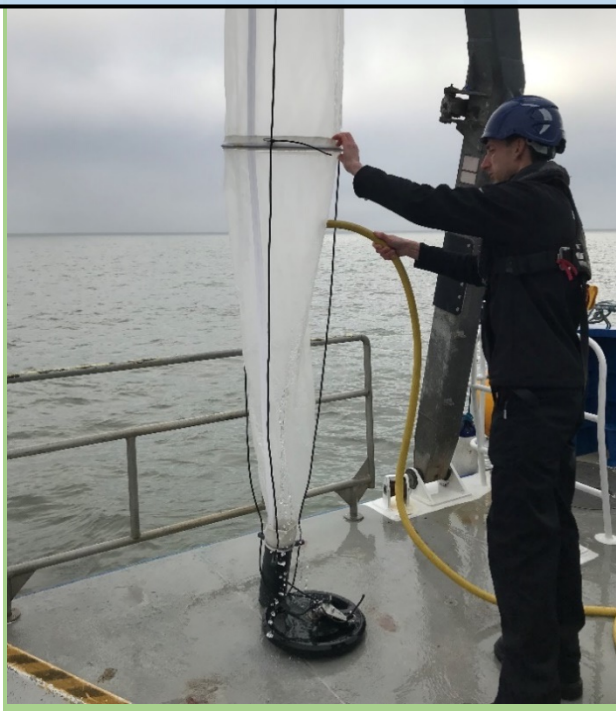
NCEA Natural Capital and Ecosystem Assessment

2023

Marine Natural Capital and Ecosystem Assessment (mNCEA) Programme – Pelagic Monitoring Programme Project Year 1

Monitoring, programme, sampling analysis, and data collection



Mike Best, Rob Hillman, Michelle Devlin & David Johns

EA, Cefas & MBA

2/24/2023

Environment Agency | Cefas | Marine Biological Association | JNCC Joint Nature Conservation Committee | Marine Management Organisation | Department for Environment Food & Rural Affairs

Project title:	NC34 Pelagic Monitoring Programme
Lead organisation	Environment Agency
Project SRO/lead:	Roger Proudfoot
Project start date:	Y1 (of 3): 01 April 2022
Project end date:	Y1 (of 3): 31 March 2023
Total cost:	Y1 (of 3): £630,854

Version	Date	Summary
Draft 1.0	21/11/2022	1st draft
Draft 2.0	05/01/2023	2nd draft
Draft 3.0	29/01/2023	3rd draft – Cefas basic info added
Draft 4.0	05/02/2023	4th draft – EA data reanalysed
Draft 5.0	06/02/2023	5th draft – MBA appendix added
Draft 6.0	07/02/2023	6th draft – MBA Chlorophyll method added
Draft 7.0	08/02/2023	7th draft – Reformat and edits
Draft 8.0	10/02/2023	8th draft – Formats, EA data appendices
Draft 9.0	23/02/2023	9th draft – Merged edits, added appendices, re-format
Draft 10.0	24/02/2023	10th draft – Cefas merged edits and reformat. Final draft from a technical perspective.
Draft 11.0	21/08/2023	11th draft – Largely formatting changes made by EA and following feedback from Defra
Final	26/02/2024	Final report – Final tweaks before publication by Defra

This project was funded by the Department for Environment, Food and Rural Affairs (Defra) as part of the marine Natural Capital and Ecosystem Assessment (NCEA) programme. The marine NCEA programme is leading the way in supporting Government ambition to integrate natural capital approaches into decision making for the marine environment. Find out more at https://www.gov.uk/government/publications/natural-capital-and-ecosystem-assessment-programme

1. Ways to read this report

Key messages and highlights

At the beginning of this report there is a summary of Key Messages of the pelagic monitoring programme showing where and when samples were collected. However, if you need to understand the development of the programme, then the Overview of Sampling would also be useful.

Understanding methods

The organisations involved in this work were:

- Environment Agency
- Centre for Environment, Fisheries, and Aquaculture Science (Cefas)
- Marine Biological Association (MBA)
- Joint Nature Conservation Committee (JNCC)
- Marine Management Organisation (MMO)

Each organisation started from a different base in methods. The development of these for each organisation is covered in the Overview of Methods. We consider both the field collection and lab analysis. However, a significant amount of detail (and all of MBA's Continuous Plankton Recording (CPR) method) has been placed in the Appendices (see Appendices 1-5 for Environment Agency, Appendix 6 for Lab Analysis, Appendix 7 for Cefas, and Appendix 8 for MBA).

Reviewing results

We were only funded for data collection in this first year [of the Pelagic Monitoring Programme] and a detailed statistical analysis would be inappropriate on less than a years' worth of data. However, some results are presented as part of methods testing (such as, 'do different sized net meshes catch different things?' and 'does the method detect differences in different regions?'). There are also some limited results in the Results section to illustrate the data and to reassure us that community patterns are as expected (such as, greatest numbers in the summer months and then tailing off). Some examples of data sheets, together with embedded Excel data tables can be found in the Appendix 9.

Data storage

Each organisation has started at a different point in storing zooplankton data and the need to develop this. Ultimately all data flows to the Plankton Lifeform Extraction Tool (PLET) system (on DASSH) for storage, analysis, and public visibility. This is briefly covered in Database and Data Flows section. Thinking and developments in this area are still very dynamic.

mNCEA Pelagic Monitoring Programme

Year 1 report on Monitoring and data

1. Key Messages

This first year of the Pelagic Monitoring Programme project, under the marine Natural Capital and Ecosystem Assessment (mNCEA) programme, concentrated on filling the sampling gaps in what will be a coordinated England (and UK) plankton monitoring programme. The main gaps were:

1. Zooplankton in the close inshore waters (that is, Water Environment (Water Framework Directive) Regulations (WER/WFD) coastal water bodies) - There has been no consistent zooplankton information taken in this area which is heavily influenced by land-based pressures. This was undertaken by the Environment Agency (EA).
2. 'Transects' from estuary mouth to offshore SmartBuoy sites for zooplankton off the Thames and the Mersey - This was undertaken by Cefas using the Cefas Endeavour and a collaborative vessel approach with the EA coastal survey vessels (CSVs) to ensure appropriate frequency of sampling.
3. Routine Continuous Plankton Recorder (CPR) collection and analysis in the English routes (North East Atlantic to Southwest Approaches, Dogger Bank, and Southern North Sea) - These were reinstated by the Marine Biological Association (MBA).

A full 12 months of sampling will not be completed at every site due to:

- delayed start dates
- methodological issues and testing
- weather issues and down time
- vessel breakdowns.

However, sufficient data has been collected over almost all at appropriate frequencies for baseline statistical analysis and quality assurance.

Over the months of May-Jan 2022-23, at time of writing we have collected:

- 156 zooplankton samples from 25 sites, in 16 waterbodies (5 regional seas, 2 OSPAR assessment areas) and supporting water quality data (Aug-Jan 2023) (EA)
- 10 extra zooplankton sites for samples at each of two SmartBuoys locations and supporting water quality data each month (Cefas)
- 8500 sampled nautical miles (nm) of CPR tows for plankton (MBA)

This represents an area of approximately 218 thousand square kilometres (km^2) of English and UK Seas:

- **EA:** 3,329 km^2, calculated from the area of 16 coastal WER water bodies reflecting 28% of England's coastal water bodies
- **Cefas:** 2,626 km^2, estimated from coastal water bodies and adjacent waterbodies + 10%)

- **MBA:** 212,500 km^2, calculated from a quarter of the 8500 nm towed (a conservative estimate of the tows on the UK continental shelf). This is multiplied by R, where R is the width of the sea-area for which a CPR sample is representative. *Ostle et al* (2017, EcApHRA D1.3) found highest correlation between Plymouth L4 and CPR copepod data in a circle radius one quarter of a degree – this would approximate to a track-width of 50 km for R (25 k either side of tow). This is minimum estimate.

The area of UK sea is calculated at 885,430 km2 so the area covered may be considered to represent about 25% of UK shelf seas, although not evenly spread.

The location of sample sites and transects are given in Figure 1. These complement historic and current UK pelagic monitoring programmes and complement further studies in water environment regulation and river plume zones (see legend in Figure 1).

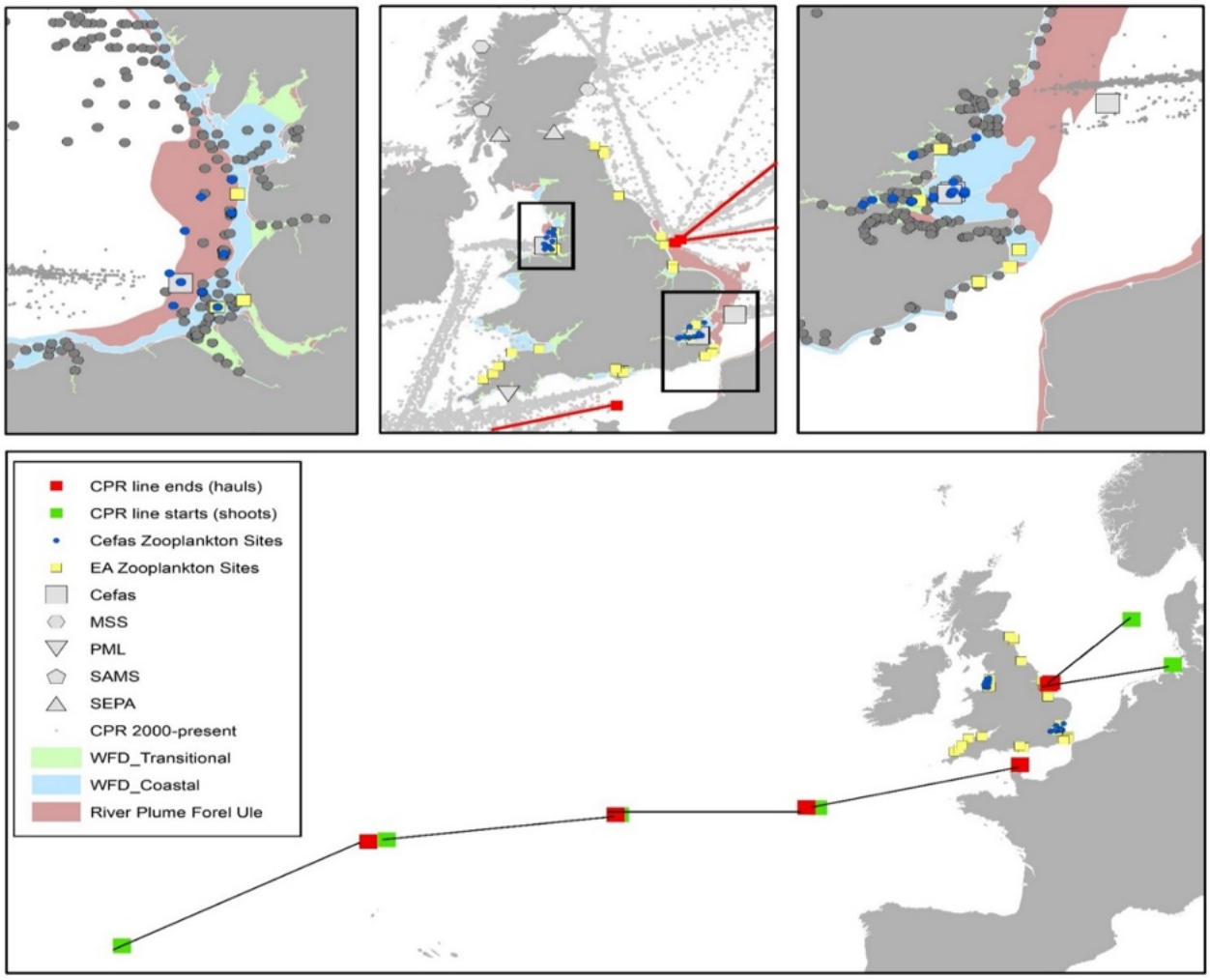


Figure 1: *Location of sampling sites and transects for EA, Cefas and MBA (indicated by red, green, blue, and yellow shapes) for this programme. The map also plots historic and current UK pelagic monitoring programmes (indicated by large grey dots, small grey dots, and grey shapes), and the water environment regulation (WFD) and river plume zones (indicated by green, blue, and brown shading).*

From the inshore sample sites over 155 different zooplankton taxa were identified from inshore waters at 25 sites sampled over 7 months at the time of writing. (the MBA samples have yet to be analysed), including the

new and invasive copepod species *Pseudodiaptomus marinus*. The total estimated count from the inshore waters was of the order of a million individuals.

The monitoring and analytical costs for these additions to existing pelagic monitoring programmes are estimated at £491K for vessel time, sample collection, sample analysis, and data storage and archiving. It is expected that this will change as the project develops – we may need more, or a different spread, of sampling points, while some efficiencies will be achieved through collaborative working and innovations. It is likely to be in a similar order of magnitude. This programme builds on current monitoring programs, which may also be at various funding risks.

2. Background: The importance of zooplankton as indicators of a changing environment

In recent decades, changes have been observed in plankton communities of the north-east Atlantic. Assessments of environmental status under the Marine Strategy Framework Directive (MSFD) include indicators for assessing changes in plankton communities. For the UK implementation, a lifeform index has been developed for Descriptor 1 (D1, Biological Diversity), D4 (Marine Food Webs) and D6 (Seafloor Integrity). The target is that the condition of the plankton community should not be significantly adversely influenced by anthropogenic drivers. Any changes may signal the need for investigative research to identify causal links with environmental change and human pressures such as fishing, nutrient enrichment, and micro-plastics.

The lifeform index is based on the use of plankton pairs (*Gowen et al. 2011, Tett et al. 2008*). The index may include different lifeform pairs for zooplankton which are derived from a range of zooplankton functional groups and features. The key functional groups may be described as follows:

- **Microzooplankton** – Invertebrate organisms which are generally < 80 µm in size and include heterotrophs such as ciliates and flagellates; samples are collected as natural seawater using Niskin bottles, buckets, or vessel mounted flow-through systems.
- **Mesozooplankto**n – Invertebrate zooplankton > 80 µm and < 4 mm in length, sampled using nets with different mesh sizes (e.g. 80 µm, 270 µm, 500 µm, 1000 µm). Mainly holoplankton (e.g. small crustaceans and their reproductive life stages) and meroplankton (e.g. reproductive stages of benthic species). Crustacean communities are generally dominated by copepods, which undertake diurnal vertical migrations in the water column. Nauplii and juveniles < 80-270 µm are also referred to as microcrustaceans.
- **Macrozooplankton** – Invertebrate zooplankton ≥ 4 mm in length; sampled using nets with mesh sizes ≥ 270 µm. Mainly holoplankton (e.g. mysids and amphipods) capable of undertaking extensive diurnal vertical migrations.
- **Gelatinous zooplankton** – Jellyfish and other gelatinous organisms (e.g. ctenophores), ranging in size from less than a millimetre to nearly 2 m in diameter; difficult to sample quantitatively.
- **Ichthyoplankton** – Fish eggs and larvae. Sampled as for zooplankton, using nets with mesh sizes ≥ 270 µm.

Within each of these functional groups, zooplankton may be assigned to additional functional groups, and to size classes within these groups. For example, copepods can be assigned to the functional groups below:

- **Calanoid copepods** – Dominant in marine waters. 'Large' calanoid copepods include *Calanus spp*, while 'small' calanoid copepods include *Temora, Centropages, Acartia, Paracalanus and Pseudocalanus* species, and juvenile stages of the 'large' copepods.
- **Cyclopoid copepods** – Include *Oithona spp*. And *Oncaea spp.* More abundant in shallow waters near the coast or overlying the continental shelf ('neritic').
- **Harpacticoid copepods** – Infrequent in water column samples but more common in estuaries (e.g. *Tisbe spp*) and near the seabed.

3. Overview of Sampling

3.1.Year 1 Objectives

1. Monthly nearshore zooplankton collection and identification from EA English inshore (WER, previously WFD) water bodies – approximately 20-25 water bodies (Approx. 300 samples per full year), covering key inshore eco-hydrographic areas. Aiming for annual data sets with monthly resolution of zooplankton taxa that will be directly comparable to current co-located phytoplankton data sets, allowing comparison of inshore changes and impacts on food webs.
2. Re-instigation of zooplankton monitoring at and around two Cefas English SmartBuoy sites together with neighbouring supporting sites supplying water quality data. Aiming for monthly sampling. Provides critical zooplankton data from Thames Estuary plume and Liverpool Bay plume, aiding the identification of impacts on key natural capital assets (shellfish and fisheries). Surveys are collaborative between EA and Cefas, undertaken on EA vessels with Cefas sampling staff.
3. Routine Continuous Plankton Recorder (CPR) collection and analysis from reinstated English routes to cover all key areas. Annual data sets resolved at the frequency of ships of opportunity (weekly/ monthly). Completes spatial coverage of England (offshore) waters. The reinstated MBA routes run alongside the existing CPR survey (part funded by Defra), and the combined dataset to be used in all assessment work. The route details are the:
    a. suspended extended B-route (since 2017), this is the area that goes towards the north-east approaches of the UK from the Atlantic, it is a key 'early warning' area as it tends to be where we first pick up new or warmer water species moving upwards from lower latitudes
    b. CPR KC route (the only CPR route to go through Dogger Bank region, important for fisheries)
    c. HE route (a southern North Sea route)

    All data from these restarted routes will be immediately comparable to all historical CPR data.

The data from these surveys will be available on both institutional and national databases, made findable by via data archive centres such as PLET: Plankton Lifeform Extraction Tool (dassh.ac.uk), DASSH: The Archive for Marine Species and Habitats Data - Home, and MEDIN: Marine Environmental Data and Information Network.

The overall sample coverage is shown in Figure 2.

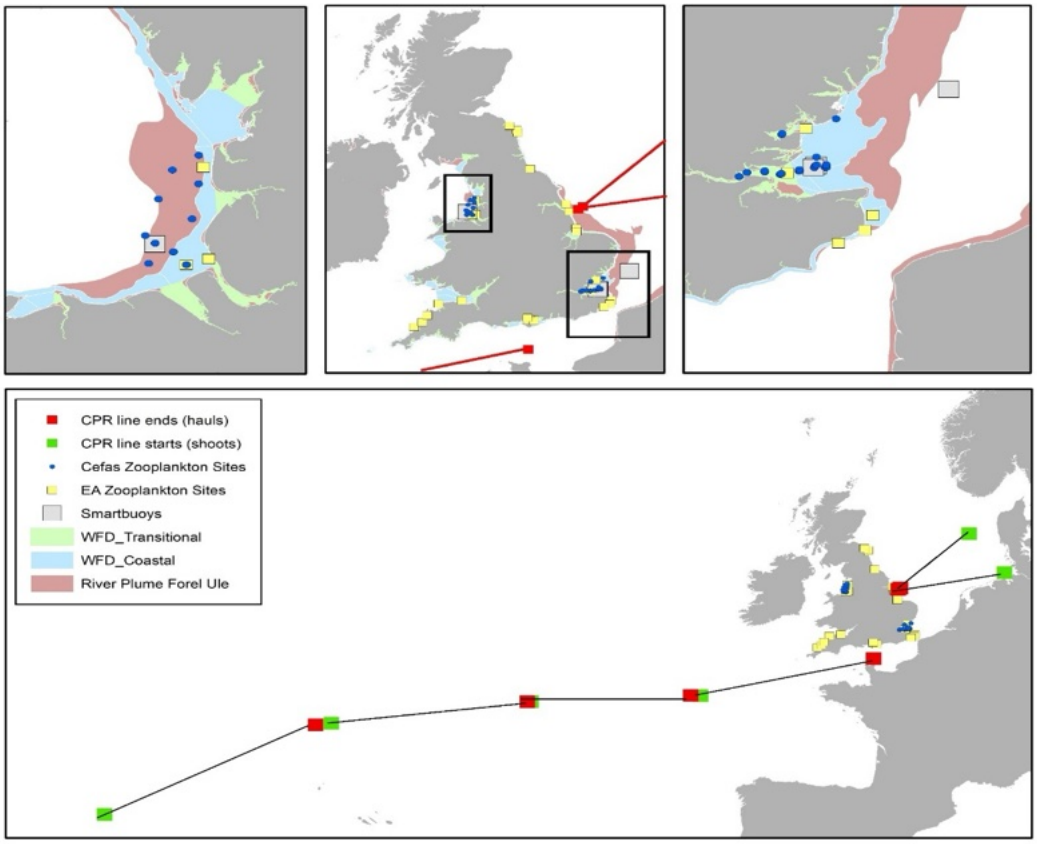


Figure 2: *Location of sites and transects for EA, Cefas and MBA*

3.2.Environment Agency sampling

Sites were selected to coincide with WER (WFD) phytoplankton sites, optimising both vessel time sampling costs and data alignment. There are currently 26 WFD sites spread across 16 coastal and transitional water bodies. For the purposes of year 1 of this programme, the sampling was focused mainly on coastal water bodies.

Zooplankton sampling is more complex than filling a sample bottle for phytoplankton analysis (see Methods section and Appendices 1-5), the size of net selected, and methodology put constraints on sample sites. The main selection criteria were:

1. Sites sampled from Coastal Survey Vessel (CSV) rather than Rigid Hull Inflatable Boat (RIB), or similar – The size of the net and the weight required to sink the net and keep it near vertical in a current means that this method cannot be used in a very small craft. Consequently, as the Southwest Channel coast is almost entirely delivered by RIB this area has initially been excluded.
2. Depth – A minimum water depth of 10m is needed to take a zooplankton sample. This will provide 5m of water column to sample, allowing for the length of the net (approximately 3.2m) plus a buffer between the drop weight and the seabed of 1-2m. For the safety of the CSV this depth was required at all states of the tide.
3. Avoiding areas of heavy vessel traffic – The zooplankton net deployment and recovery take a significantly longer time on site than collecting a water quality and phytoplankton sample, so the survey team wanted to avoid harbour entrances and areas with busy recreational traffic.

4. Good geographic coverage – Samples to be spread around east, west, and channel coasts. As the budget limited us to analysis of 25 samples a month (including any replicates) a good representative coverage is required to include areas of estuarine plumes and open coast.
5. Where possible consider areas where there is bivalve or fishery recruitment – There is a lot of interest in the recruitment of bivalves, crustacean, and fisheries, so some sites were chosen in areas of interest to these projects.
6. Opportunity to have multiple sites in a water body – This allows us to check on variability and indicate minimum sampling size.

These selection criteria are illustrated in the infographic (Figure 3) showing the different types of spatial and temporal sampling.

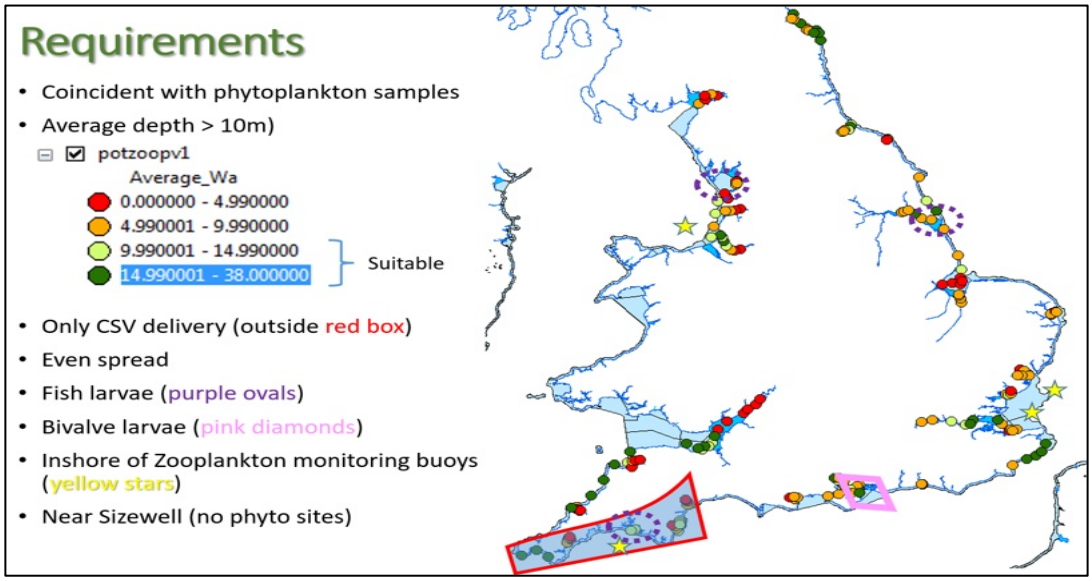


Figure 3: *Visualisation of how Environment Agency sites were selected for zooplankton monitoring*

All our live phytoplankton sites were tabulated and assessed against these criteria, so that we could select coinciding sites for zooplankton sampling (Figure 4, full table in Appendix 4).

Region	WFD water body	Spt code	BIOSYS code	NGR	Northing	Easting	CSV or RIB	Average Water Depth (m)	Spt Grid Ref	>5m ?	>15m?	> 20m?	Good for larval Fish [Y/N]	Good for Oyster (bivalve?) larvae [Y/N]	Good for other reasons (add in comment)
AN	Blackwater Outer	BE02	OBW003P	TM0400011000		211000 604000	CSV	8	TM0400011000	Y	FALSE	FALSE			
AN	Blackwater Outer	BE061099	OBW005P	TM0615009950		209950 606150	CSV	12	TM0615009950	Y	FALSE	FALSE			
AN	Blackwater Outer	NE608211	OBW001P	TM0860011700		211700 608600	CSV	5	TM0860011700	FALSE	FALSE	FALSE			
AN	Blackwater Outer	NE608214	OBW004P	TM0840914662		214662 608409	CSV	3	TM0840914662	FALSE	FALSE	FALSE			
AN	BURE & WAVENEY & YARE & LOTHING	BWY004P	BWY004P	TG4820006500		306500 648200	RIB	0		FALSE	FALSE	FALSE			
AN	BURE & WAVENEY & YARE & LOTHING	WAV179	BWY002P	TG4737004050		304050 647370	RIB	4	TG4737004050	FALSE	FALSE	FALSE			
AN	BURE & WAVENEY & YARE & LOTHING	YAR2625	BWY003P	TG4655004900		304900 646550	RIB	5	TG4655004900	FALSE	FALSE	FALSE			
AN	BURE & WAVENEY & YARE & LOTHING	YAR268	BWY005P	TG5175008000		308000 651750	RIB	3	TG5175008000	FALSE	FALSE	FALSE			
AN	BURE & WAVENEY & YARE & LOTHING	YAR270	BWY001P	TG5212007540		307540 652120	RIB	9	TG5212007540	Y	FALSE	FALSE			
AN	GREAT OUSE	57M01	GRO003P	TF6120018500		318500 561200	CSV	6	TF6120018500	Y	FALSE	FALSE			
AN	GREAT OUSE	62M01	GRO004P	TF6010023400		323400 560100	CSV	5	TF6010023400	FALSE	FALSE	FALSE			
AN	GREAT OUSE	62M31	GRO001P	TF5865026600		326600 558650	CSV	6	TF5865026600	Y	FALSE	FALSE			
AN	GREAT OUSE	62M34	GRO002P	TF5855028200		328200 558550	CSV	5	TF5882028050	FALSE	FALSE	FALSE			
AN	HUMBER LOWER	CLNMON1	HUM001P	TA2261918075		418075 522619	CSV	9	TA2261918075	Y	FALSE	FALSE		M	
AN	HUMBER LOWER	CONT29	HUM003P	TA3215015998		415998 532150	CSV	9	TA3215015998	Y	FALSE	FALSE		M	
AN	HUMBER LOWER	HUM87702	HUM006P	TA1690023400		423400 516900	CSV	16	TA1690023400	Y	Yes	FALSE		Y	
AN	HUMBER MIDDLE	HU504425	HUM009P	TA0400025000		425000 504000	CSV	8	TA0400025000	Y	FALSE	FALSE			
AN	HUMBER MIDDLE	HU506426	HUM007P	TA0658026042		426042 506580	CSV	8	TA0658026042	Y	FALSE	FALSE			
AN	HUMBER MIDDLE	HU510427	HUM008P	TA1000027000		427000 510000	CSV	8	TA1000027000	Y	FALSE	FALSE			
AN	Lincolnshire	LC544405	SYK003P	TA4450005500		405500 544500	CSV	8	TA4450005500	Y	FALSE	FALSE			
AN	Lincolnshire	LC558374	SYK004P	TF5800074000		374000 558000	CSV	9	TF5800074000	Y	FALSE	FALSE			
AN	Lincolnshire	LC560357	SYK005P	TF6058557271		357271 560585	CSV	12	TF6058557271	Y	FALSE	FALSE			
AN	ORWELL	ORW085	ORS002P	TM1760040400		240400 617600	CSV	8	TM1760040400	FALSE	FALSE	FALSE			
AN	ORWELL	ORW122	ORS001P	TM2540034800		234800 625400	CSV		TM2540034800		FALSE	FALSE			
AN	ORWELL	WFDORW03	ORS006P	TM2135938325		238325 621359	CSV		TM2135938325		FALSE				
AN	STOUR (ESSEX)	SE0310	ORS005P	TM2590132888		232888 625901	CSV		TM2590132888		FA				
AN	STOUR (ESSEX)	SE0650	ORS003P	TM191507		232850 619150	CSV								
A	STOUR (ESSEX)	SE08	ORS004P	TM1525		640	CSV								
/	Wash Outer	WA552	OWS0			200									
	Wa	WA560	OWS												

Figure 4: *Example section from Environment Agency data sheets, demonstrating how live phytoplankton sites were assessed against the criteria for zooplankton sampling (Full table in Appendix 4).*

From these 26 sites were selected. Following initial field trials modifications were made to 3 sites following information from survey officers and crew. Following data analysis on replicate sites we may be able to redistribute some of the sites.

3.2.1. Fixed sites and dates

The final sites and some of their geographic reporting contexts are shown in Table 1. Numbers in brackets are number of sites in that geographic area. Location of sites are illustrated in Figure 5.

