High temporal resolution zooplankton dynamics in the Dutch coastal zone (ID14 MONS Monitoring Zooplankton Phase 1) – Results from 1 year of Marsdiep sampling

Dick van Oevelen[1], Nadine Peck[2], Robbert Jak[2], Marcel Polling[3], Jeroen van Dalen[1], Myron A. Peck[4], Lodewijk van Walraven[2, 5]

[1] Department of Estuarine and Delta Studies, Royal Netherlands Institute for Sea Research (NIOZ), The Netherlands, E-mail: dick.van.oevelen@nioz.nl
[2] Wageningen Marine Research (WMR), Den Helder, The Netherlands
[3] Wageningen Environmental Research, Wageningen, The Netherlands
[4] Department of Coastal Systems, Netherlands Institute for Sea Research (NIOZ), The Netherlands
[5] Hydrobiological laboratory, Rijkswaterstaat, Lelystad, The Netherlands

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NOORDZEEOVERLEG

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ABSTRACT

As part of the Dutch Monitoring-Onderzoek-Natuurversterking-Soortbescherming (MONS, Monitoring-Research-Nature Enhancement-Species Protection) program, we investigated the temporal dynamics of zooplankton at the Marsdiep from the NIOZ jetty for a period of 13 months (November 2023 – November 2024). High tide sampling at the Marsdiep is representative of the coastal North Sea and is subjected to variable salinity regimes. This 'ecotype' is a rarely sampled OSPAR region and the proximity to the NIOZ institute allows for high temporal resolution sampling. Zooplankton samples were collected with 10L buckets to allow for sampling within a relatively short time frame. Sampling was done at a frequency of 42 times per year, aligned with the sampling program of nutrients and pigment dynamics maintained by the NIOZ. A total of 200L seawater was collected, pre-screened with a 2-mm sieve to collect jellyfish and stored in formaldehyde (2 samples of 50L) and the non-toxic preservative DESS (2 samples of 50L). The biovolume of jellyfish was determined and individuals were photographed for later identification. Formaldehyde-fixed samples were processed with the zooscan-method and a subset of the samples (12 per year, total of 15 samples) were analysed by microscopy by a zooplankton expert. All DESS-fixed samples were analysed for DNA metabarcoding (markers 18SV9 and COI). Overall, the data show strongly seasonal dynamics, with a peak in zooplankton abundance and diversity in spring. Interestingly, this peak was dominated by meroplankton, both in abundance and diversity. Summer zooplankton abundance, but not diversity, was lower, while the autumn peak was dominated by holoplankton.

We advise the following for the Marsdiep sampling. It is strongly advised to retain the DNA metabarcoding analysis in the program. Species phenology and diversity was followed at a very high temporal resolution and showed the high contribution of meroplankton to the species diversity. DNA metabarcoding also showed interesting temporal dynamics for several invasive species that are otherwise challenging to obtain. Analysis costs do not scale linearly with the number of samples, so we advise to keep the sampling frequency at 20x per year for biodiversity and invasive species investigations, but preferably at ±42x per year to also included phenological analysis. At this moment it is premature to give a final advise on how to use the zooscan and / or microscopy analysis in the Marsdiep monitoring. Both methods are time-consuming and therefore expensive, but both have clear advantages. The present sampling scheme will continue for another two years, and we advise to evaluate the full three-year time series to determine/investigate whether the zooscan analysis can be synchronized at the lower temporal frequency of the microscopic analysis. The pre-screening of the samples successfully kept jellyfish (prone to 'dissolving' in formaldehyde and degrading sample quality) out of the formaldehyde-fixed samples. We advise to continue this pre-screening for enhanced sample quality. The pre-screening allowed for the analysis of jellyfish biovolume and although sampling is (too) small for accurate estimates, jellyfish biovolume was high. Hence, we suggest considering using a more targeted sampling strategy (e.g. larger seawater volume, but lower temporal resolution) for jellyfish biovolume.

1 Introduction

The North Sea is under pressure of various anthropogenic activities, ranging from shipping, fishing, sand extraction, pollution to climate change. In this context, our use of ecosystem services from the North Sea is changing and increasing and is collectively framed in three transitions: 1) the energy transition, i.e. the construction of large-scale Offshore Wind Farms (OWFs), 2) the food transition, i.e. a change in fishing pressure (reduction of bottom contact fisheries and new target species) and the new prospects of aquaculture (possibly in combination with OWFs) and 3) the nature transition, to advance the conservation and restoration of the North Sea ecosystem.

The Dutch Monitoring-Onderzoek-Natuurversterking-Soortbescherming (MONS, Monitoring-Research-Nature Enhancement-Species Protection) program aims to answer the central question whether and how these transitions in the use of the North Sea fit within the ecological carrying capacity of the North Sea (Asjes et al., 2021), as emerged from the North Sea Agreement. The North Sea Agreement established the necessity of an integrated and systematic research and monitoring program due to changing use. The MONS program has been drawn up for this purpose, which focuses on making information available about various basic physical, chemical and biological parameters. The results from the MONS program will also be used to address knowledge gaps that emerged from the 2023 OSPAR assessment were the need to use national datasets with high resolution in space and time to better relate changes in plankton to changes in the environment.

Zooplankton forms an important trophic link in the pelagic food web (Steinberg and Landry 2017), with many (commercial) fish species being reliant on zooplankton for their dietary requirements (Heath 2005). Moreover, many benthic organisms have a temporary planktonic life stage, the so-called meroplankton, which can be numerically dominant in coastal seas (Kirby et al. 2007, Kirby et al. 2008) and represent a bottleneck for the settling and spatial distribution of benthic fauna. Zooplankton biomass and diversity is recognised as Essential Ocean Variable (EOV) in the Global Ocean Observing System (GOOS) and is used in several quality indicators in the latest OSPAR's quality status report (Holland et al. 2023, Louchart et al. 2023). Despite the key role of zooplankton in the pelagic food web, there is no consistent zooplankton monitoring on the Dutch Continental Shelf, which is considered a major knowledge gap.

In order to design a monitoring plan for zooplankton in the Dutch part of the North Sea, several research questions were defined (Jak et al., 2022): 1) Which monitoring strategy allows for optimal insight in the spatial and temporal resolution of zooplankton, 2) Which monitoring strategy allows for a best comparison to dynamics of other components of the marine food web, such as phytoplankton and pelagic fish, 3) Are data useful for food web models that are developed within MONS and 4) What is the most cost efficient monitoring strategy? In the context of these research questions, several activities have been initiated. Van Walraven et al. (2025) evaluated a coastal survey conducted with the R/V Tridens where zooplankton, alongside pelagic fish abundance, was monitored with the onboard plankton imager Pi-10 and net samples that were analysed with metabarcoding and microscopy. That sampling strategy was successful and showed that this would allow for data on the zooplankton distribution along the Dutch coast at very high spatial resolution. Hence, that sampling strategy will be used as a template for future zooplankton monitoring on the R/V Tridens and R/V Zirfaea.

In this report, we compare the results of sampling a full annual cycle (Nov 2023 to Nov 2024) at the Marsdiep from the NIOZ jetty, in which we sampled zooplankton with nets and analysed the community composition using microscopy, zooscan (sample scanning on a flatbed scanner and subsequent automated analysis) and DNA metabarcoding. This monitoring activity especially addresses the need for high temporal sampling, as samples are taken at around 42 times per year, and will allow for comparison with other food web components as these are also sampled from the NIOZ jetty. The results from this first year of sampling will be used to advise on the sampling methodology and frequency of the Marsdiep sampling, and we evaluate the value of the Marsdiep sampling for the MONS program.

2 Materials and Methods

2.1 Study site and sampling

The NIOZ-jetty (53°00'06" N; 4°47'21" E) is located at the Marsdiep basin (Western Wadden Sea) between the North Sea and the Wadden Sea (Fig. 1) and is approximately 4.5 m deep (Ly et al., 2014). Water samples have been collected using a bucket at a frequency of 40–60 times per year, varying from once or twice a month in winter up to twice a week during phytoplankton spring blooms at high tide from the NIOZ-Jetty for decades. The water samples are analysed for temperature, salinity, transparency (Secchi disc), turbidity, inorganic nutrients, suspended particulate matter, pigment concentration and composition (Philippart et al., 2010), phytoplankton species (Philippart et al., 2000), primary production with ^{14}C and presence/absence of bivalve larvae PCR gel electrophoresis using species-specific primers PCR gel electrophorese metabarcoding (Philippart et al., 2014).

Long-term trends in relative nutrient concentrations in the western Wadden Sea suggest that phytoplankton production during the spring and summer blooms was P-limited in the 1970s, Si-limited or N-limited in the 1980s, and then P-limited again thereafter (Philippart et al., 2007). Moreover, phytoplankton dynamics are comparable to the coastal North Sea with a succession in spring from diatoms to *Phaeocystics globosa* (Ly et al., 2014) and indicates that zooplankton data from the NIOZ-jetty samples will also be indicative for the North Sea.

Figure 1: The location of the NIOZ-jetty in the Western Wadden Sea (from Ly et al. 2014).

2.2 Sampling procedure

All required sample water for the zooplankton assessments (a total of 200L) was taken manually with 10L buckets (i.e. a total of 20 buckets per sampling event), immediately after the sample for the regular NIOZ-jetty time series had been taken. In this way, sampling time is as short as possible and closely comparable to the other variables measured. Water was sampled with buckets attached to a rope (Fig. 2A) and is poured over a metal 2-mm sieve (Fig. 2B) to remove the larger (mostly gelatinous) organisms that often complicate identification of smaller zooplankton specimens under the stereomicroscope. The sample was further concentrated using a small Hydrobios 150-µm net and rinsed into a sample jar (Fig. 2C). A total of 4 samples (4x50L) is stored as duplicate formaldehyde (4% final concentration) and duplicate DESS (see description below) samples (Fig. 2D). The total biovolume of (mostly gelatinous) organisms that were preserved on a 2-mm sieve was determined in a measuring cylinder and specimens were photographed in a Petri dish with a ruler as background for sizing. An overview of all samples that have been taken is given in Table 1.

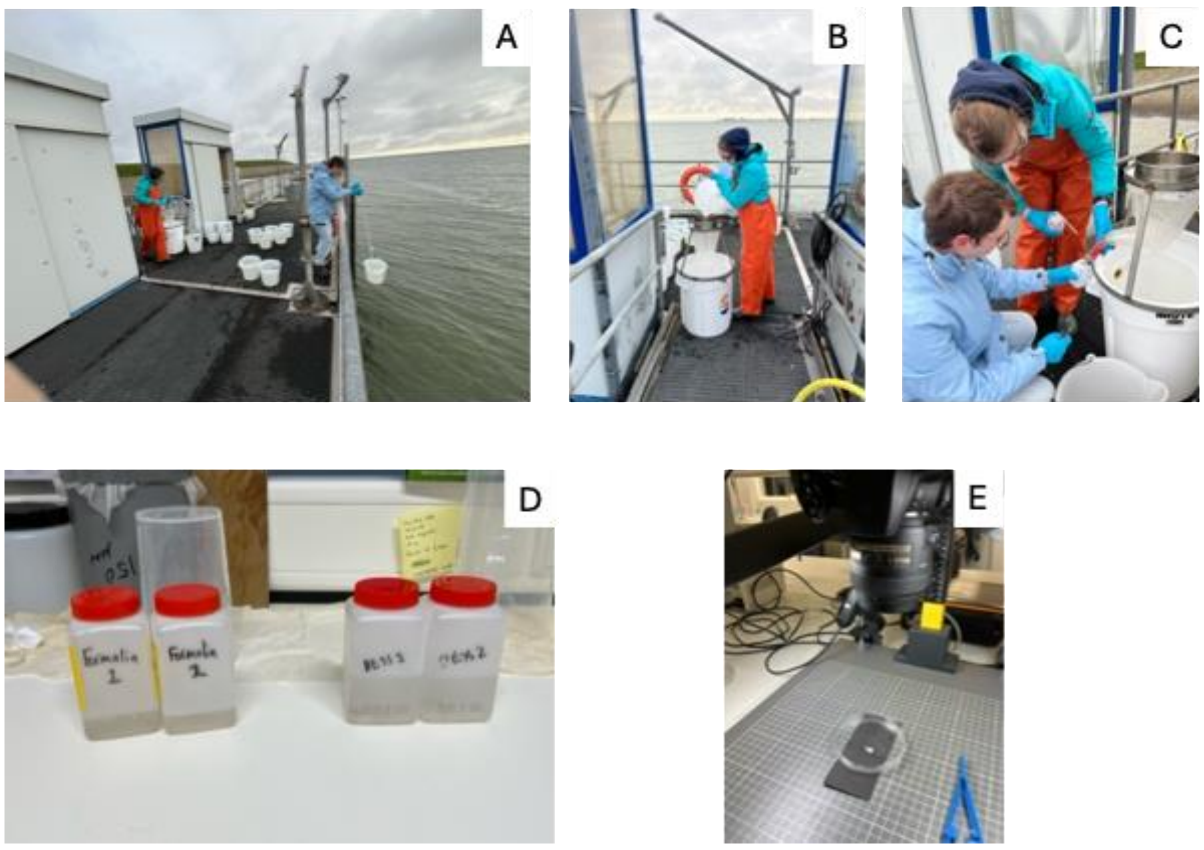
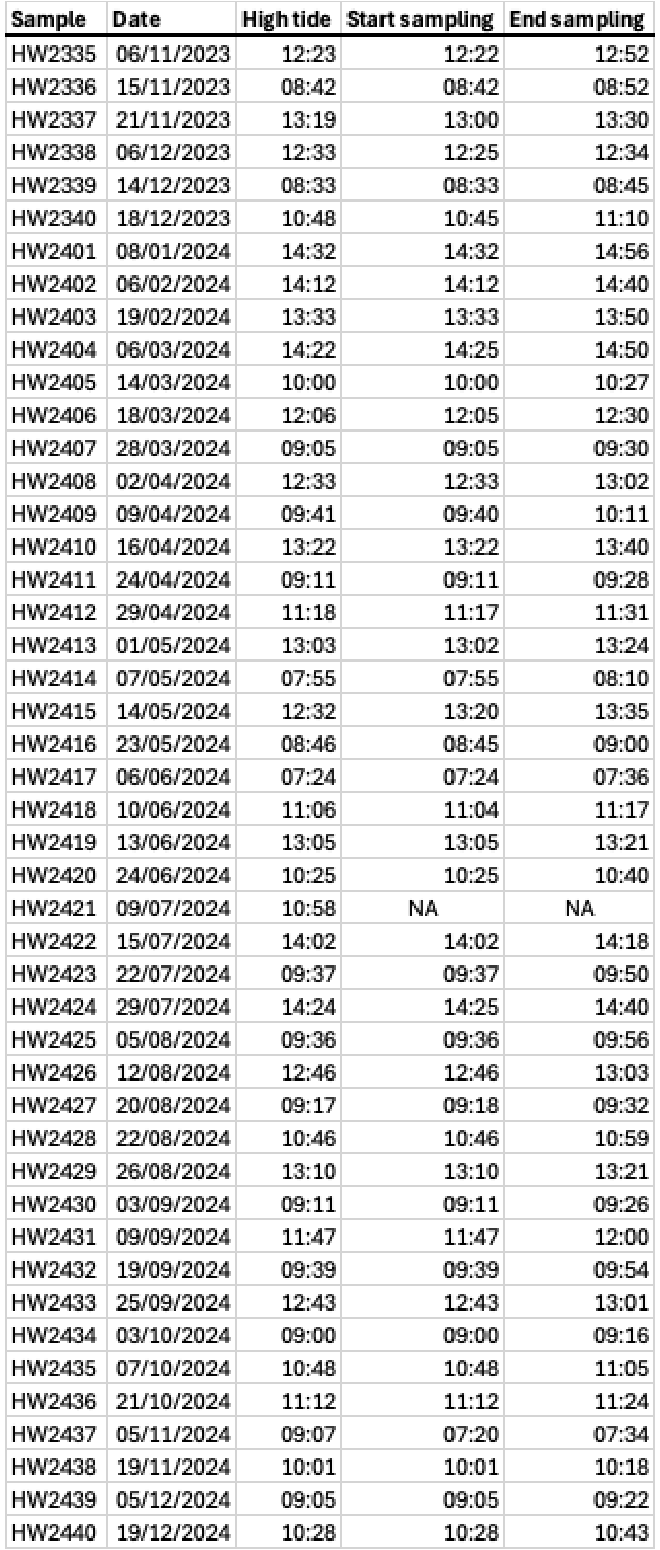


Figure 2: The zooplankton sampling procedure on the NIOZ-jetty: A) water sampling with 10L buckets, B) pre-sieving with a metal 2mm sieve to separate larger (mostly gelatinous) organisms and the sieving with a 150 um net, C) transferring the concentrated sample in a sample jar, D) a total of 4 samples, duplicate formaline and duplicate DESS sample were taken and E) for the gelatinous organisms, the total biovolume was determined and individuals were photographed with a ruler as background.

Table 1: Sample details of zooplankton samples. Note that HW2421 could not be sampled (NA = not available) due to illness in the sampling team.



