AtlantOS plankton Report

Based on observations from the Continuous Plankton Recorder survey

Sir Alister Hardy Foundation for Ocean Science 2017

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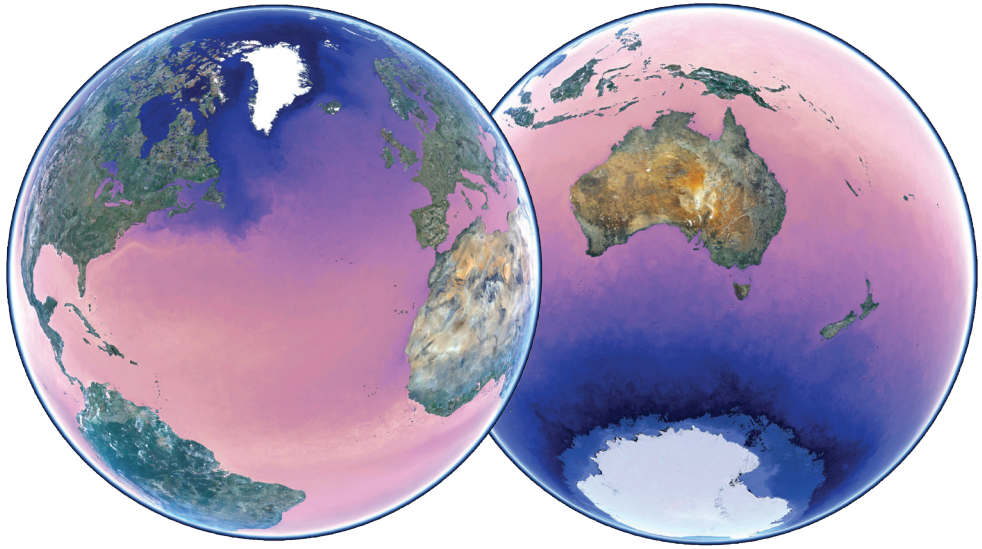
Introduction



The Continuous Plankton Recorder (CPR) survey is one of the most well established autonomous observing systems covering the North Atlantic basin-scale over multiple decades. It has 80 years of experience with working with the commercial shipping industry and is an established platform providing a global network of Ships of Opportunity for scientific research.

As part of the European project AtlantOS which aims to build a more integrated Atlantic wide observation system, the CPR survey aims to optimize and enhance its current CPR survey network. The CPR is an autonomous instrument mainly towed from ships of opportunity that has been in use for over 80 years. Currently, samples are collected monthly covering 20,000 km in the major ecosystems of the North Atlantic. Recently the network has expanded to sample in the South Atlantic and other regions globally. It has been observing over 1000 biological variables over a eighty year period as well as a number of physical variables.

There is an increasing need to monitor the marine environment for legislative reasons (e.g. MSDF Good Environmental Status targets) and at reduced costs using autonomous methods. Therefore, there are obviously huge cost benefits in incorporating new technologies and sensors into existing infrastructures like the CPR survey to optimize and enhance the Atlantic observing system. The CPR survey, managed by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) already has good interlinkage between its observations and other SOOP activities such as Carbon VOS for example.

Within AtlantOS, the CPR surveys aims to be optimized and enhanced and to make the data it collects more easily and widely discoverable. A method to more rapidly determine zooplankton abundance aims to be developed through the use of new technology complimenting the existing plankton time-series. The preliminary results of this are contained in this report. Near-real-time sensors for variables such as conductivity, temperature and chlorophyll-a fluorescence from bespoke sensors will also be developed on the CPR transects across some coastal to open ocean waters and faster quantitative molecular assays of key harmful and pathogenic organisms will be investigated using new techniques.

This report contains information on the main trends and status of plankton in the North Atlantic and the preliminary results from the new method of rapidly determining zooplankton abundance.

Plankton Essential Ocean Variables

The global network of CPR surveys now routinely monitors the North Sea, North Atlantic, Arctic, North Pacific and Southern Ocean. Recent surveys are underway in the eastern Mediterranean, Australian, New Zealand, Japanese and South African waters while Brazilian and Indian Ocean survey activities began in 2016. This global network also brings together the expertise of approximately 70 plankton specialists, scientists and technicians from 14 laboratories around the world and has established links or formal affiliations with a number of key stakeholders including, Global Ocean Observing System (GOOS), GEO-BON, the International Oceanographic Commission (IOC), the Scientific Commission on Oceanic Research (SCOR), the International Council for the Exploration of the Sea (ICES), the Partnership for Observation of the Global Oceans (POGO) and the North Pacific Marine Science Organization (PICES).

It is recognised that there is an increasing need to monitor the marine environment as part of global initiatives like the development of 'Essential Ocean Variables (EOVs) for the Global Ocean Observing System (GOOS). Of particular relevance to the CPR survey and AtlantOS at an Atlantic wide and global perspective are some of the recent recommendations given by G7 Ministers of Science to 'the future of the seas and oceans' and include:

- Continuing critical regional observing in the tropics and maintaining and enhancing our observing capacity in the marine cryosphere (Arctic and Antarctic)
- Enhancing the effective use and international coordination of research ships and satellites to leverage their unique capabilities in the ocean observing strategy
- Fostering increased collaboration with the shipping industry on ocean observations to explore increasing use of commercial fleets for observing of the ocean and seas.
- Supporting and accelerating the development and implementation of ecosystem/ biodiversity Essential Ocean Variables (EOVs) for routine implementation
- ensure sustainable science-based ocean management and provide clarity on resource-management
- promote observing and data sharing and development of products and models that provide integrated ocean state knowledge
- promote co-ordination with relevant activities of the Intergovernmental Panel on Climate Change/ Intergovernmental Platform on Biodiversity and Ecosystem Services

SAHFOS scientists have already been taking an active lead in developing ecosystem EOVs (identified in the G7 statement) through its involvement with the GOOS panel on Biology and Ecosystems (Grimes, 2014) and with the GEO-BON Working group 5 (marine ecosystem change). As part of its involvement with these organisations, SAHFOS is helping to develop biological and ecosystem Essential Ocean Variables (EOVs) through its involvement with the AtlantOS framework programme which aims to build a North Atlantic wide integrated observing system.

A key component for the success of EOVs is the need for the variable to have a high impact in responding to scientific and societal needs and crucially to have a high feasibility of sustained observation. Ocean observations are the 'bread and butter' of ocean and climate change science (Cai et al., 2014) and the network of CPR surveys operating around the world were seen as a critical ongoing network for a sustained and internationally coordinated effort for biological observation at the global scale (Constable et al., 2016).

An important goal of the global CPR programme is to develop indictors for scientists and policy makers to monitor and understand global plankton changes as well as providing the global community with useful products such as EOVs that can be used to monitor and assess marine biodiversity and ecosystem health. Once there has been international agreement on what ecosystem EOVs are required SAHFOS will primarily disseminated them through SAHFOS's Ecological Status Report and through international programmes such as GOOS, GEO-BON and the EU AtlantOS programme. Although at this stage of development the biological and ecosystem EOVs have not been formalised they will come under the general heading of phytoplankton biomass and diversity; zooplankton biomass and diversity; fish abundance and distribution; marine turtles, birds and mammals abundance and distribution; live coral; seagrass cover; mangrove cover and macroalgal canopy. In the context of the CPR survey and AtlantOS we are particularly concerned with the phytoplankton and zooplankton variables that will aim to address the biological phenomena shown in figure 1.

Developing phytoplankton and zooplankton Essential Ocean Variables for monitoring biology and ecosystems

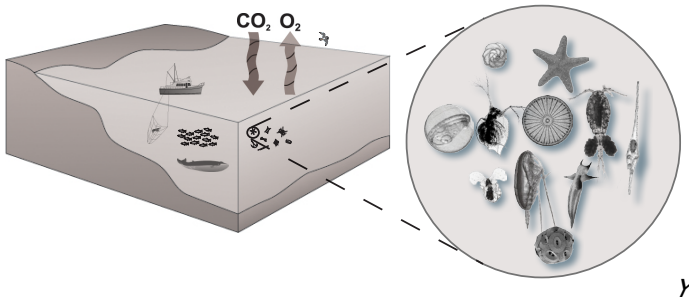
1. Societal drivers

Sustainable economic growth/development
Conservation/ biodiversity
Improved management/ ecosystem approach
Capacity building and technology transfer
Food security - fishing/aquaculture
Environmental quality and health
Energy production

