particulate organic matter in planktonic environments. *Limnol. Oceanogr.* **21**: 14–24. doi:10.4319/lo.1976.21.1.0014
- Allredge, A. L. 1998. The carbon, nitrogen and mass content of marine snow as a function of aggregate size. *Deep-Sea Res. I.* **45**: 529–541. doi:10.1016/S0967-0637(97)00048-4
- Allredge, A. L., and C. C. Gotschalk. 1989. Direct observations of the mass flocculation of diatom blooms: Characteristics, settling velocities and formation of diatom aggregates. *Deep-Sea Res.* **36**: 159–171. doi:10.1016/0198-0149(89)90131-3
- Almeda, R. A., H. van Someren, and T. Kiørboe. 2018. Prey perception mechanism determines maximum clearance rates of planktonic copepods. *Limnol. Oceanogr.* **63**: 2695–2707. doi:10.1002/lno.10969
- Arendt, K. E., T. A. Juul-Pedersen, J. Mortensen, M. Blicher, and S. Rysgaard. 2013. A five years study of seasonal patterns in mesozooplankton community structure in a sub-arctic fjord reveals dominance of *Microsetella norvegica*

- (Crustacea, Copeoda). *J. Plankton Res.* **35**: 105–120. doi:10.1093/plankt/fbs087
- Barth-Jensen, C., M. Koski, Ø. Varpe, P. Glad, O. S. Wangensteen, K. Præbel, and C. Svensen. 2020. Temperature-dependent egg production and egg hatching rates of small egg-carrying and broadcast-spawning copepods *Oithona similis*, *Microsetella norvegica* and *Microcalanus pusillus*. *J. Plankton Res.* **42**: 564–580. doi:10.1093/plankt/fbaa039
- Besiktepe, S., and H. G. Dam. 2002. Coupling of ingestion and defecation as a function of diet in the calanoid copepod *Acartia tonsa*. *Mar. Ecol. Prog. Ser.* **229**: 151–164. doi:10.3354/meps229151
- Bochdansky, A. B., and G. J. Herndl. 1992. Ecology of amorphous aggregations (marine snow) in the northern Adriatic Sea. III. Zooplankton interactions with marine snow. *Mar. Ecol. Prog. Ser.* **87**: 135–146. doi:10.3354/meps087135
- Böttger-Schnack, R. 1995. Summer distribution of micro- and small mesozooplankton in the Red Sea and Gulf of Aden, with special reference to non-calanoid copepods. *Mar. Ecol. Prog. Ser.* **118**: 81–102. doi:10.3354/meps118081
- Böttger-Schnack, R., and D. Schnack. 2005. Population structure and fecundity of the microcopepod *Oncaea bispinosa* in the Red Sea – a challenge to general concepts for the scaling of fecundity. *Mar. Ecol. Prog. Ser.* **302**: 159–175. doi:10.3354/meps302159
- Brun, P., K. Stamieszkin, A. W. Visser, P. Licandro, M. R. Payne, and T. Kiørboe. 2019. Climate change has altered zooplankton-fuelled carbon export in the North Atlantic. *Nat. Ecol. Evol.* **3**: 416–423. doi:10.1038/s41559-018-0780-3
- Brzezinski, M. A. 1985. The Si:C:N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables. *J. Phycol.* **21**: 347–357. doi:10.1111/j.0022-3646.1985.00347.x
- Cavan, E. L., S. A. Henson, A. Belcher, and R. Sanders. 2017. Role of zooplankton in determining the efficiency of the biological carbon pump. *Biogeosci.* **14**: 177–186. doi:10.5194/bg-14-177-2017
- Couch, C. A. 1989. Carbon and nitrogen stable isotopes of meiobenthos and their food resources. *Est. Coast. Shelf Sci.* **28**: 433–441. doi:10.1016/0272-7714(89)90090-5
- Daniels, C. J., A. J. Poulton, M. Esposito, M. L. Paulsen, R. Bellerby, M. St John, and A. P. Martin. 2015. Phytoplankton dynamics in contrasting early stage North Atlantic spring blooms: Composition, succession, and potential drivers. *Biogeosci.* **12**: 2395–2409. doi:10.5194/bg-12-2395-2015
- De Melo, M. J., L. K. Miyashita, and R. M. Lopes. 2021. A 3-year study of the seasonal variability of abundance, biomass and reproductive traits of *Oncaea venusta* (Copepoda, Oncaeidae) in a subtropical coastal area. *J. Plankton Res.* **43**: 751–761. doi:10.1093/plankt/fbab055
- Debs, C. A. 1984. Carbon and nitrogen budget of the calanoid copepod *Temora stylifera*: Effect of concentration and composition of food. *Mar. Ecol. Prog. Ser.* **15**: 213–223. doi:10.3354/meps015213