Sample	Date	High tide	Start sampling	End sampling
HW2335	06/11/2023	12:23	12:22	12:52
HW2336	15/11/2023	08:42	08:42	08:52
HW2337	21/11/2023	13:19	13:00	13:30
HW2338	06/12/2023	12:33	12:25	12:34
HW2339	14/12/2023	08:33	08:33	08:45
HW2340	18/12/2023	10:48	10:45	11:10
HW2401	08/01/2024	14:32	14:32	14:56
HW2402	06/02/2024	14:12	14:12	14:40
HW2403	19/02/2024	13:33	13:33	13:50
HW2404	06/03/2024	14:22	14:25	14:50
HW2405	14/03/2024	10:00	10:00	10:27
HW2406	18/03/2024	12:06	12:05	12:30
HW2407	28/03/2024	09:05	09:05	09:30
HW2408	02/04/2024	12:33	12:33	13:02
HW2409	09/04/2024	09:41	09:40	10:11
HW2410	16/04/2024	13:22	13:22	13:40
HW2411	24/04/2024	09:11	09:11	09:28
HW2412	29/04/2024	11:18	11:17	11:31
HW2413	01/05/2024	13:03	13:02	13:24
HW2414	07/05/2024	07:55	07:55	08:10
HW2415	14/05/2024	12:32	13:20	13:35
HW2416	23/05/2024	08:46	08:45	09:00
HW2417	06/06/2024	07:24	07:24	07:36
HW2418	10/06/2024	11:06	11:04	11:17
HW2419	13/06/2024	13:05	13:05	13:21
HW2420	24/06/2024	10:25	10:25	10:40
HW2421	09/07/2024	10:58	NA	NA
HW2422	15/07/2024	14:02	14:02	14:18
HW2423	22/07/2024	09:37	09:37	09:50
HW2424	29/07/2024	14:24	14:25	14:40
HW2425	05/08/2024	09:36	09:36	09:56
HW2426	12/08/2024	12:46	12:46	13:03
HW2427	20/08/2024	09:17	09:18	09:32
HW2428	22/08/2024	10:46	10:46	10:59
HW2429	26/08/2024	13:10	13:10	13:21
HW2430	03/09/2024	09:11	09:11	09:26
HW2431	09/09/2024	11:47	11:47	12:00
HW2432	19/09/2024	09:39	09:39	09:54
HW2433	25/09/2024	12:43	12:43	13:01
HW2434	03/10/2024	09:00	09:00	09:16
HW2435	07/10/2024	10:48	10:48	11:05
HW2436	21/10/2024	11:12	11:12	11:24
HW2437	05/11/2024	09:07	07:20	07:34
HW2438	19/11/2024	10:01	10:01	10:18
HW2439	05/12/2024	09:05	09:05	09:22
HW2440	19/12/2024	10:28	10:28	10:43

2.3 Analytical procedures

2.3.1 Microscopy

After the zooscan analysis (see below), a subset of 12 (once per month) out of all samples was analysed using microscopy. The samples were selected to have a good annual coverage starting in March 2024. In the lab, the samples were rinsed to remove formaldehyde and, if necessary, split with a Motoda plankton splitter to obtain a representative subsample. This sample was examined using a stereomicroscope (Zeiss Discovery.V8) and organisms present were identified, with adult Calanoid copepods being identified to the species level if possible. If necessary, copepods were dissected to examine identification characteristics using a microscope (Zeiss Observer.A1). Copepodite stages of copepods were counted separately.

2.3.2 Zooscan

The formaldehyde-fixed samples were first rinsed and then size-fractioned on two stacked sieves of 500 μm and 100 μm. The smallest size class (<500 μm) was stained with Rose Bengal for 24 hours to increase contrast of the organisms on the scan. This was not necessary for the largest size fraction. The full sample, sometimes in multiple ½ or ¼ splits to reduce number of particles per scan, was scanned on an Epson Perfection V850 Pro scanner at the recommended resolution of 2400 dpi (Gorsky et al., 2010). Each scan was fed into ZooProcess, an ImageJ based algorithm to identify and store individual segments from a full Zooscan (Gorsky et al., 2010). While care was taken to separate organisms or particles before the scanning, it is important to mention that a segment can also be composed of so-called 'multiples' which represents organisms or particles that are touching or clustered together (Fig. 3). Earlier zooscan applications have shown that these clusters can represent up to 25% of the organisms in a sample (Van Walraven et al., 2025). In a MSc thesis project, we therefore explored the use of a region-based convolutional neural network (YOLOv11-seg, Jocher and Qiu, 2024) to count and identify individual zooplankton specimens in clusters. The thesis project is still ongoing and we show preliminary results of this effort in the Results section.

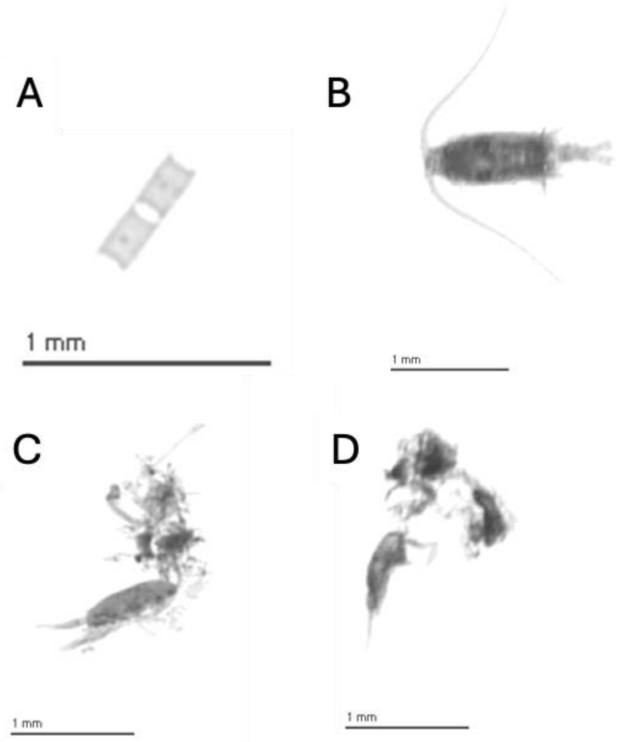


Figure 3: Examples of zooscan segments from the jetty sampling. A) Diatom, B) copepod, C) cluster of particles, with a copepod in the bottom left of the cluster and D) another cluster with a copepod in the bottom left.

For the classification of the segments, learning sets from WMR (Wageningen Marine Research) and VLIZ (Vlaams Instituut voor de Zee) were used, and these were combined into the following classes: Annelida, Appendicularia, Appendicularia_tail, Artefact, Asteroidea_larvae, Bivalvia_larvae, Brachyura_zoea, Branchiopoda, Bryozoan_cyphonaute, Bubble, Calanoida, Chaetognatha, Cirripedia_cypris, Cirripedia_nauplii, Cnidaria, Copepoda_exuvium, Copepoda_naupli, Ctenophora, Cumacea, Detritus, Echinoidea_echinopluteus, Fibre, Foraminifera, Gastropoda_veliger, Harpacticoida, Multiple, Mysida, Noctiluca, Ophiuroidea_larvae_branchiolaria_star, Pisces_egg, Pisces_larvae and Polychaete_nectochaeta. A convolutional neural network (EfficientNetV2S, Hovenkamp et al., In review) based classifier was trained and used to classify all segments in the first round. In the second round, a subset of the classified Marsdiep segments were manually verified and used to update and/or replace the original learning set. This second learning set was then used for training and classification of the unseen segments.

2.3.3 Metabarcoding

The DNA extraction and DNA amplification of the DESS samples was performed at the dedicated DNA laboratory of Wageningen Environmental Research and is identical to sample processing reported in (Van Walraven et al., 2025) and is therefore described in brief here.

First, DESS was removed from the 50 ml tube samples with centrifugation. DNA was extracted from the remaining pellet using DNeasy PowerSoil Pro Kit (Qiagen) with a slightly modified protocol. For next-generation sequencing, a two-step PCR protocol was used to create a dual index amplicon library using the primers COI (COI_mlCOIintF_v2 and COI_jgHCO2198) and 18SV9 (Euk_1391f and EukB2). All primers were flanked with Truseq adapters at their 5' ends. PCRs were performed in duplicate, the products of which were combined after amplification to take stochasticity in the reaction into account. Two PCR negative and one positive PCR control were included for each marker as well. Library preparation and addition of sample-specific barcodes ligated onto all PCR products was performed by IGAtech before sequencing on an Illumina NovaSeq PE250 bp. Raw files processing is described in detail in Van Walraven et al. (2025).

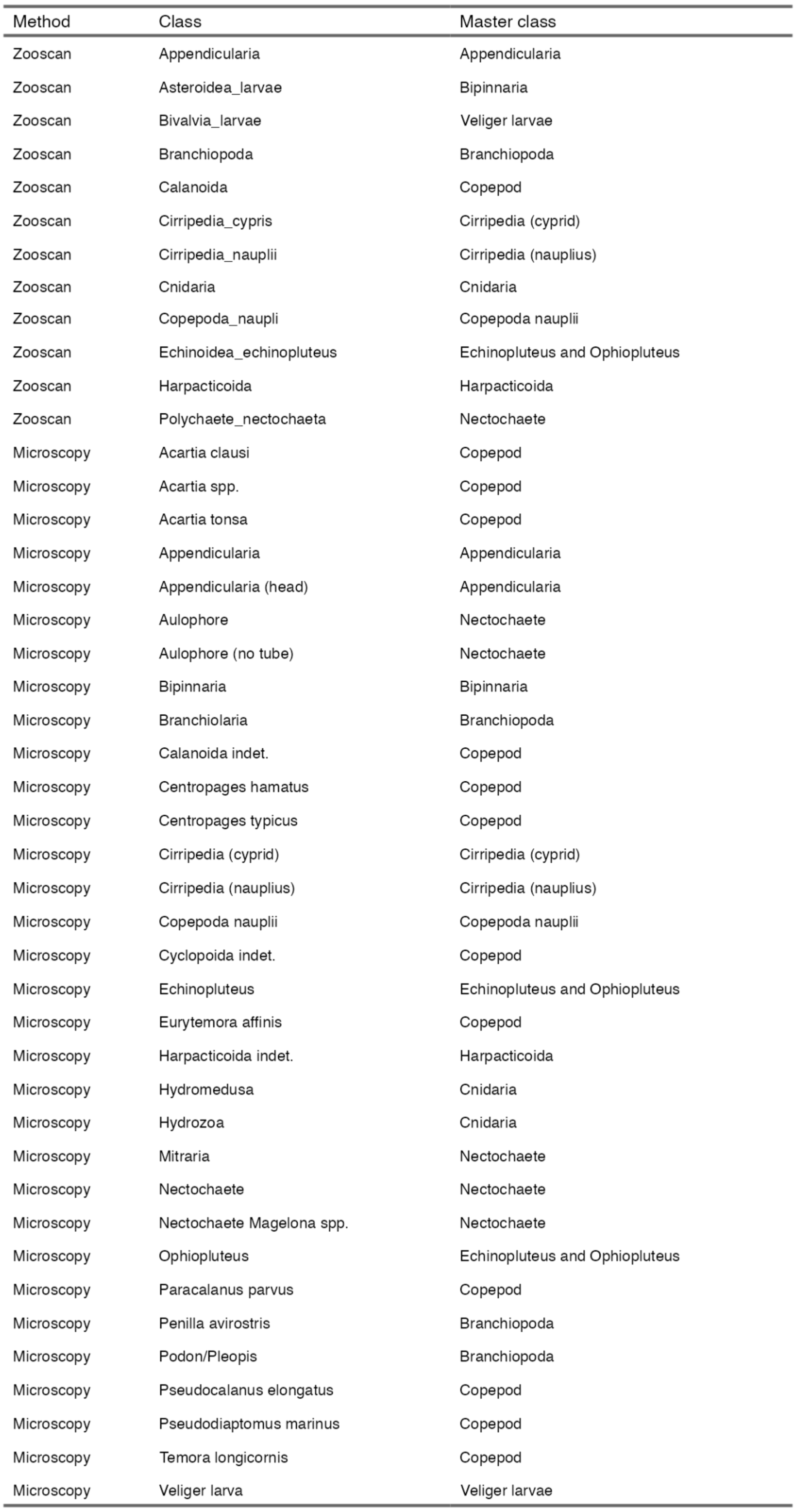
For COI, taxonomy assignment was performed using the BOLDigger package version 2.1.1 that makes it possible to access all records on the Barcode Of Life Data (BOLD) system, including early access and private records. The option digger_hit from the JAMP pipeline was used to obtain a last common ancestor of the top 20 hits returned from the BOLD website and all flagged hits were manually checked. The following thresholds were used: at least 97% sequence similarity for species level identification, 95% for genus, 90% for family and anything lower is classified to the order level. For 18SV9, sequences were classified using a BLASTn search against the NCBI GenBank nt database (downloaded 03-2024). All sequences were curated using a lowest common ancestor (LCA) approach, requiring at least 95% query coverage and 97% identity match, and collapsing to the LCA if the percentage identity between consecutive hits differed by less than 0.5%. Taxonomic assignments were manually validated for plausibility for occurring in the North Sea ecoregion and assigned a confidence label based on whether classification was plausible, doubtful, or unlikely.

For all markers, sequences were filtered in several steps (see Van Walraven et al. 2025). After filtering steps, the negative PCR control and extraction negative controls were found to be clean. Taxa with identical identifications were then merged. To account for differences in sequencing depth, for each marker we rarefied all samples to the sample with the least number of reads using the rarefy function in the 'vegan' program prior to other analyses. Consequently, the sequencing depth was 280k reads for COI and 520k reads for 18SV9.

2.4 Comparing the microscopy and zooscan data

The zooplankton concentrations between microscopy counts and the zooscan were compared by defining a ‘master class’ which was composed of counts from the zooscan and the microscopy. Such a master class definition is necessary as e.g. species resolution for Copepoda is high from microscopy counts, but low from the zooscan. The lookup table for the master class definition is shown in Table 2.

Table 2: Lookup table to quantitatively compare the microscopy and zooscan results.



Method	Class	Master class
Zooscan	Appendicularia	Appendicularia
Zooscan	Asteroidea_larvae	Bipinnaria
Zooscan	Bivalvia_larvae	Veliger larvae
Zooscan	Branchiopoda	Branchiopoda
Zooscan	Calanoida	Copepod
Zooscan	Cirripedia_cypris	Cirripedia (cyprid)
Zooscan	Cirripedia_nauplii	Cirripedia (nauplius)
Zooscan	Cnidaria	Cnidaria
Zooscan	Copepoda_naupli	Copepoda nauplii
Zooscan	Echinoidea_echinopluteus	Echinopluteus and Ophiopluteus
Zooscan	Harpacticoida	Harpacticoida
Zooscan	Polychaete_nectochaeta	Nectochaete
Microscopy	Acartia clausi	Copepod
Microscopy	Acartia spp.	Copepod
Microscopy	Acartia tonsa	Copepod
Microscopy	Appendicularia	Appendicularia
Microscopy	Appendicularia (head)	Appendicularia
Microscopy	Aulophore	Nectochaete
Microscopy	Aulophore (no tube)	Nectochaete
Microscopy	Bipinnaria	Bipinnaria
Microscopy	Branchiolaria	Branchiopoda
Microscopy	Calanoida indet.	Copepod
Microscopy	Centropages hamatus	Copepod
Microscopy	Centropages typicus	Copepod
Microscopy	Cirripedia (cyprid)	Cirripedia (cyprid)
Microscopy	Cirripedia (nauplius)	Cirripedia (nauplius)
Microscopy	Copepoda nauplii	Copepoda nauplii
Microscopy	Cyclopoida indet.	Copepod
Microscopy	Echinopluteus	Echinopluteus and Ophiopluteus
Microscopy	Eurytemora affinis	Copepod
Microscopy	Harpacticoida indet.	Harpacticoida
Microscopy	Hydromedusa	Cnidaria
Microscopy	Hydrozoa	Cnidaria
Microscopy	Mitraria	Nectochaete
Microscopy	Nectochaete	Nectochaete
Microscopy	Nectochaete Magelona spp.	Nectochaete
Microscopy	Ophiopluteus	Echinopluteus and Ophiopluteus
Microscopy	Paracalanus parvus	Copepod
Microscopy	Penilla avirostris	Branchiopoda
Microscopy	Podon/Pleopis	Branchiopoda
Microscopy	Pseudocalanus elongatus	Copepod
Microscopy	Pseudodiaptomus marinus	Copepod
Microscopy	Temora longicornis	Copepod
Microscopy	Veliger larva	Veliger larvae

2.5 Data availability

All data in this report will be made available in an online repository and to the MONS data repository.

3 Results

3.1 Microscopy

A total of 4,162 specimens were counted under the stereomicroscope over the period 09-10-2024 to 05-12-2024 (Appendix 1). In this report, we removed the 'non-zooplanktonic' class 'Insect larvae' (non-marine organisms) and focused on classes that occur on at least 2 sampling dates, resulting in specimen count of 4,143 divided over a total of 42 classes. Specimens found in the 150-500 µm and >500 µm size fraction of the same sample were combined in the analysis.

Total zooplankton abundance over the whole sampling period is shown in Fig. 4. Total abundance was strongly seasonal, with maximum abundance per sampling occurring during two spring sampling dates on 01-05-2024 (32 ind. L^{-1}) and 02-04-2024 (28 ind. L^{-1}). The abundance during the summer and autumn months ranged between 5 and 10 ind. L^{-1} and <5 ind. L^{-1} in the winter months.

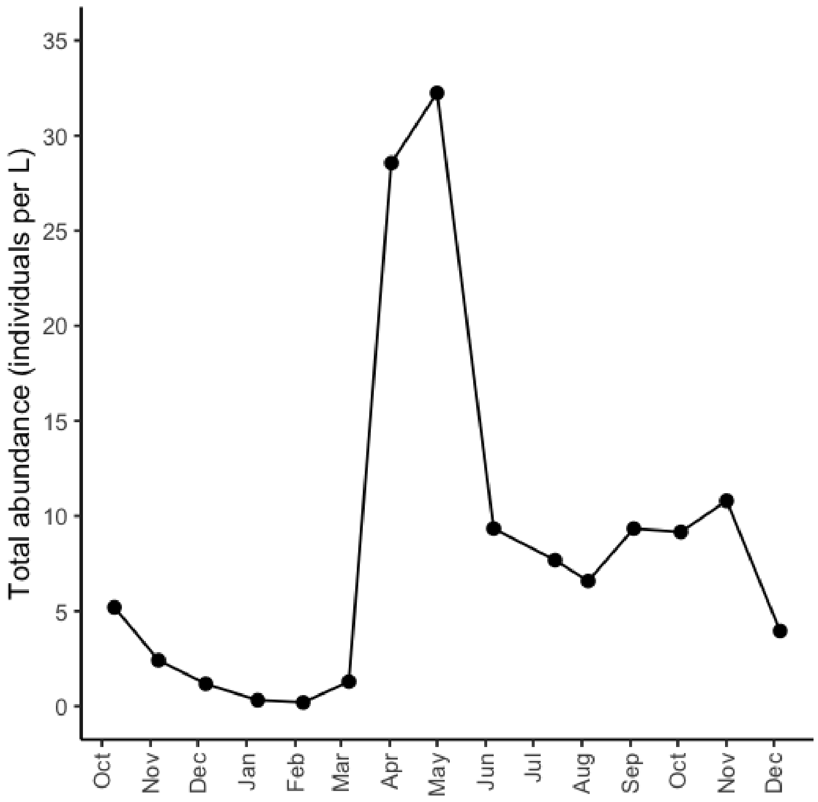


Figure 4: Total zooplankton abundance at the Marsdiep jetty based on microscopy counts.

