



Detection of foray behaviour in zooplankton of the Antarctic Polar Front region

Victoria Dewar-Fowler¹, Carol Robinson², Ryan A. Saunders¹, Geraint A. Tarling^{1,*}

¹British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET, UK

²School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, UK

ABSTRACT: In addition to diel vertical migration, individual zooplankton may also make a number of shorter-term migrations, or forays, into the surface layers from deeper depths. Direct observation of these forays is limited, particularly in the open ocean, which hinders our understanding of carbon flux via the biological carbon pump (BCP). We designed a novel net device capable of trapping zooplankton during such forays. The motion-compensated upward and downward looking (MUDL) net device consisted of 2 conical nets, one looking upwards and the other looking downwards, designed for stationary deployment at a set depth, into which migrating individuals must swim to be captured. The device was deployed at different time points within the diel cycle and at contrasting environments across the Antarctic Polar Front region in the southwestern Atlantic sector of the Southern Ocean. A range of zooplankton species were captured, with differences in abundance and species composition between times of day, net directions and sites. Of particular note was the large contribution of the copepod *Oithona* spp. to catches of both the upward and downward looking nets. Our study demonstrates the utility of our MUDL net for future ecosystem studies in the open ocean, particularly in relation to quantifying vertical carbon flux via the BCP.

KEY WORDS: Polar Ocean · Pelagic · DVM · Behaviour · Satiation sinking · Unsynchronised vertical migration

Resale or republication not permitted without written consent of the publisher

1. INTRODUCTION

Zooplankton have long been known to undertake vertical migrations, the most commonly described being diel vertical migration (DVM) (Cushing 1951). DVM is a phenomenon in which, classically, zooplankton travel in a synchronised manner to the upper water column at night to feed and reside in deeper waters during the day (Lampert 1989). Although this behaviour is often thought of simply as a feeding strategy synchronised by external light (Pearre 2003), the diversity of migration patterns that have now been resolved suggests that it may have a number of drivers. For instance, synchronised migration patterns have been shown to be disrupted or

cease altogether in the absence of predators (Gliwicz 1986), which supports the view that DVM is a response to predation threat (the predator avoidance hypothesis; Stich & Lampert 1981). Alternatively, individuals may reduce energetic costs through residing in deeper, colder waters (metabolic advantage hypothesis; Enright 1977).

In addition to classical DVM, individual zooplankton may make numerous further shorter-term migrations, or forays, into the surface layers (Pierson et al. 2009). Unlike DVM, these forays are more likely to be unsynchronised at the population level and hard to detect with traditional net sampling and active acoustic methods (Pearre 1979, Cottier et al. 2006). These forays could allow individuals to max-

*Corresponding author: gant@bas.ac.uk

imise their food intake through numerous short-term visits to the feeding layers at the surface and sinking to less risky layers during digestion (hunger–satiation hypothesis; Pearre 2003). Accordingly, variation between individuals in feeding and digestion rates, which determines the timings of upward and downward forays, results in a loss of synchronised vertical movement.

The vertical movement of zooplankton enhances the export of nutrients and carbon from the upper to the deeper layers (known as active flux), contributing to the biological carbon pump (BCP; Turner 2002). The contribution of this active flux to the total volume of particulate organic carbon (POC) is poorly parameterised but is thought to vary across seasons and oceanic regimes (Buesseler & Boyd 2009, Boyd et al. 2019), with estimates ranging from 3% (Hernández-León et al. 2001) to 70% (Dam et al. 1995) of the total POC export and up to 90% of dissolved inorganic carbon export (Boyd et al. 2019). Faecal pellets produced by zooplankton may contribute significantly to the downward flux of POC (Turner & Ferrante 1979, Cavan et al. 2015, Manno et al. 2015). Foray behaviour was found to increase the number of faecal pellets produced below 30 m (a depth at least 10 m below the highest chlorophyll [chl a] values) when compared to a population displaying no migration patterns (Wallace et al. 2013), suggesting that the presence of this behaviour may act to increase the contribution of active flux to the BCP. Greater knowledge of the prevalence of foray behaviour will allow more accurate estimates of the BCP.

Although the Southern Ocean (south of 50° S) occupies only 10% of the global ocean area, it takes up about 20% of the global ocean CO₂ uptake flux and so is a major contributor to the BCP (Takahashi et al. 2002). Furthermore, high-latitude regions undergo the largest changes in seasonal light regimes, with almost continual light during the productive summer period (Berge et al. 2015). If light is a cue to synchronise zooplankton migration, it is in these environments that synchronised migration patterns will undergo the greatest seasonal changes, possibly halting altogether during summer (Blachowiak-Samolyk et al. 2006). This may initiate greater levels of foray behaviour (Cottier et al. 2006, Wallace et al. 2010), so continuing the process of active flux.

Pierson et al. (2009) attempted to investigate zooplankton foray behaviour using net traps in Dabob Bay, an inshore bay on the west coast of the USA. Foray behaviour was observed in *Calanus pacificus* and *Metridia pacifica* females, evidenced by the

presence of individuals in the upward looking nets throughout the night, indicating that a number of zooplankton were migrating downwards. Furthermore, these individuals had increased gut contents compared to those caught swimming upwards. Nevertheless, the sampling system was subject to some methodological biases, including a selectivity for downward migrating plankton and a bias towards larger individuals in the upward looking net.

Although the system of Pierson et al. (2009) was successful in an inshore setting, the more dynamic environmental conditions of the open ocean, with greater variability in sea state and wind speeds, provide an even greater challenge. Our study aimed to build upon the work of Pierson et al. (2009) by developing a new net system to detect zooplankton foray behaviour in the open ocean environment that will help improve our understanding of carbon flux in future ecosystem studies. We developed and trialled a novel bi-directional net, the motion-compensated upward and downward looking (MUDL) net, with the intention of quantifying upward and downward travelling zooplankton simultaneously. Our net system was deployed in the Antarctic Polar Front region of the southwestern Atlantic sector of the Southern Ocean, a region where sampling conditions are challenging and very little is known about foray behaviour in the local zooplankton community. The MUDL net catches were analysed for taxonomic composition to examine which Southern Ocean zooplankton species and developmental stages undertake foray behaviour and to consider possible regional differences between taxa undertaking this behaviour across different environmental conditions. Our study aimed to demonstrate the utility of the MUDL net for examining zooplankton behaviour in open ocean environments, which will improve our understanding of the role of zooplankton vertical migration in vertical carbon flux via the BCP.

2. MATERIALS AND METHODS

2.1. Net system

The MUDL net was designed by scientists and engineers at the British Antarctic Survey and comprises 2 conical nets mounted on an aluminium frame (Fig. 1). Both nets have a rigid cylindrical opening with a diameter of 61 cm, with 100 µm nylon netting tapering to cod ends 2 m away. The nets are positioned with one net opening looking up and the other looking down. At the entrance of the cod ends is a

