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Bord Iascaigh Mhara
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Aquaculture Explained

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Cultivating *Laminaria digitata*

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Cultivating
Laminaria digitata

This document is an output of the project, PBA/SW/07/001 (01), '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.

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Foreword

In the past two decades, there has been much debate amongst industry, the state development agencies and the research institutions about the potential of the Irish seaweed sector. Seaweed gathering and processing is a traditional activity in Ireland bringing economic activity and employment to coastal communities. Ireland's seaweed and biotechnology sector is currently worth €18 million per annum and employs 185 full time equivalent people (Morrissey *et al.*, 2011). The potential to increase employment, exports and wealth from seaweed in Ireland was looked at by the National Seaweed Forum which was established in 1999 to join industry with research bodies, state agencies and departments to make recommendations for the future development of the industry.

One such recommendation was the development of seaweed cultivation. With this in mind, groups such as Bord Iascaigh Mhara (BIM), Taighde Mara Teo (TMT), National University Ireland Galway (NUIG) and Queen's University Belfast (QUB) initiated seaweed cultivation trials. These early trials had varied success and allowed for experimentation and year-on-year technique improvement. Farming seaweed as opposed to simply gathering seaweed requires a thorough knowledge of seaweeds and perfect manipulation of the seaweed life cycle. Mastering this has concerned Irish seaweed researchers and industry practitioners alike over the last decade.

The Marine Institute's, 'Sea Change' Strategy (2007-2013) further recognised the potential for seaweed farming in Ireland. This important strategy anticipated that the Irish seaweed production and processing sector would increase significantly in value by 2020 to circa. €30 million. In order for this to be achieved seaweed aquaculture will need to become firmly established here. At the same time, seaweed research must continue to identify new compounds and extraction techniques for the manufacture of human consumption products, agricultural applications, cosmetics, thalassotherapy and the bio-pharma sector.

In the same report, a stated objective is the development of hatchery techniques for cultivating seaweeds which will be critical to the success of the 2020 'Sea Change' vision. From this recommendation, the current project PBA/SW/07/001 (01) was developed. The 'Seaweed Hatchery' project aims to provide 'the development and demonstration of viable hatchery and ongrowing methodologies for seaweed species with identified commercial potential'.

The Seaweed Hatchery project has focused on developing new techniques, and improving existing knowledge of seaweed cultivation. This manual is one such output of the project. The manual is offered to the industry as a guide to the hatchery techniques required to develop new aquaculture opportunities for *Laminaria digitata*. As with all BIM 'Aquaculture Explained' manuals, it is based on the research and experiences of the group and includes both hatchery and sea trial cultivation results obtained over several years and sites. An attempt has been made to provide an easy to use document filled with practical advice for those interested in growing kelp.

Maeve Edwards, NUIG and
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May 2011

Table of Contents

Chapter 1

1.1 Introduction

Aquaculture has become an important part of overall world food production, which continues to increase by 6.2% globally per year (FAO 2010a). Production from capture fisheries has stabilised in recent years, whereas aquaculture plays an increasingly important role. Seaweed aquaculture exists as an extremely successful and important economic activity. The vast majority of aquatic plant products are now cultivated as opposed to harvested from the wild (Figure 1.1). For example, in 2008, 15.8 wet weight million tonnes of aquatic plants were cultivated, the equivalent of 93.8% of total production, the value of which was US$7.4 billion (FAO 2010a). In 2008, 23% of the quantity of all cultured organisms was seaweeds/aquatic plants (Figure 1.1). The total value of all cultured organisms was valued at US$ 105873991, of which seaweed was worth 7% of the total value (FAO 2010a).

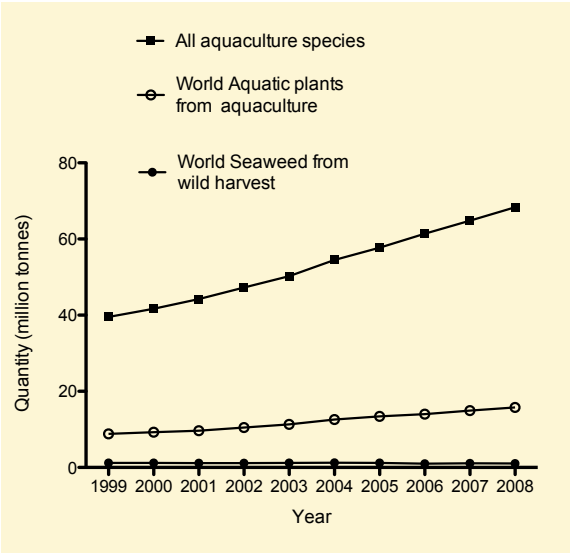


Figure 1.1.
Worldwide quantities (million tonnes) of all cultured aquatic organisms, cultured aquatic plants and wild-harvested seaweeds between 1999 and 2008. Data from FAO Annual Yearbook 2008 (FAO 2010a).

Most of the world seaweed production is centred in Asia, with China by far producing the most biomass (62%). The Philippines, Japan, the Republic of Korea and the Democratic People's Republic of Korea are also major producers (FAO 2010b). Only a few countries grow a small number of seaweed species, but do so on a large scale, accounting for most of the value of the seaweed industry (Figure 1.2).

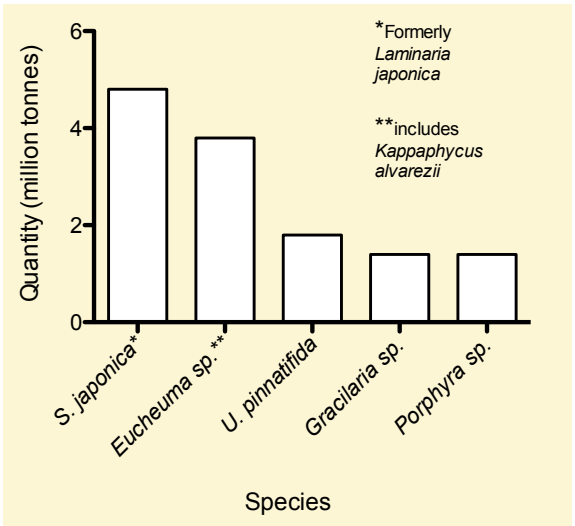


Figure 1.2.
Cultivated seaweed species with the highest world biomass production. (FAO 2010b).

Western countries do not have a tradition of cultivating seaweeds, and consequently, seaweed production (from cultivation) in the latest FAO publication (2010b) is almost non-existent (Figure 1.3). However, large amounts of seaweed biomass naturally exist in countries such as Ireland (Hession *et al.* 1998). Countries with the most active traditions of harvesting seaweed and utilising the resource include Canada, France, Scotland and Norway and Ireland (for Ireland, see Figure 1.3). Walsh (unpubl., 2006) reports on the French and Norwegian kelp industries in particular, where 50,000 tonnes of *Laminaria digitata* and 200,000 tonnes of *Laminaria hyperborea* respectively are harvested annually. In both Norway and France the majority of the weed is processed into alginates.

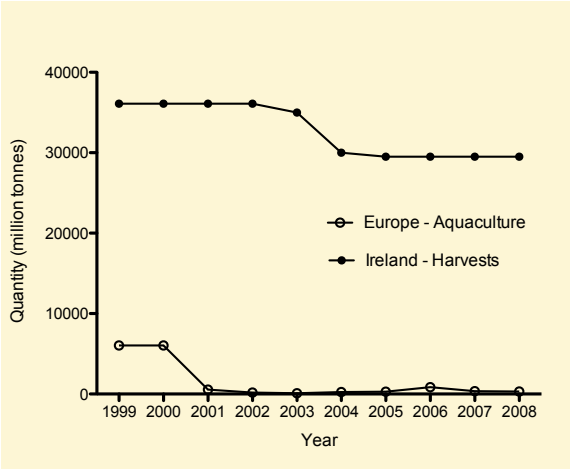


Figure 1.3.
Seaweed production (tonnes) from Ireland's wild harvests and European aquaculture (no aquaculture data available for individual countries). (FAO 2010a)

According to the marketing strategy report, 'A market analysis toward the further development of seaweed aquaculture in Ireland' (Walsh and Watson, 2011) and the FAO (2010), between 25500 and 29500 tonnes of seaweed is harvested annually in Ireland (Figure 1.3 and Table 1.1) with the seaweed and biotechnology sector valued at approximately €18 million (Morrissey *et al*, 2011). The biggest Irish seaweed markets are for agricultural, food and cosmetics/ therapies products. For the reasons explained in Chapter 2, the cultivation of seaweeds (and other algae) is now the recent focus of much research across the world. The reasons for the renewed interest in seaweed cultivation are many, but include the use of seaweeds for bioremediation purposes, as a source of food, and as novel pharmaceutical and cosmetic products. Many species with commercial potential have been identified, with Ireland also recognising the value of this marine resource.

Table 1.1.
Estimated annual Irish national seaweed harvest (Walsh and 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., Porphyra sp., F. vesiculosus, 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.

In Ireland, seaweed aquaculture has been the focus of several research groups for the past ten to fifteen years. In the National University of Ireland, Galway (NUIG), several researchers concentrated on the cultivation of the large brown alga *Alaria esculenta,* developing mass cultivation techniques (Arbona, 1997; Kraan, 2000) leading to development of specific strains of *A. esculenta* with an aim to improving overall yield (Kraan *et al.,* 2000). This initial work was continued through aquaculture development funding from Bord Iascaigh Mhara (BIM) and work carried out by Cartron Point Shellfish Ltd. The techniques required to grow *A. esculenta* have been refined, and crops have been grown annually for the last 7 years. Many aquaculture students have also had the opportunity to participate in this cultivation work, and an 'Aquaculture Explained' manual has also been produced (Arbona and Molla, 2006).

Cultivation of red algae has also been investigated. The introduced species, *Asparagopsis armata* was cultivated on an experimental scale in Co. Galway, but has since been discontinued (Kraan and Barrington, 2005). More extensive research into the cultivation of the edible red alga, *Palmaria palmata* was completed in Strangford Lough, Northern Ireland (Edwards and Dring, 2011). Tank trials of the alga were conducted through the EU-funded SEAPURA project. This was followed by cultivation trials at sea on longlines.

The experience and expertise generated from these previous research projects has provided a solid platform from which to launch the Seaweed Hatchery Project.

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 with commercial potential included 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 settlement techniques for 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 7 SMEs (Small to Medium-size Enterprise) were involved in the project (Table1.2).

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
Cartron Point Shellfish Ltd. CPS	SME	Associate. Active participation in cultivation of *L. digitata* and *P. palmata* (hatchery and sea sites)
Tower AquaProducts Ltd.	SME	Associate. Tank cultivation of *P. palmata*
Irish Seaweeds Ltd*	SME	Associate. Provision of licensed sea trial site
G + B Barger Operators Ltd.	SME	Associate. Industry partner
Roaringwater Bay Seaweed Co-operative Society Ltd.	SME	Associate. Provision of licensed sea trial site
Cleggan Seaweeds Ltd**	SME	Associate. Provision of licensed sea trial site
Dingle Bay Seaweeds Ltd.***	SME	Associate. Provision of licensed sea trial site

* Formely know as Dolphin Sea Vegetable Co. ** Cleggan Seaweeds Ltd subsequently disengaged from the project.
***Joined the project in the last year.

During the lifetime of the project two new sites were made available, namely a one-hectare site in Ard Bay, Co. Galway (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 hectares.

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 (Figure 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 (the Daithi O' Murchu Marine Research Station) in the South-West (Gearhies, Co. Cork) was owned by Fastnet Mussels Ltd., leased by the Project and BIM, and operated 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 *Laminaria digitata*.

Sea sites with aquaculture licenses 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 Figure 1.4 for the full list of sites used). Site conditions varied, with depths between 6 and 18 m, and substrate types ranged from boulders and bedrock to silt, sand and mud. Most were situated in sites sheltered from the most prevailing weather, 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 New Quay and Ventry Harbour (55 and 18 ha., respectively), while the smallest site used was in Ard Bay (1 ha.).

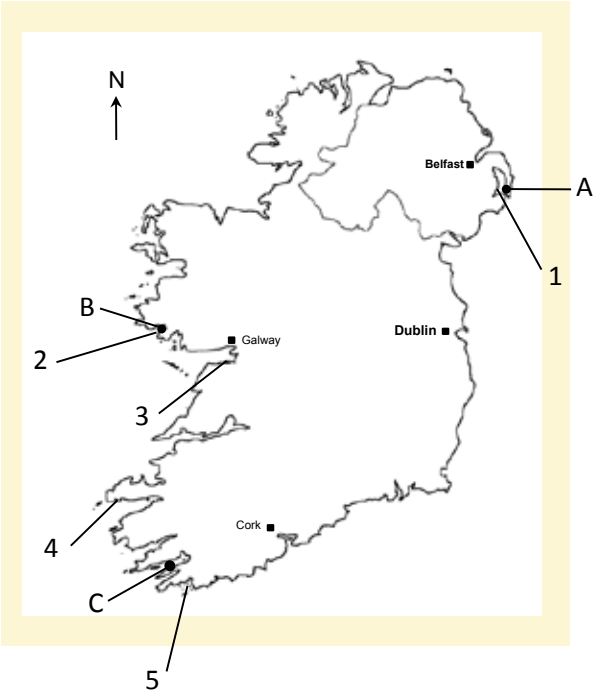


Figure 1.4.
Locations of the project hatcheries (letters), and licensed sea trial sites (numbers). **Hatcheries**: A, Queen's Univsersity, Belfast Marine Laboratory at Portaferry, Co. Down. B, National University of Ireland, Galway Carna Research Station, Carna, Co. Galway. C, The Daithi O'Murchu Marine Research Station, Gearhies, Co. Cork. **Sea sites**: 1, Strangford Lough (x2 sites), with permission from Irish Seaweeds Ltd. 2, Ard Bay, with permission from Michael Ward. 3, New Quay, with permission from Redbank Shellfish Ltd. 4, Ventry Harbour, with permission from Dingle Bay Seaweeds Ltd. 5, Roaringwater Bay, with permission from Roaringwater Bay Seaweed Cooperative Society Ltd. A licensed sixth site in Cleggan Bay, Co. Galway was not used due to the disengagement of Cleggan Seaweeds Ltd. from the project.

Chapter 2
Seaweeds, their uses and methods of cultivation

Seaweeds belong to an astonishingly diverse group of photosynthetic organisms called *algae* (Dring, 1992). Species of algae are generally referred to as macroalgae when they are multicellular, and large enough to be identified by the naked eye (e.g. the seaweeds). Microalgae are those species that exist as single, or small groups of cells that can only be identified by means of a microscope. Seaweeds/ macroalgae live almost entirely within marine environments, but a few species of brown and red algae are found in brackish water (e.g. estuarine conditions) or freshwater (Guiry and Guiry, 2011).

Algae vary greatly in their structural complexity, and historically all algae were classified within the plant kingdom due to their ability to photosynthesise. This taxonomic interpretation is currently under review, as more new species are discovered and our understanding of existing species and groups improves. In recent decades, more complex tools have been developed that shed new light on the relationships between groups of algae, and where they fit into the 'tree of life'. While the classical approach of placing all living organisms into a specific 'kingdom' still exists (for example, as used for algae in Guiry and Guiry, 2011), a new classification system is being proposed. This groups all species with a true nucleus containing DNA in chromosomes (Eukaryotes) into a 'Domain' within which 'Supergroups' are recognised on the basis of 'relatedness' (Baldauf, 2003; Keeling, 2007). Under this new proposal, the algae are now classified within several of these supergroups, and only green and red seaweeds are still considered to be related to higher plants (Keeling, 2007).

As testament to their diversity, algae can be found in almost every habitat on earth. They can survive a wide range of temperatures, salinities, light exposure and access to water (Bold and Wynne, 1978). Both micro- and macroalgae are among some of the most important primary producers on the planet, sustaining the development of entire ecosystems (Atkinson *et al.,* 2004; Tynan, 1998). These primary producers use carbon dioxide from the atmosphere for growth, and produce oxygen in the process. In oceanic environments when microalgae die, the carbon deposited in the carbonate skeletons of many algae is stored in the sediment, thus algae play an important part in regulating the effects of climate change (Falkowski *et al.,* 2004).

Seaweeds can be grouped into three taxonomic divisions known as phyla, depending on their pigmentation (Dring, 1992). These phyla are the Heterokontophyta, Rhodophyta or Chlorophyta. The species within these groups/ phyla are more commonly referred to as brown, red or green seaweeds, respectively. For example, the alga reported on in this manual (*Laminaria digitata*) is a brown seaweed of the phylum Heterokontophyta within the class Phaeophyceae (see Table 2.1 for full details).

The consumption of seaweed has been documented for thousands of years in Asia, exemplified by the well-known red seaweed *Porphyra* grown in Japan (commonly known as 'Nori'). Most Asian countries have a long history of eating many different types of seaweeds, but the most notable of these countries are Japan, China and Korea. Closer to home, another red alga, *Palmaria palmata* (Dulse) is described in an Irish poem thought to be from the 11th century (M.D. Guiry, pers. comm.). The poem describes the work of monks, who amongst other duties, gathered and dried Dulse for distribution to the poor. Although arguably one of the most popular edible seaweeds in Ireland, it is not the only one. Another well-known alga is *Chondrus crispus* (also known as Carrageen or Irish Moss), which was, and still is, prized for the gel that can be extracted from the dried seaweed. Seaweeds were known to be useful in other ways, most notably as a fertiliser for nutrient-poor soil in coastal farmland. Certain types of brown seaweeds (e.g. *Ascophyllum nodosum* or Egg Wrack) were collected and placed on the land to rot into the soil before the new season's potatoes were sown. In time, further uses were discovered for large brown seaweeds of the lower intertidal. These seaweeds were collected and burnt in kilns to produce an ash called 'kelp', which was a source of potash and latterly iodine (McErlean *et al.,* 2002). Despite the dirtiness of the job, it was lucrative for a time. The practice was so widespread, that the process gave the large brown seaweeds their generic name 'kelp' that is used today. More recently, it was discovered that 'gels' with various properties and characteristics could be produced from a number of different algal species. These gels are agars, alginates and carrageenans and form the basis of a multi-billion dollar hydrocolloid industry (Bixler and Porse, 2010). They have been used in a wide variety of food

(e.g. toothpaste, beer) and industrial processes in the paper and textiles industry etc. (McHugh, 2003). Much of the *Saccharina japonica*, *Eucheuma* sp. and *Gracilaria* sp. cultivation shown in Figure 1.2 will be used in global industries because of their gel properties. Most of the rest of the cultivated seaweeds described will be used as a food product. At present, the cosmetic industry use small amounts of high quality seaweed extracts in their products (e.g. face creams, supplement pills etc.). There is increasing interest in marine bioactive compounds for use in pharmaceutical applications. Seaweed extracts have already been shown to improve animal and human health, and show a positive effect on diseases such as cancer, AIDS, diabetes etc. (Løvstad Holdt and Kraan, 2011; Smit, 2004). Technological advances continually improve our understanding of the seaweed resources that we have, from the molecular (e.g. DNA) level to the ecosystem level. As a consequence, it is likely that research will take many new directions in the future. Even within the last 5 years, Irish and UK research projects are already focusing on using seaweeds in such diverse areas as fish feed diets and nutraceuticals/functional foods (e.g. the NutraMara[1] project), bioremediation and bioenergy (e.g. Biomara[2] and SUPERGEN[3]).

While the potential uses of seaweeds become ever more varied, environmental protection is becoming more stringent in many countries. If the demand for seaweeds (especially rare or endemic species) becomes too great, the product cannot be supplied reliably, and ecosystems can be badly affected, in some cases, irreversibly. It is clear that research and responsible cultivation of seaweeds is required to meet demand and relieve pressure on such environments (Romo *et al.,* 2006; Westermeier *et al.,* 2010).

The cultivation of any seaweed depends very much on the species in question, and the environment where the cultivation takes place (McHugh, 2003). Knowledge of the life history of the seaweed is critical if cultivation is to be attempted. Some species, such as *Eucheuma* sp. and *Kappaphycus* sp. (grown for their carrageenan content) are cultivated in a very simple manner, whereby fragments of plants are tied to lines supported by bamboo frames, and anchored in shallow water (McHugh, 2003). These fragments grow over a period of time, and at harvest, the picker will remove most of the biomass, but leave a small fragment of weed for future development. This method of cultivation is cheap, effective, and suited to shallow water where access is easy for the pickers. Due to the low cost of this system, it is now thought to be of vital importance to rural communities with low incomes in countries such as the Philippines. Once harvested and dried, *Eucheuma* sp. is processed to extract the carrageenan. This phase is much more expensive, and relies on the relatively low cost of cultivation to remain competitively priced in the commodities market.

In contrast, the cultivation process of *Porphyra* sp. in Japan is complicated, highly mechanised and expensive (Lobban and Harrison, 1997). Reliance on vegetative growth alone is not possible, and cultivation depends entirely on the knowledge of the life history of the alga. Here, the alga has an intermediate life stage known as the 'conchocelis'. This stage develops inside oyster shells, before spores are produced which are settled on nets, from which the vegetative blade develops. Wages of workers in Japan are much higher, and more equipment is required, making production costs much greater overall than in the Philippines. While post-cultivation processing exists, it is less expensive than that of *Eucheuma sp.* Due to the high production costs, it is only possible to grow *Porphyra* because it commands a high market price. It is greatly prized as a foodstuff in Japan and is an integral part of Japanese cuisine.

From these two examples, it is important to note that understanding the biology of the seaweed is vital for establishing any type of cultivation, but equally, the economics of the product also affect the success of the venture. As large amounts of labour and equipment can be invested in some types of seaweed cultivation, the return on the product must always be sufficient to make it worthwhile.

[1] http://www.teagasc.ie/ashtown/nutramara/
[2] http://www.biomara.org/
[3] http://www.supergen-marine.org.uk/drupal/

2.1 Introduction to *Laminaria digitata*
Hudson J.V. Lamouroux

2.1.1 Ecological range and habitat

Species of kelp can be found all over the world, and generally dominate lower intertidal, and shallow sub-tidal rocky shores in temperate coastal regions. The largest of these kelps, *Macrocystis pyrifera* typically grows up to 45 m in length, with a maximum growth rate of 30 cm per day (Guiry and Guiry, 2011 and references therein). Some species of kelp form monospecific stands or 'beds' and can be considered as 'keystone' species, forming essential components of important coastal habitats. Some species of kelp are annual, whereas others are perennial, and can be relatively long-lived. For example, *Laminaria hyperborea* individuals can live up to 15 years (Kain, 1979). The biodiversity within these kelp stands or forests is rich, providing both a direct and indirect source of food and habitats for organisms of all trophic levels (Birkett *et al.,* 1998). However, these habitats are sensitive to change, and the biodiversity within kelp forests can be compromised when the kelp are lost. Threats include the effects of climate change and El Niño events (warmer water temperatures, and increased storm events). Excessive grazing pressure from sea urchins also clears large areas of kelp forests if they are found in sufficient numbers (Foster and Schiel, 2010).

