# Delivrable N° 4.2 : **Protocols implemented for plankton monitoring**

**Projet:** FED/2018/402-604 - DEMERSTEM, DEMERsal ecosySTEMs
**Décision:** FED/2017/038-922 - Improved regional fisheries governance in Western Africa (PESCAO)

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**Protocols implemented for plankton monitoring within the DEMERSTEM project**

A standardised monitoring of phytoplankton and zooplankton was implemented within the project DEMERSTEM, in order to obtain quality data useful for the assessment of NW African plankton populations. Here below the adopted sampling protocols and analysis procedures are summarised. Detailed methodological protocols and procedures, together with additional resources for the taxonomic identification of main planktonic species, will be also made available through the SZN website (https://marine-observatory.szn.it/north-atlantic-cclme/).

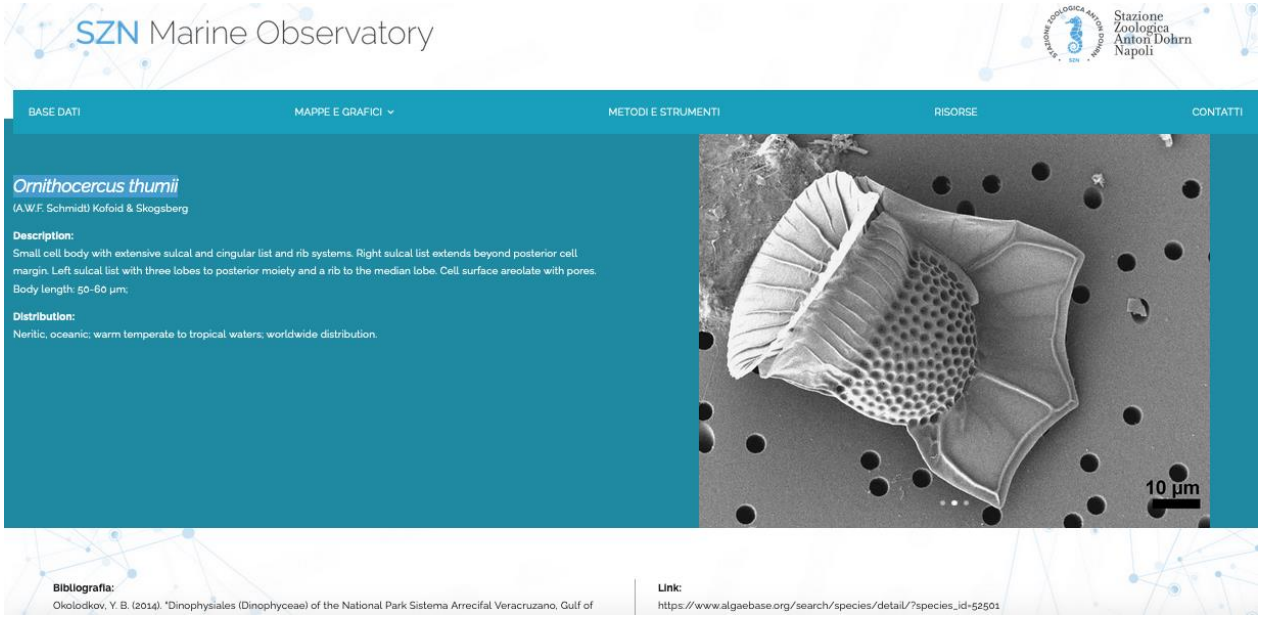


Figure 1. Key information on the taxonomy and ecology of the phytoplankton species *Ornithocercus thumii* available at SZN webpage https://marine-observatory.szn.it/north-atlantic-cclme/.

PLANKTON SAMPLING

Phytoplankton

Phytoplankton samples were collected by vertical tows using a net with a conical shape, a 40 cm diameter mouth and a 20 µm mesh size, equipped at the end with a tap (Fig. 2a-b).


