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

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**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 two conical nets, one looking upwards and the other looking downwards, designed for stationary deployment at a set depth, into which migrating individuals must swim in order to be captured. The device was deployed at different time points within the diel cycle and at contrasting environments across Antarctic Polar Front region in the southwest 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 direction 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.

## 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 a number of 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 (Cottier et al., 2006; Pearre, 1979). These forays could allow individuals to maximise 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 upwards and downwards 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 (Boyd et al., 2019; Buesseler & Boyd, 2009), 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 (Cavan et al., 2015; Manno et al., 2015; Turner & Ferrante, 1979). 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 $CO_2$ 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, USA, an inshore bay on the west coast of the USA. Foray behaviour was observed in *Calanus pacificus* and *Metridia pacificus* 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, provides an even greater challenge. Our study aims 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 southwest 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 examine possible regional differences between taxa undertaking this behaviour across different environmental conditions. Our study aims 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.

## Methods

Net system: The Motion compensated Upward and Downward Looking net (MUDL) was designed by scientists and engineers at the British Antarctic Survey. The MUDL net is comprised of two conical nets mounted on an aluminium frame (Figure 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 spherical valve. This valve is hollow with three 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 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 compensation 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 *Supplementary Information 1*.

Sampling protocol: Zooplankton sampling was carried out by deploying the MUDL from the *RRS James Clark Ross* as part of the British Antarctic Survey (BAS) long-term ecosystem monitoring programme in the vicinity of South Georgia during austral summer, December 2016-January 2017 (JR16003). The MUDL net was deployed in four locations, to the south and east of, and within, the Polar Frontal Zone (Figure 2). Stations within the Polar Front were labelled Polar Front 2 (PF2) and Polar Front 4 (PF4), while the stations to the south of the Polar Front were labelled P2 and P3.

At each station, the MUDL was deployed at two different time points separated by approximately 12 h. We have nominally categorised these as dusk and dawn although exact timings vary with regards the relationship to sunrise and sunset (the average time difference between sunset or sunrise and the start of a deployment was 2 h 20 mins while the maximum difference was 4 h 16 mins). Dual deployments were made at each time point, one 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 station P2 where further time was available.

A vertical mini-Bongo deployment was made at approximately the same time as the evening MUDL deployment at station PF4 as a means of comparing the zooplankton community captured by a traditional net to that by the MUDL (further details on the specification of the mini-Bongo, its deployment protocol and subsequent sample and statistical analyses are provided in *Supplementary Information 2*).

Environmental sampling: Information on environmental conditions at the time of sampling was provided by CTD casts. The CTD consisted of a SBE32 carousel water-sampler, a SBE9Plus CTD, containing a SBE3Plus temperature sensor, a fluorometer (Chelsea Aquatracker mark III) and a photosynthetically active radiation (PAR) sensor. The depth of the Chl-a maxima were determined from the Chl-a profile obtained during the downcast (Figure 3). Water for use in the respective MUDL deployments was obtained from 100 m 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 minutes, 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 zooplankters to swim into the cod ends. The valves remained in this open position for 20 minutes, before being closed for retrieval. Whilst open, the MUDL remained at the target depth, and with the aid of the motion compensating mechanism, 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.

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 months 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 distinguishable 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.

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 two locational groups: (1) the Polar Front group (stations PF2 and PF4) and (2) the P2 group. Due to only having one sample from 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:

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

Where $p_i$ is the proportion of individuals belonging to each species.

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 (version7.0.13, 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 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 multi-dimensional differences in the underpinning resemblance matrix.

## Results

Environmental context: Temperature, salinity and fluorescence profiles show the differing environmental conditions at the stations sampled during the cruise (Figure 3). P3 and P2 showed a similar water column structure consisting of a surface mixed layer (~0 to 80 m), winter water (~80 to 200 m) and then upper circumpolar deep water (UCDW) (below ~200 m). Temperature ranged between -0.5°C 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 Front Zone (PF) stations, the structure of the water column was less clear, most likely as a result of high levels of vertical mixing. Temperatures were higher than 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 P3 had much greater Chl-a maxima (0.7 and 0.8 mg $m^{-3}$ respectively) than P2 (~ 0.25 mg $m^{-3}$). At P3, the maximum Chl-a was found in the surface 50 m before a sharp decline to < 0.01 mg $m^{-3}$ by 200 m. At 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 mg $m^{-3}$ by 200 m.

Performance of MUDL net: All MUDL 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 (Figure 4).

A mini-Bongo vertical deployment was carried out at PF4 to compare with the PF4 MUDL deployment. 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 compared to their relative prevalence in the water column while cyclopoids were less likely to be captured (see *Supplementary Information 2* for further details).

Analyses of taxa: Across all samples, copepods accounted for over 90% of all organisms in terms of abundance (Figure 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 (Figure 5).

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

With regards Shannon diversity, values were greater in the PF stations compared to 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; Figure 6). This was not the case at 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 genus across all samples (Table 2). The other dominant taxa differed between P2 and PF stations. At P2, *Oithona*, the calanoid copepod *Ctenocalanus*, pteropods and nauplii were abundant across both net directions and sampling times while, at the PF stations, harpacticoid copepods, the calanoids *Metridia* and *Haloptilus*, the cyclopoid *Oncaea* and pteropods were most abundant. There was little difference in the taxonomic composition of organisms captured at the depth of the Chl-a max + 10 m compared with those captured deeper, at 100 m (Table 3). The major difference was the presence of pteropods in the upward looking net at Chl-a max + 10 m, which were not found at the deeper depth nor in any downward looking nets.

Multivariate analysis of all samples collected during the survey showed two broad groups in the nMDS plot (Figure 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 the 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.

## Discussion

Performance of MUDL net: 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 (PF2 and PF4) being around 1 to 2°C warmer, and with a deeper mixed layer compared to the Scotia Sea stations (P2 and P3). Across this range of conditions, the MUDL 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 captured a similar composition 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 direction bias Within nMDS analyses, the samples formed two groups. Samples from the upward looking net formed one cluster whilst 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 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 a number of 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.

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 pacifica* 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, as would be hypothesised by the hunger satiation hypotheses. Gut contents of individuals caught by the MUDL 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. A number of studies have found vertically migrating zooplankton to be present in the upper water column during daylight hours (e.g. Irigoien et al., 2004; Ohman et al., 1983; Pearre, 1970, 1973; 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, showing that these taxa were also undergoing vertical migrations and may be exerting predation pressure upon the zooplankton community.

Cyclopoid foray behaviour: The cyclopoid *Oithona spp*. were found to be the most dominant taxa captured by the MUDL across all locations and were particularly dominant at P2. This high abundance is in line with previous sampling efforts within the Scotia Sea (Atkinson & Sinclair, 2000; Ward et al., 2012). The ratio of directional movement of zooplankton at P2 was found 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 *Oithona similis* under sea ice in mid-summer. During 24-hour 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, a number of authors report no vertical migration in *Oithona spp.* species over daily (Bogorov, 1946; Irigoien et al., 2004) or seasonal cycles (Ashjian et al., 2003; Atkinson, 1998). 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 (Paffenhöfer & Mazzocchi, 2002; Saiz et al., 2003; Svensen & Kiørboe, 2000). 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' in order to catch motile and non-motile prey (Kiørboe, 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

368 dusk, further underlining the presence of foray behaviour. *Oithona similis* have been found
369 to have a passive sinking speed of 0.0237 ± 0.0042 cm $s^{-1}$ and a mean swimming velocity of
370 0.051 ± 0.030 cm $s^{-1}$ (Hubareva & Svetlichny, 2016). Using these speeds, it would take *O.*
371 *similis* between 2 and 3 hours to passively sink the 2 m distance from the top of the upward
372 looking net to the cod end, but they may be able to swim the distance in 40 minutes. While
373 this would indicate that *Oithona* could not traverse the 2 m length of the net during the 20
374 min in which the net was open, those that were already within the net system, i.e. at the
375 depth where the MUDL came to rest, would be able to travel into the cod ends. In addition
376 to this, as passive sinking is slower than active swimming, the high numbers of *Oithona spp.*
377 caught in the present study are likely to have been actively swimming in either an upward or
378 downward direction, showing that *Oithona spp.* must be undertaking vertical migrations.

