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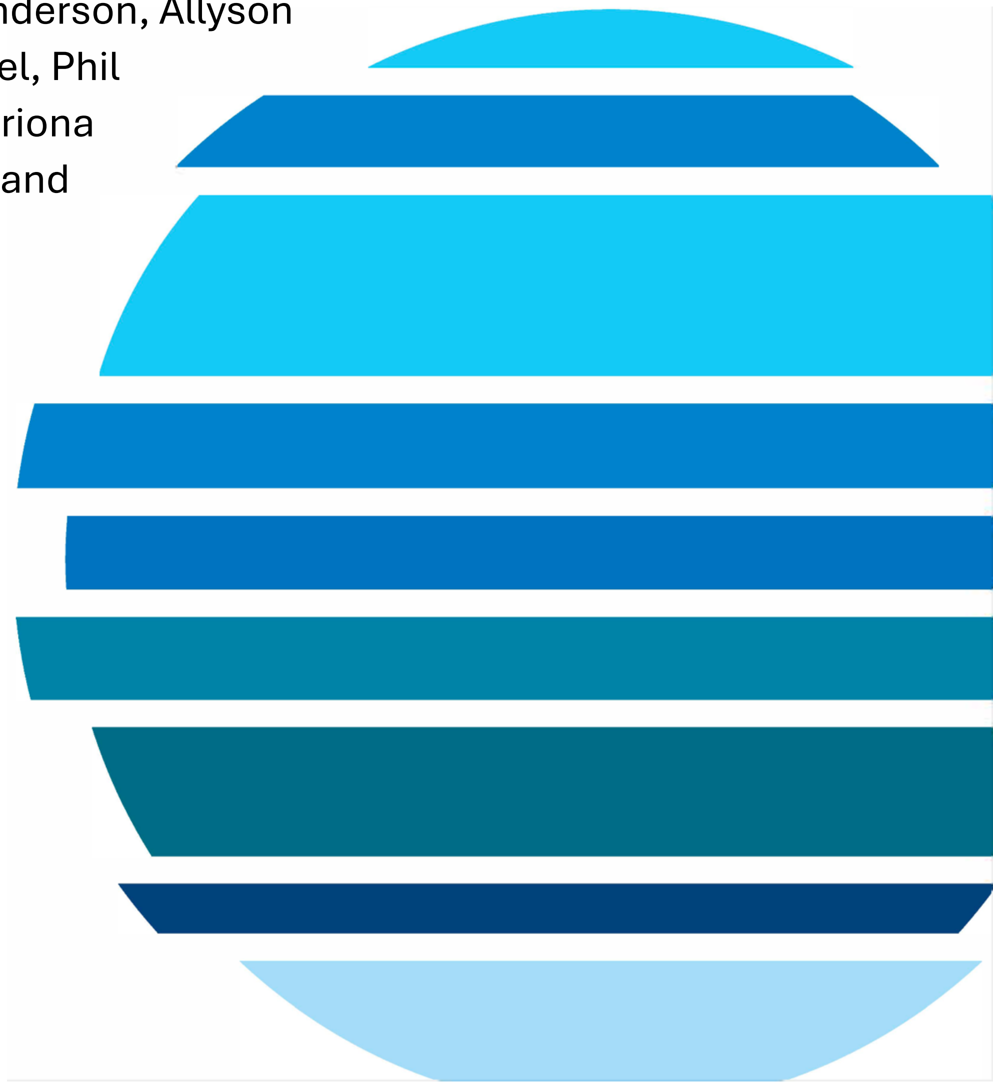
Aquaculture Production of Australian Laminarian Kelps:

A manual and research recommendations for *Ecklonia radiata, Lessonia corrugata,* and *Macrocystis pyrifera*.

Wouter Visch, Cecilia Biancacci, Glenn Farrington, John Craig Sanderson, Allyson Nardelli, Jakob Schwoerbel, Phil Lamb, Bradley Evans, Catriona L. Hurd, Alecia Bellgrove, and Catriona Macleod

May 2024



Seaweed Solutions

CRC-P

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Seaweed
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Executive Summary

This manual is as a comprehensive guide for farming laminarian kelps, specifically targeting species in southeastern Australia (*Macrocystis pyrifera*, *Ecklonia radiata*, and *Lessonia corrugata*). The manual covers various aspects of kelp farming, and it identifies knowledge gaps and opportunities for optimisation across the entire production cycle.

The manual provides detailed instructions on how to set-up and operate a kelp **nursery**, including the equipment needed. Detailing the production of seeded lines, the creation and maintenance of gametophyte stock cultures, and the appropriate nursery conditions. It explains how to monitor the growth and health of the seaweed during the nursery phase, and how to identify and address common problems that may occur during this stage, such as contaminants.

Concerning **at-sea cultivation**, the manual highlights the significance of site selection, out-planting, and monitoring of seaweed growth and biofouling of the produced biomass, along with the maintenance of the farm infrastructure. Recommendations are provided for transplanting seaweed from the nursery to the ocean. The manual also highlights the impact of buoyancy of crop and drag-forces, influencing equipment and farm design considerations. Knowledge of environmental conditions, such as seawater temperature, nutrient concentrations, light levels, and water motion, is crucial for site selection, offering opportunities for careful selection of farm sites supporting crop growth and development. Conducting small-scale pilot studies can be highly informative ground truthing the suitability of a farm site based on environmental monitoring data. Finally, it is important to comply with regulations and building positive relationships with other stakeholders when farming seaweed in the coastal environment.

Regarding **harvesting** and **processing** of the farmed kelp biomass the manual discusses drying techniques, considering the biochemical content of the produced seaweed. It highlights that processing methods may vary based on the intended final product. Processing seaweed biomass is essential for producing high-quality valuable seaweed products. Further research is needed to develop efficient and cost-effective processing techniques to enhance shelf life and product quality, such as mechanized washing and commercial-scale dryers, enabling seaweed farmers to produce high-quality products while minimizing costs.

This manual is a valuable resource for anyone interested in kelp aquaculture practices. While acknowledging the need for further research in this field, but the manual provides a solid foundation and guide for those interested in pursuing kelp aquaculture as a sustainable business.

Prelude

This manual is a comprehensive guide to the process of farming laminarian kelps, specifically targeting species native to southeastern temperate Australia. It covers various aspects of kelp aquaculture, including nursery operations, at-sea cultivation, harvesting, and processing of the produced seaweed biomass. An economic assessment of the aquaculture production of the studied kelp species was performed in association with this manual.

The nursery section provides instructions on how to set up and operate an on-land kelp nursery, including information on the equipment and materials needed, and the proper conditions for growing kelp spores, gametophytes, and juvenile sporophytes. The at-sea cultivation section covers topics such as site selection, out-planting and tending to the kelp crops, and monitoring the health and growth of the kelp as well as the farm infrastructure. The harvesting section describes appropriate techniques for collecting the kelp biomass and the processing section details the steps involved in turning the seaweed biomass into useful products by assessing how the biochemical content is affected by farming and processing practices (e.g., deployment/harvesting time, cultivation depth and/or site, drying method).

This manual is based on the results from the *Seaweed Solutions for Sustainable Aquaculture CRC-project* between industry partners Tassal Group Ltd, Spring Bay Seafoods Ltd, and researchers from the Institute for Marine and Antarctic Studies (University of Tasmania) and the DeakinSeaweed Research Group, Deakin Marine Research and Innovation Centre at Deakin University, to develop a sustainable Integrated Multi-Trophic Aquaculture (IMTA) model and support commercial seaweed production in Australia. Cooperative Research Centres (CRC) Grants provide funding for medium to long-term (i.e., ~3 years), industry-led research collaborations with research institutions.

Chapter 1. Introduction

Large brown seaweeds (Ochrophyta, Phaeophyceae) are commonly known as the 'kelps' and include species in the orders Laminariales and Fucales, both of which provide significant ecosystem services (Bennett et al. 2015; Smale et al. 2013). Moreover, laminarian kelps, in particular, also have substantial global economic importance (Bennett et al. 2015; Steneck et al. 2002; Chopin and Tacon 2021) contributing to ~53% of the annual US$15.3 billion global seaweed-aquaculture industry (FAO 2023). Globally, kelp aquaculture has expanded significantly in recent years, with a wide array of products already on the market, and new kelp-based products constantly in development (Gutierrez et al. 2006; Holdt and Kraan 2011). Whilst kelp aquaculture has historically been focused in Asia, it is gaining worldwide interest (Naylor et al. 2021). There are many benefits of seaweed farming when compared to terrestrial primary production (e.g. seaweeds do not use fertiliser, pesticides, or need freshwater irrigation), and as such kelp aquaculture is now emerging in "non-traditional" regions of production, including Australia (Kelly 2020). In southeastern Australia, seaweed aquaculture practices build on research that identified various native species from amongst the unique and diverse Australian seaweed flora (47 reds, 31 greens, 24 browns) as suitable for the edible market (Sanderson and Di Benedetto 1988; Cumming et al. 2019; 2020b; Skrzypczyk et al. 2019; Hurd et al. 2023); whilst early cultivation trials of *M. pyrifera* and red seaweeds (e.g. *Gracilaria* spp. and *Gelidium* spp.) focussed on the supply of feed for abalone in the late 80s and early 90s (Scott and Sanderson 1994).

Kelp aquaculture is typically a two-stage process, with an indoor **nursery** (hatchery) phase, and an **at-sea** grow-out phase. Optimising the production of lines seeded with juveniles in the nursery is essential for successful commercial cultivation at-sea (Camus and Buschmann 2017; Su et al. 2017; Peteiro et al. 2019; Hu et al. 2021). There are several cultivation manuals available for commercially important kelp species non-native to Australia; including *Saccharina japonica*, *S. latissima*, and *Undaria pinnatifida* (Edwards et al. 2011; Flavin et al. 2013; Redmond et al. 2014; Rolin et al. 2016; Forbord et al. 2018). Cumming et al. (2020a) developed a preliminary handbook for the mariculture of the endemic Australian fucoid *Phyllospora comosa*, but this is currently held in commercial confidence. Whilst, Le et al. (2022) recently published a review on the culture practices to support the cultivation of giant kelp (*Macrocystis pyrifera*), to date, there are no cultivation manuals available for Australian laminarian kelp species that are of commercial interest (Kelly 2020) like the ones here identified: *Ecklonia radiata* (C.Agardh) L.Agardh, *Lessonia corrugata* A.H.S. Lucas, and *Macrocystis pyrifera* (L.) C.Agardh (Scott 2017).

Laminarian kelps have an alternating, biphasic, diplohaplontic **life cycle** with two morphologically distinct stages (Figure 1): a macroscopic diploid **sporophyte** stage and a microscopic haploid **gametophyte** stage. The cultivation of laminarian kelps is largely dictated by the environmental conditions required to support growth of each life stage and the life-history. In addition to growth, reproduction and life history phase shifts are to some extent controlled by the environment. See *Box 1 "Farming requires life-cycle control"* for a more detailed description of how the life-cycle of kelp affects its cultivation in a practical sense. In this manual, we provide the synthesis of the latest scientific literature in combination with our real-world experience gained through the *Seaweed Solutions for Sustainable Aquaculture CRC project* to provide new understanding on how to cultivate laminarian kelp species native to southeastern Australia. Furthermore, we provide knowledge gaps and highlight steps in the cultivation process where opportunities for efficiencies remain.

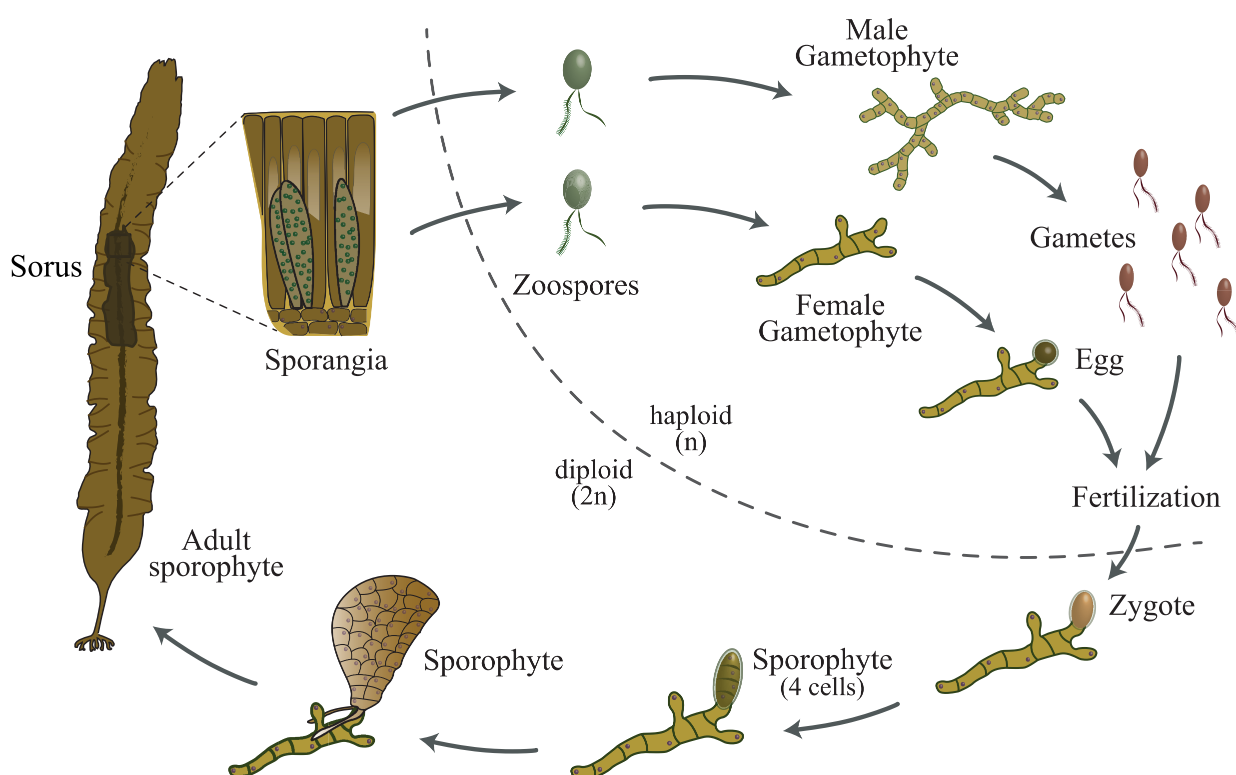

Figure 1. Kelps' life cycle. Mature sporophytes produce sporogenous tissue (a sorus) within which are uniocular sporangia that contain zoospores. Sporangia release zoospores into the surrounding water, and these settle on suitable substrates and germinate into microscopic male and female gametophytes. Gametophytes develop reproductive structures; males develop antheridia (sperm-producing cells), and females grow oogonia (egg-producing cells). The sperm fertilises the egg in situ within the female gametangium, and the zygote develops into a juvenile sporophyte, initially attached to the female gametophyte. Illustration adapted from Visch et al. (2019).

Box 1. “Farming requires life-cycle control: a practical guide to managing the life-cycle of kelps.”

The decisions made about the environmental conditions in the nursery, site selection, settlement substrata or seeding twine type, seedstock sourcing, and timing of deployment and harvest are all related to the biology of the kelp species being cultivated. This section describes the impact of the life cycle on the nursery and at-sea cultivation of laminarian kelps, including practical examples linked to variations in life cycle characteristics.

The laminarian life cycle includes two life stages, the **sporophyte** and the **gametophyte**. The transition from **spore** to embryonic sporophyte can occur in 3 to 4 weeks, depending on species and environmental conditions.

Mature sporophytes produce **sorus** tissue which contains the spore producing cells (**sporangia**) that develop on the surface of mature blades. Spore release can be artificially stimulated by desiccating the sorus tissue for >1 h and subsequently resubmerging it in sterile filtered seawater, whereby the outward-facing sporangia burst open and spores are released into the water (Figure 6E and Figure 6F). The flagellated spores swim away from light (negatively phototactic) towards suitable settlement substrata (e.g., a spool or frame with seeding twine), typically under dark or low-light conditions. The resulting substrata seeded with spores can then be further cultivated in a tank-based nursery system.

Spores germinate on the substrata into male or female **gametophytes**, which under white light and with sufficient nutrients, develop reproductive structures and undergo **gametogenesis**, continuing their life cycle. However, in unfavourable conditions, such as under red light with limited availability of trace-metals, gametophytes grow **vegetatively** and form filamentous cells that can be propagated by fragmentation. This allows for control of the timing of the nursery phase, independent of the natural reproductive cycle. Gametophytes can be stored long-term under red light and low nutrient conditions to be used for selective breeding programs and controlled gametogenesis when then given optimal light and nutrient conditions.

During gametogenesis, male gametophytes develop antheridia that produce sperm, while females grow oogonia that produce eggs. Vegetatively propagated gametophytes can be induced to reproduce by changing the culture conditions from **red to white light**, with high energy wavelengths in the blue spectrum. To improve the rate of gametophyte cells transitioning into sporophytes, it is recommended to break up gametophyte clusters into smaller filaments a few days before moving the cultures into white light, as the clusters can inhibit gametogenesis in some cells.

Distinguishing between vegetatively grown male and female gametophytes can be difficult, but the reproductive structures make it easier. In optimal culture conditions, **eggs** are extruded from the oogonia of female gametophytes after 10-14 days. Oogonia appear as darker coloured extrusions on female gametophyte cells (Figure 9). The eggs produce a sexual pheromone that triggers **sperm** release from antheridia, which are pale brush-like structures (Figure 9). The pheromone attracts the sperm towards the egg for fertilization. The egg remains connected to the oogonia ensuring orientation of the zygote and is essential for the survival and development of young sporophytes. A detached egg, even if fertilized by sperm, will not develop properly into a sporophyte.

The zygote develops into an embryonic and juvenile **sporophyte**, which then grows into an adult and mature sporophyte. Fertile sporophytes can be sourced from farmed or natural individuals for onward cultivation. It has been shown that the development of sorus tissue of laminarian kelps from the northern hemisphere can be artificially induced facilitating year-round access to spores. This involves sampling non-fertile individuals, removing the meristem region of the blade (~10-15 cm above the base), and culturing the non-meristematic blade parts under a short-day photoperiod (8h light:16h dark). After approximately 10-weeks, fertile sorus tissue appears and zoospores release can be stimulated. This method has not yet been tested for Australian kelps, however.

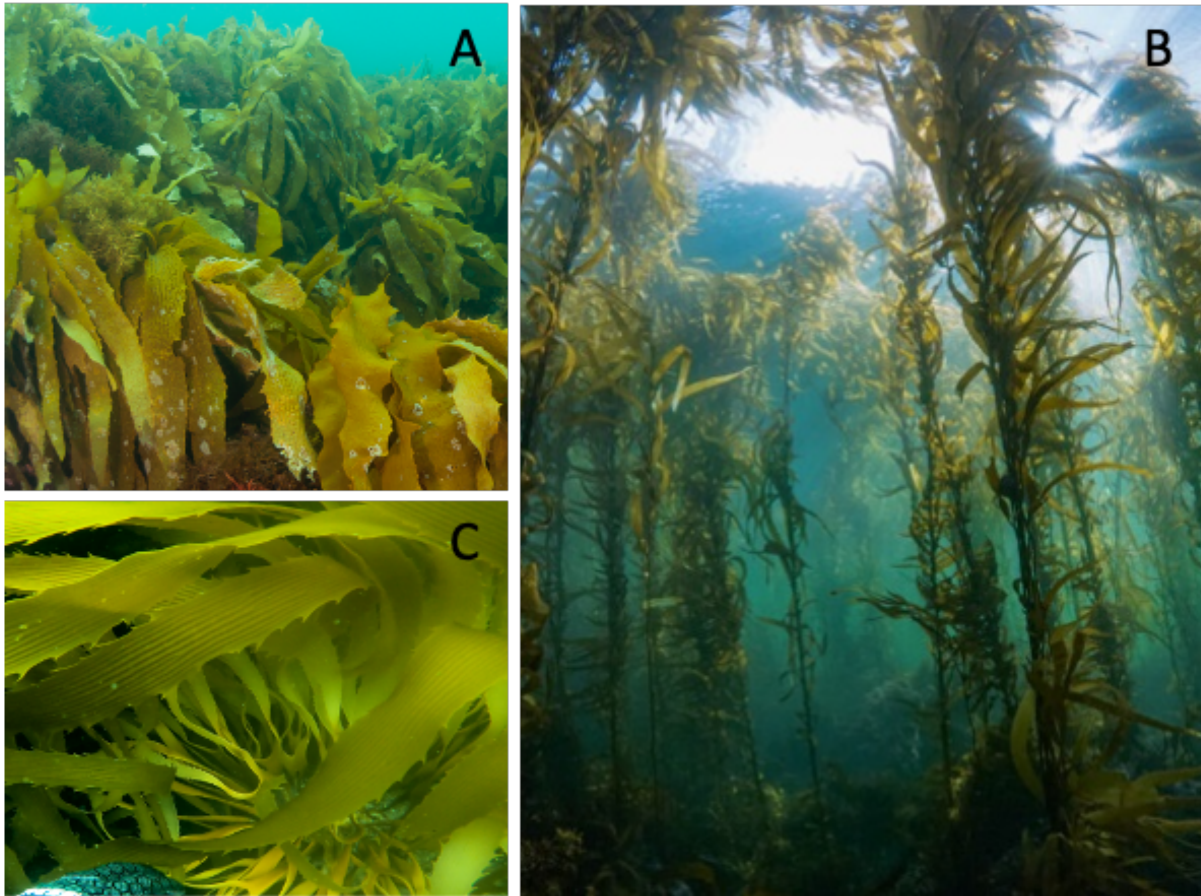

Figure 2. Photos of the three kelp species native to Tasmania. (A) *Ecklonia radiata*, (B) *Macrocystis pyrifera*, and (C) *Lessonia corrugata*. Photo credit: (A) Matthew Doggett, (B) Joanna Smart, (C) Allyson Nardelli.

