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Best Practice Guidelines for Seaweed Cultivation and Analysis

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This project has received European Regional Development Funding through INTERREG IV B.
INTERREG IVB

Best Practice Guidelines for Seaweed Cultivation and Analysis

Executive Summary

This document is a compilation of Best Practice recommendations and considerations employed by the EnAlgae macroalgal pilot facilities. EnAlgae was a four-year Strategic Initiative of INTERREG IVB North-West Europe programme. As part of a key series of outputs, three macroalgae pilot sites were developed in the UK, Ireland and France to demonstrate algal cultivation techniques for bioenergy. This integrated network of pilot sites collaborated on method development and optimal pilot operation with respect to Standard Operating Procedures (SOPs, separate document). These SOPs were combined and refined into a comprehensive overview of the Best Practices for Macroalgal Cultivation across a range of environmental conditions and species.

Best Practices (including recommendations and considerations) are presented for siting a pilot plant; macroalgae cultivation (including strain collection, preparation, maintenance and monitoring); macroalgae seeding, deployment, at-sea maintenance and monitoring and biomass harvesting. In addition, detailed technical descriptions of the different pilots and the infrastructure used in have been provided, along with key information on practices that were unsuccessful. The aim is to provide the information necessary for those new to pilot and commercial scale macroalgae cultivation, across a range of site conditions and resources available.

Acknowledgements

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Contents

1 Introduction

The cultivation of seaweed has been identified as a 'clean' industry, due to the myriad potential environmental benefits of seaweed cultivation. Macroalgal biomass is increasingly in demand across Europe, and its range of applications is moving beyond food (the main focus of production in Asian countries, the greatest producers of cultivated seaweed) and animal feed (the main focus for current production in Europe). At the moment, macroalgal biomass production is dominated (>95%) by cultivated strains, rather than wild harvests which accounted for only 4.5% of total world seaweed production in 2010 (FAO, 2012 http://www.fao.org/docrep/016/i2727e/i2727e00.html). There is a growing need for a sustainable source of macroalgal biomass. Recent years have seen a growth in the seaweed industry in Europe, through the application of seaweed extracts in nutraceuticals, pharmaceuticals and as soil enhancers, as well as becoming increasingly of value as a food source or "sea vegetable" due to increased awareness of the health benefits of eating seaweed and seaweed extracts: improved weight loss (Hall *et al*., 2012), combating mineral deficiency (Flores *et al*., 2015), antioxidant (García-Casal *et al*., 2009) and anti-tumour properties (Kuda *et al*., 2005; Rajan *et al*., 2013).

Macroalgal cultivation is regarded as environmentally benign or even beneficial. This has both direct and indirect economic benefits through e.g. provision of ecosystem services: nutrient and heavy metal uptake, habitat refuge and nursery grounds for marine animals, CO_2 sequestration and water oxygenation. Integrated Multi-Trophic Aquaculture (IMTA) applies this principle in the remediation of excess nutrients associated with shellfish or finfish aquaculture, and the rise in macroalgal biomass used for bioenergy production has a direct economic benefit. The accessibility of longline cultivation technology means that the marine industry can avail itself of the economic benefits of growing seaweed for a range of uses or products (Figure 1).

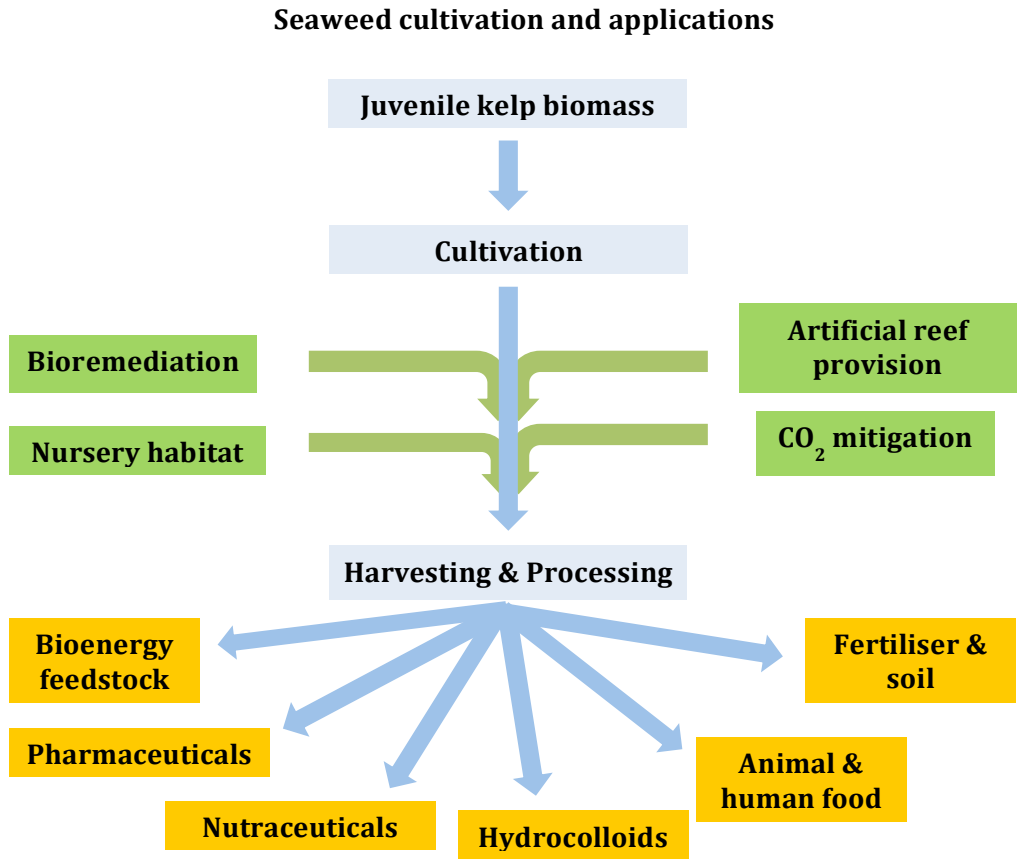

Figure 1: Seaweed cultivation potential environmental benefits (green boxes) and seaweed products (orange boxes).

The increase of seaweeds in biofuel applications largely follows on from the use of microalgae in biodiesel production, with studies examining the applicability of Anaerobic Digestion (AD) to obtain methane from macroalgae and the high productivity of some macroalgae species compared to many terrestrial crops used for biofuel (Bruhn *et al*., 2011; Lewis *et al*., 2011; Montengelli *et al*., 2015). In addition, in the UK the Crown Estate is aiming to make more commercial use of the seabed under its management and is promoting the cultivation of macroalgae for bioenergy (Fry *et al*., 2012). However, a large drawback of macroalgae for bioenergy is the vast quantity required, on top of what is already cultivated for food. In the UK, macroalgae have traditionally been wild-harvested and used for food, feed and as fertiliser in coastal communities for hundreds of years. It is estimated that 2,000- 3,000 dry tonnes (equivalent to 25 – 40,000 tonnes wet weight) of seaweed is harvested per year in the UK to produce food and feed products as well as speciality chemicals and fertilisers (Schlarb-Ridley and Parker, 2013).

The increasing use of seaweeds in a range of industries means that production needs to be increased significantly, and this can only be achieved through cultivation as wild harvest is nearing its sustainable limits in the UK (AB-SIG Roadmap, 2013). Current European cultivation techniques are struggling to cope with the increased demand, with most technology based on current, and moderately inexpensive aquaculture methods of longline deployment e.g. those used for mussel cultivation. Due to an extensive and diverse European coastline, there is high potential for availability of sites to cultivate seaweed, as well as the potential to establish stock cultures of a range of species and strains, ensuring that it is only local species that are cultivated in any area. Cultivation has the added advantage of being able to promote strain selection to obtain harvests of the most beneficial material, whether it is optimised for rapid growth, optimal biochemical composition or flavour. The EnAlgae project was developed to optimise cultivation processes for macro and microalgae with a view to enabling commercially viable production of energy from algal biomass. Three species of brown macroalgae were chosen: *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima* as these are all commonly found around the NW Europe coastlines, are very productive and have previously been cultured by the EnAlgae macroalgae partners.

1.1 Cultivation Overview

Cultivated kelp can be wild fertile material that is allowed to settle naturally on ropes and harvested when large enough; this is an indirect method which requires very little human input, other than deploying ropes and anchors, than harvesting. It is the cheapest method of cultivation, but the biomass can be a mixture of species and will likely be of little commercial value. The alternative method, and the one which this document focuses on, is a more directed approach using targeted strains of kelp, where juveniles are grown in a hatchery and then deployed at sea. While this is a more expensive method, the cost can vary, primarily depending on the juvenile development technique used. However, this approach allows particular species to be grown, targeted development of specific strains which can be tailored according to the end-products required, and much more control over the timings of the cultivation season.

When wild kelp is fertile, it develops visible patches called sori which contain reproductive cells, which themselves contain asexual zoospores (haploid, n). Figure 2 displays the life cycle of *Laminaria digitata*. When mature, these zoospores are released into the water column and develop into gametophytes, which have reproductive structures that are either male (with antheridia) or female (with oogonia). When the male gametophytes release sperm and fertilise the female gametophytes, then fertilisation has occurred and juvenile sporophytes (diploid, 2n) begin to develop.

When direct seeding is used in cultivation, the rope or seeder string is immersed in the water with zoospores (Figure 2, point A), allowing the gametophyte phase to process uninterrupted and the juvenile sporophytes to settle directly onto the seeding substrate. In contrast, Gametophyte seeding is when the

zoospores are released and held in flasks prior to seeding, at the start of the gametophyte phase (Figure 2, point B). When gametophytes are held under red light, they don't develop the male and female reproductive structures and so will grow vegetatively, increasing in number without becoming fertile. This is a very simple way to "bulk up" kelp cultures, so that a large amount of seed can be produced from a small amount of fertile adult material. Fertility can then be induced transferring flasks to blue light, where the gametophytes will then begin to develop into males and females, and produce juvenile sporophytes which are then sprayed onto the seeder substrate.

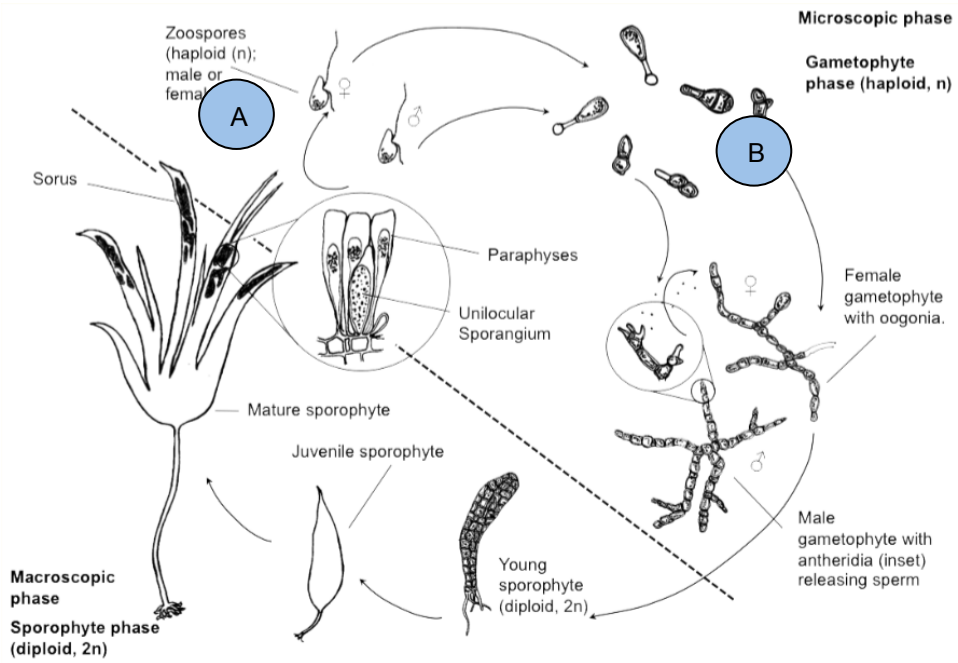

Figure 2: Life cycle of Laminaria digitata. *Point A is where direct seeding occurs, Point B is where the gametophyte cultures are started. Taken from Edwards & Watson Aquaculture Explained: Cultivating* Laminaria digitata.