- Decho, A. W., and J. W. Fleeger. 1988. Ontogenetic feeding shifts in the meiobenthic harpacticoid copepod *Nitocra lacustris*. *Mar. Biol.* **97**: 191–197. doi:10.1007/BF00391302
- Dilling, L., and A. L. Alldredge. 2000. Fragmentation of marine snow by swimming zooplankton: A new process impacting carbon cycling in the ocean. *Deep-Sea Res. I* **47**: 1227–1245. doi:10.1016/S0967-0637(99)00105-3
- Dilling, L., J. Wilson, D. Steinberg, and A. Alldredge. 1998. Feeding by the euphausiid *Euphasia pacifica* and the copepod *Calanus pacificus* on marine snow. *Mar. Ecol. Prog. Ser.* **170**: 189–201. doi:10.3354/meps170189
- Dugas, J. C., and J. A. Koslow. 1984. *Microsetella norvegica*: A rare report of a potentially abundant copepod on the Scotian shelf. *Mar. Biol.* **84**: 131–134. doi:10.1007/BF00392997
- Dutz, J., M. Koski, and S. H. Jónasdóttir. 2008. Copepod reproduction is unaffected by diatom aldehydes or lipid composition. *Limnol. Oceanogr.* **53**: 225–235. doi:10.4319/lo.2008.53.1.0225
- Fox, J., and others. 2020. Phytoplankton growth and productivity in the Western North Atlantic: Observations of regional variability from the NAAMES field campaigns. *Front. Mar. Sci.* **7**: 24. doi:10.3389/fmars.2020.00024
- Franco-Santos, R. M., H. Auel, M. Boersma, M. De Troch, C. L. Meunier, and B. Niehoff. 2018. Bioenergetics of the copepod *Temora longicornis* under different nutrient regimes. *J. Plankton Res.* **40**: 420–435. doi:10.1093/plankt/fby016
- Green, E. P., and M. J. Dagg. 1997. Mesozooplankton associations with medium to large marine snow aggregates in the northern Gulf of Mexico. *J. Plankton Res.* **19**: 435–447. doi:10.1093/plankt/19.4.435
- Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In* W. L. Smith and M. H. Chanley [eds.], *Culture of marine invertebrate animals*. Plenum Press. doi:10.1007/978-1-4615-8714-9_3
- Holling, C. S. 1965. The functional response of invertebrate predators to prey density and its role in mimicry and population regulation. *Mem. Ent. Soc. Can.* **97**: 5–60. doi:10.4039/entm9745fv
- Ikeda, T., Y. Kanno, K. Ozaki, and A. Shinada. 2001. Metabolic rates of epipelagic marine copepods as a function of body mass and temperature. *Mar. Biol.* **139**: 587–596. doi:10.1007/s002270100608
- Jackson, G. A., and D. M. Checkley. 2011. Particle size distributions in the upper 100 m water column and their implications for animal feeding in the plankton. *Deep-Sea Res.* **58**: 283–297. doi:10.1016/j.dsr.2010.12.008
- Kattner, G., C. Albers, M. Graeve, and B. Schnack-Schiel. 2003. Fatty acid and alcohol composition of the small polar

- copepods, *Oithona* and *Oncaea*: Indication on feeding modes. *Polar Biol.* **26**: 666–671. doi:10.1007/s00300-003-0540-x
- Kjørboe, T. 2000. Colonization of marine snow aggregates by invertebrate zooplankton: Abundance, scaling, and possible role. *Limnol. Oceanogr.* **45**: 479–484. doi:10.4319/lo.2000.45.2.0479
- Kjørboe, T. 2016. Foraging mode and prey size spectra of suspension-feeding copepods and other zooplankton. *Mar. Ecol. Prog. Ser.* **558**: 15–20. doi:10.3354/meps11877
- Kjørboe, T., and A. Hirst. 2014. Shifts in mass scaling of respiration, feeding and growth rates across life-form transitions in marine pelagic organisms. *Am. Nat.* **183**: E118–E130. doi:10.1086/675241
- Koski, K., T. Kjørboe, and K. Takahashi. 2005. Benthic life in the pelagial: Aggregate encounter and degradation rates by pelagic harpacticoid copepods. *Limnol. Oceanogr.* **50**: 1254–1263. doi:10.4319/lo.2005.50.4.1254
- Koski, M., J. Boutorh, and C. de la Rocha. 2017. Feeding on dispersed vs. aggregated particles: The effect of zooplankton feeding behavior on vertical flux. *PLoS One* **12**: e0177958. doi:10.1371/journal.pone.0177958