Ecklonia radiata (Figure 2A) is a stipitate kelp and typically is 0.5 – 2 m tall. The growth region (i.e., meristem) is found just above the stipe at the base of the blade. It is subtidal and found in New Zealand, Madagascar, South Africa, and in southern Australia where it is the dominant habitat-forming kelp of the "Great Southern Reef" (Bennett et al. 2015; Wernberg et al. 2019). This relatively large geographical range suggests a broad tolerance of gametophytic and juvenile sporophytic life-stages to environmental conditions, including temperature and light level (tom Dieck 1993; Mabin et al. 2013; Wernberg et al. 2019). Ammonium uptake kinetics in *E. radiata* remain consistent throughout the seasons, displaying a saturable uptake pattern with a maximum uptake rate (V_{max}) of 32 ± 7 µmol g dry weight^{-1} h^{-1} (mean ± SE) and a half-saturation constant (K_s) of 104 ± 36 µM (mean ± SE) (Smart et al. 2022). When compared to *M. pyrifera*, the data indicates that *E. radiata* exhibits a lower growth rate (as indicated by V_{max}) but is proficient in ammonium uptake at lower concentrations (as indicated by K_s). This suggests its suitability for cultivation in oligotrophic waters around Tasmania (Hurd et al. 2023).

We found that the size of female gametophytes of *E. radiata* in Tasmania varied between seasons and sites (Schwoerbel et al. 2023). While gametophytes sampled in the southern site (Lady Bay) were smallest in spring and largest in autumn, gametophytes sampled in the northern site (Bicheno) were largest in spring but smallest in winter. Gametophytes from both sites could not survive temperatures above 25 °C and grew to multicellularity very rarely at temperatures below 10 °C. The mean optimal temperature for female gametophyte growth across season and site was 19.7 °C (Schwoerbel et al. 2023), confirming earlier research that reported an optimal temperature of around 20 °C for this species in Tasmania (Mabin et al. 2013) and mainland Australia (Mohring et al. 2014). Praeger et al. (2022) found that in New Zealand sporophyte formation of sporophytes was favoured when individuals were maintained under 17 °C. Our study (Schwoerbel et al. 2023) also revealed that sporophytes from both sites grew at temperatures between 11 – 22 °C in all seasons, with peak sporophyte production observed at 16 °C.

Macrocystis pyrifera (Figure 2B) is more globally distributed, and can be found from the low intertidal zone to a depth of 30 m in all cold-temperate waters in the southern hemisphere and along the west-coast of north America (Assis et al. 2023). Its distribution is largely determined by temperature, and its upper thermal tolerance for gametophytes is higher than that of sporophytes (tom Dieck 1993; Schiel and Foster 2015; Mabin et al. 2019). Seasonal variation in ammonium uptake kinetics (V_{max} and K_s), was observed in *M. pyrifera* and displayed a saturable uptake pattern (Smart et al. 2022). During summer, the species exhibited the highest V_{max} and K_s values at 200 μmol g dry weight^{-1} h^{-1} and 362 μM, respectively. Conversely, lowest values were recorded in spring, with a V_{max} of 36 μmol g dry weight^{-1} h^{-1} and K_s of 52 μM. *M. pyrifera*'s maximum V_{max}, approximately 4.5 times greater than for *L. corrugata* and *E. radiata*, aligns with its fast growth rate. The relatively high K_s value suggests *M. pyrifera* ability to take up ammonium at higher concentrations compared to species with lower K_s values. This implies that the species is well-suited for integrated multi-trophic aquaculture (IMTA) operations in Tasmania (Smart et al. 2022).

The first trials of near-shore cultivation of *M. pyrifera* in south-eastern Tasmania as part of the *Seaweed Solutions for Sustainable Aquaculture CRC-P* indicate that to optimise yield and quality of the cultured biomass, the kelp deployed in April (autumn) should be harvested in July-August (mid to late winter) (Biancacci et al. 2022c). The nutritional composition and heavy metal profile of *M. pyrifera* cultured and wild-harvested in proximity to salmon and mussel farms in Tasmania indicate that this species is suitable for human and animal consumption, except for the iodine content, which was above the maximum tolerable level for imported dried seaweed products

established by Food Standards Australia New Zealand (Biancacci et al. 2022a; 2022b), suggesting that post-processing to reduce the iodine content may be required.

Lessonia corrugata (Figure 2C), which is endemic to Tasmania, is a seaweed species that typically grows between 0.5 – 2 m tall. It primarily thrives in wave-exposed and moderately wave-exposed areas, forming kelp forests throughout Tasmania, where it sustains unique ecosystems that support high levels of biodiversity (Hurd et al. 2023; Nardelli et al. 2023c). Its holdfast consists of branched haptera, and chimerism can occur when individual plants fuse their holdfasts (Segovia et al. 2015). This holdfast fusion is thought to play an important role in seaweed ecology, affecting reproduction, survival, and development of seaweed populations (Nardelli et al. 2023c). The stipe grows from the holdfast and produces corrugated blades that experience longitudinal growth and splitting from the base, leading to dichotomous branching of the stipe. *L. corrugata* has a narrow temperature and irradiance range for optimal gametophyte growth (tom Dieck 1993; Paine et al. 2021).

There has been a growing interest in recent years in the species, with research focusing on various aspects. For instance, studies by Durrant et al. (2015; 2018) have explored the influence of seascape habitat patchiness and hydrodynamics on the genetic structuring of *L. corrugata* populations. Additionally, the gametophyte life stage exhibits a relatively narrow range tolerance to temperature and irradiance (tom Dieck 1993; Paine et al. 2021). We investigated rates of primary production, examining seasonal and spatial patterns of biomass accumulation, blade growth, erosion, and tissue carbon content in natural populations of *L. corrugata* at three sites with varying water motion levels across four seasons in southeast Tasmania (Nardelli et al. 2023b). The study revealed spatial variation and a distinct seasonal pattern in net biomass accumulation (NBA), with the highest NBA recorded in spring and the lowest in summer. During summer, blades exhibited a negative NBA, highlighting the significant impact of seasonality and water motion on carbon dynamics in *L. corrugata* populations. The ammonium uptake kinetics, Smart et al. (2022) found that the process in *L. corrugata* does not seasonally vary and follows a saturable uptake pattern with a maximum uptake rate (V_{max}) of 34 ± 7 µmol g dry weight^{-1} h^{-1} (mean ± SE) and a half-saturation constant (K_s) of 119 ± 42 µM (mean ± SE). Comparisons with *M. pyrifera* suggest that *L. corrugata* has a lower growth rate (indicated by a relatively low V_{max}) but can uptake ammonium at lower concentrations (indicated by a relatively high K_s), making it suitable for cultivation Tasmanian waters that have low nutrient concentrations (Hurd et al. 2023).

The genus *Lessonia* is among the most harvested seaweeds from natural beds worldwide, primarily in South America, which puts pressure on wild populations in these regions (Nardelli et

al. 2023c). Like *M. pyrifera*, *L. corrugata* biomass farmed in proximity to salmon and mussel farms has suitable nutritional composition for human food and animal feed and heavy metal content below the daily intake, except for the iodine content which exceeded the maximum tolerable level established by Food Standards Australia New Zealand (Biancacci et al. 2022a). Finally, there is a growing interest in the species for its commercial aquaculture potential, with studies focusing on optimising both the nursery and at-sea phases (Nardelli et al. 2023a; Visch et al. 2023b; 2023c).

Chapter 2. Nursery Production

2.1 Laboratory and culture media preparation

Gametophyte culture requires specific scientific **equipment** such as laminar flow hoods (to minimise airborne biological contaminants whilst handling cultures and nutrient medium), culture vessels and stoppers, glass tubing, micropipettes and tips. A list of equipment can be found in Appendix 1 . There is a high risk of biological contamination during preparation and maintenance of gametophyte cultures, so all equipment must be **sterilised** (e.g., by autoclaving) and handled following aseptic techniques (Paine, 2018). Laminar flow hoods should be sterilised using UV radiation and wiped with ethanol before and after use. Glassware, stoppers, tubing and pipette tips should be by autoclave sterilized before use for cultures and nutrient media. Before autoclaving, the culture vessels should be fitted with stoppers and glass tubing. The stoppers and glass tubing should remain covered in aluminium foil during autoclaving. Once cool, the foil should be replaced with parafilm within the laminar flow hood.

The **room set-up** for gametophyte culturing should be isolated from other laboratory activities to minimise the risk of biological contamination. Isolation can be achieved by simply using white plastic/corflute sheeting (see Paine, 2018). The optimum wavelengths of light reaching the gametophytes should range between 620 nm to 750 nm (red colour spectrum) so red fluorescent lights and red cellophane paper covering lights and/or windows are needed in the room to maintain this wavelength range (Figure 3A). The surrounding surfaces of the culturing chamber/room should be cleaned with ethanol before placing the culture vessels on it. The air pump needs to be connected to each flask using silicon tubing (Figure 3B). One long piece of glass tube (internal diameter between 2-5 mm) threaded through the hole of the rubber stopper helps in passing the air in and another short glass tube is inserted in the same way for air outlet. Air filters (0.22 or 0.45 μm pore size) are attached to

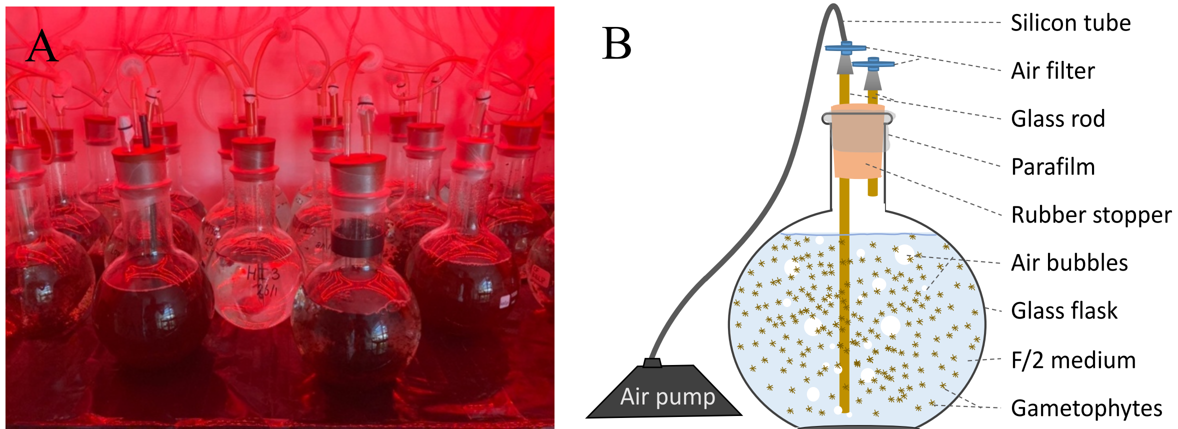

Figure 3. (A) Aerated flasks with kelp gametophytes, vegetatively cultured under red light. (B) An illustration of the flask highlighting each individual component.

each glass tube which is then connected to silicon tubing (internal diameter between 2-5 mm). The inlet glass tube is connected to the aerator using an additional piece of silicon tubing and the outlet tube is fitted with an air filter exposed to the surrounding air. For reducing biological contaminants, the stopper and glass tube should be covered with parafilm during use.

Nutrients are essential for the growth and development of any organism, including kelp. In the nursery, additional nutrients are supplied in the form of PES-medium (Provasoli enriched seawater) or **F/2-medium** (see recipe in Appendix 2). In our research we use F/2-medium (Guillard and Ryther 1962a), but PES-medium is a suitable substitute in most cases (e.g. Praeger et al. 2022). Some steps in the nursery require alteration to the nutrients supplied, which we highlight, but for all other steps full strength F/2-medium is used. It is important that the medium is not a vector for infection or contamination, and it should therefore be filtered and sterilised (e.g., UV or autoclave). We recommend filtering (0.2 µm pore size) and/or autoclaving natural seawater when making the culture medium. Where needed germanium dioxide (GeO_2) or antibiotics can be added to the culture medium to control biological contaminants (more information below). Following the results of Praeger et al. (2022), it is recommended to limit the use of GeO_2 to no more than 2 days, or as short pulses, as extended use has detrimental effects on the development of laminarian sporophytes. To reduce the workload when handling large seawater volumes, pre-made F/2-medium can be used (VariconAqua Cell-hi F2P; Appendix 3) (Figure 4).

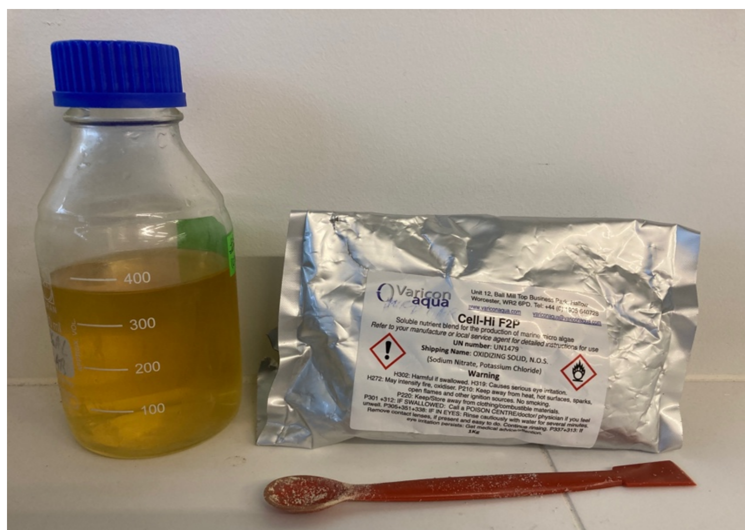

Figure 4. Flask with the F/2-medium stock solution on the left and the commercially available pre-made F/2-medium powder on the right.

Opportunities to optimise the culture media preparation:

Culture medium: The selection of a cost-effective culture medium can be optimised. In this manual, F/2 medium is used, but it can be expensive and labour-intensive to prepare from scratch. Additionally, gametophytes or juvenile sporophytes typically require fewer nutrients to support their growth and reproduction compared to what is provided by F/2 medium. Furthermore, F/2 medium contains silicate, which seaweeds do not utilize and instead promotes diatom growth, increasing the likelihood and intensity of contamination. Therefore, it could be beneficial to explore the minimum and/or optimal nutrient concentrations, especially when scaling up nursery operations.

2.2 Sporophyte collection and spore release

Mature fertile sporophytes can be observed in the field by either dark or light discolouration on the blades compared to non-fertile tissue (Figure 5 and Figure 6). **Sorus tissue** is most easily differentiated from non-fertile tissue by holding a mature blade up to the sky to check for discolouration. Interestingly, when underwater the sorus tissue appears white/silver in colour as opposed to the dark colouration when out of the water (Figure 6D). For *Ecklonia radiata,* sori can be found on both the central lamina and lateral blades (Figure 5A and Figure 6A). Sorus-bearing blades of *Macrocystis pyrifera* are called sporophylls and are differentiated/specialised blades located at the base of the thallus above the holdfast (Figure 5B and Figure 6B). But sori can also be observed on pneumatocyst-bearing blades and apical blades (Leal et al. 2021; Visch pers. obs.). Of the three species cover in this manual, fertile *Lessonia corrugata* blades are least

distinct compared to non-fertile blades. Most blades are capable of bearing sori, and mature fertile blades are almost entirely covered with sorus, which make it challenging to distinguish fertile sorus tissue compared to other laminarian kelps (Figure 5C and Figure 6C).

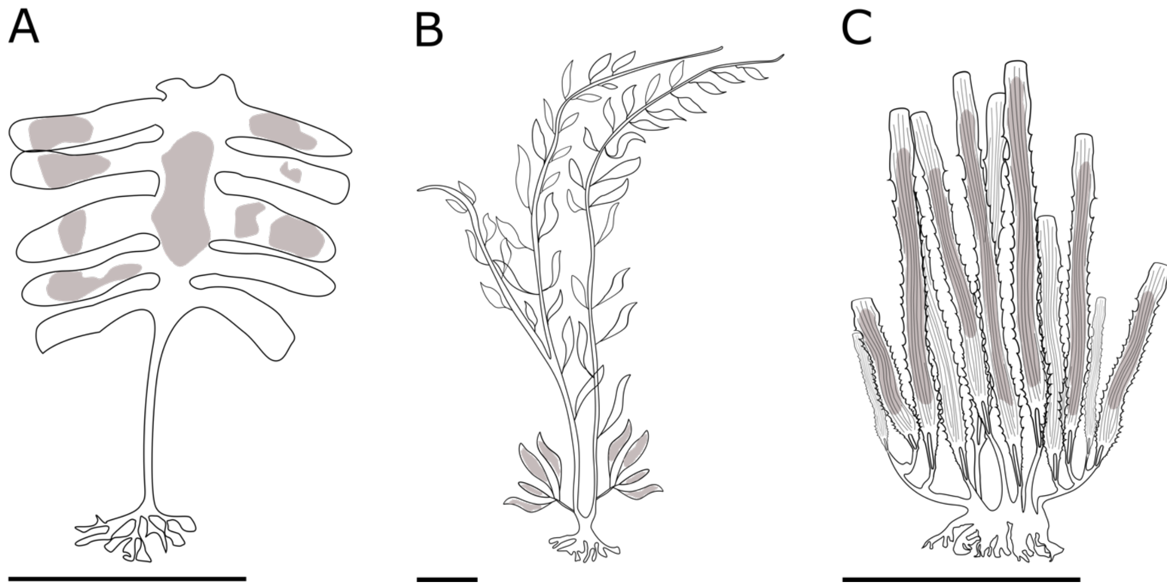

Figure 5. Mature fertile kelp sporophytes. The grey patches on the blades indicate the location where mature sorus tissue is typically found on the respective kelp species; (A) *Ecklonia radiata*, (B) *Macrocystis pyrifera*, and (C) *Lessonia corrugata*. The scale-bars indicate 50 cm.

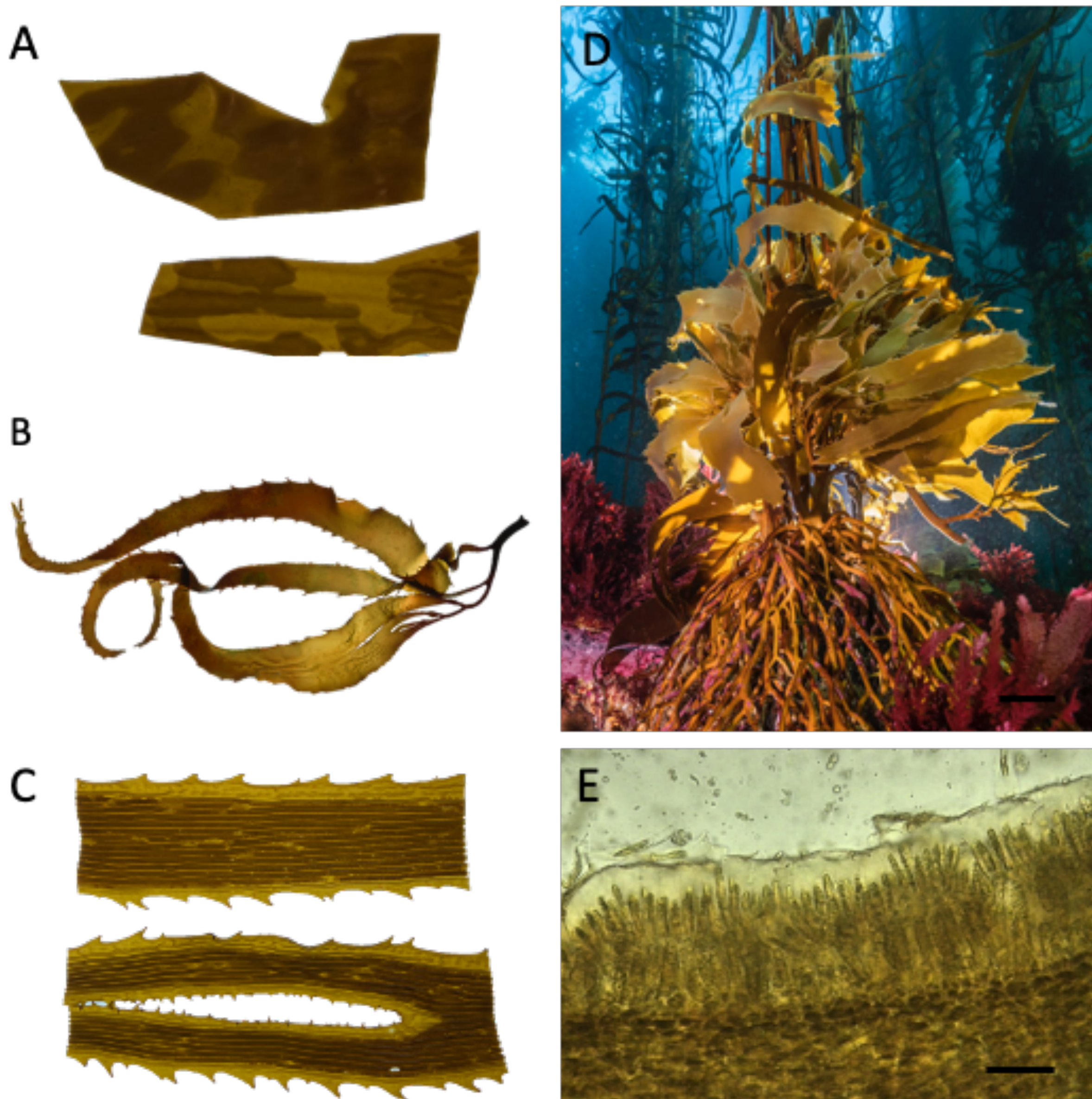

Figure 6. Illustrations of sorus tissue. (A) Dissected sorus tissue from *Ecklonia radiata* showing dark sorus tissue and lighter coloured non-fertile tissue. (B) *Macrocystis pyrifera* mature sporophyll where the dark sorus can cover the entire blade, here it covers the distal half of the blades and the lighter coloured basal parts are non-fertile tissue. (C) *Lessonia corrugata* blade where the darker sorus tissue can be found in the middle where it typically covers almost the entire length of the blade, the latera lighter coloured sections are non-fertile tissue. (D) an underwater image of a *M. pyrifera* sporophyll at the base of the plant, submerged sorus tissue is white/silver coloured. (E) an image of dissected cross mature sorus tissue showing the sporangia orientated outwards (i.e., on the top/bottom on the blade which is in contact with seawater) and the normal cells in the centre of the blade. (F) an image of dissected section of mature sorus tissue showing the outward orientated sporangia (top) and the non-reproductive cells (bottom). Scale bars represent: D, 40 mm; E, 30 µm; F, 100 µm. Photo credits: (A, C) Jakop Schwoerbel, (B) Josie Iselin, (D) Uli Kunz, (E) Wouter Visch.

