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COMPARATIVE AND INTERCALIBRATION STUDIES

# Picturing plankton: Complementing net-based plankton community assessments with optical imaging across diverse marine environments

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## *Abstract*

In recent years, optical imaging has emerged as a promising tool for in situ observations of plankton. In this study, we aimed to compare the plankton community estimates obtained from a Video Plankton Recorder (VPR) imaging device with net-based approaches. By collecting VPR and net samples in clear waters with large-sized plankton and eutrophic waters with small-sized plankton, spatial and temporal patterns in plankton densities and community composition were compared. Furthermore, it allowed the evaluation of the performance of imaging methods under diverse hydrographic conditions. We observed pronounced spatial differences in density estimates. In the eutrophic site, the WP2 net densities consistently surpassed those from a VPR, while in the clear water site the observed densities of the VPR and a MultiNet were more similar. Variations in water column turbidity, plankton body size, plankton nets and their mesh size, and the total sampled water volume were found to likely play a role in the observed inconsistencies between the sampling sites. The results suggested that a VPR is particularly well-suited for use in clear waters inhabited by large-sized plankton. The VPR demonstrated potential in enhancing density estimates of fragile (*Phaeocystis*) and gelatinous taxa (Cnidaria and Ctenophora) in specific environments being non-invasive. Overall, the VPR and other optical imaging devices show valuable insights into zooplankton ecology and distribution, complementing density estimates of traditional net sampling methods, and enhancing our understanding of the role of zooplankton in marine ecosystems.

Zooplankton communities are crucial for the functioning of marine ecosystems. They form the link between phytoplankton and higher trophic levels, such as many economically important fish (Nielsen et al. 1993) and play a pivotal role in biogeochemical cycles (Steinberg and Landry 2017). Most zooplankton species are short-lived, have a fast generation time and are sensitive to temperature fluctuations (Mackas et al. 2012; Chivers et al. 2017), making them suitable indicator organisms to evaluate the effects of environmental and anthropogenic pressures on ecosystem functioning. Gaining insight into zooplankton ecology and biology will therefore help to understand the functioning of marine ecosystems and predict their response to environmental changes.

Despite their importance, studying zooplankton can be an intricate task due to their small size, high diversity, and complex life cycles. They can vary in shape, size, and behavior, making it difficult to identify them accurately (Hablützel et al. 2021). Moreover, their patchy distribution patterns and seasonal variations are also challenging to predict, further increasing the complexity. There are various methods to study the plankton community (Hablützel et al. 2021). Typically, plankton research starts from physical samples collected by

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plankton nets followed by often time-consuming species identification and counts at the species level under a microscope. This technique has been used for over a century, and has provided valuable information about the abundance, diversity, and distribution of plankton. However, due to the labor-intensive nature of this methodology, it is often constrained to a limited spatiotemporal coverage. Moreover, net samples only represent a single point in time and space, failing to encompass the variability among biotic and abiotic components across a continuum of spatiotemporal scales.

Nowadays in situ imaging tools have been developed and are more frequently used for plankton research. Optical imaging devices such as the Video Plankton Recorder (VPR) harness unique capabilities to perform continuous in situ observations and observe planktonic organisms in their natural environmental context across various spatial scales. It has some specific characteristics that overcome the limits of traditional net sampling methods. It has the advantage of observing fragile marine diversity non-invasively without damaging it, and the simultaneous abiotic, spatial and geographic measurements allow for a tight coupling between plankton and the environment to research its vertical distribution and affinities with water quality parameters. Also having the sample in a digital format can allow for a faster processing time with automated classifiers.

The aim of this study is to evaluate the accuracy of in situ imaging estimates in assessing the plankton community compared to different net-based approaches. This study will involve a comparison of plankton abundances and short-term patterns using an in situ Real Time VPR (Davis et al. 2004), a WP2 net and a MultiNet mini under different hydrographic conditions.

## Materials and procedures

### Study area

In this study, we focus on two distinct geographical regions (Fig. 1), each presenting unique environmental characteristics. The first of these study areas is the Belgian part of the North Sea which covers approximately 3600 $km^2$. Situated in the southern North Sea, it serves as a transitional zone between the Atlantic Ocean and the North Sea. This region is relatively shallow with maximum depths of 40 m and encompasses several sandbanks (Van Lancker et al. 2012). The strong tidal currents result in a well-mixed water column with very weak salinity and temperature stratification (Fettweis and Nechad 2011) which seasonally range between 28 and 35 NTU and 6–22°C, respectively (Flanders Marine Institute 2020). The eutrophic water column sustains a dense plankton community and is characterized by a high nutrient concentration (De Galan et al. 2004) due to the outflow from the rivers as Ijzer, Scheldt and Maas (Nihoul et al. 1978). Especially the river Scheldt has a dominant influence in terms of suspended particulate matter (SPM), forming a high turbidity zone near the coast (Fettweis et al. 2007).

The second study area is the Uummannaq fjord system in West Greenland (between ca. 70–72°N; ca. 50–55°W). Inshore, the fjord system is connected to the Greenland Ice Sheet via numerous streams and tidewater glaciers, and to Baffin Bay offshore. The complex interplay between these boundaries determines the local hydrography and water masses distribution. Depending on depth and water mass, the salinity and temperature of the water column exhibit a broad range, varying between 24 and 35 and −2 to 9°C (Carroll et al. 2018), respectively. The fjord system depth varies from approximately 250 to 600 m. A key bathymetric feature is the 700–800-m deep trough that extends to the continental shelf break, through which oceanic water masses can flow into the fjords (Rignot et al. 2016).

### Data collection

Data was collected during three cruises (Fig. 1; Table 1). The first cruise ("methodological cruise") took place in March 2021 aboard the RV Simon Stevin in the southern North Sea when samples were specifically collected to compare a WP2 net (Hydro-Bios, Germany) and a Real Time VPR (Seascan, USA) samples. Different towing procedures and magnification settings of the VPR were used, since these parameters are known to affect the measurements of the VPR and therefore the zooplankton density estimates (Ollevier et al. 2022). A Real Time VPR comprises four preset magnification settings denoted as S0 till S3, each providing distinct fields of view and resulting in different imaged volumes. Three WP2 net samples were collected, followed by three downcasts with the VPR and a 1-h transect (~ 7.77 km) undulating the VPR through the water column using a high magnification setting (S1; field of view: 20.8 × 15.2 mm). After returning to the location of the WP2 haul, three more VPR downcasts and a continuous 1-h VPR transect were performed using a low magnification setting (S3; field of view: 46.5 × 34.5 mm). The continuous VPR transects allow for the assessment of the zooplankton community in a larger area around the location of the WP2 haul. During data collection of the downcasts and transects, the VPR was undulated between 3 and 34 m depth with a winch at a speed of 0.15 m $s^{-1}$, while the vessel sailed at 3–4 knots relative to the water column.

Zooplankton was sampled with a 200 $\mu$m WP2 net (57 cm diameter opening), which was deployed vertically and equipped with a flowmeter, following the protocol of Mortelmans et al. (2019). The sampled volume [$m^3$] of each WP2 haul was calculated by multiplying the surface area of the net opening [$m^2$] by the flow distance [m]. The net was lowered to just above the sea bottom and hauled up with a maximum speed of 1 m $s^{-1}$. Upon retrieval, the outside of the WP2 net was rinsed with a hose to gather the remaining zooplankton into the cod-end. Zooplankton collected in the cod-end was sedated with carbonated water and fixed in 4% formaldehyde.