1.2 Best Practice Aims

The scope of the following document is to describe the various ways the EnAlgae macroalgae partners are cultivating kelp species in a range of environments, using a range of techniques. This document is a guide to the basics of what the EnAlgae partners have found to be the most important factors affecting cultivation and what we have tried that both worked and perhaps more crucially, did not work well according to the varying resources and geographical locations used. It is designed to be read in parallel with the **EnAlgae Standard Operating Procedures (SOPs) document** which will give further details of how each procedure was carried out. Specific SOPs will be referenced in parentheses where appropriate within this document e.g. (SOP: 2.1.1). As well as providing a guideline of best practice, we have also endeavoured to include various methods that were unsuccessful, along with suggestions for improvements. The purpose of this approach is to provide practical advice to future kelp farmers who can assess the various methods described, and use a combination of those which best suit their needs and location of cultivation.

2 Infrastructure

2.1 The Pilot Plant

A seaweed cultivation system is made up of two main components: (i) a hatchery, and (ii) an on-growing site (either land based tanks or at sea). It is possible to purchase juvenile seed from an external hatchery or seed can be grown up in an on-site hatchery. This section will outline the considerations required to set up a hatchery and on-growing site at sea, with case studies from the three EnAlgae pilot sites. For full details, see SOP Chapter 2: Basic Algal Hatchery Techniques.

2.1.1 Hatchery Set-Up

There are several basic requirements for a seaweed hatchery:

- filtered seawater
- filtered air supply
- lighting
- chiller unit
- tanks
- seeders or rope
- microscope
- flasks
- storage

In order to minimise the operating costs during the hatchery phase, paying attention to energy consumption is key (Taelman *et al.*, 2015). For instance, it is important to choose an air blower of the correct power/output as because this equipment has to be operated continuously for several weeks to months and is one of the most energy consuming pieces of equipment used.

Case Study: Queen's University Belfast – Marine Laboratory, Portaferry

The Queen's University Belfast pilot site is situated on the Ards Peninsula, Northern Ireland. The hatchery is in the Queen's Marine Laboratory in Portaferry and the on-growing site is to the west of Jackdaw Island, in Strangford Lough. The hatchery is located in an outbuilding behind the main laboratory, with an area of 30.5 m². It is equipped with a filtered seawater system on tap, filtered air supply, lighting and a chiller unit, tanks for seeder cultivation and storage (Figure 3). Tank lighting is a mix of fluorescent tubes and LED strips, and is controlled via a custom-made timer system for a set light:dark cycle. The hatchery is designed as a wet room, with a drain in the floor centre to allow easy cleaning, filling and emptying of tanks. There is also a plant culture cabinet in the hatchery used for cultivating gametophyte culture flasks; the cabinet has red and blue light sections and maintains flasks under a variable light:dark cycle at 10 ^{o}C (Appendix).

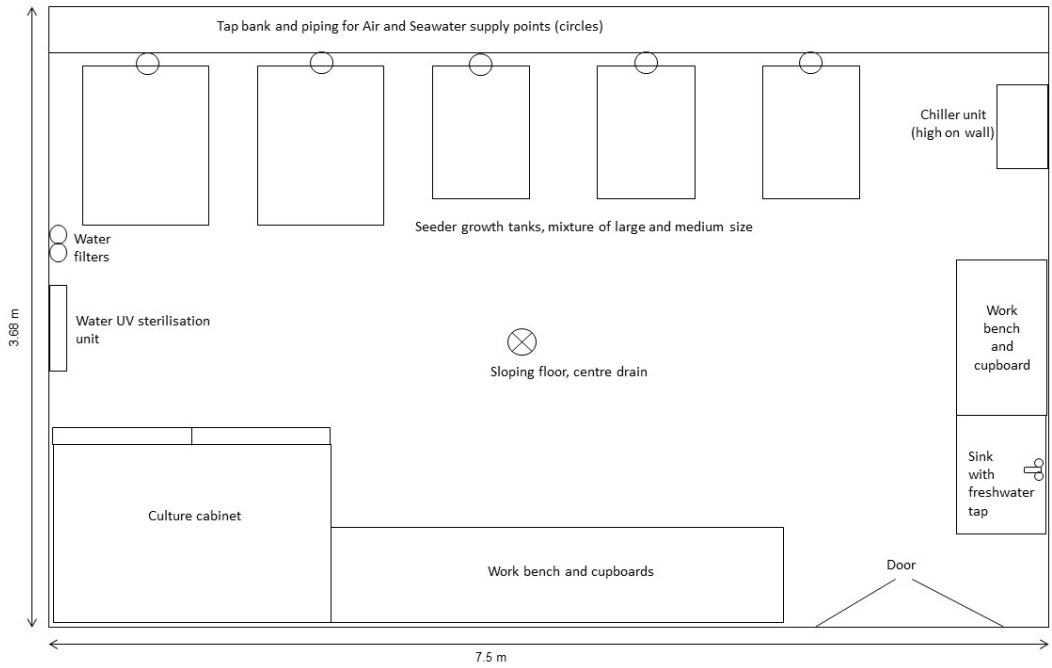

Figure 3: Hatchery schematic at QUB.

Case study: CEVA, Pleubian

The CEVA pilot site is situated in Pleubian, Brittany, France. The hatchery is located in the main building, with an area of 165 m². It has two rooms (Figure 4). The first room has a work bench for the preparation of fertile material prior to seeding and the release of spores. There are two growth cabinets for cultivating of gametophyte culture flasks. These cabinets can be operated under white or red light. The second room is a wet room with a drain in the floor to allow easy emptying of tanks and cleaning. Brittany experiences a wide tidal variation (4-7 m). When the tide is high enough, seawater is pumped ashore and stored in a 6 m^3 tank. In the hatchery, there is a filtration unit consisting of cartridge filters (10 µm, 2 µm, 1 µm, 0.22 µm) and a UV lamp. One can choose to use untreated, filtered or UV-treated & filtered seawater. There is a total capacity of 16 tanks, 300 L each. Air is supplied in each tank. Tanks are illuminated with fluorescent tubes (58W) under a given photoperiod (light:dark cycle). If the water temperature in tanks needs to be decreased, individual chiller units are used.

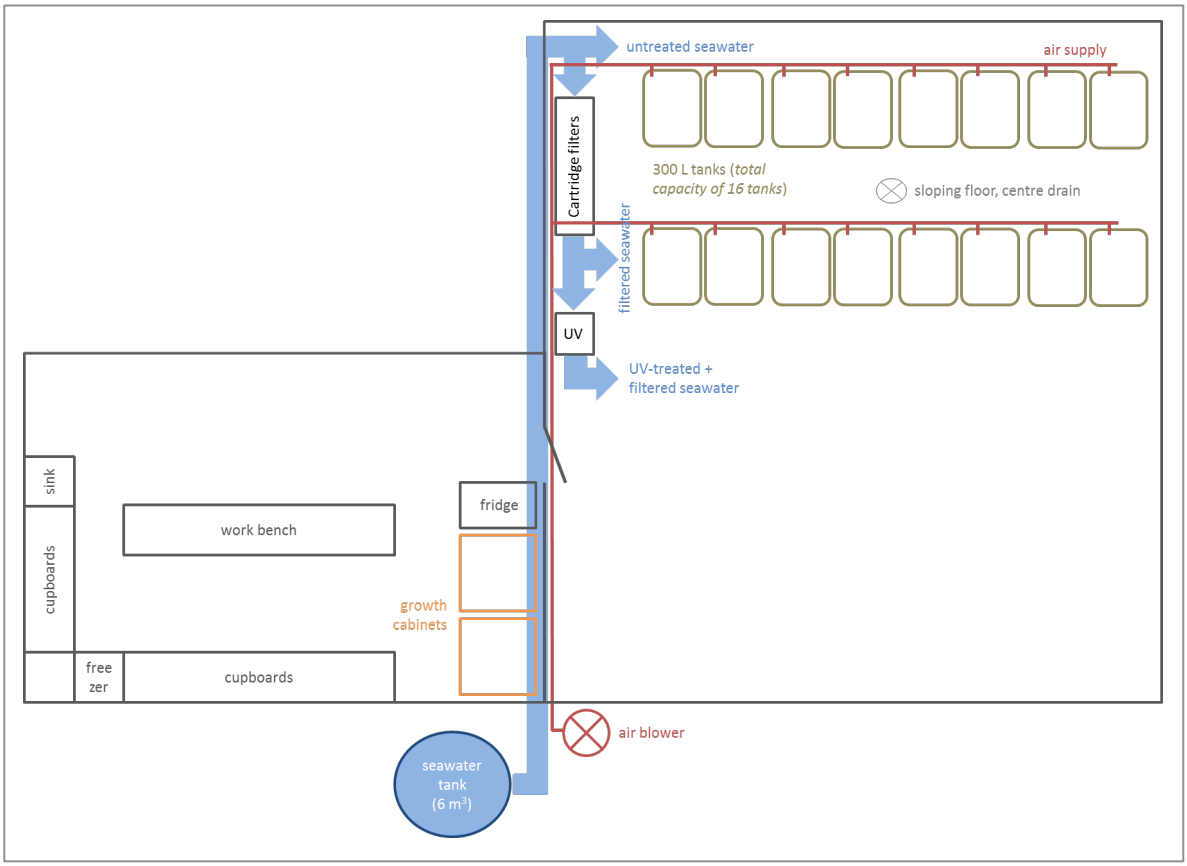

Figure 4: Hatchery schematic at CEVA.

Case study: National University of Ireland Galway, Carna Research Station, Co. Galway

The National University of Ireland Galway (NUIG) seaweed hatchery is situated on the west coast of Ireland near Carna, Co. Galway, approximately 80 km from Galway city. The hatchery is located within the Carna Research Station Marine Innovation Building, a purpose-built aquaculture research facility and has a floor area of 40.8 m² (Figure 5). This room has a constant incoming seawater supply (drum-filtered and UV-filtered to 60 µm). Water is distributed around the room by a low-level piped network (63 mm diameter) through five supply valves. Further in-room seawater processing can occur, with cartridge filtration of water to 1 µm and additional UV-filtration as required. An air blower supplies a constant unfiltered air supply around the entire room on a high-level ring mains (pipe diameter: 63 mm); individual filters are fitted on incoming culture air tubes as required. A powerful chiller unit maintains a room air temperature of 9-10 ˚C throughout the year, ensuring tank water temperature is a steady 10 ˚C.

There are two sets of cool white fluorescent lighting in the room, both on timers for photoperiod control, and both in IP65-rated waterproof housing units. One set of lights (x9 units) can illuminate the full floor area and is fixed to overhead supports. The second set of lights (x9 units) are located over the main cultivation tank space, and are adjustable up and down on chains to allow good illumination control of specific tanks during key cultivation periods. Further IP65-rated waterproof wall sockets (2 sets of 4) controlled by individual timers are provided at opposite sides of the hatchery and are used for additional lighting in the culture cabinets for gametophyte cultures under red and blue light. Tanks range from 250 L and 500 L rectangular tanks to 1000 L circular tanks made of HDPE. As the hatchery room is wet laboratory space, seawater can be directed onto the sloping floor to a central drain for removal to effluent

processing and discharge. Workbench space and a hot and cold freshwater supply at a sink are also provided within the hatchery for culture maintenance.

