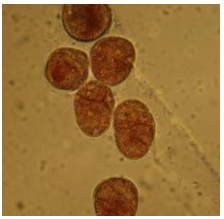
May 2011

Bord Iascaigh Mhara
Irish Sea Fisheries Board

Aquaculture Explained

No. 27

Cultivating *Palmaria palmata*



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Marine Institute Foras na Mara
SeaChange Casadh na Taoide
NDP National Development Plan 2007 - 2013

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Cultivating *Palmaria palmata*

This document is an output of the project, PBA/SW/07/001, 'Development and demonstration of viable hatchery and ongrowing methodologies for seaweed species with identified commercial potential'. This project is carried out under the Sea Change Strategy with the support of the Marine Institute and the Marine Research Sub-programme of the National Development Plan, 2007-2013.

Project Partners

Bord Iascaigh Mhara (BIM)

Queen's University Belfast (QUB)

National University of Ireland, Galway (NUIG)

Cartron Point Shellfish (CPS) Ltd.

Tower AquaProducts Ltd.

G&B Barge Operators Ltd.

Irish Seaweeds Ltd.

Roaring Water Bay Seaweed Cooperative Society Ltd.

Dingle Bay Seaweeds Ltd.

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Foreword

In this publication, methods and techniques are described for the cultivation of *Palmaria palmata* at an industrial scale. This includes growing *Palmaria* from spores in the hatchery with subsequent on-growth at sea, as well as vegetative cultivation of the alga in land-based tanks. The manual is based on the research carried out under the project, PBA/SW/07/001, 'Development and demonstration of viable hatchery and ongrowing methodologies for seaweed species with identified commercial potential', funded by the Marine Institute under the Marine Research Sub-programme of the National Development Plan, 2007-2013, under the umbrella of the Sea Change Strategy.

Palmaria palmata is one of three species of economic interest which have been investigated and tested in sea- and tank-trials. Due to its historic and economic significance in Ireland, it was felt that the time and circumstances were right to facilitate the important step-change of collection of *Palmaria* from the shores to sea-based and land-based aquaculture of this species. The methodologies have been developed specifically for use to cultivate *Palmaria* at a commercial scale. Business models are included, which are based on the results of sea- and tank-trials. Although the conclusions from this economic analysis show that cultivation of *Palmaria* in Ireland is not economically viable at present, largely because the prices currently available for *Palmaria* as a food–product or for abalone feed are so low, this detailed manual has been prepared in the expectation that increasing demand for *Palmaria* will soon be reflected in the price, and that high-value end-products based on *Palmaria* will soon be developed.

Astrid Werner & Matthew Dring
Authors and Co-editors
May 2011

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Chapter 1 - Introduction

1.1 The current status of seaweed aquaculture

Aquaculture has become an important part of overall world food production with this production continuing to increase globally by 6.2% per year (FAO, 2010a; Fig. 1.1). The production from capture fisheries has stabilised in recent years due to the decline of fish stocks, in contrast to the steady increase in aquaculture of marine animals. In the seaweed sector, the production by aquaculture exceeds by far the production from wild stocks (Fig. 1.1). In 2008, 15.8 million tonnes (wet weight) were cultivated, which equals 93.8% of total production with a value of US$7.4 billion (€5.5 billion; FAO, 2010a). Seaweeds/aquatic plants constituted 23% of the total weight of all cultured organisms, but only 7% of the total value.

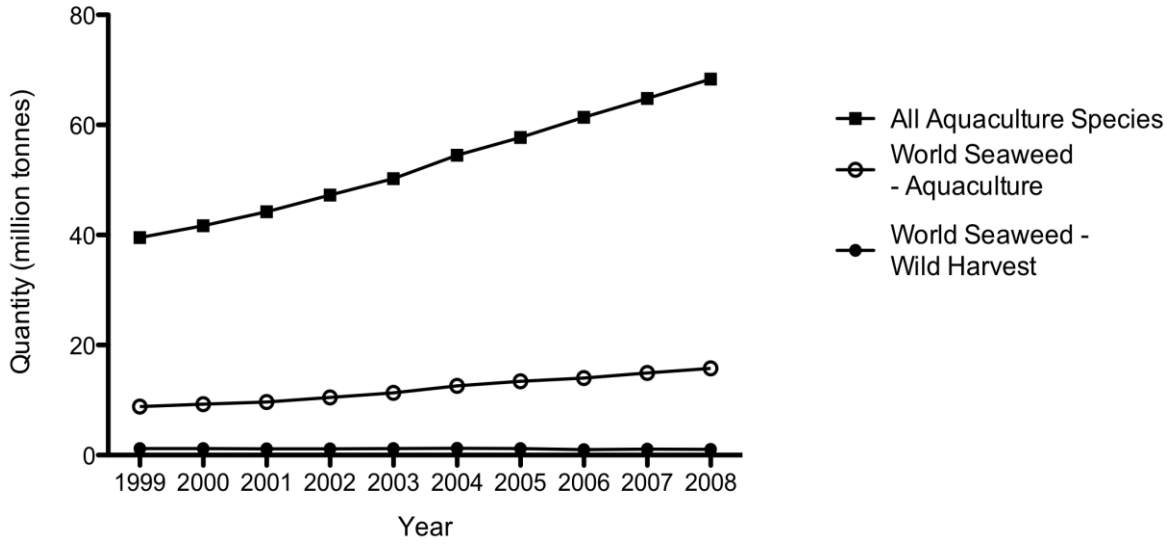


Fig. 1.1. Worldwide quantities (million tonnes) of all cultured aquatic organisms, cultured aquatic plants and wild-harvested seaweeds between 1999 and 2008. Data from FAO Annual Yearbook 2008 (FAO, 2010a).

The vast majority of the world seaweed production is centred in Asia, with China being the main producer of seaweed biomass (62%), followed by the Philippines, Japan, the Republic of Korea and the Democratic People's Republic of Korea (FAO, 2010a,b). Only a few other countries grow a small number of seaweed species. Among these, only Chile and Zanzibar produce more than 10 million tonnes wet weight per annum (FAO, 2010a). Fig. 1.2 shows highest biomass production worldwide by species.

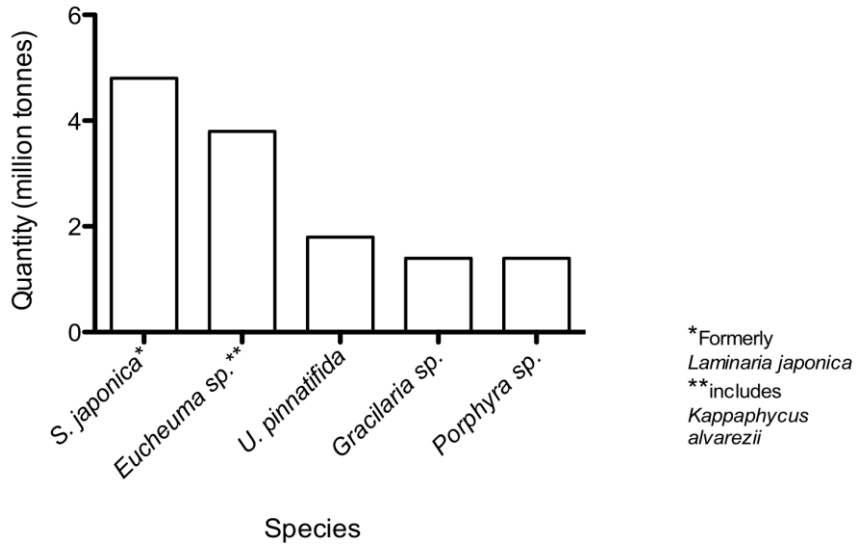


Fig. 1.2. Cultivated seaweed species with the highest world biomass production. S = *Saccharina*; U = *Undaria*. Data from FAO, 2010b.

Large amounts of seaweed biomass exist naturally in the North Atlantic, and countries such as Ireland, Scotland, France, Norway and Canada have a tradition of harvesting seaweed and utilising the resource extensively (Lüning, 1990; Hession *et al*., 1998). In France, Norway and the East coast of Canada, specific mechanical harvesting techniques and comprehensive sustainable resource management have been developed (Werner & Kraan, 2004). In contrast, cultivation of seaweeds in these countries is very limited as shown in the latest FAO publication (2010b; Fig. 1.3).

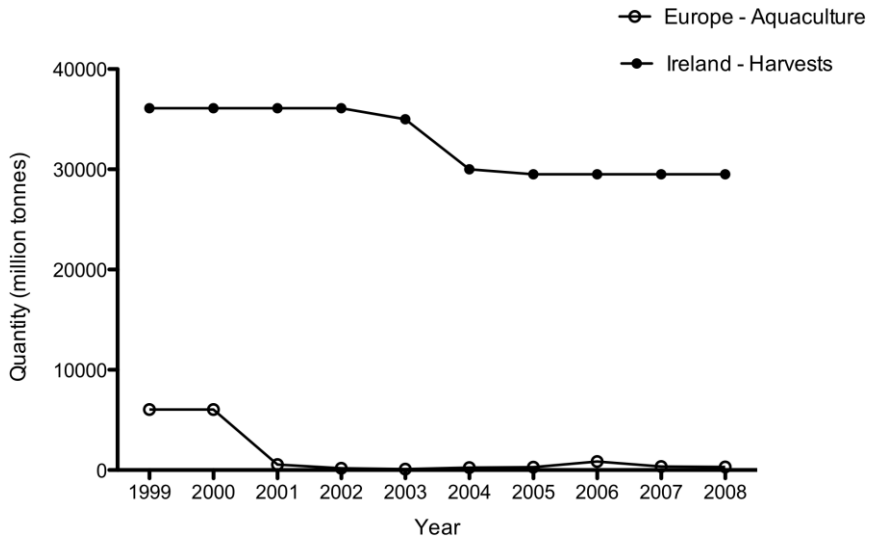


Fig. 1.3. Seaweed production (tonnes) from Ireland's wild harvests and European aquaculture (no aquaculture data available for individual countries). Data from FAO, 2010a.

The lack of seaweed aquaculture development in Western countries is largely due to a smaller demand than in Asian countries because of different traditions in food use. Seaweed cultivation in Europe has tended to develop as niche enterprises with specific applications rather than as operations for mass production of a commodity (Werner et al., 2004).

New market demands and applications are slowly transforming the seaweed sector in Europe as well as in other western countries. According to the marketing strategy report, 'A Market Analysis toward the further development of seaweed aquaculture in Ireland' (Walsh & Watson, 2011) and the FAO (2010a), between 25500 and 29500 tonnes of seaweed is harvested annually in Ireland (Fig. 1.3 and Table 1.1), and the industry is valued at approximately €10 million. The biggest Irish seaweed markets are for agricultural, food and cosmetics/therapies products. The cultivation of seaweeds (and other algae) has recently become the focus of much research across the world, including novel uses for seaweeds. Many species with commercial potential have been identified, and Ireland has recognised the potential value of this marine resource.

Table 1.1. Estimated annual harvest of seaweeds in Ireland (Walsh & Watson, 2011).

Estimated Annual National Seaweed Harvest	
Species	**Annual Harvest (tonnes)**
Ascophyllum nodosum	25,000
Fucus serratus	200
Palmaria palmata	<100
Chondrus crispus / Mastocarpus stellatus	<100
Laminaria digitata	<150
Himanthalia elongata, Saccharina latissima, L. hyperborea, Ulva sp., Pophyra sp., F. vesiculouis, Alaria esculenta etc.*	<10 each

***** Ulva harvesting may exceed 10 tonnes at times when it is removed from amenity bathing areas. There is *currently* no commercial application for such material.

The recognition of the economic potential of the seaweed industry has long been of concern to the Irish Government. More than ten years ago, the status of the seaweed industry was evaluated by the National Seaweed Forum (1999) with the objective of identifying key areas for development. Seaweed aquaculture was one of these key areas and the feasibility of developing the aquaculture sector was further assessed by a subsequent study funded by the Marine Institute (Werner et al., 2004). These studies concluded that the development of seaweed aquaculture in Ireland should mostly be aligned towards low volume – high value

products. Methods for the cultivation of *Palmaria palmata* had been investigated in previous years by Browne (2001) and Edwards (2007) at the Queen's University Marine Laboratory in Portaferry and the economics of cultivation were also assessed. This provided an important base for the research conducted in the context of the current project (see below).

1.2 The Seaweed Hatchery Project – PBA/SW/07/001 (01)

1.2.1 Project Objectives

The main objectives of this project were to develop and trial industry-scale hatchery and ongrowing methodologies for identified seaweed species and to provide a platform for transferring the technology to create new business opportunities in seaweed aquaculture within Ireland. The three species that were identified as having commercial potential were the edible red alga *Palmaria palmata,* the large brown kelp *Laminaria digitata*, and a second edible red alga, *Porphyra* sp. During the course of this project a fourth species was added, *Saccharina latissima*.

Objectives within the project were both scientific, and industry-focused. These included:

Scientific

- Establishment of optimal hatchery culture conditions for each seaweed species
- Development of techniques for the settlement of each seaweed onto suitable substrates for deployment at sea
- Monitoring and improving the yield of cultured seaweeds on culture equipment at various licensed sea sites
- Development of seaweed harvesting strategies at sea sites

Industry-focused

- Seaweed marketing strategy report
- Economic analyses for the three seaweed species
- Training courses in algal cultivation techniques
- Production of cultivation manuals under BIM's 'Aquaculture Explained' series
- Desk-based GIS study for assessing requirements for locating seaweed cultivation sites, using Bantry Bay as the study area.

1.2.2. The Seaweed Hatchery Project – Funding

This project was carried out under the Sea Change Strategy with the support of the Marine Institute and the Marine Research Sub-programme of the National Development Plan, 2007-2013. The project started in March 2008 and was completed in May 2011.

1.2.3 Partners and Industry Associates

A total of 3 partners and 6 associates (all Small to Medium-size Enterprises, SME) were involved in the project (Table 1.2).

Table 1.2. Project partners (bold) and associates, with a description of their role within the project.

Participant	Description	Role within Project
BIM **(Bord Iascaigh Mhara)**	State Agency	Lead partner and co-ordinator of project partners.
QUB **Queen's University Belfast**	University	Partner, and WP leader on *P. palmata* and *Porphyra* sp.
NUIG **National University of Ireland, Galway**	University	Partner, and WP leader on *L. digitata* and GIS site assessment study
CPS Cartron Point Shellfish Ltd.	SME	Active participation in cultivation of *L. digitata* and *P. palmata* (hatchery and sea sites)
Tower AquaProducts Ltd.	SME	Tank cultivation of *P. palmata*
Irish Seaweeds Ltd.	SME	Provision of licensed sea trial site
G + B Barger Operators Ltd.	SME	Industry partner
Roaringwater Bay Seaweed Co-operative Society Ltd.	SME	Provision of licensed sea trial site
Cleggan Seaweeds Ltd*	SME	Provision of licensed sea trial site

* Cleggan Seaweeds Ltd subsequently disengaged from the project.

During the lifetime of the project, two new sites were made available. The first was a 1-hectare site in Ard Bay, Co. Galway (licence held by Mr. Michael Ward). The second site (18 hectares) was operated by Dingle Bay Seaweeds Ltd in Ventry Harbour.

The total licensed area available to the project was 85 ha.

1.2.4 Project facilities – hatcheries and licensed seaweed trial sites

Three hatchery facilities were made available for the duration of the project. Each hatchery was strategically placed to provide seeded seaweed collectors for deployment at nearby sea sites (Fig. 1.4). In Northern Ireland (Portaferry, Co. Down), the hatchery facility was owned and operated by QUB, in the West of Ireland (Carna, Co. Galway), the hatchery was owned and operated by NUIG, while the hatchery in the South-West (at the Daithi O'Murchu Marine Research Station, Gearhies, Co. Cork) was owned by Fastnet Mussels Ltd, and operated jointly by CPS and BIM. Each facility had access to filtered seawater, an air supply and an insulated, constant temperature unit for the necessary control over the life cycle of the cultured species.

Sea sites with aquaculture licences suitable for seaweed cultivation were also made available by the SME associates (Table 1.2). Most of the sites were located in the West and South-West of Ireland. Two further longlines were sited in Strangford Lough, Northern Ireland (see Fig. 1.4 for the full list of sites used). Site conditions varied, with depths between 6 and 18 m, and substrate types ranging from boulders and bedrock to silt, sand and mud. Most were situated in sites sheltered from the prevailing wind, and experienced greater current action than wave action. Access to all sites was by boat, and it took up to 45 minutes to reach each site. The largest sites used were at Newquay and Ventry Harbour (55 and 18 ha, respectively), while the smallest site used was in Ard Bay (1 ha).

