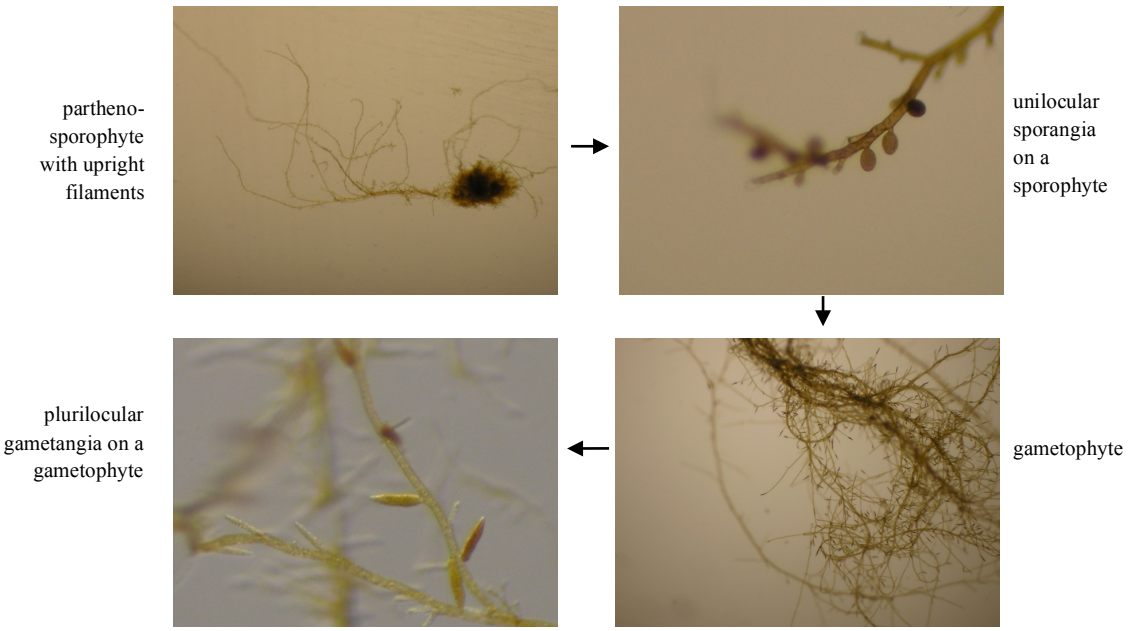
*I*solation *of* Ectocarpus *gametophytes*

Akira Peters, Laurence Dartevelle
Station Biologique de Roscoff, France

Isolation of unilocular sporangia from sporophytes

1. Cultivate sporophytes or parthenosporophytes at low density (e.g. 1-3 individuals per 55 mm Petri dish) and check for the presence of upright filaments which are required for the production of unilocular sporangia. If upright filaments have not yet developed, change the medium frequently until the uprights appear. Stop the medium changes once the uprights appear. Often, starting from minute thalli and cultivating under strong white light (30-40 $\mu molm^{-2}s^{-1}$) is helpful. Certain strains (e.g. those carrying the *imm* mutation) form uprights more readily than others. Note that at 13 ºC the unilocular sporangia develop on the erect thalli about a week after the plurilocular sporangia.



2. Prepare 55 mm Petri dishes with a coverslip stuck to the bottom by placing the cover slip on a small drop of Provosoli enriched seawater (PES). Place ≥4 additional drops of medium around rim of dish to provide some extra humidity.

3. Cut sections of upright filament carrying a single (if required, e.g. for tetrad analysis) or several unilocular sporangia using the broken end of a Pasteur pipette. Be careful not to include any plurilocular sporangia.

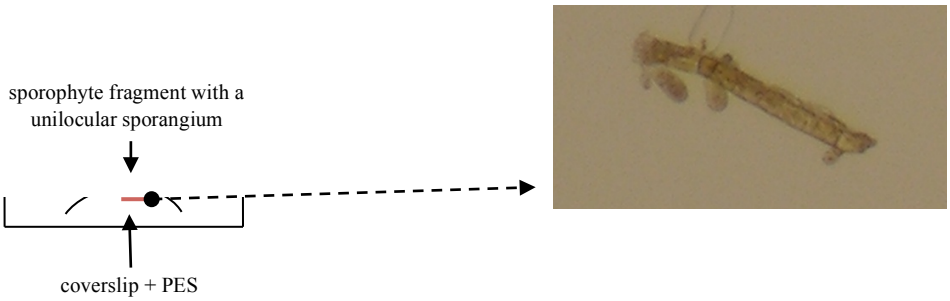
ASSEMBLE
ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES

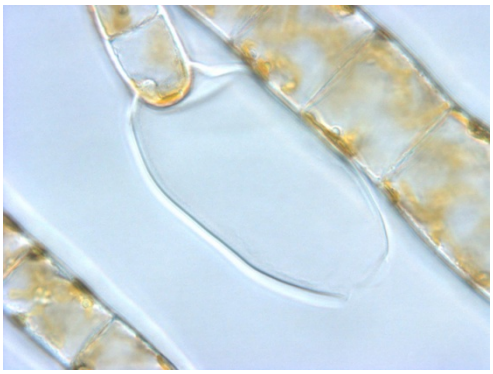
4. Transfer the fragment with the Pasteur pipette to a drop of seawater on the coverslip. It may be necessary to remove the fragment with forceps to avoid it being contaminated with other fragments of filament, etc.