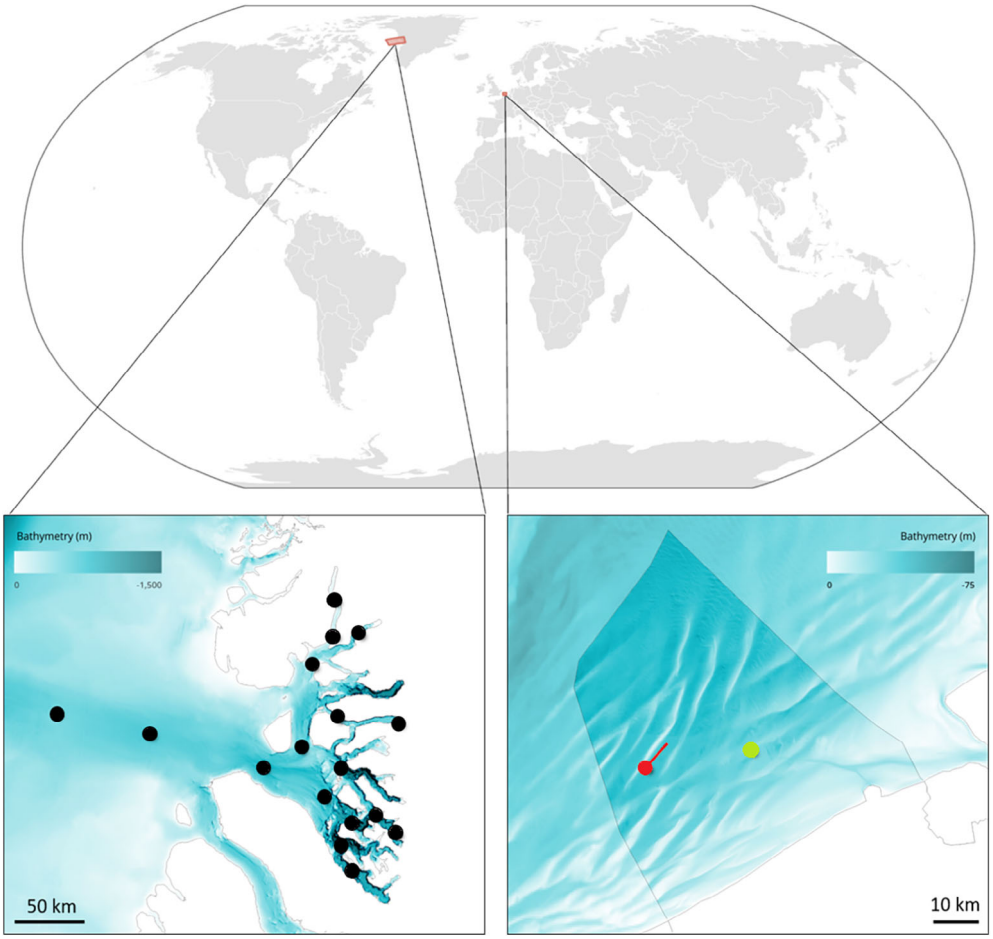


**Fig. 1.** Sampling locations in Greenland and the Belgian part of the North Sea. Red dot and transect = "methodological cruise" in March 2021; green dot = "24-h cruise" in May 2021; black dots = "Greenland cruise" in June–July 2022.

**Table 1.** Overview of the VPR methodology and settings for the three cruises. Overview of the number of repeats for each data collection method, the (mean) sampling time [min or h], field and depth of view [mm], imaged volume per frame [mL] and (mean) total sampled volume [$m^3$] per WP2 or MultiNet haul and VPR cast or transect. Note: for the MultiNet the mean sampled volume of the top 150 m layer was given in order to compare it better with the mean sampled volume of the VPR in Greenland.

| | Methodological cruise | | | | | 24-h cruise | | Greenland cruise | |
|---|---|---|---|---|---|---|---|---|---|
| | March 2021 | | | | | May 2021 | | June–July 2022 | |
| Magnification and towing procedure | WP2 haul | High downcast | High transect | Low downcast | Low transect | WP2 haul | High downcast | MultiNet haul | Low transect |
| Number of repeats | 3 | 3 | 1 | 3 | 1 | 24 | 23 | 17 | 17 |
| Sampling time | — | 6.2 min | 1 h | 5.6 min | 1 h | — | 15 min | — | ± 1.5 h |
| Field of view [mm] | — | 20.8 × 15.2 | 20.8 × 15.2 | 46.5 × 34.5 | 46.5 × 34.5 | — | 20.8 × 15.2 | — | 46.5 × 34.5 |
| Depth of view [mm] | — | 105.5 | 105.5 | 271.9 | 271.9 | — | 93.5, after 3:00 83.3 | — | 209.2 |
| Imaged volume per frame [mL] | — | 33.342 | 33.342 | 436.162 | 436.162 | — | 29.564, after 3:00 26.345 | — | 335.622 |
| Total sampled volume [$m^3$] | 8.93 | 0.31 | 3.01 | 3.67 | 38.25 | 6.17 | 0.65 | 18.75 (top 150 m) | 46.08 |

In the lab, net samples were digitized by using the ZooScan (Hydroptic, France), a benchtop plankton imaging device. To accomplish this, formaldehyde was drained from the samples, which were then rinsed. Following this, the samples were fractionated using a Motoda splitter (Motoda 1959), which served to dilute them and prevent organism overlap in the scanned images (Grosjean et al. 2004; Gorsky et al. 2010). After scanning, the fixative was changed to 70% ethanol. The obtained scans were processed in ZooProcess and Plankton Identifier software (v. 1.3.4, Gasparini and Antajan 2018) in order to detect and classify the digitized objects (Grosjean et al. 2004; Gorsky et al. 2010). Predictions were manually verified and finally assigned to one of the following taxa: Annelida, Anomura, Appendicularia, Brachyura, Branchiopoda, Calanoida, Chaetognatha, Cirripedia, Cnidaria, Ctenophora, Cumacea, Echinodermata, fish eggs, fish larvae, Harpacticoida, Mollusca, and *Noctiluca*. Non-planktonic categories included artifacts, detritus, and fibers.

Plankton was also sampled by means of a VPR. The collected image data, consisting of regions of interest (ROIs) that were automatically segmented from each image full frame by the AutoDeck software, was manually classified by sorting the zooplankton into the categories Amphipoda, Annelida, Appendicularia, Brachyura, Calanoida, Cnidaria, Ctenophora, Cumacea, Echinodermata, fish larvae, and Harpacticoida. Other particles were classified as *Noctiluca*, *Phaeocystis*, detritus, bubbles, fibers, or unknown. Plankton densities were calculated as the number of individuals per sampled volume and then linearly extrapolated to cubic meters of water [ind $m^{-3}$], to allow a comparison between VPR and nets, in which sampled volume is determined by:

$$\begin{aligned}\text{Sampled volume [mL]} = {} & \text{Imaged volume} \left[\text{mL frame}^{-1}\right] \\ & \times 25 \left[\text{frames s}^{-1}\right] \\ & \times \text{Duration of the tow [s]}\end{aligned}$$

During the methodological cruise, the imaged volume of every VPR frame corresponded to 33.342 mL at the high magnification setting and 436.136 mL at the low magnification setting, which was computed as the field of view multiplied by focal depth. This latter was determined by the parameters used with the VPR AutoDeck software (Supporting Information Table 1). A high magnification (S1) was chosen as it deemed to be a suitable setting for the general plankton community in the southern North Sea, but also a low magnification (S3), was included in the analysis because of its suitability for larger organisms (Ollevier et al. 2022).

During the second cruise ("24-h cruise"), data was collected during a 24-h campaign in May 2021 while the RV Simon Stevin laid at anchor in the southern North Sea. Every hour, a WP2 sample was collected and the VPR was deployed for approximately 15 min, while it was vertically lowered and raised between 3 and 20 m depth at a speed of 0.15 m $s^{-1}$. During the first hours the imaged volume of every VPR frame was 29.564 mL. From 3:00 (UTC + 2) onwards, these parameters were unintentionally changed, resulting in an imaged volume of 26.345 mL for the rest of the cruise. A high magnification setting (S1) was used for the whole duration of the cruise.