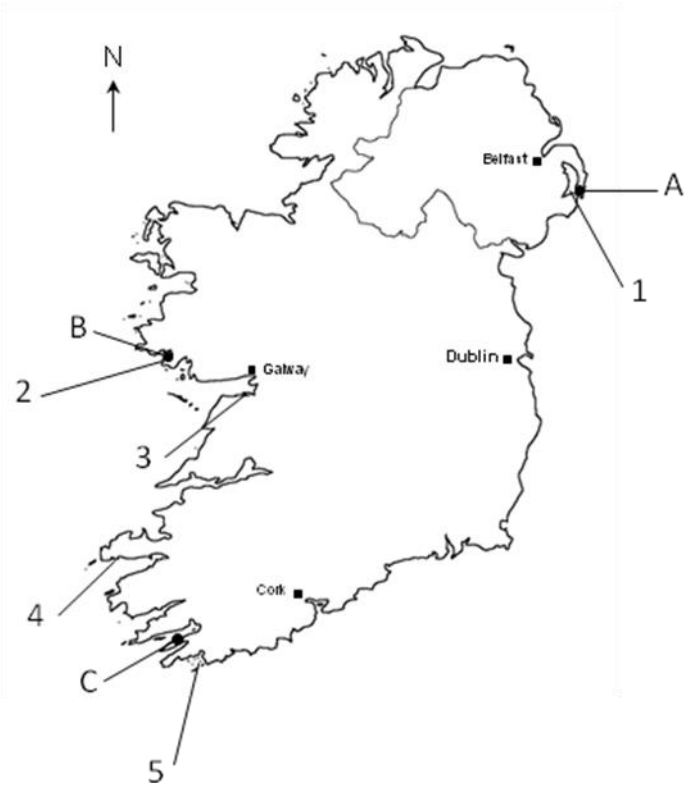


Fig. 1.4. Locations of the project hatcheries (letters), and licensed sea trial sites (numbers). **Hatcheries**: A: Queen's University Marine Laboratory at Portaferry, Co. Down. B: National University of Ireland, Galway Carna Research Facility, Carna, Co. Galway. C: The Daithi O'Murchu Marine Research Station, operated by Cartron Point Shellfish Ltd. and Bord Iascaigh Mhara, Gearhies, Co. Cork. **Sea sites**: 1: Strangford Lough, with permission from Irish Seaweeds Ltd. 2: Ard Bay, with permission from Michael Ward. 3, Newquay, with permission from Redbank Shellfish Ltd. 4: Ventry Harbour, with permission from Dingle Seaweeds Ltd. 5: Roaringwater Bay, with permission from Roaringwater Bay Seaweed Cooperative Society Ltd. A sixth licensed site in Cleggan Bay, Co. Galway was not used due to the disengagement of Cleggan Seaweeds Ltd from the project.

Chapter 2 - Biology of *Palmaria palmata*

2.1 Taxonomic classification

Phylum	Rhodophyta
Sub-phylum	Eurhodophytina
Class	Florideophyceae
Sub-class	Nemaliophycidae
Order	Palmariales
Family	Palmariaceae
Species	*Palmaria palmata* (Linnaeus) O. Kuntze (1891)

Description of *Palmaria palmata*

The red alga *Palmaria palmata* has a flat thallus which is typically divided in a fork-like manner (dichotomously) or grows palmately, i.e. into lobes radiating from the centre of the fronds. The texture of fronds is membranous or leathery. The fronds arise from a small discoid holdfast either attached singly or clustered together in groups on the substrate. Stipes are indistinct and often less than 5 mm in length. Mature fronds commonly grow to about 50 cm but can reach a length of up to one metre. Older plants often show marginal outgrowths of new fronds from the primary blade. The morphology can be very variable depending on the environmental conditions (Fig. 2.1). Finely dissected forms of *Palmaria*, known as *P. palmata* var. *sarniensis* and var. *sobolifera*, can be found as well as the typical form (var. *palmata*). These two ecotypes tend to occur in more sheltered and silty sites than var. *palmata*. *Palmaria palmata* is a perennial species showing new growth of fronds every year. The maximal lifespan of fronds is not known.

Fig. 2.1. *Palmaria palmata*. A (middle): Habit of typical tetrasporophyte plant. B (right): Cross section through thallus of tetrasporangial plant. C (left): Morphology of ecotype *Palmaria palmata var. sarniensis* (from Irvine, 1983).

2.2 Geographical distribution

Palmaria palmata is a cold temperate species of the North Atlantic and North Pacific Oceans. The area of distribution in the North Atlantic stretches from Spitzbergen and Greenland in the Arctic (80°N) to Portugal and New Jersey (40°N). *Palmaria palmata* is also found along the Pacific coasts of Canada and the USA from Alaska to northern California, and along the cold-temperate coasts of eastern Russia (Sakhalin and the Kuril Islands) and northern Japan (Hokkaido; Lüning, 1990). It is a common constituent of many rocky shores in Ireland and the UK (Bunker et al., 2010; Hardy & Guiry, 2003).

2.3 Habitat and ecology

Palmaria palmata grows in the lower intertidal and shallow subtidal zone to a maximum depth of 20 metres. In European waters, the alga is a frequent epiphyte on stipes of *Laminaria hyperborea* or *L. digitata*, but it is also found occasionally on *Fucus* species or on rocks and mussel shells (see Fig. 2.2). *Palmaria* prefers sheltered to semi-exposed sites with a moderate to strong water current. A good water flow has a very positive effect on growth, probably because of its impact on nutrient exchange. Young fronds of *Palmaria* are normally free of epiphytic growth, but older fronds are frequently covered by bryozoans and other organisms. Older thalli also frequently contain small endophytic algae, which are visible as dark brown dots in the tissue, and marine fungi.

Fig. 2.2. *Palmaria palmata* in its natural habitat. Clockwise from top left: *Palmaria* growing on the stipes of *Laminaria digitata* at spring low tide in Strangford Lough; *Palmaria* at spring low tide submerged; *P. palmata* growing on stipes of *Laminaria hyperborea* at spring low tide at Ballyrisode Beach, South West Cork; recruits of *Palmaria palmata* on a stipe of *L. digitata* in June.

2.4 Life cycle and reproduction

Like many other red algae, *Palmaria palmata* has a biphasic life cycle with a sexual, gametophyte phase alternating with an asexual, tetrasporophyte phase. The tetrasporophytes and male gametophytes are of similar morphology and are indistinguishable until they become reproductive. When the tetrasporophytes reach maturity after the first year of growth, they develop tetrasporangial sori on the fronds which are visible as dark red, irregularly shaped and slightly elevated tissue areas. Under a microscope, the spores can be seen to be clustered together in packs of four in one spore case or sporangium, hence the name "tetraspores".

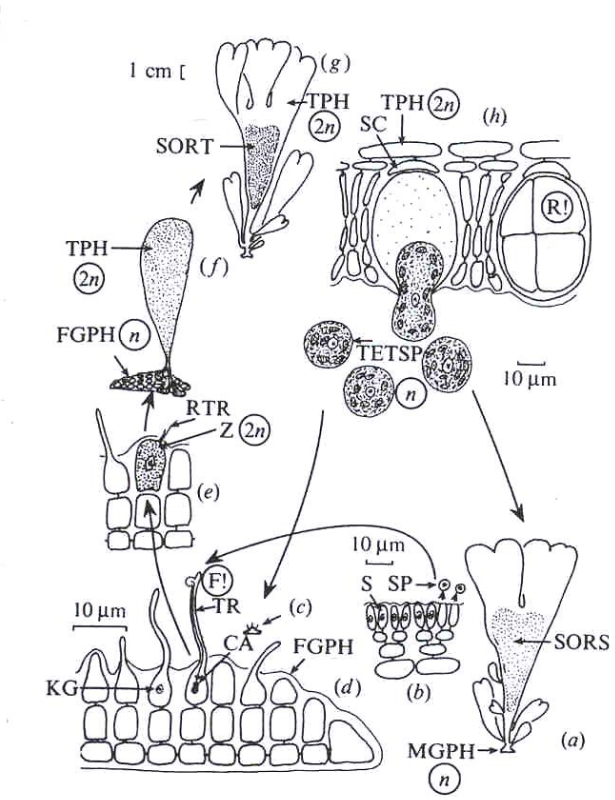


Fig. 2.3. Life cycle of *Palmaria palmata* (from van den Hoek *et al*., 1995). a) The blade-like gametophyte (n). b) Cross section through the cortex of a male gametophyte, showing the spermatangia. c) The tiny (ca 0.1 mm diameter) crustose female gametophyte (n). d) Cross section of a female gametophyte 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; F! = fertilisation; FGPH = female gametophyte; KG = karyogamy; MGPH = male gametophyte; R! = 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.

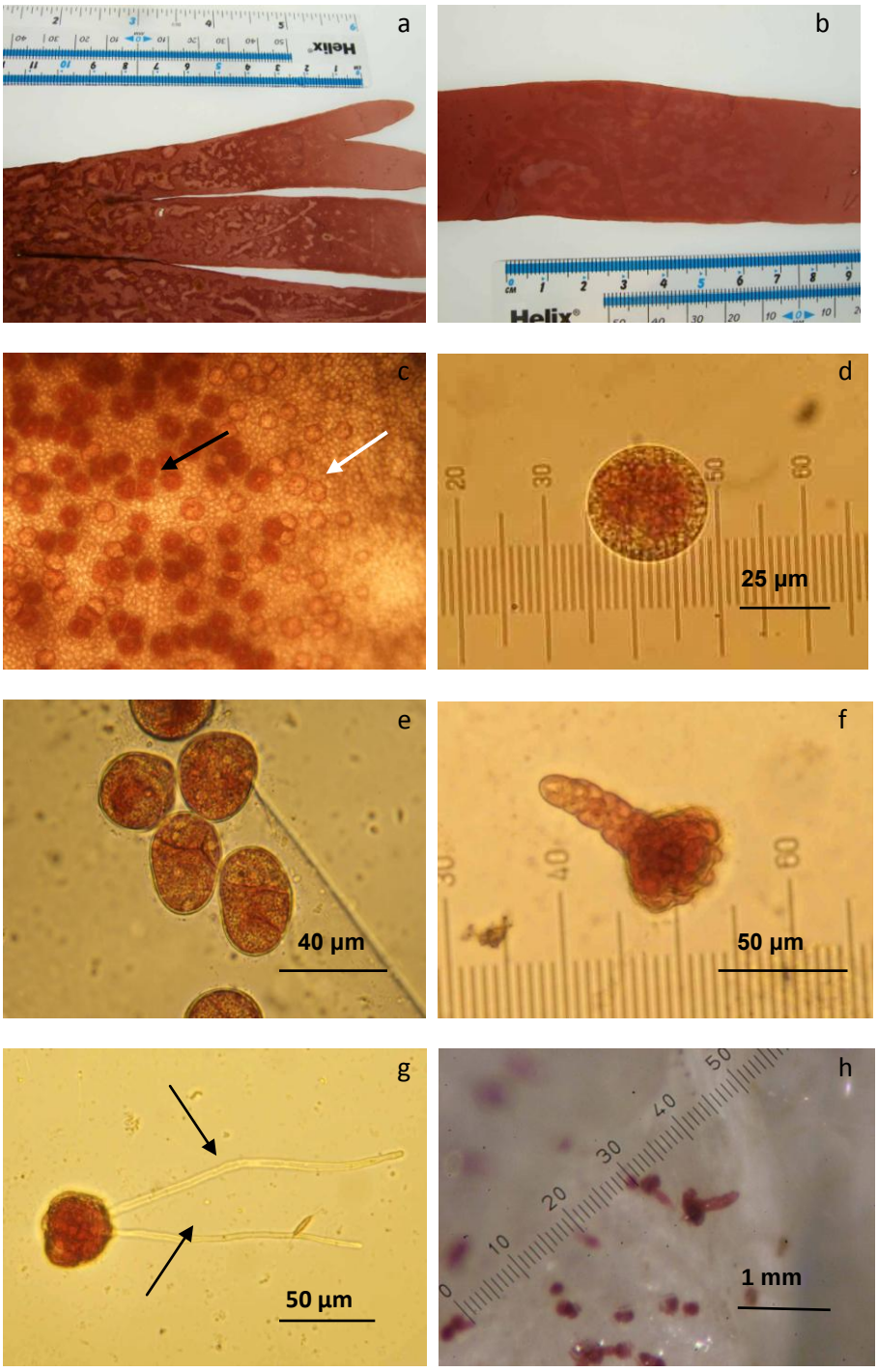


Fig. 2.4. a) Reproductive frond of a tetrasporophyte of *Palmaria palmata*. The irregularly shaped dark areas are spore-containing sori. b) Fertile frond of a male gametophyte. Lighter coloured, irregularly shaped areas contain spermatangia. c) Micrograph of fertile tissue of *Palmaria*. One tetrasporangium (spore compartment) contains four tetraspores (arrow). The white arrow points to an empty tetrasporangium after spore release. d) Spore shortly after release. e) Spores undergoing cell-division 4 days after release. f) Developing male gametophyte 10 days after spore release. g) Female gametophyte 10 days after spore release with trichogynes (arrows). h) Sporelings growing on culture string 1 month after seeding.

After release, these tetraspores settle onto a substrate and develop into male and female gametophytes in a 1:1 ratio. This gametophyte part of the life cycle is very unusual among algae in that males and females are completely different in morphology and life span. At an immature stage (i.e. before reproduction begins after about 1 year), the male gametophytes are indistinguishable from the diploid tetrasporophytes. In contrast, the female gametophytes develop into microscopic crustose thalli, no more than 3-4 cells thick and 0.1 mm in diameter (Fig. 2.3 c-e). Consequently, female gametophytes cannot be detected on the shore and they were only discovered relatively recently through culture studies (van der Meer & Chen, 1979; van der Meer & Todd, 1980). If their egg cells (carpogonia) are not fertilised within a few days, the female gametophytes die. Since the male gametophytes of the same generation are all immature at this stage, the females must be fertilised by gametes from males released in the previous year, and this ensures a good mixing of genetic material. After fertilisation, a zygote is formed and a new tetrasporophyte develops on top of the female gametophyte, and soon overgrows it (Fig. 2.3 f).

The male gametophyte reaches sexual maturity 9-12 months after germination. Spermatangial sori are formed that release spermatia which fertilise the egg cells of female gametophytes. The sori resemble the sori of the tetrasporophyte in shape but have a milky to translucent appearance.

Tetrasporophytes become fertile during winter. This coincides with the reproductive time of male gametophytes from the previous year. The reproductive season starts in November-December at most sites around Ireland and lasts until March/April.

2.5 Uses and nutritional value of *Palmaria palmata*

Palmaria palmata has a long history of use as a sea vegetable. The earliest record of the collection of *Palmaria* for food in Ireland is found in a poem written by an Irish monk in the 12th century. It is also mentioned in Icelandic sagas from the 11th century onwards but was used also in other countries such as Norway and France and on the east coast of Canada. Descriptions of *Palmaria* as a herbal remedy against, for example, worm infestation can be found in some European herbal books of the 18th century (www.algaebase.org).

In Ireland, England and Scotland, *Palmaria* is known under the name Dulse or Dillisk. In Gaelic, there are two names to describe *Palmaria*: *Creathnach* (feminine) and *Duileasc* (masculine). The first describes a smaller, narrower form that generally grows on small mussels on more exposed shores, also called “Shell-dulse”. The masculine form *Duilleasc* is used for the more commonly distributed larger, broader form that grows directly on kelp, *Fucus* or rocks (www.algaebase.org).

As one of the few seaweeds in Europe used for human consumption, the chemical composition of *Palmaria* has been of interest for a long time (Morgan et al., 1979). In recent years, the increased health awareness among the general public, and the search for new protein sources for animal feed and novel bioactive compounds, has led to further investigations into the chemicals found in seaweeds, which consequently have increased the range of applications for health foods, food supplements, cosmetics and also medical applications (Løvstad Holdt & Kraan, 2011; Indergaard & Minsass, 1991). *Palmaria palmata* has a relatively high protein content which makes it suitable for human and animal food. The protein content can even be increased when it is grown in integrated aquaculture or co-cultivated with abalone (Langdon et al., 2004). *Palmaria* is also a valuable source of vitamins and minerals as well as of antioxidants, which scavenge free radicals. The content of different chemicals, however, varies with season and location (Hagen Røgge *et al*., 2004). The average concentrations of chemical compounds found in *Palmaria palmata* are shown in Table 2.1.

Table 2.1: Chemical composition of *Palmaria palmata* (from Morrissey, Kraan & Guiry, 2001)

Protein	12 – 21 %
Fat	0.7 – 3 %
Carbohydrates	46 – 50 %
Vitamin C	150 – 280 ppm
Beta-Carotene	663 i.u.
Vitamin B1	7 ppm
Vitamin B2	2 – 5 ppm
Vitamin B3	2 – 19 ppm
Vitamin B6	9 ppm
Vitamin B12	6.6 ppb
Vitamin E	1.71 ppm
Calcium	2000 – 8000 ppm
Iodine	150 – 550 ppm
Iron	56 – 350 ppm
Magnesium	0.2 – 0.5 %
Manganese	10 – 155 ppm
Sodium	0.8 – 3 %
Zinc	3 ppm

Chapter 3: Setting up a seaweed hatchery

3.1 Hatchery facilities for seaweeds

A seaweed hatchery may be planned and built as a new unit or it may be integrated into an existing fish- or shellfish hatchery. However, certain basic facilities are required for the early stages of seaweed cultivation and this section outlines these requirements, some of which will be met by similar facilities available in other types of hatchery for aquaculture. To set up a seaweed hatchery, it is essential to have a temperature-controlled culture room, equipped with tap water, a supply of seawater and of air, a filter unit for seawater, fluorescent lamps connected to a timer and variable in height for control of intensity.

- **Temperature-controlled hatchery unit:** This can be an in-built cold-room or a refrigerated container* which can be installed outside in a yard area. The culture room should have seawater resistant fittings (including waterproof electric sockets), a non-slip waterproof floor and bottom drainage. The room should have a supply of tap water and compressed air, and convenient access to seawater.

  Since most of our native seaweed species are cultured at relatively low temperatures (5-15°C), and the hatchery must be kept very clean to avoid contamination of the cultures, it is almost essential to have additional workspace at normal room temperature that can be used as a wet-laboratory for processing collected or harvested algal material. Another warmer room for microscope work, preparation of culture media and collectors, storage etc. would be ideal.