Regional Sea	EA Region	Water body	Site Name
Northern North Sea (4)	North East (5)	Northumberland North (1)	OFFSHORE CHESWICK SANDS
		Farne Islands to Newton Haven (2)	FARNE ISLANDS 2.5KM OFF BEADNELL BAY (NORTHUMBERLAND WFD SITE 09)
			FARNE ISLANDS 2KM E OF INNER FARNE (THE BUSH) (FARNE ISLANDS WFD 03)
		Tees (1)	TEES AT REDCAR JETTY (SURFACE)
Southern North Sea (6)		Yorkshire South (1)	YORKS COAST - WITHERNSEA
	Anglian (4)	Lincolnshire (2)	LINCS COAST HAILE SAND FLAT S.YORKSHIRE LINCS.
			LINCS COAST OUTER DOGS HEAD 4.5 KM O/S
		Wash (1)	WASH SITE 33 - THE WELL/LYNN DEEPS 2 CONNECTED TO SPT WA560348 NEW SPT CREATED AS SITE MOVED
		Blackwater Outer (1)	VIRLEY CHANNEL OUTER R.BLACKWATER ST.PETER FLATS
	Thames (1)	Thames Lower (1)	THAMES AT NO.2 SEA REACH (77.6KMS BELOW LONDON BRIDGE)
Eastern Channel (8)	Southern (8)	Kent South (3)	GOODWIN FORK BUOY - INVESTIGATIONS BASELINE SURVEY
			SOUTH FORELAND - INVESTIGATIONS BASELINE SURVEY
			I KM SOUTH OF FOLKESTONE PIER, SOUTH KENT
		Portsmouth Habour (1)	PORTSMOUTH HARBOUR MOUTH SAMPLING POINT
		Solent (3)	EAST BRAMBLES SAMPLING POINT
			RYDE-SHELLFISH WATER, 50'44.750N, 01'06.340W AT NE MINING GROUND BUOY
			COWES-SHELLFISH WATER, 50'46.380N, 01'17.500W AT PRINCE CONSORT BUOY
		Southampton Water (1)	FAWLEY SOUTH SAMPLING POINT
Western Channel & Celtic Sea (5)	South West (5)	Cornwall North (3)	NORTH CORNWALL OFF HARLYN BAY (WFD02)
			NORTH CORNWALL OFF BOSSINEY (WFD05)
			NORTH CORNWALL OFF SANDY MOUTH (WFD04)
		Barnstaple Bay (1)	BARNSTAPLE BAY OFF WOOLACOMBE (WFD 01)
		Bristol Channel Inner South (1)	INNER BRISTOL CHANNEL OFF MINEHEAD B (WFD 10)
	North West		MERSEY ESTUARY AT BUOY C21 HELICOPTER POINT 5

Irish Sea (3)	North West (3)	Mersey Mouth (3)	COASTAL SURVEY NRA-173 BLACKPOOL: SITE SLC 40
			COASTAL SURVEY NRA-170 N WIRRAL: SITE SLC 55 & WLA 1

Table 1: *Geographic regions and sites sampled under the Environment Agency sampling programme*

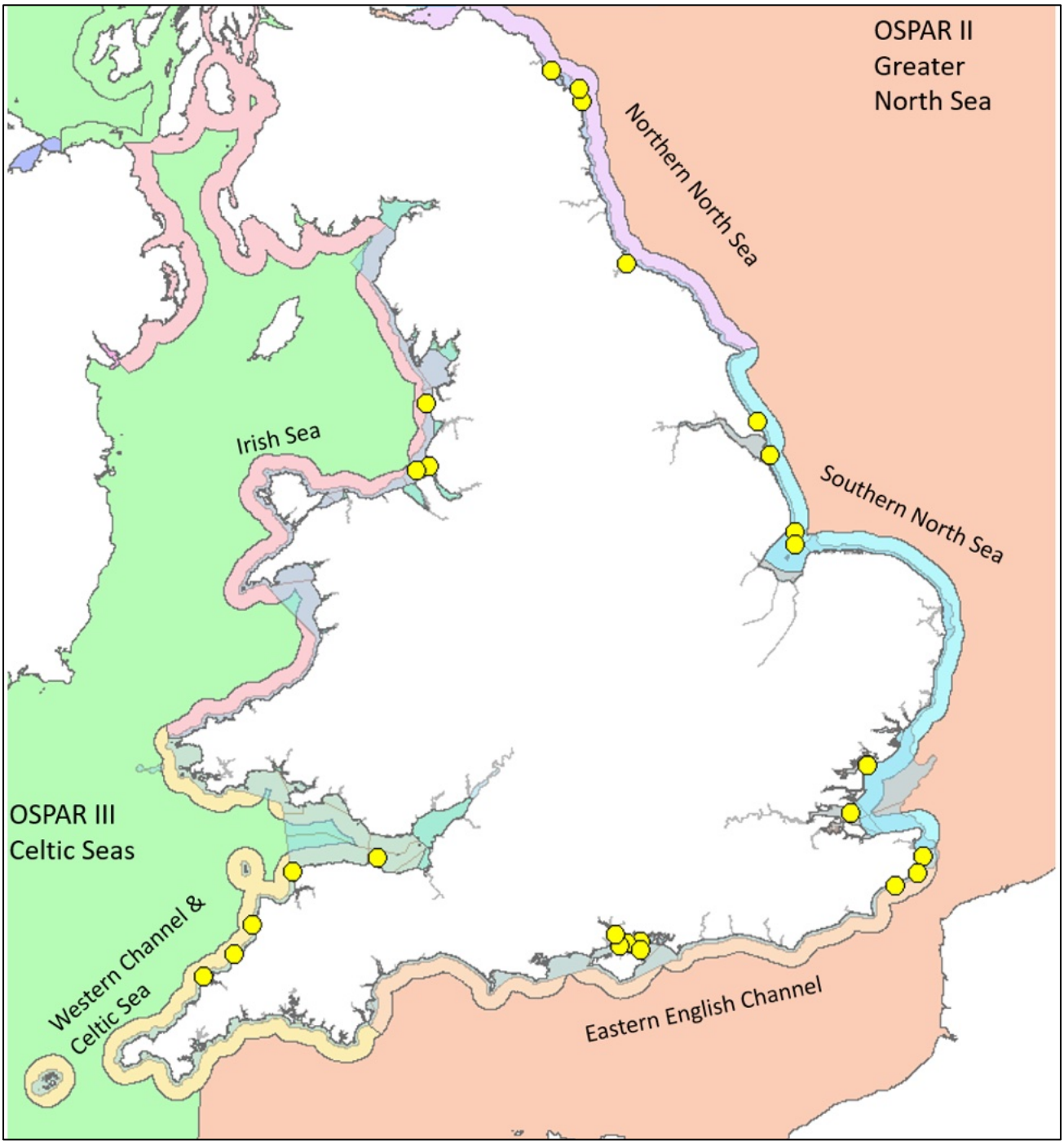
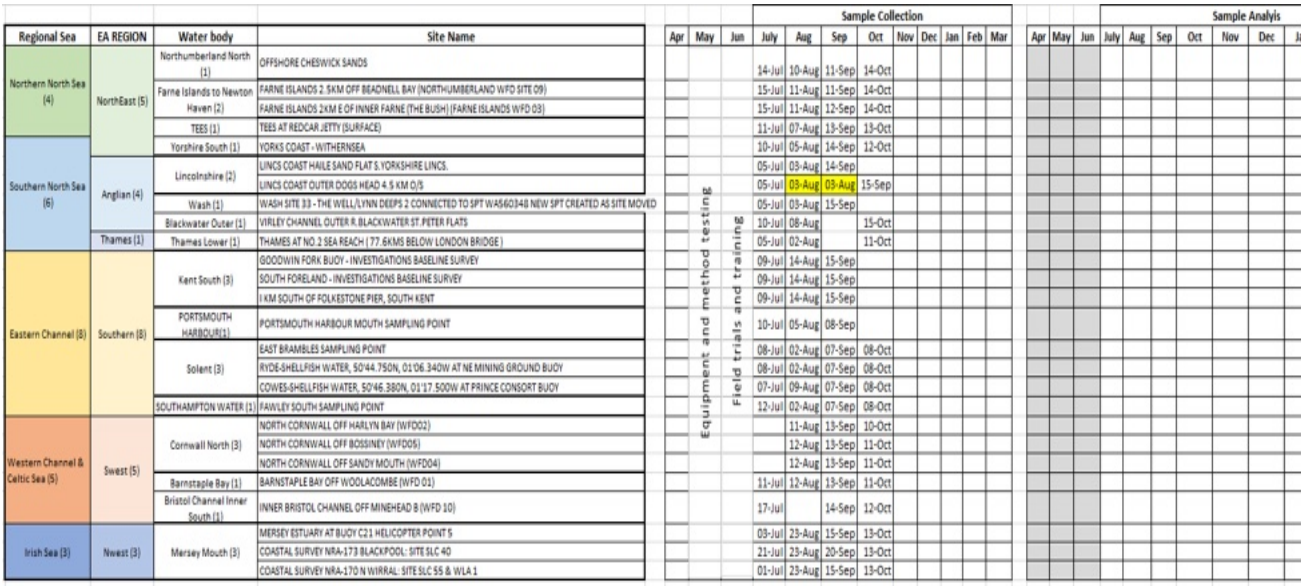


Figure 5: *Location of the Environment Agency zooplankton sampling sites (yellow circles)*

3.1.2. Temporal spread and sampling success

The beginning of the year involved equipment purchasing, method testing, field trials, and training with the first operational samples undertaken in August 2022. These details are summarised in Figure 6.



Regional Sea	EA REGION	Water body	Site Name	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
							Sample Collection								
Northern North Sea (4)	NorthEast (5)	Northumberland North (1)	OFFSHORE CHESWICK SANDS				14-Jul	10-Aug	11-Sep	14-Oct					
		Farne Islands to Newton Haven (2)	FARNE ISLANDS 2.5KM OFF BEADNELL BAY (NORTHUMBERLAND WFD SITE 09)				15-Jul	11-Aug	11-Sep	14-Oct					
			FARNE ISLANDS 2KM E OF INNER FARNE (THE BUSH) (FARNE ISLANDS WFD 03)				15-Jul	11-Aug	12-Sep	14-Oct					
		TEES (1)	TEES AT REDCAR JETTY (SURFACE)				11-Jul	07-Aug	13-Sep	13-Oct					
Southern North Sea (6)		Yorshire South (1)	YORKS COAST - WITHERNSEA				10-Jul	05-Aug	14-Sep	12-Oct					
	Anglian (4)	Lincolnshire (2)	LINCS COAST HAILE SAND FLAT S.YORKSHIRE LINCS.				05-Jul	03-Aug	14-Sep						
			LINCS COAST OUTER DOGS HEAD 4.5 KM O/S				05-Jul	03-Aug	03-Aug	15-Sep					
		Wash (1)	WASH SITE 33 - THE WELL/LYNN DEEPS 2 CONNECTED TO SPT WA560348 NEW SPT CREATED AS SITE MOVED				05-Jul	03-Aug	15-Sep						
		Blackwater Outer (1)	VIRLEY CHANNEL OUTER R.BLACKWATER ST.PETER FLATS				10-Jul	08-Aug		15-Oct					
	Thames (1)	Thames Lower (1)	THAMES AT NO.2 SEA REACH (77.6KMS BELOW LONDON BRIDGE)				05-Jul	02-Aug		11-Oct					
Eastern Channel (8)	Southern (8)	Kent South (3)	GOODWIN FORK BUOY - INVESTIGATIONS BASELINE SURVEY				09-Jul	14-Aug	15-Sep						
			SOUTH FORELAND - INVESTIGATIONS BASELINE SURVEY				09-Jul	14-Aug	15-Sep						
			1 KM SOUTH OF FOLKESTONE PIER, SOUTH KENT				09-Jul	14-Aug	15-Sep						
		PORTSMOUTH HARBOUR(1)	PORTSMOUTH HARBOUR MOUTH SAMPLING POINT				10-Jul	05-Aug	08-Sep						
		Solent (3)	EAST BRAMBLES SAMPLING POINT				08-Jul	02-Aug	07-Sep	08-Oct					
			RYDE-SHELLFISH WATER, 50'44.750N, 01'06.340W AT NE MINING GROUND BUOY				08-Jul	02-Aug	07-Sep	08-Oct					
			COWES-SHELLFISH WATER, 50'46.380N, 01'17.500W AT PRINCE CONSORT BUOY				07-Jul	09-Aug	07-Sep	08-Oct					
		SOUTHAMPTON WATER (1)	FAWLEY SOUTH SAMPLING POINT				12-Jul	02-Aug	07-Sep	08-Oct					
Western Channel & Celtic Sea (5)	Swest (5)	Cornwall North (3)	NORTH CORNWALL OFF HARLYN BAY (WFD02)					11-Aug	13-Sep	10-Oct					
			NORTH CORNWALL OFF BOSSINEY (WFD05)					12-Aug	13-Sep	11-Oct					
			NORTH CORNWALL OFF SANDY MOUTH (WFD04)					12-Aug	13-Sep	11-Oct					
		Barnstaple Bay (1)	BARNSTAPLE BAY OFF WOOLACOMBE (WFD 01)				11-Jul	12-Aug	13-Sep	11-Oct					
		Bristol Channel Inner South (1)	INNER BRISTOL CHANNEL OFF MINEHEAD B (WFD 10)				17-Jul		14-Sep	12-Oct					
Irish Sea (3)	Nwest (3)	Mersey Mouth (3)	MERSEY ESTUARY AT BUOY C21 HELICOPTER POINT 5				03-Jul	23-Aug	15-Sep	13-Oct					
			COASTAL SURVEY NRA-173 BLACKPOOL: SITE SLC 40				21-Jul	23-Aug	20-Sep	13-Oct					
			COASTAL SURVEY NRA-170 N WIRRAL: SITE SLC 55 & WLA 1				01-Jul	23-Aug	15-Sep	13-Oct					

Equipment and method testing

Field trials and training

Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
			Sample Analysis								
Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec	J		

Figure 6: *Description of field sampling sheet for EA sampling showing dates samples were taken at each site*

A more detailed breakdown shows that once routine sampling started in August 2022 most samples were successfully collected from across the 26 sites, in the order 60-80%, until the bad weather in January 2023 which removed over half the samples (an identified risk). Technical and staff issues only impacted on 5 or less samples per month (Table 2).

	Trials and testing period									
	Jun-22	**Jul-22**	**Aug-22**	**Sep-22**	**Oct-22**	**Nov-22**	**Dec-22**	**Jan-23**	**Aug-Jan Totals**	**Aug-Jan %**
Not scheduled	25	23	2	2	2	2	1	1	10	6%
Sample collected	1	3	21	22	19	16	16	5	**99**	**63%**
Health Safety & welfare			1		2				3	2%
Staff availability					3				3	2%
Technical issues				2			5	5	12	8%
Bad weather			2			8	4	15	29	19%
Total sites	**26**	**26**	**26**	**26**	**26**	**26**	**26**	**26**	**156**	**100%**

	Jun-22	**Jul-22**	**Aug-22**	**Sep-22**	**Oct-22**	**Nov-22**	**Dec-22**	**Jan-23**
Not scheduled	96%	88%	8%	8%	8%	8%	4%	4%
Sample collected	4%	12%	81%	85%	73%	62%	62%	19%

Health Safety & welfare	0%	0%	4%	0%	8%	0%	0%	0%
Staff availability	0%	0%	0%	0%	12%	0%	0%	0%
Technical issues	0%	0%	0%	8%	0%	0%	19%	19%
Bad weather	0%	0%	8%	0%	0%	31%	15%	58%
Total sites	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

***Table 2**: Success rate of EA zooplankton deployments listing main issues associated with impact on sampling*

3.3. Cefas sampling

Data were collected on a monthly basis with Cefas staff collecting samples along transects in Liverpool Bay and Thames using EA vessels. The survey location targeted two existing Cefas SmartBuoy sample sites. Priority zooplankton stations and water quality data were taken in as close as possible to the buoys. Other sites on the way to each buoy were also sampled for water quality data (Conductivity, Temperature, and Depth (CTD) dips, nutrient, and chlorophyll). High frequency data for temperature, salinity, dissolved nutrients, turbidity, and fluorescence were also collected at the SmartBuoy sites and along the transects from inshore to SmartBuoy sites.

A total of 10 surveys were run in this first year of the project (excluding those running in March 2023) (Table 3 and Figure 7). 7 surveys were completed on the Thames estuary. 3 surveys were completed on the Mersey estuary. Surveys that were not completed were due to poor weather, changes in vessel schedule and delays in providing correct vessel certification. To counter the reduced number of small boat surveys, additional zooplankton samples were taken from additional Cefas cruises when passing Thames and Liverpool Bay area. A total 81 stations were sampled across all surveys and estuaries. An additional four surveys carried out on the Endeavour were also completed where zooplankton samples were taken on the Warp and Gabbard SmartBuoy sites.

Each survey collected a suite of additional water quality and optical information that will be used in the analysis of zooplankton community. Measurements are described in Table 3.

Type of sample	Measurement	Description	Relevance to programme
Biological	Zooplankton sample	Zooplankton sample	Baseline data for zooplankton communities in Thames and Liverpool Bay
Biological	Phytoplankton sample	1 L water sample, fixed with Lugols and sampled under microscopy	Further elucidation of phytoplankton community through microscopy techniques (5 – 20 µm)
Water Quality	Total Suspended Solid	The amount of filtered (0.45 µm) total suspended solids in 1000 ml of water	Increase in particulate load in the water column, which acts to restrict light penetration impeding phytoplankton
Water Quality	Chlorophyll a	Measure of fluorescence as a proxy for phytoplankton biomass	Measure of phytoplankton abundance
Water Quality	Dissolved Nutrients (N)	Dissolved nutrients were collected through a 0.45 µm syringe tip	Released to the marine environment above natural concentrations in human waste and by human activities (agriculture and aquaculture)

Water Quality	Dissolved Nutrients (P)	0.45 µm syringe-tip polyethersulfone (PES) membrane filter	human activities (agriculture and aquaculture). High or imbalances in concentrations can impact on plankton
Water Quality	Dissolved oxygen	Dissolved oxygen concentrations are above levels required to maintain healthy marine ecosystems	Primary production increases oxygen concentrations, while (aerobic) breakdown of organic matter (remineralisation) in the water column depletes oxygen

Table 3: *List of Cefas water quality parameters and relevance to programme*

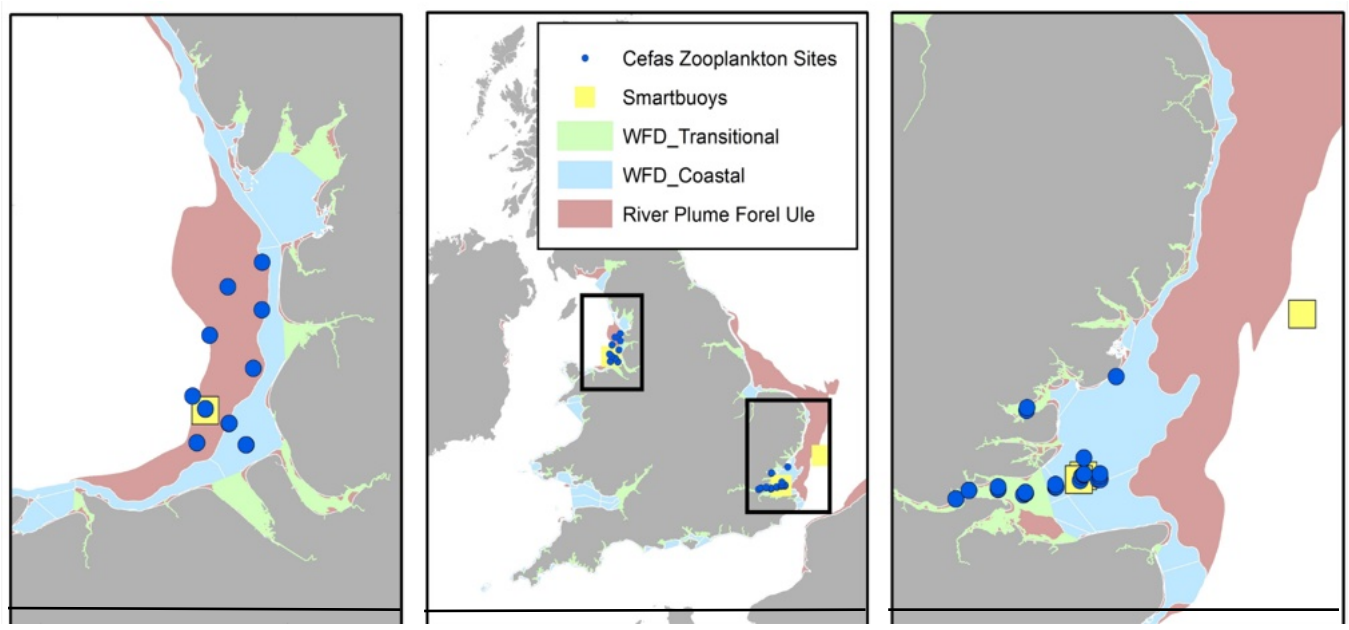


Figure 7*: Map showing location of Cefas sampling stations*

In most cases deployment of all instruments was a success. The methodology associated with the field sampling is detailed in Figure 8. Table 4 summarises the deployments at each site.

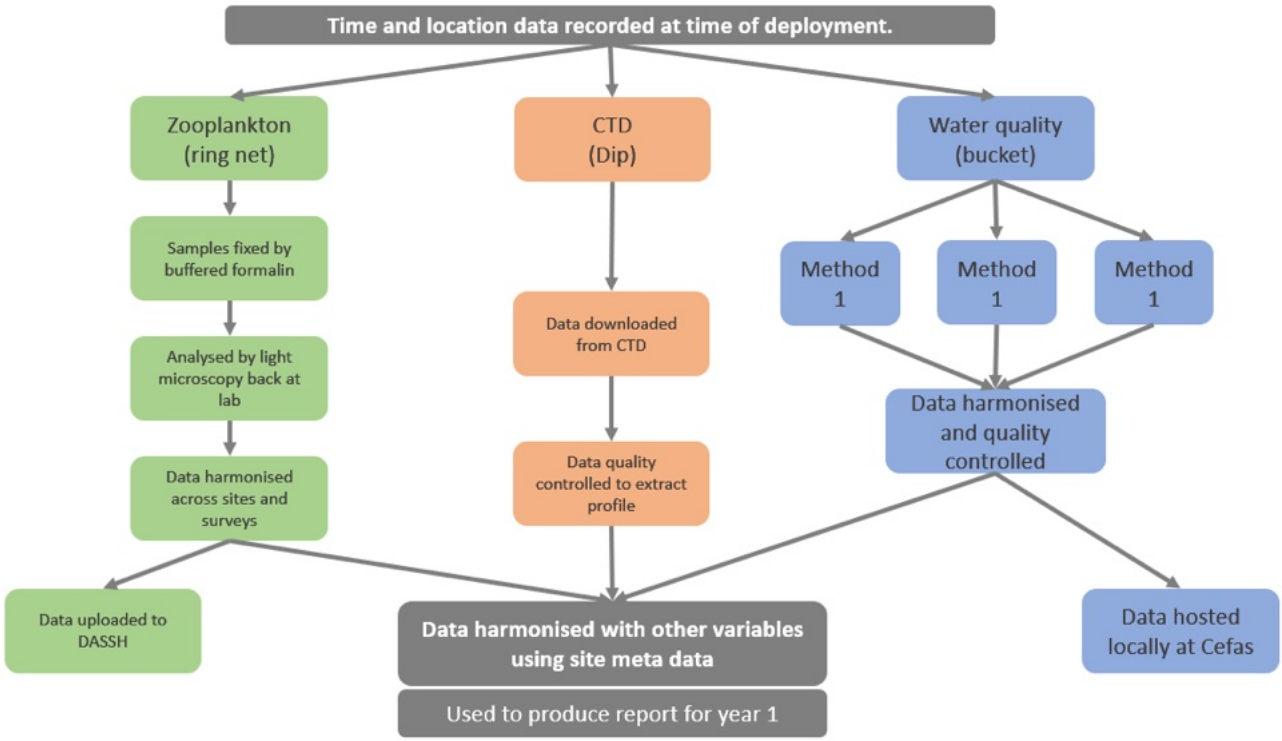


Figure 8: *Cefas field sampling methodology – Process from field sampling to data analysis*

Table 4: *Deployments at each site for each of Thames and Liverpool Bay. Full details of sites and parameters sampled at each site can be found in the Appendices*

Date	Area	Zoo-plankton	Water Quality	Phyto-plankton	Eye on water app	Secchi disk (m)	CTD Depth data
14/07/2022	Thames	2	6	1	Y	Y	5
09/08/202	Thames	4	7	7	7	7	7
21/09/2022	Thames	4	7	7	7	7	7
18/10/2022	Thames	4	7	7	7	7	7
28/11/2022	Thames						
06/01/2023	Thames	2	4	4	4	4	4
24/08/2023	Liverpool	1	4	4	4	4	6
02/12/2022	Liverpool	1	10	10	10	0	10

3.4. MBA sampling

3.4.1 Routes and dates

For this project, three routes (extended Bs, HE and KC) were reinstated, as detailed above. The initial plan was for tows to commence in June 2022, due to the planned delay in sampling. However, for the HE route we were successful in negotiating an earlier start in May 2022, which will give important data on the plankton community in the Southern North Sea during the late springtime.

The KC route is operated on the sea cargo (SC) ship Connector, which is Norway's largest sailing vessel. The vessel uses rotating cylinders that utilise the 'Magnus' effect to produce propulsion from wind. With a battery pack, the vessel can avoid the use of auxiliary engines, which means that it can be 100% emission free during sailing and whilst docked.

Figure 9: *Picture of SC Connector vessel used for collecting Continuous Plankton Recorder (CPR) tows*

The B-route is operated on the cargo ship Lombok Strait, which operates between the UK (English Channel region) and Barbados. This allows coverage from the shallow waters of the English Channel, across the continental shelf, to the deeper mid Atlantic waters at the Porcupine Abyssal Plain (PAP) Sustained Observatory. This is an important area, as changes in the plankton community in this oceanic region can give early warning regarding potential changes in UK waters.

Figure 10: *Picture of Lombok vessel used for collection Continuous Plankton Recorder (CPR) tows*

To date, routes up to December 2023 have been returned to the MBA, and a total of over 8500 nautical miles have been sampled on these routes.

Figure 11: *Transect of the MBA CPR tows*

4. Overview of Methods

1. Environment Agency monitoring, survey, and method checks

1.1. Net selection

The main objective was to select a sampling system whereby a suitable sample net and mesh size could be deployed and recovered safely in a consistent, repeatable way throughout the EA fleet, while being compatible with the methods of other organisations. Full details on net selection are provided in Appendix 1.

After consulting published standards, initial sampling ideas were discussed with other organisations with marine zooplankton sampling programmes, including Marine Scotland, MBA, Natural Resources Wales, Plymouth Marine Laboratory, Southampton University, and Cefas.

Most zooplankton are usually collected from one of 4 basic platforms:

1. Large Research Vessels with sufficient winching capability for large nets and sinking weights – Although these vessels can manage very large nets, they are restricted to deeper waters and are expensive to run. This approach is not available to EA near shore coastal waters which are rarely greater than 30m depth.
2. Coastal Survey Vessels (CSVs) / small fishing boats – These can operate from offshore to shallow estuaries and have some winching capacity, so can manage medium to small nets.
3. Small rigid hull inflatables (RIBs) and other small vessels – These generally do not have power winches and so can only use small nets which are hand hauled. Although suitable for very shallow waters they can only use small nets which are adequate for qualitative results, but they struggle to give consistent quantitative results.
4. Piers and jetties – Same issues as 3 with added disadvantage of being a fixed position and not reflecting the nearby open water.

Although the EA fleet has both CSVs and RIBs. CSVs were the preferred option providing a hydraulic winch allowing a bigger net with a heavier depressor weight. Additionally, the CSVs provide a more stable operating platform (e.g. for using preservative and washing nets).

Initially, a paired Bongo net sampling methodology was explored in which two different mesh sized nets, 68um and 200um, would be deployed in one haul and the samples combined into one pooled sample. However, although 2 nets can be hauled at once, there are several issues with this approach:

- The smaller mesh net may be liable to clogging necessitating redeployment and a wastage of the larger mesh sample.
- Different size meshes require different hauling speeds over short depths. It is hard to get a consistently optimal speed for both mesh sizes.
- Similarly, a smaller meshed net requires a longer net which may limit sampling in shallower waters.
- It is inappropriate to combine the samples from two different meshes into a single sampling pot. Therefore, each haul must be treated as separate samples, doubling the analysis costs.
- It is harder to manage two nets.

The single net option deployed by a CSV was chosen as the most flexible option allowing consistent sampling.

Detailed consideration of net dimensions and meshes suggested trialling both 100 µm and 200 µm meshes with a 400 mm aperture. The 400 mm aperture was a compromise between the risk of the finer mesh net clogging if the aperture is too large, whilst filtering enough water per haul to provide a representative sample and keeping the net length reasonably short (3.5 m). After initial field trials in July and August 2022, the 100 µm nets were dropped as they both performed in a relatively similar way. This is illustrated in Figures 12 and 13 below.

In the first box plot in Figure 12 (for the East Channel, where we tested both net mesh sizes), we can see that, unsurprisingly the finer mesh catches significantly more (and smaller) 'particles', than the larger mesh (Figure 12).

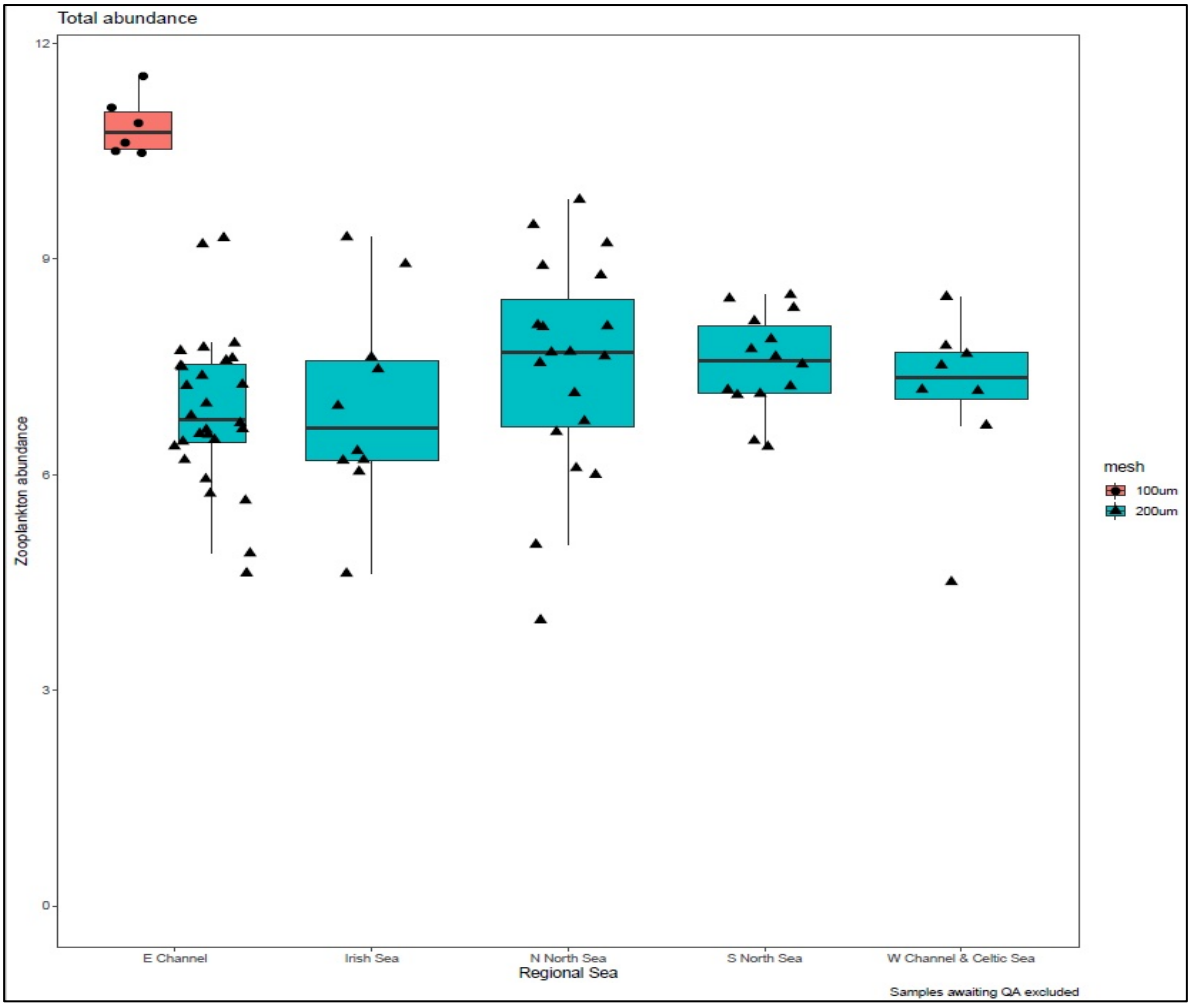


Figure 12: *Differences in zooplankton abundance for different Regional Seas, and for different net mesh size (100 µm and 200 µm) in the East channel*

Differences in catch between different mesh sizes were less clear due to the small number of 100um samples collected. However, the finer mesh seems to catch a slightly lower variety of taxa (a median of around 25 taxa, compared to the 30 or 40 taxa with the larger mesh) (Figure 13). This probably reflects clogging with small particles reducing the catching ability of the net. We considered reinvestigating this in the winter of 2022/23 if low numbers were caught with the 200 µm net. However, results in the winter appeared sufficient using only a 200 µm net, and this allowed us to be compatible with other key organisations.

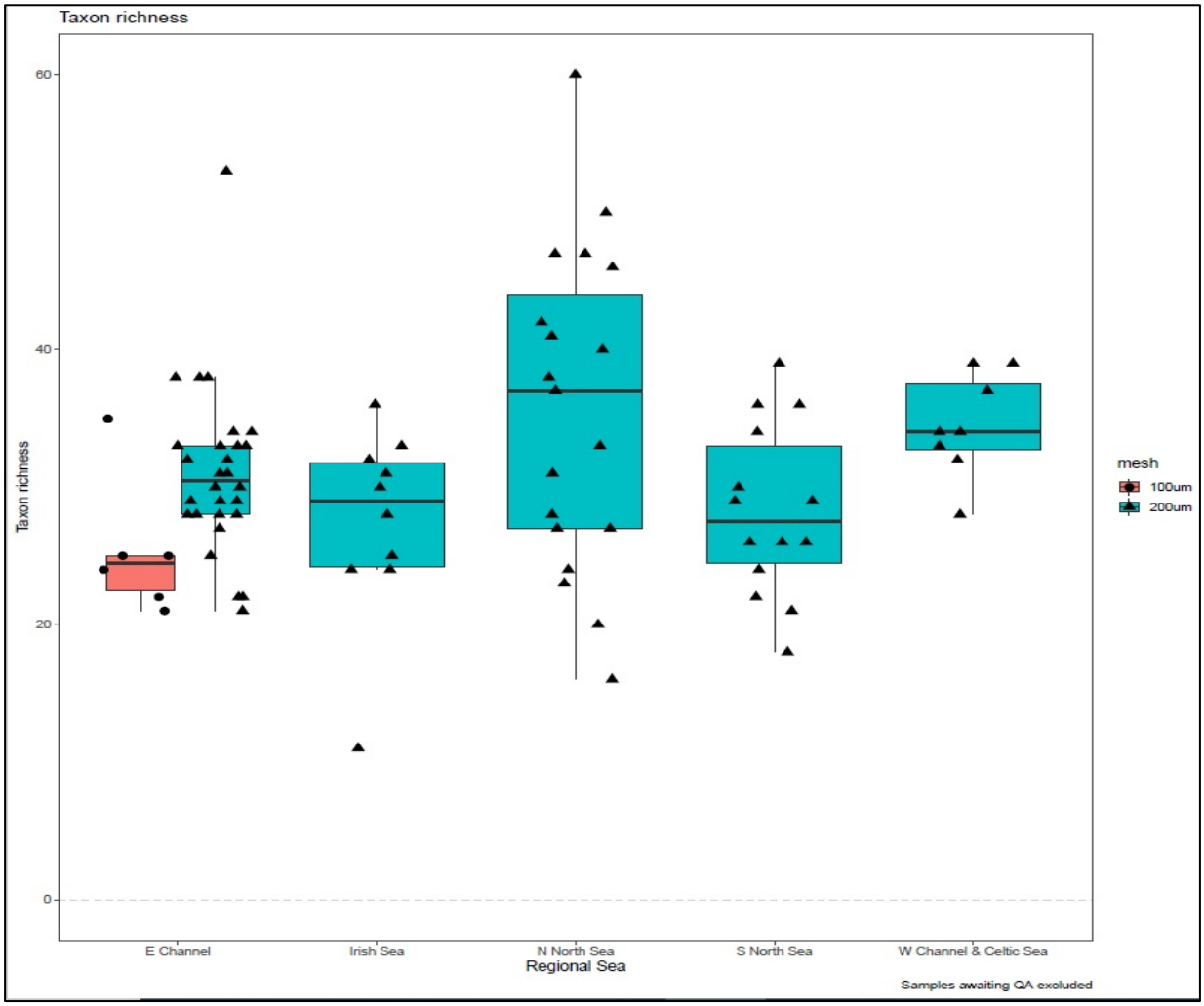


Figure 13: *Differences in taxon richness for different Regional Seas, and for different net mesh sizes in the East Chanel*

From the limited data set available, we have collected 153 out of an 'expected' 233 taxa (66%) over 5 months; it looks likely that the method has been catching most of the significantly numerical taxa (see results section for further details) (Figure 14).