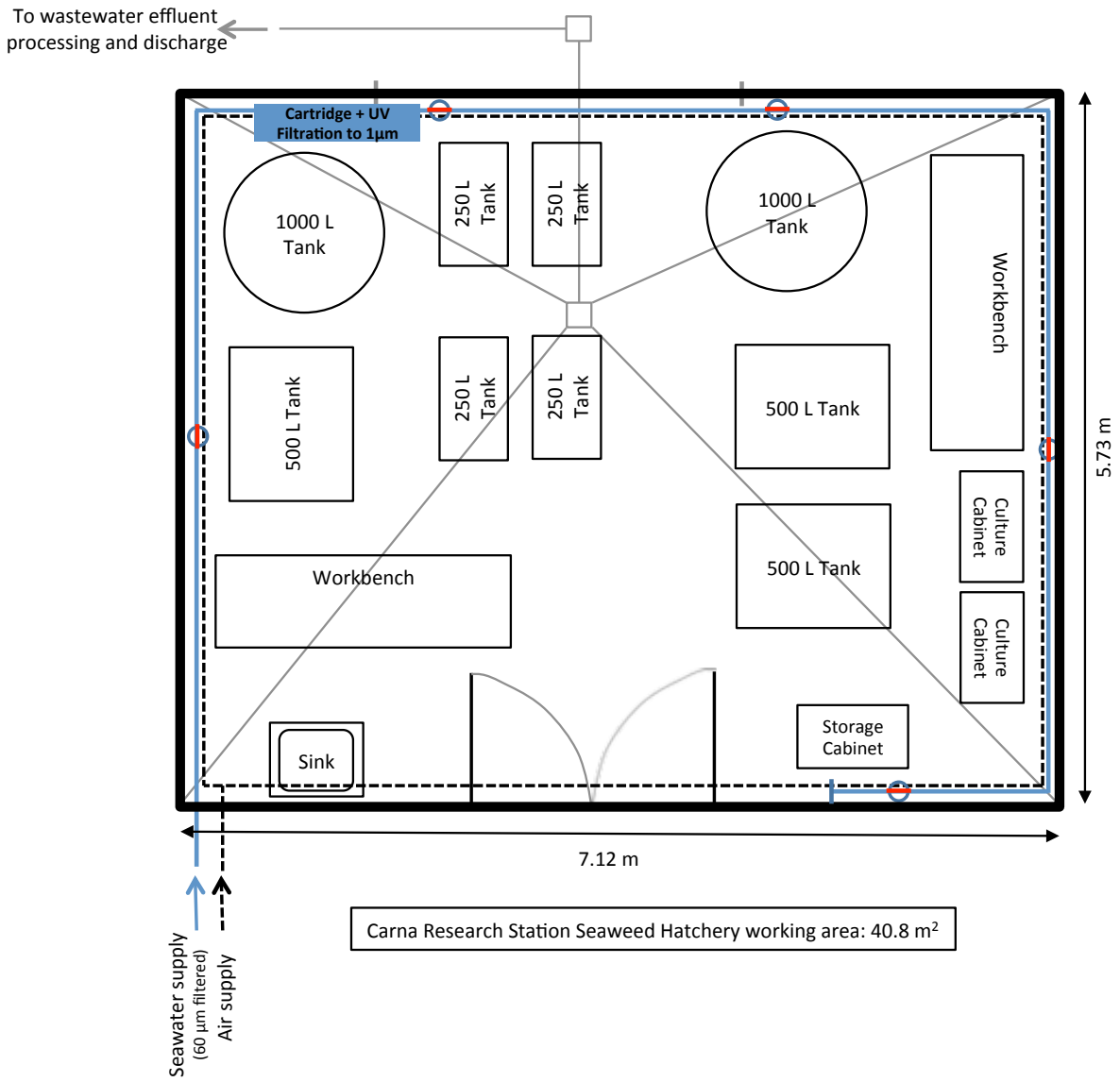

Figure 5: Hatchery schematic at Carna, NUIG.

2.1.2 At Sea On-growing Site

In general, the growth systems for kelp have certain similar characteristics including (in its simplest form): header and anchor ropes, buoys and anchors. The juvenile kelp can be either attached around the header rope (as in Figure 7a and b) or on droppers, which hang down from the header rope. Key factors which will influence the final longline system design include:

- Resources available
  - Overall cultivation budget
  - The size and type of boat used
- Size and location of site
  - Nearshore, offshore

        - Surrounding activities – other aquaculture, fishing, conservation area, recreational area
    - Type of species being cultivated
        - Must be local to the cultivation area
    - Water depth
        - Although dependent on tidal height range, water depth needs to be at least 5-6 m deep at Mean Low Water Springs (MLWS)
    - Nutrients
        - Sufficient nutrients for high density algal growth
    - Turbidity
        - Light penetration suitable for algal growth, approx. 3-4 m on average
    - Hydrodynamics (waves, currents, tides) and wind
        - What is the exposure; do lines need to go deeper to avoid excessive wave action; is access to site restricted by tide; how frequent are repairs likely to be?
        - It is recommended to align longlines running parallel with the current
        - A maximum swell of 2 m is recommended for nearshore sites.
        - A maximum current of 3 knots/1.5 m s^{-1} is recommended.
        - The cost for anchoring equipment will increase with current speed.
    - Deployment method
        - Direct seeding, gametophyte seeding
        - Droppers or headline cultivation?
    - Type of substrate
        - Important factor for anchoring. The optimal substrate is sand and/or mud.
    - Water temperature
        - Kelp species prefer low temperatures. During the growth cycle, a maximum seawater temperature of 18 °C is recommended for most European species, as exposure for any length of time to higher temperatures can be lethal (Kerrison *et al*., 2015).
    - Pollution
        - Kelps can accumulate heavy metals (Ratcliff *et al*., 2015). It is recommended to choose a growing site far from any source of heavy metals pollution if the biomass is dedicated to food or feed applications.

It is possible to situate a kelp cultivation site in areas outside of these parameters, but modifications will likely need to be made to allow for more challenging conditions. For example, longline cultivation in areas with swell of up to 7m is possible, but this is only with the use of counter-buoyancy measures. When the swell is high, the lines are counter weighted and are temporarily sunk further below the surface of the water to avoid the worst of the swell and waves.

The weather conditions are extremely important in accessing lines for deployment, maintenance and harvest. If possible, plan these activities for periods of low wind; both NUIG and QUB staff have found that during periods of wind over ~15mph it is difficult to maintain boat position for line placement and speed of deployment. This is site dependent however, and the effect of wind will vary from site to site depending on site exposure/wind direction. For an easy operation of the lines or maintenance at its site, CEVA also recommends not to work during periods of wind over 20 knots (23 mph). If the site is characterised by strong currents, such as at CEVA, it is better to work around slack tide (from 1h before slack tide to 1h after slack tide) and even better when the tide coefficients are low. For diving operations, periods of low tide slack water are recommended. Lifting heavy longlines is also much easier when there is a decreased amount of tension in the ropes as they loosen at low water (longlines are designed to be taut/tensioned at high water). Manual handling should only be attempted during this time to ensure a safer working environment and fewer potential injuries.

The QUB on-growing site is situated in Strangford Lough, east of Jackdaw Island (grid ref: point A) 54 23.02N 05 37.19W; point B) 54 23.02N 05 36.83W; point C) 54 22.92N 05 36.82W; point D) 54 22.92N 05 37.19W, Figure 6). The site is relatively sheltered with an average current speed of 0.3 m^2 but there can be moderate wave action when the wind is coming from northerly and easterly directions. The depth profile is variable, ranging from 2 m to 13 m at MLWS. The current predominantly runs in a West – East direction.

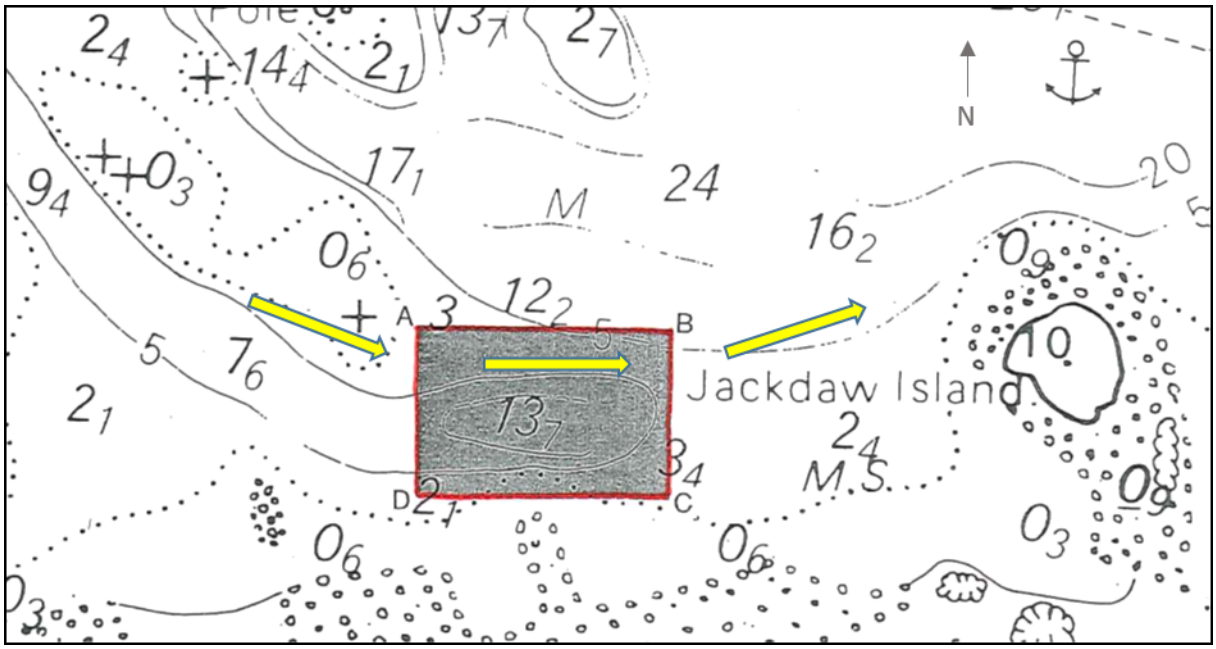

Figure 6: Location of EnAlgae QUB longline site on Crown Estate OS Map. Prevailing current direction is shown by yellow arrows.

The QUB group has trialled two different growth systems over three growth seasons. A simple longline system was used as a base structure, with no droppers as the site is shallow in some areas and previous work indicated poor growth below 3 m on droppers (Edwards and Watson, 2011). In Season One (2012-2013), *Laminaria digitata* and *Saccharina latissima* were cultivated on traditional longline header systems (Figure 7a), deployed in December 2012 and harvested in July 2013. These consist of a 100 m header rope, attached to anchor rope and 1 ton anchors at either end. In Season Two (2013-2014), *L. digitata*, *S. latissima*, and *Alaria esculenta* were all cultivated at the site, using a combination of two growth systems. The traditional 100 m header system was used, as well as a trial of a new grid system (Figure 8). Seeding was conducted in September 2013 and February 2014 and harvested in July 2014.

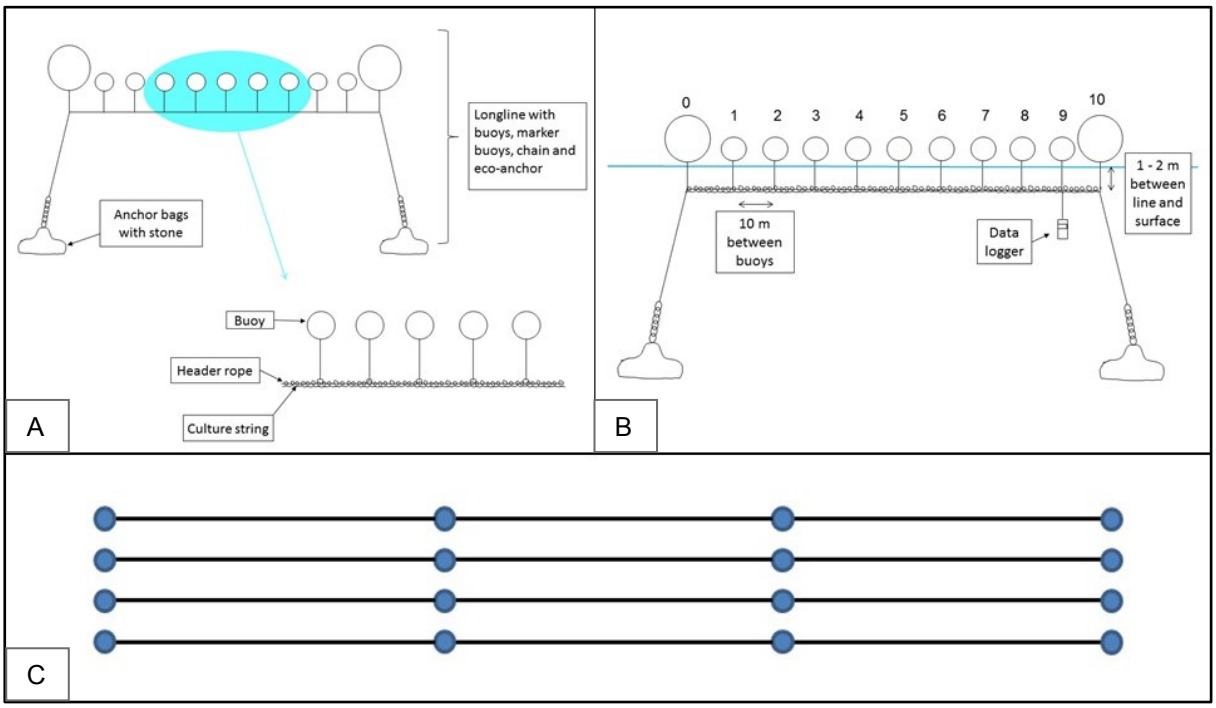

Figure 7. Longline site schematic QUB (Strangford Lough): (a) overview of longline setup and wrapping of culture string; (b) detailed longline setup; (c) birds-eye view of longlines in Season Three.