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

383 Contribution to carbon flux The present study reveals the existence of widespread foray
384 behaviour in the zooplankton communities of the Scotia Sea and Polar Frontal Zone. By
385 migrating from the upper water column to deeper waters, zooplankton transport carbon (as
386 well as nutrients) deeper in the water column, so contributing to the BCP. Wallace et al.,
387 (2013) used a model to simulate the volume of carbon exported in faecal pellets from
388 copepods undergoing different migration patterns, including foray behaviour. These authors
389 found that, in the absence of any vertical migrations, the resulting carbon export would be
390 minimal. Foray behaviour increased the volume of faecal pellet production in the deeper
391 layers although it was less than that generated by DVM. Asynchronous migrations reported
392 in the present study may therefore enhance the level of carbon flux in this region beyond
393 that generated by the DVM community. In the Scotia Sea, particulate organic carbon flux is
394 up to 22.91 mg C $m^{-2}$ $d^{-1}$ at 2000 m, with much of this being in the form of faecal pellets
395 (Manno et al., 2015), highlighting the importance of zooplankton migrations in this region.

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

cyclopoids such as *Oithona*) 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 direction of swimming although this is only over a short distance, as compared to the MUDL which integrates upwards or downwards movement over the 2 m length of the net.

A major advantage of the MUDL is that it captures live organisms displaying a particular foray behaviour (i.e. upwards or downwards 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 examination of gut contents before any further substantial amount of digestion has taken place.

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571

572

573 **Figure legends**

574 Figure 1: MUDL net being deployed from the *RRS James Clark Ross* in the Southern Ocean.
575 The main features of the net are indicated, including the motion compensatory system and
576 bi-directional nets.

577 Figure 2: Map of Polar Front region close to South Georgia, showing the four stations where
578 MUDL deployments were carried out from *RRS James Clark Ross* during 2016/17. Hatched
579 area shows location of the Polar Front Zone as determined by Orsi et al. (1995) and Trathan
580 et al. (2000). SACCF – Southern Antarctic Circumpolar Current Front

581 Figure 3: Example profiles of temperature (red line), salinity (blue line) and fluorescence
582 (green line) with depth as recorded by CTD casts at P3, P2, PF2 and PF4. Horizontal lines
583 indicate depth of MUDL deployment (Chl-a max + 10 m and/or 100 m). Note different
584 depths of Chl-a max + 10 m at station P2 resulted from being matched to different CTD
585 casts.

586 Figure 4: Abundance of organisms identified in upward looking and downward looking nets
587 across all MUDL deployments carried out during 2016/17. The boundaries of the box
588 indicate the 25th and 75$^{th}$ percentiles, the line within box, the median, the whiskers, the
589 90th and 10th percentiles, and the dots, 5$^{th}$ and 95$^{th}$ percentiles.

590 Figure 5: Percentage of individuals captured per taxa across all MUDL deployments. Each
591 stacked bar represent all deployments grouped together based on deployment time
592 irrespective of location or depth.

593 Figure 6: Abundance of individuals captured per taxa across all MUDL deployments at P2
594 (left) and combined PF stations (right). Each stacked bar represent all deployments grouped
595 together based on deployment time irrespective of depth. Numbers on top of stacked bars
596 represent Shannon's Diversity Index for each grouping (note that the Diversity Index is
597 based on lowest taxonomic level within the dataset of which some categories are grouped
598 here for presentation purposes).

599 Figure 7: nMDS (non-metric Multidimensional Scaling) of all MUDL samples. Spacing
600 between samples indicates the level of similarity, with those being located closest together
601 having the greatest similarity. Blue symbols denote samples caught by the upward looking

602 net; green symbols, by the downward looking net. Solid symbols represent dusk samples,
603 unfilled symbols, dawn samples.

604

## Tables

Table 1: MUDL deployments during cruise JR16003, December 2016 to January 2017 (in Local Time, GMT-3). Local sunset and sunrise based on times generated by the NOAA Solar Calculator https://gml.noaa.gov/grad/solcalc/sunrise.html

| Depth (m) | | Station | 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-a 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-a 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-a 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-a 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-a 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 |

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

614 a)

| P2 | Dawn | Dusk |
|---|---|---|
| Downward looking net | *Oithona spp. (40.8 ± 14.6)*<br>Unknown (copepod) (2.8 *± 3.0*)<br>*Ctenocalanus spp. (8.3 ± 4.5)*<br>Pteropod (4 *±* 1.2)<br>Nauplii (2 *± 1.3*) | *Oithona spp. (13.5 ± 28.2)*<br>Unknown (copepod) (5.5 *± 2.1*)<br>Nauplii (0 *± 0.7*)<br>*C. acutus (0.5 ± 0.7)*<br>*R. gigas (1 ± 0.7)* |
| Upward looking net | *Oithona spp. (314 ± 82.4)*<br>Unknown (copepod*) (16 ± 22.5)*<br>*Ctenocalanus spp. (51 ± 10.3)*<br>Pteropod (51.5 *± 6.4*)<br>*Oncaea spp. (21.3 ± 6.1)*<br>*Metridia spp. (39.8 ± 5.5)* | *Oithona spp. (177 ± 93.36)*<br>Unknown (copepod) (39.5 *± 1.4*)<br>*Ctenocalanus spp. (34.5 ± 9.2)*<br>Nauplii (4.5 *± 6.4*)<br>Pteropod (7.5 *± 3.5*) |

615

616 b)

| PF | Dawn | Dusk |
|---|---|---|
| Downward looking net | *Oithona spp. (37.7 ± 32.7)*<br>Harpacticoid (8.0 *± 13.9*)<br>*Ctenocalanus spp. (6.0 ± 4.6)*<br>*Metridia spp. (5.0 ± 5.0)*<br>*Halyoptus spp. (3.3 ± 5.8)* | *Oithona spp. (17.7 ± 25.4)*<br>*Oncaea spp. (8.7 ± 12.5)*<br>*Metridia spp. (7.0 ± 5.61)*<br>*Ctenocalanus spp. (5.3 ± 6.8)*<br>Calanoid (unidentified) (7.5 *± 7.8*) |
| Upward looking net | *Oithona spp. (291.67 ± 177.46)*<br>*Ctenocalanus spp. (43.33 ± 4.9)*<br>Pteropod (28.3 *± 23.5*)<br>*Metridia spp. (17.7 ± 21.953)*<br>Unknown (copepod) (15.3 *± 11.3*) | *Oithona spp. (203 ± 1629)*<br>*Ctenocalanus spp. (54 ± 23.9)*<br>Pteropod (45.3 *±* 66.5)<br>*Metridia spp. (41.3 ± 43.2)*<br>*Oncaea spp. (33.0 ± 18.19)* |

617

618

619

620

621 Table 3: Most numerous taxa, in order of abundance, across all MUDL samples collected at
622 Chl-a max + 10 m and at 100 m. Numbers in brackets denote the average abundance (± SD)
623 across all deployments at that depth in units of individuals per net.