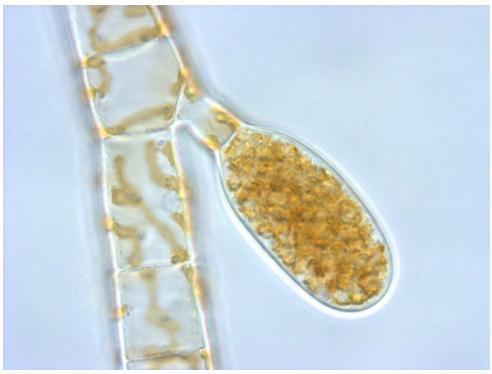
5. If required, add a drop of PES to cover the dissected fragment, seal the dish with Parafilm and incubate at 13°C. The additional drops of medium around the rim of the dish help avoid drying-out of the drop containing the unilocular sporangia. Meio-spores should be released within 1-3 days. If release does not occur, discard the material.

6. When release has occurred, remove the fragment with forceps and add enough PES to half fill the Petri dish.

7. Incubate at 13°C. The gametophytes should be large enough to isolate after about 2 weeks in culture.







Isolation of gametophytes

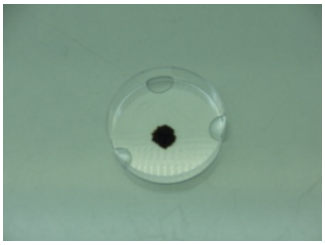
8. As soon as the gametophytes become visible under the binocular microscope (0.2 mm), transfer them to 140 mm Petri dishes (ca. 100 ml) using forceps or a Pasteur pipette (1 gametophyte per 10 ml is fine).

9. After a few weeks, when the plurilocular gametangia become dark, the gametes are ready to be released.

Gamete release

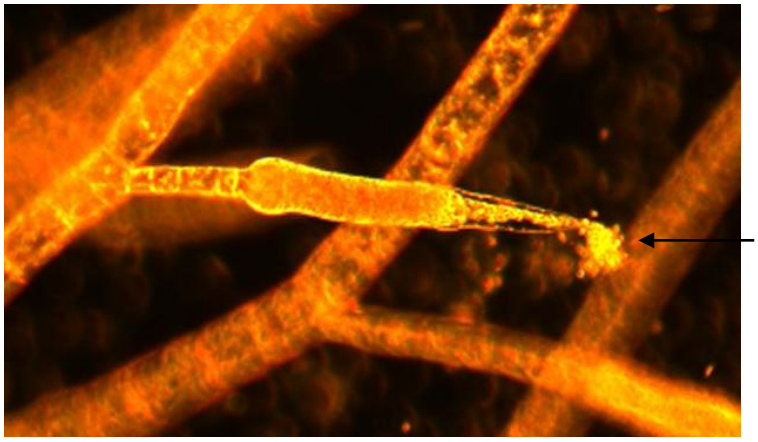
10. Simulate a low tide the day before the release by placing the gametophytes in a 55 mm Petri dish. Clump the material together and remove any excess seawater. Spot 3 drops of PES around the clump to provide some humidity.

11. Seal the dish with parafilm and place in the dark at 13°C for 10 to 18 hours (this time can be reduced to 4 hours if necessary)

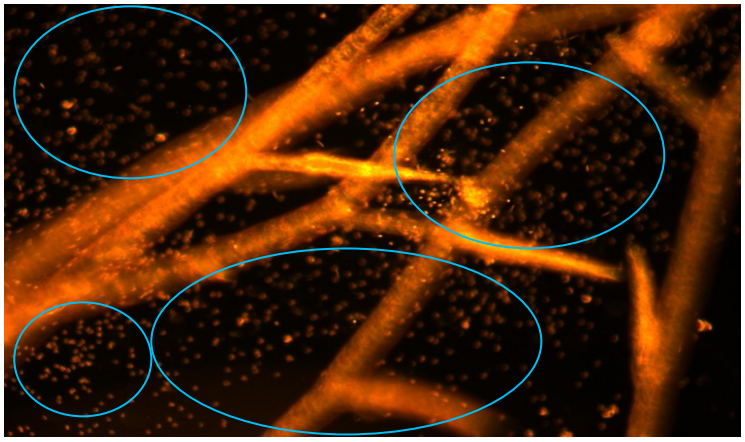


12. Simulate a high tide the day of the release by adding between 300 µl and 500 µl of PES (depending on the amount of material).

13. Wait 5 min then check for release under the binocular microscope. Do not wait longer than 20 min as the gametes may start to adhere to the bottom of the dish. The gametes can be harvested with a pipette.



plurilocular gametangium releasing gametes



released gametes

14. If the gametes are to be frozen, induce release by adding as little PES as possible. Pipette the gametes into an Eppendorf tube and centrifuge at 4°C and 12500 rpm for 5 minutes (repeat the centrifugation until a pellet forms). Remove the supernatant, freeze in liquid nitrogen and store at -80°C.

Production of parthenosporophytes

15. Pipette gametes into a 140 mm Petri dish. The number of gametes per dish can be estimated using a Neubauer cell.

16. Incubate at 13°C under normal light conditions. Change the medium every 2-4 weeks for optimal growth.