



Open-sea cultivation trial of the red alga, *Palmaria palmata* from seeded tetraspores in Strangford Lough, Northern Ireland

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ABSTRACT

Cultivation of *Palmaria palmata* was performed by a technique introduced in this paper of tetraspore release and attachment to culture string. Optimal conditions for laboratory growth were investigated with respect to irradiance and nutrient media. The length of laboratory culture required for the growth and development of attached sporelings was also investigated, prior to deployment of seeded strings on a longline structure in Strangford Lough, Northern Ireland. Of sporelings grown in 5, 10, 25 and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, those at the lower irradiances (5 and 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were significantly longer than those at higher irradiances after 12 weeks. Sporeling length was also significantly greater at lower irradiances when in F/2 nutrient media with additional vitamin solution, compared with F/2 medium without the vitamin solution. Seeded string was held in the laboratory for 2, 4, 6, or 8 weeks. After 3 months at sea, the 2-week strings had fewer visible plants and had shorter plants than the 4, 6 and 8-week strings, but plants on all strings were similar in length after 7 months at sea. The demand for *P. palmata* as a snack food in Northern Ireland regularly outstrips the supply obtainable from natural populations. The technique of seeding string with tetraspores is proposed as one method of commercially cultivating *P. palmata* on a large scale.

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1. Introduction

In comparison to capture fisheries and agricultural sectors, the aquaculture industry continues to grow at a much faster rate, with figures from 2008 showing that aquaculture products accounted for 46% of all fish destined for use as a foodstuff (FAO, 2010a). During the same period, the global aquaculture industry also showed an increase in seaweed production to 15.8 million tonnes, which was valued at US \$7.4 billion (FAO, 2010b). Countries with the largest seaweed production are China, the Philippines, the Republic of Korea and Japan, and they focus on the cultivation of four main species, namely *Laminaria japonica*, *Undaria pinnatifida*, *Porphyra tenera* and *Eucheumatoids* (McHugh, 2003).

The versatility of seaweeds, whether used whole in the food industry, or for extraction in the hydrocolloid, pharmaceutical and cosmetics industries has created a demand that cannot be satisfied by harvesting of wild stocks alone. Of the 16.8 million tonnes of seaweed produced in 2008, 93.8% were cultivated (FAO, 2010a). This shows that the cultivation of seaweeds is essential in providing a good-quality product to satisfy the demands of a variety of industries. In addition, aquaculture of seaweeds reduces the need to harvest large amounts of

biomass from wild algal populations, many of which create and support entire habitats (Birkett et al., 1998; Edwards, 1980).

Most Asian countries are much more accustomed to growing, harvesting and cooking seaweeds than Western European countries. Despite this, several of these countries also have a long tradition of eating and using seaweed (Kain, 2003). Perhaps the most notable country is Ireland, where the red algae 'dulse', 'irish moss' and 'sloke' (*Palmaria palmata*; *Chondrus crispus*; *Porphyra* sp.), amongst others, are eaten widely. *P. palmata* is especially popular in Northern Ireland and the West of Ireland, where it is hand-harvested, dried, and eaten as a savoury snack. Local small businesses collect approximately 8 tonnes per year, and find there is a constant demand for the dried product (G. Heath, personal communication). A niche has also been created in the health food market by promoting *P. palmata* as a good source of protein (Galland-Irmouli et al., 1999). Other studies identify *Palmaria* as a source of antioxidants, which scavenge free radicals (Yuan et al., 2005), while extracts from *Palmaria* have been reported to inhibit the proliferation of a cancer cell line by up to 78% (Yuan and Walsh, 2006).

Ireland's seaweed industry is currently worth €10 million per year to the economy and mainly relies on wild harvests of *Ascophyllum nodosum* (M. Walsh, personal communication). As of yet, there is no large-scale seaweed aquaculture anywhere in Ireland but the commercial importance of *Palmaria* has long been recognised (Werner et al., 2004). Cultivation of the alga has been the focus of research for years in a variety of countries including Ireland, using various cultivation techniques. For example, research has focused on

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the vegetative growth of plants in tank culture (Morgan et al., 1980a, 1980b; Le Gall et al., 2004; Pang and Lüning, 2004), induction of year-round tetrasporangia (Pang and Lüning, 2006) and on growth trials at sea with plants inserted into ropes or placed in mesh bags (Browne, 2001; Martínez et al., 2006). Work completed by Browne (2001) also included the cultivation of tetraspores under laboratory conditions and the economics of setting up and running a *Palmaria* farm. This analysis indicated that in Northern Ireland at least, the only economically feasible method of growing *Palmaria* was through the settlement of tetraspores on a suitable substrate and subsequent on-growing at sea. Although further studies of *Palmaria* tetraspore settlement were made (Browne, 2001), the aquaculture of *P. palmata* from tetraspores was not completed in open sea conditions and has formed the basis for further work, which is presented in this paper.

The objective of this paper is to demonstrate the growth and survival of the red alga *P. palmata*, firstly in the laboratory and then by cultivation at sea, and discuss its potential for commercial-scale aquaculture.

2. Materials and methods

2.1. Collection of reproductive material for cultivation techniques

All tetrasporophytes of *P. palmata* (Linnaeus) Kuntze used in the sporeling cultivation techniques were collected at low tide from various parts of Strangford Lough (Fig. 2.1), Northern Ireland (54°22'48 N, 5°33'9 W). The main site, the Walter Shore, was situated in the 'Narrows' where there is a strong current at periods of peak tidal flow. Plants were collected by hand from the shore, mainly at low water on spring tides during the main reproductive season for *Palmaria*. Although *P. palmata* can be found growing epilithically in Strangford Lough, all algal biomass was collected from stipes of *Laminaria digitata*.