624

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

625

Fig.1

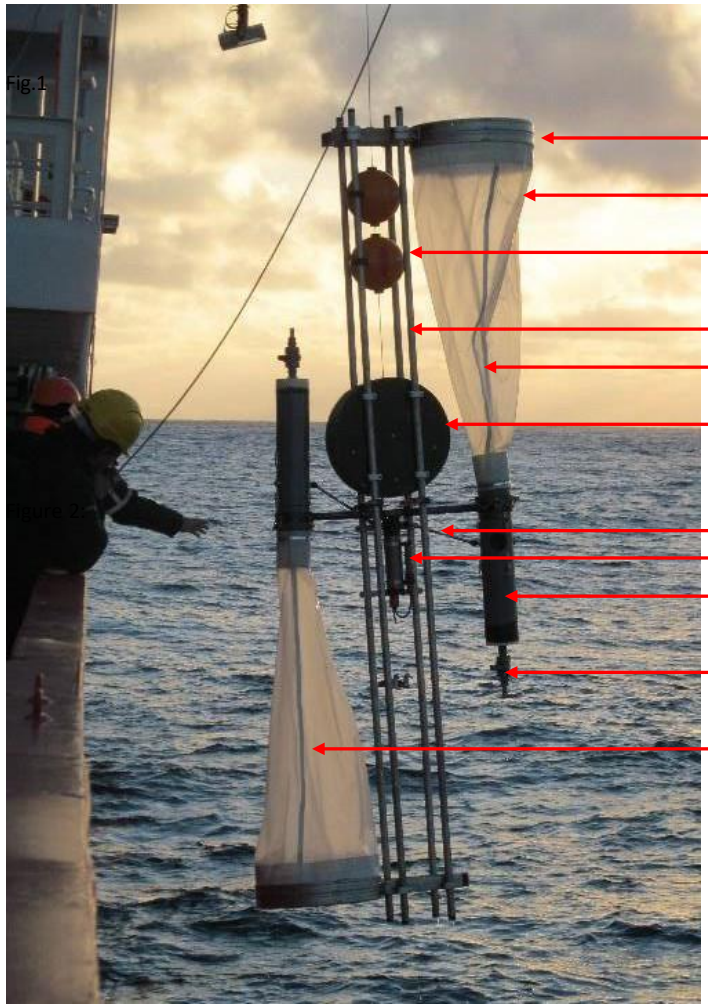
Net opening
100 µm mesh
Buoyancy floats
(attached to frame)
MUDL Frame
Upward looking net
Spring loaded motion compensatory
system containing deployment wire
Motor arm
Motor
Cod end
Tap
Downward looking net

Fig. 1

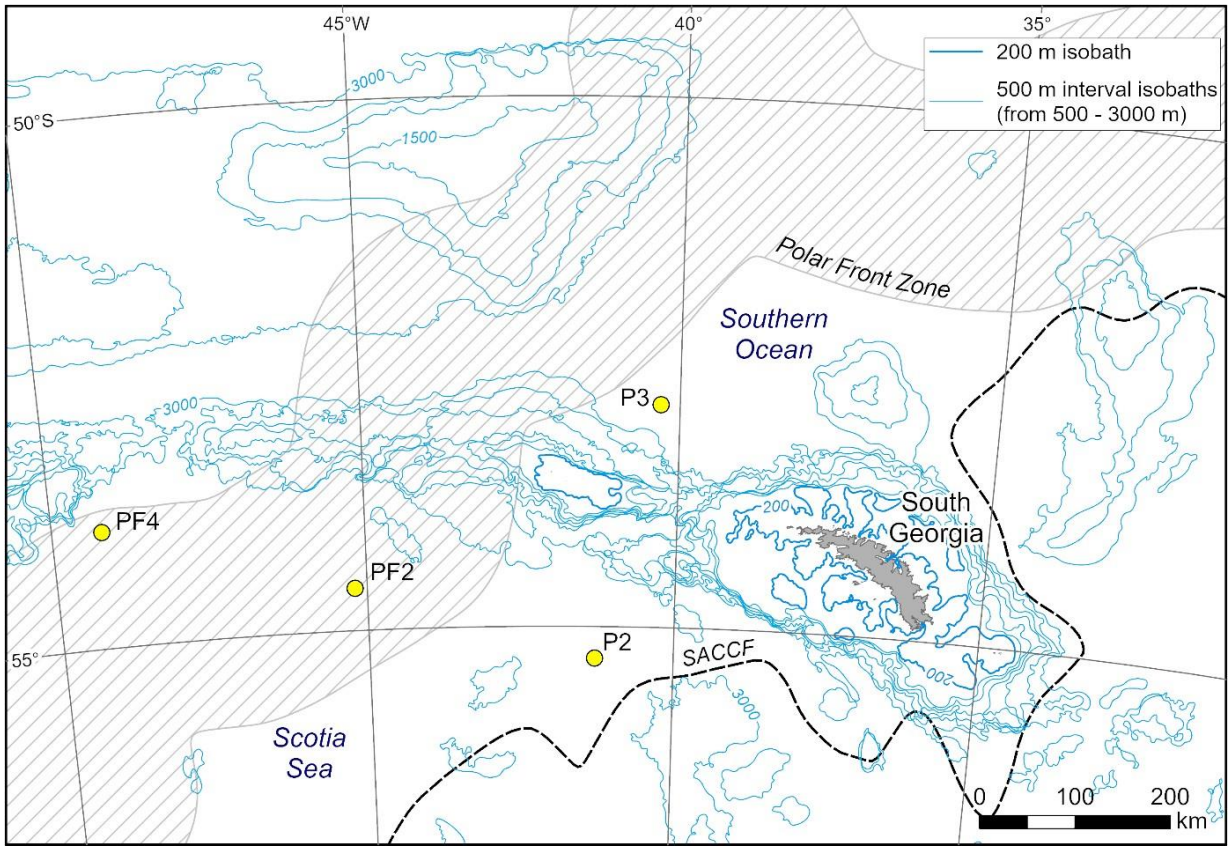
45°W
40°
35°
50°S
55°
200 m isobath
500 m interval isobaths
(from 500 - 3000 m)
3000
1500
Polar Front Zone
Southern
Ocean
P3
PF4
PF2
P2
South
Georgia
SACCF
Scotia
Sea
200
0
100
200
km