In the eastern North Atlantic, five different species of kelp dominate lower intertidal/subtidal rocky shorelines, ranging from Norway to Portugal (Lüning, 1990). These include *Laminaria hyperborea, Laminaria digitata*, *Saccharina latissima*, *Saccorhiza polyschides* and *Alaria esculenta*. The invasive species *Undaria pinntatifida* is also present in more southerly ranges (e.g. Northern France), but is not yet dominant within natural habitats around the south coast of England (Farrell and Fletcher, 2006).

Of the different kelp species, *L. digitata* mainly grows in the shallow subtidal on rocky substrates, generally only becoming uncovered on spring tides. Good water exchange determines where individuals will grow, as *L. digitata* will not grow in sheltered or muddy areas. They are also less likely to found in very exposed sites, but do require at least a moderately high level of exposure to water currents for survival.

2.1.2 Classification and description

All five kelps are members of the Class Phaeophyceae, but are classified within a number of different Orders and Families. Table 2.1. describes the currently accepted classification of *Laminaria digitata*.

Help Box: See www.algaebase.org for further taxonomic information.

Table 2.1.
Classification of *Laminaria digitata*.

Empire	Eukaryota
Kingdom	Chromista
Subkingdom	Chromobiota
Infrakingdom	Heterokonta
Phylum	Heterokontophycophyta
Class	Phaeophyceae
Order	Laminariales
Family	Laminariaceae
Genus	Laminaria
Species	digitata

Individuals can reach up to 2-3 m in length, depending on the exposure of the shore. Each plant is firmly attached to bedrock and large boulders by a substantial root-like holdfast. The stipe distinguishes *L. digitata* from *L. hyperborea* in the field, as it is flexible and smooth, whereas the stipe of *L. hyperborea* is rigid and roughly textured. The single frond that develops is thick and leathery, broad at the base, tapering towards the tip. The frond tends to split into 3-8 blades (Figure 2.1). Meristematic tissue can be found at the base of the frond, near the top of the stipe. It is from this region that a new frond regenerates during the new growing season.

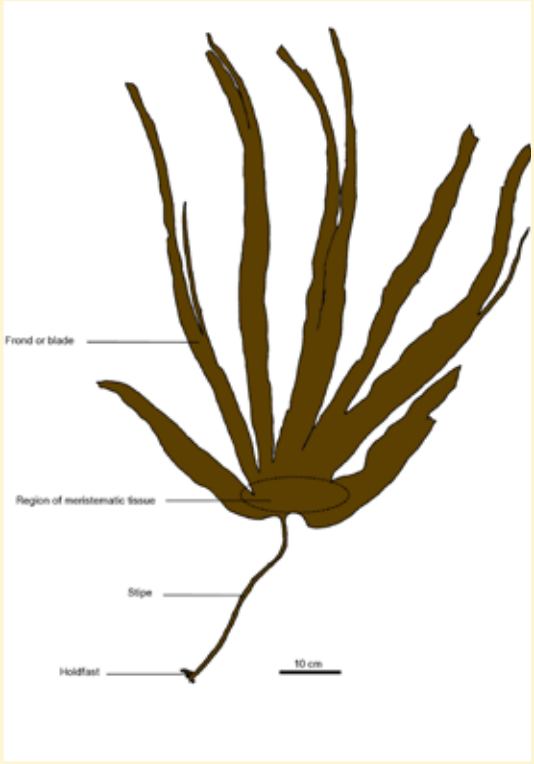


Figure 2.1.
Morphology of *Laminaria digitata*.

2.1.3 Life History

Kelps are commonly some of the largest species of seaweeds to be found growing on the rocky shore. Their reproductive cycle, or life history (Figure 2.2.), alternates between a large and structurally complex phase with a holdfast, stipe and frond, to the development of microscopic filaments that settle on rocks and other hard surfaces. The large plant is known as a 'sporophyte', while the small filaments are known as 'gametophytes'.

This cycle starts when reproductive patches ('sori') develop on the distal ends of the blades during April to October/November in the West of Ireland. The timing of sorus development can change, depending on location. The sori appear as dark, slightly raised patches on the blade. Within these sori, reproductive cells (unilocular sporangia) develop, and zoospores are released when they mature. Each cell can contain/release 32 zoospores (Kain, 1979). These zoospores are equipped with two flagella, enabling the cell to have some control over where they settle. After a period in the water column, the motile zoospore loses its motility and will settle passively onto a suitable substrate, for example, under the algal canopy where low light levels are suitable for germination (Kain, 1979; Roleda *et al.,* 2010). After the zoospore germinates, cellular division begins. This is now the gametophyte phase. Gametophytes will bear either male or female reproductive structures (antheridia and oogonia, respectively). Under the appropriate cultivation conditions, gametophyte cellular division continues unabated and individuals can become quite large. Once the environmental cues change (including changes in light quality and intensity, or quantum dose), the development of reproductive structures is triggered (Lüning, 1980). Thus, the availability of blue light in particular is important for this process (Lüning, 1980). In natural conditions, there is usually sufficient blue light available and gametophytes become reproductive almost immediately (Lüning, 1980). Female gametophytes produce ova/ eggs, while male gametophytes produce spermatozoids. The eggs remain attached to the gametophyte, and emit a pheromone, which attracts the male sperm (Maier *et al.,* 1988). Once fertilisation has taken place, a zygote develops, followed by cellular division as the new sporophyte develops. These cells of the juvenile sporophyte are differentiated at an early stage into frond cells, and rhizoid-like cells which will become the holdfast. The sporophyte also then attaches to a suitable substrate, and within the first year, will grow to approximately 50-60 cm.

In Ireland, where the main reproductive period is during the summer and autumn months, sporophyte development will start in autumn, and continue throughout the winter. Kelp such as *L. digitata* have the ability to take up and store phosphorus, which can be limiting at certain times of the year. Once established, kelps including *L. digitata* are known as 'season anticipators', meaning that they start growing during the winter. This gives them a selective advantage as they can start growing much earlier than other algal species (Bartsch *et al.,* 2008).

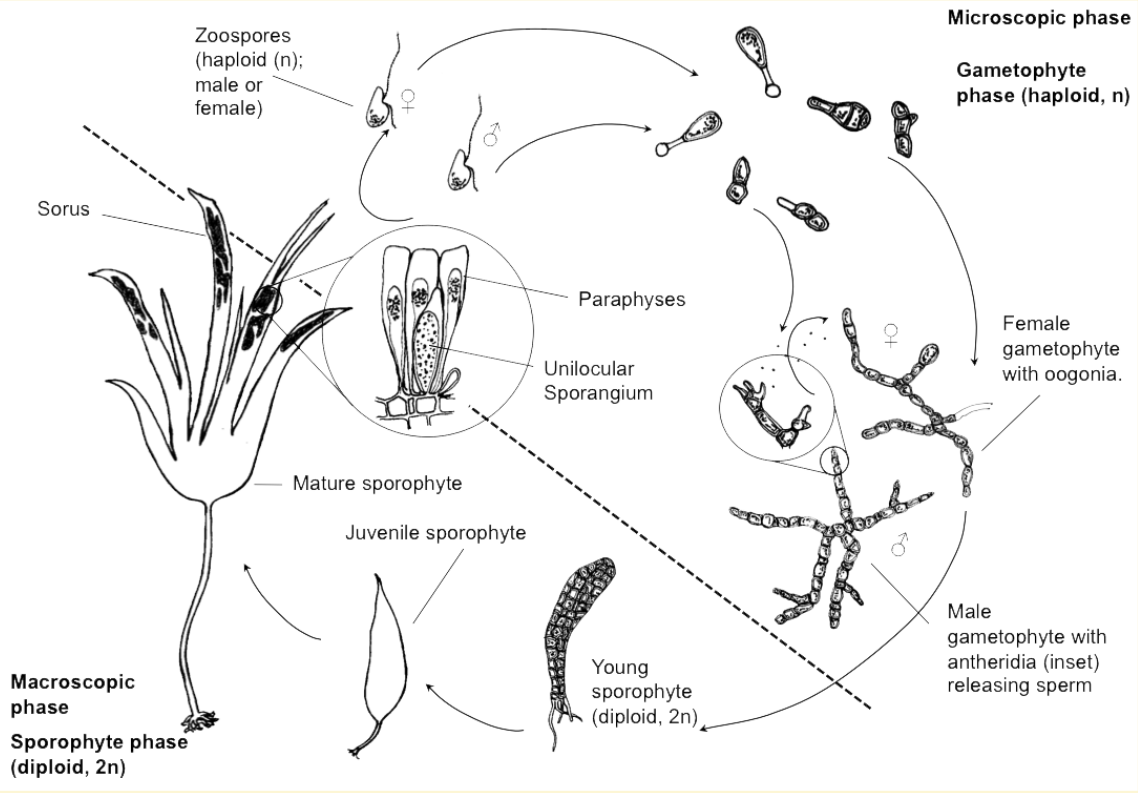


Figure 2.2.
Life history of *Laminaria digitata*

2.2 Overview of the *Laminaria digitata* cultivation process

As a brief introduction to the remainder of this manual, an outline of the procedure for cultivating *L. digitata* is described in Figure 2.3.

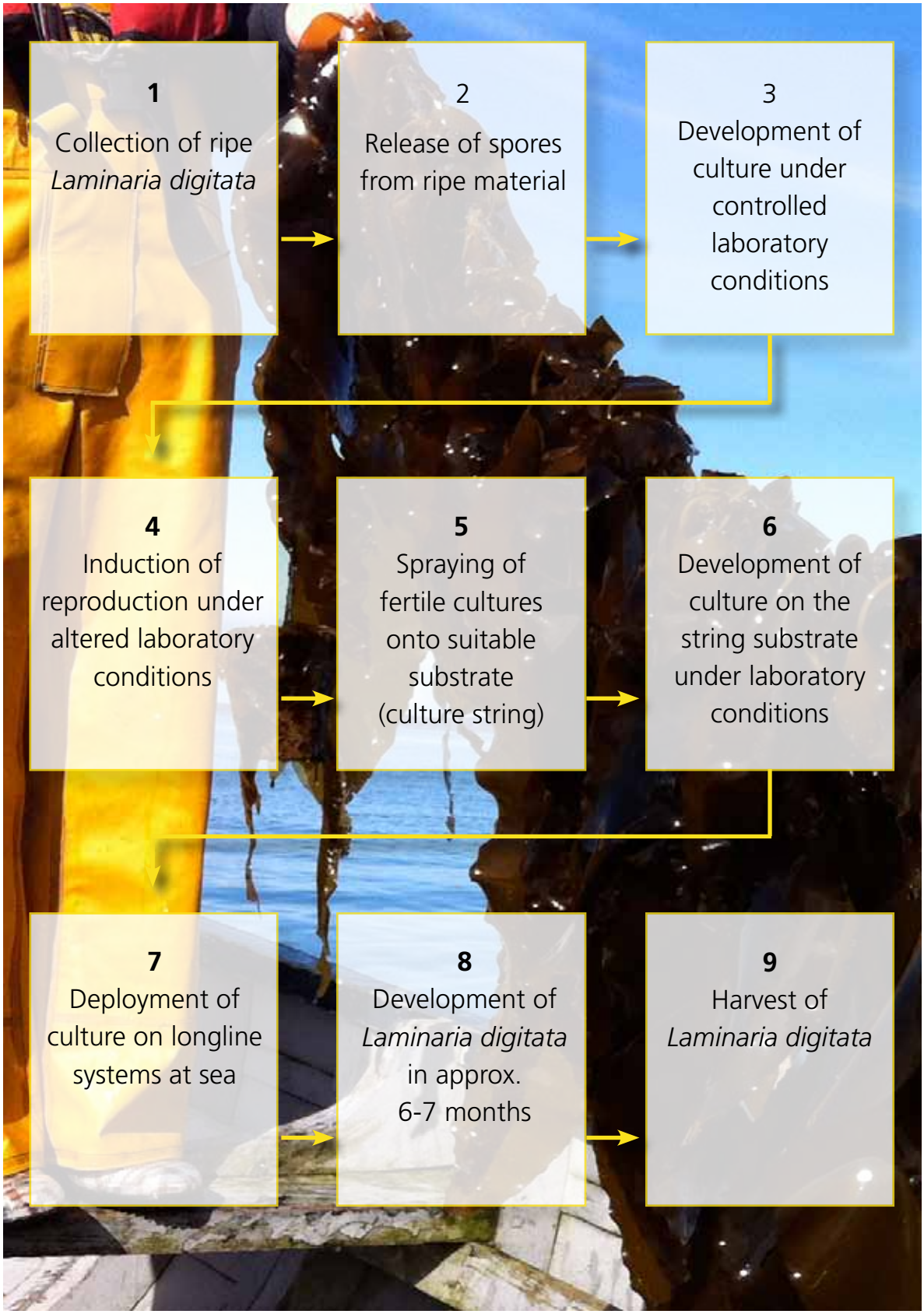


Figure 2.3.
Overview of the cultivation of *Laminaria digitata*

Chapter 3 The Hatchery and the Ongrowing Site at Sea

3.1 Facility Requirements

The hatchery must be located on flat, low-lying land adjacent to the sea with a low pumping head. It must have three-phase power, road access and sufficient space for adequate tankage, a laboratory, an office and facilities. Section 3.1 provides practical advice on the most important aspects to consider. The ongrowing sea site is also considered in Section 3.5.

In the Republic of Ireland, the Department of Agriculture, Marine and Food (DAMF) must license all land and sea-based aquaculture facilities. This includes seaweed hatcheries, which must hold an aquaculture license, a foreshore license and also an effluent discharge license obtained from the Local Authority. See the DAFF website for more information (www.agriculture.gov.ie/fisheries/aquacultureforeshoremanagement/).

In Northern Ireland, no specific license for seaweed cultivation exists, but this may change. The Department of Agriculture and Rural Development (DARD) will most likely issue licenses in the future. At present, the area of foreshore chosen for cultivation purposes must be leased from the Crown Estate. Consent must also be sought from both the Coastal and Maritime Agency and the Northern Ireland Environment Agency (NIEA).

Overview Box:

- Temperature control – either of the air temperature of the entire unit or the water of the cultures only.
- Seawater – water quality must be at least very good, if not excellent for both small and large-scale culture work, with filtration down to 1-5 µm ideal. Consider what seawater filtration and sterilisation systems will be available.
- Air supply – the main considerations are continuity of supply and quality of supply.
- Drainage – ensure discharge licenses from the hatchery have been obtained, and that drainage is sufficient for large-scale culture work without being overwhelmed.
- Light regime – the ability to manipulate both day length and light intensity is required.

Temperature control

Whether the hatchery is set up as a dedicated algal culture lab, or it is incorporated into an existing shellfish and/or finfish hatchery (which is more likely), a temperature-controlled, insulated room is required. The room should be held at a constant temperature of approximately 10°C (± 1°C). Cultures vessels can be exposed to chilled air, which ensures large amounts of water can be chilled to the same temperature at the same time. If however, several different species requiring different culture temperatures are grown in the same space, then individual water chillers and water baths should be considered for large and small culture vessels, respectively, as these will allow for greater flexibility of the work space.

Whilst chilled culture rooms are needed for the algae the environment can be difficult for the operator to work in for any length of time. Therefore, in addition to the temperature-controlled room, a warmer room is also advisable for the use of the microscope, changing culture media, storing equipment etc. For ease of use, this should be as close to the chilled room as possible. A supply of freshwater (hot and cold) and a large sink are also required in this room for the cleaning of glassware prior to sterilisation.

Seawater

Seawater pumping and filtration systems can be extremely variable and complex, depending on what the water is ultimately used for. For algal cultures, water quality is extremely important, and should reflect the species cultured. Water for *Laminaria digitata* culture for example, should be taken from fully saline (34-35 ppt) unpolluted coastal waters.

After the seawater is pumped from the sea, it should be coarse filtered, and pumped into a large reservoir for sediment to settle out. Light penetration into the reservoir should be avoided, as this will encourage unwanted algae to grow. The seawater from the reservoir should be passed through a finer filtration system, such as drum or sand filters of suitable size, followed by sterilisation in an Ultra-Violet (UV) light system. Particle filtration will now be as small as 1-5 µm, and suitable for larger scale culture work. Further sterilisation (tyndallisation) is required for smaller cultures that are held for longer periods of time in the laboratory. This is discussed in greater detail in Section 3.2. The quality of the seawater also depends on keeping the pumping and filtration systems in good working order. This includes the regular (daily) back-flushing of filters and periodically cleaning pipes with a 'pig' which will ensure no development of high bacterial loads etc.

Air Supply

A small to medium-sized air blower is recommended for an algal hatchery. While small electric 'aquarium' air pumps are useful for cultures up to volumes of 10 L, a dedicated air blower will provide the necessary air supply for much larger tanks. Increasing the air supply to these tanks over the development time of the culture is important. Lack of adequate air quickly develops 'dead spaces' in tanks, resulting in the degradation and loss of cultures. In a culture room, air can be delivered through a pipe network that runs around the room in a ring. Adjustable taps inserted into the pipe can supply individual tanks with as much air as required. Always consider what the back-up system will be, if the air blower breaks down. A standby blower connected to the system is recommended.

It is worth considering that if the air blower (which generates quite a lot of heat) is placed in warm room, and is pumping air into a much colder room, a significant amount of condensation forms inside the pipes. It is useful to incorporate a filter and a number of valves into the pipe network to drain the condensation from the system as part of a weekly routine.

Drainage

Depending on the legal requirements of the country in question, all on-shore aquaculture activities require a discharge license, which is monitored on a regular basis by the relevant authority. Ensure that these licenses are in place before starting production in an algal hatchery. In terms of drainage for the culture room, a suitably sized drain to cope with drainage from tanks of up to 1000 L tanks should be incorporated in to the design of the room. A floor that slopes slightly towards the drain is also extremely valuable. Non-slip floor surfaces should also be a standard as there can be a considerable amount of water lying on the floor at times.

Light Regime

Finally, light plays an essential role in the culture of any organisms that rely on photosynthesis for their growth and development. Algae also require different light intensities and day lengths for different periods of their development. An adaptable lighting system is of greatest benefit. For example, instead of having overhead lights permanently attached to the ceiling, suspend them on chains, so that they can be lifted closer or moved further away from tanks and culture flasks to vary light intensity. Fluorescent lighting is commonly used in culture rooms as they do not emit a large amount of heat and are relatively stable. Waterproof housings can be fitted to the bulbs so that the lights can be used near water. By attaching timers to these lights, day length can also be manipulated. The colour of the lights can also be manipulated by covering the light fitting in a layer of coloured cellophane.

Additional notes on Hatchery Facilities

The cost of setting up a hatchery facility is discussed in Chapter 8. If it is not possible to build a hatchery or adapt part of a building into one, a cost-effective suggestion is to use an insulated container unit. These containers of varying lengths are of an industry standard for the global transport of chilled or frozen goods and come with the refrigeration unit already installed. They can just as easily be connected to a generator or to the mains electricity (a qualified electrician is required for this). Some companies specialise in the supply of these containers, along with many customised changes. For example, lighting can be fitted, waterproof and water resistant plugs can be added, and even a discrete hole can be drilled for further input of services, for example air and seawater. The biggest challenge in these containers is drainage, as too many openings cut into the fabric of the container compromises its ability to maintain temperature. As the walls and floors of the container are all generally made of metal, it may increase the longevity of the unit to request an aluminium floor and wall border, which will not corrode in the same way steel does when seawater is present.

If it is not practical to have a large pumping and filtration system for the culture facility, the very least that is required for an acceptable water supply is outlined below (Figure 3.1).

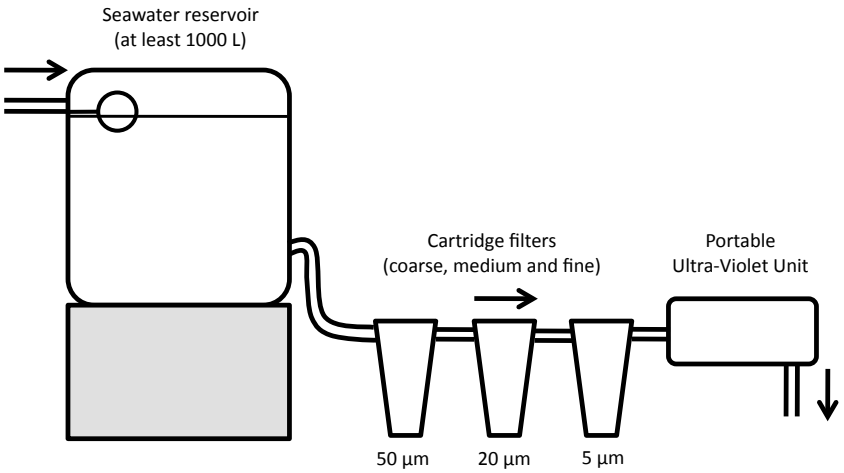


Figure 3.1.
Diagram showing system with minimum water treatment

Seawater is pumped from the source; sediment and other particulates are allowed to settle out in a reservoir tank with a lid. This lid is very important as it excludes light from the tank and therefore prevents microalgal growth. Reservoirs can either be made from very large tanks, or built from concrete blocks to whatever dimensions are required, and finished with a pond liner or similar strong waterproof lining. When water is required in the hatchery, a second pump sends water through coarse, medium and fine filters (these need to be cleaned regularly), before the water is passed through a portable UV unit for final sterilisation. Water can also be chemically sterilised with Chloros or a similar product, but this is probably not necessary for large-scale macroalgal cultivation if the water is of reasonable quality.

