

# COLLECTION STANDARD OPERATING PROCEDURE

## Zooplankton

### 1 Sample collection

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- Zooplankton is collected using a double WP2 plankton net.
- Each hoop is equipped with a WP2 net with 0.25 m<sup>2</sup> aperture and 200 µm mesh size (Fig. 1)
- This double WP2 plankton net allows duplicate samples in one tow.



*Figure 1 Zooplankton collection using WP2 plankton net*

- The net is towed vertically from 200 m depth to surface at the NEREA\_CAPRI station and from 50 m depth to surface at the NEREA\_MC station, while it is towed obliquely from 15 m depth to surface at the NEREA\_SARNO station
- A mechanic flux meter is used to calculate the water passed through the net
- The sample is collected in a 500 mL plexiglas filtering cod-end (Fig. 2)
- The sample is then disposed into clean plastic containers (500 ml) (Fig. 2)



*Figure 2 Transfer of samples into clean plastics containers*

## 2 SAMPLE PROCESSING

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Sample processing is done as soon as possible.

Of the two replicate samples one is used for community metabarcoding, metagenomics and metatranscriptomics (metaB/G/T), the other for Reference Library generation and qualitative/quantitative analyses

For community meta B/G/T:

- The sample is concentrated on a 2000  $\mu\text{m}$  mesh-size sieve (Fig.3)
- The concentrated material on the sieve is split into three subsamples using a metal spatula previously cleaned with Ethanol
- Each subsample is then disposed into 2 ml cryovials
- Cryovials are immediately frozen in liquid nitrogen on board and later stored at  $-80^{\circ}\text{C}$



*Figure 3 Sample passed through 2000  $\mu\text{m}$  sieve*

For Reference Library construction, and quali-quantitative analysis:

- The net sample is split in two equal subsamples of 250 ml each and disposed into clean plastic containers
- Each sample is concentrated on a 200 µm mesh size sieve
- Each concentrated material is then disposed in a plastic container with 95% ethanol using a squirt bottle and a clean plastic funnel
- One subsample is used for isolating selected organisms for reference library construction, the other for counts and species determination
- Samples are preserved in 95% ethanol in the fridge

### 3 List of materials, equipment and supplies

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- WP2 plankton net, with 0.25 m<sup>2</sup> aperture and 200 µm mesh size, equipped with a mechanic flux meter
- a 2000 µm mesh-size sieve
- a metal spatula
- a squirt bottle with 95% ethanol
- three 2 ml cryovials
- container with liquid nitrogen
- two 250 ml plastic bottles
- two 100 ml plastic bottles
- a clean plastic funnel
- a 200 µm mesh size sieve