The grid system used the same four header longlines and buoys spaced at 10 m as Season One, but the seaweed was grown on smaller "seeder rope" that zig-zagged horizontally between parallel header ropes, which meant that more of the space was being used. The seeder rope was attached every 10 m along the header rope (so attaching at every 5 m on alternate headers) giving 220 m of culture rope instead of 100 m (Figure 8). It was anticipated this would at least double the yield.

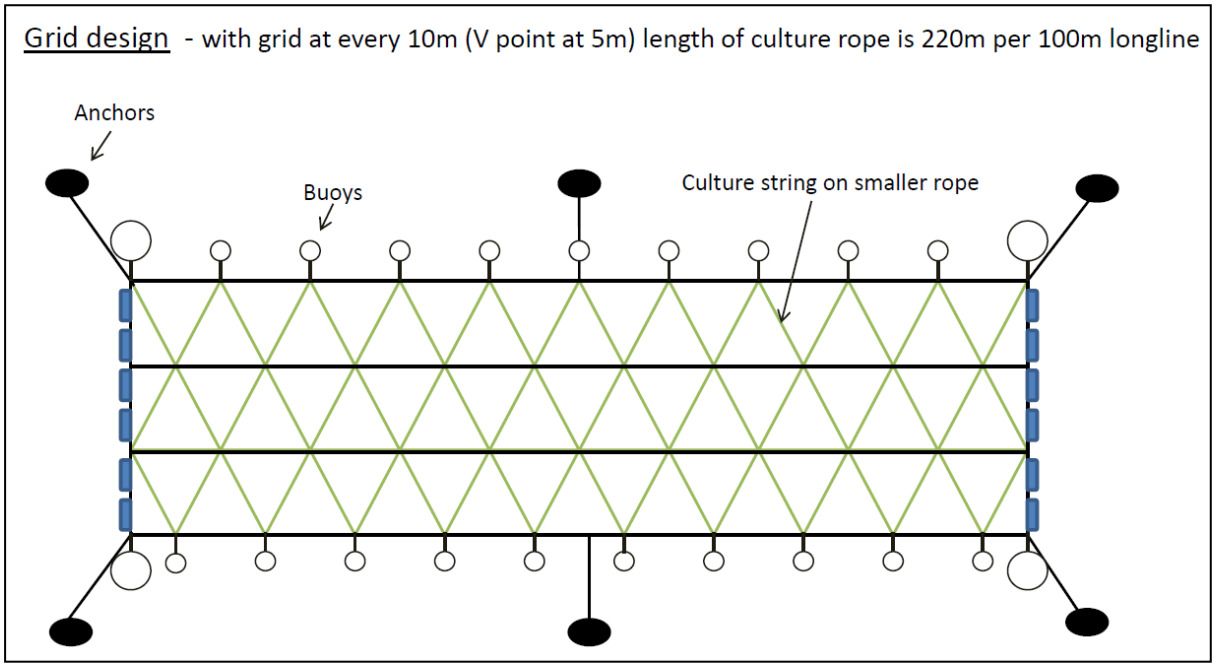

Figure 8. Season Two longline grid system, birds eye view.

Overall this longline design was unsuccessful. Deployment was much more time-consuming and difficult than expected, leading to increased damage to the delicate sporelings. The inner lines (closest to land) were in much too shallow water (High Water Depth 3-4 m) and this was reflected in poor growth/biomass at the time of harvest. On several occasions it was noted that the lines were beached during low tide, and there was a lot of frond erosion due to scraping along the seabed. The seeder rope used was not weighted and tended to float on the surface of the water, and drift seaweed became tangled in it very easily. This also meant that the rope became tangled around buoys when the wind/swell increased and led to large loss of biomass (Figure 9).

Figure 9. Season Two issues with drift seaweed and storm damage – note the extensive tangling of seeder rope around the buoy and seed string breakage.

Due to the comparative simplicity and ease of use of the traditional header longline system, in Season Three QUB decided to remain with this design and increase the number of lines deployed, and no longer trialling the grid design. The final longline layout is shown in Figure 7c. Out of 12 lines deployed, 11 were deployed using the seeder deployment method (SOP: 3.1) and a trial was carried out on one line for direct seeding the header rope (see SOP: 2.3.2, replacing seeders for header rope). The deployment of the direct seeded rope was much simpler and quicker than the seeder deployment, although more space is required per line for this method. In addition, QUB staff found it useful to use lightweight MDPE pipe (blue water pipe) over the buoy attachment rope (Figure 10). This prevents the buoy from wrapping around the header rope and causing loss of biomass. Finally, we have also found that using simple knots and cable ties for tying ropes and buoys is better overall than the use of shackles, even marine grade shackles. Although tying knots is a slightly longer process, it is cheaper and will eliminate the loss of buoys and potential disconnection of the header rope through shackles breaking or rusting. It is always good practice to secure all fastenings, especially any with shackles, with cable ties as an extra precaution.

Figure 10. Buoy with blue MDPE pipe around attachment rope.

Case study: CEVA, Pleubian

CEVA's on-growing site is located in Pleubian, 2 km south-west from the hatchery (Figure 11). It is a 6 ha site with depth between 12 and 25 m. It is located in a sheltered area with low wave action (1 to 1.5 m heights) but strong water currents up to 3-4 knots at high tide, which are challenging. Since the 1990's, seaweed production at CEVA was done on traditional longlines (Figure 12). Basically, the rope was stretched between moorings with buoys to keep the rope 1 to 2 meters below the surface. Because the currents are so strong, a large distance (50 m) was needed between the lines to avoid loss of plants through friction (Figure 13, left-hand side). This was not optimal in terms of use of space and yield of biomass per hectare. Therefore, CEVA designed a new system where the lines are set much closer (Figure 13, right-hand side), in a raft structure (Figure 14). During the EnAlgae project, growth of *Saccharina latissima* and *Alaria esculenta* was tested on both longline and raft systems and appeared to be comparable. This raft structure could be considered in the future as a model for kelp farming in locations where currents are challenging.

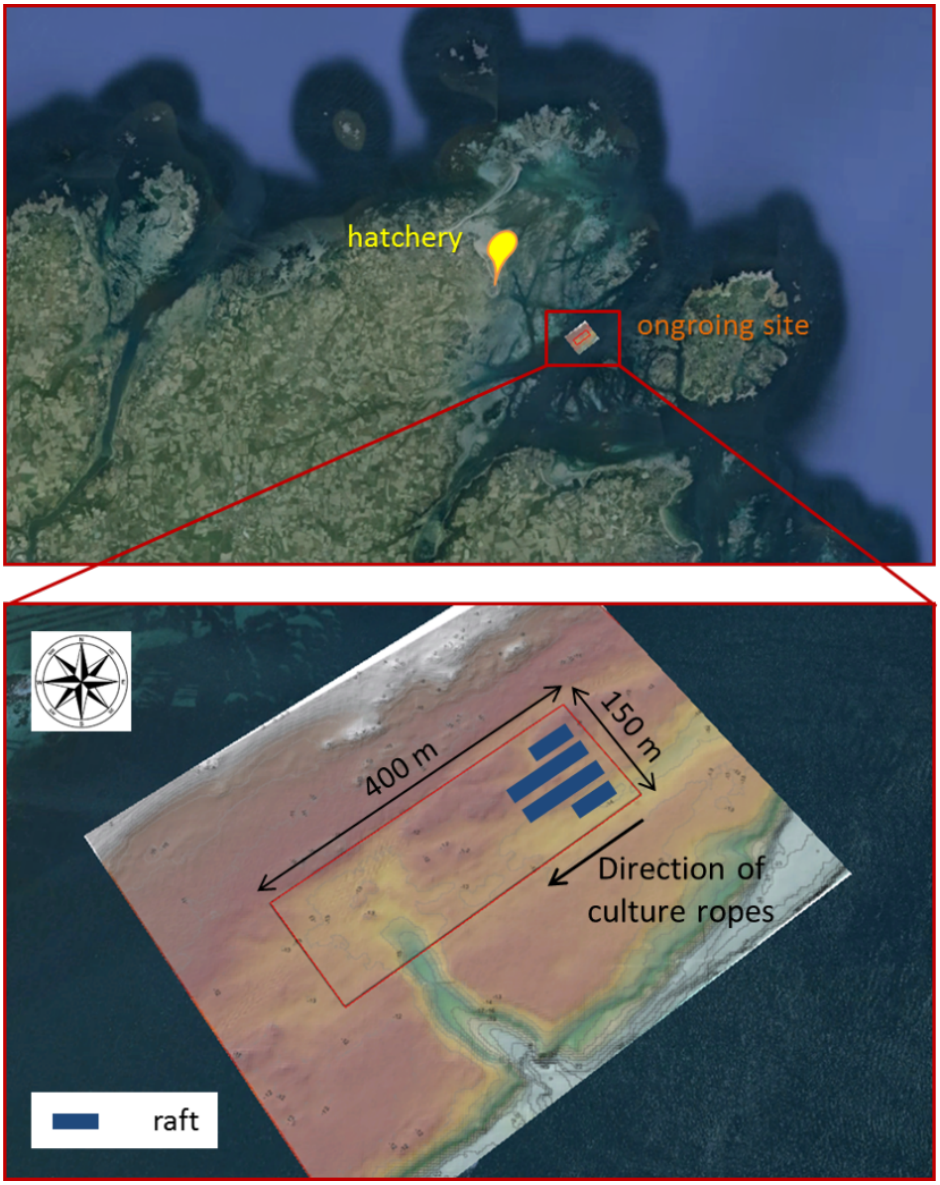

Figure 11. Location of CEVA's hatchery and on-growing site in Pleubian, France

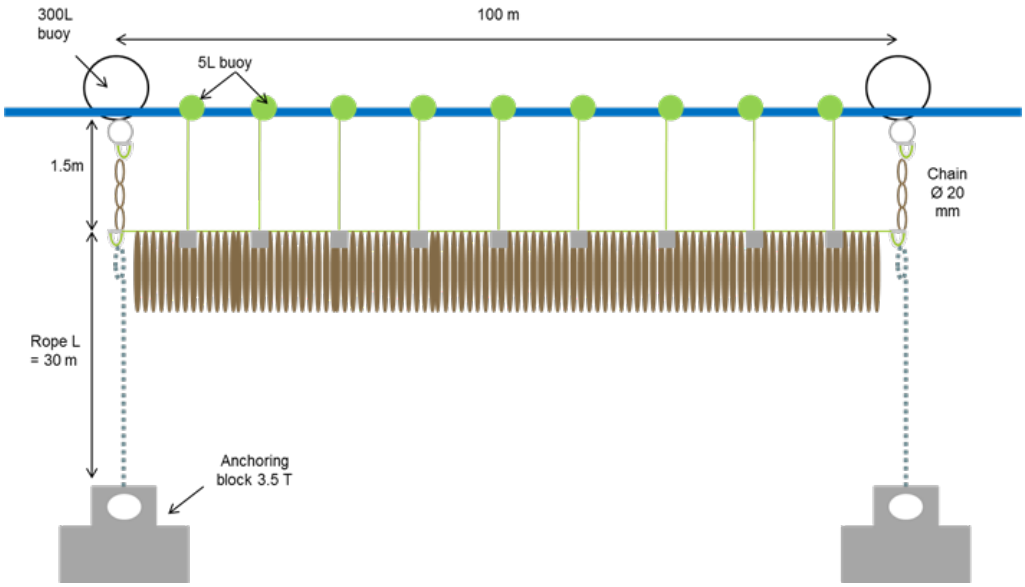

Figure 12. Schematic of longline system used at CEVA until 2013.