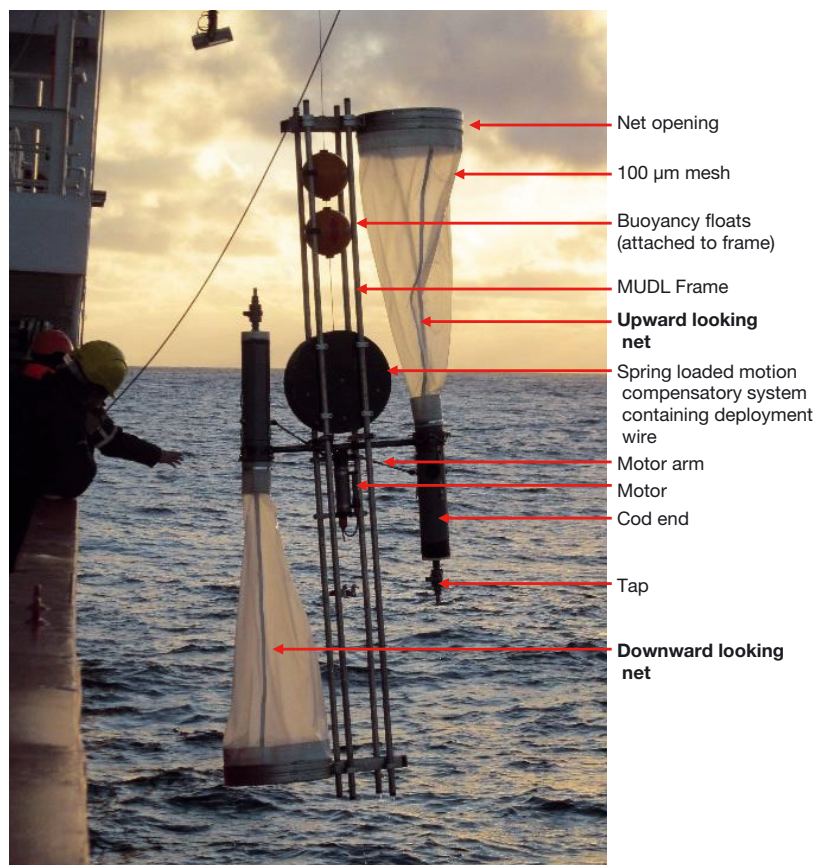


Fig. 1. Motion-compensated upward and downward looking (MUDL) net being deployed from the RRS 'James Clark Ross' in the Southern Ocean. The main features of the net are indicated, including the motion compensatory system and bi-directional nets

spherical valve. This valve is hollow with 3 circular holes cut into it and is the mechanism which allows the net to start and end sampling. The positioning of the valves determines when sampling occurs. In a closed position, the alignment of the valves results in the cod ends being closed and any water flushed through the net being released through a side opening in the cod end. Sampling commences when the valves rotate to an open position by aligning the holes to create an open passage into the cod ends. After a set time, the valves rotate back into the closed position, ensuring that no zooplankton are caught during net retrieval. The valves are rotated via arms connected to a motor located in the centre of the frame. Prior to deployment, the motor is programmed to rotate the arms and therefore the valves at set time points. In addition to the motor, a spring-loaded motion compensatory mechanism sits in the centre of the frame. This allows the net to maintain its vertical position and remain stable within the water column, independent of any ship motion. It is necessary to fill the

cod ends with water prior to deployment to avoid large differences in environmental conditions within the cod ends. This water was taken from Niskin bottles fired at the intended deployment depth during a prior CTD cast. Further details of the design and sampling protocol are provided in Supplement 1 at www.int-res.com/articles/suppl/m715p027_supp.pdf.

2.2. Sampling protocol

Zooplankton sampling was carried out by deploying the MUDL net from the RRS 'James Clark Ross' as part of the British Antarctic Survey long-term ecosystem monitoring programme in the vicinity of South Georgia during austral summer, December 2016 to January 2017 (JR16003). The MUDL net was deployed in 4 locations, 2 within the Polar Frontal Zone and 2 to the poleward side of this zone (Fig. 2). Stations within the Polar Frontal Zone were labelled Polar Front 2 (Stn PF2) and Polar Front 4 (Stn PF4), while stations to the poleward side were labelled Stns P2 and P3.

At each station, the MUDL net was deployed at 2 different time points separated by approximately 12 h. We have nominally categorised these as dusk and dawn, although exact timings vary regarding the relationship to sunrise and sunset (the average time difference between sunset or sunrise and the start of a deployment was 2 h 20 min, while the maximum difference was 4 h 16 min). Dual deployments were made at each time point, the first to a set depth of 100 m and the second to 10 m below the chl *a* maximum (typically 60–80 m depth) as determined by a prior CTD cast (Table 1). These dual deployments were repeated at Stn P2 where further time was available.

A vertical mini-Bongo deployment was made at approximately the same time as the evening MUDL net deployment at Stn PF4 as a means of comparing the zooplankton community captured by a traditional net to that by the MUDL net (further details on the specification of the mini-Bongo, its deployment protocol and subsequent sample and statistical analyses are provided in Supplement 2 at www.int-res.com/articles/suppl/m715p027_supp.pdf).

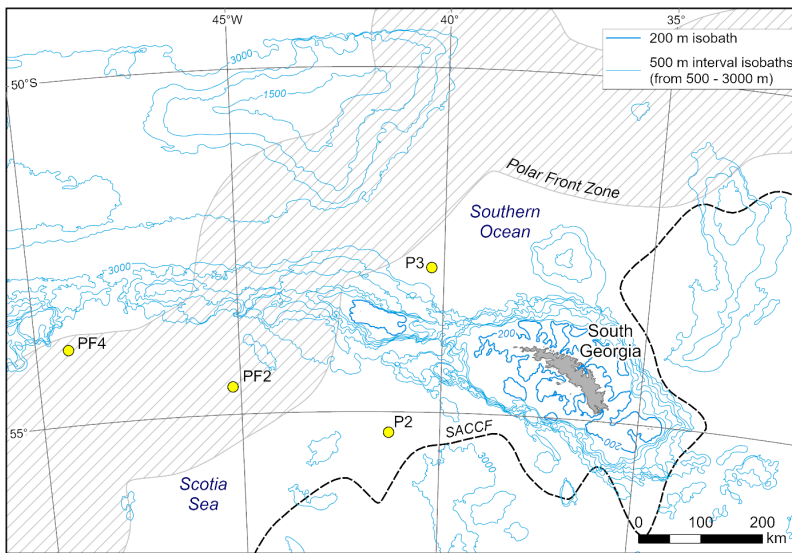


Fig. 2. Polar Front region close to South Georgia, showing the 4 stations (yellow circles) where MUDL net deployments were carried out from the RRS 'James Clark Ross' during 2016 and 2017. Hatched area shows location of the Polar Front Zone as determined by Orsi et al. (1995) and Trathan et al. (2000). SACCF: Southern Antarctic Circumpolar Current Front

2.3. Environmental sampling

Information on environmental conditions at the time of sampling was provided by CTD casts. The CTD consisted of an SBE32 carousel water sampler equipped with an SBE9Plus CTD containing an SBE 3Plus temperature sensor, a fluorometer (Chelsea Aquatracka Mark III) and a photosynthetically active radiation sensor. The depths of the chl *a* maxima

were determined from the chl *a* profile obtained during the downcast (Fig. 3). Water for use in the respective MUDL net deployments was obtained from 100 and 10 m below the chl *a* maxima during the upcast of the CTD, and the cod ends were filled with this water as close as possible to the time of deployment. The MUDL net was programmed using customised Hydrobios software and was set to rotate the valves within the cod ends to an open position after 12 min, allowing it to reach the target depth before opening. Deployments were made with the valves in a closed position, minimising contamination during this stage. Once the net was at the target depth, the valves rotated to an open position, allowing the mixing of water in the cod ends and zooplankton to swim into the cod ends. The valves remained in this open position for 20 min before being closed for retrieval.

While open, the MUDL net remained at the target depth, and with the aid of the motion compensatory system, vertical movement was minimised, decreasing the potential of zooplankton to enter the nets through intermittent vertical motions caused by the effect of oceanic swell. The net was hauled vertically to the surface in the closed position, preventing contamination of the cod end samples during this phase.

