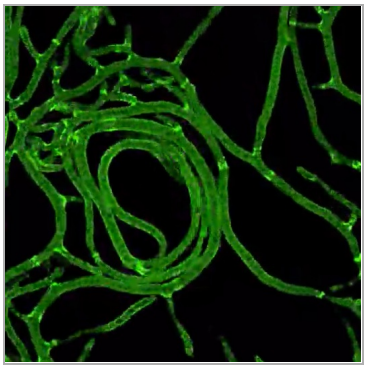

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We use this protocol and it's working

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Abundance of fungal hyphae in seawater by epifluorescence microscopy using Calcofluor White stain method

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ABSTRACT

Protocol to stain fungal hyphae from seawater with Calcofluor White.

ATTACHMENTS

Abundance of fungi in seawater.pdf

GUIDELINES

References

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SAFETY WARNINGS

See SDS (Safety Data Sheet) for hazards and safety guidelines.

Collection

1 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.

Filtration

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

Stain

3 Stain filters with the retained material from filtration by applying 600 µL of aqueous 0.1% Calcofluor White directly to sample and filter, making sure to cover the entire area of the filter.

Allow 00:05:00 to 00:10:00 then use vacuum to remove the excess stain from the filter. **Avoid any light source.**

Abundance

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

5 Count slides immediately on epifluorescent microscope or store at -20 °C.

6 Use an 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.

7 Count all hyphae identified and record their length and width. Use cylinder volume as a

morphological approximation to estimate the biovolume of fungal filaments.

Note

Biomass can be estimated based from biomass: biovolume ratios described from fungi (Newell and Statzell-Tallman, 1982).