Per taxonomic class, maximum abundances were observed for Echinopluteus (13.4 ind. L^{-1}, 01-05-2024), Veliger larva (10.4 ind. L^{-1}, 02-04-2024), Harpacticoida spp. (5.3 ind. L^{-1}, 01-11-2024) and Copepodites (4.8 ind. L^{-1}, 02-04-2024) (Fig. 5). A total of nine Calanoid copepods were identified and observed at species level: *Acartia clausi, Acartia tonsa, Centropages hamatus, Centropages typicus, Eurytemora affinis, Paracalanus parvus, Pseudocalanus elongatus, Pseudodiaptomus marinus* and *Temora longicornus*, of which *Temora longicornus* was observed most frequently (mostly due to one observation in spring). Most species-specific Calanoid copepod densities are below 0.1 ind. L^{-1} and have single sampling events in which their abundance is substantially higher. Not all Cyclopoida were identified to species level, most are *Oithona* spp., about 15-25% of the Cyclopoida are *Othoina nana*, 15-25% *Othoina davisae* and sometimes a few Poecilostomatoida specimens were observed (data not shown). Interestingly, for several classes, their maxima were either found in spring (e.g., *Acartia clausi*, *Aulophore*) or autumn (e.g., Cyclopoida, Caprellidae) or in both seasons (e.g. Copepoda nauplii), with generally comparatively low abundances in summer.

Figure 5: Seasonal abundance (individuals L^{-1}) of 38 zooplankton classes as counted by microscopy at the Marsdiep.

The taxonomic groups were assigned to either holoplankton, i.e., zooplankton that lives its entire life cycle in the water column, or meroplankton, i.e., zooplankton that spend only part of its life cycle in the water column, such as larvae of benthic fauna. These two plankton groups have a clear different ecological role and show different dynamics over the year (Fig. 6). Meroplankton abundance (19.8 ind. L^{-1} on 02-04-2025 and 19.4 ind. L^{-1} on 01-05-2025) dominates the zooplankton composition from spring to early summer, after which holoplankton takes over. In contrast to meroplankton, holoplankton has two distinct peaks, one in spring (01-05-2025, 12.8 ind. L^{-1}), but with a lower abundance than meroplankton, and a peak in autumn (01-11-2025, 10.2 ind. L^{-1}).

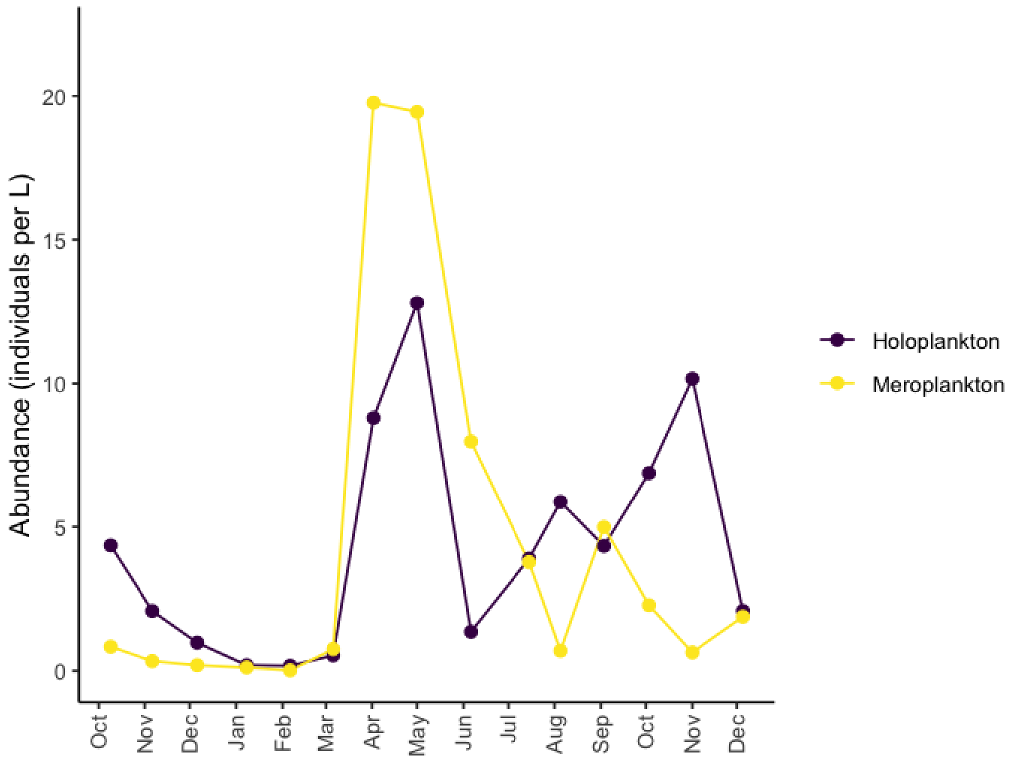


Figure 6: Seasonal abundance of holo- and meroplankton based on microscopy counts at the Marsdiep.

3.2 Zooscan

The learning set comprised 10,030 images divided over 32 classes (Table 3). An example segment of the main classes included in this report is shown in Fig. 7.

Table 3: Characteristics of the learning set for the second training and classification round of the zooscan images.

Class name	Number of images
Appendicularia	546
Appendicularia_tail	15
Artefact	500
Asteroidea_larvae	46
Bivalvia_larvae	292
Brachyura_zoea	158
Branchiopoda	545
Bryozoan_cyphonaute	27
Bubble	131
Calanoida	1,103
Chaetognatha	87
Cirripedia_cypris	362
Cirripedia_nauplii	551
Cnidaria	138
Copepoda_exuvium	222
Copepoda_naupli	30
Ctenophora	25
Cumacea	220
Detritus	500
Echinoidea_echinopluteus	240
Fibre	445
Foraminifera	169
Gastropoda_veliger	510
Harpacticoida	534
Multiple	1,414
Mysida	22
Noctiluca	655
Ophiuroidea_larvae_branchiolaria_star	93
Pisces_egg	97
Pisces_larvae	11
Polychaeta	262
Polychaete_nectochaeta	80

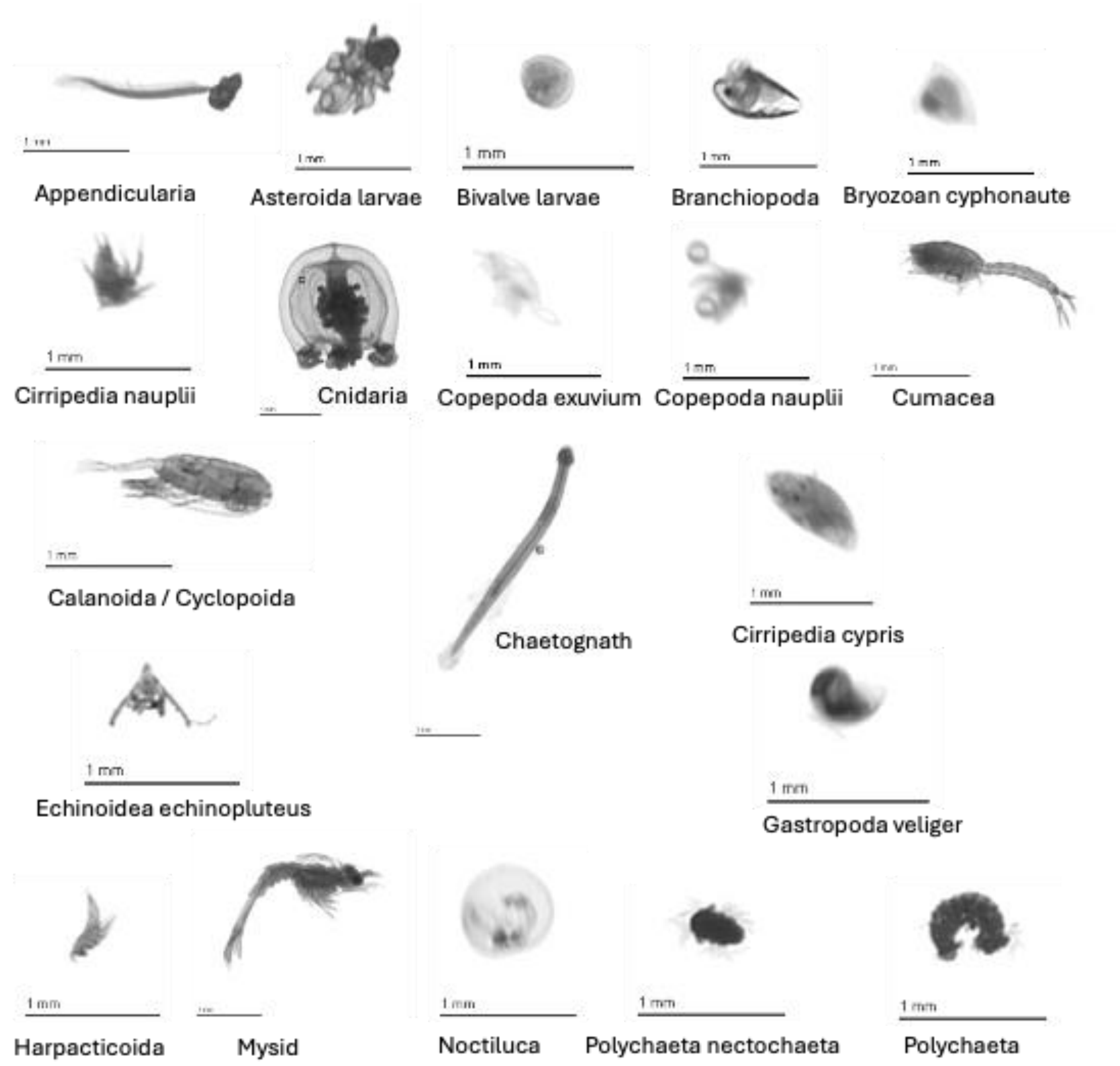
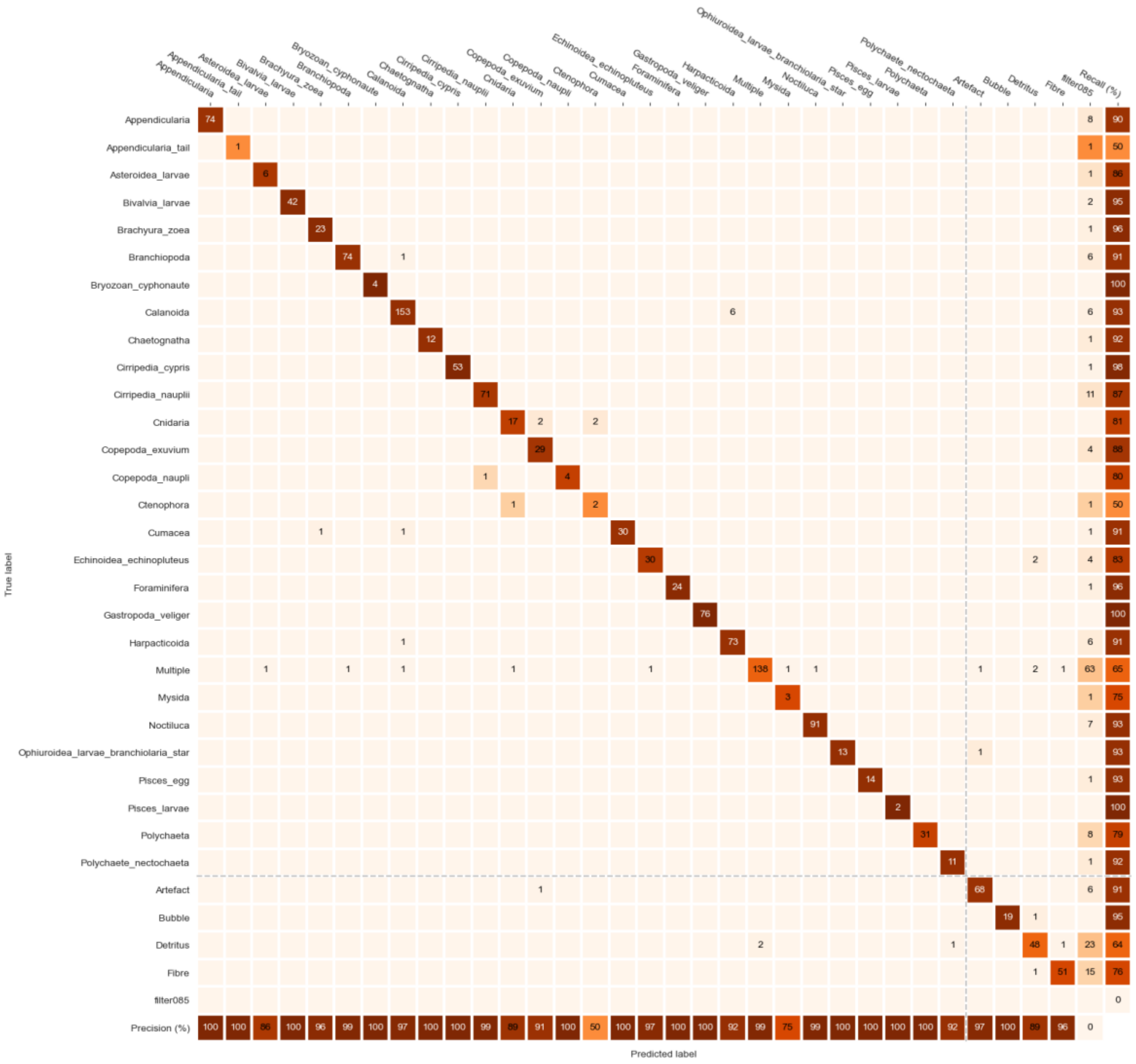


Figure 7: Example of the zooscan classes considered in the report. The scale bar on each image is 1 mm.

We applied a probability threshold of 85% to each classification. This reduces the number of segments classified to class but reduces the risk of false positives and is often a good choice in unbalanced data sets (Hovenkamp et al., unpub. data). The confusion matrix (with the 85% probability threshold applied) for the second round of zooscan classification is shown in Fig. 8. Overall, the high scores for precision (% of positive predictions) and recall (% of all relevant instances that were recovered) show that the model performs well on the learning set.



	Appendicularia	Appendicularia_tail	Asteroidea_larvae	Bivalvia_larvae	Brachyura_zoea	Branchiopoda	Bryozoan_cyphonaute	Calanoida	Chaetognatha	Cirripedia_cypris	Cirripedia_naupli	Cnidaria	Copepoda_exuvium	Copepoda_naupli	Ctenophora	Cumacea	Echinoidea_echinopluteus	Foraminifera	Gastropoda_veliger	Harpacticoida	Multiple	Mysida	Noctiluca	Ophiuroidea_larvae_branchiolaria_star	Pisces_egg	Pisces_larvae	Polychaeta	Polychaete_nectochaeta	Artefact	Bubble	Detritus	Fibre	filter085	Recall (%)
Appendicularia	74																																8	90
Appendicularia_tail		1																															1	50
Asteroidea_larvae			6																														1	86
Bivalvia_larvae				42																													2	95
Brachyura_zoea					23																												1	96
Branchiopoda						74		1																									6	91
Bryozoan_cyphonaute							4																											100
Calanoida								153												6													6	93
Chaetognatha									12																								1	92
Cirripedia_cypris										53																							1	98
Cirripedia_nauplii											71																						11	87
Cnidaria												17	2		2																			81
Copepoda_exuvium													29																				4	88
Copepoda_naupli											1			4																				80
Ctenophora												1			2																		1	50
Cumacea					1			1								30																	1	91
Echinoidea_echinopluteus																	30														2		4	83
Foraminifera																		24															1	96
Gastropoda_veliger																			76															100
Harpacticoida								1												73													6	91
Multiple			1			1		1				1					1				138	1	1						1		2	1	63	65
Mysida																						3											1	75
Noctiluca																							91										7	93
Ophiuroidea_larvae_branchiolaria_star																								13					1					93
Pisces_egg																									14								1	93
Pisces_larvae																										2								100
Polychaeta																											31						8	79
Polychaete_nectochaeta																												11					1	92
Artefact													1																68				6	91
Bubble																														19	1			95
Detritus																					2							1			48	1	23	64
Fibre																															1	51	15	76
filter085																																		0
Precision (%)	100	100	86	100	96	99	100	97	100	100	99	89	91	100	50	100	97	100	100	92	99	75	99	100	100	100	100	92	97	100	89	96	0	

Figure 8: Confusion matrix of the second round of zooscan classification showing Precision% and Recall% for each class.

From all zooscans, a total of 328,160 segments were identified, which were classified into the 32 classes (see Table 3). The classes Artefact, Bubble, Ctenophora, Detritus, Fibre and Multiple are not considered in this report as these are not zooplankton. A total of 166,038 segments were assigned to an organism class. After classification, the groups Brachyura_zoea, Bryozoan_cyphonautes, Chaetognaths, Cumacea and Gastropoda_veliger were manually verified. Especially, Bryozoan cyphonautes were very difficult to confirm and contained many false positives and therefore we decided to not include this class in the results. In addition, the classes Pisces_larvae, Ctenophora, Ophiuroidea_larvae_branchiolaria_star and Pisces_egg were found <25, so were ignored.

Total zooplankton densities, based on the zooscan, vary considerably over time with *Noctiluca scintillans* being a very dominant taxon and hence the annual dynamics are shown with *Noctiluca scintillans* (Fig. 9A) and without *Noctiluca scintillans* (Fig. 9B). Highest densities for both results are always seen in April and May, with lower densities in summer and an increase again in autumn in October and November.