Table 1. Motion-compensated upward and downward looking (MUDL) net deployments during cruise JR16003, December 2016 to January 2017 (in local time, GMT-3; dates as d/mo/yr). Local sunset and sunrise based on times generated by the NOAA Solar Calculator (<https://gml.noaa.gov/grad/solcalc/sunrise.html>)

Depth (m)		Stn	Deployment commencement	Deployment finish local	Local sunset	Local sunrise
100	Preset depth	P3	21:13, 29/12/16	21:44, 29/12/16	03:55	20:46
80	Chl <i>a</i> max + 10 m	P2	23:24, 30/12/16	00:02, 31/12/16	03:37	20:58
100	Preset depth	P2	00:13, 31/12/16	00:54, 31/12/16	03:37	20:58
80	Chl <i>a</i> max + 10 m	P2	05:09, 31/12/16	05:50, 31/12/16	03:37	20:58
100	Preset depth	P2	05:56, 31/12/16	06:38, 31/12/16	03:38	20:58
100	Preset depth	P2	07:05, 1/1/17	07:44, 1/1/17	03:39	20:57
60	Chl <i>a</i> max + 10 m	P2	07:55, 1/1/17	08:32, 1/1/17	03:39	20:57
100	Preset depth	PF2	22:05, 2/1/17	22:43, 2/1/17	03:28	20:37
80	Chl <i>a</i> max + 10 m	PF2	22:52, 2/1/17	23:29, 2/1/17	03:28	20:37
100	Preset depth	PF2	06:19, 3/1/17	06:59, 3/1/17	03:31	20:36
80	Chl <i>a</i> max + 10 m	PF2	07:08, 3/1/17	07:49, 3/1/17	03:31	20:36
100	Preset depth	PF4	21:43, 4/1/17	22:20, 4/1/17	03:20	20:16
100	Preset depth	PF4	04:41, 5/1/17	05:24, 5/1/17	03:21	20:15

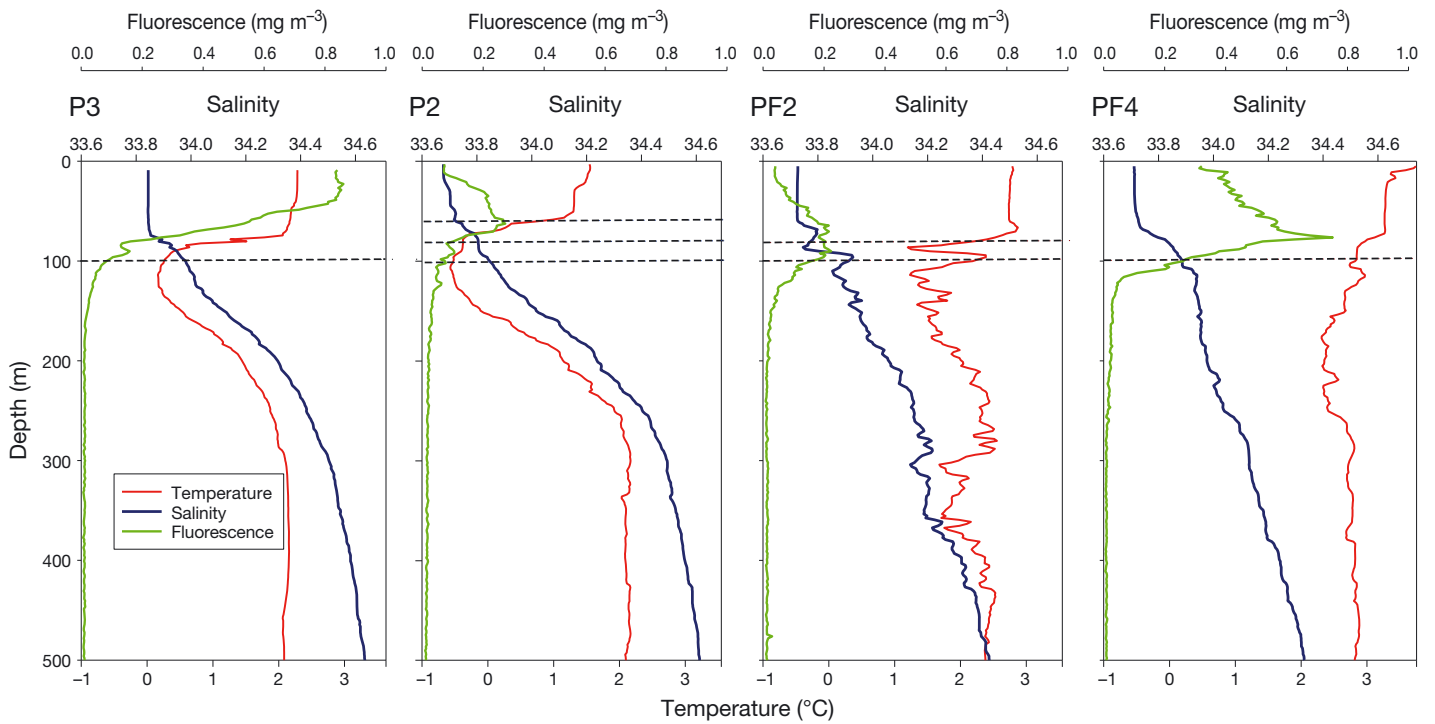


Fig. 3. Example profiles of temperature, salinity and fluorescence with depth as recorded by CTD casts at Stns P3, P2, PF2 and PF4. Horizontal lines indicate depth of MUDL net deployment (chl *a* max + 10 m and/or at 100 m). Note different depths of chl *a* max + 10 m at Stn P2 resulted from being matched to different CTD casts

2.4. Zooplankton sample processing

Once the net had been recovered, the cod ends were emptied. This was done by opening a tap valve at the base of the upward looking net, allowing the sample to be collected by placing a large bucket underneath. To collect the sample from the downward looking net, the base of the net was removed and the valve rotated to an open position. Once in an open position, the sample was collected in the same way as the upward looking net. Samples were then passed through a 100 μm nylon mesh cloth, collecting any zooplankton on the cloth. Mesh cloths were immediately frozen at -80°C for analysis back at the UK laboratory.

Once back at the UK laboratory, frozen samples from the MUDL net deployments were transferred to ethanol or 4% buffered formaldehyde (within 24 mo of collection) and the samples analysed within days of this fixation. Whole samples were analysed, and zooplankton were counted and taxonomically identified using a stereo microscope (Olympus SZX16) with a Nikon D750 camera attachment. Photos of zooplankton were taken to enable secondary verification of taxonomic identification. Easily distinguish-

able zooplankton were identified to species, such as *Rhincalanus gigas*, *Calanoides acutus* and *Calanus propinquus*. Other taxa were identified to genus level, including *Oithona* spp., *Metridia* spp. and *Thysanoessa* spp. The remainder were identified to groups, including calanoids, pteropods, ostracods, chaetognaths, euphausiid calyptopes and hyperiid amphipods.

2.5. Statistical analyses

To compare species diversity at the various locations, Shannon diversity indices were calculated. Samples were pooled by location for this analysis, with all samples across all depths at each time point pooled to form 2 locational groups: (1) the Polar Front group (Stns PF2 and PF4) and (2) the P2 group. Because only 1 sample was available from Stn P3, this sample was not included in this specific analysis (although details and analyses of the P3 catch are included where appropriate elsewhere). For each location, this enabled community structure comparisons between the upward and downward looking nets at both dusk and dawn. Shannon diversity index

measures species richness based on the total number of species found within a population and was calculated as follows:

$$H' = \sum_{i=1}^R p_i \ln p_i \quad (1)$$

where p_i is the proportion of individuals belonging to each species, i , for the total number of species, R .

Comparisons between abundances within upward looking and downward looking net catches were made using a non-parametric Mann-Whitney U -test, having first failed tests for either normality or equal variance.

A multivariate analysis was conducted using Primer 7 (version 7.0.13, PRIMER-e) (Clarke & Gorley 2015). Data were imported in a sample–variable matrix, where each net at each deployment was a sample and the taxa were variables. In total, 26 samples were included from 13 net deployments. All samples had corresponding factors of location, time, depth and net direction.