3.2 Preparation of Sterilised Seawater (SSW)

There are several different ways of sterilising seawater for culture volumes up to 15 L. The method will depend on the equipment available. For a hatchery to maintain a large number of glass and heat resistant plastic vessels (e.g. Nalgene® bottles) on a weekly basis, an autoclave is useful for the sterilisation of seawater. Larger models can take 8 to 10 vessels at a time, with an approximate cycle of 3-4 hours, making sterilisation more efficient. Fill the glass or plastic vessels with filtered and UV-sterilised water to approximately ¾ capacity, and cover the mouth either with the lid (only loosely screwed, so that steam can escape), or with an aluminium cap or a piece of strong tinfoil and set the autoclave on a standard programme, e.g. 121°C, 1.03 bar (15 lb in^{-2}) for 15 minutes.

The second method of sterilisation is known as Tyndallisation, after the Irish physicist John Tyndall. In this process, vessels are filled and capped as described above, but placed on the hob of a domestic cooker. The seawater temperature is monitored and is brought *almost* to the boil (90 ˚C). As soon as this is achieved, the heat is turned off and the seawater is left to cool. After 24 hours, the process is repeated, and the seawater can now be cooled and used. The application of heat will kill most viable organisms in the water in the first instance, but the second application of heat after 24 hours ensures that any microorganisms that have encysted and subsequently germinated within that time will be destroyed.

Both methods have their merits, but it is argued that the Tyndallisation method is better for culture work as the heat does not affect the water chemistry, whereas the higher temperatures in the autoclave do change water chemistry. Table 3.1 below compares the two methods.

Table 3.1.

Comparison of two methods of seawater sterilisation – by autoclave, and by Tyndallisation. Pros and cons of each method.

Autoclave	**Tyndallisation**
Water chemistry altered	Water chemistry not altered
Expensive equipment required: Large autoclave (thousands of euro)	Relatively cheap equipment required: standard hob either standalone, or as part of oven (hundreds of euro)
Useful for sterilising larger amounts of water	Only up to 4 vessels sterilised at a time on a domestic cooker
3-4 hours to sterilise seawater	Up to 36 hours to sterilise seawater
Automatic programme	Worker required to monitor seawater temperature (approximately 1 hour) twice over 24 hours
Glassware and heat-resistant plastic can be used	Glassware only on the hob – plastic will melt
Other culturing equipment can be sterilised at same time, e.g. glass tubing, cylinders, beakers etc.	Oven can be used to sterilise other culturing equipment at same time, e.g. glass tubing, cylinders, beakers etc. (200 ˚C for 1-2 hours, depending on item).

3.3 Choice of media – preparation and use

Like any terrestrial plant crop, the growth of seaweed benefits from the addition of nitrogen, phosphorus and potassium in specific ratios (N:P:K). These elements are most likely to be delivered in the form of nitrates and phosphates. A wide range of nutrient media has been developed over the last 50 years and tailored to deliver specific macronutrients to particular types of seaweed. Trace metals and vitamins are also required for normal algal development. The macronutrients, trace metals and vitamins are made up separately into stock solutions, from which the 'working solutions' can be made up.

3.3.1 Preparation of media

Care must be taken to make the media in clean glassware, following the recipe precisely. A fine-scale balance is required for this, as well as distilled water for making the solution up to the volume stated. It is also useful to have a magnetic stirrer to mix the solution, as some chemicals take a long time to dissolve. A heat plate may aid the dissolving of the chemicals. Take care also to add the chemicals sequentially as stated in the recipe as some will not dissolve once other chemicals have been added.

While the macronutrient stock solution will be stable in the longer term and can be autoclaved to prevent contamination (the same applies for the trace metal solutions), the vitamins in stock solutions breaks down easily, especially in light and heat. It is therefore advisable to keep all the stock solutions in the fridge, to prevent break down, and maintain maximum potency. Any stock solution that cannot be sterilised by autoclaving needs to be filter-sterilised (through a filter with a pore size of 0.2 µm), divided into aliquots (i.e. a small portion) and frozen. Each aliquot can then be defrosted and used as required.

3.3.2 Suggested media for the cultivation of *L. digitata*

Most literature describing laboratory culture of any of the Laminariales generally suggest the use of Provasoli's Enriched Seawater (PES). However, there are many variations of this recipe used, with no clear consensus of which is best (Harrison and Berges, 2005). Appendix 2 contains the recipes for the 'best formulation for general use' PES (from Harrison and Berges, 2005), and the version as described in the BIM Aquaculture Explained manual for *Alaria esculenta* (Arbona and Molla, 2006). Table 3.2 describes the amount of nutrient media to add to each litre of sterilised or filtered seawater.

The recipe for another nutrient medium called f/2 (i.e. half-strength 'f' medium, Guillard, 1962) is also contained within Appendix 2, also using the recipe from Harrison and Berges, (2005). f/2 is a versatile nutrient medium that is used for the cultivation of many different species of seaweeds, and works well with *L. digitata*.

Table 3.2

Volume of nutrient media required per litre of seawater.

Media Type	Volume of stock solution per litre of seawater (to make up medium)
PES	20 ml L^{-1} (Harrison and Berges, 2005); 4 ml L^{-1} (various stocks Arbona and Molla, 2006)
f/2	0.1 g L^{-1} (pre-prepared solid medium) or 4.5 ml L^{-1} (various stocks, Harrison and Berges, 2005)

3.3.3 Preparation from individual components and pre-prepared media

As macro- and microalgae are cultured for a variety of different reasons all over the world, it is easy to obtain pre-prepared media of your choice (see examples from the supplier list in Appendix 3). Pre-prepared media can come in concentrated powder or liquid form. While these pre-prepared media can be more expensive compared with media made up from the separate components (volume per volume), there are certain advantages to using them. For example, the media usually already contains both the vitamins and trace metals (however, it is always best to make sure before purchasing). Time and money can be saved, as no specialist laboratory equipment needs to be used in making up the solution. Inaccuracies in making the solution can also be avoided.

Help Box: It can be necessary to hold a license for the storage of sodium nitrate, (used in making up nutrient stocks) whereas the use of a pre-prepared media avoids such problems.

3.4 Cleaning and Sterilisation Techniques

In aquaculture, cleanliness of facilities and equipment is paramount to the success of the organism(s) being cultured. Allowing opportunistic and/or parasitic species to exploit the optimal conditions available results in lower productivity, slower development and in some cases, death of the cultured organism. Needless to say, this has an impact on the viability of the hatchery as a business in general.

Macroalgal culture is no different, and every effort should be made (where possible) to keep preparation and culture areas clean, and to practice 'aseptic' techniques where appropriate.

3.4.1 Preparation and culture areas

Every week, clean bench areas etc. with hot soapy water and rinse with hot water. When work is to be carried out (e.g. transfer of cultures into new glassware), swab the area down with ethanol.

3.4.2 Glassware

When choosing to grow cultures in glass, all vessels should be made from borosilicate glass. Polycarbonate containers are also useful in cultivation (e.g. Nalgene® bottles or carboys). The glassware should be thoroughly cleaned and sterilised before use. This can be done in a number of different ways. First, use hot water and a mild, phosphate-free laboratory detergent (for example, Decon 90). Scrub glassware inside and out with bottle brushes and scrubbing pads. Rinse thoroughly in hot water to remove all traces of detergent, and rinse again in distilled water. Drain glassware upside down on draining racks, and allow glassware to drip dry. It is not advisable to use cloths etc to dry the glassware as this may introduce contamination to the cleaned equipment. Once dry, cap vessels with tin foil. Smaller items, such as glass tubing, pipettes etc. can also be wrapped in tin foil in small batches, ready for sterilisation. Ideally, an autoclave is useful for this, on a standard cycle. However, as these are large and expensive pieces of equipment, a domestic oven is also very useful. Place glassware in cold oven, and set at 200 ˚C for 1 to 1.5 hours. Allow glassware to cool before removing from oven.

3.4.3 Other Equipment

Other pieces of equipment used in culture preparation, that cannot or do not necessarily need to be heat-sterilised in an autoclave/oven, can be wiped with ethanol, or flamed with a blow-torch. For example, the scalpel and blade used to cut the reproductive tissue could be cleaned in either of these ways.

Special attention should also be paid to equipment and consumables that can't be sterilised at all. For example, cotton wool used for the bungs in the culture flasks needs to be kept in a dry and dust-free place until required, for example, inside a sealed plastic bag. It should be cut to size on a clean surface and handled as little as possible, presumably while the operatives wear gloves.

3.4.4 Handling Cultures

When manipulating a new culture, or renewing an existing culture, it is very important to protect the cultures from air-borne contaminants. This is especially important in hatcheries that are used for culturing several different organisms, and when space is limited. Try to work in an area that is free from draughts. As mentioned above, clean the bench space with ethanol just before you use it. Have every item needed close to hand and try to avoid confusion and disorganisation by developing a routine when handling cultures. When pouring sterilised seawater or cultures, try to minimise contact with air by always replacing tin-foil caps after use, never touching one piece of glassware with another etc. The same applies for nutrient media, as these can become contaminated very easily. It is better practice to decant a small volume of medium into a sterile beaker and take the desired amount for the cultures from there. Discard excess medium - do not pour it back into the stock solution.

3.5 Ongrowing site requirements at sea

3.5.1 Site Selection

The assumption made in Section 3.5.1 is that the reader is looking to obtain a seaweed license for a new site, and where possible, must make some decisions about site selection to optimise the seaweed yield. It is also assumed that any site used, is covered by sufficient insurance, and safety of workers is considered above all other factors.

Parameters for good site selection can be divided into those required for the organism to grow well, and those that make the site easy to access, and work in. Of the latter, consideration should be taken for the following:

a) Access from shore –

- Is there a pier/quay nearby or will boats be deployed from a natural shoreline?
- Can the water be accessed easily at any state of the tide?
- Other licensed aquaculture activity – is it possible to create synergies between the activities by sharing boats and/ or equipment?
- Is there any road infrastucture available to launch/ recover boats from, and to remove harvests easily?

b) Site –

- How far away from the shore is the licensed site (consider fuel costs)?
- How exposed is the site – will it be easy to access and work from in most types of weather?
- What other users are within the area of the site (e.g. fishing vessels, watersports, other aquaculture, shipping lane etc.)?
- What levels of pollution may be present in the water – e.g. untreated sewage, industrial plant discharges nearby etc.?

Of the former parameters, depth, substrate type, water currents/ exposure, temperature and salinity must be considered when choosing a site:

a) *Depth* – tidal range can be large on some coastlines and must be taken into account when choosing a minimum depth for installing a structure. An absolute minimum depth of water suggested is 6 m, although 10 m depth is advisable. At the other end of the scale, very deep water is also not advised, due to increased costs of equipment, and more difficult conditions for divers to make safe inspections/carry out maintenance.

b) *Temperature* – the limiting factor for growth and development of *L. digitata* is high water temperatures. Unless there is a threat of ice formation, minimum winter temperatures are not considered to be damaging to development. Ideally, average maximum temperatures should not go above 15° C during spring/early summer. Therefore, do not choose a site that experiences large fluctuations in temperature (e.g. near a large, exposed intertidal area that will heat up rapidly during the day at the low tide). Water over sandy substrates also tends to warm up more rapidly than water over rocky substrates.

c) *Substrate* – correct location on different types of substrate is important for several reasons. For example, the 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 can also 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 plough anchors or similar can be used effectively in sandy sites. Combinations of muddy, silty and sandy sites are to be avoided as they may/ may not provide solid anchorage, and any fine sediment thrown up into the water column will settle on developing plants and reduce photosynthetic efficiency.

d) *Water currents and Exposure* – *Laminaria* culture requires sites with a suitable water exchange. Exposure to currents as opposed to waves is preferred, as areas of high flows will improve nutrient exchange, whereas increased wave action may damage the longline structures. Currents with mid to high flow rates (between 5 and 10 cm s^{-1}) are considered useful for seaweed cultivation. Carefully consider the licensed site to be used in this respect – it is worth noting areas of stronger current, and situating longlines in line with the main flow of the current available.

e) *Salinity* – Most sites around the Irish coastline will experience full salinity and are suitable sites for *Laminaria* cultivation. Avoid sites with brackish water, such as estuaries or where other large volumes of freshwater run into the system from streams and rivers.

3.5.2 Equipment and Maintenance

Cultivation of seaweeds at sea currently uses single-header longline structures, not dissimilar to mussel longlines (although these longlines generally have two header ropes). Figure 3.2 shows a simple longline construction suitable for the cultivation of seaweed. 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 will be outlined below, but longlines can vary slightly between sites and between operators due to different challenges in the deployment, and also in the type and amount of equipment available. No matter how the longline is constructed, two elements should remain the same throughout. Firstly, 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. Secondly, the header rope must always be positioned approximately 0.5 to 1 m below the surface of the water. This is necessary as *L. digitata* develops and grows better at this depth than at the surface as light levels are lower here and photosynthesis is not inhibited.

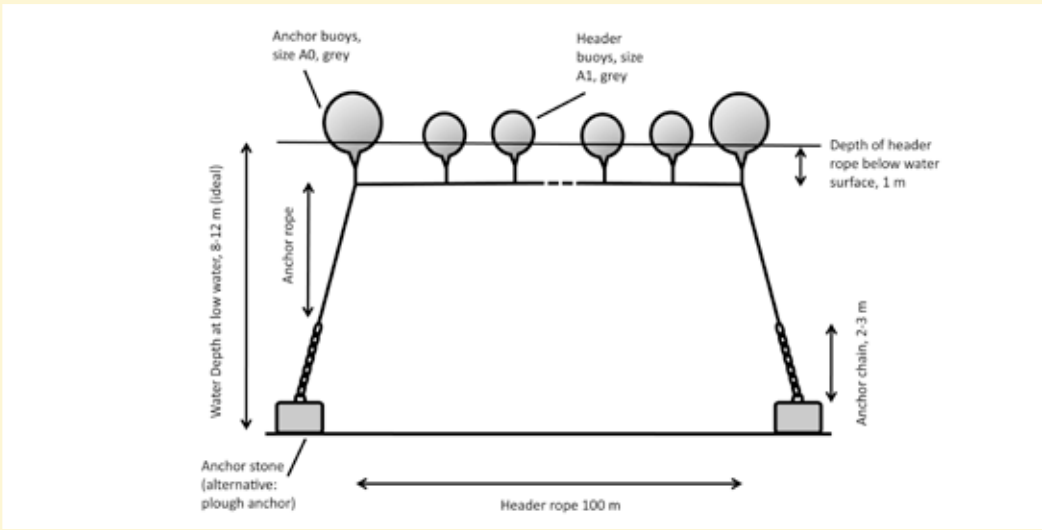


Figure 3.2.
Diagram showing construction of a 100-m longline for seaweed cultivation (not to scale).

Equipment for one 100 m longline

1. *Anchors* – these can be either concrete anchor stones of suitable size or plough anchors/ equivalent. Anchor stones must be *at least* 0.5 tonne per stone, and preferably a weight closer to 1 tonne for a 100-m longline. Size/ weight of stone may depend on what vessel can be used to deploy the stones. Some method of attachment is required on the stone, e.g. heavy metal ring, or eye. Anchors must also be of suitable size, and be able to lodge securely in the substrate to provide maximum stability.

2. *Heavy Link Chain* – several meters of chain are required to connect anchors/ stones to the anchor rope. Heavy chain with at least 10-20 cm links is suggested for use.

3. *Anchor Rope and Buoy* – measure depth of site at high tide adding an extra 1-1.5 m to obtain the length of anchor rope required, allowing a small amount of slack. 25-mm braided polypropylene rope is suggested for use. As the header rope will be periodically removed for cleaning, it is imperative to have the anchors marked with a buoy to ensure later identification.

Help Box: *Although each operator may deploy lines to his/her personal method, it is suggested to splice an eye at each end of the anchor ropes (preferably with metal eyelets), so that shackles can attach the bottom of the rope to the chain, and the top of the rope to the header rope.*

4. *Header rope* – measure the header rope to provide the length of longline required (e.g. 100 m) and add several metres to allow for movement or 'give'. Heavy, sinking rope is suggested for use, for example, 25 mm as per anchor ropes. Header ropes must be easily detached for seeding and removal during summer months.

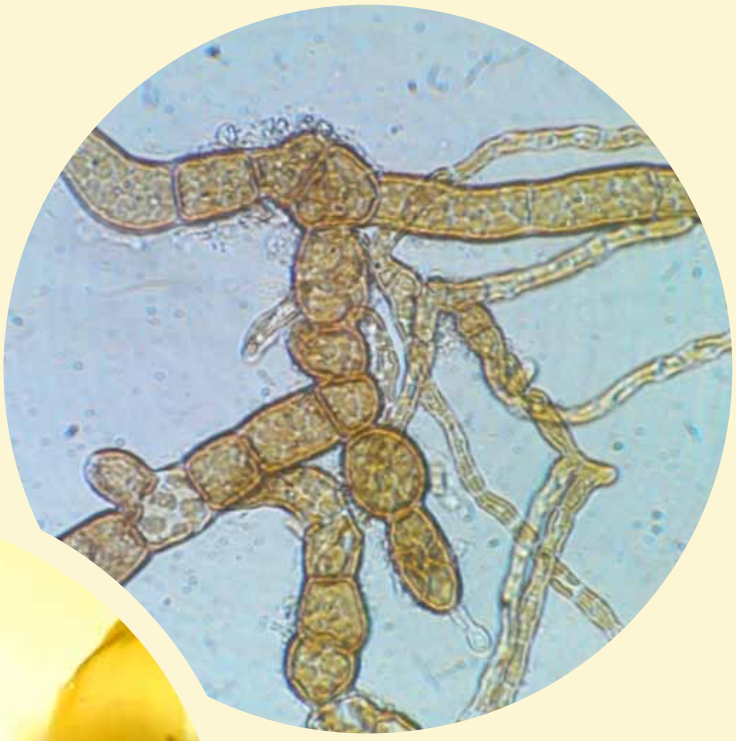
5. *Buoys* – while cultivated seaweeds do not exert the same force on the header rope as mussels, regular buoyancy is required to maintain the structure at 0.5 - 1 m below the water's surface. Again, access to resources may influence the type of buoy used, but they needn't be very large. Rather, smaller buoys work well, as long as they are plentiful and evenly spaced. This gives the advantage of providing well-balanced buoyancy, which will support the header rope, even if one or two buoys are lost over time. Buoys should be attached to the header line by 1-m lengths of 12-mm polypropylene rope. The buoys therefore support the header rope approximately 1 m below the surface of the water (Figure 3.2).

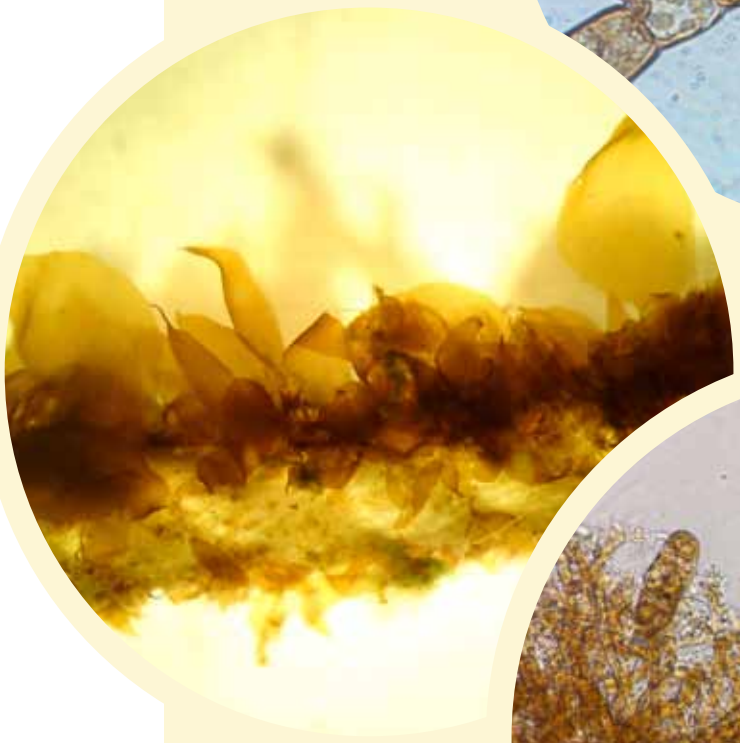
6. *Marker Buoys* - all sites must have an Aquaculture License and a Foreshore License. The sites are also required to be marked by standardized marker buoys with radar reflectors etc. the Marine Surveyor's Office in Ireland, should be consulted about these requirements.

Maintenance and cleaning of longlines

Maintenance can be considered in terms of monthly checks, and periodic inspections. During the growing season, it is advisable to check anchor ropes connections to the header rope, and the connections of the buoys to the header rope for wear and tear. Replace buoy attachment ropes if they are worn or frayed. Periodic checks of the anchors and chain connections by divers are advisable to ensure no sudden catastrophic failures of parts. It is advisable to replace shackles every 1-2 years if required. A longline can be moved or dragged if a medium-large vessel ties up to it. It is therefore advisable to assess the tension of the header rope occasionally and either reposition the anchors, or tighten the header rope where necessary.

The amount and type of cleaning of longlines will depend on the site. For example, at some sites, time may be taken up in removing flotsam and jetsam that have tangled in the lines, whereas there will be very little of this work to do at other sites. Settlement of mussel spat is also common, and unless it is removed from anchor and header ropes (without disturbing developing plants), the longline will become significantly weighed down. Header ropes should be removed at the end of the growing season. Storage of the ropes on land will ensure that the fouling organisms will have dried up and can be easily removed before re-deployment for the following culture season.





Chapter 4
Laminaria digitata gametophyte cultures

Once a suitable hatchery facility has been set up, culture work can begin for any number of different algal species, for example, the large brown alga, *Laminaria digitata*. The equipment list below covers all of the items required for successful gametophyte culture, but additional glassware etc. can be added over time as culture facilities expand. Some of the more expensive pieces of equipment such as a microscope and light meter are invaluable but expensive. Initially, if it is not possible to buy these items, access to one locally should be arranged.