  *Industrial refrigerated containers used for global transport are equipped with effective cooling units for a temperature range between -10 and 20°C. They are of standard width and height but are available in different lengths and can be purchased second hand. The interior can be customised to the requirements of a seaweed hatchery, e.g. lining floor and possibly walls with saltwater-resistant materials such as aluminium or synthetic materials to minimise corrosion. Any holes that are drilled through the hull of the container for electricity, water and air supplies or for drainage will need to be carefully insulated to maintain a constant temperature in the container throughout the year. Rupture of pipes due to frost needs to be avoided, as well as heavy condensation in the aeration pipes in summer due to temperature differences in and outside the container. Such condensation can collect in U-bends and block air flow. To deal with this problem, outdoor air pipes should be insulated and a water outlet should be built into the aeration line before the supply pipes are divided to serve the culture tanks, etc.

- **Seawater access:** A supply of good quality seawater is one of the most essential and most expensive elements of a hatchery and needs careful consideration. Seawater should be pumped from a fully marine and unpolluted site. The treatment should comprise coarse filtering, sediment settlement in a

storage tank, followed by fine filtering using, for example, cartridge filters (10, 5 and 1 µm pore size) and a final Ultra-Violet (UV) sterilisation unit with 2-3 UV light units in a row to kill microorganisms. Seawater treated in this way is suitable for *Palmaria* cultivation, including the processes of spore release and settlement, as well as cultivation of seeded material.

- **Drainage:** All on-shore aquaculture facilities require an aquaculture licence and a seawater discharge licence. Information on legal requirements can be obtained from the relevant authorities such as the Department of Agriculture, Fisheries and Food, Ireland (DAFF) in the Republic of Ireland, and the Department of Agriculture and Rural Development in Northern Ireland (DARD; see Appendix 1). Once a licence is granted, discharge activities will be monitored on a regular basis by the responsible authorities.

  The capacity of the drainage system needs to be carefully considered. It should be over- rather than under-sized and should cope with drainage of 1000-L tanks. In a flow-through system, a suitable capacity would be 5-10 times the overflow.

- **Light:** Light units for culture tanks are fitted with cool white fluorescent tubes providing an even illumination of tanks or areas with smaller culture vessels. The height of the light units should be adjustable or they should be connected to a dimmer so that the light intensity can be changed during cultivation. Light periods, i.e. day lengths, are controlled by timers.

- **Air:** Aeration of culture vessels is essential. Without proper aeration and the resulting movement of water around the algal culture vessels and tanks, the algal material will start to deteriorate quickly.

  An airblower needs to be installed in a separate, well aerated shed or separate room because it generates noise and heat. The capacity of airblower required depends on the size of the hatchery facility.

  From the airblower, the air is conducted to the culture room via PVC pipes. Multiple outlet sites are necessary to serve a large culture room. Therefore, the room should be fitted with a ring system of pipes with a number of outlet valves which can, in turn, be fitted with multiple outlets to which 3-4 mm tubing can be connected for aeration of the culture vessels and tanks. The diameter of the aeration pipe is progressively reduced to allow the pressure to be maintained.

3.2 Culture equipment and indoor cultivation tanks

The initial phase of *Palmaria* aquaculture is the settlement of spores onto a suitable substrate, and the cultivation of this seeded substrate in tanks. *Palmaria* spores are obtained from freshly collected plant material from the shore and seeded directly onto the culture string attached to structures/collectors which are then maintained in tanks. Spore suspensions can be cultured only for short periods of time to assess the viability of spores by monitoring germination success. This means that the requirements for *Palmaria* cultivation at a hatchery stage differ substantially from those for kelps such as *Alaria esculenta*, *Laminaria digitata* or *Saccharina latissima*. The basic equipment, however, is similar for any sort of hatchery.

Equipment for algal cultivation:

- Stereo-microscope: this is essential equipment for assessing spore release and monitoring the development of spores on the culture substrates into young plants.
- Light meter: different stages of *Palmaria* cultivation require different light levels. Therefore it is essential to be able to measure irradiance in the hatchery and adjust light levels accordingly.
- Salinity meter
- Balances: two different types of balance are needed. A fine balance (weighing up to about 500 g, with an accuracy of 0.01 or 0.001 g) is required to weigh out chemicals for media preparation, etc. A handheld spring balance (up to 5 or 10 kg) is useful for field work, for example to weigh freshly collected *Palmaria* in mesh bags.
- Glass vessels, e.g. 1- & 2-L flasks and beakers
- Tubing for aeration lines and disposable pipettes: aeration of cultures of any size (i.e. from 250-mL to 5-L culture flasks up to 200-L tanks) can be achieved simply by using clear flexible PVC tubing (a bore size of 5 mm is usually adequate) and 2-mL disposable plastic pipettes (see Fig 3.1a). For aerating small tanks, a weight (e.g. a rubber bung with a hole bored through it) should be attached to the pipette. For larger tanks, a pipe (e.g. electricity conduit) with a weight has to be used to ensure proper aeration (see Chapter 8.1).
- String collectors: the collectors to hold the culture string or nets are rectangular with a size of ca 50 x 40 cm. Collectors can be self-made by using plastic pipes (e.g. electricity conduit) to make a simple frame to which the string can be attached (see Fig. 3.1b).

- Plastic trays of different sizes: smaller trays (e.g. 30 x 20 cm, 15-20 cm in height) are useful for processing collected algal material. Large shallow trays (approx 80 x 50 cm) can be used for seeding collectors wit*h Palmaria* spores.

- Tanks for the cultivation of seeded collectors: the size and shape of tanks depends on the space available and amount of seeded material required. The tanks can be between 200 and 1000 litres. The tanks should be fitted with a drain valve at one side close to the bottom of the tank or in the floor to make them easy to empty. Either round or rectangular tanks are possible, preferably with a height of 60–100 cm, and can be made from any smooth material, such as glass fibre or PVC, as long as it is non-corrosive, non-abrasive and non-toxic. Smooth surfaces are less prone to fouling and are easier to clean.

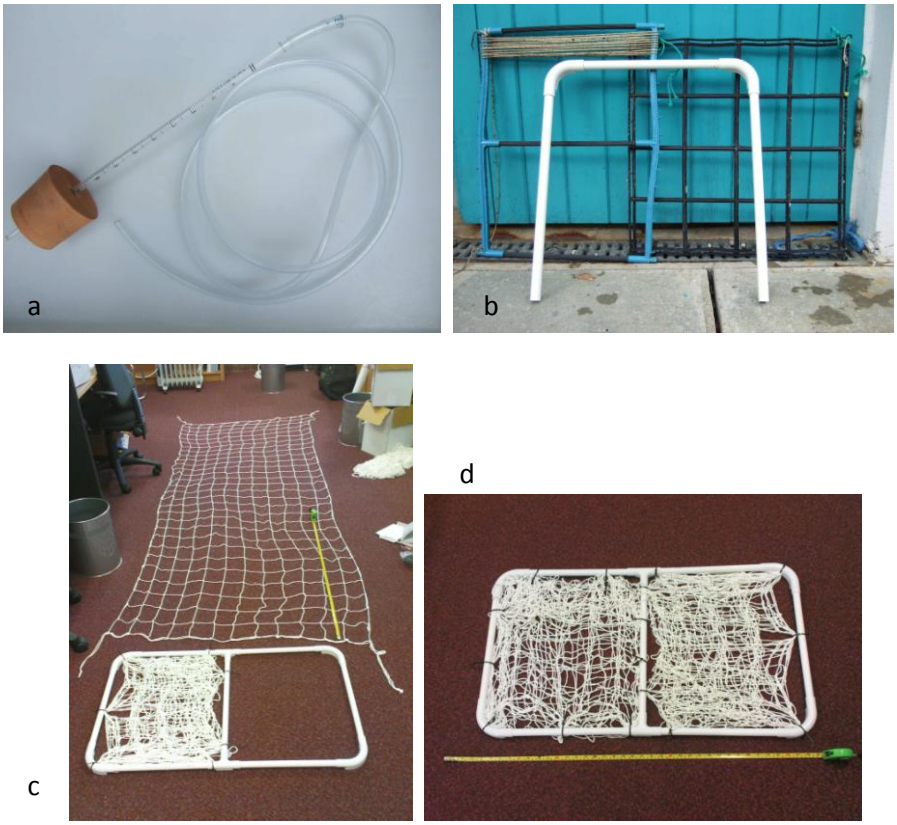


Fig. 3.1. a) Aeration tube for indoor culture tanks, consisting of PVC tubing, a disposable 2-mL pipette and a rubber bung as weight. b) Different types of culture string collectors. In the foreground, a home-made collector to which nets can be attached; in the background, collectors for culture string. c) Collector with one net attached and one spread out. d) Two nets folded and tied to a collector. Size of culture net: 1.2 x 3.0 m.

3.3 Culture medium

The developing sporelings of *Palmaria* are cultivated in seawater supplemented with nutrients. The nutrient enrichment medium can be prepared in the laboratory or bought as a ready-prepared mixture. All media contain macro-nutrients such as nitrogen, phosphorus, potassium, magnesium, calcium and sulphur, and micro-nutrients such as cobalt, iron, manganese, zinc, and vitamins. They vary slightly in their composition (Andersen et al., 2005; Appendix 2). A common enrichment medium used for red algae is von Stosch. "f2" is a medium frequently used for cultivating microalgae, but it is also commonly used for a range of macroalgae including *Palmaria*. These media can be purchased from different aquaculture-supply companies or culture collections (see Appendix 3).

3.4 Culture substrate and collector preparation

The substrate on which the *Palmaria* tetraspores are settled is an extremely durable and long-lasting string of 2 mm diameter. In Asia this string is commonly known as Kuralon (Fig. 3.2) and frequently used in seaweed aquaculture. It is made of polyvinyl or polyvinylalcohol fibre (PVA), woven or spun, but has the appearance of cotton string, i.e. slightly fluffy. To purchase original Kuralon string from Asia (e.g. China, Japan, Korea) is complicated unless commercial links are established. Other types of string were tested in sea trials during the "Seaweed Aquaculture Project and found suitable, e.g. 1-2 mm polyester string, spun or woven. Such string can be purchased in Ireland, the U.K. and other European countries (see supplier list, Appendix 3).

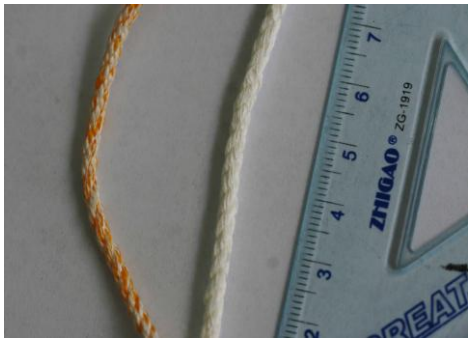


Fig. 3.2. Two types of Kuralon culture string used in China.

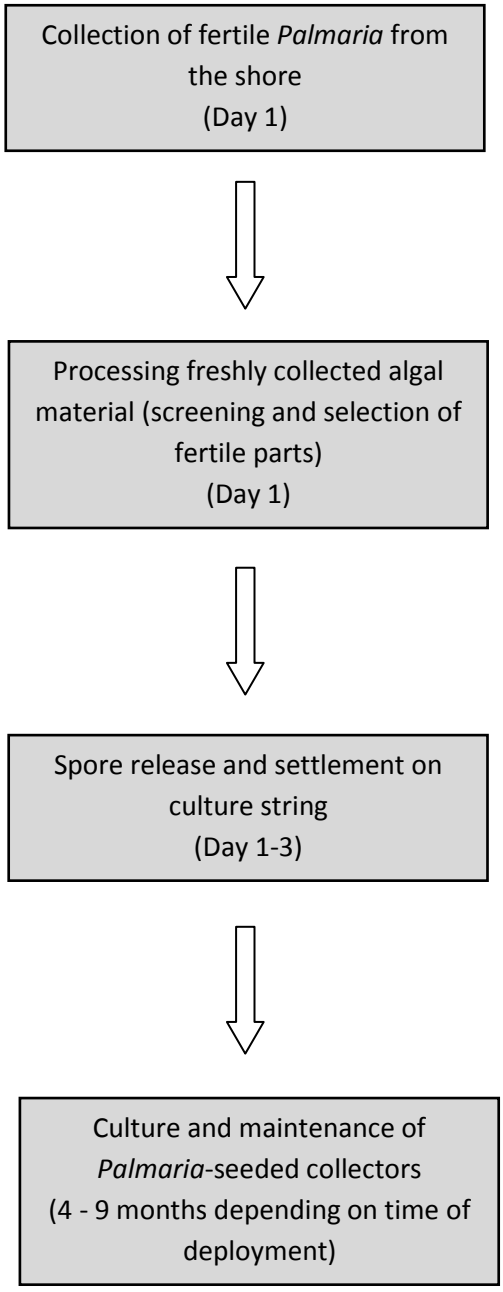
The most space-efficient deployment of *Palmaria*-seeded string is in the form of nets. Using the string described above, nets can be made with any desired dimension and mesh size. A size of net that has been extensively tested in the current project is 1.2 m wide x 3 m long, with a 10 x 10 cm mesh size. The length of the net is limited by the depth to which sufficient light penetrates to support the growth of *Palmaria*. A width of 1.2 m is suitable because, when seeding the net with spores, it must be folded a few times and then tied to a frame.

Seeded string can also be deployed as droppers. The string is then wrapped around a frame-shaped collector, with small spaces left between the parallel strings to allow spores to fall through to the string on the underside of

the frame. Prior to deployment, the string is cut into shorter lengths. The total length of string that can be accommodated on a frame collector of 55 x 40 cm is about 50-60 m (see Chapter 3.2).

Chapter 4: Cultivation of *Palmaria palmata* – nursery stage

The procedure for obtaining spores of *Palmaria* and cultivating sporelings in the hatchery until deployment at sea involves four major steps which are described in detail below. Two processes are crucial for successfully growing *Palmaria* on culture string: the selection of clean fertile algal material, and setting the right conditions for spore release and the survival of spores during the first two weeks. The flow-chart gives an overview of the major steps involved and a time frame.



4.1 Collection and processing of reproductive *Palmaria* material

Collecting fertile *Palmaria palmata*:

Palmaria tetrasporophytes are reproductive during winter. The best time for collecting reproductive material (i.e. when large numbers of spores are released and the settling and development of spores is most successful) differs from site to site around the coast of Ireland. The main period of reproduction around Ireland's coasts is between December and March/April. This period may be extended in the North, especially along the Irish Sea coasts, where water temperature is a few degrees lower than in the South and Southwest.

As *Palmaria palmata* is most abundant at or below the boundary between the lower intertidal and upper subtidal zones, the only time to collect fronds from the shore is during the low waters of spring tides. *Palmaria* is removed from the stipes of kelps or other substrate and taken to the hatchery for further processing. Usually 3-4 kg wet weight (1.5 – 2 onion bags) will provide enough material to seed 2-3 collectors. The collected material should be kept moist and cool, preferably by immersion in seawater at ambient temperature (e.g. in an outdoor tank with a continuous flow of seawater and/or aeration) until further processing.

Processing of collected material:

The algal material should be processed as soon as possible after return from the shore when the fronds are still in good condition. It is possible to keep *Palmaria* in the bags for up to 24 hours, provided that the bags are fully submerged in seawater with high water flow-through. If the fronds are closely packed and there is insufficient water flow along the surface of each thallus, they will die quickly and the colour will change from dark red to orange. The algal material should be processed at a relatively low room temperature (10-15°C) to avoid stress, which may trigger early spore release. Clean pieces of frond showing dark red areas of sori are selected for spore release (Fig. 4.1a-b). Any parts of *Palmaria*, which show attachment of other algae or animals, or unusual coloration (e.g. brown spots indicating endophytic infection), which are commonly found on the oldest parts of the algae, should be discarded (Fig. 4.1c-f).