For all analyses, data were fourth root transformed to reduce the influence of highly abundant taxa. Shade plots were used to confirm that such taxa had been sufficiently down-weighted before proceeding with further analyses (Clarke & Gorley 2015). Subsequently, a resemblance matrix was generated, calculating Bray-Curtis similarities between samples.

The above similarity matrix was analysed through non-metric multidimensional scaling (nMDS; Kruskal stress formula 1) to provide further context on similarities and differences between sample sets. nMDS generates ordination plots where the distance between samples is a representation of the multidimensional differences in the underpinning resemblance matrix.

3. RESULTS

3.1. Environmental context

Temperature, salinity and fluorescence profiles showed the differing environmental conditions at the stations sampled during the cruise (Fig. 3). Stns P3 and P2 showed a similar water column structure consisting of a surface mixed layer (~0–80 m), winter water (~80–200 m) and then upper circumpolar deep water (UCDW) (below ~200 m). Temperature ranged between -0.5 and 2°C , reaching a minimum in the winter water layer and a maximum in the UCDW. Salinity ranged between 33.7 and 34.6, being freshest at the surface and most saline at depth. At the Polar Frontal Zone stations (Stns PF2 and PF4), the

structure of the water column was less clear, most likely as a result of high levels of vertical mixing. Temperatures were higher than at Stns P2 and P3, varying between 2.4 and 3.6°C . Salinity was 33.8 in the upper mixed layer, increasing to 34.4 by 500 m. Chl *a* derived from fluorescence measurements reached its maximum value within the surface mixed layer at all locations. The PF stations and Stn P3 had much greater chl *a* maxima (0.7 and 0.8 mg m^{-3} , respectively) than Stn P2 ($\sim 0.25 \text{ mg m}^{-3}$). At Stn P3, the maximum chl *a* was found in the surface 50 m before a sharp decline to $<0.01 \text{ mg m}^{-3}$ by 200 m. At Stn P2, a subsurface maximum was present, with the highest values being around 70 m followed by a sharp decline to around 0.02 mg m^{-3} by 150 m. The same pattern of chl *a* abundance was seen in the PF stations, with subsurface maxima being present in the mixed layer at ~ 70 m before declining rapidly to $<0.02 \text{ mg m}^{-3}$ by 200 m.

3.2. MUDL net performance

All MUDL net deployments resulted in zooplankton being caught in both nets, indicating that the net was effective in catching both upward and downward swimming zooplankton. However, the upward looking nets caught significantly more than the downward looking nets (Mann-Whitney U -test, $p < 0.001$), with the downward looking net catching an average of 24% (SD 23%) of the corresponding upward looking net catch (Fig. 4).

A mini-Bongo vertical deployment was carried out at Stn PF4 to compare with the MUDL net deployment at this station. Both devices captured a similar range of organisms, dominated by calanoid and cyclopoid copepods. However, calanoids were more likely to be captured by the MUDL net compared to their relative prevalence in the water column, while cyclopoids were less likely to be captured (see Supplement 2 for further details).

3.3. Analyses of taxa

Across all samples, copepods accounted for over 90% of all organisms in terms of abundance (Fig. 5). Cyclopoids were the most common copepod followed by calanoids. Cyclopoid dominance was slightly higher in dawn samples than dusk samples, with taxa such as calanoids, ostracods, annelids and pteropods having a greater proportional abundance at dusk (Fig. 5).

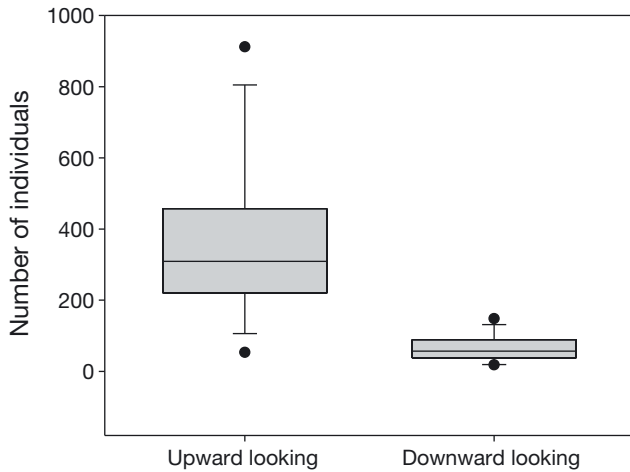


Fig. 4. Abundance of organisms identified in upward looking and downward looking nets across all MUDL net deployments carried out during the present study. The boundaries of the box indicate the 25th and 75th percentiles, the line within the box indicates the median and the whiskers indicate the 90th and 10th percentiles and the dots indicate the 5th and 95th percentiles

The pattern of much higher abundances in the upward looking compared to downward looking net was apparent both at the PF stations and at Stn P2 (Fig. 6). However, between dusk and dawn, abundances in nets of the same direction were comparatively similar. For instance, across Stn P2 and the PF stations, dawn and dusk upward looking nets all had averages of between 200 and 500 individuals, while

averages of downward looking nets ranged between 40 and 125 individuals. Within these ranges, overall abundance was higher at the PF stations compared to Stn P2 in both upward and downward looking nets.

Regarding Shannon diversity, values were greater at the PF stations compared to Stn P2. Furthermore, the diversity of the community moving at dusk at the PF stations was greater than at dawn (dawn: upward looking $H = 1.72$, downward looking $H = 1.84$; dusk: upward looking $H = 2.13$, downward looking $H = 2.24$; Fig. 6). This was not the case at Stn P2, where dawn samples had marginally higher Shannon diversity indices than samples collected at dusk (dawn: upward looking $H = 1.52$, downward looking $H = 1.44$; dusk: upward looking $H = 1.22$, downward looking $H = 1.42$).

In terms of taxonomic composition, the cyclopoid copepod *Oithona* spp. was found to be the most dominant taxon across all samples (Table 2). The other dominant taxa differed between Stn P2 and the PF stations. At Stn P2, *Oithona* spp., the calanoid copepod *Ctenocalanus* spp., pteropods and nauplii were abundant across both net directions and sampling times, while at the PF stations, harpacticoid copepods, the calanoids *Metridia* spp. and *Haloptilus* spp., the cyclopoid *Oncaea* spp. and pteropods were most abundant. There was little difference in the taxonomic composition of organisms captured at the depth of chl *a* max + 10 m compared with those captured deeper, at 100 m (Table 3). The major differ-