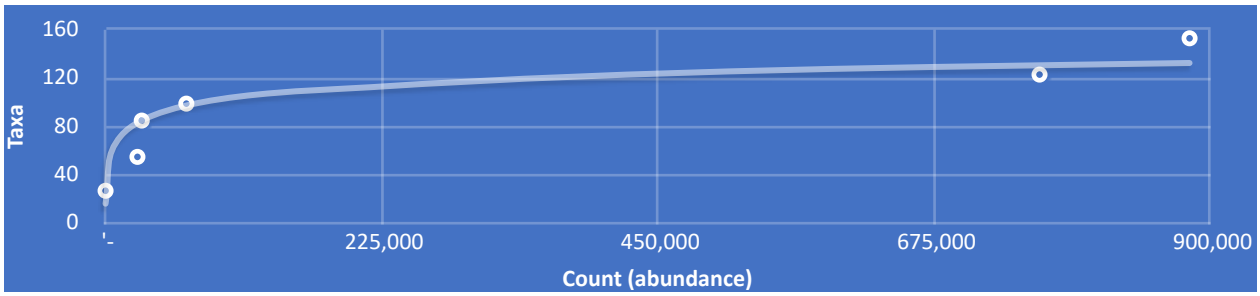


Figure 14: *The number of zooplankton taxa against the total abundance of zooplankton*

Preliminary analysis with a limited data set showed that this sample collection methodology effectively separated out samples by 'Regional Seas' (Figure 15).

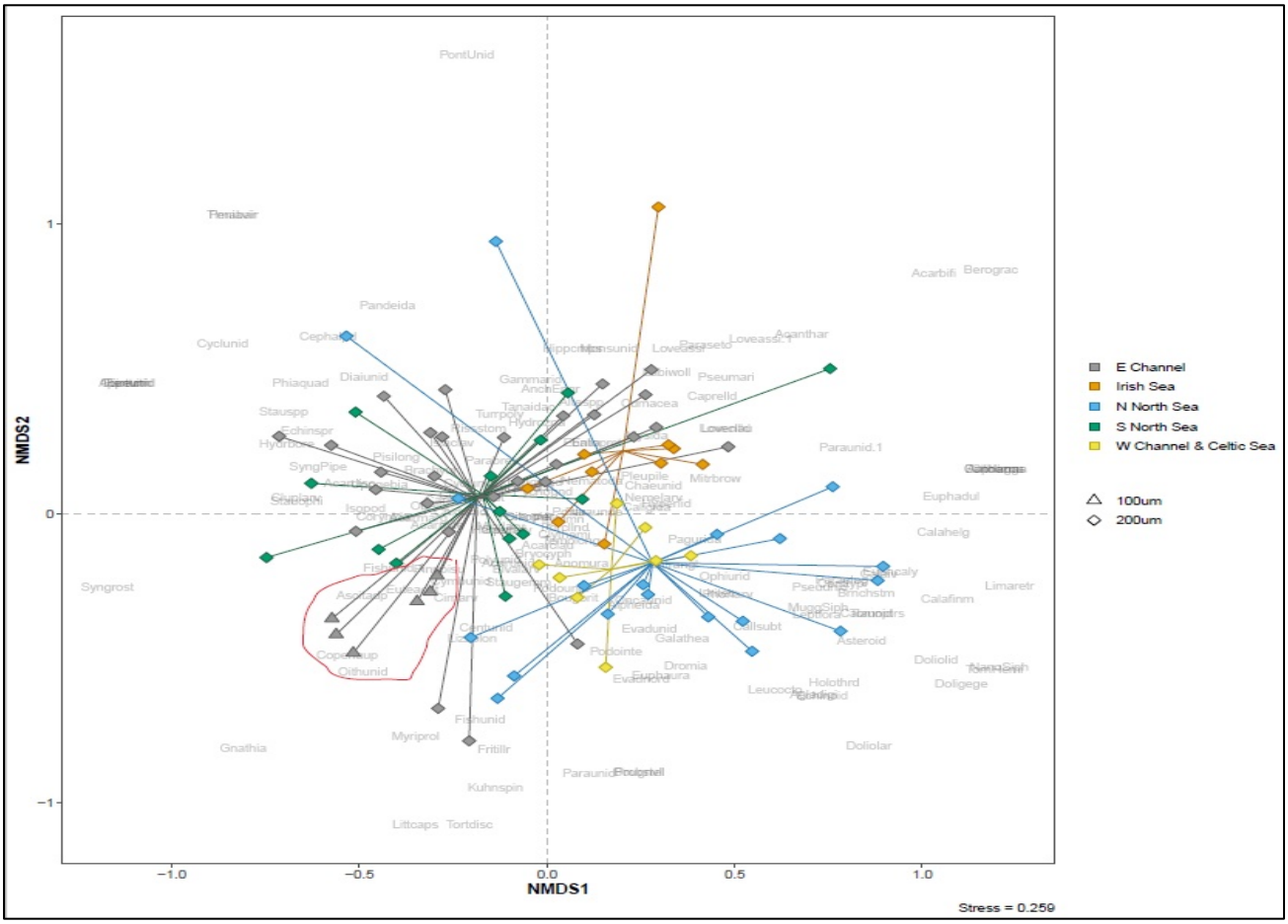


Figure 15: *Zooplankton samples separated out by regional seas*

The preliminary analysis suggests that the data allow us to detect regional high-level differences in zooplankton assemblages. This indicates the method is effective and can pick up geographical differences (Figure 15).

Consequently, it was concluded that the lack of clogging gives preference to the 200 µm nets. This was identified as a good catch-all mesh size to use to characterise the zooplankton community. Moreover, 200 µm is a commonly used mesh size used by other organisations in UK inshore waters. Despite the zooplankton community being very variable it was felt that replicate samples were sufficiently similar that we could use a single sample per site. Future work will look at comparing different mesh sizes to assess how historical zooplankton data (collected with a variety of methods) can be 'standardised' and incorporated into the programme.

4.1.2. Depth and volume calculations, preservation, and sample logs

Trials with flowmeters to record the net volume sampled were unsuccessful as the data were inconsistent and unreliable and the deployment and maintenance of the equipment was disproportionately time-consuming. A decision was made to estimate net volume sampled using the net dimensions and the length of rope deployed instead; with the winch cable angle less than 25° from vertical (Figure 16); this is effective in shallow waters and has also been used by other organisations.

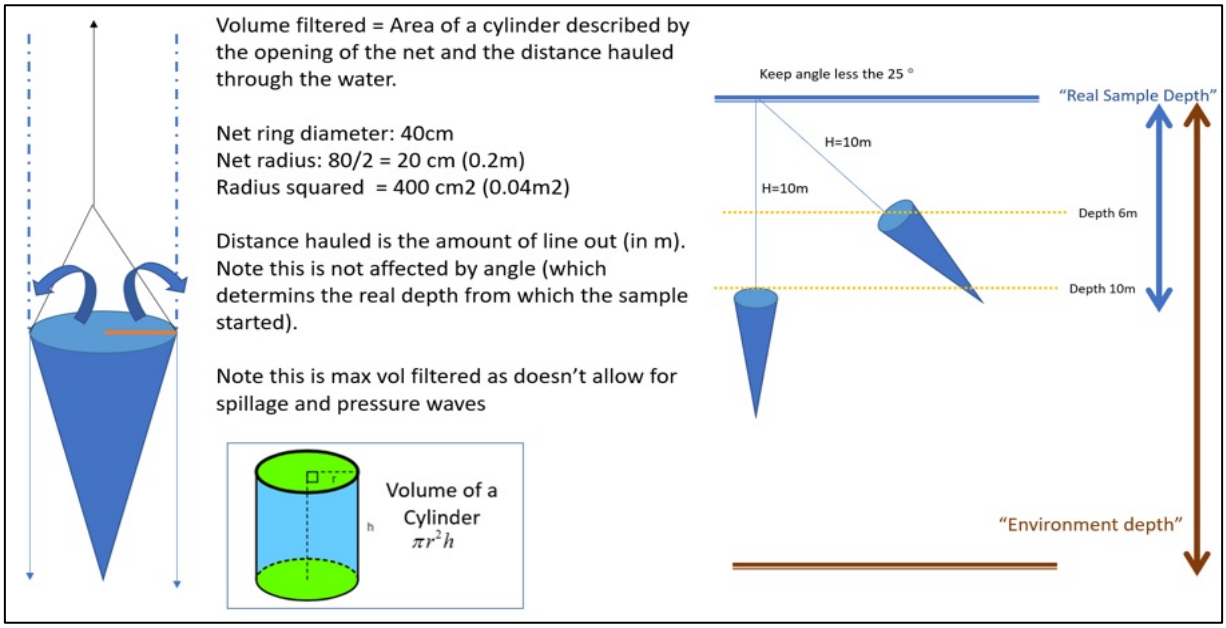
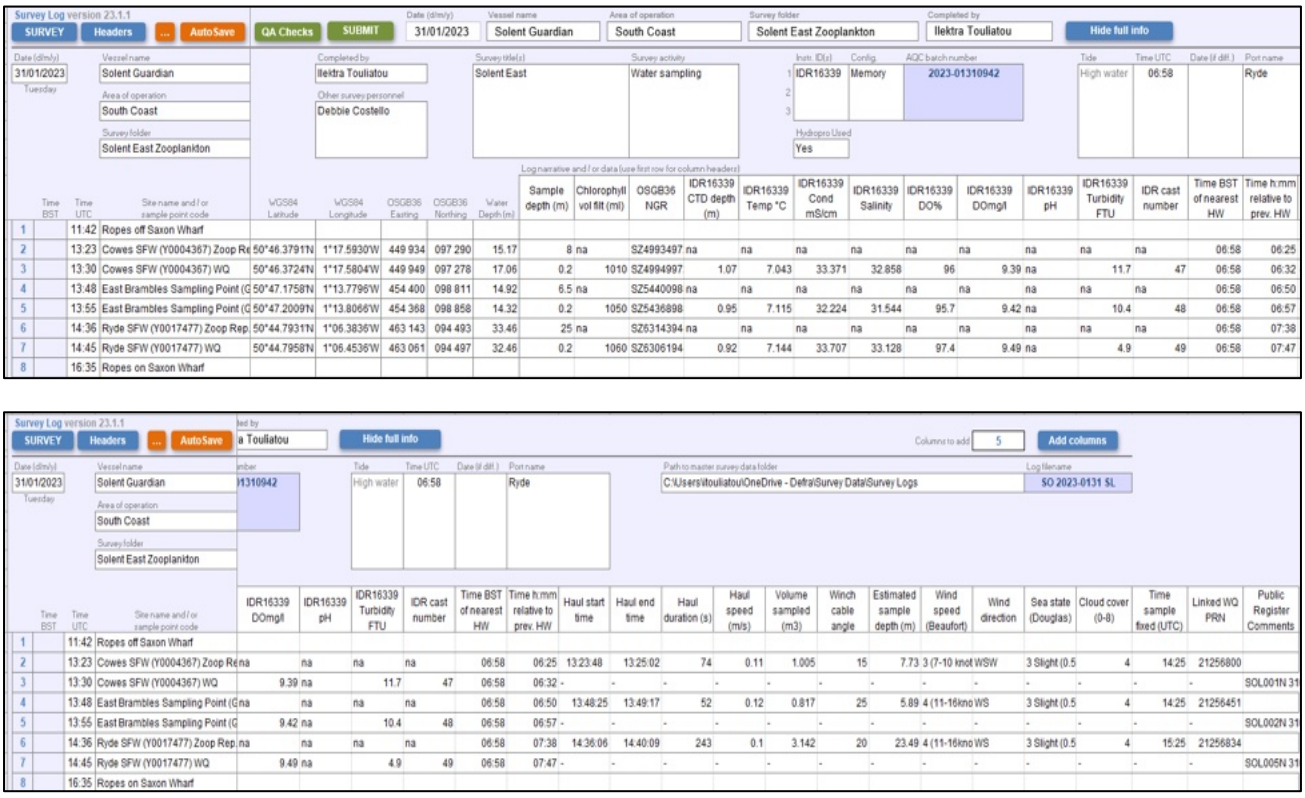


Figure 16: *Flow diagram for the analysis of net volume for zooplankton sampling*

Collected zooplankton are stored in 1:1 by volume formalin (10% borax buffered formalin solution) in line with other organisations. All the field details are stored in the modified survey log (screenshot Figure 17, and Appendix 3) developed to enable consistent data collection by sampling teams.



	Time BST	Time UTC	Site name and / or sample point code	WGS84 Latitude	WGS84 Longitude	OSGB36 Easting	OSGB36 Northing	Water Depth (m)	Sample depth (m)	Chlorophyll vol filt (ml)	OSGB36 NGR	IDR16339 CTD depth (m)	IDR16339 Temp °C	IDR16339 Cond mS/cm	IDR16339 Salinity	IDR16339 DO%	IDR16339 DOmg/l	IDR16339 pH	IDR16339 Turbidity FTU	IDR cast number	Time BST of nearest HW	Time h:mm relative to prev. HW
1		11:42	Ropes off Saxon Wharf																			
2		13:23	Cowes SFW (Y0004367) Zoop Re	50°46.3791'N	1°17.5930'W	449 934	097 290	15.17	8	na	SZ4993497	na	na	na	na	na	na	na	na	na	06:58	06:25
3		13:30	Cowes SFW (Y0004367) WQ	50°46.3724'N	1°17.5804'W	449 949	097 278	17.06	0.2	1010	SZ4994997	1.07	7.043	33.371	32.858	96	9.39	na	11.7	47	06:58	06:32
4		13:48	East Brambles Sampling Point (G	50°47.1758'N	1°13.7796'W	454 400	098 811	14.92	6.5	na	SZ5440098	na	na	na	na	na	na	na	na	na	06:58	06:50
5		13:55	East Brambles Sampling Point (G	50°47.2009'N	1°13.8066'W	454 368	098 858	14.32	0.2	1050	SZ5436898	0.95	7.115	32.224	31.544	95.7	9.42	na	10.4	48	06:58	06:57
6		14:36	Ryde SFW (Y0017477) Zoop Rep	50°44.7931'N	1°06.3836'W	463 143	094 493	33.46	25	na	SZ6314394	na	na	na	na	na	na	na	na	na	06:58	07:38
7		14:45	Ryde SFW (Y0017477) WQ	50°44.7958'N	1°06.4536'W	463 061	094 497	32.46	0.2	1060	SZ6306194	0.92	7.144	33.707	33.128	97.4	9.49	na	4.9	49	06:58	07:47
8		16:35	Ropes on Saxon Wharf																			

	Time BST	Time UTC	Site name and / or sample point code	IDR16339 DOmg/l	IDR16339 pH	IDR16339 Turbidity FTU	IDR cast number	Time BST of nearest HW	Time h:mm relative to prev. HW	Haul start time	Haul end time	Haul duration (s)	Haul speed (m/s)	Volume sampled (m3)	Winch cable angle	Estimated sample depth (m)	Wind speed (Beaufort)	Wind direction	Sea state (Douglas)	Cloud cover (0-8)	Time sample fixed (UTC)	Linked WQ PRN	Public Register Comments
1		11:42	Ropes off Saxon Wharf																				
2		13:23	Cowes SFW (Y0004367) Zoop Re	na	na	na	na	06:58	06:25	13:23:48	13:25:02	74	0.11	1.005	15	7.73	3 (7-10 knot	WSW	3 Slight (0.5	4	14:25	21256800	
3		13:30	Cowes SFW (Y0004367) WQ	9.39	na	11.7	47	06:58	06:32	-	-	-	-	-	-	-	-	-	-	-	-	-	SOL001N 31
4		13:48	East Brambles Sampling Point (G	na	na	na	na	06:58	06:50	13:48:25	13:49:17	52	0.12	0.817	25	5.89	4 (11-16kno	WS	3 Slight (0.5	4	14:25	21256451	
5		13:55	East Brambles Sampling Point (G	9.42	na	10.4	48	06:58	06:57	-	-	-	-	-	-	-	-	-	-	-	-	-	SOL002N 31
6		14:36	Ryde SFW (Y0017477) Zoop Rep	na	na	na	na	06:58	07:38	14:36:06	14:40:09	243	0.1	3.142	20	23.49	4 (11-16kno	WS	3 Slight (0.5	4	15:25	21256834	
7		14:45	Ryde SFW (Y0017477) WQ	9.49	na	4.9	49	06:58	07:47	-	-	-	-	-	-	-	-	-	-	-	-	-	SOL005N 31
8		16:35	Ropes on Saxon Wharf																				

Figure 17: *Example of survey log data submission sheets for zooplankton samples*

Further details and the operational instructions can be found in Appendices 1 to 5 inclusive.

4.1.3. Laboratory analysis methods

EA zooplankton samples were analysed by the MBA, following their standard methods for net samples; details are provided in Appendix 6 and 8.

Routine samples for WER (WFD) were also collected for phytoplankton, preserved, and sent to contracting labs, water quality samples particularly nutrients and chlorophyll were sent to the EA National Laboratory Service (NLS), physico-chemical instrument samples (e.g. conductivity, salinity, temperature, dissolved oxygen, turbidity, and depth) were collected on board, entered into the survey log, and registered with the NLS. This will not be considered further in this report. Additional measurements may be made depending on the other reasons for sampling at each site. A full list of determinands is given in Table 5 and the draft data set can be found in the Appendix 9.

Table 5: *List of determinands for Environment Agency sampling*

Determinand description	Unit
Ammoniacal Nitrogen, Filtered as N	mg/l
Chlorophyll : Acetone Extract	ug/l
NGR : Easting	NGR
NGR : Northing	NGR
Nitrate, Filtered as N	mg/l
Nitrite, Filtered as N	mg/l
Nitrogen, Dissolved Inorganic : as N	mg/l
Nitrogen, Total Oxidised, Filtered as N	mg/l
Orthophosphate, Filtered as P	mg/l
Oxygen, Dissolved as O2	mg/l
Oxygen, Dissolved, % Saturation	%
Phytoplankton	Coded
Salinity : In Situ	ppt
Sample Depth below surface	M
Silicate, Filtered as SiO2	mg/l
Temperature of Water	CEL
Time of high tide	hh.mm
Time of sampling relative to previous high	hh.mm
Turbidity : In Situ	FTU
Volume of Sample Filtered	ml
Water Depth	M
Benzo(a)Pyrene	ug/l
Benzo(b)Fluoranthene	ug/l
Benzo(g,h,i)Perylene	ug/l
Benzo(k)Fluoranthene	ug/l
Cadmium, Dissolved	ug/l

Determinand description	Unit
GCMS Screen : Target Based multi-residue screening : Semi Quantitative	Text
Indeno(1,2,3-cd)pyrene	ug/l
Lead, Dissolved	ug/l
Nickel, Dissolved	ug/l
Perfluorooctanoate anion	ug/l
Perfluorooctylsulphonate anion	ug/l
Tributyl Tin as Cation	ug/l
Triphenyl Tin as Cation	ug/l
Zinc, Dissolved	ug/l
4-Nonylphenol Branched	ug/l
4-tert-Octylphenol :- {p-tert-Octylphenol}	ug/l
Arsenic, Dissolved	ug/l
Chromium Hexavalent, Dissolved :- {Cr VI}	ug/l
Iron, Dissolved	ug/l
Mercury, Dissolved	ug/l
Cyanide : Free as CN	mg/l
Cyanide as CN	mg/l
LCMS Screen : Semi Quantitative	Text
2,2,3,4,4,5,6-Heptabromodiphenyl ether :- {PBDE 183}	ug/l
2,2,3,4,4,5-Hexabromodiphenyl ether :- {PBDE 138}	ug/l
2,2,3,4,4-Pentabromodiphenyl ether :- {PBDE 85}	ug/l
2,2,4,4,5,5-Hexabromodiphenyl ether :- {PBDE 153}	ug/l
2,2,4,4,5,6-Hexabromodiphenyl ether :- {PBDE 154}	ug/l
2,2,4,4,5-Pentabromodiphenyl ether :- {PBDE 99}	ug/l
2,2,4,4,6-Pentabromodiphenyl ether :- {PBDE 100}	ug/l

Determinand description	Unit
Carbon, Organic, Dissolved as C :- {DOC}	mg/l
Copper, Dissolved	ug/l
Fluoranthene	ug/l

Determinand description	Unit
2,2,4,4-Tetrabromodiphenyl ether :- {PBDE 47}	ug/l
2,3,4,4-Tetrabromodiphenyl ether :- {PBDE 66}	ug/l
2,4,4-TriBromoDiphenylEther	ug/l

4.2. Cefas survey methods

The analysis of the three different types of data (zooplankton, phytoplankton, and water quality) are resolved by a workflow within Cefas and harmonised with linked-up databases on completion of analysis. The steps for the analysis of the zooplankton sample are detailed in Figure 17.

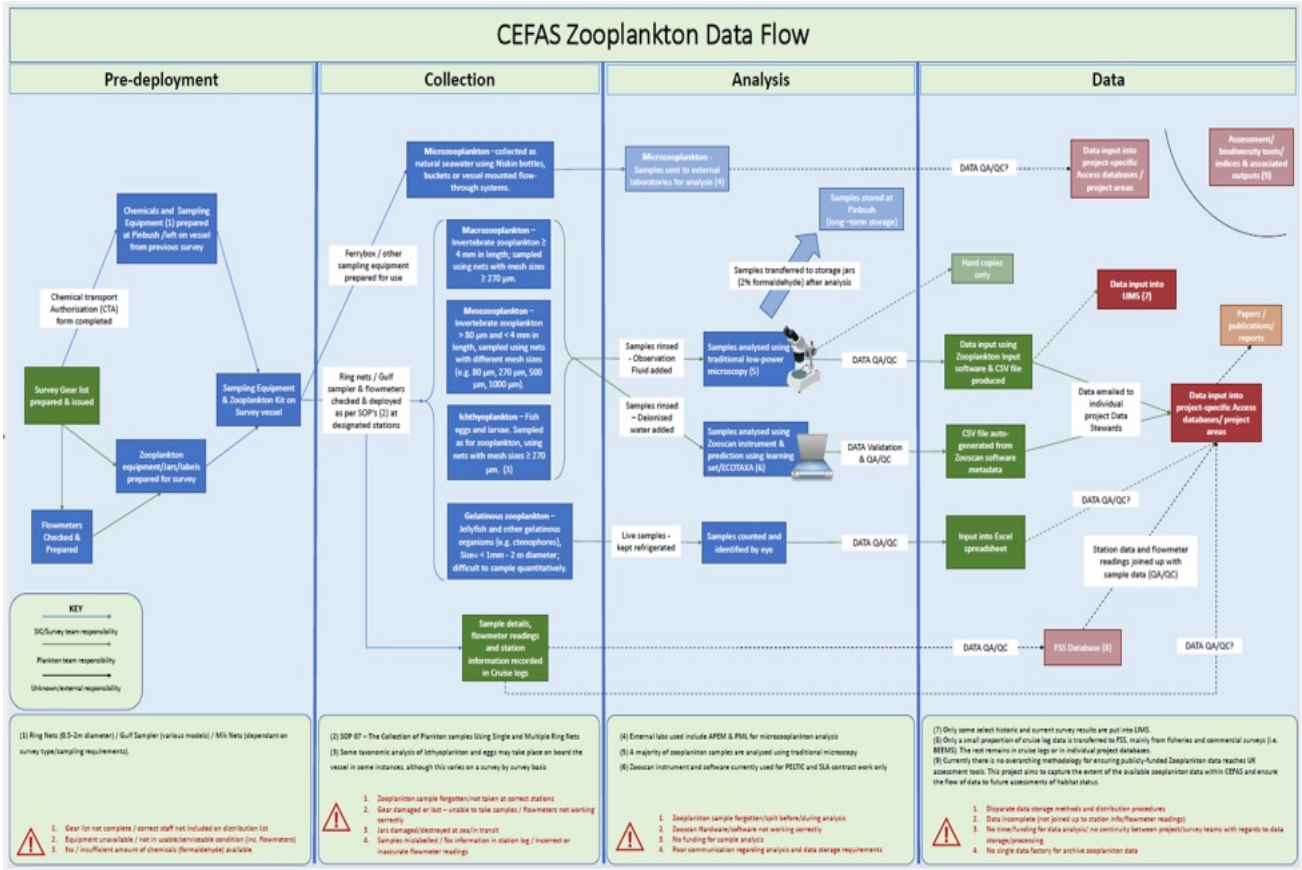


Figure 17: *Flow chart of various methods and analysis used within Cefas for the pre-deployment, collection, analysis, and data flows associated with zooplankton sampling*

For our full methodology, there are Standard Operational Procedures (SOPs) for zooplankton, phytoplankton, water quality collected by CTD and in-situ water quality. SOPs for the following are included in the Appendix 7:

- Capture and fixation of zooplankton samples and zooplankton analysis by light microscopy
- Conductivity, Temperature, and Depth (CTD) instrument
- 'Water Quality'

Most plankton data previously collected by Cefas are either for phytoplankton or ichthyoplankton. Relatively few data appear to have been collected on zooplankton, although they have been collected by some monitoring programmes and research projects (Table 6). Sampling has focussed on mesozooplankton, which are relatively easy to sample, preserve and quantify. In contrast, data on gelatinous zooplankton are largely qualitative, due to the difficulty of sampling and preserving gelatinous organisms.

Table 6: *Different Cefas zooplankton data surveys prior to mNCEA project*

Survey / Project	**Area**	**Years**	**Gear**	**MDR Links; Comment**
Dove Station	North Sea	1964-2002	Ring net, 46 cm diameter, naked, 80µm mesh	http://mdrviewer/#/View/2634
Blackwater Herring	Blackwater Estuary	1959-1999	Gulf VII, Pup net, naked, 5cm diameter nosecone opening, 80µm mesh	http://mdrviewer/#/View/2632
Jonus II	Irish Sea	1996-1997	Ring net, 50 cm diameter, naked, 270µm mesh	http://mdrviewer/#/View/2637
Irish Sea AEPM	Irish Sea	2000	Gulf VII, Pup net, Naked, 5cm diameter nosecone opening, 80µm mesh	http://mdrviewer/#/View/2641
SmartBuoy LPB	Eastern Irish Sea	2004-2011	Mesozooplankton. Ring net, 50 cm diameter, naked, 80µm mesh and, Ring net, 100 cm Diameter Naked 270µm	http://mdrviewer/#/View/2643
MEMO	North Sea, English Channel	2011-2012	Ring net, 50 cm diameter, naked, 200µm mesh	http://mdrviewer/#/View/2603
Ecosystem Connections	North Sea	2007-2008	3 sites sampled over one to two annual cycles for microzooplankton (Niskin) and mesozooplankton: ring nets 50 cm 80 µm mesh, 1m 270 µm mesh	Data held locally.
Poseidon	Celtic Sea	2013-2015	70 stations in south west regions: ring nets 50 cm 80 µm mesh, 1m 270 µm mesh. 18 prime stations: water samples (from Niskin or FerryBox) for micro zooplankton. Mesozooplankton analysed by zooscan; microzooplankon by flowcam	Sampling in October only. Data held locally

This programme will focus on the collection of zooplankton data that can be used directly for the analysis of lifeforms by applying common methodology that aligns with EA and MBA. However, time will also be allocated to ensure that any additional zooplankton data held by Cefas will also be considered for submission into DASSH. This will require some additional thought on the feasibility of merging zooplankton data collected for different purposes and by different methodology. Similar issues were faced in the lifeform analysis of UK phytoplankton (see Bedford et al., 2020), with varying degrees of success of combining data or combining outcomes.

4.3. MBA monitoring and survey method

CPR samples collected on the extended Bs, HE and KC routes were analysed using the standard CPR method. This method is detailed in Appendix 8.

On typical CPR routes, alternate samples are analysed, where phytoplankton and zooplankton are identified to the highest taxonomic resolution and enumerated, using a standard methodology. In addition, for every sample a value of 'greenness' is taken, known as Phytoplankton Colour Index (PCI). This is a coarse indicator of phytoplankton biomass and has been used in comparison studies with satellite ocean colour variables.

For the mNCEA routes, we have collected PCI values for all routes which have been processed – as it is for every sample, this gives a higher resolution than routine CPR phytoplankton data.

Sample and data analysis from year 1 have not been completed and therefore not analysed but are already showing late spring blooms in the English Channel (likely diatoms), with lower values in the summer (and moving into off-shelf areas). In addition, there appears to be a bloom in the central and southern North Sea in September, which typically we would suppose would be more dinoflagellate dominated.

Once all routes are processed, we will be able to compare PCI values with historical data from these routes and surrounding areas. Although there will be a gap in recent years, we can likely put these new results within a context of multiple decades-worth of previously analysed data.

5. Results

1. Overview

The first year of the project was NOT funded for data analysis and, at the time of writing, with only 7 months of data collected, only limited comment and conclusions can be supplied. Amongst the 160 taxa identified so far, we have seen a seahorse fry (probably accidental) and new invasive copepod species *Pseudodiaptomus marinus* (which is now confirmed inshore as well as previously offshore (Figure 18).

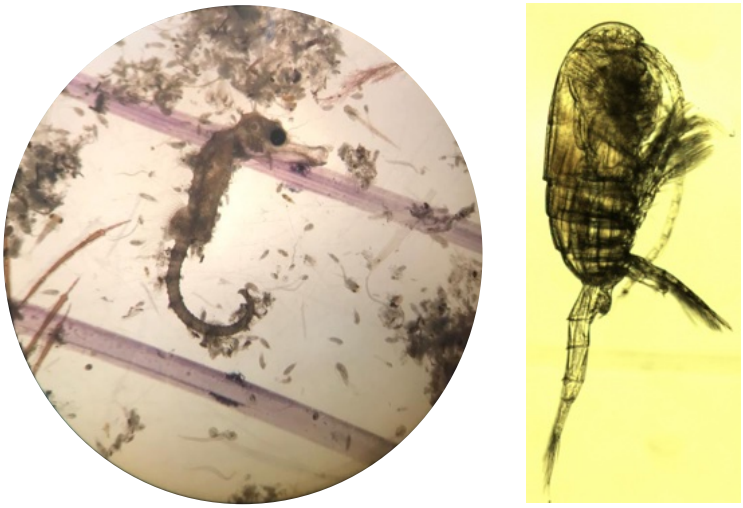


Figure 18: *Images of* Pseudodiaptomus marinus

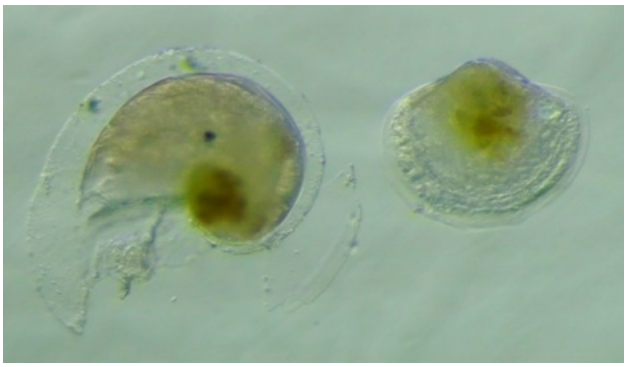


Figure 19: *Image of gastropod (left) and bivalve (right) larvae*

2. Environment Agency samples

From our limited data set we have identified 152 taxa from a 'potential' list of 231 held by MBA analysts. This list has been expanded with additional new taxa found inshore from this project (in locations not previously sampled). For our 200 µm nets, of the total estimated count of 199,861 organisms, over 70% is represented by just 10 taxa, while only 19 taxa contribute more than 15 of the total count (Table 7).

Considering the most frequently encountered taxa groups, across all water bodies and sampling occasions (49 in this analysis), gastropod larvae (Figure 19), occurred in all samples, while 5 taxa occur in over 95% of samples, and 19 taxa in over 70% of samples (Table 7). Twelve taxa occur in both groups and can be considered the 'most common' in these samples (highlighted in bold in Table 7, together with some example images in Figure 20).