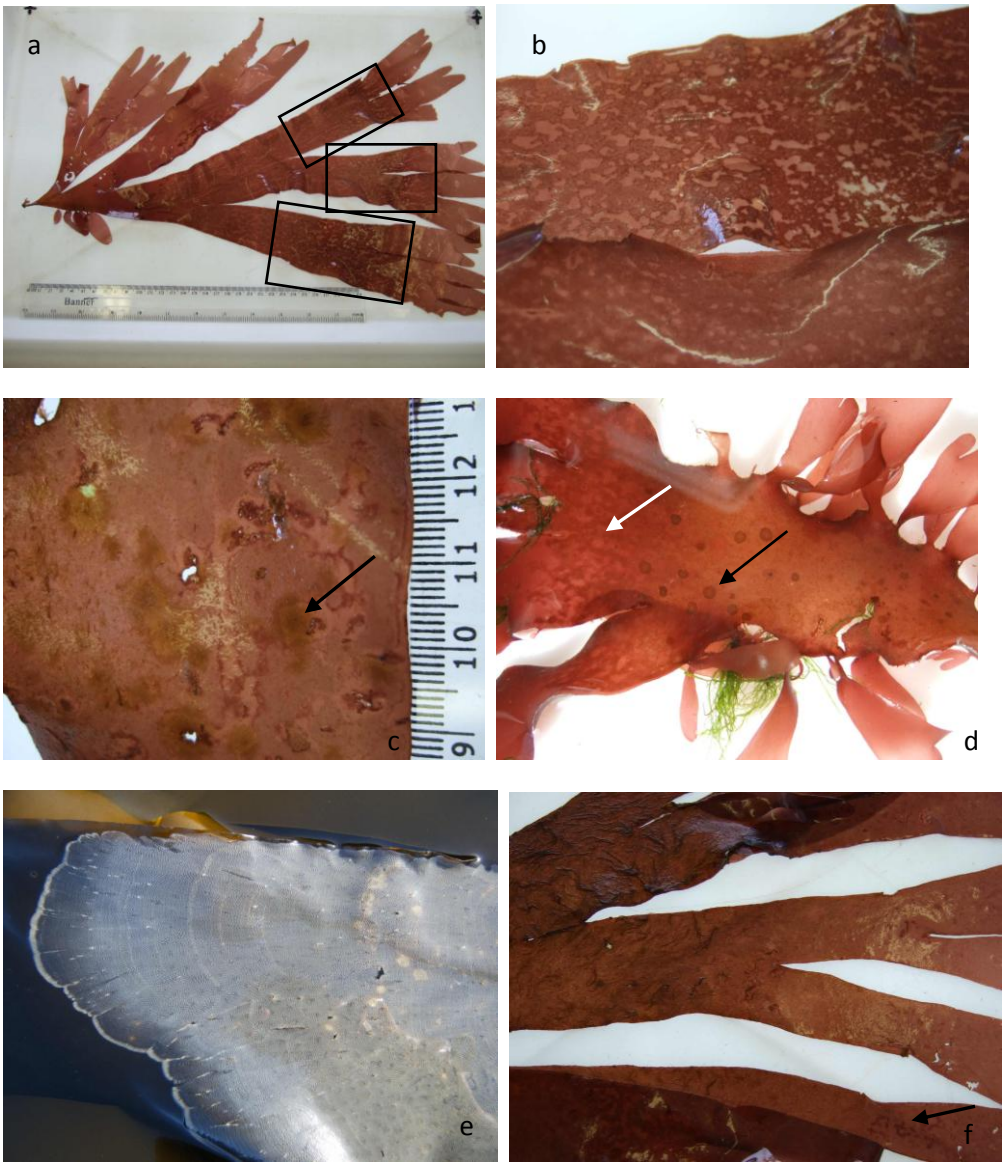


Fig. 4.1. Selecting fertile *Palmaria* fronds for spore release. a) Young reproductive tetrasporophyte – areas of mature sori marked by rectangles are suitable for spore release. b) Fertile section of *Palmaria* frond at higher magnification. c) Older part of *Palmaria* frond with endophytes - brown areas indicated by arrow. d) Young *Palmaria* frond with out-growths at the margins, spore-containing sori (white arrow) and patches with endophytes (black arrow). Some epiphytic green algae are also attached to the frond. e) Bryozoans growing on a *Palmaria* frond. f) Filamentous algae growing on a thallus of *Palmaria*; arrow indicates small area of developing sori. *Palmaria* fronds shown in c-f should not be selected for spore release.

The selected material can then be collected in a tray with a little seawater. When each batch of fronds has been processed, the material is briefly washed twice in UV-filtered seawater.

4.2 Spore release and seeding collectors

Kuralon culture string wrapped around a frame, or nets made with Kuralon (see Chapter 3.4), are used as collectors. The frames or 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 collectors or placed on a net lying on the collector which allows easier removal of the frond parts after spore release (Fig. 4.2). During the process of spore release, the containers are kept under low light (i.e. 5-10 µmol m^{-2} s^{-1}) at 10°C with gentle aeration and a light:dark period of 12:12 hours. 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.

Fig. 4.2. Spore release from *Palmaria*: fertile pieces of fronds placed on top of a collector carrying 2 nets.

4.3 Cultivation of seeded collectors

After three days of spore release, the algal material is removed and the seeded collectors are transferred to larger holding tanks (capacity: 200 L or more) filled with UV-filtered seawater and supplemented with enrichment medium (see Chapter 3.3 and Appendix 2). The light:dark period and temperature should be maintained but the light intensity can be increased slightly to 15-20 µmol m^{-2} s^{-1}.

Spore development: Firm settlement of spores on the string occurs in a few days. Spores can be seen under the microscope as small red dots. After settlement, the spores start to divide, and divisions are visible 3-4 days after spore release. After about 7 days, female gametophytes are recognisable as small cell aggregates with a filamentous outgrowth, the trichogyne, through which the spermatia from the male gametophyte will travel to fertilise the egg cell at the base of the trichogyne. The male gametophyte shows at this stage some hump-like outgrowths which develop into fronds (see Chapter 2.4 for development of spores).

An even distribution of settled spores over the whole collector is desirable for successful ongrowth in the sea, ideally at a density of 50 or more spores per cm of string. During the first two weeks after settlement, however, large numbers of spores (60-90%) frequently die off naturally. The reasons for this high mortality of spores are

not clear. In the worst cases, the numbers of spores left on the string are very low (10 per cm, or less) and, if this occurs, the collector should be discarded or re-seeded.

Light intensity: Light is essential for the growth of *Palmaria*, and the optimal light levels change as young plants develop. In its natural habitat, *Palmaria* grows in the dense shade created by the canopy of kelp fronds and it is not adapted to grow under high light levels. During the earliest phase of development, *Palmaria* spores and sporelings are even less tolerant of high light levels. Therefore, lower light is required during the first few weeks. For ongrowth, the light intensity can be increased to accelerate the growth of small *Palmaria* plants. If the collectors are to be kept in the hatchery for more than 6 months (i.e. for deployment in autumn), an irradiance of about 20 µmol m^{-2} s^{-1} will maintain a slow rate of growth and reduce fouling by more light-hungry competitors.

Maintenance: Tanks should be cleaned and the tank water renewed every two to three weeks. Aeration of the tanks is essential to supply the growing sporelings with CO_2 for photosynthesis and to enhance nutrient uptake. If fouling organisms start to settle on the culture string and the holding structure of the collector, the collectors should be gently rinsed with UV-sterilised water and fouling algae may be wiped off the holding structure/frame with a cloth. Larger algae may be removed individually.

Chapter 5: Seaweed farm design and set-up

Establishing a seaweed farm is a complex task which needs to take account of many different aspects from physical and biological parameters to legal requirements, some of which require advanced planning.

5.1 Site selection

The criteria for selecting a good site include technical, economic, physical, hydrobiological and legal issues. The different aspects are described below, starting with access and economic considerations, followed by physical parameters and the hydrobiology of the site.

- **Technical and economic selection criteria**
  - Utilisation of the area:
    - Does the area have any designation status, such as Special Area of Conservation (SAC), Special protection area (SPA), Area of Special Scientific Interest (ASSI), etc.? New activities in such areas are restricted and the responsible Environment Agency must be consulted.
    - Is the area close to shipping lanes or extensively used for recreational activities?
    - Are there other licensed aquaculture facilities near by? This may have synergistic effects, for example sharing of equipment, boats etc.
  - Access and workability: Access and travel distances are important factors affecting costs for labour, fuel etc.
    - How far is the site from the land-based business? Is the site within reach of a harbour?
    - Is there a pier or quay close to the site for launching a boat? And can the harvest be easily unloaded from the boat?
    - Does the road infrastructure provide easy access to the site of boat launching?
    - How exposed is the site? Can it be reached and worked on in most weather conditions?

- **Hydrobiological aspects:** The selection of a site for optimal growth of *Palmaria* is most important for successful aquaculture. At a site selected for *Palmaria palmata*, other seaweeds, such as *Laminaria digitata*,

Saccharina latissima and *Alaria esculenta*, can generally be cultivated as well. But *Palmaria* cannot necessarily be grown successfully at a site selected for other species.

- **Water current and exposure:** *Palmaria* requires a site with high water current. This is essential for good and healthy growth of the seaweed as it facilitates good nutrient and CO_2 exchange across the surface of the fronds. A strong water current is also essential for *Palmaria* to keep the fronds relatively clear from settlement of sediment and fouling organisms. Currents with flow rates of 5-10 cm s^{-1} are considered useful for *Palmaria* cultivation. The longlines should be in line with the main flow of the current. The exposure of the site to wave action should be moderate. This accommodates the biological requirements of *Palmaria* but is equally important for the accessibility of the site by boat and the stability of the farm structures.

- **Depth and tidal range:** The tidal range must be considered when choosing the minimum depth for installing farm structures. The depth of the site at low water should be at least 10 metres. The maximum depth should not exceed 20-25 metres to ensure better stability of the structures and keep installation and equipment costs down. It is also easier for divers to make safe inspections or carry out maintenance work.

- **Temperature:** The limiting factor for growth and good development is high water temperatures in summer and autumn. For example, in the South and Southwest coast of Ireland, summer temperatures may be too high to permit summer deployment of *Palmaria*, whereas in the North and along the Irish Sea, such deployment is feasible. Minimum winter temperatures are not considered to be damaging unless there is a threat of ice-formation which could affect the structures of the seaweed farm and cause abrasion of the cultures. Sites that experience large fluctuations in temperature (e.g. near a large, exposed intertidal area that will heat up rapidly during the day at low tide) should be avoided. Water over sandy substrates also tends to warm up more rapidly than water over rocky substrates.

- **Substrate:** Correct location on different types of substrate is important for several reasons. substrate will indicate the amount of flow of water and exposure of the site (e.g. deposition of mud indicates a low flow of water). It can also affect the turbidity of the water during stormy weather, and influence the water temperature (see above). Finally, it will determine what sort of anchoring equipment is needed. For example, a large heavy anchor stone is more appropriate for a rocky substrate, whereas lighter plough anchors or similar can be used effectively in sandy sites. Combinations of muddy, silty and sandy sites should be avoided as they may not provide solid anchorage, and any fine sediment thrown up into the water column may settle on developing plants and reduce photosynthetic efficiency

- **Salinity:** Most sites around the Irish coastline will experience full salinity and are suitable sites for *Palmaria* cultivation. Sites with brackish water, such as estuaries or where other large volumes of freshwater run into the system from streams or rivers, as well as outflows from wastewater treatment works or industrial sites must be avoided.

5.2 Materials for longline installation

The set-up of a seaweed farm varies with the demands of the seaweed species, the type of seeded collectors used and economic considerations. For *Palmaria* cultivation at sea, a single-header longline structure is currently used. Figure 5.1 shows a basic but versatile longline construction suitable for the cultivation of a range of seaweeds. The structure consists of a method of anchorage, connected to a header rope on or near the surface of the water, which is supported by buoys. The details of the equipment are outlined below, but longlines can vary slightly between sites and between operators because of different challenges in the deployment, and also in the amount of resources/materials available. No matter how the longline is constructed, two elements should remain the same throughout. First, care should be taken over the tensioning of the header rope – this must never be too slack as entanglement of the line becomes a problem. If it is too tight, however, rubbing can occur, with the breakup of the line likely, and loss of the harvest. Second, the header rope must always be positioned approximately approx. 0.5 m below the surface of the water. This is necessary as *Palmaria* develops and grows better at this depth than at the surface since light levels are lower here and bleaching is less likely.

Equipment for one 100-m longline

- **Anchors/mooring blocks:** Depending on the current at the site, the bottom substrate and the length of the longline, the anchor devices can be either concrete anchor stones of suitable size, plough anchors or other devices such as railway wheels. For a 100-m longline, the anchor stones should weigh at least 0.5 tonne, and preferably close to 1 tonne. Depending on the size, weight and number of anchor stones, a barge may be required for deployment. Some method of attachment is required on the stone, e.g. heavy metal ring, or eye.

- **Heavy link chain:** Several metres of galvanised heavy-duty chain are required to connect anchors/stones to the anchor rope. Chain with at least 16-20 cm links is suggested for use.

- **Anchor and header ropes:** The length of anchor rope which is attached to the chain from the mooring block or anchor is adjusted so that it is tight at high water without allowing too much slack at low tide. This must be checked after installation at different states of the tide. A marker buoy is attached to the end of each anchor rope. The headrope is then installed between the two marker buoys. Durable ropes such as 3-core polypropylene rope (e.g. 32-mm for mooring, 16-mm for headrope) should be used.

- **Buoys:** While cultivated seaweeds do not exert the same force on the header rope as mussels, regular buoyancy is required to maintain the stability of the structure . Marker buoys are attached to the end of each anchor rope. Over the length of each headrope smaller buoys are attached evenly spaced. (e.g. 25L barrels or smallerA rope length of about 30-50 cm should be allowed when tying the buoys to the headrope. Buoyancy should be monitored during the growing season. Additional buoys should be deployed if buoyancy is lost due to increasing weight of the collectors. There may be a legal requirement to install buoys of certain size and colour to the corners of the seaweed farm for designation.

5.3 Installation of longlines

The header ropes or longlines for *Palmaria* cultivation are set up in parallel. Usually the longlines are 50-100 m long and spaced about 10 m apart. Spacing of the longlines depends obviously on the size of licensed site and the number of longlines to be installed. The distance should be a minimum of 5 m to allow for boats working on maintenance and harvest to get access. Spacing that is too narrow may also affect the water flow. The longlines should be aligned with the tidal current. This facilitates the flow along the cultured seaweed without slowing down the water current too much.

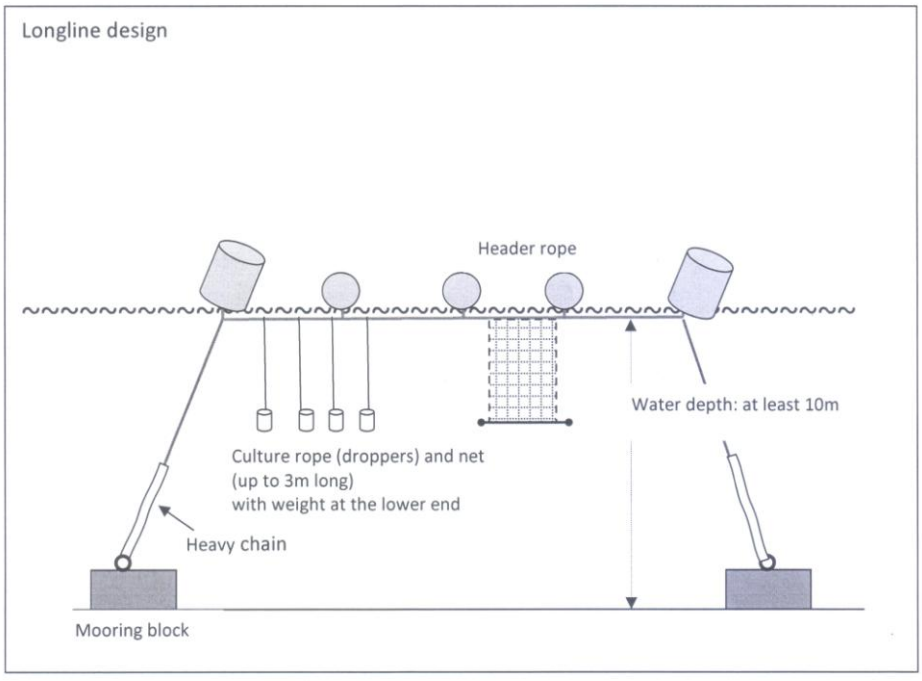


Fig. 5.1. Diagram of longline suitable for ongrowing *Palmaria* at sea

5.4 Maintenance and cleaning of longlines

Monthly checks and periodic inspections are necessary for maintenance. During the growing season, it is advisable to check connections of anchor ropes to the header rope, and attachment of buoys to the ropes for wear and tear. Buoy attachment ropes should be replaced if they are very worn or frayed. Periodic inspection of the anchors and chain connections by divers are required as metal parts corrode in seawater and attachments can break. Consequently, shackles should be replaced every 1-2 years as failure during the growing season can end in loss of whole longlines. Longlines can be moved or dragged out of position if a medium-sized or large vessel ties up to it. It is therefore advisable to assess the tension of the headrope occasionally and either reposition the anchors, or tighten the header rope if necessary.

During the routine visits, the header ropes should be checked for growth of fouling algae and entangled floating seaweed. These should be removed frequently as they add surplus weight to the longlines and increase the drag. The kelps *Saccharina latissima* and *Saccorhiza polyschides* are often found as fouling algae, as well as smaller red algae. Other species of seaweeds frequently become entangled with the headrope, e.g. *Ascophyllum nodosum*, *Fucus* species, *Himanthalia* and *Chorda*. The species composition depends on the site.

Settlement of mussel spat and sea squirts is also common (especially on the weights attached to droppers or nets) and should be removed as the longlines will become significantly weighed down. Headropes may be removed at the end of the growing season if the lines are not used for cultivating of other species. Storage of the ropes on land will ensure that fouling organisms will be completely dried up, so that they can be easily removed before re-deployment for the following culture season.

Chapter 6: Deployment and ongrowth of seeded collectors at sea and harvesting methodology

6.1 Deployment of seeded substrate

Transfer of seeded collectors: For the transfer of the seeded collectors from the hatchery to the site of deployment it is essential to keep the cultures string under damp and cool conditions. The nets or droppers should be removed from their holding structures in the hatchery. For the transport they should be loosely packed in large plastic boxes and covered with wet cloth (dipped in seawater!). For long journeys and/or hot days, some ice packs may be added but wrapped in damp cloth before placing them beside the culture string. The collectors must be handled with care and as less as possible because *Palmaria* sporelings detach easily from the string when touched.

Method: Seeded string is deployed either in the form of droppers or as nets. The droppers are spaced about one metre apart along the header rope, and are attached with thin ropes (5 mm) or cable ties, which are passed through the lay of the header rope (Fig. 6.1). A weight is attached to the end of the dropper to keep it vertical in the water column and to avoid entangling with neighbouring droppers. Suitable weights for droppers and nets will be about 500 g. They can easily be made by filling 500 mL-disposable plastic cups with concrete after inserting a loop of string for attachment. Other materials such as pieces of metal or stones can also be used.