Figure 2. Plankton nets used in the DEMERSTEM Cruises. Phytoplankton net with weight attached at the bottom (A) and detail of the collector, with tap in closed position (B); Zooplankton WP2 net with weight attached at the bottom (C) and detail of the collector, with rectangular mesh window (D).

In deep stations, where the bottom was ≥200m, the net was towed between 200m depth and the surface, otherwise it was towed from the bottom to the surface.

Once the net was recovered on board, the sample gathered in the collector was poured into a plastic bottle (500 ml) containing 10 ml of 37% buffered formalin (2% final concentration of formaldehyde), stored in a cool place and then at 4°C until microscope examination.

Zooplankton

Mesozooplankton samples were collected by vertical tows using a WP2 standard net, with a 57cm diameter mouth and 200 µm mesh size, equipped with a filtering cod-end (Fig. 2c). Once the net was recovered on board, the sample gathered in the collector was poured into a plastic bottle (500 ml), paying attention to carefully wash the cod-end and its rectangular mesh window (Fig. 2d), in order to collect all the organisms attached to it. 37% buffered formalin (20 ml) was added to fix the sample at 4% final concentration of formaldehyde. Samples were stored in a cool place and then at 4°C until microscope examination.

Large jellyfish

Scyphozoan jellyfish sampled by fish trawls were counted and weighted (wet weight) directly on board. Pictures of each scyphozoan species were taken and their semi-quantitative number was recorded in terms of class of abundance (Frontier, 1969) (Fig.3). Whenever possible, bell diameter was measured in at least ~10-20 individuals of each species. Selected specimens (whole individual and/or a fragment of the umbrella including gonads) were also preserved in ethanol 97% or 99%, and stored in a cool place, for future molecular analysis.

**CAMPAGNE 2020 PLATEAU GUINEEN. MÉDUSES DANS LES FILETS DE PÊCHES**

| Station de pêche | Date | Heure | Méduses (espèces/type) | Classe d'abondance | Numéro d'individus | Photo (nom du fichier) | Echantillons en éthanol pour analyse génétique) |
|---|---|---|---|---|---|---|---|
| 94 | 25/9/2020 | 11:43 | Type 1 | 1 | 1-3 | St.94_Type1.jpg | Oui (1 flacon) |
| 94 | 25/9/2020 | 11:43 | Type 2 | 3 | 18-80 | St.94_Type2.jpg | Oui (2 flacons) |
| 96 | 26/9/2020 | 16:15 | Type 2 | 4 | 80-350 | St.96_Type2.jpg | No |

| Classes d'abondance | Numéro d'individus |
|---|---|
| 1 | 1-3 |
| 2 | 4-17 |
| 3 | 18-80 |
| 4 | 80-350 |
| 5 | 350-1500 |
| 6 | 1500-6500 |

Figure 3. Example of annotation of scyphozoan jellyfish onboard the DEMERSTEM Cruises. Classes of abundance (class 1=1-3 individuals; class 2= 4-17 individuals; class 3= 18-80 individuals etc.) according to Frontiers, 1969, are indicated per jellyfish species in each tow.

PLANKTON ANALYSIS

Phytoplankton

Phytoplankton net samples were analysed by light microscopy, at 40x magnification, using an inverted light microscope Zeiss Axiovert 200 (Carl Zeiss, Germany) equipped with a Zeiss Axiocam digital camera in order to estimate phytoplankton relative abundance (%). Variable amount (0.5-3 ml) of the sample were allowed to settle in sedimentation chambers for at least a couple of hours. Specimens' identification and counts were performed according to the Utermöhl method (Edler and Elbraechter, 2010). Cells falling in 1 or 2 transect of the sedimentation chamber were identified and enumerated in order to estimate the relative abundance of phytoplankton taxa. Taxonomic identification was performed at the species level whenever possible according to the most relevant and updated taxonomic literature (e.g., Tomas 1997) and other specific literature.

