HANDBOOK FOR THE CULTURE OF *MACROCYSTIS PYRIFERA*

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INSTRUCTIONS for culture of *Macrocystis* spp.

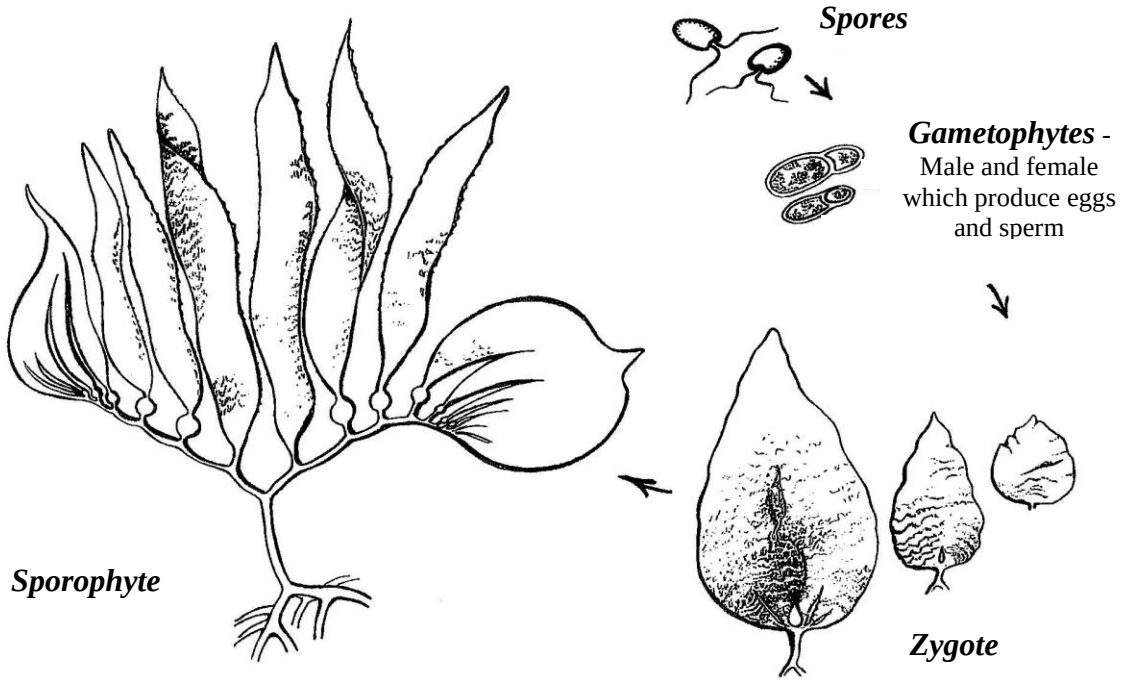
The aim of this handbook is to present methods for culture of *Macrocystis* species so that community groups or other interested parties can ‘grow their own’. This handbook follows on from and should be read in conjunction with the '*Macrocystis* Transplant Handbook'.

INTRODUCTION

The lifecycle of *Macrocystis pyrifera* has two phases, similar to ferns. One is the large plant we can see; the second is a microscopic filamentous phase.

The large plant (sporophyte) gives rise to motile spores. The spores are released from specialised blades near the base of the plant. These blades are readily identifiable, as they do not have vesicles (bubbles/bladders) at their base near the stem.

In Tasmania, plants have sporophylls all year round so are likely to have the capacity to release spores throughout the year although the quality and quantity is likely to vary with season. The spores are very small with a diameter of less than 10µm. 'Ripe' sporophylls are identifiable by a raised surface, which has a furry appearance and is darker in colour, but lighter and tattered where spores have been released.



Studies in California show spores can be motile for as much as 24 hours. Once landed on suitable substrate, the spores develop into microscopic filamentous plants called gametophytes. Gametophytes are believed to be hardier than the parent plants, able to resist adverse conditions such as higher temperatures and low nutrient concentrations.