2. Societal pressures

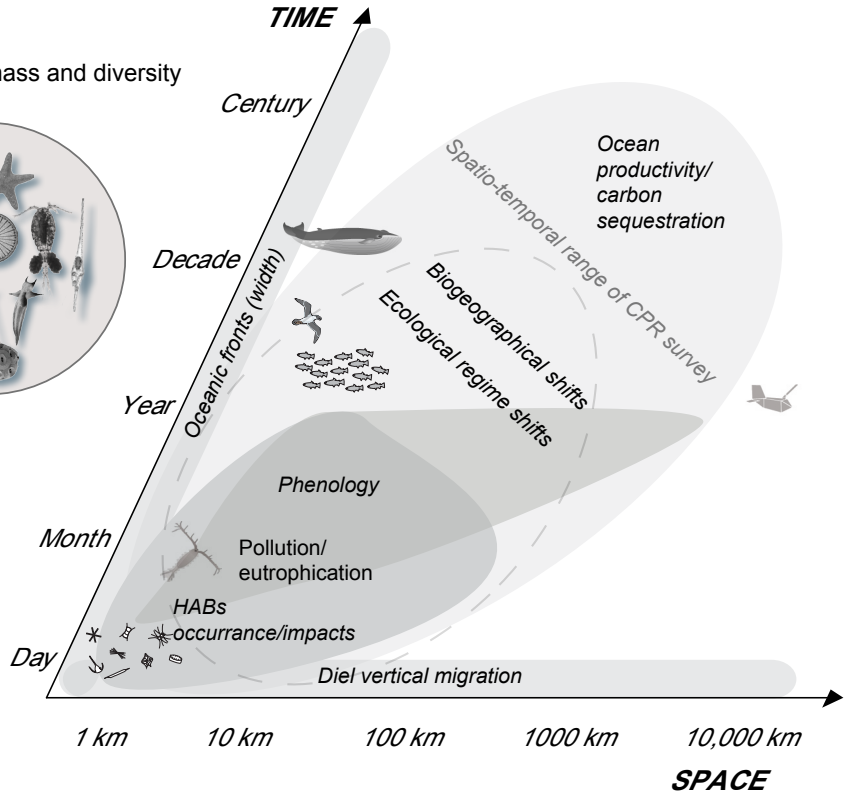
Climate change
Ocean acidification
Extreme weather events
Loss of resources/habitats
Overfishing
Seabed mining
Solid wastes/ marine litter
Pollution/eutrophication
Invasive species
Coastal development

Biological EOVs: phytoplankton and zooplankton biomass and diversity



3. Scientific questions

What is the current status of life and biodiversity in the oceans?
How is life in the oceans changing?
What are the natural and anthropogenic drivers of change?
Are HAB events increasing in frequency or spatial location?
Are invasive species increasing?
How does the changing life in the oceans affect ecosystem function (health and services)?



4. Biological phenomena to capture (plus spatio/temporal scales)

Phenology
Occurrance of Harmful Algal Blooms (HABs)
Biogeographical shifts
Biodiversity/invasive species
Impact on calcareous organsims
Ecological regime shifts
Ocean productivity
Carbon sequestration

Fig.1. A schematic representing the main societal drivers and pressures and the biological phenomena used to capture these changes in our oceans. From a plankton and monitoring perspective many of the processes to be addressed occur on a number of spatial and temporal scales which equally needs a monitoring system operating on similar scales such as the CPR Survey network.

Current and historic CPR sampling in the North Atlantic

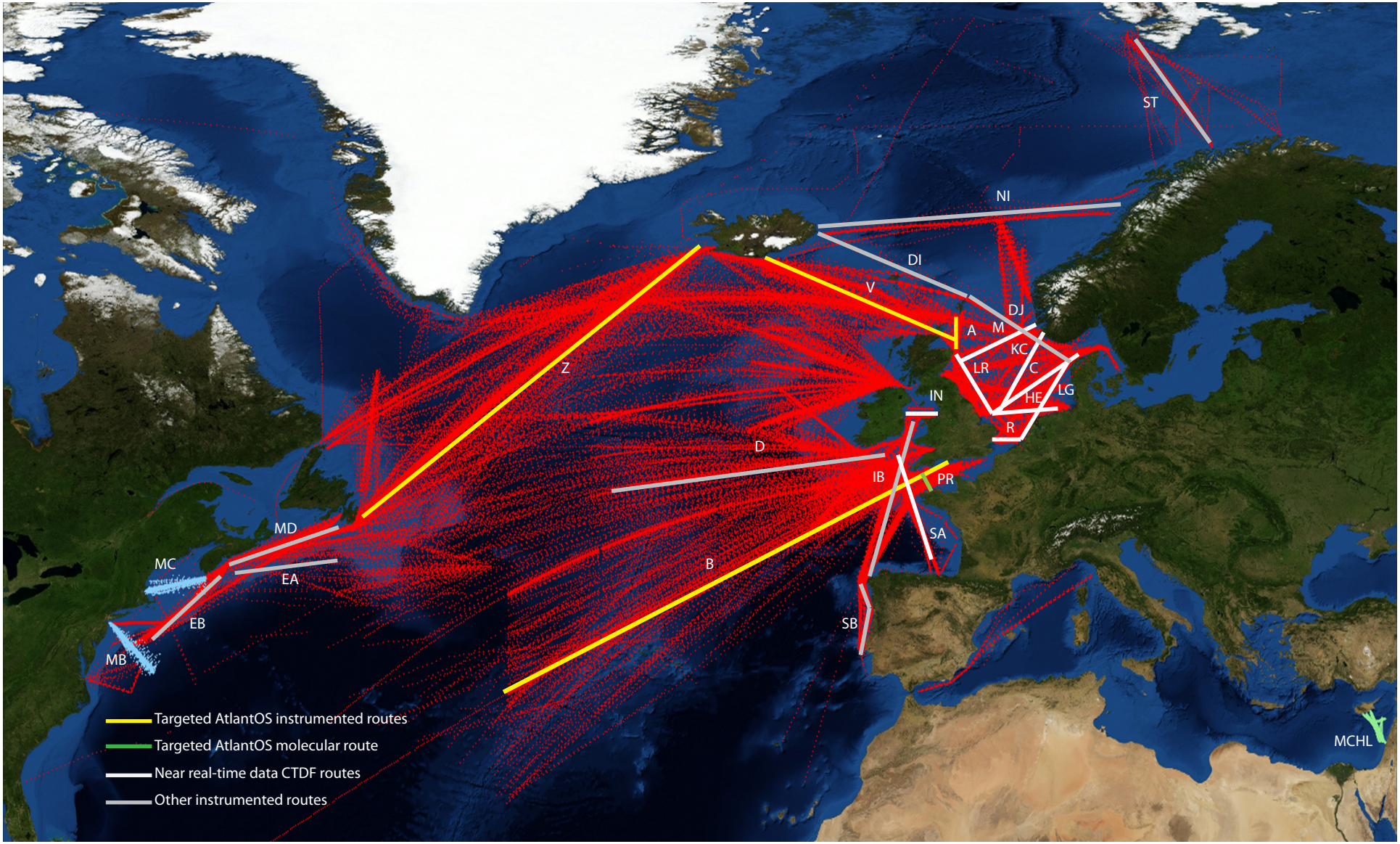
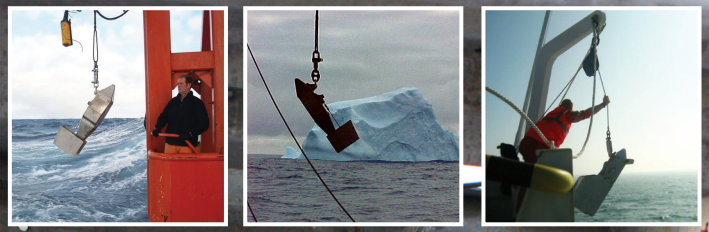


Fig. 2. Historic sampling in the North Atlantic by the CPR survey (red samples) and current CPR routes (lines). Letters refer to CPR route identification. Targeted AtlantOS instrumented routes are in yellow and targeted molecular route in green.



CPRs are operated by ships of opportunity and research vessels throughout the world's oceans. Here is a CPR being deployed on the research sailing vessel *Tara* on the CPRs first total polar circumnavigation (photo credit: Vincent Hilaire).

Enhancing North Atlantic Observations using the CPR network as an operational research platform

With an increasing need to monitor the marine environment for legislative reasons (e.g. EU delivery of the MSDF Good Environmental Status targets) and at reduced costs using autonomous methods there are obviously huge cost benefits in incorporating new technologies into existing infrastructures like the CPR survey autonomous sampling network. For example, the CPR network covers around 20,000 km of ocean per month and in offshore areas that rarely or consistently sampled (Fig.2). One of the aims of AtlantOS in a CPR perspective is to help develop this existing network and help enhance its operations.

Cost effective physical and chemical monitoring

There is considerable scope for the further development of the CPR instrumentation programme to provide synoptic physical/biogeochemical measurements with the plankton for use in global climate change and ecological models and satellite calibration as well as to help interpret causes of plankton and fisheries variability. Variability in ocean chemistry – nutrients, pH, C02 concentration and other dissolved gas measurements – provide crucial constraints to plankton growth rates and survival as well as insight into the impact of global climate change on the ocean.

Good links already exist with the physical oceanographic community and pCO2 ship-of-opportunity communities and through AtlantOS these links will be further strengthened. These contacts will in addition be used to keep abreast of relevant new measurement technologies that could be fitted to CPRs and further add to the value of the autonomous survey. As part of the AtlantOS's integrated observing system the CPR survey could act as an essential regional and long-term backbone covering multiple observational scales. Currently near-real-time sensors for variables such as conductivity, temperature and chlorophyll-a fluorescence from bespoke sensors are being operated on CPR transects across some coastal to open ocean waters and faster quantitative molecular assays of key harmful and pathogenic organisms are being investigated using new molecular techniques (see Fig.3).