Selected net samples were examined using scanning electron microscopy and/or transmission electron microscopy for species identifications.

For the examination of diatoms in TEM, samples were treated with nitric and sulphuric acids (1:1:4, sample:$HNO_3$:$H2SO_4$), boiled for some seconds in order to remove the organic matter and washed with distilled water. Acid-cleaned material was mounted on Formvar-coated grids. All grids were observed with a Philips 400 TEM (Philips Electron Optics BV, Eindhoven, Netherlands).

For scanning microscope observation samples were placed on 3µm-pore size nucleopore filters, washed in distilled water, dehydrated in an ethanol series (25%, 50%, 75%, 95% and 100%) and critical point-dried. Dried filters were mounted on stubs, sputter-coated with gold-palladium and observed using a JSM 6700F (JEOL Ltd, Tokyo, Japan). Some acid-cleaned material was also directly mounted on stub and processed as above for SEM examination.

Zooplankton

Fixed zooplankton samples were filtered using a 100 µm mesh net and re-suspended in filtered sea water up to 200 ml volume to get rid of formaldehyde. Two aliquots of 5ml each were then taken after careful mixing using a large-bore graduated pipette.

Each aliquot was transferred in a mini-Bogorov chamber (10 ml) (Fig.4) and examined under a stereomicroscope Leica MZ125 at 0.8x and 10x magnification, for the identification

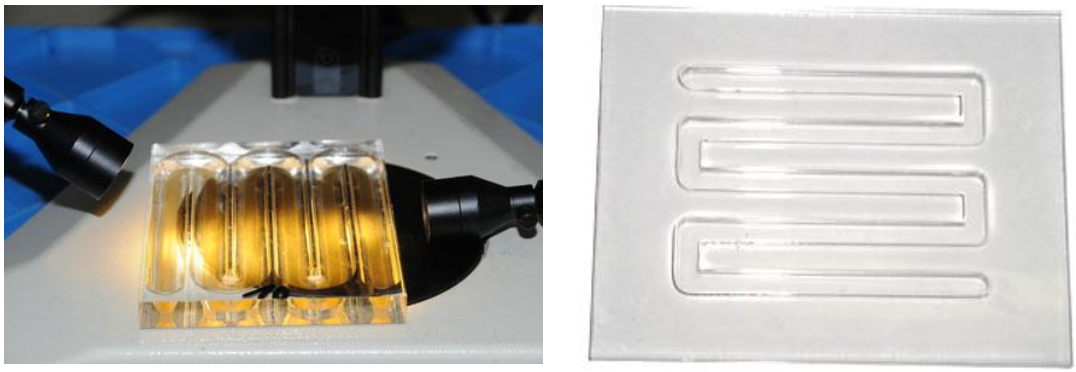

Figure 4. Mini-Bogorov chamber under the binocular (left panel) and in frontal view (right panel). The Mini-Bogorov chamber is a rectangular plastic tray (8 X 70 X 40 mm), with a 6 mm deep path.

and count of all zooplankters in it. A microscope Zeiss Axioskop 2 Plus (10x, 20x and 40x magnification) was also used to verify morphological details needed for the taxonomic identification of the species.
Counts obtained from the analysis of the aliquots were then reported to the total volume. Gelatinous zooplankton species, in particular hydrozoans, ctenophores, pteropods, pelagic tunicates and fish larvae, were identified and counted in the whole sample.
Taxonomic identification of zooplankton was performed at the species level, whenever possible according to the most relevant and updated taxonomic literature.

## List of References

Edler, L. and M. Elbrächter (2010). The Utermöhl method for quantitative phytoplankton analysis. Microscopic and Molecular Methods for Quantitative Phytoplankton Analysis. B. Karlson, C. K. Cusack and E. Bresnan. Paris, UNESCO. 55: 13-20.

Frontier, S. (1969.) Calcul de l'erreur sur un comptage de zooplankton. Journal of Experimental Marine Biology and Ecology, Vol3: 18-26.

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