4.1 Equipment List

- Autoclave*
- Dissection/Field microscope*
- Fine-scale balance*
- Light meter*
  (measuring PAR - Photosynthetically Active Radiation. Avoid using a light meter that measures in LUX
- Dissection kit
  (including spatula, scalpel and blades, fine-nosed tweezers, coarse and fine scissors etc.)
- Blow-torch
  (models with their own mini gas canister are useful, as are the smaller catering versions that can be re-filled with butane)
- Sample bags
- Cool box and ice blocks (optional, but useful)
- Permanent marker
- Assortment of glassware
  (to include x3 or 4 1-L beakers, x4 or more 6-L flat-bottomed round culture flasks; other flasks, such as 4-L flat-bottomed round culture flasks, 1-L conical flasks, funnel and a 1-L graduated cylinder are useful, but optional)
- Glass tubing, outer diameter 4-6 mm
- Glass tubing cutter
- Plastic air tubing/hose to fit glass tubing
- 'Whatman' Hepa-Vent air filters (autoclavable)
- Fluorescent lighting, e.g. T5 tubes in 'Linda' housing
- Light timer
- Cellophane (red)
- Variable pipette (in the dispensing range of 100-1000 µL)
- Nutrient medium (e.g. Provasoli Enriched Seawater (PES) or F/2)
- Phytoplankton net (30 CM^2), with mesh size 30-60 µm.
- Parafilm
- Cotton Wool
  (hospital grade roll, not cotton wool balls or pads. Non-absorbent if possible)
- Tinfoil
- Laboratory tissue
- Ethanol

*or access to one

4.2 Establishment and maintenance of L. digitata gametophyte cultures

Fertile *Laminaria digitata* can be found during April to November around Ireland. It grows on rocky shores (bedrock and or large boulders) in the lower intertidal and subtidal regions. Collection and release of zoospores from fertile material is a two-day process, with collection and preparation of the *L. digitata* completed on day 1, followed by zoospore release on day 2.

4.2.1 Collection of fertile *L. digitata*

1. When collection of fertile material is required, use the relevant tide and correction tables for your area of coastline, and identify the spring tides (see help box below). Choose a day when the height of water above chart datum at low tide is approximately 0.6-0.9 m. Collection of kelp is easiest on these days, as kelp beds are not generally exposed when the tidal height is above 0.9 m (Figure 4.1).

Figure 4.1.
A kelp bed during a spring low tide, exposing *Laminaria digitata*. Kelp beds are generally accessible only when tide tables calculate low water is 0.9 m above chart datum or less.

Help Box : *Two useful websites for obtaining tide times and heights include:*
a) http://www.sailing.ie Choose the 'Racing' web page for tide tables around Ireland for the whole year and click on 'Tides' on the navigation bar.
b) http://easytide.ukho.gov.uk/EasyTide/EasyTide/index.aspx , which gives free tidal predictions for the next seven days, anywhere in the world.

2. Taking care on slippery rocks and being aware of the state of the tide, identify an accessible kelp bed. Inspect the distal ends of *L. digitata* blades for raised patches of sori (Figure 4.2). Choose the cleanest blades that can be found, i.e. with little or no attached organisms visible.

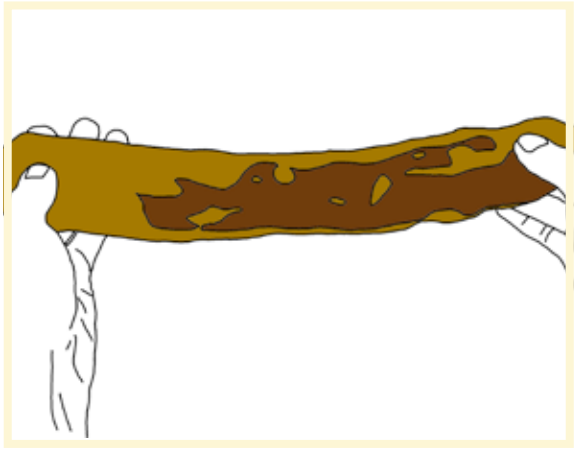


Figure 4.2.
Inspect the blades of *L. digitata* for raised patches of sori (dark brown) against the lighter brown of the sterile tissue.

3. Cut off only the pieces of blade with the sori that are required – it is not necessary to remove the whole plant. If the available kelp bed is far from the hatchery/laboratory, wrap the pieces of blades in laboratory tissue soaked in seawater and keep cool by placing in a sample bag in a cool box containing frozen ice blocks.

4.2.2 Cleaning and preparation of *L. digitata* sori

4. Back in the laboratory, the pieces of thalli/blades are prepared for spore release by selecting only the reproductive sori and cleaning these areas. Use a scalpel to cut around large areas of sorus roughly, to remove as much sterile tissue as possible (Figure 4.3). Ensure any small patches of fouling organisms are excised from the pieces. Discard the sterile tissue.

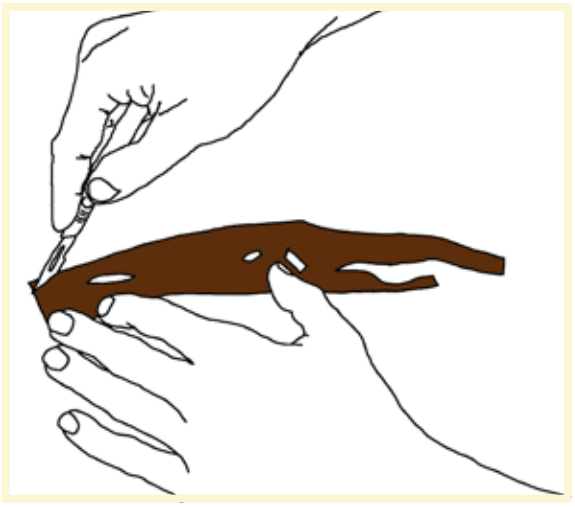


Figure 4.3.
Use a scalpel to cut away excess tissue from around the sorus, also making sure to remove fouling organisms from the sorus.

5. Clean the sori by dipping laboratory tissue into sterilised seawater (SSW) and wiping both sides of the

piece of sorus firmly (Figure 4.4). Discard the tissue, and using a clean, dry piece, firmly wipe the sorus dry. Repeat this process 3-4 times. Once all pieces of sorus are prepared, place between several layers of tissue, and keep in a dark, cool place (~ 10°C) for 18 to 24 hours.

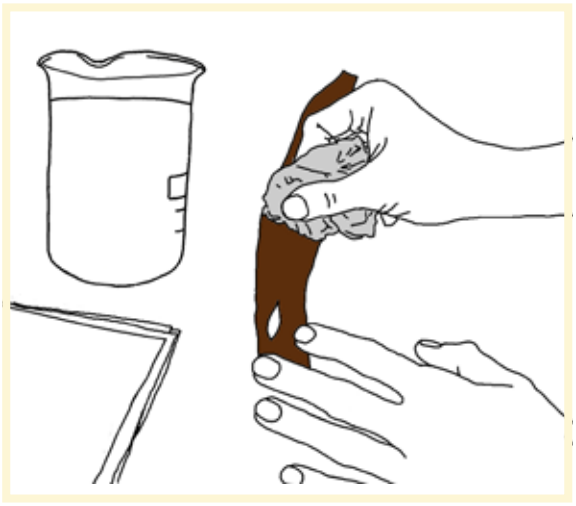


Figure 4.4.
Clean the sorus with laboratory tissue and sterilised seawater 3-4 times on each side.

4.2.3 Release of zoospores

6. Remove the prepared pieces of sori from the dark cupboard or box and cut them into smaller pieces (4 or 5 cm^2). Place in a 1-L beaker and add cool, sterilised seawater (Figure 4.5). Cover the beaker (e.g. with tinfoil or parafilm) and leave the sori to release zoospores for 30-45 minutes, stirring occasionally with a clean spatula. The seawater becomes cloudy from spores released from the pieces of sori. Using the phytoplankton netting in a funnel to remove the pieces of blade, strain the zoospore suspension into a suitable culture vessel. Discard the rehydrated sori.

Figure 4.5.
Cut pieces of sori are placed in cool sterilised seawater and zoospores are released within 45 minutes.

4.2.4. Setting up the culture – nutrients, aeration, light and temperature

7. Add further SSW to the spore suspension to fill the vessel. Don't overfill the vessel - leave enough space (approximately 10-15 cm) between the water level and the neck of the vessel to ensure the culture does not come in contact with the bung when the culture is aerated (examples in Figures 4.6-4.8). Having determined which medium to use, and at what concentration (see Chapter 3.3), add sufficient nutrients for the volume of culture used. Swirl the vessel to disperse nutrients throughout the culture. Figure 4.6 shows the equipment required to set up a gametophyte culture.

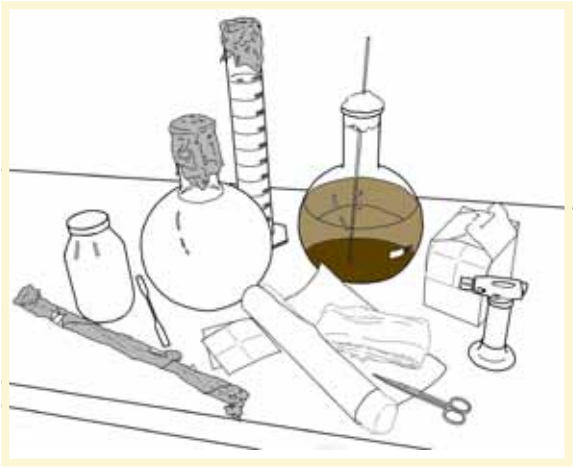


Figure 4.6.
Equipment required for setting up a new, or cleaning an existing gametophyte culture. Equipment from left to right includes: autoclaved packet of glass tubing in tinfoil, jar of nutrients and spatula, sterilised seawater with tinfoil lid, sterilised graduated cylinder (1 L), parafilm and non-absorbent cotton wool roll and scissors, an existing gametophyte culture, with gametophytes settling to the bottom of the flask, roll of parafilm, and blow torch.

8. Insert a pre-sterilised glass tube into the culture vessel, and secure with a cotton wool bung (Figure 4.7). If there is a doubt about the sterility of the glass tubing, pass it through a flame before use. Stretch a piece of parafilm over the bung and glass tubing to seal the top of the vessel. Connect the glass tubing to an air-supply via a length of plastic tubing and an air filter.

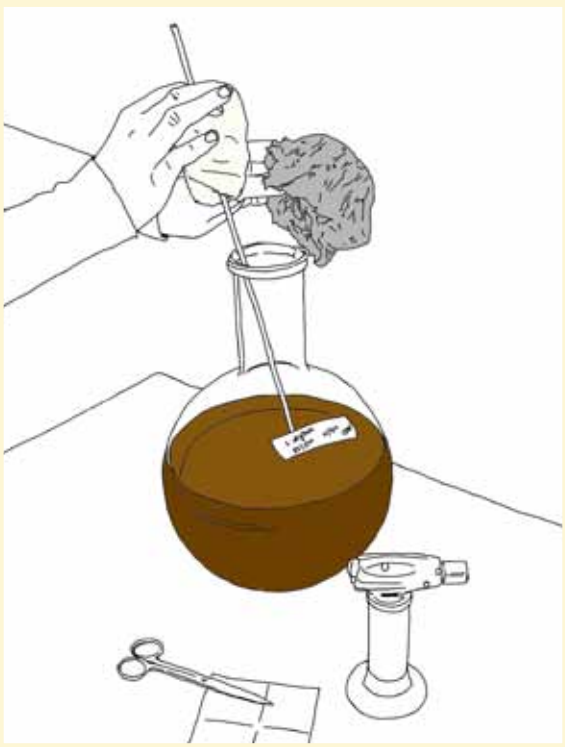


Figure 4.7.
Assembly of the culture glassware prior to aeration.

9. Adjust the position of the glass tubing within the culture vessel to obtain a steady, moderate aeration. For example, lift the tubing off the base of the vessel slightly to increase the airflow, or use a clip or an adjustable tap on the plastic tubing from the air supply to reduce the airflow.

Help Box: ***Air*** *can be supplied either by a small aquarium pump, or off a main line, supplied by a central air blower. Use an in-line filter, such as a Whatman Hepa-Vent™ to prevent contamination of the culture from air-borne pathogens, microalgae etc.* ***The light requirements*** *for the vegetative development of L. digitata gametophytes require the installation of either single or double strip lighting, such as T5 Lindas. These are moisture-proof units, which house fluorescent bulbs that emit relatively low levels of heat. Measure the light intensity (PAR) with a meter calibrated in µmol* m^{-2} s^{-1}.

10. Cover the light units in red cellophane, and place them at an approximate distance of 50 cm above or behind the cultures (Figure 4.8). The irradiance at the surface of the glassware should be 15-20 µmol m^{-2} s^{-1}. Adjust the culture to this value by moving the light source closer or further away from the culture. Irradiance can also be reduced by adding layers of good quality tracing paper under the cellophane. Connect the light source to a time-switch set to long days; 16 to 24 hours of light per day are sufficient.

Figure 4.8.
Cabinet with cultures at various stages of vegetative development. Cultures held in red light under long days, at 10 °C with aeration.

11. Regardless of the design of the hatchery, cultures should be kept in constant temperature conditions of 10 ˚C. This can be at room level, where air is chilled to the desired temperature, or in a water bath, where cultures are surrounded by chilled water, maintaining 10 ˚C culture temperatures, despite ambient room conditions.

4.2.5 Maintenance of gametophyte cultures

The amount of biomass per culture flask must be increased in the next 3 to 5 months. This is achieved by keeping the cultures in motion and regularly exchanging the media, every 10-14 days.

12. Prepare SSW and glassware/equipment as described in Chapter 3 and follow the advice on handling cultures.

Remove the air tubing and cotton wool bung, but retain the parafilm cover over the mouth of the flask. If the gametophytes have adhered to the flask, use a sterilised spatula or piece of modified glass tubing to scrape the gametophytes into the main culture. Allow the gametophytes to settle onto the bottom of the culture vessel, which may take approximately 30 min, depending on amount of biomass in the vessel (e.g. Figure 4.6).

Figure 4.9.
Modified glass tubing for scraping gametophytes from the sides of glass flasks.

13. Once the gametophytes have settled, gently pour off the seawater without pouring off too many gametophytes with it. If the biomass has been disturbed, allow it to settle again, before continuing to pour off as much of the excess seawater as possible. For example, in a 6-L flask containing approximately 5 L of culture, it is possible to remove 3.5 to 4 L of seawater, leaving the biomass remaining in the final litre.

14. Decant the gametophytes into newly sterilised glassware, and fill with new SSW. Add the appropriate amount of nutrients for the volume, all the while maintaining a cover on the new flask between additions of seawater, nutrients and culture. Introduce an air supply to the culture and replace the vessel in the light regime described above (e.g. Figure 4.8).

Chapter 5
Development of *Laminaria digitata* sporophyte cultures

After 3-5 months, the gametophyte cultures have developed a sufficient biomass to be used for spraying onto the culture string for deployment at sea. Before this happens however, the gametophyte cultures are placed in new culture conditions for a short period to induce fertility. In this period, the female gametophytes develop ova, or eggs, and the male gametophytes produce sperm. Fertilisation occurs and a zygote is formed, from which the juvenile sporophytes develop. Additional equipment is required for this process, as the cultures are moved from glassware to tanks.

5.1 Equipment List

(In addition to the equipment required for gametophyte cultivation, see chapter 4.1)

- Medium to large-sized tanks (250 L up to 1000 L, depending on space and budget)
- Blender
- Plant mister/spray bottle
- Plastic box (30-50 L appropriate)
- Electric drill with round-hole cutter bit (diameter 4 cm)
- Long poles (> 1.5 m, preferably plastic, e.g. electrical conduit piping)
- Culture string (> 100 m)
- Square drainpipe, cut into 30-cm lengths
- Air tubing, air stones
- Cellophane (blue)

5.2 Induction of reproduction

1. Induction of a *L. digitata* sporophyte culture begins by refreshing the medium in the culture flask of gametophytes as described in chapter 4.2.5. to ensure that development is not affected by any lack of nutrients.

2. The light requirements for sporophyte development also require fluorescent lighting but the units are covered in blue cellophane. Irradiance at the surface of the glassware should also be 15-20 µmol m^{-2} s^{-1}. Adjust this value by moving the light source closer or further away from the culture. Connect the light source to a timer, with equal light and dark periods, i.e. 12:12 hours, light:dark. The temperature of the culture should be kept at 10 °C as before and aeration should be provided.

Maintain the culture flask(s) in these conditions for 12-15 days or until reproductive structures can be observed. These will either be the developing unfertilised eggs still attached to the female gametophyte (Figure 5.1b), or the fertilised egg/developing sporophyte (Figure 5.1c). The reproductive state of the culture is assessed by following egg development, as it is much more difficult to observe the smaller male reproductive structures.

3. Once a large number of reproductive structures are observed, the culture is prepared for spraying onto culture string.

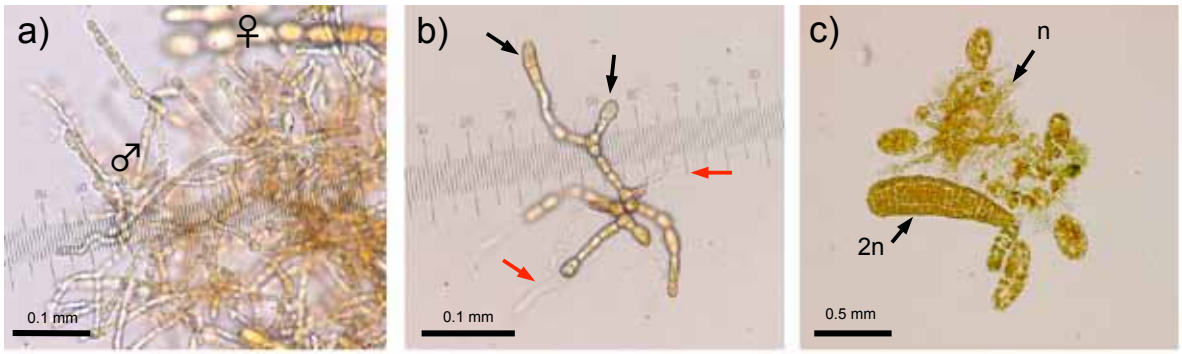


Figure 5.1 (a-c).

Stages of gamete and sporophyte development. Male (foreground, ♂) and female (background, ♀) gametophytes showing differences in cell size (a). Reproductive female gametophyte with developing oogonia and eggs (black arrows), and previously released eggs (red arrows) (b). Clump of gametophytes (n) with developing juvenile sporophytes (e.g. 2n) (c).

5.3 Spraying sporophyte cultures onto collectors

5.3.1 Preparation of the culture string collectors, and culture tanks

4. Prepare the culture string collectors by cutting 65-mm square drainpipe into 30-cm lengths. Use the hole cutter bit on the electric drill/ pillar drill to cut 4- or 5- cm holes out of the drainpipe in as many areas as possible without reducing the structural integrity of the collector (Figure 5.2).

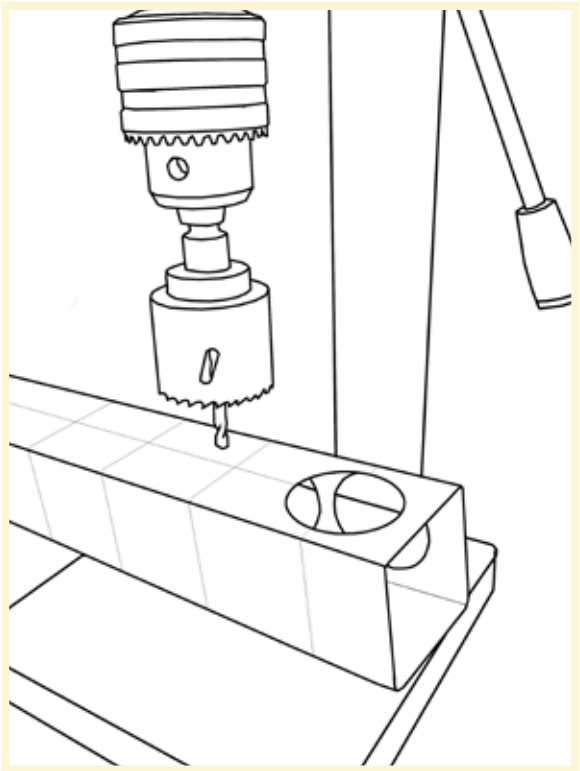


Figure 5.2.
Making a collector from a square drainpipe using a pillar drill and hole-cutter.

Help Box: *Collectors are placed vertically in the tanks, and so they can be longer if the tank is deeper. Cutting holes in the collector will improve water exchange with the string from both sides of the collector and reduces the possibility of culture decomposition.*

5. Contaminating chemicals may be present in the culture string as a result of production, transport or storage, and these may be detrimental to the culture development. To reduce these, steep the hanks of culture string in a bucket of warm or hot fresh water overnight. Do not use boiling water as this may denature the shape/characteristics of the string. Refresh the hot water in the bucket each day for 4-5 days, before hanging the string up to dry thoroughly before use. If this process is done well in advance of the culture spraying, store in a clean dry place, protected from dust etc. until the string is required.

6. Tie one end of the cleaned culture string to the collector and wrap the string around the outside of the collector in a neat and even single layer all the way to the other end of the collector, then cut the string and tie off.

Figure 5.3
Wrapped collector with hairs being burnt off by blow-torch.

Using the blow-torch, lightly singe off any loose fibres from the string without actually burning or discolouring the string itself (Figure 5.3). Sporophytes that would settle on these loose fibres would have a weaker attachment to the string, and would be more likely to fall off.

Help Box: *It is advisable to leave an extra length of string (10-20 cm) after the knot at the end of collector, which can be snipped off at regular intervals to inspect the development of the sporophytes (Figure 5.3).*

7. Once sprayed, collectors need to go immediately into culture tanks, so the tanks should also be set up prior to use. The collectors will be suspended vertically in the tanks. Fill the tanks with sufficient filtered and UV-sterilised seawater to cover the collector, and add the appropriate amount of nutrients for the volume of water used.

Position a light source over the tank which can be lowered or raised depending on the amount of light the cultures require (see Table 5.1 for light intensities).

Ensure that the tank has an adjustable source of aeration (filtered if possible), which is capable of providing a strong airflow at later stages in the cultivation process.

8. Remove the culture flasks from the blue light conditions, and take out the glass tubing and cotton wool, but retain the parafilm as a covering over the mouth of the flask.

Allow the culture to settle to the bottom as when changing the media. Once this has occurred, slowly pour off the excess seawater media and retain a concentrated suspension of cultured gametophytes.

9. Pour the culture into a blender flask or into a beaker for blending with a handheld blender, (either way works well; Figure 5.4). Carefully blend for 45-60 seconds. This ensures that the culture material can be evenly sprayed across the culture string and does not affect the developing sporophytes.