Monitoring and collecting additional biological information

Under this area SAHFOS has focused on continued deployment of the Water and Microplankton Sampler (WaMS) and developing quantitative molecular methods for Harmful Algal Blooms and pathogens. The water sampler opens up new opportunities to identify additional HABs as well as important smaller or delicate plankton and pathogenic species that may be missed or damaged by CPR tows. Rapid cell identification methods will continued to be explored using flow cytometry to sort cells on size and pigment for further to classify and quantify cells by size and pigment which can be isolated for later molecular analysis. The microsampler is seen as adding huge value in contributing to the the EU Marine Strategy Framework Directive and also complimenting the molecular analysis already currently being done at SAHFOS. The main objective of the water and microplankton sampler is to enable the CPR survey to monitor the full size range of plankton in the oceans from the larger plankton (which the CPR already samples) to the nano and pico plankton size ranges. The water and microplankton sampler is also aimed at monitoring the smaller Harmful Algal Bloom (HAB) species. New automatic visual identification methods will also be continued to be developed to speed up components of the traditional taxonomic analysis (e.g. quick estimates of zooplankton biomass/ size structure, see final section of this report 'Rapid optical assessment of zooplankton abundance').

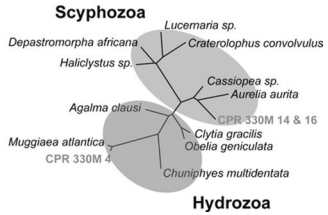
SIZE RANGES

Genetic analysis

Light microscopy

Flow cytometry

Mega and macro plankton

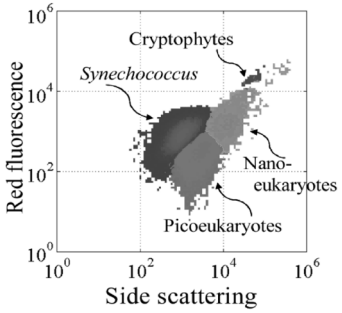


Meso and micro-plankton

Upto 1000 taxa routinely recorded making the CPR database one of the richest ecological datasets in the world

Nano and pico-plankton

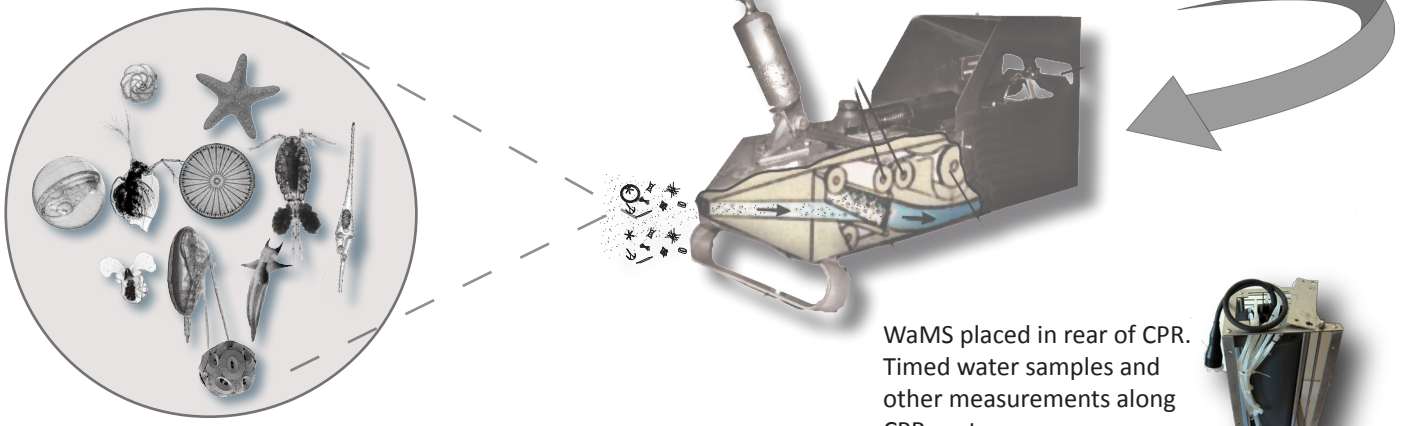
(example)



BIOLOGICAL MEASUREMENTS 1931-

(a) Continuous Plankton Recorder (CPR)

(i) Longest sustained marine biological time-series in the world (1931-). Routine analysis of ~1000 plankton taxa.
(ii) Multi-decadal sample and molecular archive at ocean-basin scale (1960-).



WaMS placed in rear of CPR. Timed water samples and other measurements along CPR route.

(b) Water and Microplankton Sampler (WaMS)

Aimed at smaller size-fraction nano and pico plankton community.
(i) Flow cytometry (2010-)
(ii) Molecular probes and barcoding (2010-)
(iii) Harmful Algal Bloom microarrays (2010-)

PHYSICAL MEASUREMENTS 1991-

(i) In situ instrumentation (1991-) Sea surface temperature, salinity, depth and chlorophyll.
(ii) Marine microplastics (2004-).
(iii) Other measurements and collaboration with other parties: pCO2, flowrate, Dissolved Inorganic Carbon, Alkalinity, oxygen content, nutrients.

Fig.3. A schematic showing significant milestones through time for the CPR survey from the first collection of plankton data from the North Sea in 1931 to the development of modern molecular methods. The CPR continues to collect over 1000 taxonomic entities using traditional methods but now employs a number of modern methods from flow cytometry to molecular probes to capture the whole size range and biodiversity of the planktonic system. Biological data is further complimented with the additional measurement of physical variables using instrumented CPRs.

CPR plankton observations

The North Atlantic and Arctic

The Continuous Plankton Recorder Survey is a long-term, sub-surface marine plankton monitoring programme consisting of a network of CPR transects towed monthly across the major geographical regions of the North Atlantic (Fig.4). It has been operating in the North Sea since 1931 with some standard routes existing with a virtually unbroken monthly coverage back to 1946. The CPR instrument is towed at the surface behind volunteer-operated vessels (ships of opportunity), sampling plankton onto a moving 270 µm (micrometre) band of net silk as the vessel and CPR unit traverse the North Atlantic and/or North Sea. Within the CPR instrument, the net silk and its captured plankton are preserved in formalin until they are returned to SAHFOS for routine analysis including the estimation of phytoplankton biomass (Phytoplankton Colour Index), and the identification of up to ~1000 different phytoplankton and zooplankton taxa. Direct comparisons between the phytoplankton colour index and other chlorophyll *a* estimates including SeaWiFS satellite estimates indicate strong positive correlations (Batten *et al*. 2003; Raitsos *et al*. 2005). During the processing, the net silk is divided into sections representing 10 nautical miles of towing, and each section is analysed for plankton composition and abundance.

Due to the mesh size of CPR silks, many phytoplankton species are only semi-quantitatively sampled owing to the small size of the organisms. There is thus a bias towards recording larger armoured flagellates and chain-forming diatoms and that smaller species abundance estimates from cell counts will probably be underestimated in relation to other water sampling methods. However, the proportion of the population that is retained by the CPR silk reflects the major changes in abundance, distribution and specific composition (i.e. the percentage retention is roughly constant within each species even with very small-celled species) (Edwards, *et al*. 2006). The addition of a water sampler onboard certain CPRs can provide information on the whole size-spectrum of plankton using molecular techniques from bacteria and viruses to flagellates and other taxa not normally identified using standard CPR analysis. For the purpose of this assessment, the North Atlantic Basin has been geographically subdivided into different ecoregions. The 40 geographical regions shown in the

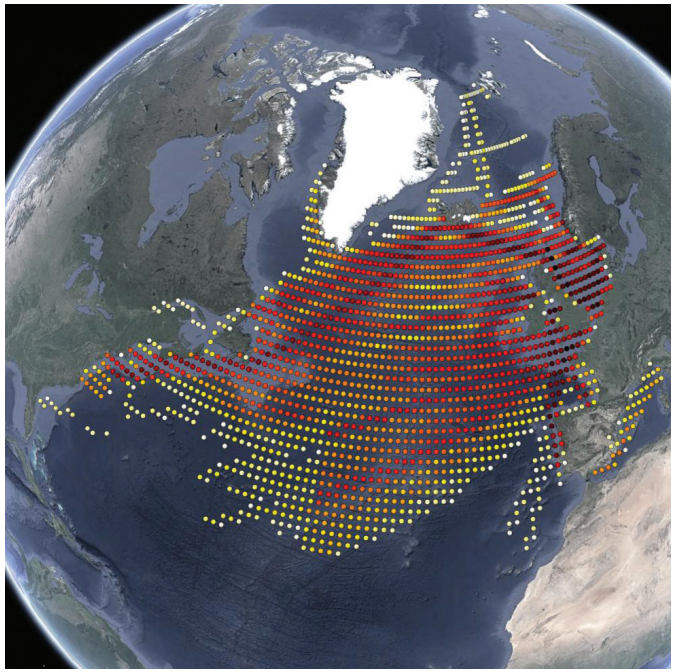


Fig.4. Gridded CPR sample effort (1° by 1°) for the North Atlantic. Map by Google Earth.

