Development of integrated zooplankton monitoring for the Dutch North Sea within the MONS project

ID14 MONS Monitoring Zooplankton Phase 1

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Dit onderzoek legt de basis voor de monitoring van zoöplankton op de Noordzee. Zoöplankton monitoring is van belang voor de beoordeling van de goede milieutoestand en de bepaling van de ecologische draagkracht van de Noordzee. Het onderzoek wordt medegefinancierd door de Europese Unie via het European Maritime Fisheries and Aquaculture Fund (EMFAF).

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Photo cover: A collage of images of organisms as imaged using the Pi-10 Plankton Imager on RV Tridens. Collage by L. van Walraven.

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Leeswijzer

Deze versie van de rapportage over MONS Zoöplankton fase 1 betreft de rapportage waarbij, zoals afgestemd met de opdrachtgever, een aantal onderdelen van het MONS Zoöplankton onderzoek Fase 1 wel en een aantal onderdelen (nog) niet uitgewerkt zijn.

De onderdelen die in deze rapportage zijn verwerkt betreffen:

- Een zelfstandig leesbare samenvatting in het Nederlands en Engels.
- Een beschrijving van de verzamelde data en de methodes waarmee ze verzameld zijn;
- Een presentatie van de data van de juni survey in 2023 met uitzondering van de hieronder genoemde onderdelen.
- Een analyse van CPR data.
- Het onderdeel Deskstudy KRM/OSPAR met informatie over indicatoren die vanuit de monitoring toegepast kunnen worden voor beleidsrelevante vragen en ecosysteemmodellering.
- Aanbevelingen voor vervolgmonitoring en –onderzoek.

Deze onderdelen zijn (nog) niet in het concept verwerkt:

- De in september 2023 geplande offshore survey met de Zirfaea. Dit is niet doorgegaan in verband met het niet op tijd gereed zijn van de apparatuur voor continue monitoring aan boord. Dit onderdeel is daarom niet uitgevoerd.
- De resultaten van de hoog-frequente monitoring in het Marsdiep; deze zullen in 2025 als apart rapport worden gepubliceerd omdat er dan een jaarronde bemontering kon worden gedaan tot en met oktober 2024.

Samenvatting

In het zoöplankton fase 1 onderzoek is in het kader van het MONS programma een onderzoek gedaan naar de diversiteit, verspreiding en samenstelling in ruimte en tijd van zoöplankton in de Nederlandse Noordzee. Hiervoor is een analyse gedaan van bestaande gegevens, o.a. uit het Continuous Plankton Recorder (CPR) programma, om inzicht te krijgen in de veranderingen in zoöplankton in het Nederlandse deel van de Noordzee en de mogelijke oorzaken daarvan. In enkele delen van de Nederlandse Noordzee, zoals westelijke Noordzee en Doggersbank, is de CPR-dekking onvoldoende voor een goede trendanalyse. Voor de overige delen is in het algemeen een afname te zien van kleine en grote copepoden en een toename van larven van benthische organismen (het meroplankton), met de sterkste veranderingen in de zuidelijke Noordzee. Enkele permanente stations (2xUK, 1xGER) laten zien dat een toenemende temperatuur hierin bijdraagt, maar niet alle veranderingen kan verklaren. De veranderingen in de planktongemeenschap betekenen dat de structuur van voedselweb waarschijnlijk complexer is dan voorheen, met een belangrijkere rol voor het meroplankton en waarschijnlijk ook voor de weinig bestudeerde kwalachtigen.

Daarnaast is een deskstudie uitgevoerd om inzicht te krijgen hoe zoöplanktonmonitoring is geïntegreerd in beoordelingen voor OSPAR en de Kaderrichtlijn Mariene Strategie, welke kennisleemtes er uit deze beoordelingen naar voren komen en hoe nieuw op te zetten Nederlandse zoöplankton monitoring kan bijdragen aan het invullen van deze kennisleemtes. De planktonindicatoren binnen OSPAR zijn voornamelijk gebaseerd op veranderingen in functionele groepen plankton en niet op veranderingen op soortniveau. Deze indicatoren worden ontwikkeld op basis van bovengenoemde CPR gegevens en permanente stations. Kennisleemtes die naar voren kwamen uit de OSPAR beoordeling van 2023 waren de noodzaak tot gebruik van nationale datasets met hoge resolutie in ruimte en tijd om veranderingen in plankton beter te kunnen relateren aan veranderingen in de omgeving, iets waar de toepassing van innovatieve technieken een oplossing voor kan zijn. Ook is er een gebrek aan gegevens voor rivierpluimen zoals langs de Nederlandse kust, en is er een gebrek aan kennis over groepen zoals kwalachtigen en het kleinste plankton (microplankton en kleiner).

Binnen dit onderzoek is ook een veldstudie uitgevoerd in de Nederlandse Noordzeekustzone in mei en juni 2023 tijdens een MONS kustsurvey voor pelagische vis om, met inzet van innovatieve technieken, inzicht te krijgen in de huidige verspreiding en samenstelling van de zoöplankton gemeenschap. Hierbij zijn verschillende innovatieve technieken ingezet. Aan boord van RV Tridens II is een Plankton Imager geïnstalleerd die tijdens het varen continu de planktonsamenstelling meet. Daarnaast zijn er monsters genomen met het WP2 planktonnet welke met een combinatie van drie technieken zijn geanalyseerd: DNA metabarcoding met markers COI, 18SV4 en 18SV9, plankton scanner (zooscan) en microscopische analyses.

De kustsurvey leverde belangrijke inzichten op over zoöplankton in de Noordzee en de integratie van innovatieve technieken. Continumetingen met de Plankton Imager langs het transect laten een grote ruimtelijke variatie zien in planktonsamenstelling. De verspreiding van vrijwel alle planktongroepen kon met deze methode met hoge resolutie in kaart worden gebracht. Een opvallende waarneming hierbij was dat de dichtheid van mantelvisjes (larvacea) meer dan dubbel zo groot was dan die van copepoden. Met DNA metabarcoding kon de verspreiding van honderden verschillende soorten holo- en meroplankton in kaart worden gebracht, wat nieuwe inzichten kan leveren over de link tussen plankton en benthos en kan bijdragen aan vroege detectie van Niet Inheemse Soorten (NIS). Verschillende mogelijke NIS werden dan ook gedetecteerd. De analyse met de plankton scanner kostte meer tijd dan verwacht en de toepassing van zooscan is daarom vooral aanvullend in het geval er geen Pi-10 kan worden ingezet. Microscopische analyses bleken bruikbaar bij het valideren en kalibreren van de andere technieken, zoals de Plankton Imager en DNA metabarcoding.

Op basis van de inzichten uit de diverse deelonderzoeken is een advies gegeven over hoe een nieuw op te zetten zoöplankton monitoring in het kader van MONS eruit zou moeten zien.

Samenvatting van het monitoringsadvies.

Monitoringsactiviteit	Beschrijving
Hoogfrequente metingen vanaf de NIOZ-steiger	- Voortzetting van de huidige metingen, waaronder: - DNA metabarcoding met behulp van COI- en 18SV9-markers - Microscopie (selectie van monsters) - Zooscan
Monitoring op MTWL-transecten	- Doorlopende metingen met FerryBox-container, inclusief Plankton Imager - Verticale WP2-netmonsters op MTWL-punten (afhankelijk van budget/tijd), geanalyseerd met: - DNA metabarcoding met behulp van COI- en 18SV9-markers - Microscopie (selectie van monsters)
Doorlopende monitoring tijdens RV Tridens WOT-visserijonderzoek	- Continumetingen met Plankton Imager - Continue omgevingsmetingen (minimaal watertemperatuur en zoutgehalte)

Summary

As part of the MONS program, the zooplankton phase 1 study investigated the diversity, distribution and composition in space and time of zooplankton in the Dutch North Sea. To this end, an analysis of existing data, including those from the Continuous Plankton Recorder (CPR) program, was done to gain insight into the changes in zooplankton in the Dutch part of the North Sea and its possible causes. In some parts of the Dutch North Sea, such as western North Sea and Dogger Bank, CPR coverage is insufficient for proper trend analysis. For the remaining parts, in general a decrease in small and large copepods and an increase in larvae of benthic organisms (the meroplankton) can be seen, with the strongest changes in the southern North Sea. Some permanent stations (2xUK, 1xGER) show that increasing temperature contributes to this but does not explain all the changes. The changes in the plankton community suggests that the structure of food webs is probably more complex than before, with a more important role for the meroplankton and probably also for the understudied jellyfish.

In addition, a desk study was conducted to gain insight into how zooplankton monitoring is integrated into assessments for OSPAR and the Marine Strategy Framework Directive, what knowledge gaps emerge from these assessments, and how newly established Dutch zooplankton monitoring can contribute to filling these knowledge gaps. The plankton indicators within OSPAR are mainly based on changes in functional groups of plankton and not on changes at the species level. These indicators are developed based on the CPR data and permanent stations mentioned above. 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, something that the application of innovative techniques can address. There is also a lack of data for river plumes such as along the Dutch coast, and a lack of knowledge about groups such as jellyfish and the smallest plankton (microplankton and smaller).

Within this project, a field study was also carried out in the Dutch North Sea coastal zone in May and June 2023, during a MONS coastal survey for pelagic fish, to gain insight into the current distribution and aggregation of the zooplankton community using innovative techniques. Several innovative techniques were employed for this purpose. A Plankton Imager was installed on board RV Tridens II, which continuously measured the plankton composition while sailing. In addition, samples were taken with the WP2 plankton net which were analysed with a combination of three techniques; DNA metabarcoding with markers COI, 18SV4 and 18SV9, plankton scanner (zooscan) and microscopic analyses.

The coastal survey provided important insights about zooplankton in the North Sea and the integration of innovative techniques. Continuous measurements with the Plankton Imager along the transect showed a large spatial variation in plankton composition. The distribution of almost all plankton groups could be mapped with high resolution using this method. A striking observation here was that the density of mantle fish (larvacea) was more than double that of copepods. DNA metabarcoding allowed the mapping of the distribution of hundreds of different species of holo- and meroplankton, which can provide new insights on the link between plankton and benthos and contribute to early detection of Non-Indigenous Species (NIS) Several possible NIS were indeed detected. The analysis with the plankton scanner took more time than expected and the use of zooscan is therefore mainly supplementary in the event that Pi-10 cannot be used. Microscopic analyses proved useful in validating and calibrating the other techniques.
Based on the insights from the various sub-studies, an advice was given on what a new zooplankton monitoring system to be set up in the framework of MONS should look like.

The monitoring advice summarised.

Monitoring Activity	Description
High-frequency measurements from the NIOZ jetty	- Continue current measurements, including: - DNA metabarcoding using COI and 18SV9 markers - Microscopy (selection of samples) - Zooscan
Monitoring on MTWL[1] transects	- Continuous measurements with FerryBox container, including Plankton Imager - Vertical WP2 net samples at MWTL points (budget/time dependent), analyzed by: - DNA metabarcoding using COI and 18SV9 markers - Microscopy (selection of samples)
Continuous monitoring on RV Tridens WOT fisheries surveys	- Continuous operation of the Plankton Imager - Continuous environmental measurements (at minimum, water temperature and salinity)

[1] MWTL = Monitoring Waterstaatskundige Toestand des Lands, https://waterinfo-extra.rws.nl/monitoring/

1 Introduction

1.1 Background

The Monitoring-Onderzoek-Natuurversterking-Soortbescherming) (Monitoring-Research-Nature Enhancement-Species Protection; MONS) program aims to answer the central question of whether and how 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 (Physical Environment Consultation Body, 2020).

The North Sea Agreement established that there is a great need for an integrated and systematic research and monitoring program due to changing use. The Monitoring-Research-Nature Restoration-Species Protection (MONS) program has been drawn up for this purpose, which focuses on making information available about various basic physical, chemical and biological parameters.

Policy and management of the North Sea requires a good insight into the consequences of human use and other drivers on the carrying capacity of the North Sea ecosystem and how this affects the protection of areas and animal species and the food supply through fishing and mariculture.

Although zooplankton is not part of the current monitoring on the Dutch Continental Shelf, it is highly necessary to be able to monitor, understand and, if possible, predict changes in the zooplankton in the North Sea in order to determine the effects of human activities and climate change on the carrying capacity of the North Sea ecosystem and to assess interactions and consequences in the food web. Zooplankton forms an essential link within the North Sea food web.

A plan for monitoring and supporting research of zooplankton in the Dutch part of the North Sea has been drafted (Jak et al., 2022) that should provide answers to the following questions in due course:

- What is the composition and distribution of zooplankton in space and time?
- What are the trends (years and decades) in composition and distribution of zooplankton in space and time?
- What are the effects of new human use on zooplankton?

The results from this proposed monitoring should enable to understand and predict changes in zooplankton in the North Sea, so that validated scenario studies can be performed. All this in order to be able to assess the ecological carrying capacity and the effects of individual and cumulative use thereon.

The monitoring plan included two phases. This 1-year inventory study exploring the application of innovative techniques and gaining initial insight in zooplankton distribution in space and time to be able to design a monitoring programme that can adequately be used to answer the above mentioned questions (phase 1). Following this, a 4-year monitoring programme will be planned which will be designed taking into account the results of this 1-year study (phase 2).

Phase 1 focuses on the following components:

- An analysis of literature and information from experts and OSPAR,
- An analysis of existing data (including CPR) and available samples,
- Development and implementation of innovative techniques in a field survey
    - collection of reference data for:
        - list of (potential) species
        - DNA metabarcoding
        - Plankton Imaging
- A monitoring campaign based on a selection of locations and frequency based on MWTL cruises
- Design of a monitoring plan for Phase 2.

This report takes into account these issues in the following chapters.
In chapter 2 an overview of existing data will be presented bases on results from the Continuous Plankton Recorder, with a focus on sub-areas within the Dutch Continental Shelf and trends in species abundances.
In Chapter 3 Zooplankton-based indicators are discussed that are being developed and applied in assessing the status of marine biodiversity in relation to Good Environmental Status within the EU Marine Strategy Framework Directive (MSFD), and how zooplankton monitoring and zooplankton-based indicators can contribute to this.
In Chapter 4 the results from a coastal survey are presented where continuous sampling using a Plankton Imager was combined with analysis of net samples with DNA metabarcoding, zooscan and microscopy to gain insight in current zooplankton composition and distribution and to investigate how the different techniques used can complement each other.
In Chapter 5 an overview is given of the species found in the different activities.
In Chapter 6 A review is performed on how zooplankton monitoring is currently integrated in MSFD and OSPAR, what the main knowledge gaps are and how new zooplankton monitoring could address these.
Finally, conclusions and recommendations are given in chapter 7.

1.2 Research questions

The research questions as formulated in the original project plan were:

- Which monitoring design provides the most optimal insight into distribution and dynamics in space and time of the zooplankton community?
- Which set-up is most optimal in relation to other components of the food web (phytoplankton, pelagic fish)?
- Are the data useful in relation to the food web models to be developed within MONS?
- What is the most cost-effective monitoring design?

2 Existing data

The North Sea is under pressure of various anthropogenic activities, ranging from shipping, fishing, sand extraction, pollution and climate change. In this context, our use of ecosystem services from the North Sea is changing and increasing and are framed in 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 Monitoring-Onderzoek-Natuurversterking-Soortbescherming (MONS) program has the goal to determine whether these transitions are within the ecological carrying capacity of the Dutch North Sea (Asjes et al., 2021). In this chapter, we present a brief literature and data analysis of ongoing environmental changes in the North Sea and changes, based on existing data, in the zooplankton community.

2.1 Environmental changes in the North Sea due to climate change

The transitions on the North Sea happen in a time of climate change, which leads to increasing sea surface temperatures, temperature extremes and ocean acidification. Here, we summarise the main changes that have been documented for the North Sea.

2.1.1 Sea surface temperature and temperature extremes

Understanding and forecasting the consequences of coastal warming requires knowledge of the near-shore temperature changes that have occurred in the last decades (Lima and Wethey, 2012). A global analysis on data from 1982 to 2010 showed that 71% of the coastlines are significantly warming and that the North Sea is amongst the fastest changing coastal seas in the world. On average, the North Sea warms at a rate of ~0.5^{o}C warming per decade (Lima and Wethey, 2012). Similar warming is also seen in the long-term monitoring from NIOZ jetty in the Marsdiep (https://www.nioz.nl/en/expertise/wadden-delta-research-centre/data-tools/long-term-ecological-time-series/sea-water-temperature).

Not just the monthly temperatures have increased. The frequency of extremely cold events (±30 days dec^{-1}) has decreased, while extremely hot days are becoming more common at 0-5 days in 1982 to >20 in 2021 (Thoral et al., 2022). Finally, Lima and Wethey (2012) showed that the onset of the warm season, relevant for the phenology of organisms, is significantly advancing to earlier in the year at a rate of ±8 days dec^{-1}.

2.1.2 Ocean acidification

Ocean acidification, sometimes called the 'evil twin of climate warming', is the decrease in pH due to the absorption of CO_2 by the oceans and concomitant formation of H^+-ions by the formation of various inorganic carbon species (Doney et al., 2009). In contrast to the open ocean, the dynamics of pH in coastal seas displays a broad range of temporal trends and are as likely to show a long-term increase as decrease in pH (Carstensen and Duarte, 2019). The pH of many coastal ecosystems displays nonlinear trends, with seasonal and interannual variations even exceeding 1 pH unit. The main reasons for these variable dynamics are primarily driven by inputs from land, including freshwater (typically diluting seawater alkalinity and thereby reducing proton buffering), nutrients (enhancing productivity and pH), as well as organic matter supporting excess respiration driving acidification. Of these factors, nutrients are particularly relevant as reduced (or unbalanced) nutrient supply reduces eutrophication and reduces thereby the primary production-enhanced pH (Carstensen and Duarte, 2019). This latter factor is directly relevant for management purposes as

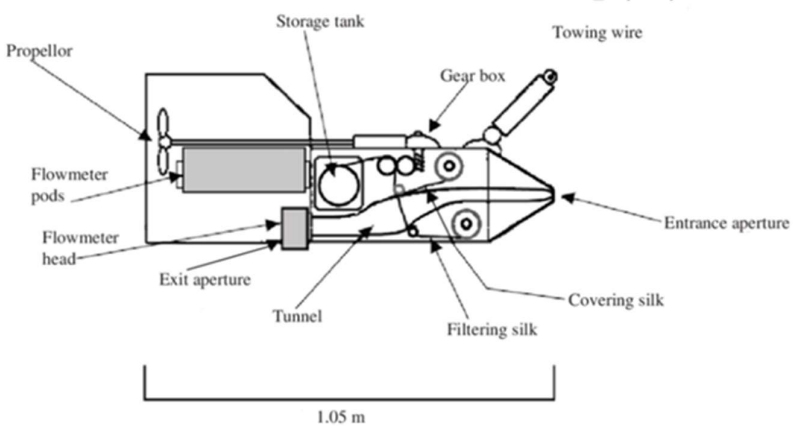
reduced (or possibly unbalanced) nutrient supply may lower the removal of CO_2 by primary production and therefore may lead to a lower pH.
Provoost et al. (2010) analysed pH changes in the Dutch coastal zone since 1975 (based on data from Rijkswaterstaat). Expected pH declines based solely on the increase in atmospheric CO_2 can be relatively narrowly constrained to 0.0013 to 0.002 pH units y^{-1} (Orr et al., 2005). Provoost et al. (2010) found pH changes exceeding 0.02 pH y^{-1} for several North Sea stations, thus grossly exceeding pH declines expected from enhanced CO_2 uptake alone. They argued that this long-term trend is linked to the modified balance between primary production and respiration. A decrease in primary production (i.e., CO_2 removal thus pH increase) or increase of respiration (i.e., CO_2 formation thus pH decline) will shift the balance to enhanced pH decline. Primary production has indeed been shown to decline in the last decades (Capuzzo et al., 2018), likely due reductions in nitrogen and phosphorus runoff mostly in the 80s- and 90s and later reductions in water clarity (Capuzzo et al., 2015). The explanation of enhanced acidification linked to reduced primary production was also postulated in a modelling study of the North Sea (Borges and Gypens, 2010).

2.2 Changes in zooplankton composition and abundance

2.2.1 Large-scale changes in the Atlantic and Greater North Sea based on data from the continuous plankton recorder (CPR) and long-term monitoring sites

Zooplankton plays a key role in the marine food web as the primary node of organic matter transfer from primary producers to higher trophic levels (Steinberg and Landry, 2017). A particularly important trophodynamic process is the predation of fish larvae and small pelagic fish on copepods. For example, Heath (2005) calculated the dependency of commercially landed fish on various food sources and concluded that >60% of their biomass production is supported by feeding on zooplankton, mostly copepods. Cappuzzo et al. (2018) directly correlated decadal declines in primary production to declines in small copepods and fish recruits. In a seminal paper, Beaugrand et al. (2003) found that the higher seawater temperature since the 1990s has changed the composition of calanoid copepods causing a mismatch in time with the recruitment of young North Sea cod and therefore lower recruitment success. The view that this classical herbivorous food chain (Legendre and Rassoulzadegan, 1995), of fish (larvae) feeding on copepods, dominates the North Sea food web is, however, changing.

Box 1: Continuous Plankton Recorder survey



The continuous plankton recorder (CPR) survey is the world's largest and longest-running plankton monitoring program, providing data on phyto- and zooplankton abundance in the North Sea and North Atlantic (Richardson et al., 2006). The CPR program was already active before 1958, but since then the counting procedure changed, so often only data >1958 are considered. The CPR (see figure below, from Richardson et al., 2006) is towed by commercial vessels and operates at a standard depth of around 7 meters. Water is sampled through a 1.27x1.27 cm sampling port and captured plankton is trapped between moving band of silk and fixed with formaline. The mesh size of the silk is 270 µm, thereby focussing on phytoplankton blooms and zooplankton individuals (copepods, Cladocera, pteropods, and chaetognaths). Each sample represents ±10 nautical miles and ±3 m3 and the central location is recorded at geographical sample location. From each sample, the silk colour (representing phytoplankton density), phytoplankton species, zooplankton <2mm (through a standard traverse across the silk) and large zooplankton (all individuals counted by eye) are recorded. Counts are translated into categories (e.g. between 12 and 25 individuals = category 5 with an accepted abundance of 17) to speed up the sample processing but means that counts are semi-quantitative. A full description can be found in (Richardson et al., 2006).

In autumn 2023, OSPAR released their latest Quality Status report in which the status of the NE Atlantic and Greater North Sea have been assessed through various variables and indicators (https://oap.ospar.org/en/ospar-assessments/quality-status-reports/qsr-2023/). Findings specifically for phyto- and zooplankton dynamics are based on data collected with the continuous plankton recorder (CPR) (see Box 1). Overall, Holland et al. (2023b) reported significant decreases in abundance for most planktonic lifeforms over the period 1960 - 2019 for the Atlantic region, including holoplankton (median decrease of 7% decade^{-1}), small (8% decade^{-1}) and large copepods (9% decade^{-1}). Conversely, fish larvae/egg abundance increased (3% decade^{-1}) and especially meroplankton demonstrated a very different pattern with a median increase in abundance of 12% decade^{-1}. There were however clear spatial differences among assessment regions. Three OSPAR regions cover the DCS (Dutch Continental Shelf), i.e. the Southern North Sea, Eastern North Sea and Doggerbank region (Figure 1). The dynamics over the full 60-year time series at these smaller spatial scales can be summarized as follows (Holland et al., 2023a, 2023b):

1. The abundances of small and large zooplankton are decreasing in the more offshore Doggerbank region, while they are stable or slightly increase at the Eastern and Southern North Sea, respectively.
2. Holoplankton, i.e. zooplankton that spends its entire life cycle in the water (including copepods, Cladocerans and pteropods), are also declining in the Doggerbank, while their abundance decreases and remains stable in the Eastern and Southern North Sea, respectively.
3. Meroplankton, i.e. zooplankton that spends only part of its lifecycle in the water column and includes larvae of benthic organisms such as sea urchins, bivalves and crabs, is (strongly) increasing in abundance in all three assessment areas of the DCS.
4. The temporal dynamics of gelatinous zooplankton cannot be assessed as insufficient data are available because the CPR doesn't adequately sample soft-bodied specimens. Evidence of abundance changes in the larger gelatinous zooplankton is inconclusive on a global scale, but jellyfish blooms have become more common in several coastal regions in recent decades (Condon et al., 2013), possibly exacerbated by the increase in ocean sprawl (Duarte et al., 2013).

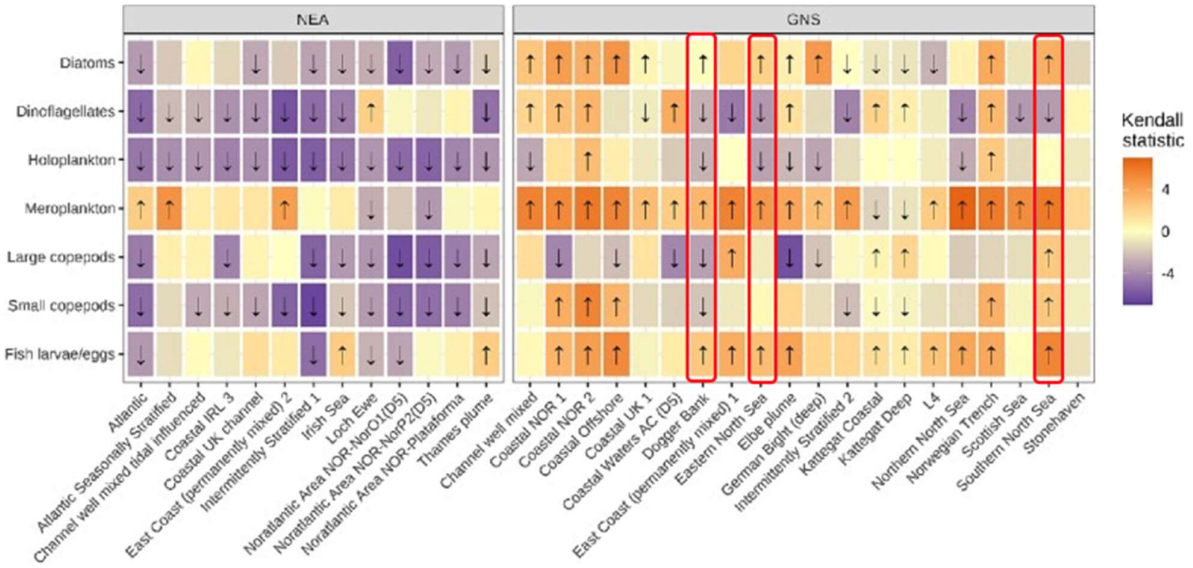


Figure 1: Heatmap displaying the distribution of Kendall statistics for the 30 COMP4 assessment units (NEA: north-east Atlantic, GNS: Greater North Sea) and 3 fixed-point stations for each lifeform. Kendall statistic indicates an increase (positive values) or decrease (negative values) of the abundance over the investigated period. The arrow symbols indicate whether a lifeform abundance time-series was significantly ($p \leq 0.05$) increasing (↑) or decreasing (↓). Red boxes indicate the three assessment regions that are relevant for the DCS. Modified from Holland et al. 2023b.

In addition to the CPR data, which cover a broad spatial scale, there is also information from several zooplankton time-series from various monitoring stations in the North Sea and NE Atlantic. Corona et al. (2024) recently published the main findings of these long time-series, in particular the stations Stonehaven (SH), Helgoland Roads (HR), and Plymouth (L4) (Figure 2). These time-series are already in operation for several decades and the data were used to determine the 'realised thermal niche', i.e. in situ temperature during peak occurrence, and 'phenology', i.e. seasonal timing of species abundance, of 7 dominant copepods taxa *Acartia clausi*, *Calanus helgolandicus*, *Centropages typicus*, *Oithona* spp., *Paracalanus parvus*, *Pseudocalanus elongatus*, and *Temora longicornis*.

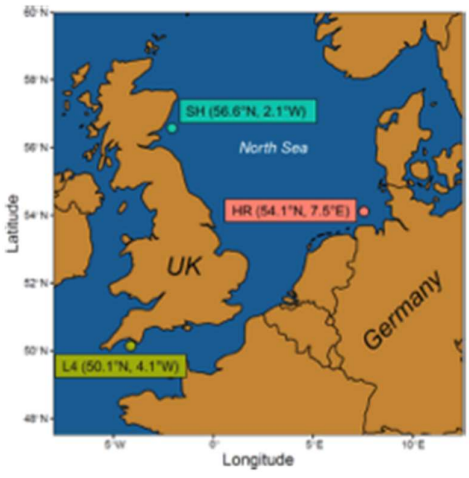


Figure 2 Location of the three stations (L4, HR, and SH) in the North Sea and English Channel. Figure from Corona et al. (2024).

Their analysis confirmed the overall copepod decline, except for *Oithona* spp, at the stations which were also reported in the CPR-based study by Holland et al. (2023b). Surprisingly, these was no evidence of that the realised thermal niches changed over time, despite the temperature increase that has occurred at the research stations. Realised thermal niches may therefore be quite conservative. Moreover, the realised thermal niche was strongly site-specific, which indicates that a species' response to temperature is location dependent and cannot be easily generalized from e.g. laboratory studies. This will be important to consider for future modelling studies. Finally, various indices on the ecological dynamics such as phenology (i.e. events in the life cycle of an organism) and egg production changed between the first and second decade of the time series and these changes were larger than that could be explained by temperature alone, suggesting an influence of other factors.

Information on gelatinous zooplankton is scarce (Holland et al., 2023b) and the temporal analysis of time-series is inconclusive on whether gelatinous zooplankton abundance in increasing (Condon et al., 2013). Experimental mesocosm studies, however, suggest that larvaceans (or Appendicularia) increased in abundance with increased temperature and thereby took up food particles at the expense of food availability for the copepods (Winder et al., 2017). While temporal studies are inconclusive, environmental factors (e.g. higher temperature, reduced oxygen concentration), fishing and marine sprawl (Duarte et al., 2013) are likely to increase gelatinous zooplankton in the future, which can affect food web dynamics as metabarcoding studies show that gelatinous zooplankton are a more important food source for higher trophic levels than previously thought (Jaspers et al., 2023).

2.2.2 Changes in the zooplankton community for the Dutch Continental Shelf based on additional CPR-based analysis

Below, we use the CPR data of this broadscale analysis (available in Djeghri et al., 2023) to zoom in specifically on the trends in CPR-data availability and zooplankton composition and abundance to establish a baseline of zooplankton dynamics for the various regions of the DCS.

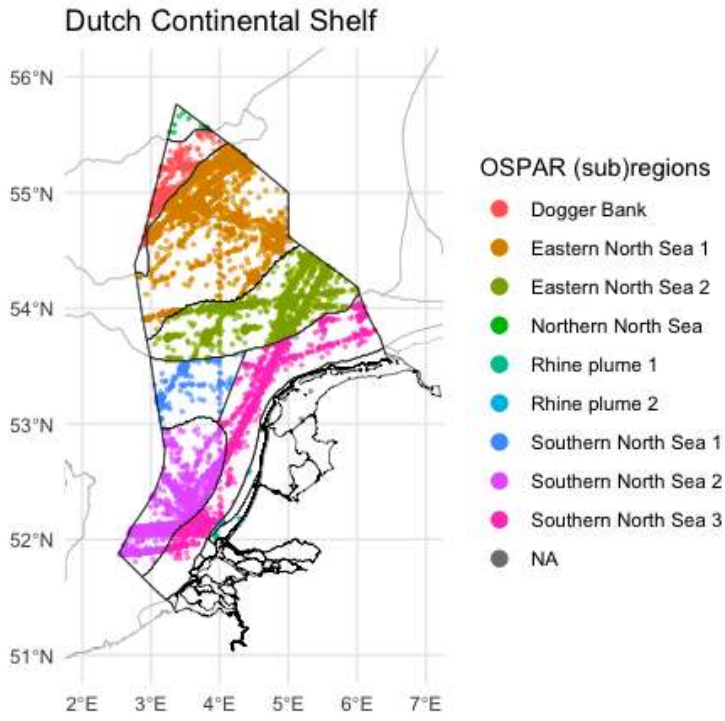


Figure 3 Distribution of the CPR samples in the various subregions of the OSPAR areas.