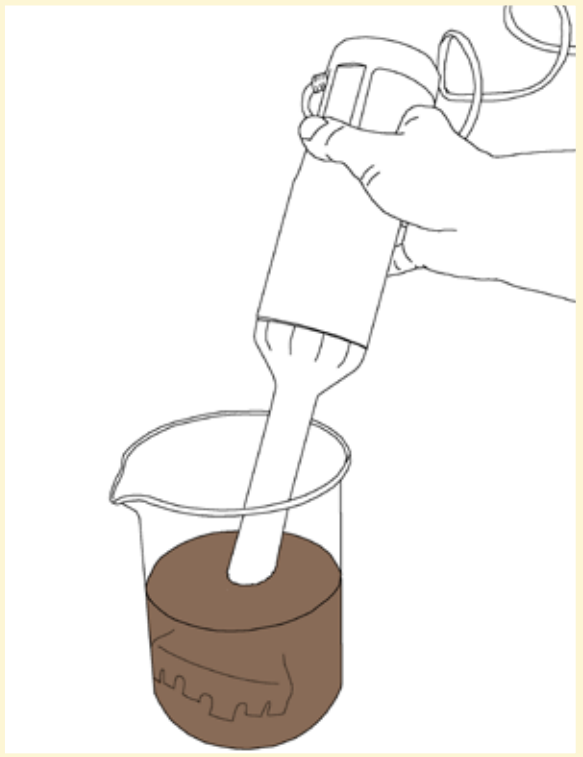


Figure 5.4.
Blending the sporophyte culture

10. Set up the collectors in a clean plastic box in preparation for spraying. Pour the blended culture into the plant spray bottle (Figure 5.5). Adjust the nozzle of the bottle to ensure that the culture is delivered as gently as possible (i.e. misted). Spray each side of the collector(s) evenly until they are well covered. If the spray action is too forceful, the culture will be washed off the collector.

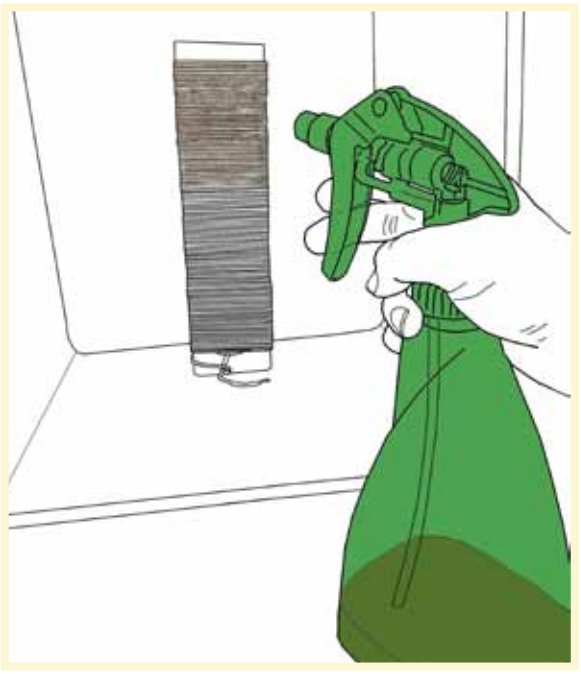


Figure 5.5.
Spraying collectors in the clean plastic box.

11. Once the culture string has been sprayed gently several times, allow the collector to be exposed to 10 °C air for 10-15 minutes. This allows better attachment of the sporophytes to the string.

5.4 Nursery culture of juvenile sporophytes

12. Attach the sprayed collectors to the long poles so that they hang freely and will not touch each other in the tank (Figure 5.6). Slowly lower the pole with the collectors into the tank to ensure that as few gametophytes are washed off as possible at this stage.

Courtesy of Freddie O'Mahony

Figure 5.6.
Collectors suspended in tanks.

Leave the collectors in the tank with *no aeration* for the first 2-3 days. This allows the sporophytes to develop their attachment onto the string. After this initial period, aeration is added in increasing amounts.

The light regime remains at 12:12 hours, light:dark over the duration of the nursery period. However, the light intensity is increased steadily over the period to improve the development of sporelings. See Table 5.1 for the progression of the amount of air and light intensity that is required over 30-45 days.

Help Box: *Increasing the strength of the aeration allows sporophytes to have a better attachment to the string by developing stronger holdfasts. Survival at sea is improved by using this technique.*

13. Clean tanks and change the medium every 3-4 days to ensure the healthiest development of the sporophytes. During the cleaning process, carefully remove the collectors and place them in a different tank with UV-filtered seawater. Return them to the original tank after the cleaning and refilling has occurred.

Cultures must be allowed to develop in the laboratory (Figure 5.7) for at least a month before deployment at sea, but can be held in the laboratory for up to 2 months if weather conditions are not suitable for deployment.

Figure 5.7.
Development of juvenile sporophytes on culture string, approximately 3 weeks after spraying.

Table 5.1.
Air and light over culture period for sporophytes in tanks.

Day Number	Light Intensity at surface of tank (µmol m^{-2} s^{-1})*	Aeration
0-3	35-40	None
3-14	60-70	Low, increasing to moderate
14+	60-70	Moderate increasing towards high

*Double bulb fluorescent light suggested for use.

Chapter 6 Deployment of *Laminaria digitata* at sea

6.1 Transfer of sporophyte culture from laboratory to sea site

Choosing a suitable sea site at which to grow *L. digitata* has already been discussed in Chapter 3.5.1 and longline design and maintenance has been discussed in Chapter 3.5.2. It is advisable to have the anchors and anchor ropes in position well in advance of the deployment of culture string. Once a deployment is scheduled, the header rope should be connected to the anchor ropes (e.g. 1-7 days in advance). Once the deployment of the seeded culture string has been made, the buoys should be attached, spaced at suitable distances (e.g. every 5-8 m). The buoys will therefore maintain the position of the header rope in the water column as the seaweed grows and becomes heavier.

A suitable vessel is also required to access the longline(s) for deployment of culture string, for maintenance and for harvest. Choice of boat can be important for comfort and safety during both of these activities. For example, too large a vessel will impede access to the lines, as the operator will not be able to reach comfortably over the side to deploy or clean the header rope. At harvest, if the boat is too small, many more trips will have to be made to the lines to remove the biomass. There could even be a risk of the boat being swamped if too much kelp biomass is loaded on board. Two people working together make the boat work most efficient and safe.

Transfer of cultures to sea occurs after a laboratory period of 1-2 months. Deployment of *L. digitata* around Ireland occurs between October and December (see cultivation Gantt chart, Table 6.1). Deployment will require a calm sea, as the cultures should be handled as little as possible. The change in culture conditions will be stressful, so anything that can reduce this stress should be taken into account. For example, light intensity will be much higher outdoors than in the laboratory. In this case, deployment on an overcast day is likely to be less stressful than on a bright day with clear skies.

Transportation conditions on the boat are also important for any seaweed deployments, especially if takes more than 15 minutes to arrive at the site. Collectors are usually removed from the culture tanks, and transported in plastic boxes or cool boxes in damp, but not wet conditions. Collectors can be loosely wrapped in a wet cloth to ensure cultures don't dry out or move around too much in the boxes. If the deployment day is unseasonably hot for the time of year, an ice block near, but not touching the cultures could be added to the box.

Help Box: *A boat box containing useful items for deployment and running repairs or emergencies should be brought on every trip. Some suggestions for contents include: twine or string, lengths of 8- or 10-mm polypropylene rope for replacing worn buoy ropes, long cable ties, knife/ scissors, marlin spike, sample bags, first aid kit, bottle of water, mobile phone.*

Table 6.1.
Cultivation of *L. digitata* Gantt chart

Availability of fertile material				■	■	■	■	■	■	■	■	
Initiation of gametophyte cultures					■	■	■					
Gametophyte culture development (increase in gametophyte biomass)						■	■	■	■	■	■	
Initiation of sporophyte cultures								■	■	■		
Spraying of culture collectors and tank cultivation								■	■	■		
Deployment of culture at sea										■	■	
Maintenance of longline structure	■	■	■	■	■	■				■	■	■
Harvest of *L. digitata*			■	■	■							
Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**

6.2 Deployment of *L. digitata* cultures at sea

1. On arrival at the longline, tie the boat to the header rope at one end in such a way that the boat can be quickly detached when needed.

2. Detach the header rope from the anchor rope, making sure that the anchor rope has a buoy attached to the end, or that it can be secured to the boat temporarily to avoid loss.

3. Remove damp cloths carefully from the first collector, and insert the header rope through the middle of the collector.

4. Untie the end of the culture string from the end of the collector closest to the detached end of the header rope, and secure through the lay of the header rope, using the marlinspike if necessary. Try to hold the collector at either end, rather than touching the small, delicate plants on the culture string (Figure 6.1).

5. Re-attach the anchor rope to the header rope, ensuring a strong connection. Untie the boat from the line, and slowly pull the boat down the length of the header rope hand over hand. The collector should also be pulled down the length of the header rope, the culture string spiralling around the larger diameter header rope.

Figure 6.1.
Collector inserted onto header rope (photograph courtesy of Freddie O'Mahony).

Figure 6.2 (a-b).
a) Deployment of culture string along header rope. **b,** above) Developing *L. digitata* culture string, wound around header rope (photograph courtesy of Freddie O'Mahony).

6. Make sure that the string is not coiled too loosely around the rope, as the small plants develop on the string first, followed by further growth on the rope itself (Figure 6.2 a-b).

Upon reaching the other end of the longline, or the end of the culture string supply (whichever comes first), tie the end of the culture string through the lay of the rope once again, as at the start.

7. If possible, detach the anchor rope and header line and remove the collector. If this is not possible, leave the collector tied to the very end of the line for retrieval after the harvest. Before leaving the site, ensure that the header rope is submerged to a minimum of 0.5 m below the surface.

8. Attach the buoys to the header rope, spacing them evenly down the length of the line. See chapter 3.5.2 for further details.

6.3 Growth at sea and harvesting

9. Once each cohort of seaweed is deployed, visit the cultivation site once per month for maintenance and monitoring of growth. Take representative monthly samples to follow the growth and development of the crop by removing all *L. digitata* plants from 15 cm of header rope in at least 3 randomly chosen areas of header rope. Per 15-cm sample, weigh the total wet biomass, and measure the length of the 10 longest blades present. A monthly set of samples over the growing period will allow comparisons of the cultivation progress to be made, year on year.

10. It is expected that the maximum yield per site will occur after 5-6 months growth. Harvest will occur around April-May, before sea water temperatures rise significantly, and epiphytes reduce the quality of the product.

11. For large sea sites with many longlines, harvesting will require a vessel that is easy to work from, and can store the biomass until it is delivered to shore. As the seaweed longlines are very similar to mussel longlines, mussel barges/ boats will also be quite appropriate for efficiently harvesting seaweed. The header rope of the longline can be lifted out of the water, and held at waist-height for processing the seaweed (Figure 6.3). Operators work their way down the longline, cutting away the weed, before sending it to a large storage bag via a conveyor belt.

12. Once all seaweed from a line has been removed, the header rope can also be taken in. The harvested weed can then be transported by road for further processing at another facility.

Figure 6.3.
Header rope of a seaweed longline lifted onto a mussel barge platform for processing. Photograph courtesy of Dave Millard (BIM).

Chapter 7
Results from *L. digitata* trials, 2009-2011

7.1 Experiences of developing hatchery cultures, substrate types and deployment strategies

Gametophyte cultures of *Laminaria digitata* were established in the hatchery at Gearhies and Carna within a few months of the start of the project in 2008. Cultures were established within the year at Portaferry. In general, few difficulties were experienced during the set up of these cultures. Once established, the cultures continued to increase in biomass if the optimal, vegetative conditions remained constant. Attention to detail, and cleanliness of the reproductive sori prior to release became paramount to establishing a good culture. The very few *L. digitata* cultures that had to be discarded were due to contamination with diatoms, ectocarpoid species, and high numbers of ciliate species (protozoans that graze on algal spores and detritus). While it is impossible to obtain an axenic culture of *L. digitata* using the methods described in this manual, with enough care and attention, it is possible to culture a large biomass of *L. digitata* gametophytes quickly and easily with no detrimental effect to the final yield or product.

Methods for chemically and physically cleaning the sori were investigated, including exposing sori to low concentrations of sodium hypochlorite, iodine solution (e.g. the commercially available disinfectant Betadine), distilled water and scraping the sori with a scalpel blade. None of these treatments markedly improved the cleanliness of the culture, and in some cases, adversely affected spore release. Consequently, these treatments were not included in Chapter 4.

Some small trials were also carried out to test the efficacy of direct zoospore release onto culture string and onto polypropylene rope. While this method was generally considered successful, patchy development of gametophytes was obvious, implying that there is a greater control of gametophytes if they are sprayed onto the ropes after being cultured in glassware.

The largest collection of longlines was sited in Roaring Water Bay, and was seeded as described in Chapter 6 (i.e. horizontal deployment of seeded string along the header rope). Longlines at New Quay and Ventry Harbour were also deployed in this way. Seeded material was deployed differently at smaller longlines in Ard Bay and Strangford Lough to economise on space. At these sites, 3-m rope droppers with 0.9-kg concrete weights were attached vertically to the header rope. Culture strings were simply wound around the droppers instead of the header rope (Figure 7.1).

Figure 7.1.
Rope dropper (3-m) with developing *Laminaria digitata* that hangs vertically from the header rope. Seeded string has been wound around the rope. A 0.9-kg concrete weight helps to keep the rope vertical in the water column.

In addition to the 220-m longline in Ventry Harbour, a 30- x 30-m grid, made up of 9 smaller 10-m^2 squares was also deployed (for details, see Figure 8.2). This grid could hold up to 450-m of seeded string. Two different species of kelp were deployed (*L. digitata* and *S. latissima*) on two separate substrates (either kuralon or polypropylene). Directly seeded/ sprayed rope was used instead of sprayed kuralon string (as used on all of the other longlines.

Results from the experiments conducted at these sea sites between 2009 and 2011 described above are presented in Section 7.2.

7.2 Growth trial data

All experiments described in the following sub-sections were derived from cultures of *L. digitata* cultivated and sprayed as described in Chapters 3-5 of this manual. Deployment and sampling techniques differed, and are described separately for each experiment.

7.2.1 Growth trials in Roaringwater Bay and New Quay, 2009 – horizontal deployment of *L. digitata*

Deployment and sampling technique

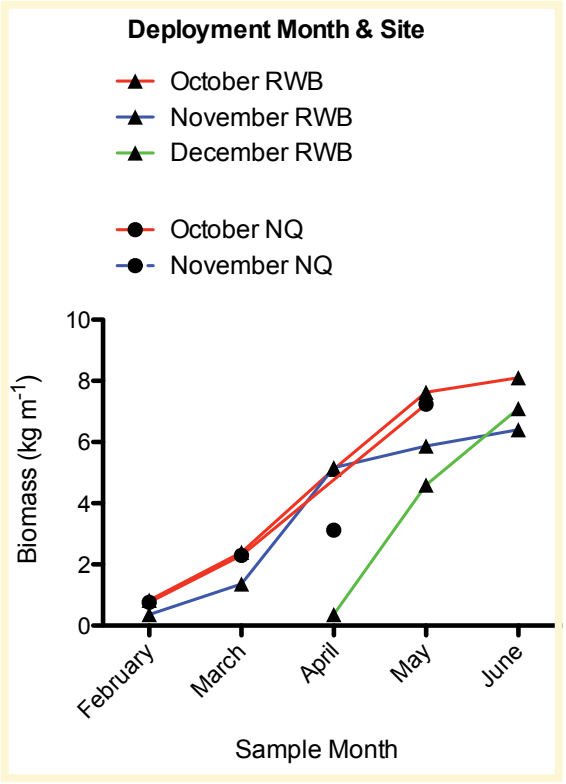
Cultures developed at the Daithi O' Murchu Marine Research Station (Co. Cork) were used at both Roaringwater Bay (RWB) and New Quay (NQ) sites. One 30-cm sample of biomass was taken at monthly intervals at both sites to measure the yield of horizontally deployed culture string (kg m^{-1}).

Results

Estimates of yield were similar in RWB and NQ, and showed a steady increase over the late winter to early summer (Figure 7.2a). Biomass appears to reach a maximum yield (kg m^{-1}) between May and June. The October deployment yielded 7.6 to 8.1 kg m^{-1} during this period (7-8 months at sea).

Frond length also increased significantly over 8 months at sea at both sites (Figure 7.2b). Average frond length measured in the October deployment was 29.5 cm after 4 months at sea (February), and reached 92.6 cm after 8 months at sea (June). Plants deployed in December were significantly shorter than those plants deployed in October.

a)



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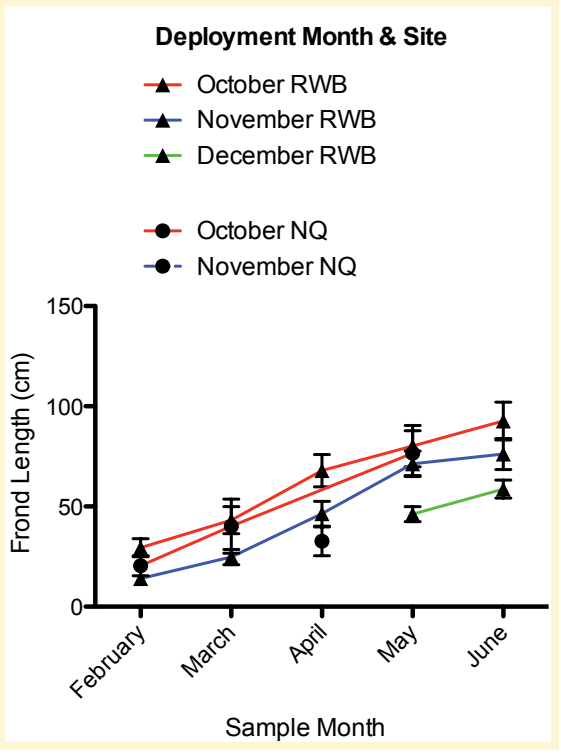


Figure 7.2 a) and b).
Cultivation of *L. digitata* in Roaringwater Bay (RWB; triangles), Co. Cork and New Quay (NQ; circles), Co. Clare. Deployments made in October (red lines), November (blue lines) and December 2008 (green lines), and sampled between February and June 2009. **a)** Yield of biomass per metre of header rope for the multiple deployments at both sites. **b)** Maximum frond lengths achieved at each sample period for all deployments. Error bars represent 95% confidence limits.

7.2.2 Growth trials in Strangford Lough and Ard Bay, 2009 – vertical deployment of *L. digitata*

Deployment and sampling technique

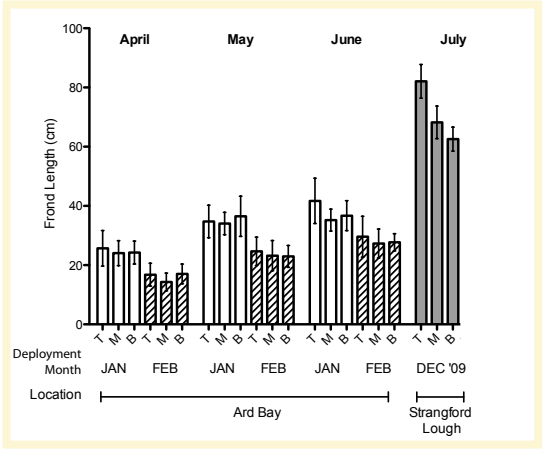
Laminaria digitata was grown on droppers in Ard Bay (AB), Co. Galway and Strangford Lough (SL), Co. Down during 2009. Seeded culture string was wrapped around 3-m polypropylene droppers and weighted by a 0.9-kg concrete weight. In AB, three replicate droppers were deployed in January and a further three in February. Four replicate droppers were deployed in SL in December. Monthly samples were taken in AB, measuring the 10 largest plants in the top, middle and bottom sections of the droppers. A sample of the biomass yield (10-cm sample) was taken from each dropper in June 2009. In SL, the ten largest plants were measured from the top, middle and bottom of droppers at the final harvest (July 2010).

Results

There were no differences in frond length on the top, middle or bottom sections of 3-m droppers deployed in Ard Bay (Figure 7.3a). In Strangford Lough, plants were significantly longer in the first metre of the dropper, compared with the lower portions (Figure 7.3a). These results indicate, that while using seeded droppers can increase the amount of *L. digitata* grown per metre of longline header rope, the use of droppers may not be appropriate in all sites. The January deployment of droppers in Ard Bay was harvested after 5 months at sea, with plants reaching maximum frond length of 37-42 cm (Figure 7.3a). The December deployment of droppers in Strangford Lough was harvested after 7 months at sea, with frond lengths of 63-82 cm. As shown in other growth trials in this manual, it appears that deployments of *L. digitata* made as early as possible (up to December) yield larger plants and therefore greater biomass.

Biomass per metre of dropper was low in most samples from Strangford Lough and Ard Bay in 2009 (0.6-1.3 kg m^{-1}; Figure 7.3b). The best biomass came from the December 2009 deployment in Strangford Lough (5.1 kg m^{-1}).

a)



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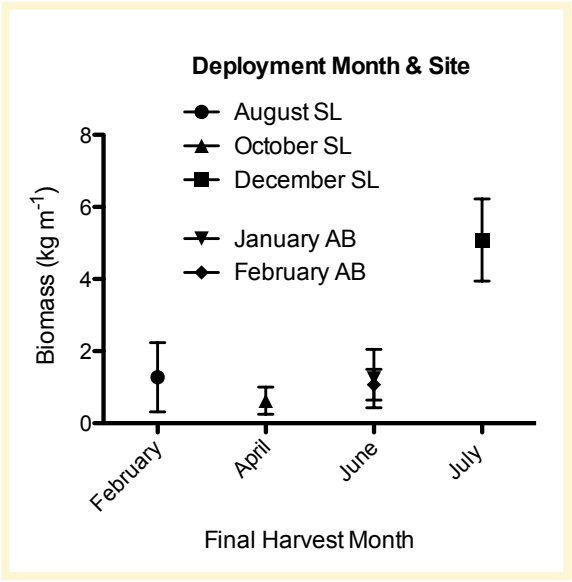


Figure 7.3 a and b).
Cultivation of *L. digitata* in Ard Bay (AB), Co. Galway and Strangford Lough (SL), Co. Down 2009/2010. **a)** Averaged frond lengths of largest plants measured in Top (T), Middle (M) and Bottom (B) 1 m sections of 3-m droppers. AB deployments made in January (white columns) and February (striped columns) 2009, and sampled between April and June 2010. SL deployment (grey columns) made in December 2009 and harvested July 2010. **b)** Biomass per metre of dropper at final harvest for SL (August, October and December), and AB (January and February). Error bars in both graphs represent 95% confidence limits.