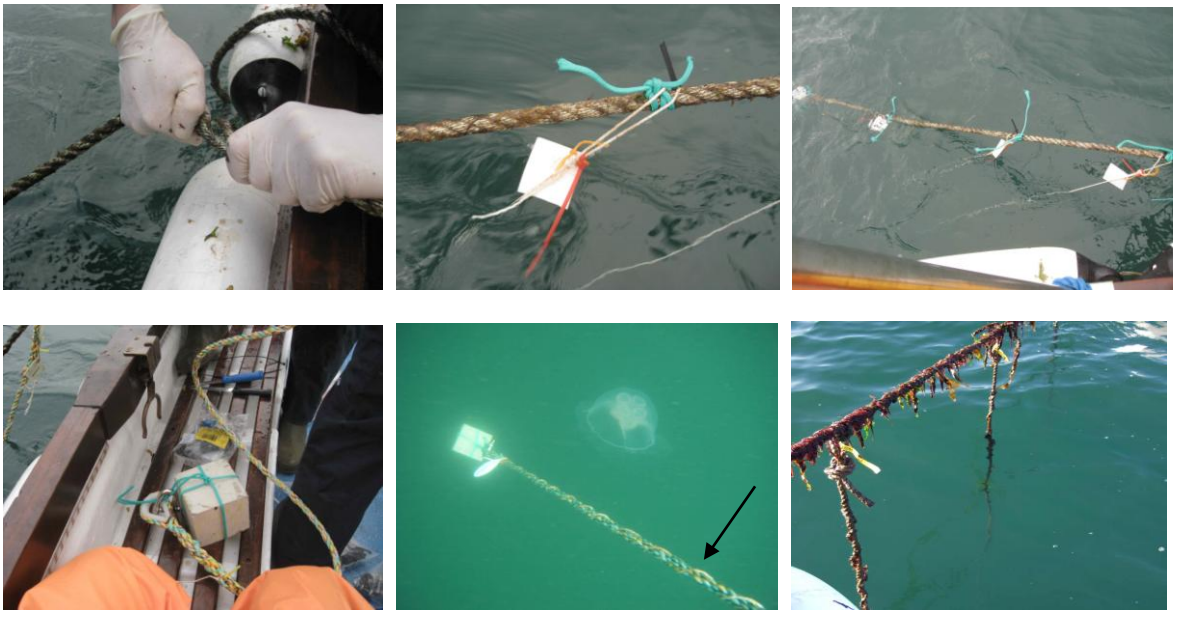


Fig. 6.1. Deployment of *Palmaria* droppers. Top row: Opening of the lay of the header rope to insert small rope or cable tie for attachment of dropper; experimental culture string tied to the header rope; droppers tied to the header rope. Bottom row: Weight attached to a dropper; seeded culture string (white colour; arrow) wrapped around 8-mm rope with an attached weight, suspended from the header rope; droppers in December at Strangford Lough two months after deployment - small plants are visible (NB fouling on the header rope).

Nets are attached directly to the header rope in a similar way to the droppers but with multiple attachment points. To keep a 3-m long net straight and unfolded in the water, a metal or plastic rod should be attached to the bottom end of the net and 2-3 weights attached.

Timing: The best season for deployment is late autumn/winter. At this time of the year, the growth of *Palmaria* will be reduced by the short daylengths and low temperatures but these factors will also inhibit the growth of opportunistic fast-growing fouling algae. Consequently, the *Palmaria* will be able to grow sufficiently to develop a good cover of the string, and this will make it more difficult for fouling organisms to settle when growth conditions improve at the start of spring in the following year.

Palmaria collectors can also be deployed in late spring/early summer to exploit the natural period for optimal growth. The material used for spring deployment should be well developed, i.e. *Palmaria* fronds should be at least 1 mm in length, and the culture string should be densely seeded. This is essential to prevent the settlement of opportunistic algae which can out-compete *Palmaria* plants by very fast growth. Another factor to consider is light. In summer, light intensities can be very high and small *Palmaria* plants can be easily and irreversibly damaged by bleaching (Fig. 6.2). In their natural habitat, this doesn't occur because *Palmaria* plants are shaded by the canopy of kelp blades. Lowering the nets or droppers to a greater depth (e.g. 1 m below the surface) may prevent bleaching over the summer.

Fig. 6.2. *Palmaria palmata* on a longline in Strangford Lough in June. The green tips indicate bleaching by high light. At this stage affected tissue can still recover.

6.2 Monitoring of plant development

Biomass increase should be monitored frequently, at least once a month. Fouling algae should be removed from the header ropes and from the droppers or nets. Depending on the time of deployment and the size of plants required, the growing season at sea will be between 5 and 8 months (e.g. May to September for spring deployment, October to April or May for winter deployment). Fronds should be harvested before fouling starts to affect the quality of the plants.

6.3 Harvesting methods

The optimal size at which *Palmaria* fronds should be harvested is 30-40 cm in length. It is crucial to monitor the fronds during the final stages of ongrowth at sea (i.e. after 5 months) to ensure that *Palmaria* is harvested before the fronds are overgrown by fouling organisms such as bryozoans. This is especially important when *Palmaria* is to be used for human consumption and the top quality is required. It is also important to monitor the sizes of fronds; with increasing time at sea and increasing size of fronds, individual *Palmaria* become more easily detached from the string.

Fig. 6.3. *Palmaria* cultivated in Strangford Lough. Left: *Palmaria* on a dropper after 5 months at sea. Right: *Palmaria* on a net after 5 months at sea; in some parts of the net, the *Palmaria* was heavily grazed.

Depending on the development and density of *Palmaria* fronds on the collectors, either one harvest or multiple harvests are possible. If the fronds are densely spaced and healthy, it is advisable to harvest the largest fronds by pulling them off the string to make space which will allow the smaller, suppressed plants to grow out. If this is done, the size of the *Palmaria* being harvested may be smaller than the ideal size, i.e. about 15-20 cm, but two or even three harvests may be possible if the quality of the collectors is good. If the density of the fronds is too low to support multiple harvests, or growth on the collector is very patchy, all the fronds should be harvested at one time. Collectors (nets or droppers) are untied from the header rope and brought to the hatchery, where all the fronds are removed from the string. Further processing depends on the intended use of the *Palmaria*.

Chapter 7: Results of *Palmaria* longline cultivation trials 2009/2011 and economic analysis

7.1 Case study: cultivation of *Palmaria* at four Irish sites: Summary of results

7.1.1 Spore release, settlement on culture string and ongrowth of sporelings

In every reproductive season of *Palmaria palmata* during the course of the project, i.e. winter 2008/09, 2009/10 and 2010/11, fertile tetrasporophytes of *Palmaria* were found in abundance for sporulation trials at each hatchery site (DOMMRS, MRI Carna Laboratories, QUB Portaferry). These trials were carried out in order to build up stocks of seeded string during the period of optimal spore production, and to test the ongrowing potential in the hatchery and later at sea. Reproductive material was collected fortnightly during low spring tides between January and May. The core time for reproduction is February to April. Fertile material found before February is often not fully mature, whereas material collected in May (this is only possible on the colder east coast of Ireland, i.e. Irish Sea coasts) tends to release only low numbers of spores with low viability (Fig. 7.1).

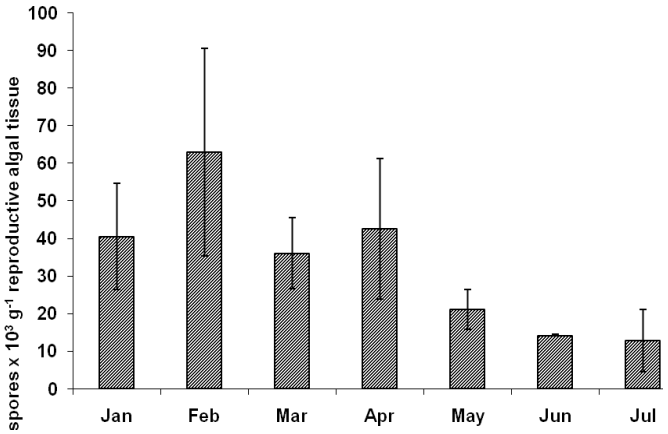


Fig. 7.1. Released spores per gram of fertile *Palmaria* fronds during the reproductive season. All algal material was collected during low spring tides in Strangford Lough. Error bars indicate standard deviations.

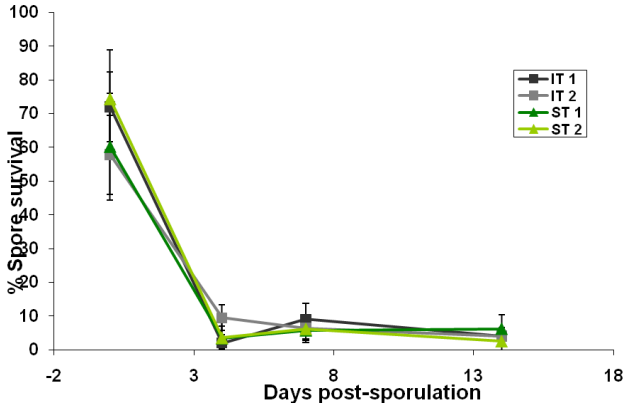


Fig. 7.2. An example of spore survival during the first two weeks after release. Reproductive *Palmaria* material was collected during low spring tides in Strangford Lough in April 2010 from different hieghts of the shore. Algae were collected from the lower intertidal (IT) and upper subtidal (ST). Error bars indicate standard deviation.

All hatcheries reported excellent spore release for most sporulation trials in each reproductive season. However, frequently - but not always - a mass mortality of spores was observed during the first 7 days after spore release (Fig. 7.2). Comparison of procedures, spore release rates and subsequent sporeling growth among the three hatcheries during the reproductive season of 2008/09 showed little correlation with factors that might affect the success or loss of seeded spores. In the following reproductive season (2009/10), a comprehensive programme of experiments was carried out by all partners to investigate the factors affecting spore survival and development, including water quality, nutrient and oxygen supply, harmful chemicals, grazers, and exposure of reproductive thalli to air on the shore prior to collection.

The results from all hatcheries showed that spores survive under a range of conditions after release. Overall, spore survival varied with the source of collected algal material but was generally low, i.e. 20-40%. None of the factors investigated appeared to be a 'key' factor that ensured a high survival rate. Mass mortality thus seems to be natural for *Palmaria* and is counterbalanced by high numbers of spores produced over a fertile season of about 4 months. To ensure a dense and even settlement of spores on the culture string, it was found that about 150 g of reproductive *Palmaria* tissue is needed for seeding a net or frame collector.

7.1.2 Development of spores on culture string in the hatchery

The spores which survived the first week after release settled well and firmly on culture string. Different culture string (Kuralon string from Korea and from China, spun polyester string, PV string) was used for settlement trials (Fig. 7.3). No significant differences in settlement and ongrowth were found. The string, however, should be washed thoroughly before use to leach out potentially hazardous chemicals, which may remain after manufacture and storage. Spores usually settled well on the conduit pipe used as frames for holding the culture string, and the frames became overgrown by sporelings.

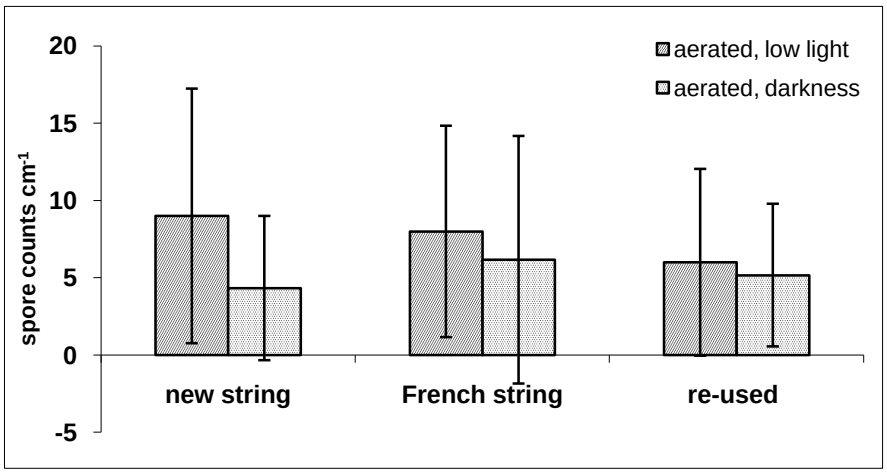


Fig. 7.3. Spore settlement on different substrates (new string: Kuralon, washed in warm water; "French" string: different type of string, smoother than Kuralon string, washed in warm water; re-used Kuralon string: washed in warm water before use). The culture media were aerated during spore release. Sori-containing *Palmaria* fronds were left on top of culture string in the test trays for 3 days. Spores were counted per linear cm of culture string after 7 days. Large standard deviations (n=10) are due to unequal distribution of spores on the string. "Nests" of spores are found on the string in comparison to stretches with only very low numbers of spores or none.

7.1.3 Ongrowth of sporelings at sea

At different times of the year, seeded culture string (either as nets or droppers) was deployed at the four trial sites (i.e. RWB, New Quay, Ard Bay and Strangford Lough). For the summer deployment, seeded material obtained from sporulation trials of the previous winter was used (i.e. after 4-5 months in the hatchery). For the autumn/winter deployments, however, the seeded culture string was kept for up to 12 months in the hatchery.

Deployment of seeded material in October-December was most successful at all sites tested. The sporelings developed slowly but well during the winter, especially because the competition from fouling organisms was low. In Ard Bay, *Palmaria* sporelings on 3-m droppers deployed at the end of November 2009 produced a biomass of 750 g per linear metre of culture string after about 5 months. Samples taken from the droppers at different time intervals showed maximum growth rates in February and March with a 3-fold increase in biomass per month. In April, growth rates decreased to 53% per month. Similar yields were obtained in Strangford Lough. One set of *Palmaria* droppers deployed at the end of October 2010 was harvested in March 2011 after 5 months at sea, and another set one month later. The average yields (wet weight) were 510 g m^{-1} and 880 g m^{-1}, respectively (Table 7.1). Droppers deployed in November 2010 had an even higher yield of 1190 g m^{-1} of high quality *Palmaria* fronds. Despite the promising development of *Palmaria* sporelings in RWB and New Quay in the winter of 2010-11, no crop could be harvested because of heavy fouling of the longlines in early spring, which overgrew the *Palmaria* plants.

Table 7.1. Yield of *Palmaria palmata* on droppers deployed in Ard Bay and Strangford Lough.

Deployment	Location	Days at sea	Months at sea	Yield (g FW m^{-1})	Standard-deviation
Nov. 2009	Ard Bay	151	5	750	156
Oct. 2010	Strangford Lough	153	5	510	101
Oct. 2010	Strangford Lough	180	6	880	460
Nov. 2010	Strangford Lough	158	5	1190	530

Droppers deployed in the early spring or summer of most years of the project were heavily fouled in a very short time, and did not produce a substantial amount of crop.

The results for biomass production using nets are more limited. In Ard Bay, a crop of *Palmaria* could be successfully harvested in 2010. The nets were deployed in December 2009 and the largest plants were removed from the nets in three successive harvests between March and May 2010 (Table 7.2). The total harvest was 25 kg from each net after 6 months at sea. A net deployed in November 2010 in Strangford Lough produced only 5.5 kg after 5 months at sea due to heavy grazing and areas of the net where no *Palmaria* sporelings had developed (Fig. 6.3).

Table 7.2. Yield of multiple harvests of *Palmaria palmata* on nets (3 x 1.3 m) deployed in Ard Bay.

Deployment	Location	Days at sea	Months at sea	Yield (g FW m^{-1})	Total yield (kg FW)
Dec. 2009	Ard Bay	111	4	91	7.1
		147	5	150	11.7
		183	6	80	6.3

The trials using nets seeded with *Palmaria* spores proved more challenging for obtaining good yield. Nets as a substrate for *Palmaria* spores are potentially more economic than droppers because they can provide more metres of seeded string per meter of longline (i.e. two 3-m droppers spaced 1 m apart provide 6 m of substrate per metre of longline, whereas one net with a width of 1.2 m and 3 m in length provides approx. 78 m of substrate). The procedures for producing seeded nets and deploying them are also less labour intensive. However, it is more difficult to achieve a dense and even settlement of spores on the string of the net than on long lengths of string wound around a frame. Consequently, the yield per linear metre of culture string was

usually less for nets than for droppers, but the total yield per metre of longline was higher, especially when multiple harvests could be taken.

On the basis of our trials of *Palmaria* ongrowth at sea, which have been conducted over 3 years at four different sites, the following conclusions can be drawn:

- **Site of cultivation:** The choice of site is critical for successful grow-out of *Palmaria* at sea. Strangford Lough and Ard Bay seem to provide the right habitat, probably because strong currents ensure good water exchange and favourable water temperatures. Although the site at New Quay has a relatively high current flow, *Palmaria* production was not satisfactory. *Palmaria* trials have been conducted at RWB in all 3 years of the project, but it is evident that this site is not suitable for *Palmaria* cultivation. The sheltered position of the site means that water currents are relatively weak, so that summer water temperatures can exceed the optimum for *Palmaria*, and the potential for fouling also seems to be high. It was shown, however, that the site is excellent for the cultivation of several kelp species.