Fig. 2

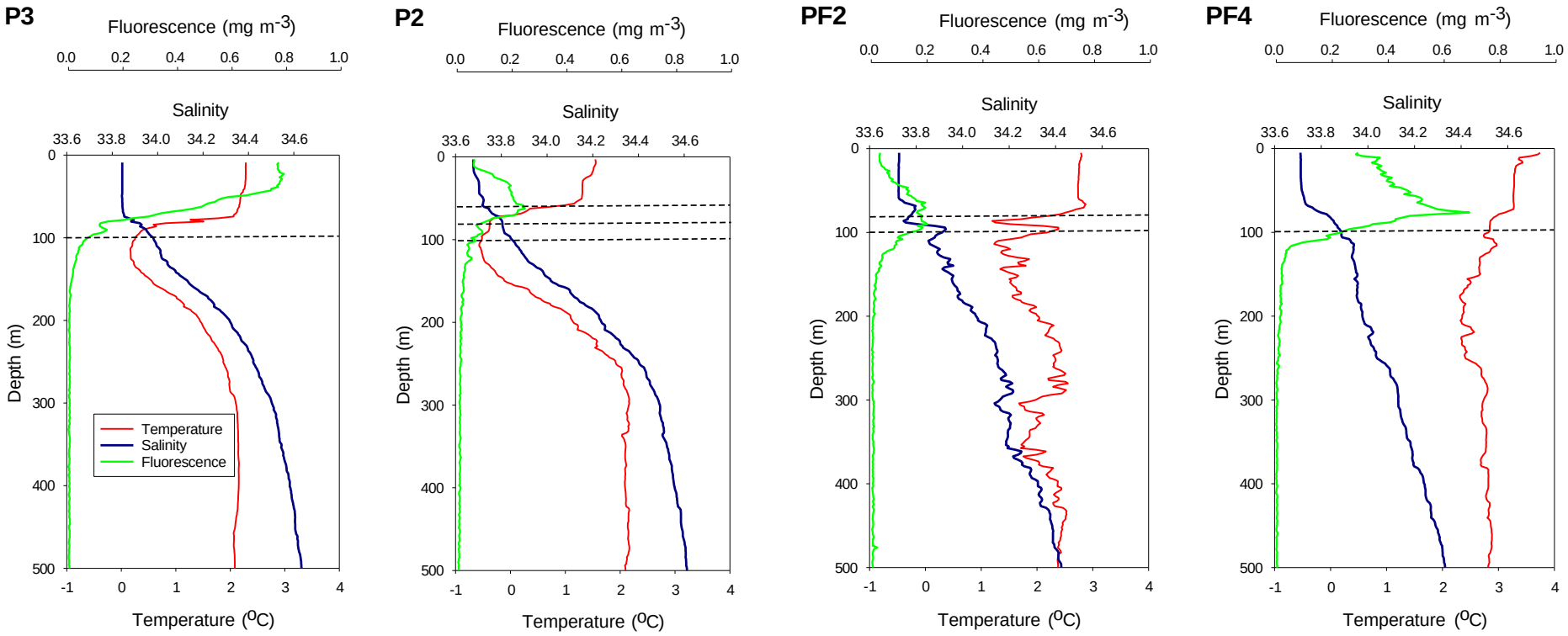
P3
P2
PF2
PF4
Fluorescence (mg m-3)
0.0 0.2 0.4 0.6 0.8 1.0
Salinity
33.6 33.8 34.0 34.2 34.4 34.6
Depth (m)
0 100 200 300 400 500
Temperature
Salinity
Fluorescence
-1 0 1 2 3 4
Temperature (°C)

Fig. 3

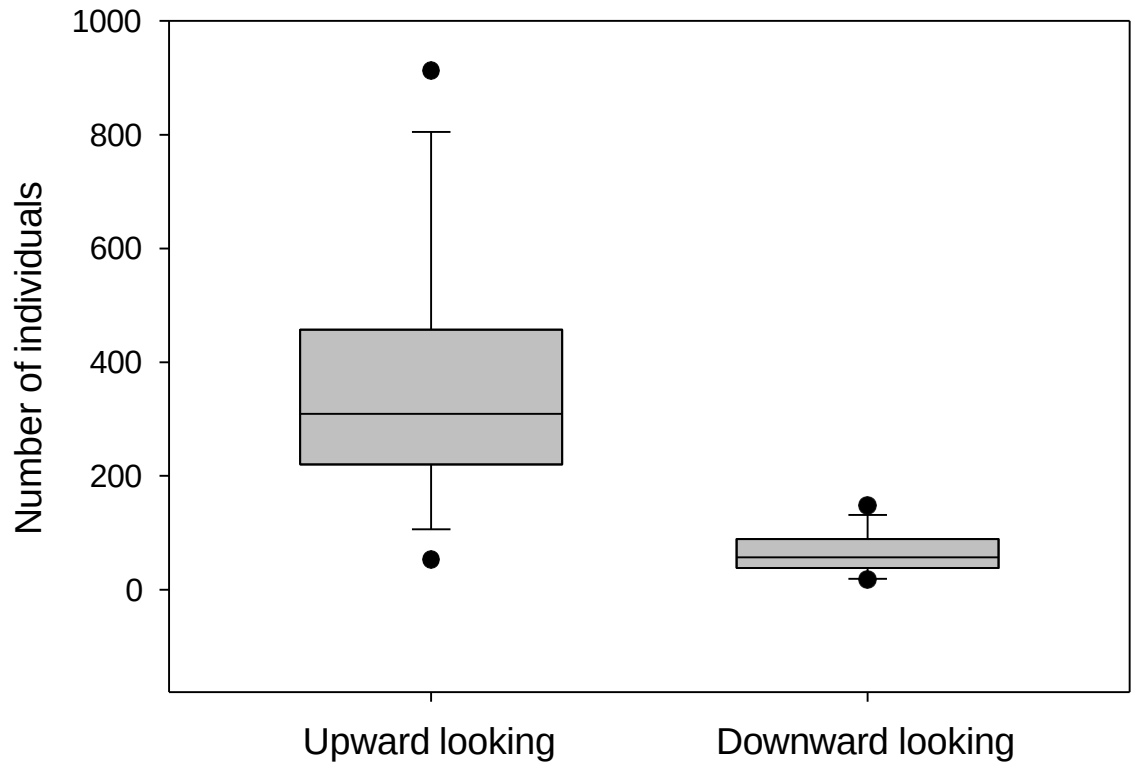
1000
800
600
400
200
0
Number of individuals
Upward looking
Downward looking

Fig. 4

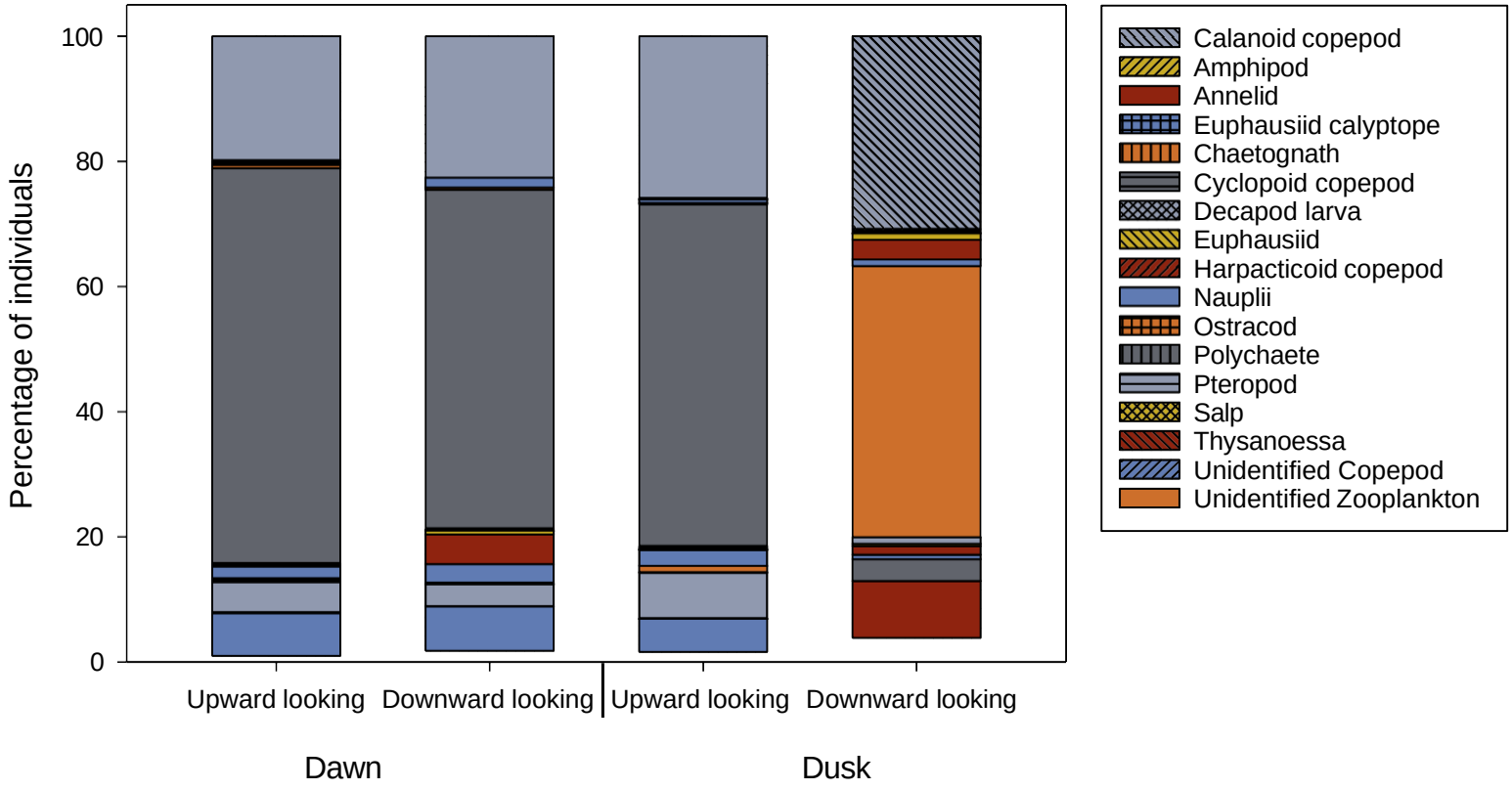
Percentage of individuals
100
80
60
40
20
0
Upward looking
Downward looking
Upward looking
Downward looking
Dawn
Dusk
Calanoid copepod
Amphipod
Annelid
Euphausiid calyptope
Chaetognath
Cyclopoid copepod
Decapod larva
Euphausiid
Harpacticoid copepod
Nauplii
Ostracod
Polychaete
Pteropod
Salp
Thysanoessa
Unidentified Copepod
Unidentified Zooplankton