The sampling coverage during the 60-year CPR program in the various OSPAR regions indicates a seemingly good coverage of the DCS (Figure 3). However, a more detailed breakdown of CPR-sampling effort to years

and months, shows that while subregions SNS1, SNS2, ENS1, ENS2 are sufficiently covered, especially since the 1980s, the Northern North Sea, Doggers Bank and Rhine Plume show large gaps which precludes a detailed temporal analysis of zooplankton dynamics for the latter regions (Figure 4). The MONS Zooplankton monitoring program is designed to fill these gaps. The spatial and temporal coverage of the DCS will be increased by the implementation of the Plankton Imager (Pi-10) during the regular Zirfaea cruises that cover large parts of the DCS, see Van Walraven et al. (2023) for preliminary results. Coastal zooplankton sampling coverage will be increased with regular (40x per year), high tide (i.e. largely representing North Sea water) zooplankton sampling from the NIOZ jetty.

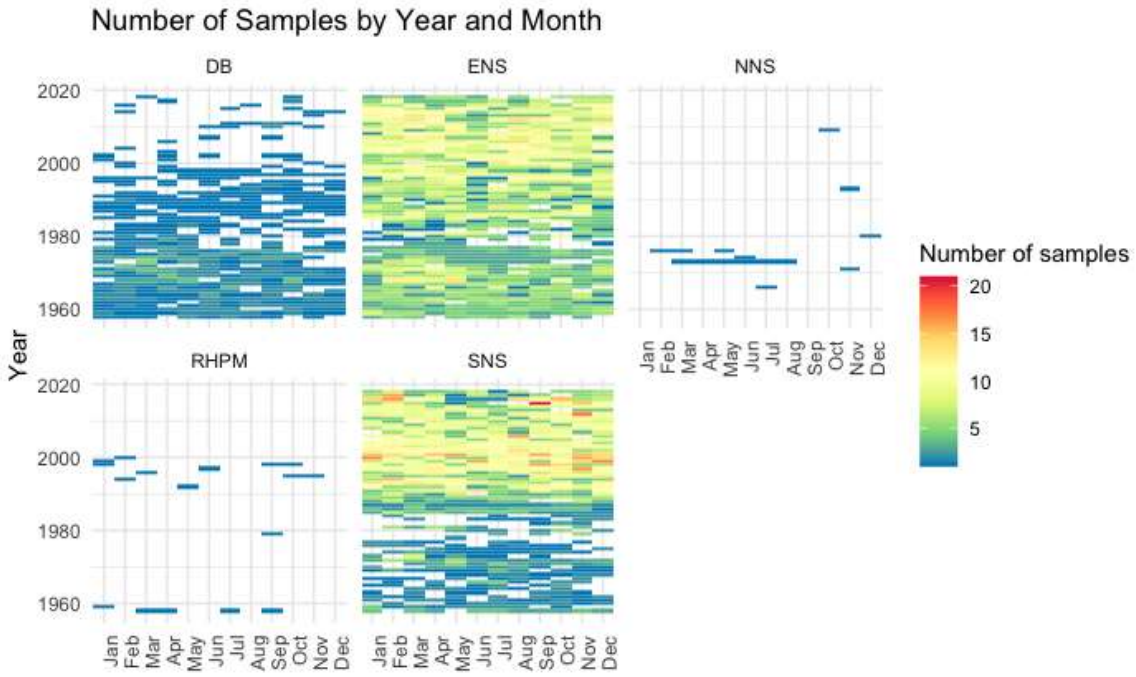


Figure 4 Distribution of the number of CPR samples by year and month over the different OSPAR subregions that cover the DCS. Abbreviations are DB = Doggers Bank, ENS = Eastern North Sea, SNS = Southern North Sea.

The CPR data of the well-covered subregions, i.e. Southern North Sea 1 & 2 and Eastern North Sea 1 & 2, are used in several analyses to how the large-scale patterns summarized above hold for the DCS. Earlier analysis of the plankton dynamics in the NW Atlantic Ocean and the Greater North Sea has identified two periods of 'regime shifts' ('RS'): RS1 in the 1980s and RS2 from 1996 to 2003, interluded with two relatively stable periods (Djeghri et al., 2023). In this analysis, we have used these regime shifts and interludes (i.e. 'dynamic equilibria', DE) to investigate the zooplankton dynamics in the various subregions of the DCS. We employed identical statistical methods as Holland et al. (2023b). In brief, the non-parametric Kendall test is used to test whether there is an up- downward trend in species/group abundance over time for the specific period. The resulting Kendall's τ is converted into a Z-score to correct for the number of samples and to test for statistical significance. In *Figure* 5, we show these results for the various periods, plankton taxa (split into 'holoplankton' and 'meroplankton') and DCS subregions, in which the Z-score is proportional to the magnitude of change and hatching indicates significance.

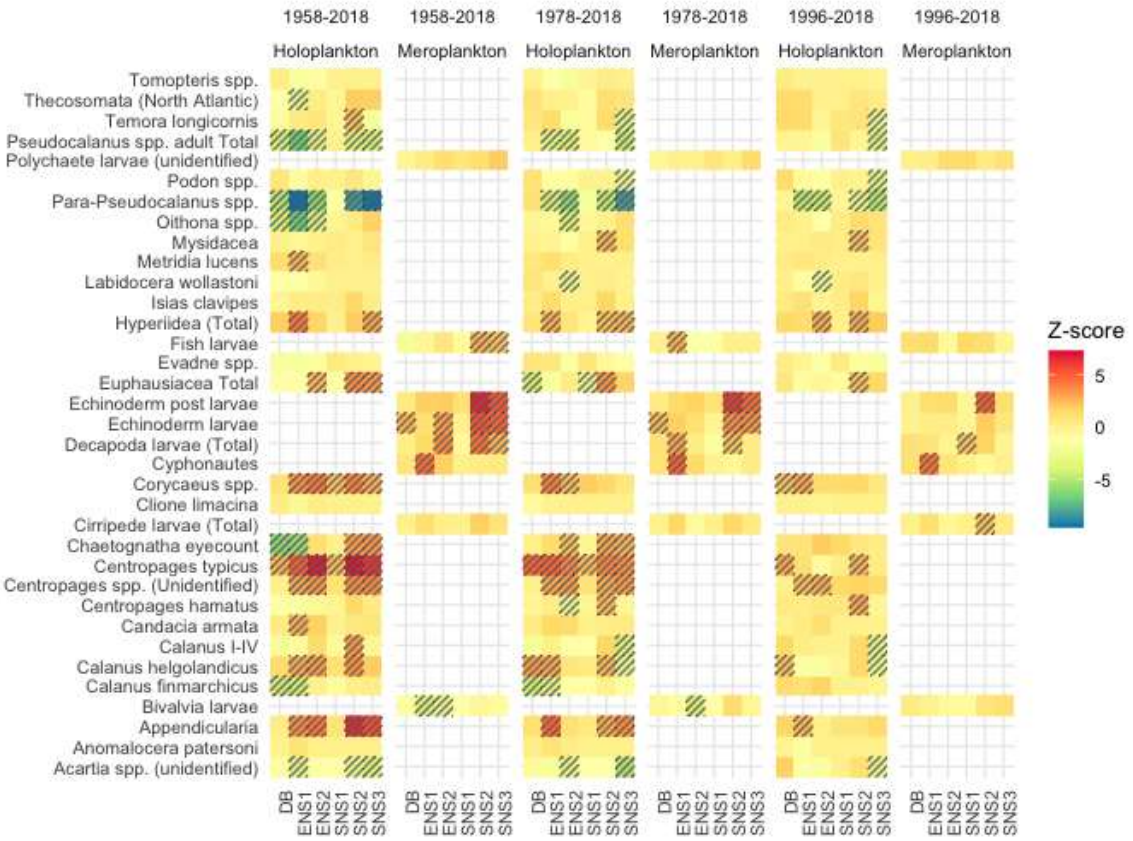


Figure 5 Z-scores of the temporal trend analysis for plankton taxa and functional group (holo- and meroplankton) during various periods and in the DCS subregions. The Z-score indicates the magnitude (colour) and direction of change (<0 = downward (decrease), >0 = upward (increase)). Statistical significance is indicated with hatching.

In general, we see strongest temporal changes during the longest period studied 1958-2018 with significant declines in abundance for the copepods *Para-Pseudocalanus* spp. (a group of copepods that includes *Paracalanus* spp. and *Pseudocalanus* spp.), *Pseudocalanus* spp., *Oithona* spp., *Calanus finmarchicus* and *Acartia* spp. The downward trend for some taxa even extends until the last period (1996-2018), especially in the Southern North Sea subregions. At the same time the abundance of the copepods *Calanus helgolandicus*, *Centropages typicus* (a species that prefers warmer waters) and *Corycaeus* spp. and of larvacea (Appendicularia) and several meroplankton taxa like echinoderm larvae (e.g. common heart urchin), decapod larvae (e.g. crabs) and fish larvae substantially increased. Over the whole period, temporal changes are strongest in the more southern North Sea subregions like SNS2, SNS3 and ENS2. Some changes are apparent during all periods, like for echinoderm larvae, while some changes, such as the increase in cirripede larvae (i.e. barnacles) are only seen more recently.

We also inspected changes in the average plankton community composition, based on abundance, for the various 'stable dynamic' periods. An important note here is that some species are known to be under-sampled with the CPR as compared to e.g. zooplankton nets. Pitois and Fox (2006) provide correction factors for this under-sampling for several copepod species which range from 1.7 for *Centropages typicus* to 45.4 for *Oithona* spp. As correction factors are not available for all species, we base our analysis on the raw data but highlight that for future MONS research it is important to determine such correction factors for all taxa. In addition, abundance data should be converted to units that are relevant for food web and ecosystem dynamics, e.g. carbon, so also these conversion factors need to be determined. The imaging-based MONS zooplankton monitoring lends itself well for size-based conversions to e.g. C units and aligns well with approaches that are developed within several ICES-WGEs (ICES-Working Group for Zooplankton Ecology). We emphasize here that this issue will be a focal point for the PhD-students that are appointed within MONS.

Despite these data limitations, clear shifts can be seen in the species composition (Figure 6). Especially apparent is a strong increase in echinoderm larvae and a strong decrease of *Para-Pseudocalanus* spp.

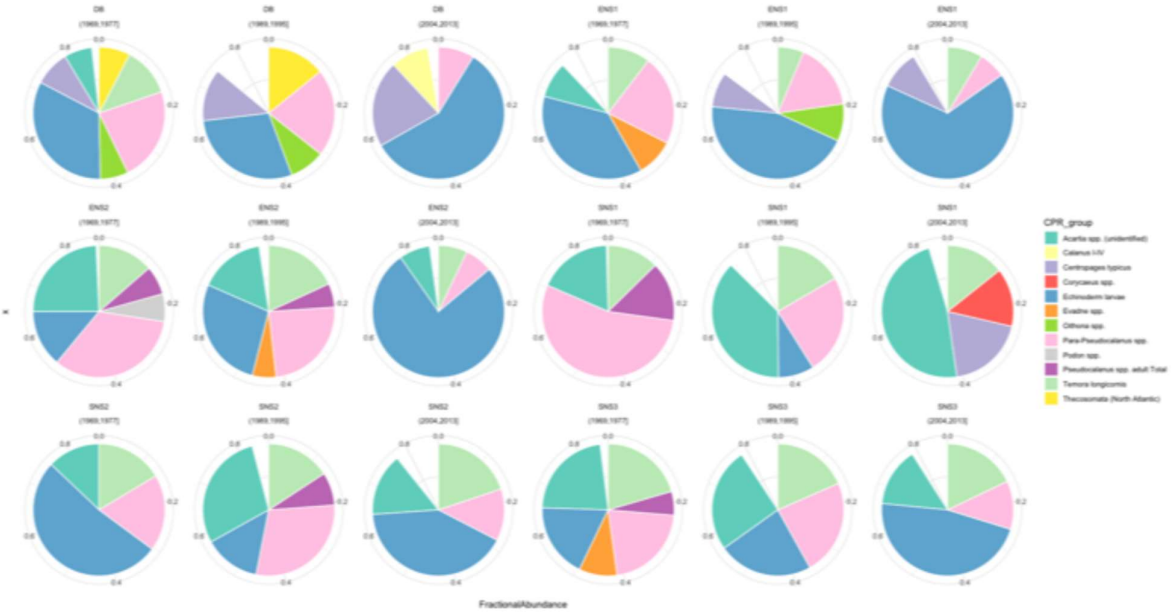


Figure 6 Relative abundance of dominant zooplankton taxa during the 'stable equilibria' periods in the various subregions of the DCS.

2.2.3 Possible impacts of transitions on the North Sea on the zooplankton community

The long-term, decadal, changes of zooplankton abundances described above are mostly attributed to nutrient dynamics (Holland et al., 2023b), particle concentrations (Capuzzo et al., 2018), and climate change, including increasing sea temperature (Kirby et al., 2008). A major transition that is expected in the North Sea concerns the large-scale construction of offshore wind farms (OWFs) on the Dutch Continental Shelf. Direct observations on the consequences of this transition are largely unavailable (Hogan et al., 2023). Floeter et al. (2017) performed in situ observations on, amongst others, zooplankton distribution around and within an OWF using a Video Plankton Recorder (VPR) during several tidal cycles and revealed a distinct meroplankton composition within or downstream of OWF as opposed to an otherwise copepod-dominated community. The elevated meroplankton concentrations were attributed to the high numbers of organisms, including sea urchins, starfish and mussels, on the pillar foundations. These unique data show that OWFs can directly modify the zooplankton community at a local scale.

Other effects on zooplankton concentration and composition are much more complex to assess and are largely based on modelling studies. The OWF-turbines and foundations provide a habitat for an epifaunal community that is often composed of filter feeders (Degraer et al., 2020). These filter feeders may directly depredate on zooplankton in the water-column or reduce phytoplankton in the water column, and thereby could indirectly reduce the zooplankton concentration. A modelling study by Slavik et al. (2019) indicates that mussel densities will indeed increase in the North Sea due to mussel growth on turbines which could lead to higher mussel larvae concentrations and local reductions of phytoplankton concentration of 3-8%. Some studies further suggest an increase in water column turbidity due to OWF, which may lead to reduced primary production, while a reduction in water column stratification may increase primary production (van Duren et al., 2021). It remains challenging at this point to assess how these complex interactive processes, which can be antagonistic or synergistic, will affect the zooplankton community.

2.2.4 A new view on the zooplanktonic food web of the North Sea

It appears that the classical herbivorous view on the marine food web (Legendre and Rassoulzadegan, 1995) is changing. Given the changes in the planktonic food web of the North Sea documented above and that are expected given the continuing change in the environmental conditions, the zooplanktonic community in the North Sea should not be viewed as a solely herbivorous component of the food web (left frame in Figure 7), but as a multitrophic food web in which meroplankton and gelatinous zooplankton should be explicitly considered (right frame in Figure 7). The MONS zooplankton monitoring program and the PhD students that are recruited for the MonZooSS (MONS-Zooplankton proceSS studies) have taken this new view as starting point already.

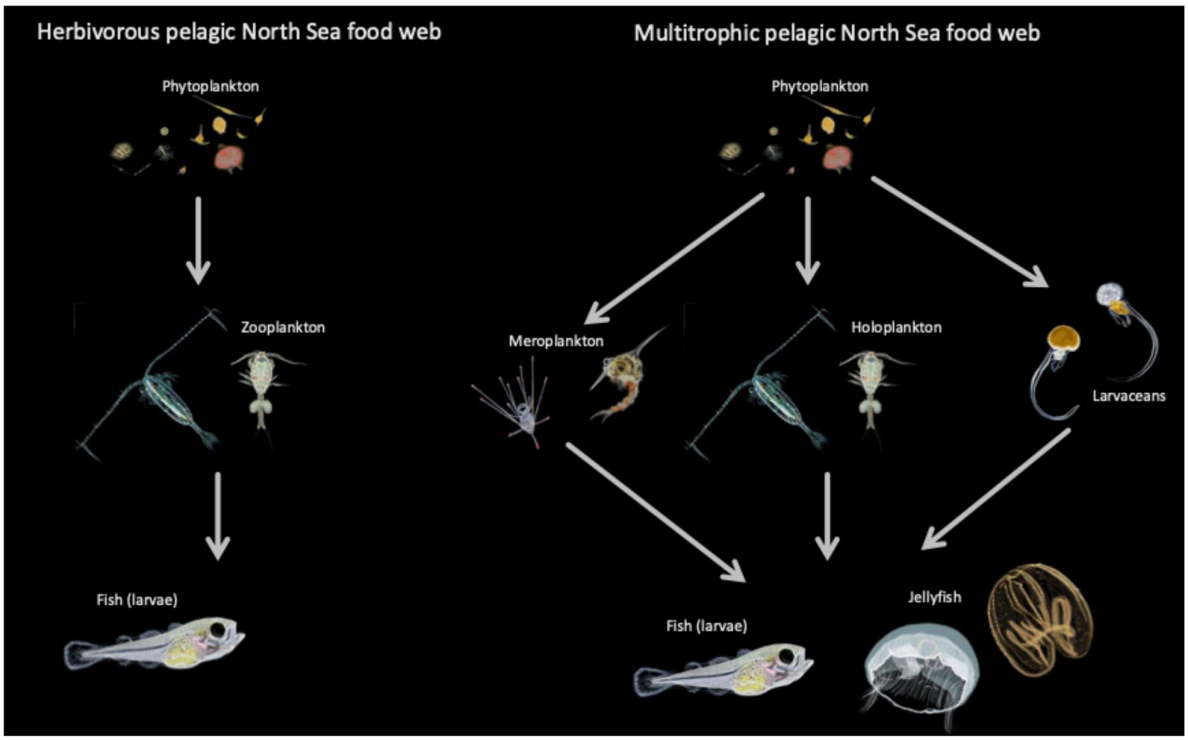


Figure 7 Schematic representation of the classical herbivorous (left) and multitrophic (right) pelagic food web of the North Sea.

3 Zooplankton monitoring in international policies

3.1 Introduction

Zooplankton are an important component of ecosystems and form the link between the base of the food web and higher trophic levels. Zooplankton often have a high reproduction rate and a fast generation time and can therefore respond quickly to environmental changes. As a result, zooplankton can be useful and important indicators of the environmental status of ecosystems (Pitois and Yebra, 2022). Zooplankton-based indicators are being developed and applied in assessing the status of marine biodiversity within the EU Marine Strategy Framework Directive (MSFD), for the first time for the Baltic Sea (Gorokhova et al., 2016). Recently, a wide range of indicators have been developed within OSPAR working groups for the Northeast Atlantic (McQuatters-Gollop et al., 2022) for pelagic habitats, food webs and invasive species. The 2023 OSPAR Quality Status Report reports on the status of these indicators for the Northeast Atlantic Ocean, including the North Sea region (M. Holland et al., 2023; Louchart et al., 2023a, 2023b ; OSPAR, 2023a, 2023b).

The design of the zooplankton monitoring that is currently being developed and implemented within the MONS program takes into account the current monitoring for the MSFD and recent developments such as the OSPAR indicators, for example, through the design of the sampling program, the choice of the measured parameters such as the average size of copepods (Pitois et al., 2021), the selection of sampling techniques and equipment and by aligning the sampled areas with the assessment areas used by OSPAR.

Now that monitoring has started and the first data have been collected, we are examining how it can be used for the various OSPAR/MSFD indicators for pelagic habitats and food webs, invasive exotic species and eutrophication. These activities are coordinated with experts from RWS and LNV who are involved in the MSFD assessment. To coordinate the work internationally, gather input and share the results, Lodewijk van Walraven and Robbert Jak participate in the OSPAR pelagic habitats expert group and the ICES Working Group for Zooplankton Ecology (WGZE). Dick van Oevelen joined WGZE as well in 2024. Aside from desk research, the following activities were undertaken which are relevant for this chapter:

- Lodewijk van Walraven presented and discussed the design and initial results of the MONS zooplankton monitoring during the **ICES WGZE meeting** on 6-8 February 2024 in Plymouth, United Kingdom. Discussions were held here about the development of zooplankton-based indicators and about possible international coordination and cooperation in monitoring. Authors Dick van Oevelen and Lodewijk van Walraven are participating in several Terms of References (ToRs) of WGZE (found at: https://wgze.net/tors/) aimed at, among other things, integrating automated image analysis into zooplankton monitoring (ToR E) and improving representation of under-surveyed macrozooplankton and non-crustacean taxa (ToR F).
- Lodewijk van Walraven and Dick van Oevelen participated in **the ICES/PICES zooplankton production symposium** in Hobart, Australia on 17 – 22 March 2024, where the design and initial results of the MONS zooplankton monitoring were presented.
- Lodewijk van Walraven, Jeroen Hoekendijk and Dick van Oevelen are participating in the **Plankton Imager User Group (PIUG)** which consists of users and future users of the Pi-10 Plankton Imager and is aimed at developing common analysis pipelines and ecological indicators for the Pi-10 Plankton Imager. Aside from NIOZ and WMR, this group contains members from the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Plymouth Marine Laboratory (PML), Turing Institute and British Antarctic Survey (BAS). A first workshop was held in Lowestoft on 24 and 25

September during which research plans were discussed and agreements were made for the development of common research papers.

3.2 Zooplankton in OSPAR

Zooplankton-based indicators are mainly used within OSPAR to assess the status of marine food webs (OSPAR 2023b). Three different indicators (abbreviated as PH1/FW5, PH2 and PH3) are reported, generally looking at the extent at which the indicator for the assessment period (2015-2019 for QSR 2023) has changed compared to a previous reference period. These indicators are described below. Suggestions on how MONS monitoring can contribute to further development and evaluation of these indicators are made later in this document.

3.2.1 Zooplankton-based indicators

PH1/FW5 (Holland et al. 2023b) describes changes in plankton communities. This indicator does not look at trends at species level, but at trends in functional groups of "lifeforms" or groups of planktonic organisms with similar functional properties. Examples include diatoms, dinoflagellates, holoplankton, meroplankton (larvae), large copepods and small copepods. By using lifeforms instead of species, trends can be compared between areas with different properties and species composition. In addition, the variation in abundance within plankton species is often so high that this variation makes it difficult to link trends in abundance of a species to changes in environmental factors. For the various COMP4 sub-areas (Common Procedure for the fourth assessment of the degree of eutrophication), trends in abundance of the various functional groups are determined and these trends are linked to environmental variables.

PH2 (Louchart et al. 2023b) describes changes in phytoplankton biomass and zooplankton abundance. The Chl *a* concentration and/or the Phytoplankton Colour Index (PCI) are used as a measure of phytoplankton biomass. PCI is a semi-quantitative measure of phytoplankton biomass based on "greenness" of the filter from the Continuous Plankton Recorder (Batten et al. 2003). For zooplankton abundance, only the number of copepods is considered, and other plankton are not included. As with PH1, both variables are analysed for each OSPAR COMP4 sub-area to assess to what extent there are trends between the assessment period and the previous reference period. Here too, we examine which environmental factors appear to be the most important drivers for changes in phytoplankton biomass and zooplankton abundance.

PH3 (Louchart et al. 2023a) describes changes in plankton diversity, looking at both α-diversity (the diversity within a certain area) and β-diversity (here β-diversity refers to the change in diversity of a plankton community over time). This indicator was first tested on a limited number of phytoplankton time series (Rombouts et al. 2019) but expanded in QSR2023 to a wider range of time series of both phytoplankton and zooplankton from different COMP4 sub-areas. The data sources mainly consist of CPR data and longer-term point monitoring such as Plymouth L4 station. For the North Sea, this indicator still has the status of a pilot assessment.

The above indicators have also been used in the OSPAR QSR to estimate the consequences of reduced eutrophication (OSPAR 2023c). Logically, only the phytoplankton components of the indicators were considered in the QSR because there is a direct link with eutrophication.
Another indicator assessment for which zooplankton monitoring is relevant is the assessment of trends in new observations of Non-Native Species (NIS). For this purpose, a "New Introductions" parameter (P1) is determined that looks at the number of new introductions of NIS over a period of 6 years (Stæhr et al. 2022).

3.2.2 Zooplankton knowledge gaps and research suggestions from OSPAR 2023 QSR

For the various OSPAR indicator assessments, the QSR provides an overview of the most important knowledge gaps for the various indicators and suggestions to address them. An OSPAR Science Agenda will

soon be published based on the 2023 QSR (pers. comm Jos Schilder). Below is an overview of the knowledge gaps relevant to the MONS zooplankton monitoring and the Dutch North Sea from the 2023 OSPAR QSR with suggestions *in italics* on how the MONS zooplankton monitoring can contribute to addressing these knowledge gaps.

For **PH1/FW5**, additional research is recommended by OSPAR to explore direct mechanistic links between changes in environmental factors and changes in lifeform abundances. It is also recommended to investigate the consequences of changes in lifeform abundances for higher trophic levels.
Even though it is not specifically mentioned as a knowledge gap, data on the functional group of jellyfish is very limited. Therefore, no trend of jellyfish could be reported in the OSPAR QSR for virtually any COMP4 sub-area.

Specific suggestions include:

- Using national datasets with finer spatial and temporal resolution to explore the link between changes in pelagic habitats and plankton in relation to changes in climate and anthropogenic pressures*. The innovative techniques used in MONS plankton monitoring make it possible to map variation in plankton composition and abundance as well as environmental factors at a very fine spatial and temporal resolution. The MONS zooplankton research planned for PhD 1 and 2 will make an important contribution to this analysis.*
- There is a lack of monitoring data for variable salinity habitats (river plumes) in, among others, the North Sea. The CPR monitoring does not sufficiently cover these areas. *The MONS monitoring will make an important contribution to this by ensuring that the Rhine, Meuse and Scheldt plumes are sufficient covered by monitoring.*
- Basic properties such as diet are still unknown for many plankton species. *The planned MONS zooplankton PhDs can contribute here. Filling this knowledge gap is also on the agenda of ICES WGZE (ToR D and F).*
- Better understanding of the dynamics of pico- and nanoplankton is required as these are now excluded from CPR monitoring*. The Cytosense measurements as planned with RV Zirfaea on the MWTL can contribute to this.*

For **PH2**, OSPAR recommends adding additional datasets and comparing the indicators with the other indicators within pelagic habitats and food webs to get a more holistic picture of changes. It is also recommended to further improve the methodology, make better use of remote sensing data and refine the link between PH2 and pressure factors such as eutrophication.

Specific suggestions include:
As with PH1, address the lack of zooplankton data in variable salinity habitats.

- To further develop indicators, rely more on semi-automatic methods such as in vivo fluorescence and flow cytometry within existing monitoring programs. *The primary production and flow cytometry measurements as planned with RV Zirfaea on the MWTL sites can contribute to this.*
- To further develop indicators, rely more on automated imaging methods that enable higher spatial and temporal resolution within existing seasonal or monthly monitoring programs. *The planned and proposed continuous measurements with the Plankton Imager, such as with RV Zirfaea on the MWTL and RV Tridens on the WOT/ICES surveys, can contribute to this. This is also being worked on within WGZE ToR E.*
- Improve coherence and integration with eutrophication indicators. *This mainly applies to phytoplankton monitoring, but the MONS zooplankton monitoring also collects high-resolution data on* Phaeocystis *and* Noctiluca *abundance, which are interesting candidates for additional indicators linked to eutrophication.*

For **PH3**, OSPAR recommends that monitoring should be more tailored and structured at a regional scale, for example, in terms of taxonomic expertise and resolution, and to integrate semi-automated sampling methods such as flow cytometry and image analysis. Molecular methods such as DNA metabarcoding are also mentioned as potential methods to achieve high taxonomic resolution in analyses at lower costs. *The flow cytometry measurements as planned with RV Zirfaea on the MWTL can contribute to this. The data from*

plankton sampling with both DNA metabarcoding and microscopy from the MWTL sampling and also the NIOZ jetty Marsdiep sampling will probably be very useful in a number of years for the further development of this indicator.

Various knowledge gaps and recommendations are relevant for the assessment of the number of new Non-Native Species (NIS). It is recommended, among other things, to improve the speed of early detection of NIS and to expand the quality and quantity of NIS monitoring. It is also recommended to promote the use of new detection methods such as molecular methods*. The DNA metabarcoding data from both the MWTL sampling and the NIOZ jetty Marsdiep sampling can be very valuable for the early detection of NIS, both for plankton and benthos, as the majority of benthos have meroplanktonic larvae. The detection of potential NIS in the DNA metabarcoding data as reported in the 2023 progress report is an example of this.*

3.3 Zooplankton in the MSFD

The OSPAR indicators PH1/FW5, PH2 and PH3 are also used for **MSFD descriptor D1 biodiversity** for **pelagic habitats criterion D1C6**, with PH3 still being a pilot assessment. The knowledge gaps and recommendations for these OSPAR indicators are therefore directly relevant to the MSFD assessment.

The OSPAR indicator PH1/FW5 is also used for **MSFD descriptor D4** (Good Environmental Status (GES) of **food webs**), **criterion D4C1** (diversity of trophic guilds) and **D4C2** (balance between trophic guilds is not adversely affected by anthropogenic pressures). Indicators PH2 and PH3 are not used for MSFD descriptor D4.

Plankton indicators are not part of MSFD **criterion D4C3** (length distribution within trophic guilds is not adversely affected by anthropogenic pressure factors). In the Baltic Sea, a zooplankton-based indicator is used by HELCOM for MSFD descriptor D4C3; Zooplankton mean size and total stock (MSTS). This indicator is based on studies in which zooplankton biomass increases but average zooplankton size decreases with increasing nutrient input, which can have adverse effects on food availability for pelagic fish (HELCOM 2023). A zooplankton-based indicator may also be relevant for the Dutch MSFD assessment of D4C3. *Within the MONS zooplankton monitoring it will be possible to collect zooplankton length distribution data with very high resolution in space and time using Plankton Imaging. This data can be used for the assessment of D4. The results of the MONS zooplankton PhD projects will contribute to this. Work is also being done within ICES WGZE ToR A on size-based indicators, while within ToR E work is being done on the use of Plankton Imaging for, among other things, size-based indicators.*

The **MSFD descriptor for Non-Native Species (D2)** may also be important for MONS zooplankton monitoring because, as mentioned above, the analysis of zooplankton samples with DNA metabarcoding can potentially detect NIS at an early stage of introduction.

Finally, zooplankton monitoring within MONS can contribute to **MSFD descriptor for Eutrophication (D5)**, by mapping changes in zooplankton biomass and composition that could possibly be the result of eutrophication. Specifically for D5C3 (blooming of pest algae), zooplankton monitoring can contribute because Plankton Imager sampling with RV Tridens and Zirfaea can probably also be used to monitor the density of pest algae such as foam algae (*Phaeocystis*) and sea sparkle (*Noctiluca*).

3.3.1 Integration of MONS zooplankton monitoring into MSFD/OSPAR summarized

As indicated above, MONS zooplankton monitoring can make important contributions to the assessment of the Good Environmental Status of the North Sea by filling knowledge gaps within the OSPAR and MSFD assessments. To determine trends, monitoring will first have to be carried out for a number of years. However, prior to this, monitoring is already valuable for the research and development of indicators, especially because of the high spatial and temporal resolution of data on plankton composition. Data on plankton size distribution will hopefully allow a detailed analysis of the relationship of plankton indicators with

changes in environmental factors and anthropogenic pressures. Due to the high taxonomic resolution of the DNA metabarcoding monitoring, Non-Indigenous Species can probably be detected more quickly.

4 Coastal survey

4.1 Introduction

For the MONS projects Monitoring pelagic fish phase 1 and Monitoring zooplankton phase 1, Wageningen Marine Research conducted a survey with RV Tridens II which was made available by RWS (Van Walraven et al., 2023). The purpose of the survey was to sample small pelagic fish in the Dutch coastal zone to collect data on the distribution of pelagic fish in space and time, particularly aiming to understand the distribution of food for seabirds and marine mammals. In addition, the time at sea was used to deploy innovative techniques for sampling zooplankton aiming to develop and test these techniques for use within the MONS monitoring and to gain insight into the spatial distribution and composition of zooplankton in the Dutch coastal zone.

Conducting zooplankton sampling at the same time as sampling pelagic fish may provide insight into the relationship between the presence of pelagic fish and their food, the zooplankton.

Pelagic fish are found in the water column where they swim freely, often in rapidly moving (large) schools. This rapidly changing heterogeneous distribution requires a monitoring design in which large portions of the sampling area are systematically mapped reasonably quickly. Therefore, when sampling pelagic fish, a design is usually chosen in which transects are sailed while an acoustic method is used to map the distribution of fish in the water column. To validate the acoustic data, nets are used to collect fish regularly of which species and length are determined.

For the MONS monitoring, transects were chosen to sail in a zigzag pattern covering the entire coastal zone from the Belgian to the German border. The fishing trawl stations were systematically planned in space. The purpose of the plankton sampling was to collect samples and data to investigate the spatial distribution of zooplankton, partly in relation to the occurrence of pelagic fish. For this purpose, the standard method of net sampling was extended to include continuous measurements of zooplankton by plankton imaging. Plankton net sampling was scheduled at the same locations as the fish sampling so that the catches could be linked.