- **Time of deployment:** The best time for deployment of *Palmaria* sporelings is late autumn/early winter. Before December, the sporelings reach a size (approx 5 cm) that enables them to compete with the fast-growing fouling algae, which start to settle from late January onwards. Rapid growth of *Palmaria* is observed in early spring until early summer

- **Length of cultivation:** The fastest growth of *Palmaria* is observed from early spring until early summer. The results indicate that *Palmaria* fronds of the highest quality are harvestable in early-mid spring. The material can quickly deteriorate, due mainly to settlement of fouling organisms, grazing, bleaching of fronds due to high light in late spring/summer, and detachment or dislodgement of fronds when they reach lengths of over 40 cm and weights of over 100 g. An ongrowth time of 5 months from November until April seems to be optimal.

In conclusion, despite some encouraging results in the project, it has not been possible – between 2008 and mid 2011- to demonstrate year-on-year a consistently successful culture methodology for the sequence of *Palmaria* sporulation – settlement on string – sea deployment – grow-out to harvest.

7.2 A seaweed production model

This section examines the costs of setting up a seaweed hatchery and a seaweed grow-out farm, and the possible income using the best results of the work undertaken by the Project Team. It seems clear that the tonnage of *Palmaria* required to meet Ireland's needs for abalone and urchin feed will not be achieved from seaweed aquaculture unless techniques are developed to overcome the problems currently encountered. For example, the amount of *Palmaria* required annually to feed Ireland's macroalgivores at full production is estimated to be around 1,000 tonnes. If the predicted abalone and urchin tonnage is harvested (27 tonnes of sea urchins, 43 tonnes of *Haliotis discus hannai* and 37 tonnes of *H. tuberculata*), utilising an all-macroalgal diet, the required tonnage of *Palmaria* will be between 750 and 1,200 tonnes per annum.

Measured Yield of *Palmaria palmata*

In previous work in Strangford Lough, Edwards (2007) demonstrated growth of 0.583 kg *Palmaria*/linear metre on droppers by day 150, and achieved a yield of 1.724 kg/linear metre, after 4 sequential harvests of *Palmaria* on droppers. However, during the recent work at various sites around Ireland, the good yields that have been obtained were:

a) 0.321 kg/linear metre on nets (1.3x3 m with 10-cm mesh) in Ard Bay by June 2010.

b) 0.75 kg/metre of string dropper after 151 days' growth in Ard Bay in April 2009.

It is these values that have been used in the following economic analysis.

Assumptions

Assumption 1: *Palmaria* grow-out on nets

A 100-m longline can hold up to 70 nets (1.3 m wide with 12 cm between nets for attachment). Since the nets are 3 m long and have a 10-cm mesh, the total length of string per net is 82.3 m. Assuming a yield of 0.321 kg m^{-1}, each net will yield 82.3 x 0.321 = 26.4 kg, and a 100-m longline with 70 nets will produce 1.8 tonnes using this technique.

Assumption 2: *Palmaria* grow-out on droppers

Using 3-m droppers, an average coverage of 0.75 kg m^{-1} has been achieved, so that the total potential yield from a 3-m dropper is 2.25 kg. It is possible to deploy up to 80 droppers on a 100-m longline, allowing space for tying and buoyancy. A 100-m longline with 80 droppers will produce only 180 kg of *Palmaria* using this technique.

Conclusion

Based on the best results obtained during the current project, the use of nets results in much higher yields of *Palmaria* than the use of 3-m droppers.

Requirement for fertile material to seed collectors

The results to date have indicated a very poor survival of spores in the hatchery and this is the subject of much intensive analysis. Based on the project's experience, there is an average requirement to collect 1.875 kg of wet *P. palmata* to provide enough ripe material to seed one net of the size 3x1.3 m with 10-cm mesh. Only 8% of that amount will be usable for sporulation work, i.e. 150 g ripe material per net. On this basis, 10.5 kg of ripe material will be needed to seed the 70 nets for a 100-m longline. This will mean that 131.25 kg of *Palmaria* will need to be collected from the shore for each 100-m longline.

Since the yield from one 100-m longline containing 70 nets is only 1.8 tonnes, the complete cultivation process is increasing the starting material by only 13.7 times. Unless sporulation success can be improved and better yields obtained, there appears to be little point in culturing *Palmaria* in the hatchery for deployment to sea on nets for grow-out.

Hatchery set up costs

In spite of these disappointing results, we have continued with the preliminary work to design and cost a hatchery for the supply of *Palmaria* seeded nets. There is an absolute requirement for certain pieces of equipment regardless of the level of production, i.e. the cold room, autoclave and microscope. It is envisaged that the hatchery will be multi-disciplinary with bivalve production taking place and other seaweed species being produced. The hatchery unit costs include a cold room capability in an insulated container with all of the equipment items required (Table 7.3). A laboratory, office, toilet and canteen are not costed. The cold room (12.19x2.7x2.43 m) would contain a single layer of 24 tanks at floor level with a total tank volume of 16.2 m^3. These tanks can be used to supply either *Laminaria* collectors or *Palmaria* seeded nets. Unfortunately, folded nets (3x1.3 m) for *Palmaria* do not make efficient use of the available space, since only two nets could be fitted in each tank. Therefore, if all 24 tanks were used for *Palmaria*, only 48 nets could be held in the system, and these would cover only 70% of a 100-m longline. Because *Palmaria* must be maintained in the hatchery through the summer for sea deployment in the autumn, culture of this species will occupy the complete hatchery capacity until deployment. Forty eight nets deployed at sea would yield 1.26 tonnes of *Palmaria palmata*.

Hatcheries of this size and with this amount of equipment exist in Ireland at the moment and these are mainly used for shellfish production work (oysters, scallops, clams). The costs given above are an accurate reflection of the current situation. A hatchery of this size could be also be used for bivalves in association with seaweed work.

Production from Hatchery

As discussed, this hatchery could produce 48 nets for *Palmaria* grow-out in just one batch and for sea deployment in October, November, December and January.

Table 7.3. Indicative Hatchery set-up and operational costs year 1

Hatchery Costs	**€**
1 x insulated room	8,500
with AC and control panel	
Autoclave	14,000.00
Microscope	1,500.00
Precision balance	1,500.00
Pipework	2,000.00
Tankage16.2m³	14,000.00
UV	1,000.00
Consumables	
Glassware	1,500.00
Fluorescent lamps	500.00
Nutrients	750.00
Nets (48 @ €10 each)	480.00
Total	**45,730.00**
Electricity per annum	30,000.00
Labour per annum	60,000.00
Total	**135,730.00**

At-sea costs

The total cost of one 100-m longline is €1,817. A stand alone longline unit with ten 100-m longlines would cost €30,010 (Table 7.4).

Table 7.4. Cost of seaweed longline

Cost of seaweed longline		
Nylon rope 110mx28mm	€	350.00
Anchor rope 90m	€	200.00
Chain 5m	€	100.00
Blocks 2x2tonne concrete	€	600.00
Buoys 20xA2 buoys with spliced ropes	€	400.00
Trawl floats 2	€	27.00
Shackles	€	40.00
Tying rope	€	100.00
Total	**€**	**1,817.00**
Cost of 10 longlines	€	18,170.00
4 Navigation buoys and anchors	€	11,340.00
Deployment	€	500.00
Total cost 10x100m longline unit	**€**	**30,010.00**

***Palmaria* seaweed farm productivity and value**

A 100-m longline with capacity of 70 nets x 26.4 kg each will produce 1.8 tonnes wet *Palmaria* using this technique. NB. The hatchery described has capacity for only 48 nets.

Dry yield from 70 nets is 20% of 1.8 tonnes, i.e. 0.36 tonnes

Assumption. Value in the market place

Palmaria

Price per kg wet €2.50 per kg

Price per kg bulk, dry and bagged €16 - 19 per kg

Sales value ex farm 100-m longline with 1.8 tonnes @ €2,500 per tonne wet weight is €4,500

Sales value dry weight product 0.36 tonnes @ €16-19 per kg is €5,760-6,840

The potential value of sales of bulk, dried and bagged *Palmaria* from 48 nets is €4,055-4,815

This suggests that cultivation of *Palmaria* using these techniques will not provide the basis for a viable business.

Chapter 8: Land-based tank cultivation of *Palmaria palmata*

Tank cultivation provides another means of cultivating *Palmaria palmata*. The advantage of this cultivation method is that the nursery phase is omitted because harvestable biomass of *Palmaria* is grown vegetatively from an initial stock of *Palmaria* collected from the shore. Once the initial biomass is growing in the tanks, the surplus material is harvested at frequent intervals throughout the year. Therefore, there is no need for a hatchery or for on-growing structures in the sea, and the limitations imposed by boat availability and weather are also avoided. However, this type of cultivation does require considerable space on land to set up the tanks, and land rental may represent a significant cost. Tank cultivation is most appropriate, therefore, if a site is available with seawater access and discharge facilities, or if existing tanks can be used in a shellfish or fish hatchery. Depending on the available facilities and the purpose of cultivation, tanks can be installed outdoors or indoors. The cultivation system can be designed as a modular system in which a variable number of tanks of different sizes can be added up to a certain limit determined by the capacity of the seawater supply and drainage systems, and the aeration system on site.

8.1 Equipment and tank set-up

The descriptions given in the following section refer primarily to outdoor tank cultivation. Indoor cultivation will probably be considered only if tanks can be integrated into an existing indoor aquaculture facility, thereby reducing investment and running costs. Tank set-up for indoor cultivation is described in paragraph 8.1.2.

8.1.1 Outdoor tanks for algal cultivation

- A circular tank fitted with a central aeration pipe (see below) provides the best pattern of water circulation, in which the fronds are continuously tumbled around. Water movement in rectangular tanks is substantially reduced in the corners of the tank, and this can lead to the accumulation of algae in areas of restricted water flow (“dead corners”). In rectangular tanks, different types of aeration device are needed to produce a circular water flow (see Fig 8.1).

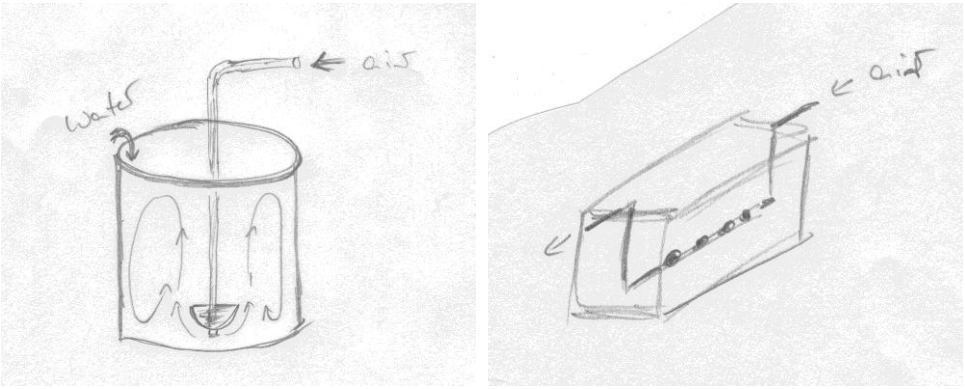


Fig. 8.1. Aeration devices for circular and rectangular tanks. The air bubbles create a circular water flow which carries the algal fronds with it.

- **Tank sizes:** The most appropriate size depends on how much biomass is to be produced and how much space is available. However, common sizes used for aquaculture are 1000-L and 2000-L tanks. Tank dimensions and are given in Table 8.1.

Table 8.1. Dimensions for a 1000-L and 2000-L tank – an example.

Tank volume	Tank depth	Tank diameter	Tank surface area
1000 L	90 cm	119 cm	1.112 m^2
2000 L	110 cm	160 cm	2.01 m^2

- **Tank material:** Tanks made of PE/HDPE (High Density Polyethylene) are commonly used. Fibreglass tanks (i.e. glass reinforced polyester (GRP) resin tanks) can be used but may not provide a very smooth surface and this may encourage the settlement of fouling organisms. PE tanks can be single- or double-walled, colourless or black. Double-walled tanks may be particularly valuable for outdoor cultivation because of the insulation they provide.

- **Seawater supply:** A seawater supply system will usually comprise pumps, filter units, storage tank and pipe network for seawater distribution, and a drainage system. For the tank cultivation of seaweeds, a coarse filter system such as sand filters will probably be sufficient. Depending on the seawater access and pumping mode, seawater will probably need to be stored after filtering and before it is supplied to the tanks. The volume of holding tanks required depends on the volume used for cultivation and the set-up of the system, i.e. flow-through or interval-flushing.

  Seawater can be pumped continuously through the tanks. The turn-over rate (the number of times that the full volume of the tank is replaced each day) depends on the sizes of tanks and the amount of seawater available for continuous supply. Working with a continuous flow-through certainly involves

higher running costs than does interval flushing, especially when bigger tanks are used. Interval flushing involves pumping seawater into the tanks at intervals to exchange a certain volume every hour (or longer time period). High and continuous flow-rates have the most positive effect on growth of *Palmaria*, but interval flushing combined with strong water circulation, driven by aeration in the tank, can be nearly as efficient as continuous flow-through.

- **Aeration:** Aeration is essential for good growth and health of *Palmaria*. The water movement created by aeration fulfils two important tasks: a) movement of algae in the water to enhance nutrient uptake and thus growth, and b) movement of algae in a circular flow from the surface to the bottom and up again to minimise exposure to high light intensities, especially in summer, and thus to avoid bleaching of *Palmaria* fronds. Bidwell et al. (1985) were the first to develop the principle of tumble culture using specific air agitation for the cultivation of *Chondrus crispus* but it has been applied to the culture of other algal species, including *Palmaria* (Pang & Lüning, 2004). Fig. 8.1 shows types of aeration suitable for circular and rectangular tanks. Circular tanks are best aerated by a central pipe with an air outlet at the bottom. A weight will hold the pipe in place (Fig. 8.2). Aeration devices can be home-made quite easily or bought in. Rectangular tanks should be fitted with a pipe running along the whole length of the tank in the middle of the floor. Holes should be drilled at intervals in this pipe for the air to escape and bubble up to the surface.

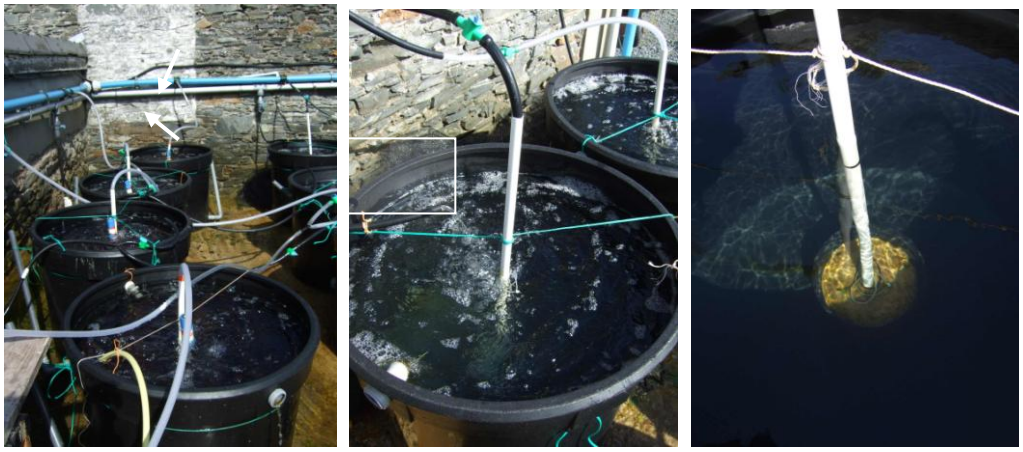


Fig. 8.2. Experimental 200-L tanks for *Palmaria* cultivation. Left: Overview of set-up. Arrows indicate the main supply pipes for air (blue) and seawater (grey). Middle: Seawater is supplied through a tube (2 cm diameter; white box) and air by a central pipe. Right: Aeration pipe with weight at the bottom made by filling a flowerpot with concrete.

The capacity of the air blower required depends on the size of the aquaculture system. It may be advisable to build in some surplus capacity, since that will allow the tank system to be expanded if required. A stand-by air blower should also be purchased to replace the main one in emergency. Without aeration of tanks, *Palmaria* starts to decay in a few days. Air is distributed from the air blower to

the tanks via a pipe system where the final diameter of the pipes is 2.0-2.5 cm when they reach the aeration device. Valves in each line enable adjustment of air flow into each individual tank (Fig. 8.2).

- **Light:** Outdoor tanks may need semi-transparent screens or blinds to reduce light levels and thus avoid bleaching of *Palmaria* fronds. A black mesh with small mesh size may be sufficient to prevent bleaching. Shade cloth mesh tarpaulins used in fish aquaculture are, for example, available in different grades of shading. Such screens will also reduce the growth of high-light requiring, opportunistic algae that may compete with the *Palmaria*, although such competitors should not be a problem if the initial stocking densities are high (see Chapter 8.2).

- **Temperature:** Optimal temperatures are between 8 and 12°C, and *Palmaria* will not grow well if the water temperature exceeds 15°C. In some parts of Ireland, therefore, it may be necessary to cool the seawater going into the tanks in summer. Cooling units can be inserted into the seawater supply system. Holding tanks should be well insulated in order to maintain the ambient temperature of seawater. Any periods of low water temperature in winter should not cause problems in a flow-through system but, with interval-flushing, there may be significant decreases in water temperature when the air temperature falls below freezing, because of the large surface area of tanks containing 1000 litres or more. Variations in water temperature may be reduced by double-walled tanks, or by covering the tanks at night.

8.1.2 Indoor tanks for algal cultivation

Culture tanks for seaweed may be integrated into an existing shellfish or fish farm with indoor cultivation tanks. The set-up of algal culture tanks depends strongly on the existing facilities and the space available. The requirements with respect to tank size, shape, aeration devices, temperature control and drainage are the same as for outdoor cultivation but there will be differences in seawater supply and light.