A third cruise ("Greenland cruise") took place in the Uummannaq fjord system in West Greenland aboard the RV Sanna in June–July 2022. On 17 locations inside the fjords and the connecting shelf area, samples were collected with a MultiNet mini (Hydro-Bios, Germany) and VPR. The MultiNet consists of five 50 $\mu$m nets with openings of 35.5 × 35.5 cm that open and close at predefined depth intervals. The topmost net sampled the upper 50 m, the second topmost net the upper 50–100 m, and the remaining net intervals varied according to the water column depth respectively. The sampled volume [$m^3$] for each net was calculated by multiplying the surface area of the net opening [$m^2$] by the distance of the depth interval [m]. It was deployed vertically at a speed of 0.4 m $s^{-1}$. During retrieval, the outside of the net was rinsed to wash the organisms into the cod-end. The zooplankton collected in the cod-end was transferred to a recipient and was fixed in 4% borax-buffered formaldehyde. Using microscopy, the samples were subsequently counted, identified at species or genus level, and classified by stage by the Arctic Agency in Poland. To allow for density estimations between the MultiNet and VPR, species or genera were relabeled according to the VPR taxa, which have lower taxonomic levels. The VPR was deployed for approximately 1.5 h at each station, while it was vertically lowered and raised through the water column between 3 and 250 m (restricted by the length of the winch cable) at a winch speed of 0.15 m $s^{-1}$. The imaged volume of every VPR frame was 335.622 mL based on the low magnification setting (S3). A low magnification setting was chosen due to the large size of the plankton. During deployment, maximum depth of the VPR was limited by the length of the winch cable and resulted in maximum depths of 150–250 m for individual stations, depending on the current and vessel speed. To match sampled depths between the two sampling methods, only the top 150 m of each dataset was included in further analysis.

## Data analysis

Data from the methodological cruise (including WP2 samples and four deployment methods of the VPR) and the Greenland cruise (involving MultiNet and VPR data) was evaluated by comparing plankton density estimates, zooplankton relative abundances and presence/absence of taxa. The taxa *Noctiluca* (dinoflagellates) and *Phaeocystis* (algae) were included in the analysis because their size range aligns with the mesoplankton fraction, allowing their observation through nets and/or the VPR. A Kruskal–Wallis was performed to test if the Shannon diversity index significantly differed between sampling methods. Because this analysis yielded non-significant results, further pairwise comparisons using a Dunn test were

deemed unnecessary. Taxon-specific Wilcoxon tests were performed on the density estimates to test if density estimates are significantly different between sampling methods. A regression was plotted for each comparable taxon and a correlation analysis using Kendall's rank correlation coefficient (tau) was conducted to examine the relationship between density estimates of the WP2 and VPR for each taxon. Only taxa present in more than five of the VPR and net samples were included in the regression plot. For data from the methodological cruise, the Shannon diversity index was calculated for each sample as a proxy for the capability of a method to observe a portion of diversity of the plankton community.

To assess temporal patterns of density, the data from the 24-h cruise was used. For each taxon, the temporal autocorrelation was calculated using the tscount package (Liboschik et al. 2017), focusing on a single 1 h time lag. This approach allowed to capture the immediate temporal dependency in the time series of WP2 and VPR data and to evaluate how strongly the current counts are predicted by preceding counts. Additionally, cross-correlation analysis was performed to examine relationships between sampling methods, with log transformation applied to the data to mitigate the influence of outliers and normalize distributions. Data exploration, representation, and analysis were done in R v4.0.3 (R Core Team 2020). Shannon diversity indexes were generated using the vegan v. 2.6-2 package (Oksanen et al. 2022).

Length measurements of the observed organisms in the southern North Sea, based on data from the methodological cruise, were made to assess the size range of the sampling methods. Size estimations of the body length by the ZooScan were based on the major axis of the best fitting ellipse (Gorsky et al. 2010). It should be noted that fixation in formaldehyde causes shrinkage of specimens, for example, crustaceans (Caspers 1978) but in particular of soft-bodied organisms such as Appendicularia, Chaetognatha, Ctenophores, and Cnidaria (Thibault-Botha and Bowen 2004; Nishikawa and Terazaki 1996; De Lafontaine and Leggett 1989), causing length measurements from the ZooScan to be smaller compared to the ImageJ measurements from living organisms. To determine the size ranges of the VPR data, a total of maximum 10 images per taxon and per tow was selected and measured in ImageJ software. First, the scale was set depending on the magnification setting of the VPR. The size for the VPR images was calculated by measuring the maximum length of the specimen on the image without excluding appendages.

## Assessment

### Density estimates and community composition

Zooplankton densities obtained from the WP2 net and VPR during the methodological cruise varied substantially. Taxa observed by both methods had consistently higher density estimates for the WP2 net (Fig. 2; Table 2), with the three most abundant taxa (Calanoida, Cirripedia, and Appendicularia) having densities exceeding 418 ind $m^{-3}$. In contrast, the VPR data at a high magnification recorded densities ranging from 3.33 to 144 ind $m^{-3}$, while the densities of these taxa were less than 12 ind $m^{-3}$ at a low magnification. There is however one taxon, *Phaeocystis*, that reaches high abundances in the VPR samples with densities up to 532 ind $m^{-3}$, suggesting that there was a *Phaeocystis* bloom at the moment of sampling. VPR downcasts showed slightly higher densities than continuous transects, but observed fewer taxa. Only for the less abundant taxa Amphipoda, Cumacea, Harpacticoida, Anomura, Branchiopoda, Chaetognatha, Mollusca, and fish eggs statistically significant differences (Krukall–Wallis, chi-squared = 9.7059–10, df = 4, $p < 0.05$) between density estimates of sampling methods were found.

Certain taxa were only observed by one sampling method or magnification setting of the VPR. The VPR clearly observed *Phaeocystis*, whereas this taxon was not observed by the WP2 net. Furthermore, there are a few taxa such as Amphipoda and fish larvae which were recorded in low abundances by the VPR, but not by the WP2 net. On the contrary, the WP2 showed low densities of Anomura, Branchiopoda, Chaetognatha, Mollusca, and fish eggs which were not captured by the VPR. The VPR only observed Brachyura, Cirripedia, Cumacea, and Harpacticoida with the high magnification setting.

Count data (Table 2) indicated that the number of organisms collected in a WP2 sample is much higher than the number of plankton ROIs collected by a cast or transect from the VPR, when considering the entire collected WP2 sample (images scanned by the ZooScan multiplied by its dilution factor of 5–6 times for the samples collected in March 2021). Comparing the VPR towing procedures shows that tows with a similar sampling time (VPR downcast high magnification vs. downcast low magnification; VPR transect high magnification vs. transect low magnification) yielded similar counts, but that Cnidaria and Ctenophora were observed more frequently with a low magnification compared to a high magnification and that *Noctiluca* was not observed with the low magnification. A longer sampling time (transect vs. downcasts) resulted in a higher count of individuals. The total sampled volume in the methodological campaign was 8.93 $m^3$ for the WP2 hauls and 0.31, 3.01, 3.67, and 38.25 $m^3$ for the VPR high magnification downcast, high magnification transect, low magnification downcast, and low magnification transect, respectively (Table 1).

Relative abundances of zooplankton taxa differed between the WP2 and VPR sampling methods, and between the magnification settings of the VPR (Table 2). The most abundant zooplankton taxon, that is, with the exclusion of the taxa *Noctiluca* and *Phaeocystis*, across all sampling methods was Calanoida, accounting for 24.97% of the zooplankton community in WP2 nets and for 51.41–64.23% as observed by the VPR. Cirripedia and Appendicularia were the second and third most abundant in the WP2 data, with relative densities of 20.58% and 14.23%, respectively, followed by Harpacticoida,