Table 7: *Common taxa collected in EA samples, with total count in the left-hand table and taxa that occur most frequently across the 49 samples in the right-hand table. The 12 taxa that are considered the 'most common' are indicated in bold. Note that Total (count) is per m³ multiplied up from the subsample.*

Taxa (greater than 1% of total count)	Total	% of total
Acartia spp. unident	41,606.06	20.82%
P-P small unident calanoid	26,989.53	13.50%
Temora longicornis	14,309.01	7.16%
Acartia clausi	12,714.31	6.36%
Centropages hamatus	10,727.07	5.37%
Cirripede larvae	9,491.29	4.75%
Gastropod larvae	7,361.40	3.68%
Bivalve larvae	7,079.49	3.54%
Podon spp. (unident)	6,714.79	3.36%
Oikopleura	6,704.07	3.35%
Oithona spp. (unident)	6,460.00	3.23%
Pisidia longicornis	5,712.09	2.86%
Isias clavipes	3,999.28	2.00%
Euterpina acutifrons	3,529.42	1.77%
Evadne nordmanni	3,281.54	1.64%
Paracalanus spp. (undeint)	3,046.25	1.52%
Foraminifera	2,610.34	1.31%
Brachyura	2,604.44	1.30%
Copepod nauplii	2,213.65	1.11%

Taxa (occuring in more than 70% of samples)	Occurance	% Occurance
Gastropod larvae	49	100.00%
Acartia spp. unident	48	97.96%
P-P small unident calanoid	48	97.96%
Polychaete larvae unident	48	97.96%
Oikopleura	47	95.92%
Brachyura	46	93.88%
Temora longicornis	44	89.80%
Acartia clausi	44	89.80%
Bivalve larvae	44	89.80%
Centropages hamatus	43	87.76%
Cirripede larvae	43	87.76%
Caridea	43	87.76%
Bryozoa larvae (cyphonautes)	39	79.59%
Hydrozoa	39	79.59%
Pisidia longicornis	38	77.55%
Euterpina acutifrons	35	71.43%
Cyclopoida (unident)	35	71.43%
Chaetognath (unident)	35	71.43%
Pleurobrachia pileus	35	71.43%

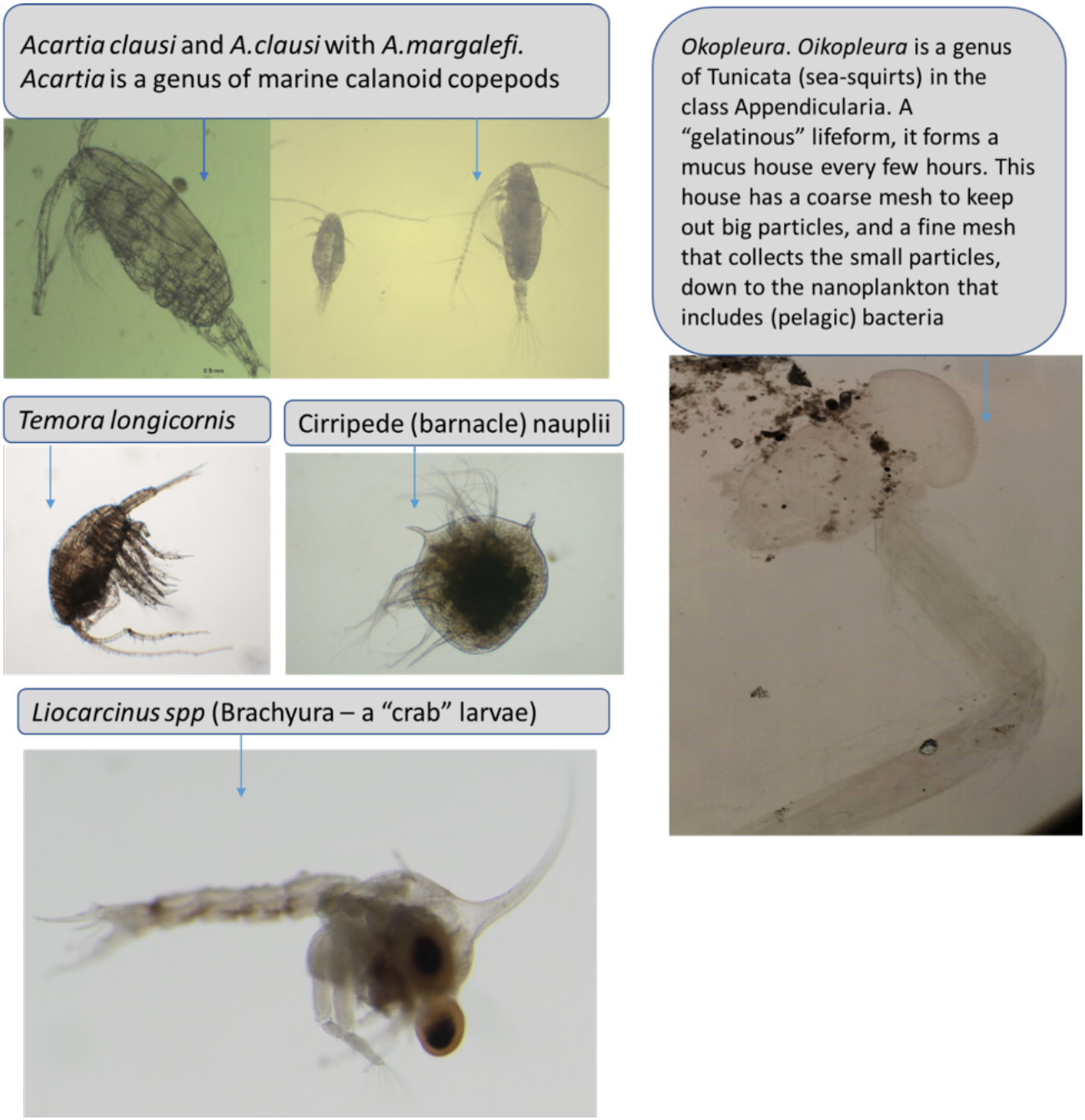


Figure 20: *Examples of the diverse range of zooplankton caught in EA samples*

Table 8 summarises the abundance and diversity of taxa collected from our main sites between August and October 2022. Geographically the most diverse sites tended to be the same as those sites that had the highest numbers. The highest counts were around the Farne Islands in the Summer. However, this is a very limited data set which will repay more detailed study when we have a complete years' worth of data.

***Table 8**: Summary of abundance and diversity of taxa for the main sites in EA sampling. Note that Total (count) is per m³ multiplied up from the subsample*

Site	Month	Total	% total	Number of taxa	Percent of total taxa
Farne Islands to Newton Haven	8	20,571.51	10.29%	65	28.14%
Farne Islands	9	4,006.65	2.00%	61	26.41%
Cornwall North	8	10,124.25	5.07%	56	24.24%
Ryde	9	2,043.17	1.02%	52	22.51%
Mersey Mouth	10	14,559.36	7.28%	51	22.08%
Cowes	9	4,180.60	2.09%	45	19.48%
Barnstaple Bay	8	3,227.31	1.61%	45	19.48%
Tees: Redcar Jetty	8	22,024.03	11.02%	44	19.05%
Thames Lower	8	8,111.02	4.06%	42	18.18%

Combining and summarizing the data from all sites, we can see that total zooplankton follows expected patterns with the summer months seeing the largest numbers (note the variable number of sites and months limits conclusions that can be drawn from this data set (Figure 21).

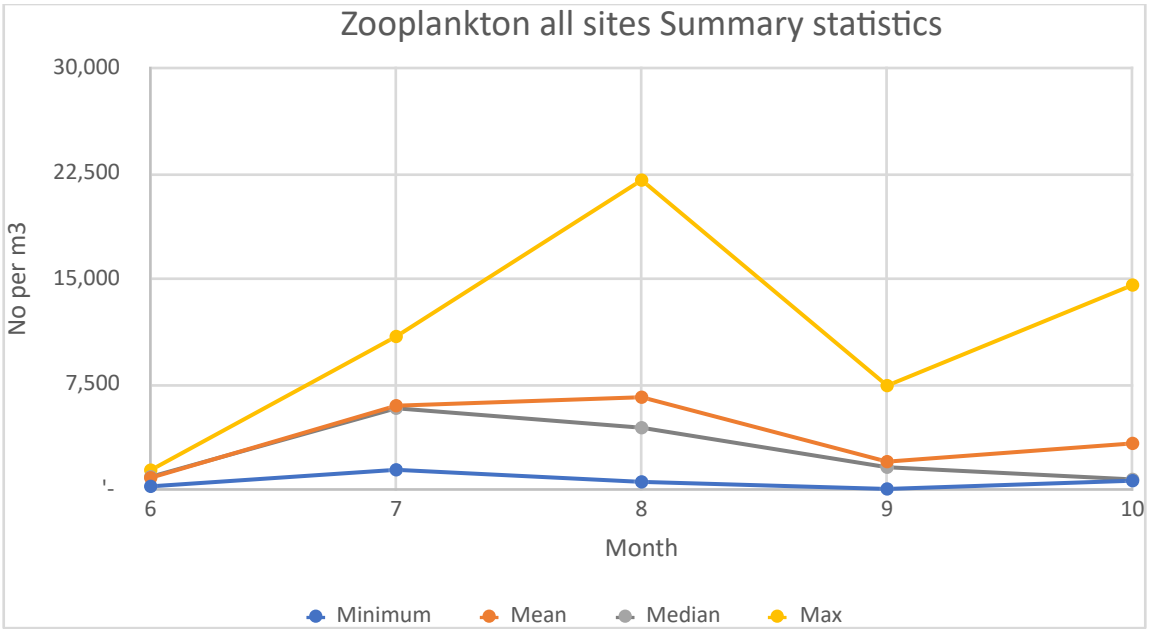


***Figure 21:** Minimum, mean, median and maximum zooplankton numbers in EA samples per m3 for each month, where month 6 is June 2022, month 7 is July 2022 etc.*

Summer and autumn appear to have more outliers, with Tees: Redcar jetty in August having the highest counts (Figure 22). Further details and raw data tables can be found in Appendix 9.

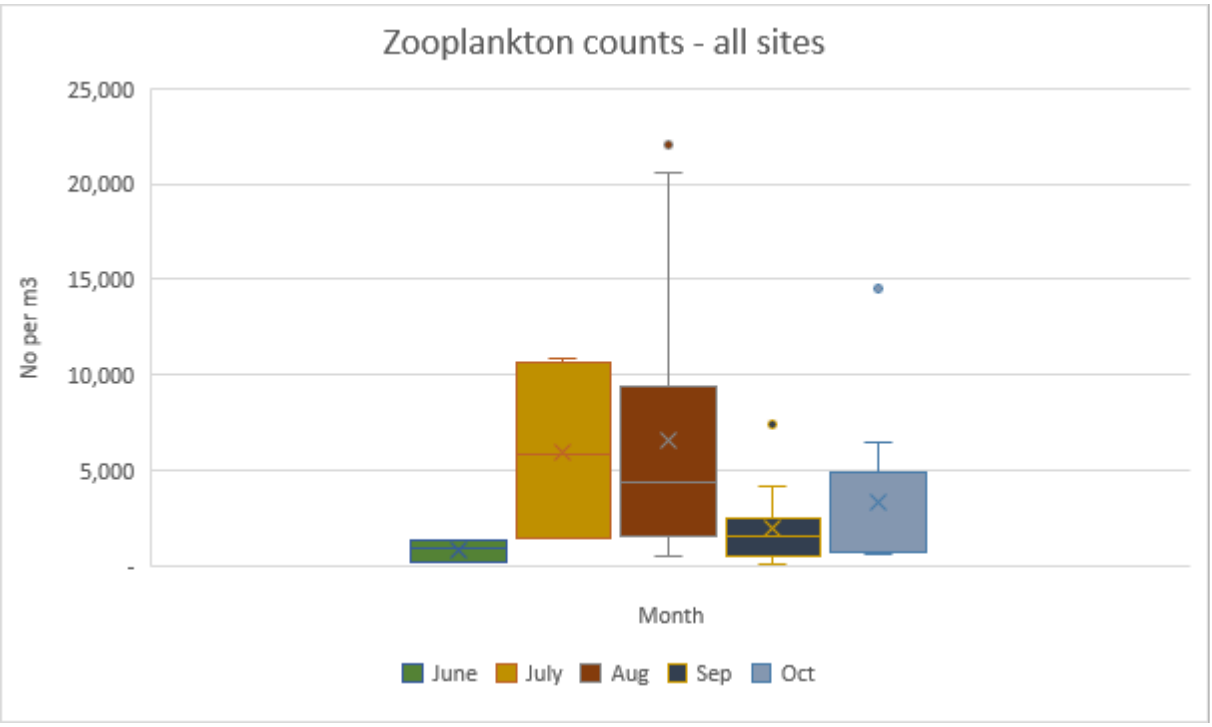


Figure 22: *Number of zooplankton counts in EA samples per m3 for all sites, between June and October 2022*

3. Cefas samples

A total of 56 different species were identified in Mersey samples and 51 in the Thames samples. Data analysis is still very much ongoing, and there are only a limited number of samples for data analysis.

As with the EA data, the preliminary assessment suggests strong geographical differences between west (Liverpool) and East (Thames) (Figures 23 and 24) with higher abundance measured in the Thames, both overall and across monthly samples.

Monthly abundances also suggest a strong seasonal pattern, following the spring plankton blooms typically seen in coastal and offshore UK waters. Abundance of zooplankton peaks in August and September for the Thames, and August in Liverpool Bay, indicating these high numbers are closely linked to the higher plankton numbers through spring and summer. Abundances decrease into the winter months, with lowest abundances measured in the January cruises.

Further sampling and data analysis will be carried out on the spring -summer months to follow the seasonal cycle. In the next year of sampling and data analysis, we will look to integrate phytoplankton and zooplankton over the annual cycle to increase our understanding of the linkages between nutrients, phytoplankton, and zooplankton abundances.

Figure 23: *Mean zooplankton abundance plots for both estuaries sampled by Cefas. Zooplankton mean abundance are Log10(x+1)*

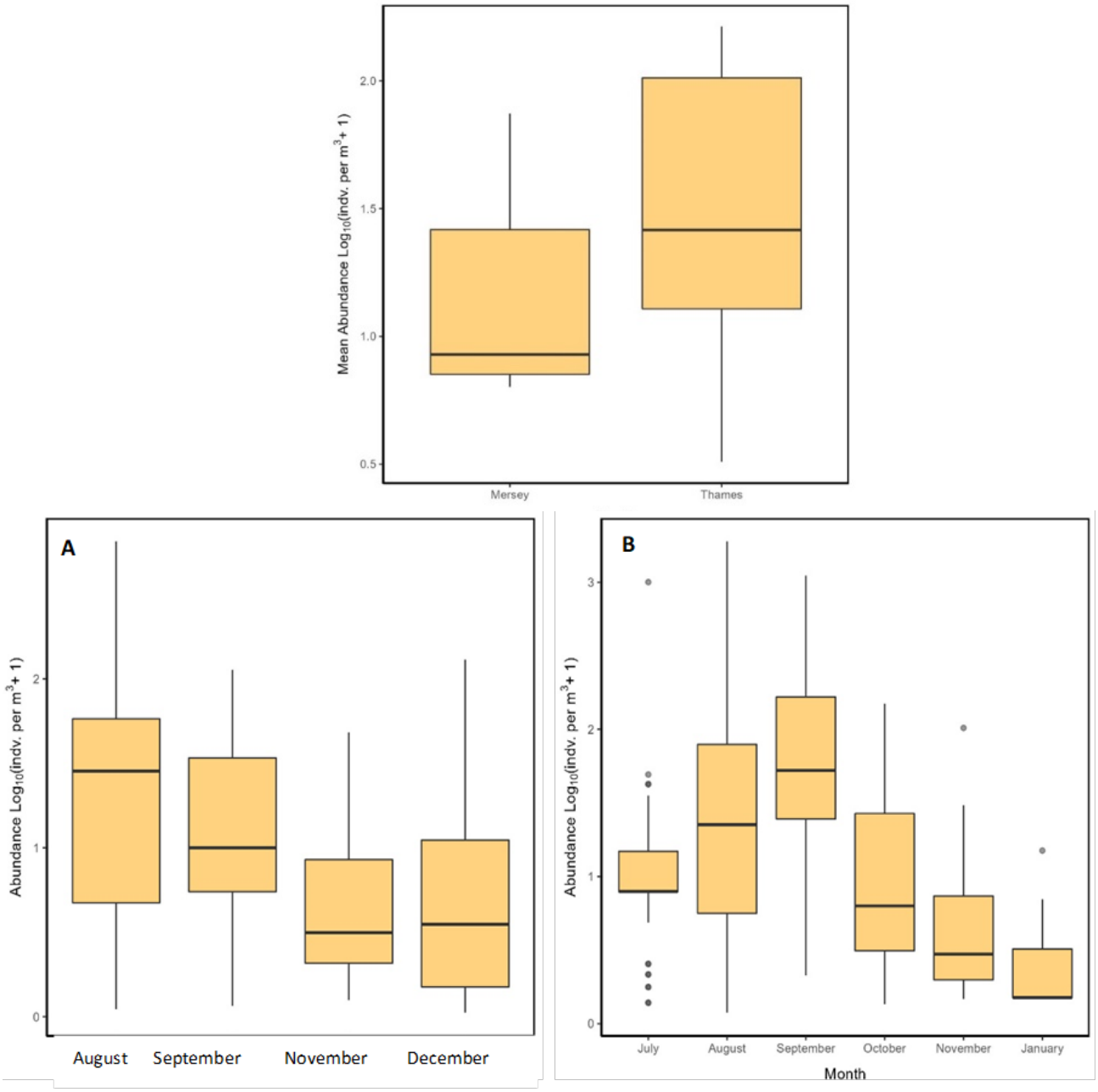


Figure 24: *Monthly abundance plots for Mersey (A) and Thames (B) samples collected by Cefas between July 2022 and Jan 2023. Zooplankton mean abundance data are Log10(x+1)*

6. Database and Data Flows

1.1. Plankton Lifeform Extraction Tool

Each institution has its own data procedures and internal databases to store the data it has collected. After internal data quality control, the data is stored on the institutes databases (both internal and public facing) and then the dataset assigned a Digital Object Identifier (DOI) and combined with the data from other UK marine monitoring institutions for further analysis. This is the publicly available Plankton Lifeform Extraction Tool (PLET, Plankton Lifeform Extraction Tool (dassh.ac.uk)), hosted by DASSH at the MBA. DASSH is part of the UK's network of marine data archive centres. PLET data is not yet automatically findable on MEDIN due to metadata restrictions, but we are looking at many other ways to make the data more discoverable such as through DOIs and the Pelagic Habitat Expert Group (PHEG) website (Figure 25).

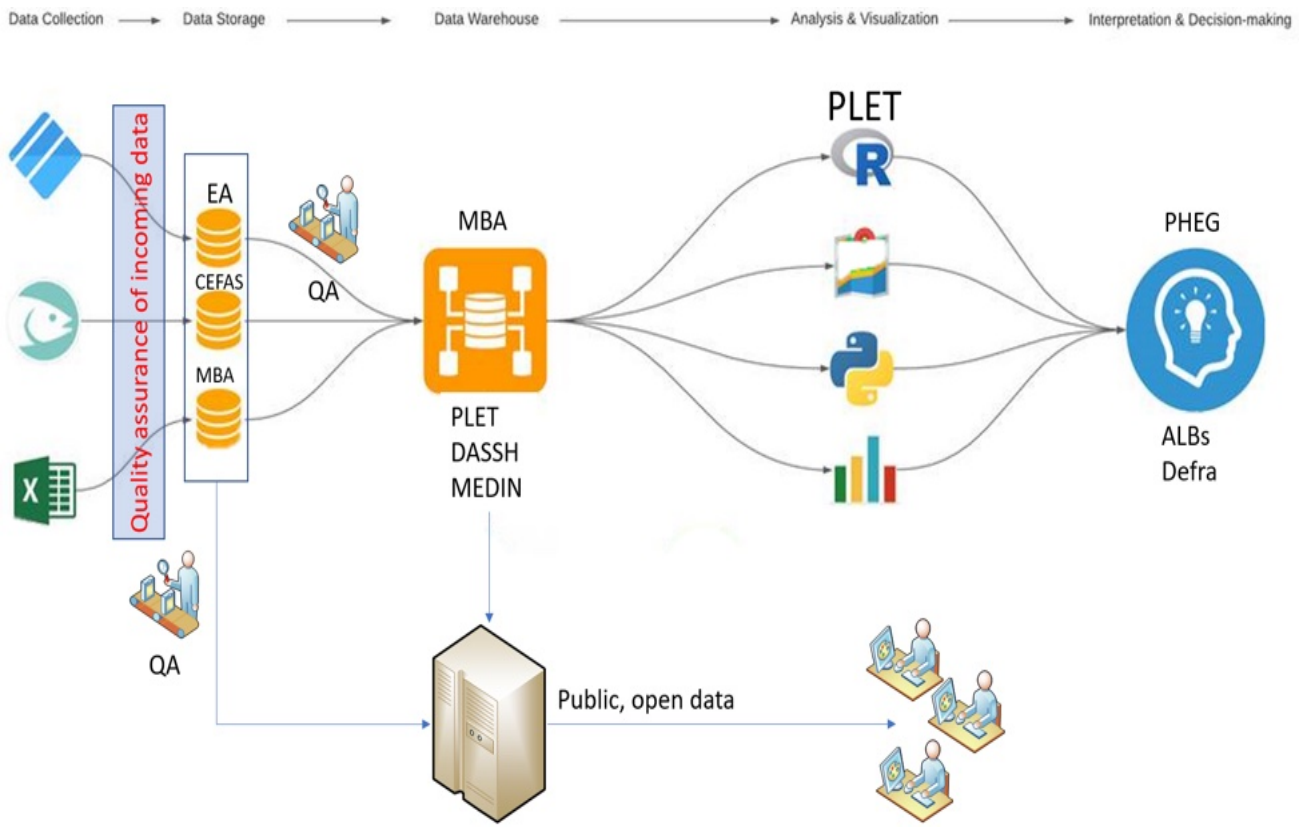


Figure 25: *Data flows between EA, Cefas, MBA and PLET for lifeform analysis*

6.2 Data flows for inshore sampling

The EA holds biological data on its 'Biosys' database, and water quality data (including Chlorophyll) on its WIMS (Water Information Management) database. Biological and chemistry data from the same survey can be linked through code. The sampling, analysis, and data storage flows within the EA are complex (Figure 26) but Open data is available from the Defra data services platform hosting both Open WIMS data (Open WIMS data) and Open biology (Biosys) data (EA Ecology & Fish Data Explorer (Figure 26). Biosys was not designed to store zooplankton data but will be upgraded in year 2 of the project (refer to 6.1.1). All biological data from the project will also be hosted on the publicly available PLET.

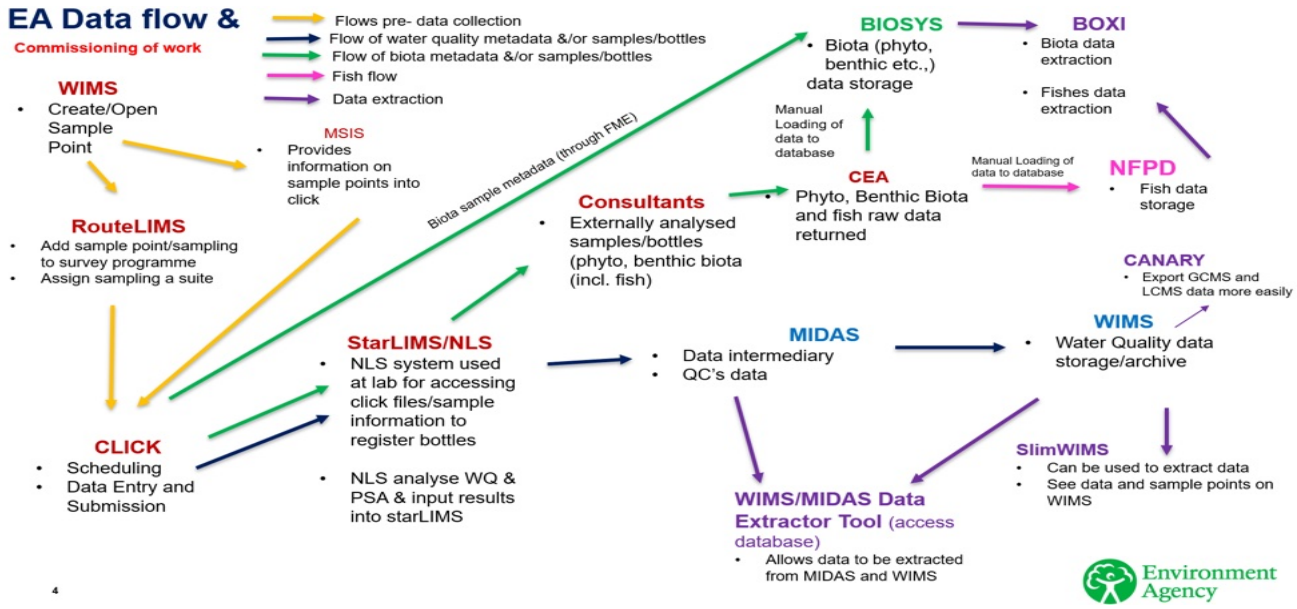


Figure 26: *Example of EA's multiple data levels between sampling and analysis and data storage*

6.1.1. Developing EA data archive to hold zooplankton

Currently the EA's biology database Biosys is not ready to ingest zooplankton data. The database and its taxa list are maintained by several internal and external parties (e.g. the Natural History Museum maintains and validates our taxa) and the approvals and test processes is quite involved – part of the process is simplified in the illustration below (Figure 27). System developments will take place in year 2 of the project.

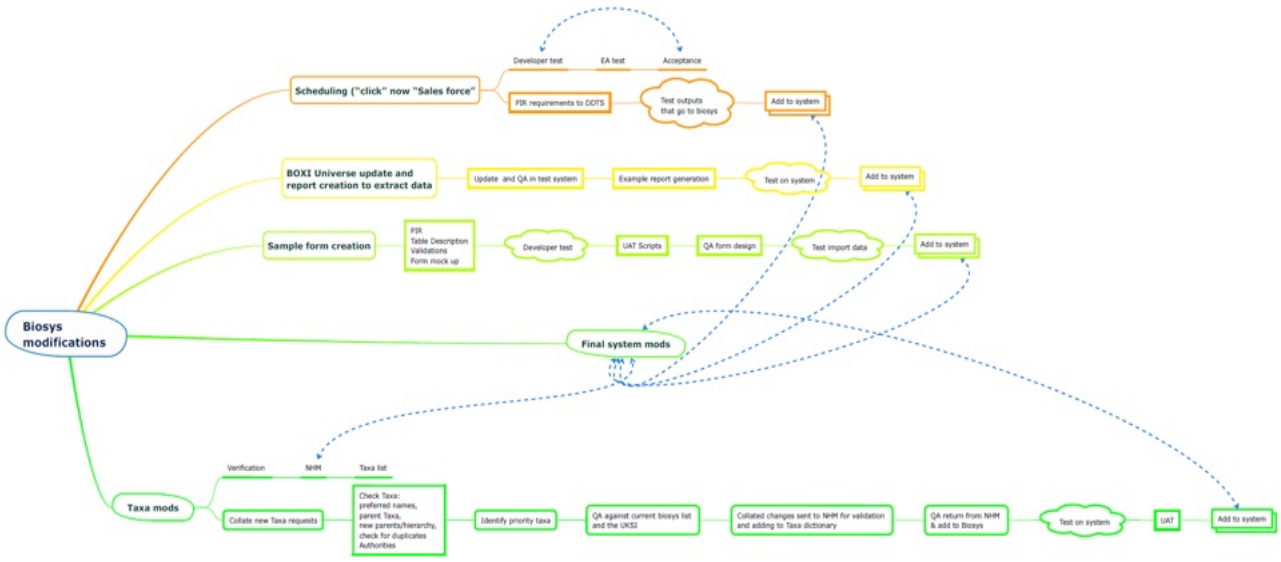


Figure 27: *Approval and test processes for EA for the collection and storage of zooplankton data*

2. References

BEDFORD, J., OSTLE, C., JOHNS, D.G., ATKINSON, A., BEST, M., BRESNAN, E., MACHAIROPOULOU, M., GRAVES, C.A., DEVLIN, M., MILLIGAN, A., PITOIS, S., MELLOR, A., TETT, P. AND MCQUATTERS-GOLLOP, A. 2020. Lifeform indicators reveal large-scale shifts in plankton across the North-West European shelf. Global Change Biology, 26: 3482-3497.

BENDSCHNEIDER, K., AND ROBINSON, R.J. 1952. A new spectrophotometric method for the determination of nitrite in sea water. Journal of Marine Research, 1 (11): 87-96.

CARPENTER, J.H. 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnology and Oceanography, 10, 141-143.

GOWEN, R., MCQUATTERS-GOLLOP, A., TETT, P., BEST, M., BRESNAN, E., CASTELLANI, C., COOK, K., FORSTER, R., SCHERER, C., & MCKINNEY, A. 2011. The Development of UK Pelagic Plankton Indicators and Targets for the MSFD.

GRASSHOFF, K., EHRHARDT, M., AND KREMLING, K. 1983. Methods of Seawater Analysis. Second revised and extended edition.

KEROUEL R., AND AMINOT, A. 1997. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow. Marine Chemistry Vol. 57, no. 3-4, pp. 265-275.

LINCOLN, R., AND SHEALS, J. 1979. Invertebrate animals: collection and preservation. British Museum Natural History) Cambridge University Press. Pages 127, 135-136.

MURPHY J., AND RILEY, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31–36.

OMIRI M., AND IKEDA, T. 1984. Methods in marine zooplankton ecology. John Wiley and Sons, Chichester. Pages 59, 61-62.

OSTLE, C., ARTIGAS, F., AUBERT, A., BUDRIA, A., GRAHAM, G., JOHANSEN, M., AND ROMBOUTS, I. 2017. Programming outputs for constructing the plankton lifeform indicator from disparate data types, EcApRHA Deliverable WP1.1. London, UK: OSPAR: 30.

RAITSOS, D.E., REID, P.C., LAVENDER, S.J., EDWARDS, M., AND RICHARDSON, A.J. 2005. Extending the SeaWiFS chlorophyll data set back 50 years in the northeast Atlantic. Geophysical Research Letters, Vol. 32.

REID, P.C., COLEBROOK, J.M., MATTHEWS, J.B.L., AIKEN, J., AND THE CONTINUOUS PLANKTON RECORDER TEAM. 2003. The Continuous Plankton Recorder: concepts and history, from Plankton Indicator to undulating recorders. Progress in Oceanography, 58: 117-173.

RICHARDSON, A.J., WALNE, A.W., JOHN, A.W.G., JONAS, T.D., LINDLEY, J.A., SIMS, D.W., STEVENS, D. AND WITT, M. 2006. Using Continuous Plankton Recorder data. Progress in Oceanography, 68: 27-74.

STEEDMAN, H.F. (Ed) 1976. Zooplankton fixation and preservation. Unesco, Paris.

TETT, P., CARREIRA, C., MILLS, D. K., VAN LEEUWEN, S., FODEN, J., BRESNAN, E., AND GOWEN, R. J. 2008. Use of a Phytoplankton Community Index to assess the health of coastal waters. ICES Journal of Marine Science, 65: 1475–1482.

WINKLER, L.W 1888. Die Bestimmung des im Wasser gelosten Sauerstoffes. Ber. Dtsch. Chem. Ges., 21, 2843-2855.

YEATS P.A., AND BRUGMANN, L. 1990. Suspended particulate matter: collection methods for gravimetric and trace metal analysis. ICES Techniques in Marine Environmental Sciences, No. 7. 9 p.

Appendix 1. Development of the EA inshore zooplankton sampling methodology (as of February 2023)

The development of an inshore zooplankton sampling method began by discussing initial sampling ideas with other organisations with marine zooplankton sampling programmes, including Marine Scotland, Marine Biological Association (MBA), Plymouth Marine Laboratory, Southampton University, Cefas, and Natural Resources Wales (NRW). The main considerations when developing a suitable sampling method were finding a suitable sample net and mesh size which could be deployed and recovered safely in a repeatable way throughout the EA fleet, while being compatible with other organisations. The samples needed to reflect the coastal zooplankton community using a cost-effective analysis approach.

Initially, a paired Bongo net sampling methodology was explored in which two different mesh sized nets, 68um and 200um, would be deployed in one haul and the samples combined into one pooled sample. This idea was dropped in favour of a single-net sampling approach for several reasons, including advice from other samplers. Firstly, the issue of clogging in the finer mesh net was a concern and the deployment of two nets simultaneously might be restrictive if one net consistently clogged whilst the other didn't. The ability to haul the different net sizes at different speeds might be needed, which Bongos wouldn't allow. Secondly, the use of a fine-mesh sampling net such as 68um in shallow waters requires the net to be relatively long, preventing sampling at the shallowest sites. This is further complicated in that the catches from the two nets cannot be mathematically combined.

Other organisations sample zooplankton from either a coastal survey vessel (CSV) equipped with a hydraulic winch/davit, or sample using a smaller rib and hauling the, usually smaller, net by hand. At an early stage, the CSV was considered a better option, either hauling using the winch, or by davit if the minimum winch speed was too fast. Unlike the rib option, a CSV provides a stable sampling platform for using preservative, a safer method of deploying the net using a winch and a means of rinsing the sampling net. Although NRW hand-haul a sampling net from a rib, other organisations advised that a winch was better; a sampling trip with MBA revealed that the depressor weight needed to keep the net deployed near-vertical was significant and could not be safely hauled by hand. NRW use a very light depressor weight when deploying nets from a rib but use a real-time depth probe to avoid net collision with the seabed. Using a real-time depth probe was not favoured by the EA monitoring survey team as it is an additional consideration for samplers; it also poses a snagging risk. Furthermore, sampling using a light depressor weight can take longer for the net to reach the required depth as more line has to be paid out, during which time the vessel has drifted a considerable distance away from the survey site.

Having decided that single-net sampling from a CSV, using a davit or winch was the best approach, there were several technical considerations such as best mesh size, aperture size, detailed sampling method, preservative and supporting data. Following advice on the risk of fine mesh nets (e.g. 68um) clogging, a decision was made to use 100um and 200um sampling nets of 400mm aperture; 400mm was selected as a compromise between the risk of the finer mesh net clogging if the aperture is too large, whilst filtering enough water per haul to provide a representative sample. A larger aperture drum would also have necessitated a longer net for effective filtering, especially the 100um, and 400mm was a good compromise without making the net so long as to present problems for deployment from the CSVs using the winch, and for sampling at shallow sites. Nets were sourced from Aquatic Biotechnology. Field trials in Southampton Water on board Solent Guardian confirmed that the required haul speed could be achieved using the winch and the davit-deployment option was discarded.