Fig. 5

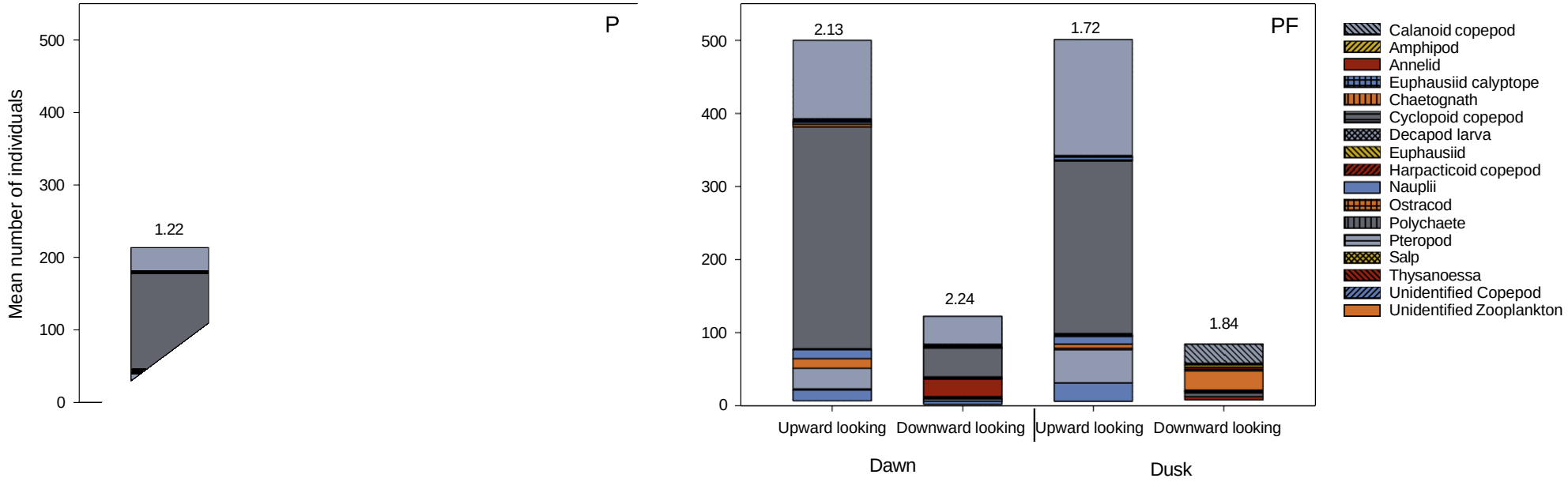
P
PF
Mean number of individuals
0
100
200
300
400
500
1.22
2.13
2.24
1.72
1.84
Upward looking
Downward looking
Upward looking
Downward looking
Dawn
Dusk
Calanoid copepod
Amphipod
Annelid
Euphausiid calyptope
Chaetognath
Cyclopoid copepod
Decapod larva
Euphausiid
Harpacticoid copepod
Nauplii
Ostracod
Polychaete
Pteropod
Salp
Thysanoessa
Unidentified Copepod
Unidentified Zooplankton

Fig. 6

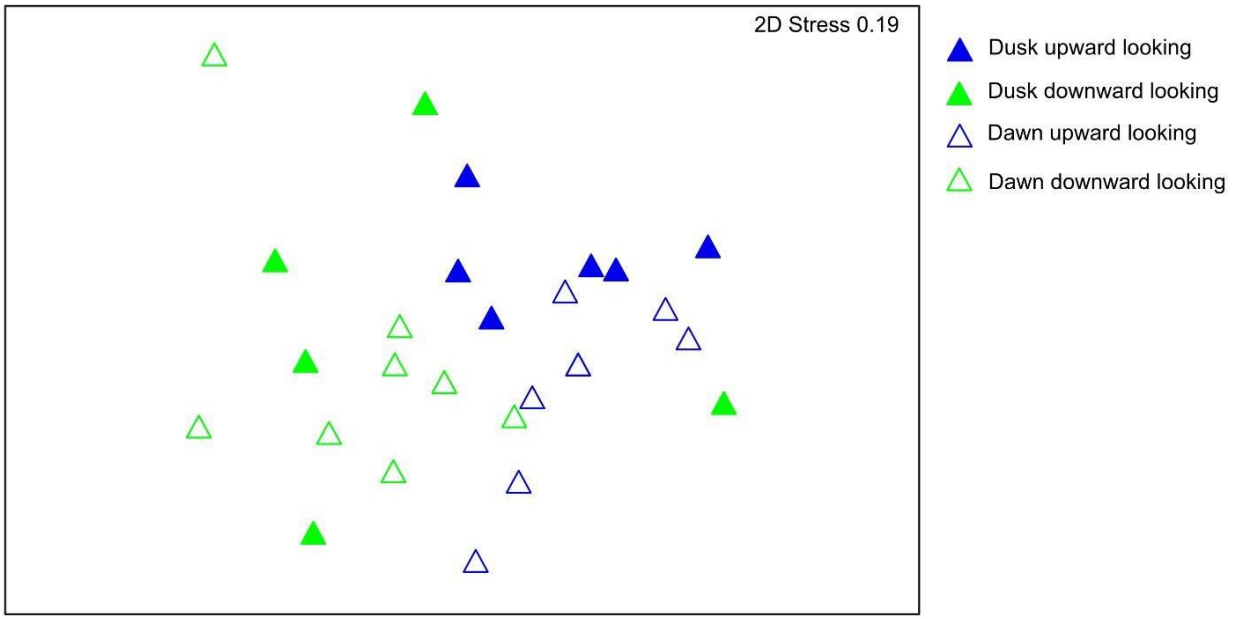
2D Stress 0.19
Dusk upward looking
Dusk downward looking
Dawn upward looking
Dawn downward looking

Fig. 7

# Supplementary Information 1: Further technical details of the Motion compensated Upward and Downward Looking (MUDL) net

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The Motion compensated Upward and Downward Looking (MUDL) net was designed by scientists and engineers at the British Antarctic Survey. It is comprised of two conical nets mounted on an aluminium frame with the deployment wire attached to a spring loaded mechanism (Figure SI1.1). It is deployed tethered to a deployment wire that lowers it to a predetermined depth where it remains for a set period before recovery back onto deck. The purpose of the MUDL is to trap mesozooplankton that enter the net through their own upwards or downwards swimming. This type of trapping allows the level of simultaneous upward and downward flux of these organisms to be determined over a designated period of time.