7.2.3 Vertical and/or horizontal deployment of L. digitata in Roaringwater Bay, New Quay, Ard Bay, Ventry Harbour and Strangford Lough (2010/2011)

Deployment and sampling technique

Multiple deployments of seeded culture string were made during 2010, and these were sampled during 2011. Seeded string was deployed horizontally across the header rope in Roaringwater Bay, New Quay and Ventry Harbour (VH), while vertical droppers (3-m lengths, 5 replicates per deployment) were used in Ard Bay and Strangford Lough. Random sampling of three 15-cm sections occurred at the RWB, NQ and VH longlines, whereas one 15-cm sample was randomly selected from each replicate dropper in AB and SL.

Additional measurements of dry weight and epiphyte load were measured using plants in AB. Epiphyte load was assessed by measuring the length of L. digitata frond covered with epiphytes from the tip towards the stipe. This may be used in the future to assess the quality of the harvest.

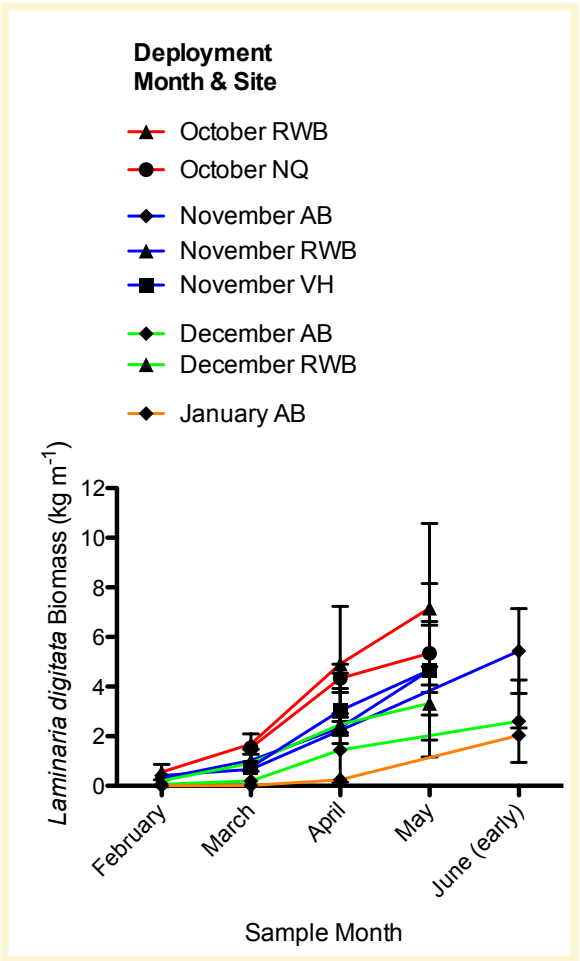
Results

Measurements were made in all sites up until May/ June. It was not possible to collect data from all sites in May due to persistent bad weather. Therefore, May results are presented for RWB, NQ and VH while early June results are presented for AB only.

Biomass of *L. digitata* increased in all deployments at each site between February and May and/ or June. The smallest increase in biomass occurred in the AB January deployment (Figure 7.4a). Yields from different sites appear to be similar (especially November deployments), but the variation in biomass is large, which may mask any significant differences. Overall, larger yields of *L. digitata* were obtained from earlier deployments made in October and November, but again, variation in yield is large.

Relative growth rates (RGR) of deployments (measuring frond length) were variable after 2-4 months at sea and were in the range of 0.02-0.04 cm cm^{-1} day^{-1} (equivalent to a 2-4% increase in frond length per day; Figure 7.4b). Relative growth rates (RGR) decreased over time in most deployments particularly between April and May/ June. This is most likely due to increased degradation of the frond tips, which occurs more frequently in early summer (Figure 7.4b). It is likely that the meristematic tissue was still actively producing new tissue, but the tips degraded at the same rate or slightly faster than this production, which results in a slowing of the RGR.

a)



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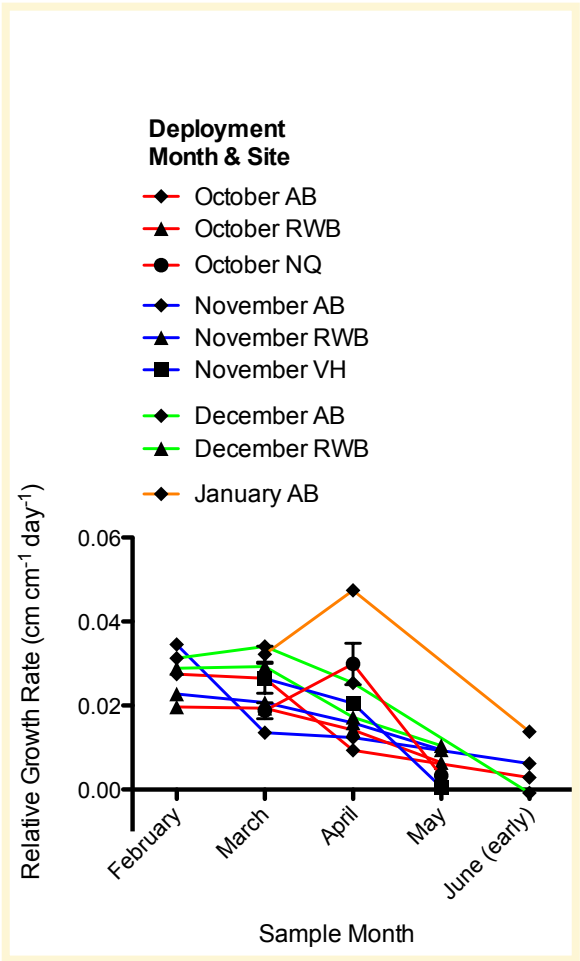


Figure 7.4 a) and b).

Cultivation of L. digitata at multiple sites during 2010/2011, including Ard Bay (AB), New Quay (NQ) Roaringwater Bay (RWB) and Ventry Harbour (VH). Horizontal longlines deployed in NQ (October only) RWB (October-December) and VH (November only). Vertical droppers deployed in AB (October-January). October = red lines, November = blue lines, December = green lines and January = orange lines. a) Yield of biomass per metre of header rope or dropper as appropriate for the multiple deployments at both sites. b) Average relative growth rates (for length) of each deployment. Error bars in **a**) and **b**) represent 95% confidence limits.

Frond lengths increased significantly in length in all deployments at each site between February and April (Figure 7.5), however plants grown in October and November deployments were significantly longer than those in the December and January deployments (Figure 7.5). Frond lengths did not increase greatly in length between April and May/ June for most deployments and also became increasingly variable. Maximum frond lengths were achieved from plants in October deployments and were approximately 90-100 cm by May/ June.

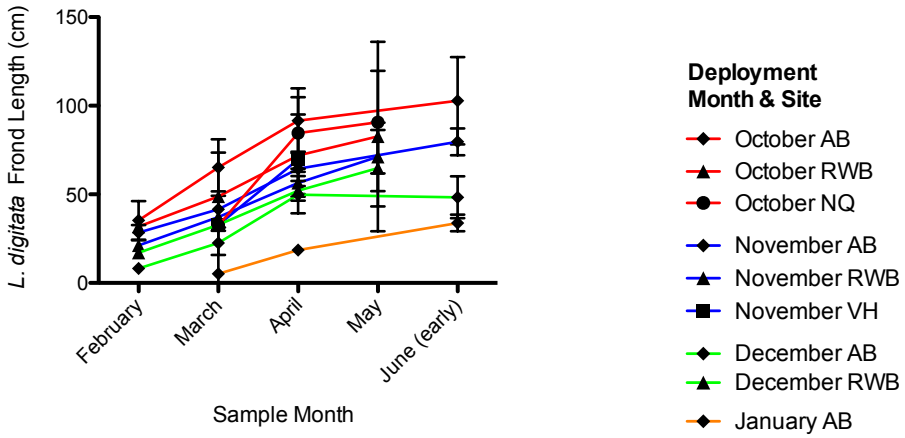


Figure 7.5.

Cultivation of *L. digitata* in Ard Bay (AB), New Quay (NQ), Roaringwater Bay (RWB) and Ventry Harbour (VH) during 2010/2011. Average maximum frond lengths achieved at each sample period for all deployments. Error bars represent 95% confidence limits.

Figure 7.6.

L. digitata deployed horizontally on the Ventry Harbour longline. A 15-cm section has been taken for analysis. Plants are 5 months old (deployed November 2009, sampled in April 2010).

Epiphyte cover (%) on fronds in Ard Bay in October, November and December deployments sampled between February and April showed little or no increase over this period (Figure 7.7). Epiphyte cover did not exceed 20% during this time. While fouling was easily measured during February to April, it was not heavy, and mainly consisted of diatoms and filamentous red algal species developing on the very tip of the frond. No encrusting or settling animals such as bryozoans or hydroids were present. By early June, a significant increase in the amount of epiphytes on fronds of October and November deployments were recorded (~ 60-70%). The amount of epiphytes also increased on plants in the later deployments of December and January, but plants were significantly cleaner with ~22-28% of the fronds covered in epiphytes. Fouling on all plants by early June was much heavier than during February to April. Bryozoans and hydroids were very common and accounted for most of the fouling during this period.

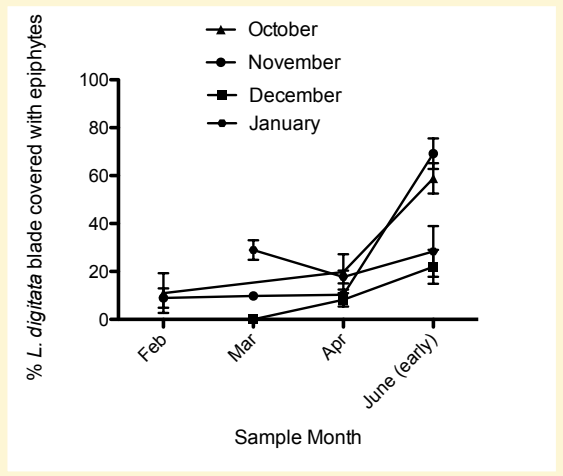


Figure 7.7.

Epiphyte coverage of L. digitata fronds (%) in Ard Bay, for separate deployments made in October, November and December 2010. Error bars represent 95% confidence limits.

Reproductive plants were recorded at Ard Bay for the first time. Two of the longest plants from the October deployment were reproductive in April. The frond lengths measured 126 cm and 120 cm, respectively. Tissue containing the sori were taken back to the laboratory to release spores, however, the effort was unsuccessful.

7.2.4 Ventry Harbour seaweed grid, 2011 – horizontal deployment of directly sprayed *L. digitata* and *S. latissima*

Deployment and sampling technique

As described in Section 7.1 and Figure 8.2, rope (8-mm diameter) was used instead of culture string as the method of seeding an ongrowing structure at sea (i.e. the seaweed grid instead of linear longlines). 11-m (10 m plus an extra 1 m for attachment to the grid) lengths of kuralon and polypropylene ropes were wound around large collectors (length approx. 90-cm), Figure 7.8. In total, 450-m of rope were sprayed with L. digitata (250-m), and S. latissima (200-m). The collectors were then transported to the site (approx. 4.5 hours) wrapped in layers of damp tissue and kept moist during the journey. They were deployed in Ventry Harbour in January, 41 days after spraying. Deployment of 450-m seeded string took 1.5 hours. Of the five squares containing L. digitata on ropes, three were randomly chosen. One rope in each square was randomly selected and taken back to shore for measuring. The 10 largest plants were measured in each of three 15-cm samples taken from each rope. Total biomass per rope was also measured.

Figure 7.8.

Large collector with kuralon and polypropylene rope being sprayed in the hatchery.

Results

On a brief inspection of the grid in March, no plants were visible. One month later in April, growth of *L. digitata* was confirmed. During deployment, it became apparent that the polypropylene rope was very buoyant, and was therefore deemed unsuitable for horizontal deployment. Plantlets of both species were exposed to high levels of light at the surface of the water, and did not develop further (Figure 7.9). The kuralon rope was negatively buoyant and maintained position approx. 1 to 1.3 m below the surface of the water. Kuralon ropes seeded with *S. latissima* did not develop, despite visual observations suggesting the plantlets were more developed than those on the *L. digitata* droppers. It is possible that the *S. latissima* plantlets (sprayed, transported and deployed exactly like the *L. digitata*) were more sensitive to the transport conditions than the *L. digitata* and did not survive the trip.

Figure 7.9.

Comparison of deployed polypropylene (top rope) and kuralon ropes (bottom rope) seeded with *L. digitata* after 3 months (seaweed grid, Ventry Harbour).

Although *L. digitata* settled and developed well on the kuralon ropes, growth of the was disappointing overall, and yield was no more than 1 kg m^{-1}. Largest frond length was approximately 16 cm, and most plants were heavily covered in epiphytes (e.g. hydroids and bryozoans). As a concept, the seaweed grid works well. It was relatively straightforward to deploy and work on, and can potentially hold much more seaweed per square metre than linear longlines. However, results from this initial prototype confirm that *L. digitata* should be deployed before Christmas for good development of seaweed biomass.

7.2.5 Growth trials of *S. latissima* in Strangford Lough (2009/2010), and Ard Bay, 2010/2011.

Deployment and sampling technique

In the hatchery, *Saccharina latissima* was cultured in the same way as *L. digitata* (as described in this manual). Gametophytes were also sprayed on culture string and deployed on 3-m droppers in Strangford Lough and Ard Bay as described in Section 7.2.2. SL deployments were made in March, 2010 and harvested in July 2010 (5 replicate droppers). Ard Bay deployments were made in October and January 2010 (5 replicate droppers each), and harvested from February 2011 onwards. One 15-cm section per replicate was taken. One AB dropper was destructively harvested in April to measure the biomass on the whole 3-m dropper, while a further two droppers were destructively sampled in early June.

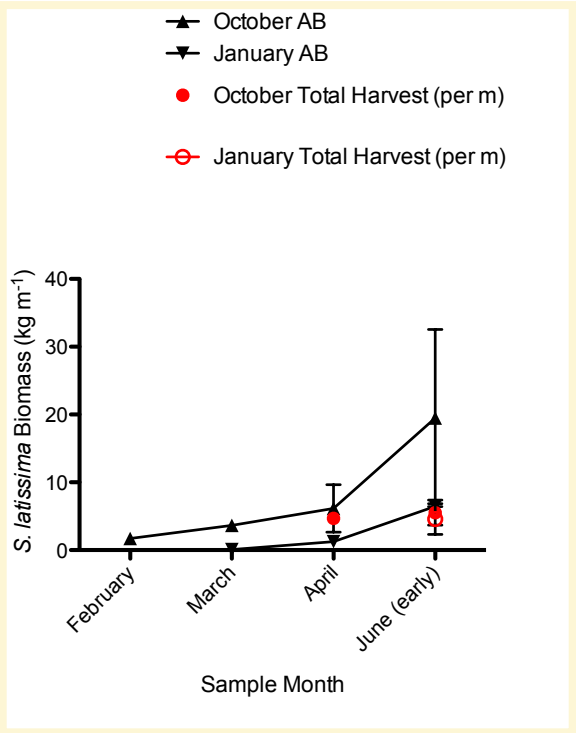
Results

The first deployment of *S. latissima* using the *L. digitata* cultivation method was successful in SL. The final averaged biomass in July resulted in 6.04 kg m^{-1} (± 1 kg) rope dropper. Average frond lengths of the largest plants from top, middle and bottom sections of the droppers were in the range of 74-80 cm.

The biomass yield of *S. latissima* in AB rose steadily between February and April, with a maximum biomass of 6.1 kg m^{-1} achieved in April (Figure 7.10a). Variability of the biomass sampled from droppers in June was extremely large, averaging 19 kg m^{-1} (± 13 kg). The average biomass extrapolated yield from a 15-cm sample must therefore be treated with caution. As a comparison, the single dropper harvested in April gave a biomass yield of 4.7 kg m^{-1}, while the two droppers harvested in June give an average total biomass of 5.5 kg m^{-1} (± 1.9 kg), which is a much more conservative figure (Figure 7.10a).

Relative growth rates of plants in both deployments in AB decreased steadily over the same period (Figure 7.10b). There was much greater variability in the RGR of frond length for the January deployment than for the October deployment. Average frond length in the October deployment increased between February and March, but has not increased significantly between March and April (Figure 7.10c). Frond length increased significantly again between April and June for the January deployment. Fronds from the October deployment grew longer, but ever more variable. An indication of the size of plants on an October dropper in early June is shown in Figure 7.11.

a)



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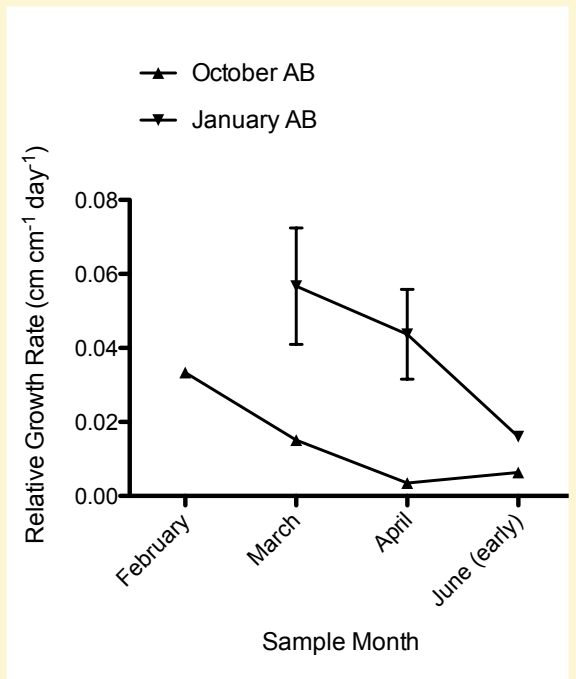

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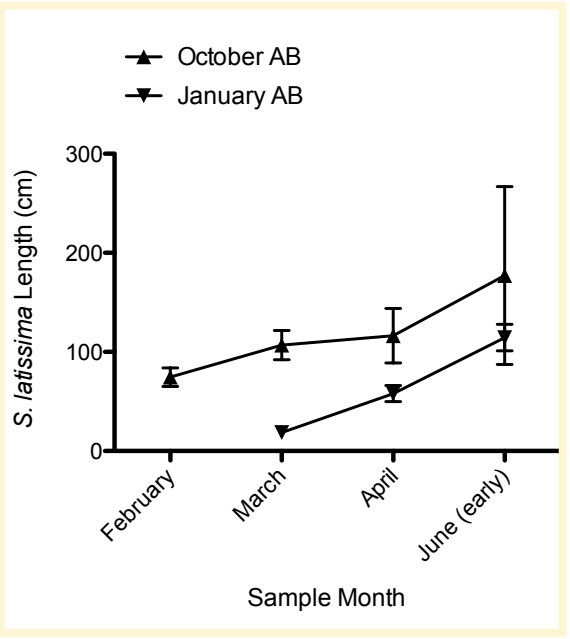


Figure 7.10 a-c).

Cultivation of *S. latissima* at Ard Bay (AB) during 2010/2011, Vertical droppers deployed in AB (October and January). **a**) Yield of biomass per metre of dropper. **b**) Average relative growth rates (for length) of each deployment. **c**) Average maximum frond lengths achieved at each sample period for all deployments. Error bars in **a**), **b**) and **c**) represent 95% confidence limits.

Figure 7.11.

Harvested dropper of *S. lattisima*. Length, 3-m. Deployed October 2010, harvested April 2011.

7.3 Conclusions and future improvements

Deployments of *Laminaria digitata* collectors have been made in the same sites over several years. The seeded string has been deployed over a range of months, mainly from October to February at different sites around Ireland. Three different deployment methods have been trialled at sea, e.g. horizontal and vertical deployment, either on longlines or a seaweed grid. Several different seeding substrates have been used, i.e. culture string or rope collectors.

From all of these results in trials over several years and sites, maximum yields of *L. digitata* were approximately 7-8 kg m^{-1} after 7-8 months at sea. From the biomass results, and frond length development, it is clear that deployments made before December are most productive in most sites.

Variations in deployment, such as using droppers, were successful in some sites (e.g. Ard Bay and Strangford Lough). Use of droppers on linear longlines increases the productivity or yield per metre of header rope three-fold, assuming growth of plants is equal from top to bottom on 3-m droppers. In sites with high or variable turbidity such as Roaringwater Bay, the use of horizontal longlines may be more appropriate than dropper deployment. Consequently, the characteristics of each site must be considered. These include understanding patterns in current and wave exposure, temperature and salinity fluctuations, turbidity etc.

The continuous grow-out unit, or seaweed grid placed in Ventry Harbour, in January 2011, was a successful demonstration of the structure itself with respect to deployment, access and durability. The structure supported 450-m seeded culture rope, suspended in single 10-m lengths across each square. The grid could have held even more culture rope, if the rope deployment 'zig-zagged' across each square on a continuous culture rope (see Figure 8.2). Plants of *L. digitata* sprayed onto kuralon culture rope developed well, demonstrating the potential of significantly increasing the amount of seaweed grown per hectare. Unfortunately, due to project constraints, the deployment of grid and seeded rope was not made until January, therefore the full potential and productivity of the crop could not be realised as per the other sea trials.

The seaweed grid represented a new approach to seaweed deployment within the project, where the largest amount of seeded material was deployed in the shortest time to date. The practical advantage of spraying *L. digitata* directly onto ropes in the hatchery appears to be the time saved at sea during deployment. However, as the collectors are much larger than the string collectors, they take up more space in the laboratory.

The results obtained through the Seaweed Hatchery Project, indicate that the hatchery and ongrowing methods for *Laminaria digitata* are reliable and productive. However, further research is necessary to improve various aspects of the whole process, leading to increased yield at sea. These may include:

- Gametophyte density on collectors – spraying a culture that is too dilute results in poor coverage, whereas spraying too concentrated a culture results in too many plants per area of string and too much competition for space, light and nutrients.
- Plant size – is the developmental success of the *L. digitata* culture at sea related to the size of plants initially deployed?
- Culture rope vs. culture string – investigate advantages and disadvantages of both, test new (cheaper and more readily available?) types of rope/string.
- Deployment time – how early can cultures be deployed? Is it possible to deploy in September, or will water temperature or irradiance be too high?
- Increased productivity per hectare – more work is required to establish how much seeded rope the seaweed grid used in growth trials (Section 7.2.4) can support.
- Environmental monitoring – try to link growth and development of seaweeds with the prevailing culture conditions at sea for a greater understanding of the effects of water temperature, current and wave exposure, turbidity, nutrient availability etc.
- Harvesting – investigate and/or develop new methods of taking large amounts of biomass from longlines/grids for processing.