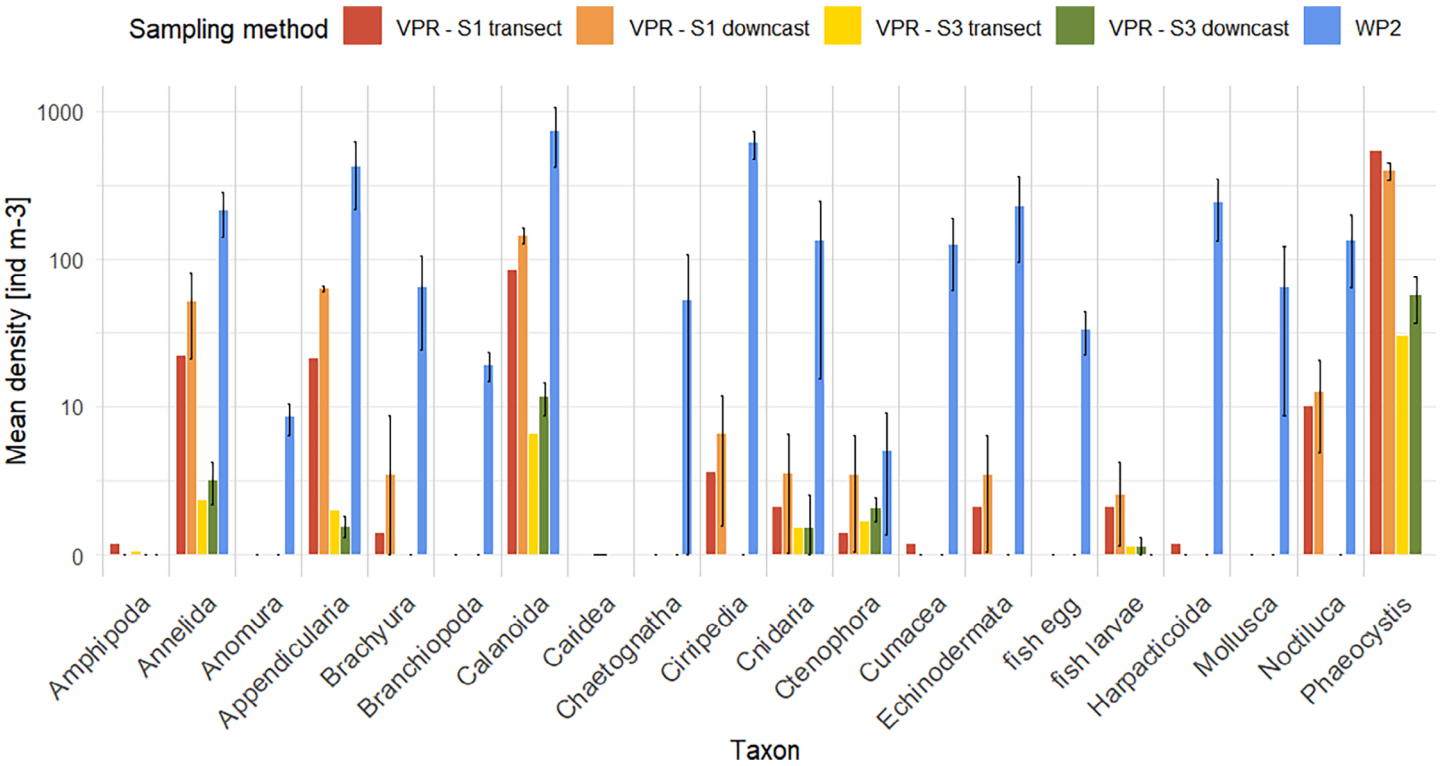


**Fig. 2.** Plankton taxa densities estimated by the WP2 net and different settings of the VPR. The mean density (with error bars) of WP2, VPR—S1 (high magnification), and VPR—S3 (low magnification) were based on three net samples for the first, and three downcast profiles for the two latter. Mean density is presented with a log10 scale.

Echinodermata, and Annelida contributing between 7.20% and 8.13% to the zooplankton community. For the VPR, Appendicularia and Annelida were frequently observed and contributed to the zooplankton community with relative abundances of 5.09–22.64% and 15.81–18.30%, respectively. Cirripedia, Harpacticoida, and Echinodermata were, however, rarely or never observed by means of the VPR in this study. Cnidaria and Ctenophora accounted for a higher percentage in the VPR data with magnification S3 compared to other samples. The remaining taxa had low relative densities across all sampling methods.

The highest Shannon diversity was found in WP2 samples (Fig. 3), but the Shannon diversity index did not differ significantly between sampling methods and VPR settings (Kruskal–Wallis, chi-squared = 8.8485, df = 4, $p > 0.05$).

Plankton density estimates of samples taken by the WP2 net and VPR were plotted against each other, based on the data from the May 2021 cruise (Fig. 4A; Supporting Information Table 2). Of the taxa in the regression plot, the correlation analysis revealed a significant positively related rank correlation between WP2 and VPR densities for the taxon *Noctiluca* ($z = 2.5464$, $p < 0.05$, tau = 0.3639). The tau correlation coefficient (Supporting Information Table 3) suggests a moderate positive relationship. This means that as the plankton density recorded by WP2 increases, there is a corresponding tendency for an increase in density as measured by VPR.

Plankton densities of the top 150 m of the water column in Greenland estimated by the MulitNet and VPR are often in the same order of magnitude. The three most abundant groups in the MultiNet were Copepoda, other meroplankton, and Euphausiacea together contributing 71.33% to the plankton community. Average densities of these groups ranged between 6 and 19 ind $m^{-3}$ (Table 3). Other meroplankton categories included Gastropoda, Bivalvia, Echinodermata, Bryozoa, and Pilidium, of which Bivalvia larvae had the most important contribution to this group. In the VPR samples none of the taxa grouped under other meroplankton were observed, nor were Cirripedia, other crustaceans, and Rotifera. In the collected VPR data, Copepoda, Cnidaria, and Chaetognatha were the most observed taxa, together contributing 78.91% to the mesozooplankton community and having average densities between 6 and 17 ind $m^{-3}$. These latter two taxa also had higher relative abundances in the VPR data (15.53% and 13.45%, respectively), compared to the MultiNet data (3.72% and 1.63%, respectively).

A Wilcoxon test per taxon (Supporting Information Table 4) showed significant differences ($W = 18–306$, $p < 0.05$) for less abundant taxa such as Amphipoda, Annelida, Appendicularia, Chaetognatha, Cirripedia, Cnidaria, other meroplankton, Ostracoda, other crustaceans, fish larvae.

**Table 2.** Average counts (± stdev) [ind], average density (± stdev) [ind $m^{-3}$], and relative abundance [%] as estimated by the WP2 net and various deployment methods of the VPR from the methodological cruise. The counts for the WP2 net represent the counted individuals by the ZooScan of a fraction of the sample (fractionated 5 to 6 times). Counts and density are given for the mesoplankton community, relative abundances were based on the zooplankton community (i.e., excluding the taxa Noctiluca and Phaeocystis).