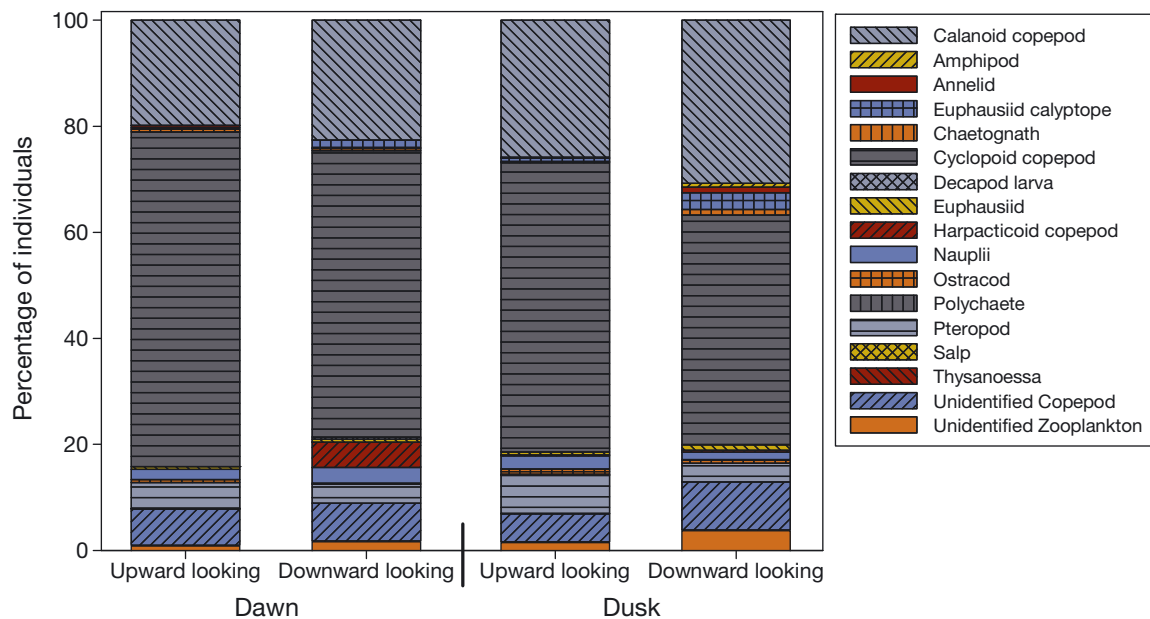


Fig. 5. Percentage of individuals captured per taxa across all MUDL net deployments. Each stacked bar represents all deployments grouped together based on deployment time irrespective of location or depth

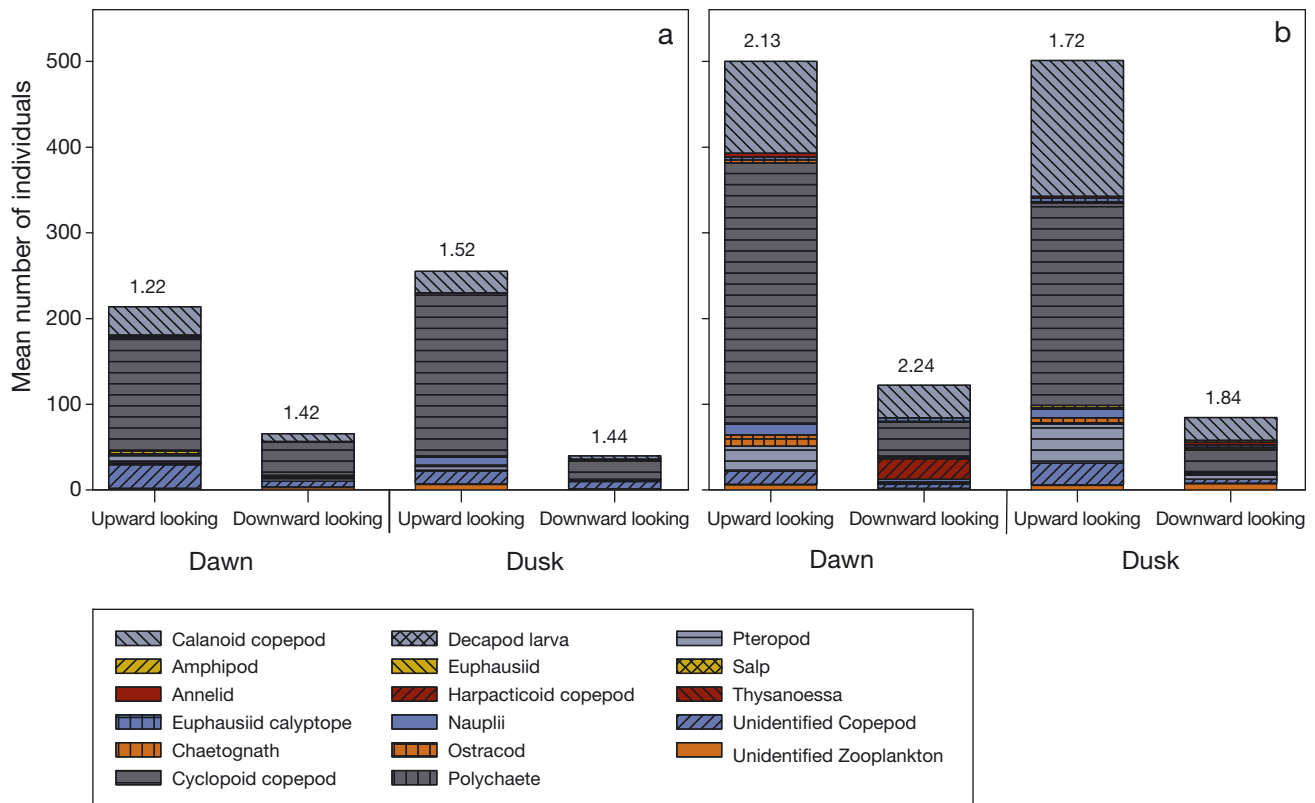


Fig. 6. Abundance of individuals captured per taxa across all MUDL net deployments at (a) Stn P2 and (b) combined PF stations (Stns PF2 and PF4). Each stacked bar represents all deployments grouped together based on deployment time irrespective of depth. Numbers on top of stacked bars represent the Shannon diversity index for each grouping (note that the diversity index is based on lowest taxonomic level within the dataset of which some categories are grouped here for presentation purposes)

ence was the presence of pteropods in the upward looking net at chl *a* max + 10 m; pteropods were not found at the deeper depth or in any downward looking nets.

Multivariate analysis of all samples collected during the survey showed 2 broad groups in the nMDS plot (Fig. 7), separating the downward looking samples from the upward looking samples. Furthermore, the upward looking samples grouped closer together than the downward looking samples, indicating a greater degree of variation between deployments in the latter. In addition, when dawn and dusk samples were analysed without considering net direction, dawn samples grouped closer together than dusk samples, indicating that similarity between dawn samples was comparatively greater. However, there was a large degree of overlap within the groupings, suggesting high variability and the presence of common taxa across all samples. Location did not have a major influence on the pattern of grouping despite differences in water column structure between stations.

4. DISCUSSION

4.1. MUDL net performance

The system was successful in capturing a range of zooplankton taxa, swimming both upwards and downwards, at a range of depths within the epipelagic layers. These taxa were captured swimming in both directions at both dusk and dawn.

Our environmental analyses showed particular spatial contrasts in water column structure, with the Polar Frontal Zone stations (Stns PF2 and PF4) being around 1 to 2°C warmer and with a deeper mixed layer compared to those stations to the poleward side of this zone (Stns P2 and P3). Across this range of conditions, the MUDL net successfully captured similar amounts of organisms in both the upward and downward looking nets, showing that the prevalence of these behaviours was widespread.

Compared to a traditional vertically deployed mini-Bongo net, the MUDL net captured a similar compo-

Table 2. Most numerous taxa, in order of abundance, within upward looking and downward looking nets for (a) Stn P2 and (b) PF stations. Numbers in brackets denote the average abundance (\pm SD) across all deployments at dusk and dawn in units of individuals per net