Chapter 8 Economic Analysis

8.1 Introduction

This chapter presents an economic analysis of the production of *Laminaria digitata*, based on work carried out within Project PBA/SW/07/001 (01), including the growth results described in Chapter 7. Further information on seaweed marketing and economics can be found in Walsh and Watson (2011) and Watson and Dring (2011), respectively.

8.2 Current demand for cultivated macroalgae in Ireland

Ireland's seaweed and biotechnology sector industry is currently worth €18 million (Morrissey *et al, 2011*), and processes 36000 tonnes of wild seaweed annually. The main market for this wild-harvested crop is in high-volume, relatively low-value agricultural products such as fertiliser and animal feeds. Expansion and development of the industry is required to attain an estimated worth of €30 million by 2020 (Sea Change Report, 2007). Developing seaweed aquaculture has been recommended as one way of delivering a sustainable supply of higher-value, high-quality algal species to the industry. Two seaweeds in particular, have been identified for their commercial potential, namely, *Laminaria digitata* and *Palmaria palmata*.

In addition to the current production of fertilisers and animal feeds from algae, algal product is also required to feed farmed abalone (*Haliotis discus hannai* and *Haliotis tuberculata*) and sea urchin (*Paracentrotus lividus*). Within these valuable industries, macroalgae are used as an important component in the diet of the abalone and urchins. Macroalgae are used either as a sole foodstuff, or in addition to a formulated diet.

At full production capacity, the estimated wet weight of *Laminaria digitata* required for the Irish abalone industry growing *H. discus hannai* is between 645 and 860 tonnes based on an FCR of 15-20:1 and an annual harvest of 43 tonnes of abalone. For an estimated annual harvest of 37 tonnes of *H. tuberculata,* the volume of *P. palmata* required would be between 555 and 925 tonnes based on FCR's of 15-25:1 With a predicted annual harvest of 27 t of sea urchins, the algal requirement is 400 to 540 t based on an FCR of 15-20:1. The species requirement is approximately a 50:50 split between *L. digitata* and *P. palmata*

The supply of cultivated macroalgae for abalone and sea urchin feed has several advantages over wild harvested material. Cultivated seaweeds provide a sustainable way of sourcing feed, at a location that is always accessible (ideally near to the abalone hatchery). In contrast, harvesting of wild biomass can be hampered by tides, weather and local availability. Seaweed quality changes over the course of the year, and can vary from location to location. Cultivated seaweeds from one location may provide a more standardised crop with respect to epiphyte control, and nutritional content.

8.3 Considerations for the establishment of seaweed aquaculture

Before seaweed cultivation can be established as a new commercial aquaculture technique, certain requirements must be met. Initially, seaweed hatchery facilities need to be set up as described in Chapter 3. It is suggested that the seaweed aquaculture sector could be established quite quickly in the near future by using some of the many available and licensed shellfish hatcheries, which already have many or all of the facilities required for seaweed growth.

8.4 Seaweed production model for *Laminaria digitata*

The seaweed production model for *L. digitata* will describe the cost and capacity of a seaweed hatchery and grow-out farm, based on the tonnage required to satisfy the feed supply demands of the abalone industry.

8.4.1 Assumptions

Hatchery facility description

The hatchery facility costed in the exercise below, is for a temperature-controlled container unit (12.2 x 2.7 x 2.43 m; Figure 8.1), fitted with 24 tanks (16.2 m^3 total volume). The costings includes all the equipment that is required for seaweed cultivation but does not include a laboratory, office, toilet and canteen.

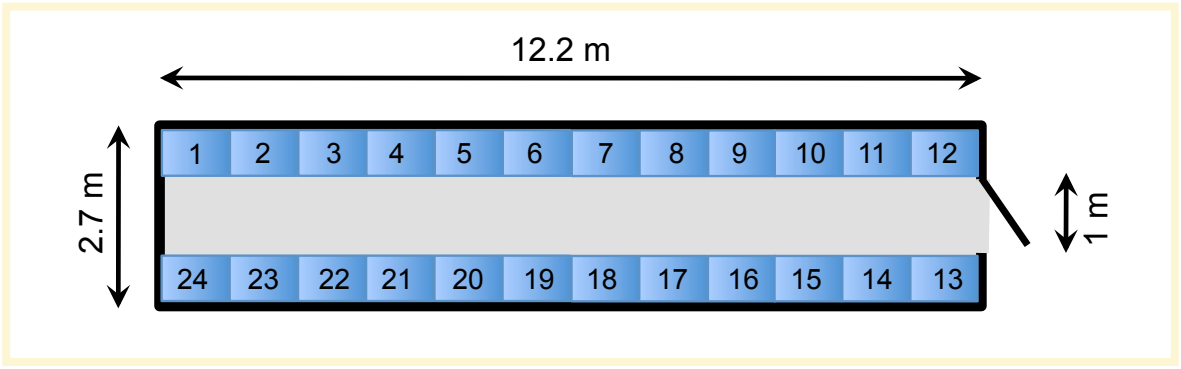


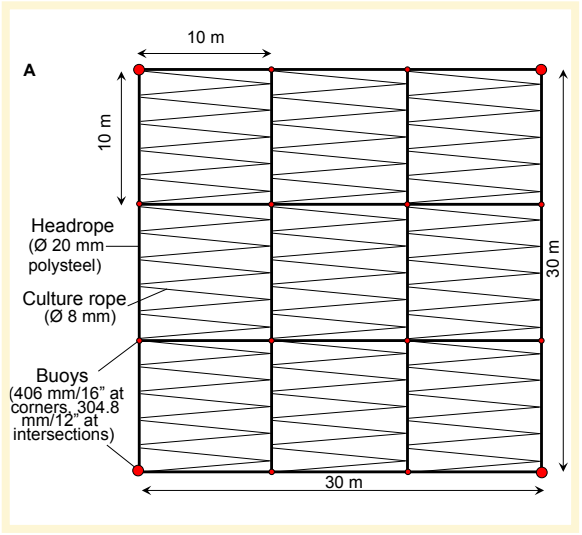
Figure 8.1.
Schematic of a container unit/ hatchery facility with 24 tanks.

Production capability of hatchery facility

Deployment of seeded material to grow-out facilities takes place between October and January. The seeded string/ rope requires a minimum of 30 days in the hatchery before deployment. It is therefore envisaged that the hatchery could be used to produce two batches of seeded string per growing season.

Grow-out facility

Two different types of grow-out facility have been described in Chapter 7 (individual longlines of 100 m length, and a continuous grow-out culture unit of 30 m^2 containing 450 m of header rope). While the costs of a 100-m longline are presented as a comparison, the continuous grow-out unit is used in the final model (Figure 8.2).



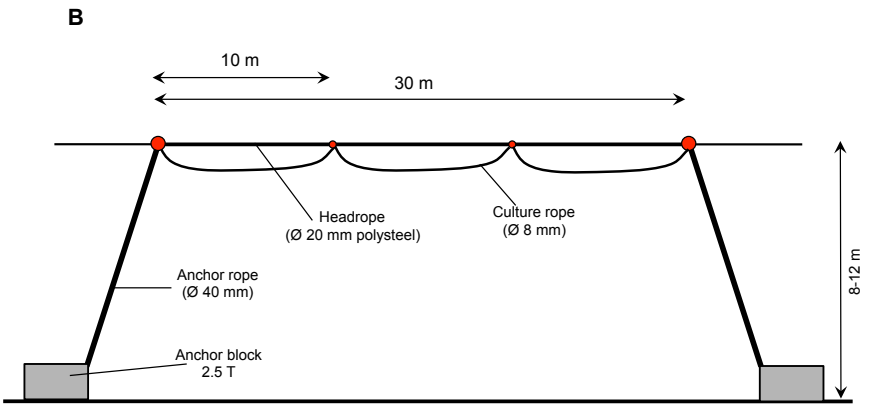


Figure 8.2.
Schematic of a 30-m^2 prototype continuous grow-out culture unit for seaweed (seaweed grid). Overview of grid (A), transverse view (B).

Yield of cultivated L. digitata

Within the period of 2008 to mid 2011, Project PBA/SW/07/001 (01) carried out several sea trials of *L. digitata*. A yield of 6.4 to 8.1 kg m^{-1} has been achieved. An average yield of 7 kg m^{-1} is therefore assumed for the purposes of the model.

8.4.2 The Hatchery

Production of seeded string

Each hatchery unit tank (dimensions described in Section 8.4.1) can hold up to 10 collectors. Each collector is wound with 30 m culture string. With a full hatchery containing 24 tanks, the total amount of collectors sprayed per batch is 240 collectors, collectively holding 7200 m seeded string (240 collectors x 30 m).

As assumed in Section 8.4.1, two batches of seeded string can be produced per growing season, therefore the total productivity of the unit is 7200 m x 2 batches, or 14400 m seeded string.

Set-up and operational costs

Hatchery costs for set-up and production of two batches of seeded string per growing season are shown in Table 8.1.

Table 8.1.
Costs for a self-contained seaweed hatchery (Year 1 only).

Facility Costs	**Cost (€)**
Insulated room, with air-conditioning unit and control panel	8500
Autoclave	14000
Microscope	1500
Precision scales	1500
Pipework	2000
Tankage (16.2 m^3)	14000
Ultra-Violet unit	1000
Consumables	
Glassware	1500
Fluorescent tubes	500
Nutrients	750
Collectors for *L. digitata* = 480 (240 x 2, at €5 each)	2400
Total	**47650**
Electricity per annum	30000
Labour per annum	60000
Total	**137650**

8.4.3 Grow-out Facility at sea

Production of a L. digitata *crop*

The annual production of *L. digitata* is estimated from the observed average yield of 7 kg m^{-1} (Section 8.4.1), and a total amount of seeded string per growing season of 14400 m. The total yield from one hatchery per growing season will be 14400 m x 7 kg m^{-1} or 100.8 tonnes.

Set-up and operational costs

To deploy 14400 m of cultured string at sea would require 144 x 100-mlonglines would be required. Alternatively, 32 of the prototype seaweed grids described in Figure 8.2 would be needed (14400 m/450 m = 32).

As a practical indication of how much space is required for the deployment of gear in a licensed area, a one-hectare site of 200 x 50 m is considered. It would be feasible to fit in a total of five 100-m longlines within this space (allowing for anchorage and access) or 11 seaweed grids (900 m^2 as described in Figure 8.2.). Once again, due to the space requirements for anchorage and access, it would be more realistic to envisage 6 seaweed grids per hectare.

The cost of a traditional single-header rope longline of 100 m (Figure 3.2) is compared with the continuous-rope culture unit, or seaweed grid (Figure 8.2). Table 8.2 and 8.3 detail costs of the linear longline and the seaweed grid, respectively.

Table 8.2.

Breakdown of the costs associated with one 100-m longline, and with a 10 100-m longline unit.

Cost of seaweed longline	**Cost (€)**
Header Rope (Nylon rope 110mx28mm)	350
Anchor rope (90m)	200
Chain (5m)	100
2 Anchor blocks (concrete, 2 tonne ea.)	600
20 Buoys (A2, with spliced ropes)	400
2 Trawl floats	27
Shackles	40
Tying rope	100
Total	**1,817**
Cost of 10 longlines	**18,170**
4 Navigation buoys and anchors	11,340
Deployment	500
Total cost x10 100m-longline unit	**30,010**

Table 8.3.

Breakdown of the costs associated with one continuous grow out unit (seaweed grid).

Cost of seaweed grid	**Cost (€)**
16 Buoys (A2/LD)	464
Header rope (Nylon rope, 18mm 440 m)	560
3 Nylon (twisted twine 9mm, 8.5 kg spool)	255
4 Anchor blocks (concrete, 2.5 tonne ea.)	1,350
Total	**€2,629**

(Total inc. 21% VAT = € 3,181.09)

Total for 32 prototype grids to support 14400 m seeded culture string from hatchery unit as described: €2629 x 32 = €84128.

Labour costs for the hatchery and seaweed farm

The hatchery will employ one full time and one part time person per annum, at a total cost of €60000 (Table 8.1). The seaweed farm will require one part time person per annum (€25000).

8.5 Cost analysis for production of *Laminaria digitata*

8.5.1 Financial Appraisal

A hatchery and grow-out facility as described in Section 8.4 will yield 100 wet tonnes *L. digitata*. The lifecycle is of the order of 5 to 7 months. This is a straightforward growth cycle offering an annual return on investment to the farmer. In calculating the dry weight yield, a value of 15% has been applied, allowing for the variability in the wet to dry weight ratio. Therefore, 15 tonnes dried *L. digitata* is achieved from the facility.

Market place value for a high quality wet product (for human consumption only) is €1 kg^{-1}. Assuming the same high quality end product, the value of bulk dried and bagged *L. digitata* is €10-16 kg^{-1}.

*The potential value of **sales** from a 100 tonne seaweed facility for a high quality dried and bagged product is €150000 to €240000.*

Drying costs (i.e. primary processing) have to be taken into consideration to achieve a market value of €10-16 dry kg^{-1}. If this process is contracted out, it would cost approximately €150 dry $tonne^{-1}$ of seaweed for industrial scale drying. The cost of drying 15 tonnes of seaweed is €2250.

Table 8.4 details the investment in capital and running costs required to dry seaweed if the processing is not contracted out.

Table 8.4.
Costs associated with dryer technology for seaweed primary processing.

Dryer facility costs	**Cost (€)**
Container unit (length, 12.19 m), fitted with humidistat, fans and heaters (excl. VAT) and a 2 wet tonne capacity	10000-12000
Dehumidifier (optional and excl. VAT)	3000
Dryer running costs	
Electricity – 3 phase generator (per hour) OR	10
Electricity – 3 phase mains power (per hour)	7.50
Total	**€2,629**

Premises would also be required to site the dryer, to receive deliveries of seaweed biomass, and to process the product (i.e. bagging and distribution). A 2-tonne facility as outlined in Table 8.4 could process the biomass in 6-10 hours, therefore costing €60-100 or €45-75 on 3 phase generator or mains electricity, respectively.

For a 100-tonne seaweed farm, the cost of running the dryer unit to process this harvest would cost in the range of €3000-5000 and €2250-3750 for 3 phase generator or mains electricity, respectively. This cost is realised over the harvest period between March and April.

8.5.2. Case studies

As already described in Section 8.3 above, there are many licensed shellfish hatcheries in the country that could be used as seaweed hatcheries. Costs of a seaweed hatchery could therefore be kept to a minimum by using these existing facilities and investing in an insulated tanked container.

To assess the financial performace of a 100 tonne seaweed grow-out farm and associated hatchery unit, two separate strands of financial data are analysed by Watson and Dring (2011). The cost of an insulated 'hatchery' as an add-on to an existing hatchery building is examined, and the cost of setting up a new seaweed grow-out site comprising a set of 30 x 30-m grids.

The view held is that adaptation of an existing hatchery unit is the most likely scenario for an Irish seaweed promoter. As mentioned previously, it is unlikely that a proprietary hatchery will be built. There are a number of existing marine pump-ashore hatcheries around the Irish coast. It is our view that the addition of an insulated tanked container is the optimum way to provide the additional space that is needed without going into the significant expense of a new build.

The farm set up and associated cost analysis is considered as 'self-start' in isolation. In this regard it is assumed that bank borrowings are the main source of financial support coupled with grant aid on eligible capital items.

Indicative financial analyses are presented for four case studies (Watson and Dring, 2011) in which different scenarios are envisaged for setting up a farm to grow 100 tonnes of *Laminaria* per year. These scenarios are:-

Case Study 1. A new seaweed hatchery with a new grow-out site.

Case Study 2. A new seaweed hatchery and an existing mussel site partially used for seaweed grow-out.

Case Study 3. A new seaweed and scallop combined hatchery with a new seaweed grow-out site.

Case Study 4. A new seaweed and scallop hatchery with an existing mussel site partially used for seaweed grow-out.

In identifying the costs associated with these options, common costs are isolated (Table 8.5 and Table 8.6). These are costs that will be incurred regardless of the eventual seaweed production method chosen. The common or fixed costs are associated with both the hatchery and the sea site set-up and operation. In addition, there are variable costs, which depend on the type of farm option chosen. The variable costs are labour in the hatchery and at sea, vessel and skipper hire and also bank interest which itself is dependent on the capital required for the undertaking.

Table 8.5.

Summary profit and loss statements for a 100-tonne *Laminaria digitata* farm and hatchery unit. Costs common to all scenarios:

3 Year projections	5 months to Dec-10	Year 2 Dec-11	Year 3 Dec-12	7 months to Jul-13
COGS				
Hatchery				
Nutrients	750	750	750	0
Collectors	2,400	2,400	2,400	0
Electricity	12,500	30,000	30,000	17,500
Total common hatchery costs	**15,650**	**33,150**	**33,150**	**17,500**
Sea site & General				
Regulation 6-ha. seasite	340	309	309	309
Van Lease	1,625	3,900	3,900	2,275
Depreciation	10,719	25,726	25,726	15,007
Diving	1,600	3,200	3,200	1,600
Protective clothing	1,000	1,000	1,000	0
Repairs/maintenance	600	1,800	1,800	1,200
Audit and accounts	0	1,000	1,000	0
Insurance	2,000	2,000	2,000	0
Telephone/postage	750	1,800	1,800	1,050
Total common sea site costs	**18,634**	**40,734**	**40,734**	**21,440**

Table 8.6.

Summary cash flow statement for a 100-tonne *Laminaria digitata* grow-out farm and hatchery unit including capital budgets and grants. Costs common to all scenarios:

3 Year Projections	5 mths to Dec-10	Year 2 Dec-11	Year 3 Dec-12	7 mths to Jul-13
Cash Out				
Hatchery				
Insulated room plus equipment	8,500	0	0	0
Autoclave	14,000	0	0	0
Microscope	1,500	0	0	0
Precision balance	1,500	0	0	0
Pipework	2,000	0	0	0
Tankage	14,000	0	0	0
UV steriliser	1,000	0	0	0
Glassware	1,500	0	0	0
Fluorescent tubes	2,000	0	0	0
Nutrients	750	750	750	0
Collectors	2,400	2,400	2,400	0
Electricity	12,500	30,000	30,000	17,500
Total common hatchery costs	**61,650**	**33,150**	**33,150**	**17,500**
Sea site & General				
Regulation 6ha seasite	340	309	309	309
Van Lease	1,625	3,900	3,900	2,275
Diving	1,600	3,200	3,200	1,600
Protective clothing	1,000	1,000	1,000	0
Repairs/ maintenance	600	1,800	1,800	1,200
Audit and accounts	0	1,000	1,000	0
Insurance	2,000	2,000	2,000	0
Telephone/ postage	750	1,800	1,800	1,050
Total common sea site costs	**7,915**	**15,009**	**15,009**	**6,434**

Labour costs in the hatchery are associated with work on seeding collectors, and represent the greatest monetary outlay for the hatchery, apart from the initial capital costs. One full-time and one part-time worker are allowed for. When scallop cultivation is added for Case Studies 3 and 4, two full-time workers are required for the hatchery. At sea, the major costs are associated with labour (one part-time worker) and with hiring a vessel and skipper for the initial establishment of the grow-out units, and the annual deployment of seeded collectors and subsequent harvest of material. These costs are reduced however, when the seaweed is grown out using and sharing an existing mussel farm set-up. The necessary vessels for this work are available around the coast on mussel and salmon farms. It is more effective to hire this equipment than it is to purchase it. 'Diving' is required for the placement of the sea structures and 'Repairs and maintenance' refers to both the hatchery and the sea site.

In our analysis we have used two potential sales prices for *Laminaria digitata*, €1 kg^{-1} and €2 kg^{-1}, and, in the scallop hatchery examples, we have applied a price of €0.05 per scallop spat.

Capital items are grant supported at 40%. For illustrative purposes, the remaining funding is sourced from bank loan arrangements. In this regard a long-term loan over ten years in envisaged. The interest cost indicated is commercially available at the current time.

The financial analysis starts from August in Year 1 and runs until July of Year 4. This allows three full growth cycles of seaweed. All the required hatchery work takes place during the latter part of the year and, for all the scenarios studied, it is during the last 5 months of Year 1 that there will be the greatest monetary outlay and there will be no income.

Taking the scenarios in turn, the farm in Case Study 1 performs comparatively poorly. At a sales value of €1 kg^{-1}, the venture is loss making for all three years analysed. At a sales value of €2 kg^{-1}, the profit and loss statement shows a small positive balance in Year 3 , and a healthy profit is demonstrated in the 7 months to July of Year 4 (€90982). The cash flow shows the large cash outlay required in Year 1 to buy the 32 grow-out grids (€84128). Net cash is positive from Year 2 at €2 kg^{-1} (€25421) but is only marginally positive by Year 4 at €1 kg^{-1} (€5988).

For Case Study 2, in respect of the corresponding cash flow statements, when the grow-out of seaweed is combined with a mussel farm, we see a reduction in the total overheads because of reduced costs for labour and vessel/skipper hire, together with lowered bank interest charges. These savings result from the sharing of manpower and vessel time between the mussel farm and the seaweed farm. In addition, the very costly outlay on the 32 grids is avoided completely, which reduces bank borrowings. Nevertheless, the undertaking is still loss making until Year 4 at €1 kg^{-1}, but it performs significantly better at a sales price of €2 kg^{-1}. Mussel longlines are ideal for growing seaweed, and they are located in near- shore licensed areas that are suitable for both seaweed and mussel growth.

Case Study 3 examines the potential profit in carrying out both scallop and seaweed cultivation in the same new hatchery. Hatchery work for scallops takes place from February to June with a nursery phase from July onwards, whereas *Laminaria digitata* is worked on in the hatchery from June to October and is deployed to sea in November. The hatchery production capacity is estimated to be 1.5 million juvenile spat per annum. At a nominal €0.05 each, this returns €75000 to the hatchery each year. The annual cost of consumables to produce 1.5 million scallop is estimated at €3000 to include algae, culture bags, netlon mesh, green and white bags. Extra labour is also allowed for, bringing the total hatchery labour to two full time persons (€80000 in total). The indicative cash flows are much improved by the revenue from the scallop production. Profit is achieved in Year 2 (€51696), at a sale price of €2 kg^{-1} for the seaweed and €0.05 for scallop spat but is delayed until Year 4 at only €1 kg^{-1} for seaweed (€51320).