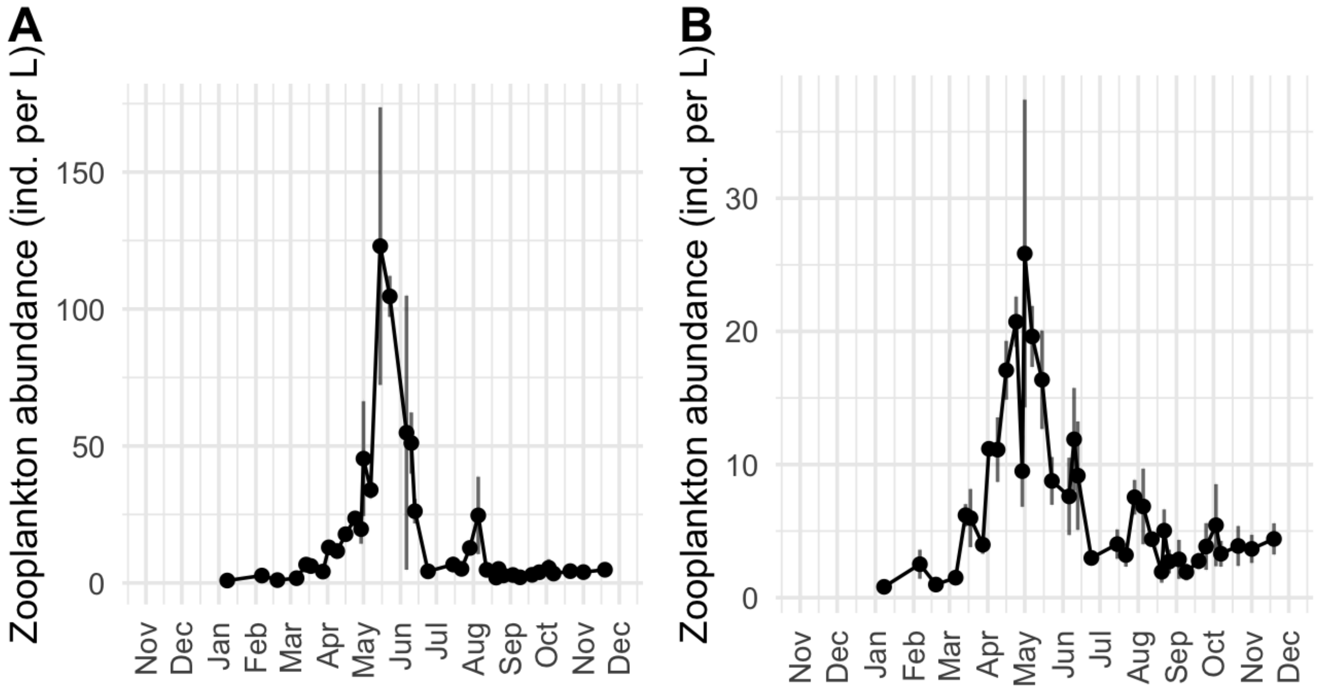


Figure 9: Total zooplankton abundance (mean ± range) at the Marsdiep jetty based on zooscan analysis, A) total abundance with Noctiluca and B) without Noctiluca.

The annual cycle of each class shows considerable variation with a presence over the whole year and clear spring peak for Appendicularia, Calanoida and Branchiopoda and a temporally short spring peak for Bivalve larvae and *Noctiluca scintillans* (Fig. 10).

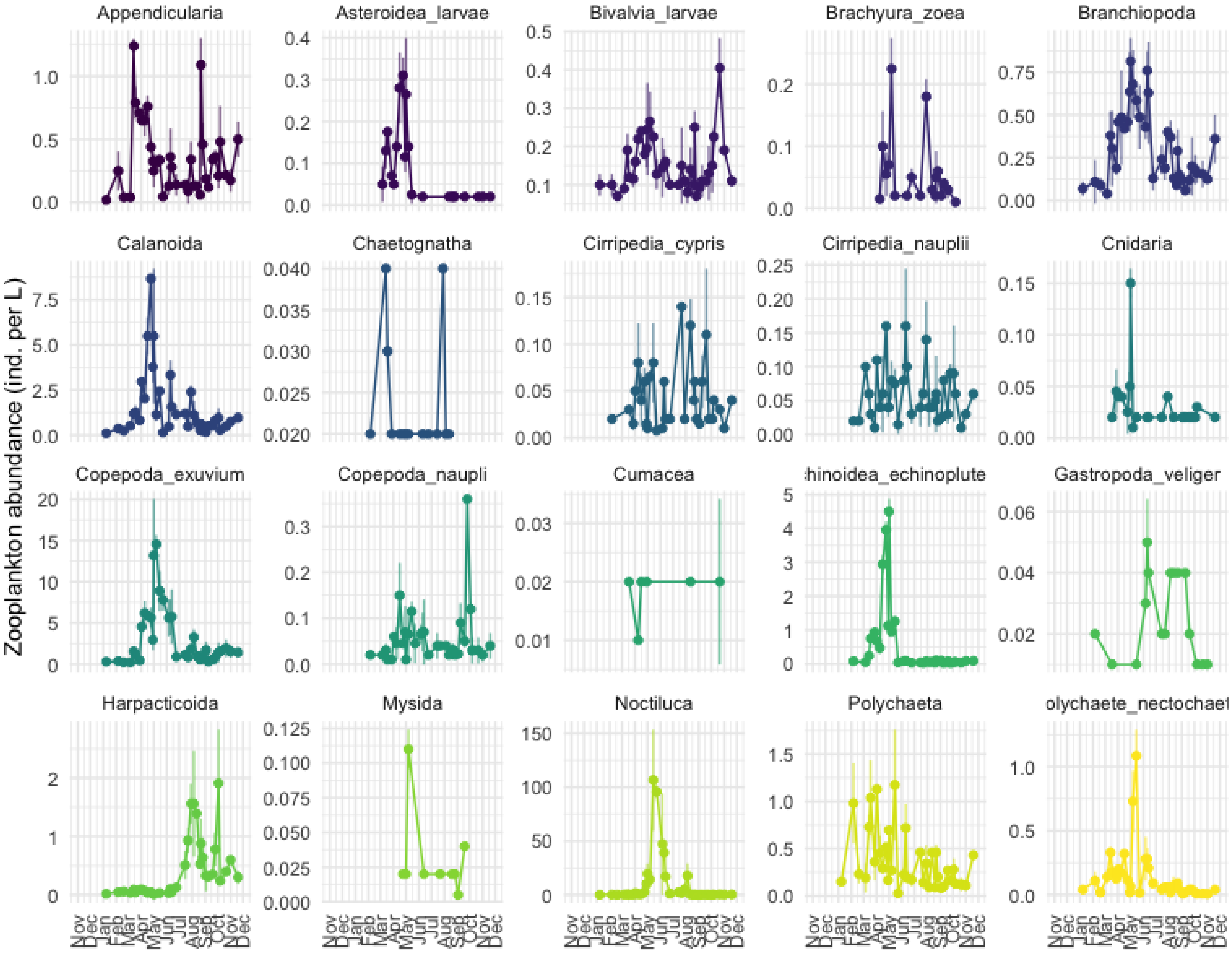


Figure 10: Seasonal zooplankton dynamics (mean ± range) at the Marsdiep based on the zooscan method.

A total of 19,068 segments were classified as 'multiples' (or clusters) by the neural network algorithm. In the analysis above, these clusters are not considered as the MSc project to automatically count and classify organisms in the clusters is still ongoing, but preliminary results are promising (Fig. 11). In several clusters, copepods, Branchiopoda (Evadne) and Echinoderm larvae are correctly identified. Also some incorrect identifications are made, including an *Oikopleura* identification (3rd row, 3rd column), but overall results are encouraging. Therefore, in the next progress report, we will include results of this project.

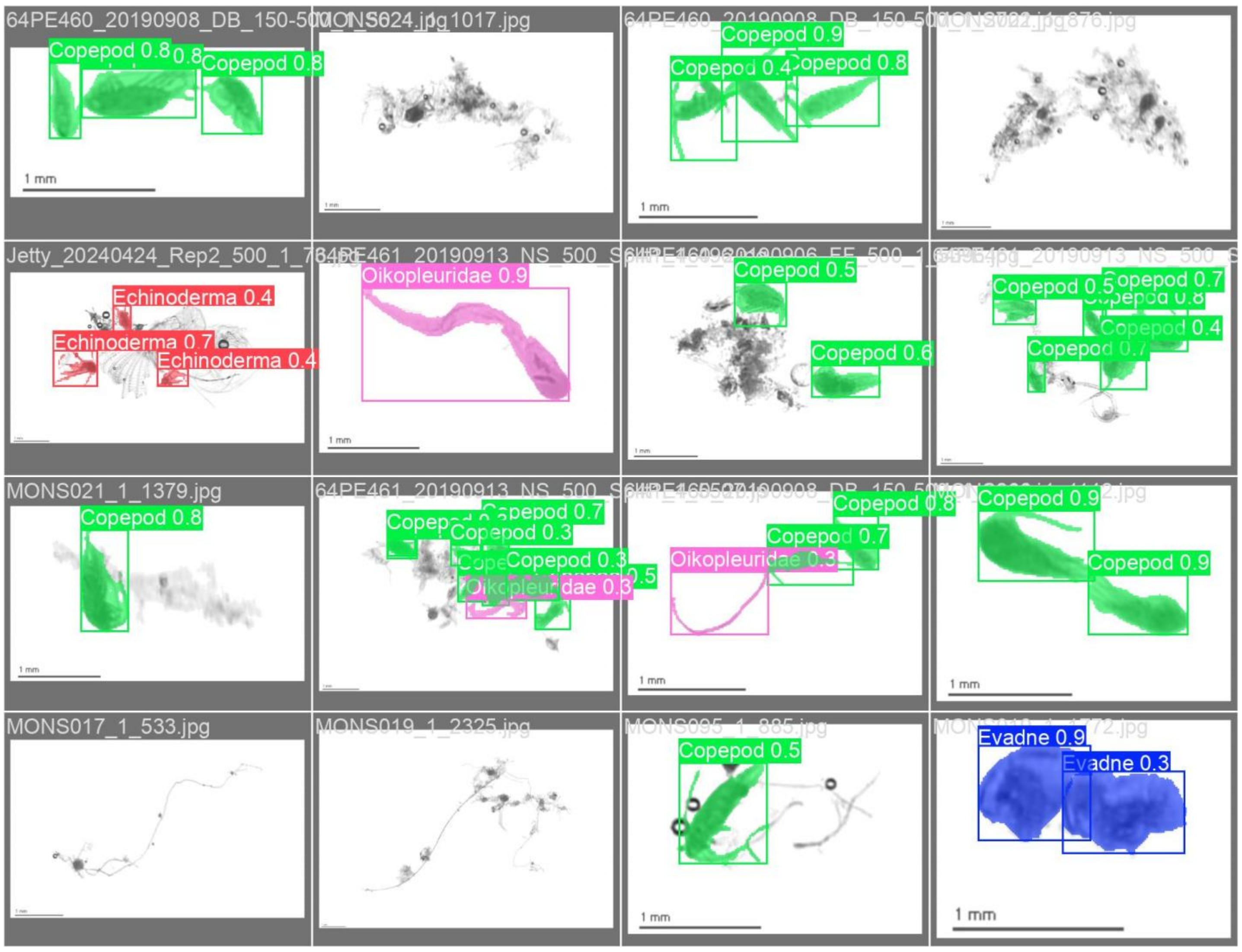


Figure 11: Preliminary results of the YOLO instance segmentation of zooscan clusters.

3.3 Metabarcoding

The metabarcoding analysis returned more than 1,000 'species' for both primer regions (18Sv9 and COI), in which also many phytoplankton taxa are present. In our analysis we focused therefore on the zooplankton species in the following classes Anthozoa, Appendicularia, Asteroidea, Bivalvia, Copepoda, Echinoidea, Gastropoda, Hydrozoa, Polychaeta and Scyphozoa. For each species in these classes, we determined whether their occurrence was 'plausible' in Dutch coastal waters using Van Walraven et al. (2025) and resources on the World Register of Marine Species (https://www.marinespecies.org/). This resulted in a total unique species count of 131. The presence of each species for each primer region is shown in the Appendix 2 and the temporal dynamics of each species is shown in Appendix 3. Very recently, also data of the metabarcoding samples of the primer region 18SV4 have become available. Due to the late delivery, these data could not be included in the present report. A recent report on metabarcoding of zooplankton in the coastal zone of the North Sea showed that the 18SV4 was the least informative primer (Van Walraven et al., 2025), so we do not expect that the 18SV4 would have changed the results considerably.

Total zooplankton diversity is low in winter months with a presence of 20-30 species (Fig. 12A). Species diversity quickly increases to 60 in spring and summer and gradually decreases back to ±20 in autumn/winter. Highest diversity is seen in the Polychaete class (Fig. 12B). When split over mero- and holoplankton, it becomes clear that species diversity in the holoplankton in relatively constant over the year (Fig. 13). By far the highest species diversity is seen in the meroplankton of which the diversity sharply increases in spring and decreases in September (Fig. 13).

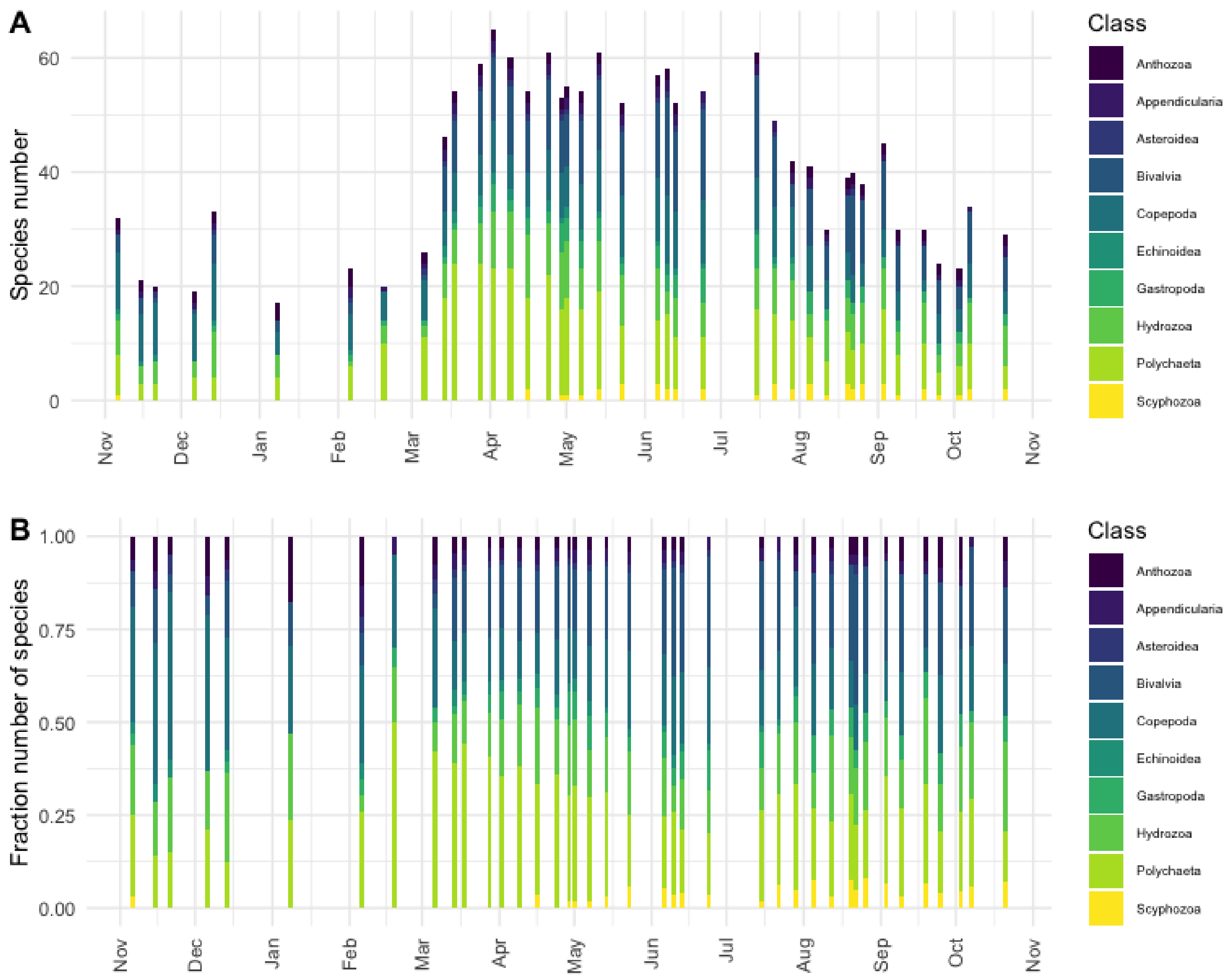


Figure 12: A) Absolute and B) relative zooplankton species number at the Marsdiep.