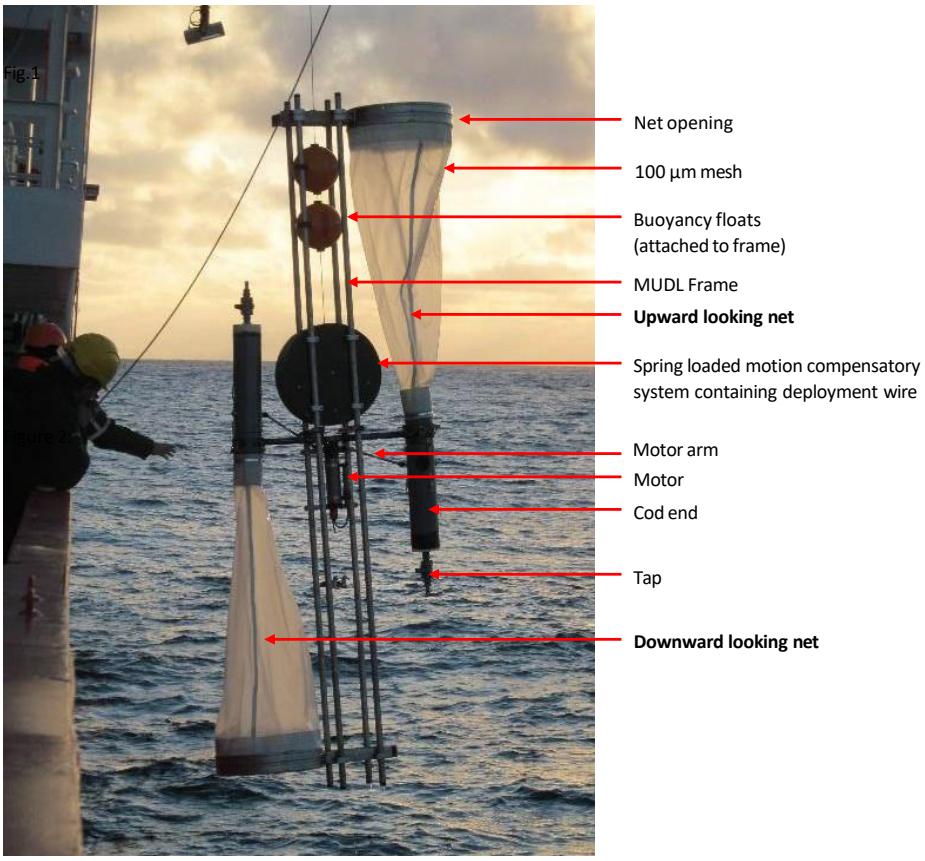


*Fig. SI1.1. Motion compensated Upward and Downward Looking (MUDL) net being deployed in Scotia Sea (Dec 2016)*

Both the upward-looking and downward-looking nets have a rigid cylindrical opening with a diameter of 61 cm, with a 100 µm nylon netting tapering to cod ends 2 m away. The spring loaded motion compensation mechanism that sits in the centre of the frame allows the net to maintain its vertical position and remain stable during deployment. In essence, it compensates for the effect of swell on the deployment vessel that would otherwise be transmitted down the deployment wire to which the MUDL remains tethered throughout the period of deployment. Buoyancy floats positioned towards the upper part of the main frame are there to keep the MUDL as vertical as possible during deployment.

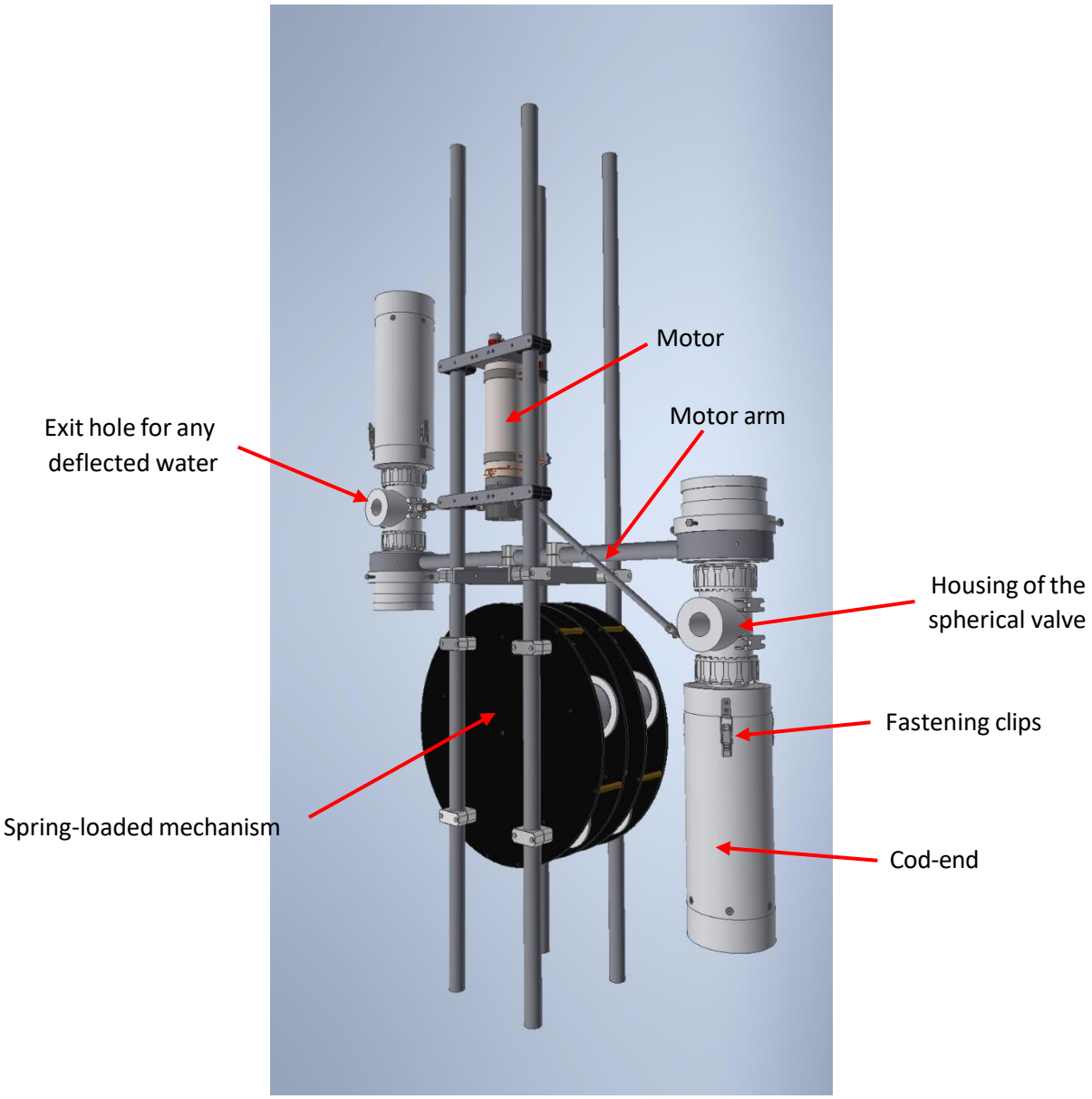


*Fig. SI1.2: Schematic of the cod-end mechanism (NB. Taps at ends of cod-ends not shown). Figure courtesy of Scott Polfrey and Daniel Ashurst, British Antarctic Survey, Engineering and Technology*

At the entrance of the cod ends is a spherical valve contained within a housing (Fig SI1.2). This valve is hollow with three circular holes cut into it. The rotation of this valve starts and ends sample collection. In one position, a valve is opened into the cod-end that allows organisms to swim in. In other positions, this cod-end is sealed. There are exit holes next to the spherical valve that allows

any water funnelling through the net (for instance, during downward deployment and upward recovery) to be deflected away. The two spherical valves (one in each cod-end) are rotated by a single motor which is connected to the spherical valves via motor arms.

