Abundance of fungal hyphae din seawater by epifluorescense microscopy using Calcofluor White stain method

Prepared by M.H. Gutiérrez, May 2018

- To collect samples in sterile 50 mL polypropylene tubes and fix with formaldehyde or glutaraldehyde (2% final concentration). Store samples at 4 °C in the dark.

- To filter 5 to 30 mL (depends on the environment) of seawater by 0.22-µm mesh black 25 mm diameter polycarbonate filters (Millipore Corp.).

- To stain filters with the retained material directly with 600 µL of aqueous 0.1% Calcofluor White, making sure to cover the entire area of the filter. After a few minutes (5-10 m) use vacuum to remove the excess of stain from the filter. Avoid any light source.

- Place the filter (sample side up) onto slide and add 1 drop of non-fluorescent immersion oil on top of the filter and cover with a cover slip.

- The slides can be counted immediately on the epifluorescent microscope or stored frozen (-20 °C).

- In a epifluorescence microscope equipped with UV filter (used for DAPI, eg. filter set 49 Carl Zeiss Ltd., 365 nm excitation and 445-450 nm emission band pass) to examine at 1000X the entire effective area of the filters.

- To count all hyphae identified and record their length and width, use cylinder volume as a morphological approximation to estimate the biovolume of fungal filaments. Biomass can be estimated based from biomass:biovolume ratios described fro fungi (Newell and Statzell-Tallman, 1982).

References

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