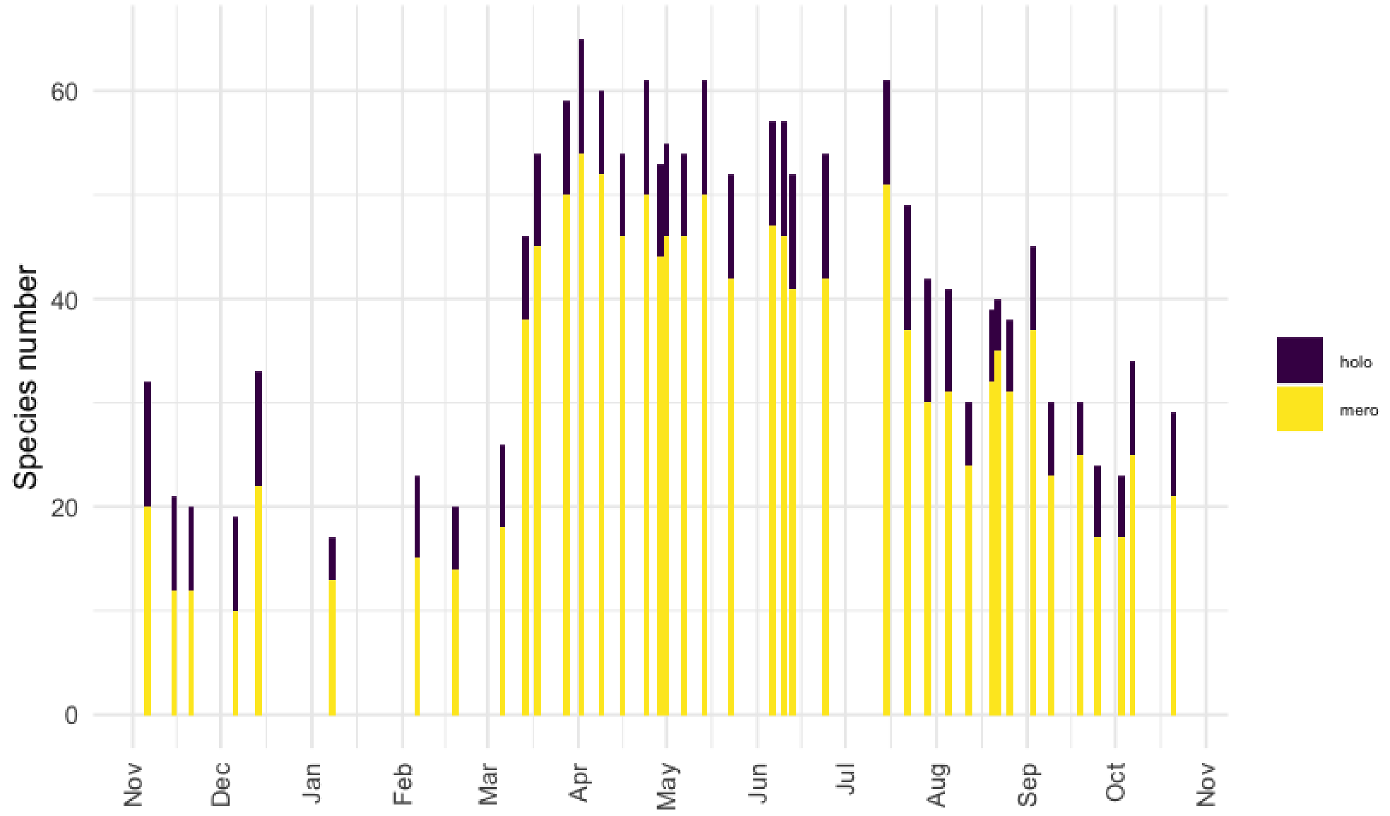


Figure 13: Species diversity at the Marsdiep split over holo- and meroplankton.

To illustrate the different dynamics of mero- and holoplankton, we included the dynamics of relative reads for both primer regions for 6 species (Fig. 14). The three holoplankton species show presence during most of the year and have clear peaks in spring and autumn (*Oikopleura dioica*) or summer (*Centropagus hamatus* and *Temora longicornis*).

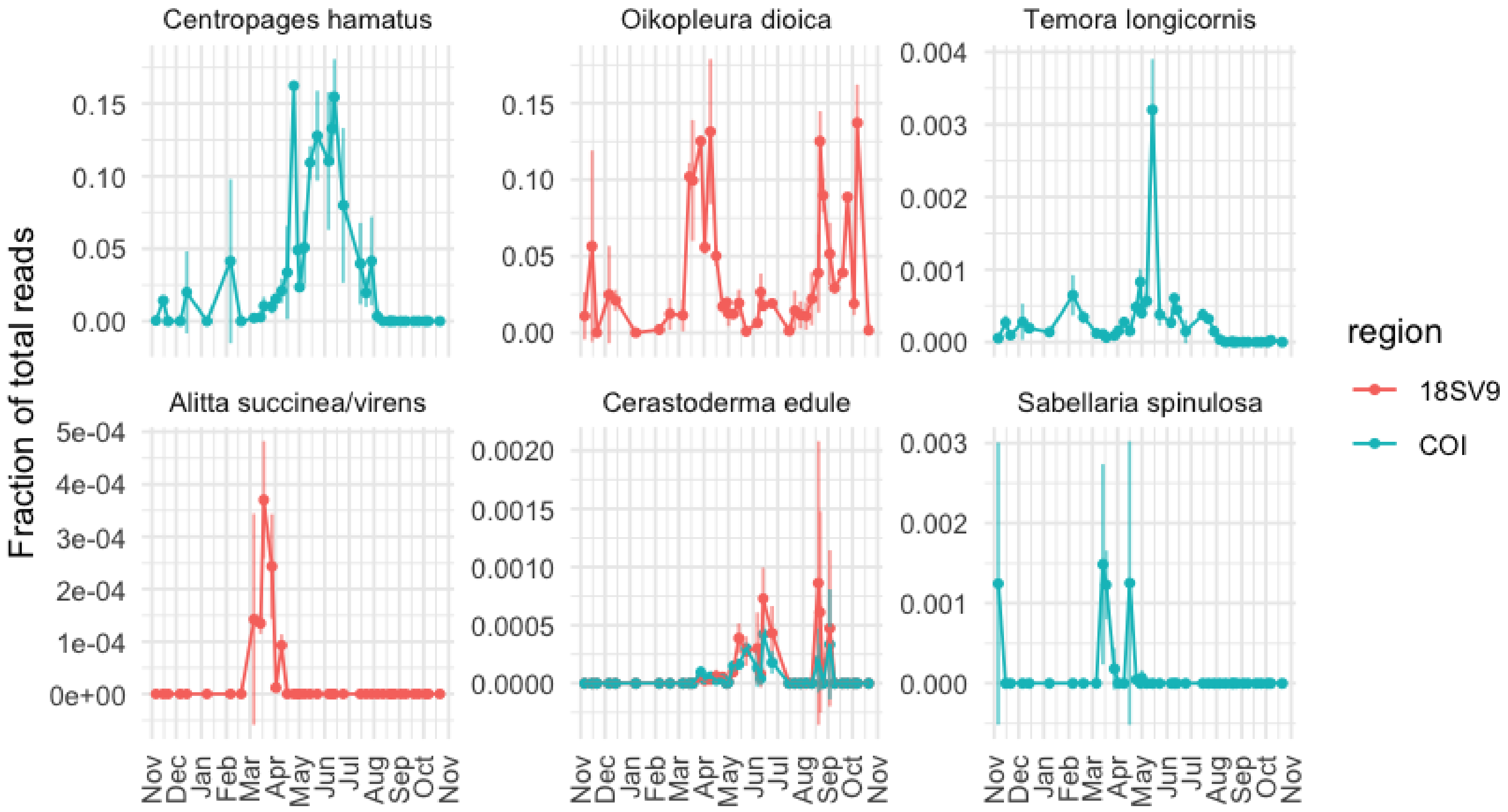


Figure 14: Temporal dynamics, based on fraction of total reads (mean ± range), of 6 zooplankton species with the top row 3 holoplankton species (i.e. copepod Centropagus hamatus, *appendicularian* Oikopleura dioica *and the copepod* Temora longicornis) *and bottom row 3 meroplankton species (i.e. the polychaete* Alitta succinea/virens, *the bivalve* Cerastoderma edule *and the polychaete* Sabellaria spinulosa*).*

To analyse whether the sampling frequency is sufficient to capture the zooplankton species diversity, we constructed species accumulation curves over time for 4 sampling frequency scenarios: 43 (= present sampling effort), 20 (50% of present sampling effort), 12x (once per month and 4x (once per season). The different sampling events were equally distributed over the year. We compare the cumulative species diversity captured by both primers for both holo- and meroplankton (Fig. 15). The COI primer clearly captures highest zooplankton diversity detecting up to almost 100 meroplankton and 15 holoplankton species, while the 18SV9 primer detects almost 50 meroplankton species and 10 holoplankton species. Reducing the sampling frequency has comparatively limited consequences for detecting species diversity in the holoplankton. This is different for meroplankton, up to 40% of the meroplankton species will be missed when the sampling frequency is reduced to 4x per year. With a sampling frequency of 12x and 20x per year, about 80% of the meroplankton species are detected.

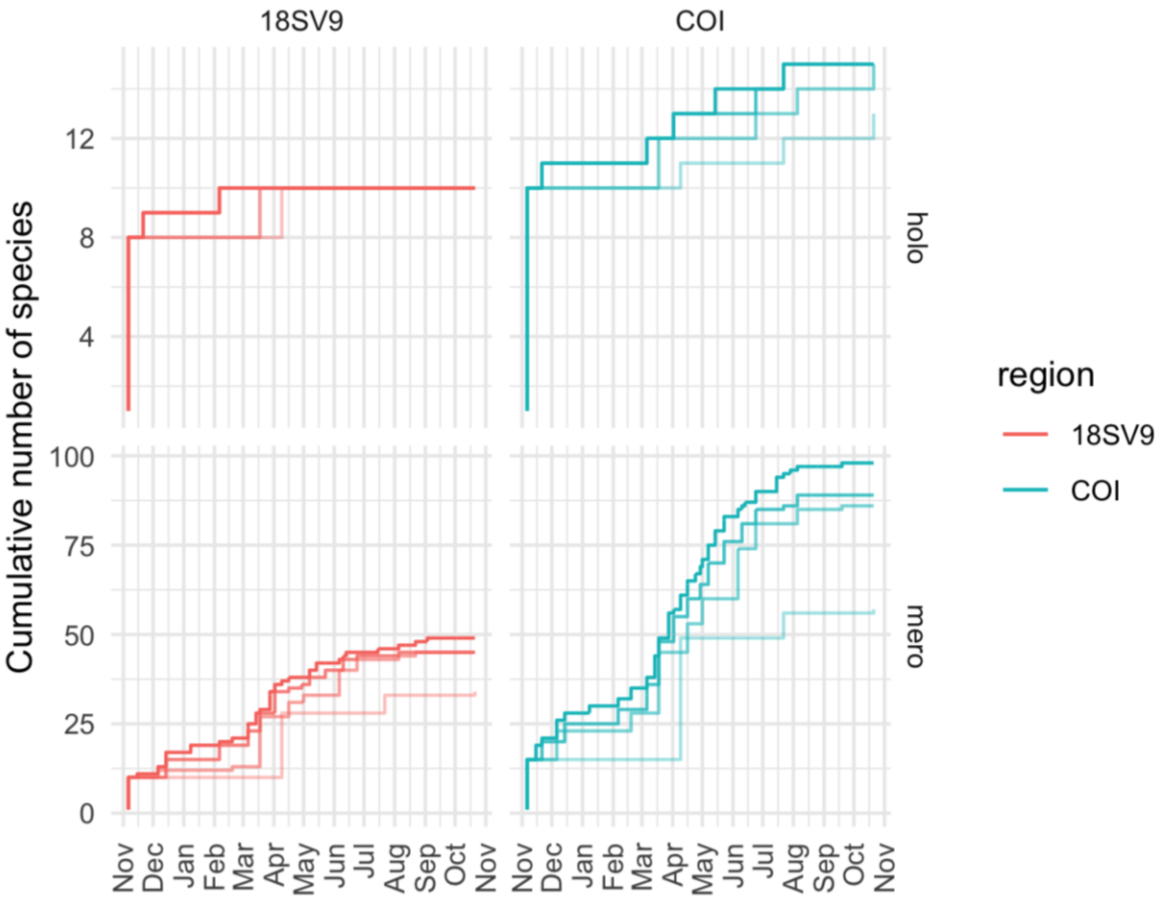


Figure 15: Species accumulation curves for holo- and meroplankton taxa for the two primer regions. The sampling frequency ranges from 43x (uppermost line, darkest colour), 20x, 12x to 4x (lowest line, lightest colour) per year.

3.4 Jellyfish biovolume

Jellyfish biovolume was variable throughout the year, ranging from undetectable, particularly in winter months, up to 75 ml biovolume m^{-3} (Fig.16A). The taxonomic composition was identified from images and the jellyfish sampled were mostly composed of *Mnemiopsis leidyi* and *Eucheilota maculata* (Fig. 16B).

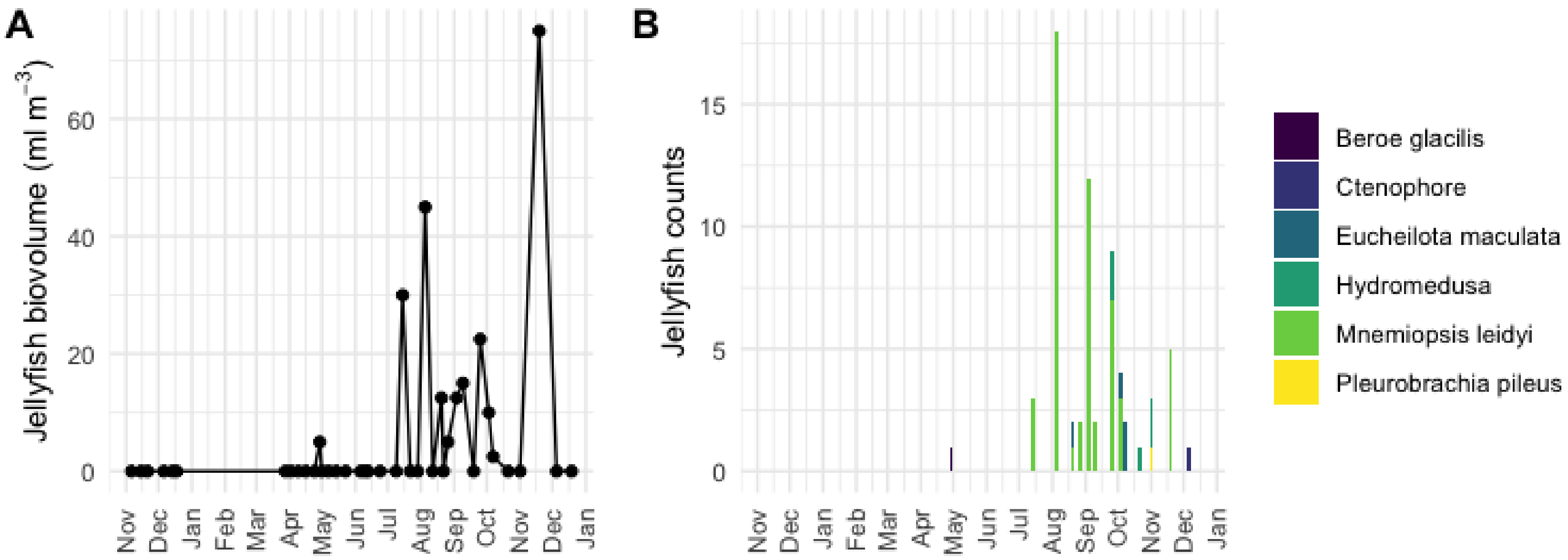


Figure 16: A) Jellyfish biovolume and B) jellyfish specimen counts at the Marsdiep.

3.5 Comparing data sets

Based on the lookup table aggregation, concentrations between the microscope and zooscan counts were made (Fig. 17). We plot values separate for each master class to ease comparison. Overall, we see that count for Appendicularia and Cnidaria compare reasonably well. However, most master classes have higher counts based on the zooscan (e.g. Copepod, Bipinnaria, Branchiopoda and 'Echinopluteus and Ophiopluteus') or based on microscopy (e.g. Cirripedia (nauplius), Harpacticoida, Nectochaeta and Veliger larvae). Several classes were counted by the zooscan but were missing from the microscopy counts, including Brachyura (zoea), Chaetognath, Cumacea and Gastropoda veliger. Of these classes, Brachyura (zoea), Chaetognath and Gastropoda veliger occurred in samples that were also inspected with microscopy, but Cumacea only occurred in samples that were analysed by the zooscan. As Copepod (exuvium) and *Noctiluca scintillans* were purposedly not counted under the microscope these were also missing from the microscope data set.

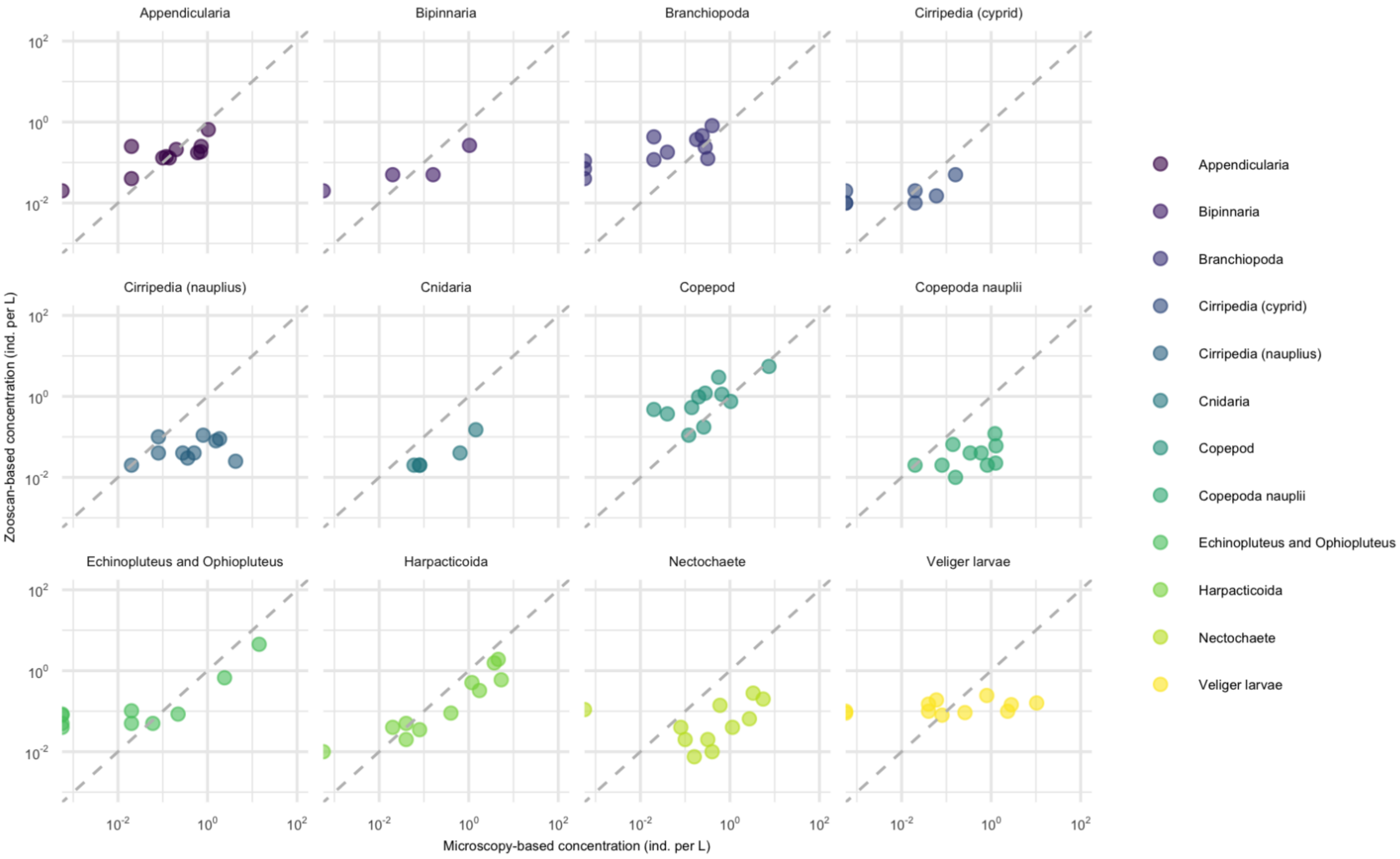


Figure 17: Comparison between the zooscan and microscopy counts. Note the log$_{10}$-scales for the x- and y-axis. Symbols 'sticking' to the y-axis imply that these were counted by the zooscan but were zero in the microscopy counts. The grey dashed line is the 1:1 relation.