4.2 Materials and Methods

4.2.1 Acoustics

Acoustic recordings took place with a Simrad EK80 echo sounder with so-called "splitbeam transducers" with frequencies of 38, 70, 120, 200 and 333 kHz. The transducer is located in the drop keel of the Tridens and was lowered 0.5m below the ship (draft 4.2m) during use, so the total depth of the transducers was 4.7m. The echosounder was used in wideband mode for all frequencies except 38 kHz. A ping interval of 0.4 seconds was targeted, but failed during the first two weeks. The actual ping interval was about 1.2 seconds. Starting midway through the third week, recordings were made at 0.4 seconds. The pulse length was 0.256 seconds. The data were stored on an on-board NAS.

4.2.2 Fish sampling

During the first fishing haul, the Pelagic Flex Net 480m, which had been developed by the Rijksrederij for this monitoring, was used. Because it was damaged during the first haul of the survey, a GOV net was subsequently used. This is the standard gear in the IBTS survey (ICES, 2020). The GOV net is a bottom net with a net height of ~5.5m. 15-minute hauls were conducted at the pre-planned locations. The catches were

brought in and processed in the fish processing deck. Here, the catch was sorted by species and then a representative subsample of specimens were measured to the nearest mm. The benthos species in the catch were counted and weighed. The jellyfish were also identified to species and from a (sub)sample the weight and number of each species was determined.

4.2.3 Secchi disk

Prior to each fish sampling, a Secchi disk measurement of water transparency was performed. This was done by lowering the Secchi disk on the shadow side of the vessel until it was no longer visible. Then the disk was raised, and the moment the disk became visible, the total length of line from the disk to the surface of the water was examined and recorded.

4.2.4 Plankton Imager

4.2.4.1 Image acquisition

A Pi-10 Plankton Imager was borrowed from Plankton Analytics because the newly ordered device could not be delivered and installed on time. This system has been deployed on the British research vessel Endeavour in recent years (Scott et al., 2021). The Tridens is the second ship on which the Plankton Imager has been deployed.

The Plankton Imager, supplying pump and control box are installed in the back of the port side net hold. Fiber optic connections run from the Plankton Imager to the dry- and hydrographic labs. From these labs, the Plankton Imager's pump can be turned on and off and the pump speed controlled.

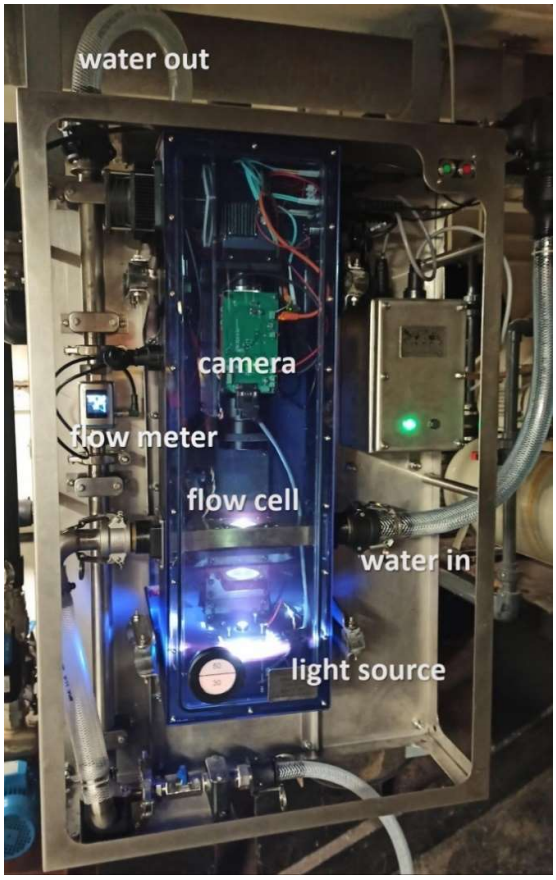


Figure 8 Overview of the Pi-10 system.

For the Pi-10, water is pumped from the intake point port side centre aft of the vessel at a depth of about 4.5 m depending on the draft of the vessel. This water passes at a rate of about 34 L/min through a flow cell

in the Plankton Imager so that about 2 m^2 is sampled per hour. The flow rate is controlled by the pump's variable speed drive and by partially closing the valve in the outlet. After passing the Plankton Imager, the water is discharged.

The Plankton Imager contains an LED light source that projects a parallel light beam onto the flow cell. A camera on the other side of the flow cell receives this beam. Particles in the flow cell (partially) block the light source, casting a shadow on the camera's image sensor; this technique is called "shadowgraph." The image data from the Plankton Imager was sent via the fibre optic link to a receiving PC (PI-PC) in the dry lab. This PC extracted images of individual particles from the continuous stream of image data from the Plankton Imager based on preset values for minimum and maximum particle size, among others. A selection of images was sent via a UDP data stream over a LAN network to a second computer that converts the data stream back into individual images and wrote them to an external Solid State Drive (SSD). On the survey transect, the Plankton Imager ran continuously, except for during the nights when no activity was taking place.

For this cruise, the number of images collected was set at 10,000 per minute. The device logged how many images were collected per minute and how many are discarded so that a subsample factor can be calculated. The size range was set to 200 µm – 2 centimetres.

4.2.4.2 Image processing and analysis

During and after the survey, collected images were evaluated and an initial set of classes was selected based on taxonomy, life stage, appearance and condition of the organisms. Images were selected for these classes non-randomly with the aim of having as wide a range of image quality, size and orientation as possible and a minimum of 100 images per class. A first learning set of 9855 images of 58 classes was used in an initial training experiment where different network sizes and configurations were evaluated for their performance. The ResNet deep learning architecture was chosen for this task because it is widely applied for similar problems and CEFAS also used ResNet for their Pi-10 classifier.

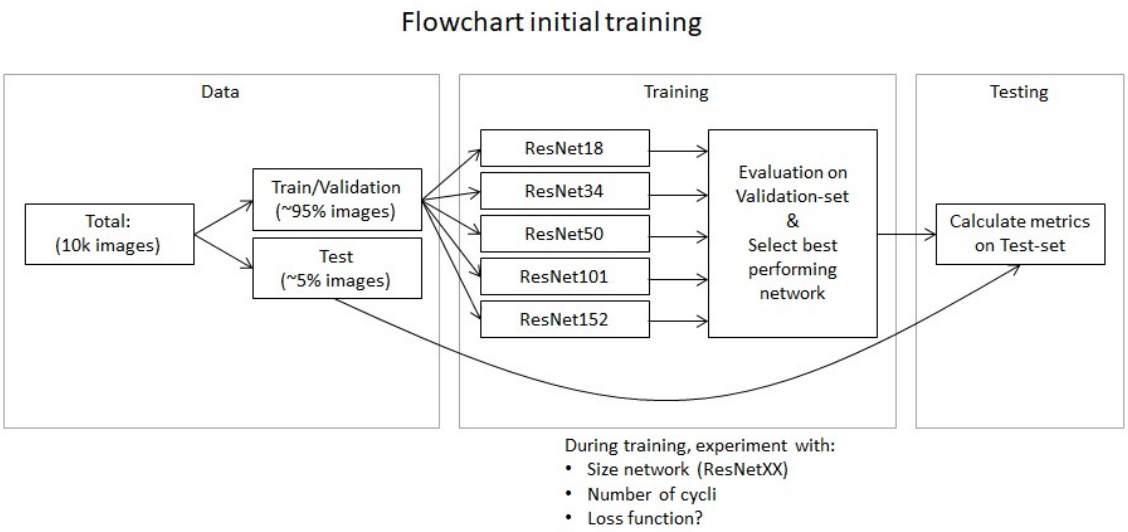


Figure 9 Flowchart showing the process used for the initial training cycle for the Plankton Imager classifier.

Network training and image classification was performed on the WUR Anunna HPC cluster on 2 NVIDIA A100 processing units. After the initial round of training it was decided to use a ResNet 50 network and a model was trained using a batch size of 600, 20 cycles of training only on fully connected layers and 10 cycles of training on the whole network (Figure 9).

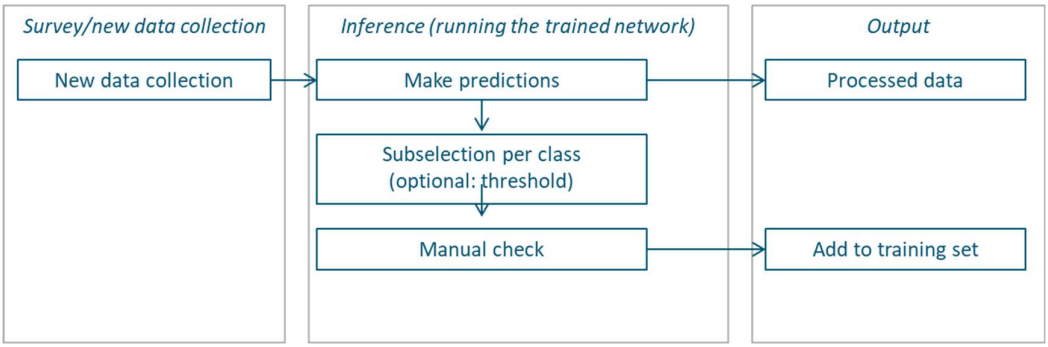


Figure 10 Flowchart of the process used for validating the model results and extending the learning set.

After each training round the network was used to classify the unlabelled images in the dataset (Figure 10). A random subsample of images of each class was selected and manually evaluated for classification accuracy. These manually classified images were added to the next iteration of the learning set to increase the amount of images available and improve performance on rare classes. When necessary, classes were added, combined or removed. Following this, the network using the new learning set was retrained and the process was repeated. This was done a total of three times to maximize the amount of images of rare classes in the learning set. A selection of rare classes images from later surveys were added to the learning set as well.

For analysis the amount of images per class were counted for each 10 minute of transect. This was converted to estimated density by dividing with the estimated volume imaged per 10 minutes which was 340 L. No analysis of biomass based on organism sizes on the images has been performed as part of this study.

4.2.5 WP2 plankton net and CTD

A WP2 net with a diameter of 57 cm, a length of 2.6 m and a mesh size of 200 µm was used to collect zooplankton samples. Due to supply problems, a flow meter could not be mounted in the net. As a substitute, the sampled water volume was estimated by using the length of the payed out cable as the tow length. A MiniCTD (Valeport) was attached above the WP2 net for profiling depth, temperature and salinity.

The WP2 net was deployed from starboard using a winch used for CTDs. Sampling was conducted to a depth of 5 m above the bottom, measured from the top of the net. The rate of lowering and retrieval of the net did not exceed 0.5 m/s. Upon surfacing, the net was hosed down with salt water from the outside. The collection jar containing collected plankton was exchanged for a clean one after which a second sample was taken.

On board, the samples taken were split into two halves with a Motoda box splitter. One half, or a further subsample thereof, if necessary, was concentrated over a sieve and fixed on "Steve 1" solution (formalin). The other half was further split into a smaller subsample of $^1/_8$ or $^1/_{16}$, concentrated and stored on the preservative DESS (Yoder et al 2006) in 50ml Greiner centrifuge tubes for DNA metabarcoding analysis. If jellyfish were present in the samples, they were removed by pouring the sample through a colander, after which their volume, species and individual length in cm were determined.

4.2.6 Transect

A total of 980 nmi of survey transect was covered (Figure 11). In addition to the sea miles travelled on the planned transect, another approximately 300 nm was travelled for logistics such as getting to- and from the start and end of the transect.

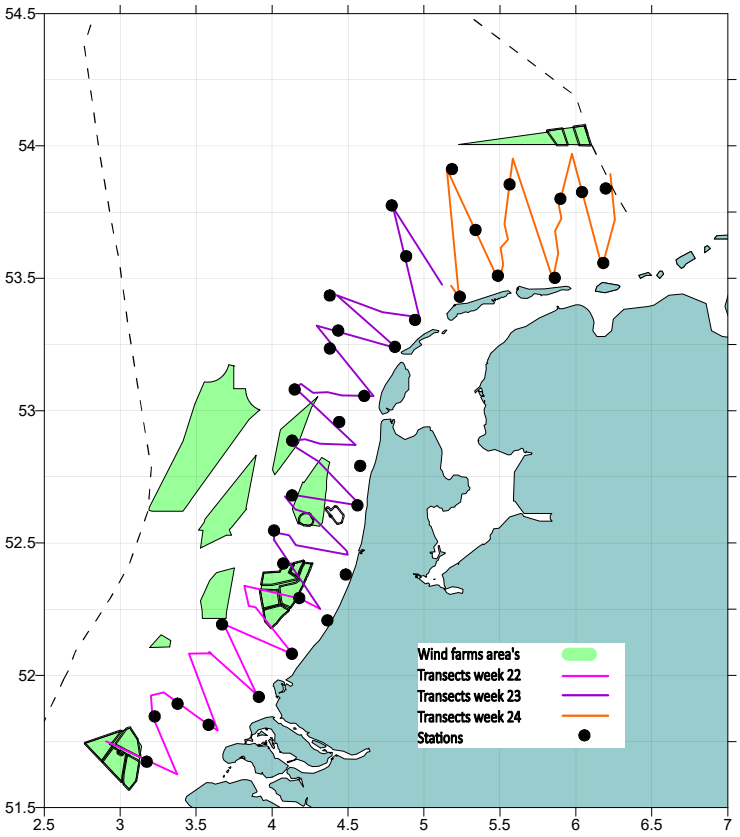


Figure 11 The sailed transects with the sampled stations indicated by black dots.

There were 36 stations sampled on the transects. At each station, a fish tow was conducted, a Secchi disk measurement was taken, and two WP2 samples and CTD casts were taken. At one station,, near-shore west point Terschelling, zooplankton samples were taken before and after the fish haul, resulting in an additional zooplankton sample. Additional samples from WP2 catches were preserved with DESS at a number of sites to investigate the suitability of this agent for morphological analysis with zooscan and/or microscopy. Due to a dead battery, no CTD recordings were stored for the stations on June 13 and the first station on June 14.

4.2.7 WP2 net samples: microscopic analysis

Eight net samples were analysed using microscopy. These were selected to have a good coverage of the whole transect. In the lab, the samples were rinsed to remove formaldehyde and, if necessary, split with a Folsom plankton splitter to obtain a representative subsample. This sample was examined using a stereomicroscope and organisms present were identified, with copepods being identified to the species level if possible. If necessary, copepods were dissected to examine identification characteristics using a microscope.

4.2.8 WP2 net samples: DNA metabarcoding

The DNA extraction and DNA amplification of the DESS samples was performed at the dedicated DNA laboratory of Wageningen Environmental Research. First, to remove DESS from the sample, the 50ml tubes containing the zooplankton were spun at full speed for 2 min, after which DESS was gently poured off keeping a close eye on the pellet. DNA from the zooplankton was extracted using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's protocol, but excluding the bead-beating step and vortex adapter, adding a 2 hour incubation step with proteinase K at 56 °C and including negative extraction controls in each extraction batch of 23 samples.

For next-generation sequencing, a two-step PCR protocol was used to create a dual index amplicon library using the following primers:

- COI - COI_mlCOIintF_v2, COI_jgHCO2198 (modified from Geller et al. 2013)
- 18SV4 - 18Sv4_TAReuk454FWD1, 18Sv4_TAReukREV3_v1 (Stoeck et al., 2010, Van den Heuvel et al., 2021)
- 18SV9 - Euk_1391f, EukB2 (Amaral-Zettler et al, 2009)

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. Each reaction consisted of 12,5 µl, including 1 U Platinum Taq (Fisher Scientific), 1× PCR buffer, 2.5 mM $MgCl_2$, 5 % (m/m) Trehalose, 200 ng/µl BSA, 200 µM dNTP, 250 µM water and 2.0 µl of sample DNA extract. The program consisted of 2 min activation at 94 °C, followed by 15 cycli of 30 s denaturation at 94 °C and annealing for 3 min using a touchdown program starting at 56 °C (for both 18S markers) or 53 °C (COI) and decreasing by 1 °C each cycle (touchdown PCR), 1 min elongation at 72 °C, followed by 20 cycli of 30 s at 94 °C, 3 min at 39 °C and 10 min final extension at 72 °C. 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 fastq files were demultiplexed by IGAtech, who provided R1 and R2 files for each sample. All files were renamed into the QIIME2 format and imported into the QIIME2 platform version 2023.2 (Bolyen et al., 2019). The cutadapt plugin (Martin, 2011) was used to delete forward and reverse primers from both the R1 and R2 sequences, using a minimum sequence length of 80 bp and a maximum error rate of 0.15, discarding any reads that were untrimmed. The sequences were subsequently joined, denoised into ASVs (Amplicon Sequence Variants) and chimeras removed using the DADA2 plugin (Callahan et al., 2016). After visual inspection, the forward and reverse reads were truncated during this step at 200 bp (COI) or 90 bp (18SV9), while no read truncation was applied for 18SV4 due to the large length variation at this locus (Callahan et al., 2016). After this step, only for COI, only ASVs with a length of 3 bp shorter and longer than the expected 313 bp amplicon size were kept. For COI, the VSEARCH tool was used to cluster the resulting ASVs into 98% clusters (OTUs; Operational Taxonomic Units) to reduce the number of sequences with identical taxonomic identifications (Rognes et al., 2016). Since we did not know the barcoding gap for the 18S markers, we did not cluster these ASVs into OTUs. Finally, to determine whether a sequence represented an actual biological sequence or actually a sequencing or PCR error, the LULU algorithm was used applying the standard settings (Frøslev et al., 2017).

For COI, taxonomy assignment was performed using the BOLDigger package version 2.1.1 (Buchner and Leese, 2020) 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 and 18SV4, 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% (Mousavi-Derazmahalleh et al., 2021). 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 using the following steps: all unassigned and non-target identifications were removed, including Archaea, Bacteria, Fungi and Viridiplantae since the selected primers are not optimized for these groups. All sequences with less than 10 reads in total were removed from the dataset. If a sequence had more reads in any of the negative controls than in any of the samples, it was removed from the dataset. No tag-switching was detected. Subsequently, the maximum remaining read count of any sequence in a negative control was subtracted from all samples. After these 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 rrarefy function in the 'vegan' program prior to other analyses. Consequently, the sequencing depth was 280k reads for COI, 303k reads for 18SV4 and 520k reads for 18SV9.

To test whether the recovered communities at the different distances from the coast were significantly different, the Bray-Curtis dissimilarity index was calculated using the relative abundance data between each pair of samples using the vegdist function within vegan in R (Oksanen et al., 2018). The corresponding values were ordinated with a NMDS and visualized using the ordiplot function in vegan, grouped per distance from coast-category. The statistical significance was tested using a permutational multivariate analysis of variance (perMANOVA) with 999 permutations, using the adonis2 function in vegan.

4.2.9 WP2 net samples: sample scanning

Formaldehyde preserved samples were stained with Bengal Rose a day prior to sample analysis to enhance contrast of smaller and transparent organisms on the scans. An Epson perfection v850 flatbed scanner was used for scanning the samples. Samples were size-fractionated using a calibrated 500 µm sieve, and fractions larger- and smaller than 500 µm were scanned separately. Prior to scanning, samples were split using a Motoda box splitter if necessary. For scanning, samples were poured into custom made glass trays, and organisms where separated and distributed along the tray as much as possible. Samples were scanned in 8 bit grayscale at 3200 dpi. Further processing was done with the standard ZooProcess analysis pipeline for zooscan images (Gorsky et al.2010) which extracts Regions Of Interest (ROIs) from the scans. These ROIs were uploaded to EcoTaxa (https://ecotaxa.obs-vlfr.fr/) where images can be pre-classified using a classification algorithm, after which all the predictions were manually checked and corrected by experts.

All samples were scanned using the zooscan, but because the setting up of the pipeline and the analyses took much more time than expected, only eight scans were selected to allow for comparison with the microscopic analyses.

4.2.10 Comparison of techniques

Comparison of the estimated abundance of different taxa with different techniques was done between zooscan and microscopy and between microscopy and Plankton Imager. If one technique had a higher taxonomical resolution than the other, the abundance estimates for the different taxonomical subgroups were combined. To get an estimate of density by Plankton Imager at each sampling station the average density per Plankton Imager group was calculated for the 10-minute averages closest to the sampling time at the station.
The relationship between organism density, method used for density estimation and organism group was investigated using generalized linear mixed models (GLMM) with a Tweedie distribution and a log link function to model the densities. Sampling station was included as a random effect. Models of increasing complexity were compared by their AIC (Akaike's Information Criterion) values with the model with the lowest AIC value chosen as the best fit.
These models compared:
M1: *organism density ~ organism group*
M2: *organism density ~ method*
M3: *organism density ~ method + organism group*
M4: *organism density ~ method + organism group + method * organism group*
The package glmmTMB (Brooks et al. 2017) was used with R 4.3.3. Validation of residuals versus predicted values was done using the R DHARMa package (Hartig, 2024).

4.3 Results

Data on acoustics and fish sampling will be reported separately (Couperus et al. in prep).

4.3.1 Abiotic parameters

Secchi depth at the sites sampled for zooplankton ranged from 1.8 m to over 6.5 m. Depths greater than 6.5 m could not be measured. Secchi depths averaged about 5 m and visibility was generally lower near the (shallower) shore than in deeper water further offshore (Figure 12).

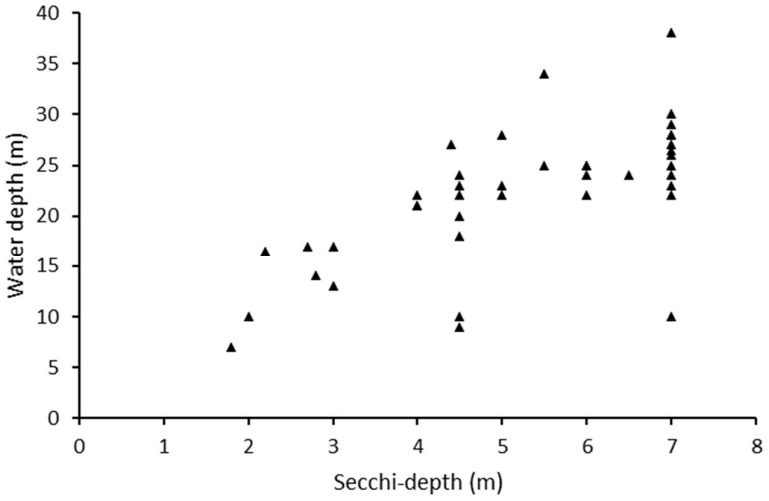


Figure 12 Relationship between Secchi-depth (m) and water depth (m), where Secchi-depths higher than 6.5 m are indicated as 7 m.

4.3.2 WP2 net samples: DNA metabarcoding

All 86 samples were analysed successfully for the three markers COI, 18SV4 and 18SV9 (Figure 13). In total 319 taxa were detected with COI of which 238 were merozooplankton, 42 were holozooplankton, 28 were phytoplankton, 7 were parasites and 5 were macroalgae. With 18SV4, 276 taxa were detected of which 115 were merozooplankton, 30 were holozooplankton, 67 were phytoplankton, 60 were protists, 3 were parasites and 5 were macroalgae. Finally, with 18SV9 300 taxa were detected of which 131 were merozooplankton, 32 were holozooplankton, 67 were phytoplankton, 58 were protists, 12 were parasites and 5 were macroalgae. COI thus detected the most merozooplankton as well as holozooplankton taxa, followed by 18SV9.

Not all taxa could be detected to the species level for all markers. Selecting only taxa that could be identified to species level, the amount of species detected per marker can be compared, as is summarised in Figure 13. for holoplankton and Figure 14. for meroplankton. 257, 89 and 67 species-level taxa were detected for COI, 18SV9 and 18SV4, respectively. 53 unique species were detected for holoplankton and 243 unique species were detected for meroplankton. 7 species of holoplankton were detected by all markers and 20 species of meroplankton were detected by all markers. When looking at species that were only detected by a single marker; 25 holoplankton and 150 meroplankton species were detected only with COI, 6 holoplankton and 16 meroplankton species were detected only with 18SV9 and 2 species of holoplankton and 7 species of meroplankton were only detected with 18SV4.

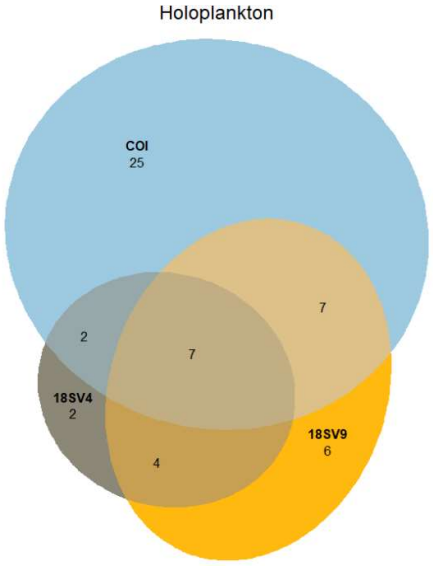


Figure 13 Venn diagram showing the number of species of holoplankton detected with the three different markers, and how many species were detected by multiple markers

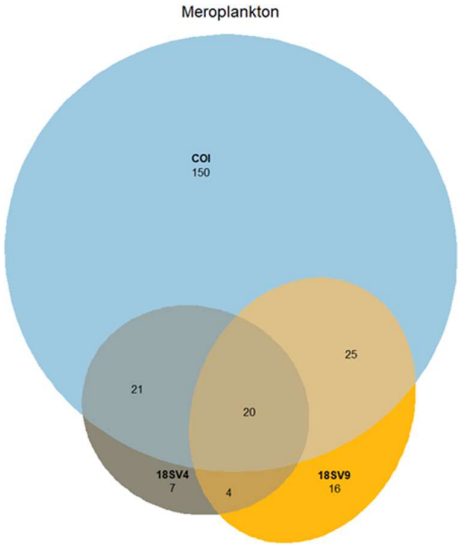


Figure 14 Venn diagram showing the number of species of meroplankton detected with the three different markers, and how many species were detected by multiple markers.

4.3.2.1 Species composition

With a total of 413 species level detections across each three markers, and 895 taxa in total, it is impossible to describe all the interesting results and patterns present in the data. For all taxa detected by the three markers, a map was created showing the number of reads per stations. All these maps (n = 895) are supplied as supplementary reports per marker. To summarise DNA metabarcoding data, interactive Krona plots can be used to get an overview of the community composition found for each marker. These plots are presented below for each marker (Figure 15, Figure 16, Figure 17), and are also available as interactive documents. Following, the findings for some commonly found zooplankton taxa are described, and plots for all species are reported in the Supplement-DNA reports.

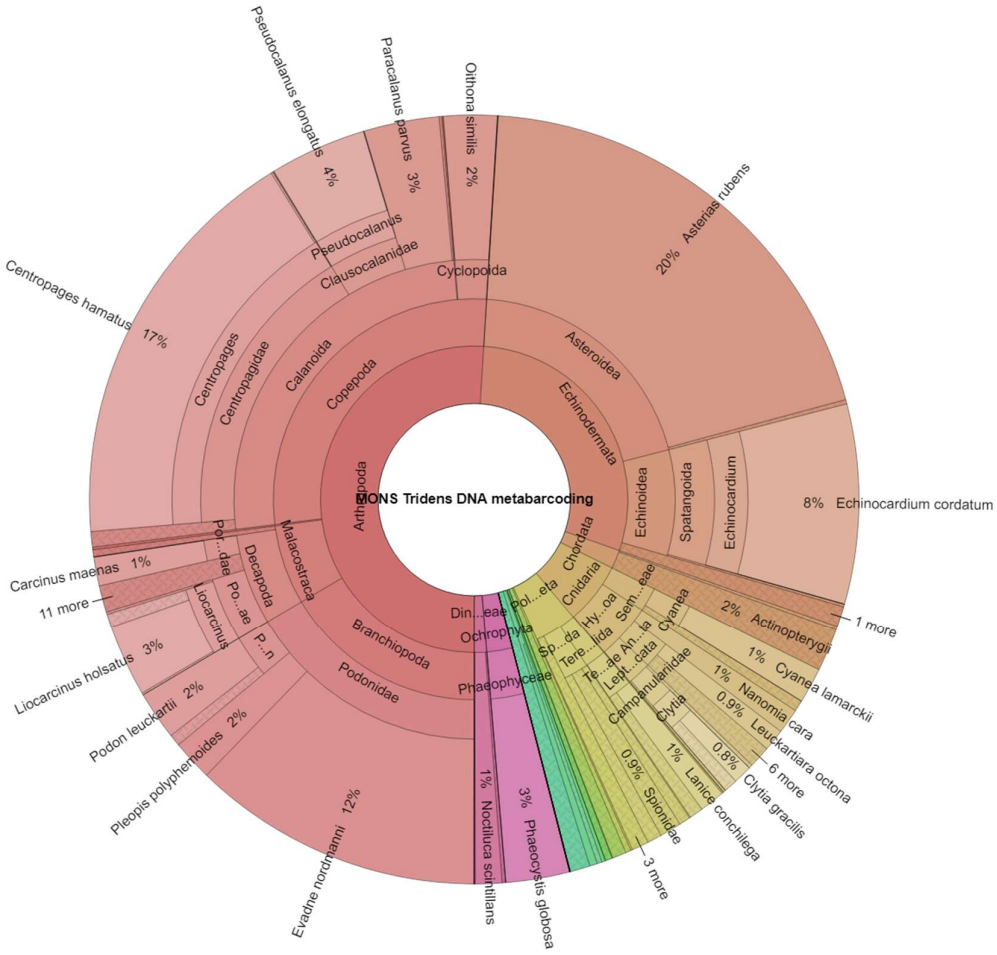


Figure 15 Krona plot of COI data showing the average composition of the samples based on the mean number of reads per taxon.

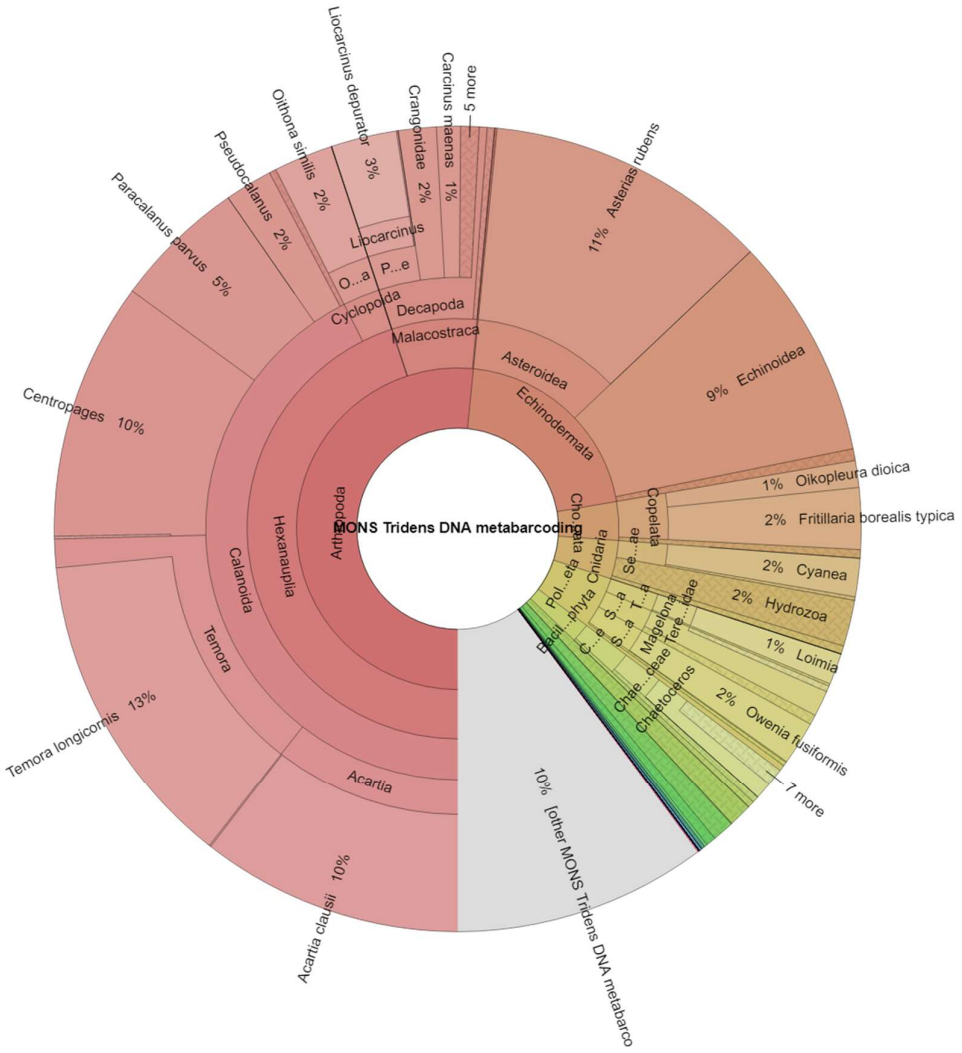


Figure 16 Krona plot of 18SV4 data showing the average composition of samples based on the mean number of reads per taxon.