	Dawn	Dusk
(a) Stn P2		
Downward looking net	<i>Oithona</i> spp. (40.8 \pm 14.6) Unknown (copepod) (2.8 \pm 3.0) <i>Ctenocalanus</i> spp. (8.3 \pm 4.5) Pteropod (4 \pm 1.2) Nauplii (2 \pm 1.3)	<i>Oithona</i> spp. (13.5 \pm 28.2) Unknown (copepod) (5.5 \pm 2.1) Nauplii (0 \pm 0.7) <i>C. acutus</i> (0.5 \pm 0.7) <i>R. gigas</i> (1 \pm 0.7)
Upward looking net	<i>Oithona</i> spp. (314 \pm 82.4) Unknown (copepod) (16 \pm 22.5) <i>Ctenocalanus</i> spp. (51 \pm 10.3) Pteropod (51.5 \pm 6.4) <i>Oncaea</i> spp. (21.3 \pm 6.1) <i>Metridia</i> spp. (39.8 \pm 5.5)	<i>Oithona</i> spp. (177 \pm 93.36) Unknown (copepod) (39.5 \pm 1.4) <i>Ctenocalanus</i> spp. (34.5 \pm 9.2) Nauplii (4.5 \pm 6.4) Pteropod (7.5 \pm 3.5)
(b) Stns PF2 and PF4		
Downward looking net	<i>Oithona</i> spp. (37.7 \pm 32.7) Harpacticoid (8.0 \pm 13.9) <i>Ctenocalanus</i> spp. (6.0 \pm 4.6) <i>Metridia</i> spp. (5.0 \pm 5.0) <i>Haloptilus</i> spp. (3.3 \pm 5.8)	<i>Oithona</i> spp. (17.7 \pm 25.4) <i>Oncaea</i> spp. (8.7 \pm 12.5) <i>Metridia</i> spp. (7.0 \pm 5.61) <i>Ctenocalanus</i> spp. (5.3 \pm 6.8) Calanoid (unidentified) (7.5 \pm 7.8)
Upward looking net	<i>Oithona</i> spp. (291.67 \pm 177.46) <i>Ctenocalanus</i> spp. (43.33 \pm 4.9) Pteropod (28.3 \pm 23.5) <i>Metridia</i> spp. (17.7 \pm 21.953) Unknown (copepod) (15.3 \pm 11.3)	<i>Oithona</i> spp. (203 \pm 1629) <i>Ctenocalanus</i> spp. (54 \pm 23.9) Pteropod (45.3 \pm 66.5) <i>Metridia</i> spp. (41.3 \pm 43.2) <i>Oncaea</i> spp. (33.0 \pm 18.19)

Table 3. Most numerous taxa, in order of abundance, across all MUDL net samples collected at chl *a* max + 10 m and at 100 m. Numbers in brackets denote the average abundance (\pm SD) across all deployments at that depth in units of individuals per net

	Chl <i>a</i> max + 10 m	100 m
Downward looking net	<i>Oithona</i> spp. (35.6 \pm 22.5) <i>Ctenocalanus</i> spp. (6.8 \pm 5.9) <i>Oncaea</i> spp. (5.2 \pm 10.0) Unknown (copepod) (4.8 \pm 3.6) <i>Metridia</i> spp. (3.8 \pm 5.0)	<i>Oithona</i> spp. (25.8 \pm 22.4) Unknown (copepod) (5.6 \pm 3.4) Harpacticoid (3.1 \pm 8.4) <i>Metridia</i> spp. (2.7 \pm 4) <i>Oncaea</i> spp. (1.2 \pm 1.6)
Upward looking net	<i>Oithona</i> spp. (243.3 \pm 130.9) Pteropod (38.8 \pm 50.5) <i>Ctenocalanus</i> spp. (36.8 \pm 25.8) Unknown (copepod) (31.0 \pm 15.1) <i>Metridia</i> spp. (22.4 \pm 38.6)	<i>Oithona</i> spp. (176.4 \pm 126.1) <i>Ctenocalanus</i> spp. (25.2 \pm 20.4) Unknown (copepod) (18.3 \pm 13.0) <i>Oncaea</i> spp. (12.0 \pm 11.7) <i>Metridia</i> spp. (10.4 \pm 15.2)

sition of taxonomic groups, dominated by calanoid and cyclopoid copepods. However, in comparative terms, the more motile calanoids were more likely to be captured by the MUDL net.

4.2. Net direction bias

Within the nMDS analyses, the samples formed 2 groups. Samples from the upward looking net formed one cluster, while the other cluster was

formed of a majority of samples from the downward looking net. However, although these groups were broad, with a high degree of overlap, net direction appeared to be a major driver of multivariate structure. One explanatory factor may be differences in net capture efficiency between upward and downward looking nets. The MUDL net consistently caught a larger number of downward migrating than upward migrating zooplankton, with the downward looking net catching around a quarter of the corresponding upward looking net catch. It is not clear

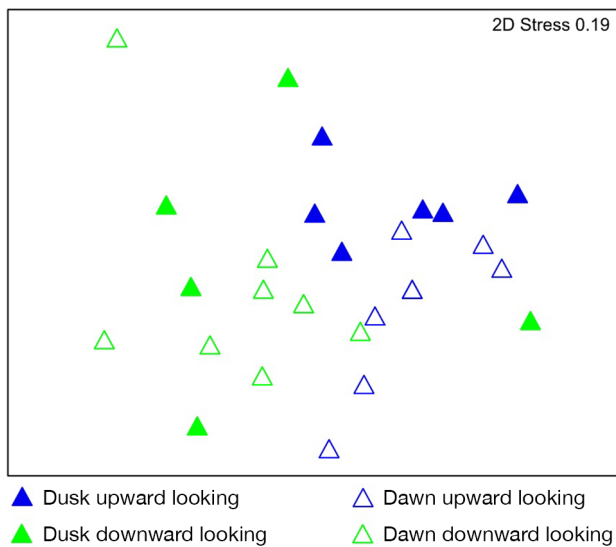


Fig. 7. Non-metric multidimensional scaling of all MUDL net samples. Spacing between samples indicates the level of similarity, with those located closest together having the greatest similarity

why this was the case, although this pattern is consistent with Pierson et al. (2009), who also caught larger numbers in the upward looking net when using net traps similar to those used in the present study. Pierson et al. (2009) suggested several reasons for this, including differences in behavioural and sensory responses of migrating organisms. For instance, organisms swimming upwards into the downward looking net may encounter the side panels of the net as they enter and respond by swimming downwards. If this is the case, organisms that come into contact with the net at any point during upward migration may not enter the cod end and hence avoid capture. Organisms entering the upward looking net may be doing so by passive sinking and may be less inclined to swim upwards and out of the net on encountering any side panels. In addition to this, if the main response to sensing the equipment is to swim down, then this will only act to increase the funnelling of naturally downward swimming organisms into the cod end, resulting in a bias in numbers caught by the upward looking net.

Pierson et al. (2009) further suggested light attenuation caused by the net may be a factor in reducing the capture efficiency of downward looking nets. As zooplankton migrate upwards, the net may block or alter the light signal they receive from the surface. This alteration may result in avoidance behaviour by the zooplankton, reducing the capture efficiency of the downward looking net. If this is the case, a greater difference between the nets between night

and day would be observed. Due to the deployments in this study occurring at dawn and dusk, it is not possible to resolve this, as light levels may have been similar at both time points. Irrespective of the different capture efficiency rates, it is clear from this study that zooplankton are migrating in both directions at these times.

4.3. Asynchronous migrations

The present study found migrations in both directions at both dawn and dusk, implying that migrations were asynchronous and occurring throughout a daily cycle, supporting the presence of foray behaviour. Very few studies have successfully detected foray behaviour due to difficulties in detecting individual zooplankton movements (Pearre 2003). However, Pierson et al. (2009, 2013) were able to demonstrate foray behaviour in the copepods *Calanus pacificus* and *Metridia pacifica*. This was evidenced by vertical migrations throughout the night, with those individuals migrating downwards having fuller guts than those migrating upwards, suggesting satiation-associated downward migration, consistent with the hunger-satiation hypothesis. Gut contents of individuals caught by the MUDL net were not measured in the present study, so direct attribution to hunger and satiation processes cannot be made. However, the similarity in migration patterns seen within this study to those carried out by Pierson et al. (2009, 2013) suggests that similar mechanisms may be responsible.