- **Seawater supply:** Algal culture tanks that have been installed in a shellfish hatchery for the purpose of producing algae as shellfish feed may be integrated into an existing seawater re-circulation system. This will enable the seaweed tanks to function as a water cleaning unit for the wastewater from the shellfish or fish tanks, which will contain nutrients released from the feed residues and faeces of the fish or shellfish. The combined use of fish/shellfish and seaweed species in one aquaculture operation permits the waste produced by one aquatic species to be transformed into a product, which can either be recycled within the operation or sold as a separate product. This is called “Integrated Multi-Trophic Aquaculture” (IMTA; see Appendix 4, Glossary, for further information).

In such an integrated system, the waste water from the fish or shellfish tanks should be filtered before it is pumped into the algal tanks to reduce the particulate load, since this cannot be removed by the seaweed and is likely to settle on the fronds and inhibit light absorption and hence growth. The efficiency of nutrient extraction by *Palmaria* depends on the concentration of nutrients in the water, the stocking density of algae in the tanks, the growth rate of the algae and the light provided. These need to be tested in the system as it is installed.

- **Light:** Appropriate levels of lighting are essential for achieving good growth rates of *Palmaria*. Since artificial light sources will never reach the intensity of sunlight, there is no danger of exposing the algae to irradiances that could cause bleaching in outdoor tanks. The lamps that are most commonly used for illuminating algal tanks are cool-white fluorescent tubes. The light tubes are housed in waterproof casings and hung over the tanks in a way to provide an even light field. Other light sources used for seaweed cultivation which may provide higher irradiances than cool-white fluorescent tubes are sodium high-pressure lamps and metal-halide lamps. An irradiance of 200-400 µmol photons m^{-2} s^{-1} and a light-dark cycle of 16:8 hours would be suitable for achieving relatively high growth rates.

8.2 Algal material for tank cultivation and stocking densities

The cultivation of seaweeds in tanks relies entirely on vegetative growth, i.e. the ongrowth of small plants or fragments of plants that have been collected from the shore or grown within the system. Consequently, the life cycle and reproductive state of *Palmaria* have no influence on tank cultivation.

- ***Palmaria* material for tanks:** For the initial stocking of the tanks, *Palmaria palmata* must be collected from the shore. The material collected should be screened carefully before use to ensure that the fronds are healthy, in good condition and do not carry any organisms on their surface. Young plant material, i.e. the tips of newly grown fronds, provides the best starting material for ongrowth in the tanks. Such fronds usually grow rapidly and are free from fouling organisms, either on the surface (epiphytes) or within the tissue (endophytes). Large fronds can be torn into smaller fragments, so that the youngest, apical parts, which contain the growth meristem at the edges of the frond, can be used. The older parts of the frond, i.e. the base with the holdfast, tend to grow more slowly and frequently carry epiphytes or endophytes (Fig. 4.1), and these parts of the fronds should be removed. The fronds will be circulating freely in the tanks, carried by the water flow and aeration, and do not need attachment points.

- **Stocking densities:** Stocking densities are usually expressed as kg fresh weight per square metre of tank surface (kg FW m^{-2}). The effects of different stocking densities between 2 and 6 kg FW m^{-2} on growth under different light and nutrient conditions have been investigated for *Palmaria palmata* as well as for *P. mollis*, a species similar to *P. palmata* from the north east Pacific. The densities commonly

recommended are relatively high, i.e. 3-4 kg FW m^{-2} (Demetropoulos & Langdon, 2004; Pang & Lüning, 2004; Morgan et al., 1980) because it is only with such dense cultures that the growth of opportunistic algae can be suppressed. For a specific site and tank set-up, however, stocking densities may be adjusted depending on the tank size and shape, light exposure and season, and nutrient application and seawater flow-through rate. High stocking densities create more shading in the tank, and this is advantageous for two main reasons: opportunistic algae are shaded out, and individual blades of *Palmaria* are only briefly exposed to sunlight when tumbled in the water flow, so that there is less danger of bleaching. On the other hand, high biomass in the tank reduces the flow along the fronds of individual *Palmaria* plants, thereby reducing nutrient uptake and in turn reducing growth. Selecting a stocking density therefore requires a balance between good growth rates stimulated by high nutrients at low densities versus the potential damage to fronds caused by high light, and the competition from opportunistic algae. The biomass per tank should certainly be adjusted to the available light, with higher stocking densities in summer and lower densities in winter.

8.3 Nutrient supply

The enrichment of seawater with nutrients increases the growth of cultured algae in the same way that fertilisers affect land plants. Several aspects have to be considered about nutrient addition, e.g. which nutrients to add, at what concentration, how they can be applied in a flow-through system, the potential side effects of seawater fertilisation.

- **Nutrients:** A range of nutrients is essential for the growth of seaweeds, and these can be divided into macro-nutrients such as nitrogen (N), phosphorus (P), potassium, calcium and magnesium, which are required in higher concentration, and micro-nutrients such as cobalt, iron, manganese, zinc and vitamins, which are needed in only very small amounts. Seawater provides all these nutrients, but the concentrations of N and P and many of the micro-nutrients will be much lower during the summer months than in the winter. The addition of nutrients ensures that all nutrients are in excess, so that growth is not limited by a shortage of any nutrient. The application of N especially, in the form of either nitrate or ammonium or both, has a visible effect on the appearance of *Palmaria*: it increases the pigmentation of the fronds and thus intensifies the colour of the tissue to dark purple-red.

  A mixture of all the essential nutrients can be applied in the form of self-prepared or ready-mixed fertiliser solution or as a slow-release solid fertiliser. Some agricultural fertilisers may be useful. However, the addition of nitrate and phosphate at a 10:1 ratio can be as effective as a complete nutrient mix, because seawater contains an adequate supply of most of the metals required.

- **Nutrient concentrations:** Pre-prepared fertiliser usually provides very high concentrations of nutrients compared to the concentrations found in ambient seawater. The concentrations available from slow-release fertilisers are less controllable, but they provide a low-maintenance, easy-to-use source of nutrients. If only N and P are added to the seawater, suitable concentrations for enhancing growth are 0.5 - 1.0 mmoles N L^{-1} seawater and 0.05 – 0.1 mmoles P L^{-1} seawater (N:P ratio of 10:1).
  If *Palmaria* culture tanks are integrated into a re-circulating system with a double purpose of supplying feed for the shellfish (e.g. abalone) and bioremediation, the nutrients present in the wastewater will be taken up by the algae, thus cleaning the effluent. The major N source here is ammonium.

- **Nutrient application in a flow-through system:** In a flow-through system the easiest way to provide a continuous supply of nutrient is to use a slow-release fertiliser. This can be prepared by adding a fertiliser solution or a mixture of crystalline salts to a porous clay vessel, which is tightly closed so that the nutrients are released through the pores of the pot when submerged in the tank water.
  Fertiliser can also be added at intervals by stopping the water-flow for a period, e.g. for 2-6 hours every day. The algal fronds will rapidly take uo the nutrients during this period, and such “pulse-feeding” using a high concentration of nutrients is just as effective as continuous application of fertiliser at a lower concentration. Pulse-feeding may also be appropriate when seawater is supplied to the tanks by “interval flushing” (see Chapter 8.2, Seawater supply), adding the nutrients just after the seawater has been exchanged.
  In winter, when nutrient levels in seawater are high and the growth rates of *Palmaria* are limited by low light and temperatures, application of fertiliser has only a very limited effect.

- **Potential side effects of fertilisation:** Nutrients added to the seawater stimulate not only the growth of *Palmaria* but also that of other algae, which may be brought into the tanks either attached to the fronds of collected *Palmaria* or through the seawater supply. Fouling algae with especially rapid growth rates, such as some fine filamentous brown algae and some green algae, may develop quickly under these conditions. Such species are generally adapted to higher irradiances than *Palmaria*, so that reduction of light and/or increase in stocking density can reduce their growth.

8.4 Monitoring and maintenance

To produce high quality biomass, factors such as light, stocking densities and nutrient supply should be adjusted as the cultures develop during the course of the year. Several factors should be monitored on a frequent basis:

- **Growth of *Palmaria*:** Growth is measured as increase in biomass over time (e.g. kg wet weight per week). The driving factors for growth are light, nutrients and, to a lesser extent, temperature, as described above.

- **Colour and integrity of fronds:** The algal fronds should show a rich red-purple colour. A change in colour to green and disintegration of fronds due to parts dying off indicates that the culture conditions are not right (see Fig. 8.3). The reasons could be: too much light; nutrients depleted in times of rapid growth; over-fertilisation at times of slow growth; aeration too low and insufficient to tumble the fronds; fronds too large and too heavy to tumble; flow rate of seawater too small, resulting in too little flushing.

- **Epiphytes and endophytes:** The growth of epiphytes is easily detected. Small green or brown fine filamentous algae settle preferentially around the stipes of *Palmaria* and on old thallus parts (see Fig. 8.3) or along the edges, especially where fronds have been torn into smaller pieces. Endophytes are visible as dark brown spots inside the tissue of older parts of *Palmaria* thalli. Although endophytes do not cause any harm to consumers, infected tissue parts will not meet the quality demands for foodstuff and have to be removed. Epiphytes appear mainly from the spring until autumn. Growth of epiphytes can be reduced by lowering light intensity and nutrient concentrations in the seawater.

Fig. 8.3. *Palmaria* from tank cultivation. Left: Individuals which are damaged and/or bleached. Right: Epiphytic algae growing on the older parts of fronds; new fronds have grown out from the margins of the lower part of a frond

- **Movement of algae in the tanks:** As *Palmaria* is a species which grows best at sites with a strong current, it is essential to ensure that the water in the tank is agitated by strong aeration and that the tanks are flushed sufficiently. This should be checked daily because *Palmaria* thalli die off quickly without proper movement.

- **Cleaning of tanks:** The tanks should be cleaned frequently. In summer, under higher light intensities, longer days and higher temperatures, biofilms quickly build up on the walls of outdoor tanks and fouling algae may develop at the level of the water surface. These should be removed frequently. Fouling organisms growing on the walls will, sooner or later, affect the fronds of *Palmaria* as well. Therefore, the tanks should be cleaned at 1- or 2-week intervals. This is best done when *Palmaria* is harvested. Indoor tanks supplied with effluent water should be cleaned once a week depending on the load of particulate matter and microalgae in the effluent. Power-washing is the best method of cleaning and removing biofilms, especially for large tanks.

8.5 Harvesting of cultivated *Palmaria palmata*

The production of biomass changes with season. Highest growth rates can be expected from spring through the summer and into early autumn. With decreasing daylength, growth rates go down significantly and there is very little growth during the winter months. Therefore the frequency of harvests must be adjusted to the growth rate.

- **Harvesting intervals:** During periods of rapid growth, surplus biomass can be removed every week. In times of slow growth (0-10% increase in biomass per week), *Palmaria* should be harvested only every fortnight.

- **Harvesting of *Palmaria*:** All *Palmaria* material which exceeds the initial stocking density should be removed from the tanks at appropriate intervals. Removing surplus material increases the space for the remaining fronds in the tank and thus "re-sets" the supplies of light and nutrients to the initial conditions. Small tanks (200-500 L) can be easily drained, the whole biomass removed from the tanks for weighing and screening of the fronds for those of highest quality. The tanks can also be cleaned before re-stocking with *Palmaria*. If larger tanks (1000-2000 L) are used, it is less easy to empty the tanks fully. This should be done only once a month for a full measurement and quality check of the biomass, and for cleaning (best to power-wash) the tanks. On a weekly basis, part of the *Palmaria* biomass (e.g. in summer an estimated 40-60% of the initial stocking weight) should be removed. The remaining fronds should be screened, removing older, fouled and damaged frond parts and dividing larger plants into smaller ones. It is essential to keep a healthy and well growing stock in the tanks.

- **When to re-stock the tank:** With frequent screening and quality checks, the initial stock of *Palmaria* fronds can continue to grow for weeks. Especially during the main growing season, however, bleaching and fouling can affect the quality quite substantially. Even if the fronds are fully intact and have a rich

red colour, microscopic algae may have settled on the surface of the fronds without being detectable. To test the quality, small volumes of fronds should be removed from the tanks for drying. Healthy, high quality fronds keep a dark red colour when dried, whereas fouled fronds appear to have a green, slightly fluffy cover, especially on the older parts of fronds (Fig. 8.4). If the number of fouled fronds increases significantly, fresh *Palmaria* should be collected from the field and the tanks re-stocked.

Fig. 8.4. Dried *Palmaria* samples after three weeks of tank cultivation. Sample T10 (bottom right) shows a surface cover of green microscopic algae.

Chapter 9: Results of tank cultivation trials of *Palmaria palmata* in 2010/2011 and economic analysis

9.1 Case study: land-based tank cultivation of *Palmaria palmata*: Summary of results

Trials on land-based tank cultivation were started in August 2010 at the three hatcheries (DOMMRS, MRI Carna Laboratories, QUB Marine Laboratory Portaferry) and at Cartron Point Shellfish Ltd., Co. Clare. The tank set-up varied depending on the facilities and equipment available (Table 9.1) but followed the recommendations in Chapter 8.1 and 8.2. Different tank sizes were used, and growth in both natural and nutrient-enriched seawater was compared.

Table 9.1 Tank set-up at the different hatchery sites.

	QUB Portaferry	NUIG Carna Laboratories	DOMMRS	Cartron Point Shellfish
No of tanks	8 small	4 large	2 large	2 large
Tank volume	230 L	1000 L	1000 L	1000 L
Tank depth	60 cm	90 cm	90 cm	90 cm
Tank diameter	70 cm	119 cm	119 cm	119 cm
Tank surface area	0.385 m^2	1.112 m^2	1.112 m^2	1.112 m^2
Stocking density	0.8 – 3.3 kg m^{-2}	3.6 kg m^{-2}	3.6 kg m^{-2}	1.8 kg m^{-2}
Flow-through (turn-over day^{-1})	17 – 20 times	7 - 15	8 - 9 times	8 - 9 times
Nutrient supply	Sand-filtered seawater; Nutrient applications	2x wrasse effluent 2x natural seawater	Raw seawater + slow release fertiliser	Sand-filtered seawater + slow release fertiliser

First trials starting in late summer were conducted at QUB Portaferry, using high seawater flow rates, different stocking densities (0.78 kg m^{-2}, 1.3 and 1.82 kg m^{-2}) and no additional nutrient supply. The biomass approximately doubled (i.e. about 100% increase) every 14 days over a 2-month period between August and October without significant differences among the stocking densities (Table 9.2). A maximum yield of 2 kg FW m^{-2} $14d^{-1}$ was achieved at the highest stocking density in September. From October onwards, yield dropped sharply to an average of 15-20% per 14 days. In contrast, at DOMMRS and Cartron Point Shellfish, yields of approx. 30% and 40% per 14 days were achieved during November and December 2010. Additional nutrient

supply enhanced growth by about 20% in trials performed at Cartron Point Shellfish (Table 9.3). Trials at MRI Carna Laboratories showed good yields in September, when the increase in biomass over 14 days was 25% and 50% using raw seawater and the effluent from wrasse tanks, respectively.

Table 9.2 Growth of *Palmaria* in tanks at QUB Protaferry. The increase of biomass in % calculated for a period of 14 days is shown.

	Stocking densities		
Date	**T1 1.3 kg m^{-2}**	**T2 0.78 kg m^{-2}**	**T3 1.82 kg m^{-2}**
20.08.10	87.91		
03.09.10	96.54	126.47	
17.09.10	69.89	105.52	111.65
06.10.10	54.16	65.66	73.62
28.10.10	40.00	33.38	0.50
04.11.10	2.25	17.67	29.29
17.11.10	13.02	21.01	50.80
02.12.10	14.57	7.53	-2.42

Table 9.3 Yield of *Palmaria* cultivated in tanks at DOMMRS and Cartron Point Shellfish Ltd. (stocking densities: 3.6 kg m^{-2} at DOMMRS and 1.8 kg m^{-2} at Cartron Point Shellfish: nutr. = nutrient application as slow release fertiliser).

	% increase 14 d^{-1}			
	DOMMRS		**Cartron Point Shellfish**	
Date	**TANK1 + nutr**	**TANK 2**	**TANK1 + nutr**	**TANK 2**
01/11/2010	36.50	35.50	49.88	0.00
08/11/2010	32.00	30.85	44.80	27.30
15/11/2010	22.50	28.00	42.00	27.00
22/11/2010	17.00	19.00	40.00	6.00
30/11/2010	23.92	28.00	31.50	8.75
13/12/2010	33.15	35.05		

At QUB Portaferry, growth of *Palmaria palmata* increased significantly from January until March 2011. By mid March, yields of up to 80-100% increase in biomass per 14 days of culture were obtained. In May, growth rates increased even further to 100-150% increase in biomass per 14 days.

Conclusions from preliminary work on land-based tank cultivation of *Palmaria palmata*

During the limited time period of these trials, the considerable potential of tank cultivation of *Palmaria* became clear. High growth rates between early spring and autumn result in high biomass production per unit of tank surface area. Adjustment of stocking densities allows optimal production. In times of rapid growth and high light during spring and summer months, stocking densities can be raised to increase the shading effect and thus reduce bleaching of *Palmaria* fronds. Addition of fertilisers enhances growth of *Palmaria*, but may increase the growth of fouling algae as well. The main advantage of this cultivation method is that a hatchery is no longer required, and the expenses and weather dependence of boat work at sea are avoided. Biomass can be produced continuously at an accessible site, provided that the land area required is available adjacent to the sea.