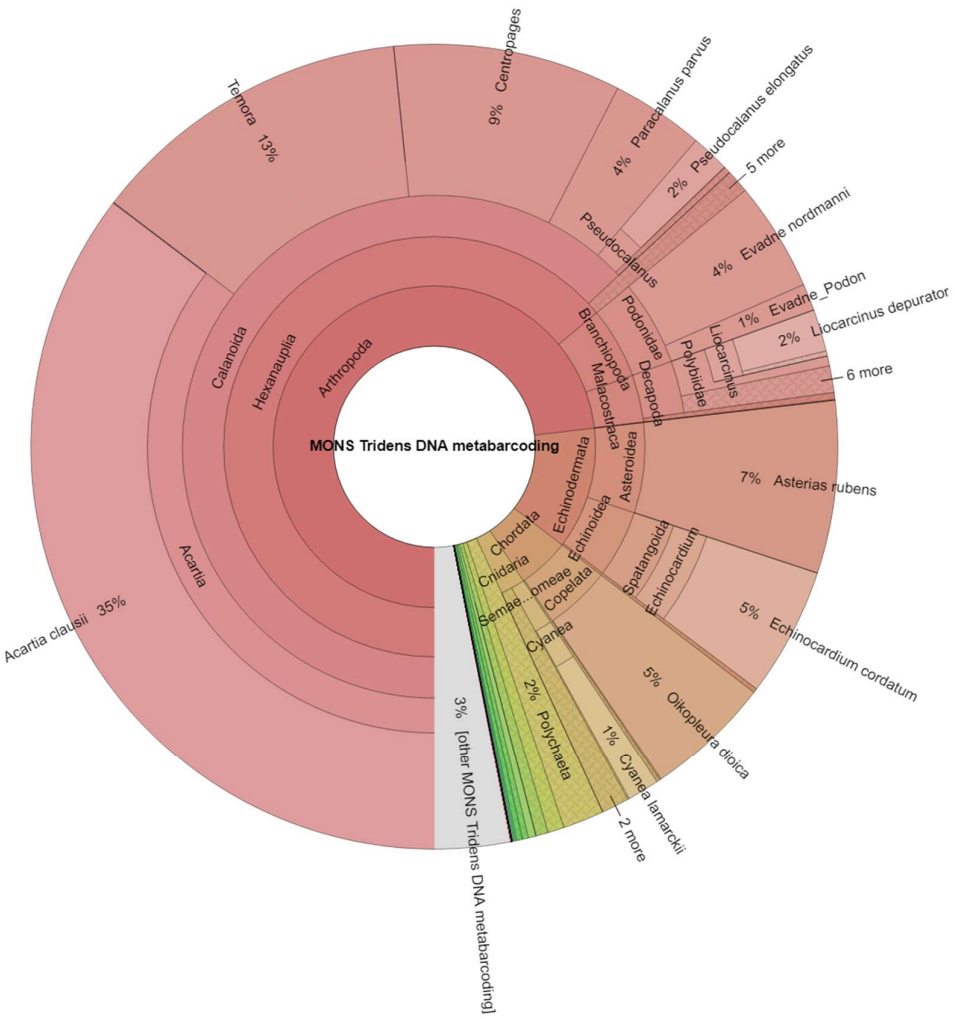


Figure 17 Krona plot of 18SV9 data showing the average composition of samples based on the mean number of reads per taxon.

Copepods

With COI 34 copepod taxa were detected (Table 1); 16 Calanoida, 3 Canualloida, 3 Cyclopoida, 10 Harpacticoida and 2 Siphonostomatoida. 28 of these were species-level detections. When verifying the data, two of these species were considered doubtful. For *Pseudocalanus mimus* only 15 reads were found, which suggests that these might be sequencing errors in commonly found *Pseudocalanus elongatus* sequences. For *Oncaea waldemari* the sequence coverage was only 98.71%, making this identification doubtful.

After verification, several possible new species for the Dutch North Sea remained. *Pseudocalanus moultoni* was detected at 10 stations. This species was already detected earlier in the Dutch North Sea by Laakmann et al. (2013). Samples were checked to confirm the presence of *Pseudocalanus moultoni* but this was not successful, as identification of *Pseudocalanus* species is often not possible (Castellani & Edwards,2017). *Tortanus discaudatus,* a western Atlantic species, was detected at 7 stations (Figure 18). The presence of this species could be confirmed using microscopy.

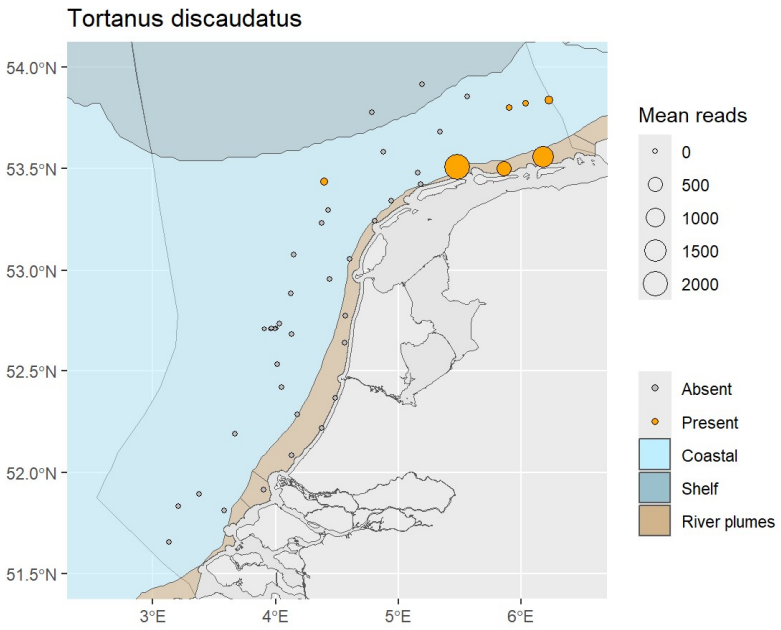


Figure 18 Map showing mean number of COI reads of Tortanus discaudatus *in WP2 net samples. At gray points the taxon was not detected at the station. OSPAR Eutrophication areas are plotted in the background as different colour shades*

Table 1 overview of copepod taxa detected with COI. Uncertain identifications are highlighted orange (see text)

Order	Family	Genus	Species
Calanoida	Acartiidae	Acartia	NA
Calanoida	Acartiidae	Acartia	*Acartia bifilosa*
Calanoida	Acartiidae	Acartia	*Acartia clausi*
Calanoida	Acartiidae	Acartia	*Acartia tonsa*
Calanoida	Calanidae	Calanus	*Calanus helgolandicus*
Calanoida	Centropagidae	Centropages	*Centropages hamatus*
Calanoida	Centropagidae	Centropages	*Centropages typicus*
Calanoida	Centropagidae	Isias	*Isias clavipes*
Calanoida	Clausocalanidae	Microcalanus	*Microcalanus pusillus*
Calanoida	Clausocalanidae	Pseudocalanus	*Pseudocalanus elongatus*
Calanoida	Clausocalanidae	Pseudocalanus	*Pseudocalanus mimus*
Calanoida	Clausocalanidae	Pseudocalanus	*Pseudocalanus moultoni*
Calanoida	Paracalanidae	Paracalanus	*Paracalanus parvus*
Calanoida	Pseudodiaptomidae	Pseudodiaptomus	*Pseudodiaptomus marinus*
Calanoida	Temoridae	Temora	*Temora longicornis*
Calanoida	Tortanidae	Tortanus	*Tortanus discaudatus*
Canuelloida	Canuellidae	Canuella	*Canuella perplexa*
Canuelloida	Longipediidae	Longipedia	NA
Canuelloida	Longipediidae	Longipedia	*Longipedia coronata*
Cyclopoida	Corycaeidae	Ditrichocorycaeus	*Ditrichocorycaeus anglicus*
Cyclopoida	Oithonidae	Oithona	*Oithona similis*
Cyclopoida	Oncaeidae	Oncaea	*Oncaea waldemari*
Harpacticoida	Ameiridae	NA	NA
Harpacticoida	Cylindropsyllidae	Evansula	*Evansula pygmaea*
Harpacticoida	Ectinosomatidae	NA	NA
Harpacticoida	Ectinosomatidae	Microsetella	*Microsetella norvegica*
Harpacticoida	Leptastacidae	Leptastacus	*Leptastacus aff. laticaudatus*
Harpacticoida	Leptastacidae	Paraleptastacus	*Paraleptastacus espinulatus*
Harpacticoida	Miraciidae	Amphiascopsis	*Amphiascopsis cinctus*
Harpacticoida	Paramesochridae	NA	NA
Harpacticoida	Tachidiidae	Euterpina	*Euterpina acutifrons*
Harpacticoida	Tisbidae	Tisbe	*Tisbe elegantula*
Siphonostomatoida	Caligidae	Caligus	NA
Siphonostomatoida	Pennellidae	Lernaeenicus	*Lernaeenicus sprattae*

Of the Cyclopoida, Oithona similis was the most common species having a distribution restricted to the northern part of the surveyed area (Figure 19). Most of the species of Harpacticoida are assumed members of the meiofauna and are often not considered in zooplankton studies, making validation of the status of these species in the Dutch fauna difficult.
Other species of copepods were species already observed in the Dutch North Sea before, as described in the species list in this report.

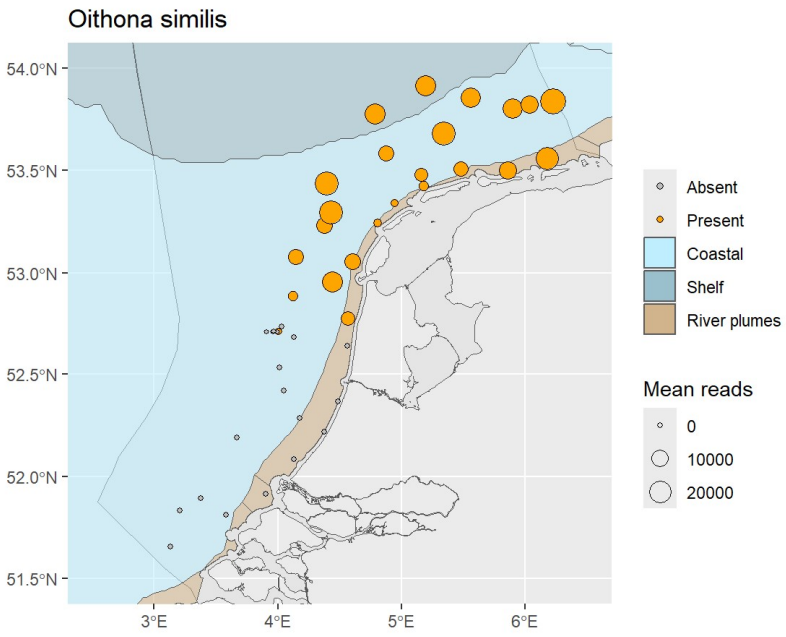


Figure 19 Map showing mean number of reads of Oithona similis *in WP2 net samples. At gray points the taxon was not detected at the station. OSPAR Eutrophication areas are plotted in the background as different colour shades*

Larvaceans

Interestingly, no larvacean taxa were detected with COI, even though they were abundantly found with other methods. With 18SV4 and 18SV9 both *Fritillaria borealis* and *Oikopleura doica* were detected. These species exhibited a marked difference in distribution patterns with *F. borealis* restricted to the northern part of the survey (Figure 20) and *O. doica* being ubiquitous throughout the surveyed area.

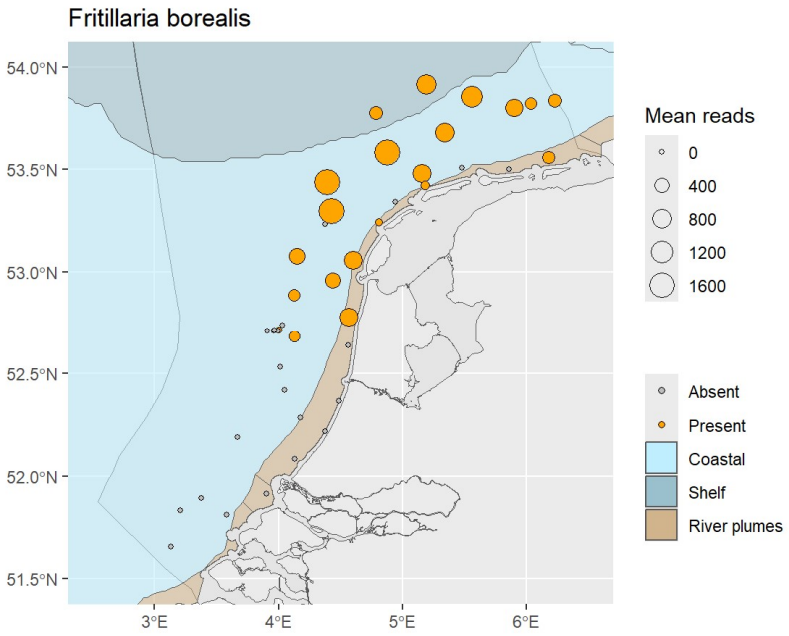


Figure 20 Map showing mean number of 18SV9 reads of Fritillaria borealis *in WP2 net samples. At gray points the taxon was not detected at the station. OSPAR Eutrophication areas are plotted in the background as different colour shades.*

Cladocera

With COI four different species of cladocera were detected; *Pleopis polyphemoides*, *Podon intermedius*, *Podon leuckartii* and *Evadne nordmanni*. *Evadne nordmanni* and *Podon leuckartii* were found throughout the surveyed area, while *Podon intermedius was* found only in the southern part (except for a single northern station), and *Pleopis polyphemoides* (Figure 21) was mainly observed close to the coast. With 18SV9 only *Evadne nordmanni* could be detected to species level with the other species likely aggregated in the sequence for "*Evadne/Podon* sp.". With 18SV4 no cladocera were detected.

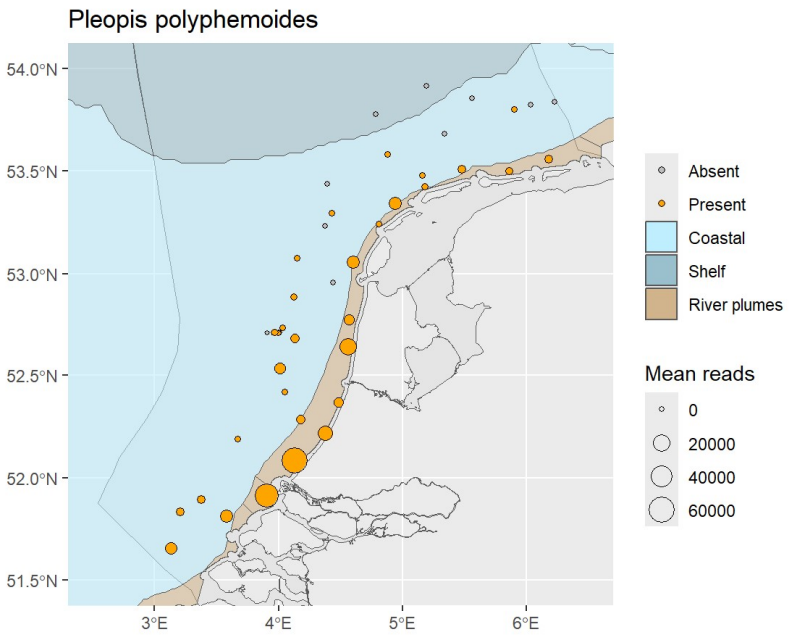


Figure 21 Map showing mean number of COI reads of taxon Pleopis polyphemoides *in WP2 net samples. At gray points the taxon was not detected at the station. OSPAR Eutrophication areas are plotted in the background as different colour shades.*

Chaetognaths

COI detected both *Parasagitta elegans* and *Parasagitta setosa*, though both species appeared rare during the survey. With 18SV4 these species were detected as well, and on a single station *Parasagitta bipunctata* was detected. With 18SV9 only *Parasagitta elegans* was found despite reference sequences for *Parasagitta setosa* being available.

Ctenophora

Five different ctenophore taxa were found with COI of which one (*Beroe* sp.) was only to genus level. Species found were native species *Pleurobrachia pileus*, *Bolinopsis infundibulum* and *Beroe cucumis*, and the invasive species *Mnemiopsis leidyi*. *Pleurobrachia* was found at stations throughout the surveyed area while *Beroe cucumis* and *Bolinopsis infundibulum* were mainly found in the northern part. *Mnemiopsis leidyi* was limited to the nearshore stations.

Cnidaria

With COI 33 Cnidaria taxa were detected; four of these were Scyphozoa (jellyfish), 25 were hydrozoa with an alternating jellyfish-polyp life cycle, and three were likely meroplanktonic larvae; *Sagartia troglodytes*, *Alcyonium digitatum* and *Cerianthis lloydii*). With COI also one siphonophore species was detected, *Nanomia* cara, which was found on northern, offshore stations. 18SV9 and 18SV4 both detected only 21 cnidaria, including siphonophores to the order and family level, respectively.

Other arthropods

Aside from cladocera and copepoda COI detect 50 different crustacea taxa. 16 of these were amphipods, 4 were cumacea, 21 were decapods, 3 isopods, one mysid, 4 barnacles and one euphausid. The euphausid *Euphausia recurva* was likely a result of an error in the reference database and was omitted from further analysis. The decapods (crabs and shrimp) spanned a wide range of species known for the area, including

commercially important species *Crangon crangon* and *Cancer pagurus*. With 18SV4 22 other arthropods were detected, though only 12 of these at species level. 18SV9 detected 25 other arthropods, of which 16 at species level.

Polychaetes

With COI 56 polychaete taxa were detected of which 51 to species level, with 18SV4 29 (14 to species level) and with 18SV9 24 (9 to species level). All of these except *Tomopteris helgolandica* are benthic species and thus meroplanktonic. Species of note were the reef-building polychaetes *Sabellaria alveolata* and *Sabellaria* spinulosa (Figure 22). Interestingly, with COI *Sabellaria spinulosa* was detected and with 18SV9 *Sabellaria alveolata,* at exactly the same stations. A check revealed that it is likely that the 18S sequence is not species specific in this case, and that the COI identification of *S.* spinulosa is the correct one. The recently discovered large terebellid *Loimia ramzega* was also detected at 17 stations with COI (Figure 23).

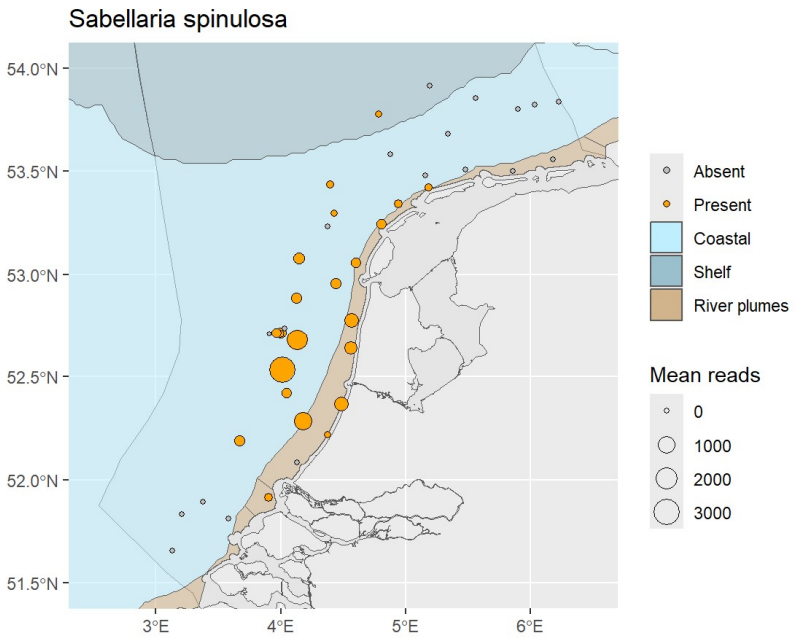


Figure 22 Map showing mean number of COI reads of Sabellaria spinulosa *in WP2 net samples. At gray points the taxon was not detected at the station. OSPAR Eutrophication areas are plotted in the background as different colour shades.*

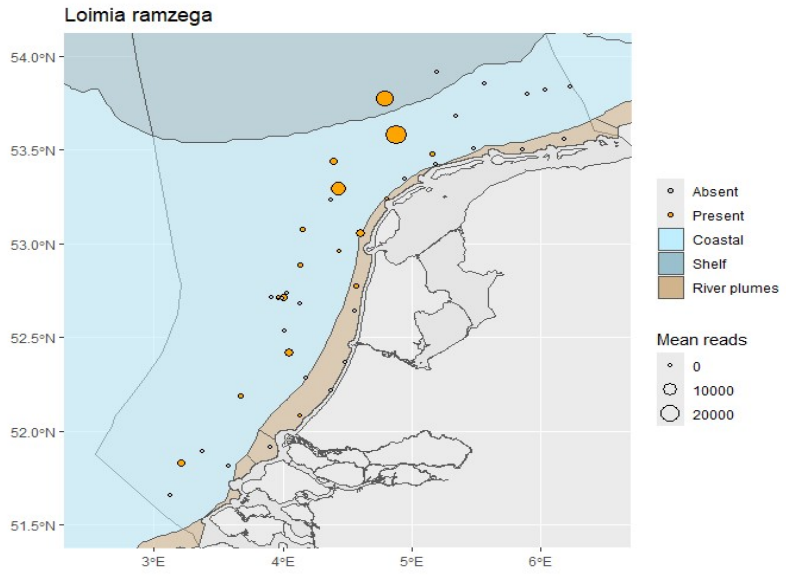


Figure 23. Map showing mean number of COI reads of taxon Loimia ramzega *in WP2 net samples. At gray points the taxon was not detected at the station. OSPAR Eutrophication areas are plotted in the background as different colour shades.*

Molluscs

COI detected 54 mollusc taxa of which 31 were bivalves, 2 were cephalopods and 21 were gastropods. 43 of these were species-level taxa. 18SV9 detected 31 mollusc taxa; 14 bivalves and 17 gastropods, of which 11 to species level. 18SV4 detected 19 mollusc species; 7 bivalves and 12 gastropods, of which 9 to species level. No holoplanktonic molluscs like *Limacina* sp. Were detected, all molluscs were likely meroplanktonic larvae. With COI, taxa relevant for policy like *Spisula* and *Ensis* could be identified to species level with COI.

Many taxa that were found in the DNA metabarcoding data are poorly studied and it was thus difficult to judge whether these were new to Dutch waters. Table 2 provides an overview of detections rare and possibly new species for Dutch waters, for benthos as well as plankton.

Table 2 Overview of new and rare species detected in the coastal survey

Species	Group	Marker
Trizona brandti	Acantharia	18SV4
Hyperoche medusarum	Amphipoda	COI
Themisto sp. *(abyssorum)*	Amphipoda	COI
Heliospora caprellae	Apicomplexa	18SV9
Tortanus discaudatus	Copepoda	COI
*Tisbe elegantula (*uncertain*)*	Copepoda	COI
Amphiascopsis cinctus	Copepoda	COI
Loxosomella stomatophora	Entoprocta	18SV4
Helgicirrha cari	Hydrozoa	COI
Nanomia cara	Hydrozoa	COI
Paramunna bilobata	Isopoda	COI
Calliopaea bellula	Mollusca	18SV4, 18SV9
Embletonia pulchra	Mollusca	COI
Atalodoris inconspicua/sparsa	Mollusca	COI
Alloteuthis media	Mollusca	COI
Cephaloidophora cf. *communis*	Myzozoa	18SV9
Callinera grandis	Nemertea	18SV4
Siphonenteron bilineatum	Nemertea	18SV4
Tenuilineus albocinctus	Nemertea	COI
Hubrechtella dubia	Nemertea	COI
Stylochus zebra	Platyhelminthes	18SV9
Prosorhynchoides megacirrus	Platyhelminthes	18SV9
Bucephalus minimus	Platyhelminthes	18SV9
Polygordius lacteus	Polychaeta	COI
Polydora onagawensis	Polychaeta	COI
Meiodrilus adhaerens	Polychaeta	COI
Protodrilus oculifer	Polychaeta	COI
Loima ramzega	Polychaeta	COI

4.3.2.2 Quantitative comparison between markers

The number of reads detected per species can give an indication of the abundance of species, though at the moment this should be considered semi-quantitative at best, as the resulting amount of reads in the dataset can be influenced by many factors in the analysis pipeline of the samples (van der Loos & Nijland 2020). However, especially within single taxa between locations, spatial patterns in read abundance could be observed in the DNA data (as seen in the example maps presented below) that were similar to patterns observed in the other data sources.

The Krona plots (Figure 15, Figure 16, Figure 17) and the tables in the supplementary data report provide a highest read abundance followed by *Centropages hamatus, Evadne nordmanni, Echinocardium cordatum , Pseudocalanus elongatus and Paracalanus parvus.* For 18SV9 *Acartia clausii* had the highest mean read abundance*, followed by Temora sp., Centropages sp., Asterias rubens, Echinocardium cordatum, Oikopleura doica and Evadne nordmanni.* For 18SV4 *Temora longicornis* had the highest mean read abundance, followed by *Asterias rubens, Acartia clausii, Centropages sp., Noctiluca scintillans, Echinoidea* indet (this might be *Echinocardium cordatum*) and *Paracalanus parvus.* Although the ranking of the most abundant species differed, the taxa that were most abundant partly matched between markers. One notable exemption was *Temora longicornis* which was one of the dominant taxa according to the 18S markers, but had very low read abundance (201 reads on average per sample) for COI. Looking at the amount of reads for Calanoid copepods, for COI 26 % of reads belonged to Calanoida, while for 18SV4 and 18SV9 this was much higher, at 43 % and 63%, respectively.

4.3.2.3 Sampling effort

The relationship between the sampling effort and the amount of taxa detected was investigated for the marker COI using a Species Accumulation Curve (Figure 24). For this curve a number of samples are analysed randomly from the total sample pool, with the number of taxa detected for a given amount of samples determined. The curve is levelling off at the maximum number of samples and suggests that at half the sampling effort most of the taxonomic diversity would still be detected.

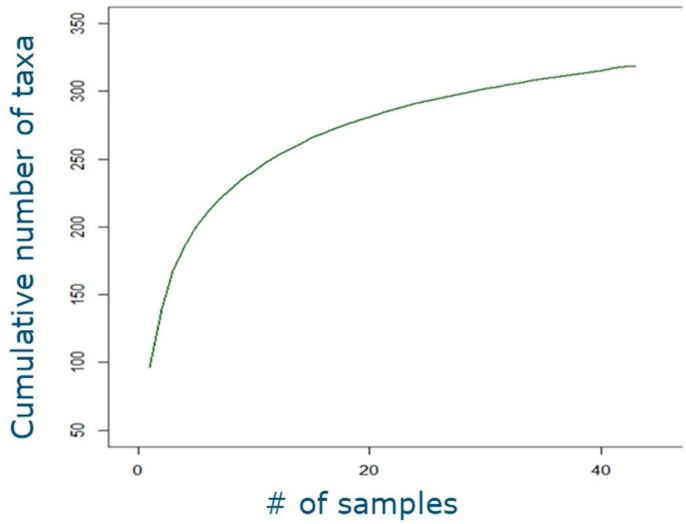


Figure 24 Species Accumulation Curve showing the amount of taxa detected with COI with increasing sampling effort (amount of samples).

4.3.2.4 Spatial variation in zooplankton communities

Variation in community species composition was assessed where two geographic areas were distinguished, a near shore area, comprising the OSPAR Eutrophication areas categorised as "river plume", and an offshore area, categorised by OSPAR as "coastal" and "shelf" (see also Figure 18 - Figure 23). Based on CO1 data, the NMDS plot as presented in Figure 25 shows a clear distinction in community composition between these two areas. Future monitoring results may be able to distinguish different communities between the OSPAR eutrophication areas.

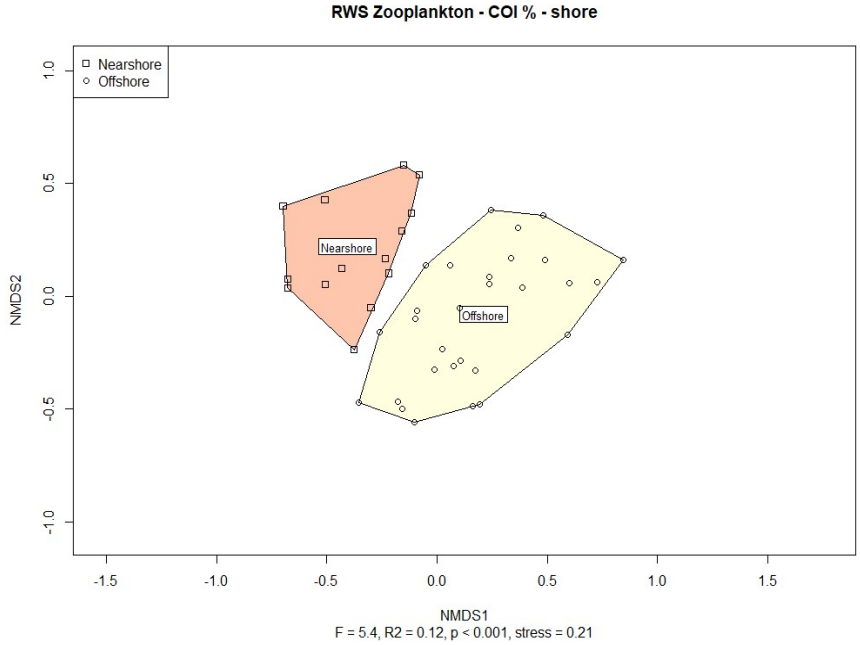


Figure 25 NMDS plot showing the presence of different communities based on COI data, where nearshore include the stations in the "river plume" area and offshore include the "coastal" " shelf" stations as indicated in Figure 18 - Figure 23.

4.3.3 WP2 net samples: microscopic analysis

In total, 45 taxa and/or life stages were distinguished with microscopy of which 19 were merozooplankton and 26 were holozooplankton. The ten most abundant taxa in the samples were *Acartia* sp. copepods (mean density 1.7 ind L^{-1}, mainly *A. clausii*), echinoderm pluteus larvae (mean density 1.5 ind L^{-1}), *Evadne* sp. Cladocera (mean density 1.3 ind L^{-1}), larvaceans (mean density 1 ind L^{-1}), *Temora longicornis* copepods (mean density 1 ind L^{-1}), *Podon/Pleopis* cladocera (mean density 0.6 ind L^{-1}), *Oithona* sp. copepods (mean density 0.5 ind L^{-1}), sea star branchiolaria larvae (mean density 0.3 ind L^{-1}), *Centropages* sp. copepods (mean density 0.3 ind L^{-1}, mainly *C. typicus*) and unidentified Calanoid copepods (mean density 0.2 ind L^{-1}). A Krona plot based on the average abundance of all microscopically identified taxa is presented in Figure 27.

The full list of copepod taxa identified were:

- *Acartia clausii*
- *Calanus helgolandicus*
- *Centropages hamatus*
- *Centropages typicus*
- Harpacticoida
- *Isias clavipes*
- *Labidocera* sp.
- *Oithona similis*
- Oncaeidae
- *Paracalanus* sp.
- *Pseudocalanus* sp.
- *Temora longicornis*
- *Tortanus discaudatus*

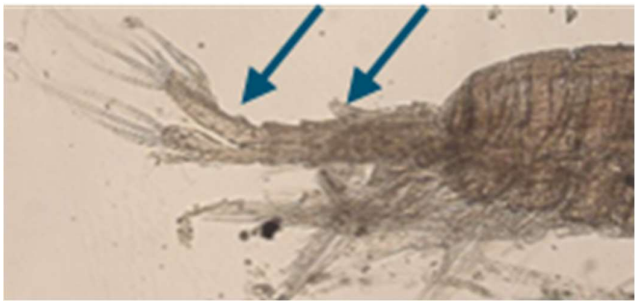


Figure 26 Microscopic identification of Tortanus discaudatus*, showing discriminating characteristics of the asymmetric urosome.*

Tortanus discaudatus was not found in routine microscopic sample analysis, but was detected in the DNA metabarcoding data (Figure 26) and subsequent targeted searching also revealed the species in the microscopic samples.