There are several things to consider when collecting seedstock for onward cultivation: (a) the months/season when the seaweed is reproductive at a given sampling location; (b) the number of sampling sites and individuals sampled within each site; and (c) the distance of the sampling location with respect to the farm site.

a) As a seaweed farmer it is useful to have a good understanding of seaweed **reproductive phenology** when sourcing seedstock for onward cultivation. Like plants, laminarian kelps go through cycles of growth and reproduction (i.e., reproductive phenology). Timing of reproduction of temperate seaweeds is typically mediated by seasonal changes in temperature (de Bettignies et al. 2018) and/or day-length (Lüning 1988), provided sufficient nutrients are available (Nimura and Mizuta 2002). Figure 7 provides an estimate of the reproductive phenology of the three kelp species targeted in this manual. In Tasmania, *E. radiata* is generally fertile throughout the year with a peak in autumn/early winter (April-June) (Tatsumi et al. 2022). Similarly, fertile *M. pyrifera* can be found year-round in Tasmanian waters; however, it peaks in later winter/spring (Oct-Dec) (Cayne Layton and Craig Sanderson, pers. obs). In contrast, *L. corrugata* is reproductive year-round except for spring (Nov-Jan) (Allyson Nardelli et al., in prep).

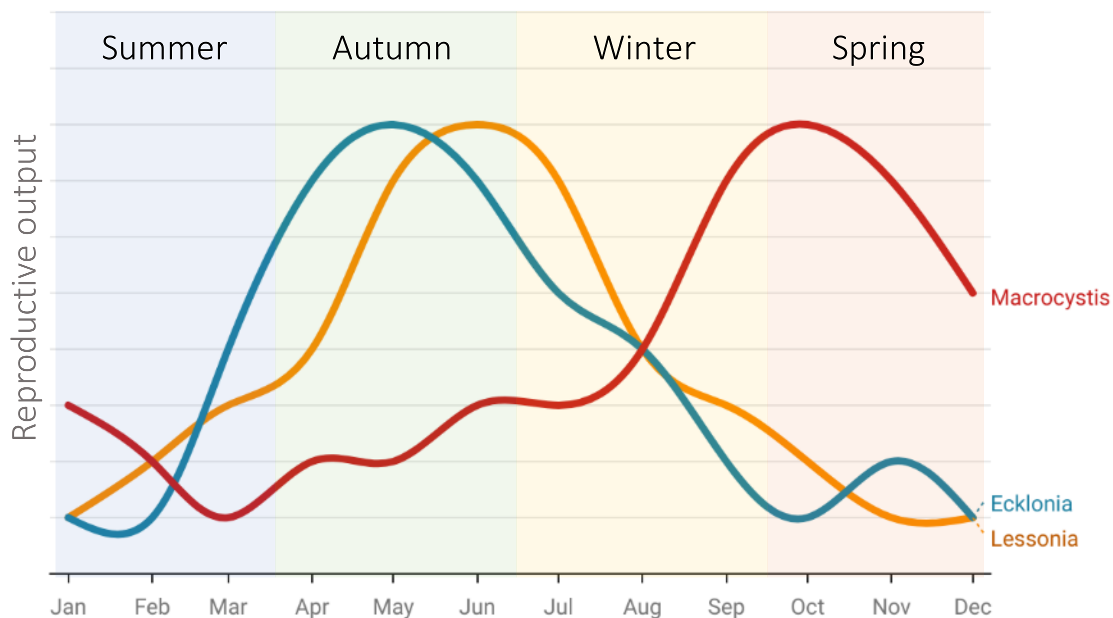

Figure 7. Seasonal variation in reproductive output in Tasmanian waters for *Macrocystis pyrifera* (red), *Ecklonia radiata* (blue), and *Lessonia corrugata* (yellow). Data presented may vary between sites and years, and is based on Tatsumi et al 2022 for *E. radiata*, Nardelli et al. (this project; in prep) for *L. corrugata*, and personal observations for *M. pyrifera* (this project).

b) A **genetically diverse**, and therefore resilient crop can be achieved by including a sufficient genetically diverse seedstock. How much is 'sufficient' depends on the management practices and breeding strategies that allow the maintenance of the seedstocks' (i.e., domesticates') evolutionary potential. Valero et al. (2017) advise that this may include the choice of source populations and the selection of varieties suitable for sustainable aquaculture systems in the context of spatial and temporal environmental variability, given the difficulty to standardize cultivation conditions in the open sea. Finally, genetic diversity is tightly linked to the provenance of seedstock.

c) Sourcing of seedstock in relation to proximity to wild populations of conspecifics is important to consider for the potential for genetic contamination of wild populations from cultivated thalli or *vice versa*. Currently in Tasmania, cultivation permits may be issued for native species only, with the exception of *Undaria pinnatifida* provided it is harvested before it become fertile, and it is encouraged to source seedstock locally. However, there is no specific legislation that precludes translocation of seed stock from other regions, and how 'locally' is defined may be species specific. For example, previous studies have shown low genetic diversity amongst *Macrocystis pyrifera* populations in Australia and the southern Pacific, likely due to air-filled vesicles that allow giant kelp to have a wide dispersal range and population connectivity (Durrant et al. 2015). In contrast, *Ecklonia radiata* and *Lessonia corrugata* are both negatively buoyant species, resulting in reduced population connectivity and relatively high genetic diversity compared to *M. pyrifera* (Durrant et al. 2015). To maximise the genetic diversity of the cultured crop it is advised to collect between 10 - 20 individuals collected at least 1 m apart. This provides opportunities to select locally sourced, high-performing strains of *E. radiata* and *L. corrugata*.

After transporting the fresh mature sorus tissue to the laboratory, there are several steps involved with the **release of spores**. The sorus tissue needs to be cleaned to reduce the likelihood of biological contamination of cultures. This can be achieved by various methods and differs slightly among the three species. Typically, the sorus tissue is cleaned from any visible fouling by wiping the sorus thoroughly with paper towel and rising it in filtered (0.2 μm pore size) or autoclaved seawater. Note that the heavily corrugated blades of *L. corrugata* need extra care and sometimes a small soft brush (e.g., toothbrush) can be helpful here. *E. radiata* can be extremely slimy after transport, which makes cleaning sometimes challenging. Also note that this is especially marked when *E. radiata* blades are resubmerged after desiccation, which therefore has to be avoided at this stage of the process. The sorus bearing blades (i.e., sporophylls) located at the base of a *M. pyrifera* plant are relatively small in size and easy to clean.

Then the tissue is rinsed and washed three times for 10 s in a Betadine® (iodine) solution with sterile seawater (5 mL L^{-1}) and further rinsed with sterile seawater. After cleaning, the sorus tissue is **desiccated** to artificially stimulate spore release. Typically, this is done by storing the sorus tissue in damp paper towel, in the dark, overnight between 12 - 15 °C. Depending on how the gametophytes are cultured there are two methods to **release the spores** from the cleaned sorus tissue:

(1) fully submerge all the cleaned, desiccated sori in sterile seawater – this ensures the mass release of all the spores present thus generating the greatest number of spores,
(2) pipet small droplets of sterile seawater on the sorus tissue – this provides more control over the spore density and is therefore the preferred option for male or female gametophyte isolation and/or clone cultures.

In the **first method**, spore release is triggered by submerging the sorus tissue in filtered (0.2 µm pore size) or sterilised seawater with F/2-medium and germanium dioxide (0.02 mmol L^{-1}) (see Chapter 2.7 for details) in dark or low-light conditions (>50 µmol photons m^{-2} s^{-1}) – see Figure 8A. The seawater temperature is typically similar to the temperature at which the gametophytes will be kept in culture. After 20 – 30 min, most viable spores are released and the empty sorus tissue can be removed using sterilised forceps (e.g., ethanol sprayed). Filtering the spore solution through a 50 - 100 µm sterilised sieve can further improve purity of cultures as spores (~5 µm in diameter) pass through, whilst any larger debris dislodged during spore release can be removed. Note that spore solutions of *E. radiata* are typically slimy, which makes sieving the solution important to remove excess polysaccharides (slime). This will however reduce the total spore amount as many spores will be removed with the mucus. This is less of an issue for *M. pyrifera* and *L. corrugata*. The number of spores (i.e. **spore density**) can be assessed using a haemocytometer under a normal light microscope (100× magnification) (Figure 8B and Figure 8C). Combined, the initial volume of the medium in which the spores release and the total number of spores released by sorus tissue determine the highest possible concentration of spores. Note that the spore density can be diluted but not concentrated (see Figure 8A as an example of the typical volumes and amount of sorus tissue used.).

In the **second method**, is particularly useful when aiming to store gametophyte cultures for extended periods of time in relatively low quantities, for example in a seedbank or in a selective breeding program. Here spore release is triggered by pipetting droplets of sterile seawater onto the sorus tissue (Figure 8D). This way, only the sporangia associated with the droplet will release spores, typically within 5-10 min. This is beneficial because, despite cleaning, the sorus tissue

can still be a source of biological contaminants. This method reduces the likelihood of contamination by reducing the surface area in contact with the culture medium. It also allows for better control over the concentration of spores, which is particularly important when isolating gametophytes at a later stage. The concentration of spores can be checked by taking sub-samples of known volume (e.g., 10-20 μL) from the droplets with a micro-pipette and placing these onto a petri-dish (Figure 8D). This allows the assessment of the viability of the released spores (i.e., moving or not) and, if the sub-sample is small enough, it is possible to estimate the total number of spores per droplet under a light-microscope – a haemocytometer is not needed here. If the spores are viable (i.e., motile), a small droplet can be added to a petri-dish with sterilized seawater and nutrient medium. The spores settle and develop further in the petri-dish (Figure 8E- Figure 8H). Adjust the number of spores added by changing the volume of the droplet. To prevent the gametophytes from growing over each other and ensure proper isolation at a later stage, we recommend 30-50 spores per petri-dish and, periodically, gently swirling the culture medium.

Opportunities for sporophyte collection and spore release:

Collecting fertile sorus tissue: This manual provides instructions to acquire seedstock by collecting fertile sorus tissue from natural populations. This process involves snorkelling/diving, and depending on the sampling site, it may require the use of boats and a qualified field team. An alternative method, which has been proven successful for species from the genus *Saccharina* and *Laminaria*, involves inducing fertile tissue through laboratory manipulations. This enables year-round access to sori, even outside the reproductive season in the field.

Zoospore release: Optimisations can be implemented during the release of zoospores from fertile sorus tissue, particularly for *Ecklonia radiata*, which tends to also release a significant amount of mucus (i.e., polysaccharides) during the process. This results in a slimy solution where the zoospores often become trapped, making it challenging to accurately determine zoospore concentrations, seed twine, or establish clean/contaminant-free gametophyte cultures.

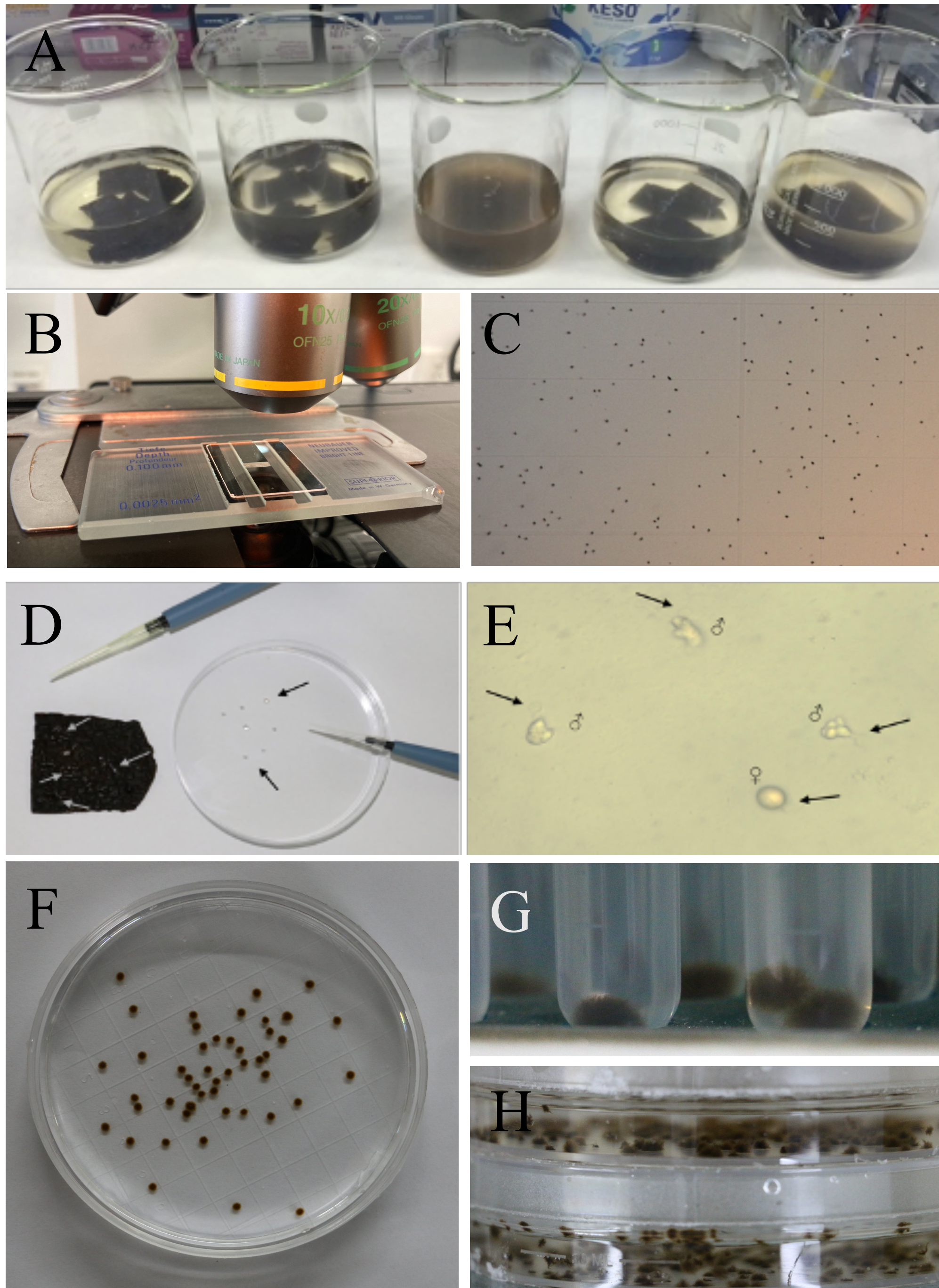

Figure 8. Images showing the methods for spore release and gametophyte isolation. (A) 2-L beakers with submerged rehydrated sorus tissue. The dark and mirky solution indicates the mass release of spores. (B) A haemocytometer used under a light microscope. (C) Microscopic spores (black dots) under the microscope at 100x magnification. (D) Sorus tissue with spores released in the droplets (grey arrows) and a petri-dish with small droplets of spore solution (black arrows) to check spore viability and number of released spores. (E) Low-density settled male (♂) and female (♀) spores with germination tube (black arrows). (F) Top view of a petri-dish with vegetatively growing individual gametophytes ("pom-pom-like blobs") that each has developed vegetatively from a single spore. (G) Isolated gametophytes on the bottom of a culture tube after they were vegetatively cultured in a petri-dish. (H) Side view of a petri-dish with vegetatively cultured gametophytes. Photo credits: Wouter Visch.

2.3 Gametophyte stock cultures and maintenance

The goal of producing and maintaining gametophyte stock cultures is to suspend the gametophyte development and **vegetatively propagate** the gametophytes through fragmentation. This can be achieved by creating gametophyte cultures in sterile glassware and manipulating their light exposure. After spores are released into the culture medium, they settle onto the culture flask/container/petri dish, germinate, and develop into gametophytes. While a small portion of the free-floating spores may mature into gametophytes, more research is needed to confirm this. Allow approximately one day for the spores to settle and germinate and 2-3 days for the gametophytes to develop before gently scraping them off from the inside of the culture flask/container with a sterilised spatula, allowing the gametophytes to then float freely. The light irradiance at the surface of the glassware should be 15-20 μmol m^{-2} s^{-1} (**red light spectrum**), adjusted depending on the density of gametophytes (Figure 3). Higher gametophyte densities can handle higher irradiances (up to 30 μmol m^{-2} s^{-1}) due to self-shading. Irradiances greater than 20 μmol m^{-2} s^{-1} will trigger gametogenesis, and reproductive structures (oogonia and antheridia) will be formed. Typically, gametophytes are cultured in a 12:12h or 16:8h (light:dark) photoperiod. Gently aerate the culture flask/container after 1-2 days. If aeration of the culture flask/container is postponed for too long, fluxes in pH can be detrimental to spore development and gametophyte growth.

Change the **culture medium** every 2-3 weeks to ensure availability of sufficient nutrients for growth. Adjust the frequency of media changes depending on the culture density, with more frequent media changes for dense cultures. Full or partial media changes are performed in a sterile environment in a laminar flow hood using sterilised equipment. For full media changes, pour the gametophyte culture through a sterile sieve (~100 μm mesh size). For partial media changes, decant the top layer of the culture medium and replace it with fresh sterile culture media. Occasionally, transfer the remining gametophyte solution into a new sterilized culture flask/container.

Fragmentation is used to increase the gametophyte biomass. For relatively dense cultures this is typically done using a handheld kitchen blender for approximately 10-30 seconds. Small gametophyte cultures can be fragmented using the back of a pipette-tip or squeezed in between microscope slides. Use sterile equipment and perform fragmentation in a laminar flow hood to avoid cross-contamination. Sterilize all equipment using ethanol between fragmenting multiple cultures in one session. There are several ways to ensure no is ethanol remaining on the

instruments to avoid damaging gametophytes. One can simply wait to ensure the ethanol has evaporated off or burn off over a flame and then cool in sterile filtered seawater to ensure sterile but no ethanol remaining.

Opportunities for further optimisation of gametophyte stock cultures and maintenance:

Frequency of fragmentation: The optimal frequency for fragmenting gametophytes to enhance biomass was not tested in this study but is a potential area for exploration to optimise productivity of kelp nurseries.

Culture density: Apart from culture conditions such as temperature, light, and nutrients, the density at which free floating gametophytes are cultured can significantly influence their growth and productivity. Although not specifically investigated in this project, it is crucial to study the appropriate culture density for each of the target species mentioned in this manual.

2.4 Induce gametogenesis and fertilisation

Gametogenesis (i.e., the development of gametes) can be induced approximately two weeks before seeding/spraying. Fragment the gametophyte cultures using a sterilised (e.g., ethanol-sprayed) handheld kitchen blender for approximately 10-30 seconds and transfer them from **red light into white light** (~60 - 70 μmol photons m^{-2} s^{-1} and 16h light:8h dark). The other culture parameters (e.g., aeration, temperature) should remain the same as during the vegetative growth period. Culture media should be replaced with F/2-medium in sterilized seawater with the use of a laminar flow cabinet weekly. This can be achieved by either removing the aeration, allowing the gametophytes to sink to the bottom of the flask and removing as much excess culture medium as possible or by filtering the gametophytes through a sterilized filter (mesh size <10 μm). The former method is quicker and has a lower chance of introducing unwanted contaminants. The latter method has the benefit that the old glassware can be replaced with new sterilised glassware. In summary, transferring from red to white light combined with the addition of fresh nutrients stimulates gametogenesis (gamete production), facilitating fertilisation, and ensures that the gametophytes develop into sporophytes.

After fragmentation they can be placed in a new culture environment (e.g., flask), it is important to monitor gametophyte cultures under a microscope periodically to observe the emergence of small sporophytes (as shown in Figure 9). The appearance of these sporophytes indicates that the gametophyte/sporophyte culture is ready to be sprayed onto the spools. Typically, this occurs during the *c* and *d* stages depicted in Figure 9 and usually takes 10 to 14 days.

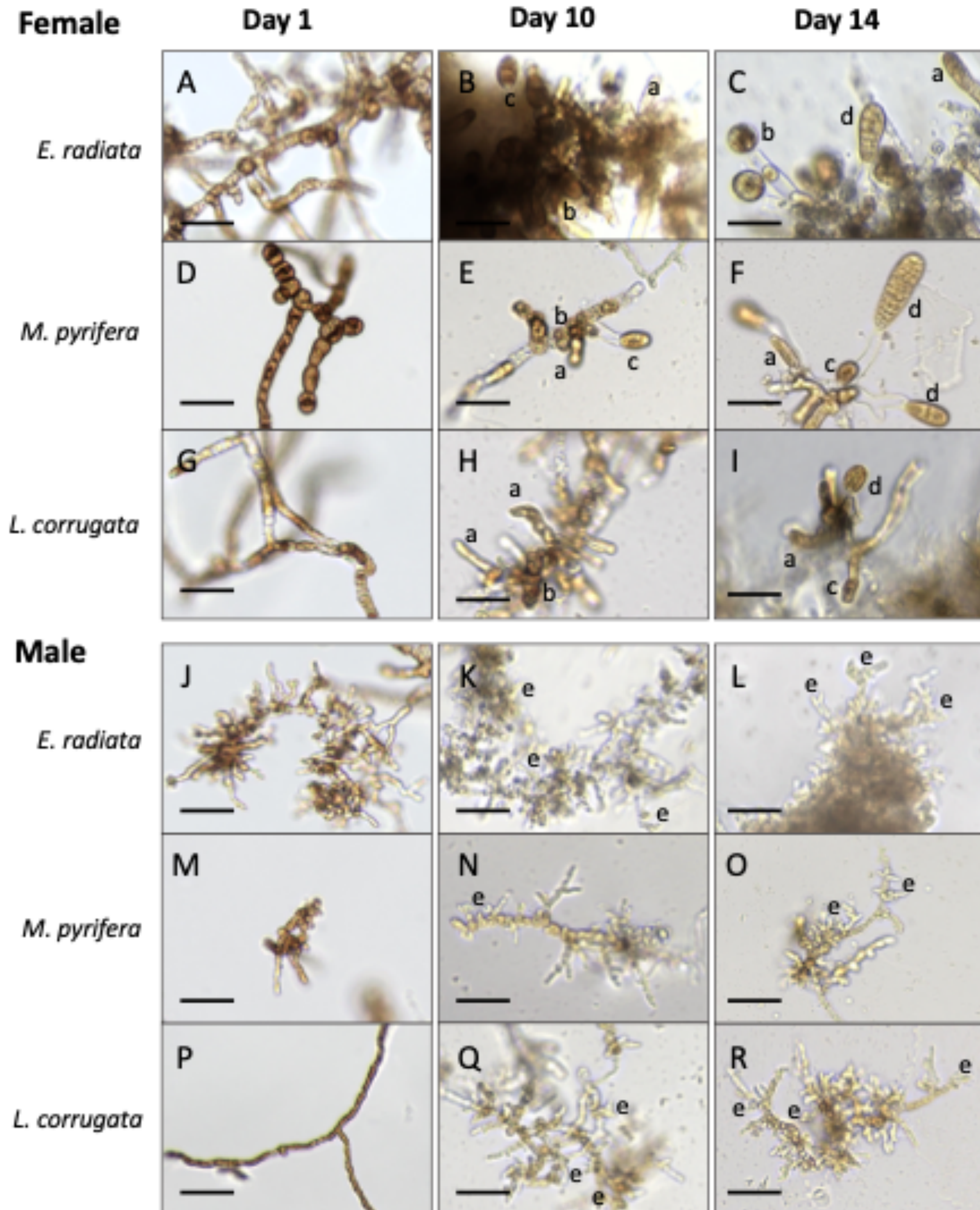

Figure 9. Developmental stages of female and male gametophytes and early sporophytes of *Ecklonia radiata*, *Macrocystis pyrifera* and *Lessonia corrugata* at 1, 10 and 14 days after transition of the gametophyte cultures from the red to white light conditions. The lowercase letters in each panel highlight reproductive structures that indicate the stage of development for female gametophytes (A-I): (a) oogonia, (b) eggs, (c) zygote, and (d) microscopic sporophyte and male gametophytes (J-R): (e) antheridia. Images obtained with the aid of an inverted light microscope (magnification 200×) fitted with a camera (Nikon Eclipse TS2-P-CF); black scale-bars are 50 μm.