4 Discussion

We present results of a high-resolution full year zooplankton sampling from the NIOZ-jetty in the Marsdiep based on sample analysis with microscopy, zooscan and metabarcoding. These data add to an important goal of the MONS program, which is to gain insight in the temporal abundance and diversity of zooplankton in the (coastal) Dutch North Sea. We here use these data to discuss and highlight the value of the three methods that were used using this year-round sampling and we evaluate the ecological implications of the observed temporal dynamics of zooplankton.

4.1 Method comparison

Three different methods were used to determine the temporal zooplankton dynamics at the Marsdiep. Due to time constraints, microscopy counts were done on 15 samples (n=1, i.e. monthly resolution) and 41 samples were analysed for metabarcoding (n=2 per sampling) and 37 sample dates for zooscan (n=2 per sampling).

The practical sampling procedure at the jetty has been optimized over time and now takes about 3 hours per sampling event by a technical assistant of the NIOZ department Coastal Systems (COS), who is typically accompanied by a student for safety reasons and help with the bucket sampling. We do not see expect that major improvements can be made here. Samples, also those fixed with formaldehyde, can be transport from NIOZ-Texel to WMR or NIOZ-Yerseke by regular package delivery services, which is cheap and efficient. The microscopic analysis was conducted by Nadine Peck (WMR) and this takes about 5 hours per sample. Nadine is a zooplankton expert, and no major improvements are to be expected anymore. The metabarcoding was done by WER and they deal with these samples on a routine basis, so we expect no major improvements in this pipeline either. The spin-up time of the zooscan analysis proved to be time-consuming, as a learning set needed to be developed and several rounds of training the EfficientNet18 classifier were needed. However, sample analysis, including scanning, segmentation and classification, is now limited to 1.5 - 2 hours per sample and many classes can be recognised. For future studies, we plan to extend the learning set refine the classes included. Based on their distinct shape, it should also be possible to define separate classes for the copepod *Temora longicornis* and cyclopoid copepods (e.g. *Oithona* spp.).

4.1.1 Microscopy

Microscopy counts show the typical zooplankton dynamics for the coastal North Sea (Fransz et al., 1991), with a strong increase and dominance of holoplankton, in particular Copepoda, in the spring months. Sample analysis is however time consuming (~5 hours per sample) and therefore time resolution and replication are more limited compared to zooscan and metabarcoding. Microscopic analysis allowed detecting most holoplankton species and especially the diversity of Copepoda.

4.1.2 Zooscan

A direct comparison of taxa abundance estimates between microscopy and zooscan shows that for some classes, both methods broadly agree with some systematic biases for various taxonomic classes. For example, Copepoda, Harpacticoida, Nectochaetes and Cirripedia (nauplius and cyprids) were consistently higher based on microscopy counts compared to the zooscan. At the same time several classes that were identified with the zooscan were missing in the microscope analysis, due to the lower temporal resolution and replication, which includes Brachyura (zoea), Chaetognath, Cumacea and Gastropoda veliger.

Part of the reason for the under-estimation zooplankton abundance by the zooscan can be due to clustering of organisms, which can be addressed by visual counting under the miscroscope but so far not by the Zooprocess method for the zooscans. Such clusters are classified as 'Multiples' and cannot be separated yet by machine learning approaches, but see a first approach on https://www.imagine-ai.eu/case-study/zooscan-ecotaxa-pipeline-taxonomic-identification-of-zooplankton-using-zooscan. In the frame of this MONS project, an MSc project was done by Maud Overbeek, which aimed to classify zooplankton specimens in such Multiples using instance segmentation and this showed promising results. We will extend this experimental approach and report on the results in future progress reports on the Marsdiep sampling.

4.1.3 Metabarcoding

The metabarcoding data revealed a substantially higher diversity in the zooplankton composition (>130 species) as compared to the microscopy (38 classes) and zooscan (20 classes) sample analysis. In addition, by far highest biodiversity was seen in the meroplankton, especially with the COI marker, while the diversity of meroplankton is equal (zooscan) or lower in the microscopy counts due to the difficult or impossible identification at high taxonomic resolution. The North Sea experiences a shift from a holoplankton to a meroplankton dominated zooplankton community (Holland et al., 2023; Kirby et al., 2008) and meroplankton is often underrepresented in ecological studies, so metabarcoding fills an important gap in understanding the phenology of the North Sea ecosystem.

In line with the recommendations from van der Loos and Nijland (2021) and Van Walraven et al. (2025), we used COI and the more conservative marker 18SV9 in our study. Van Walraven et al. (2025) showed that the marker 18SV9 detected more zooplankton taxa compared to 18SV4, so we prioritised the 18SV9 marker over the 18SV4 marker. Several zooplankton taxa, including the relevant Appendicularia and several polychaete species were only recorded with the 18SV9 marker.

Species accumulation curves proved to be a very useful method to investigate how metabarcoding sampling frequency influences the species diversity that will be detected. A reduction from the present ±42x per year to 20x is not expected to reduce species detection for holoplankton and around 10-15% for meroplankton. Of course, any reduction in sampling frequency will reduce the ability to follow holo- and meroplankton phenology. Corona et al. (2024) recently analysed zooplankton phenology in the Celtic Sea and Northern North Sea for which time series with a 1-3x per week sampling frequency was used. Given that analysis costs of DNA metabarcoding samples does not increase linearly with sample number, we advise to keep the sampling frequency at least at 20x per year for biodiversity investigations and at ±42x per year for phenological analysis.

4.2 Ecological implications

The nutrient status and phytoplankton dynamics have been researched extensively at the NIOZ jetty and has provided insights in control of nutrient inputs on lower trophic level dynamics of a coastal ecosystem (Philippart et al., 2007). Overall, the chlorophyll-a concentration at the Marsdiep station is characterised by a distinct spring and autumn peak (Philippart et al., 2010). Decadal time series analysis by these authors showed that the timing and magnitude of the spring bloom has not changed since the 1970s, but the autumn bloom ends sooner with repercussions of the length of the growth season. Despite seasonality in the chlorophyll-a concentration, the composition of the phytoplankton community has remained stable after 1988, with a dominance of the diatoms *Leptocylindrus minimus* and small *Thalassiosira* (Philippart et al., 2000). Many of these dynamics are thought to be controlled by riverine inputs of N and P that have changed considerably over the last decades. Currently, primary productivity and phytoplankton community composition are thought to be primarily controlled by the P-limited conditions (i.e. high N:P ratios) following the reduction in P loads to the coastal zone with riverine inputs at the end of the 1980s and early 1990s (Philippart et al., 2007). Higher trophic level ecosystem components such as macrobenthos and estuarine birds were weakly correlated with this decline in nutrient reduction (Philippart et al., 2007) and have been associated with a change in its nursery function (Van Der Veer et al., 2022). For example, the biomass and filtration capacity of bivalves in the western Wadden Sea has decreased fourfold from ±0.15 m^3 m^{-2} d^{-1} at the end of the 1980s to 0.04 m^3 m^{-2} d^{-1}, with an average water depth of 3.3m (Philippart et al., 2000), this amounts to 0.012 m^3 m^{-3} d^{-1} in 2000 (Philippart et al., 2007). The role of zooplankton in grazing of phytoplankton (Philippart et al., 2010) and food web dynamics (Maathuis, 2025) is unclear due to a lack of sampling of these groups. We believe that the monitoring data of the Marsdiep can be used to assess this impact in the future.

Microscope counts show typical zooplankton dynamics for the Wadden Sea, with a strong increase and dominance of holoplankton, especially copepods, in the spring months. These dynamics deviate somewhat from the generalized schematic proposed by Jak and Slijkerman (2023). These authors suggest that holo- and meroplankton increase in roughly the same proportion in spring and peak in May / June after which zooplankton abundance steadily declines towards the winter months. Our results however show that zooplankton peaks already in April and May with a clear dominance of meroplankton. Both holo- and meroplankton have a relatively low abundance in summer, and holoplankton shows an autumn peak in October and even into November. The autumn zooplankton peak is likely triggered by the autumn bloom of chlorophyll-a, which is a known phenomenon in the Wadden Sea (Philippart et al., 2010). Recently, Maathuis et al. (2024) showed that the small pelagic fish in the Wadden Sea also feed on meroplankton, emphasizing the role that meroplankton plays in the coastal North Sea / Wadden Sea food web.

An extensive review by Fransz et al. (1991) showed that total copepod abundance in the eastern North Sea peaks in June/July at an abundance 0.6 – 0.8 copepods L^{-1}. While this peak is lower and later than our results show, there are methodological explanations for these differences. Fransz et al. (1991) used data from the Continuous Plankton Recorder (CPR) for their analysis, which collects data further offshore and with a mesh size of 270 µm. Our sampling was done with a mesh size of 150 µm which collects the more abundant smaller copepods and our sampling site is under influence of the shallower (so warmer) and more nutrient-rich Wadden Sea. However, Fransz et al. (1992) specifically measured the temporal dynamics of the copepod *Temora longicornis* in the periods 1973 to 1991 (data availability: 1973-1978, 1983 and 1990-1991) at the Marsdiep and found abundances of the adult stage to increase from ±0.5 ind. L^{-1} (1973-1976), 1-2 ind. L^{-1} (1977-1976) to 2-3 ind. L^{-1} (1990-1991). They concluded that eutrophication stimulated the abundance of *T. longicornis*. Our metabarcoding data show year-round presence of *T. longicornis*, but, apart from a highest abundance of 4 ind. L^{-1} in May, abundances based on microscopic counts were substantially lower at <0.2 ind. L^{-1}, so nutrient reduction may have reduced the occurrence of this copepod species in present day compared to 3 decades ago.

Van Walraven (2016) studied the gelatinous fraction of the zooplankton extensively in 2009 to assess the effect of the invasive species *Mnemiopsis leidyi* on the Wadden Sea ecosystem. Our jellyfish biovolume, dominated by *Mnemiopsis leidyi* are comparable to the results from Van Walraven (2016). However, it must be considered that our sampling volume (4x50=200 L for jellyfish) is likely too low for an accurate sampling of large gelatinous zooplankton abundance such as *Mnemiopsis leidyi*, which means that higher sampling volumes may reveal that their abundance has increased in the last 10 years.

The tubeworm *Lanice conchilega* forms extensive reefs in the sub- and intertidal zone in the Wadden Sea (Ricklefs et al., 2022) and these reefs are known for disproportionally high organic carbon processing rates (De Smet et al., 2016). Both the COI and 18SV9 marker regions show that *Lanice conchilega* has 2 to 3 peaks in reproduction. The first reproduction peak is in March/April, potentially also one in July and another one in September/October.

The metabarcoding data also detected temporal dynamics of several invasive species in the North Sea / Wadden Sea. For example, the invasive calanoid copepod *Pseudodiaptomus marinus* (also present in the microscopy counts) and cyclopoid *Oithona davisae* (possibly in the *Oithona* spp. counts) were detected. Surprisingly, both *Pseudodiaptomus marinus* and *Oithona davisae* showed clear abundance at the end of 2023, but COI-reads (both species are not detected by 18SV9) in 2024 were very low or absent in 2024. Microscopic counts of *Pseudodiaptomus marinus* confirmed their near absence in 2024, so we conclude that both invasive species may not have found a dominant and lasting niche in the coastal North Sea / Wadden Sea food web. DNA of other exotic species was detected, such as of the polychaete *Polydora onagawaensis*, a species that drills in oyster shells, the polychaete *Marenzelleria neglecta* and the bivalves *Mulinia lateralis* and *Ruditapes philippinarum*.

4.3 Advise for the Marsdiep monitoring in context of the MONS program

The Marsdiep sampling site can play an important role in the OSPAR sampling regions as it is located in an ‘ecotype’ of coastal regions with variable salinity due to river plumes, which is not yet covered by the OSPAR time series (Louchart et al., 2023; OSPAR, 2023). Philippart et al. (2010) claims that even with a sampling frequency of 60x per year it is challenging to establish the wax and wane of chlorophyll-a at this coastal site due to high growth rates. The sampling sites of the Dutch monitoring for the North Sea (MWTL sites) are sampled at most 19x per year. So, for both the MONS and OSPAR programs, the NIOZ jetty provides a unique opportunity to study the phenology of holo- and meroplankton at high temporal resolution for North Sea coastal plankton. Moreover, further integration of the MONS sampling program integration with the ongoing NIOZ-sampling, which targets nutrients, phytoplankton and fish (i.e. through the NIOZ Fyke program), will allow to address the role of zooplankton in relation to other components of the marine food web.

We employed three different methods to follow up the zooplankton dynamics. Of these, it is strongly advised to retain the DNA metabarcoding analysis. The temporal dynamics for meroplankton, a typically under-sampled and -appreciated group of zooplankton were very revealing. Species diversity was dominated strongly by meroplankton, and this was missed by both microscopy and zooscan. Also, the DNA metabarcoding showed interesting dynamics for the several invasive species that are otherwise challenging to obtain. Given that analysis of DNA metabarcoding samples does not increase linearly with sample number, we advise to keep the sampling frequency at least at 20x per year for biodiversity and invasive species investigations and at ±42x per year for phenological analysis. Like the advice in Van Walraven et al. (2025), we advise to use at least the markers COI and 18SV9. The marker COI clearly covers most species diversity, but some important species, like *Fritillaria borealis, Oikopleura dioica, Alitta succinea, Heteromastus filiformis, Crassostrea* and *Sabellaria* and only recorded with 18SV9.

It is at this moment challenging to advise on whether to include zooscan and / or microscopy as methodology in the sampling strategy and in what frequency in the MONS program. The zooscan method proved to be time-consuming in the initial phase. With the continued development of the learning set and machine-learning method to also count and classify the so-called clusters, we believe that especially the zooscan will be particularly useful for plankton size spectrum and biomass (not yet pursued in this report) analysis. As microscopy remains the only method to conclusively establish the presence of a species, we advise to continue the collection of formaldehyde-fixed samples for both zooscan and microscopic analysis. The present sampling scheme will continue for another two years (ending in 2026), but we advise to investigate on the full three-year time series whether zooscan analysis can be synchronized at the lower temporal frequency of the microscopic analysis.

The pre-screening of the zooplankton samples with a 2-mm mesh was very successful in keeping jellyfish out of the formaldehyde-fixed samples, where their tissue often breaks up and makes sample sorting cumbersome. Hence, we advise to continue this pre-screening for enhanced sample quality. The subsequent jellyfish biovolume estimation and photography, however, takes up time and it is unclear whether the sampling volume is sufficient to provide a good estimate of the jellyfish biovolume. As these limited data indicate that jellyfish are abundant and the biovolume has potentially increased in the last decade, we advise to continue the present methodology. We also advise to evaluate after three years whether jellyfish sampling should be done at a lower frequency, but with a more targeted sampling method (e.g. larger net from a small vessel), focused on summer and autumn when their abundance is highest.

5 Conclusions

The combination of microscopy, zooscan analysis and metabarcoding revealed detailed insight in the zooplankton dynamics in the Wadden Sea. Holo-, but especially meroplankton abundance increases quickly in spring and are lower in summer, after which especially holoplankton shows a peak in autumn. Meroplankton dominates overall species diversity and strongly increases in spring and remains high over the summer. Meroplankton phenology is highly variable as species show great variation in the timing and number of reproduction periods. The dominance of meroplankton abundance and diversity suggests an important role in food web dynamics. We conclude that the unique positioning the NIOZ jetty sampling site will play a valuable role in the MONS project and for KRM (Kader Richtlijn Marien) / OSPAR assessments.

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7 References

Asjes, J., Merkus, H., Bos, O.G., Steenbergen, J., Stuijfzand, S., van Splunder, I., van Kooten, T., Rivero, S., Vis, G.A.J., 2021. Monitoring en Onderzoek Natuurversterking en Soortenbescherming (MONS). Definitieve versie: 4 oktober 2021.

Corona, S., Hirst, A.G., Atkinson, D., Renz, J., Boersma, M., Atkinson, A., 2024. Long-term shifts in phenology, thermal niche, population size, and their interactions in marine pelagic copepods. Limnol. Oceanogr. lno.12499. https://doi.org/10.1002/lno.12499

De Smet, B., van Oevelen, D., Vincx, M., Vanaverbeke, J., Soetaert, K., 2016. Lanice conchilega structures carbon flows in soft-bottom intertidal areas. Mar. Ecol. Prog. Ser. 552, 47–60.

Fransz, H.G., Colebrook, J.M., Gamble, J.C., Krause, M., 1991. The zooplankton of the north sea. Neth. J. Sea Res. 28, 1–52. https://doi.org/10.1016/0077-7579(91)90003-J

Fransz, H.G., Gonzalez, S.R., Cadée, G.C., Hansen, F.C., 1992. Long-term change of Temora longicornis (copepoda, Calanoida) abundance in a dutch tidal inlet (marsdiep) in relation to eutrophication. Neth. J. Sea Res. 30, 23–32. https://doi.org/10.1016/0077-7579(92)90042-D

Gorsky, G., Ohman, M.D., Picheral, M., Gasparini, S., Stemmann, L., Romagnan, J.-B., Cawood, A., Pesant, S., Garcia-Comas, C., Prejger, F., 2010. Digital zooplankton image analysis using the ZooScan integrated system. J. Plankton Res. 32, 285–303. https://doi.org/10.1093/plankt/fbp124

Holland, M.M., Louchart, A., Artigas, L.F., Ostle, C., Atkinson, A., Rombouts, I., Graves, C.A., Devlin, M., Heyden, B., Machairopoulou, M., Bresnan, E., Schilder, J., Jakobsen, H.H., Llody-Hartley, H., Tett, P., Best, M., Goberville, E., McQuatters-Gollop, A., 2023. Major declines in NE Atlantic plankton contrast with more stable populations in the rapidly warming North Sea. Sci. Total Environ. 898. https://doi.org/10.1016/j.scitotenv.2023.165505

Hovenkamp, P., Van Walraven, L., Ollevier, A., Van Oevelen, D., Van der Stappen, F., In review. Optimising automated classification for zooxoplankton in coastal conditions: impact of model selection, imaging instruments, and colour information.