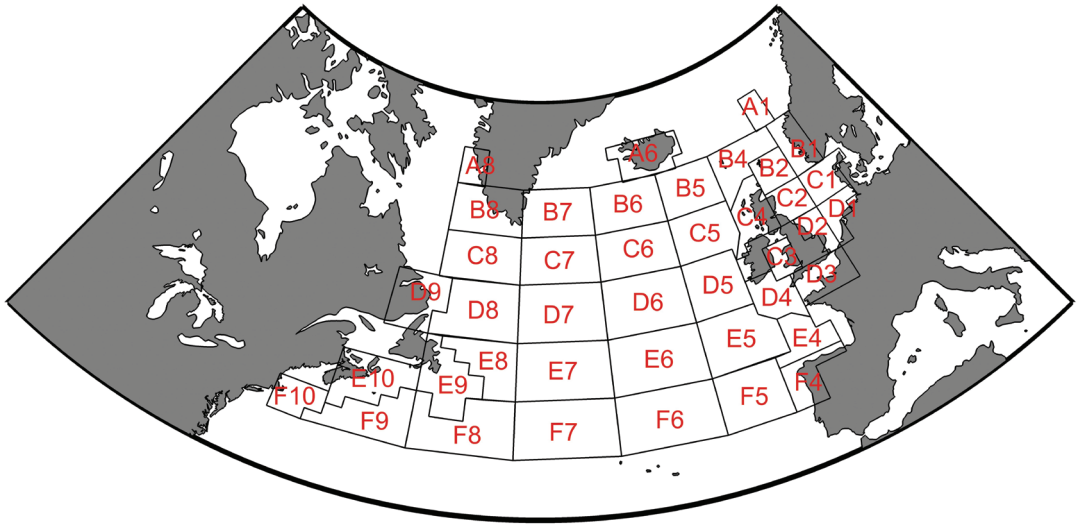
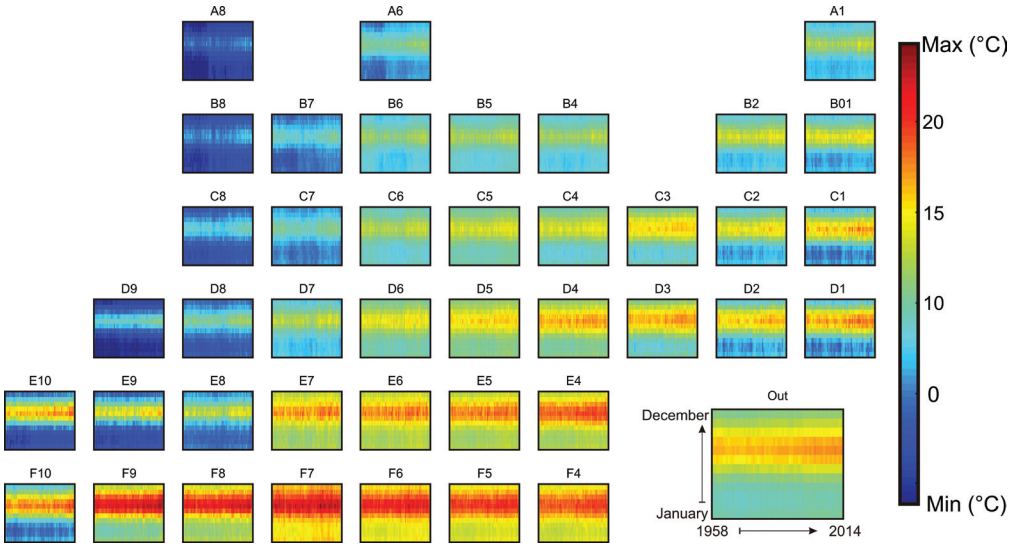


Fig. 5. CPR Survey standard areas used in the analysis of regional patterns of plankton for the North Atlantic.

Sea Surface Temperature



Phytoplankton abundance (PCI)

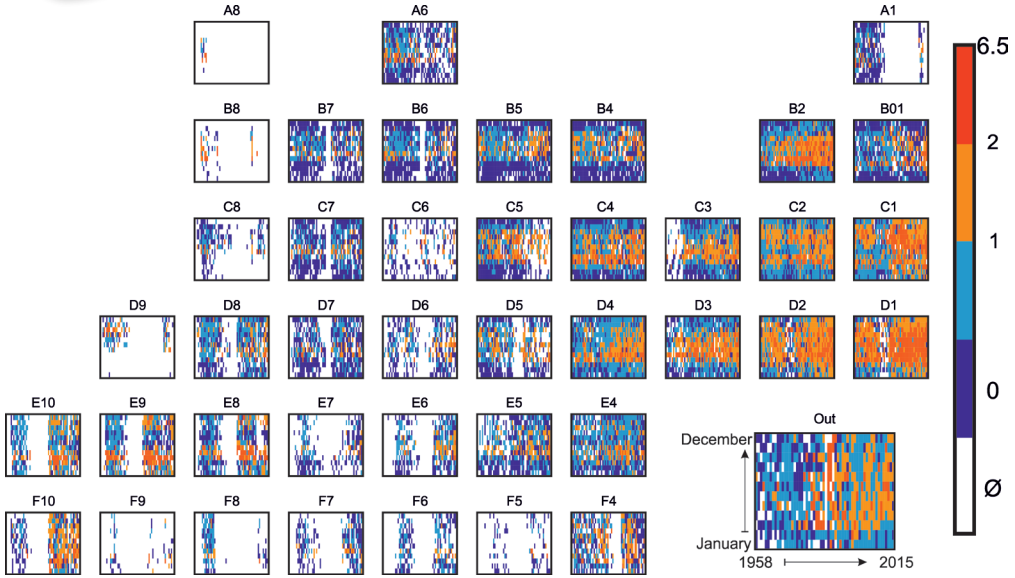


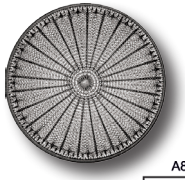
Fig. 6. Long-term trends in Sea Surface Temperature (1958-2014) and Phytoplankton abundance in standard CPR regions of the North Atlantic from 1958-2015. Data available online www.sahfos.ac.uk.

figures are known as the CPR standard areas (Fig.5). The Figures 6-8 show regional trends in standard areas generated using standard statistical methods for calculating annual means. As part of the AtlantOS project these standard CPR regions will become redefined based on the biological communities there and become new ecoregional areas for the North Atlantic.

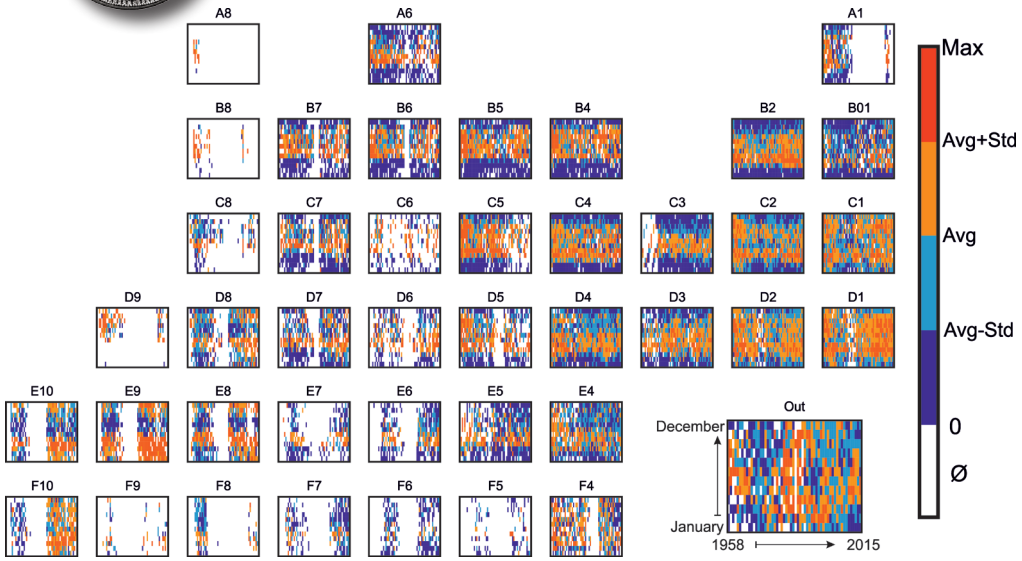
Basin scale trends in plankton and natural variability

To summarise the long-term trends in plankton in the North Atlantic Basin we used indices of plankton that included the CPR Phytoplankton Colour Index (PCI) and the sum of the abundance of all counted diatoms and all counted dinoflagellates and total copepod numbers and mean copepod size. Using bulk indices like this are less sensitive to environmental change and will quite often mask the subtleties that individual species will give you; however, it is thought that these bulk indices represent the general functional response of plankton to the changing environment. In the North Atlantic, at the ocean basin scale and over multidecadal periods, changes in plankton species and communities have been associated with Northern Hemisphere Temperature (NHT) trends, the Atlantic Multidecadal Oscillation (AMO), the East Atlantic Pattern (EAP) and variations in the North Atlantic Oscillation (NAO) index. These have included changes in species distributions and abundance, the occurrence of sub-tropical species in temperate waters, changes in overall plankton biomass and seasonal length, changes in the ecosystem functioning and productivity of the North Atlantic (Beaugrand, *et al.* 2003; Edwards, *et al*. 2001; Edwards, *et al.* 2002; Edwards & Richardson, 2004; Reid & Edwards, 2001). Of particular recent note is the emergence of a cold water anomaly in the North Atlantic south of Greenland (sub-polar gyre region) since 2014. This area experienced record cold conditions in 2015 thought to be driven by melt water discharges from the Greenland Ice Sheet and possible Atlantic wide circulation changes. The consequences of this anomaly on the plankton of the North Atlantic are currently being investigated.