Studies in closely related species have revealed that gametophytes can survive lengthy periods (more than 8 months) without light. This means that they can easily be transported within ship's hulls such as for the introduced alga *Undaria pinnatifida*. A

study currently (1999) underway in Mexico is examining the potential for gametophytes of *Macrocystis pyrifera* to remain dormant for a number of years until conditions are suitable and then give rise to the parent plants. This may explain why beds appear to spring spontaneously in areas where there have been none previously for a number of years.

The gametophytes produce gametes – a motile male spore (approx. 10μm in diameter) and a larger female egg. Because gametophytes are either male or female, chances for fertilisation success is enhanced when the male and female gametophytes are in close proximity. This means that fertilisation success is usually greatest close to the parent plant/plants as the further away from the parent plant, the further the gametophytes are likely to be from each other. They rarely produce plants further away than 5m from the source plant.

CULTURE

One of the primary aims when culturing *Macrocystis* and other algae from spores is to maintain conditions that are as clean and sterile as possible. This limits the chances of other algae and microorganisms being initiated within the cultures that would then compete for space, light and nutrients.

CULTURE PREMISES

Culturing should be done where there is plenty of bench space. This area should be cleaned prior to and during spore release. Wiping with alcohol (70% isopropanol or ethanol) or a chlorine solution (approx. 400ppm available chlorine) ensures minimal possible accidental infection from either other algae or bacteria. Where the intended culture areas are close to the coast or flowing seawater – extra care should be taken, as some algae can be transported through the air. Ideally all work should be done in a laminar flow cabinet. This ensures there is minimal risk of contamination from air borne algae or bacteria.

CONTAINERS FOR CULTURE

Glass culture vessels are the easiest to keep clean, however as the intention is to grow the alga in large quantities, plastic and polyetheylene containers can be used. I am currently using cheap 15l plastic see-through vessels with lids obtained from a discount shop, and similar can be found at the local supermarket. The advantage of having see-through lids is that is possible to provide light for growth whilst minimising the chances of contamination through airborne algae; and loss of seawater through evaporation; while allowing light through for growth of the algae.

STERILISATION OF SEAWATER

Common practice for sterilisation of seawater is to pass the seawater through successively finer filters with the aim of excluding all particles greater than 0.2 - 0.5 μm in diameter. This effectively keeps out all organisms with a cellular structure ie phytoplankton and potentially herbiverous zooplankton without significantly altering the chemistry of the seawater.

Other methods for the sterilisation of seawater include autoclaving, boiling, the addition of chlorine and then neutralisation with sodium thiosulfate and exposure to UV light (up to 1200 W).

Heating to 73^{o}C usually is effective for removing algal contaminates, heating to 73^{o}C on three successive days with intermittent cooling at room temperature usually kills all bacteria.

STERILISATION OF CONTAINERS AND OTHER EQUIPMENT

After washing in a bio-friendly detergent, containers and other equipment can either be autoclaved, heat treated, soaked with a chlorine solution (approximately 400 ppm available Chlorine), microwaved or exposed to UV light to sterilise.

Chlorine: The chlorine solution can be made from the Chlorine available for treating swimming pool water or common bleach used for cleaning in-door household surfaces. Chlorine treated articles should be air-dried allowing Chlorine to escape before use.

Autoclaved: 121^{o}C in pure saturated steam at 15lb/in^{2} above atmospheric pressure for at least 10 min.

Heat treatment: in a hot air oven: 2 hours at 160^{o}C.

UV Treatment: surfaces require exposure to a 20-40W lamp. Media should be put into quartz test tubes and irradiated for 2-4 hours. Note that UV radiation can cause severe eye damage, therefore protective glasses should be worn.

Microwave: Place containers and/or other gear in microwave for 5 min on 'High'.

SPOROPHYLL COLLECTION

Sporophylls are collected from the base of adult plants. They should be placed in seawater and kept cool as possible (5-10^{o}C) immediately after collection, particularly if spore release is not to be effected on the same day.