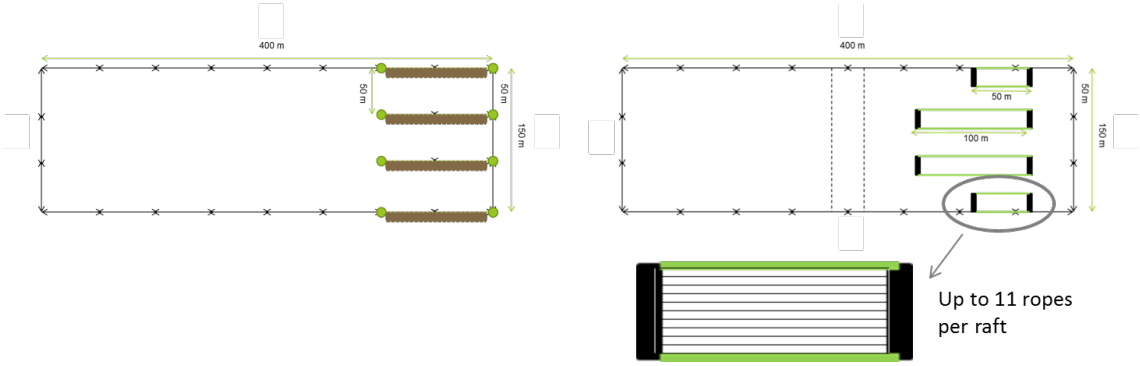

Figure 13. Past (left) and current (right) occupation of lines at CEVA's site; details of a raft (bottom right).

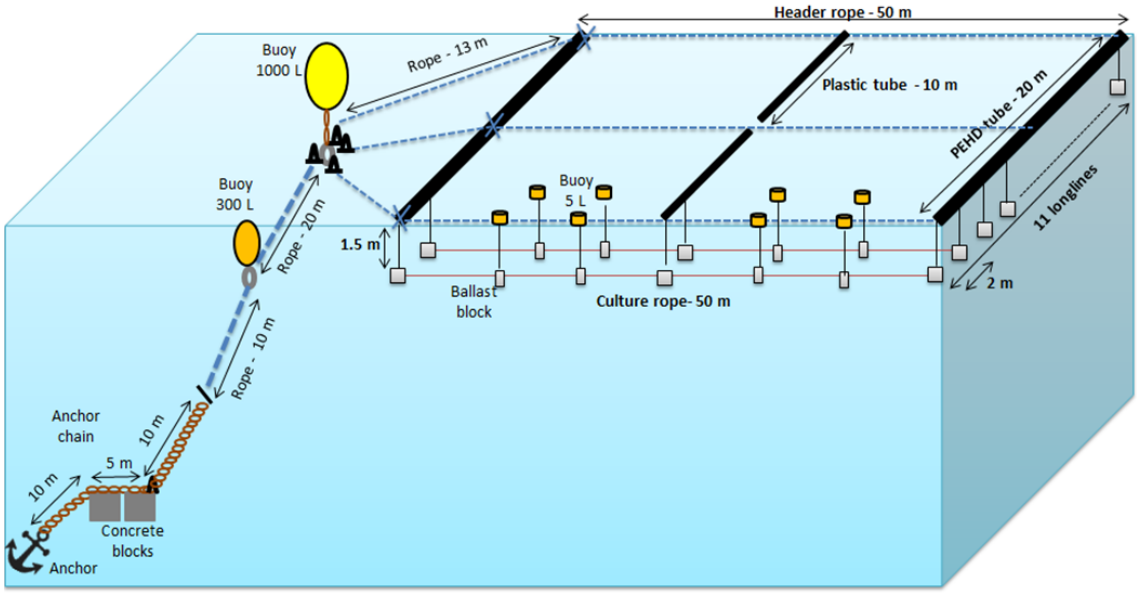

Figure 14. Raft system developed at CEVA (diagram reproduced from Taelman et al., 2015).

In 2014, a license for IMTA production was obtained. The aim is to grow salmon, mussels and kelp seaweeds at the same current site. IMTA is considered as (i) more eco-friendly than only fish farming as fish wastes are remediated by shellfish and seaweed and (ii) more economically profitable than only seaweed farming as fish and shellfish are high-value products, adding weight in the business model.

Case study: National University of Ireland Galway, Carna Research Station, Co. Galway

The NUIG EnAlgae pilot on-growing site is located on the west coast of Ireland, approximately five hours drive south from the Carna Research Station hatchery and is situated in Ventry Harbour, Co. Kerry (Figure 15). It is an 18 ha. licensed site (N 52.115883, E 10.356623) in commercial use and is located to the western side of Ventry Harbour, south and east of Cuan pier. The site is 200 m x 900 m, with the length/main orientation lying NW/SE. The Ventry Harbour site is approximately 7 m deep at the NW end, and remains at this depth for at least half of the length of the site, before depth increases to 17-20 m at the SE end of the site. The substrate is mainly sandy, however boulders and seaweed are present in the furthest SE corner. The site is relatively protected from all but SE and N winds, but access can be difficult after stormy conditions due to a large swell that makes launching from Cuan pier difficult. The growth systems in use are 'traditional' longlines. EnAlgae has five longlines within the site, which can contain up to 45 lines. Each EnAlgae longline is 280 m long (header rope), giving a total header rope length of 1400 m (Figure 16). Distance between each longline is 10 m, and all longlines are placed in parallel with longest side of site (i.e. the lines lie NW/SE). Collectors containing juvenile sporophytes on culture string are wound around the header rope, with an average of seven collectors required to seed each 220 m header rope. For the EnAlgae project, longlines are most commonly seeded linearly (i.e. only the header rope is seeded, droppers or nets are not used). Header ropes have buoys (~23 L) placed every 14 m. Header ropes are attached to anchor rope and chain (~2 m), which is attached to concrete anchor blocks (1-1.5 t).

The species used at the Ventry Harbour site are *Alaria esculenta* and *Saccharina latissima*. Seeding method is either by spraying gametophytes onto collectors, or by allowing direct seeding by zoospores. Overall, the traditional longlines worked well at this site, which is why they have not been modified greatly over the course of the project. Regular visits to the site and a maintenance schedule helped greatly to anticipate failure of components before this occurred. An example of one major failure that resulted in a loss of many buoys from the site and the sinking (but not loss of) longlines was in the use of large metal swivel clips. These were used to clip buoys onto the header rope and was much speedier than tying them on at deployment time. However, during significant '100 year' storm events during winter 2013/2014, almost all of these clips sheared off, with instant loss of buoyancy to the line. In contrast, all buoys tied on with rope weathered the storms and remained attached. Over the years it has been recognised that simpler longline equipment, with fewer inflexible (i.e. metal) parts survived much better from year to year.

Figure 15: NUIG on-growing site location in Ventry Harbour, Co. Kerry, Ireland.

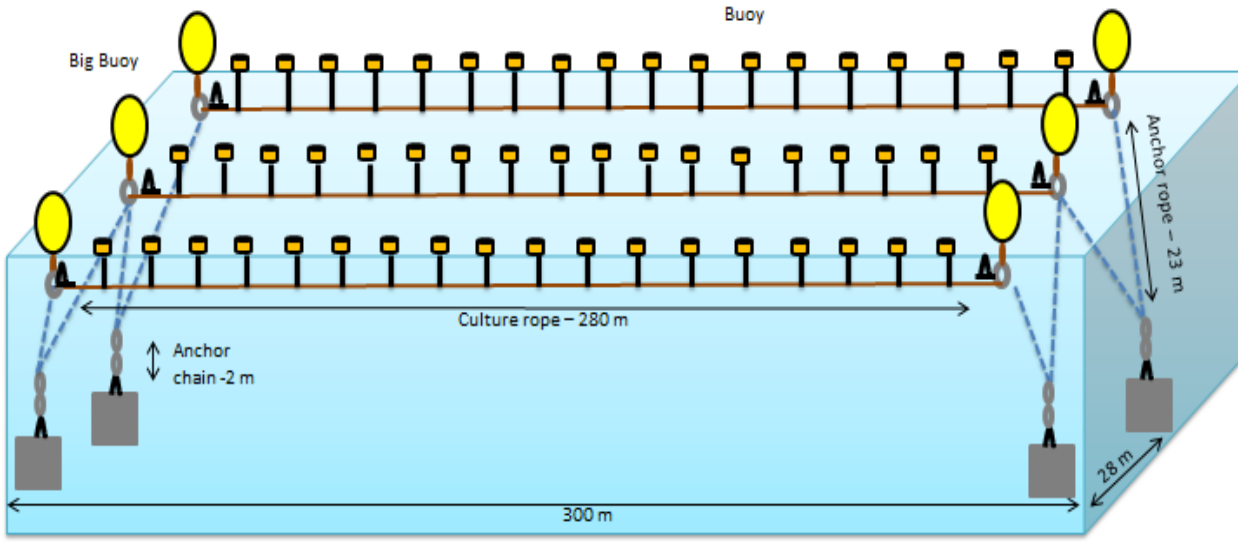

Figure 16. Traditional longline system developed at NUIG (diagram reproduced from Taelman et al., 2015).

2.2 Licensing and Permits

The licensing and permits regarding seaweed cultivation and harvesting will vary according to region and local Government. A full breakdown of the licensing and permits is in the EnAlgae WP2A10.01 Report - Regulations and Permitting concerning algal cultivation in North West Europe (Parker *et al*., 2014), with a case study of the QUB EnAlgae pilot site in this Appendix. Briefly, some of the main considerations for obtaining a cultivation license will be:

- Contact relevant local authority for permission to use seabed
- Contact relevant local authority for license to cultivate seaweed
- There may be a formal assessment of the impacts on:
    - Navigation
    - Environment (e.g. seabed, light penetration, nutrients, other flora and fauna, hydrodynamics)
    - Other users of the sea (e.g. fishermen, aquaculture, recreational users, tourism)

3 Best Practices for Macroalgae Cultivation

This section will detail the processes involved in the collection of fertile wild material to use as starter seed for cultivation, how to prepare this material, induce reproduction and maintenance for growth of juvenile plants and how to deploy juveniles at sea. The process is roughly split into two parts: the hatchery phase and the on-growing phase. Depending on the resources available and methods chosen for seeding and deployment, the amount of time each phase takes can vary. However, the timeline below (Figure 17) gives an indication of the yearly breakdown of work for kelp deployed in October and harvested in June/July.

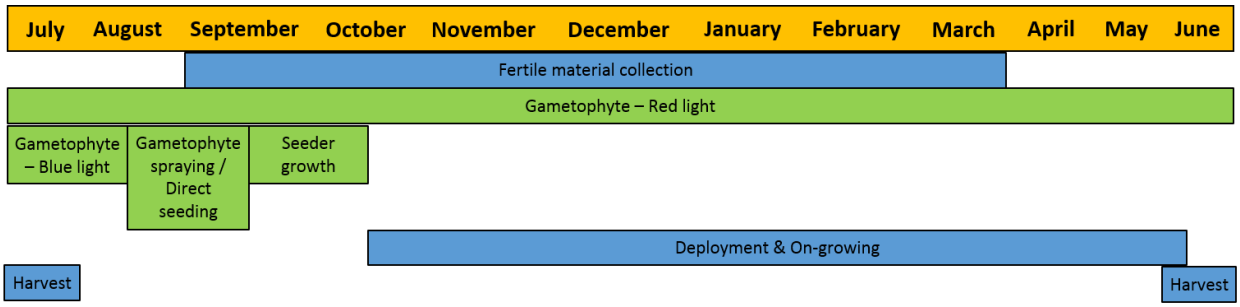

Figure 17: Ttimeline overview of kelp cultivation activities. Green boxes indicate Hatchery *phase activities, blue boxes indicate field-work (algae collection) and* On-growing *phase activities.*

In the UK and Brittany, fertile kelp can be collected during the autumn through to spring. Depending on the species and location, fertile material can be found during the summer e.g. *S. latissima* can be found in Northern Ireland during December through to July, so it is best to survey your sampling area to see when is the best time for collections, or contact your regional seaweed expert for more information (available via the Algal Information Network (AIN), http://www.algae-network.eu/).

Once fertile material is collected, spores can be kept in red light gametophyte culture all year round. Depending on the seeding method and deployment time, red gametophyte cultures need to transfer to blue light prior to seeding, or fertile material can be directly seeded onto seeders or rope. Once juveniles have had sufficient time to develop on seeders in the hatchery, they can then be deployed at sea and left until harvest (usually around June – July time).