Contemporary observations over a 10 year period of satellite *in situ* blended ocean chlorophyll records indicate that global ocean net primary production has declined over



Diatom abundance



Dinoflagellate abundance

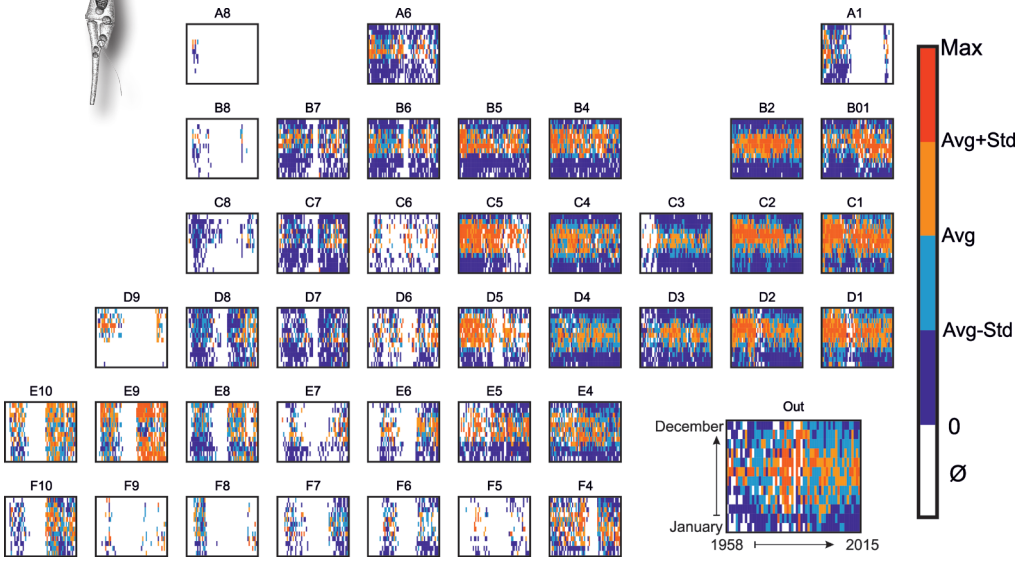


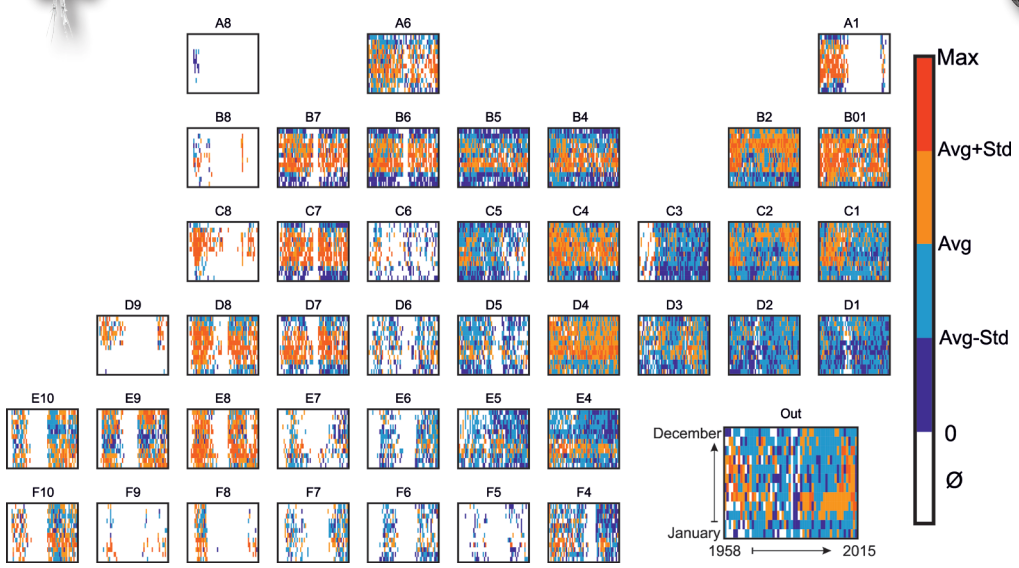
Fig. 7. Long-term trends in diatom and dinoflagellate abundance in standard CPR regions of the North Atlantic from 1958-2014. Data available online www.sahfos.ac.uk

the last decade, particularly in the oligotrophic gyres of the world's oceans (Behenfeld *et al*. 2006). However, over the whole temperate NE Atlantic there has been an increase in phytoplankton biomass with increasing temperatures but a decrease in phytoplankton biomass in warmer regions to the south (Richardson & Schoeman, 2004), as shown in Figure 6. Presumably this is a trade-off between increased phytoplankton metabolic rates caused by temperature in cooler regions but a decrease in nutrient supply in warmer regions.

It must be noted, however, that climate variability has a spatially heterogeneous impact on plankton in the North Atlantic and not all regional areas are correlated to the same climatic index. For example, trends in the AMO are particularly prevalent in the oceanic regions and in the sub-polar gyre of the North Atlantic and the NAO has a higher impact in the southern North Sea where the atmosphere-ocean interface is most pronounced (Harris *et al.* 2013). This is also apparent with respect to the Northern Hemisphere Temperature where the response is also spatially heterogeneous with areas of the North East Atlantic and shelf areas of the North West Atlantic warming faster than the North Atlantic average and some areas like the sub-polar gyre actually cooling. Similarly, regime shifts or abrupt ecosystem shifts do not always occur in the same region or at the same time. The major regime shift that occurred in plankton in the late 1980s was particularly prevalent in the North Sea and was not seen in oceanic regions of the North Atlantic. However, a similar regime shift occurred in the plankton colour index 10 years later in the Icelandic Basin and in oceanic regions west of the British Isles. The different timing and differing regional responses to regime shifts have been associated with the movement of the 10°C thermal boundary as it moves northwards in the North Atlantic (Edwards *et al*. 2013).

In examining the long-term trends in the plankton indices, the general pattern is an increase in PCI for most regions in the North Atlantic with differing timings for the main step-wise increase being later in oceanic regions compared to the North Sea. For the dinoflagellates there has been a general increase in abundance in the North West Atlantic and a decline in the North East Atlantic over a multi-decadal period (see Fig. 7). In particular, some regions of the North Sea have experienced a sharp decline over the last decade. This decline has been mainly caused by the dramatically reduced abundance of the *Neoceratium* genus in the North Sea. However, *Neoceratium* abundance has recovered in the North Sea over the last

Copepod abundance



Copepod mean size

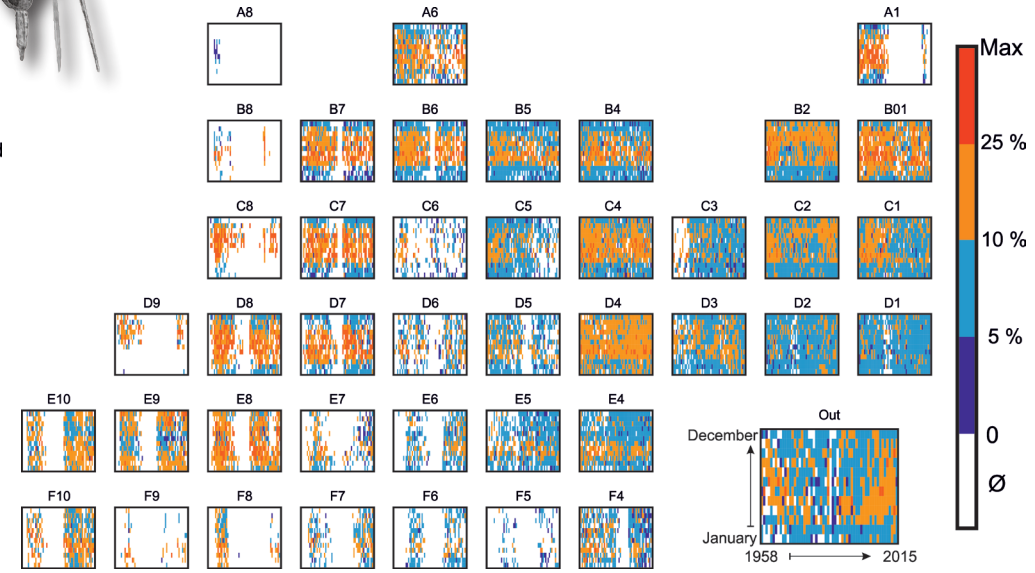
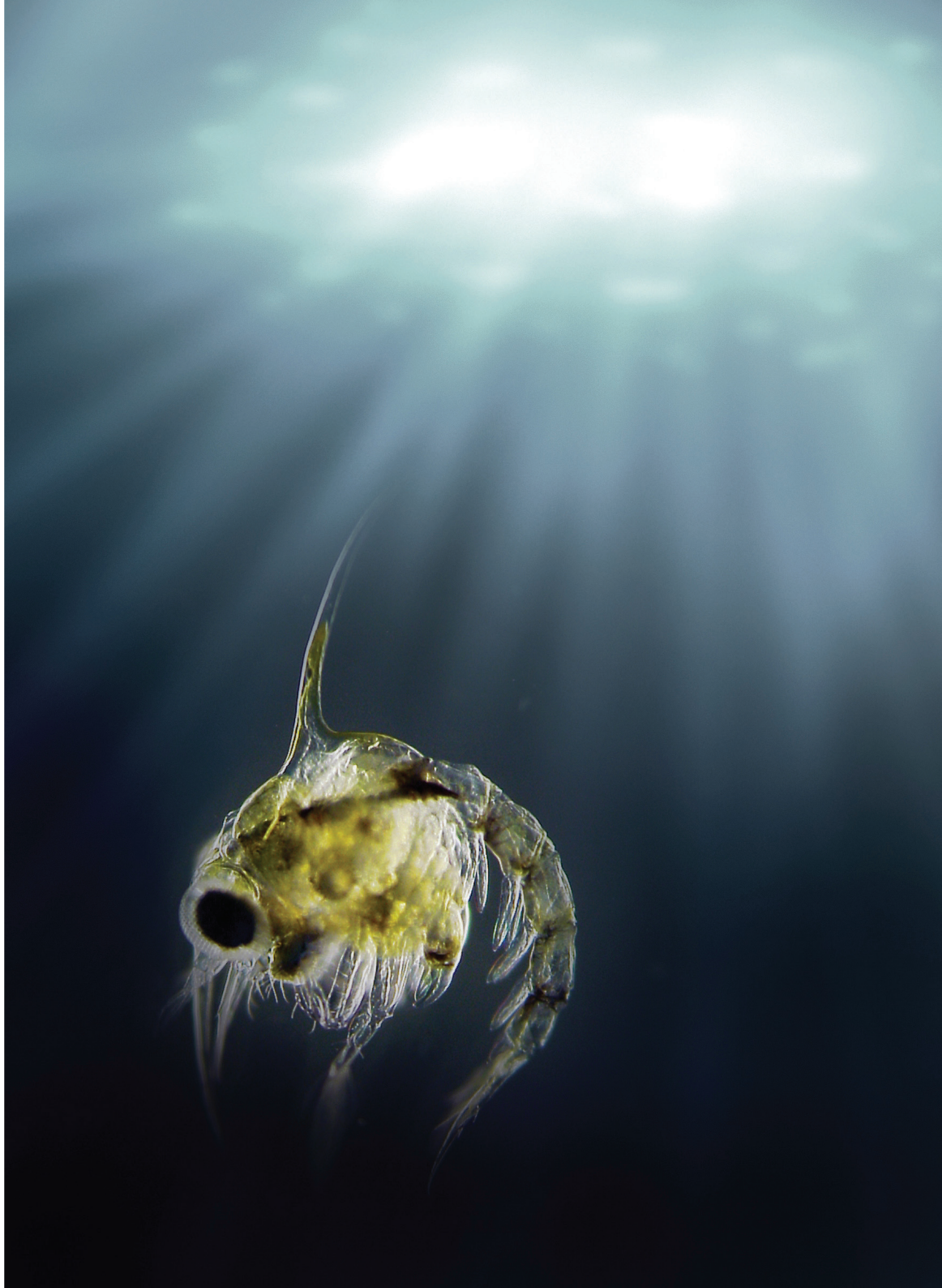