However, after initial field trials in July and August 2022 the 100um nets were dropped and sampling proceeded using the 200um nets only; this was seen as a good catch-all mesh size to use to characterise the zooplankton community. Combining two samples, one each from a 100um and 200um net, into one pot was

not considered good practice by MBA and, in any case, the lab analysis approach to the fine mesh sample is different to the coarse-mesh sample so combining the net contents wasn't possible. Continued sampling with both net sizes would have increased analysis costs beyond the scope of the project budget. Preliminary analysis of the data from field trials with the 100um and 200um mesh nets (6 and 35 samples, respectively) showed that we can distinguish between geographical areas around the coast quite reasonably. Given that most samples were collected using the 200um net, this suggested that continuing with this mesh size only would enable effective monitoring of the zooplankton community.

Regarding differences in catch between different mesh sizes, data analysis results were less clear due to the small number of 100um samples collected, all from the same geographical area. Taxon richness tended to be a bit lower in the 100 µm mesh, but total abundances in this mesh were higher.

When sampling began in July and August 2022, two replicate samples were collected using each net mesh size to test inter-sample variability as a measure of how representative the sampling method was. Analysis showed that the replicate samples were similar and contained abundant zooplankton. In simple terms, this showed that the sampling method worked (it caught abundant zooplankton) and that the zooplankton community could be monitored without the need to collect a second sample at each site. Replicate sampling was therefore dropped in September 2022 to maintain affordable sampling and analysis costs.

Field trials in Southampton Water suggested that a minimum sampling depth of 10m still provided a viable zooplankton sample in which the net drum is hauled from 5m deep to the surface (the net length and a safety margin taking up the remaining 5m depth). Sites of mean depth greater or equal to 10m were selected when designing the monitoring network.

All other sampling organisations in the UK use formalin solution to preserve zooplankton samples and therefore formalin was selected. A safe method for sample preservation within an acceptable sampling interval was developed.

Throughout September to November 2022, numerous method-refinements were made. Trials with flowmeters to record the net volume sampled were unsuccessful as the data was inconsistent and unreliable and the deployment and maintenance of the equipment was disproportionately time-consuming. A decision was made to estimate net volume sampled using the net dimensions and the length of rope deployed instead; this option has also been adopted by other organisations for similar reasons. The net volume sampled is calculated within the survey log and sent to the MBA to enable the analysis results to be provided to the EA as an estimated count per taxa, per unit volume sampled.

Initially a stopwatch was used to record the haul duration and check the haul velocity but subsequently dropped as the same information could be obtained from the start/end fixes in the survey log. Further streamlining of the method also included dropping the collection of a position fix at the end of a haul, as only one position fix, taken as the haul begins, can be entered onto Biosys in any case. The survey log has been developed to enable consistent data collection by sampling teams.

Winch cables have been fitted with ropes marked at metre intervals to facilitate easy recording of the net volume sampled; further work and training is planned in January 2023 to ensure that a consistent approach is being used on every vessel.

Trials are planned in Jan/Feb 2023 for using a lighter net depressor weight; currently 20kg is being used which works well to maintain a near-vertical winch angle but this makes net deployment difficult for some samplers. This may help on board Thames Guardian in particular, where the shorter height of the A-frame compared to other CSVs has made sampling difficult using a 20kg weight.

The smaller A-Frame and lack of safety barrier at the stern on Thames Guardian (TG) has proved an issue when trying to safely deploy and retrieve the zooplankton net. As a result, zooplankton sampling was 'red

carded' on TG in September 2022 with sampling in October and November taking place on a hired survey vessel. The vessel crew and Scientific Officer on Thames have trialled a variety of methods for the safe retrieval of the net but to no avail. In early 2023, the crew will try one last method for safe net deployment and retrieval using the vessels moon pool and top cabin winch. We are currently waiting for a part to be fabricated that will raise the height of the top winch so that the net will clear the deck as it is retrieved through the moon pool.

Appendix 2. Example review of zooplankton sampling survey logs from EA Vessels

Based on the review of samples taken in August and September 2022.

Focus of review

1. Are flowmeters providing useful data on volume sampled? Is the volume estimate from flowmeters comparable to the estimate from cable length deployed and net dimensions?
2. Is there any difference between the haul time measured using the survey log fix at the start and end of the haul, compared to a stopwatch?
3. Have we observed winch cable angles greater than 25 degrees?
4. The variation between site naming convention
5. The variation in recording of zooplankton samples between the different CSV teams
6. The recording of maximum sample depth (aka cable length deployed) in the survey log
7. The haul speed: does it exceed the 0.2 m/s in the OI? Does it exceed the maximum recommended of 0.5m/s?
8. Are the zooplankton code and PRN number provided in the Public Register Comments?
9. Check the recording of environmental conditions
10. Sample preservation interval; <3 hours?

Results

1. Flowmeters

As observed in the initial field trials, there is generally low consistency between the flowmeter and cable length methods for estimating net volume sampled. The flowmeter estimates are erratic, imparting low confidence in the results. Conclusion: Should we stop using flowmeters based upon these results?

2. Survey fix times versus stopwatch

Apart from the first sample collected on 3 August, the haul duration on all subsequent surveys on Humber Guardian are the same. This suggests that the stopwatch method may have been dropped after the first survey.

Surveys on Solent Guardian and Thames Guardian show similar haul durations from the fixes versus stopwatch methods, suggesting that it is unnecessary to continue with the stopwatch method. Conclusion: Should we stop using the stopwatch to time haul duration and use the fixes instead?

3. Winch cable angle

Winch cable angles varied between 0 and 30 degrees. On 3 hauls, the cable angle was estimated at 30 degrees; on 24 hauls the cable angle was less than or equal to 25 degrees. Conclusion: Method is successful at keeping below 25 degrees.

4. Site naming convention

There is currently variation in the naming convention of zooplankton samples between sites and vessels. For example:

- Site code, zooplankton, Replicate (Humber Guardian)
- Site name, (Site code), Zoop, Replicate (Some Solent and Thames Guardian samples)
- Site name, (Site Code), Haul Start/End
- Site name, (Site code), Zoop, Haul/On Surface, Replicate (some Solent and Thames samples)

Conclusion: Should we have a standard approach to recording site name and sample code in the log?

5. How are zooplankton samples recorded in the survey log?

- Humber Guardian samplers have recorded zooplankton samples as one sample per row in the survey log.
- Solent Guardian samplers have recorded zooplankton samples over two lines in the survey log, with a position fix at the start and end of the haul
- Thames Guardian samplers have recorded one sample using one line per haul; two samples on 2 lines per haul.

Conclusion: Should we have a standard approach to recording zooplankton samples in the survey log? The draft OI specifies taking a fix at the start and end of the haul.

6. Observations of maximum sample depth (cable length deployed)

- Samples from Humber Guardian have followed the OI and deployed to a maximum sample depth (cable length) rounded to the nearest metre.
- Samples from Solent Guardian and Thames Guardian always record the cable length deployed as 'Water depth minus 5m'. However, this deviates from the OI which specifies rounding down to the nearest whole metre of the depth and then deploying to 5m less than that figure. In reality, because the markings on the winch cable are to the nearest metre and the OI guidance is to round down to the nearest metre, the cable length deployed won't automatically be exactly 5m less than the water depth. We can't be that accurate as the markings on the winch cable are only every metre.
- On two surveys from Solent Guardian on 15 Sept 2022, the sample depth at end of haul is recorded in the log as 5m. This isn't needed in the survey log.

Recording the true cable length deployed (not just water depth minus 5m) should be the standard method going forward. This is especially important if we are using cable length deployed to calculate net volume sampled (and not flowmeters).

7. Does haul speed exceed guidance?

In most cases, the haul speed was below the maximum speed of 0.2m./s in the OI. However, in 9 samples, the haul speed was >0.2m/s. In two samples on board Thames Guardian, the haul speed exceeded 0.5m/s; this is above the maximum recommended towing speed for vertical zooplankton hauls and may have impacted the zooplankton sampling at these sites.

8. Are the zooplankton code and PRN number provided in the Public Register Comments?

All samples had this information supplied but there was variation in the format. Samples from Humber Guardian had the PRN in a separate column and the zooplankton codes for all sample replicates collected written in the WQ sample row. Samples from Solent Guardian and Thames Guardian had the zooplankton code and PRN written in the row relevant to the zooplankton sample.

Does this need to be standardised to make data extraction straightforward?

9. Wind speed (Beaufort), wind direction, sea state (Douglas) and Cloud cover

These details were consistently recorded for all samples, except there was some variation in wind direction; some entries were abbreviations whilst others were written in full.

Zooplankton samples have only been collected when the sea state is slight (Douglas scale 3), and wind is Beaufort scale 5 (17-21 knots).

Would it be helpful for samplers to create a drop down of abbreviations or just record the bearing as a number?

Thinking ahead to when zooplankton samples can be scheduled automatically, are these all fields that are already contained on WIMS? Or are the scales for wind and sea state different? It makes sense to get samplers used to using these if we intend to adopt the fields which are already in WIMS.

10. Sample preservation interval

None of the samples exceeded the maximum sample preservation interval of 3 hours, although 4 samples were preserved between 2 and 3 hours after collection.

None of the samples from Solent Guardian had a preservation time recorded so check of preservation interval not possible; all samples had a 'within 10 minutes' comment.

Two samples from Thames Guardian did not have a preservation time entered in the log.

It seems that the preservation time as entered within the log is in GMT (UST), but this isn't made clear in the log.

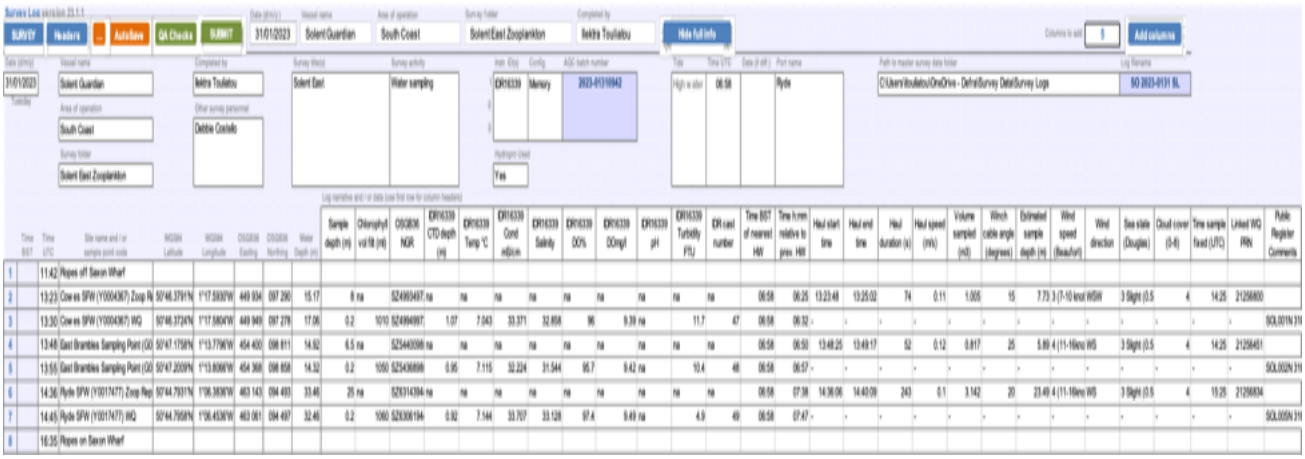
Preservation time should be recorded as an actual time and defined as GMT in the column title of the log.

Recommendations

1. Stop using flowmeters to estimate net volume sampled; use the cable length deployed instead.
2. Stop using a stopwatch to time haul duration; use the start and end fixes in the survey log instead .
3. Check the sample results of the three samples where the winch cable angle was 30 degrees; if all ok this suggests that a cable angle of 30 degrees or slightly more will provide representative zooplankton samples.
4. Standardise the naming convention for the sample name and sample code in the survey log.
5. Standardise the recording of zooplankton samples over two lines within the survey log with a position fix at start and end of haul.
6. Record the cable length deployed to the nearest metre; do not auto-populate this column in the survey log with the water depth minus 5m.
7. Remind samplers that the haul speed should be as slow as possible, aiming for <0.2m/s.

8. Consider standardising the format that the zooplankton code and PRN numbers are provided in the survey log.
9. Make recording environmental conditions as easy as possible for samplers e.g., wind direction. Also, consider using scales that are already WIMS fields so these can easily be scheduled dets for collection in future.
10. Preservation time should be recorded as an actual time and defined as GMT in the column title of the log.

Appendix 3. Survey log example



	Time BST	Time UTC	Site name and / or sample point code	WGS84 Latitude	WGS84 Longitude	OSGB36 Easting	OSGB36 Northing	Water Depth (m)	Sample depth (m)	Chlorophyll vol fit (nl)	OSGB36 NGR	CTD depth (m)	Temp °C	Cond mS/cm	Salinity	DO%	DOmg/l	pH	Turbidity FTU	CR cast number	Time BST of nearest HW	Time h:mm relative to prev. HW	Haul start time	Haul end time	Haul duration (s)	Haul speed (m/s)	Volume sampled (m3)	Winch cable angle (degrees)	Estimated sample depth (m)	Wind speed (Beaufort)	Wind direction	Sea state (Douglas)	Cloud cover (0-8)	Time sample fixed (UTC)	Linked WQ PRN	Public Register Comments
1		11:42	Ropes off Bacon Wharf																																
2		13:23	Cowes SPW (Y0004367) Zoop Ne	50°46.3791'N	1°17.593'W	449 934	097 290	15.17	8 na		524993497	na	na	na	na	na	na	na	na		06:58	06:25	13:23:48	13:25:02	74	0.11	1.055	15	7.73 3 (7-10 knot	WSW	3 Slight (0.5	4	14:25	2125680		
3		13:30	Cowes SPW (Y0004367) WQ	50°46.3724'N	1°17.580'W	449 949	097 279	17.06	0.2	1010	524994997	1.07	7.843	33.371	32.858	96	9.39 na		11.7	47	06:58	06:32	-	-	-	-	-	-	-	-	-	-	-	-	-		SOL001N 21
4		13:48	East Brambles Sampling Point (QC	50°47.1758'N	1°13.779'W	454 400	098 811	14.92	6.5 na		525440098	na	na	na	na	na	na	na	na	na	06:58	06:50	13:48:25	13:49:17	52	0.12	0.817	25	5.89 4 (11-16kn	WS	3 Slight (0.5	4	14:25	2125645		
5		13:55	East Brambles Sampling Point (QC	50°47.2039'N	1°13.806'W	454 368	098 858	14.32	0.2	1050	525436898	0.95	7.115	32.224	31.544	95.7	9.42 na		10.4	48	06:58	06:57	-	-	-	-	-	-	-	-	-	-	-	-	-		SOL002N 21
6		14:36	Ryde SPW (Y0017477) Zoop Rep	50°44.7931'N	1°06.383'W	463 143	094 493	33.46	25 na		525314394	na	na	na	na	na	na	na	na	na	06:58	07:38	14:36:06	14:40:09	243	0.1	3.142	20	23.49 4 (11-16kn	WS	3 Slight (0.5	4	15:25	2125685		
7		14:46	Ryde SPW (Y0017477) WQ	50°44.7958'N	1°06.453'W	463 061	094 497	32.46	0.2	1060	525306194	0.92	7.144	33.707	33.128	97.4	9.49 na		4.9	49	06:58	07:47	-	-	-	-	-	-	-	-	-	-	-	-	-		SOL005N 21
8		16:35	Ropes on Bacon Wharf																																

Appendix 4. EA Sample site selection

Region	WFD water body	Spt code	BIOSYS code	NGR	Northing	CSV or RIB	Average Water Depth (m)	Spt Grid Ref	>5m ?	>15m?	> 20m?	Good for larval Fish [Y/N]	Good for Oyster (bivalve?) larvae [Y/N]	Good for other reasons (add in comment)	Comment	Potential to Use	Use
AN	Blackwater Outer	BE02	OBW003P	TM0400011000	211000	CSV	8	TM0400011000	Y	FALSE	FALSE						
AN	Blackwater Outer	BE061099	OBW005P	TM0615009950	209950	CSV	12	TM0615009950	Y	FALSE	FALSE					Y	Yes
AN	Blackwater Outer	NE608211	OBW001P	TM0860011700	211700	CSV	5	TM0860011700	FALSE	FALSE	FALSE						
AN	Blackwater Outer	NE608214	OBW004P	TM0840914662	214662	CSV	3	TM0840914662	FALSE	FALSE	FALSE						
AN	BURE & WAVENEY & YARE & LOTHING	BWY004P	BWY004P	TG4820006500	306500	RIB	0		FALSE	FALSE	FALSE						
AN	BURE & WAVENEY & YARE & LOTHING	WAV179	BWY002P	TG4737004050	304050	RIB	4	TG4737004050	FALSE	FALSE	FALSE						
AN	BURE & WAVENEY & YARE & LOTHING	YAR2625	BWY003P	TG4655004900	304900	RIB	5	TG4655004900	FALSE	FALSE	FALSE						
AN	BURE & WAVENEY & YARE & LOTHING	YAR268	BWY005P	TG5175008000	308000	RIB	3	TG5175008000	FALSE	FALSE	FALSE						
AN	BURE & WAVENEY & YARE & LOTHING	YAR270	BWY001P	TG5212007540	307540	RIB	9	TG5212007540	Y	FALSE	FALSE						
AN	GREAT OUSE	57M01	GRO003P	TF6120018500	318500	CSV	6	TF6120018500	Y	FALSE	FALSE						
AN	GREAT OUSE	62M01	GRO004P	TF6010023400	323400	CSV	5	TF6010023400	FALSE	FALSE	FALSE						
AN	GREAT OUSE	62M31	GRO001P	TF5865026600	326600	CSV	6	TF5865026600	Y	FALSE	FALSE						
AN	GREAT OUSE	62M34	GRO002P	TF5855028200	328200	CSV	5	TF5882028050	FALSE	FALSE	FALSE						
AN	HUMBER LOWER	CLNMON1	HUM001P	TA2261918075	418075	CSV	9	TA2261918075	Y	FALSE	FALSE		M				
AN	HUMBER LOWER	CONT29	HUM003P	TA3215015998	415998	CSV	9	TA3215015998	Y	FALSE	FALSE		M				
AN	HUMBER LOWER	HUMB7702	HUM006P	TA1690023400	423400	CSV	16	TA1690023400	Y	Yes	FALSE		Y		too turbid	Y	
AN	HUMBER MIDDLE	HU504425	HUM009P	TA0400025000	425000	CSV	8	TA0400025000	Y	FALSE	FALSE						
AN	HUMBER MIDDLE	HU506426	HUM007P	TA0658026042	426042	CSV	8	TA0658026042	Y	FALSE	FALSE						
AN	HUMBER MIDDLE	HU510427	HUM008P	TA1000027000	427000	CSV	8	TA1000027000	Y	FALSE	FALSE						
AN	Lincolnshire	LC544405	SYK003P	TA4450005500	405500	CSV	8	TA4450005500	Y	FALSE	FALSE						
AN	Lincolnshire	LC558374	SYK004P	TF5800074000	374000	CSV	9	TF5800074000	Y	FALSE	FALSE				Check depth	Y?	Yes
AN	Lincolnshire	LC560357	SYK005P	TF6058557271	357271	CSV	12	TF6058557271	Y	FALSE	FALSE						
AN	ORWELL	ORW085	ORS002P	TM1760040400	240400	CSV	3	TM1760040400	FALSE	FALSE	FALSE						
AN	ORWELL	ORW122	ORS001P	TM2540034800	234800	CSV	9	TM2540034800	Y	FALSE	FALSE						
AN	ORWELL	WFDORW03	ORS006P	TM2135938325	238325	CSV	8	TM2135938325	Y	FALSE	FALSE						
AN	STOUR (ESSEX)	SE0310	ORS005P	TM2590132888	232888	CSV	5	TM2590132888	FALSE	FALSE	FALSE						
AN	STOUR (ESSEX)	SE0650	ORS003P	TM1915032850	232850	CSV	8	TM1915032850	Y	FALSE	FALSE						
AN	STOUR (ESSEX)	SE08	ORS004P	TM1525032640	232640	CSV	5	TM1525032640	FALSE	FALSE	FALSE						
AN	Wash Outer	WA552342	OWS002P	TF5200042000	342000	CSV	0		FALSE	FALSE	FALSE						
AN	Wash Outer	WA560340	OWS004P	TF6000040000	340000	CSV	0		FALSE	FALSE	FALSE						
AN	Wash Outer	WA560348	OWS003P	TF6000048000	348000	CSV	0		FALSE	FALSE	FALSE				see below	Y	Yes (moved)
AN	Wash Outer	WA562344	OWS005P	TF6200044000	344000	CSV	0		FALSE	FALSE	FALSE						
AN	Wash Outer	WA565346	OWS001P	TF6400046000	346000	CSV	0		FALSE	FALSE	FALSE						

Zoop Sampling SitesV4 and working

Appendix 5. Draft EA Zooplankton Operational Instruction

Please contact the EA if you would like the latest version of this controlled document

Collecting and handling of zooplankton samples

Instruction: LIT Ref. no yet assigned **Date published:** Not yet published

What's this document about?

How to sample, preserve and transport zooplankton samples from coastal and estuarine waters for analysis. It ensures that we collect samples for the marine Natural Capital Ecosystem Assessment (NCEA) programme, and potentially other programmes, and send them to the analysis contractor with the correct documentation and properly preserved.

There is a national contract for the analysis of marine zooplankton specifically collected for NCEA monitoring. Additional samples following this guidance may be able to use this contract.

Who's this document for?

This document is aimed at survey officers in the Estuarine and Coastal Monitoring Survey Team involved in collecting, preserving, and handling marine zooplankton samples.

Background

Zooplankton are a diverse assemblage of predominantly microscopic animals that drift in the water column in freshwater, brackish and fully marine environments. These organisms are an important part of the pelagic food web forming a link between primary producers (phytoplankton) and higher trophic levels, such as fish.

Zooplankton (and phytoplankton) communities respond to changes in their environmental conditions ranging from nutrient availability, temperature, light, pollution, food quality and degree of predation by other animals. Indices of zooplankton abundance and species diversity will be used to measure the health of the inshore coastal ecosystem.

The zooplankton community ranges from organisms a few micrometres long to giant jellyfish. This methodology focuses upon sampling the mesoplankton (0.2-20mm). The main mesozooplankton groups include rotifers, crustacean holozooplankton and merozooplanktonic larvae of other taxa such as echinoderms, bivalves, and crustaceans.

This methodology provides guidance for sampling zooplankton communities by nets, collection of supporting information, preservation of samples, transportation of samples and data handling responsibilities. Sampling will occur at the same site and time as phytoplankton and nutrient sampling.

Zooplankton samples are sent to a specialist marine contractor for species identification and abundance. Larger individuals (e.g. jellyfish, fish larvae, euphausiids etc) are picked out, counted, and identified; from the remaining sample between 200 and 400 organisms are identified and counted to provide a representative subsample.

Zooplankton are identified to the lowest taxonomic level possible.

Health and Safety

Samplers must apply the required risk control measures in the:

- Working in or near water instruction and Working in or near water risk assessment
- Taking a sample from water risk assessment
- When taking samples from a vessel, sampler and vessel crew must also refer to the Boat work instruction and Boat work risk assessment. Note: boat work contractor crew will have their own risk assessments to follow
- Zooplankton sampling risk assessment (insert link)
- Generic COSHH risk assessment for using 4-10% formaldehyde solution in the field
- Generic risk assessment for transporting samples

Collecting samples

Sample replicates

Routinely, collect one zooplankton sample from each survey site, labelling the sample A.

In the early stages of the sampling programme, collect two samples at each site and label them A and B. Both samples will be analysed to provide quality assurance of the sampling and analysis methods.

Rules

You must collect a phytoplankton and chlorophyll sample at each zooplankton sample site on each sampling occasion (Collection and handling of marine phytoplankton samples and Collection and handling of marine chlorophyll samples for Water Framework Directive). Only one phytoplankton and one chlorophyll sample are required per sample site, even if multiple zooplankton samples are collected at that site.

Water quality data should also be collected at each zooplankton sample site on each sampling occasion. Water quality data should be collected in between phytoplankton/chlorophyll and zooplankton sampling to minimise the interval between water quality sampling and zooplankton sampling. Only one water quality sample is required per zooplankton sample site, even if multiple zooplankton replicate samples are collected.

Competence of samplers

- All samplers collecting marine zooplankton samples must complete the Environment Monitoring-Ecology module in the Learning Zone
- Samplers must receive practical training from experienced colleagues, before leading a zooplankton monitoring survey.
- Samplers must be at TDF capability level 3 to operate without supervision.
- Samplers should also complete the Environment Monitoring - Chemistry module in the Learning Zone.
- All samplers must have read and understood the operational instruction Chemical and microbiological sampling of water.

Collection frequency

NCEA requires sample collection each calendar month. Ideally, samples should be 28-31 days apart throughout the year. This is because zooplankton have a natural seasonal variability, dependent on the phytoplankton community and other environmental factors. There must be at least a 14-day interval between sampling occasions at each site.

Note: There is no value in taking 'catch-ups' beyond this period as data needs to be representative of changes in the zooplankton community throughout the year at as near monthly intervals as possible.

Time of day dependency

Zooplankton sampling should take place during daylight hours (between first and last light) in all sample months, where possible. This will allow effective comparison of samples between months. Zooplankton sampling should always coincide with phytoplankton and water chemistry sampling at zooplankton sampling sites.

Although the phytoplankton sampling OI advises that phytoplankton sampling during winter months can occur during normal working hours due to shorter daylight hours, where possible zooplankton sampling should occur during daylight hours only.

The level of ambient light influences the degree of net avoidance by the larger forms of macrozooplankton and fish larvae, so to provide comparable data between sample months, sampling should be done during daylight hours.

Minimum sampling depth

A minimum water depth of 10m is needed to take a zooplankton sample. This will provide 5m of water column to sample, allowing for the length of the net (from the drum to the drop weight, approximately 3.2m) plus a buffer between the drop weight and the seabed of 1.8m. Important! Sampling should not be undertaken in heavy groundswell conditions as there is a risk that the net will touch the seabed.

When measuring the vertical distance sampled in the water column, measure from the top of the net drum to the surface of the water.

Biosecurity

To prevent cross-contamination of organisms between sites use only sampling equipment that has been thoroughly cleaned. If multiple sites are sampled for zooplankton on the same survey day, the same net can be used at different sites but only if the net is thoroughly rinsed using the deck hose and then immersed in freshwater to soak between survey sites.

When sampling different estuary systems on the same day, different nets must be used to mitigate biosecurity risk and prevent the cross-contamination of organisms between samples.

Nets must be thoroughly cleaned at the end of each survey day- see 'Post survey equipment maintenance'.

Further information on biosecurity can be found in Biosecurity for field and monitoring work guide and the Coastal survey vessel biosecurity guidance.

Equipment

- 2 x 40cm diameter 200um mesh zooplankton nets
- 2 x 200um detachable filtering cod-ends
- Drop weights (20kg, kept on board CSV)
- Swivel link for net attachment
- Vessel winch with cable marked at 1 metre intervals (or coloured electrical tape for marking the cable)
- Pencil
- Waterproof notebook
- Sample bottles (1 litre Kautex wide mouth bottles)
- Small plastic funnel (for use with formalin only)
- 10% formalin solution (in 5 litre bottle)
- Deck hose
- Cable ties
- Measuring board or tape measure (for recording jellyfish size)
- NLS crate (for adding formalin and shipping)
- Field guides for jellyfish identification
- Field guides for Beaufort wind scale and Douglas Sea state scale

Sample pot type

Zooplankton samples will be collected in 1 litre pots: Kautex 1000ml Capacity Polypropylene Wide Mouth Bottles from Fisher Scientific.

Configuring the sampling net

1. Screw on the cod end sample collector, taking care not to overtighten.
2. Use cable ties to create three stoppers on the attachment cords approximately 10cm above the ‘arms’ of the cod end; this prevents the cod-end from sliding up the cords, which can constrain the flow of water through the net if the net folds over.
3. Attach the base plate to the lower ends of the three attachment cords running lengthways up and down the outside of the net.
4. Secure the base plate shackles with cable ties.
5. Tie the drop weight to the base plate attachment point and secure the knot with cable ties.
6. Ask the deck hand to secure the net bridle to the end of the short rope which links between the bridle and the winch cable, using a D-shackle on either side and a swivel link between the two shackles. Important! Avoid touching the net with the winch cable as this can clog the net mesh with grease.
7. Secure the shackles with cable ties.

8. With the net set up fully extended, measure the distance from the bottom of the drop weight to the top of the net drum. This should be approximately 3.2m. Add 1.8m to this measurement to provide a safety margin between the drop weight and seabed.
9. Measure 5.0m up the rope between the net drum and the winch cable, starting from the top of the net drum and remembering to include the net bridle within this measurement. There should be a marker on the winch cable at this point to mark the minimum sampling depth of 10m. If there isn't a marker on the winch cable add one.
10. The winch cable should be marked at 1m intervals. If not, from the first marker add another marker at 1 metre intervals up the winch cable. There should be enough markers along the winch cable for the water depth at the sampling site. For example, if the water depth is 20m then the cable will need a further 10 markers after the first one.

Figure 1: *Zooplankton sampling net ready for use*

Figure 2: *Close up of net drum, bridle and shackles linking to winch rope*

Figure 3: *Close up of net weight, base plate attached to net guide ropes*

Method

Sample A

1. Once the net is configured for sampling, inform the skipper that sampling is about to begin.
2. The survey officer and the deck hand are needed to deploy the plankton net. The deck hand will raise the net on the winch until the net and drop weight is just above deck level. Ensure that the net is not twisted, and the attachment cords aren't tangled around the cod end.
3. The survey officer will manoeuvre the net and drop weight between the stern safety rail and gantry and stand clear (Figures 4 and 5).
4. The deck hand will operate the winch to lower the net into the sea, keeping the net drum above the surface (Figure 6).
5. Let the net settle in the water for a minute. This allows any air bubbles in the cod-end and net mesh to escape, making the net less buoyant. Check again that the net hasn't twisted or tangled, and the net is sitting correctly in the water with the cod end lowermost.
6. When ready to deploy the net, the sampler will observe the water depth from the survey log.
7. Important: Determine the length of winch cable to deploy by rounding down from the water depth to the nearest whole number and subtracting 5 metres (to allow for the net length (~3.1m) and safety margin (~1.9m). For example, if the water depth reading is 15.4m then the net will be deployed to the 10m depth marker on the cable.
8. Inform the deck hand of the required deployment depth.

Figure 4: *Survey officer manoeuvring the net and drop weight between the stern safety rail*

Figure 5: *Survey officer standing clear of manoeuvred net and drop weight*

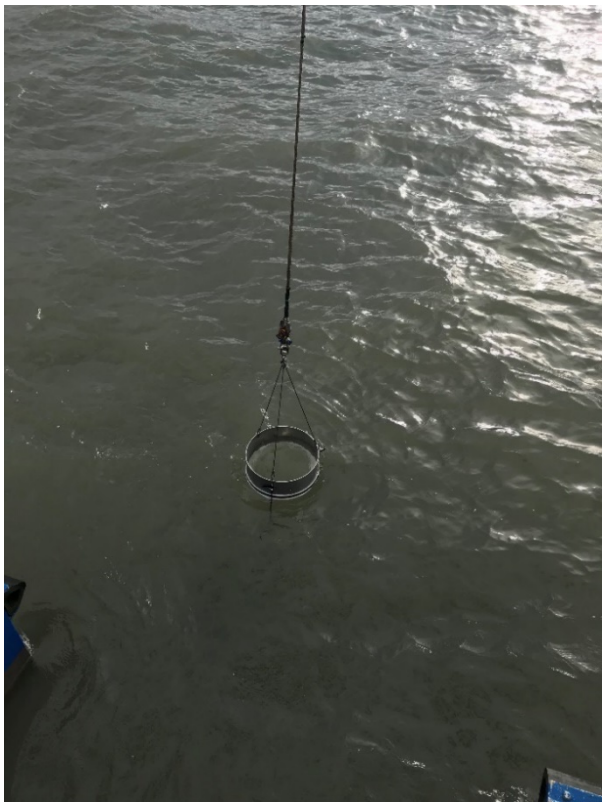


Figure 6: *The zooplankton net lowered into the water, keeping the drum above the surface*

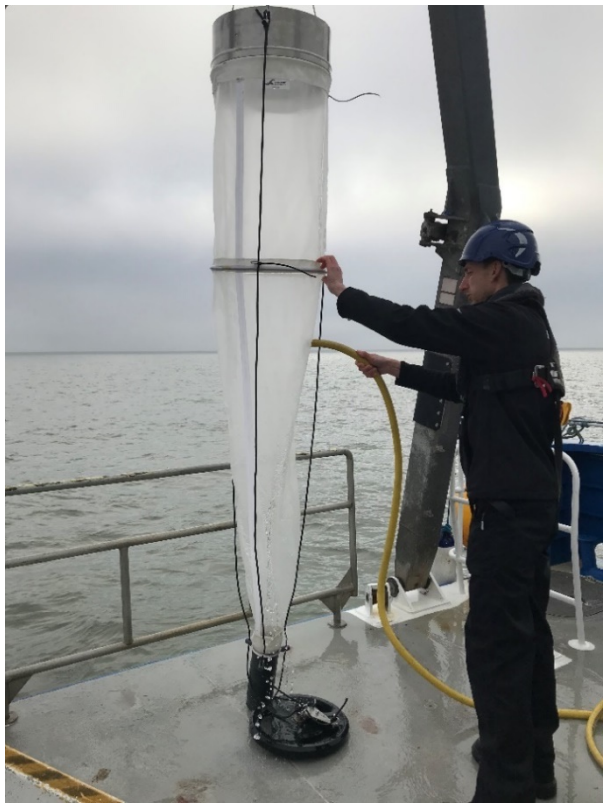


Figure 7: *Rinsing the sampling net using the deck hose*

9. Important: The deck hand will lower the net using the winch, whilst the sampler watches the winch cable and calls a halt when the net has been deployed to the correct depth marker on the cable (this should be to within 0.5m of the required cable length when deploying in choppy conditions)
10. When ready to haul the net and begin sampling, simultaneously:
    a. take a fix on the survey log to capture survey time, location, and water depth
    b. instruct the deck hand to begin hauling on the slowest winch speed. Important: the haul speed must not exceed 0.2m/s.

