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Department:
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REPUBLIC OF SOUTH AFRICA

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South African Kelp Farming Project (Phase 2 Feasibility study)

Standard Operating Procedure (SOP): Sporulation

SOP number: BSASA-4	**Date:** 1 March 2025
Compiled by: The Project Manager (Dr Lizeth Botes) on behalf of BSASA	**Version:** 1 **Review date:** to be adapted and revised by industry

Introduction

The overall goal of the South African Kelp Farming Project (SA KFP) was to gather, analyse and disseminate evidence and research results to a broad stakeholder base, including the existing aquaculture industry and new potential entrants, to lay the foundations toward building a sustainable Kelp Aquaculture Industry in SA and the region.

One of the project objectives was to investigate and tailor the hatchery and nursery methods for our local kelp species (based on the kelp farming manuals from elsewhere that are already publicly available and accessible on the SA KFP webpage), which can then be adapted and revised by industry to suit their own needs. Although the temporary set-ups that were used in Phase 2 of the SA KFP were aimed at achieving the short-term project objectives, it was the first successful attempt to cultivate *Macrocystis pyrifera*, *Ecklonia maxima* and *Laminaria pallida* in South Africa, and also the first successful attempt to cultivate *E. maxima* and *L. pallida* anywhere.

Purpose of SOP:

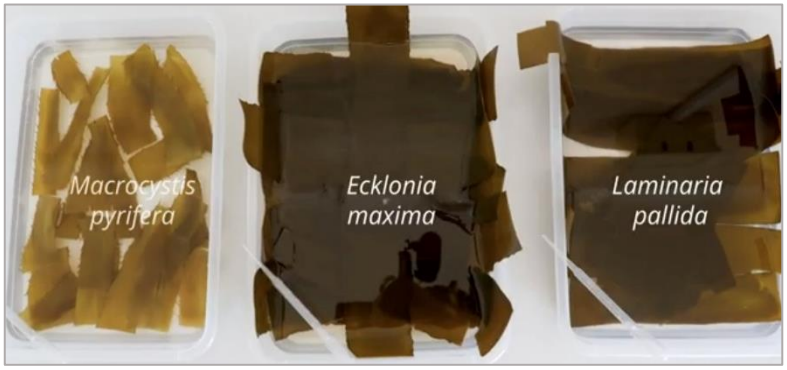
The purpose of this SOP is to provide a breakdown of steps to sporulate the kelp blades to obtain a spore solution that will be used for the inoculation of spools (see SOP 5). This procedure should be executed in a temperature-controlled room of ≤15°C.

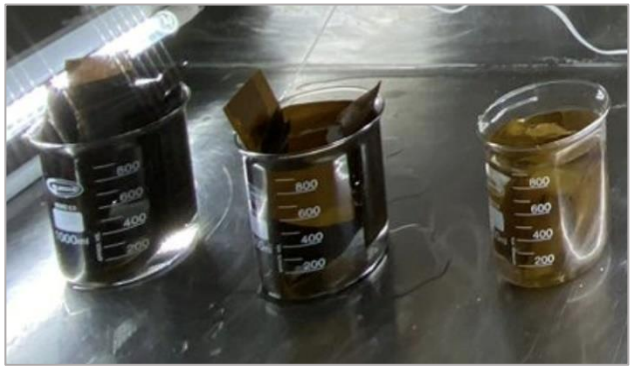
Preparation for sporulation:

1. Wear powder-free surgical gloves when preparing for and during the sporulation procedures.
2. Clean the work surface and make sure enough paper towels are available for use during the procedure.
3. Sterilize all utensils and beakers that will be needed for the procedure.

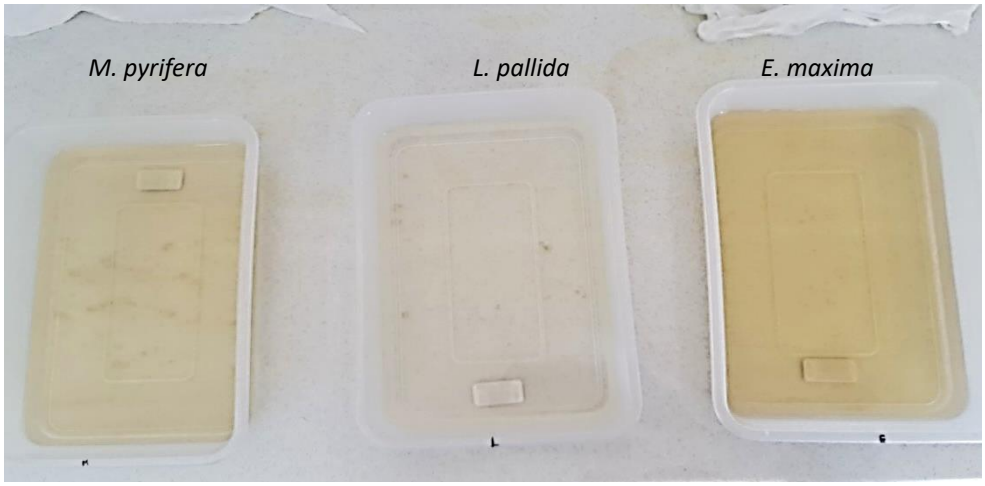
Sporulation of blades:

1. After 24 hrs, while wearing surgical gloves, open the foil parcels that were prepared. If some browning is visible on the paper towel, this is evidence of early spore release.
2. Immediately remove the blades from the paper towel and place each species in its own sterilised container (can be glass jars or plastic trays) containing sterilised seawater in the 15°C temperature-controlled room for 1 hr (stirring every now and again). If possible, try keep the cut ends above the water to prevent mucilage/mucus from going into the water in which the spores will be released (especially in the case of *Ecklonia* and *Laminaria*).





3. After 1 hour, remove the blades and discard. The remaining spore solution may have a slight discolouration. Of the three species, the water from *Laminaria* is generally the lightest in colour.



4. Optional step- pour the spore solution through a 0.5mm mesh to remove mucilage/mucus and any debris from the spore solution.
5. Gently stir the spore solution containing the spores and put a drop of the solution onto a microscope slide to do a quick check on a microscope to see if the spores are present and looking healthy. Spores that swim around are healthy, but those that are spinning around its own axes or stationary are not healthy and should be omitted from the count.
6. Mix well and then take a drop of spore solution with a pipette and place it onto a haemocytometer slide to calculate the spore density and volume to be added to the inoculation container. Ideally 2000 spores per ml for *E. maxima* and *L. pallida* is the required spore density, and 5000 spores per ml for *M. pyrifera* is the required spore density.
   Note: A detailed description of the method of how to count and calculate the spore density is available on pages 64-69 of the Ocean Approved Kelp Farming Manual.
7. Repeat the above for each species

Cleaning workplace after Sporulation:

1. Clean the workplace and discard in the appropriate manner all used paper towels, kelp blade cut-offs and gloves.