| | Counts [ind] | | | | Density [ind $m^{-3}$] | | | | Relative abundance [%] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | WP2 | VPR low, downcast | VPR low, transect | VPR high, downcast | VPR high, transect | WP2 | VPR low, downcast | VPR low, transect | VPR high, downcast | VPR high, transect | WP2 | VPR low, downcast | VPR low, transect | VPR high, downcast | VPR high, transect |
| Amphipoda | 0 | 0 | 1 | 0 | 4 | — | — | 0.33 | — | 0.1 | 0 | 0 | 0.24 | 0 | 0.83 |
| Annelida | 36 ± 5 | 16 ± 10 | 66 | 10 ± 4 | 74 | 211.54 ± 69.98 | 51.14 ± 30.13 | 21.96 | 2.86 ± 1.14 | 1.93 | 7.2 | 18.3 | 15.87 | 15.81 | 15.99 |
| Anomura | 2 ± 1 | 0 | 0 | 0 | 0 | 8.37 ± 2.07 | 0 | 0 | 0 | 0 | 0.28 | 0 | 0 | 0 | 0 |
| Appendicularia | 70 ± 21 | 20 ± 1 | 63 | 3 ± 1 | 56 | 418.3 ± 202.11 | 63.26 ± 2.66 | 20.96 | 0.92 ± 0.35 | 1.46 | 14.23 | 22.64 | 15.14 | 5.09 | 12.1 |
| Brachyura | 12 ± 9 | 1 ± 2 | 2 | 0 | 0 | 64.54 ± 39.93 | 3.17 ± 5.5 | 0.67 | 0 | 0 | 2.2 | 1.13 | 0.48 | 0 | 0 |
| Branchiopoda | 4 ± 2 | 0 | 0 | 0 | 0 | 19.12 ± 4.14 | 0 | 0 | 0 | 0 | 0.65 | 0 | 0 | 0 | 0 |
| Calanoida | 125 ± 35 | 45 ± 6 | 255 | 42 ± 10 | 245 | 733.82 ± 314.4 | 143.66 ± 18.02 | 84.84 | 11.62 ± 3.05 | 6.4 | 24.97 | 51.41 | 61.3 | 64.23 | 53.02 |
| Chaetognatha | 9 ± 7.55 | 0 | 0 | 0 | 0 | 52.58 ± 55.7 | 0 | 0 | 0 | 0 | 1.79 | 0 | 0 | 0 | 0 |
| Cirripedia | 106.33 ± 23.8 | 2 ± 1.73 | 10 | 0 | 0 | 604.74 ± 129.14 | 6.4 ± 5.46 | 3.33 | 0 | 0 | 20.58 | 2.29 | 2.41 | 0 | 0 |
| Cnidaria | 21.67 ± 14.57 | 1 ± 1 | 5 | 3 ± 4.36 | 32 | 131.47 ± 116.14 | 3.24 ± 3.18 | 1.66 | 0.86 ± 1.28 | 0.84 | 4.47 | 1.16 | 1.2 | 4.75 | 6.96 |
| Ctenophora | 1 ± 1 | 1 ± 1 | 2 | 5.67 ± 1.53 | 41 | 4.78 ± 4.14 | 3.2 ± 3.12 | 0.67 | 1.56 ± 0.49 | 1.07 | 0.16 | 1.15 | 0.48 | 8.62 | 8.86 |
| Cumacea | 21 ± 6 | 0 | 1 | 0 | 0 | 125.49 ± 63.43 | 0 | 0.33 | 0 | 0 | 4.27 | 0 | 0.24 | 0 | 0 |
| Echinodermata | 38 ± 16 | 1 ± 1 | 5 | 0 | 0 | 227.08 ± 132.68 | 3.2 ± 3.12 | 1.66 | 0 | 0 | 7.73 | 1.15 | 1.2 | 0 | 0 |
| Fish eggs | 6 ± 4 | 0 | 0 | 0 | 0 | 33.46 ± 10.96 | 0 | 0 | 0 | 0 | 1.14 | 0 | 0 | 0 | 0 |
| Fish larvae | 0 | 1 ± 1 | 5 | 1 ± 1 | 9 | 0 | 2.16 ± 1.88 | 1.66 | 0.27 ± 0.27 | 0.24 | 0 | 0.77 | 1.2 | 1.49 | 1.99 |
| Harpacticoida | 41 ± 11 | 0 | 1 | 0 | 0 | 239.03 ± 105.71 | 0 | 0.33 | 0 | 0 | 8.13 | 0 | 0.24 | 0 | 0 |
| Mollusca | 10 ± 7 | 0 | 0 | 0 | 0 | 64.54 ± 56.01 | 0 | 0 | 0 | 0 | 2.2 | 0 | 0 | 0 | 0 |
| *Noctiluca* | 23 ± 11 | 4 ± 3 | 30 | 0 | 0 | 131.47 ± 67.78 | 12.69 ± 8.02 | 9.98 | 0 | 0 | — | — | — | — | — |
| *Phaeocystis* | 0 | 123 ± 21 | 1598 | 209 ± 80 | 1142 | 0 | 394.25 ± 51.29 | 531.64 | 56.59 ± 19.95 | 29.86 | — | — | — | — | — |

Plankton density estimates of MultiNet and VPR samples were plotted against each other (Fig. 4B). However, the correlation analysis yielded results indicating that there is no statistically significant rank correlation between MultiNet and VPR densities for the taxa depicted in Fig. 4B (Supporting Information Tables 2 and 5). This implies that there is no consistent association between MultiNet and VPR densities for the taxa under consideration in Fig. 4B and that these two methods may not provide matching estimates of plankton density for these taxa.

### Size measurements

Length measurements (Supporting Information Table 6) of the WP2 sample and the VPR data at a high magnification generally yielded similar size measurements for the plankton, except for ctenophores that were larger in the VPR data. In the case of the low magnification of the VPR, larger specimens were consistently observed compared to the WP2 and VPR high magnification data, particularly among Ctenophora and Cnidaria. Notably, images captured by the low magnification of the VPR often included the tentacles of ctenophores, and cnidarians with larger hoods compared to the high magnification.

### Comparison of plankton patterns over time

The densities of Calanoida, *Noctiluca*, Echinodermata, and Cumacea taxa abundantly present in both WP2 and VPR data from the 24-h cruise, significantly differed between sampling methods (Fig. 5). Densities differed some orders of magnitude. Changes in plankton density for these taxa over a 24-h period, however, followed similar patterns where peaks and valleys generally coincide between methods, except for a few high-density outliers. Cross-correlation analysis revealed significant relationships for certain taxa: *Noctiluca* and Cumacea had a significant correlation coefficient of 0.6751 and 0.3926, respectively, indicating a strong association between the two sampling methods. Calanoida had a correlation coefficient of 0.3901 (not significant), indicating some degree of correspondence but not enough to be deemed statistically significant. Echinodermata exhibited a correlation of 0.2939, which was not significant. Calanoida displayed three peaks, *Noctiluca* and Echinodermata two peaks, whereas Cumacea were mostly observed during the night.

The autoregression coefficients (Supporting Information Table 7) were relatively high in both WP2 and VPR methods for Amphipoda, indicating strong positive temporal autocorrelation. Past counts of Amphipoda seem to be a good predictor of its later counts, gradual changes over time. Other taxa such as *Phaeocystis*, *Noctiluca*, and Annelida in the VPR dataset and Cirripedia and Harpacticoida in the WP2 dataset had moderate coefficients and signify some level of temporal autocorrelation. These values imply that there might be some relationship between previous counts and current counts. The VPR

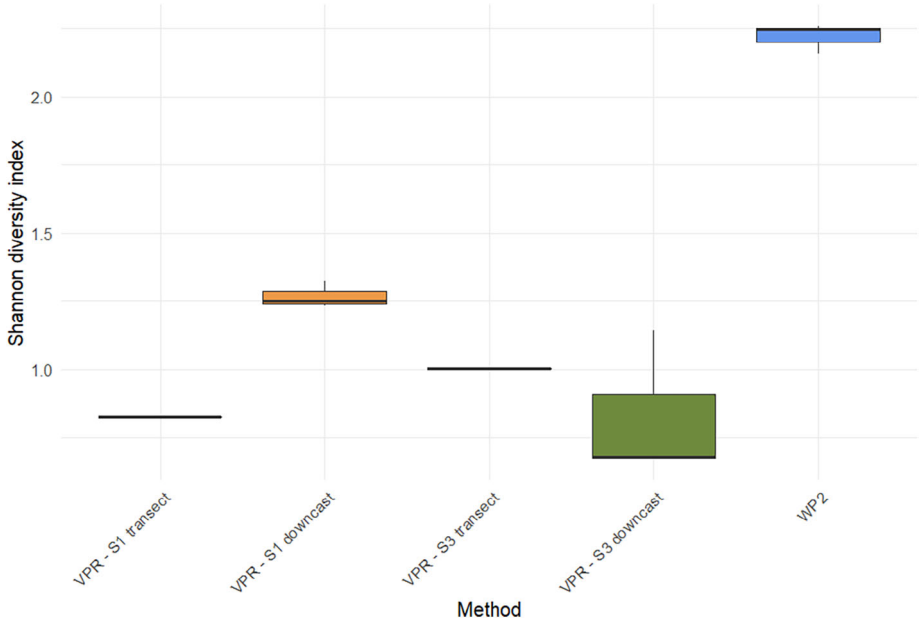


**Fig. 3.** Shannon diversity index of mesoplankton community observed by the WP2 and VPR sampling methods.

tends to have higher autocorrelation coefficients for specific taxa compared to the WP2 net.