Opportunities for further optimisation of gametogenesis and fertilisation:

Culture density: Further exploration is required to investigate the impact of gametophyte density when inducing gametogenesis. Issues related to shelf shading, which can hinder synchronised gametogenesis of gametophyte cells, need to be addressed, particularly when scaling up operations.

Culture conditions: The optimisation of environmental variables and how to manipulate them for inducing gametogenesis and subsequent fertilisation can be further improved. Some work has been carried out during this project, but more work is needed when upscaling nursery production of the target species discussed in this manual.

Parthenosporophytes: Parthenogenesis, the development of unfertilised female gametes into new sporophytes, is a reproductive phenomenon often observed in some species of the Laminariales. However, this phenomenon has not been systematically studied for the target species mentioned in this manual, and therefore necessitates further exploration, particularly in the context of cultivation and/or breeding.

Male to female ratio: Currently, it is assumed that a surplus of male gametes yields maximum fertilisation rates when producing sporophytes from clonally cultured male and female gametophytes. However, the fertilization of an egg by more than one sperm (polyspermy), is lethal in most organisms. This is not well studied in laminarian kelp species, but its negative effects are observed in the Fucalian seaweed that have a diplontic life cycle. Irrespective, there is an opportunity cost associated with using excessive gametophytes. Thus, it is necessary to experimentally determine the optimal ratio of male to female gametophytes mixed before fertilisation and subsequent sporophyte production in nurseries.

2.5 Preparation of seeded twine on spools

Juvenile sporophytes can be cultured in the nursery on **seeding twine spun onto PVC pipes** to make a so called “spool” (like a large cotton reel) for efficient and scalable deployment at sea. There are many types of culture twine available on the market, and the choice of twine can potentially affect the cultivation success (Cumming et al. 2020b; Kerrison et al. 2017). We used ~1-mm diameter rough texturized seeding twine specifically developed for kelp aquaculture (AtSeaNova, AlgaeTex®) (as per Biancacci et al. 2022c). Re-usable spools can be simply and cheaply constructed from PVC pipe available from plumbing suppliers. We used 75-mm diameter pipe cut into 50-cm lengths. It is important to ensure the cut edges of the spools are filed to smooth to eliminate rough surfaces that may promote colonisation of biological contaminants.

To prepare the spools with culture twine, it is important to work in a **clean environment**. Hand-spinning spools with culture twine is a laborious process, but it can be mechanised for increased efficiency (Figure 10) (Solvang et al. 2021). For monitoring purposes, leaving a few centimetres of excess twine at the end is recommended. After the twine has been spun onto the PVC pipes, soak

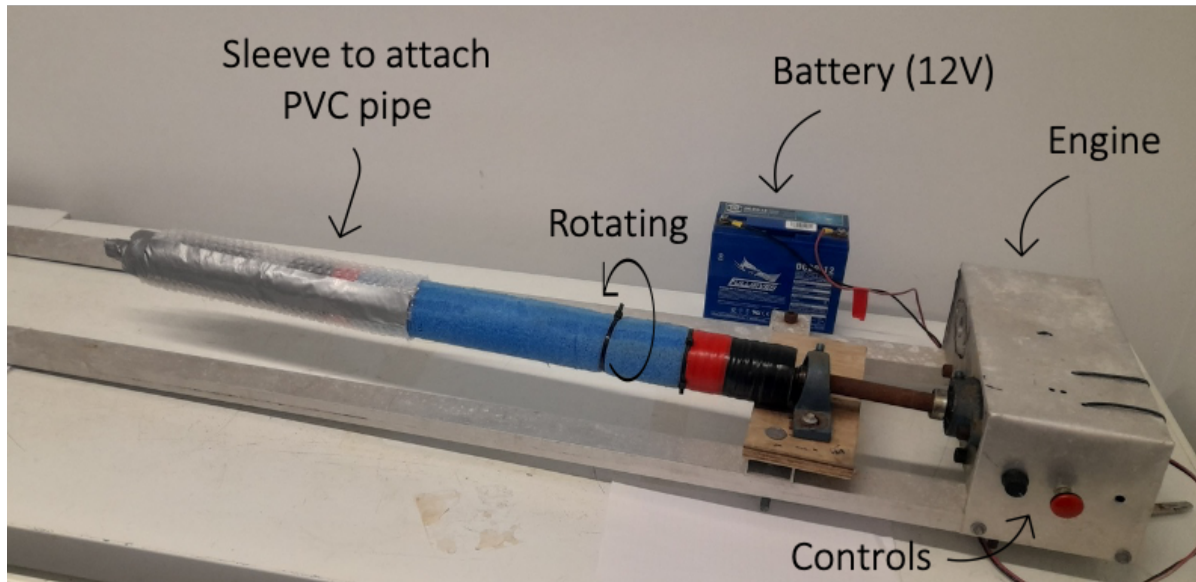

Figure 10. The spooling machine that aids in the winding of twine around PVC pipe to automate spool preparation. This has substantially reduced both time and labour costs. The PVC pipe is inserted onto the sleeve, crafted from a foam pool noodle to hold the spool firmly in place, which is set in motion by a 12-volt DC engine. The rotational speed (rotations per minute, rpm) can be regulated using the buttons on the right side in the photo. Generally, twine spooling starts at the lowest rpm to ensure proper alignment of the twine onto the PVC pipe.

the spools in a 5% v/v freshwater solution of either Decon-90 or bleach for at least 12 hours. Then, rinse and soak the spools thoroughly in freshwater for at least 12 hours. Before inoculation, allow the spools to air dry for at least 12 hours to facilitate the uptake of the inoculant. If long-term storage of the sterilised spools required, this can be done by placing them in a closed sterile container or by wrapping them in aluminium and placing them in a dry space.

An example of how spools can be pre-treated is as follows: (1) soak the spools in a 5% v/v solution of Decon-90 or bleach first thing in the morning; (2) rinse them thoroughly and soak them in freshwater at the end of the day; (3) air dry them in a sterile environment (e.g. laminar flow) the next morning; (4) the spools are then ready to be inoculated/sprayed in the afternoon or the day after. In summary, allocate at least 2 days for the pre-treatment of the spools before inoculation with spores, gametophytes, or small sporophytes.

There are three main methods for **seeding** the culture twine to cultivate sporophytes in the nursery (see Figure 11A , Figure 11B, and Figure 11C).

The first method takes advantage of the natural swimming ability of **spores**. A spore solution containing around 2000 spores mL^{-1} is added to spools that are completely submerged in culture

medium (filtered or sterilized seawater with F/2-medium), and the spores settle onto the spool (Figure 11A). This is typically done in the dark, under the same environmental culture conditions as the gametophytes (as described above). This method often yields a high and evenly distributed density of sporophytes on the spools. However, it relies on the availability of sori, which may be difficult to obtain for some species and may not be available year-round. Additionally, there is a higher risk of biological contamination introduced by the sorus tissue.

The second method involves using **gametophytes** or **small sporophytes** to seed the spools (Figure 11B). Gametogenesis can be induced in free floating gametophyte cultures 7-10 days before they are applied onto the spools by exposing the gametophyte culture to white light in order to reduce the time in the nursery. The developing gametophyte solution is concentrated to 25 filaments μL^{-1} using a cell counting chamber (Sedgewick Rafter S50). This gametophyte culture can then be evenly sprayed using a sterilized 1.25-L handheld pressure sprayer (Hozelock Ltd. Viton®) (e.g. Visch et al. 2023c). This approach allows for a consistent source of seedstock throughout the year and greater control over the genetics of the crop. Furthermore, the nursery period is reduced by a few weeks compared to seeding with spores. However, this method requires additional lab space and skilled personnel to maintain the gametophyte stock cultures in sterile conditions. To ensure optimal seeding, the gametophytes should be sprayed evenly onto pre-treated spools (as per above), and the spools should be uniformly wet after spraying. It is recommended to record the number of gametophytes and volume of the solution used to inform future seedings.

The third method eliminates the requirement for spools and the associated necessity of large tanks during the nursery phase. Gametophytes are cultivated in flasks and combined with a **binder solution** to facilitate direct attachment to the grow line (Figure 11C) (Sioen Industries 2013; Kerrison et al. 2018; Visch et al. 2023a). This approach is designed to conserve resources and enhance productivity by promoting the adhesion of microscopic propagules, such as gametophytes, embryonic sporophytes, or a combination of both, onto specially designed substrates using binding agents, serving as an alternative to conventional techniques used in kelp cultivation (Kerrison et al. 2018). Seeding can be performed on-site, typically on board a vessel at the farm site (i.e., *in-situ*), by immersing the cultivation line in the gametophyte-binder solution or by gently applying the gametophyte-binder solution to the grow line using a brush. This method requires minimal laboratory space and resources during the nursery phase. However, in high-energy environments, the crop may not attach securely to the grow line or may detach during the initial days after deployment (Visch et al. 2023a).

<u>Opportunities for further optimisation for seeding of spools:</u>

Automation: The machine that aids winding twine around PVC pipe to automate spool preparation has significantly reduced labour costs. However, further development and optimisation can be pursued, such as improving twine guidance (currently done by hand) to enhance spinning speed and increase the number of spools produced per hour.

Twine type: Numerous studies have been conducted globally to investigate various twine types in kelp aquaculture. However, a systematic examination of twine types specifically for the target species discussed in this manual has not been carried out.

Binders: The use of binder or adhesives to assist gametophyte adherence to the cultivation line has been tested with varying success in other locations and for different species. It is worth exploring this approach for the target species and farm sites discussed in this manual.

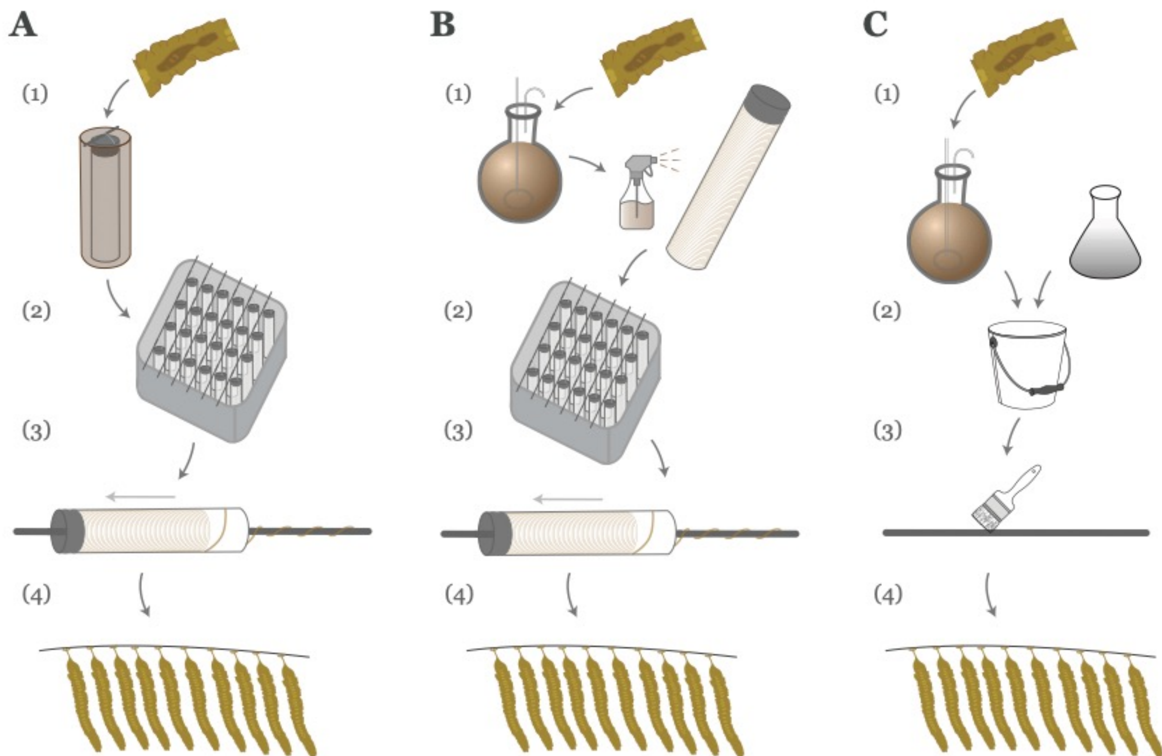

Figure 11. This illustration depicts the three distinct methods for producing seeded long lines. (A) involves allowing motile spores to settle onto the spool. (B) gametophytes are initially cultured in a flask and then sprayed them onto the spool. (C) involves mixing gametophytes and a binder to create a viscous solution that is used to seed the cultivation line directly by either brushing it on or soaking the cultivation line in the solution. Illustration adapted from Visch (2019).

2.6 Culture conditions of the spools

The culture conditions required for a successful nursery depend on several factors, such as target species, pools' size, culture tank volume, lighting configuration (e.g., overhead or from one/two sides), time since inoculation. In a study by Visch et al. (2023c), the optimal nursery conditions for three Tasmanian kelp species, *Ecklonia radiata*, *Lessonia corrugata*, and *Macrocystis pyrifera*, were identified. The study explored the interactive effects of **temperature** (12 °C, 15 °C, and 18 °C) and **light intensity** (~30 μmol photons s^{-1} m^{-2}, and ~60 μmol photons s^{-1} m^{-2}) on sporophyte length, sporophyte density, and contamination on spools were examined over a 34-day period – Table 1 present the results.

Water motion in a traditional kelp nursery is solely provided by aeration. However, we found that **rotating** the spools at a rate of 15 rpm (approximately 7 cm s^{-1}) leads to significantly improved nursery outcomes (as seen in Figure 11) (Nardelli et al. 2023a; Lush et al., in prep) (see Figure 12). Interestingly, the rotating method resulted in notably longer sporophytes for *E. radiata*, *L. corrugata*, and *M. pyrifera*, with a respective increase in factors of approximately 2, 4, and 2 (Lush et al., in prep). Density was not impacted by the rotating treatment, indicating that the sprayed gametophytes were firmly attached.

Furthermore, Nardelli et al. (2023a) found that rotation led to an increase in blade and holdfast size of juvenile *L. corrugata* sporophytes during the nursery phase, and that biomass production doubled for the rotated spools during the at-sea growth phase. These findings were supported by Lush et al. (in prep), who reported a substantial increase in yield (kg wet weight per metre) for all tested species, with respective fold increases of 2, 5, and 1.2 for *E. radiata*, *L. corrugata*, and *M. pyrifera*. The density on the long line seems one of the major explanatory factors in the rotating treatment, with 2, 3.5, and 3 fold increase for *E. radiata*, *L. corrugata*, and *M. pyrifera* respectively (Lush et al. in prep).

Table 1. The temperature and light levels during the nursery phase for *Ecklonia radiata*, *Lessonia corrugata*, and *Macrocystis pyrifera* yielding best results based on a combination of the longest juvenile sporophytes, highest density on the seeded twine, and least contaminants (Visch *et al.* 2023). Throughout the cultivation a long-day photoperiod (16 h light:8 h dark) provided by LEDs (Fluval aquasky®).

Species	Temperature (°C)	Light level (μmol photons m^{-2} s^{-1})
Ecklonia radiata	15	30
Lessonia corrugata	12	60
Macrocystis pyrifera	12	30 or 60

Figure 12. Image of spools with juvenile *Lessonia corrugata* sporophytes after being cultured between 6 to 8 weeks in the nursery. (A) the rotation treatment and (B) the traditional methods with only aeration. Photo credit: Allyson Nardelli.

Optimisation of nursery culture conditions (spools):

Alteration of culture conditions: Throughout this project, diverse environmental conditions have been manipulated to achieve the optimal culture conditions during the nursery phase. As microscopic sporophytes grow and develop, their requirements change accordingly. Hence, it is necessary to investigate the modification of culture conditions such that they are optimal throughout the nursery.

Acclimation prior to deployment at sea: Prior to deployment, juvenile sporophytes can be acclimatised to the culture conditions at sea. Previous studies on other seaweed species have demonstrated that this practice can enhance survival rates, consequently positively affecting farm productivity. Although this approach has not been tested for the target species mentioned in this manual, it is interesting to test.

Horizontal orientation spools: The nursery cultivation of seeded spools is typically done by vertically placing them in seawater, with overhead lighting. To enhance space efficiency, arranging the pools horizontally can be more effective. However, in the case of overhead lighting, the spools must rotate to ensure uniform light distribution and balanced growth around the spool. In this manual, spool rotation showed great potential as a cultivation technique, but the efficacy of this method in a horizontal orientation still requires evaluation.

2.7 Contamination and biofouling

Controlling **contamination** in kelp aquaculture nurseries is similar to controlling contamination in microalgal cultivation (Richmond 2004; Beyter et al. 2016). In microalgal cultivation, contaminants can reduce growth and quality of the culture species, and instabilities in the culture can cause it to collapse entirely (Elisabeth et al. 2021).

Several decontamination methods have been tested in microalgal cultures, including chemical treatments (Guillard 2005), and more ecological approaches (Kazamia et al. 2012; Shurin et al. 2013). For instance, Shurin et al. (2013) suggest that a community engineering approach, which manages algal diversity, species composition, and environmental conditions, can enhance the resilience and productivity of algal culture systems. Kazamia et al. (2012) explore how a better understand of algal ecology can ensure more dependable raceway-based algal cultivation, drawing from established community ecology principles to identify protective practices against unwanted contaminants in algal cultures. In kelp aquaculture, few studies have focused on the nursery phase. As discussed earlier, there are two nursery methods used in kelp aquaculture: (1) mass release of motile zoospores from fertile parts on the thallus that settle to rope and develop into gametophytes and juvenile sporophytes that are deployed at-sea (Su et al. 2017), or (2) zoospores are released into media and developed into free-floating gametophytes that are vegetatively propagated under red-light conditions until they are manually adhered to rope (e.g., sprayed or brushed) and developed into juvenile sporophytes that are deployed at-sea.

Both methods are susceptible to contamination, and various methods have been tried to deal with contamination, such as **mechanical removal** of epiphytic contaminants, **alterations in culture conditions** in favour of the culture species over the contaminant (Su et al. 2017), or **chemical disinfection** of the sorus tissue prior to spore release (Rød 2012). Su *et al.* (2017) observed reduced contamination when the light level was limited to below 20-25 µmol photons m^{-2} s^{-1}, combined with relatively low levels of nutrients (8 – 11.8 µmol L^{-1} NO_3 and 0.7 – 1.5 µmol L^{-1} PO_4) compared to nutrient levels typically employed (F/2-medium: 882 µmol L^{-1} NO_3 and 36.2 µmol L^{-1} PO_4). Both Lush et al. (in prep) and Nardelli et al. (2023a) noted a significant reduction in contamination on the spools when they were **rotating** compared to when they only received water motion through aeration. Furthermore, closed culture systems are also being developed to avoid contamination during media changes when vegetatively propagating kelp gametophytes (Ebbing et al. 2022).

Chemical de-contamination strategies are commonly used to manage or eliminate contamination. For example, infestations of zooplankton can be managed by **lowering the pH** (Becker 1994) or adding **ammonium hydroxide** thereby temporarily raising the free ammonia concentration to approximately 20 mg L^{-1} (as N) (Lincoln et al. 1983). Diatom growth can be reduced by adding **germanium dioxide** (GeO_2) at a concentration between 0.02 and 0.11 mmol L^{-1} seawater (Shea and Chopin 2007). However, as mentioned earlier, it is advisable to restrict the utilization of GeO2 to a maximum of 2 days or as brief intermittent treatments, as prolonged exposure can negatively impact the growth and maturation of sporophytes (Praeger et al. 2022).

Cyanobacteria contamination can be treated with **antibiotics** (Andersen 2005). However, the use of antibiotics must be balanced between contamination control and nursery success, as microbial interactions are complex (Li et al. 2020; Li et al. 2021) and likely a prerequisite for sporophyte growth and development (Charrier et al. 2017; Wichard 2022). A dose response experiment of gametophytes to the application of antibiotics (per mL consisting of 10,000 units penicillin, 10 mg streptomycin, and 25 µg amphoteric-B) indicated its utility against common types of bacterial contamination in these cultures (e.g., cyanobacteria) (see Table 2).