Fig. 8. Long-term trends in copepod abundance and copepod mean size in standard CPR regions of the North Atlantic from 1958-2014. Data available online www.sahfos.ac.uk

5 years. For the diatoms there is not really a predominant trend for the North Atlantic Basin as a whole (Fig. 7) but some regions show a strong cyclic behaviour over the multidecadal period. The time signal resembles an oscillation of about 50-60 years and a minimum around 1980 reflecting changes in the AMO signal. Trends in copepod abundances have been more stable in offshore regions but have shown a decrease in abundance, particularly in the southern North Sea (Fig.8). In summary, while climate warming is a major driver for the overall biomass of phytoplankton, diatoms are less influenced by temperature and show a strong correlation with the AMO signal and wind intensity in many regions (Harris *et al.* 2013). The increase in diatoms associated with the positive phase of the AMO and the decline in dinoflagellate abundance over the last 10 years in the NE Atlantic can be reflected in the diatom/dinoflagellates ratio favouring diatoms.

Indirectly the progressive freshening of the Labrador Sea region, attributed to climate warming and the increase in freshwater input to the ocean from melting ice, has resulted in the increasing abundance, blooms and shifts in seasonal cycles of dinoflagellates due to the increased stability of the water-column. Similarly, increases in coccolithophore blooms in the Barents Sea and HABs in the North Sea are associated with negative salinity anomalies and warmer temperatures leading to increased stratification (Edwards *et al*. 2006). It seems likely that an important environmental impact caused by climate change is an increase in the presence of haline stratification in regions susceptible to fresh-water inputs resulting in an increased potential for bloom formation. Other trends including anthropogenic pressures such as ocean acidification and eutrophication are summarised in the next section on applied ecological indicators of the NE Atlantic.


LEICA MZ6



Rapid optical assessment of zooplankton abundance

Introduction

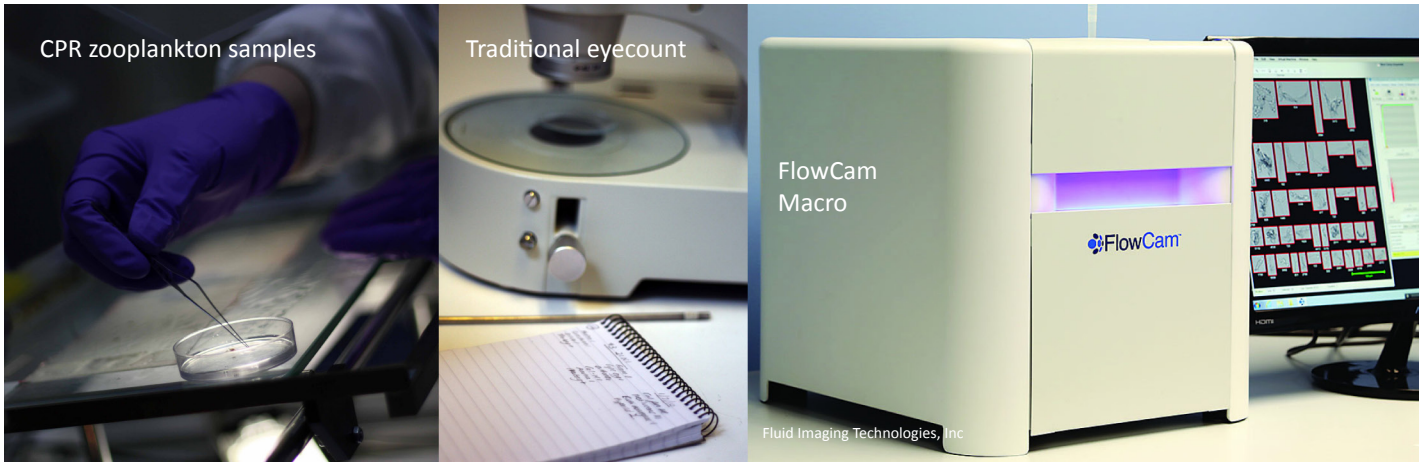
To further enhance its observational capabilities, SAHFOS is also exploring the latest in autonomous technology for rapid particle counting (abundance estimation) and discrimination (identification and speciation) in order to improve monitoring and reporting speed of zooplankton observations. As part of the AtlantOS project, SAHFOS are investigating the feasibility of using the new Fluid Imaging Inc. FlowCam Macro for the rapid determination of zooplankton abundance to complement the manual taxonomic analysis using conventional microscopy that the organisation traditionally undertakes. In this report we explore some of the initial development work that is being carried out to ascertain how the Flow Cam might be used for rapid zooplankton monitoring in order to complement traditional SAHFOS analysis.

Combining high speed imaging, flow cytometry and microscopy in a single unit, the FlowCam Macro is designed to automatically detect individual particles in an aqueous sample, take high resolution digital images of particles and derive more than 30 different types of measurements per particle. The main difference between the traditional FlowCam VS used in phytoplankton analysis and the FlowCam Macro (FCM) is the targeted size range, with the FCM aimed at the range between 50 micros and 5 mm which fits the size-range of the mesozooplankton. Parameters include count, size and volume and advanced, morphological measurements such as circle fit, perimeter and roughness. The system is capable of imaging and characterising thousands of particles per second in real-time and of differentiating particle types in a heterogeneous sample. Utilising image libraries containing similar particles types, the FlowCam can automatically identify and classify the particles as they are imaged.

Traditional CPR sample analysis is conducted in two stages to examine phytoplankton and zooplankton. For the zooplankton eyecount stage of traditional CPR analysis, identification and quantification is performed ‘off-silk’, all material ≥≈2 mm is removed from the filtering and covering silks, transferred to a Bogarov tray or watchglass and analysed using different microscopes than used for the phytoplankton and traverse zooplankton analysis stages. Both the very small sized phytoplankton and some of the very small microzooplankton stages of traditional CPR sample analysis could be considered semi-quantitative making direct comparison with FlowCam Macro counts problematic. Because the material identified and quantified for the zooplankton eyecount stage is removed from the silk, it presents a perfect opportunity to interpret the traditional process (where, once analysed, the eyecount material would be returned to the silk and the sample labelled, wrapped and stored) and analyse this material using FlowCam Macro. A proportion of the traverse zooplankton analysed using traditional methods fall below the 250µm lower recommended operational limit stated by the manufacturer. SAHFOS have investigated this lower detection limit and found acceptable particle identification down to 150µm for some CPR species / groups). For these reasons the decision was taken to focus on the zooplankton eyecount stage of traditional CPR analysis, where counts are fully-quantitative and the minimum particle size counted is ≈2 mm, therefore direct comparison is potentially achievable.

System Setup

A funnel is attached to the inlet tubing, which runs vertically down to the flow cell, held in place by the flow cell holder. Positioned to the right of the flow cell is the light source, and to the left is the fast repetition rate (FRR) camera. The outlet tubing then runs vertically downwards and turns 90° to exit the FlowCam Macro. The outlet tubing then passes through the peristaltic pump, and attached to the end is an inline 63µm mesh filter. The end of the tubing, including filter is placed inside a collection vessel to catch the sample in case of filter failure. Prior to running CPR samples through FlowCam Macro, a number of performance tests were undertaken to determine the most suitable hardware and software configurations, balancing ease of use, quality of image capture and reproducibility of results.