## *Discussion*

### Imaging and net sampling methods for plankton estimates

Density estimates varied across different study areas for the various sampling methods employed. Results from the two sampling campaigns in the southern North Sea showed that the VPR in general underestimates plankton densities compared to WP2 net hauls for the majority of the taxa. The most abundant non-gelatinous taxa in the WP2 sample (Calanoida, Cirripedia, Harpacticoida, Echinodermata, and Annelida), together accounting for more than 65% of the total zooplankton community, all had densities that were considerably higher, although not statistically significant, than those observed in the VPR sample and often differed one order in magnitude. This is in contrast to the density estimates of the

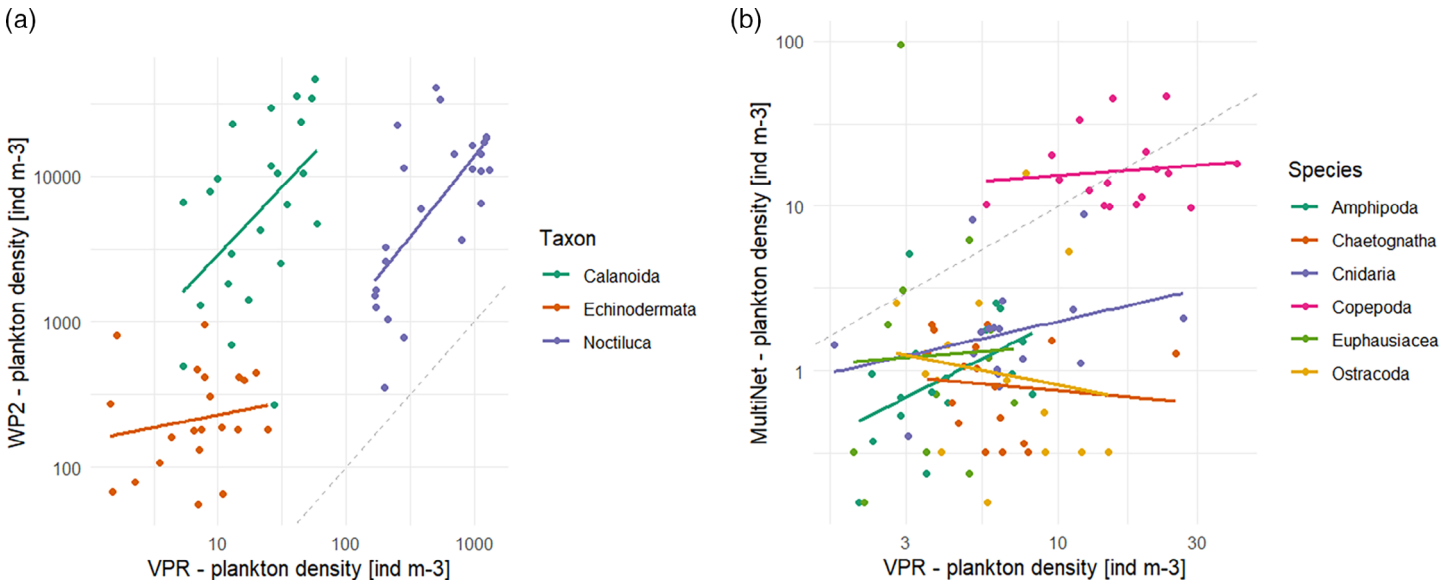


**Fig. 4.** Regression plots to compare density estimates between (**A**) a WP2 net and VPR based on data from a 24-h campaign in the southern North Sea and (**B**) a MultiNet mini and VPR based on data from Greenland. Points and lines are colored per taxon. Solid lines show the line of the best fit. The dashed line represents the 1 : 1 line.

**Table 3.** Average density (± stdev) [ind $m^{-3}$], relative abundance [%], and average counts (± stdev) [ind] per sampling station as estimated by the MultiNet net and the VPR in Greenland. The MultiNet counts represent the individuals counted through microscopy within a subsample that underwent fractionation (1 to 6 times). This representation serves to clarify the specific values used for calculating the actual densities.

| | Density [ind $m^{-3}$] | | Relative abundance [%] | | Count [ind] | |
|---|---|---|---|---|---|---|
| **Taxon** | **MultiNet** | **VPR** | **MultiNet** | **VPR** | **MultiNet** | **VPR** |
| Amphipoda | 1.28 ± 1.17 | 4.16 ± 2.18 | 2.12 | 8.72 | 7.33 ± 4.81 | 7.59 ± 6.65 |
| Annelida | 2.6 ± 4.24 | 0.42 ± 1.23 | 4.32 | 0.88 | 14 ± 16.81 | 1.5 ± 0.71 |
| Appendicularia | 3.28 ± 8.42 | 0.53 ± 1.23 | 5.44 | 1.11 | 11.08 ± 18.28 | 1.33 ± 0.58 |
| Chaetognatha | 0.98 ± 0.56 | 6.42 ± 5.18 | 1.63 | 13.45 | 8.72 ± 5.68 | 14.41 ± 9.82 |
| Cirripedia | 0.28 ± 0.66 | 0 ± 0 | 0.46 | 0 | 2.17 ± 1.94 | 0 ± 0 |
| Cnidaria | 2.24 ± 2.4 | 7.41 ± 5.83 | 3.72 | 15.53 | 22.83 ± 17.71 | 55.76 ± 29.71 |
| Copepoda | 18.74 ± 11.39 | 17.02 ± 9.19 | 31.11 | 35.67 | 1039.61 ± 143.47 | 599.29 ± 360.12 |
| Ctenophora | 0.06 ± 0.17 | 1.6 ± 2.94 | 0.1 | 3.35 | 1 ± 0 | 5 ± 5.39 |
| Euphausiacea | 6.49 ± 22.3 | 3.05 ± 2.43 | 10.77 | 6.39 | 25.36 ± 51.38 | 9.62 ± 10.52 |
| Fish larvae | 0 ± 0 | 0.73 ± 1.69 | 0 | 1.53 | 0 ± 0 | 1.25 ± 0.5 |
| Other meroplankton | 17.74 ± 40.67 | 0 ± 0 | 29.45 | 0 | 22.69 ± 28.31 | 0 ± 0 |
| Ostracoda | 2.67 ± 4.34 | 5.71 ± 4.22 | 4.43 | 11.97 | 11.22 ± 15.48 | 6.33 ± 5.55 |
| Other | 2.97 ± 7.2 | 0 ± 0 | 4.93 | 0 | 20.6 ± 21.45 | 0 ± 0 |
| Other crustaceans | 0.26 ± 0.29 | 0 ± 0 | 0.43 | 0 | 1.77 ± 1.09 | 0 ± 0 |
| Pteropoda | 0.51 ± 0.74 | 0.67 ± 1.67 | 0.85 | 1.4 | 2.4 ± 1.26 | 2 ± 1 |
| Rotifera | 0.14 ± 0.33 | 0 ± 0 | 0.23 | 0 | 1 ± 0 | 0 ± 0 |

VPR and MultiNet in Greenland, where density estimates were more similar and often lay within the same order of magnitude. The observations from the Greenland campaign are in line with previous studies with nets and optical imaging devices, which generally showed similar density estimates for abundant taxa in net and VPR samples (Benfield et al. 1996; Broughton and Lough 2006; Strand et al. 2020; Beroujon et al. 2022). The VPR and MultiNet data showed similar density estimates for the most abundant taxon, that is, Copepoda. However, the VPR did not observe any of the taxa grouped under other meroplankton (Gastropoda, Bivalvia, Echinodermata, Bryozoa, and Pilidium) which were abundant and accounted for almost a third of the plankton community in the MultiNet samples. The predominant constituents of this group were identified as Bivalvia. The predominant members of this group were identified as Bivalvia. It is likely that the VPR failed to capture certain taxa either because their small size placed them outside the VPR's effective magnification range, or due to the rarity of these taxa, with some observed only once.