Nevertheless, mechanical removal of contaminants is still a common practice in large-scale kelp hatcheries in China, where seeded culture ropes containing juvenile sporophytes are washed periodically (usually between 1 and 3 days) to eliminate ungerminated spores, malformed sporophytes, and other contaminants that negatively affect the nursery's productivity (Su et al. 2017).

Employing a combination of the methods mentioned above may yield the most effective results; mechanical removal can be followed by a chemical treatment, and culture conditions can be adjusted (such as temperature or light levels) to promote the growth of target kelp juveniles while discouraging contaminant growth. Figure 13 depicts various types of contaminants and their adverse effects on the quality of seeded spools.

Regularly monitor the spools' **quality and sporophyte density** for any contaminants and record the growth of small sporophytes. To do this, snip a small sample from a culture string that has been deliberately left hanging from each spool and examine it under a binocular microscope (Figure 14). Take a photo of the sample with a scale bar to note any changes that occur throughout the nursery period.

Table 2. The recommended antibiotic* concentration showed no negative signs on gametophyte growth nor on their rate of transition into sporophytes of the tested kelp species. A dose response experiment testing 0, 1, 5, 10, 25, 50, 100, 200, 400, 800, 1200, and 2000% of the recommended concentration by the manufacturer is 10 mL/L for 50 days (n=8 per species treatment combination).

Species	Concentration (% of 10 mL/L)	Volume (mL/L)
Ecklonia radiata	5	0.5
Lessonia corrugata	10	1
Macrocystis pyrifera	10	1

* Antibiotic Antimycotic Solution (100x), per mL consisting of 10,000 units penicillin, 10 mg streptomycin, and 25 µg amphoteric B.

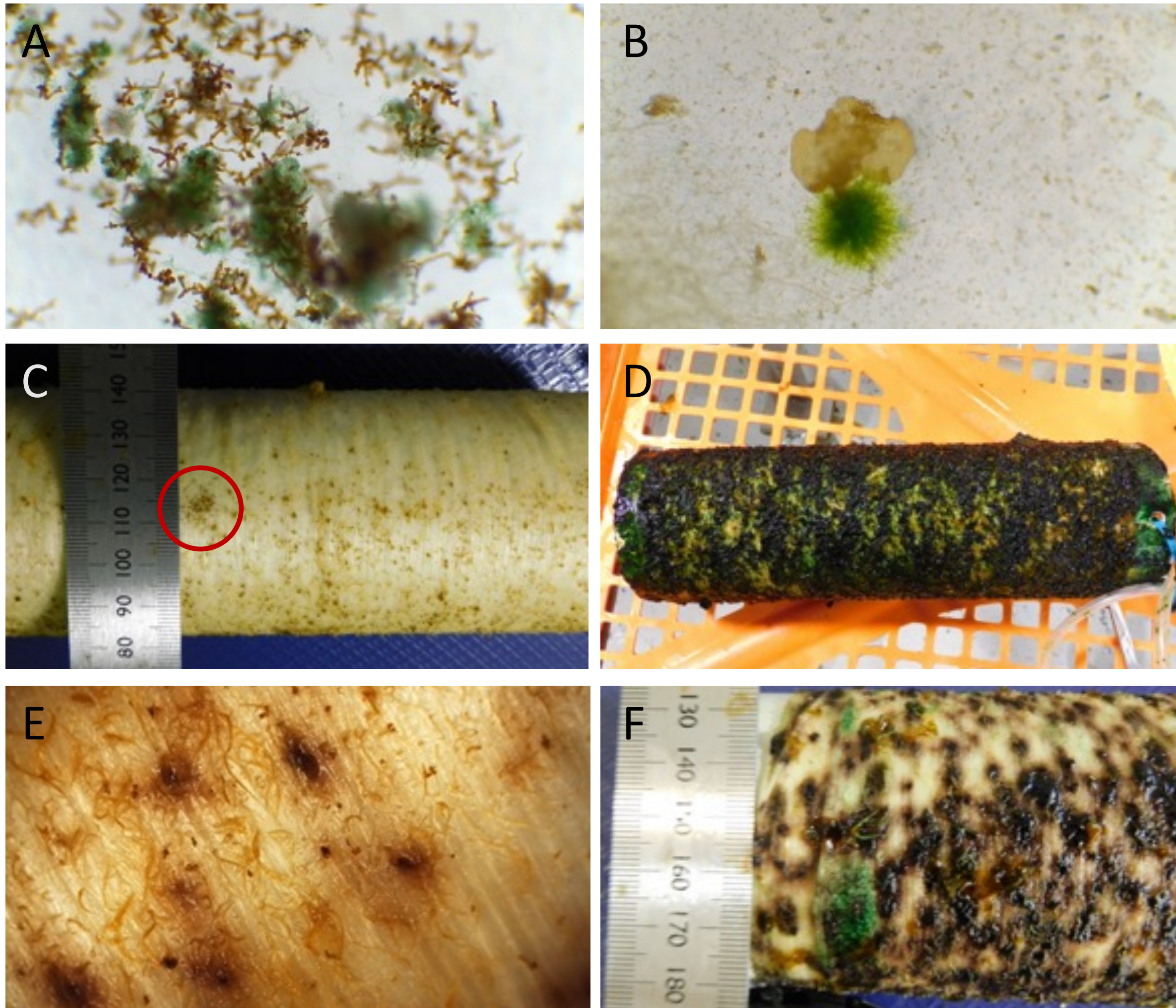

Figure 13. Various types of contaminants observed during the nursery phase. (A) Free floating gametophyte culture infested with blue-green microalgae (i.e. cyanobacteria). (B) A juvenile kelp sporophyte infested with a green alga. (C) Diatom contamination on a seeded spool with an example encircled in red. (D) The result of a contaminated spool left untreated in culture for several weeks. (E) A close-up image of diatom contamination on a seeded spool. (F) A close-up image of a spool contaminated with cyanobacteria and *Ectocarpus* left untreated and in culture for several weeks with juvenile sporophytes only sporadically present. These types of contaminants are common worldwide and not confined to Tasmania. Photo credits: Craig Sanderson.

Figure 14. Sample of the seeded string from a culture spool showing kelp sporophytes growing along its length. Photo credit: Harry Lush.

Opportunities for contamination and biofouling management:

Contamination: It is essential to conduct systematic studies on the treatment of various forms of contaminants that have a negative impact on nursery outcomes. Particularly when scaling up nursery operations to larger volumes where controlling contaminants is generally more challenging compared to smaller scales.

Combining methods: There exist several methods that can enhance efficiency in kelp aquaculture nurseries by reducing contamination. These methods encompass mechanical removal of contaminants, adjustment of culture conditions, or employment of chemical treatments. The most effective outcomes may be achieved by employing a combination of these methods. However, further evaluation is required to determine the optimal combination of methods for specific contaminants.

Monitoring: It is recommended to regularly monitor the quality of the spools and the density of sporophyte to identify any contaminants and record the growth of small sporophytes. By implementing these strategies, the nursery's productivity can be increased, and the growth and quality of the cultured species can be enhanced.

2.8 Summary of knowledge gaps and opportunities for nursery production

This chapter described the necessary equipment and steps for a successful production of seeded line in a nursery. It covers various aspects, including the collection of fertile kelp tissue in the field, gametophyte culturing, appropriate culture condition for growing small kelp on twine, and the management of biofouling and contaminants. While this manual outlines these processes for the target species for the first time, there are still gaps in our current understanding and opportunities for improvement.

Regarding **laboratory and culture media preparation** (Chapter 2.1), a cost-effective culture medium tailored to the target species can be developed. For example, most manuals use either F/2-medium (e.g., this manual) or Provasoli Enriched Seawater (PES). However, given a diverse range of kelp species and strains are cultured or newly brought into culture, each with their specific nutrient requirements, it is worth exploring the use of different receipts and nutrient concentrations throughout each step of the nursery phase.

The optimisations in **sourcing of seedstock** and **spore release** (Chapter 2.2) include inducing fertile sorus tissue in kelp sporophytes through laboratory manipulations, enabling a year-round supply of spores. This is particularly important as kelp reproduces seasonally. Additionally, efforts should be made to reduce or handle the amount of polysaccharides released during the spore release process, which is particularly significant in *Ecklonia radiata* and to a lesser extend *Macrocystis* and *Lessonia*.

Maintenance of gametophyte stock cultures (Chapter 2.3) typically involves breaking up the gametophyte into smaller fragments to maximise biomass production for onward cultivation. The frequency, the fragment size, and the optimal culture density in relation to gametophyte size are unknown, and this knowledge could significantly improve nursery outcomes.

Efficiencies for the **stimulation of reproduction in kelp gametophytes** (Chapter 2.4) include the optimisation of the environmental conditions such as light, temperature, and nutrients in relation to the culture density. Exploring optimal male to female ratios in kelp nurseries, as well as studying the contribution and potential negative effects of parthenosporophytes on nursery outcomes, are also necessary.

Efficiencies in **the preparation of seeded line** (Chapter 2.5) in the nursery involve improving the automating the twine winding around pipes, investigating different types of twine that improve nursery outcomes and the sustainability by potentially reducing the amount of plastic-based rope in kelp aquaculture, and exploring the use of binders that aid gametophytes in adhering to cultivation line at sea.

One of the knowledge gaps in our understanding of the **culture condition of spools in the nursery** (Chapter 2.6) is whether modifications can be made optimise them for the microscopic sporophytes as they grow and develop throughout the entire nursery phase. Another gap determining whether acclimating the seeded spools in the nursery to the environmental condition at-sea prior deployment is beneficial. Additionally, the potential benefits of horizontally rotating spools to improve space utilisation till needs to be tested.

There are various knowledge gaps and efficiencies regarding **contamination and biofouling** (Chapter 2.7) in kelp nurseries, especially when scaling up. These gaps range from studying treatment types for the different forms of contamination and finding preventative and reactive treatment combinations that improve nursery outcomes. Moreover, methods for regular monitoring to prevent contamination or facilitate prompt handling when it occurs need to be developed.

Finally, even if all the suggested knowledge gaps and efficiencies for the nursery phase are resolved and implemented, new ones are likely to arise. These gaps will typically emerge during crucial phases or transitions in the developmental biology and life cycle of kelp, as they require changes in culture conditions, environment, and manual handling. Therefore, Figure 15 provides a comprehensive timeline, highlighting these crucial phases or transitions and their respective duration and timing.

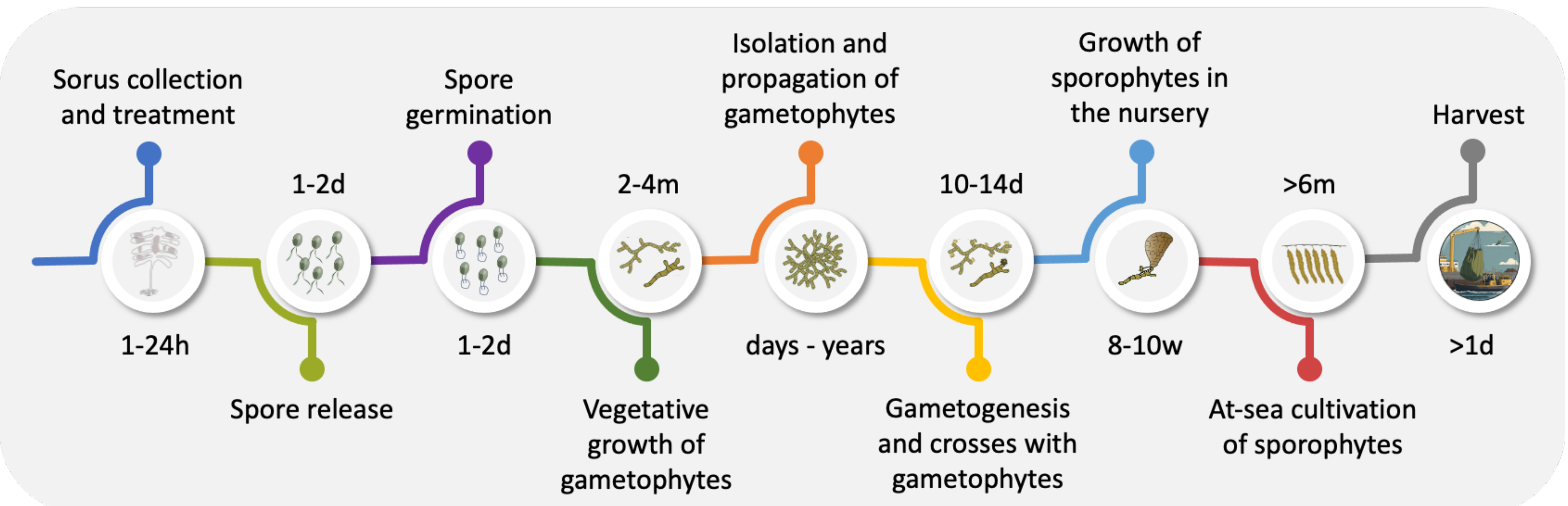

Figure 15. A comprehensive timeline of the crucial phases or phase-transitions of spore development into a gametophyte and sporophyte in an aquaculture setting, both when spores are mass-released and/or clonal gametophyte cultures are utilised. The timeline outlines the duration from one phase to the next, starting with the collection of mature sorus tissue and spore release, and ending with the harvest of kelp biomass following the deployment of juvenile sporophytes. It is important to note that the duration of each phase may vary depending on the culture species or the culture conditions. With h = hour; d = day; w = week; m = month.

Chapter 3. At-sea cultivation

3.1 Equipment

The equipment needed to deploy the seeded grow line at sea depends on the layout and structure of the farm, as well as the bathymetry and exposure of the site (Figure 16). A comprehensive list of the equipment required for cultivating kelp at sea can be found in Appendix 4.

Constructing the farm involves two phases: deploying and setting the mooring/anchors, bridle, backbones, and buoys in the first phase, and seeding the grow lines and setting them at the farm infrastructure that was placed at sea in the first phase. Generally, a farm design consists of three components:

1) Mooring/ anchors
2) Bridle, backbones, and buoys
3) Winding seeded twine and setting the grow line

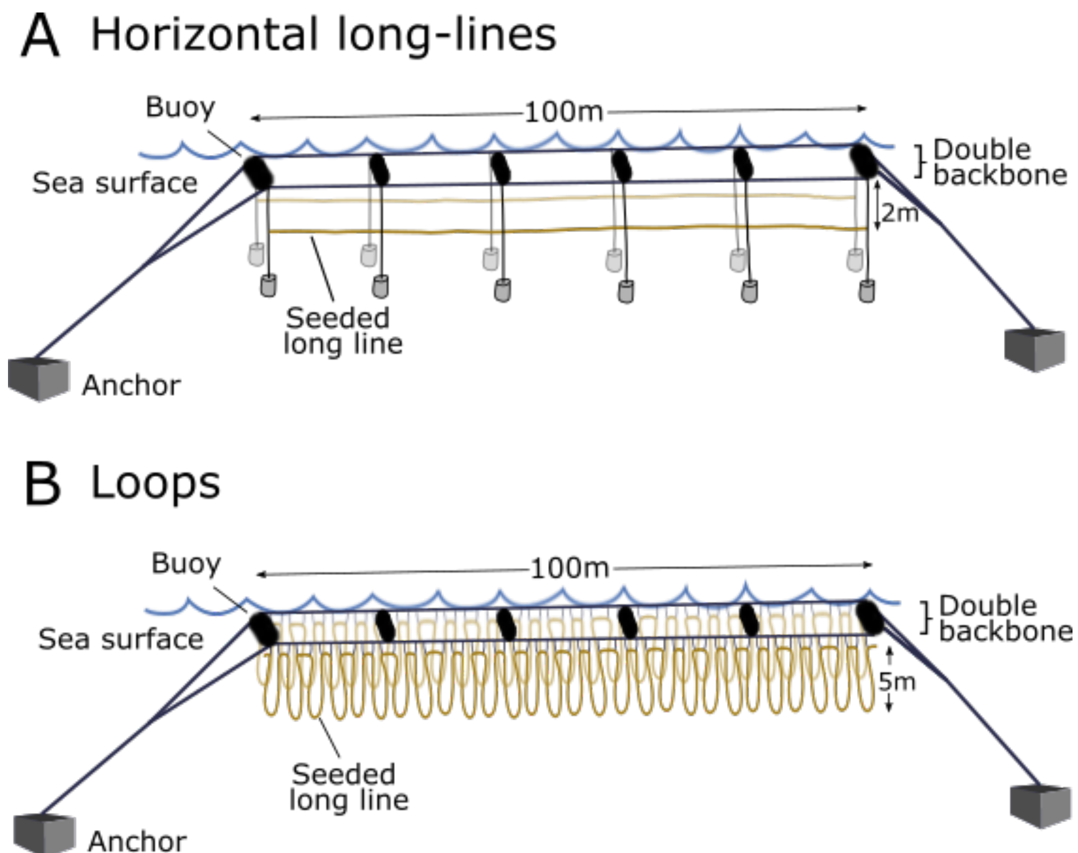

Figure 16. Two examples of farm designs used in the Seaweed Solutions for Sustainable Aquaculture CRC-project. Both arrangements make use of the same infrastructural design, such as anchors, buoys, and double backbone. (A) grow-line system horizontally suspended at 2-m

depth (B) grow-line system placed in the looped arrangement suspended between 0.5 to 5-m deep, adapted from mussel aquaculture.

To arrange grow lines in a **horizontal farm layout** (Figure 16A), the seeded twine is tied onto the grow line and wound around it by pulling it through the spool (see step 3 of Figure 11). It is important to make sure that the twine is tightly wound around the grow line to allow for firm attachment of the seedling holdfasts/haptera. Regularly spaced zip-ties (every 5 to 10 m) can be used along the grow lines to ensure proper contact and prevent unwinding in case of twine breakage. For large-scale operations, grow lines are wound at sea either by lifting them onto a vessel or running a smaller vessel along the line. In small-scale operations such as R&D or test sites, the grow lines can be prepared onshore to avoid exposing juvenile seedlings to stressful temperatures or air for extended periods. For example, by regularly keeping the seedlings wet and submersing the seeded grow lines directly in seawater until deployment at sea. After preparation, the grow lines can be transported in tubs filled with seawater or covered in damp cloths and deployed at the farm site. Although not quantified here, seedling loss from the twine or mortality, possibly due to water movement and/or abrasion of moving twine/grow line, during transport need to be taking into consideration. These factors highlight the logistical challenges around moving seeded twine from the nursery out at sea.

In the **looped farm arrangement** (Figure 16B), grow lines are deployed in loops at 1-5 m depth onto 100 m double-backbone long lines made of 32 mm polypropylene rope. This setup maximizes the available space in the marine lease area and is suitable where the seawater is relatively clear enabling light to penetrate deeper. In our test trials, we had three double-backbones aligned parallel and separated by ~25 m (to allow for passage of service vessels) at each of the three sites: IMAS R&D farm at Tower Bay, and the Okehampton Bay and Great Taylor Bay marine leases. The grow lines are looped parallel along each backbone and attached to the backbone at ~1 m intervals, secured with 5-mm nylon rope and submerged at 1 m depth. This arrangement allows for a significantly longer length (approximately 2000 m) of grow line on each 100 m double-backbone, but the light environment is more variable than for horizontal lines submerged at a given depth; and as the sporophytes grow, shading of the bottom of the loops can occur creating uneven biomass and quality along the grow line. Each double-backbone is kept afloat and separated by 1.3 m with 300 L buoys on either end and four 110 L buoys spaced along the backbone, secured at the sites by 350 kg concrete anchors. Winding seeded twine around the looped grow lines can be done using a modified electric motor with a spool spinning around the grow line – this method is applicable both to horizontal and looped farm arrangements (Figure 17) (Solvang et al. 2021).

In both the horizontal and looped farm arrangements changes may be required as the cultivation season progresses. For example, additional weights to keep the positively buoyant grow lines with *M. pyrifera* submerged, or additional buoys to maintain the optimal growth depth of neutrally buoyant species like *E. radiata* and *L. corrugata*.

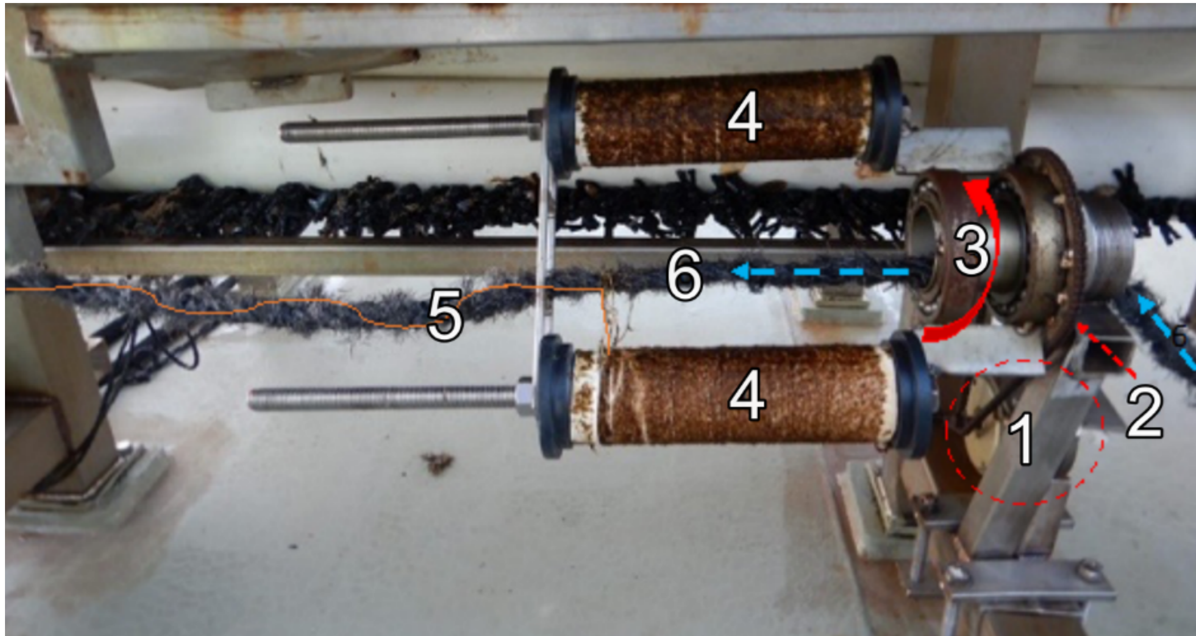

Figure 17. Seeded twine deployed onto grow lines (polypropylene mussel rope) spinning the spools around the rope with the help of a modified electric motor. (1) An electric motor (dashed red circle) that drives (2) a belt (dashed red arrow) and spins (3) two (4) seeded spools around the grow line, thereby winding (5) the seeded twine (orange line) around (6) the grow line (dashed blue arrows) that is subsequently deployed at sea. This method was used in Biancacci et al. (2022c).