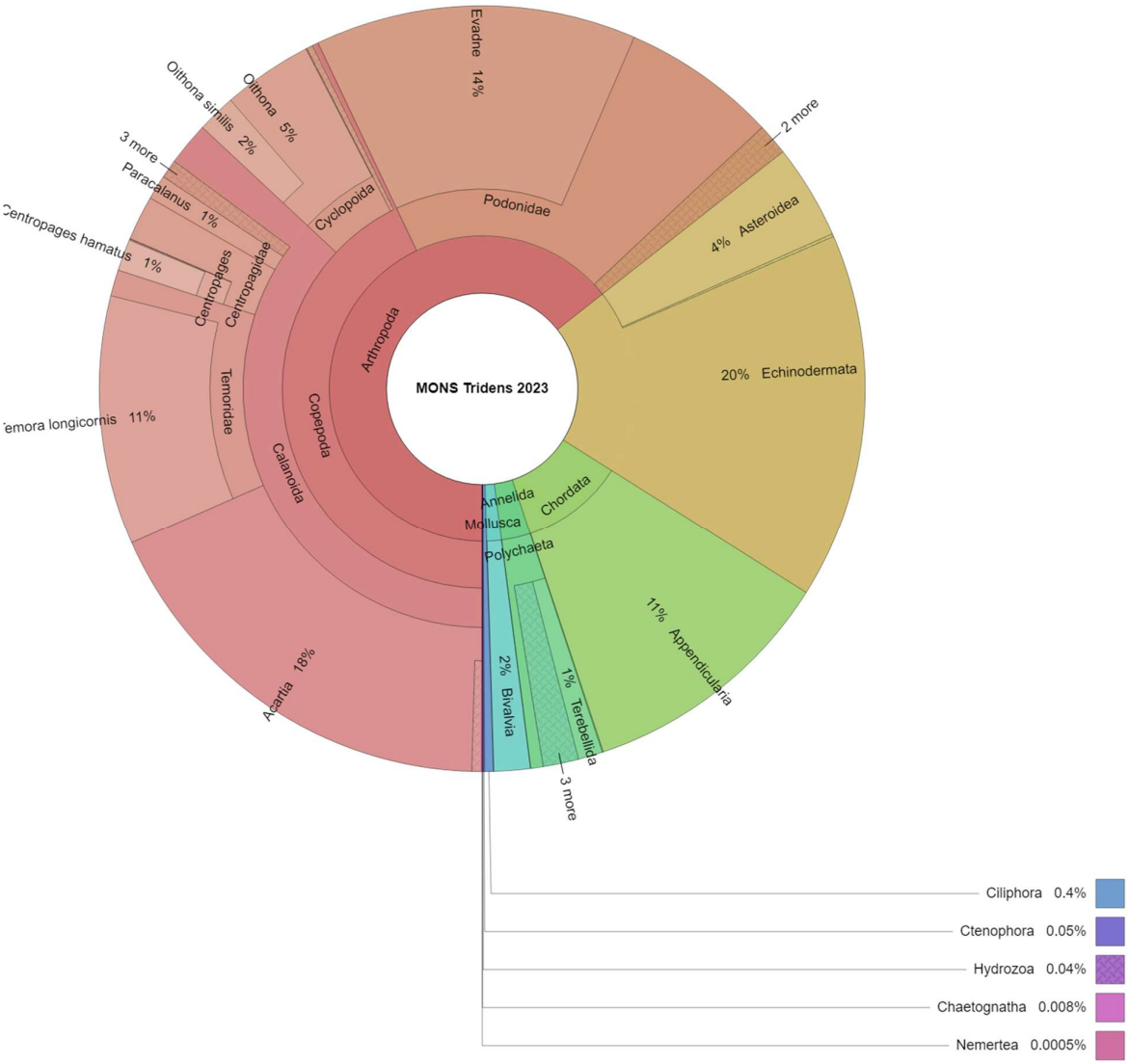


Figure 27 Krona plot of average density of different taxa in microscopy samples.

4.3.4 WP2 net samples: zooscan

Figure 29 shows the mean density in numbers per L for each group found in the zooscan samples. All taxonomic groups included in the training set are presented, and the results show that some of these groups were not present in the analysed samples.

High density of echinoderm larvae and appendicularian houses caused issues in preparing and processing the scans. Echinoderm larvae and appendicularian houses may form dense clusters in the samples which also entangle organisms like copepods (Figure 28). Separating these clusters is very time consuming and often not possible without damaging the organisms. The ZooProcess pipeline extracts these clusters as single Regions Of Interest (ROI) which poses challenges in the automated EcoTaxa classification, and individuals in clusters are not counted automatically. To get an estimate for the amount of organisms that is currently missed in this routine zooscan analysis due to their inclusion in clusters, the number of individuals in these clusters were manually counted (by vision). It is shown in Figure 29 (in red) that for some taxa such as *Noctiluca*, copepods and the cladocerans *Evadne* sp. and *Podon* sp. a large fraction of individuals would be missed in routine classification, in case organisms are stuck together in clusters.

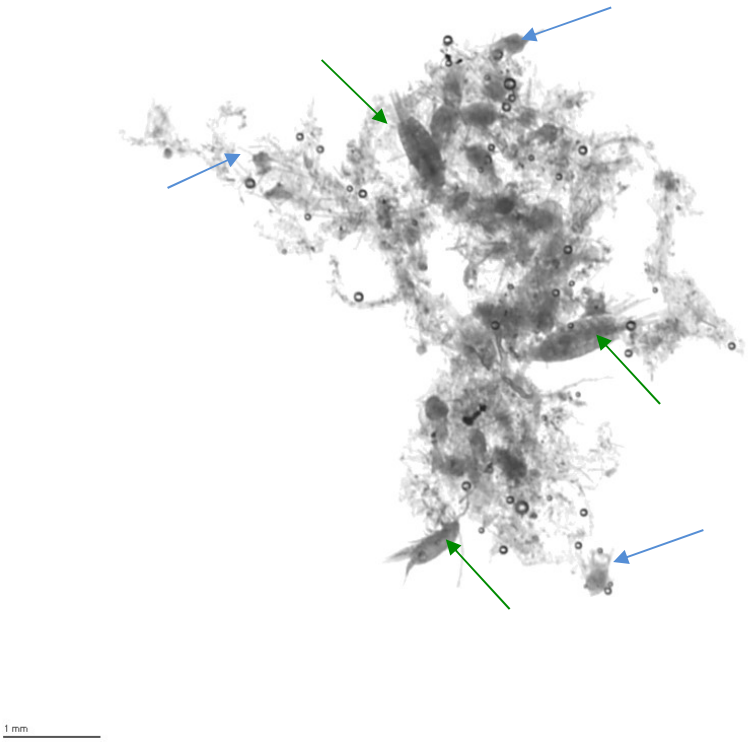


Figure 28. An example of a cluster of organisms in a scanned sample, including echinoderm larvae (some indicated with blue arrows) and copepods (some indicated with green arrows).

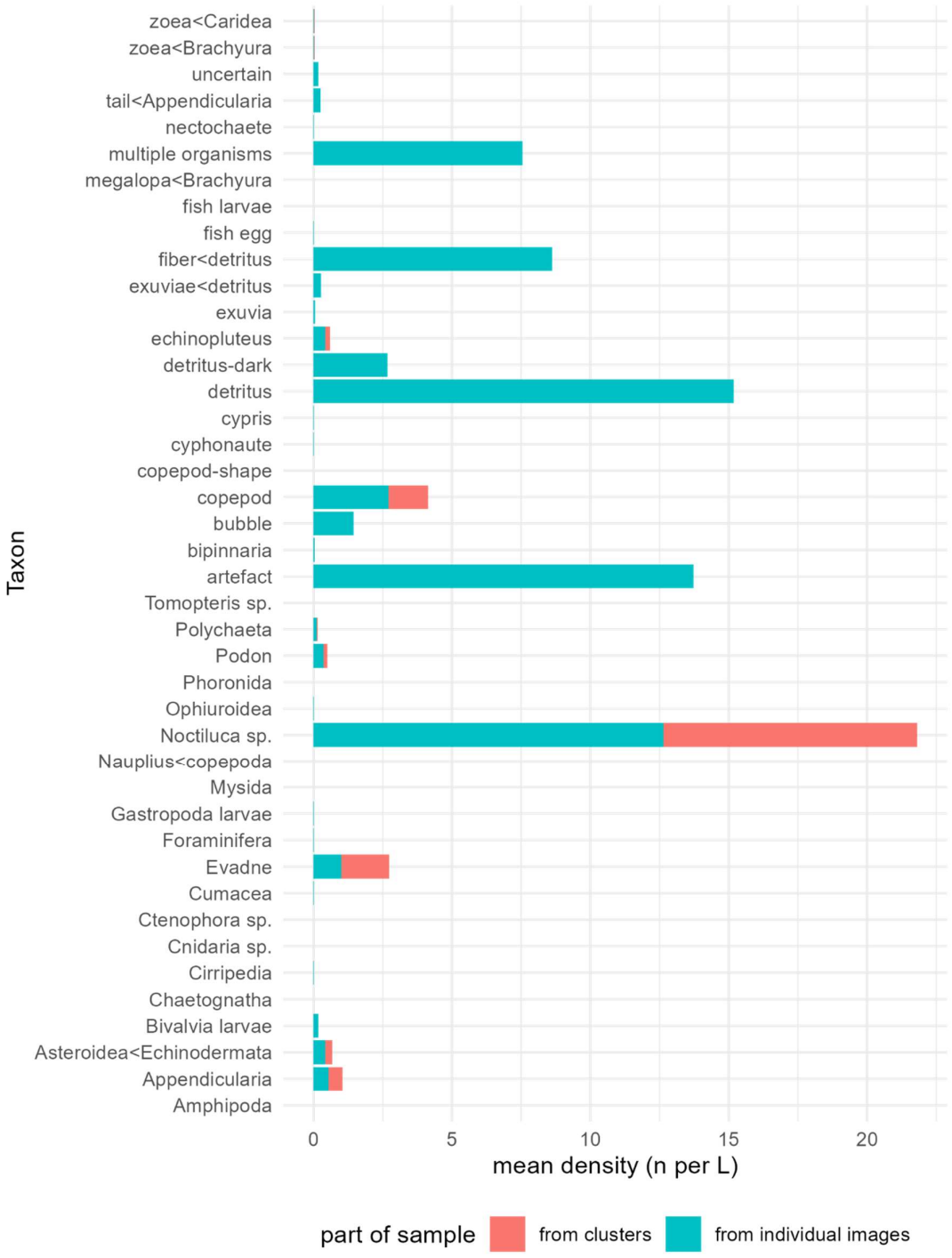


Figure 29. Mean density for different zooscan groups. The red part of the bar shows the additional density estimated from organisms counted in clusters that were missed in the routine analysis.

4.3.5 Plankton Imager

4.3.5.1 Learning set and model training

The Plankton Imager ran continuously during the survey, but experienced some issues with overheating of the PC when particle density was very high (this PC has since been replaced by a more powerful one). On 12 June there was an issue with fogging of the flow cell, and no usable data could be collected. This was resolved in the morning of June 13.

The total amount of images collected along the transect was 85,124,560. From these images an initial learning set (V1) of 9,855 images belonging to 58 classes was collected. This classifier was used to perform a set of experiments to find the optimal training parameters and model size.

After training the ResNet 50 model, 100 images per class were randomly sampled from the classified data. This revealed that about 50% of images were detritus that was incorrectly classified as another class, as detritus was not yet included as a class. Subsequently detritus was included as an additional class and the manually validated images included in the learning set (V2). For rare classes that were uncommon in the data of the coastal survey, such as fish eggs and fish larvae, additional images from other surveys (where these were more commonly detected) were added to the learning set (V3) and the model retrained with a learning set of 17,350 images in 63 classes. Again, a random sample of images per class, this time 300, were manually validated and included in the learning set, increasing it to 32,132 images (V4), after which the model was retrained again and the process was repeated. The results in this report are based on learning set V4 of 32,132 images. Using our iterative approach of growing the learning set resulted in a learning set with 100+ images for the majority of classes (Figure 31). The resulting ResNet 50 model achieved good results on the test set (Figure 30) with a mean precision of 93.1 %, mean recall of 91.0% and mean F1 score of 91.5. Precision is the percentage of images in the test set that the model assigned to a class where this was the correct class. A high precision means fewer false positives (an image was incorrectly classified as belonging to the class). Recall is the percentage of images of a certain class that were classified correctly. A high recall means fewer false negatives (an image was not classified as belonging to the class). F1 score combines the precision and recall scores and shows the balance between precision and recall. The confusion matrix (Figure 32) showed that for most classes the predictions are good, but that organisms, or fragments thereof, are still often classified as detritus.

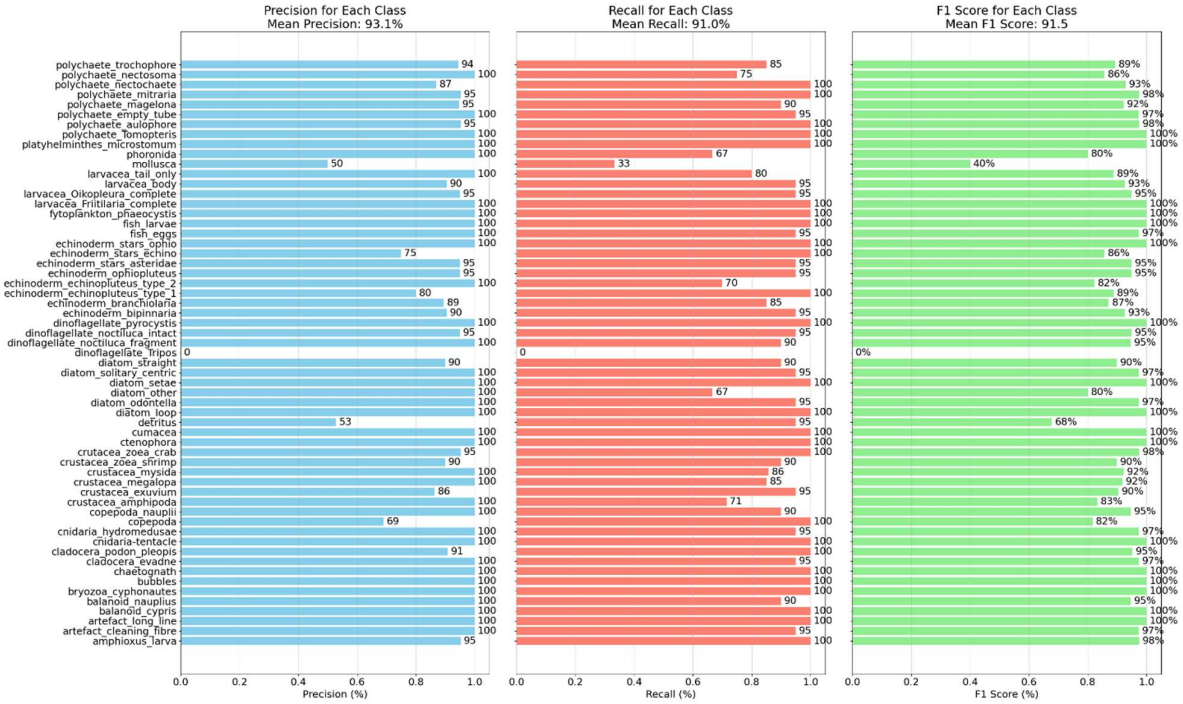


Figure 30. Precision, recall and F1 score (see text) for each class in the ResNet50 model used for classification of the data in this report.

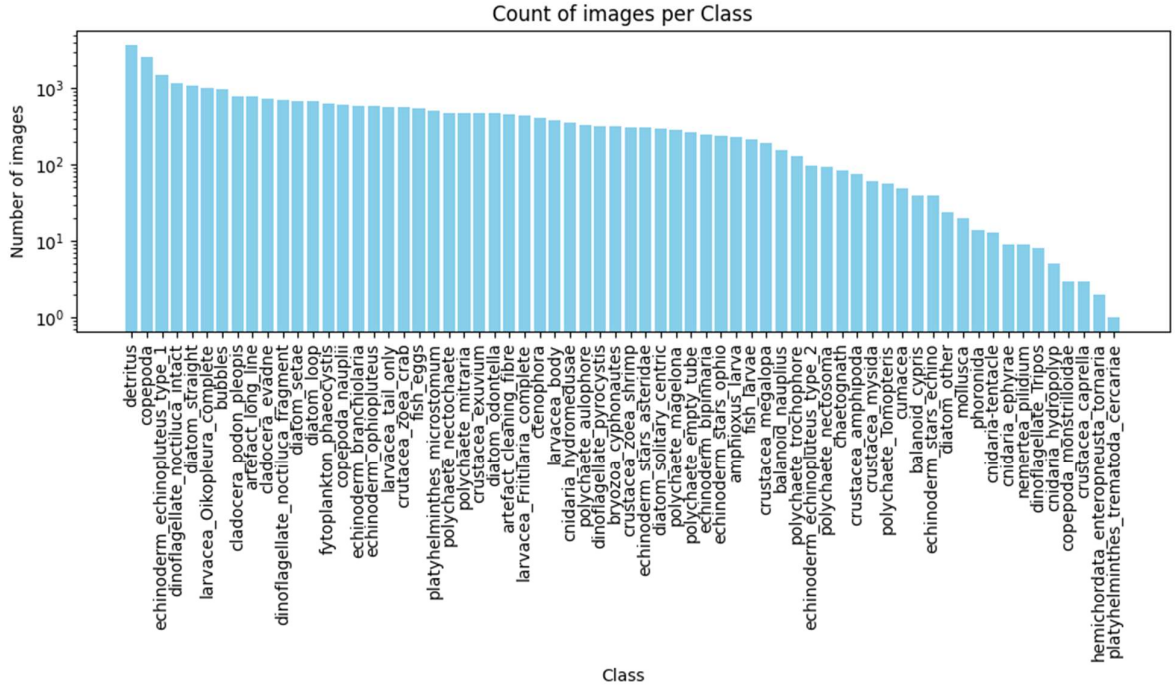


Figure 31 Number of images per class in the Pi-10 learning set.

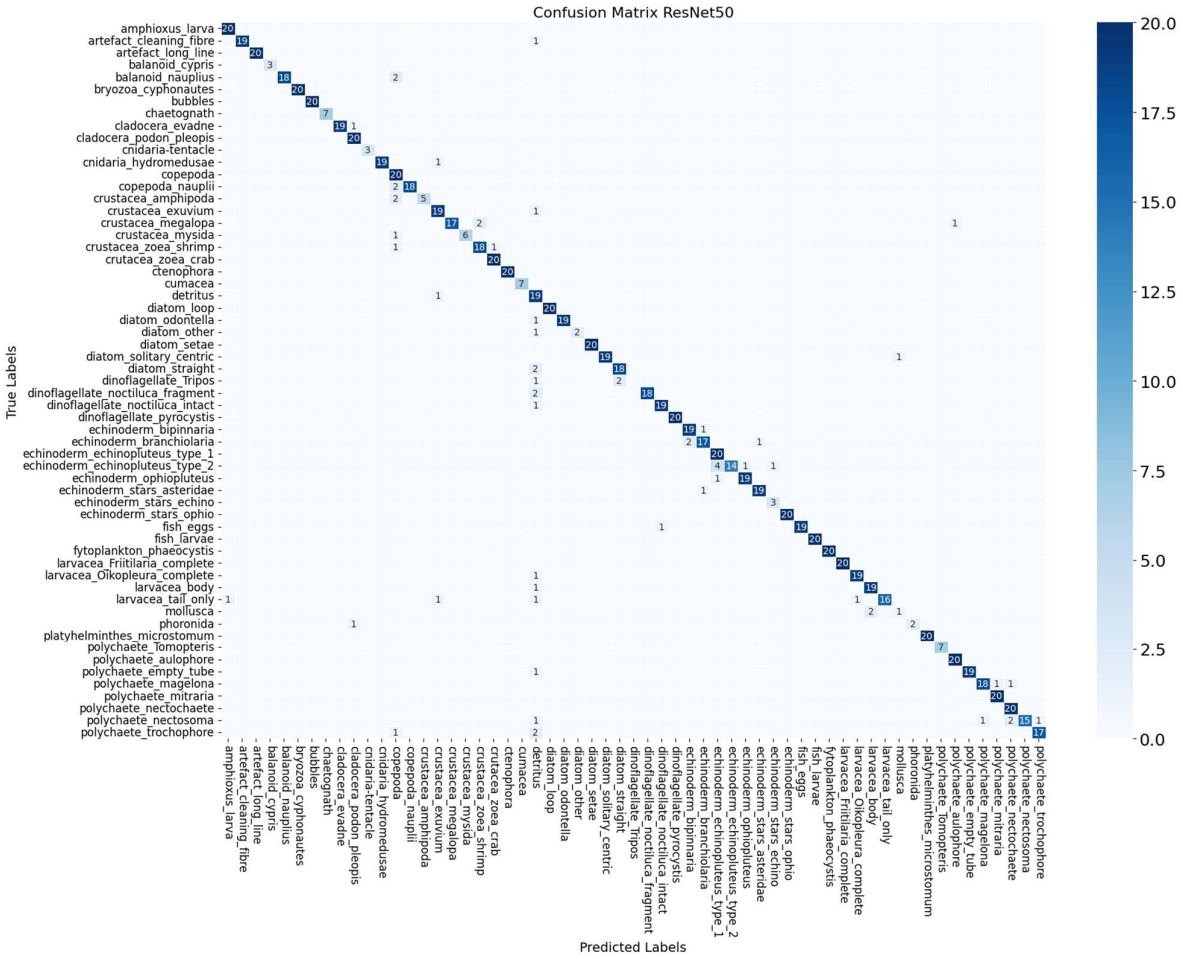


Figure 32 Confusion matrix of the ResNet50 model used for classification of the data in this report. The true label of the images (the label assigned by the expert) is shown on the Y axis and the label assigned to the images is shown on the X axis. The numbers show the amount of test set images assigned to the class by the expert and the model, respectively.

4.3.5.2 Spatial distribution

Examples of the horizontal distribution of zooplankton taxa identified with Pi-10 imaging are shown in Figure 33 to Figure 36. The plots show variations in densities (number per litre) which differ between the identified taxa. Copepods show high densities all over the surveyed area with little horizontal variation (Figure 33), although the species composition may vary. Surprisingly, also larvaceans show high densities (Figure 34), especially off the mouth of the Westernscheldt and along the coast of North-Holland and the Wadden isles of Texel, Vlieland and Schiermonnikoog. Larvae of echinoderms were very abundant in the southern part of the surveyed area (Figure 35), and zoea larvae of crabs show low abundances with no clear trends in distribution (Figure 36). Figures for all identified taxa are presented in the Supplement Plankton Imager report.

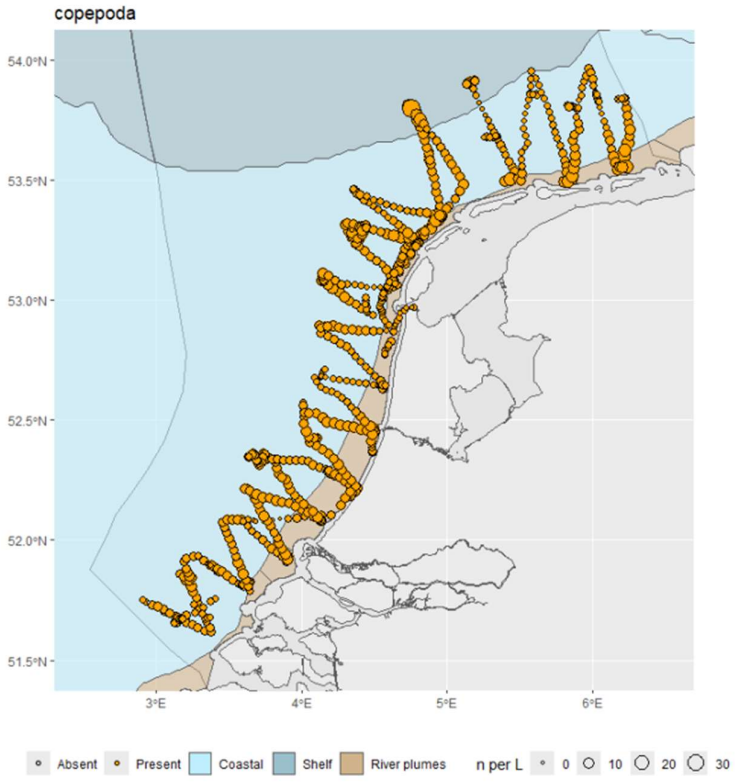


Figure 33 Map showing density estimates (n per L) averaged per 10 minutes for class copepoda along the transect. When the class was absent points are grey. OSPAR Eutrophication areas are plotted in the background as different colour shades.

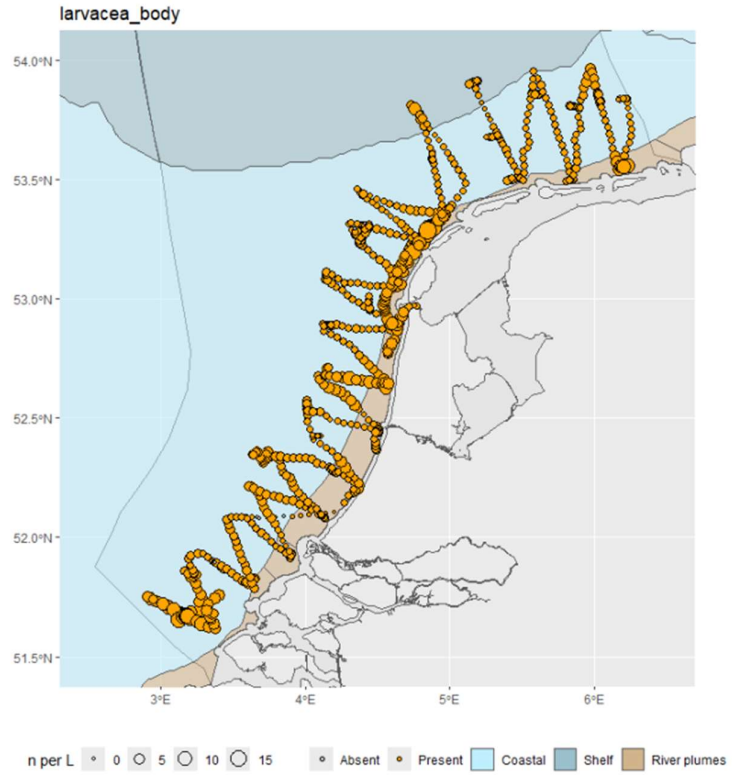


Figure 34 As in Figure 33, for identified body parts of Larvaceans.

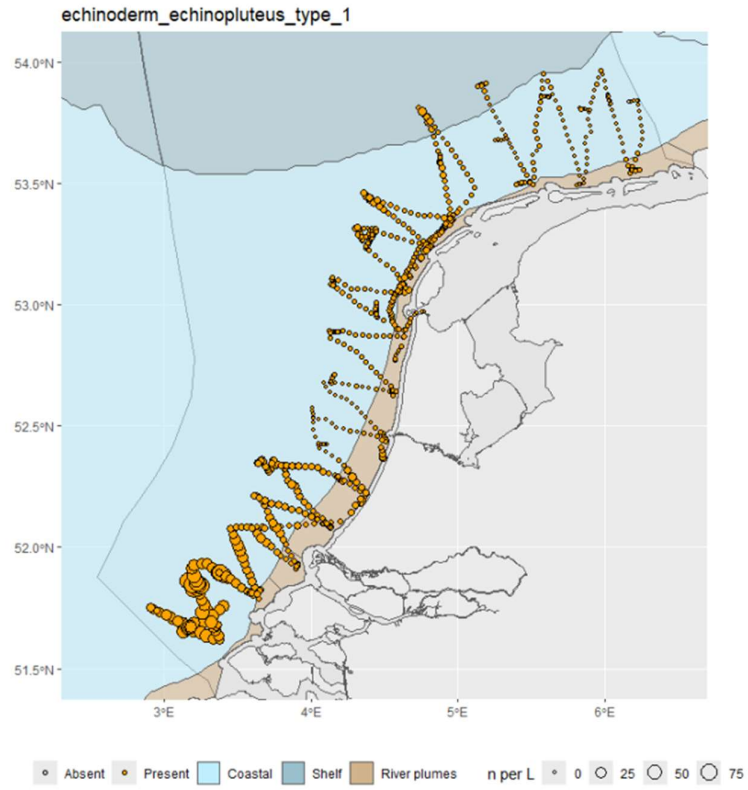


Figure 35 As in Figure 33, for identified echinopluteus larvae (Echinoderma).

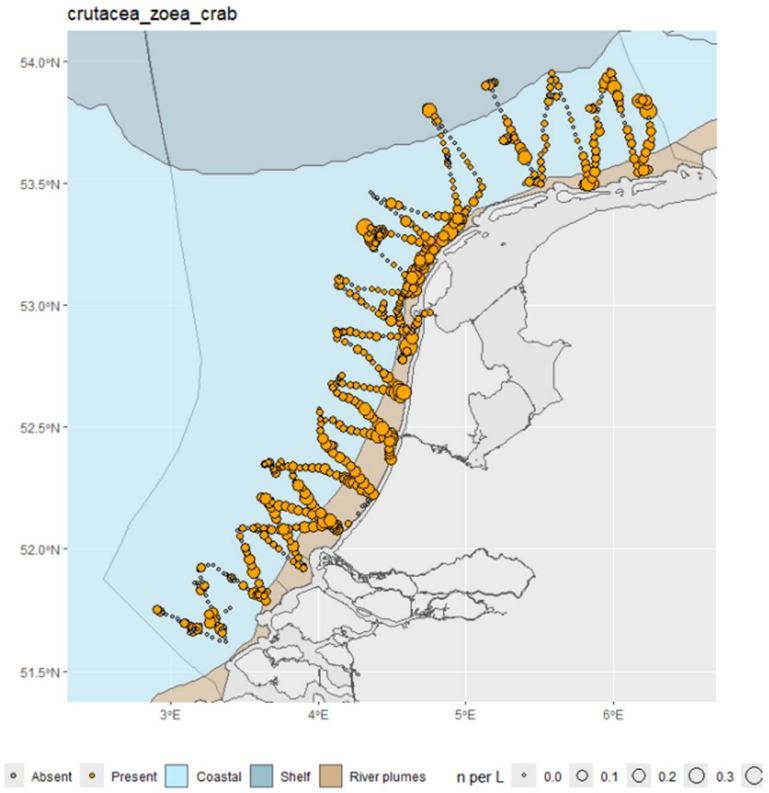


Figure 36 As in Figure 33, for identified zoea larvae of crabs (Crustacea)

4.3.6 Comparison of techniques

4.3.6.1 Microscopy vs Zooscan

For most plankton groups microscopy and zooscan produced comparable density estimates when the manually counted organisms in the "multiple organisms" clusters were included (Figure 37).

A comparison of different generalised linear mixed models applied to 336 observations distributed across 7 sampling stations showed that the simplest model M1 (*organism density ~ organism group*), where organism density was predicted by organism group only, was the best fit. The analysis identified significant differences in density among zooplankton groups. Adding analysis method as a predictor did not improve the model and in the models where analysis method was included as predictor it did not have a significant effect.
Model performance was evaluated using AIC (AIC = -71.3), Bayesian Information Criterion (BIC = 31.8), and log-likelihood (logLik = 62.6). The dispersion parameter for the Tweedie family was estimated at 0.906, and the variance of the random intercept for stations was 0.594 (standard deviation = 0.771). Validation of residuals versus predicted values showed homogeneously distributed residuals with no significant deviations.