The VPR is thought to be suitable for fragile and gelatinous taxa as in situ optical imaging devices can observe particles inside the water column without damaging or destroying them. Other studies with a high resolution sampler (SIPPER/OPC) indicated that nets underestimate Cnidaria and Ctenophora abundance by 1200% (Remsen et al. 2004). In the samples from Greenland, the density and relative abundance estimates of the fragile and gelatinous taxa Cnidaria, Ctenophora, and Chaetognatha ranged from 3 to 33 times higher in the VPR samples in terms of density and relative abundance compared to the MultiNet samples. This corroborates observations of Beroujon et al. (2022) in East Greenland where gelatinous taxa were underestimated by a WP2 net compared to an autonomous VPR. In the samples from the southern North Sea, however, densities of gelatinous species were undersampled by the VPR, but regarding relative abundances, Cnidaria and Ctenophora made a slightly greater contribution to the plankton community in the VPR data, particularly in magnification setting S3 (4.75–8.86%), compared to the WP2 data (0.16–4.47%). This, however, may be because the WP2 net observed a higher number of taxa and thus a higher proportion of the biodiversity. The tendency to have higher (relative) abundances with the VPR compared to net sampling methods highlights the potential advantage of the VPR in observing delicate and gelatinous species. This is further supported by the observations of *Phaeocystis*, a fragile colonial forming phytoplankton species, that was very abundant in the VPR data, but absent in the WP2 net data in the southern North Sea despite being in the size range of the WP2 net as the colonies are likely destroyed during net sampling (Bender et al. 2018).

### Discrepancies between sampling areas

The comparison of net hauls and the VPR yielded different results between sampling campaigns in the southern North Sea and Greenland. Because the findings from the Greenland campaign are in line with previous studies, it raises the question why the VPR underestimated most plankton densities in the southern North Sea compared to net-based approaches.

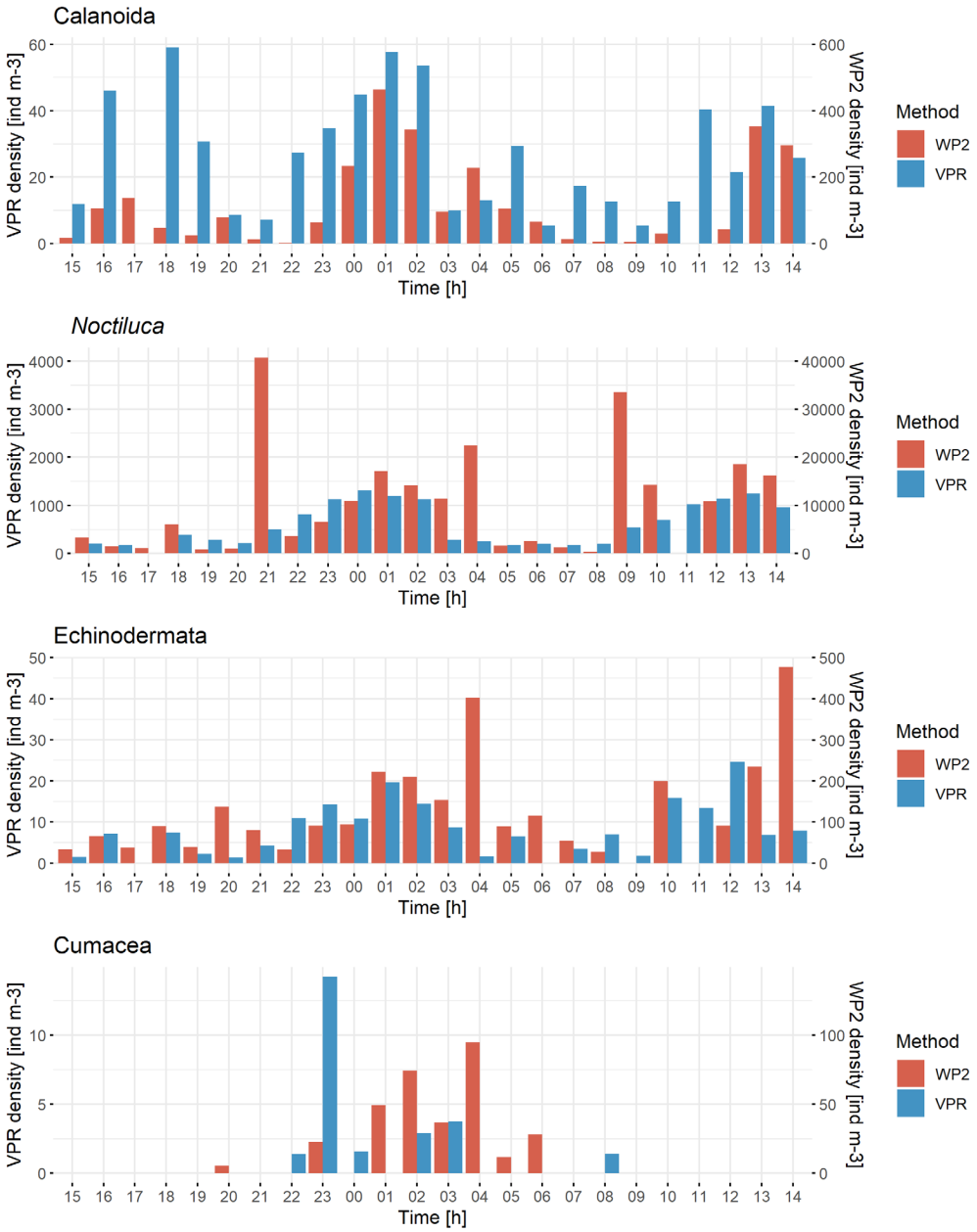


**Fig. 5.** Density estimates [ind $m^{-3}$] of Calanoida, Noctiluca, Echinodermata, and Cumacea over a 24-h period as observed by the WP2 net and VPR with the high magnification setting. Note that two y-axes are used, one for each sampling method.

First of all, it is important to note that these comparisons involve two hydrologically distinct ocean areas, each studied using different net sampling methods, characterized by variations in mesh sizes, net openings and towing speeds. These methodological differences inherently lead to variation in how the plankton community is sampled and estimated (Skjoldal et al. 2013, 2019), thereby introducing variation in the community we compare to the VPR results. As a result, this adds complexity to the comparison between regions and emphasizes the need for careful interpretation when contrasting the outcomes of the VPR with traditional net-based approaches. Nevertheless, considering that both WP2 and MultiNet nets are widely used in plankton research, this underscores the significance of comparing the outcomes of a VPR to these established methods. The intercomparison of net sampling methods by Skjoldal et al. (2013) indicated that the mesh size had a major influence on the biomass and species composition of the zooplankton community. Reducing the mesh size may increase the retention of small organisms, but may lower the filtration efficiency and increase the avoidance

of large organisms. Conversely, increasing the towing speed and mesh size may improve the catch efficiency of larger zooplankton, but results in greater loss of small individuals by extrusion through the mesh of the net. Additionally, nets are susceptible to clogging, particularly during events like phytoplankton blooms (e.g., of *Phaeocystis*), which can prevent the passage of plankton and result in an undersampling of the community. During the May campaign *Phaeocystis* was blooming, but not to the extent that the nets were clogged.

Second, there exists a substantial contrast in the plankton communities between the two sampling locations. The VPR was originally designed for open ocean use with low densities of copepods (Davis et al. 1992, 1996). However, conditions in the southern North Sea are very different with high densities of smaller-sized plankton. Conversely, the plankton community in Greenland is typified by larger specimens, such as *Calanus hyperboreus*, capable of reaching a body length of 7 mm (Leinaas et al. 2016), yet they exist in lower densities. The plankton size itself could affect the density estimations. Small plankton will appear smaller and less distinctive on the ROIs, causing them to be potentially overlooked and harder to identify during the manual validation process depending on the magnification used. Additionally, the AutoDeck software's extraction process for ROIs may further impact the density estimations of smaller organisms. User-defined parameters in AutoDeck, such as segmentation thresholds (low and high), focus settings (sobel and standard deviation), growth scale, minimum blob size (area), and minimum join distance, determine whether an image is saved as an ROI. Adjusting these parameters manually could introduce errors. For instance, settings designed to filter out unwanted small detritus particles might inadvertently exclude more small organisms than larger ones. Since this VPR setup saves only the selected ROIs and not full frames, we were unable to fully assess the extent of this potential issue. Inherently connected to the magnification setting is the size range wherein particles are observed. The low magnification setting is mainly suitable for larger organisms as seen in the results, causing the lack of observing small taxa. In the studies of Strand et al. (2020) and Beroujon et al. (2022), densities of smaller organisms were also generally underestimated.