    Haul speed can be calculated by dividing the maximum sampling depth by the haul duration in seconds; a trial deployment must be done on new winches or vessels.
11. The winch cable angle should not be more than 25° from vertical. Record the estimated cable angle (to the nearest degree) in the survey log using a phone app called 'Bubble Level- Super Simple' to measure the cable angle.
12. When the net drum reaches the surface the survey officer will take a fix in the survey log to capture haul end time. Note: the drum must be sufficiently raised above the waterline to prevent waves from washing into the net.
13. Tell the skipper that the net is at the surface, so the vessel can be manoeuvred to minimise the net swinging upon retrieval to the deck.
14. The deck hand will winch the net to the stern of the vessel, with the drop weight just below deck level. The survey officer, under instruction from the deck hand, will guide the net through the gap

between the stern safety rail and the gantry, with assistance from the deck hand operating the winch to raise the net as needed.

15. Keeping the net raised on the winch, but with the drop weight securely on the deck, take care to keep the cod-end upright to avoid the contents washing back into the net.
16. The sampler will use the deck hose to rinse the net with a standardised approach to ensure similar rinsing effort between samples (Figure 7). Rinse from the drum to the cod-end, taking care not to spray the hose into the mouth of the net. Starting at the top and working in a clockwise direction, spray the net mesh with the deck hose from the drum to the reinforcement hoop midway down the net. The three attachment cords can be used as a guide rinse the upper net panel in three stages. Then rinse around the lower half of the net from the hoop to the cod-end, working around the net in an anti-clockwise direction to get the deck hose back to the start position and avoid twisting around the net. On occasion, if the net mesh is covered in phytoplankton, the net will need a longer rinsing time.
17. Check that the net hasn't become clogged during sampling. On occasions a large jellyfish may obstruct the net and the tow must be repeated with a clean net and cod-end. The tow must also be repeated if the net has hit the sea floor and the sample contains sediment. Record jellyfish species and notes in the survey log as described in the 'Net clogging section'.
18. Allow the cod-end to drain of water, after which it can be unscrewed from the net.
19. Turning the cod-end sample collector so the mesh panel is uppermost, hold the cod-end at a slight angle from vertical.
20. With the deck hose on a low setting, from the outside and working top to bottom, rinse the mesh panel in the cod-end sample collector once to remove any organisms stuck to the mesh panel (Figure 8). Note: this won't remove all organisms from the mesh panel but ensures most of them will be collected.

Figure 8: *Rinsing the mesh panel of the sample*

collector

21. Turning the cod-end sample collector to pour from the side away from the mesh panel, pour the sample into a 1 litre sample bottle, topping up the sample with sea water to 500ml. If the sample volume exceeds 500ml, use a larger (1.5l) sample bottle. Avoid splitting the sample between more than one sample bottle.
22. Ensuring the sample bottle is labelled on the outside and add an internal label within the sample bottle, secure the lid and store in a cool, dark place (ideally a sample refrigerator).
23. Rinse the cod-end sample collector with the deck hose and screw the cod-end back on to the net.
24. The deck hand will now lower the net slightly on the winch until the drum is at eye level.
25. Record the final flowmeter reading in the survey log.
26. Enter all notebook entries (if applicable) and populate the survey log with all required information (see Information Required), including:
    - estimated cable angle
    - maximum sampling depth Important: the maximum sampling depth is calculated by rounding down the water depth to the nearest whole number and subtracting 5m
    - record any comments specific to the first sampling haul, e.g. jellyfish in the net.

Sample B (only relevant if replicate samples are required)

Repeat the steps described for Sample A, labelling the second sample 'B'.

When sampling is concluded, rinse the nets thoroughly (see 'post-survey equipment maintenance'). To avoid the net mesh becoming permanently clogged, do not allow the nets to dry out until they have thoroughly cleaned. Nets can be kept in a tub of seawater until they can be cleaned thoroughly in freshwater at the end of the survey day.

Net clogging

If the net becomes clogged during sampling (i.e. the cod-end sampler becomes so clogged that water can no longer filter through the net) discard the sample and repeat the sampling. Clogging can often be due to phytoplankton blooms, especially in spring, which cover the net mesh in a film that prevents water from filtering through the net. If the cause of clogging was due to jellyfish, note the species and estimate the number in the net before discarding.

Important! Take care and wear gloves when handling jellyfish as some species have a severe sting.

When it is impossible to avoid jellyfish (for example, due to a jellyfish bloom) they should be rinsed from other zooplankton. The zooplankton occurring in the rinse water should be returned to the sample and the jellyfish can be discarded. Record the species, bell diameter and number of any jellyfish discarded.

Post-survey equipment maintenance

Raise the nets one by one, without cod-ends or drop weight, on the winch and rinse the nets and deck (if necessary) using the deck hose. This can also be done between hauls if there is a lot of phytoplankton in the water and the nets are getting clogged.

Following every survey the nets and cod ends should be visually inspected for damage. Nets and cod ends should be washed thoroughly in freshwater and hung up to dry to minimize the risk of clogging and to ensure optimum filtration capacity. This is especially important after sampling in waters with high concentrations of large diatoms or other organisms that can stick to the mesh and permanently clog the net if dried.

Nets should be dried at the end of the survey day and stored dry. If the nets are washed thoroughly with the deck hose and afterwards immersed in freshwater whilst on board the survey vessel, the net can be hung up by the bridle to dry under the covered area on deck.

During periods without use nets should be hung up in storage to ensure they dry completely.

Information required

To enhance the value of the zooplankton data and aid interpretation of the results, the following supporting information should be collected and recorded in the Survey Log for each sample:

- Survey date
- Vessel name
- Area of operation
- Survey folder
- Samplers
- Zooplankton site name and sample point code. The correct format should be: 'Site name (WIMS sample point code) Zooplankton BIOSYS ID Zooplankton replicate'. For example, Withernsea (YC536426) SYK006N Zoop Rep A
- Sample position fix at start of sampling, as the net haul begins. Recorded in survey log as Latitude, Longitude, Easting, Northing and NGR (from position fix).
- Water depth (to nearest 0.1m at start of haul)
- Maximum sample depth (the depth that the net drum is hauled from to nearest 0.5m; this is the length of cable deployed) Important: this should be calculated by rounding Water Depth down to nearest whole number and subtracting 5.0m. It should not be an auto-populated field in the survey log calculated by subtracting 5.0m from Water Depth (Note: this field is called Sample Depth in survey log)
- Time of nearest high water
- Haul start time
- Haul end time
- Haul duration (s) (calculated by the survey log)
- Haul velocity (m/s) (calculated by survey log from Maximum sample depth (m) and haul duration (s)) Important: Check this did not exceed 0.2m/s
- Water volume sampled (m3) (calculated in survey log from net area and Maximum sample depth)
- Winch cable angle to nearest degree (measured using Bubble Level-Super Simple phone app)
- Estimated sample depth (to nearest 0.1m calculated by the survey log)

- Weather and water conditions:
    - Cloud cover in eighths (0-8)
    - Wind conditions using Beaufort scale (0-12)
    - Sea state using Douglas scale (0-9)
    - Wind direction
- Preservation time (the time that the sample was fixed)
- Linked Water Quality sample PRN
- Zooplankton sample code(s) (recorded in Public Register Comments) (See Sample label and documents for details)
- Notes (this could include capture details of jellyfish in the net) (recorded in Public Register Comments)

Supporting data

- Phytoplankton sample
- Chlorophyll (recorded for phytoplankton sampling)
- Sea surface temperature (°C)
- Field salinity (at 0.2m depth)
- Field dissolved oxygen (% saturation at 0.2m depth)
- Turbidity (recorded for phytoplankton sampling)

Preservation

Important safety information for using formaldehyde

It is not feasible to travel to a laboratory to preserve zooplankton samples and therefore, fixing must be done on board the coastal survey vessel (CSV).

- Observe the following safety information when onboard a CSV
- seek the master's permission ahead of time to bring formaldehyde on board and provide a COSHH assessment
- the leader of the team bringing formaldehyde on board must identify themselves to the master and survey officer. The leader is responsible for the safe use of the chemical (and safe clear up of chemical if a spill occurs)
- only use or store formaldehyde (securely, within a secondary container) on the open deck (in an area designated by the master), it must never be brought into the cabins or hold
- only competent persons are to use formaldehyde and only when wearing full PPE (chemical resistant gloves (EN374, Class III), eye protection (goggles/face mask – EN166), full wet weather gear/ footwear)
- a spill kit must be to hand
- you must only carry out the dispensing of formaldehyde from a stable platform in calm conditions, non-essential individuals to the task must stay clear. Only use plastic containers

- all chemical work to be carried out on deck
- ensure the deck hose is on when fixing samples in case a spill occurs
- always ensure lids are secured on any sample and chemical bottle, especially once on an open deck. Make other members of staff aware that you are working with the chemical
- label all sample containers with 4-10% formaldehyde as HARMFUL
- ensure solid waste such as gloves and blue roll are placed in appropriate waste bags.

Spills

Observe the following safety information for spills and spillages:

- when on-board a CSV, a spill kit must always be to hand
- you must alert all personnel immediately when a spill occurs. Wear full PPE (chemical resistant gloves (EN374, Class III), eye protection (goggles/face mask – EN166), full wet weather gear/ footwear). Dispose of solid waste in appropriate waste bags

Use the following procedure for dealing with spills:

- Respond to a spill rapidly using either:
    - large-scale dilution with available hoses to open water
    - or, in contained areas such as marinas, by using Spill-X-FP. Spill-X-FP formaldehyde polymerizer is contained in the Formaldehyde and solvent spill treatment kit
- mop up small spills and splashes with blue roll and discard in an appropriate waste bag
- any spills on wet weather clothing, rinse off with the deck hose.
- When fully absorbed, sweep up the Spill-X-FP formaldehyde polymerizer. Seal in a plastic bag using the equipment provided in the spill kit.
- Label the bag as hazardous, transport it to a laboratory following the measures laid out for transport of formaldehyde (see section on Transport below). Arrange disposal, following the method set out in the instruction 52_05 Ecology laboratory safety.

Competence of samplers

All samplers must be familiar with the COSHH assessment for formaldehyde. They must wear chemical resistant gloves and safety goggles/face shield when adding concentrated formaldehyde solution to a sample.

Actions

1. Once in a bottle, zooplankton start to die and degrade; there is also an element of predation within the sample. It is therefore critical for species identification that samples are preserved as soon as possible; this must be in the field and ideally should be done before the bottle top is replaced.
2. Important! Once collected, samples should be stored in a cool dark place, ideally in a refrigerator, and fixed as soon as possible. Samples must be preserved within one hour if kept in a cool dark place or within 3 hours if kept in a refrigerator.

3. Once all zooplankton samples have been collected at a site, samples can be preserved at the same time to simplify the process, provided this doesn't exceed a 3-hour interval between sample collection and fixing.
4. Follow the steps in the table below to add formalin (10% formaldehyde solution) to preserve the sample.
5. Obtain the formalin as a pre-prepared solution only (10% borax buffered formalin solution).
6. Note the volume of the zooplankton sample within the sample bottle (check that this is 500ml).
7. Place the sample bottle within one of the compartments created by the fixed dividers in a National Laboratory Service (NLS) shipping crate.
8. Place the funnel dedicated for use with formalin on top of the crate dividers with the tail of the funnel in the sample bottle.
9. Apply appropriate safety precautions and PPE, add 500ml of 10% formalin solution to each sample bottle, using the funnel. In other words, formalin solution is added to the zooplankton sample at a 1:1 ratio to the sample volume. If the sample size exceeds 500ml, use a larger 1.5 litre sample bottle and adjust the amount of preservative accordingly - Do not split zooplankton samples between multiple sample bottles.
10. Add the interior label to the sample bottle and replace the sample bottle top securely to prevent any leakage and gently agitate contents to ensure complete mixing.
11. Ensure each sample bottle is clearly labelled with an exterior label.
12. Place the sample bottles in an NLS crate. Secure the lid with cable ties and/or duck-tape if ready to send, ensuring the sample submission form and packing list have been added. Ensure the crate is securely stowed to prevent movement on deck.
13. Rinse the funnel using the deck hose and dry before storing.

Sample labels and documents

Samples collected by MSEC Team are sent directly by courier from MSEC to the analysis contractors. Each sample bottle must have an external label and contain a waterproof label inside the bottle. A sample submission form and packing list should accompany batches of samples sent to the contractor.

Sample labelling

Each zooplankton sample bottle should be labelled with:

- Zooplankton Sample Code consisting of:
    - Zooplankton BIOSYS ID (e.g. POH001N, POH002N…) – this code will be the same as the phytoplankton code but ending in 'N' instead of 'P'
    - Sample date (In the format DDMMYY)
    - Sampling net mesh size (200um)
    - Replicate code (A or B) – if taking 2 samples A is the first sample taken and B is the second sample taken
- Programme Reference Number (PRN) – can be found on the Water Quality Click task
- Safety information ('Contains 3-5% formalin' and 'HARMFUL')

For example, POH001N 010622 200A PRN: XXXXXX

Sample submission form

A sample submission form should be sent as a paper copy with each batch of samples sent by courier to the analysis contractor. In addition, this should be emailed to the contractor.

The form should include the following information which is included as standard unless stated as requiring completion by samplers:

- Contractor details: Contractor, Contact name(s), Telephone number, Email, Address
- Survey Officer details: Environment Agency Team, Contact name, Phone number, Email
- Project details: Project title, Waterbody/waterbodies, Purpose, Survey date(s), Number of zooplankton samples, Sampling device, Net mesh (mμ), Sample type, Safety information
- National Contract details: Contract, Contact name, Phone, Email

Packing list

A packing list should be sent as a paper copy with each batch of samples sent by courier to the analysis contractor. In addition, this should be emailed to the contractor.

The packing list should include the following information for each sample submitted:

- Zooplankton sample name and PRN (e.g. POH001N 010622 200A PRN: XXXXXX)
- Site name
- BIOSYS ID
- Sample date (DDMMYY)
- Net mesh size (μm)
- Replicate code (A or B)
- PRN number
- Net volume sampled (m3)
- Safety information (type and concentration of preservative)
- Number of sample pots (should always be 1 per sample)
- Sent by (initials)

Storage and transport

Storage requirements

You must ensure the following storage requirements are met:

- fixed samples stored on board survey vessels, prior to sending by courier, should be stored within the chemical cabinet.
- storage of stock formaldehyde is in the corrosive chemical cabinet, never store with strong oxidising agents, such as hydrochloric acid
- samples preserved in 3-5% buffered formalin can be kept at room temperature
- log sheets must accompany each container of samples.

How to transport formaldehyde samples

Observe the following safety information when transporting formaldehyde:

- plan to ensure that you transport only the minimum amount of stock solution;
- in case of leaks or spills you must wear the correct protective clothing (eye protection, gloves, and footwear) when loading/unloading sealed fixed samples or dilute/undilute formaldehyde in their primary containers;
- you must follow the instructions in 'Transportation of dangerous goods by road' when transporting formaldehyde.

If a spill should occur, use the same procedure and equipment as detailed above under 'Spills'.

How to send formaldehyde samples by courier

MSEC team will be responsible for arranging transport of zooplankton samples by courier from the survey vessels to the analysis contractor.

Samples can be retained in the chemical storage cabinet on board the survey vessel and sent as a batch, provided the total package weight doesn't exceed 25kg (if using DPD).

Next-day delivery service should be used when sending zooplankton samples by courier. Avoid sending samples on a Friday, to ensure that samples arrive with the analysis contractor on a day that the laboratory is open and there is someone available to receive the package.

Action

1. Ensure sample bottles are fully labelled, including with '5% formaldehyde' contents description
2. Pack sample bottles in an NLS crate with dividers
3. Include a sample submission form in the crate and email a copy of the form to the analysis contractor
4. Use duct tape to seal the crate
5. Add an 'UPRIGHT' label to the crate

Data handling roles and responsibilities

- MSEC Team will collect all field data for zooplankton samples via the zooplankton survey log and send samples and a sample submission form by courier to the analysis contractor.
- Important! MSEC Team will record that a zooplankton sample has been collected in the Sampler's Comments Field of Click within the associated water quality sample information by recording the zooplankton sample code(s) for zooplankton samples collected at that site.
- MSEC will record the PRN number for the water quality sample in the zooplankton survey log to enable CEA to keep track of the samples.
- MSEC will send the zooplankton survey log to the Marine Monitoring email inbox at the end of each survey day, including labelling as 'zooplankton'. If after QA checks by MSEC there are changes made to the survey log, an updated copy of the log should be sent to the MM email inbox.
- CEA Team will register zooplankton sample collection on BIOSYS by manually creating a 'Sample Collect' form for each sample; CEA will reference the PRN number of the associated water quality sample within each 'Sample Collect' form for zooplankton samples on BIOSYS.
- CEA Team will enter sample collection details on BIOSYS, referencing the information in the survey log. Initially, the sampling details will be entered in the 'Comments' field of the Sample Collect form if there is not an existing field on the form.

- CEA Team will track the collection, transport, and analysis of zooplankton samples via spreadsheet created and shared by the analysis contractor.
- CEA Team will archive the sample analysis results data on a shared network drive at Kingfisher House in the folder: G:\N_Marine\06 Delivering Marine Monitoring\All Survey data\2022\Zooplankton\Raw\MBA Data Returns.

BIOSYS requires some amendments before zooplankton data can be archived, so initially the results data cannot be entered on to BIOSYS.

Data entry on to BIOSYS

To enter data, you must:

- Refer to the Biosys Marine Techniques data entry instruction
- set up stations on BIOSYS, using the site data corresponding to the linked WIMS sites
- zooplankton sampling will take place at existing phytoplankton sites so CEA Team will not need to set up sample stations. However, if new sites are required CEA Team are responsible for setting up these sites.

Sample collection must be registered manually on BIOSYS by CEA Team as soon as possible after collection, but no longer than one month after the survey.

The method (Zooplankton 200um net) should be entered for each sample, along with the replicate code (A or B).

Date, time and NGR should be populated within the Sample Collect form.

The comments field in the Sample Collect form should be used to add the sampling details against the sample. Each of the following pieces of information should be entered in a new row in the comments form using the abbreviation in brackets:

1. Water depth (metres to 1 decimal place) (WD)
2. Maximum sample depth (metres to nearest 0.5m, 1 decimal place) (MSD)
3. Estimated sample depth (metres to nearest 0.5m, 1 decimal place) (ESD)
4. Net volume sampled (cubic metres to 2 decimal places) (NVS)
5. Haul velocity (m/s to 1 decimal place) (HV)
6. Time of nearest high water (THW)
7. Wind conditions (Beaufort scale 0-12) (WBS)
8. Wind direction (1-3 characters) (WIND)
9. Sea state (Douglas scale 0-9) (SSD)
10. Cloud cover (Eighths 0-8) (CC)
11. Notes: jellyfish species and number recorded; bell diameter measured (cm)

Appendix 6. EA/MBA laboratory methods

As the EA inshore zooplankton work has been undertaken for the first-time samples have been sent the MBA for analysis. The MBA protocol is given below, it is like that from many other institutions, although there are some differences to the CEN (BS) standard mainly due to the flexibility and alternatives provided by the latter. The MBA protocol is presented below.

Net Caught Analysis Protocol

The following protocol has been used in recent years by the MBA Continuous Plankton Recorder (CPR) analysis team to undertake various zooplankton contract work, including work with the Agri-Food and Biosciences Institute (AFBI), Natural Resources Wales (NRW) and most recently, the Environment Agency. Note the protocol below is used for samples typically from around the UK, samples from different regions may require a slight adjustment to procedure to better reflect the zooplankton composition of the area.

Typically, samples arrive already fixed in formalin solution and are in pots, often of various sizes. Accompanying this, is a list of samples collected with all the information needed to proceed.

Taxa are identified to the lowest taxonomic level possible and as per the UK PHEG Master Taxa List. The protocol is to also provide a species voucher collection (one of each taxa seen needs to be tubed in a small amount of formalin and sent back to the client, if requested, for QA/QC purposes). There is an abundance of key literature on site to ensure accurate identification, along with a wealth of experience (including accreditation) across the team. All work is carried out under the supervision of the senior analyst with unusual taxa removed for confirmation.

For the Environment Agency contract, we are providing further QA/QC through reanalysis of 1 in 10 of the samples received. This will be undertaken internally by one of our more experienced analysts who is proficient in their accuracy of both CPR and contract net-caught samples and furthermore, assists the Zooplankton Technical Manager in providing specimens for the NMBAQC Zooplankton ring test.

Methodology

1. Using a notebook, take out designated pot and record the sample information i.e. station number, date, depth etc. from the sample label and note down the date of sample analysis.
2. Under a fume cupboard hood - take the sample bottle and using filter mesh, funnel, and beaker, gently rinse the sample with freshwater to remove most of the formalin. NB the filter mesh aperture needs to be smaller than that of the net that the sample was collected with (see Figure 1).

Figure 1: *Rinsing of sample*

Figure 2: *Contents within petri dish*

3. Lay the mesh out onto a microscope stage or flat surface for examination.

4. Carefully remove the larger organisms that might interfere with sub sampling such as large medusa, shrimps etc. and identify, recording results legibly in notebook.
5. Empty the contents from the mesh into a marked/lined petri dish(es) (Figure 2) and examine under a stereo microscope. Aggregations of plankton clumped together can be gently separated using fine forceps or mounted needles, to aid an even a distribution of specimens and to ensure effective subsampling in later stages.
6. Pick out carefully any organisms >= 2mm e.g. larger adult copepods (such as *Calanus finmarchicus/ helgolandicus, Pontellidae, Candacia, Metridia* etc), decapods, larger chaetognaths, gelatinous taxa and fish larvae etc. Organisms high in abundance may remain in the sample if deemed they will not interfere with subsampling. Identify and count organisms, recording legibly in a notebook. At this stage, also be on the lookout for rarer smaller taxa, which might not be represented accurately in any subsampling. Larger benthic artefacts can also be removed here, as they can often hinder subsampling (e.g. barnacle exuvium, macroalgae).
7. You are then left with the remaining organisms from the sample, the majority of which are typically small and can be adequately subsampled. We are now aiming to count approximately 200-400 organisms from the remaining sample. Anything less than this would require steps 7-10 being repeated until this is achieved. However, sometimes, particularly during winter months, there may be low numbers of organisms – if this is the case, no sub sampling is needed and a count and identification of the whole sample adequate.
8. Carefully take out the organisms from the dish and, depending on sample size, place into an appropriate sub-sampling container (e.g. Folsom splitter, rounded flask etc.), topping up to a known volume (Figure 3 - marked on beaker below) with freshwater.

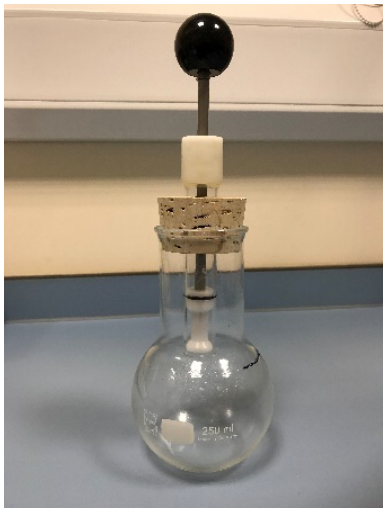


Figure 3: *Sub-sampling*

9. Gently agitate the sample in a random pattern of movement (i.e. avoiding swirling) to homogenise the organisms within the pot. Whilst the pot/beaker is still well mixed, take your subsample, e.g. if using a sample splitter, a half sample; if using a pipette, 5ml etc. The method of subsampling chosen will depend on the density of the sample and is very much reliant on an analyst's judgement.
10. Check your sample contains 200-400 organisms: re-filter the subsample using the same apparatus as in Point 2, removing the contents from the mesh but this time placing them into a Bogorov tray (Figure 4). Scan the contents using a stereo microscope checking you have between 200-400 organisms for counting (and which have not been counted in any previous stage). If >400 organisms are present re-subsample; likewise if <200 organisms are present, subsampling will need to be adjusted accordingly, this on occasion requires counting the entire sample.

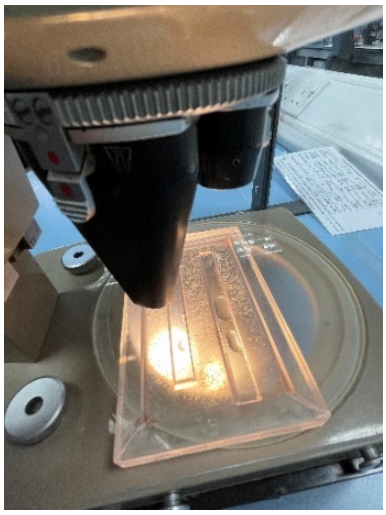


Figure 4: *Sample within Bogorov tray*

11. Starting at one end of the Bogorov channel, work your way through the sample identifying and counting each organism, record results (along with the subsample fraction used) in a notebook.
12. An example of each taxa identified must be retained for QC purposes in a separate vial (including label and pot found in) and sent back if requested with other 'voucher specimens'.
13. Once the sub-sample has been analysed, the contents can be returned to the main sample, and all washed back into the original sample pot along with the original formalin (if kept) or fresh 4% formalin added. This must be undertaken under an extraction hood.
14. The results will then need to be transferred into the appropriate database and re-analysis (repeating steps 1-13) may be necessary.

Appendix 7. Cefas field methods

Stage 1. Capture and fixation of zooplankton samples

1. Attach ring net to the hydro wire. In the case of multi-net deployments, attach the ring nets to opposite ends of the bridle bar frame. Attach bar to hydro wire. It is advisable to request the crew to do this.
2. Attach lead weights to the strain loop(s) at bottom of each ring net.
3. Prepare sample jars, add details of survey no, station no, and gear with China graph pencil on the lid, and copy details onto plankton label with a 2B pencil (there may be times when more than 1 jar is required, so add jar 1 of xx to the lid and label and repeat on subsequent jars.
4. In the case of the GO flowmeter fill with fresh water via the screw hole on the back of the flowmeter. There is no need to fill the KC flowmeter with water.
5. Attach flowmeter(s) to each ring-net bracket, so the nose faces the top of the ring net.
6. Record the flow-meter reading(s), usually in the red logbook.
7. Slowly lower the ring-net(s) to within 2-5 m of seabed.
8. Record time of sampling, this is when the net is being hauled up, position, water depth, warp angle and any other relevant details in the red log book.
9. Slowly bring the ring net back to surface at approximately 1m/second.
10. Record the flow meter reading(s) in the red log book.
11. Unscrew end bag(s) and place in separate bowls or jugs marked with the net identification, they are usually different mesh sizes.
12. Transfer sample from each end bag into a plastic jug. Label each jug with ring net size / mesh size to identify each sample.
13. Wash each sample into the sieve of the same mesh size as the end bag and swirl gently until most of seawater has gone.
14. Whilst wearing PPE and in a well-ventilated deck space, wash each sample into a relevant prepared jar using a funnel and a wash-bottle filled with 4% formaldehyde. Refer to- RA2100 The collection and preservation of Plankton and Marine Litter Samples and remember any spill should be washed with copious amounts of sea water.
15. Top up jar with 4% formaldehyde to just below the ‘shoulder’ and add plankton label to jar.
16. Fit lid securely to jar as soon as possible and write details.
17. Record the same details in the survey (red) log book.

Stage 2. Analysis by light microscopy

This is like the MBA standard in Appendix 6, but with subtle differences which are unlikely to affect the comparability of results.

1. Place a large funnel into a 10-litre waste container and put a sieve within the funnel and pour the sample into the sieve to decant off the formaldehyde and retain the plankton sample. Wash the sample thoroughly through the sieve with fresh water into the container, until satisfied it has been sufficiently washed of formaldehyde (Figure 1).

Figure 1: *Decanting and washing of sample*

2. Rinse the plankton into a jug with observation fluid (Figure 2).

Figure 2: *Rinsing of sample into jug*

3. Pour some of the sample into observation trays (Figure 3) and under a binocular microscope remove any specimens over 2mm and any rare species the taxonomist feels will not be sampled during analysis, then pour the remainder into a separate jug. If the project requires, remove all fish eggs and larvae too. Repeat until all the sample has been observed. Identify all specimens to the lowest taxonomic level of confidence. If there are lots of a given taxa, they may remain in the sample as these should be sufficiently sampled (Figure 4).

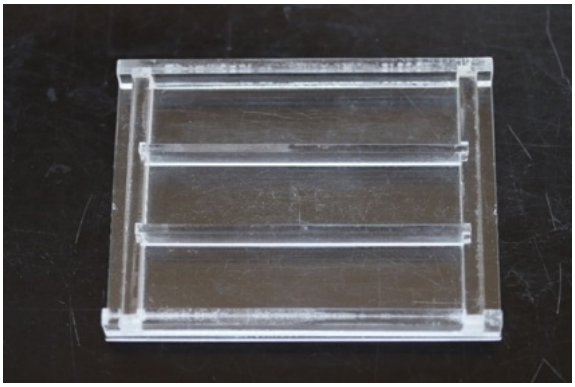


Figure 3: *Sample within observation trays*

4. The remainder of the sample can then be poured into a Florence Flask and toped up to the given mark. 150 ml, 250ml or 500ml (Figure 4).

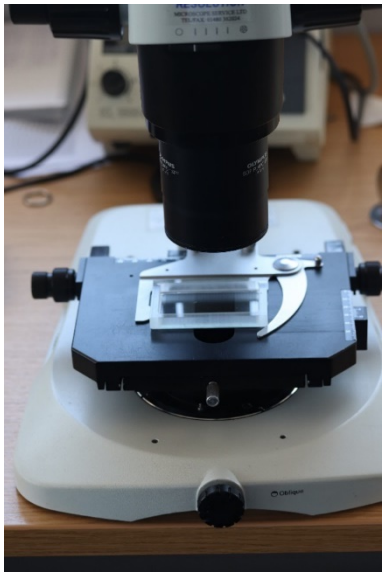


Figure 4: *Sample within Florence Flask* ***Figure 5:*** *Stager attached to the microscope*

5. Attach the stager to the binocular microscope (Figure 5).
6. Swirl the Florence Flask in a figure of eight motion with at least 20 cycles to mix the sample thoroughly, do not swirl in a circular motion.

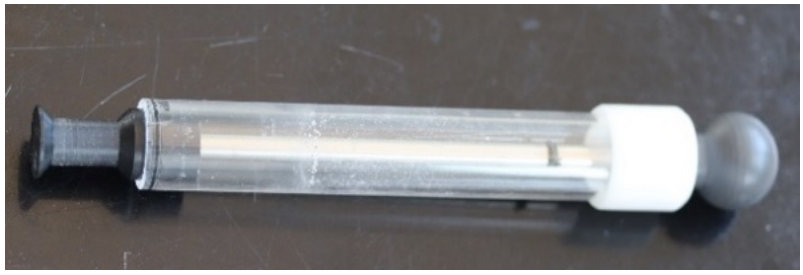


Figure 6: *Stempel Pipette*

7. Insert the Stempel Pipette (Figure 6) into the centre of the sample within Florence Flask and remove the given sub sample and eject it into a 2mm grided analysis tray (Figure 7).

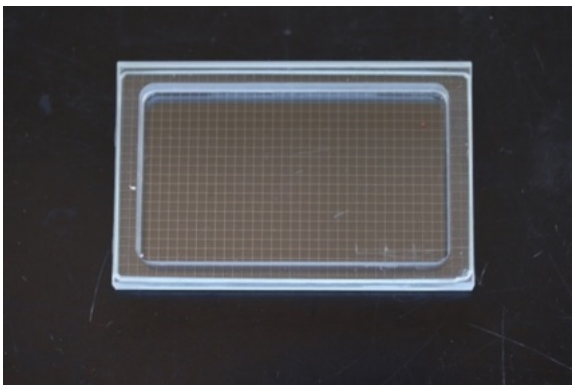


Figure 7: *Sample within 2mm grid analysis tray*

8. Place the tray with sample into the stager on the microscope (Figure 5) and set the field of view to view two or three of the squares at the top left, and work your way from left to right, then down and right to left etc, or down then right up etc, until all grid has been scanned and the specimens within the sample have been identified, and mark onto an analysis sheet.
9. The aim is to collect less than 9 taxa 100 specimens, or over 9 taxa 200 specimens. If there is not enough in the first sub sample, then steps 6-9 must be repeated until all the specimens in the sub sample are analysed. Sub sample raising factors are calculated- Sample Volume/ Sub- sample Volume.

Conductivity, temperature, and depth (CTD)

A RBR CTD logger (Figure 8) is used to capture conductivity, temperature, depth, salinity, etc. The CTD is deployed by hand over the side of the vessel and takes a surface sample only. Below is the manufacturer's quick start guide. The CTD is deployed at each station if possible.

RBR CTD Deployment Quick Start

It is important to know that to switch from 'Stopped' to 'Enabled' requires that any data on the instrument is deleted. If you connect to the RBR when it is enabled the configuration cannot be changed. Each time the instrument is enabled any previous data will be removed and a new file generated. We suggest this done once per day of sampling, so each file will contain one day of data. Alternatively, you may wish to have a single file per survey, it will depend on how many stations you are collecting.