4.3.6.2 Microscopy vs Plankton Imager

Density estimated using Plankton Imager and microscopy showed clear differences between different groups (Figure 38). Density of abundant groups such as cladocerans and copepods appeared similar between the different methods with some exceptions such as Appendicularia, echinopluteus and copepod nauplii which were estimated at densities of an order of magnitude or higher by the Plankton Imager than using microscopy. Density of organisms occurring at lower densities appeared much more variable between methods with groups like fish larvae, chaetognaths and crustacean larvae being detected less frequently by the Plankton Imager than with microscopy.
The glmm analysis of the relationship between n organism density, analysis method and plankton group showed the model with the best fit was model M3 being *organism density ~ method + organism_group*, whereby density differs between methods and between organism groups. The more complicated model M4 where the difference in density between methods was different for different organism groups was also attempted but this failed to converge, possibly because of sample size being too low.
Model fit was assessed using Akaike's Information Criterion (AIC = 213.3), Bayesian Information Criterion (BIC = 360.6), and log-likelihood (logLik = -71.7). The dispersion parameter for the Tweedie family was estimated at 1.4, and the variance of the random intercept for station was 0.225 (standard deviation = 0.475). The coefficient of the method (Plankton Imager) was estimated at 1, with a standard error of 0.17 ($z = 5.91$, $p < 0.001$), indicating that the Plankton Imager provides significantly higher density estimates overall compared to microscopy. As expected most of the variation was explained by the high variation in density of the different plankton classes which were all significantly different at the $p < 0.05$ level or lower. Validation of residuals versus predicted values showed homogeneously distributed residuals with no significant deviations except for a slight pattern in the rank transformed DHARMa residuals.

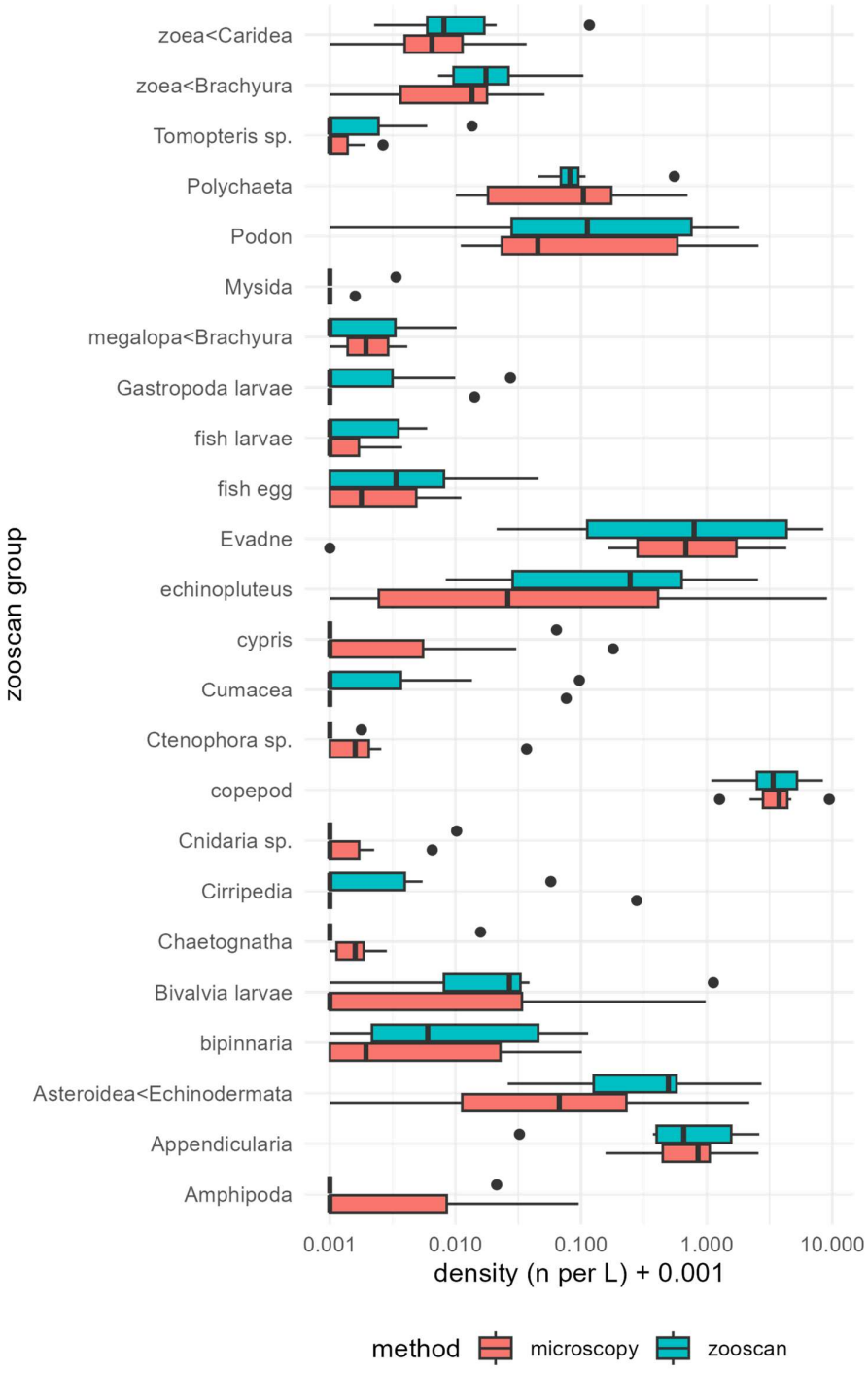


Figure 37 Boxplots of density of different plankton groups estimated by microscopy and zooscan. A value of 0.001 was added to the density to allow plotting on a log scale.

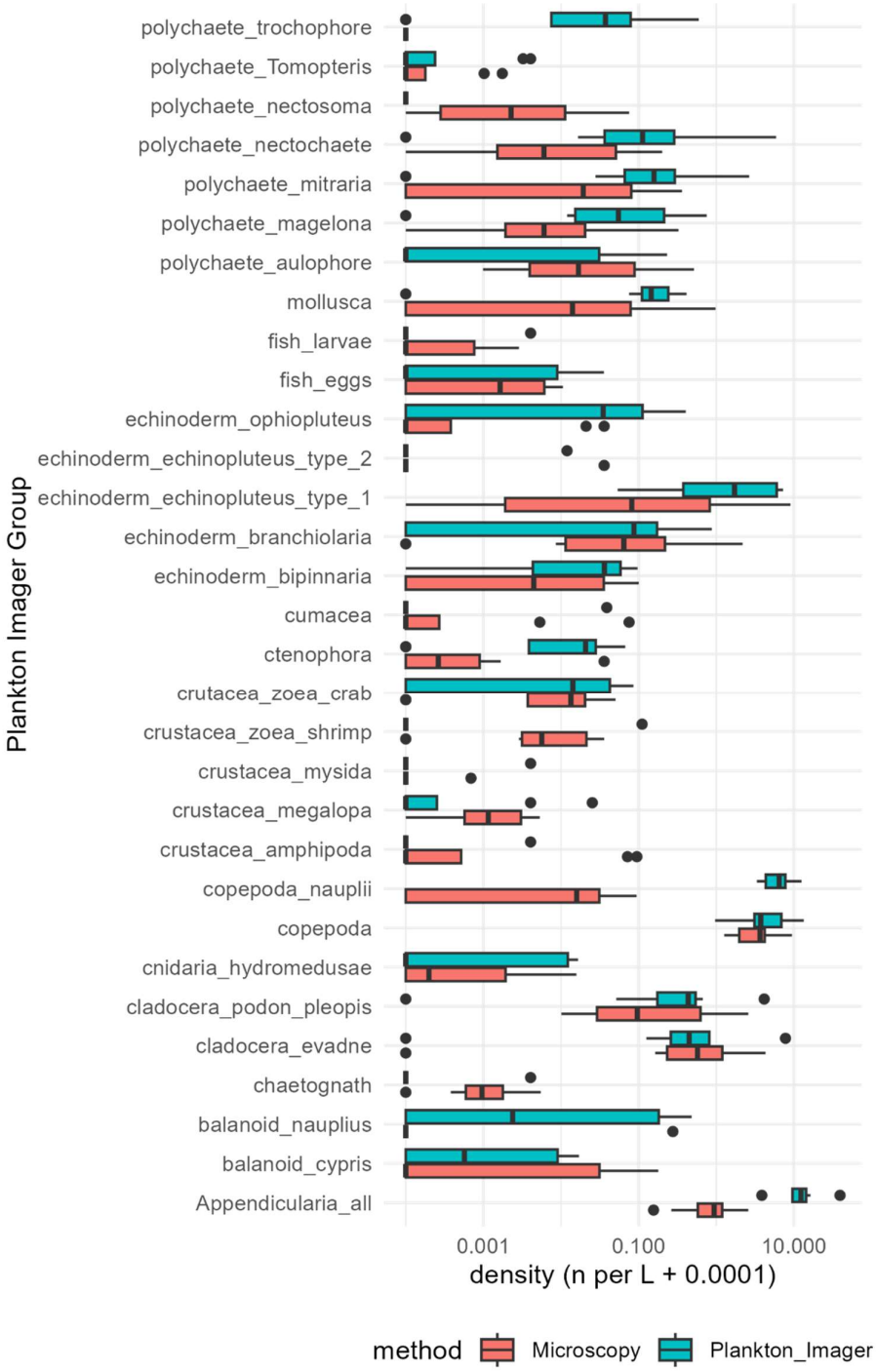


Figure 38 Boxplots of density of different plankton groups estimated by microscopy and Plankton Imager. A value of 0.001 was added to the density to allow plotting on a log scale.

4.4 Discussion

4.4.1 DNA metabarcoding

Our results show that DNA metabarcoding is a useful tool that allows for highly detailed sampling of zooplankton composition for both holoplankton as well as meroplankton.
With the three different markers used for metabarcoding, COI, 18SV9 and 18SV4 we were able to detect a wide range of plankton taxa, including most species of copepods expected in the area. All 86 samples successfully yielded data for the three markers (COI, 18SV4, and 18SV9). COI identified the highest number of merozooplankton and holozooplankton taxa, followed by 18SV9 and then 18SV4. The COI marker appeared to detect the most meroplanktonic species to species level.

Because there is always an error rate with DNA sequencing, minor sequence variation can result in incorrect identification of species, especially when species with highly similar sequences are present in the area. Furthermore, a species identification is only as good as the identification of the reference DNA barcode in the reference database. Taking this into account, DNA metabarcoding results were manually evaluated for the reliability of the detection for zooplankton species.

A striking result was the high number of meroplanktonic taxa sampled. Many larvae of taxa such as crustaceans, bivalves, gastropods, polychaetes and fish could be identified to species level and linked to known benthos species in the area as e.g. available on the Dutch Species Register (https://www.nederlandsesoorten.nl/). Most meroplanktonic larvae are typically difficult or even impossible to identify by microscopic identification, so identification of meroplankton in zooplankton time series such as the CPR has traditionally been mostly limited to the phylum, class or order level.

With regards to the choice of markers for a zooplankton monitoring programme, we recommend, similar to van der Loos and Nijland (2020) that not only COI should be chosen but also another, more conserved marker. In our case 18SV9 might be preferred over 18SV4 as 18SV9 detected more relevant zooplankton taxa compared to 18SV4. The addition of 18SV9 allows for the detection of taxa that are currently missed with COI such as larvaceans. Also, when DNA metabarcoding data is used for (semi)quantitative analysis, it appears that the read abundance for different taxa for the 18S markers better reflects the relative abundance of taxa found in microscopy and Plankton Imager data, than COI.

The application of DNA metabarcoding of samples on zooplankton monitoring can enable the assessment of detailed temporal and spatial patterns for meroplankton at the species level. This data can be useful to investigate the role of zooplankton in food webs but also has several possible applications beyond this. Using DNA metabarcoding of meroplankton, Non- Indigenous Species (NIS) that might turn invasive, can be detected in an early stage, also for benthic organisms that are hard to find because they are cryptic or live in difficult to sample habitats. Knowledge about the presence of possible NIS species in the plankton can allow for targeted searching for the species in the benthos. As an example, we detected DNA of the polychaete *Polydora onagawaensis* which is a possible nuisance species as it drills in oyster shells, and has already been found in Normandy, France (Sato-Okoshi et al 2022), but might now also be present in the Netherlands.

Another application of DNA metabarcoding might be more detailed insight into the spatial distribution and spawning phenology of benthic species of interest in ecosystem restoration. Knowing whether or not there is a natural source of larvae in a proposed area for restoration, e.g. for the reef-building polychaete *Sabellaria alveolata* or the flat oyster *Ostrea edulis* will allow for better informed decisions on the feasibility of ecosystem restoration and the selection of possible sites.

4.4.2 Plankton Imager

The approach we took to expand the learning set for the classification algorithm allowed us to rapidly increase the image count for rarer classes in the learning set and thus train a ResNet 50 network that achieved a high accuracy for most classes present in the dataset. The constant measurements of the

Plankton Imager along the transect provided high resolution spatial data on the distribution of many taxa and provided important insights in the zooplankton composition along the Dutch coast.

An important find was that the density of larvaceans was high, often double that of copepods. As this understudied group can achieve production rates orders of magnitude higher than that of copepods and can be an important food source for fish (Jaspers et al. 2023) it might be an important part of the North Sea food web that should be incorporated into food web models. This finding will be investigated further within the MONS PhD projects.

The presence of *Microstomum* sp. turbellaria in high densities in Plankton Imager images was an interesting discovery. These predatory pelagic flatworms consume zooplankton. Greve and Reiners (1996) found that *Microstomum* (then named *Alaurina*) consumed copepods in the German Bight and suggested that, at similar high densities to what was found in the MONS survey, the species might have an important role as a predator of copepods in the North Sea ecosystem.

The Plankton Imager data also provided interesting insights in spatial patterns and abundance of phytoplankton. *Noctiluca* heterotrophic dinoflagellates, an important component of the food web which is considered a potential Harmful Algal Bloom (HAB) species (Ollevier et al, 2021), could be detected in high densities on the images. *Phaeocystis* colonies could also be imaged quantitatively. *Phaeocystis* is considered a nuisance species which can form massive foam banks on the coast that can pose a threat to water sports and lead to the deaths of five surfers in the Netherlands in 2020 (reviewed in Peperzak & van Wezel 2023). Currently there is no monitoring for *Phaeocystis*, and the Plankton Imager data can fill this gap. High densities of larger solitary and chain-forming diatoms are also observed with the Plankton Imager.

With enough data available, the taxonomic resolution of several classes could be increased. Copepods could be further split to the order level, and for some taxa possibly even genus levels, although the challenge here would be that this does not apply to all the images of copepods, as identification characteristics cannot be seen on all images, depending on orientation on the images and image quality. Also, for other taxa like crab and shrimp zoe larvae and phytoplankton the taxonomic resolution of the classification might still be increased.

Integrating continuous measurements of abiotic parameters like water temperature and salinity on RV Tridens will allow detailed linking of plankton composition to abiotic parameters to distinguish different water masses and document whether changes in plankton composition can be related to changing environmental conditions.

4.4.3 Microscopical identification

With microscopic identification we were able to identify most, but not all, of the copepod species found in the DNA metabarcoding data. One genus, *Labidocera*, was only found in microscopy and not in DNA metabarcoding. This is likely because the expected species *Labidocera wollastoni* was not present in the reference database. The samples contained high numbers of juvenile copepods which often cannot be identified to species level and some genera like *Paracalanus* and *Pseudocalanus* can be highly difficult to distinguish morphologically (Castellani & Edwards 2017).

4.4.4 Zooscan

The zooscan method turned out to be much more time-consuming than expected based on previous analyses of pump-collected samples in the Wadden Sea (Maathuis et al., 2024). The North Sea samples collected with the WP2 plankton net contained clusters of clumped organisms, probably caused by Appendicularia houses, made of gelatinous material and the presence of pluteus larvae, which have long protrusions. Manually separating individuals from these clusters was time-consuming and often not possible without damaging the organisms. This meant that many organisms were identified as clusters of "multiple organisms". This made it necessary to manually count the individuals within the clusters to get an accurate estimate of the density of

the different plankton groups. This also limits the possibility for making accurate estimates of the biomass of organisms from the images, as this requires one organism per image.

4.4.5 Comparison of techniques

4.4.5.1 Microscopy versus Zooscan

The comparison between microscopy and zooscan showed that a higher taxonomical resolution could be achieved using microscopy than using zooscan, although the taxonomical resolution of the zooscan analyses could be further improved as copepods could be identified to the order level and often to the genus level from the images. Comparing the density estimates between zooscan and microscopy for the samples analysed shows that the zooscan method provides comparable density estimates to microscopy as long as all organisms in clusters are also counted. If the current problem with the automated analysis of images of organisms clustering together is fixed, the zooscan method could be a useful alternative for the analysis of samples using microscopy, provided species-level identification is not necessary. At the moment however, zooscan is not a cost-effective alternative to microscopical analysis for coastal North Sea zooplankton samples.

4.4.5.2 Microscopy versus Plankton Imager

It appeared that taxa detected by microscopy were also imaged by the Plankton Imager, the latter albeit often at a lower taxonomic resolution. Density estimates by Plankton Imager were comparable to microscopy for abundant and important crustacean taxa such as cladocerans and copepods. Some taxa densities were an order of magnitude higher with the Plankton Imager compared to microscopy, namely copepod nauplii and Appendicularia. Copepod nauplii are likely undersampled by the WP2 net because of their size being smaller than the 200 µm mesh size, although the resolution of Pi-10 images is also limited for organisms smaller than 200 µm. Appendicularia are fragile and often destroyed in plankton net sampling and thus underestimated by net sampling (Jaspers et al. 2023).
The increased variation at lower organism densities and lower density estimates for taxa like chaetognaths, fish larvae and crustacean larvae for the Plankton Imager is expected since the sampled volume of the WP2 net is several cubic metres per sample whereas the Plankton Imager samples 34 L of water per minute.

Differences in density estimates between Plankton Imager might also be caused by vertical variation in plankton densities, as the Plankton Imager samples at a fixed depth whereas vertical tows are taken with the WP2 net.

4.4.6 Integrated use of techniques

Some clear examples of the advantage of integrating different techniques are provided below.

Plankton imaging has shown to provide quantitative information on the spatial distribution of major groups within the plankton at a high resolution. The taxonomic resolution is relatively low, although in some cases, species or genera could be identified.

The identity of worm-like organisms on the Plankton Imager images was suggested to be *Microstomum* sp. turbellaria based on the appearance of the organisms. This identity could be confirmed by the DNA metabarcoding 18SV9 data where *Microstomum* sp. DNA was found in almost every sample. The species was not detected in the samples selected for microscopy, but would probably have been detected and identified when present.

DNA metabarcoding provides information on the composition of the zooplankton community at a high taxonomic resolution. Like microscopical analyses, the spatial resolution is determined by the sampling effort. In addition to DNA metabarcoding, microscopy can be used to confirm identification of rare species. The western Atlantic copepod species *Tortanus discaudatus* was first detected in the COI DNA metabarcoding data, but not in the microscopic analyses of the same sample, probably because of subsampling. When we looked specifically for *Tortanus discaudatus in* the samples, it was found and its presence in the samples

could be confirmed. Without DNA metabarcoding data the presence of this non-indigenous species, found only once before in the Dutch EEZ in the Continuous Plankton Recorder data, would have been missed. This shows that the combined application of microscopy and DNA metabarcoding is the best choice for early detection of new non-indigenous species.

DNA of *Sabellaria* sp. reef building polychaetes was found in a large number of samples with DNA metabarcoding. Evaluation of samples revealed that these larvae could also be detected using microscopy, owing to the conspicuous serrated morphology of the setae as seen in Lezzi et al. (2015). The newly found tubeworm species *Loimia ramzega* was abundantly detected in DNA metabarcoding of COI, and larvae of *Loimia* could also be identified in the microscopy samples.

As shown by these examples, next to the innovative techniques DNA metabarcoding (high taxonomic resolution) and Plankton Imaging (high spatial resolution), microscopical identification still remained a useful tool, to be able to investigate to what extent the other techniques accurately capture the abundance and composition of the zooplankton in the area. In comparison with Plankton Imager data, it could provide a check to see whether some taxa might be missed by the Plankton Imager because their density is too low, they are too big, or they are destroyed by the sampling pump.

Collecting samples and saving samples for microscopic analysis also allows for revisiting samples if later it turns out that an identification was unclear, and for training of researchers in plankton taxonomy and identification.

5 Species list

Several sources were identified in scientific literature, including historical data, being relevant to the taxonomic composition of the zooplankton of the Dutch Continental Shelf.

In 1983, Fransz et al. published an overview of zooplankton taxa that had been reported for the Dutch, German or Danish Wadden Sea, and includes also taxa entering the Wadden Sea from the North Sea. The list covers the common Wadden Sea species, including brackish water species, and contains 260 taxa. The list covers both holoplanktonic taxa and meroplanktonic taxa.

Van Ginderdeuren et al. (2012) drew up a list of marine species that were found in the Belgian part of the North Sea in 2009-2010. This concerns 137 taxa, a number of which were not previously known for this part of the North Sea. A distinction has been made between holoplankton, meroplankton and ichtyoplankton (fish larvae). In addition to invertebrates, fish were included as meroplankton, having a planktonic stage. For each taxon it is indicated what the average and maximum density was, in which season the taxon was found and whether it was found along the coast, offshore and / or in between. The list is an up-to-date list of the most common species in the shallow coastal zone that can be identified at a taxonomic level by microscopy.

Greve et al (2004) distinguished 76 mesozooplankton taxa and 367 macrozooplankton taxa from around the isle of Helgoland in the German Bight of the North Sea. The list also contains length classes or larval stages for some species. A number of taxa were only identified to the genus level. The list contains taxa that mainly occur somewhat further offshore.

Soesbergen drafted an annotated species lists for ciliates, rotifers and copepods based on reported species in scientific literature and other sources. The ciliates are generally too small to be sampled by WP2 nets (200 µm) and are therefore not covered in the mesozooplankton size range investigated in the MONS Zooplankton sampling. Only 8 species of rotifers are indicated as marine species (Soesbergen, 2022). Soesbergen (2023) listed 40 species of marine or brackish copepod species. These sources can be considered to be the most updated lists for these species groups.

Also the Continuous Plankton Recorder forms a source of taxonomic data. A list of taxa present at the North Sea was extracted from the Global Biodiversity Information Facility. The list included only taxa identified at the species level, mainly copepods.

The coastal survey reported in Chapter 4 provides an extended list of species as identified by DNA metabarcoding, using CO1, 18SV4 and 18SV9 as makers. A number of 822 taxa were identified, of which 631 were identified at the species level, while 191 taxa were only identified at a higher level (genus, family), or for which identification was unclear. The DNA metabarcoding data show a clear overlap with the common species listed in the previous mentioned sources (see Annex2), however, there are several reasons why there is no complete matching. First of all, the coastal survey was carried out within a time period of 3 weeks, and many species may be only present during another time of the year. For instance, the larvae of the lugworm *Arenicola marina* were not present at all. The year round sampling at the NIOZ jetty will likely add species to the list. Second, a WP2 net was used with a mesh size of 200 µm, and smaller species, like tintinnids and rotifers, may have passed the net. Third, the survey only included the coastal zone, and therefore species occurring offshore may have been missed. Fourth, the DNA metabarcoding database may not be complete for all taxa. Fifth, DNA metabarcoding may identify species which can hardly be identified by microscopy, which are therefore grouped at a genus or higher level of identification or may be misidentified. In addition, some species may have been introduced to the Dutch Continental Shelf recently or may have disappeared.

6 Advice for future monitoring and research

6.1 Advice on a monitoring plan for zooplankton in the Dutch North Sea

This report also provides an impetus for the zooplankton monitoring plan. Because part of the project (the offshore survey) could not take place, we do not have all the knowledge we expected to need to advise on what a further MONS zooplankton monitoring plan might look like. However, with the currently available information and data we think there is a good picture of the advantages and disadvantages of the different methods and we can therefore propose a well-founded monitoring plan.

This advice is largely consistent with the previous proposal for monitoring zooplankton in the North Sea, MONS ID14. In estimating the spatial and temporal coverage of the sampling program, it was assumed that the combined May-June coastal survey for pelagic fish with RV Tridens as conducted in 2023 will not be continued. It is now clear that this survey will be continued, but RV ISIS will be deployed for this MONS coastal survey instead of RV Tridens. For zooplankton monitoring, options on the ISIS are limited due to the absence of the Plankton Imager and lack of space for personnel, so additional zooplankton monitoring on this survey is currently not included in our advice.

For this advice we considered the following research questions as defined in the original proposal:

- Which monitoring design provides the most optimal insight into distribution and dynamics in space and time of the zooplankton community?
- Which set-up is most optimal in relation to other components of the food web (phytoplankton, pelagic fish)?
- Are the data useful in relation to the food web models to be developed within MONS?
- What is the most cost-effective monitoring design?

Below, the various techniques deployed are evaluated and whether the spatial and temporal coverage of the recommended monitoring for the various subareas of the Dutch North Sea area is sufficient.

6.1.1 Key principles of advice

This advice for a new zooplankton monitoring programme has been drafted considering the following key principles:

1. Zooplankton monitoring should be integrated into existing national and international monitoring programmes and surveys to link data with data on other ecosystem components,
2. Proposed methods and techniques should be matched with existing surveys of North Sea countries (as reviewed in MONS ID 14),
3. Innovative techniques can be used to efficiently obtain data on zooplankton biomass, composition and diversity at the high spatial, temporal and/or taxonomical resolution needed in zooplankton monitoring but,
4. These innovative techniques should be used together in an integrated approach, making use of the strengths of techniques to compensate for weaknesses of other techniques and using traditional analysis of net samples to calibrate and validate the results.

With respect to these principles we recommend that zooplankton monitoring should be integrated as follows:

- National monitoring should consist of the following elements:
    - Integration of zooplankton monitoring on the national water quality monitoring surveys (MWTL),

    - Integration of zooplankton monitoring in the NIOZ long term Marsdiep jetty time series.
- International monitoring should consist of the following elements:
    - Integration of zooplankton monitoring on ICES fisheries surveys using continuous measurements of the Pi-10 Plankton Imager combined with abiotic parameters on RV Tridens,
    - Joint analysis of Pi-10 data collected by different North Sea countries to match methodologies and increase spatial and temporal coverage,
    - Continuation of Continuous Plankton Recorder (CPR) measurements on current North Sea transects.

In below section the different techniques applied are evaluated.

6.1.2 Evaluation of different techniques

6.1.2.1 Plankton Imager (Pi-10)

The Plankton Imager is a device that can be installed on a ship or other platform to continuously image plankton and other particles during surveys. For this, water is pumped from outside of the vessel and passed through a flow cell equipped with a camera which collects images of all particles within set size thresholds. Images are saved on disk drives for later analysis. The hundreds of millions of images are identified using an AI-based classifier. A Convolutional Neural Network is trained on a set of expert-labelled images of different classes, which is then used to classify the remaining data. By measuring the sizes of the organisms on the images, biomass can also be estimated.

Measurements with the Plankton Imager on RV Tridens appear to be able to provide useful data on zooplankton composition and size distribution at very high spatial and temporal resolution as indicated in the chapter reporting on the coastal survey. Following this survey the Plankton Imager has been in operation on WOT fisheries surveys in 2024, at the request from MONS. In Q1 and Q2 of 2024 some ongoing issues with the stability and reliability of the onboard data acquisition were resolved and starting from Q3 2024 the Plankton Imager has been collecting data on all WOT fisheries surveys. The Plankton Imager can now run continuously on these surveys with minimal effort needed from crew and WMR personnel.

As mentioned earlier, in 2024 an international Plankton Imager User Group was formed which consists of users and future users of the Pi-10 Plankton Imager and is aimed at developing common analysis pipelines and ecological indicators for the Pi-10 Plankton Imager. Aside from NIOZ and WMR this group contains members of the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), the Plymouth Marine Laboratory (PML), the Turing Institute and the British Antarctic Survey (BAS). Collaboration within this group will ensure that data can be used and shared between different parties in the OSPAR area and beyond.

With the Plankton Imager on RV Zirfaea sampling along MWTL transects, we expect to collect similar data as with the Tridens, but at higher temporal resolution (4 to 19x per year, depending on how frequent stations are visited) and only within the Dutch EEZ. Since many abiotic parameters and phytoplankton data are also collected here at the same time, these data will be important for developing and validating plankton-based indicators.

6.1.2.2 Plankton scanner (zooscan)

The analysis of net samples with the plankton scanner has not yet been completed. Processing samples and images using current analysis methods takes more time than anticipated. The experience at both NIOZ and WMR is that organisms cluster together in the samples. Manually spreading out all organisms in the samples before scanning is often not possible or takes too much time. The problem with this is that the Zooprocess segmentation method currently used for zooscan does not see these organisms as separate Regions of Interest (ROI). The moment an ROI consists of multiple organisms then automatic classification with machine learning is greatly hampered because it works on the principle that only one organism is represented on each ROI. The accuracy of the classification algorithms used in the standard analysis method in EcoTaxa is low which still requires extensive manual quality control. The current analysis method for zooscan samples takes far more time than expected for the above reasons.

A different image analysis pipeline based on direct classification of images on scans using Object Detection and/or Instance Segmentation algorithms might be better suited to this data and could contribute to a higher quality and faster analysis method, but this was outside of the scope of this project. It appears that currently an improved classification pipeline (ZooProcess V10) is being developed which incorporates Instance Segmentation as part of the iMagine project (imagine-ai.eu). Also with the plankton scanner, exchange of knowledge and data is possible with neighbouring countries where this method is used, such as in the Belgian Lifewatch monitoring of the VLIZ.

When organisms in clusters are processed correctly and do not require manual counting the zooscan might be a useful addition, also because the density estimates obtained for most zooplankton groups were found to be comparable to those obtained using microscopy. In its current stage, we think the zooscan is not a cost-effective solution for analysing high numbers of coastal North Sea zooplankton net samples. The plankton samples taken at the NIOZ jetty from the island of Texel have a much lower sampled volume, and might be better suited for zooscan analysis than the large-volume WP2 net samples. The analysis of the NIOZ jetty plankton samples will be reported in a later report.

6.1.2.3 DNA metabarcoding

In DNA metabarcoding, a select gene region is amplified, with the resulting DNA fragments sequenced. The obtained sequences are cross-referenced with DNA sequence libraries and, if possible, assigned to taxonomical classes. This can provide a detailed overview of the taxa present in a sample and can be scaled up in a cost-effective way, enabling detailed analysis of high numbers of samples.

DNA metabarcoding can be performed on water samples containing environmental DNA (eDNA), or on bulk samples containing the actual organisms themselves. We used the latter approach and analysed WP2 net samples using DNA metabarcoding for three markers; COI, 18SV9 and 18SV4 to investigate which combination of markers was best suited for North Sea zooplankton.

Analysis of samples using DNA metabarcoding provided valuable data on species composition of zooplankton, especially also for the meroplankton (larvae) component where, for example, larvae of reef-forming species are also found. Non-native species can also be detected at an early stage with this technique. We found that DNA metabarcoding results can be considered semi-quantitative. Different markers yielded widely different proportions of DNA reads for different taxa. However, within single species the amount of reads in samples showed spatial patterns similar to those observed in the Plankton Imager data.

The recommendation is to make DNA metabarcoding an integral part of the monitoring. We advise to use both CO1 and S18V9 as genetic markers, since these appear to have a complementary value in the identification of marine zooplankton species and omitting one of three markers saves on sequencing and analysis costs. 18SV4 yield only a few additional zooplankton species not being identified by CO1 or S18V9 and is not recommended for future use. For a cost-effective monitoring we also advise to combine the analyses of DNA metabarcoding samples in larger batches, possibly also combined with samples for other zooplankton projects, such as the NIOZ jetty samples, as the costs of DNA metabarcoding sample analyses decreases with increasing amount of samples. Combining the analyses of samples from different monitoring programmes or areas also facilitates the consistency and comparison of results.

6.1.2.4 Microscopic analysis

A limited number of samples were analysed with microscopic analysis, mainly to validate the other methods. Microscopic analysis allowed for detailed analysis of individual organisms in samples. Many organisms could be identified to species level. Due to time constraints we only identified a subsample of copepods to species level. A limitation of microscopic analysis is that it is often only possible for the adult forms. Juveniles and larvae are often not possible to identify to species level. For example, the coastal survey samples contained high numbers of juvenile copepods which could not be identified to species level.

The combination of microscopic analysis and metabarcoding proved to be useful. Potentially new (invasive) species found with DNA metabarcoding were confirmed with microscopic identification.

We advise that microscopic analysis of a selection of samples should still be performed in the monitoring programme, mainly as validation of the results of the other techniques and for confirmation of the presence of new species.

6.1.2.5 In-situ imaging

Although originally planned for, in-situ imaging was not used within this project for logistic reasons. This technique is particularly valuable in mapping the vertical distribution of plankton in stratified systems. Although a valuable technique, it is challenging to use so we do not foresee easy application during routine monitoring cruises. In future research by the MONS zooplankton PhDs, for example, this will be a valuable technique.

