Recommendations for optimal techniques for obtaining spores of *Palmaria palmata*, settling and maintaining them prior to outplanting at sea

Principal authors
Astrid Werner, QUB
Matthew Dring, QUB
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Astrid Werner & Matthew Dring

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At three hatchery sites (i.e. DOMMRS, MRI Carna Laboratories, QUB Portaferry) numerous trials were conducted under the project PBA/SW/07/001 ‘Development and demonstration of viable hatchery and ongrowing methodologies for seaweed species with identified commercial potential’ in order to optimise the techniques for obtaining spores of *Palmaria palmata*, and settling and maintaining them. The techniques summarised here represent the conclusion of these trials and are considered to be the most suitable for achieving spore release and maintaining sporelings in the hatchery until they are ready for transfer to the sea for on-growing.

1. **Optimal technique for obtaining spores of *Palmaria palmata***

The season for obtaining spores of *Palmaria palmata* is winter to spring. Fertile *Palmaria* tetrasporophytes, i.e. the life stage of *Palmaria* which produces spores (see Fig. 2), are recognisable by dark red, slightly raised, irregularly shaped areas on fronds of *Palmaria* (Fig. 1). The optimal time for collecting fertile material for spore release differs from site to site along the coast of Ireland and is slightly extended in coastal areas with lower water temperatures, e.g. the Irish Sea. In general the best months for collection is between January and March/April.

*Palmaria* is collected at low spring tides when kelp beds, the main natural habitat of *Palmaria* of most sites around Ireland, are exposed. *Palmaria* can be also found growing on *Fucus* species in the lower intertidal.
The collected fronds should be processed directly after collection at a relatively low room temperature to avoid stress for the algae which can trigger early spore release. Fertile and clean (i.e. without fouling organisms) areas of *Palmaria* are torn off from the fronds and collected in a container. About 150 g of fertile tissue are needed for seeding one net.

2. Optimal technique for seeding culture string with Palmaria spores

Seeding of culture string should be conducted in the hatchery under low light and low temperature conditions (i.e. 5-10 µmol m\(^{-2}\) s\(^{-1}\) at 10°C) under a light:dark period of 12:12 hours. For spore release, not more than two nets are placed in a tank or a large tray (approx. 50x70 cm) and covered with UV-filtered seawater to a depth of about 20 cm. The reproductive material (i.e. the pieces of frond with sori) is then placed directly on top of the

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**Fig. 2.** Life cycle of *Palmaria palmata* (from van den Hoek et al. 1995). a) The blade-like gametophyte (n). b) Cross section through the cortex of a male gametophyte, showing the spermatangia. c) The tiny (ca 0.1 mm diameter) crustose female gametophyte (n). d) Cross section of a female gametophyte and fertilisation of a carpogonium by a spermatium (n). e) Cross section of a female gametophyte, with zygote (2n, stippled). f) Young blade-like tetrasporophyte (2n). This grows directly from the zygote, which is retained in the gametophyte. g) Fully grown tetrasporophyte. h) Cross section through the cortex of a tetrasporophyte, showing tetrasporangia; one tetrasporangium is releasing its four tetraspores (n).

CA = carpogonium; F1 = fertilisation; FGPH = female gametophyte; KG = karyogamy; MGPH = male gametophyte; R1 = reduction division (meiosis); RTR = remnant of the trichogyne; S = spermatangium; SORS = sorus of spermatangia; SORT = sorus of tetrasporangia; SP = spermatium (n). TETSP = tetraspore; TPH = tetrasporophyte; TR = trichogyne; Z = zygote.
collectors or placed on a net lying on the collector which allows easier removal of the frond pieces after spore release. The algal material is left in the container for three days, but should be gently stirred in the tank by hand once every day to promote mixing and more even settlement of spores on the collectors.

After removal of reproductive tissue, the collector is placed in a tank under the same conditions as described for the spore release. By this time spores have attached to the culture string. Spore development can be monitored under the microscope as shown in Fig. 3. Successful spore release should result in a settlement of approx. 100 or more spores per cm culture string. These numbers will decrease over the first 7 – 14 days as natural spore mortality is high (60-80%).

![Fig 3. a) A spore shortly after release; b) Spores 4 days after release undergoing cell-division; c) Female gametophyte 10 days after spore release; d) Male gametophyte 10 days after spore release; e) sporeling growing on culture string 1 month after seeding; f) Culture string with *Palmaria* sporelings 4 months after seeding.](image)
3. Maintaining seeded culture string of *Palmaria palmata* in the hatchery

After spore release the collectors are removed from the trays and transferred into larger holding tanks (capacity: 200 L or more). Tanks and collectors should be kept in a very clean state avoiding contamination with other algal cultures. As a medium, cartridge-filtered and UV-sterilised seawater is used and a nutrient enrichment is added (e.g. f2 medium). The tanks are aerated to increase CO₂ supply and the flow of water along the developing sporelings, which enhances nutrient uptake and consequently growth. The water temperature should be kept at 10°C. Light intensity is low during the first weeks of cultivation (15-20 µmol m⁻² s⁻¹) but can be increased to 30-40 µmol m⁻² s⁻¹ at a later stage. The tanks should be cleaned and tank water exchanged every 2-4 weeks.

Growth can be manipulated to match the intended time of deployment of *Palmaria* culture string in the sea. For autumn/winter deployment, seeded collectors will need to be maintained in the hatchery for 6-9 months. Therefore growth of sporelings should be restricted so that they do not grow to more than 5-8 mm in length, which has been found to be the most favourable size for deployment. For summer deployment, the cultivation time in the hatchery is shorter (only 2-4 months) and therefore growth should be enhanced. Manipulation of light (light period and intensity) and the frequency of water exchange and renewal of culture medium can either increase or restrict the growth of sporelings. It is advantageous to increase water motion a few weeks before deployment to stimulate the sporelings to attach more firmly to the culture string.

For successful on-growth in the sea, collectors should be deployed with culture string that is evenly and densely seeded with *Palmaria* sporelings, the largest of which are 5-8 mm in length.
4 Reference

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