Jak, R.G., Slijkerman, D.M.E., 2023. Short review on zooplankton in the Dutch Wadden Sea : considerations for zooplankton monitoring. Wageningen Marine Research, Den Helder. https://doi.org/10.18174/586428

Jocher, G., Qiu, J., 2024. Ultralytics yolo11.

Kirby, R.R., Beaugrand, G., Lindley, J.A., 2008. Climate-induced effects on the meroplankton and the benthic-pelagic ecology of the North Sea. Limnol. Oceanogr. 53, 1805–1815. https://doi.org/10.4319/lo.2008.53.5.1805

Louchart, A., Holland, M.M., McQuatters-Gollop, A., Artigas, L.F., 2023. Changes in Phytoplankton Biomass and Zooplankton Abundance. In: OSPAR, 2023: The 2023 Quality Status Report for the Northeast Atlantic. OSPAR Commission, London.

Ly, J., Philippart, C.J.M., Kromkamp, J.C., 2014. Phosphorus limitation during a phytoplankton spring bloom in the western Dutch Wadden Sea. J. Sea Res. 88, 109–120. https://doi.org/10.1016/j.seares.2013.12.010

Maathuis, M.A.M., 2025. Pelagic fish in shallow waters. Wageningen University & Research.

Maathuis, M.A.M., Tulp, I., Valk, S., Van Den Brink, X., Couperus, A.S., Keur, M.C., Nijland, R., Sakinan, S., van der Vorst, V., Poos, J.J., 2024. Small pelagic fish in the shallow Wadden Sea show opportunistic feeding with a strong benthic link. ICES J. Mar. Sci. 81, 1521–1535. https://doi.org/10.1093/icesjms/fsae096

OSPAR, 2023. Pelagic Habitats Thematic Assessment. In: OSPAR, 2023: Quality Status Report 2023. OSPAR Commission, London.

Philippart, C.J.M., Beukema, J.J., Cadée, G.C., Dekker, R., Goedhart, P.W., Van Iperen, J.M., Leopold, M.F., Herman, P.M.J., 2007. Impacts of Nutrient Reduction on Coastal Communities. Ecosystems 10, 96–119. https://doi.org/10.1007/s10021-006-9006-7

Philippart, C.J.M., Cadée, G.C., Van Raaphorst, W., Riegman, R., 2000. Long-term phytoplankton-nutrient interactions in a shallow coastal sea: Algalcommunity structure, nutrient budgets, and denitrification potential. Limnol. Oceanogr. 45, 131–144. https://doi.org/10.4319/lo.2000.45.1.0131

Philippart, C.J.M., Van Bleijswijk, J.D.L., Kromkamp, J.C., Zuur, A.F., Herman, P.M.J., 2014. Reproductive phenology of coastal marine bivalves in a seasonal environment. J. Plankton Res. 36, 1512–1527. https://doi.org/10.1093/plankt/fbu073

Philippart, C.J.M., Van Iperen, J.M., Cadée, G.C., Zuur, A.F., 2010. Long-term Field Observations on Seasonality in Chlorophyll-a Concentrations in a Shallow Coastal Marine Ecosystem, the Wadden Sea. Estuaries Coasts 33, 286–294. https://doi.org/10.1007/s12237-009-9236-y

Ricklefs, K., Franken, O., Glorius, S., Mascioli, Francesco, Nielsen, P., Reimers, H.-C., Trampe, A., Franken, O., Glorius, Sander, Mascioli, F., Nielsen, P., Reimers, H.-C., Trampe, A., 2022. Wadden Sea Quality Status Report: Subtidal habitats. Common Wadden Sea Secretariat. https://doi.org/10.5281/ZENODO.15209437

Van Der Loos, L.M., Nijland, R., 2021. Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. Mol. Ecol. 30, 3270–3288. https://doi.org/10.1111/mec.15592

Van Der Veer, H., Tulp, I., Witte, J., Poiesz, S., Bolle, L., 2022. Changes in functioning of the largest coastal North Sea flatfish nursery, the Wadden Sea, over the past half century. Mar. Ecol. Prog. Ser. 693, 183–201. https://doi.org/10.3354/meps14082

Van Walraven, L., 2016. Flexible filter feeders: The gelatinous zooplankton community in the Netherlands after the invasion of the ctenophore Mnemiopsis leidyi. University of Groningen.

Van Walraven, L., Hoekendijk, J.P.A., Jak, R.G., Keur, M.C., Peck, N., Polling, M., Van Dalen, J., Van Oevelen, D., 2025. Development of integrated zooplankton monitoring for the Dutch North Sea within the MONS project - ID14 MONS Monitoring Zooplankton Phase 1 (No. C013/25). Wageningen University.

Appendix 1: Summary of microscopic counts

Taxonomic class	Total microscopic counts	No. days that class was counted
Acartia clausi	3	2
Acartia spp.	5	4
Acartia tonsa	25	9
Amphipoda	3	2
Appendicularia	116	24
Appendicularia (head)	23	12
Appendicularia (tail)	15	12
Aulophore	53	8
Aulophore (no tube)	26	7
Bipinnaria	17	4
Branchiolaria	8	2
Calanoida indet.	4	4
Caprellidae	2	2
Centropages hamatus	7	4
Centropages typicus	2	2
Chaetognata	5	3
Cirripedia (cyprid)	11	7
Cirripedia (nauplius)	480	22
Copepoda nauplii	331	22
Copepodites	459	27
Cumacea	2	2
Cyclopoida indet.	258	19
Cyphonautes	3	3
Echinopluteus	196	6
Eurytemora affinis	3	3
Harpacticoida indet.	906	25
Hydromedusa	44	13
Hydrozoa	27	8
Megalopa	6	5
Mitraria	35	5
Nectochaete	395	23
Nectochaete Magelona spp.	8	4
Nematoda	7	3
Ophiopluteus	28	4
Paracalanus parvus	54	9
Paracalanus spp.	9	3
Penilla avirostris	17	7
Podon/Pleopis	27	6
Pseudocalanus elongatus	5	2
Pseudodiaptomus marinus	13	8
Temora longicornis	65	10
Veliger larva	440	24

Appendix 2: Summary of metabarcoding data

Taxa	Primer region	Presence (% of sampling days)
Abra alba	COI	83.7
Acartia bifilosa	18SV9	95.3
Acartia bifilosa	COI	81.4
Acartia clausii	18SV9	111.6
Acartia hudsonica/tonsa	COI	37.2
Acartia tonsa	18SV9	100.0
Acartia tonsa	COI	100.0
Actiniaria	18SV9	118.6
Actiniaria	COI	2.3
Aeolidia papillosa	COI	7.0
Alcyonidioides mytili	18SV9	34.9
Alitta succinea	COI	30.2
Alitta succinea/virens	18SV9	25.6
Alitta virens	COI	25.6
Ammodytes tobianus	COI	9.3
Ammodytidae	18SV9	4.7
Ampharete finmarchica	18SV9	4.7
Amphiascopsis cinctus	COI	14.0
Amphibalanus improvisus	COI	130.2
Aora gracilis	18SV9	2.3
Aora gracilis	COI	2.3
Arenicola defodiens	COI	30.2
Arenicola marina	18SV9	53.5
Arenicola marina	COI	23.3
Asterias rubens	18SV9	74.4
Asterias rubens	COI	81.4
Aurelia aurita	18SV9	11.6
Aurelia aurita	COI	18.6
Austrominius modestus	COI	148.8
Balanomorpha	18SV9	181.4
Balanus glandula	COI	114.0
Barnea candida	18SV9	44.2
Barnea candida	COI	41.9
Bathycoccus prasinos	18SV9	4.7
Bathyporeia elegans	COI	2.3
Beroe sp.	COI	48.8
Botrylloides violaceus	18SV9	2.3
Botrylloides violaceus	COI	2.3
Calanus helgolandicus	COI	79.1
Cancer pagurus	COI	2.3
Canuella perplexa	18SV9	44.2
Canuella perplexa	COI	39.5
Caprella equilibra	COI	27.9
Caprella mutica	COI	7.0

Taxa	Primer region	Presence (% of sampling days)
Carcinus maenas	COI	65.1
Centropages	18SV9	118.6
Centropages hamatus	COI	111.6
Centropages typicus	COI	20.9
Cerastoderma edule	18SV9	62.8
Cerastoderma edule	COI	65.1
Chaetoceros socialis	18SV9	158.1
Chamelea striatula	COI	2.3
Chrysaora	18SV9	4.7
Chrysaora hysoscella	COI	18.6
Chydorus sphaericus	18SV9	7.0
Chydorus sphaericus	COI	9.3
Ciliata mustela	COI	2.3
Clupea harengus	COI	16.3
Clytia hemisphaerica	18SV9	111.6
Clytia hemisphaerica	COI	114.0
Clytia languida/gracilis	COI	100.0
Crangon crangon	18SV9	23.3
Crangon crangon	COI	27.9
Crepidula fornicata	COI	16.3
Cuthona nana	18SV9	7.0
Cuthonella concinna	COI	7.0
Cyanea lamarckii	18SV9	44.2
Cyanea lamarckii	COI	25.6
Cylista troglodytes	COI	125.6
Dendronotus frondosus	COI	2.3
Didemnum vexillum	COI	0.0
Dipolydora	18SV9	120.9
Ditrichocorycaeus anglicus	COI	11.6
Donax vittatus	COI	7.0
Dulichia falcata	COI	2.3
Dynamena pumila	COI	23.3
Echinocardium cordatum	18SV9	81.4
Echinocardium cordatum	COI	93.0
Ectocarpus fasciculatus	COI	30.2
Ectocarpus siliculosus	18SV9	4.7
Ectocarpus siliculosus	COI	16.3
Ectopleura dumortierii	18SV9	4.7
Ectopleura dumortierii	COI	2.3
Electra pilosa	18SV9	32.6
Electra pilosa	COI	51.2
Emplectonema gracile	18SV9	7.0
Emplectonema gracile	COI	4.7
Ensis directus/leei	COI	167.4
Ensis siliqua	COI	7.0
Eriocheir sinensis	18SV9	53.5

Taxa	Primer region	Presence (% of sampling days)
Eteone flava	COI	25.6
Eteone longa	18SV9	2.3
Eteone longa	COI	11.6
Eubranchus exiguus	COI	39.5
Eucheilota maculata	18SV9	79.1
Eucheilota maculata	COI	79.1
Eulalia viridis	18SV9	16.3
Eunereis longissima	COI	53.5
Euterpina acutifrons	18SV9	134.9
Euterpina acutifrons	COI	116.3
Eutonina indicans	18SV9	7.0
Eutonina indicans	COI	0.0
Evadne nordmanni	18SV9	30.2
Evadne nordmanni	COI	34.9
Evansula pygmaea	COI	7.0
Fabricia stellaris	COI	2.3
Fabulina fabula	COI	55.8
Facelina bostoniensis	18SV9	2.3
Facelina bostoniensis	COI	2.3
Fibrocapsa japonica	18SV9	69.8
Fibrocapsa japonica	COI	76.7
Fritillaria borealis	18SV9	88.4
Gammarus	18SV9	7.0
Gammarus crinicornis	COI	2.3
Gammarus locusta	COI	2.3
Gastrosaccus spinifer	18SV9	11.6
Gastrosaccus spinifer	COI	11.6
Gonothyraea loveni	18SV9	46.5
Gonothyraea loveni	COI	58.1
Halichondria panicea	COI	11.6
Haliclona	COI	23.3
Hemigrapsus sanguineus	COI	25.6
Hemigrapsus takanoi	COI	39.5
Heteromastus filiformis	18SV9	53.5
Hysterothylacium aduncum	COI	4.7
Jassa marmorata	COI	16.3
Kurtiella	18SV9	16.3
Kurtiella bidentata	COI	16.3
Labidocera wollastoni	COI	4.7
Lanice conchilega	18SV9	165.1
Lanice conchilega	COI	167.4
Laomedea flexuosa	COI	2.3
Leptastacus aff. laticaudatus	COI	4.7
Lernaeenicus sprattae	COI	2.3
Leuckartiara octona	18SV9	14.0
Leuckartiara octona	COI	11.6

Taxa	Primer region	Presence (% of sampling days)
Leucocryptos marina	COI	4.7
Limanda limanda	COI	9.3
Liocarcinus depurator	18SV9	2.3
Liocarcinus holsatus	COI	7.0
Lipophrys pholis	COI	9.3
Littorina littorea	COI	123.3
Lizzia blondina	18SV9	41.9
Lizzia blondina	COI	11.6
Loimia ramzega	COI	30.2
Lutraria lutraria	COI	79.1
Macoma balthica	COI	37.2
Macomangulus tenuis	COI	7.0
Mactra stultorum	COI	9.3
Mactridae	18SV9	118.6
Magallana/Crassostrea	18SV9	72.1
Magelona filiformis	COI	9.3
Magelona johnstoni	COI	69.8
Magelona mirabilis	18SV9	23.3
Magelona mirabilis	COI	23.3
Malmgrenia lunulata	COI	32.6
Marenzelleria viridis	COI	30.2
Margelopsis haeckelii	18SV9	23.3
Margelopsis haeckelii	COI	25.6
Metridium senile	COI	14.0
Microphthalmus listensis	COI	2.3
Microphthalmus similis	COI	16.3
Microprotopus maculatus	COI	18.6
Mnemiopsis leidyi	COI	90.7
Monocorophium acherusicum	COI	37.2
Monopseudocuma gilsoni	COI	4.7
Mulinia lateralis	COI	4.7
Mya arenaria	18SV9	95.3
Mya arenaria	COI	65.1
Mycale	COI	2.3
Mytilus	18SV9	144.2
Mytilus sp.	COI	144.2
Necora puber	COI	2.3
Nemopsis bachei	COI	48.8
Nephtys assimilis	COI	11.6
Nephtys cirrosa	COI	30.2
Nephtys hombergii	COI	20.9
Noctiluca scintillans	18SV9	179.1
Noctiluca scintillans	COI	125.6
Nototropis swammerdamei	18SV9	2.3
Nototropis swammerdamei	COI	2.3
Obelia bidentata	COI	90.7

Taxa	Primer region	Presence (% of sampling days)
Obelia dichotoma	COI	95.3
Obelia dichotoma/geniculata	18SV9	114.0
Obelia longissima	COI	41.9
Oerstedia dorsalis	COI	9.3
Oikopleura dioica	18SV9	172.1
Oithona	18SV9	7.0
Oithona davisae	18SV9	60.5
Oithona davisae	COI	58.1
Oncaea	18SV9	2.3
Onchidoris bilamellata	COI	2.3
Ophiocten affinis	COI	2.3
Ophiothrix fragilis	COI	23.3
Ophiura albida	COI	107.0
Ophiura ophiura	COI	100.0
Ophiuroidea	18SV9	127.9
Orchestia mediterranea	COI	2.3
Owenia fusiformis	18SV9	116.3
Owenia fusiformis	COI	118.6
Pagurus bernhardus	COI	2.3
Paracalanus parvus	18SV9	148.8
Paracalanus parvus	COI	148.8
Paraleptastacus espinulatus	COI	2.3
Paramunna bilobata	COI	2.3
Paraonis fulgens	COI	2.3
Parasagitta setosa	COI	55.8
Pariambus typicus	COI	4.7
Pectinaria koreni	18SV9	60.5
Pectinaria koreni	COI	39.5
Peringia ulvae	18SV9	55.8
Peringia ulvae	COI	55.8
Petricolaria pholadiformis	18SV9	62.8
Petricolaria pholadiformis	COI	69.8
Phaeocystis globosa	COI	137.2
Phaxas pellucidus	18SV9	2.3
Phyllodoce groenlandica/mucosa	COI	30.2
Phyllodoce rosea	COI	7.0
Pleopis polyphemoides	COI	53.5
Pleurobrachia pileus	18SV9	46.5
Pleurobrachia pileus	COI	55.8
Podon intermedius	COI	2.3
Polydora cornuta	COI	134.9
Protodrilus oculifer	18SV9	2.3
Psammechinus miliaris	18SV9	39.5
Psammechinus miliaris	COI	44.2
Pseudo-nitzschia delicatissima	18SV9	60.5
Pseudocalanus elongatus	18SV9	95.3

Taxa	Primer region	Presence (% of sampling days)
Pseudocalanus elongatus	COI	93.0
Pseudochattonella verruculosa	COI	14.0
Pseudodiaptomus marinus	18SV9	83.7
Pseudodiaptomus marinus	COI	74.4
Pygospio elegans	18SV9	111.6
Pygospio elegans	COI	114.0
Pylaiella littoralis	18SV9	23.3
Pylaiella littoralis	COI	23.3
Rhizosolenia setigera	COI	174.4
Rhizostoma octopus	COI	25.6
Rhizostoma pulmo	18SV9	51.2
Ruditapes philippinarum	18SV9	16.3
Ruditapes philippinarum	COI	20.9
Sabellaria	18SV9	23.3
Sabellaria spinulosa	COI	20.9
Sacculina carcini	COI	4.7
Sardina pilchardus	COI	2.3
Schistomysis kervillei	18SV9	2.3
Schistomysis kervillei/ornata	COI	2.3
Scolelepis	18SV9	53.5
Scolelepis bonnieri	COI	53.5
Scolelepis neglecta	COI	20.9
Scolelepis squamata	COI	60.5
Scoloplos cf. armiger 'intertidal clade'	18SV9	9.3
Scoloplos cf. armiger 'subtidal clade'	18SV9	4.7
Scrobicularia plana	COI	7.0
Semibalanus balanoides	COI	51.2
Sigalion mathildae	COI	2.3
Skeletonema dohrnii	COI	48.8
Spio decorata	COI	53.5
Spio symphyta	COI	93.0
Spiophanes bombyx	COI	83.7
Spisula solida	COI	9.3
Spisula subtruncata	COI	95.3
Sprattus sprattus	18SV9	4.7
Sprattus sprattus	COI	7.0
Sthenelais boa	COI	2.3
Synchaeta grimpei	18SV9	32.6
Synchaeta grimpei	COI	62.8
Tellimya ferruginosa	COI	20.9
Temora longicornis	COI	141.9
Tergipes tergipes	18SV9	62.8
Tergipes tergipes	COI	58.1
Terschellingia longicaudata	COI	2.3
Trisopterus luscus	COI	7.0
Tubularia indivisa	COI	48.8