**Prior to deployment**

1. Motor and programming: The motor was custom supplied by Hydro-Bios Apparatebau GmbH and is based around the motor that drives their MultiNet system. To the motor, we fitted a bespoke gearing mechanism to drive two motor arms that rotate the spherical valves. The Hydro-Bios motor was programmed via a customised version of their Ocean-Lab 3 software where times were set for the motor to rotate first to move from a closed to open position and then to move from an open to closed position.
2. Cod-end preparation: The spherical valves sealed off the cod-ends prior to deployment. To avoid pressure differentials during downward deployment, it was necessary to fill the cod-ends with water prior to deployment. For the upward looking cod-end, clips could be unfastened, the cod-end detached from the device and filled with water, and then re-attached and the clips refastened. For the downward looking cod-end, it was necessary to fill the cod-end via a tap at the end of the cod-end (see Fig. SI1.1). We used seawater taken from a CTD water sample taken just prior to the MUDL deployment, at a similar depth to the intended MUDL collection depth.

**Deployment**

The net is deployed using the same method as for a standard WP2 or Bongo net, with the net being lowered vertically over the side of the ship and then the deployment wire paid out at an approximate rate of 0.3 m $s^{-1}$. Once reaching its maximum designated depth, the MUDL remains there for a preset period during which time the entrances to the cod-ends open and organisms can swim in. For the present study, this collection period was set to 20 mins. The net was then recovered at a hauling-in rate of approximately 0.3 m $s^{-1}$ and secured upright on deck.

**Post-deployment**

The sample within the upward looking cod-end could be recovered through simply opening the tap and letting the contents gently drain into a bucket partially prefilled with filtered seawater to cushion the flow of organisms into it. For the downward looking cod-end, firstly it was necessary to untether the bottom narrow end of the net from the cod-end. A partially prefilled bucket was then held underneath the cod-end. Direct communication with the motor was established and it was instructed to rotate and unseal the cod-end, allowing the contents to flow out into the bucket.

**Further details on the spherical valve mechanism**

The different positions of the spherical valve are illustrated in Figs. SI1.3 to Fig SI1.4, in which the outer housing has been cut away to show the valve itself. At the start of the deployment, the valve in the closed position (Fig SI1.3). The cod-end is sealed and any water flowing through the net, as it travels downward, is deflected through the exit holes.

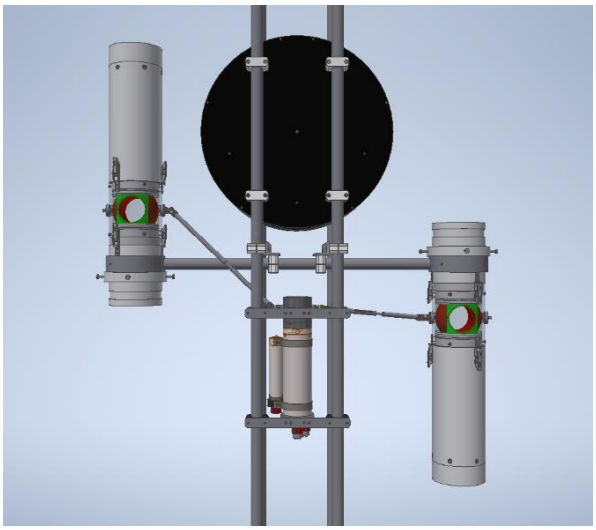

*Fig. SI1.3. Position during downward deployment. Cod ends are sealed, water is deflected out of exit hole (NB. Taps at ends of cod-ends not shown). Figure courtesy of Scott Polfrey and Daniel Ashurst, British Antarctic Survey, Engineering and Technology*

Once the net reaches the deployment depth, the valve rotates, which opens up a conduit from the net to the cod-end and seals the exit holes (so avoiding any individuals taking a short-cut into the cod-end; Fig SI1.4)

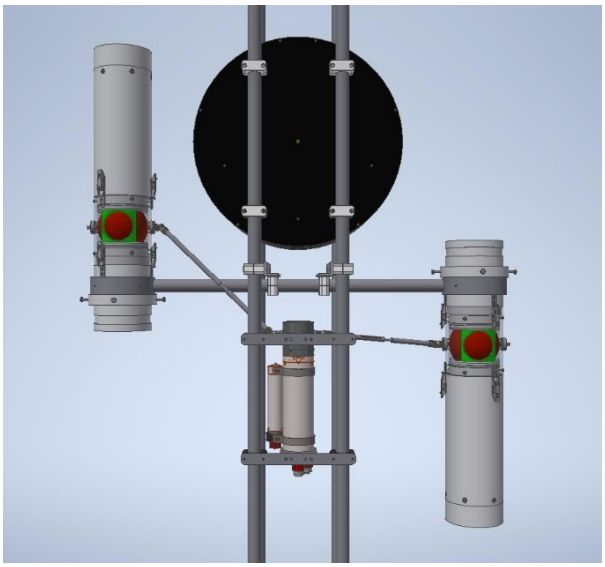

*Fig SI1.4. Position during sample collection. The entrance to the cod-end is now open, allowing organisms to swim in (NB. Taps at ends of cod-ends not shown). Figure courtesy of Scott Polfrey and Daniel Ashurst, British Antarctic Survey, Engineering and Technology*

At the end of a preset time (see above), the valve rotates once more, sealing off the cod-end so that it is in the same conformation as during downward deployment (Fig SI1.3). This means that any water passing through the net is deflected through the exit holes during upward recovery and the sample within the cod-ends is sealed from any contamination.

# Supplementary Information 2: Comparison of catches between MUDL net and vertically deployed mini Bongo net

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**Rationale**

To provide a context for the capture efficiency of the MUDL Net, a mini Bongo net was deployed vertically to a maximum depth of 70 m at approximately the same time as a MUDL deployment at Polar Front station PF4. We report on the total number of organisms captured by the respective nets as well as the proportional composition of different zooplankton taxonomic groups.

**Methods**

The design and method deployment of the MUDL net is described in detail in the main manuscript and will not be further described here. The mini-Bongo net had an 18 cm mouth diameter with 53 µm meshed net tapering to the cod end. Both nets were deployed at Polar Frontal Zone station PF4 (53.930°S, 49.154°W) within one hour of each other on 4/1/2017 (mini-Bongo between 21:32 and 21:37 GMT; MUDL between 21:42 and 22:20). The mini-Bongo went to a maximum depth of 70 m, the MUDL was sent to a depth of 100 m and opened for 29 mins before subsequent recovery. Both nets were within the mixed layer as defined by the temperature and salinity profiles (Fig. 3). Upon retrieval, the mini-Bongo sample was filtered and preserved in 95% ethanol. The preserved sample was sent to Morski Instytut Rybacki, Poland for zooplankton taxonomic analysis, using the following protocol: any organisms larger than 10 mm were removed from the aliquot and recorded before the sample was sorted; the aliquot was then sorted and all plankton identified to the lowest possible taxonomic level; the raw counts of each species were multiplied by the inverse of the sample fraction to give a whole sample count for each species/ stage recorded. The MUDL net samples were frozen and subsequently preserved back at the home laboratory before taxonomic identification and abundance analyses under a light microscope (see the main manuscript for full details).