9.2. Economic assessment of tank cultivation of *Palmaria palmata*

In a preliminary assessment of the economics of the type of tank cultivation of *Palmaria* that has been attempted in the last few months of the project, we have assembled the approximate costs of the capital equipment required, and estimated the electricity demands of a farm with either 40 or 80 tanks of 1000 litres (Table 9.4).

Table 9.4 Estimates of costs of establishing and running a farm for cultivation of *Palmaria palmata* on land with either 40 or 80 tanks.

Capital costs (€):			
	Unit cost	40-tank total	80-tank total
Tanks, 1000 L, polyethylene			
Natural	254	10164	20328
Ball valves, 2 cm	18	720	1440
Aerators	6	240	480
Air blower: BBC	960	960	
Air blower: Rieschle			1450
Submersible seawater pump	800	800	800
Pipework	300	300	600
Joints, glue, sundries	200	200	400
Switch gear (installed)	300	300	300
Shading net (50 m^2)	120	120	240
Total capital cost		13804	26038
Depreciation (20%/year):		2761	5208
Electricity costs (€):			
	Rating (kW)	Cost/year	
Seawater pump (40% of time)	1.5	778	778
Air blower: BBC	0.85	1102	
Air blower: Rieschle	1.5		1944
Total running costs		1879	2722
Running + depreciation costs:		**4640**	**7930**

The production to be expected from a 40-tank farm has been estimated from the data obtained in the project, and from the experiences of Klaus Lüning at Sylter Algenfarm in Denmark. The maximum growth rate observed was 2 kg fresh weight m^{-2} 14 $days^{-1}$, and this value is in close agreement with the general observation at Sylter Algenfarm that, at a stocking density of 4 kg m^{-2}, *Palmaria* doubles in weight every 4 weeks (i.e. the growth rate is 4 kg m^{-2} $month^{-1}$). If this rate can be maintained throughout the year, the annual production will be 48 kg m^{-2}, and the total production from 40 tanks will be 1920 kg.

The current value of wet Palmaria in Ireland is €2.50 per kg (see Section 4.5), so that the total value of full production from a 40-tank farm would be €4800. Although this return would be just enough to cover the electricity costs and the depreciation on the equipment (20% of value per year), there would be very little left over (€160 per year!) to pay anyone to do the work of harvesting the *Palmaria* and maintaining the tanks. Consequently, if it were to be economic, the farm would need to have more than 40 tanks, and the final column of Table 9.4 shows the capital and running costs for 80 tanks. A more powerful air blower (Rieschle, 1.5 kW) would be needed, with a consequent increase in both capital and running costs. Although the total production would be 3840 kg with a value of €9600, giving an excess over running and depreciation costs of nearly €1700, this is still not enough to cover a significant amount of labour. Further increases in the number of tanks would require a considerable area of land, so that the rent might add significantly to the costs.

An alternative approach, as with all seaweed species, is to try to obtain a higher value for the cultivated material. A small company in northern Spain (Cultivos Marinos del Cantábrico) started to sell *Palmaria* directly into restaurants in 2000 and was able to demand at least 5 times the price that was being paid in Ireland at that time. A similar strategy of obtaining a premium price by selling directly to restaurants is currently operated by Sylter Algenfarm. Such prices would clearly transform the prospects for tank cultivation of *Palmaria* (or any other similar seaweed) at the scale envisaged here.

Appendix 1 – References and useful links

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1.2 Useful links

The following web sites provide useful information about seaweeds, seaweed aquaculture and seaweed related issues. In addition, web sites of relevant development agencies and research institutions in Ireland, the UK and other countries are given.

- www.algaebase.org: Information about seaweed taxonomy, nomenclature, photos of algal species, utilisation of seaweed and publications
- www.bim.ie: Bord Iascaigh Mhara, Irish Sea Fisheries Board, Ireland
- www.ceva.fr: Centre d'Etude et de Valorisation des Algues (Seaweed Manufacturing Technology Centre). CEVA is a unique centre for algal research in France providing service in seaweed analysis, product development and feasibility studies for aquaculture, and market analysis.

- http://agriculture.gov.ie/: Department of Agriculture, Fisheries and Food (DAFF), Ireland
- www.dard.gov.uk: Department of Agriculture and Rural Development, Northern Ireland
- www.doeni.gov.uk/niea/: Northern Ireland Environment Agency
- http://www.epa.ie/: Environmental Protection Agency, Ireland
- www.fao.org: Food and Agriculture Organisation
- www.ifremer.fr: French Research Institute for Exploitation of the Sea. Fields of activities are coastal environment management, marine living resource management, ocean research, engineering and marine technology, and managing ocean research vessels and tools for underwater invention.
- www.irishseaweed.com: Irish Seaweed Research Group (formerly the Irish Seaweed Centre)at the National University Ireland in Galway, Ireland
- www.marine.ie: Marine Institute, Ireland
- www.marlin.ac.uk: Marine Life Information Network for Britain and Ireland; provides information about taxonomy, biology and habitats of seaweeds, and their importance
- www.patents.com: Intellectual property resource, worldwide data base for patents
- www.seaweed.ie: Source for general information about seaweed species, their utilisation and cultivation
- www.weedseen.co.uk: Website designed to supplement and complement available keys and guides to marine algae of the Britain and Ireland

Appendix 2 – Recipes for culture media

2.1 Von Stosch (Grund) Medium (after Guiry and Cunningham 1984)

This enriched seawater medium was modified from Grund Medium by von Stosch in 1963 and further modified by Guiry and Cunningham (1984). It is suitable for growing many red seaweeds.

Prepare stock solutions in distilled or deionised water (dH_2O). The stock solution should be autoclaved. The vitamin stock solution is prepared separately and is not autoclaved. For final concentration add 10 ml of stock solution to 1 litre of the final medium.

Component	**Stock solution (g L^{-1} dH2O)**	**Quantity used for final medium**	**Concentration in final medium (µmol)**
Na_2 ß-glycerol PO_4*H_2O	5.36	10 ml	248
$NaNO_3$	42.52	10 ml	5000
$FeSO_4$*7 H_2O	0.28	10 ml	10
$MnCl_2$*4 H_2O	1.96	10 ml	100
Na_2EDTA* 2 H_2O	3.72	10 ml	100
Vitamins stock solution	See recipe below	10 ml	

Vitamins stock solution

For the vitamins stock solution, dissolve the thiamine*HCl in 950 ml of dH_2O. Then add 1ml of each of the two other stock solutions. The final vitamins stock solution should be partitioned and kept in the freezer until use.

Component	**Stock solution (g L^{-1} dH2O)**	**Quantity used for final medium**	**Concentration in final medium (µmol)**
Thiamine * HCl (vitamin B_1)	-	200 mg	5.9
Biotin (vitamin H)	0.1	1 ml	0.0041
Cyanocobalamin (vitamin B_{12})	0.2	1 ml	0.0015

2.2 f/2 Medium (Guillard and Ryther 1962, Guillard 1975)

This is a common and widely used general enriched seawater medium designed for growing coastal marine algae, especially diatoms. The original medium created by Guillard and Ryther 1962 was double concentrated (f medium) and later reduced to half the concentration (f/2; Guillard, 1975).

Component	**Stock solution** (g L^{-1} dH2O)	**Quantity used for final medium**	**Concentration in final medium (µmol)**
$NaNO_3$	75	1 ml	882
$NaH_2HPO_4 * H_2O$	5	1 ml	35.2
$Na_2SiO_3 * 9\ H_2O$ *	30	1 ml	9
Trace Metals solution	See following recipe	1 ml	-
Vitamins solution	See following recipe	0.5 ml	-

*Silicate used in enrichment medium for diatom cultivation. It is not required for macroalgal cultivation and can be omitted.

f/2 trace metals solution

Dissolve the EDTA and other components In 950ml of dH_2O and bring the final volume to 1L.

Component	**Stock solution** (g L^{-1} dH2O)	**Quantity used for final medium**	**Concentration in final medium (µmol)**
$FeCl_3 * 6\ H_2O$	-	3.15 g	11.65
$Na_2EDTA * 2\ H_2O$	-	4.36 g	11.7
$MnCl_2 * 4\ H_2O$	180.0	1 ml	0.91
$ZnSO_4 * 7\ H_2O$	22.0	1 ml	0.077
$CoCl_2\ 6\ H_2O$	10.0	1 ml	0.042
$CuSO_4 * 5\ H_2O$	9.8	1 ml	0.039
$Na_2MoO_4 * 2\ H_2O$	6.3	1 ml	0.031

f/2 Vitamins solution

Dissolve thiamine*HCL in 950 ml of dH_2O. Add 1 ml of each primary stock and bring final volume to 1L with dH_2O. Store frozen.

Component	**Stock solution** **(g L^{-1} dH2O)**	**Quantity used for final medium**	**Concentration in final medium (µmol)**
Thiamine * HCl (vitamin B_1)	-	200 mg	0.296
Biotin (vitamin H)	0.1	1 ml	0.002
Cyanocobalamin (vitamin B_{12})	0.2	1 ml	0.00037

These recipes were compiled by Andersen et al. (2005) and published in "Algal Culturing Techniques".

2.3 f/2 Medium – ready-to-use granular pre-mix

F/2 can be bought as a ready-to use granular or liquid mix containing all the required chemicals. See Appendix 3 for suppliers.

An example for preparation using granular mix: Following supplier's information, e.g.: 1kg of f/2 pre-prepared mix for 10,000 L of final medium. For preparation of stock solution dissolve 100 g of granular mix in 950 ml dH_2O. Bring to final volume of 1 L. Add 1 ml of stock solution to 1L of medium.

Appendix 3 – Suppliers list

The list is compiled to help readers to find equipment based on our own experience or recommendations by others. It is not comprehensive and does not reflect special preferences of the authors.

- Chandleries, general aquaculture supplies
  - Clare Aquaculture Supplies (Jim Simmons). Tel: 00353 (0)65 7078080 *
  - Swan-Net Gundry Ltd. Killibegs and Rossaveal. Tel: 00353 (0)74 9731180 Fax: 00353 (0)74 9731100, http://www.swannetgundry.com
  - Northern Ireland Fish Producers Organisation Ltd, Ardglass Tel. 0044 (0)28 4484 2144; Kilkeel Tel. 0044 (0)28 4176 2901; Portavogie Tel. 0044 (0)28 4277 1601
  - Down Marine Co. Ltd., 163 Comber Road, Dundonald, Belfast, BT16 0BU. Tel.: 0044 (0)2890 480247; http://www.downmarine.com

- Chemicals for nutrient media
  - Sigma-Aldrich Ireland, http://www.sigmaaldrich.com/ireland.html
  - See VWR International website below

- Culture string
  - PT Winchester Ltd. Broadgauge Business Park, Bishops Lydeard, Taunton, Somerset, TA4 3RU, Tel: 0044 (0)1823431983; http://www.ptwinchester.co.uk/

- Filters (cartridge)
  - Hall Pyke. Walkinstown, Dublin 12. Tel: 00353 (0)1 4501411, Fax: 00353 (0)1 4507960, http://www.hallpyke.ie/index.html
  - FSUK Filter Specialists (UK) Ltd, Unit H1, Taylor-Business Park, Risley, Warrington, Cheshire, WA3 6BL, UK. Tel: 0044 (0) 19 25 76 76 67, Fax: 0044 (0) 19 25 76 38 75; E-Mail: fsuksales@fsifilters.com; http://www.fsifilters.com

- Laboratory consumables (e.g. glassware, filtration equipment etc.)
  - Davidson & Hardy (Laboratory Supplies) Ltd. Aravon House, 8 Pembroke Road, Dublin 4, Tel: 00353 (0)1 6600725 & 1800 709080, Fax: 1800 409028; http://www.dhlab.com

- Davidson & Hardy (Laboratory Supplies) Ltd. 453-459 Antrim, Road, Belfast, BT15 3BL, Tel: 0044 (0)28 90 781611 028 90 781611, Fax: 0044 (0)28 90 772801; http://www.dhlab.com
- Fisher Scientific Ireland. Ballycoolin, Dublin 15. Tel: 00353 (0)1 885 5854, Fax: 00353 (0)1 899 1855, http://www.ie.fishersci.com/
- VWR International, http://ie.vwr.com/app/Home
- Lennox Laboratory Supplies. Naas, Dublin 12. Tel: 00353 (0)1 4552201, Fax: 00353 (0)1 4507906, http://www.lennox.ie/

- Light Meter
  - Skye Instruments. Powys, Wales. Tel: 0044 (0) 1597824811, Fax: 0044 (0) 1597824812, http://www.skyeinstruments.com/

- Microscope – see laboratory consumables above

- Pre-prepared media
  - Varicon Aqua Solutions. Malvern, UK. Tel: 0044 (0) 1684 312980, Fax: 0044 (0) 1684 312981, http://www.variconaqua.com/
  - Culture Collection of Algae and Protozoa (CCAP). Oban, Scotland. http://www.ccap.ac.uk/index.htm

- Temperature controlled units
  - Boxtainer Ltd., Europa Trading Centre, London Road, Grays, Essex, RM20 4DB, UK, http://www.boxtainer.co.uk
  - Containers for Sale [with agents in the UK and Ireland], Unit 2b Eddystone Road, Southampshire Business Park, Calmore, Southampton, SO40 3SA, UK, http://www.containersforsale.co.uk/

- Tanks
  - The Irish Box Company. Gorey, Co. Wexford. Tel: 00353 (0)53 9481262, Fax: (053) 9481594, http://www.irishboxcompany.ie/
  - TMC Marine Ltd. London, Manchester, Bristol. Tel: 0044 (0)1923 284151 (London), Fax: 0044 (0) 1923 285840, http://www.tmc-ltd.co.uk/

Appendix 4 - Glossary

Carposporangium, carposporangia (pl.): Carpospore-producing cell cluster of red algae.

Carpospore: A spore released from a carposporangium of a red alga.

Carposporophyte: Microscopic diploid life phase of red algae, which develops attached to the female gametophyte.

Dichotomous: Type of branching where a growing point divides into two equal growing points, which can, in turn, divide into a further two new growing points.

Diploid: Having twice the basic (i.e. haploid) number of chromosomes (2n).

Distal: Away from the base or point of attachment (e.g., the tip of a branch relative to its point of origin on the axis); also the converse of proximal.

Endophyte: An organism living within another plant which can have beneficial or harmful effects on the host plant.

Epifauna: Aquatic animals living on or attached to the surface of algae, other animals or the seabed.

Epiphyte: An organism hat grows on another plant in a non-parasitic relationship.

Euphotic zone: Upper layer of a water body, which receives sufficient light to satisfy the requirements for photosynthesis, and can be inhabited by autotrophic algae and plants.

Eutrophication: Process in which a water body becomes overloaded with inorganic nutrients, such as nitrate, ammonium and phosphate. This can cause planktonic blooms and massive growth of certain seaweeds (e.g. green tides), and possibly subsequent oxygen depletion of the water body.

Frond: Leaf-like or erect portion of a thallus; often used to define the entire erect portion of a foliaceous or foliose thallus other than the attachment structure.

Gamete: A cell (sperm or egg) capable of fusing with the complementary gamete to form a zygote.

Gametophyte: Haploid phase of the life cycle of a seaweed which produces gametes.

Haploid: Having one complete set (n) of chromosomes.

Holdfast: Disc or root-like structure with which an alga attaches to the substratum.

Hydrocolloids: Generic term for commercially important polysaccharides extracted from seaweeds (phycocolloids) and higher plants.

Integrated Multi-Trophic Aquaculture, IMTA: The concept of IMTA is provision of by-products/wastes from one culture species as feed or fertiliser input for another/other species. Fed aquaculture (e.g. fish or

shrimps) is combined with organic extractive (e.g. shellfish) and inorganic extractive (e.g. seaweed) aquaculture species to counterbalance environmental impacts by reducing waste (e.g. nutrient-rich effluents from fish) through re-use by shellfish (particulate matter) and seaweeds (dissolved inorganic components of effluent such as nitrates and phosphate). Ref., for example: Nobre *et al*., 2010; Neori, 2008; Chopin *et al.*, 2001; Chopin & Yarish, 1999).

Karyogamy: The fusion of the two nuclei as a result of the union of a male and a female gamete.

Meiosis: A type of cell and nuclear division in which the number of chromosomes is halved, e.g. from diploid to haploid.

Meristem: An area or aggregation of cells which are not specialised but divide frequently to produce new tissue; an area of growth.

Phycoerythrin: A red, water-soluble pigment, in red algae. It is utilised as a fluorescent tag by the medical diagnostic industry.

Phycocolloids: Economically important polysaccharides extracted from seaweeds with gelling properties (agar, carrageenan, alginates; see also **Hydrocolloids**). These are widely used by the food and pharmaceutical industry.

Polysaccharide: A large carbohydrate polymer built of simple sugar molecules, to which specific chemical groups may be attached.

Sorus, sori (pl.): A cluster of spore-containing structures (sporangia) found in algae, fungi and lichens. The spores that are produced are haploid meiospores.

Spermatangium, spermatangia (pl.): Sex organ of a male gametophyte in red algae which develops a single non-motile spermatium.

Spermatium, spermatia (pl.): A non-motile male gamete, released by a male gametophyte.

Sporangium, sporangia (pl.): A cell that divides to form spores.

Spore: An asexual reproductive cell produced by sporangia.

Sporophyte: Diploid phase in the life cycle of seaweed and plants, producing spores for reproduction.

Tetrasporophyte: Diploid phase of the life history of many red algae, which produces tetraspores for reproduction.

Thallus, thalli (pl.): The undifferentiated body of an alga.

Zygote: A diploid cell formed by the union of a male and female haploid gamete.

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