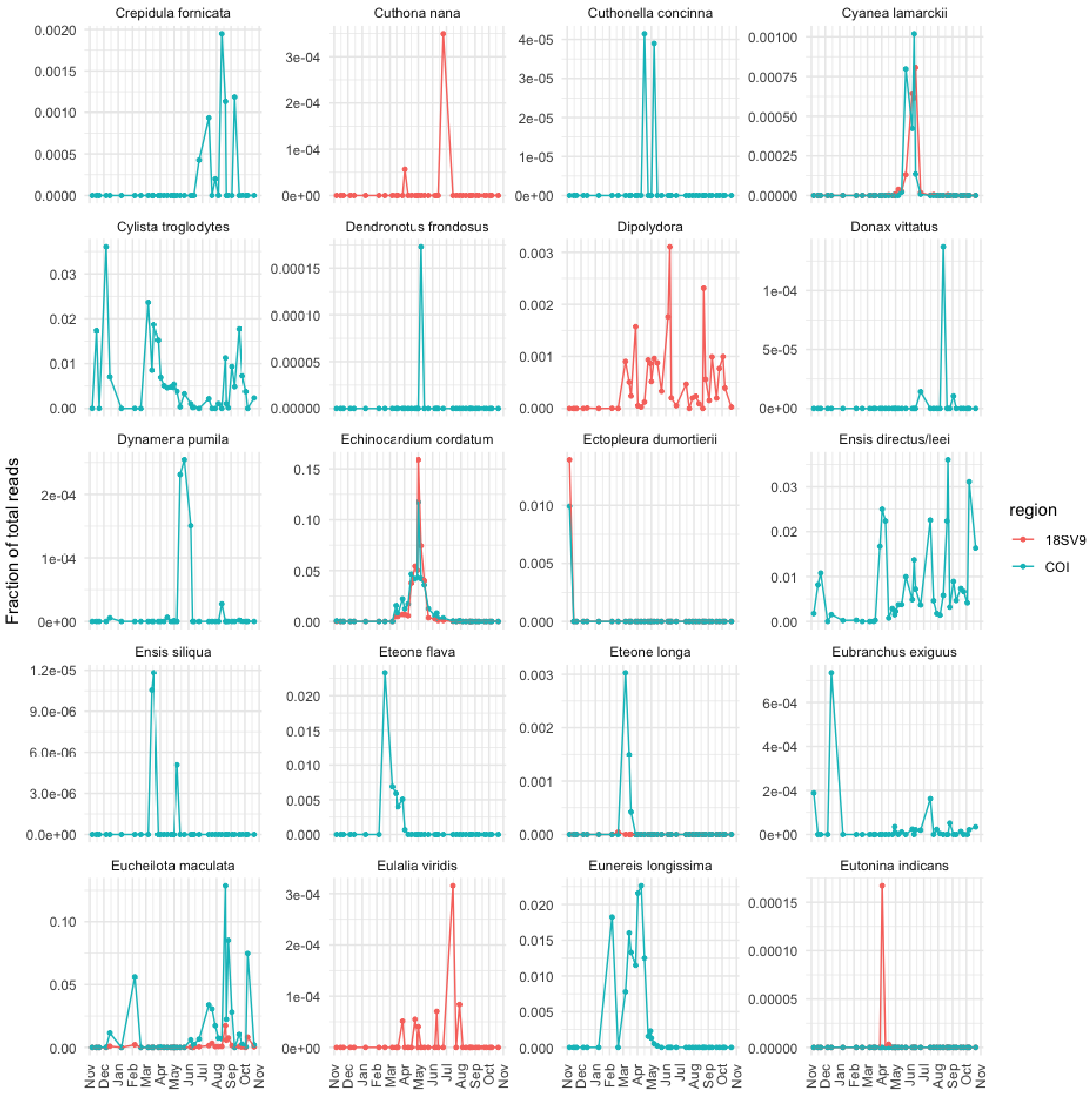
Taxa	Primer region	Presence (% of sampling days)
Veneridae	18SV9	32.6
Venerupis corrugata	COI	30.2
Verruca stroemia	COI	23.3

Appendix 3: Temporal dynamics based on metabarcoding data

Temporal dynamics of each species recorded with both primer regions split between holoplankton (first page) and meroplankton (next pages). Only the mean is show to improve readability.

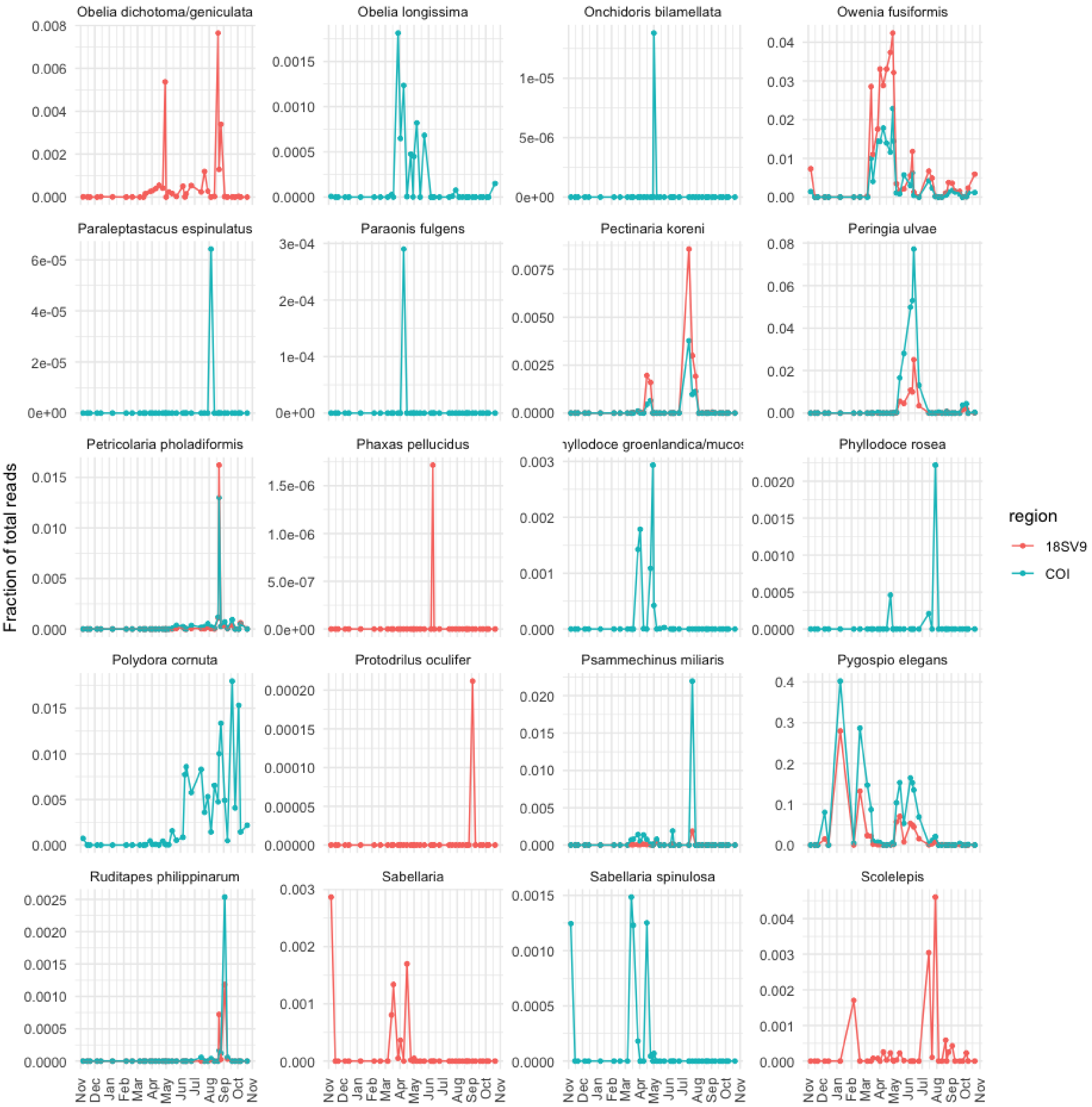
Holoplankton

Meroplankton


Crepidula fornicata
0.0020
0.0015
0.0010
0.0005
0.0000
Cuthona nana
3e-04
2e-04
1e-04
0e+00
Cuthonella concinna
4e-05
3e-05
2e-05
1e-05
0e+00
Cyanea lamarckii
0.00100
0.00075
0.00050
0.00025
0.00000
Cylista troglodytes
0.03
0.02
0.01
0.00
Dendronotus frondosus
0.00015
0.00010
0.00005
0.00000
Dipolydora
0.003
0.002
0.001
0.000
Donax vittatus
1e-04
5e-05
0e+00
Fraction of total reads
Dynamena pumila
2e-04
1e-04
0e+00
Echinocardium cordatum
0.15
0.10
0.05
0.00
Ectopleura dumortierii
0.010
0.005
0.000
Ensis directus/leei
0.03
0.02
0.01
0.00
region
18SV9
COI
Ensis siliqua
1.2e-05
9.0e-06
6.0e-06
3.0e-06
0.0e+00
Eteone flava
0.020
0.015
0.010
0.005
0.000
Eteone longa
0.003
0.002
0.001
0.000
Eubranchus exiguus
6e-04
4e-04
2e-04
0e+00
Eucheilota maculata
0.10
0.05
0.00
Eulalia viridis
3e-04
2e-04
1e-04
0e+00
Eunereis longissima
0.020
0.015
0.010
0.005
0.000
Eutonina indicans
0.00015
0.00010
0.00005
0.00000
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov


Evansula pygmaea
Fabricia stellaris
Fabulina fabula
Facelina bostoniensis
Gonothyraea loveni
Heteromastus filiformis
Kurtiella
Kurtiella bidentata
Lanice conchilega
Laomedea flexuosa
Leptastacus aff. laticaudatus
Leuckartiara octona
Littorina littorea
Lizzia blondina
Loimia ramzega
Lutraria lutraria
Macoma balthica
Macomangulus tenuis
Mactra stultorum
Mactridae
Fraction of total reads
region
18SV9
COI
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov


Magallana/Crassostrea
0.015
0.010
0.005
0.000
Magelona filiformis
3e-05
2e-05
1e-05
0e+00
Magelona johnstoni
0.004
0.003
0.002
0.001
0.000
Magelona mirabilis
0.0015
0.0010
0.0005
0.0000
Malmgrenia lunulata
0.0100
0.0075
0.0050
0.0025
0.0000
Marenzelleria viridis
0.15
0.10
0.05
0.00
Margelopsis haeckelii
0.05
0.04
0.03
0.02
0.01
0.00
Metridium senile
0.0020
0.0015
0.0010
0.0005
0.0000
Microphthalmus listensis
9e-05
6e-05
3e-05
0e+00
Microphthalmus similis
5e-04
4e-04
3e-04
2e-04
1e-04
0e+00
Mulinia lateralis
1.0e-05
7.5e-06
5.0e-06
2.5e-06
0.0e+00
Mya arenaria
0.002
0.001
0.000
Mytilus
0.04
0.02
0.00
Mytilus sp.
0.020
0.015
0.010
0.005
0.000
Nemopsis bachei
0.020
0.015
0.010
0.005
0.000
Nephtys assimilis
0.0015
0.0010
0.0005
0.0000
Nephtys cirrosa
0.08
0.06
0.04
0.02
0.00
Nephtys hombergii
3e-04
2e-04
1e-04
0e+00
Obelia bidentata
0.2
0.1
0.0
Obelia dichotoma
0.04
0.03
0.02
0.01
0.00
Fraction of total reads
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
region
18SV9
COI


Obelia dichotoma/geniculata
0.008
0.006
0.004
0.002
0.000
Obelia longissima
0.0015
0.0010
0.0005
0.0000
Onchidoris bilamellata
1e-05
5e-06
0e+00
Owenia fusiformis
0.04
0.03
0.02
0.01
0.00
Paraleptastacus espinulatus
6e-05
4e-05
2e-05
0e+00
Paraonis fulgens
3e-04
2e-04
1e-04
0e+00
Pectinaria koreni
0.0075
0.0050
0.0025
0.0000
Peringia ulvae
0.08
0.06
0.04
0.02
0.00
Petricolaria pholadiformis
0.015
0.010
0.005
0.000
Phaxas pellucidus
1.5e-06
1.0e-06
5.0e-07
0.0e+00
yllodoce groenlandica/mucos
0.003
0.002
0.001
0.000
Phyllodoce rosea
0.0020
0.0015
0.0010
0.0005
0.0000
Polydora cornuta
0.015
0.010
0.005
0.000
Protodrilus oculifer
0.00020
0.00015
0.00010
0.00005
0.00000
Psammechinus miliaris
0.020
0.015
0.010
0.005
0.000
Pygospio elegans
0.4
0.3
0.2
0.1
0.0
Ruditapes philippinarum
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
Sabellaria
0.003
0.002
0.001
0.000
Sabellaria spinulosa
0.0015
0.0010
0.0005
0.0000
Scolelepis
0.004
0.003
0.002
0.001
0.000
Fraction of total reads
Nov
Dec
Jan
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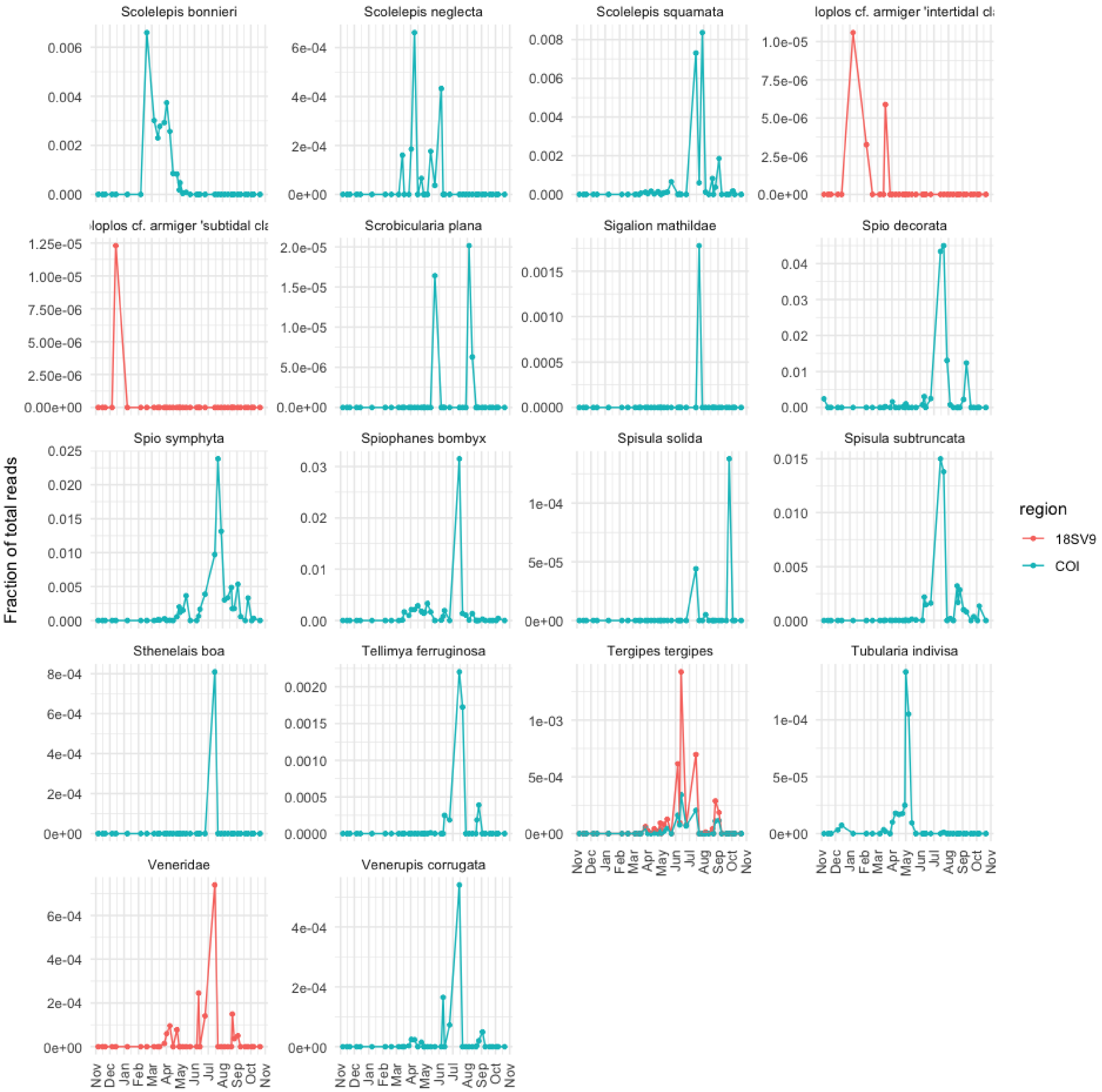
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
region
18SV9
COI


Scolelepis bonnieri
Scolelepis neglecta
Scolelepis squamata
loplos cf. armiger 'intertidal cl
loplos cf. armiger 'subtidal cl
Scrobicularia plana
Sigalion mathildae
Spio decorata
Spio symphyta
Spiophanes bombyx
Spisula solida
Spisula subtruncata
Sthenelais boa
Tellimya ferruginosa
Tergipes tergipes
Tubularia indivisa
Veneridae
Venerupis corrugata
Fraction of total reads
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
region
18SV9
COI