ABSTRACT

Protocol to stain fungal hyphae from seawater with Calcofluor White.

ATTACHMENTS

Abundance of fungi in seawater.pdf

GUIDELINES

References


SAFETY WARNINGS

See SDS (Safety Data Sheet) for hazards and safety guidelines.
**Protocol Integer ID:**
17442

**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, e.g. 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).