<u>Considerations for equipment, setting the farm infrastructure, and deploying the grow lines:</u>

Equipment: The choice of equipment use is closely tied to the environmental conditions at the farm site, as well as the selected species and scale of the farming operations. Typically, safety factors are applied when determining the appropriate rope size, anchors, and other farm infrastructure to ensure they can withstand the forces they will encounter.

Buoyancy of crop: *Ecklonia* and *Lessonia* are stipitate seaweeds that exhibit neutral buoyancy and are typically harvested at lengths not exceeding 1.5 metres. In contrast, *Macrocystis* can grow up to 6 metres in cultivation, with multiple fronds containing blades every 10-15 cm and air-filled bladders. The variation in growth and buoyancy among the target species discussed in this manual has significant implications for farm design, use of weights/floats, and the type of grow line employed. Further research is required to develop species-specific farm designs and associated equipment.

3.2 Site selection

Careful site selection is crucial for successful at-sea kelp cultivation, and it depends on a variety of factors such as seawater temperature, nutrient concentrations, light levels, water motion, salinity, and how these factors interact and vary with the season and growth requirements of the target kelp species (i.e., *Ecklonia radiata, Lessonia corrugata,* and *Macrocystis pyrifera*). Furthermore, it is important to consider the minimum and maximum values – not just averages – and the time the crop is exposed to these extremes during the cultivation season. The map (Figure 18) below displays the geographic locations of the three farm sites in south-eastern Tasmania, Australia, where the data on which this manual is based were collected.

The **timing of deployment and harvest** is crucial for producing commercial-grade biomass with a desirable biochemical composition and minimal biofouling (see Chapter 4 for more detailed description). In the southern hemisphere, kelp cultivation typically occurs from autumn (March, April, May) through winter (June, July, August), with harvesting in spring (September, October, November).

Table 3. Physical and chemical conditions of the seawater that can affect the growth and productivity of seaweeds at the three farm sites (mean ± SD) during a typical cultivation period in Tasmania (i.e., March – November). The values for temperature, salinity, and Secchi depth are from 2014 to 2020, while the values for nitrates, and phosphate are from 2020. Wave action/exposure level is subjectively classified.

Environmental variable	Okehampton Bay	Great Taylor Bay	Tower Bay
Bathymetry / depth (m)	27	35	20
Wave action / exposure level	Moderately exposed	Moderately exposed	Exposed
Mean annual temperature (ºC)	12.9 ± 1.5	12.6 ± 1.6	12.6 ± 1.3
Max annual temperature (ºC)	17.6	16.7	16.4
Mean nitrates (µmol L^{-1})	2.5 ± 1.3	2.3 ± 1.2	2.3 ± 1.0
Max nitrates (µmol L^{-1})	5.1	5.8	4.1
Mean phosphate (µmol L^{-1})	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Max phosphate (µmol L^{-1})	0.7	1.0	0.7
Mean salinity (ppm)	33.4 ± 1.5	31.2 ± 1.1	32.5 ± 0.7
Min salinity (ppm)	29	28.0	30.0
Secchi depth (m)	9.5 ± 3.0	5.9 ± 0.8	6.0 ± 1.2

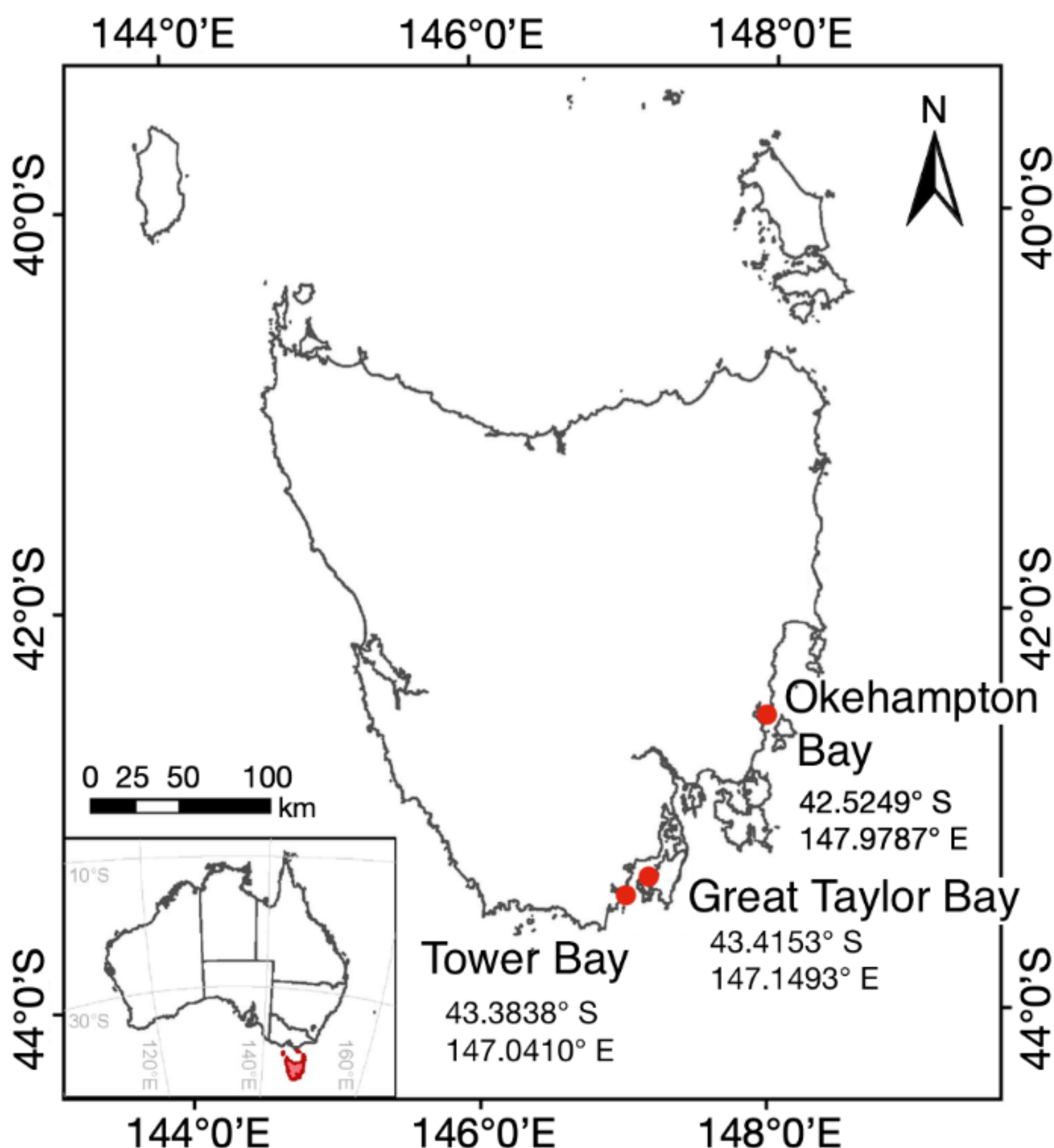

Figure 18. The geographic locations of each farm site in south-eastern Tasmania, Australia. Where Okehampton Bay and Great Taylor Bay are aquaculture lease sites that have salmon pens nearby. Tower Bay is a research and development aquaculture lease in which currently only seaweeds are allowed. See Table 3 for a detailed description of the environmental conditions at each site.

However, other researchers have explored an 18-month cultivation cycle for *Macrocystis pyrifera* cultured at a depth of 9 meters in Tasmania (Cayne Layton, pers. com.). Combining the results of yield, biofouling, and biochemical composition Biancacci et al. (2022c) suggested a mid-late winter (July-August) harvest for *M. pyrifera* to optimise yield and quality of the cultured kelp biomass when farmed between 1-5m depth in south-eastern Tasmania. Finally, Nardelli et al. (2023a) explored year-round cultivation of *Lessonia corrugata* at Tower Bay and found that spring produced the greatest biomass and survival at 3m depth.

The cultivation period/season interacts with site selection, as well as the physical and chemical properties of the sea at the cultivation site (see Table 3 for more details of the sites used in this project). Generally, kelps prefer high-nutrient clear waters and temperatures below 18 ºC, although this varies by species. *E. radiata* can tolerate the highest temperatures compared to *M. pyrifera* and *L. corrugata*, based on the culture conditions in the nursery. Smart et al. (2022) observed higher ammonium uptake rates for *M. pyrifera* than for *L. corrugata* and *E. radiata*, indicating that it is the most suitable species for an integrated multi-trophic aquaculture context where elevated ammonium concentrations are expected. Furthermore, under ambient temperature conditions, ammonium uptake showed little to no seasonal pattern, indicating that the timing of deployment is not limited by nutrient uptake rates for the kelp species targeted in this manual (Smart et al. 2022).

Wave exposure is a crucial factor to consider when selecting an optimal farm location. The site must not only suit the biology of the kelps but also be practical and safe to work at. The farm infrastructure must withstand the hydrodynamic forces of the waves. However, working around the weather can be challenging, and more exposed sites typically require larger vessels to operate safely in high wave conditions, which can further limit the days available for deployment, harvesting, and farm maintenance. In addition, wave exposure can affect the nutrient uptake and erosion rate of the tip while decreasing biofouling (Visch et al. 2020b). More exposed sites typically have increased nutrient uptake, which can enhance kelp growth. However, it can also increase the apical erosion rate, leading to a reduction in biomass. Nonetheless, it can decrease biofouling, which can improve the overall quality of the biomass. Therefore, it is essential to strike a balance between all these factors to select the optimal farm location (Visch et al. 2020b; Visch et al. 2020a). For more detailed information, please refer to Chapter 4.1.

Finally, regarding site selection, there has been a driving interest to relocate farming operations from near-shore to **off-shore environments**. However, Visch et al. (2023b) have identified several challenges and knowledge gaps for cultivating seaweed in offshore environments in southern Australia, including Tasmania. Some of these challenges include the need for an improved understanding of cultivation biology, the development and maintenance of appropriate infrastructure and technology, and the identification of suitable species for offshore cultivation. Of the kelp species discussed in this manual, this study rates *M. pyrifera* the highest, followed by *E. radiata*, and then *L. corrugata* in terms of potential for offshore cultivation. This is primarily driven by a higher market potential, biomass production, and current cultivation knowledge for *M. pyrifera*. Whereas *L. corrugata* scores moderate to moderate low on these metrics in addition

to a restricted distribution and temperature tolerance, which suggests it may be more vulnerable and less resilient compared to *M. pyrifera* and *E. radiata*.

3.3 Farm maintenance throughout the cultivation period

Regular farm maintenance is a crucial aspect of seaweed farming in Tasmania. According to the current **permit regulations**, farm infrastructure must be checked monthly to ensure its integrity. This includes inspecting the buoys, which are prone to detachment during storms. Failure to report any damage to infrastructure can result in hefty fines. The accumulation of detached **drift-weed**, such as *Phyllospora comosa* and/or *Macrocystis pyrifera*, around the farm also needs attention as it can entangle and damage the infrastructure, and increase drag forces. To prevent this, the drift-weed must be regularly removed to ensure the farms integrity and prevent the buoys from sinking and impacting the depth of the cultivation lines (Figure 19). Additionally, biofouling, mainly mussels/invertebrates and other seaweeds (e.g., *Ulva* spp., *Durvillaea* spp., and various filamentous red seaweeds), must be removed from the buoys and back-bones to minimise drag and negative impacts on the quality of kelp biomass. One effective method of cleaning the back-bones is to attach a rope to a boat's hull and run the rope alongside the back-bones, stripping away any fouling organisms. Biofouling growing underneath the buoys can be removed from a boat or by divers using a shovel or dive knife by tilting the buoy slightly so it can be reached from either side. It is also important to monitor the moorings and anchors regularly, which involves SCUBA diving or a submersible remotely-operated-vehicle (ROV).

To carry out fieldwork, it is essential to have access to vessels and **skilled personnel**; for example, at least one skipper and two or three other qualified individuals, depending on the size of the vessel used. Harvesting (see Chapter 4) can be done from the boat, but in certain situations, such as tangled lines or dislodged buoys, snorkelling or SCUBA diving may be necessary. It is also necessary to have a towing vehicle with a boat trailer to transport the boat to the launch point.

Maintaining a good relationship with **local stakeholders**, such as fishermen and other aquaculturists, who use the coastal waters nearby the seaweed farm lease, can be very beneficial. They can provide valuable information about abnormalities of the farm infrastructure. It is also important to regularly check the crop, which can be aligned with farm maintenance throughout the cultivation period. Monitoring of infrastructure is still necessary when no crop is present, as per the permit requirements. Overall, regular farm maintenance is crucial to ensure the success and sustainability of seaweed farming in Tasmania.

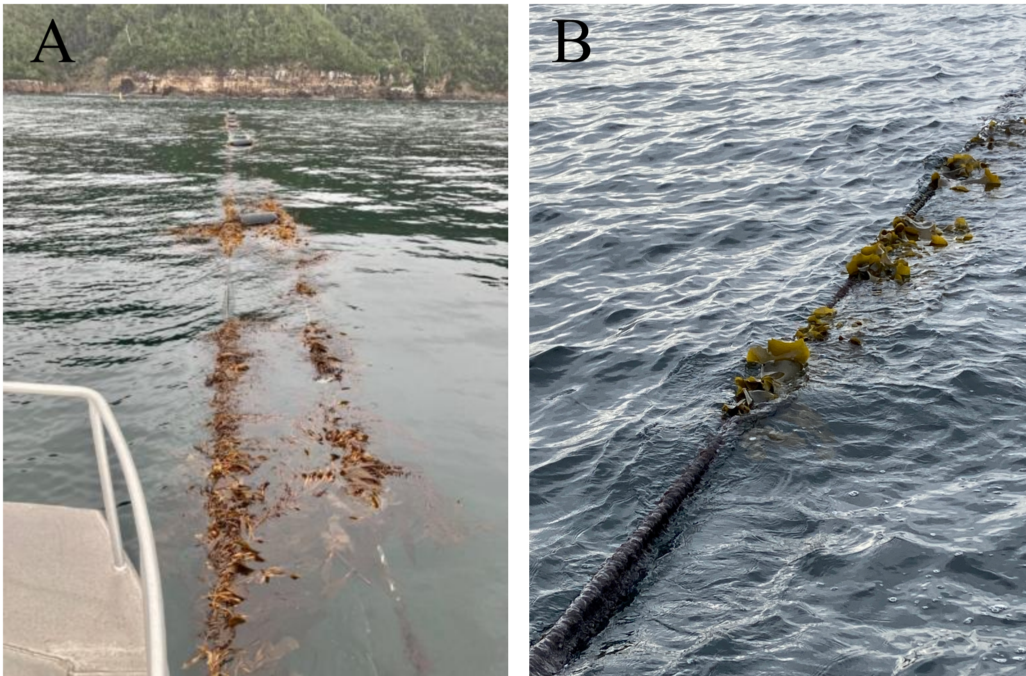

Figure 19. Examples of fouling seaweed observed on the farm infrastructure at Tower Bay, Tasmania. The fouling seaweed either drifted or settled into the lines that float on the waterv surface and increases drag and reduces the buoyancy of the structure, making it instable. (A) *Phyllospora comosa*, a brown seaweed with long, strap-like fronds, drifted into the lines. (B) Naturally recruited *Durvillaea* spp., a large, brown seaweed with thick, leathery fronds, settled onto the lines. Photo credit: Wouter Visch.

Summary of requirements for farm maintenance:

Regular farm maintenance: Ensuring the integrity and sustainability of the seaweed farming infrastructure is crucial. By conducting regular inspections and removing biofouling, drift-weed, and other growth from the buoys and backbones, farms can maintain their productivity and prevent costly damages.

Compliance with regulations: The current permit regulations in Tasmania require monthly checks of the farm infrastructure to ensure its integrity. By adhering to these regulations and promptly reporting any damages, farmers can avoid hefty fines and ensure the long-term sustainability of their operations.

Monitoring of moorings and anchors: Annual inspections conducted through SCUBA diving or ROV can prevent the buoys from sinking and ensure the stability of the farm infrastructure.

Building good relationships with local stakeholders: Maintaining positive relationships with local fishermen and other aquaculturists can provide valuable information about abnormalities of the farm infrastructure. This can help prevent damages and enhance the overall sustainability of the farms.

Crop monitoring: Regular monitoring of the crop throughout the cultivation period allows farmers to identify any issues early on and take appropriate measures to ensure the success of the farming operations.

3.4 Summary of knowledge gaps and opportunities for at-sea cultivation

In chapter 3, the necessary equipment, location considerations, and farm maintenance for cultivating kelp at sea is discussed. Various aspects are covered, including the required equipment specifically in relation to the farm design (e.g., lines arranged either horizontally or in loops) and site selection, based on factors such as seawater temperature, nutrient concentrations, light levels, and water motion. Similar to the nursery phase, this manual outlines these processes for the target species for the first time, but there are still gaps in our current understanding and opportunities for improvement.

Considerations for **equipment, setting the farm infrastructure, and deploying the seeded long-lines** (Chapter 3.1) primarily revolve around the local environmental conditions at the farm site and the scale of the farming operations, making generalisations challenging. In addition, the morphology and buoyancy of the crop have significant implications for the equipment employed and farm design.

Regarding **site selection** (Chapter 3.2), opportunities for improvement exist concerning the cultivation season, prior knowledge of the environmental conditions at the farm, and careful selection of farm sites that support growth and development of the targeted crop during the cultivation period. Conducting a small-scale pilot study can be highly informative. In certain instances, nearby environmental monitoring stations can provide baseline data to support the suitability of a potential farm site.

Requirements around **farm maintenance** (Chapter 3.3) involve regular inspections of both the crop and the farm infrastructure, compliance with regulations, and building positive relationships with other stakeholders.

These knowledge gaps, challenges, and opportunities are tightly linked to the scale of the farming operations and future farmers should not forget to incorporate them into the business model. In particular, farm maintenance and monitoring can lead to unexpected costs as they are typically not the primary focus of aquaculturists.

Chapter 4. Product harvesting and primary processing and stabilisation

Seaweeds are important marine bioresources that have been traditionally used for various applications including food, feed, fertilizer, and the extraction of valuable compounds such as agar and alginates. They are also sources of biologically active compounds including antioxidants, fatty acids, polysaccharides, and sources of important nutrients including minerals and vitamins (Kadam et al. 2015; Skrzypczyk et al. 2023).

Interest in the commercialization of seaweed products for cosmetics, nutraceuticals, food, and feed are growing worldwide. This is due to the increasing awareness of the numerous health benefits associated with use of seaweeds. However, producing and developing high-quality and consistent seaweed-based products can be challenging. This is because the biochemical profile of seaweeds can not only vary depending on the season, species, and site, but also depend on the harvesting, processing, and storage of the biomass (Kadam et al. 2015). In addition, processing of the seaweed biomass for a particular product (e.g. food applications) can lead to the production of significant “waste”. To maximize biomass utilization and promote the circular economy, the biorefinery processing approach should be adapted to local conditions to minimize and/or exploit waste fractions (Torres et al. 2019). Understanding how to implement and optimize these processes is fundamental for the successful development of the seaweed industry in Australia and worldwide.

4.1 Harvesting

To minimize the risks associated with working on the boat during harvesting, calm and stable **weather conditions** with low winds and swell should be sought. As alluded to in Chapter 3.2, **wave exposure** is a crucial factor in **site selection**, as it not only affects crop yields but also impacts the safety of working on the boat and the efficiency of harvesting. While off-shore cultivation may pose greater logistical, operational, and economic challenges than seaweed farming in coastal waters, it may be the preferred option where possible, particularly for high-end products that justify the additional costs (Visch et al. 2023b). Off-shore cultivation typically results in reduced conflicts with existing coastal activities (e.g. marine protected areas, fisheries, etc.), higher water mixing and reduced biofouling (Bak et al. 2018).

The **harvesting time** for seaweed is heavily dependent on the cultured species and the environmental conditions, such as irradiance and temperature, at the farm site. For the investigated sites on the southeastern coast of Tasmania, we recommend harvesting the biomass of *Macrocystis pyrifera* and *Ecklonia radiata* towards the end of winter or at the start of spring (between July and September-October in Australia) before the seawater temperature increases and promotes the growth of biofouling and epiphyte communities on the cultured seaweed (Biancacci et al. 2022c). Furthermore, our data suggests a similar harvest time for *Lessonia corrugata* for optimal biomass between late spring and early summer (Nardelli et al. 2023a). This would prevent negative impacts on both the seaweed biomass and farm infrastructure caused by biofouling and thereby reduce the labour and maintenance costs associated with the maintenance of the grow lines and other infrastructure. Previous studies on other seaweed species have recommended harvesting before rising seawater temperatures increase biofouling (Park and Hwang 2012; Ateweberhan et al. 2015; Førde et al. 2015; Marinho et al. 2015; Keesing et al. 2016), which is consistent with our findings, especially for *M. pyrifera* and *E. radiata*. Nonetheless, regular monitoring, such as periodic biomass assessments and deploying loggers to measure water parameters like temperature and light, should be carried out to ensure the best harvesting time, as this will vary depending on the local culture conditions (Forbord et al. 2020). Harvesting time will also vary depending on the intended market for the collected biomass. For example, if the biomass is supplied as fertiliser or building material, fouling may not impact the final application, and harvesting might occur later in the season.

Mechanical harvesting provides a more efficient means of collecting biomass, especially when dealing with large quantities. However, this can only be done on vessels of a certain size (such as **Figure 20**) that are equipped with the appropriate gear for anchoring the lines, lifting them out of the water (as shown in **Figure 20**A) and manually stripping the attached biomass (**Figure 20**B) or mechanically pulling the lines through a circular cutter.