3.1 Hatchery Operation and Maintenance

3.1.1 Species Selection

Macroalgae species selection is mostly dependent on two factors:

1. what species are local to the area you intend to cultivate in
2. what you intend to do with the biomass i.e. what end-product you require

For all locations in NW Europe, as yet there has been no research carried out on the impact of large-scale seaweed cultivation on local wild populations (e.g. genetics, disease transfer and non-native species transportation), and so a precautionary approach is taken whereby only local strains of a species can be cultivated. This means for each site, either direct seeding of fertile wild material must be carried out, or gametophyte cultures of local fertile strains can be maintained and used for cultivation when ready.

For point 2, as different species of seaweed have different physical and biochemical properties, each one may have a different optimal use after harvest (see Figure 1). The most commonly cultivated seaweeds in NW Europe can be found in Table 1 with some of their most common uses. Depending on the type of local species, the end product may be restricted if it requires a species of kelp not local to the intended cultivation site.

Table 1: Some commonly cultivated kelp species, their usage and location of cultivation.

Kelp species	Usage	Cultivation location
Alaria esculenta	Food (human and animal), Cosmeceuticals	Ireland, UK (Northern Ireland, Scotland), France
Laminaria digitata	Food, Cosmeceuticals, Abalone food	Ireland, UK (Northern Ireland, Scotland), France
Laminaria ochroleuca	Cosmeceuticals	France
Saccharina latissima	Food (human and animal), Bioplastics	Ireland, UK (Northern Ireland, Scotland), France
***Undaria pinnatifida* (not native)**	Food	France, Spain

Undaria pinnatifida, also known as Wakame, is listed in the table because it has been the most cultivated species in terms of volume in Europe, particularly in France and Spain (Peteiro and Freire, 2011). However, this species is not native to North West Europe and most countries prohibit its cultivation. In 2012, Ifremer, the French research institute for exploitation of the sea, recommended the prevention of further development of Wakame cultivation along French coastline (Ifremer, PDG/DCB/2012-05). Thus, only the current existing farms can continue to produce this species and it is likely that these licenses will not be renewed.

3.1.2 Hatchery Preparation for Seeding

It is best to have the hatchery facility fully set up and well stocked prior to sampling for fertile material and in readiness for seeding lines (Figure 18). The seeding method chosen will influence the amount of advance preparation required. For both methods, the sampling protocol for fertile material is in SOP 2.2.1.

Direct Seeding

The necessary requirements are a cold room/seawater maintained at 10°C, lights on a 12:12 light:dark cycle, tanks to hold header rope coiled or seeders, nutrients and equipment for doing a spore release from fertile material. In the case of seeding onto seeders/collectors, these seeders need to be prepared prior to seeding.

Gametophyte seeding

The necessary requirements are a cold room/seawater maintained at 10°C, lights on a 12:12 light:dark cycle, tanks to hold seeders, nutrients and equipment for doing a spore release from fertile material. Once the spores are released and developing under red light, then the seeders will need to be prepared.

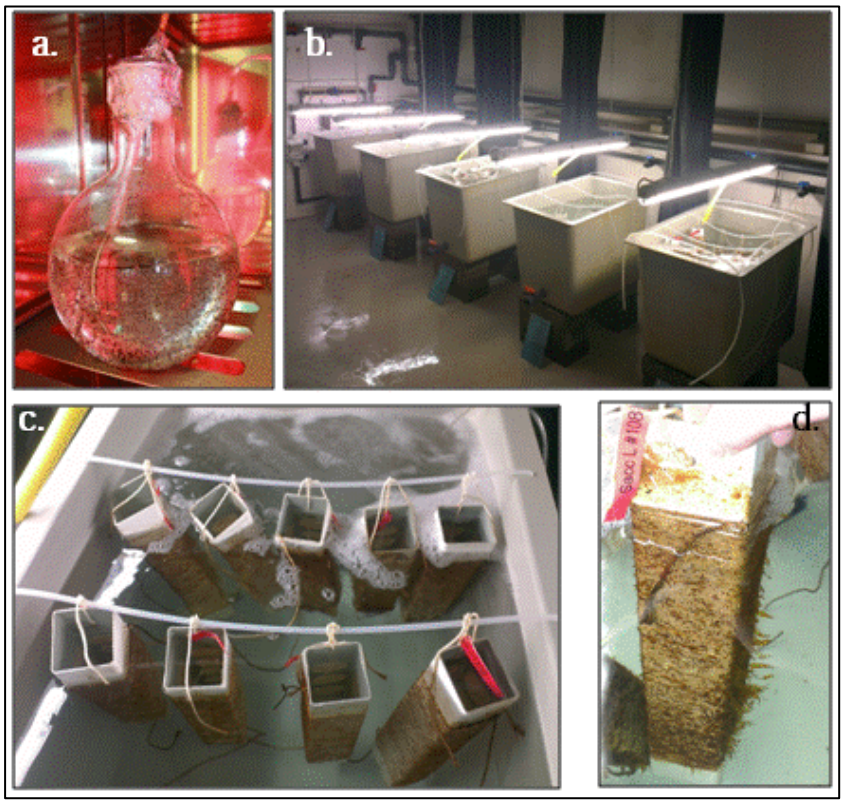

Figure 18: Current QUB hatchery facility. a: kelp gametophytes in flask; b: Light and seawater tank setup; c: seeders in tanks; d: mature seeder ready for deployment.

It is generally good practice to have a cupboard full of clean autoclaved glassware (flasks, beakers, stirrers), with the openings closed to ensure the insides are kept as sterile as possible. A selection of glass and plastic pipettes, tubing, microscope slides, coverslips, gloves, cotton wool, autoclave tape, phosphate-free laboratory detergent (e.g. Decon-90), bottle brushes, cleaning cloths, laboratory roll, parafilm, petri dishes (varying sizes), tin foil, scalpels/blades and scissors should be kept well stocked and accessible. We have also found it is useful to have a back up air supply (an aquarium pump is sufficient) in case of an air failure, and spare bulbs.

If making up your own nutrient media, it is important to ensure you have adequate stocks of all ingredients; likewise if using pre-prepared media, ensure there is enough for any water changes required. Ethanol is also required for sterilising and wiping down workbenches, there may be a license required to store this onsite which you will need to check with the local authority.

3.1.3 Seeding Strategies

Seeding strategies will vary depending on the site used, the hatchery facilities and timeline of deployment.

Direct seeding is often the cheapest and simplest seeding method, as once the fertile material is cleaned and prepped (SOP 2.2.1), spores can be released directly into seawater containing either ropes or seeders (SOP 2.3.2). The downside of this method is that the timing of seeding is dependent on access to fresh, fertile material – if there is no material to be found, seeding cannot happen. One way around this is to artificially induce fertility in fresh, infertile material (SOP 2.2.6). In this way, it is possible to ensure access to fresh fertile material year round, although it is much more labour and resource intensive than collecting from the shore. Another downside of the direct seeding method is that more fertile material is required to do the seeding as there is no possibility of bulking up gametophyte cultures in flasks.

Gametophyte seeding uses the gametophyte stage in the kelp lifecycle. When zoospores are released from fertile material (SOP 2.2.2), instead of attaching directly onto seeders or rope, they are kept in culture in flasks of aerated seawater medium. When the cultures are kept under red light (Figure 19), the gametophytes will increase in density through cellular division, and will not become fertile. After keeping the gametophyte cultures under red light for a minimum of 3-6 months, reproduction and growth of juvenile sporophytes can begin under blue light (SOP 2.2.5). This stage takes at least 2-3 weeks, before the culture is ready for spraying onto seeders or rope (SOP 2.3.1). While this whole process takes longer and is more labour intensive and expensive due to the hatchery maintenance phase, there is greater control over the timing of seeding and deployment and it is possible to maintain selected kelp strains for cultivation instead of relying on use of wild types as in direct seeding methods.

Figure 19: QUB Cultivation Cabinet. a: closed unit with temperature reading, timer switches and alarm (lights inset into doors); b: flasks in red (top two shelves) and blue light (bottom shelf) stage with individual air supply.

The type of seeding substrate (e.g. culture string or rope) used is also dependent on facilities and budget. Culture string seeders/collectors will allow a greater amount of sprayed material to be maintained in a small space, but do take longer to prepare (SOP: 2.3.1) and deploy (SOP: 3.1). Seeding directly onto rope is much easier for deployment at sea, but requires a larger hatchery space and seeded ropes are more difficult to transport to sea without damaging juveniles.

3.1.4 Culture Maintenance

To maintain effective and robust cultures, avoiding contamination and maintaining a stable hatchery environment are key to successful culture maintenance. There is a wealth of information available on good culture techniques; one recommended book is Algal Culturing Techniques (2005). The following information below is a summary of key issues encountered by EnAlgae staff.

Contamination

It is very easy for biological contaminants to make their way into the culture system, whether in the gametophyte/flask phase or in the sporophyte/tank development stage. While it is not possible to eliminate all contamination from a mass release of spores from wild tissue and the process is not aseptic, it is possible to create very clean and healthy gametophyte cultures using care in cultivation processes. The most common sources of contamination include the culture medium (sea water and nutrients), the air (from the air supply as well as the environment), the culture vessel and the starter culture.

Common contaminants include a filamentous brown algae called *Ectocarpus spp.*, a green algae from the Ulvales family, and diatoms (Figure 20). For brown and green algal contaminants, the most effective practice is to ensure adequate seawater filtration and to practice (close to) aseptic techniques and avoid cross-contamination as much as possible. It is beneficial to add Germanium Dioxide (GeO_2) to the nutrient media, which will suppress diatom growth (Shea and Chopin, 2007).

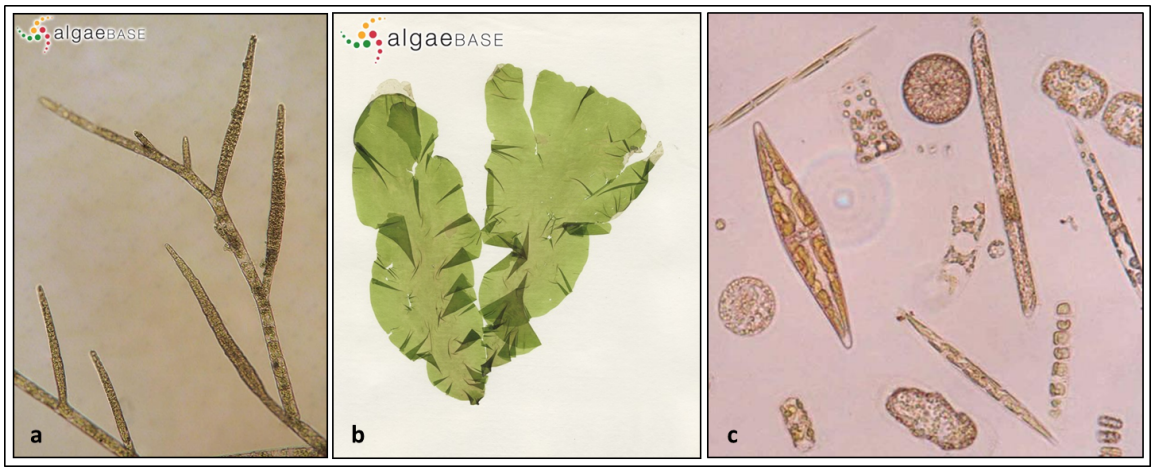

Figure 20: Three common algae culture contaminants. a: Ectocarpus siliculosus *(Dillwyn) Lyngbye; b:* Ulva lactuca *Linnaeus; c: selection of marine diatoms (taken from Marine Scotland blog http://blogs.scotland.gov.uk/coastal-monitoring/2012/02/10/monitoring-marine-phytoplankton-4/).*

Basic good laboratory practice can reduce the establishment of many of these contaminants. When doing a spore release from wild material, it is best to minimise the amount of non-fertile tissue present, the cleaning stages have been properly carried out and the culture flasks are properly prepared and ready to go. Having all necessary equipment close to hand during any procedure will reduce the amount of time required and the possibility of contamination. During water changes, it is best to have everything prepared in advance and be sure that all equipment, media etc is available. Once culture flasks have

been established, don't transfer any cotton wool, pipettes or tubing between flasks and replace tin foil caps if damaged. When the juvenile sporophytes are developing in tanks, keep batches of seeders together and wash hands when moving from one tank to another. If there is contamination of seeders by *Ectocarpus* spp. or Ulvales, QUB staff have found that it can be greatly reduced by leaving the seeders in the open air for a short period (10-15 mins) during water changes and reducing the light intensity during the tank cultivation stage.