2.2. Selection and preparation of reproductive material

Reproductively mature tetrasporophytes with no visible epiphytes were selected and the maturity of fertile tetrasporophytes was

assessed by eye. Reproductive sori had a dark red, raised texture when mature. Tetrasporophytes were torn by hand into small pieces (approximately 4 × 4 cm) to select only the reproductive areas of sori (tearing the thalli was as effective as and quicker than using a knife or scissors). These pieces were placed into clear plastic aquaria containing UV-filtered seawater.

2.3. Settlement of released tetraspores on coverslips and culture string

The reproductive pieces of thalli were placed above glass coverslips (22 × 22 mm) on the bottom of a 1 l aquarium and was placed in an illuminated incubator (LMS cooled incubator) at 10 °C (± 0.5 °C). The cultures were maintained in the incubator for several days and exposed to short days (8 h:16 h light:dark) at an irradiance of 10–20 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and received moderate aeration. The glass coverslips were inspected for released tetraspores after 24 h using a dissecting microscope (Wild, M3). When a large number of released tetraspores were found, plant material was removed from the aquarium and the aeration was stopped for approximately 12 h to allow the spores to adhere properly to the substrata. After this period, cultures received gentle aeration for one to two days until spores did not detach from the glass surface when it was shaken gently. The aquarium containing seeded coverslips was then cleaned thoroughly and re-filled with UV-filtered seawater. Additional nutrients were added (full strength F/2 and vitamins at a rate of 1 ml and 0.1 ml/l seawater, respectively – Guillard and Ryther, 1962). The light regime was changed to long days (16:8, light:dark), with moderate aeration also provided. Subsequently, the aquarium was cleaned and re-filled with UV-filtered seawater and nutrients/vitamins added once per week.

2.4. Growth of *P. palmata* sporelings in the laboratory on coverslips

Twelve coverslips settled with *Palmaria* tetraspores were placed individually into 5-cm diameter plastic, sterile Petri dishes containing autoclaved, UV-filtered seawater. Each Petri dish was placed in an illuminated incubator (LMS cooled incubator) and exposed to different irradiances and culture media.

Irradiances of 5–25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were supplied by the light source contained within the illuminated incubator (two vertically mounted fluorescent lamps (General Electric, F30W/35) behind a translucent protective screen). An additional lamp was used to achieve 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A car inspection lamp (8 W fluorescent lamp manufactured by 'Ring', type RIL 100) provided bright light while still being cool enough to avoid heating the water in the Petri dishes. The amount of light reaching the sporelings, was measured with a cosine-corrected Skye photometer (model SKP 200). The sensor of the photometer had a Petri dish lid placed over it so that any attenuation of light by the plastic lid in the Petri dishes was allowed. The following seawater media were used:

1. 'O'; Seawater from Strangford Lough – unenriched, UV-filtered and autoclaved.
2. 'F/2'; As "O", but enriched with full strength F/2 medium (1 ml/l seawater) and with trace metal solution (recipe as described in Guillard and Ryther, 1962).
3. 'F/2+'; As "F/2", but with vitamin solution added (recipe as described in Guillard and Ryther, 1962).

Temperature and daylength remained constant throughout the experiment at 10 °C (± 0.5 °C) and 16:8 h light:dark, respectively. Petri dishes were cleaned and the media replaced once a week. Any large epiphytes growing on the coverslips were carefully removed with fine-nose forceps. No chemicals were used to eliminate unwanted epiphytes in case they affected the growth of the sporelings. In later weeks, many sporelings became detached from the coverslip. Once this happened, the coverslip was lifted out as usual

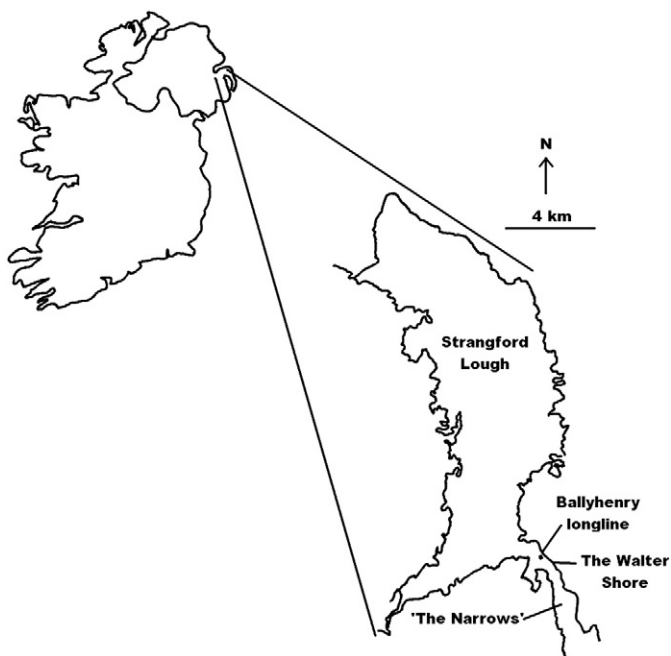


Fig. 2.1. Map of Ireland, including Northern Ireland. Expanded box shows Strangford Lough and shows the main areas of interest. These include the main collection site for wild *Palmaria palmata* and the sea cultivation trial site in Ballyhenry Bay.

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