Third, the environmental conditions and physical characteristics of the two regions may also play a role in the discrepancy. In particular, turbidity of the water column strongly impacts the performance of the VPR (Ollevier et al. 2022), resulting in less or no capturing of images when turbidity levels are too high (values exceeding 6.2 NTU). Turbidity of the water column inside the fjords in West Greenland is, except for areas with freshwater runoff or glacial influence (Holinde and Zielinski 2016; van Genuchten et al. 2022), generally low. In contrast, the southern North Sea receives a lot of SPM from rivers which raises the turbidity in the coastal areas. The sampling location within the southern North Sea was specifically chosen to be located in an area below the turbidity threshold value mentioned above to minimize the impact of turbidity on the capture of images, resulting in only small disparities in average water column turbidity compared to sampling locations in Greenland.

Next, another factor that could contribute to differences in plankton densities between nets and the VPR is the sampling depth. The top three meters of the water column were sampled during a net haul, but not during VPR deployment as a safety range is kept to avoid collision with the vessel and to avoid turbulence in the ship's wake. Organisms are not homogeneously vertically distributed in the water column (Conway and Minton 1976; Jónasdóttir and Koski 2011; Ollevier et al., unpublished) and in case they aggregate in high density patches in these zones, this can also contribute to the undersampling of the plankton community by the VPR.

Finally, variations in the total sampled volume, coupled with potential errors in volume estimations, may contribute to the observed disparities. The imaged volume per frame of the VPR is low, particularly at higher magnification settings, resulting in a generally low total sampled volume compared to net sampling methods. This discrepancy becomes more pronounced in brief deployments, as evidenced during campaigns in the southern North Sea (Table 1). Contrastingly, in Greenland, the total sampled volume exceeded that of the surface 150 m sampled by the MultiNet. This is due to the employment of lower magnification settings, leading to a larger imaged volume per frame, and a longer deployment of the VPR. The increased total sampled volume in Greenland might ensure a more comprehensive representation of the water column and contribute to more comparable density estimates between methods and highlights the need of a sufficient total sampled volume. Further, Basedow et al. (2013) pointed out that small errors in the estimated sampling volume of the VPR can give rise to large errors in the estimated densities. The VPR's sampling volume relies on the user-defined AutoDeck setting which determines the depth of view, the magnification which determines the field of view and the duration of the VPR deployment. Alterations in these settings can substantially affect the sampling volume, yet the direct translation of these changes to corresponding alterations in densities remains unclear. Broughton and Lough (2006) also highlighted the importance of accurate calibration of the VPRs field of view, as inaccurate calibration in their study was potentially the reason for errors in density estimates. Calibration in their paper had to be done manually by gluing a copepod to a hair and moving it back and forth and judging when the plankton imaged was too dark or out of focus to be readily identified, whereas now Seascan is using targets with drilled holes with a constant speed which is less subjective. The calibration of the VPR was not reevaluated after the sampling campaign in this study because the findings from the Greenland campaign aligned with outcomes from other studies, indicating that the system's

calibration was not the reason for the undersampling observed in the southern North Sea.

### Temporal patterns

Despite the VPR's tendency to underestimate density in the southern North Sea, consistent patterns were observed in density variations of abundant taxa over a 24-h period, showing that both the VPR and WP2 net capture the underlying dynamics of zooplankton communities. To address density discrepancies between sampling methods, the implementation of a calibration factor has been suggested (Broughton and Lough 2006). Previous studies used a correction factor for certain specific taxa (Ohman and Lavaniegos 2002) or for the whole community (Remsen et al. 2004). Based on our observations, determining a calibration factor for the whole community or even specific taxa might be difficult due to variations in the regression lines between taxa and study regions. It should also be determined whether a region specific correlation factor would remain valid during different seasons. Furthermore, the examination of the temporal autocorrelation of taxa-specific densities indicated that the VPR tends to have higher autoregression coefficients, suggesting higher measurement precision or reduced sensitivity to small-scale density variations compared to WP2 samples. This is likely because the VPR continuously samples a smaller volume of water along a vertical profile or undulating transect, which helps smooth out localized fluctuations. Consequently, high-density patches have a smaller effect on the overall density estimates than they would in the WP2 net hauls.

### Added value of optical methods

Both optical imaging devices and net sampling methods possess unique advantages and drawbacks. Net methods present a straightforward and standardized approach for collecting discrete plankton samples and have become widely adopted. An advantage is that physical net samples, identifiable through microscopy and expert classification, can be identified to low taxonomic levels that in situ images, constrained by their low resolution, may not reach. On the other hand, optical imaging methods provide researchers a multitude of benefits, such as the ability to employ various magnifications and both discrete and continuous deployment methods. This flexibility allows researchers to tailor their investigations to meet specific needs. Additionally, the combination of optical imaging devices with CTD sensors facilitates in situ sensing and allows to investigate plankton distribution with a high spatial resolution in the order of centimeters and allows for a tight coupling with simultaneously collected abiotic, spatial, and geographic measurements. In previous studies, imaging devices such as a VPR have been deployed to study the interaction between marine snow and zooplankton (Möller et al. 2012), the aggregation of hydromedusae around a density continuity (Jacobsen and Norrbin 2009), the diel vertical migration of zooplankton (Sainmont et al. 2014; Pan et al. 2018), and the formation and life cycle of a bloom (Takahashi et al. 2015). Optical imaging methods therefore are crucial for studies focusing on the vertical distribution of organisms through the water column and those requiring high spatial and temporal resolutions, which cannot be achieved with traditional net sampling methods. Additionally, ongoing advancements in automated classifiers will facilitate the more accurate, rapid, and cost-effective identification of images generated by optical methods. This progress will enable the (real-time) handling of extensive data volumes without a proportional increase in resources, meeting the rising demand for more data in plankton monitoring and research.

## Conclusion

The aim of this study is to evaluate the accuracy of in situ imaging estimates in assessing the plankton community compared to different net-based approaches. The comparison between the VPR and net-based approaches resulted in different plankton density estimations depending on the environmental characteristics of the study area and the type of plankton community. Whereas the VPR consistently underestimated zooplankton densities compared to the WP2 net in the southern North Sea, density estimates in Greenland were similar to those from the MultiNet. A combination of factors linked to water column turbidity, plankton body size, plankton nets and its net mesh size, and the total sampled water volume probably contributed to the discrepancies between study areas. It suggests that a VPR is more suitable in clear waters with low plankton densities.

For a long time, our knowledge of plankton relied on plankton nets. However, our results indicate that the VPR, in certain environments, can improve estimates of gelatinous or fragile taxa that are often damaged and consequently under sampled by net sampling methods. Furthermore, despite the differences in absolute density estimates between optical devices and nets, both methods captured similar patterns over a 24-h period, suggesting that the density fluctuations of zooplankton were consistent regardless of the sampling approach. This consistency highlights the ability of both methods to capture comparable temporal variations in zooplankton populations. All these aspects, together with the higher spatiotemporal resolution, concurrent measurements with additional sensors, and the expedited identification process of extensive data volumes, signify the potential of optical methods to significantly contribute to plankton research. By leveraging both net-based and optical imaging approaches, future studies can improve spatial coverage and enhance the accuracy and comprehensiveness of zooplankton assessments, leading to a deeper understanding of their ecological significance in marine ecosystems.

## Author Contributions

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Leandro Ponsoni. Formal analysis: Anouk Ollevier. Writing – original draft: Anouk Ollevier. Writing – review & editing: Anouk Ollevier, Jonas Mortelmans, Wieter Boone, Klaas Deneudt, Marleen De Troch, Roeland Develter, Lorenz Meire, Klas Ove Möller, Leandro Ponsoni, Pascal I. Hablützel. Supervision: Jonas Mortelmans, Klaas Deneudt, Marleen De Troch, Wieter Boone, Pascal I. Hablützel.

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## Conflicts of Interest

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

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## Supporting Information

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

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