- Koski, M., B. Valencia, R. Newstead, and C. Thiele. 2020. The missing piece of the upper mesopelagic carbon budget? Biomass, vertical distribution and feeding of aggregate-associated copepods at the PAP site. *Prog. Oceanogr.* **181**: 102243. doi:10.1016/j.pocean.2019.102243
- Koski, M., R. Swalethorp, S. Kjellerup, and T. G. Nielsen. 2021. Aggregate-colonizing copepods in a glacial fjord: Population dynamics, vertical distribution and allometric scaling of growth and mortality rates of *Microsetella norvegica* and *Oncaea* spp. *Prog. Oceanogr.* **197**: 102670. doi:10.1016/j.pocean.2021.102670
- Koski, M., E. FriisMøller, M. Maar, and A.W. Visser. 2007. The fate of discarded appendicularian houses: degradation by the copepod, *Microsetella norvegica*, and other agents. *J. Plankton Res.* **29**: 641–654. doi:10.1093/plankt/fbm046
- Lampitt, R. S., W. Hillier, and P. Challenor. 1993a. Seasonal and diel variation in the open ocean concentration of marine snow aggregates. *Nature* **362**: 737–739. doi:10.1038/362737a0
- Lampitt, R. S., K. F. Wishner, C. M. Turley, and M. V. Angel. 1993b. Marine snow studies in the Northeast Atlantic Ocean: Distribution, composition and role as a food source for migrating plankton. *Mar. Biol.* **116**: 689–702. doi:10.1007/BF00355486
- Lehtimäki, J., K. Sivonen, R. Luukkainen, and J. Niemelä. 1994. The effects of incubation time, temperature, light, salinity, and phosphorus on growth and hepatotoxin production by *Nodularia* strains. *Arch. Hydrobiol.* **130**: 269–282. doi:10.1127/archiv-hydrobiol/130/1994/269
- Lombard, F., and T. Kjørboe. 2010. Marine snow originating from appendicularian houses: Age-dependent settling characteristics. *Deep-Sea Res. I* **57**: 1304–1313. doi:10.1016/j.dsr.2010.06.008
- Lombard, F., M. Koski, and T. Kjørboe. 2013. Copepods use chemical trails to find sinking marine snow aggregates. *Limnol. Oceanogr.* **58**: 185–192. doi:10.4319/lo.2013.58.1.0185
- Martin, P., R. S. Lampitt, M. J. Perry, R. Sanders, C. Lee, and E. D'Asaro. 2011. Export and mesopelagic particle flux during a North Atlantic spring diatom bloom. *Deep. Res. Part I* **58**: 338–349. doi:10.1016/j.dsr.2011.01.006
- Metz, C. 1998. Feeding of *Oncaea curvata* (Poecilostomatoida, Copepoda). *Mar. Ecol. Prog. Ser.* **169**: 229–235. doi:10.3354/meps169229
- Möller, K. O., M. St John, A. Temming, J. Floeter, A. F. Sell, J. P. Herrmann, and C. Möllmann. 2012. Marine snow, zooplankton and thin layers: Indications of a trophic link from small-scale sampling with the video plankton recorder. *Mar. Ecol. Prog. Ser.* **468**: 57–69. doi:10.3354/meps09984
- Moriceau, B., and others. 2018. Copepods boost the production but reduce the carbon export efficiency by diatoms. *Front. Mar. Sci.* **5**: 82. doi:10.3389/fmars.2018.00082
- Nishibe, Y., and T. Ikeda. 2008. Metabolism and elemental composition of four oncaeid copepods in the western subarctic Pacific. *Mar. Biol.* **153**: 397–404. doi:10.1007/s00227-007-0816-8
- Ohtsuka, S., N. Kubo, M. Okada, and K. Gushima. 1993. Attachment and feeding of pelagic copepods on larvacean houses. *J. Oceanogr.* **49**: 115–120. doi:10.1007/BF02234012
- Paffenhöfer, G. A. 1993. On the ecology of marine cyclopoid copepods (Crustacea, Copepoda). *J. Plankton Res.* **15**: 37–55. doi:10.1093/plankt/15.1.37
- Paffenhöfer, G. A., and M. G. Mazzocchi. 2003. Vertical distribution of subtropical epiplanktonic copepods. *J. Plankton Res.* **25**: 1139–1156. doi:10.1093/plankt/25.9.1139
- Ploug, H., M. H. Iversen, M. Koski, and E. T. Buitenhuis. 2008. Production, oxygen respiration rates, and sinking velocity of copepod fecal pellets: Direct measurements of ballasting by opal and calcite. *Limnol. Oceanogr.* **53**: 469–476. doi:10.4319/lo.2008.53.2.0469