The presence of downward migrating zooplankton at dusk suggests that a number of zooplankton were in the surface waters during the day. Several studies have found vertically migrating zooplankton to be present in the upper water column during daylight hours (e.g. Pearre 1970, 1973, Ohman et al. 1983, Irigoien et al. 2004, Sims et al. 2005). Lampert (1989) and Ohman (1990) argue that a flexibility in vertical migration and potentially a reverse migration is due to the presence of larger invertebrate predators such as chaetognaths and euphausiids. Predatory chaetognaths and euphausiids were caught in the MUDL net, showing that these taxa were also undergoing vertical migrations and may be exerting predation pressure upon the zooplankton community.

4.4. Cyclopid foray behaviour

The cyclopoids *Oithona* spp. were found to be the most dominant taxa captured by the MUDL net

across all locations and were particularly dominant at Stn P2. This high abundance is in line with previous sampling efforts within the Scotia Sea (Atkinson & Sinclair 2000, Ward et al. 2012). We found the ratio of directional movement of zooplankton at Stn P2 to be dependent upon time, and *Oithona* spp., as the most abundant zooplankton taxa, often dominated these community-level patterns in vertical movements. Often regarded as the most ubiquitous copepod in many oceans (Bigelow 1926), *Oithona* spp. are an important component of the zooplankton community and exhibit an omnivorous diet (Ward & Hirst 2007). However, little is known about vertical migration behaviour in *Oithona* spp. Tanimura et al. (2008) investigated the vertical positioning of *O. similis* under sea ice in mid-summer. During 24 h daylight, *O. similis* undertook a reverse migration, inhabiting surface waters during the day and migrating to slightly deeper waters at night (Tanimura et al. 2008). However, some authors report no vertical migration in *Oithona* spp. over daily (Bogorov 1946, Irigoien et al. 2004) or seasonal (Atkinson 1998, Ashjian et al. 2003) cycles. Our study shows that *Oithona* spp. in the Southern Ocean are undertaking foray-style vertical migrations in the water column, which would be difficult to resolve using traditional netting methods.

Oithona spp. are ambush predators, detecting prey particles from hydrodynamic signals (Svensen & Kjørboe 2000, Paffenhöfer & Mazzocchi 2002, Saiz et al. 2003). However, it has been reported that signals can only be perceived by stationary *Oithona* spp. (Paffenhöfer & Mazzocchi 2002), with individuals passively sinking until prey is detected, at which point they undertake an active jump to catch motile and non-motile prey (Kjørboe 2007). This feeding strategy indicates that vertical movements within the water column are a necessary part of *Oithona* spp. feeding behaviour and may lead to the observed foray behaviour. The present study found that, in contrast to previous studies, *Oithona* spp. were not vertically stable but were moving vertically within the water column at both dawn and dusk, further underlining the presence of foray behaviour. *O. similis* have been found to have a passive sinking speed of $0.0237 \pm 0.0042 \text{ cm s}^{-1}$ (\pm SD) and a mean swimming velocity of $0.051 \pm 0.030 \text{ cm s}^{-1}$ (\pm SD) (Hubareva & Svetlichny 2016). Using these speeds, it would take *O. similis* between 2 and 3 h to passively sink the 2 m distance from the top of the upward looking net to the cod end, but they may be able to swim the distance in 40 min. While this would indicate that *Oithona* spp. could not traverse the 2 m

length of the net during the 20 min in which the net was open, those that were already within the net system, i.e. at the depth where the MUDL net came to rest, would be able to travel into the cod ends. In addition to this, as passive sinking is slower than active swimming, the high numbers of *Oithona* spp. caught in the present study are likely to have been actively swimming in either an upward or a downward direction, showing that *Oithona* spp. must be undertaking vertical migrations.

This study provides strong evidence that *Oithona* spp. are vertically mobile within the water column at different time points in the day–night cycle. This is an important finding given their ubiquitous nature and the major position they occupy within global zooplankton communities and linked processes, including the BCP (Giering 2013).

4.5. Contribution to carbon flux

The present study reveals the existence of widespread foray behaviour in the zooplankton communities of the polar Scotia Sea and Polar Frontal Zone. By migrating from the upper water column to deeper waters, zooplankton transport carbon (as well as nutrients) deeper in the water column, so contributing to the BCP. Wallace et al. (2013) used a model to simulate the volume of carbon exported in faecal pellets from copepods undergoing different migration patterns, including foray behaviour. These authors found that in the absence of any vertical migrations, the resulting carbon export would be minimal. Foray behaviour increased the volume of faecal pellet production in the deeper layers, although it was less than that generated by DVM. Asynchronous migrations reported in the present study may therefore enhance the level of carbon flux in this region beyond that generated by the DVM community. In the Scotia Sea, POC flux is up to $22.91 \text{ mg C m}^{-2} \text{ d}^{-1}$ at 2000 m, with much of this being in the form of faecal pellets (Manno et al. 2015), highlighting the importance of zooplankton migrations in this region.

4.6. Future outlook

As this trapping technique becomes more established, certain improvements will be required in both the design and implementation of this technique. Longer deployments would allow a greater representation of slower swimming organisms (e.g.

cyclopoids such as *Oithona* spp.) in the catches. Also, deployment at frequent intervals across the 24 h cycle would allow the influences of classical DVM, reverse DVM, midnight sinking and daytime avoidance to be accounted for. Technical improvements could be made to alter the time-intensive way in which cod ends are filled and emptied. Further technology could also be integrated onto its robust net frame, particularly autonomous imaging instruments (Picheral et al. 2022). Such devices could provide a simultaneous view of what is in the community outside of the nets to compare directly with what is captured. To a certain extent, such imaging devices can also resolve the direction of swimming, although this is only over a short distance as compared to the MUDL net, which integrates upward or downward movement over the 2 m length of the net.

A major advantage of the MUDL net is that it captures live organisms displaying a particular foray behaviour (i.e. upward or downward swimming). Quick and sensitive retrieval of these individuals will allow meaningful physiological measurements to be made, such as on respiration and gut evacuation rates. This will allow direct comparisons of the respective states of individuals during the upward and downward phases of forays. For other individuals within the catch, instant preservation will allow the examination of gut contents before any further substantial amount of digestion has taken place.

Acknowledgements. We are grateful to the officers and crew of the RRS 'James Clark Ross' for ably assisting with deployment of the MUDL net during cruise JR16003. Engineers Scott Palfrey, Dan Ashurst and Peter Enderlein helped design and construct the net and assisted with deployments. Peter Ward provided invaluable taxonomic training to V.D.-F. and further quality control on the identification of zooplankton captured by the MUDL net. The contribution of G.A.T. and R.A.S. was supported by the NERC National Capability for sustained observations POETS programme carried out by the Ecosystems team at the British Antarctic Survey. V.D.-F. was funded by the EnvEast Doctoral Training Partnership.