1. Connect to the RBR either through Wi-Fi or USB cable
2. Check the battery status, replace if needed
3. Set the time
4. Ensure the configuration is correct
5. Click 'Enable', you will be prompted to delete any data, make sure you have already downloaded any data you need
6. The instrument should now display Enabled (Paused) and is ready to use

Figure 8: *RBR Concerto3 Conductivity, Temperature and Depth logger*

Deployment

The oxygen sensor should be two-point calibrated before each survey. If deploying by hand with a marked rope: wear gloves to avoid rope burn and make sure the rope is secured.

1. Ensure you are in the correct location, fill in your logbook
2. Confirm what depth you are deploying to.
3. Remove the Oxygen sensor lens cover.
4. If using the phone app, connect to the instrument and tag the location.
5. Turn the RBR endcap a quarter turn clockwise to Run, the instrument will vibrate. It is now recording. The oxygen sensor LED will flash. If it does not vibrate, it is not recording! It may need to be 'Enabled'.
6. Put the RBR into the water, hold it submerged at the surface for 1 minute. This ensures the instrument is the same temperature as the water.
7. Lower the RBR to the desired depth, do this smoothly but quickly. Aim for 0.5 meters per second.
8. Hold the RBR at the maximum depth for 20 seconds.
9. Bring the RBR back to the surface and hold it at the surface again for another 20 seconds.
10. Remove from the water, turn the twist collar a quarter turn anti-clockwise back to 11.

Download

Data can be downloaded over Wi-Fi during a deployment. Each new set of a data will be appended to the same .RSK file. This can be done with the phone app or PC. Do not Stop the logger unless you do not want to collect any more data. Upload your data to the RBR Dropbox as soon as possible.

Water Quality

Each parameter has its own method which are detailed below. The highly detailed SOPs are available on request.

Chlorophyll

A known volume of water is filtered through Glass Microfibre (GF/F) 47mm Filters, using a glass filtration system attached to a vacuum pump. The filters are then carefully folded and wrapped in foil before being stored. Samples are stored in a portable freezer at -20 °C (whilst on the vessel and during transit time) and then transferred to a -80 °C upon return to the lab. Samples are extracted in a known volume of acetone (90%) for a minimum of 18hrs overnight and then centrifuged prior to analysis using a Turner Trilogy

Fluorometer. Method is a modification of that described within The Determination of Chlorophyll a in Aquatic Environments (1980).

Cefas SOP Reference:

- SOP 2253 Fluorometric determination of chlorophyll and phaeopigments using a Turner10AU-005-CE fluorometer (needs updating to account for new instrument)

Dissolved Inorganic Nutrients

Approximately 60ml of water is filtered through Sartorius Minisart PES 0.45µm 2mm syringe filters into 60ml polycarbonate pots. Samples are stored in a portable freezer at -20 °C (whilst on the vessel and during transit time) and then transferred to a -20 °C freezer upon return to the lab. Analysis is performed using a Seal Analytical HR-AA3 Continuous Flow Analyser (CFA) for TOxN (TOxN = Total Oxidised Nitrogen = Nitrate + Nitrite), nitrite, silicate, phosphate, and ammonia.

Cefas SOP References:

- SOP2257 The preparation of reagents for the determination of dissolved inorganic nutrients using a Seal AA3 CFA
- SOP2258 The Simultaneous Determination of Inorganic Dissolved Nutrients using a Seal AA3 CFA

Nitrite

Nitrite reacts under acidic conditions with sulfanilamide to form a diazo compound which then couples with N-1-naphthylethylenediamine dihydrochloride (NEDD) to form a reddish-purple azo dye that is measured at 520 - 560 nm.

Method Reference:

- Seal Analytical Method - G173 R12 Nitrite MT18. Bendschneider, K, and Robinson, R.J. 1952. A new spectrophotometric method for the determination of nitrite in sea water. Journal of Marine Research, 1 (11): 87-96.

TOxN

Nitrate is reduced to nitrite at pH 7.5 in a copperized cadmium reduction coil. The nitrite is then measured as per the nitrite method.

Method References:

- Seal Analytical Method - G384 R6 NO2-NO3 MT19B
- Bendschneider, K, and Robinson, R.J. 1952. A new spectrophotometric method for the determination of nitrite in sea water. Journal of Marine Research, 1 (11): 87-96.

Silicate

The determination of soluble silicates is based on the reduction of silico-molybdate in acidic solution to molybdenum blue by ascorbic acid which is measured at 820 nm.

Method References:

- Seal Analytical Method - G177 R13 Silicate MT19
- Methods of Seawater Analysis, K. Grasshoff, M. Ehrhardt, K. Kremling, second revised and extended edition, 1983

Phosphate

The determination of soluble phosphate is based on the reaction with molybdate ion and antimony ion followed by reduction with ascorbic acid at a pH<1. The reduced blue phospho-molybdenum complex is measured at 880 nm.

Method References:

- Seal Analytical Method - G297 R7 Phosphate MT19
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31–36

Ammonia

The sample is reacted with o-phthalaldehyde (OPA) at 75°C in the presence of borate buffer and sodium sulfite to form a fluorescent species proportional to the ammonia concentration. The fluorescence is measured at 460 nm following excitation at 370 nm.

Method References:

- Seal Analytical Method - G32705 R6 Ammonia in seawater by fluorometry
- Kerouel, R, Aminot, A. Jul 1997 Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow. Marine Chemistry Vol. 57, no. 3-4, pp. 265-275. Jul 1997

Salinity

Water is collected in 125ml narrow mouthed glass bottles and sealed with a plastic insert and screw cap. Bottles are kept upright during storage and transport and no specialised storage is required. Analysis uses the Practical Salinity Scale 1978 and is based on measurements of conductivity referenced against IAPSO (International Association for the Physical Sciences of the Oceans) Standard Sea Water using a Guideline 8410A Portasal Salinometer.

Cefas SOP Reference:

- SOP 2266 Analysis of salinity samples using a Portasal™ Salinometer 8410A

Dissolved Oxygen

Samples are collected in stoppered glass bottles, then fixed by the addition of 2M manganous sulphate solution, followed by (8M sodium hydroxide, 4M sodium iodide) alkaline iodide solution. Samples inverted and stored under water in the dark. For analysis, samples are acidified with 5M sulphuric acid and titrated against 0.2M sodium thiosulphate using a SiS (Sensoren Instrumente Systeme GmbH) Dissolved Oxygen Analyser.

Cefas SOP Reference:

- SOP2262 The determination of dissolved oxygen using an SiS endpoint detector and the Winkler titration method

Method References:

- Carpenter, J.H., 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnology and Oceanography, 10, 141-143
- Winkler, L. W., 1888. Die Bestimmung des im Wasser gelosten Sauerstoffes. Ber. Dtsch. Chem. Ges., 21, 2843-2855

Suspended Particulate Matter

A known volume of water is filtered through pre-weighed Whatman cyclopore track etched 47mm 0.4µm membrane filters, using a glass filtration system attached to a vacuum pump. No specialised storage is required. The filter papers are weighed on an antistatic source (Polonium-210) after being dried in vacuum sealed desiccator over a period of week. The measurement is repeated until a stable weight is achieved and averaged over 3 measurements.

Cefas SOP Reference:

- SOP3037 The analysis of filter papers for particulate loading determination

Method Reference:

- Yeats, PA; Brugmann, L. (1990). Suspended particulate matter: collection methods for gravimetric and trace metal analysis. ICES Techniques in Marine Environmental Sciences, No. 7. 9 p.

Appendix 8. MBA CPR sample methodology

Further details regarding CPR historical developments and methods can be found in Reid et al., 2003. Prog in Oceanog 58. 117-173 and Richardson et al. 2006, Prog in Oceanog 68. 27-74. The following covers the methodology for the analysis of the collected samples, this methodology has remained unchanged since 1958.

Analysis of Samples

Before samples are analysed, each analyst must be at the correct level of competence (authorised by Senior Analyst), ensuring they have understood and able to comply with Health and Safety practices. It is already assumed that an induction programme, along with an initial but comprehensive training programme, has been followed successfully.

This section describes the stages of analysis:

1. Colour assessment
2. Phytoplankton examination
3. Zooplankton traverse examination
4. Zooplankton eye count examination

The stages are carried out in the above order. The 'cutter' carries out stage 1 before the rolls of silk are cut into samples. Stages 2, 3 and 4 are carried out by the whole analysis team; each member being given a random selection of the samples.

Basic Equipment for Standard CPR Analysis

- Large fume cupboard fitted with formalin filter, daylight bulbs and black out blinds for colour examination of silks
- CPR Analysis microscope – including light sources for microscope and eye count
- Dissecting microscopes (highest magnifications at least 100X)
- Acrylic hood for each microscope
- Stage micrometers
- Spare eyepieces with graticules for CPR and dissecting microscopes
- Stage with x and y movement housing glass plate (360mm ´ 180mm) and 'Eye count' mirror or light or both
- Fume extraction system serving the microscope hoods, fume cupboard and ventilation cupboards
- Scissors, forceps, mounted needles, wash bottles containing 4% formalin mix, PGP, water, waterproof labels for samples and permanent marker
- PPE

Colour Assessment

The assessment of Phytoplankton Colour, or the 'Green-ness Index', in the CPR Survey has remained unchanged since at least 1946. This is carried out on all analysed and non-analysed records. When the record is unrolled prior to being cut, the colour is compared with the colour of a set of specially prepared colour standards by laying the silk against a white background and using daylight bulbs and black out blinds. The colour estimates are related to the numbered divisions of the graduated silk and subsequently to the samples when the record has been cut. The colour categories are given in Table 1.

Category	**Recorded**
No colour	NIL (0)
Very pale green	VPG (1)
Pale green	PG (2)
Green	G (3)

Table 1: *Colour categories used for phytoplankton colour estimation*

The values are then recorded on the cutting point sheet and later transferred to the analysis sheet. A note is also kept of the cutter that undertook the colour assessment along with the date the cut took place.

Originally these numerical values were derived at by doing an acetone extraction for chlorophyll on a range of silks that had been assessed as having the four different colour categories. As a result of the acetone extractions, a 'colour card' was created that showed the different colour categories.

For new cutters to become trained in assessing Phytoplankton Colour on the silks, they are taught over a period of approximately one year to assess colour based on the four categories listed above. This process has continued since 1958, with successive cutters being trained 'by eye'. Initially while cutting silks, the new cutter merely observes an experienced cutter assessing the colour on the silks. Then the new cutter attempts their own assessments, but with an experienced cutter in close supervision. Finally, the new cutter becomes a 'fully qualified' colour assessor and can operate independently. Cutters use the colour card when in doubt and as a 'backup' for assessing colour levels.

To tie down the assessment of phytoplankton colour to a definite reproducible colour, levels of colour based on 'Pantone' colour cards were chosen (example in Figure 1), these are as follows:

Pantone Colour Tint Selectors numbers:
376U-378U
383U-385U
390U-392U
398U
399U
3985U
3995U

For each of these colour ranges, 10% tint = VPG, 30% tint = PG and 60% tint = G.

It must be checked that colour is due to phytoplankton colouration and not due to a high abundance of zooplankton organisms. There is a strong link between phytoplankton colour and the amount of plankton on the sample see: Dionysios E. Raitsos, Philip C. Reid, Samantha J. Lavender, Martin Edwards and Anthony J. Richardson. Extending the SeaWiFS chlorophyll data set back 50 years in the northeast Atlantic. Geophysical Research Letters, Vol. 32, March 2005.

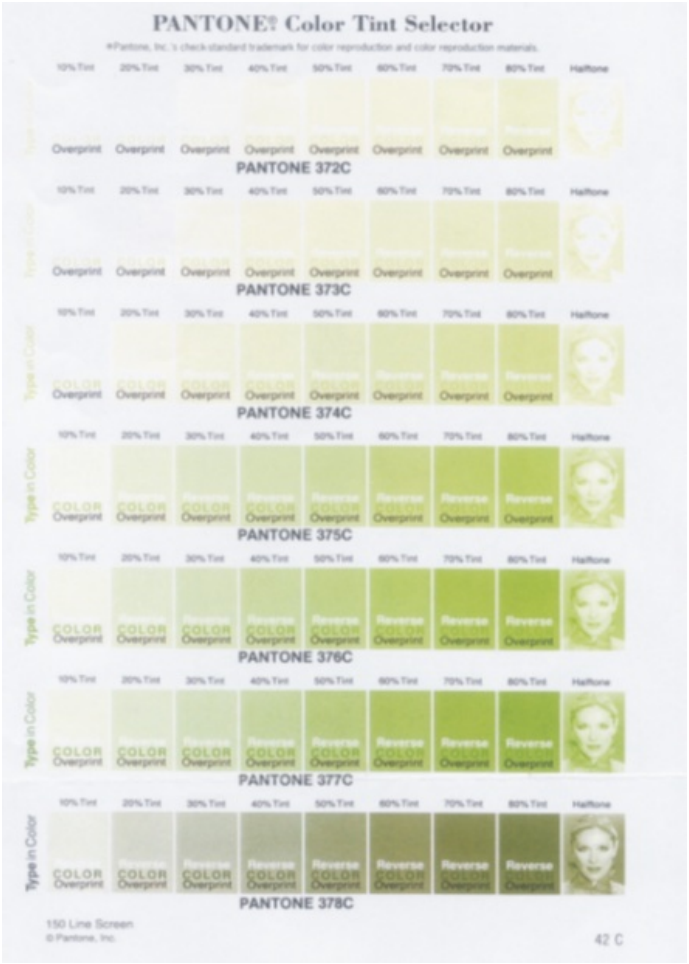


Figure 1: *Example of chosen Pantone Colour chart*

Recording of Results

Each analyst has an analysis notebook used to record results; once full, these notebooks are also kept for future reference. An example is given in Figure 2.

- The front cover of each analysis book must have clearly written in pen: the individual analyst's name, the start-date of the notebook and a notebook number (notebooks are numbered consecutively, for example: the first analysis notebook an analyst records their analysis data in would be Book 1).
- Analysis results are always written with a HB pencil. At the top left of the page record: the route name, tow number and sample number. At the top right of the page record: the analysis book page number (used for indexing), the number of the microscope the analysis was carried out on and the date the analysis was carried out.
- The three stages of the CPR plankton analysis - phytoplankton traverse, zooplankton traverse, and zooplankton eye count must be separated and clearly recognisable, i.e. either as 3 separate columns or rows.
- Quantities of taxa must be recorded using the five-bar tally system (four vertical lines cancelled diagonally or horizontally by a fifth line) or by writing down actual numbers (if using a hand counter). Written taxon names/entities and counts must be legible. Some taxa may be recorded in more than one taxonomic group, usually a 'total' grouping.
- Clearly record the relevant 'total' taxonomic grouping whenever a 'child' taxa is also recorded.

- Clearly write down any other notes or observations relevant to:
    - The overall condition of the sample (this information is used to produce the preservation data, e.g. sample poorly preserved, oil on sample, sample dried up etc). The condition of a specific taxon or group of taxa e.g. Chaetognaths all clumped together so difficult to count, Copepods eggs = 28 but 19 in one clump and copepods in poor condition, etc.
    - If the presence of unusual taxa e.g. if a rare taxon is observed or if a taxon is found in an unusual geographic region, seek verification from the on duty Senior Analyst and record the verification. If a taxon has been identified for the first time, the reference used for its identification must also be recorded.
- When the analysis is complete enter the results onto the CPR Console programme. Once the results are entered onto the CPR Console, the data entry is cross-checked against the notebook entry with another analyst or suitably qualified person. Record in the analysis notebook the initials of the person who cross-checked the data entry of the analysis results and the date the sample was checked and finalised.

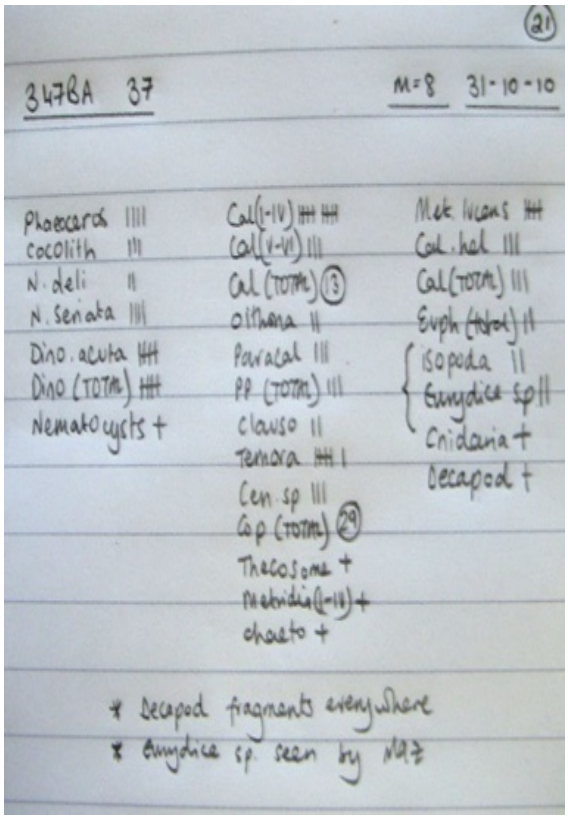


Figure 2: *Example of sample write up in analysis notebook*

Phytoplankton Count

Microscope

Using the CPR microscope, giving a field diameter of 0.295mm (with a permissible variation of plus or minus 0.01mm) using ~x500 magnification.

Procedure

The covering and graduated (filtering) silk sample is opened out (plankton uppermost) and laid out on a polythene sheet (16.51cm x 22.86cm), placed on the clean glass plate (that has been sprayed with a little

water) of the microscope stage (Figure 3). PGP solution can be used to moisten the sample while examined under the microscope.

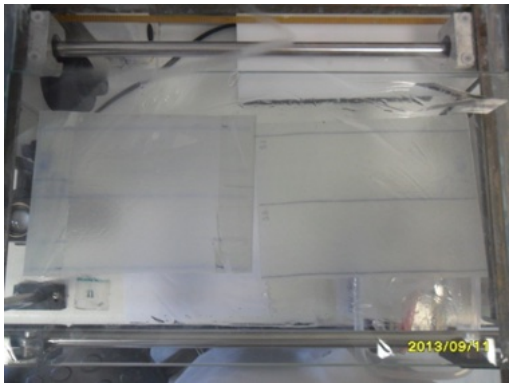


Figure 3: *A sample laid out on the microscope stage*

Of the filtering area of the filtering silk only, examine 20 fields under CPR microscope (Figure 4).

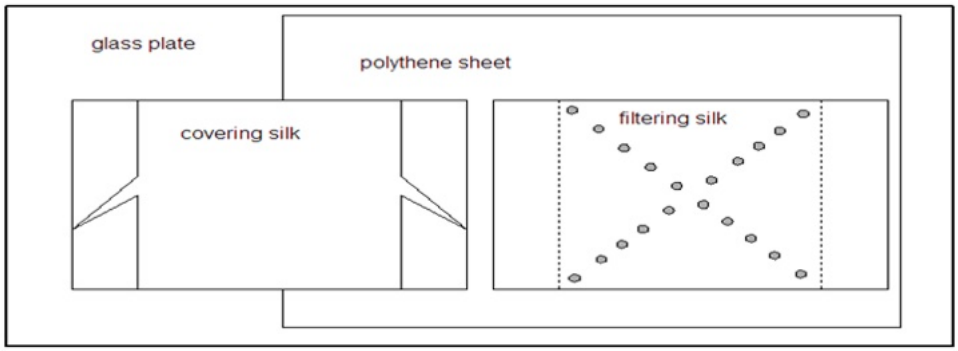


Figure 4: *Layout of silk sample showing the 20 phytoplankton fields*

How to count:

- To count an organism, its counting point should be within the field of view (see Table 2 for counting points).
- For each field of view, record (only once), each taxon seen. We record the presence of each species only. For example, if there are 3 whole *Thalassiosira* seen in one field of view, *Thalassiosira* are recorded in notebook as 1 not 3.
- Some organisms may be recorded in more than one taxonomic group (e.g. *Dinophysis tripos* would be recorded as such but also in *Dinophysis spp.* Total).
- For each field centre on a single mesh. If the field is blocked by a large piece of zooplankton move to the next mesh and if still blocked repeat once more (3x in total). If still blocked, count as one of the 20 fields, and move on.
- When the phytoplankton analysis is completed the maximum count for any taxon must be ≤ 20.
- Taxa can also be recorded as a '+' rather than an actual number. This is used when Phytoplankton cells are recognisable but incomplete, broken or with counting point outside field of view.
- At this stage you may also see non-phytoplankton taxa, that may not be seen during the traverse or eye count stages. If this is the case, make a + in your notebook to remind yourself.

- Record results legibly and logically in Analysis notebook.

Organism	Counting point
Elongate Diatoms e.g. *Rhizosolenia/Proboscia*	The end of the cell
Other single celled Diatoms	The body of the cell
Diatom chains	A cell of the chain
All Dinoflagellates	The part of the cell containing the girdle
All other phytoplankton e.g. *Pterosperma*, Silicoflagellates and Coccolithophores	If more than 50% of the cell is in view
Phaeocystis	Presence only recorded as '+'
Other non-phytoplankton organisms recorded in Phytoplankton Traverse e.g. 'Nematocyst phytoplankton field count'	If more than 50% of the organism/ cell is in view

Table 2. *Phytoplankton Counting Points*

Therefore, if the below field of view (Figure 5) was observed during one of the phytoplankton counts, the taxa shown in the table (Figure 5) below would be recorded as:

Phytoplankton field of view	Table of how each taxa is recorded
	**Taxonomic group** **Recorded as** a. Chain of *Chaetoceros* (Phaeoceros) 1 b. Chain of *Thalassiosira* 1 c. *Thalassiothrix longissima* (broken cell with tip outside field of view) + d. Chain of *Nitzschia seriata* 1 e. *Rhizosolenia hebetata semispina* 1 f. *Rhizosolenia imbricata* 1 g. *Rhizosolenia styliformis* (tip outside field of view) + h. *Asteromphalus* 1

Figure 5: *Example of sample count*

Initial Recording of Phytoplankton Examination

The results of phytoplankton analysis are first recorded in each individual analyst's notebook.

The phytoplankton sub-sample represents 1/10, 000th of the silk when the section or sample is 10.16 cm (4') long.

Zooplankton Traverse Examination

Microscope

Using the CPR microscope, giving a field diameter of 2.06mm (with a permissible variation of plus or minus 0.05mm) using ~x60 magnification.

Procedure

Examine a stepped track across filtering and covering silk under CPR microscope.

Identify, count, and record each taxon/organism seen in each traverse step.

Some organisms may be recorded in more than one taxonomic group (e.g. *Calanus* CI-IV would be recorded as such but also in Calanus Total traverse and Total Copepods).

To count an organism more than 50% must be present and its counting point must be within the field of view:

- Count *Halosphaera* cells provided at least 50% is in field of view
- Small pieces (<50%) of traverse zooplankton that are recognisable or that have the counting point outside of the field record as '+'
- Phytoplankton not recorded previously must be recorded as a '+'
- Note eye count zooplankton taxa as a reminder for eye count analysis

For the taxa counted in this stage of analysis see the Taxon List (Table 2). Record results legibly and logically in Analysis notebook.

Traverse Zooplankton Identification Points

The organisms are counted during the traverse only if the parts shown in Table 3 appear in the field of the microscope.

Organism	Identification Point
All Crustacea	Base of the antenna
Appendicularia	Body mass
Chaetognatha	The head
Cyphonautes larvae	Apex
Echinoderm larvae	Dorsal Apex
Bivalvia larvae	Hinge of shell
Thecosomata	Apex of shell
All other zooplankton organism	If more than 50% of view

Table 3: *Traverse zooplankton identification points*

Initial recording of Zooplankton Traverse Examination

The results of the zooplankton traverse analysis are first recorded in each individual analyst's notebook.

The traverse sub-sample is only 1/50th of the total area of the silk when the section or sample is 10.16cm (4') long.

Zooplankton Eye count Examination

Microscope

Normally use CPR and Stereo microscopes.

Procedure

Remove organisms over 2mm in size from the filtering and covering silks and place in a Petri dish/Bogorov tray containing PGP.

Identify and count each eye count taxon/organism that is whole or incomplete provided the greater part of the organism is present.

- Record as '+' Cnidaria tissue (if nematocysts recorded previously then Cnidaria must be '+')
- Record as '+' Tunicates, though Salps and Doliolids are counted
- Record as '+' small pieces of eye count zooplankton that are recognisable
- Record as '+' any zooplankton traverse or phytoplankton traverse taxa not previously recorded

For high numbers of eye count organisms, sub-sample by taking a proportion of the organisms from the silk or remove all organisms and use a Motoda box splitter or Fulsom splitter (Make sure a comment is noted for this in CPR Console). Of course, if a proportion is taken, it must be ensured that each number of taxa is multiplied accordingly to get the same result as if the whole sample was counted.

Record results legibly and logically in Analysis notebook.

During analysis, analysts must make a note (to be added as a comment in CPR Console) of anything that is unusual or of importance. For example, poor preservation of the plankton, or confirmation by a Senior Analyst for unusual taxa (both rare taxa and occurrence in any area outside usual distribution) etc. There is a comprehensive list of regular comments used in Appendix 6.

Zooplankton Eye count Taxa Identified

All countable organisms are identified down to the taxonomic level shown in the following lists: Organisms not on the lists should be identified, to species if possible or to genus.

Initial Recording of Zooplankton Eye Count Examination

The results of the zooplankton eye count analysis are first recorded in each individual analyst's notebook.

The numbers of organisms counted during the eye-count examinations can be estimated if they are present in large quantities, however actual numbers are entered into CPR Console.

Again, if subsampling make a note of this in CPR Console as a comment.

Metadata for New Taxa

The CPR taxa list can be continually added to (as new areas are sampled and new taxa appear). In addition, taxa identified to a low taxonomic resolution can, depending on scientific requirements, be identified to a higher resolution.

It is essential to keep accurate metadata on these changes, including the date of such change. Taxon names may also change due to new work by external taxonomists.

Identification of Taxon New to the survey

If a new taxon is created within the survey (this could be brought about by analysis occurring in a new region or the introduction of a new species to a previously sampled area), a Senior Analyst will confirm identification, with an external expert if necessary, and decide how the taxon will be recorded – i.e. phytoplankton, zooplankton traverse or zooplankton eye count. The Senior Analyst will also decide if the new taxon will form part of a larger group (a 'child' of a larger group, e.g. copepod to be included in Total Traverse Copepods). A suitable authority for the new taxon will be identified. The counting point for the new taxon will be determined based on other similar organisms. A date of first recording will be assigned and from the time decided, all analysts will record the new taxon as routine. A training session will be given to the Analysis team to ensure all are familiar with the identification of the new taxon.

Changes to Resolution of Taxa

Several taxonomic entities are recorded to a coarse taxonomic level (for example, all decapoda larvae are simply recorded as Decapoda Larvae, regardless of the genera / species). According to new scientific research priorities or contractual obligations, coarse taxonomic groups can be further resolved if required. All staff must be trained to identify the organisms to the new taxonomic resolution. The start date for the new level of identification is noted. The Senior Analyst will discuss the changes with the Database Manager to ensure that the new taxa are still recorded as part of the previous coarse taxonomic group, allowing a continuation of the time series.

An example: prior to 2004, all *Dinophysis* taxa were recorded only as *Dinophysis spp* Total. It was decided in 2003 that *Dinophysis* could be identified to species level where possible, and should be, due to their links to Harmful Algal Blooms. Staff were trained in *Dinophysis* species identification, and, from January 2004 several *Dinophysis* species were recorded. As well as the new taxa being identified, the old group of *Dinophysis spp.* Total was continued (i.e. all *Dinophysis* were also added to this group).

Change of Taxon Name

The Senior Analyst will routinely check taxonomic nomenclature of the CPR Species list. Any definite changes (that is, changes that are agreed by the taxonomic community as a whole) will be noted and, if necessary, the CPR taxon name will be changed to reflect the new name. A note of the date of change will be kept, and the Database Manager informed.

Labelling, Wrapping and Storing Samples

- Return all plankton to filtering silk
- Place covering silk back over filtering silk
- Fold sample in half and centre on plastic sheet
- Complete waterproof paper label (using a soft pencil (B) or Indian ink pen) with Sample ID and Analyst number
- Add label to top of silk

- Treat sample with 4% formalin
- Wrap sample in the appropriate manner
- Store in appropriate box – each box contains the samples of several consecutive records with the same route letter
- Refer to Figure 6

NB - Each analyst is responsible for the labelling, folding, and boxing in the correction position of all the samples that he or she has analysed. The method of labelling and folding is shown below.

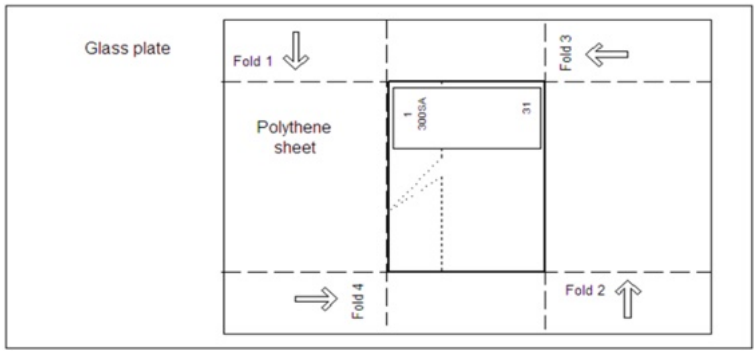


Figure 6: *Labelling and wrapping a CPR sample*

Storage of Samples and their Preservation

Storage of Samples

- All samples (analysed and non-analysed) are stored in a special archive. Samples are wrapped up in a 22.86cm x 16.51cm (9in x 6.5in) piece of polythene film, together with a note of the route letter and number and sample number. All the samples in a record are arranged consecutively in a plastic box. Each box contains the samples of several consecutive records with the same route letter.
- Each analyst is responsible for the labelling, folding, and boxing in the correction position of all the samples that he or she has analysed. The method of labelling and folding is shown above.

Getting Ready for Long Term Storage

- Check that the sample box is ready for transfer into long-term storage by consulting the check block progress folder. A tow is only ready for transfer into long-term storage when it has undergone both the check block and finalising procedures and has thus been signed off, or unless otherwise indicated by the Senior Analyst or Laboratory Manager.
- NB: there is usually more than one tow in a sample box. Analysts must make sure that all tows in the sample box have undergone the check block and finalising procedures before transfer into long term storage.
- Take the sample box ready for long-term storage and place under suitable fume hood extraction.
- Methodically check through each of the samples in the box: check that the route name and tow numbers match the label on the outside of the box; check that the samples are in the correct numerical sequence; check that the total number of samples for each tow is correct; check for absent or duplicate samples.
- If duplicate samples are found you must unwrap the sample, identify and correct the problem. If needed, the cutting point file should be consulted, together with a member of the cutting team.

- Amend the sample label and, if needed, the silk label appropriately. Inform the Senior Analyst and Senior Cutter if the silk has been incorrectly labelled.
- If a sample is absent, find the relevant cutting point file and check to see if the sample has been discarded. Missing samples may have been temporarily removed from the sample box for further study, outside of the normal plankton analysis procedure and put into a separate specialist sample box. For example, samples may be used for: training purposes; young fish collection, Cnidaria collection etc. Before recording a sample as absent you must check through the specialist sample boxes as indicated above.
- Record on a waterproof paper label: if the tow has a 'start' sample; the first sample number of the tow; the last sample number of the tow; if there is an 'end' silk sample and/or any absent samples and place on top of the samples in the sample box. Inform the Senior Analyst or Laboratory Manager of any missing samples. Record the routes checked and any missing samples and enter into the 'archive' database.
- To maximize storage capacity, the samples should be placed in two rows (if the samples are exceptionally wide this may not be possible). The maximum number of samples in a sample box is currently approximately 170. Check that there is sufficient space in the sample box for handling.
- Remove or add extra tows as appropriate to make the best use of space. Check the sample box and lid for cracks, replace if necessary.
- Squirt approximately 10-20ml of 4% Steedman's solution over the samples.
- Cover the samples with a thick black plastic sheet and secure the lid. Make sure that the sample box has a SAHFOS formaldehyde health and safety sticker on the lid and on the box itself. Remove any stickers not concerning the laboratory from the outside of the box.
- Make sure the name of the route and tow numbers are written in permanent marker on the front and side of the box and are legible. The route name should be top central, with the tow numbers along the bottom. Each tow number should be separated by a comma and the final tow should followed by two back slashes.
- Each box number must also have a unique serial number added at this stage. The number is taken from the Formalin database by the Curator and is the next sequential number. This also denotes its placement on the storage racks.

Fixation and preservation of plankton

All the chemicals which are used for the CPR Survey require mixing from the stock ingredients. In general, the recommendations of UNESCO/SCOR/WG23 are followed. The following mixtures are used in the preservation/ processing of the CPR plankton samples:

- 40% Formaldehyde (100% Formalin)
- Steedman's solution 'Concentrate' approx. 13% Formaldehyde (32% Formalin)
- 4% Formaldehyde (10% Formalin)
- PGP mix

IMPORTANT: READ HEALTH AND SAFETY NOTES BEFORE WORKING WITH THESE CHEMICALS

<u>40% Formaldehyde</u>

This solution is used to fill the storage tank of the internal mechanism of the CPR before deployment

and as a stock to mix 13% and subsequently 4%.

Chemicals required:

- 30 grams of Sodium Tetraborate (Borax)
- 1000ml of 37-41% Formaldehyde solution

Method:

- Dissolve the Borax powder into the Formaldehyde through agitation to allow the powder to dissolve.
- Store in suitable plastic container in a cool place.
- The Borax acts as a buffer maintaining the pH at around 7.

13% Formaldehyde Steedmans Solution

A concentrate solution which can be further diluted to make 4% Formalin.