Case Study 4 is the most attractive and profitable option because it combines the advantages seen in Case Studies 2 and 3 (i.e. a combined scallop and seaweed hatchery with the seaweed grow-out at an existing mussel site) . Greater income is realised from the two products, and cost savings are achieved through reduced labour at sea and vessel and skipper hire. Since there is no large monetary outlay for grids, bank interest charges are reduced. These savings are partially offset, however, by the increased costs for labour and consumables in the hatchery. At a sale price of €2 kg^{-1} for the seaweed and €0.05 for scallop spat, a substantial profit is achieved in Year 2 (€76668) but, again, profit is delayed until Year 4 at only €1 kg^{-1} (€62220).

8.5.3 Conclusions

It is clear from these analyses and the sensitivity analysis (Table 8.7) that the opportunity for profit lies in increasing the sales price above €1 kg^{-1} and/ or including an alternative income stream from sales of scallop spat (€0.05 each). The scenarios presented show substantially improved cash flows at €2 kg^{-1} wet weight of product. Any economies of scale to be achieved by increasing the size and capacity of the farm are likely to be in the costs of labour both in the hatchery and at sea, and in the co-use of vessels, in particular where a combined mussel/ seaweed farm type activity is carried out. It is unlikely that many other economies will be found as additional scale will result in a proportionate increase in costs for capital items, such as the bespoke grow-out seaweed grids and associated moorings. In the hatchery, increased capacity will require additional containerised hatchery units plus associated fit-out costs, together with extra costs for electricity and consumables.

Table 8.7.

Sensitivity analysis: 3-year break-even point for *Laminaria digitata* (fresh weight) under Case Studies 1-4.

Case Study	Description	Break even price (€ kg^{-1})
1	Seaweed hatchery and grow out farm	€2.15
2	Seaweed hatchery and existing mussel site	€1.65
3	Seaweed and scallop hatchery and grow out farm	€1.63
4	Seaweed and scallop hatchery existing mussel site	€1.12

Ultimately, new markets should be sought that add value to the seaweed biomass cultivated. It is anticipated that markets such as human specialist nutrition, medicine and functional foods may be prepared to pay a premium for a high-quality product. In the meantime, it is hoped that synergies with other seaweed projects, such as the Biodiscovery project, NutraMara, will lead to a greater understanding of the potential of seaweed products, and those from aquaculture in particular.

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The project's boatmen provided a skillful and invaluable service, taking care of longline maintenance and sampling. These patient people include Vincent Roantree, Philip Johnston and Antaine Conneely. Within the project partners, Dave Millard, Iarfhlaith Connellan, Paul Flannery and Michael Murphy are not forgotten for their involvement in the sea trials!

Appendix 1 – References

(suggested reading in bold)

Arbona J-F (1997) *A routine method for the mass cultivation of Alaria esculenta (Greville 1830).* MSc, National University of Ireland, Galway.

Arbona J-F, Molla M (2006) Cultivation of brown seaweed *Alaria esculenta*. In: *Aquaculture Explained* (ed. Watson L), p. 50. Bord Iascaigh Mhara, Dublin.

Atkinson A, Siegel V, Pakhomov E, Rothery P (2004) Long-term decline in krill stock and increase in salps within the Southern Ocean. *Nature* 432, 100-103.

Baldauf SL (2003) The deep roots of eukaryotes. *Science* **300**, 1703-1706.

Bartsch I, Wiencke C, Bischof K, *et al.* (2008) The genus Laminaria sensu lato: Recent insights and developments. *European Journal of Phycology* 43, 1-86.

Birkett DA, Maggs CA, Dring MJ, Boaden PJS (1998) Infralittoral reef biotopes with kelp species, an overview of dynamic and sensitivity characteristics for conservation management of marine SACs. In: *Natura 2000 report prepared by Scottish Association of Marine Science SAMS for the UK Marine SACs Project.* .

Bixler HJ, Porse H (2010) A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, 1-15.

Bold HC, Wynne MJ (1978) *Introduction to the algae*, 1st edn. Prentice-Hall Inc., New Jersey.

Dring MJ (1992) *The biology of marine plants*, 1 edn. Cambridge University Press, Cambridge.

Edwards MD, Dring MJ (2011) Open-sea cultivation trial of the red alga, Palmaria palmata from seeded tetraspores in Strangford Lough, Northern Ireland. . *Aquaculture*.

Falkowski PG, Katz ME, Knoll AH, *et al.* (2004) The evolution of modern eukaryotic phytoplankton. *Science* 305, 354-360.

FAO (2010a) FAO yearbook. Fishery and Aquaculture Statistics (ed. FAO), p. 72. FAO, Rome.

FAO (2010b) The State of World Fisheries and Aquaculture. (ed. FAO), p. 197. FAO, Rome.

Farrell P, Fletcher RL (2006) An investigation of dispersal of the introduced brown alga Undaria pinnatifida (Harvey) Suringar and its competition with some species on the man-made structures of Torquay Marina (Devon, UK). *Journal of Experimental Marine Biology and Ecology* 334, 236-243.

Foster MS, Schiel DR (2010) Loss of predators and the collapse of southern California kelp forests (?): Alternatives, explanations and generalizations. *Journal of Experimental Marine Biology and Ecology* 393, 59-70.

Guillard, R.R.L. and Rhyther, J.H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacaea* Cleve. *Canadian Journal of Microbiology* 8, 229-39

Guiry MD, Guiry W (2011) Algaebase.

Harrison PJ, Berges JA (2005) Marine culture media. In: *Algal culturing techniques* (ed. Andersen RA), pp. 21-33. Elsevier Academic Press, London.

Hession C, Guiry MD, McGarvey S, Joyce D (1998) Mapping and assessment of the seaweed resources (*Ascophyllum nodosum*, *Laminaria* spp.) off the west coast of Ireland.

Kain JM (1979) A view of the genus *Laminaria*. *Oceanography Marine Biology Annual Review* 17, 101-161.

Keeling PJ (2007) Genomics: Deep questions in the tree of life. *Science* 317, 1875-1876.

Kraan S (2000) *The genus Alaria (Alariaceae, Phaeophyceae) explored : phylogenetics, hybridization and aquaculture* PhD, National University of Ireland, Galway.

Kraan S, Barrington KA (2005) Commercial farming of Asparagopsis armata (Bonnemaisoniceae, Rhodophyta) in Ireland, maintenance of an introduced species? *Journal of Applied Phycolo*gy 17, 103-110.

Kraan S, Verges Tramullas A, Guiry MD (2000) The edible brown seaweed Alaria esculenta (Phaeophyceae, Laminariales): Hybridization, growth and genetic comparisons of six Irish populations. *Journal of Applied Phycology* 12, 577-583.

Lobban CS, Harrison PJ (1997) *Seaweed ecology and physiology* Cambridge University Press, Cambridge.

Løvstad Holdt S, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*.

Lüning K (1980) Critical levels of light and temperature regulating the gametogenesis of three *Laminaria* species (Phaeophyceae). *Journal of Phycology* 16, 1-15.

Maier I, Müller DG, Schmid C, Boland W, Jaenicke L (1988) Pheromone receptor specificity and threshold concentrations for spermatozoid release in Laminaria digitata. *Naturwissenschaften* 75, 260-263.

McErlean T, McConkey R, Forsythe W (2002) *Strangford Lough: an archaeological survey of the maritime cultural landscape*, 1st edn. Blackstaff Press Limited, Belfast.

McHugh DJ (2003) A guide to the seaweed industry. In: *FAO Fisherie's Technical Paper*, p. 105. FAO, Rome.

Morrissey K., O'Donoghue C., Hynes S. *Quantifying the value of multi-sectoral marine* commercial activity in Ireland. 2011. *Marine Policy* 35 721-727.

Roleda MY, Lv°der UH, Wiencke C (2010) UV-susceptibility of zoospores of the brown macroalga Laminaria digitata from Spitsbergen. *Polar Biology* 33, 577-588.

Romo H, Avila M, Núñez M*, et al.* (2006) Culture of Gigartina skottsbergii (Rhodophyta) in southern Chile. A pilot scale approach. *Journal of Applied Phycology* 18, 307-314.

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology* 16, 245-262.

Tynan CT (1998) Ecological importance of the Southern Boundary of the Antarctic Circumpolar Current. *Nature* 392, 708-710.

Watson, L and Dring, M J (2011). Part 2: Business plan for the establishment of a seaweed hatchery and grow-out farm. BIM, unpublished report. 37pp

Walsh, M and Watson, L (2011). Part 1A: Market analysis towards the further development of seaweed aquaculture in Ireland. BIM, unpublished report. 49pp

Werner, A, Clarke, C and Kraan, S (2004) Strategic review of the feasibility of seaweed aquaculture in Ireland. NDP Marine RTDI Desk Study Series, DK/01/008.2004

Westermeier R, Patiño DJ, Müller H, Müller DG (2010) Towards domestication of giant kelp (Macrocystis pyrifera) in Chile: Selection of haploid parent genotypes, outbreeding, and heterosis. *Journal of Applied Phycology* 22, 357-361.

Appendix 2 – Media recipes

Provasoli Enriched Seawater Medium (PES) – Version in Harrison and Berges (Harrison& Berges 2005).

To prepare: In a volumetric flask take 900 ml distilled water and add the components in the 'enrichment stock solution' list. It is advisable to add the vitamins last after all of the other ingredients have been added. Stir the contents with a magnetic stirrer and apply *a little* heat if necessary to dissolve the ingredients. Bring the final volume to 1 litre with distilled water. Pasteurize the solution. *Do not autoclave,* as the extreme heat of the autoclave will denature the vitamins in solution. Store the solution in a sterile dark glass bottle and keep refrigerated.

To use: Make the enriched seawater medium by adding 20 ml enrichment stock solution to 980 ml sterilised seawater.

Enrichment Stock Solution

Component	Stock Solution ($g\ L^{-1}\ dH_2O$)	Quantity Used	Concentration in Final Medium (*M*)
Tris Base	-	5.0 g	8.26×10^{-4}
$NaNO_3$	-	3.5 g	8.24×10^{-4}
Na_2 β-glycerophosphate • H_2O	-	0.5 g	4.63×10^{-5}
Iron-EDTA solution	(see following recipe)	250 ml	-
Trace Metals solution	(see following recipe)	25 ml	-
Thiamine • HCl (vitamin B_1)	-	0.500 mg	2.96×10^{-8}
Biotin (vitamin H)	0.005	1 ml	4.09×10^{-10}
Cyanocobalamin (vitamin B_{12})	0.010	1 ml	1.48×10^{-10}

Iron-EDTA Solution

To prepare: In a volumetric flask take 900 ml distilled water and dissolve the components in the order shown in the table before bringing the volume to 1 litre. Pasteurise and store refrigerated.

Component	*1°* Stock Solution ($g\ L^{-1}\ dH_2O$)	Quantity Used	Concentration in Final Medium (*M*)
Na_2EDTA • $2H_2O$	-	0.841 g	1.13×10^{-5}
$Fe(NH_4)_2(SO_4)_2$ • $6H_2O$	-	0.702 g	1.13×10^{-5}

Trace Metals Solution (from Provasoli 1968)

To prepare: In a volumetric flask take 900 ml distilled water dissolve the EDTA first followed by the other components in the order shown before bringing the volume to 1 litre. If using natural seawater leave out the boron. Pasteurise and store refrigerated.

Component	*1°* Stock Solution ($g\ L^{-1}\ dH_2O$)	Quantity Used	Concentration in Final Medium (*M*)
Na_2EDTA • $2H_2O$	-	12.74 g	1.71×10^{-4}
$FeCl_3$ • $6H_2O$	-	0.484 g	8.95×10^{-6}
H_3BO_3	-	11.439 g	9.25×10^{-5}
$MnSO_4$ • $4H_2O$	-	1.624 g	3.64×10^{-5}
$ZnSO_4$ • $7H_2O$	-	0.220 g	3.82×10^{-6}
$CoSO_4$ • $7H_2O$	-	0.048 g	8.48×10^{-7}

Provasoli Enriched Seawater Medium (PES) – Version in Arbona and Molla (2006).

To prepare: Three separate stock solutions are made (one solution recipe described per table). For each stock take 900 ml distilled water (in a volumetric flask) and add the ingredients in the order that they are listed in each table before bringing the volume to 1 litre. Use a magnetic stirrer and apply *a little* heat if necessary to dissolve the ingredients. Filter-sterilise each stock solution by passing it through a 0.2 µm pore size filter (see 'laboratory consumables' in Appendix 3 for suppliers).

To use: Use 2 ml of 'Miquel A' solution per litre of culture along with 1 ml each of 'Miquel B' and 'Provasoli 6' solutions per litre of culture.

Miquel 'A' Solution

Component	Quantity Used
$MgSo_4$	100 g
NaCl	100 g
Na_2SO_4	50 g
NH_4NO_3	10 g
KNO_3	20 g
$NaNO_3$	20 g
KBr	2 g
KI	2 g

Miquel 'B' Solution

Component	Quantity Used
$NaH_2HPO_4 \bullet 12H_2O$	24.83 g
$CaCl_2 \bullet 6H_2O$	33.55 g
HCl (concentrated)	25 ml
$FeCl_3$	25 ml

Provasoli '6' solution

Component	Quantity Used
NaEDTA	3 g
$FeCl_3 \bullet 6H_2O$	0.39 g
$MnCl_2 \bullet 4H_2O$	0.42 g
$ZnCl_2$	0.03 g
$CoCl_2 \bullet 6H_2O$	0.01 g
$CaSO_4 \bullet 5H_2O$	0.004 g
Na_2MoO_4	0.11 g
H_3BO_3	3.43 g

f/2 Medium – Version in Harrison and Berges (2005).

To prepare and use: In a volumetric flask take 950 ml *filtered seawater* and add the components in the following lists. The trace metal solution and the vitamins solution can be autoclaved and filter-sterilised respectively and stored (preferably frozen). Bring the final volume of the medium to 1 litre with filtered seawater and pasteurize before use. *Do not autoclave* as the extreme heat of the autoclave will denature the vitamins in solution.

Component	Stock Solution (g L^{-1} dH_2O)	Quantity Used	Concentration in Final Medium (*M*)
$NaNO_3$	75 g	1 ml	8.82×10^{-4}
$NaH_2HPO_4 \bullet H_2O$	5 g	1 ml	3.62×10^{-5}
$Na_2SiO_3 \bullet 9H_2O$ *	30	1 ml	1.06×10^{-4}
Trace Metals solution	(see following recipe)	1 ml	-
Vitamins solution	(see following recipe)	0.5 ml	-

*Silicate is omitted as it is not required for macroalgal growth and will only encourage diatom growth in the culture

f/2 Trace Metals Solution

Dissolve EDTA and other components in 950 ml distilled water and bring to 1 litre.

Component	1˚ Stock Solution (g L^{-1} dH_2O)	Quantity Used	Concentration in Final Medium (*M*)
$FeCl_3 \bullet 6H_2O$	-	3.15 g	1.17×10^{-5}
$NaEDTA \bullet 2H_2O$	-	4.36 g	1.17×10^{-5}
$MnCl_2 \bullet 4H_2O$	180	1 ml	9.10×10^{-7}
$ZnSO_4 \bullet 7H_2O$	22	1 ml	7.65×10^{-8}
$CoCl_2 \bullet 6H_2O$	10	1 ml	4.20×10^{-8}
$CuSO_4 \bullet 5H_2O$	9.8	1 ml	3.93×10^{-8}
$Na_2MoO_4 \bullet 2H_2O$	6.3	1 ml	2.60×10^{-8}

f/2 Vitamins Solution

In 950 ml distilled water dissolve the thiamine × HCl and add 1 ml of the primary stocks before bringing the final volume to 1 litre with distilled water. Filter-sterilise and store frozen.

Component	1˚ Stock Solution (g L^{-1} dH_2O)	Quantity Used	Concentration in Final Medium (*M*)
Thiamine × HCl (vitamin B_1)	-	200 mg	2.96×10^{-7}
Biotin (vitamin H)	1.0	1 ml	2.05×10^{-9}
Cyanocobalamin (vitamin B_{12})	1.0	1 ml	3.69×10^{-10}

Appendix 3 – Supplies Lists (A-Z)*

- Chandleries, general aquaculture supplies
    - Clare Aquaculture Supplies (Jim Simmons). Tel: (065) 7078080
    - Swan-Net Gundry Ltd. Killibegs and Rossaveal. Tel: (074) 9731180 Fax: (074) 9731100, http://www.swannetgundry.com
- Chemicals for nutrient media
    - Sigma-Aldrich Ireland, http://www.sigmaaldrich.com/ireland.html
    - See VWR International website below
- Filters (cartridge)
    - Hall Pyke. Walkinstown, Dublin 12. Tel: (01) 4501411, Fax: (01) 4507960, http://www.hallpyke.ie/index.html
- Laboratory consumables (e.g. glassware, filtration equipment etc.)
    - VWR International, http://ie.vwr.com/app/Home
    - Lennox Laboratory Supplies. Naas, Dublin 12. Tel: (01) 4552201, Fax: (01) 4507906, http://www.lennox.ie/
    - Fisher Scientific Ireland. Ballycoolin, Dublin 15. Tel: (01) 885 5854, Fax: (01) 899 1855, http://www.ie.fishersci.com/
- Light Meter (PAR)
    - Skye Instruments. Powys, Wales. Tel: 0044 (0) 1597824811, Fax: 0044 (0) 1597824812, http://www.skyeinstruments.com/
- Lighting – any commercial wholesale electrical supplies
    - e.g. Flaherty Markets, Ballybane Industrial Estate, Galway. Tel: (091) 756000, Fax: (091) 756001, http://www.flahertymarkets.com/about.html
- Microscope – see laboratory consumables above
- Phytoplankton netting
    - Hall Pyke. Walkinstown, Dublin 12. Tel: (01) 4501411, Fax: (01) 4507960, http://www.hallpyke.ie/index.html
- Pre-prepared media
    - Varicon Aqua Solutions. Malvern, United Kingdom. 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
- Tanks
    - The Irish Box Company. Gorey, Co. Wexford. Tel: (053) 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/

***** Telephone numbers with area codes for Ireland, international numbers supplied with country code.

Appendix 4 – Glossary of common terms (A-Z)

Terminology kindly borrowed with permission from www.algaebase.org. Please use 'Glossary' search engine on the website for further/additional descriptions.

- **Antheridium, antheridia (pl.)** - the male reproductive organ (gametangium) that produces sperm in oogamous sexual reproduction; also the mother-cell for antherizoids
- **Antherizoid** - a sperm or motile male gamete participating with an oogamous taxon (see sperm).
- **Axenic culture** - a culture of an organism that contains no other organism, not even bacteria; also a laboratory culture free from all other organisms than the one of interest
- **Diploid** - having twice the basic (i.e. haploid) number of chromosomes
- **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
- **Egg** - a female gamete, normally nonmotile and larger than a sperm
- **Epifauna** – animals living on and sometimes attached to the surfaces of aquatic plants, other animals or the seabed.
- **Epiphyte** - a plant growing on the outside of another plant in a non-parasitic relationship; a plant epibiont
- **Frond** - a 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 Greek word meaning, wife; in English, a sexual reproductive cell with a single set of chromosomes (haploid) that fuses with another of opposite sex or mating strain to form a zygote
- **Gametophyte** - a haploid generation (plant) on which gametangia are produced; also the phase of a life history (i.e. alternation of generations)
- **Haploid** - having one complete set (n) of chromosomes
- **Holdfast, or haptera** - the attachment organ of a seaweed- i.e. a root-like, single cell or group of cells that anchors an alga to the substratum
- **Intertidal** - the region of shore lying between high and low tide levels- i.e. a physical delineation or term
- **Meristem, meristematic tissue** - a group or region of cells dividing rapidly and initiating growth. Within some seaweeds, specific tissue sites where most cell division for growth occurs
- **Oogamous** - sexual reproduction in which a small, usually motile male cell fuses with a much larger non-motile female cell
- **Oogonium, oogonia (pl.)** - a single celled female gametangium; also a swollen cell containing one or more ova or eggs
- **Phaeophyta** - a division of the Plant Kingdom reproducing by spores emergent from vegetative tissue and possessing the brown accessory pigment fucoxanthin
- **Pheromone** - a chemical substance emitted by an organism into the environment as a specific signal to another organism, usually but not always species-specific; they usually elicit some definite developmental response
- **Photosynthesis** - the process of utilizing water, CO2, and chlorophyll to trap energy from light, which is ultimately stored in reduced carbon compounds

- **Photosynthetically Active Radiation (PAR)** - the part of the electromagnetic spectrum used in photosynthesis (400-700 nm)
- **Proximal** - referring to the attached end; also meaning, towards the base or near the point of origin or attachment
- **Sessile** - a Latin word literally meaning, fit for sitting upon; also stalkless, low, dwarf, or spreading; in botany, the term sessile primarily means, lacking a stalk, being born directly on a plant (thallus), or sitting close upon the body that supports it
- **Sorus, sori (pl.)** - a Greek word meaning, a spore case; in botany, a group (cluster) of surficial reproductive structures, sometimes occurring in specialized structures & frequently with their bases united
- **Sperm** - a motile male gamete
- **Sporophyte** - the diploid generation (phase) in a plant's life history that produces spores
- **Stipe** - a basal stalk-like portion of a thallus or any stem-like portion of a thallus that is either cylindrical or flattened
- **Subtidal** - at a depth below the lowest level of low tide- a physical term
- **Thallus, thalli (pl.)** - a vegetative plant-body that has relatively simple internal differentiation of tissues and especially lacks vascular elements; also an algal body with no differentiation into true roots, stems or leaves
- **Unilocular sporangium, sporangia (pl.)** - a single celled (loculed) sporangium in brown algae (Phaeophyta) that either contains many spores biflagellate zoospores
- **Zoospores** - motile, flagellated, naked protoplasts formed generally in undifferentiated vegetative cells; on release from a parent plant it gives rise directly to a new plant, with the period of dormancy, if any, being very short
- **Zygote** - a cell formed by the union of two gametes