Typically, mussel harvesting boats are repurposed for seaweed harvesting. However, recent investigations have been focused on developing specialised vessels for seaweed farming (Solvang et al. 2021), which will enhance the overall process, making it more efficient and customised to the target species, local environmental conditions, and local infrastructure. Consideration should also be given to whether the target species can be repeatedly partially harvested before reseeding for economic efficiency (Bak et al. 2018), and whether mechanical harvested can achieve this. Depending on the size of the vessel, movement within the farm and between cultivation lines may be limited, so the farm design should align with the available vessel size and the harvesting method (manual or mechanical). The duration of the harvesting

Figure 20. Harvesting of kelp biomass using a repurposed mussel harvesting vessel at Okehampton Bay, Tasmania, in September 2020. (A) The mussel boat geared to anchor and lift the seeded lines deployed in a looped arrangement, and (B) harvesting the biomass manually by cutting the seaweeds from the cultivation lines.

process will also depend on various factors such as processing capacity, biomass quantity, logistics, and weather conditions. The harvesting strategy depends on the expected production volume, and may be completed in a single day, or spread out over multiple days or even months.

Manual harvesting is typically done for smaller volumes and requires more time and personnel per unit wet weight compared to mechanical harvesting, but it allows for the use of a smaller vessel, and potentially more selective partial harvesting. For this project we opted to move the boat along the lines (Figure 21A) so the seeding lines could be pulled onto the boat (Figure 21B) for cutting, sorting, and storage (Figure 21C).

The **yield** and **production volume** of harvested biomass can vary greatly depending on factors such as the species, nursery practices, environmental conditions (e.g., temperature, light, depth, water motion), farm setup, deployment and harvest time, and site selection. These numerous factors can make it challenging to predict the biomass yield (Forbord et al. 2018). Therefore, regular monitoring throughout the at-sea cultivation period is recommended to assess the growth rate of the cultivated biomass and, when possible, in comparison to growth patterns in wild populations. We recorded a modest average yield of around 1.2 - 1.5 kg m^{-1} for the Australian species investigated in this project. However, higher yields have been reported for the same species cultivated in Chile (e.g., *M. pyrifera*) (Gutierrez et al. 2006; Westermeier et al. 2006; Buschmann et al. 2007). Such differences in yield are likely related to geographical variations, such as nutrient concentrations or temperature, as well as different cultivation methods.

Figure 21. Manual harvesting at Great Taylor Bay in November 2020. (**A)** the boat is connected at two points (arrows) and move along the lines; (**B)** the ropes are manually pulled on the boat and (**C)** the biomass is cut off the rope and stored into tubs on the boat.

Transport and storage of the harvested biomass is another crucial step. Seaweeds are highly perishable, with a moisture content of up to 80%. Therefore, care and timing should be applied to the transport, processing, and storage of the cultivated biomass to prevent degradation. Once the biomass is harvested, it is advisable to rinse it with seawater on board of the vessel to remove loosely associated biofouling and other epiphytic organisms. Depending on the quantity of biomass, time until processing, and ambient temperature, it can be stored without water, either in bags, tubs or cool boxes, and transported to the shore for further processing. Smaller bags/tubs may be preferred to avoid stacking too much biomass at once, resulting in the compression of biomass and an increased temperature inside the bags/tubs. To avoid degradation, the biomass may need to be kept cool or refrigerated during transport. The biomass can be harvested together with the cultivation lines and stripped once on the shore (Figure 22), or stripped on the boat (Figure 21C).

If the biomass is not **processed immediately**, it is advised to keep it cool, for example on ice or in a temperature-controlled room set at ~4 °C until processed. Processing should start within 48 hours upon harvesting, which can trigger wound response, increase oxygen exposure and activates enzymes linked with degradation (Amarowicz et al. 2009) to avoid further degradation of the biomass and leaching of valuable compounds, such as pigments, antioxidants and fatty acids. In this project, we assessed the effect on quality of storing the harvested and washed biomass of *M. pyrifera* on ice for 0, 1, 2, and 3 days. At the end of each time interval, the biomass w as freeze-dried, ground, and analysed for biochemical profiling. Preliminary results showed that the lipid content was significantly affected by the storage conditions, with the lowest lipid content at the longest storage time, whilst the moisture content increased with the increased storage time (unpublished data).

Figure 22. Harvested *E. radiata* biomass from Tower Bay, Tasmania, October 2022, still attached to the ropes ready to be cut off and sorted at the processing facility on land.

Challenges and opportunities for harvesting:

Timing: Regarding the cultivation season, both the timing of deployment and harvest are important. Understanding of when to harvest is crucial, not in relation to yield and quality of the produced biomass. In this manual, a cultivation season is proposed for *Macrocystis pyrifera*, based on a limited number of sites and years. Further research is required to generalise these findings across years and farm sites over a range of environmental conditions. Modifying the farm design, such as cultivation depth, may lead to changes in the deployment and harvest time. Our observed differences in optimal harvest time for *M. pyrifera* and *Lessonia corrugata* suggest that these aspect needs to be investigated for each target kelp species.

Crop monitoring: To make accurate predictions regarding crop yield and quality, regular monitoring and visual inspections of the biomass using loggers, remotely operating vehicles, or direct observations can be useful to improve cultivation outcomes. One major concern is the loss of crop quality and, therefore, revenue for the farmer due to biofouling. Crop monitoring can assist in determining the optimal timing of harvest.

Mechanisation: A specifically designed vessel tailored for kelp harvesting is paramount and must align with the species-specific farm design. For instance, the buoyancy and morphology of the harvested crop, such as the neutrally buoyant *E. radiata* and *L. corrugata*, or floating species like *M. pyrifera,* may influence the optimal mechanical design. Additional research is necessary to assess the efficacy and sustainability of developing mechanised, species-specific, harvesting methods and the accompanying equipment, that require minimal personnel.

Periodical pruning: This project has not investigated the periodical pruning of cultivated biomass. However, this harvesting strategy has been trialled with other species and involves cutting the blades or plants with knives or scissors, leaving small sections that contains the growth region (i.e. meristem) that subsequently regenerates. This enables multiple partial harvests from a single seeding, potentially increasing profits. The implementation of this method depends on the growth strategy of the crop. For example, *E. radiata* and *L. corrugata* have a basal meristem from which new growth arises, whereas *M. pyrifera* and most fucoid kelps have apical meristems, where each frond grows from the tip. For species with basal growth, care needs to be taken to ensure the meristem is not removed entirely during cropping. For species with apical growth such as *M. pyrifera*, some small fronds must be left intact to allow regrowth.

4.2 Primary processing

Primary processing of kelp biomass involves careful and hygienic handling, transport, and storage to maintain its quality and biochemical characteristics. The drying, milling, and storing processes are critical steps to ensure the preservation and optimization of the harvested kelp biomass.

Preventing microbial contamination of the harvested biomass prior to, during and after processing is of the utmost importance. The requirements for preventing microbial contamination will vary depending on the intended use of the biomass and are particularly critical in the processing of food-grade products for human consumption or for nutraceutical and pharmaceutical applications.

During the various stages of processing, it is crucial to apply **general hygiene measures**. These measures include handling the biomass with clean hands and wearing plastic/neoprene gloves to prevent contamination. It is also important to operate in a clean environment and avoid working when unwell. To prevent the spread of microbial contaminants, one should avoid touching their face, hair, etc. while handling the biomass and ensure thorough hand-washing after toilet breaks during the handling process. Additionally, the equipment used during the processing should be cleaned and/or disinfected after each use to avoid cross-contamination. The facility and equipment used should be food-grade and comply with food safety standards directives for food production, such as Hazard Analysis Critical Control Point (HACCP). Where seaweed biomass is being processed from human consumption (including food, nutraceuticals, and pharmaceuticals), it is recommended that all personnel involved in processing undertake certified training in safe-food handling.

Once the biomass has been collected, it is important to **wash and rinse** it with fresh water to remove excess saltwater, which could render it unsuitable for downstream uses. A common method is to dip the seaweed in tubs filled with fresh water, using 3 to 5 tubs per washing. In this project, a method was trialled where 3 tubs were washed with food-grade sanitiser detergent, thoroughly rinsed, and filled with fresh water. The biomass was then rinsed in each tub in sequence to remove sand, epibionts and other debris, with the last tub ensuring that the biomass was thoroughly cleaned, excess salt removed, and any associated organisms detached (Figure 23A). Because degradation is accelerated once the biomass is exposed to freshwater (Liot et al. 1993), if a saltwater source is available, the biomass should ideally be thoroughly cleaned in salt

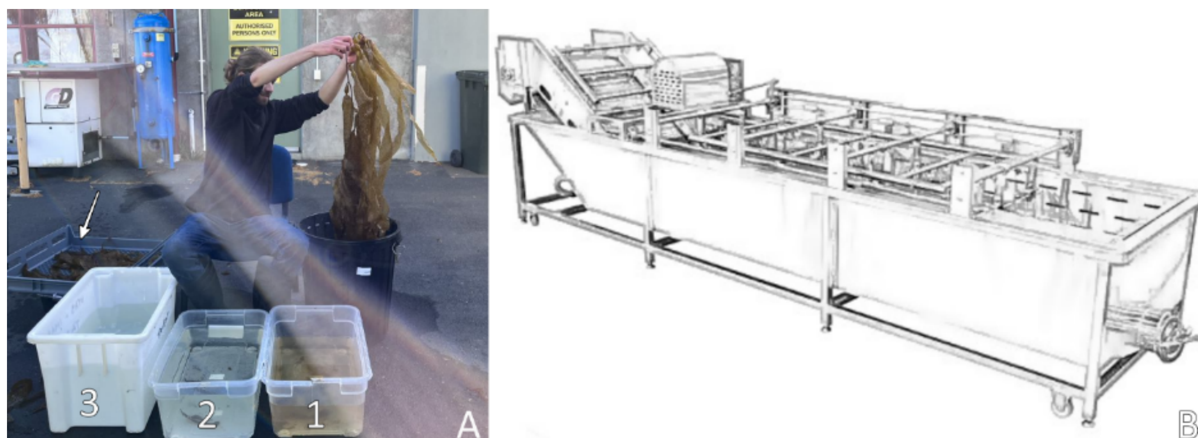

Figure 23. (A) Washing procedure applied for the harvested biomass in October 2022. The biomass was sorted and washed in three-tub system and then placed on trays to be loaded up in the dryer; (B) example of automatic washing machine normally used for vegetables but that in Asia is repurposed for seaweed processing (model taken from www.fruitok.com).

water (i.e., first two tubs) before a final freshwater rinse. However, this 3-tub method may not be practical for commercial-scale production of large volumes of seaweed, as it is labour-intensive and requires a large number of personnel, thereby increasing processing costs. In such cases, the system should ideally be mechanised, with a series of tubs at chest height and flow-through water or belt washing systems, similar to those used for washing and sorting fruit and vegetables (Figure 23B). Regardless of the system employed, the biomass should not be left to soak in the fresh water to prevent degradation.

Furthermore, the biomass can undergo a brief **blanching process** in hot water (80-90 °C) for approximately 30 seconds, followed by cooling in ice-cold water prior to drying. The necessity of this step will vary based on the intended use of the harvested biomass and may be recommended for human consumption or specific food products (Stévant et al. 2018). Depending on the final applications, the biomass can be frozen or dried. Blanching may both improve shelf-life of dried seaweed (Zhu et al. 2021) and reduce concentrations of potentially toxic minerals, such as iodine and arsenic (Dagostin 2017; Nielsen et al. 2020).

Seaweeds and seaweed products commonly undergo a **drying process** to reduce the moisture content so that the degradation of the biomass is limited and slowed down, and the products can have a longer shelf-life (Pradana et al. 2019). Drying is a delicate process that will impact the overall final quality and nutritional composition (e.g., fatty acid, amino acid, and vitamin content) of the biomass. Hence, the most appropriate drying method should be applied considering the final use of the biomass, the resources available, the quantity of the biomass harvested, and environmental sustainability (e.g., energy usage and CO_2 emissions). Overall, it is important to

dry seaweeds quickly to minimise the effects of drying on biomass quality. Various drying equipment have been applied and tested for effects on seaweed quality, including ovens, solar dryers, freeze-dryers, convective dryers, rotary dryers, and dehydrators (non-vented, partially vented, and vented) (Kadam et al. 2015). While each of these systems will vary in cost and operational requirements, they will also have limitations; and can vary in efficiency, energy consumption, and overall final dried products. For example, while solar drying is a simple and cost-effective method, it is highly variable depending on the weather and environmental conditions (e.g., humidity and temperature) and might be not ideal for food applications due to limited control of the hygiene conditions during the processing. Conversely, freeze drying generally maintains the biochemical composition of the fresh biomass but is energy intensive, expensive and results in products with very different texture to other drying methods that limits application.

Here we describe a **partially vented dehydrator system** that was specifically designed for this project, with the aim of being a modular and replicable unit for controlled drying of seaweeds and other marine bioproducts, facilitating scalability as a business grows. The base system consists of two modified shipping containers (a passive drip-dry unit and an active dehydrating dryer unit) with custom-built moveable trolleys, each with 18 trays on which the seaweed biomass is dried (Figure 24A). Both modified shipping containers have been designed to food safety standards. The drip-dry unit has sliding fine-mesh doors on either side to allow for air circulation whilst excluding insects (Figure 24C). The dehydrating dryer unit is explained in detail below. Once the seaweeds have been washed, they are **evenly distributed on trays** to ensure air circulation and faster drying. Once a trolley is filled it can be moved directly into the drying unit or placed temporarily in the drip-dry until a full batch is ready to dry. If the amount of biomass being processed exceeds the capacity of the dryer, multiple batches can be prepared and temporarily left in the drip-dry container. The dryer unit can hold six trolleys at a time unit (Figure 24B). Alternatively, the seaweeds could be hung vertically within the dryer.

The dehydrator supplied by Agridry Systems Pty. Ltd. consists of a partially vented, heat-pump dryer measuring 1.9 x 2.4 x 2.0 m. It is designed to be inserted into an opening at the end of a drying room or shipping container (Figure 25A). In the case described here, the unit was assembled inside a 20-foot shipping container placed outdoors and connected to an electric power source (Figure 25B). Additionally, the fans at the end of the unit are fitted with mesh grates (Figure 25B) to prevent dried seaweed fragments that may be blown around (especially if air flow not adequately controlled) from being caught in the fan and damaging the system.

Figure 24. (A) A tray with washed *E. radiata* biomass harvested in October 2022 ready to be loaded in the dryer, **(B)** an example of a trolley with 18 trays custom made for the dryer, and (**C)** an example of the custom-made drip-dry container with mesh doors on either side.

The system is relatively **efficient and cost effective** since one cycle of 24-72 hours can consume between AU$20 to AU$60 of electrical power. If solar panels were to be incorporated into the system, this might become even more cost-effective and environmentally sustainable. The temperature and air flow in the unit can be adjusted as required. In this project, temperatures between 30 – 50°C and airflow between 30 – 60% were experimentally tested, with the optimal temperature for drying kelps of 50°C (accounting for time and quality if dried biomass). The air flow needs to be tailored according to the type of biomass. For example, *M. pyrifera* blades are typically smaller than *E. radiata* and *L. corrugata* and thus required lower air flow to avoid the dispersion by the vigorous airflow, especially once the biomass is dried. The system can also be linked to a computer, allowing for the tracking of parameters within the unit over time, such as temperature, humidity, and airflow. An alarm can also be set to detect deviations in temperature and humidity from a set threshold.

It is important to have a solid understanding of the **technical details of the partially vented dehydrator** to effectively operate the system. The main heat source for dehydration is a Copeland scroll compressor, which runs on R134A refrigerant. The dryer is based on a partially recirculated system. The vented air exits the drying chamber through a centrally located rear vent after passing through an air-to-air heat exchange with the incoming air. The air inlets and outlets are positioned externally on opposite sides of the rear of the unit. The inlet for the evaporator is located near the switchboard, whilst a speed-controlled evaporator fan discharges the air from the opposite corner of rear of the unit. As the seaweed biomass dries, the humidity in the heated drying chamber lowers such that eventually, the humidity within the chamber may be lower than that in the ambient air. At this point, the low-humidity-mode setting triggers the low-humidity exhaust air to be recirculated via a damper and supply the chamber. This increases the efficiency

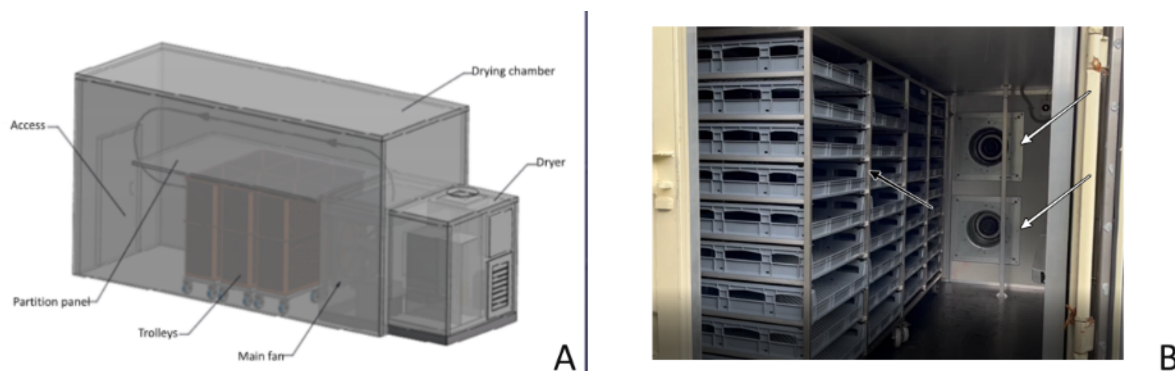

Figure 25. (A) Schematic of the dryer unit, and (**B)** dryer unit inside a 20-foot container at the processing facility at IMAS-Taroona, Tasmania. White arrows indicate the fans, and the black arrow highlights the trolleys and trays custom-made for the unit on which the seaweed is placed.

of drying, reducing drying time and costs. The heated fresh air introduced in the dryer displaces the moist air that is then discharged.

When operating at full capacity, the dryer has the ability to **process between 250 and 300 kg of wet biomass per drying cycle**. This is however depending on the volume of the biomass and how wet the biomass is before going into the dryer. However, the system can be used with smaller amounts of biomass when required. Typically, a cycle lasting 24-48 hours with an air flow of 30% and a temperature of 40-50 °C may be most effective for drying kelps. It is important to monitor and record the final moisture content of the dried seaweed, which should not exceed 22% of the dried biomass. This is a commonly recommended moisture level for the storage and use of seaweed biomass (Philippsen et al. 2014). The moisture content of the biomass dried in the system during this project ranged between 4.6 and 8% (with an average of 5.5%), significantly below the recommended moisture level. This highlights the efficacy of the presented system which will ensure a stable and consistent final product.

Once the biomass has been dried, it can be **stored and packaged** in various ways, depending on its intended used. Here, we present the process used in this project, which can be amended as required. The biomass was packed into plastic bags containing food-grade silica gel packs (Figure 26). Alternatively, vacuum-sealed bags and opaque bags (to avoid degradation by light) could be used. The material strength of the bags used needs to be considered to avoid perforation by the sharp edges of dried seaweeds. The sealed bags should be boxed to exclude light and stored in a dry and cool place. For commercialised products, depending on the final target market, labelling should also be considered. For example, in case of a food product, the label should be comprehensive and include the dry weight/volume, the scientific name of the seaweed species and their “Australian Aquatic Plant Names Standard” name (FRDC 2023), a

Figure 26. An example of packed seaweed with silica gel for further processing for biochemical analyses.

list of main nutritional components (such as protein, lipid, carbohydrates, minerals, etc.) and any relevant information on elements of interest (such as iodine, heavy metals and chemical contaminants).

An additional processing step after drying may be milling or grounding the seaweed into a fine powder. Whether or not this step is applied depends on the final product. For the purpose of this project, a coffee grinder and commercial food blender (Figure 27A and Figure 27B) were used to process the biomass prior to the biochemical analyses, but the application of grain mill machines (Figure 27C) should be considered depending on the overall quantity of the biomass processed. Deakin University is working towards developing a biorefinery at the Waurn Ponds campus to facilitate industrial-scale of milling and processing seaweeds for bioproducts in line with the goals of the MB-CRC (https://mbcrc.com/). While this step is fundamental if the biomass needs to be analysed for its nutritional profile or for extracting compounds with commercial interest, such as antioxidants and fatty acids. Processing the biomass into a powder may not be relevant if the end product consists of whole blades or sheets of seaweed rather than powder.

Understanding the **biochemical profile** of seaweeds and their **pre-treatment** for extracting specific biochemical compounds that are used in various downstream applications is important. Typically, the powdered biomass undergoes pre-treatment to improve extraction yield and make the targeted compounds more bioavailable. Depending on the target compounds, the extraction solvent and protocol will change, and optimal conditions must be selected to prevent coextraction of other bioactives.

Analyses of the overall nutritional profiling are recommended, particularly if the final product is for human and/or animal consumption, or for nutraceutical and pharmaceutical applications. Analyses conducted within this project included proximate composition (total lipid, protein and

Figure 27. An example of equipment that can be used for milling seaweed biomass (A) Coffee and spice grinder (Breville®), (B) Food blender (Vitamix®, total nutrition centre, 5200), (C) Industrial grain milling machine for 10 tonnes per day (KMEC, Engineering).

carbohydrates, ash and moisture content), fatty acid, dietary minerals and metals, total phenolic compounds, antioxidant activity, pigments, total carbon content, total nitrogen content, total hydrogen content, and the C:N-ratio. Analyses were conducted on both air-dried samples using the dehydrator system presented above and freeze-dried biomass to explore the effects of drying temperatures and methodologies on the overall biochemical composition of the final product.

Here, we present a more **description of the analytical methods** utilised to investigate the biochemical profile of the farmed seaweeds. For a more detailed and comprehensive description, we refer the reader to the references cited herein.

- **Proteins** are extracted with 98% sulphuric acid, following a modified method from AOAC (2006) using an automated KjelFlex K-360 (BUCHI, Switzerland) with N × 5 as conversion factor for seaweeds (Angell et al. 2016).
- **Total lipid content** is determined by cold extraction, using dichloromethane: methanol (2:1), according to Folch et al. (1957), modified by Ways and Hanahan (1964).
- **Residual moisture** is determined by oven drying at 105°C for at least 18 hours until weight stabilised (Atkinson et al. 1984).
- **Total ash** is determined by incineration of samples in a muffle furnace at 550 °C for 18 h (Atkinson et al. 1984).
- **Carbohydrate content** is included in the nitrogen-free extract (NFE), and calculated by subtraction, as per Skrzypczyk et al. (2019): NFE = 100 − % Protein − % Total lipid − % Ash.
- **Fucoidan content** is analysed using extraction methods developed by Marinova Ltd., and due to confidentiality reasons, cannot be disclosed.
- **Fatty acids** are analysed following Mock et al. (2020) and as described in Biancacci et al. (2022a).