A sample of 63 adult stage VI *Calanus helgolandicus* (firstly counted and speciated by SAHFOS analysts) were analysed to investigate particle capture consistency. The sample was passed through the FlowCam Macro 10 times. Despite efforts to pass all particles through the FlowCam, filter the effluent and recapture all the particles, a discrepancy between input and re-captured particle numbers was observed between runs. The discrepancies were not consistent, indicating that the FlowCam was randomly retaining some particles within the fluidics system. On investigation, there appeared to be a number of reasons why this particle loss was occurring. Some were adhering to the tubing either on the line in or line out, making those particles unavailable for the next run, or released during a later run to further skew the data. Additionally, if this occurred on the line in, the result was no image capture for that run. Some particles were missed during input, and some were lost due to errors in post-run filtration before the next run began.

In an attempt to minimise these problems the setup described above was chosen. The line in and line out tubing was reduced to an absolute minimum to avoid particle adherence. A funnel delivery system allowed the line in to be vertical and of minimal length prior to the flowcell, adding gravity assist to particle flow and reducing turbulence, and ensuring all particles entered the system. Placing the peristaltic pump on the line out rather than the line in allowed the funnel delivery system to be used, and an inline filter captured all particles onto a small filter mesh allowing complete capture and ease of handling for the next sample run.

In a second experiment, a sample of 70 *Calanus* spp. were analysed to investigate the use of a sample injection system to ensure all available particles were passed through the system. Whilst this setup improved the time efficiency of sample handling prior to a sample run, loss of particles was still encountered and particle recovery post-analysis was cumbersome. Reproducibility of results was improved but ultimately use of this system was rejected because of the increase in post-analysis sample handling time. In a third test, a sample of 50 *Calanus* spp. were analysed to investigate the use of a funnel delivery system to ensure all available particles were passed through the system. An additional benefit of this setup was a significant reduction in fluidics path length between the point of particle introduction and the FlowCam Macro imaging flow cell. Whilst this setup improved sample handling prior to a sample run, loss of particles was still encountered and particle recovery remained cumbersome. This test illustrated that without a robust method of particle recovery, reproducibility of results could remain problematic. The funnel delivery system was accepted as the preferred method of sample introduction.

In further tests, a sample of 50 *Calanus* spp. were analysed to investigate the use of an inline post-analysis filtering system using a micron mesh. Loss of particles was virtually eliminated and particle recovery greatly improved. To achieve the closest possible correlation between number of particles introduced and number of particles imaged, a range of flow rates and camera frame rates were investigated. A high flow rate can be used with a high frame rate but the speed of sample throughput makes the processing of small volume samples

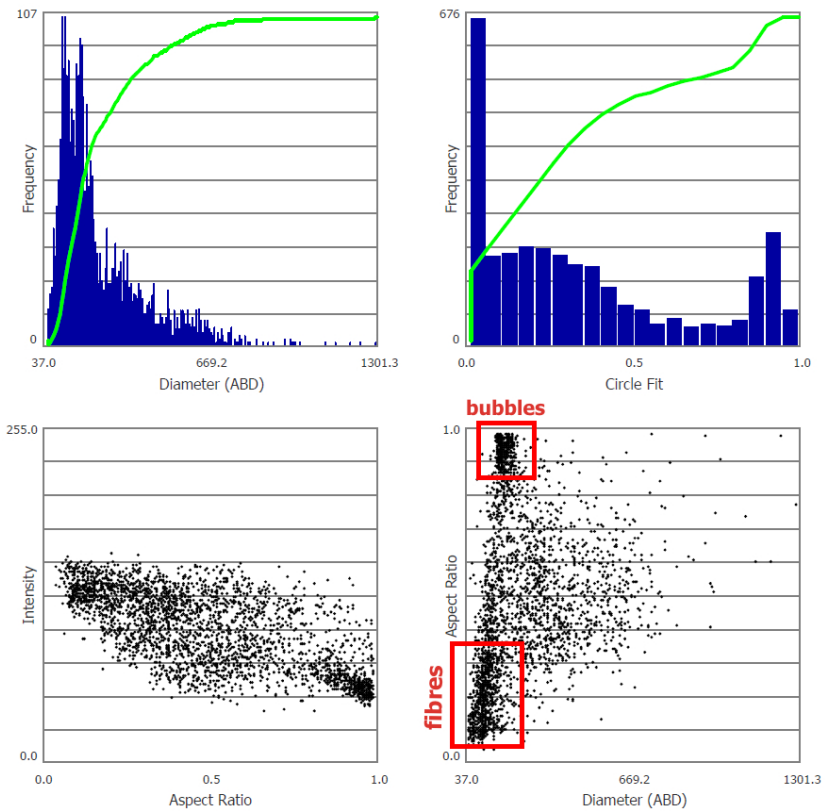


Fig 9: An example of bar and scatter plots of plankton sample particle properties. Aspect-Ratio versus diameter scatterplot shows the partitioning and clustering of bubbles and fibres. This is used to differentiate plankton from extraneous particles.

problematic. To overcome this, flow rate can be reduced but keeping the same frame rate can lead to the generation of duplicate images as particles are imaged multiple times as the pass through the flow cell. Reducing the frame rate to overcome this can lead to particles passing through the flow cell without being captured. Flow rates between 26-200 ml/min were investigated combined with frame rates between 1-40 FPS.

A sample of 10 *Calanus* spp. were passed through the system multiple times whilst changing the flow rate and frame rate until consistency of particle counts was achieved and missed / duplicate particles were reduced as far as possible during imaging. It is difficult to completely remove all risk of an underestimation of particle abundance (missed particles) or an overestimation (duplicate imaging of particles) using the FlowCam system. The decision was taken that it is favourable to generate some duplicate images which can potentially be

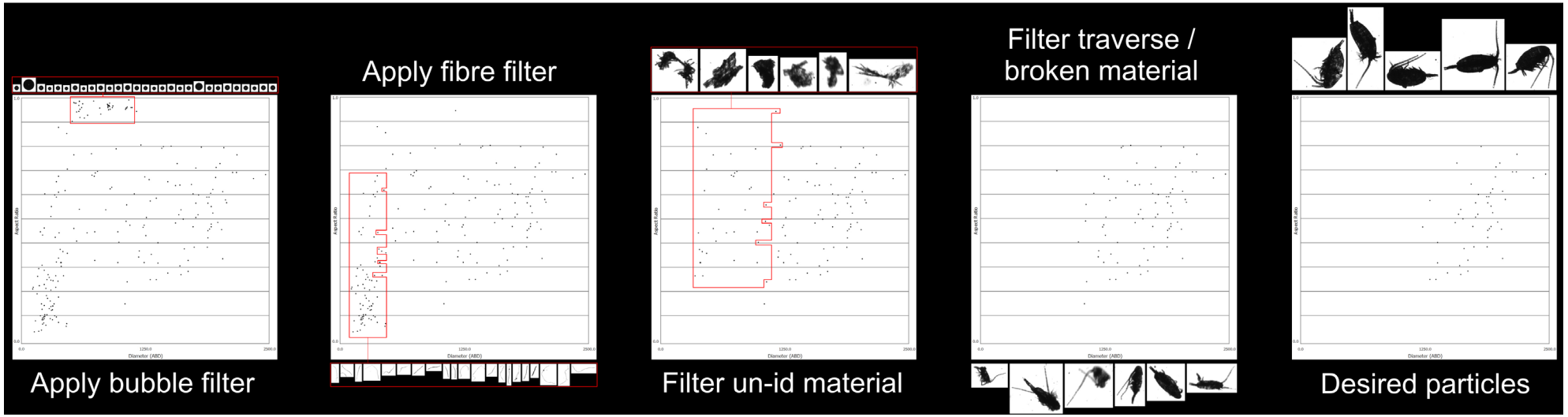


Fig. 10: An example of a CPR sample processed using the FlowCam Macro to first remove extraneous/unwanted particles by step 1 applying bubble and step 2 fibre filters. Step 3: removing unidenfied material. Step 4: splitting traverse and broken biological material. Step 5: Quantification and identification of desired zooplankton component.

removed from the data in post-processing than to miss particles completely.

Data Processing Methodology

Once the particle capture is completed, the first step is to remove unwanted particles such as air bubbles and fibres to leave a cleaner subset of images (Fig.9). Bubbles can be isolated using a number of particle properties – their aspect ratio, circularity and circularity (Hu) is close to 1.00 therefore within a sample they can be ranked accordingly using any of these properties and removed. With fibres, in regards to particle properties, the reverse is true – their aspect ratio, circularity and circularity (Hu) are usually in the range 0.01-0.10 and again, within a sample they can be ranked accordingly using any of these properties and removed.

For the benefits of this comparison of analysis methods, all particles significantly smaller (<1000µm) than the zooplankton eyecount minimum size value of 2mm can then be separated from the dataset, leaving a subset containing only the larger zooplankton traverse and the desired zooplankton eyecount images. Duplicate images are generally easy to identify and remove by using a combination of the particle I.D. number (sequential numbering of captured particles) and comparing particle properties, with a visual check to confirm. The remaining cleaner subset of images can then be ranked using any number of available particle properties in an attempt to show differences between taxonomic groups, genera and species (Fig.10). A combination of this ranking and expert taxonomic analysis can then be used to identify and count the particles. As the different sections of the training samples are classified, the resulting images and their particle properties can then be used to create reference libraries with which to interrogate other datasets. Once the above steps are observed to be robust, they can be employed in advance to automatically remove, group or identify particles as desired. Circularity / aspect ratio / image library filters can be pre-selected to remove bubbles and fibres, and a minimum particle size limit set so that all particles below a threshold are not captured. The result is a subset of all potential particles, containing only those particles with a realistic chance of identification and classification. This subset can automatically interrogate any pre-selected image libraries in an attempt to best-fit the remaining particles into taxonomic groups / genera or species (Fig.11).