Stable environment

Maintaining a hatchery and/or culture cabinet temperature of 10^{o}C (SOP 2.1.2) with a thermostat alarm, maintaining a clean and steady air supply (SOP 2.1.3), keeping lights and light fittings in good order to ensure they provide adequate lighting (SOP 2.1.4), keeping filters cleaned, changing the water (SOP 2.1.1) and keeping tanks cleaned and regular monitoring of cultures are all essential activities to maintain an optimal hatchery environment. It is good practice to draw up a list of daily, weekly and monthly activities to ensure checks are occurring and recorded (see Appendix).

3.1.5 Culture Monitoring

There are several points during cultivation that biomass can be sampled, both in the hatchery and during on-growing at sea. During the hatchery phase, it is good to build up a profile of each new culture started, keeping track of spore density from initial release to what is finally sprayed onto seeders. This can be useful to track growth, identify periods of slow growth or possible contamination factors and know when sub-cultures need to be started.

Hatchery

1. At the spore release phase: zoospores counted using a Coulter Counter or haemocytometer (SOP 2.2.3). This is beneficial to keep a record of the density of zoospores released from fertile material, particularly when identifying the best time for collection of fertile material.

2. At the gametophyte phase: gametophytes counted using the Wintrobe tube method (SOP 2.2.4). This method is useful to determine the density of asexual gametophytes (in the red light phase) and track the speed at which a culture is bulking up. It is useful in identifying particularly fast growing cultures and in indicating when sub-culturing (splitting a dense culture into two less dense ones) is needed, to avoid potential crashes.

3. At the seeder phase: regular counts of plantlets on string/seeder (SOP 3.1.4). This is useful during juvenile development to track the number of plantlet attached and will give an indication of seeding success and density of plantlets to be deployed.

At sea

1. Regular samples of plants on rope (SOP 4.1). This biomass monitoring will provide information of number of plants deployed, an indication of loss of plants during the on-growing phase and an estimate of the final biomass to be expected.

3.1.6 Hatchery Shutdown

Post deployment, the main tasks are to clean and sterilise all tanks and seeders, and store equipment for future use. If maintaining gametophyte cultures year-round, these can be kept in a smaller incubation cabinet to minimise space and energy used. Stocks of nutrient media, string, cable ties etc. should be checked and maintained, and any replacements made in preparation for the next hatchery and deployment season.

3.1.7 Other Considerations

A considerable energy investment is required to maintain cultures year round. To minimise this, it is best to use a smaller separate incubation cabinet where stock cultures can be kept in low levels of red light, thus minimising, but maintaining growth for when cultures are required again.

In addition, as light is an integral part of cultivation, use of low energy LED bulbs is beneficial. The QUB hatchery uses a combination of fluorescent strip lights and LED strip lights, and intends to move to solely LED lights in the future (Figure 21).

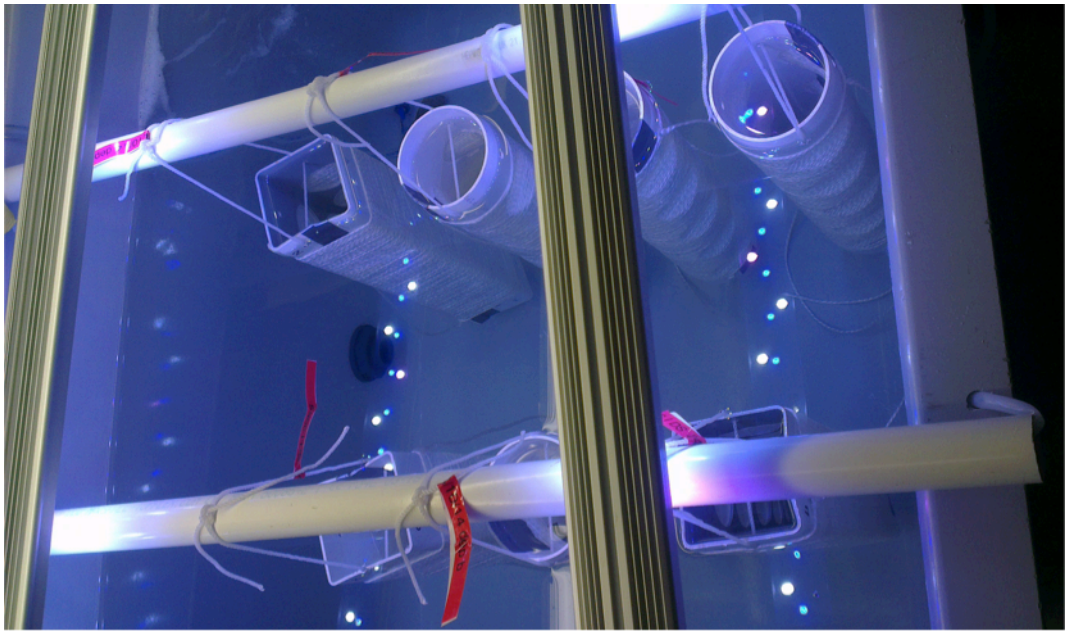

Figure 21: LED strip lights (blue and white marine grade) over a culture tank in the QUB hatchery.

3.2 Deployment and On-Growing

3.2.1 Deployment

Deployment of seeded material is best carried out during the late autumn/winter months, in keeping with the biology of the seaweed cultivated. Juvenile kelps tend to establish around this time in the wild, as this will allow for optimum environmental conditions for settlement – low light, low temperature, reduced competition for nutrients and low epiphyte risk. If possible, it is best to have the anchors deployed at the site in advance of seeder deployment, as this will minimise the time seeders are kept out of the water. On the boat, ensure that all header rope, buoys, tying rope, knives, fid/marlinspike (for splicing rope) and cable ties are present. It is useful to take a bottle of seawater to soak seeders if they begin to dry out on the journey. Finally, all health and safety guidelines should be followed – adequate warm and waterproof clothing, lifejackets, proper footwear and a first aid kit are essentials. The weather for deployment needs to be calm with no to very low wind, with a calm sea-state and no strong tides. Ideally it will be overcast and dry, but it is possible to deploy on bright or wet days.

On the day of deployment, seeders will be removed from hatchery tanks and placed into a transportation box (SOP 3.1.1 and 3.1.2). This can range from cool boxes lined with damp tissue (QUB) to large tanks with individually wrapped seeders if the journey is long (NUIG). It is also possible to wrap seed string around rope on land and then transport this to sea (CEVA).

Once at the site, the seeders are deployed onto the header rope and directly into the seawater. The final technique for this will depend on the size of boat and facilities available, and two methods are described in SOP 3.1.3. The boat is hooked up to the first anchor buoy at one end of the header rope. The rope is fed through the seeder, the culture string is cut at the top end (closest to the end of the header rope) and tied around. At this stage it is useful to splice the string through the header rope and cable tie the string to ensure it will not work loose. Once the string is secured, then the bottom of the seeder is held steady (**without** touching the string) and the header rope is fed through the seeder, over the end of the boat (Figure 22). The string will unravel around the rope and sink into the water. The speed can be controlled by the boat, with occasional stoppages to attached buoys at pre-marked spots on the header rope. It is useful to have several people for this method of deployment – one to hold the seeder, one to feed the rope and one to ready and attach the buoys. If more than one seeder is required per header rope, then several can be attached at the start and simply cut off once all the string has been deployed. When the full header rope is deployed, it is attached to the anchor buoy and the next can be started.

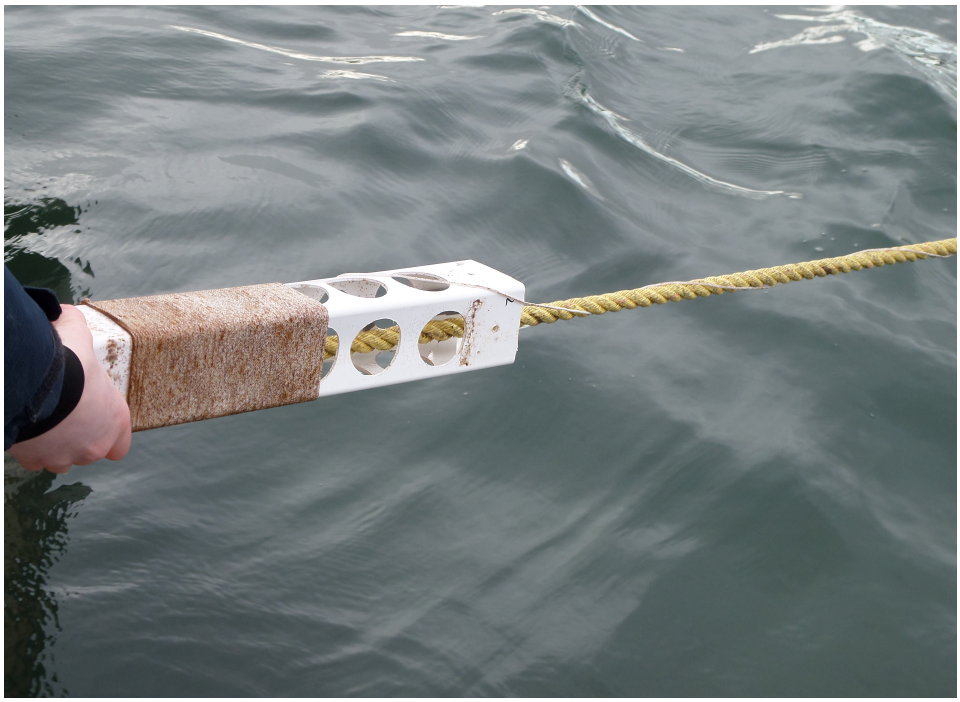

Figure 22: Seeder being deployed onto header rope at sea.

3.2.2 Monitoring and Maintenance

It is good practice to monitor the site and seaweed growth during the on-growing season, both to determine if there is any damage to the lines and to provide a real-time estimate of the final biomass yield. For site monitoring, this can be carried out as frequently as resources allow, but no less than a minimum of once a month and immediately after storm events (SOP 3.2.1). With the infrastructures described earlier in this document, the key issues tend to be loss of buoys, culture string breaking or entanglement of lines, although these all occur very infrequently. Splicing buoy rope through the header rope and securing knots with cable ties can greatly reduce any loss. To avoid culture string breaking, ensure the tension is neither too tight nor too loose when deploying, and use a good quality string. If string is loose, then cable tying it to the rope will minimise breakage. To minimise line entanglement, ensure the distance between header lines is appropriate and keep lines clean of any floating seaweed

and debris which may cause lines to tangle. At very low tide it is possible to see header ropes merge together but as long as the lines are clean they will separate again as the tide rises.

Monitoring of the environmental conditions at the on-growing site are useful, and may be a requirement of obtaining a license. The key conditions are: seawater temperature (SOP 3.4.1), underwater PAR (SOP 3.4.2), seawater nutrient concentrations (SOP 3.4.3) and turbidity (3.4.4). These are the seawater factors which can influence growth, and long term tracking will provide an invaluable database for building up an optimised kelp strain selection for a given at-sea site. They are also important considerations in assessing the influence of cultivation on the surrounding environment, and will all be useful in future Environmental Impact Assessment (EIA) applications.

Biomass monitoring of the seaweed is key to track the growth and gain a good indication of what the final harvest will be. It is not essential, but it is good practice, especially when establishing a seaweed farm. Sampling can be carried out monthly or more frequently, and as with all boat work, it is best to do it on a calm, overcast day with little or no wind (Figure 23). Sampling and analysis methods are detailed in SOPs 4.1 to 4.4.

Figure 23: Biomass sampling of Saccharina latissima after five months at sea.

3.2.3 Harvest

The final harvest is usually around June-July, when maximum biomass has been reached and before there is sufficient warm sunny weather to promote epiphyte communities. The method is described in SOP 3.3. It is possible to do an earlier harvest, or “crop” where the kelp fronds are cut just above the stipe. This can happen in mid-late spring, and the remaining biomass will regrow for a later full harvest in summer. The key factor in timing of harvest is ensuring that the seaweed is harvested just before it begins to degrade in the seawater. If regular monitoring is occurring, then it is easy to pinpoint the best time for harvest, but as a general rule: a very good spring and early hot summer will necessitate an earlier harvest, with a poor spring or late summer start will permit a later harvest.

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Appendix 1: Case Study - Licensing and Permits, Queen's University Pilot site at Strangford Lough

Appendix 1.1. Harvesting of Seaweed

The Environment and Heritage Service are the lead agency advising and implementing government's environmental policy and strategy in Northern Ireland, and are responsible for enforcing any and all legislation. It is important to make the distinction between harvesting wild seaweed and cultivation of seaweed for harvest.