- Roman, M. R. 1978. Ingestion of the blue-green alga *Trichodesmium* by the harpacticoid copepod, *Macrosetella gracilis*. *Limnol. Oceanogr.* **23**: 1245–1248. doi:10.4319/lo.1978.23.6.1245
- Roman, M. R. 1984. Utilization of detritus by the copepod, *Acartia tonsa*. *Limnol. Oceanogr.* **29**: 949–959. doi:10.4319/lo.1984.29.5.0949
- Satapoomin, S. 1999. Carbon content of some common tropical Andaman Sea copepods. *J. Plankton Res.* **21**: 2117–2123. doi:10.1093/plankt/21.11.2117
- Shanks, A. L., and E. W. Edmondson. 1990. The vertical flux of metazoans (holoplankton, meiofauna, and larval invertebrates) due to their association with marine snow. *Limnol. Oceanogr.* **35**: 455–463. doi:10.4319/lo.1990.35.2.0455

- Shanks, A. L., and K. Walters. 1997. Holoplankton, meroplankton, and meiofauna associated with marine snow. *Mar. Ecol. Prog. Ser.* **156**: 75–86. doi:10.3354/meps156075
- Sheridan, C., D. Steinberg, and G. Kling. 2002. The microbial and metazoan community associated with colonies of *Trichodesmium* spp.: A quantitative survey. *J. Plankton Res.* **24**: 913–922. doi:10.1093/plankt/24.9.913
- Sousa-Santoz, L. P., and J. Castel. 2013. Comparison of four methods to estimate meiobenthic copepod *Amonardia normani* ingestion rates. *Mar. Biol.* **160**: 2395–2404. doi:10.1007/s00227-013-2234-4
- Steinberg, D., B. A. S. Van Mooy, K. O. Buesseler, P. W. Boyd, T. Kobari, and D. M. Karl. 2008. Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. *Limnol. Oceanogr.* **53**: 1327–1338. doi:10.4319/lo.2008.53.4.1327
- Straile, D. 1997. Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. *Limnol. Oceanogr.* **42**: 1375–1385. doi:10.4319/lo.1997.42.6.1375
- Svensen, C., N. Morata, and M. Reigstad. 2014. Increased degradation of copepod faecal pellets by co-acting dinoflagellates and *Centropages hamatus*. *Mar. Ecol. Prog. Ser.* **516**: 61–70. doi:10.3354/meps10976
- Svensen, C., M. T. Antonsen, and M. Reigstad. 2018. Small copepods matter: Population dynamics of *Microsetella norvegica* in a high-latitude coastal ecosystem. *J. Plankton Res.* **40**: 446–457. doi:10.1093/plankt/fby019
- Tiselius, P., and M. Kuylenstierna. 1996. Growth and decline of a diatom spring bloom phytoplankton species composition, formation of marine snow and the role of heterotrophic dinoflagellates. *J. Plankton Res.* **18**: 133–155. doi:10.1093/plankt/18.2.133
- Turner, J. T. 1986. Zooplankton feeding ecology: Contents of fecal pellets of the cyclopoid copepods *Oncaea venusta*, *Corycaeus amazonicus*, *Oithona plumifera*, and *O. simplex* from the Northern Gulf of Mexico. *Mar. Ecol.* **7**: 289–302. doi:10.1111/j.1439-0485.1986.tb00165.x
- Turner, J. T. 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. *Prog. Oceanogr.* **130**: 205–248. doi:10.1016/j.pocean.2014.08.005
- Uye, S., and K. Kaname. 1994. Relations between fecal pellet volume and body size for major zooplankters of the Inland Sea of Japan. *J. Oceanogr.* **50**: 43–49. doi:10.1007/BF02233855
- Uye, S., I. Aoto, and T. Onbé. 2002. Seasonal population dynamics and production of *Microsetella norvegica*, a widely distributed but little-studied marine planktonic harpacticoid copepod. *J. Plankton Res.* **24**: 143–153. doi:10.1093/plankt/24.2.143
- Van der Jagt, H., I. Wiedmann, N. Hildebrandt, B. Niehoff, and M. H. Iversen. 2020. Aggregate feeding by the copepods *Calanus* and *Pseudocalanus* controls carbon flux attenuation in the Arctic shelf sea during the productive period. *Front. Mar. Sci.* **7**: 543124. doi:10.3389/fmars.2020.543124
- Wu, C.-H., J.-S. Hwang, and J.-S. Yang. 2004. Diets of three copepods (Poecilostomatoida) in the southern Taiwan Strait. *Zool. Stud.* **43**: 388–392. //WOS:00022285350025://WOS:000222853500025

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Conflict of Interest

None declared.

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