At present FlowCam Macro is not a complete replacement for traditional CPR analysis which currently identifies ~1000 taxonomic entities many to species level. For example, subtle morphological differences between important indicator species such as *Calanus helgolandicus* and *Calanus finmarchicus* are unlikely to be visible on imaged particles. On occasion when these features are visible, they will not produce a difference in particle statistics that allows for these species to be separated.

A combination of traditional microscopic analysis to determine species ratios within a sample, combined with rapid assessment of abundance/biomass using FlowCam Macro

could be used to reach a more satisfactory result. The bulk categories that exist within the CPR database present an opportunity to provide meaningful data from FlowCam Macro that could be incorporated into CPR datasets and time series. Where particle identification cannot be taken to species, genus level or higher taxonomic groups can provide a directly comparable category between the two analysis methods, for example:

- For a number of taxonomic groups, such as the Euphausidae and Hyperidae, traditional CPR sample analysis usually does not attempt to speciate observed organisms. Automatic particle classification to this higher level should be possible, although confirmation of this should be achieved by the processing of a larger number of CPR samples.

- For a number of organisms identified to species-level by the CPR survey (particularly within the Copepoda), categories exist within the database that sit above the species-level (i.e. *Calanus* V-VI Total) which should allow direct comparison between traditional analysis counts and FlowCam Macro counts. Automatic particle classification to these categories should be possible, although again, confirmation of this should be achieved by the processing of a larger number of CPR samples.

- With further development and testing FlowCam Macro should be able to provide a number of zooplankton metrics/indices in a more rapid manner including estimates of biomass/ biovolume, size-ranges of community; higher taxonomic level biodiversity data and coarse functional type based information.

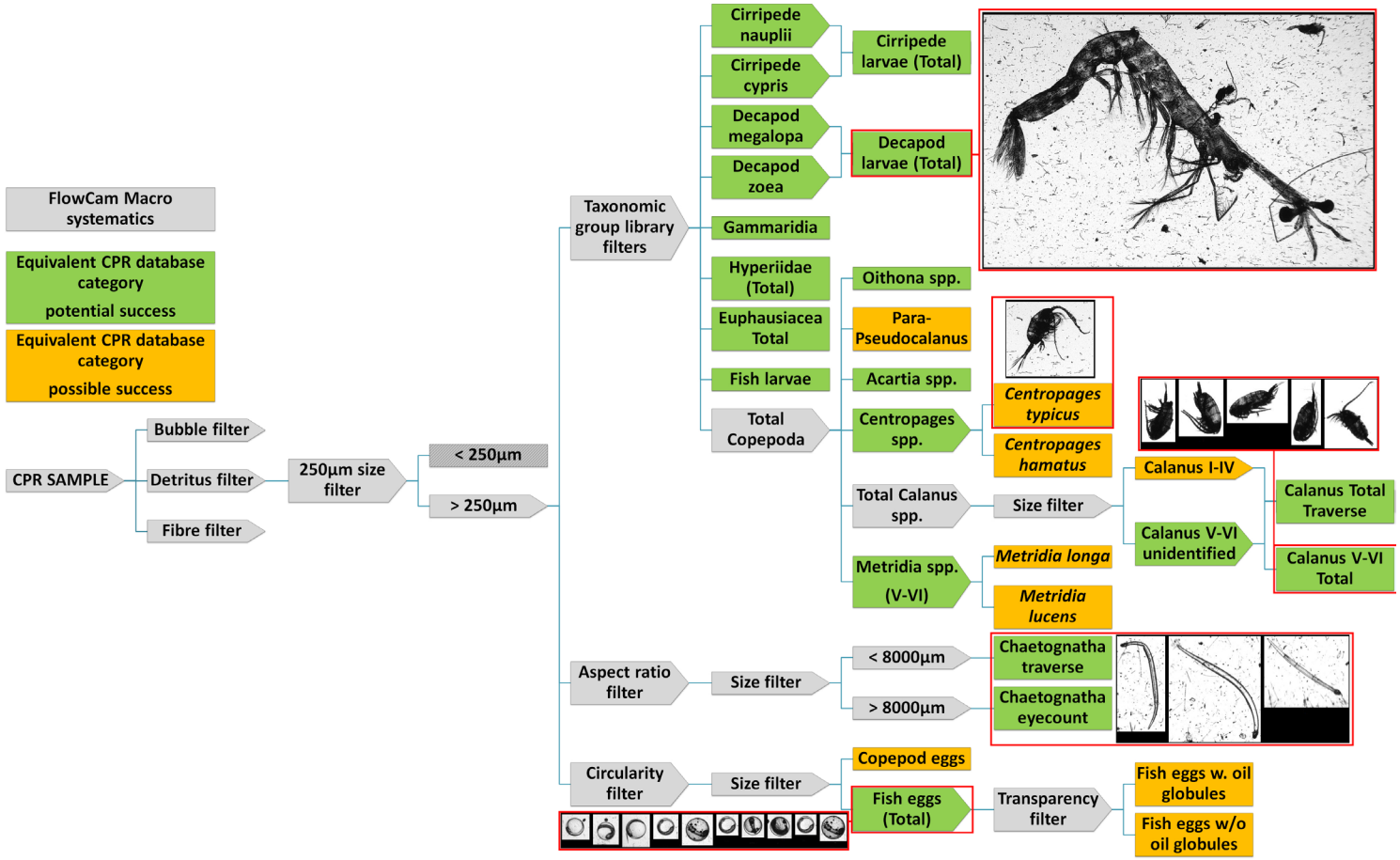


Fig 11: A schematic of how to process a CPR sample using the Flowcam Macro based on a hierarchical approach and employing both a taxonomic library filter and a hard property filters.

In summary, the FlowCam Macro has proven to consistently produce high quality images of the main components of the mesozooplankton including euphausiids, decapods, copepods and hyperiids. The information obtained from samples run on the FlowCam could compliment and contribute to the marine observation work carried out by SAHFOS and the collection of bulk zooplankton data needed to support the AtlantOS project in answering challenging questions about the impact of climate change on marine ecosystems. Rapidly and automatically determining the abundance and bio-volume of different zooplankton improves calculations of total carbon concentrations and estimates of carbon transport from the surface to the deep sea. The speed, efficiency and reliability of data acquisition are paramount and automated systems such as the FlowCam are helping to accelerate the pace of research into the health of fundamental components of the marine ecosystem. Ongoing tests and research at SAHFOS will further investigate the potential of the FlowCam to obtain fast and reliable estimates of zooplankton biomass and other plankton metrics.

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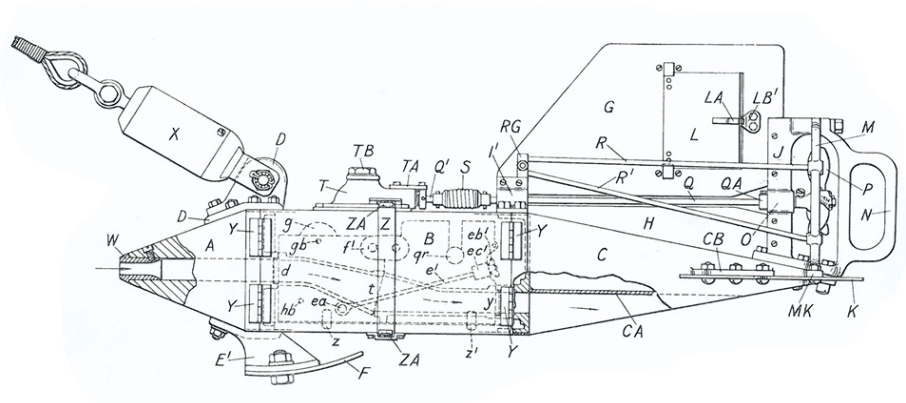

The Sir Alister Hardy Foundation for Ocean Science (SAHFOS) is an internationally funded independent research organisation (Canada, Norway, UK and the USA) that operates the Continuous Plankton Recorder (CPR) survey. The Foundation has been collecting data from the North Atlantic and the North Sea on biogeography and ecology of plankton since 1931. More recently, work has been expanded to include other regions and organisations around the globe to create a global cooperative. The results of the survey are used by marine biologists, scientific institutes and in environmental change studies across the world. The SAHFOS team is based in Plymouth, England and consists of analysts, technicians, researchers and administrators, who all play an integral part in the running of the survey.

AtlantOS is an EU research and innovation project that proposes the integration of ocean observing activities across all disciplines for the Atlantic. The vision of AtlantOS is to improve and innovate Atlantic observing by using the Framework of Ocean Observing to obtain an international, more sustainable, more efficient, more integrated, and fit-for-purpose system. The overarching target of the AtlantOS initiative is to deliver an advanced framework for the development of an integrated Atlantic Ocean Observing System that goes beyond the state-of –the-art, and leaves a legacy of sustainability after the life of the project. The project consists of 62 (research institutes, universities, marine service providers, international partners, private sector) from 18 countries (13 EU & 5 non-EU) plus supporters.

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