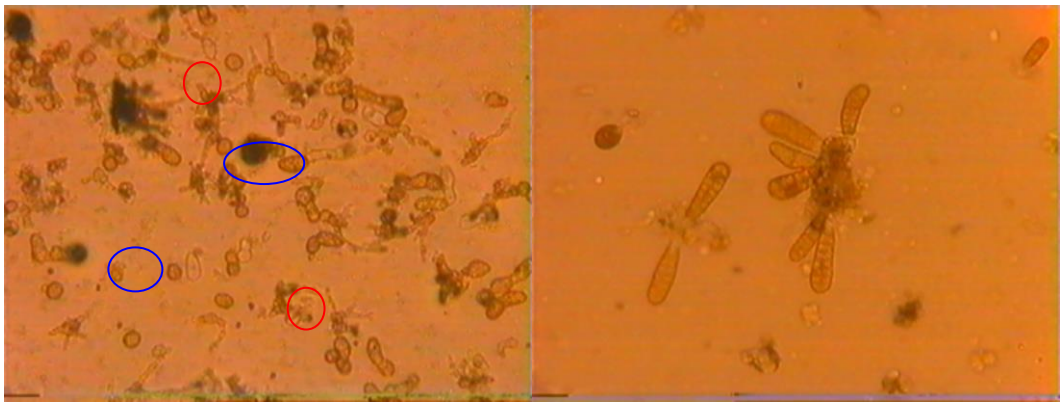
SPORE RELEASE

This should be done as soon as possible after collection of plants. Outer surfaces of the sporophylls should be sterilised to minimise the chance of introduction of other species to the culture, such as epiphytic species. This is done by wiping the outside surfaces with an antiseptic solution which may be either a 10% Betadene (hospital antiseptic) or an alcohol (70%) solution.

The sporophylls are then kept in a cool dark place (such as a fridge) for approximately two hours. This dehydrates the sporophylls. The sporophylls are then introduced to sterilised seawater. Ensure temperature is less than 18^{o}C. Spore release will then occur over the next 30 minutes. Spores may be evident in the solution as a murkiness or a light brown discolouration.

If a microscope and a graticuled slide are available, spore density can be calculated. Spore densities per area substrate that are generally between 2 and 10/mm^{2} should be aimed at to prevent overcrowding. Otherwise a ‘stab in the dark’ may be necessary. Spore solutions that result in clouding of the water in inoculated solutions should be

avoided as this indicates high spore densities and the possible introduction of foreign materials, which may enhance bacterial production.



Pictures taken at the Marine Discovery Center showing gametophytes (female:- blue circles and male:- red circles) on the left and sporophytes (beginning of large plants) on the right.

CULTURE SOLUTION

Most culture solutions for macroalgae include nutrients in proportions that are variations of what is known as a 'PES' recipe (Provosali's Enrichment Solution). This is a combination of chemicals that include nutrients critical to the growth and well being of the algae. Without laboratory facilities these solutions can be difficult to formulate. Success in hatcheries in growing microalgae has been achieved with two products commonly used to fertilise land plants. These are Aquasol and chelated iron. The Aquasol is added at the concentration of 50g/1000litres and the chelated iron at a concentration of 6g/1000litres.

The growth medium should be changed weekly for growing plants

SUBSTRATE

A substrate convenient for transplanting into the field should be used for growing plants to attach to. Commercial culture of similar algae in south east Asian countries is done on cotton twine. The cotton twine is wound on to a frame that is then placed into the stock solution. Sections of the twine are cut off afterwards and placed within rope twists for placement in the field. Other substrates that could be considered are sections of PVC pipe and rocks or gravel that may be freely distributed in the field. Remember that to optimize chances of success, these should be sterilised as above.

