

Maintenance of marine ciliates

PREAMBLE. The methods outlined below are based on those routinely employed at the Culture Collection of Algae and Protozoa (CCAP).

Planktonic ciliates are important in the transfer of material through coastal food webs; they act as a link between small phytoplankton and larger zooplankton. Ciliates graze between 30 - 50 % of primary production by microalgae and cyanobacteria in many temperate coastal waters (Pierce & Turner 1992). Furthermore, marine ciliates form a diverse assemblage, and different species may primary and/or secondary consumers (Pierce & Turner 1992).

1. Prepare and sterilise medium in advance, aseptically decant sterile medium into tissue culture flasks (50 ml), adding 20-25 ml per flask. For all strains, a surface sterilised (by boiling for ~1 min) wheat grain should be added to the medium.

Alternative culture vessels, such as test-tubes, Petri dishes etc., may be used, but cultures in tissue culture flasks remain viable for longer, are easier to check by microscopy and are more suitable for transportation.

Medium recipe:

<http://www.ccap.ac.uk/media/documents/ASWP.pdf>

Addition of a surface sterilised, previously dry, grain ensures a slow release of carbon/nutrients into the medium, thus facilitating steady growth of "food" bacteria / other smaller protists. If possible grains that have not been treated with any pesticides etc. should be used.

2. Select a dense culture from existing stocks. The state of a culture is ascertained by examination of the culture using an inverted microscope (100 - 200 x magnification) under phase contrast.

In general cultures where actively motile trophs are readily observed will be selected as an inoculum. Stability of ciliate cultures varies between taxa and even between strains, and cultures chosen to provide the inoculum for sub-culture will normally be 4 -20 weeks old.

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Protists: Preferably there should be only one ciliate in the culture, or an environmental sample containing significant numbers of the target protists.

Apparatus: a class I biological safety cabinet; temperature controlled incubator; inverted microscope with 100-500 x magnification equipped with phase-contrast and bright field optics.

Cell culture medium: ASWP, or other appropriate medium.

Plasticware: Tissue culture flasks; sterile disposable pipettes; sterile loops.

Chemicals used routinely are of Analar grade purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Day JG, Achilles-Day UEM, Brown S & Warren A (2007) Cultivation of algae and protozoa. In: manual of environmental microbiology. Hurst CJ, Kudsens GR, McInerney MJ, Stezenbach LD & Walter MV (eds). ASM Press, Washington DC. pp 79-92.

Pierce RW & Turner JT (1992) Ecology of planktonic ciliates in marine food webs. *Reviews in Aquat Sci* 6:139-181

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3. To sub-culture, ensure uniform distribution of the ciliate trophs by inverting the tissue culture flasks several times. Then aseptically pour an inoculum (>5 ml) into the new culture vessel.

Always ensure that the tissue culture flasks are fully labelled with the organisms' name, strain designation, date of inoculum and medium prior to transferring the inoculum.

The food source for the ciliates is usually the (unidentified) bacteria and other microorganisms with which the strains were isolated originally. These bacteria and smaller protists are co-transferred each time a strain is sub-cultured, and multiply on the agar surface.

4. Incubate inoculated cultures static, in the dark or low light, in an appropriate incubator under a controlled temperature regime.

For most marine taxa 15°C is the most appropriate temperature; however, for polar isolates lower temperatures (5-10°C) may be optimal and for hypersaline, thermophilic taxa 35-40°C may be optimal.