Results

We found the MUDL net to catch approximately 4.5 % and 0.3 % of the total mini Bongo catch in the downward looking and upward looking nets, respectively (Figure SI2.1). In terms of the patterns of proportional taxonomic composition of the two net types, catches were broadly similar, with cyclopoid copepods being the most abundant taxa across all samples (Figure SI2.2a). Nevertheless, certain taxa found in the mini-Bongo were absent from the MUDL, including annelids, appendicularians and tunicates. Furthermore, an ANOVA on Ranks found significant differences in the composition of the two net types ($F_{2,26}$=21.29, P <0.001), further resolved by Dunn's pairwise tests (mini-Bongo versus downward looking MUDL: P < 0.001; mini-Bongo versus upward looking MUDL, P=0.001). Most notably, the proportional abundance of the dominant taxonomic groups, calanoid copepods, was significantly greater in the MUDL samples, accounting for almost 40 % and 30 % of the total catch in the downward and upward looking nets as compared to less than 20 % in the mini Bongo sample (Figure SI2.2b). There were other minor differences between net types in the proportional abundance of rare taxa such as Euphausiidae, Gastropoda and Harpacticoids, but this is as likely to be influenced by natural variation as opposed to instrumental selectivity.

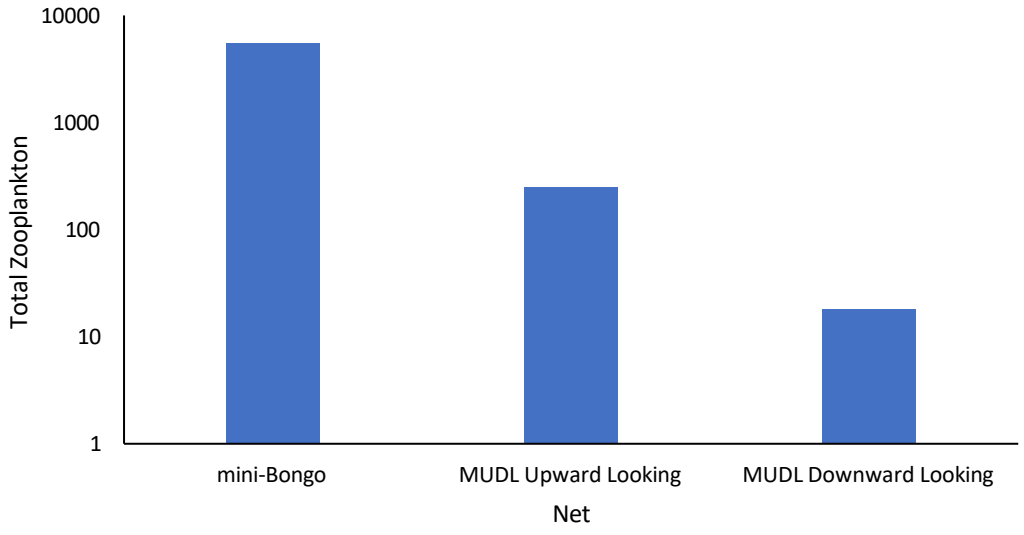


Figure SI2.1: Total number of zooplankton caught in each of the mini-Bongo, upward and downward looking nets at PF4. All nets were deployed to 100 m. mini- Bongo net was vertically hauled to the surface from 100 m, while the MUDL net only sampled whilst stationary at 100 m.

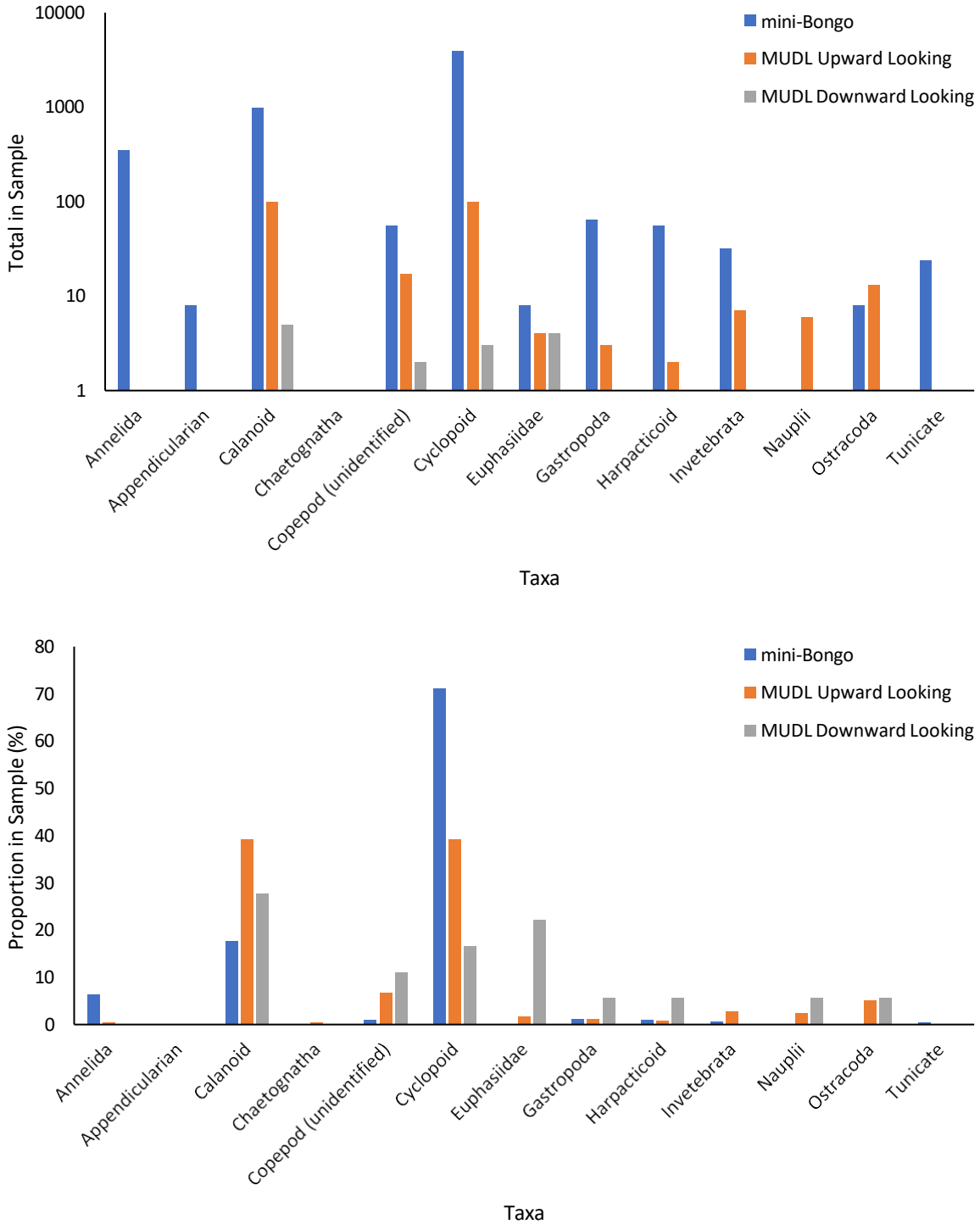


Figure SI2.2: Comparisons of MUDL and Bongo nets. (Upper) Total abundance of zooplankton in each taxa found in the mini Bongo (blue), the upward looking MUDL net (orange) and the downward looking MUDL net (grey). (Lower) Relative proportions of each zooplankton taxa found within each of the net samples for the mini Bongo (blue), the upward looking MUDL net (orange) and the downward looking MUDL net (grey).

## Conclusions

It is unsurprising that vertical integration of the water column, as performed by the mini-Bongo, will collect far more organisms per unit area compared to the MUDL into which organisms must swim in order to be captured. However, the proportional composition of different taxonomic groups was comparatively similar between the two net types. Differences between dominant taxa were found, with the MUDL more likely to catch a greater proportion of calanoid copepods and the mini-Bongo, a greater proportion of cyclopoid copepods. This may reflect the greater swimming capabilities of the calanoids, making them more likely to move into the net than the less motile cyclopoids.