Chemicals required:

- 1200ml of 37-41% Formaldehyde solution
- 250g of Sodium Tetraborate (Borax) powder
- 240ml Propylene Phenoxytol
- 2400ml of Propylene Glycol

Method:

- Add the Propylene Phenoxytol to the Propylene Glycol and stir well.
- Dissolve the Sodium Tetraborate in the 37-41% Formaldehyde and add this to the Propylene Phenoxytol and Propylene Glycol mix.
- Store in a suitable plastic container at room temperature.

4% Formalin mix

Used in the following ways:

- For applying to the silk in the storage tank of the internal mechanism of the towed CPR
- To moisten the lint surrounding the silk spool when removed from the internal mechanism of the towed CPR
- To moisten the silks at cutting and distribution
- To apply to the samples before storage

Method:

- Take 1 litre of the 13% Formalin mixture (concentrate) and add 2 litres of tap water and mix well.
- Store in suitable plastic containers at room temperature.

PGP Mix

Used to moisten the samples while cutting or undergoing analysis.

Chemicals required:

- 150ml Propylene Phenoxytol
- 1500ml Propylene Glycol
- 6350ml tap water (2000ml hot water +4350ml cold water

Method:

- Dissolve the Propylene Phenoxytol in the Propylene Glycol. Add the Propylene solution to the water and mix well.
- Store in suitable plastic container at room temperature. Mixture can become cloudy and separate if it gets cold. To remedy agitate mixture and warm slightly under a hot tap.

Propylene Phenoxytol is a very powerful bactericide and Propylene Glycol a powerful fungicide.

Propylene Phenoxytol dissolves easily in Propylene Glycol. Both these chemicals increase humectants, freezing point depression, stability, clarity, and specimen flexibility effects of the solution.

References:

- Lincoln, R., Sheals, J. 1979. Invertebrate animals: collection and preservation. British Museum (Natural History) Cambridge University Press. Pages 127, 135-136
- Omiri,M., Ikeda, T. 1984. Methods in marine zooplankton ecology. John Wiley and Sons, Chichester. Pages 59, 61-62.
- Steedman, H.F. (Ed) 1976. Zooplankton fixation and preservation. Unesco, Paris

Quality Control of Analysis

When all the analysis data for a tow has been entered by all analysts onto Console, quality checks can commence. The checks are carried out by the more experienced analysts. The decision to issue check blocks is influenced by factors such as phytoplankton colour, night/day sample, condition of plankton, comments (e.g. confirmed by …, etc), analyst experience and individual strengths and weaknesses as well as the scenarios described below.

General route and sample information checks:

- Number of samples analysed is correct
- Each sample has a valid analyst id number (i.e. not analyst 99)
- Each sample has a microscope number
- Each sample has been assigned a phytoplankton colour value
- Sequence of day/night values is appropriate for latitude and time of year of tow

Phytoplankton Checks:

- Look for unusual taxa: warm water species identified on a cold-water route and vice versa; oceanic species found on a coastal sample and vice versa, other geographic anomalies (e.g. *Neodenticula seminae* found in the East Atlantic, *Coscinodiscus wailesii* found in the West Atlantic). Check block triggered for counts of presence and above.
- Incorrect taxa selected during data entry e.g. *Corethron* Antarctic in the North Sea. Check block triggered for counts of presence and above.
- *Phaeocystis* presence: check blocks triggered on samples (who have not recorded *Phaeocystis*) adjacent to those with positive presence of *Phaeocystis*. If presence is recorded on only one sample on the entire route, then a check block will also be issued to that sample.
- Anomalous counts: anomalously high counts of taxa amongst general low counts of taxa and vice versa. In particular see details below:

- Possible undercounts of easily overlooked taxa, for example *Pseudo-nitzschia delicatissima*, Coccolithophores (*Emiliania huxleyi* type). Check block triggered for absence or low counts on samples adjacent to samples with counts over 4.
- Possible over counts of taxa counted incorrectly (not used counting point). For example, *Thalassiothrix longissima*. Check block triggered on samples recording counts of over 4 adjacent to samples with absence or low counts of taxa.
- Mis-identified taxa: alternating sequence of taxa easily confused. For example, *Rhizosolenia spp., Ceratium spp., Dinophysis spp.*
- Total groupings: taxa whose counts also need to be recorded in a 'total'. For example, *Dinophysis spp., Prorocentrum spp., Coccolithaceae, Pterosperma spp.* etc. In particular presence or '+' may also need to be recorded under more than one taxon name. E.g. *Dinophysis acuta* +, also needs to be recorded as *Dinophysis* spp. Total +.

Zooplankton Traverse Checks:

- Look for unusual taxa: warm water species identified on a cold-water route and vice versa; coastal species found on an oceanic sample (e.g. *Pseudocalanus* found in the sub-tropical mid North Atlantic) and vice versa, and other geographic anomalies. Check block triggered for counts of presence and above.
- Incorrect taxa selected during data entry e.g. *Acartia* spp. Antarctic in the North Sea. Check block triggered for counts of presence and above.
- Anomalous counts: anomalously high counts of taxa amongst general low counts of taxa and vice versa. In particular see details below:
  - Possible undercounts of easily overlooked taxa, for example Appendicularia, Tintinnids, *Penilia* and copepod nauplii. Check block triggered for absence or low counts on samples adjacent to samples with categories over 4.
  - Possible over counts of taxa counted incorrectly (not used counting point). For example, Echinoderm larvae. Check block triggered on samples recording categories of over 4, adjacent to samples with absence or low counts of taxa.
  - Mis-identified taxa: alternating sequence of taxa easily confused. For example, Para-*Pseudocalanus spp*. /other small copepods. *Isias clavipes/Centropages spp*., Acantharia/ Echinoderm larvae.
  - Total groupings: taxa whose counts also need to be recorded in a total, for example: Calanus total traverse, *Metridia* total traverse, Total Copepods, Radiolaria total etc. Also check total entries, in particular for '+''s, for non-routine taxon entries. For example taxa associated with: Cirripede larvae, *Tintinnida* and *Foraminifera spp.,* etc.

Zooplankton Eye count Checks:

- Look for unusual taxa: cold water species identified on a warm water route and vice versa (e.g. *Heterorhabdus norvegicus* found in the sub-tropical mid North Atlantic); coastal species found on an oceanic sample (e.g. *Paraeuchaeta hebes* found in the sub-tropical mid North Atlantic) and vice versa, and other geographic anomalies (e.g. *Labidocera wollastoni* found in the West Atlantic).
- Check block triggered for counts of presence and above.
- Incorrect taxa selected during data entry (e.g. *Paraeuchaeta norvegica* in the Pacific). Check block triggered for counts of presence and above.

- Anomalous counts: anomalously high counts of taxa amongst general low counts of taxa and vice versa. In particular see details below:
    - Possible undercounts of easily overlooked taxa, for example *Nannocalanus minor*, Echinoderm post larvae, *Lepas nauplii, Atlanta spp.* and Ostracods. Check block triggered for absence or low counts on samples adjacent to samples with counts over 4 (sometimes below 4 depending on the taxa).
    - Possible over counts of taxa counted incorrectly, for example Chaetognath eye count (must be ≥ 8mm), overestimating copepod abundance from subsample on samples rich in zooplankton. Check block triggered on samples recording categories of over 4 and adjacent to samples with a category difference of 2 or more.
    - Mis-identified taxa: alternating sequence of taxa easily confused. For example, *Branciostoma lanceolatum*/fish larvae, Thaliacea/Cnidaria, *Centropages bradyi*/violaceus, decapoda/ euphausiid furcilia, *Calanus finmarchicus/helgolandicus* etc.
    - Total groupings: taxa whose counts also need to be recorded in a total, for example: Decapoda, Sergestidae, Cnidaria, Calanus V-VI Atlantic, Fish larvae etc. Also check total entries, in particular for +', for non-routine taxon entries. For example taxa associated with: Salpidae, Decapoda, Hyperiidea etc.
    - Taxa recorded/counted incorrectly: Siphonophora and Thaliacea do not have abundance data and recorded as presence only.

Cross Counting Category Checks:

- Mis-identified taxa: for example tintinnid cyst (zoo traverse) mis-identified and counted as dinoflagellate cyst (phyto), Calanus I-IV (zoo traverse) mis-identified and counted as Calanus V-VI Atlantic (zoo eye count).
- Inconsistencies between zooplankton traverse and eye-count: for example if a count is made for *Metridia* total traverse, without a count for *Metridia* I-IV, then there must be an appropriate abundance for a *Metridia spp.* in eye count. This also applies to *Calanus* total traverse and *Calanus* V-VI in eye-count.

Comments Check:

- Comment is situated next to appropriate taxon, or general sample comment
- Comments which can be removed and recorded under a taxon name as non-routine analysis. For example, Hydroids, Nematocysts, Filamentous algae, and many species names now have a CPR number. Check non-routine analysis sheet for details.

Checks via Console:

- Checks for a particular sample are not carried out by the original analyst but by another on the team.
- An analysis check issued through Console enables a record to be kept.

Using Console to issue check block and amend results:

- The tow for which checks are to be issued or data edited is selected from the Console 'Inbox' and 'Check Blocks' selected from the 'Management' drop down menu.
- The relevant sample/plankton id box is chosen, the query entered (e.g. 'Any Thalassiosira spp.?') and the check analyst selected from the drop-down menu.

- As each check block is issued the relevant Sample/Plankton id box turns yellow, and the checking analyst will receive an electronic copy of the check request.
- If a check is reissued, then the Sample/Plankton id box turns red.
- When an analyst replies with their check result the Sample/Plankton id box turns blue.
- The issuer of the checks can, by selecting the relevant Sample/Plankton id box, see the result.
- If necessary, the original analysis entry can be edited by double clicking on the relevant sample to open up an 'Analysis Result Entry' form.
- Once the correct analysis result is entered the check block result 'error code' can be selected and the Sample/Plankton id box will turn dark green.

Error Codes:

- There are currently 6 error codes: No Change, Acceptable Error, Misidentified, Error With Count, Miscount and Misidentified, Data Entry Error, Could not be Confirmed, Error – Not Seen in Original Analysis.
- The error codes are assigned for several reasons:
    - To provide data as part of improving our quality assurance.
    - To provide Analysts with feedback.
    - To provide trainers with information regarding training requirements.
    - To assess progress by Analysts and Trainers.

Phytoplankton Taxa error code examples:

- No change: Count the same.
- Acceptable error: Where count is within 3 fields of original count.
- Misidentified: Clearly a mistaken id e.g. *Scrippsiella /Protoperidinium*. For 'difficult' taxa may be recorded as Acceptable error.
- Error with count: For fields where phytoplankton is counted a difference of more than 3 or 4 fields observed (may be greater if a densely covered block or many broken cells).
- Miscount and Misidentified: If it is apparent the analyst has misidentified and miscounted (e.g. *Proboscia indica* mistaken for *P. alata* and counts are more than 3 or 4 different).
- Data entry error: When Analyst has recorded in book but not cross-checked data in Console.
- Could not be confirmed: When (usually singular) taxa cannot be found on reanalysis.
- Error not seen in original analysis: for those occasions when taxa not originally recorded are seen in sufficient numbers to indicate they should have been seen. Would also apply to *Phaeocystis* if covering sample.

Zooplankton Traverse Taxa error code examples:

- No change: Count the same.
- Acceptable error: A reasonable difference from the original. Depends on the taxon e.g. copepod eggs a difference of 15 is acceptable as the eggs can clump. A difference of 5 or 6 in counts would be acceptable. For Nematocysts, a few small clumps would be acceptable. If the plankton is in poor condition, then counts are likely to be more variable.

- Misidentified: Clearly a mistaken id. This may be indicated by a reasonable count (5 or more of an organism not recorded by another analyst on that tow). If none of that organism can be found and 3 or more recorded on the sample when checked.
- Error with count: A difference of more than 5 or 6 for copepods, more for copepod eggs.
- Miscount and Misidentified: Where counts are very different compared with the original for more than one taxa (e.g. original count *C. typicus* 17, *C. hamatus* 0 on checking *C. typicus* 9, *C. hamatus* 10).
- Data entry error: When Analyst has recorded in book but not cross-checked data in Console.
- Could not be confirmed: When (usually singular) taxa cannot be found on reanalysis.
- Error not seen in original analysis: For taxa that should have been seen and counted.

Zooplankton Eye count Taxa error code examples:

- No change: Count the same.
- Acceptable error: Could be used if there are high counts and a sub sample taken. Analysts should state if sub sampled.
- Misidentified: Clearly a mistaken id (e.g. *Neocalanus gracilis* mistaken for *Calanus helgolandicus*).
- Error with count: For samples with small amounts of eye count plankton the counts should match. For samples with high number of zooplankton a difference of 1 or 2 may be acceptable and for sub-samples the counts should be within reasonable bounds.
- Miscount and Misidentified: Where counts are very different compared with the original for more than one taxa (e.g. *Pleuromamma borealis* 30, *P. gracilis* 5, *P. piseki* 0 - on checking *P. borealis* 10, *P. gracilis* 20, *P. piseki* 15).
- Data entry error: When analyst has recorded in book but not cross-checked data in Console.
- Could not be confirmed: When (usually singular) taxa cannot be found on reanalysis.
- Error not seen in original analysis: For taxa that should have been seen and counted or '+' if material plentiful e.g. large pieces of Cnidarian, nectophores of Siphonophora.

Appendix 9. EA example results and embedded spreadsheets

Important: These are provisional and should not be used for decision making purposes

1. Example of returned Zooplankton Return sheet form MBA to EA:

Environment Agency 2022						
Pot Number			11	12	17	18
Numbers per sample/pot	sample date		02/08/2022 Fawley	02/08/2022 Fawley	02/08/2022 Fawley	02/08/2022 Fawley
	station name/WIMS code		SOU001N	SOU001N	SOU001N	SOU001N
	Analyst Initial		JB	SH	SH	JB
	Sample Depth m		10-30m	10-30m	10-30m	10-30m
	Time of sampling (GMT)?					
	Is a replicate?		Rep A	Rep B	Rep A	Rep B
	any other comments on sample label		PRN 21204073	PRN 21204073	PRN 21204073	PRN 21204073
	Sample comments		200um mesh Detritus present	200um mesh	100um mesh	100um mesh Algae on sample, sediment everywhere
	Taxa	Aphia ID				
Copepods- Calanoid	Acartia clausi	149755	4	5	100	250
	Acartia discaudata	234125	72	18	100	1500
	Acartia margalefi	346039	4			
	Acartia spp. unident	104108	344	83	1000	1250
	Anomalocera patersonii	364368				
	Calanus i-iv	104152				
	Calanus finmarchicus	104464				
	Calanus helgolandicus	104466				
	Calanus V-VI unident	104152				
	Candacia armata	104474				
	Candacia spp.	104157				
	Centropages spp. (unident)	104159				
	Centropages hamatus	104496	20	26	1	
	Centropages typicus	104499				
	Diaixis spp. (unident)	104167				
	Clausocalanus	104161				
	Ctenocalanus vanus	104510				
	Isias clavipes	104501				
	Labidocera wollastoni	104736				
	Metridia lucens	850801				
	Microcalanus spp. (unident)	104164				
	Paracalanus spp. (undeint)	104196				
	Paramisophria cluthae	104359				
	Parapontella brevicornis	104686				
	Pseudocalanus spp. (unident)	104165				
	P-P small unident calanoid	1100	48		100	

SOU001N Fawley Aug 22 | SOL002N E Brambles Aug 22 | SOL005N Ryde Aug 22 | SOL001N Cowes Aug

1 tab per site/date, replicates taken in this example

Results Aug 22 per station.xlsx

2. Example of collated Water Quality data for QA and initial analysis:

SAMP_SMPT_USER_REFERENCE	REGION	IP_AREA_C	BIOSYS ID	SMPT_LONG_NAM	SMPT_TYP	SMPT_GRI	SAMP_ID	SAMP_LAE	SAMP_COI	SAMP_SCI	SAMP_SCI	P_NAME	SAMP_SAMPLE_DATE	SAMP_SAT	SAMP_MA	SAMP_PUI	MEAS_AN	MEAS_DE	DETE_DESC	Status
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	37	9993	Ammoniacal Nitrogen, Filtered as N	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	21	7887	Chlorophyll : Acetone Extract	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	6019	NGR : Easting	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	6020	NGR : Northing	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	25	9853	Nitrate, Filtered as N	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	37	6485	Nitrite, Filtered as N	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	25	4925	Nitrogen, Dissolved Inorganic : as N	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	37	9943	Nitrogen, Total Oxidised, Filtered as N	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	37	9856	Orthophosphate, Filtered as P	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	25	9924	Oxygen, Dissolved as O2	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	9901	Oxygen, Dissolved, % Saturation	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	21	4574	Phytoplankton	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	7608	Salinity : In Situ	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	6	Sample Depth below surface	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	37	9857	Silicate, Filtered as SiO2	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	76	Temperature of Water	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	4	Time of high tide	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	7342	Time of sampling relative to previous high wate	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	3976	Turbidity : In Situ	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	4865	Volume of Sample Filtered	
88007172	NWest	3	LIV003N	COASTAL SURVEY	CD	SJ2164495	5324758	467721		1	21194658	RICHARD PRITCHARD	01/07/2022	1350	2IZZ	MS	26	3428	Water Depth	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	37	9993	Ammoniacal Nitrogen, Filtered as N	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	21	7887	Chlorophyll : Acetone Extract	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	6019	NGR : Easting	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	6020	NGR : Northing	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	25	9853	Nitrate, Filtered as N	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	37	6485	Nitrite, Filtered as N	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	25	4925	Nitrogen, Dissolved Inorganic : as N	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	37	9943	Nitrogen, Total Oxidised, Filtered as N	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	37	9856	Orthophosphate, Filtered as P	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	25	9924	Oxygen, Dissolved as O2	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	9901	Oxygen, Dissolved, % Saturation	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	21	4574	Phytoplankton	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	7608	Salinity : In Situ	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	6	Sample Depth below surface	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	37	9857	Silicate, Filtered as SiO2	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	76	Temperature of Water	
88002837	NWest	3	LIV002N	MERSEY ESTUARY	CD	SJ2961898	5324754	467647		1	21194634	RICHARD PRITCHARD	03/07/2022	909	2IZZ	MS	26	4	Time of high tide	

Data collected at the same time as Zooplankton and Phytoplankton data, but store on different EA database and warehouse.

Zooplankton
WIMS-MIDAS Extract

3. Example of combining Zooplankton and Water Quality data:

Pot Number	sample.c	station.n	Analyst.I	Sample.E	Net.volur	Time of s	is a repli	any.othe	PRN	Sample.c	CEA Note	date	sea	site	Tima bai	Tortanus	Acartia b	Lovenelli	Hippocar	Doliolidi	Syngnath	Paracala	Lovenelli	Lovenelli	Ammoni	Chloroph	NGR : Eas	NGR : No	Nitrate, F	Nitrite, F	Nitrogen	Nitrogen	Orthophc	Oxygen, [	Oxyge
54	03/08/20	SYK003N	JB	10-30m	1.00531	NA	Rep B	PRN 2120	2.1E+07	200um	NA	03/08/2022	S North S	Lincs Coa	0	0	0	0	0	0	0	0	0	0	<0.007	4	544495	405528	0.137	0.0035	0.147	0.34	0.012	8.76	11
55	05/08/20	SYK006N	SH	10-30m	1.25664	NA	Rep A	PRN 2120	2.1E+07	200um	NA	05/08/2022	N North S	Yorkshire	0.79577	0	0	0	0	0	0	0	0	0	<0.007	0.58	536865	426332	<0.0063	<7e-04	<0.014	<0.007	0.0049	7.98	10
56	05/08/20	SYK003N	JB	10-30m	1.25664	NA	Rep B	PRN 2120	2.1E+07	200um	NA	05/08/2022	N North S	Yorkshire	0	0	0	0	0	0	0	0	0	0	<0.007	0.58	536865	426332	<0.0063	<7e-04	<0.014	<0.007	0.0049	7.98	10
57	07/08/20	TEE002N	SH	10-30m	1.63363	NA	Rep A	PRN 2120	2.1E+07	200um	NA	07/08/2022	N North S	Tees: Rei	0	0.61213	0	0	0	0	0	0	0	0	0.23	2.6	454478	525697	0.301	0.0086	0.54	0.31	0.053	7.64	9
58	07/08/20	TEE002N	UJ	10-30m	1.63363	NA	Rep B	PRN 2120	2.1E+07	200um	NA	07/08/2022	N North S	Tees: Rei	0	0	0	0	0	0	0	0	0	0	0.23	2.6	454478	525697	0.301	0.0086	0.54	0.31	0.053	7.64	9
59	10/08/20	NNC005N	SH	10-30m	1.75929	NA	Rep A	PRN 2120	2.1E+07	200um	NA	10/08/2022	N North S	Northum	0	0	0	0	0	0	0	0	0	0	<0.007	1.1	406912	647089	<0.0063	<7e-04	<0.014	<0.007	<0.002	8.94	11
60	07/08/20	TEE002N	JB	10-30m	1.75929	NA	Rep B	PRN 2120	2.1E+07	200um	NA	07/08/2022	N North S	Tees: Rei	0	0	0	0	0	0	0	0	0	0	<0.007	1.1	406912	647089	<0.0063	<7e-04	<0.014	<0.007	<0.002	8.94	11
61	11/08/20	FNH002N	SH	10-30m	3.39282	NA	Rep A	PRN 2120	2.1E+07	200um	NA	11/08/2022	N North S	Farne Isl	0	0	0	0	0	0	0	0	0	0	<0.007	0.87	424542	635856	0.0058	0.0015	0.0143	0.0073	0.0052	8.15	9
62	11/08/20	FNH002N	JB	10-30m	3.26726	NA	Rep A	PRN 2120	2.1E+07	200um	Rep A sh	11/08/2022	N North S	Farne Isl	0	0	0	0	0	0	0	0	0	0	<0.007	0.87	424542	635856	0.0058	0.0015	0.0143	0.0073	0.0052	8.15	9
63	11/08/20	FNH004N	SH	10-30m	2.51327	NA	Rep A	PRN 2120	2.1E+07	200um	NA	11/08/2022	N North S	Farne Isl	0	0	0	0	0	0	0	0	0	0	<0.007	0.67	425858	627691	<0.0063	<7e-04	<0.014	<0.007	0.0028	8.37	10
64	11/08/20	FNH004N	JB	10-30m	2.51327	NA	Rep B	PRN 2120	2.1E+07	200um	NA	11/08/2022	N North S	Farne Isl	0	0	0	0	0	0	0	0	0	0	<0.007	0.67	425858	627691	<0.0063	<7e-04	<0.014	<0.007	0.0028	8.37	10
40	07/09/20	SOL001N	JB	10-30m	1.56451	NA	Rep A	PRN 2121	2.1E+07	200um	NA	07/09/2022	E Channe	Fawley	0	0	0	0	0	0	0	0	0	0	0.057	1.7	447827	104299	0.133	0.0075	0.197	0.14	0.019	6.83	9
41	07/09/20	SOL002N	SH	10-30m	1.08196	NA	Rep A	PRN 2121	2.1E+07	200um	m NA	07/09/2022	E Channe	East Brar	0	0	0	0	0	0	0	0	0	0	0.021	1.1	454387	98839	0.0351	0.0029	0.059	0.038	0.01	6.92	9
42	07/09/20	SOL005N	UJ	10-30m	3.48968	NA	Rep A	PRN 2121	2.1E+07	200um	NA	07/09/2022	E Channe	Ryde	0	0	0.28656	3.43871	0	0	0	0	0	0	<0.007	1.3	463090	94471	0.0053	0.0017	<0.014	<0.007	0.0048	7.02	
43	07/09/20	SOL001N	SH	10-30m	1.3823	NA	Rep A	2.1E+07	2.1E+07	200um	m NA	07/09/2022	E Channe	Cowes	0	0	0	0	0.72343	0	0	0	0	0	0.022	1	449958	97432	0.0396	0.0034	0.065	0.043	0.01	6.86	
44	13/09/20	NCW002P	JB	10-30m	2.51327	NA	Rep A	PRN 2121	2.1E+07	Insects	Name ins	13/09/2022	E Channe	Cowes	0	0	0	0	0	0	0	0	0	0	0.013	1.8	187437	77434	0.0214	0.0036	0.038	0.025	0.0078	7.26	9
45	13/09/20	BRB001N	SH	10-30m	1.00531	NA	Rep A	PRN 2121	2.1E+07	Insects o	NA	13/09/2022	W Chann	Barnstag	0	0	0	0	0	0	0	0	0	0	0.0084	1.5	243478	143543	0.0126	0.0034	0.0244	0.016	0.0074	7.15	9
46	14/09/20	IBS002N	SH	10-30m	3.39292	NA	Rep A	PRN 2121	2.1E+07	Detritus	NA	14/09/2022	W Chann	Inner Bri	0	0	0	0	0	0	0	0	0	0	<0.007	1.6	297424	152501	0.274	0.0056	0.287	0.28	0.031	7.19	9
47	15/09/20	SKT004N	JB	10-30m	3.18296	NA	Rep A	PRN 2121	2.1E+07	200um	m The wate	15/09/2022	E Channe	S Folk Pe	0	0	0	0.31616	0	0	0	0	0	0	0.0097	1.7	623497	134439	0.0086	0.0024	0.0207	0.011	0.0067	6.72	8
48	15/09/20	SKT002N	UJ	10-30m	2.47809	NA	Rep A	PRN 2121	2.1E+07		NA	15/09/2022	E Channe	Goodwin	0	0	0	0.40354	0	0	0	0	0	0	0.018	1.9	641803	152782	0.065	0.024	0.107	0.089	0.022	6.62	8
65	11/09/20	FNH004N	SH	10-30m	2.51327	NA	Rep A	PRN 2121	2.1E+07		NA	11/09/2022	N North S	Farne Isl	0	0	0	0	0	0	0	0	0	0	0.028	1.2	425845	627698	0.0123	0.0027	0.043	0.015	0.012	7.6	9
66	11/09/20	NNC005N	JB	10-30m	1.75929	NA	Rep A	PRN 2121	2.1E+07		NA	11/09/2022	N North S	Northum	0	0	0	0	0	7.95775	0	0	0	0	0.018	1.5	406886	646995	0.0127	0.0013	0.032	0.014	0.0092	7.8	9
67	12/09/20	FNH002N	SH	10-30m	3.14159	NA	Rep A	PRN 2121	2.1E+07	200um	m NA	12/09/2022	N North S	Farne Isl	0	0	0	0	0	1.90986	0	0	0	0	0.021	1	424478	635942	0.0076	0.0017	0.0303	0.0093	0.011	7.54	8
68	13/09/20	TEE002N	SH	10-30m	1.25664	NA	Rep A	PRN 2121	2.1E+07		NA	13/09/2022	N North S	Tees: Rei	0	0	0	0	0	0	0	0	0	0	0.32	1.2	454493	525708	0.659	0.011	0.99	0.67	0.083	7.23	
69	14/09/20	SYK006N	SH	10-30m	0.87965	NA	Rep A	PRN 2120	2.1E+07	200um	NA	14/09/2022	N North S	Yorkshire	0	0	0	0	0	0	0	0	0	0	0.008	1.3	536885	426310	0.0058	0.0012	0.015	<0.007	0.0073	7.63	9
70	14/09/20	SYK003N	JB	10-30m	0.75398	NA	Rep A	PRN 2121	2.1E+07		NA	14/09/2022	S North S	Lincs Coa	0	0	0	0	0	0	0	1.32629	0	0	0.012	5.4	544526	405448	0.295	0.0053	0.312	0.3	0.031	8.04	10
72	15/09/20	OWS003P	JB	10-30m	5.02655	NA	Rep A	PRN 2122	2.1E+07		Water de	15/09/2022	S North S	Outer We	0	0	0	0	0	0	0	0	0	0	0.0098	2.3	560910	348717	0.0114	0.0016	0.0228	0.013	0.0089	7.28	9
71	15/09/20	SYK005N	SH	10-30m	1.3823	NA	Rep A	PRN 2121	2.1E+07	200um	m There is	15/09/2022	S North S	Lincs Coa	0	0	0	0	0	0	0	0	0	0	0.024	2.5	560551	357303	0.0107	0.0023	0.037	0.013	0.013	7.1	9
77	20/09/20	LIV006N	SH	10-30m	0.75398	NA	Rep A	PRN 2121	2.1E+07	200um	m NA	20/09/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	0	0	0	0.027	1.7	527753	438038	0.015	0.003	0.045	0.018	0.057	7.25	9
78	15/09/20	LIV003N	JB	10-30m	0.87965	NA	Rep A	PRN 2121	2.1E+07	200um	The wate	15/09/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	0	0	0	0.067	5	321888	395310	NA	0.0078	NA	0.073	0.052	7.01	8
79	15/09/20	LIV002N	SH	10-30m	1.39381	NA	Rep A	PRN 2121	2.1E+07	200um	Water de	15/09/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	0	0	0	0.13	2.2	529664	397995	0.568	0.052	0.75	0.62	0.12	6.35	7
73	08/10/20	SOL001N	SH	10-30m	1.50796	NA	Rep A	PRN 2122	2.1E+07	200um	m NA	08/10/2022	E Channe	Fawley	0	0	0	0	0	0	0	26.5258	0.66315	0	0.032	1.1	447850	104278	0.105	0.0051	0.142	0.11	0.016	7.78	9
74	08/10/20	SOL002N	JB	10-30m	1.13097	NA	Rep A	PRN 2122	2.1E+07	200um	m NA	08/10/2022	E Channe	East Brar	0	0	0	0	0	0	0	171.534	0.88419	0	0.031	1.5	454450	98953	0.125	0.0054	0.161	0.13	0.017	7.9	9
75	08/10/20	SOL005N	SH	10-30m	3.64425	NA	Rep A	PRN 2122	2.1E+07	200um	m NA	08/10/2022	E Channe	Ryde	0	0	0	0	0	0	0	28.5381	0.82322	0	0.016	1.2	463134	94446	0.0472	0.0038	0.067	0.051	0.011	7.82	
76	08/10/20	SOL001N	UJ	10-30m	1.75929	NA	Rep A	PRN 2122	2.1E+07	200um	Lab upda	08/10/2022	E Channe	Cowes	0	0	0	0	0	0	0	227.364	0	0	0.024	1.6	449994	97300	0.0719	0.0041	0.1	0.076	0.014	7.79	9
80	13/10/20	LIV006N	JB	10-30m	1.3823	NA	Rep A	PRN 2122	2.1E+07	200um	m NA	13/10/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	21.7029	0	0	0.051	1.9	527735	438050	0.13	0.01	0.191	0.14	0.039	8.61	9
81	13/10/20	LIV003N	SH	10-30m	0.50265	NA	Rep A	PRN 2122	2.1E+07	200um	Tow leng	13/10/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	7.95775	0	1.98944	0.071	3.8	321632	395483	0.0759	0.0081	0.155	0.084	0.04	8.37	9
82	13/10/20	LIV002N	JB	10-30m	0.12566	NA	Rep A	PRN 2122	2.1E+07	200um	NA	13/10/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	254.648	0	0	0.19	5.2	329577	398083	0.823	0.077	1.09	0.9	0.12	7.85	8
83	11/10/20	TMT005N	SH	10-30m	2.26195	NA	Rep A	PRN 2122	2.1E+07	200um	Mersey	11/10/2022	Irish Sea	Mersey N	0	0	0	0	0	0	0	7.07355	0	0	<0.02	4	596182	180725	0.228	0.012	0.26	0.24	0.042	7.88	9
84	15/10/20	OBW005P	JB	10-30m	1.25664	NA	Rep A	PRN 2122	2.1E+07	200um	Net Volu	15/10/2022	S North S	Blackwat	0	0	1.59155	0	0	0	0	1.59155	0	0	0.014	3.7	606142	209928	0.176	0.004	0.194	0.18	0.039	7.86	9
85	14/10/20	FNH004N	SH	10-30m	2.13628	NA	Rep A	PRN 2122	2.1E+07	200um	This PRN	14/10/2022	N North S	Farne Isl	0	0	0	0	0.46811	0	0	14.9793	0	0	0.011	1.3	425893	627671	0.0218	0.0042	0.037	0.026	0.012	8.08	9
86	14/10/20	NNC005N	JB	10-30m	1.63363	NA	Rep A	PRN 2122	2.1E+07	200um	NA	14/10/2022	N North S	Northum	0	0	0	0	2.44854	0	0	254.648	0	0	<0.007	1.3	406889	647124	0.0289	0.0051	0.041	0.034	0.011	8	9
87	14/10/20	FNH002N	UJ	10-30m	3.26726	NA	Rep A	PRN 2122	2.1E+07	200um	Lab upda	14/10/2022	N North S	Farne Isl	0	0	0	0	0	0	0	117.53	0	0	<0.007	1.5	424494	635922	0.0219	0.0041	0.033	0.026	0.011	7.99	9
88	13/10/20	TEE002N	JB	10-30m	1.50796	NA	Rep A	PRN 2122	2.1E+07	200um	NA	13/10/2022	N North S	Tees: Rei	0	0	0	0	0	0	0	63.662	0	0	0.16	1.1	454481	525672	0.224	0.0062	0.39	0.23	0.047	7.12	8
89	12/10/20	SYK006N	SH	10-30m	1.25664	NA	Rep A	PRN 2122	2.1E+07	200um	NA	12/10/2022	N North S	Yorkshire	0	0	0	0	0	0	0	25.4648	0	0	0.0093	1.8	536887	426286	0.0434	0.0076	0.0603	0.051	0.013	8.31	9

Currently this is a complex exercise to match physical, chemical, and biological data sets the example above is a 'wide format' where all taxa and dets have their own column. We are still trying to design the best process for this. Note the attached file has not been quality assured and is for demonstration purposes only.

zoopWIDEAbund_
m3_WIMS_USE.csv