Coverslips can be included on the bottom of the containers so that development of the gametophytes can be monitored. These are introduced prior to innoculation with the spores (remember they have to be sterilised as well) and on a regular basis, can be individually sacrificed for examination of gametophytes. Measurments can be done on

the gametophytes to determine growth rates and maturity. Coverslips can be removed with sterilised fine forceps.

AERATION

As the algae get larger they will require circulation of the medium mostly to ensure adequate supply of nutrients to all parts of the plant. The currents ensure proper development of the plant so that it is hardier and able to resist adverse environmental conditions such as wave action. Currents also ensure proper development of the holdfast so the plant can properly attach. In ideal circumstances the *Macrocystis* gametophytes may produce fertilised gametes within 2-4 weeks of innoculation. Introduction to a circulating medium should occur after 4-6 weeks. Circulation of the medium can be provided with an air pump and stone. Remember to also sterilise this before introduction to the culture medium.

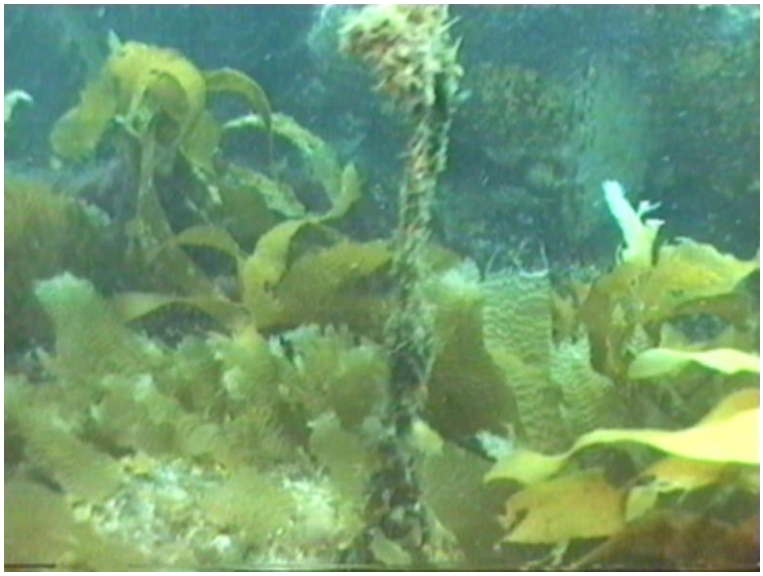
TEMPERATURE

Growing temperatures for *Macrocystis* gametophytes should be within 10-18^{o}C. Temperatures over 22^{o}C are likely to result in mortality. Cooling units or cooling baths are required if temperatures are likely to get higher than 20^{o}C.

LIGHT

Common light levels quoted for the growth of gametophytes range up to 4000 lux. Light levels from neon lights are unlikely to result in photoinhibition so the more light the better. Light from multiple (>2) flourescent tubes from a maximum distance of 30cm will result in adequate light levels.

Best growth is obtained from a 12/12 light: dark regime. Continuous light (or dark) is not recommended. A common household timer can be used to provide this regime.



Juveniles (small sporophytes) growing at the base of a concrete tyre with sub surface buoy marking the site at Cape Paul Lemenon. Plants growing on gravel had been placed here a few weeks previously.

GLOSSARY

Autoclave - Sterilization Equipment for heat - steam treatment under pressure

Culture solution - for plants from the marine environment this will be in a seawater base with added nutrients to provide best plant nutrition.

REQUIREMENTS FOR INOCULATION AND CULTURE.

Betadene (obtainable from chemists) sterilizing solution
Containers for spore release - sterilised
Containers for growth of the alga - sterilized
Sterilised seawater
Tweezers suitable for handling clean sporophyll blades
Tweezers suitable for picking up coverslips/microscope slides
Tissues/paper towels for cleaning surfaces
Sterilising solution for working surfaces
Clean workspace
Sporophylls
Lights - 2 x 30W flourescent sufficient
Cool place for culture containers (<18^{o}C)
Aerators - sterilised for growth in cultures after 4 weeks.