LITERATURE CITED

- Ashjian CJ, Campbell RG, Welch HE, Butler M, Van Keuren D (2003) Annual cycle in abundance, distribution, and size in relation to hydrography of important copepod species in the western Arctic Ocean. *Deep Sea Res I* 50: 1235–1261
- Atkinson A (1998) Life cycle strategies of epipelagic copepods in the Southern Ocean. *J Mar Syst* 15:289–311
- Atkinson A, Sinclair JD (2000) Zonal distribution and seasonal vertical migration of copepod assemblages in the Scotia Sea. *Polar Biol* 23:46–58
- Berge J, Renaud PE, Darnis G, Cottier F and others (2015) In the dark: a review of ecosystem processes during the Arctic polar night. *Prog Oceanogr* 139:258–271
- Bigelow HB (1926) Plankton of the offshore waters of the Gulf of Maine. Bull US Bur Fish, Doc 968. US Gov Printing Off, Washington, DC
- Blachowiak-Samolyk K, Kwasniewski S, Richardson K, Dmoch K and others (2006) Arctic zooplankton do not perform diel vertical migration (DVM) during periods of midnight sun. *Mar Ecol Prog Ser* 308:101–116
- Bogorov BG (1946) Peculiarities of diurnal vertical migrations of zooplankton in polar seas. *J Mar Res* 6:25–33
- Boyd PW, Claustre H, Levy M, Siegel DA, Weber T (2019) Multi-faceted particle pumps drive carbon sequestration in the ocean. *Nature* 568:327–335
- Buesseler KO, Boyd PW (2009) Shedding light on processes that control particle export and flux attenuation in the twilight zone of the open ocean. *Limnol Oceanogr* 54: 1210–1232
- Cavan EL, Le Moigne FAC, Poulton AJ, Tarling GA and others (2015) Attenuation of particulate organic carbon flux in the Scotia Sea, Southern Ocean, is controlled by zooplankton fecal pellets. *Geophys Res Lett* 42:821–830
- Clarke KR, Gorley RN (2015) Getting started with PRIMER v7: user manual/tutorial. PRIMER-e, Plymouth
- Cottier FR, Tarling GA, Wold A, Falk-Petersen S (2006) Un-synchronized and synchronized vertical migration of zooplankton in a high Arctic fjord. *Limnol Oceanogr* 51: 2586–2599
- Cushing DH (1951) The vertical migration of planktonic Crustacea. *Biol Rev Camb Philos Soc* 26:158–192
- Dam HG, Zhang X, Butler M, Roman MR (1995) Mesozooplankton grazing and metabolism at the equator in the central Pacific: implications for carbon and nitrogen fluxes. *Deep Sea Res II* 42:735–756
- Enright JT (1977) Diurnal vertical migration: adaptive significance and timing. Part 1. Selective advantage: a metabolic model. *Limnol Oceanogr* 22:856–872
- Giering SLC (2013) The role of mesozooplankton in the biological carbon pump of the North Atlantic. PhD thesis, University of Southampton
- Gliwicz MZ (1986) Predation and the evolution of vertical migration behavior in zooplankton. *Nature* 320:746–748
- Hernández-León S, Gómez M, Pagazaurtundua M, Portillo-Hahnefeld A, Montero I, Almeida C (2001) Vertical distribution of zooplankton in Canary Island waters: implications for export flux. *Deep Sea Res I* 48:1071–1092
- Hubareva ES, Svetlichny LS (2016) Copepods *Oithona similis* and *Oithona davisae*: two strategies of ecological-physiological adaptation in the Black Sea. *Oceanology* 56:241–247
- Irigoién X, Hulsman J, Harris RP (2004) Global biodiversity patterns of marine phytoplankton and zooplankton. *Nature* 429:863–867
- Kjørboe T (2007) Mate finding, mating, and population dynamics in a planktonic copepod *Oithona davisae*: there are too few males. *Limnol Oceanogr* 52:1511–1522
- Lampert W (1989) The adaptive significance of diel vertical migration of zooplankton. *Funct Ecol* 3:21–27
- Manno C, Stowasser G, Enderlein P, Fielding S, Tarling GA (2015) The contribution of zooplankton faecal pellets to deep-carbon transport in the Scotia Sea (Southern Ocean). *Biogeosciences* 12:1955–1965

- Ohman MD (1990) The demographic benefits of diel vertical migration by zooplankton. *Ecol Monogr* 60:257–281
- Ohman MD, Frost BW, Cohen EB (1983) Reverse diel vertical migration: an escape from invertebrate predators. *Science* 220:1404–1407
- Orsi AH, Whitworth T III, Nowlin WD Jr (1995) On the meridional extent and fronts of the Antarctic Circumpolar Current. *Deep Sea Res I* 42:641–673
- Paffenhöfer GA, Mazzocchi MG (2002) On some aspects of the behaviour of *Oithona plumifera* (Copepoda: Cyclopoida). *J Plankton Res* 24:129–135
- Pearre S (1970) Light responses and feeding behavior of *Sagitta elegans* Verrill. PhD thesis, Dalhousie University, Halifax
- Pearre S (1973) Vertical migration and feeding in *Sagitta elegans* Verrill. *Ecology* 54:300–314
- Pearre S (1979) Problems of detection and interpretation of vertical migration. *J Plankton Res* 1:29–44
- Pearre S (2003) Eat and run? The hunger/satiation hypothesis in vertical migration: history, evidence and consequences. *Biol Rev Camb Philos Soc* 78:1–79
- Picheral M, Catalano C, Brousseau D, Claustre H and others (2022) The Underwater Vision Profiler 6: an imaging sensor of particle size spectra and plankton, for autonomous and cabled platforms. *Limnol Oceanogr Methods* 20: 115–129
- Pierson JJ, Frost BW, Thoreson D, Leising AW, Postel JR, Nuwer M (2009) Trapping migrating zooplankton. *Limnol Oceanogr Methods* 7:334–346
- Pierson JJ, Frost BW, Leising AW (2013) Foray foraging behavior: seasonally variable, food-driven migratory behavior in two calanoid copepod species. *Mar Ecol Prog Ser* 475:49–64
- Saiz E, Calbet A, Broglio E (2003) Effects of small-scale turbulence on copepods: the case of *Oithona davisae*. *Limnol Oceanogr* 48:1304–1311
- Sims DW, Southall EJ, Tarling GA, Metcalfe JD (2005) Habitat-specific normal and reverse diel vertical migration in the plankton-feeding basking shark. *J Anim Ecol* 74: 755–761
- Stich HB, Lampert W (1981) Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature* 293:396–398
- Svendsen C, Kjørboe T (2000) Remote prey detection in *Oithona similis*: hydromechanical versus chemical cues. *J Plankton Res* 22:1155–1166
- Takahashi T, Sutherland SC, Sweeney C, Poisson A and others (2002) Global sea–air CO₂ flux based on climatological surface ocean pCO₂, and seasonal biological and temperature effects. *Deep Sea Res II* 49:1601–1622
- Tanimura A, Hattori H, Miyamoto Y, Hoshiai T, Fukuchi M (2008) Diel changes in vertical distribution of *Oithona similis* (Cyclopoida) and *Oncaea curvata* (Poecilostomatoida) under sea ice in mid-summer near Syowa Station, Antarctica. *Polar Biol* 31:561–567
- Trathan PN, Brandon MA, Murphy EJ, Thorpe SE (2000) Transport and structure within the Antarctic Circumpolar Current to the north of South Georgia. *Geophys Res Lett* 27:1727–1730
- Turner JT (2002) Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. *Aquat Microb Ecol* 27:57–102
- Turner JT, Ferrante JG (1979) Zooplankton fecal pellets in aquatic ecosystems. *Bioscience* 29:670–677
- Wallace MI, Cottier FR, Berge J, Tarling GA, Griffiths C, Brierley AS (2010) Comparison of zooplankton vertical migration in an ice-free and a seasonally ice-covered Arctic fjord: an insight into the influence of sea ice cover on zooplankton behavior. *Limnol Oceanogr* 55: 831–845
- Wallace MI, Cottier FR, Brierley AS, Tarling GA (2013) Modelling the influence of copepod behaviour on faecal pellet export at high latitudes. *Polar Biol* 36:579–592
- Ward P, Hirst AG (2007) *Oithona similis* in a high latitude ecosystem: abundance, distribution and temperature limitation of fecundity rates in a sac spawning copepod. *Mar Biol* 151:1099–1110
- Ward P, Atkinson A, Tarling G (2012) Mesozooplankton community structure and variability in the Scotia Sea: a seasonal comparison. *Deep Sea Res II* 59–60:78–92

Editorial responsibility: Deborah K. Steinberg,
Gloucester Point, Virginia, USA
Reviewed by: J. J. Pierson and 2 anonymous referees

Submitted: September 24, 2022
Accepted: June 9, 2023
Proofs received from author(s): July 24, 2023