6.1.3 Monitoring advice

6.1.3.1 Spatial and temporal coverage of planned zooplankton monitoring

To get an impression of whether the spatial coverage of zooplankton monitoring at MWTL stations is sufficient, the locations and frequency of MWTL chemistry samplings were consulted in the IHM Monitoringsagenda. When integrating zooplankton monitoring at MWTL North Sea Transects (*Figure **39***), the OSPAR COMP4 areas Southern North Sea (8 stations), Eastern North Sea (4 stations), Dogger Bank (1 station), Rhine Plume (2 stations), Meuse plume (2 stations) and Scheldt plume 1 (1 station) are sampled.

In some OSPAR COMP4 subareas, limited or no MWTL sampling points are present. However, as the sampling consists partly of continuous FerryBox measurements, data are collected not only at the sampling points themselves but also during navigation in subareas to and between the sampling points. Assuming the full cruise track of Zirfaea in 2023 (*Figure **40***) is representative for yearly operations, it appears that the continuous measurements allow for extensive coverage in poorly covered nearshore COMP4 areas such as Rhine Plume. As OSPAR has highlighted the need for additional data in areas of variable salinity such as River Plumes, this data could be a valuable addition to the assessment.

Running the Plankton Imager continuously on RV Tridens WOT fisheries surveys will allow for an extensive spatial coverage of the North Sea (*Figure 41*), although these surveys often take place only once a year, so the temporal coverage is limited. This coverage could be improved by collaborating with other parties using the Plankton Imager, such as CEFAS.

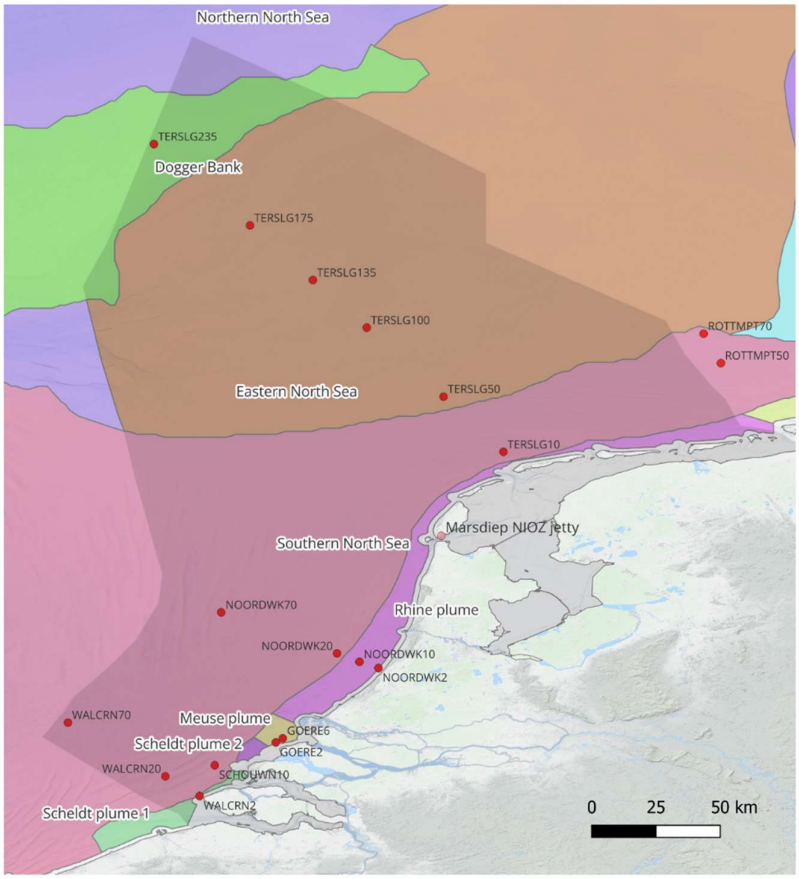


Figure 39. Map showing MWTL points in red, the Marsdiep measurement point and OSPAR COMP4 subareas of the Dutch North Sea.

Integrating the zooplankton monitoring into existing surveys is deemed feasible for both the integration with the RV Tridens WOT fisheries surveys and the RV Zirfaea MWTL monitoring surveys. Integrating the monitoring on these surveys saves on shiptime costs and allows for the comparison of the results and linking of data with the data obtained for other biotic and abiotic parameters such as water temperature, salinity, algae composition and pelagic fish composition and biomass. Below maps give an impression of the spatial coverage attained by integrating the monitoring on existing surveys.

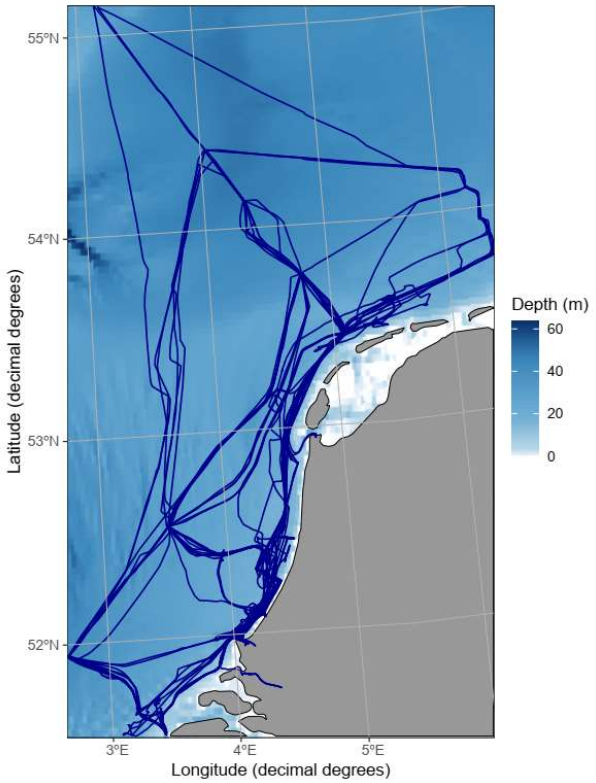


Figure 40. 2023 track of RV Zirfaea based on AIS data from globalfishingwatch.org

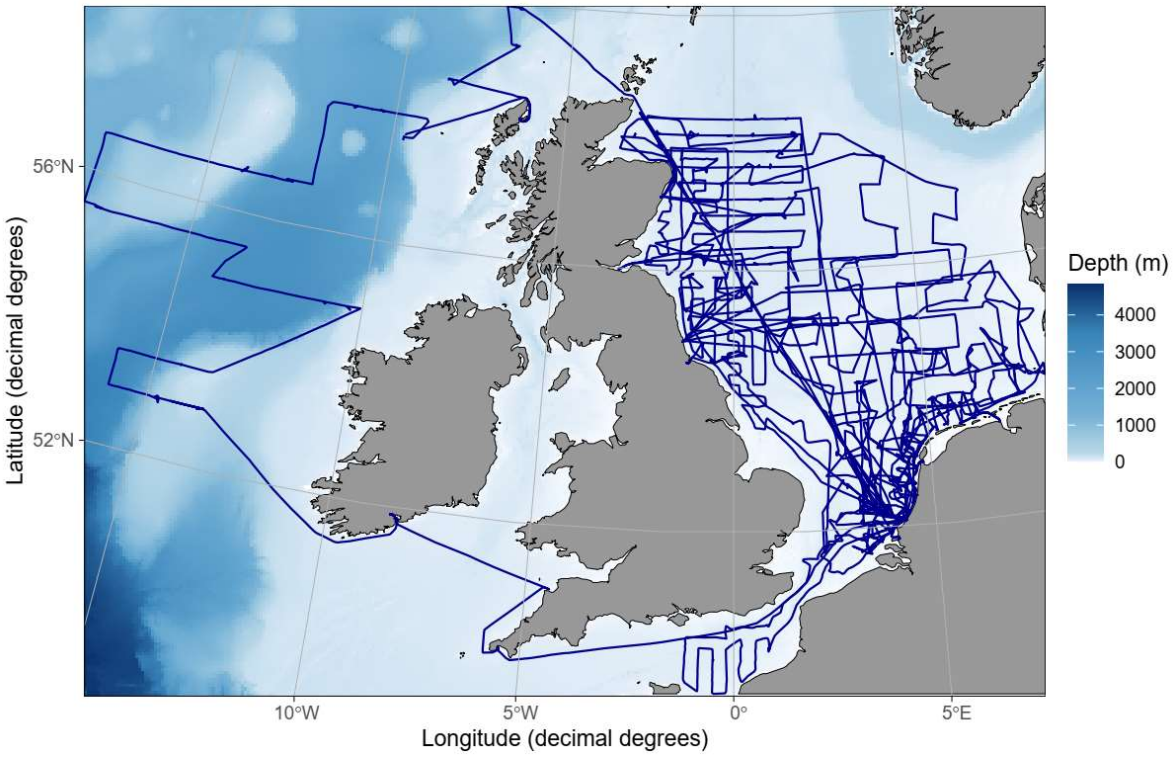


Figure 41. 2023 track of RV Tridens based on AIS data from globalfishingwatch.org

6.1.3.2 Summary of monitoring recommendations

Summarising above mentioned information, our preliminary advice is that MONS zooplankton monitoring consists of the following components:

- High-frequency measurements from the NIOZ jetty:
  - The recommendation is to continue these measurements as they are currently carried out, consisting of:
    - DNA metabarcoding using COI and 18SV9 markers,
    - Microscopy (selection of samples),
    - Zooscan (based on experience with samples currently being analysed)
    - Investigate potential for the Pi-10
- Monitoring on MTWL transects North Sea.
  - This monitoring should consist of:
    - Continuous measurements with FerryBox measurement container including Plankton Imager
  - Vertical WP2 net samples at as many MWTL sample points as possible within budget and time constraints, where the samples are analysed with a combination of:
    - DNA metabarcoding using COI and 18SV9 markers,
    - Microscopy (selection of samples),
    - Zooscan (when analysis pipeline is improved).
- Continuous monitoring on RV Tridens WOT fisheries surveys.
  - These measurements consist of:
    - Continuous operation of the Plankton Imager
    - Continuous measuring of environmental variables, at minimum water temperature and salinity.

Based on budget availability, choices may have to be made as to which and how often the MWTL points will be sampled and which techniques will be used to analyse which samples.
In addition to the regular monitoring, additional activities will be needed for research of the MONS zooplankton PhDs, among others, and deployment of in situ imaging, for example.

7 Conclusions

The MONS study reported here intended to develop a set-up for a long-term monitoring programme for zooplankton, that would give insight to changes in the zooplankton community related to the ongoing transitions in food, energy and nature at the Dutch North Sea.
Basically, the monitoring programme should provide answers to:

- What is the composition and distribution of zooplankton in space and time?
- What are the trends (years and decades) in composition and distribution of zooplankton in space and time?
- What are the effects of new human use on zooplankton composition and distribution?

Data collected covered existing data, especially from the continuous plankton recorder (CPR), species listed in literature, and from a survey along the Dutch coastline. This survey made use of innovative techniques, including plankton imaging and DNA metabarcoding. Using data from these techniques was also encouraged by OSPAR in developing indicators for zooplankton in relation to descriptors of the Marine Strategy Framework Directive. Collaboration with international partners was established to bring this further.

Although data from the CPR covers a wide range of the Greater North Sea over a long time period, the Dutch coastal area is poorly investigated. Furthermore, the taxonomic resolution of (microscopic) identification seems poor, with only dominant species being present in the dataset. However, since long time-series are available over a greater range, the data can be used to investigate long-term changes over a wider spatial scale and can thus be used for the interpretation of smaller scale high resolution monitoring aimed within MONS.

The coastal survey, being part of this study, provided data with a high spatial resolution on zooplankton species. However, the survey was limited to the coastal zone and was carried out within a three-week period and not covering seasonal dynamics, which is an important feature in zooplankton communities. The horizontal distribution was investigated by making use of a Plankton Imager, which collects data in the form of images during shipping. Since 10.000 images are stored each minute, a large data set was collected during the survey. For the interpretation of images, algorithms for the autonomous identification of species groups were developed by establishing a so-called learning set including about 50.000 images. The images also allow for measuring the size of organisms from which biomass can be calculated. The survey shows high variability in the number of particles in general and in the distribution of species groups. More specific species composition was investigated from vertical net samples. The collected zooplankton was split, and part of the sample was used for DNA metabarcoding, providing information of taxa on the species level, so at a high taxonomic resolution. Another part of the sample was stored for microscopical validation of DNA results and imaging with a flatbed scanner (zooscan). Results from the DNA metabarcoding provided semi-quantitative distribution maps for numerous species, including the larvae of benthic organisms and non-indigenous species. Since samples were only taken in June, the larvae of species spawning in other parts of the year were missed. This may also be the case for zooplankton with an offshore distribution.

We recommend to extent the developing of zooplankton monitoring by studying the wider distribution of zooplankton and their seasonal dynamics in the Dutch North Sea and also other parts of the Greater North Sea by making use of the Pi-10 Plankton Imager, combined with samples analysed using microscopy and DNA metabarcoding of samples for markers 18SV9 and CO1. Analyses using zooscan are currently not deemed cost-effective and of sufficient quality, but this could improve in the future as analysis techniques are currently being refined. The monitoring should be established by making use of the existing surveys MWTL surveys and the WOT Fisheries survey, ensuring the survey is cost-effective and the results can be integrated and compared with abiotic and biotic data collected on these surveys. Data collection during surveys intended for fish stock-assessments (WOT programme) and MWTL will allow for a North-Sea wide

coverage of zooplankton sampling by sampling on WOT surveys, with the Dutch EEZ sampled at high temporal frequency on MWTL surveys. The evaluation of the needed temporal sampling resolution is also based on the results of the Marsdiep high frequency sampling, which will be reported in a follow up report.

The MWTL surveys are carried out every year with regular intra-annual frequencies. All WOT surveys with the exception of the mackerel egg survey are carried out annually as well, and in fixed seasons. As illustrated in the discussion the combination of these surveys covers a large proportion of the Greater North Sea ecoregion. By integrating the zooplankton monitoring on WOT and MWTL surveys we think the monitoring programme will be able to answer the aforementioned questions and provide information on the composition and distribution of zooplankton in space and time, as well as annual and decadal trends.

8 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

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Justification

Report: C013/25
Project Number: 4315100213

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved:	Dr. F.L. Schaafsma WR Researcher Marine Ecology
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Date:	3 March 2025

Annex 1. Plankton Imager per class data

Table A1. Plankton Imager: Total amount of images per class ordered highest to lowest, together with min, mean and maximum observed densities. Densities are per Liter.

Class name	Total imaged	% of total	Min. density	Mean density	Max density
diatom_straight	33277126	39.09	0.04	1792.82	15584.1
detritus	17377154	20.41	0.15	669.03	6449.35
dinoflagellate_noctiluca_fragment	11965195	14.06	0	265.22	2530.71
diatom_loop	8625564	10.13	0	485.21	6366.43
dinoflagellate_noctiluca_intact	6082358	7.15	0	121.1	1268.16
diatom_setae	2712754	3.19	0	128.04	4868.37
fytoplankton_phaeocystis	2398473	2.82	0	126.31	3685.8
artefact_long_line	472384	0.55	0	27.28	4676.82
larvacea_Oikopleura_complete	305158	0.36	0	6.63	22.52
copepoda_nauplii	295645	0.35	0	6.86	23.62
copepoda	277816	0.33	0	5.23	38.31
larvacea_tail_only	233686	0.27	0	5.06	21.97
echinoderm_echinopluteus_type_1	143386	0.17	0	5.71	95.79
larvacea_body	136419	0.16	0	2.9	19.03
artefact_cleaning_fibre	135751	0.16	0	3.81	481.84
cladocera_evadne	113823	0.13	0	2.23	25.82
bubbles	98716	0.12	0	2.8	158.58
larvacea_Friitilaria_complete	84698	0.1	0	1.65	14.19
crustacea_exuvium	65306	0.08	0	1.7	7.33
platyhelminthes_microstomum	58042	0.07	0	1.89	24.47
cladocera_podon_pleopis	39649	0.05	0	1.01	25.5
diatom_solitary_centric	35414	0.04	0	0.86	13.93
dinoflagellate_pyrocystis	27869	0.03	0	0.79	5.98
diatom_other	27327	0.03	0	0.88	8.81
dinoflagellate_Tripos	18292	0.02	0	0.43	3.45
polychaete_mitraria	16505	0.02	0	0.41	7.35
polychaete_nectochaete	14453	0.02	0	0.48	20.66
diatom_odontella	13742	0.02	0	0.28	13.62
mollusca	13350	0.02	0	0.3	3.81
echinoderm_ophiopluteus	10890	0.01	0	0.41	13.8
polychaete_magelona	8708	0.01	0	0.16	1.41
echinoderm_branchiolaria	5200	0.01	0	0.11	1.33
polychaete_trochophore	4897	0.01	0	0.1	2.2
echinoderm_stars_asteridae	3689	0	0	0.08	1.72
balanoid_nauplius	3209	0	0	0.07	5.26
bryozoa_cyphonautes	3132	0	0	0.05	0.58
polychaete_empty_tube	2890	0	0	0.07	3.99
crutacea_zoea_crab	2668	0	0	0.05	0.55
echinoderm_bipinnaria	2483	0	0	0.07	2.78

polychaete_aulophore	1900	0	0	0.04	2.16
phoronida	1491	0	0	0.03	0.36
ctenophora	1462	0	0	0.03	0.26
amphioxus_larva	1348	0	0	0.02	0.63
fish_eggs	703	0	0	0.02	0.32
cnidaria_hydromedusae	664	0	0	0.01	0.34
echinoderm_stars_ophio	600	0	0	0.02	0.24
crustacea_zoea_shrimp	434	0	0	0.01	0.32
balanoid_cypris	402	0	0	0.01	0.2
echinoderm_echinopluteus_type_2	341	0	0	0.01	0.22
crustacea_megalopa	208	0	0	0	0.14
fish_larvae	198	0	0	0	0.17
cnidaria-tentacle	175	0	0	0	0.17
crustacea_amphipoda	172	0	0	0	0.07
echinoderm_stars_echino	157	0	0	0	0.17
cumacea	137	0	0	0	0.16
polychaete_nectosoma	105	0	0	0	0.09
chaetognath	95	0	0	0	0.12
crustacea_mysida	52	0	0	0	0.07
nemertea_pilidium	41	0	0	0	0.05
polychaete_Tomopteris	30	0	0	0	0.1
copepoda_monstrilloidae	12	0	0	0	0.06
crustacea_caprella	5	0	0	0	0.04
cnidaria_hydropolyp	3	0	0	0	0.02
cnidaria_ephyrae	2	0	0	0	0.01
hemichordata_enteropneusta_tornaria	1	0	0	0	0.06
platyhelminthes_trematoda_cercariae	1	0	0	0	0.02

Annex 2 Species list

The list below contains species identified by DNA metabarcoding with 3 markers, being part of this study. The numbers refer to literature sources. 1 = Fransz, 1983; 2 = Van Ginderdeuren et al., 2012; 3 = Greve et al., 2004; 4 = Soesbergen, 2023; 5= Soesbergen, 2022; CPR = Continuous Plankton Recorder data (see Chapter 2). Numbers in brackets refer to literature data where identification was only at genus level. maxid = maximum level of identification. Taxa are listed per Phylum in alphabetical order.

phylum	maxid	CO1	18S V9	18S V4	1	2	3	4	5	CPR
Amoebozoa	Squamamoeba japonica	CO1	18SV9							
Annelida	Ampharete finmarchica		18SV9							
Annelida	Ampharete sp.	CO1								
Annelida	Anaitides maculata				1					
Annelida	Arenicola marina				1					
Annelida	Autolytus branchycephalus				1		(3)			
Annelida	Autolytus edwardsi				1		(3)			
Annelida	Autolytus prolifer				1		(3)			
Annelida	Capitella capitata	CO1		18SV4	1					
Annelida	Capitella sp.		18SV9							
Annelida	Chaetopterus sarsi	CO1								
Annelida	Chaetopterus sp.		18SV9	18SV4						
Annelida	Chaetopterus variopedatus				1					
Annelida	Chaetozone pugettensis	CO1								
Annelida	Eteone longa				1					
Annelida	Eulalia viridus				1					
Annelida	Eumida mackiei	CO1								
Annelida	Eumida ockelmanni	CO1								
Annelida	Eunereis longissima	CO1								
Annelida	Ficopomatus enigmaticus	CO1								
Annelida	Gattyana cirrhosa	CO1								
Annelida	Glycera alba	CO1								
Annelida	Glycinde nordmanni	CO1								
Annelida	Glycinde sp.			18SV4						
Annelida	Goniadidae indet.		18SV9							
Annelida	Harmothoe clavigera	CO1								
Annelida	Harmothoe glabra	CO1								
Annelida	Harmothoe imbricata				1					
Annelida	Harmothoe impar				1					
Annelida	Harmothoe sp.	CO1								
Annelida	Hesionura elongata	CO1								
Annelida	Heteromastus filiformis	CO1			1					
Annelida	Heteromastus sp.	CO1								
Annelida	Lanice conchilega	CO1	18SV9		1		(3)			
Annelida	Laonice cirrata				1					
Annelida	Lepidonotus squamatus				1					
Annelida	Loimia ramzega	CO1								
Annelida	Loimia sp.			18SV4						
Annelida	Magelona filiformis	CO1								
Annelida	Magelona johnstoni	CO1								
Annelida	Magelona mirabilis	CO1	18SV9	18SV4						

Annelida	Magelona papillicornis				1		(3)
Annelida	Magelona sp.		18SV9	18SV4			
Annelida	Malmgrenia lunulata	CO1					
Annelida	Maupasia gracilis		18SV9				
Annelida	Maupasia sp.		18SV9				
Annelida	Meiodrilus adhaerens	CO1					
Annelida	Myrianida edwarsi	CO1					
Annelida	Nephtys assimilis	CO1					
Annelida	Nephtys caeca				1		
Annelida	Nephtys ciliata				1		
Annelida	Nephtys cirrosa	CO1					
Annelida	Nephtys hombergii	CO1			1		
Annelida	Nephtys sp.		18SV9				
Annelida	Nereididae indet.			18SV4			
Annelida	Nereis diversicolor				1		
Annelida	Nereis succinea				1		
Annelida	Nereis virens				1		
Annelida	Nereis zonata				1		
Annelida	Nicolea zostericola				1		
Annelida	Oligochaeta sp.					2	
Annelida	Owenia fusiformis	CO1	18SV9	18SV4	1		
Annelida	Oxydromus flexuosus/vittatus	CO1					
Annelida	Paraonidae indet.			18SV4			
Annelida	Pectinaria auricoma	CO1		18SV4	1		(3)
Annelida	Pectinaria belgica				1		(3)
Annelida	Pectinaria koreni	CO1	18SV9	18SV4	1		(3)
Annelida	Pectinaria sp.		18SV9				
Annelida	Pholoe minuta				1		
Annelida	Phyllodoce groenlandica/mucosa	CO1					
Annelida	Phyllodoce lineata	CO1		18SV4			
Annelida	Phyllodoce longipes	CO1					
Annelida	Phyllodoce rosea	CO1		18SV4			
Annelida	Phyllodoce sp.	CO1	18SV9	18SV4			
Annelida	Poecilochaetus serpens	CO1	18SV9	18SV4			
Annelida	Polydora caeca				1		
Annelida	Polydora ciliata				1		
Annelida	Polydora cornuta	CO1					
Annelida	Polydora hermaphroditica				1		
Annelida	Polydora ligni				1		
Annelida	Polydora onagawaensis	CO1					
Annelida	Polydora pulchra				1		
Annelida	Polydora quadrilobata				1		
Annelida	Polydora redekei				1		
Annelida	Polydora sp.		18SV9	18SV4			
Annelida	Polygordius appendiculatus	CO1					
Annelida	Polygordius lacteus	CO1					
Annelida	Polynoidae indet.			18SV4			
Annelida	Prionospio cirrifera				1		
Annelida	Prionospio malmgreni				1		
Annelida	Prionospio steenstrupi				1		
Annelida	Proceraea cornuta				1		
Annelida	Proceraea prismatica				1		
Annelida	Protodrilus oculifer	CO1					
Annelida	Protodrilus sp.		18SV9	18SV4			
Annelida	Pseudopolydora pulchra			18SV4			

Annelida	Pygospio elegans	CO1		18SV4	1					
Annelida	Sabellaria alveolata		18SV9							
Annelida	Sabellaria sp.			18SV4						
Annelida	Sabellaria spinulosa	CO1			1					
Annelida	Scolelepis bonnieri	CO1		18SV4						
Annelida	Scolelepis foliosa				1					
Annelida	Scolelepis neglecta	CO1								
Annelida	Scolelepis sp.		18SV9	18SV4						
Annelida	Scolelepis squamata	CO1		18SV4	1					
Annelida	Scoloplos armiger	CO1			1					
Annelida	Sigalion mathildae	CO1								
Annelida	Spio decorata	CO1								
Annelida	Spio filicornis				1					
Annelida	Spio sp.		18SV9	18SV4						
Annelida	Spio symphyta	CO1								
Annelida	Spiophanes bombyx	CO1			1					
Annelida	Spiophanes cf. bombyx VVP-2020		18SV9							
Annelida	Spiophanes cf. convexus VVP-2020		18SV9							
Annelida	Spiophanes kröyeri				1					
Annelida	Spiophanes sp.		18SV9	18SV4						
Annelida	Sthenelais boa	CO1		18SV4						
Annelida	Sthenelais limicola	CO1		18SV4						
Annelida	Streblospio shrubsoli				1					
Annelida	Tomopteris									
Annelida	Tomopteris helgolandica	CO1			1	2	3			
Annelida	Tomopteris septendrionalis						3			
Annelida	Tomopteris sp.		18SV9	18SV4						
Apicomplexa	Cephaloidophora cf. communis		18SV9							
Apicomplexa	Heliospora caprellae		18SV9							
Arachnida	Acari sp.					2				
Arthropoda	Acanthomysis longicornis					2				
Arthropoda	Acartia bifilosa	CO1	18SV9	18SV4	1			4		
Arthropoda	Acartia clausi	CO1	18SV9	18SV4	1	2		4		
Arthropoda	Acartia discaudata				1			4		
Arthropoda	Acartia hudsonica/tonsa	CO1								
Arthropoda	Acartia longiremis				1		(3)	4		CPR
Arthropoda	Acartia tonsa	CO1	18SV9	18SV4	1		(3)	4		
Arthropoda	Ameiridae indet.	CO1								
Arthropoda	Amphiascopsis cinctus	CO1								
Arthropoda	Amphibalanus improvisus	CO1		18SV4						
Arthropoda	Amphilochus neapolitanus					2				
Arthropoda	Amphipoda indet.		18SV9							
Arthropoda	Ampithoe sp.		18SV9							
Arthropoda	Anchialina agilis					2	3			
Arthropoda	Anomalocera patersonii				1		3	4		CPR
Arthropoda	Anomura megalopa					(2)	3			
Arthropoda	Aora gracilis	CO1	18SV9							
Arthropoda	Apherusa bispinosa					2				
Arthropoda	Apherusa clevei				1					
Arthropoda	Apherusa ovalipes					2				
Arthropoda	Apherusa sp.		18SV9							
Arthropoda	Argissa hamatipes	CO1								
Arthropoda	Atelecyclus undecimdentatus	CO1								

Arthropoda	Athanas nitescens						3		
Arthropoda	Athelges paguri	CO1							
Arthropoda	Atylus falcatus					2			
Arthropoda	Atylus swammerdami					2			
Arthropoda	Austrominius modestus	CO1		18SV4					
Arthropoda	Balanomorpha indet.		18SV9	18SV4					
Arthropoda	Balanus balanoides				1		(2)		
Arthropoda	Balanus crenatus	CO1		18SV4	1		(2)		
Arthropoda	Balanus improvisus				1		(2)		
Arthropoda	Bathyporea pelagica				1	(2)			
Arthropoda	Bathyporea pilosa				1	(2)			
Arthropoda	Bathyporea sarsi				1	(2)			
Arthropoda	Bathyporeia elegans	CO1							
Arthropoda	Bodotria arenosa					2			
Arthropoda	Bodotria pulchella				1				
Arthropoda	Bodotria scorpioides				1	2			
Arthropoda	Bopyridae indet.		18SV9						
Arthropoda	Brachyura sp.					2			
Arthropoda	Bryocamptus sp.		18SV9						
Arthropoda	Calanoides carinatus								
Arthropoda	Calanus finmarchicus				1		(3)	4	CPR
Arthropoda	Calanus glacialis								CPR
Arthropoda	Calanus helgolandicus	CO1			1	2	(3)	4	CPR
Arthropoda	Calanus sp.			18SV4					
Arthropoda	Calanus/Neocalanus sp.		18SV9						
Arthropoda	Caligus elongatus		18SV9						
Arthropoda	Caligus rapax				1		(3)		
Arthropoda	Caligus sp.	CO1							
Arthropoda	Callianassa subterranea	CO1	18SV9	18SV4		(2)	3		
Arthropoda	Calliopius rathkei				1				
Arthropoda	Cancer pagurus	CO1		18SV4	1		3		
Arthropoda	Candacia armata					2	3	4	CPR
Arthropoda	Candacia simplex								
Arthropoda	Canuella perplexa	CO1	18SV9						
Arthropoda	Caprella linearis				1	2	(3)		
Arthropoda	Carcinus maenas	CO1	18SV9	18SV4	1		3		
Arthropoda	Caridea sp.					2			
Arthropoda	Caridion steveni						3		
Arthropoda	Centropages hamatus	CO1			1	2	3	4	CPR
Arthropoda	Centropages sp.		18SV9	18SV4					
Arthropoda	Centropages typicus	CO1		18SV4	1	2	3	4	CPR
Arthropoda	Clausidiidae indet.		18SV9						
Arthropoda	Corophium arenarium				1				
Arthropoda	Corophium insidiosum				1				
Arthropoda	Corophium lacustre				1				
Arthropoda	Corophium sextoni				1				
Arthropoda	Corophium volutator				1	(2)			
Arthropoda	Corycaeidae indet.		18SV9						
Arthropoda	Corystes cassivelaunus	CO1					3		
Arthropoda	Crangon allmanni	CO1					3		
Arthropoda	Crangon crangon	CO1	18SV9		1	2	3		
Arthropoda	Crangon sp.			18SV4					
Arthropoda	Crangonidae indet.		18SV9	18SV4					
Arthropoda	Cumopsis goodsirii				1				
Arthropoda	Cyclopina gracilis							4	
Arthropoda	Cyclopinoides littoralis				1	2	(3)	4	

Arthropoda	Cymbasoma germanicum					2		4		
Arthropoda	Decapoda indet.			18SV4						
Arthropoda	Decapoda sp.					2				
Arthropoda	Diaixis hibernica							4		CPR
Arthropoda	Diastylis bradyi	CO1								
Arthropoda	Diastylis rathkei		18SV9		1	2	3			
Arthropoda	Diogenes pugilator	CO1								
Arthropoda	Ditrichocorycaeus anglicus	CO1			1	2	(3)	4		
Arthropoda	Ebalia spp.						3			
Arthropoda	Ectinosomatidae indet.	CO1								
Arthropoda	Elminius modestus				1					
Arthropoda	Eriocheir sinensis		18SV9							
Arthropoda	Erythrops spp.						3			
Arthropoda	Eualus occultus						3			
Arthropoda	Eualus pusiolus						3			
Arthropoda	Euchaeta acuta									CPR
Arthropoda	Eupagurus bernhardus				1					
Arthropoda	Euphausia recurva	CO1								
Arthropoda	Eurydice pulchra				1		3			
Arthropoda	Eurydice spinigera					2				
Arthropoda	Euryte longicauda							4		
Arthropoda	Eurytemora affinis				1			4		
Arthropoda	Eurytemora americana							4		
Arthropoda	Eurytemora carolleeae							4		
Arthropoda	Eurytemora hurindo							4		
Arthropoda	Euterpina acutifrons	CO1	18SV9		1	2	3	4		CPR
Arthropoda	Evadne nordmanni	CO1	18SV9		1	2				
Arthropoda	Evadne spinifera				1		(3)			
Arthropoda	Evadne/Podon sp.		18SV9							
Arthropoda	Evansula pygmaea	CO1								
Arthropoda	Galathea spp.						3			
Arthropoda	Gamarellus angulosus				1					
Arthropoda	Gammarus crinicornis					2				
Arthropoda	Gammarus locusta				1					
Arthropoda	Gammarus oceanicus				1					
Arthropoda	Gammarus salinus				1	2				
Arthropoda	Gammarus zaddachi				1					
Arthropoda	Gastrosaccus sanctus					2	3			
Arthropoda	Gastrosaccus spinifer	CO1	18SV9	18SV4	1	2	3			
Arthropoda	Giardella callianassae					2				CPR
Arthropoda	Giardella callianassae					2				
Arthropoda	Giardella thompsoni									CPR
Arthropoda	Harpacticoida indet.			18SV4						
Arthropoda	Hemicyclops aberdonensis									CPR
Arthropoda	Hippolyte varians	CO1	18SV9				3			
Arthropoda	Hippomedon denticulatus				1					
Arthropoda	Homarus gammarus						3			
Arthropoda	Hyas araneus				1		(3)			
Arthropoda	Hyperia galba				1	2				
Arthropoda	Hyperoche medusarum	CO1								
Arthropoda	Idotea baltica						3			
Arthropoda	Idotea linearis				1		3			
Arthropoda	Idothea balthica				1					
Arthropoda	Idothea chelipes				1					
Arthropoda	Idothea granulosa				1					