Seaweed can either be harvested manually using hand held tools or mechanically using machinery. In Northern Ireland, wild seaweeds are largely harvested using hand held tools, a technique which the EHS considers to be the least damaging ecologically, as mechanical harvesting techniques could threaten the marine ecosystem, undermining the sustainable use of the seaweed resource (Environment & Heritage Service, 2007). Seaweed harvesting is not currently regulated through specific licensing or permit system but is controlled mainly by impact on the environment, and falls under (i). The Environment (NI) Order 2002; (ii). The Habitats Regulation 1995; (iii). Wildlife Order 1985 and (iv). Marine Licensing (Part 4) (Environment & Heritage Service 2007). Designated sites of protection can be found on the EHS website; owners and occupiers of land within a site (under or considered protected) must obtain written consent by EHS before undertaking 'potentially damaging activities' – seaweed harvesting is considered under this (details of plan required, including baseline info, harvesting plan and measures put in place to minimize damage).

Appendix 1.2. Seaweed Cultivation

Currently, the cultivation of seaweed doesn't require any license relating specifically to the growth of algae, but only regarding the placement of growth systems in the water/on the seabed. There are two main steps to obtaining permission to cultivate seaweed:
1. Obtain permission to use the seabed – lease area from Crown Estate. The Crown Estate manage all the seabed from the Low Water Mark to 12 nautical miles offshore. To conduct any sort of activity on the seabed in this area, a lease must first be obtained. The general process for this is as follows:

- Contact your local Crown Estate representative/Aquaculture division
- Send grid references of the proposed site, along with a description of what cultivation work you intend to carry out and the growth system to be used.
- When the lease has been obtained, then approach the relevant Permitting authority (currently DOE Marine Division).

Marine Division is the branch of the Northern Ireland Department of the Environment (NI DOE) responsible for issuing licenses for aquaculture. As seaweed cultivation itself is not regulated and so does not require a license for the organism, the license needed is for placement of equipment on the seabed. The steps for this process are as follows:

- Inform Marine Division of lease obtained, and complete an Application for Marine Construction Works, Land Reclamation or Beach Replenishment in the Territorial Sea and UK Controlled Waters adjacent to Northern Ireland.
- A Marine Navigational Risk Assessment (MNRA) will need to be carried out to determine risk cultivation site poses to ships and boat traffic.

- You may be requested to conduct an Environmental Risk Assessment, or to survey for the presence of any key species which may preclude siting a seaweed farm in your proposed area; e.g. Strangford Lough is home to a protected species of mussel, the horse mussel *Modiolus modiolus*. Any new site leases can only be used once appropriate assessments are carried out to ensure no *Modiolus* are present in the area and there will be minimal negative environmental impact.
- A public consultation will begin, with details of the project being advertised in local newspapers and a consultation period (usually about one month).
- At the end, Marine Division will take a decision on the license permit for placing equipment on the seabed.

Once both a lease and license have been obtained, then cultivation can begin. It is possible to obtain a Section 14 Scientific Exemption for cultivation, which will not require a MNRA or specific license to install a growth system, but this is only granted in certain circumstances and the seaweed grown cannot be used for commercial purposes:

NIEA (2011): in most cases the removal or addition of 'scientific equipment' does not require special licensing. This does not apply if:

1. It is likely to cause significant effect on Special Area of Conservation designated under the EU habitats directive or Special Protection Area designated under the Birds Directive.
2. Likely to have 'significant effect' on a Ramsar Site (wetlands of international importance).
3. Capable of affecting the protected features of a Marine Conservation Zone (MCZ) or any ecological or geomorphological process on which the conservation of any protected features of a MCZ is dependent.

Each case is taken individually and so it is the decision of the Marine Division to decide as the applications are made. In relation to significant effect/ impact for protected areas, a test of Likely Significance is the tool that is used to determine if there is a likely significant effect on a European Site. This method screens the proposed activity by measuring the potential impacts on the features of the site whether or not it is likely to have a significant impact. This will take into consideration details such as proposed mitigation measures as part of the methodology and any other details that may have a bearing on the outcome, for example, the time of the year when the activity will be carried out (NIEA, pers comm. 2014). It is the role of the applicant to declare any work to either NIEA (inshore waters 12 nautical miles) or the Joint Nature Conservation Committee for off shore waters.

Appendix 2: Sample QUB Protocol Guides

Appendix 2.1. Recurring Tasks in the Macroalgae Labs

Recurring tasks in the Macroalgae Labs

Daily

- Visual inspection of electronics/room and housekeeping (10 mins)
- Swirl cultures samples for aeration (5 mins)
- Visual inspection of the plantlets to make sure air/light ok and no contamination (5 mins)

More than once a week

- Water change of tanks – cleaning, disinfecting, removal of samples for microscope inspection, refilling and adding culture medium. Mon and Fri. (1hr 30 mins)
- Washing, refilling and autoclaving any glassware used (1hr 30 mins)
- Inspect microscope samples from seeders. Perform density counts and measurements Inspection: 30 mins – 1hr.

Weekly

- Perform density counts and measurements on seeder samples; measurements: approx half a day depending on sample size and no. of seeders.

Fortnightly

- Change culture medium of macro cultures (2 hrs)
- Take samples from medium changed macro cultures for wintrobe density analysis (up to half a day depending on number of cultures)
- Photograph blue light samples to track change (1 hr)
- Clean filters on UV

Monthly

SEASONALLY: harvesting and processing longline samples (4-5 days). From March – July

Time consuming but infrequent tasks:

- Transferring cultures to blue light
- Making seeders
- Spraying seeders
- Modifying, cleaning and stringing seeders
- Washing, burning and drying seeders
- Shore collection of fertile material
- Spore releases and subsequent density counts
- Preparing boat and materials for boat work

	Mon	Tue	Wed	Thu	Fri
Wk1	Water change Density/measurement counts on seeder samples	Culture media change Wintrobe density analysis	Wash glassware and prepare for autoclave	Clean UV filters	Water change Visual inspection of seeder samples
Wk2	Water change Density/measurement counts on seeder samples				Water change Visual inspection of seeder samples
Wk3	Water change Density/measurement counts on seeder samples	Culture media change Wintrobe density analysis	Wash glassware and prepare for autoclave	Clean UV filters	Water change Visual inspection of seeder samples
Wk4	Water change Density/measurement counts on seeder samples				Water change Visual inspection of seeder samples

Appendix 2.2. Hatchery Daily Checklist and Troubleshooting

Hatchery Daily Checklist and Troubleshooting

Check chiller unit is operating and room is between 9-12 ^{o}C.

Tanks with plantlets should have:

- Overhead lighting
- Aeration
- Plantlets should be covered by water

Tanks with plantlets should have overhead lighting during daylight hours. If lights are not on the controls are in the box below. Check timers are correctly set before changing bulbs. Hold light switches down to dim, push once for off/on.

Aeration is controlled at the blue taps on the grey pipes behind the tanks. Green control taps attached to aeration line can be used to fine tune air supply. If central air supply has failed, attach tanks to one of two mobile air pumps on the shelf above the sink. In this scenario the incubator will also require an air pump to be attached to its air lines.

If water level has dropped, check outflow tap on tanks for leaks. To top up water supply, first turn on UV steriliser on back wall, then turn the taps attached to yellow hoses above individual tanks. Run the standing water from the pipes onto the floor initially to ensure that tanks are receiving UV sterilised water, and not residual water lying in the pipes. Turn UV off after use to prevent bulbs from shattering.

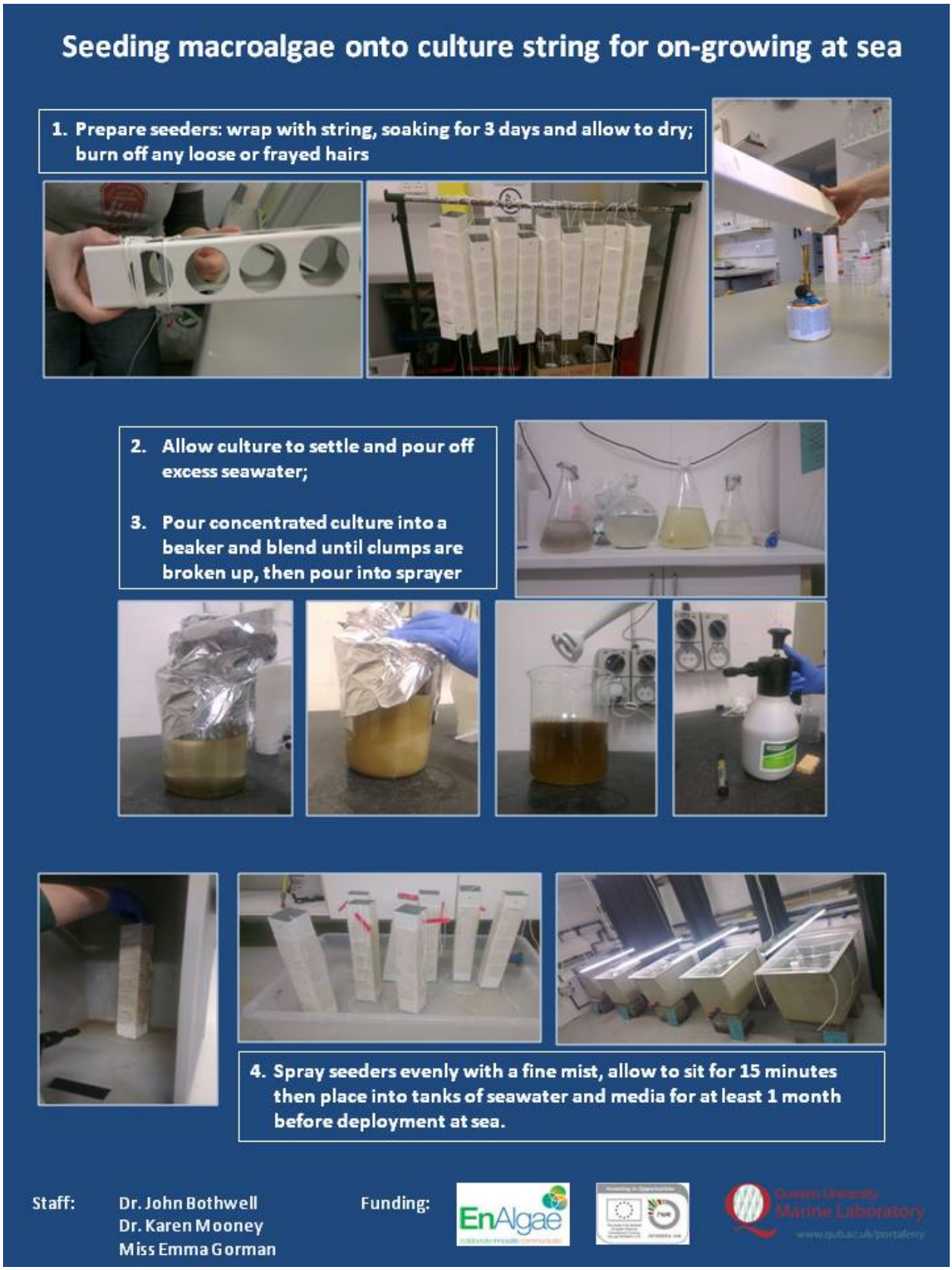
Gametophytes in Incubator should have:

- Aeration
- Light
- May need a shake to dislodge any clumps which have become stuck to the glass

Air feeds into the incubator from the top of the right hand side panel. If main air supply fails connect a mobile air pump to the two inflowing lines. If main air supply is active but flasks are not bubbling, check that a connection has not dropped off one of the flasks (this may stunt aeration in all other flasks on the same line), and re-attach if this is the case. If there are no loose connections and main air is ok, but flasks are still not bubbling, check the white round filters on the line, as these may be clogged. Water can also block the lines, so allow it to drain from any line you observe water in.

If you need any help or advice, contact XXX on XXX.

Appendix 2.3. Seeding Protocol Poster



Investing in Opportunities

This project has received European Regional Development Funding through INTERREG IVB.

INTERREG IVB

EnAlgae is a four-year Strategic Initiative of the INTERREG IVB North West Europe programme. It brings together 19 partners and 14 observers across 7 EU Member States with the aim of developing sustainable technologies for algal biomass production.

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This report has been produced by Swansea University, lead partner of the EnAlgae project.
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