Arthropoda	Idothea pelagica				1				
Arthropoda	Iphinoe trispinosa						3		
Arthropoda	Isias clavipes	CO1			1	2		4	CPR
Arthropoda	Isopoda indet.		18SV9						
Arthropoda	Isopoda sp.					2			
Arthropoda	Jassa falcata		18SV9		1		3		
Arthropoda	Jassa herdmani	CO1				2			
Arthropoda	Jassa marmorata	CO1							
Arthropoda	Labidocera acutifrons								CPR
Arthropoda	Labidocera wollastoni				1	2	3	4	CPR
Arthropoda	Lamprops fasciata				1				
Arthropoda	Leptastacus aff. laticaudatus	CO1							
Arthropoda	Leptomysis mediterranea				1		3		
Arthropoda	Lernaeenicus sprattae	CO1							
Arthropoda	Leucothoe incisa					2			
Arthropoda	Liocarcinus depurator	CO1	18SV9	18SV4					
Arthropoda	Liocarcinus holsatus	CO1							
Arthropoda	Liocarcinus maculatus		18SV9						
Arthropoda	Liocarcinus marmoreus	CO1							
Arthropoda	Liocarcinus navigator	CO1							
Arthropoda	Liocarcinus sp.						3		
Arthropoda	Longipedia coronata	CO1							
Arthropoda	Longipedia sp.	CO1	18SV9						
Arthropoda	Longipediidae indet.		18SV9						
Arthropoda	Macropipus holsatus				1				
Arthropoda	Macropodia rostrata						3		
Arthropoda	Marinogammarus marinus				1				
Arthropoda	Megaluropus agilis	CO1				2			
Arthropoda	Melita palmata				1				
Arthropoda	Menigrates obtusifrons		18SV9						
Arthropoda	Mesocalanus tenuicornis								CPR
Arthropoda	Mesopodopsis slabberi				1	2	3		
Arthropoda	Metridia longa							4-	
Arthropoda	Metridia lucens					2		4	CPR
Arthropoda	Microarthridion littorale							4	
Arthropoda	Microcalanus pusillus	CO1						4	
Arthropoda	Microprotopus maculatus	CO1			1	2			
Arthropoda	Microsetella norvegica	CO1	18SV9		1		(3)	4	
Arthropoda	Monocorophium acherusicum	CO1							
Arthropoda	Monoculodes carinatus				1				
Arthropoda	Monopseudocuma gilsoni	CO1				2			
Arthropoda	Monstrilla longiremis								CPR
Arthropoda	Monstrilla spp. (helgolandica?)						3	4	
Arthropoda	Mysis mixta				1				
Arthropoda	Nannopus sp.		18SV9						
Arthropoda	Necora puber	CO1		18SV4					
Arthropoda	Neomysis integer				1		3		
Arthropoda	Neomysis longicornis						3		
Arthropoda	Nototropis falcatus	CO1			1				
Arthropoda	Nototropis swammerdamei	CO1	18SV9		1				
Arthropoda	Nototropis vedlomensis				1				
Arthropoda	Nyctiphanes couchii					2	3		
Arthropoda	Oithona davisae							4	
Arthropoda	Oithona helgolandica							4	
Arthropoda	Oithona nana				1	2		4	
Arthropoda	Oithona similis	CO1	18SV9	18SV4	1	2	(3)		

Arthropoda	Oithona sp.		18SV9						
Arthropoda	Oncaea sp.		18SV9						
Arthropoda	Oncaea waldemari	CO1							
Arthropoda	Oncea venusta				1	(2)		4	
Arthropoda	Orchomenella nana					2			
Arthropoda	Paguridae indet.			18SV4					
Arthropoda	Pagurus bernhardus	CO1					3		
Arthropoda	Palaemon adspersus				1		(3)		
Arthropoda	Palaemon elegans				1		(3)		
Arthropoda	Palaemon longirostris				1		(3)		
Arthropoda	Pandalina brevirostris						3		
Arthropoda	Pandalus montagui				1		3		
Arthropoda	Paracalanus parvus	CO1	18SV9	18SV4	1	2	3	4	
Arthropoda	Paraeuchaeta hebes								CPR
Arthropoda	Paraleptastacus espinulatus	CO1							
Arthropoda	Paramesochridae indet.	CO1							
Arthropoda	Paramunna bilobata	CO1							
Arthropoda	Paramysis arenosa						3		
Arthropoda	Paramysis helleri						3		
Arthropoda	Paramysis kervillei				1		3		
Arthropoda	Paramysis ornata				1		3		
Arthropoda	Paramysis spiritus				1		3		
Arthropoda	Parapontella brevicornis				1			4	CPR
Arthropoda	Parathemisto oblivia				1				
Arthropoda	Pariambus typicus	CO1				2			
Arthropoda	Penilia avirostris					2	3		CPR
Arthropoda	Philocheras bispinosus	CO1					3		
Arthropoda	Philocheras trispinosus	CO1					3		
Arthropoda	Phthisica marina				1				
Arthropoda	Pilumnus hirtellus						3		
Arthropoda	Pinnotheres pisum						3		
Arthropoda	Pisidia longicornis	CO1				2			
Arthropoda	Pleopis polyphemoides	CO1			1				
Arthropoda	Pleuromamma robusta								CPR
Arthropoda	Podon intermedius	CO1			1				
Arthropoda	Podon leuckartii	CO1			1	2	(3)		
Arthropoda	Polybiidae indet.		18SV9						
Arthropoda	Pontellina plumata								CPR
Arthropoda	Pontocrates altamarinus	CO1			1	2			
Arthropoda	Pontocrates arenarius					2			
Arthropoda	Portumnus latipes	CO1							
Arthropoda	Praunus flexuosus				1		3		
Arthropoda	Praunus inermis				1		3		
Arthropoda	Processa modica	CO1				2	3		
Arthropoda	Processidae indet.		18SV9	18SV4					
Arthropoda	Pseudocalanus elongatus	CO1	18SV9		1	2	3	4	
Arthropoda	Pseudocalanus mimus	CO1							
Arthropoda	Pseudocalanus moultoni	CO1	18SV9					4	
Arthropoda	Pseudocalanus sp.			18SV4					
Arthropoda	Pseudocuma longicorne	CO1			1	2			
Arthropoda	Pseudocuma similis	CO1				2			
Arthropoda	Pseudocuma sp.					2	3		
Arthropoda	Pseudocumatidae indet.			18SV4					
Arthropoda	Pseudocyclops crassiremis							4	
Arthropoda	Pseudodiaptomus marinus	CO1	18SV9					4	

Arthropoda	Pygolabis humphreysi	CO1								
Arthropoda	Rhincalanus nasutus									CPR
Arthropoda	Rivulogammarus duebeni				1					
Arthropoda	Sacculina carcini			18SV4						
Arthropoda	Schistomysis kervillei		18SV9	18SV4		2				
Arthropoda	Schistomysis ornata					2				
Arthropoda	Schistomysis spiritus					2				
Arthropoda	Scopelocheirus hopei	CO1								
Arthropoda	Semicytherura striata			18SV4						
Arthropoda	Siriella armata				1	2	3			
Arthropoda	Siriella clausi						3			
Arthropoda	Siriella norvegica				1					
Arthropoda	Tachidius discipes							4		
Arthropoda	Taeniacanthidae indet.		18SV9							
Arthropoda	Tanais dulongii					2				
Arthropoda	Temora longicornis	CO1		18SV4	1	2	3	4		CPR
Arthropoda	Temora sp.		18SV9	18SV4						
Arthropoda	Themisto abyssorum		18SV9							
Arthropoda	Themisto sp.	CO1								
Arthropoda	Thia scutellata						3			
Arthropoda	Thysanoessa inermis						3			
Arthropoda	Thysanoessa longicaudata						3			
Arthropoda	Thysanoessa raschii						3			
Arthropoda	Tisbe elegantula	CO1								
Arthropoda	Tisbe furcata				1		(3)			
Arthropoda	Tortanus discaudatus	CO1						4		CPR
Arthropoda	Tryphosella sarsi	CO1								
Arthropoda	Upogebia deltaura	CO1								
Arthropoda	Upogebia sp.			18SV4			3			
Arthropoda	Upogebiidae indet.			18SV4						
Arthropoda	Verruca stroemia	CO1	18SV9	18SV4						
Bryozoa	Bryozoa sp.					2				
Bryozoa	Conopeum reticulum			18SV4						
Bryozoa	Electra crustulenta				1					
Bryozoa	Electra pilosa	CO1	18SV9	18SV4	1					
Bryozoa	Membranipora membranacea	CO1			1					
Bryozoa	Triticella flava		18SV9							
Cercozoa	Allas sp.		18SV9	18SV4						
Cercozoa	Botuliforma benthica			18SV4						
Cercozoa	Cercozoa indet.			18SV4						
Cercozoa	Cryomonadida indet.			18SV4						
Cercozoa	Cryothecomonas aestivalis		18SV9							
Cercozoa	Cryothecomonas longipes		18SV9	18SV4						
Cercozoa	Cryothecomonas sp. APCC MC5-1Cryo		18SV9							
Cercozoa	Discomonas retusa			18SV4						
Cercozoa	Ebria tripartita			18SV4						
Cercozoa	Helkesimastix marina			18SV4						
Cercozoa	Massisteria marina		18SV9							
Cercozoa	Massisteria sp.		18SV9							
Cercozoa	Minorisa sp. SRT705		18SV9							
Cercozoa	Minorisa sp. SRT71			18SV4						
Cercozoa	Minorisa sp. SRT75			18SV4						
Cercozoa	Norrisiella sphaerica		18SV9							
Cercozoa	Pseudopirsonia mucosa		18SV9							
Cercozoa	Rhogostoma sp.			18SV4						

Cercozoa	Rhogostomidae sp.		18SV9					
Cercozoa	Thaumatomastix sp.		18SV9					
Cercozoa	Thaumatomastix sp. CC2-Boundary Bay			18SV4				
Cercozoa	Trachyrhizium urniformis		18SV9					
Cercozoa	Ventrifissura artocarpoidea			18SV4				
Chaetognatha	Parasagitta elegans		18SV9	18SV4		2		
Chaetognatha	Parasagitta setosa	CO1		18SV4		2		
Chaetognatha	Sagitta bipunctata			18SV4				
Chaetognatha	Sagitta elegans	CO1			1		3	
Chaetognatha	Sagitta setosa				1		3	
Chaetognatha	Sagitta sp.			18SV4				
Chordata	Actinopteri indet.		18SV9					
Chordata	Agonus cataphractus						3	
Chordata	Ammodytes marinus	CO1				2		
Chordata	Ammodytes tobianus	CO1				2		
Chordata	Ammodytidae indet.			18SV4				
Chordata	Ammodytidae sp.					2		
Chordata	Anguilla anguilla						3	
Chordata	Appendicularia						3	
Chordata	Arnoglossus laterna	CO1				2		
Chordata	Bothidae indet.			18SV4				
Chordata	Branchiostoma lanceolatum	CO1				2	(3)	CPR
Chordata	Branchiostoma sp.		18SV9					
Chordata	Buglossidium luteum	CO1	18SV9			2		
Chordata	Callionymus lyra	CO1	18SV9					
Chordata	Callionymus reticulatus	CO1						
Chordata	Callionymus sp.					2		
Chordata	Chelidonichthys lucerna	CO1						
Chordata	Clupea harengus	CO1				2		
Chordata	Clupeidae indet.		18SV9	18SV4				
Chordata	Crystallogobius sp.							
Chordata	Cyclopterus lumpus						3	
Chordata	Dicentrarchus labrax	CO1						
Chordata	Doliolum nationalis						3	
Chordata	Echiichthys vipera					2		
Chordata	Engraulis encrasicolus	CO1				2		
Chordata	Entelurus aequoreus	CO1						
Chordata	Entelurus aequoreus							CPR
Chordata	Fritillaria borealis		18SV9		1		3	
Chordata	Fritillaria borealis typica			18SV4				
Chordata	Gadiformes indet.		18SV9	18SV4				
Chordata	Gobiidae indet.			18SV4				
Chordata	Gobiidae sp.					2		
Chordata	Hyperoplus lanceolatus					2		
Chordata	Limanda limanda	CO1				2		
Chordata	Merlangius merlangus	CO1				2		
Chordata	Oikopleura dioica		18SV9	18SV4	1	2	3	
Chordata	Oikopleura labradorensis				1			
Chordata	Osmerus eperlanus					2		
Chordata	Pisces sp.					2		
Chordata	Pleuronectes platessa					2		
Chordata	Pleuronectidae indet.		18SV9					
Chordata	Pleuronectiformes indet.			18SV4		2		
Chordata	Pomatoschistus lozanoi	CO1						

Chordata	Pomatoschistus microps/minutus	CO1			
Chordata	Pomatoschistus norvegicus	CO1			
Chordata	Pomatoschistus sp.				2
Chordata	Sardina pilchardus	CO1			2
Chordata	Scomber scombrus	CO1	18SV9		
Chordata	Scombridae indet.			18SV4	
Chordata	Solea solea	CO1	18SV9		2
Chordata	Soleidae indet.			18SV4	
Chordata	Sprattus sprattus	CO1	18SV9		2
Chordata	Syngnathus rostellatus				2
Chordata	Trachurus trachurus	CO1		18SV4	2
Chordata	Triglidae sp.				2
Chordata	Trisopterus luscus	CO1			
Ciliophora	Acineta sp.			18SV4	
Ciliophora	Biggaria bermudensis		18SV9		
Ciliophora	Citrithrix smalli		18SV9		
Ciliophora	Codonellopsidae indet.			18SV4	
Ciliophora	Cyclotrichium cyclokaryon		18SV9	18SV4	
Ciliophora	Ephelota sp.		18SV9	18SV4	
Ciliophora	Eutintinnus cf. apertus		18SV9		
Ciliophora	Helicostomella subulata		18SV9		
Ciliophora	Hemiophrys macrostoma			18SV4	
Ciliophora	Hypocoma acinetarum		18SV9	18SV4	
Ciliophora	Laackmanniella prolongata			18SV4	
Ciliophora	Mesanophrys carcini		18SV9		
Ciliophora	Mesanophrys sp.			18SV4	
Ciliophora	Oligohymenophorea sp.			18SV4	
Ciliophora	Paracineta limbata		18SV9	18SV4	
Ciliophora	Paralembus digitiformis		18SV9		
Ciliophora	Pelagostrobilidium neptuni		18SV9		
Ciliophora	Pelagostrobilidium sp.			18SV4	
Ciliophora	Philaster sinensis		18SV9		
Ciliophora	Philasterida indet.			18SV4	
Ciliophora	Pseudotontonia sp. JG-211a			18SV4	
Ciliophora	Sessilida indet.			18SV4	
Ciliophora	Spathidium foissneri			18SV4	
Ciliophora	Spathidium sp.		18SV9		
Ciliophora	Spirotrichea indet.			18SV4	
Ciliophora	Strombidinopsis sp.			18SV4	
Ciliophora	Strombidinopsis sp. NSMS0601		18SV9		
Ciliophora	Strombidium capitatum			18SV4	
Ciliophora	Strombidium cf. basimorphum			18SV4	
Ciliophora	Strombidium sp.			18SV4	
Ciliophora	Strombidium sp. ZS-2015		18SV9		
Ciliophora	Strombidium sp. ZS-215			18SV4	
Ciliophora	Tintinnida indet.		18SV9	18SV4	
Ciliophora	Tintinnidium mucicola		18SV9		
Ciliophora	Tintinnopsis sp.			18SV4	
Ciliophora	Vampyrophrya pelagica		18SV9	18SV4	
Ciliophora	Vorticella sp.		18SV9		
Ciliophora	Zoothamnium intermedium		18SV9		
Ciliophora	Zoothamnium sp.		18SV9	18SV4	
Cnidaria	Actiniaria indet.		18SV9	18SV4	
Cnidaria	Aequorea forskalea		18SV9	18SV4	
Cnidaria	Aequorea vitrina	CO1			

Cnidaria	Agalmatidae indet.			18SV4			
Cnidaria	Agastra mira						3
Cnidaria	Aglantha digitale				1		3
Cnidaria	Alcyonium digitatum	CO1			1		
Cnidaria	Amphinema dinema					2	3
Cnidaria	Amphinema rugosum						3
Cnidaria	Anthoathecata indet.			18SV4			
Cnidaria	Arachnactis bournei				1		(3)
Cnidaria	Aurelia aurita	CO1	18SV9	18SV4	1	2	3
Cnidaria	Bougainvillia britannica	CO1					3
Cnidaria	Bougainvillia macloviana						3
Cnidaria	Bougainvillia muscus	CO1	18SV9				
Cnidaria	Bougainvillia principis						3
Cnidaria	Bougainvillia ramosa				1		3
Cnidaria	Campanulariidae indet.			18SV4			
Cnidaria	Cerianthus lloydii	CO1					
Cnidaria	Chrysaora hysoscella	CO1			1	2	3
Cnidaria	Cladonema radiatum						3
Cnidaria	Clytia gracilis			18SV4			
Cnidaria	Clytia hemisphaerica	CO1	18SV9	18SV4		2	
Cnidaria	Clytia languida/gracilis	CO1					
Cnidaria	Clytia sp.		18SV9				
Cnidaria	Corymorpha nutans	CO1	18SV9				
Cnidaria	Corymorpha sp.			18SV4			
Cnidaria	Cosmetira pilosella	CO1					3
Cnidaria	Cyanea capillata	CO1			1		3
Cnidaria	Cyanea lamarckii	CO1	18SV9		1	2	3
Cnidaria	Cyanea sp.			18SV4			
Cnidaria	Ectopleura dumortierii	CO1	18SV9	18SV4	1		3
Cnidaria	Ectopleura larynx	CO1					
Cnidaria	Eirene viridula						3
Cnidaria	Eucheilota maculata	CO1	18SV9	18SV4	1	2	3
Cnidaria	Euphysa aurata						3
Cnidaria	Euphysa tentaculata	CO1					
Cnidaria	Eutima gegenbauri				1		3
Cnidaria	Eutima gracilis	CO1				2	3
Cnidaria	Eutima insignis						3
Cnidaria	Eutima sp.			18SV4			
Cnidaria	Eutonina indicans	CO1	18SV9			2	3
Cnidaria	Gonothyraea loveni	CO1					
Cnidaria	Helgicirrha cari	CO1	18SV9				3
Cnidaria	Helgicirrha schulzei						3
Cnidaria	Hybocodon prolifer				1		3
Cnidaria	Hydractiniidae indet.	CO1	18SV9	18SV4			
Cnidaria	Laodicea undulata						3
Cnidaria	Leuckartiara octona	CO1	18SV9	18SV4			3
Cnidaria	Lizzia blondina	CO1	18SV9	18SV4	1		3
Cnidaria	Lovenella clausa					(2)	3
Cnidaria	Margelopsis haeckelii	CO1	18SV9	18SV4	1	2	3
Cnidaria	Melicertum octocostatum	CO1	18SV9	18SV4			3
Cnidaria	Mitrocomella brownei						3
Cnidaria	Mitrocomella polydiademata						3
Cnidaria	Mitrocomella sp.			18SV4			
Cnidaria	Muggiaea atlantica						3
Cnidaria	Nanomia cara	CO1					3

Cnidaria	Nemopsis bachei	CO1			1	2	3
Cnidaria	Obelia				1	(2)	(3)
Cnidaria	Obelia bidentata	CO1					
Cnidaria	Obelia dichotoma	CO1		18SV4			
Cnidaria	Obelia dichotoma/geniculata		18SV9				
Cnidaria	Obelia longissima	CO1					
Cnidaria	Obelia sp.			18SV4			
Cnidaria	Peachia spp.						3
Cnidaria	Phialella quadrata						3
Cnidaria	Phialidium haemisphaericum				1		3
Cnidaria	Podocoryna sp.		18SV9				
Cnidaria	Podocoryne borealis						3
Cnidaria	Podocoryne carnea						3
Cnidaria	Rathkea octopunctata				1	2	3
Cnidaria	Rhizostoma octopus				1		3
Cnidaria	Rhizostoma pulmo					2	
Cnidaria	Sagartia troglodytes	CO1					
Cnidaria	Sagitaria				1		
Cnidaria	Sarsia eximia						3
Cnidaria	Sarsia gemmifera				1		3
Cnidaria	Sarsia prolifera						3
Cnidaria	Sarsia tubulosa				1	2	3
Cnidaria	Siphonophorae indet.		18SV9				
Cnidaria	Steenstrupia nutans				1		3
Cnidaria	Tiaropsis multicirrata						3
Cnidaria	Tima bairdii	CO1	18SV9				3
Cnidaria	Tubularia larynx				1		
Cnidaria	Turritopsis matriculata				1		
Cnidaria	Zanclea costata						3
Ctenophora	Beroe cucumis	CO1			1		3
Ctenophora	Beroe cucumis/gracilis		18SV9	18SV4			
Ctenophora	Beroe gracilis			18SV4	1	2	3
Ctenophora	Beroe sp.	CO1					
Ctenophora	Bolinopsis infundibulum	CO1	18SV9		1		3
Ctenophora	Lobata indet.			18SV4			
Ctenophora	Mnemiopsis leidyi	CO1				2	
Ctenophora	Pleurobrachia pileus	CO1	18SV9		1	2	3
Ctenophora	Pleurobrachia sp.			18SV4			
Ctenophora	Tentaculata indet.		18SV9				
Discosea	Paramoeba pemaquidensis		18SV9				
Discosea	Vermistella antarctica		18SV9				
Discosea	Vexillifera sp. K9		18SV9				
Echinodermata	Acrocnida brachiata	CO1					
Echinodermata	Amphilepidida indet.			18SV4			
Echinodermata	Amphipholis squamata		18SV9				
Echinodermata	Amphiura filiformis	CO1					3
Echinodermata	Amphiuridae indet.			18SV4			
Echinodermata	Asterias rubens	CO1	18SV9	18SV4	1	2	3
Echinodermata	Astropecten irregularis	CO1	18SV9				
Echinodermata	Echinocardium cordatum	CO1	18SV9		1	(2)	
Echinodermata	Echinocardium pennatifidum	CO1					
Echinodermata	Echinocyamus pusillus				1		
Echinodermata	Echinoidea indet.			18SV4			
Echinodermata	Echinus esculentis				1		
Echinodermata	Ophiocten affinis	CO1					
Echinodermata	Ophiothrix fragilis	CO1			1	2	

Echinodermata	Ophiura albida	CO1			1	(2)	(3)
Echinodermata	Ophiura ophiura	CO1					
Echinodermata	Ophiura texturata				1		
Echinodermata	Ophiuroidea indet.		18SV9				
Echinodermata	Psammechinus miliaris	CO1			1	2	3
Echinodermata	Solaster papposus				1		
Echinodermata	Spatangus purpureus	CO1					
Entoprocta	Loxosomella stomatophora			18SV4			
Euglenozoa	Bodonidae indet.		18SV9				
Euglenozoa	Hemistasia phaeocysticola			18SV4			
Euglenozoa	Neobodo sp.		18SV9				
Euglenozoa	Procryptobia sorokini		18SV9				
Euglenozoa	Rhynchomonas sp.		18SV9				
Hemichordata	Ptychoderidae indet.			18SV4			
Mollusca	Abra alba	CO1					
Mollusca	Acanthocardia sp.	CO1					
Mollusca	Aequipecten opercularis	CO1					
Mollusca	Alloteuthis media	CO1					
Mollusca	Amphorina linensis	CO1					
Mollusca	Angulus tenuis				1		
Mollusca	Aporrhais sp.		18SV9				
Mollusca	Atalodoris inconspicua/sparsa	CO1					
Mollusca	Barnea candida	CO1					
Mollusca	Bivalvia sp.					2	
Mollusca	Calliopaea bellula		18SV9	18SV4			
Mollusca	Cardiida indet.			18SV4			
Mollusca	Cardiida indet.1			18SV4			
Mollusca	Cardiidae indet.		18SV9	18SV4			
Mollusca	Cerastoderma edule	CO1	18SV9	18SV4	1		
Mollusca	Chamelea striatula	CO1					
Mollusca	Clione limacina						
Mollusca	Coecum glabrum				1		
Mollusca	Crepidula fornicata	CO1		18SV4	1		
Mollusca	Cylichna cylindracea	CO1	18SV9				
Mollusca	Cyrenidae indet.		18SV9				
Mollusca	Donax sp.	CO1					
Mollusca	Elysia viridis	CO1	18SV9				
Mollusca	Embletonia pulchra	CO1		18SV4			
Mollusca	Embletonia sp.		18SV9				
Mollusca	Ensis directus/leei	CO1					
Mollusca	Ensis ensis	CO1					
Mollusca	Ensis magnus	CO1					
Mollusca	Ensis siliqua	CO1					
Mollusca	Ensis sp.		18SV9			2	
Mollusca	Eubranchus exiguus	CO1		18SV4			
Mollusca	Eubranchus rupium	CO1					
Mollusca	Euspira nitida	CO1					
Mollusca	Euspira sp.		18SV9	18SV4			
Mollusca	Euthyneura indet		18SV9				
Mollusca	Fabulina sp.	CO1					
Mollusca	Facelina bostoniensis	CO1	18SV9				
Mollusca	Gari sp.	CO1					
Mollusca	Gastropoda indet.			18SV4			
Mollusca	Gastropoda sp.					2	
Mollusca	Hiatella arctica		18SV9				

Mollusca	Hydrobia ulvae	CO1			1				
Mollusca	Hydrobia ventrosa				1				
Mollusca	Kellia sp.		18SV9						
Mollusca	Kellia suborbicularis	CO1							
Mollusca	Kurtiella bidentata	CO1							
Mollusca	Kurtiella sp.		18SV9						
Mollusca	Laevicardium crassum	CO1							
Mollusca	Lamellaria sp.	CO1							
Mollusca	Lasaeidae indet.		18SV9						
Mollusca	Limapontia capitata				1				
Mollusca	Littorina littorea				1				
Mollusca	Loligo sp.					2			
Mollusca	Loligo vulgaris	CO1							
Mollusca	Lora turricola				1				
Mollusca	Lutraria angustior	CO1							
Mollusca	Lutraria lutraria	CO1							
Mollusca	Macoma baltica				1				
Mollusca	Macoma sp.	CO1							
Mollusca	Mactra stultorum	CO1							
Mollusca	Mactridae indet.		18SV9						
Mollusca	Mya arenaria	CO1			1				
Mollusca	Mya truncata				1				
Mollusca	Mysella bidentata				1				
Mollusca	Mytilus edulis				1				
Mollusca	Mytilus sp.	CO1	18SV9	18SV4					
Mollusca	Nassarius reticulatus				1				
Mollusca	Natica catena				1				
Mollusca	Natica poliana				1				
Mollusca	Naticidae indet.		18SV9	18SV4					
Mollusca	Neogastropoda indet.			18SV4					
Mollusca	Nucula nitidosa	CO1							
Mollusca	Nudibranchia indet.		18SV9	18SV4					
Mollusca	Onchidoris bilamellata	CO1	18SV9	18SV4					
Mollusca	Onchidoris muricata		18SV9						
Mollusca	Onoba vitrea				1				
Mollusca	Pectinidae sp.					2			
Mollusca	Peringia ulvae		18SV9	18SV4					
Mollusca	Petricola sp.		18SV9						
Mollusca	Petricolaria pholadiformis	CO1							
Mollusca	Phaxas pellucidus	CO1	18SV9						
Mollusca	Philine quadripartita				1				
Mollusca	Philinoglossa praelongata		18SV9						
Mollusca	Piliscus sp.						3		
Mollusca	Placida dendritica	CO1							
Mollusca	Pneumodermopsis paucidens								CPR
Mollusca	Polycera capitata	CO1							
Mollusca	Polycera capitata/norvegica	CO1							
Mollusca	Polycera quadrilineata	CO1							
Mollusca	Pusillina inconspicua	CO1							
Mollusca	Retusa retusa				1				
Mollusca	Rissoidae indet.		18SV9						
Mollusca	Scrobicularia plana				1				
Mollusca	Spisula solida	CO1							
Mollusca	Spisula subtruncata	CO1			1				
Mollusca	Spisula/Mulinia sp.			18SV4					
Mollusca	Tellimya ferruginosa	CO1		18SV4					

Mollusca	Tellinoidea indet.		18SV9					
Mollusca	Teredo navalis				1			
Mollusca	Tergipes tergipes	CO1	18SV9					
Mollusca	Tritia reticulata	CO1		18SV4				
Mollusca	Turritellidae indet.		18SV9					
Mollusca	Turritellinella tricarinata	CO1						
Mollusca	Varicorbula gibba	CO1						
Mollusca	Veneridae indet.		18SV9					
Mollusca	Venerupis corrugata	CO1						
Mollusca	Venus striatula				1			
Mollusca	Zirfaea crispata				1			
Mollusca	Zirfaea sp.	CO1						
Nematoda	Enoplus brevis		18SV9					
Nematoda	Hysterothylacium aduncum	CO1						
Nematoda	Sabatieria sp.		18SV9					
Nematoda	Subsphaerolaimus sp.		18SV9	18SV4				
Nematoda	Viscosia sp.			18SV4				
Nemertea	Callinera grandis			18SV4				
Nemertea	Cephalothrix	CO1						
Nemertea	Cerebratulus fuscus	CO1						
Nemertea	Cerebratulus sp.		18SV9	18SV4				
Nemertea	Hubrechtella dubia	CO1						
Nemertea	Hubrechtella sp.		18SV9					
Nemertea	Lineus bilineatus	CO1						
Nemertea	Nemertea sp.					2		
Nemertea	Oerstedia dorsalis	CO1						
Nemertea	Poseidonemertes sp.			18SV4				
Nemertea	Siphonenteron bilineatum		18SV9	18SV4				
Nemertea	Tenuilineus albocinctus	CO1						
Nemertea	Tetrastemma sp.		18SV9	18SV4				
Phoronida	Phoronis muelleri	CO1	18SV9		1	(2)		
Phoronida	Phoronis pallida	CO1						
Phoronida	Phoronis sp.		18SV9	18SV4				
Picozoa	Picomonas judraskeda		18SV9					
Picozoa	Picomonas sp.			18SV4				
Platyhelminthes	Alaurina composita				1		3	
Platyhelminthes	Bucephalus minimus		18SV9					
Platyhelminthes	Cestoda indet.		18SV9					
Platyhelminthes	Derogenes sp.		18SV9					
Platyhelminthes	Duploperaclistus circocirrus			18SV4				
Platyhelminthes	Hemiuridae indet.		18SV9					
Platyhelminthes	Kuhnia scombri							CPR
Platyhelminthes	Lecithaster gibbosus		18SV9					
Platyhelminthes	Microstomum sp.		18SV9	18SV4				
Platyhelminthes	Paromalostomum sp.		18SV9					
Platyhelminthes	Plagiostomum vittatum				1			
Platyhelminthes	Platyhelminthes sp.					2		
Platyhelminthes	Polycladida indet.			18SV4				
Platyhelminthes	Prosorhynchoides megacirrus		18SV9					
Platyhelminthes	Stylochus zebra		18SV9					
Porifera	Swartschewskia papyracea			18SV4				
Porifera	Verongida indet.	CO1						
Prasinodermophyta	Prasinoderma coloniale			18SV4				
Retaria	Trizona brandti			18SV4				

Rotifera	Brachionus mülleri				1	5
Rotifera	Keratella cruciformis				1	5
Rotifera	Notholca acuminata				1	5
Rotifera	Synchaeta grimpei	CO1				
Rotifera	Synchaeta littoralis				1	5
Rotifera	Synchaeta sp.	CO1				
Rotifera	Synchaeta triophtalma				1	5
Rotifera	Synchaeta vorax				1	5
Rotifera	Testudinella clypeata				1	5
Rotifera	Trichocerca marina				1	5
Tubulinea	Nolandella abertawensis		18SV9	18SV4		

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