- **Dietary minerals** (including iodine) and **metal ion content** are acid-extracted and analysed with an inductively coupled plasma-mass spectrometer (ICP-MS, NexION 350X, PerkinElmer, USA) as described in Biancacci et al. (2022a).
- **Phenolic compounds** are extracted with 70% ethanol (ethanol:MilliQ water 70:30, v:v) and analyses for phenolic compounds were performed following a modified Folin-Ciocalteu colorimetric assay (Dang et al. 2017; Marine Environmental Sciences Laboratory, IUEM, Brest, France).
- **Antioxidant analyses** included **phenolic content** and **DPPH radical-scavenging activity** from 70% ethanol (ethanol:MilliQ water 70:30, v:v) extracts. Analyses for phenolic compounds were performed following a modified Folin-Ciocalteu colorimetric assay (Dang et al. 2017; Marine Environmental Sciences Laboratory, IUEM, Brest, France) and DPPH analyses Brand-Williams et al. (1995) modified by Fukumoto and Mazza (2000).
- **Pigments** are extracted in dimethyl sulfoxide (DMSO) and determined spectrophotometrically following Seely et al. (1972).
- **Total carbon, nitrogen, C:N ratio, sulphur and hydrogen** are analysed from oven-dried samples using an elemental analyser (NA1500) coupled to a Thermo Scientific Delta V Plus via a Conflo IV.

Results for the average **proximate composition** and **antioxidant content** (as total phenolic content, TPC) of freeze-dried cultivated seaweed from this study is reported in Table 4. The species investigated were also rich in interesting **fatty acids** (e.g., eicosapentaenoic acid, EPA, 20:5n-3) and **minerals** (e.g., potassium, magnesium, calcium, phosphorous, iron, etc.) (Biancacci et al. 2022a).

Table 4 Summary of the proximate composition and total phenolic content of cultivated and freeze-dried *M. pyrifera*, *E. radiata* and *L. corrugata*. Data are average of the three seasons ± SD.

Species	Lipid (% DW)	Protein (% DW)	Ash (%DW)	NFE (% DW)	Moisture (% DW)	TPC (mg/g)
M. pyrifera	1.23±0.39	9.16±1.10	42.31±4.50	44.28±2.99	4.45±0.35	2.49±1.61
E. radiata	1.32±0.12	8.79±1.09	33.27±1.92	52.89±2.58	5.70±0.30	5.10±3.11
L. corrugata	0.87±0.18	8.06±0.51	38.55±2.50	46.51±2.66	6.47±0.17	2.30±1.13

Preliminary results provide insights into the **fucoidan composition** of the examined seaweed samples (**Error! Reference source not found.**). It's crucial to note that these findings are indicative due to the exploratory nature of the extraction protocol and the limited sample size per site and species. For *M. pyrifera* samples, a noticeable decrease in fucoidan yield occurred between July and November, particularly pronounced in Great Taylor Bay, with only a small decrease in biomass from Okehampton. Mannose proportion, which is indicative of mannitol content, was ~60% higher in November samples, suggesting seasonal variation in mannitol production. *E. radiata* and *L. corrugata*, harvested in November, exhibited lower extract yields than *M. pyrifera*. *L. corrugata* from Okehampton had a higher yield, while *E. radiata*'s was higher in Great Taylor Bay. Both had consistent carbohydrate profiles. Interestingly, *E. radiata* had the highest mannose/mannitol content, while *L. corrugata* the lowest. In terms of the sugars typically found in fucoidan (fucose, xylose, galactose, arabinose, rhamnose), the *L. corrugata* showed the highest overall fucoidan purity.

Yields of fucoidan extracted from seaweeds can significantly vary based on tissue type, species, harvest location, harvest time (within a season) and extraction methodology (Wang and Chen 2016; Fitton et al. 2015). For example, Zhao et al. (2018) reported extract yields from *Undaria pinnatifida* ranging from 4-33%, depending on the extraction method, emphasising that high values often result from other secondary metabolites such as mannitol, laminarin or polyphenols. In addition, fucoidan is a complex polysaccharide composed of various monosaccharaides. The molecular weight can range from a few thousand to several hundred thousand Daltons, therefore it is expressed as % dry weight (DW) in **Error! Reference source not found.**. The fucoidan yields observed in this project align with typical fucoidan content found in *U. pinnatifida* sporophyll (~ 8%) or the blade part of the plant (~2%) (pers. comm. Sam Karpiniec, Marinova Ltd.).

The average **iodine** content varied from 1,350 mg kg^{-1} (reported for *M. pyrifera* and *E. radiata*) to 283 mg kg^{-1} for *L. corrugata*, while all the **heavy metals** (e.g., Al, As, Cd, Hg, Pb) were below the risk limits for human and animal consumption (Biancacci et al. 2022a).

Preliminary results on the **effects of drying** (air dried vs freeze dried) on the overall biochemical composition of *E. radiata* and *M. pyrifera* showed that, while the levels of iodine in freeze-dried samples were above the recommended limit (e.g. 1,000 mg kg^{-1} dry weight, FSANZ), air-dried samples for the same species cultivated and harvested at the same time and site were almost halved and fell below the recommended limit (680 mg kg^{-1} dry weight and 790 mg kg^{-1} dry weight

for *E. radiata* and *M. pyrifera*, respectively), where air dried samples also retained higher levels of lipids and did not differ from the freeze dried for protein or carbohydrates.

Table 5. Preliminary results of the fucoidans present in cultivated and subsequently freeze-dried *M. pyrifera*, *E. radiata* and *L. corrugata* collected from Great Taylors Bay (GTB) and Okehampton Bay (OKE) in July and November 2020. The fucoidan yield is expressed as % dry weight (DW), all other carbohydrates are expressed as mg/g DW.

Species	Site	Month	Fucoidan (% DW)	Fucose	Xylose	Mannose	Galactose	Glucose	Arabinose	Rhamnose
M. pyrifera	GTB	Jul	7.0	10.5	1.0	3.2	1.0	0.5	0.3	0.2
		Nov	3.6	9.4	0.5	5.0	1.0	0.4	0.4	0.1
	OKE	Jul	8.2	10.9	0.3	2.8	2.0	0.3	0.3	0.1
		Nov	7.5	7.6	1.1	4.5	1.0	1.5	1.0	0.1
E. radiata	GTB	Nov	3.2	8.1	0.3	6.0	1.6	0.5	0.5	0.1
	OKE	Nov	4.8	7.6	0.2	6.2	1.4	1.0	0.6	0.1
L. corrugata	GTB	Nov	4.7	12.3	0.4	1.5	1.6	0.5	0.2	0.1
	OKE	Nov	4.4	12.6	1.0	1.3	1.0	0.5	0.1	0.1

Australia has established **food standard codes** (FSANZ) to regulate the permissible limits of certain heavy metals (such as arsenic, cadmium, lead and mercury) and minerals in seaweed products intended for **human consumption**. Excessive amounts of these elements, including iodine, could pose health risks. Therefore, special attention should be given to the analysis of these elements. Hence, the drying method should be chosen carefully, considering that the final quality and biochemical profiling of the biomass produced will be significantly affected by the modality selected.

Finally, the Seaweed Solutions for Sustainable Aquaculture CRC project aimed to develop a sustainable **Integrated Multi-Trophic Aquaculture** (IMTA) model and support commercial seaweed production in Australia, including the effects of cultivation of seaweeds in proximity to salmon and mussel farms (i.e., an IMTA model) on the overall nutrient profile of the harvested seaweed biomass. As highlighted earlier in this manual, different seaweed farming techniques, farm sites, seawater variables (such as dissolved nutrients, temperature, and salinity) can affect the biochemical composition of the cultivated biomass. Results from wild *M. pyrifera* populations harvested at sites nearby and away from salmon farms showed that specimens harvested in sites in close proximity to the fish farms had **improved nutritional properties**, such

as increased lipid content and fatty acid profiles, and **no increased uptake of heavy metals** (such as As, Cd, Hg and Pb) was detected (Biancacci et al., 2022b). Hence, opportunities exist to explore these observations further in Australia and apply them to multi-species farming systems with increased profit, sustainability, social acceptance, and productivity.

Challenges and opportunities for primary processing:

Post-harvest processing of wet biomass: The methods used in processing the harvested crop may vary based on the intended final product. Currently, processing fresh biomass upon harvest is labour-intensive, not cost-effective, and often results in variable biomass quality, hindering high-quality product assurance. Further research is needed to explore automated post-harvest processing, and the development of crop-specific procedures and equipment to improve the shelf life and product quality. Alternatively, investigating opportunities to link social benefits, such as jobs for under-employed minority groups, to labour-intensive processing is warranted.

Crop drying: The developed dryer in this project efficiently dries seaweed biomass to a high quality, and the modular design allows producers to enter in at a small scale and expand by adding modules or relocate them as their business grows. However, drying freshly harvested crop can still be logistically challenging since processing of the biomass should ideally be performed within 48 hours from harvesting to limit degradation and loss of valuable compounds. The dryer may have potential applications beyond seaweed and may require addition trails. Moisture content in the dried seaweed biomass must not exceed 20%, preferably staying below 10%. Finally, not only should the nutritional properties of the biomass be considered in the drying process, but also hygiene practices throughout the entire process, product packaging, and product quality to ensure long-lasting shelf life through the stabilisation of the product.

Efficient and replicable extractions: There is a lack of advanced biochemical analytical techniques for extracting and purifying valuable compounds from the seaweeds targeted in this manual. The development of novel, replicable, and efficient extraction methods and purification protocols that result in high value-added products would therefore make a valuable contribution towards the commercialisation of more seaweed-based products and the further development of a seaweed aquaculture industry.

Analysis of biochemical composition: A thorough understanding of the nutrient profile of the produced crop is essential but can be costly and requires skilled personnel and laboratory facilities when conducted in-house. Based on the findings of this project, there are a few target compounds that may serve as "indicator" compounds. Particular attention should be given to identifying and quantifying compounds that might pose health risks, such as iodine and heavy metals. Focussing on these compounds would reduce analytical expenses without compromising safety, particularly concerning human consumption. To achieve this, additional research is required to determine which chemical compounds should be analysed for each species individually.

4.3 Summary of knowledge gaps and opportunities for optimisation of product harvesting and primary processing and stabilisation

There are various difficulties and knowledge gaps throughout the entire seaweed aquaculture production process. Producing and developing seaweed-based products can present challenges due to the variability in biochemical profiles of seaweeds, influenced by species, farming technique, seasons, and sites as well as the methods used for harvesting, processing, and storage. This chapter described the necessary equipment and steps involved in harvesting and processing fresh kelp biomass. Processing the harvested biomass can be laborious and expensive, and current methods require optimisation of procedures and the development of replicable and scalable protocols to ensure high-quality biomass, energy efficiency, and waste minimisation. The main knowledge gaps and constraints identified in this project are outlined below, along with suggestions for optimisation strategies.

Harvesting involves multiple interacting factors requiring careful consideration. Key constraints include understanding and identifying the optimal harvest time for each species and site. Harvesting, whether manual or mechanical, presents challenges and can be costly. Optimization may be achieved through the development of more advanced and efficient methods for crop monitoring, informing the ideal harvest time based on optimal yield and biofouling, while considering interannual variability. Additionally, mechanization of the harvest and alternative strategies such as partial cropping should be explored to enhance production and cost-efficiencies.

Variability in biomass production can be significant and depend on several different factors that can be either linked to the species' characteristics, nursery production, environmental condition at the cultivation site, the farm layout, and are often in interaction, resulting in suboptimal culture conditions. This variability constrains up-scaling production and post-harvest processing. Optimisation strategies include developing tailored species-specific nursery protocols, contamination protocols both in the nursery and at sea, and identifying optimal farm sites and seasons for deployment and harvesting of each species of interest.

Primary processing is another crucial part of the production cycle, involving various steps from washing to drying and packaging, each with its complexities. Processing of the harvested biomass is often laborious and relatively expensive, especially with substantial quantities of biomass. Infrastructure and equipment, such as mechanised washing stations and commercial-scale dryers, represent significant investments. However, tailored operational procedures and

equipment, automation of processing procedures, and the use of renewable energy sources could optimise this step, improving efficiency and reducing costs.

Biochemical analyses are necessary to characterise the biomass to inform downstream applications and product development. These also can be laborious, expensive and require skilled personnel. Developing faster and cheaper analytical techniques could facilitate rapid screening of the nutritional profiles and food safety of the produced crop, enabling dynamic decision-making and ensuring accessibility to farmers and regulators. Moreover, optimising techniques for the extraction and purification of valuable compounds, while minimising waste fractions during these processes, can enhance environmental and economic sustainability.

Addressing the highlighted inefficiencies and knowledge gaps in the harvesting and primary processing of seaweed biomass must connect to upstream processes, including the nursery phase and at-sea cultivation practices such as site selection and farm design. Implementing and optimising these processes, alongside an improved understanding of their linkages, is essential for the successful development of the seaweed industry in Australia and globally.

Chapter 5. Recommendations for future research and development

By addressing the research gaps and implementing the recommended improvements as highlighted at the end of each chapter, the understanding and efficiency of kelp cultivation can be enhanced. In this chapter, we provide recommendations for further research that may lead to the successful development of an Australian seaweed industry.

Future research should further investigate the **linkages across the entire production chain** (from the nursery phase and at-sea cultivation practices to harvesting and primary processing) to optimise the overall development of the seaweed industry. Such research could include, for example, experimental tests of the effects of seeding density in kelp nurseries on at-sea growth and the biochemical profile of the produced biomass.

Explore **co-locating seaweed cultivation** with aquaculture production of other species (e.g., IMTA, 3D ocean farming, regenerative ocean farming) to ensure the diversification of seasonal farming activities and increase profits. It could also offer fishermen an opportunity to incorporate seaweeds into their operations without substantial investments, as existing leases and infrastructure can be adapted or modified. Moreover, seaweeds can serve as a means of

bioremediation, absorbing excess nutrients from feeds and faeces, leading to increased yield and biomass production. While this project has taken the first steps to understanding the potential to integrate Australian laminarian kelps with salmon and shellfish farming, it is recommended that future research delves further into the nutrient-uptake potential of cultivated Australian laminarian kelps and investigates, in detail, variations in biomass production and quality resulting from the co-cultivation of seaweeds with other organisms.

While there are recognised challenges – such as logistical, financial, operational, and legal as highlighted by Visch et al. (2023b) – associated with locating aquaculture farms in **high-energy offshore** environments, there is a pressing need to examine the potential integration of seaweed aquaculture into these systems. Given the anticipated rapid development of offshore renewable wind/solar energy farms in Australia to meet ambitious targets (Briggs et al. 2021), future work should explore the potential of integrating seaweed aquaculture into these systems and test candidate species, that can withstand or thrive in this environment.

We recommend that future research regarding kelp **nurseries** focuses on improvement of existing culture protocols for laminarian kelps in the Australian context, and the potential to adapt nursery cultivation protocols for the high diversity of Australian fucoid kelps with commercial potential (Skrzypczyk et al. 2019; 2023). For example, valuable research foci could include: 1) optimisation of culture conditions tailored to the specific nutrient requirements of different kelp species and strains; 2) detailed investigation of biological contaminants in the nursery phase and development of preventative and reactive treatment combinations to improve nursery outcomes; 3) investigation of male to female gametophyte ratios and the potential negative effects of parthenosporophytes; 4) exploration of the potential benefits of horizontally rotating spools to improve space utilisation within the nursery and 5) innovative biodegradable seeding twines that could improve nursery outcomes and reduce the amount of plastic-based rope in kelp aquaculture during the preparation of seeded line.

The rapid development of multi-scale seaweed aquaculture farms in Australia necessary to realise the potential of ambitious seaweed production goals ($100 million gross value of production by 2025; (Kelly 2020) is dependent on an adequate supply of seeded line for native seaweeds. Thus, research and investment that progresses the development of a multi-species National Hatchery Network (Kelly 2022) is critically important.

Farming outcomes during at-sea cultivation can be improved by integrating spatial ecology modelling approaches with small-scale R&D pilot studies for **site selection** to gather baseline

data on environmental conditions and assess the suitability of potential farm sites for species new to cultivation. This can greatly reduce upfront costs associated with farm infrastructure, licencing, and other requirements pre-commercialisation.

Harvesting should become more practical and cost-effective, accomplished with tailored technology/equipment that ensures the sustainability, efficiency, and profitability of the process. Therefore, investments should be directed toward developing mechanised systems capable of operating efficiently at a commercial scale, enhancing industry performance or capable of pruning the biomass (i.e. partial harvest throughout the cultivation season). Research leading to the development of more advanced, efficient methods for crop monitoring should facilitate the optimisation of the timing of harvest with respect to biomass yield and quality.

The infrastructure costs and logistical challenges associated rapid **primary processing** of large amounts of wet seaweed biomass may be a major obstacle for emerging seaweed aquaculture producers, especially in Australia where there may be vast distances between aquaculture leases such that centralised processing facilities may be unrealistic. The scalability and potential portability of the modular drying system developed in this project and described above should alleviate some of these pressures, but further research into cost-effective primary processing methods and procedures, and the integration with renewable energy sources, may make it easier for producers to enter the industry. Furthermore, research that tailors processing to the target species and intended final products to reduce variability in product quality, should be encouraged. Research that focuses on holistic sustainability (environmental, social, and economic) solutions to biomass processing and application (e.g., recycling of freshwater used in the washing phase, particularly in a dry continent like Australia; integration with renewable energy sources; collaboration with Indigenous knowledge holders; diverse job creation for remote coastal communities and minorities, profitable use of waste streams etc.) should also be prioritised. It is recommended that investments in seaweed processing facilities and technologies be strengthened and diversified, thereby unlocking greater market potential. This will also create local employment opportunities and yield social benefits for the coastal communities involved.

Australia holds an advantage over other seaweed-producing nations due to the abundance of clean coastal and offshore areas (Halpern et al. 2008). Additionally, southern Australia has an enormous diversity of endemic seaweed species (Phillips 2001; Hurd et al. 2023). However, research on the **nutritional profiles** of Australian seaweeds with commercial potential, and drivers of variability therein (e.g., seasonal, species-specific, site-specific, etc.), has only

recently begun (Schmid et al. 2018; Skrzypczyk et al. 2019; 2023; Biancacci et al. 2022a; 2022b; 2022c). Consequently, further research should be conducted to better understand the drivers of the nutritional and bioactive profiles of native Australian seaweeds, such as the kelps investigated here, along with the identification of any potential health risks associated with their consumption; and how to replicate the production of beneficial biochemicals in aquaculture production. Such research could include, for example, investigation of methods to enhance the content of valuable compounds such as protein and fatty acids (e.g., incorporating seaweed farming in integrated multi-trophic systems) and reduce the levels of contaminants (e.g., As, Cd, Hg, Pb) or elements potentially toxic in high concentrations (e.g., reduce iodine below FSANZ limits through different drying or pre-processing techniques), to maximise the safety of the final products and ensure successful commercialisation. This will pave the way for the development of a diverse range of new products, ultimately fostering industry growth.

Lastly, the success of an Australian seaweed industry is likely dependent on the development of a diversity of locally-grown-seaweed based products across the value chain with minimal to zero waste and measurable sustainability claims. Thus, research into close-loop, seaweed-biorefinery processing, where **high-value products** such as nutraceuticals and pharmaceuticals and lower-value products such as organic fertilisers, and a focus on whole-of-biomass processing lead to an economically viable industry that can contribute to the UN's Sustainable Development Goals (Spillias et al. 2022).

References

Amarowicz R, Carle R, Dongowski G, Durazzo A, Galensa R, Kammerer D, Maiani G, Piskula MK (2009) Influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods. Molecular Nutrition & Food Research 53 (S2):S151-S183. doi: 10.1002/mnfr.200700486

Andersen RA (2005) Algal Culturing Techniques; Elsevier: Amsterdam, The Netherlands, 2005. 596pp.

Angell AR, Mata L, de Nys R, Paul NA (2016) The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. Journal of Applied Phycology 28 (1):511-524. doi:10.1007/s10811-015-0650-1

AOAC (2006) Official methods of analysis, 18th edition. Association of Official Analytical Chemists, Gaithersburgs, MD

Assis J, Alberto F, Macaya EC, Castilho Coelho N, Faugeron S, Pearson GA, Ladah L, Reed DC, Raimondi P, Mansilla A, Brickle P, Zuccarello GC, Serrão EA (2023) Past climate-driven range shifts structuring intraspecific biodiversity levels of the giant kelp (*Macrocystis*

pyrifera) at global scales. Scientific Reports 13 (1):12046. doi:10.1038/s41598-023-38944-7

Ateweberhan M, Rougier A, Rakotomahazo C (2015) Influence of environmental factors and farming technique on growth and health of farmed *Kappaphycus alvarezii* (cottonii) in south-west Madagascar. Journal of Applied Phycology 27 (2):923-934. doi: 10.1007/s10811-014-0378-3

Atkinson JL, Hilton JW, Slinger SJ (1984) Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 41 (9):1384-1386. doi:10.1139/f84-170

Bak UG, Mols-Mortensen A, Gregersen O (2018) Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. Algal Research 33:36-47. doi:10.1016/j.algal.2018.05.001

Becker EW (1994) Microalgae: biotechnology and microbiology, Vol. 10. Cambridge University Press, 304pp.

Bennett S, Wernberg T, Connell SD, Hobday AJ, Johnson CR, Poloczanska ES (2015) The 'Great Southern Reef': social, ecological and economic value of Australia's neglected kelp forests. Marine and Freshwater Research 67 (1):47-56. doi:10.1071/MF15232

Beyter D, Tang P-Z, Becker S, Hoang T, Bilgin D, Lim YW, Peterson TC, Mayfield S, Haerizadeh F, Shurin JB (2016) Diversity, productivity, and stability of an industrial microbial ecosystem. Applied and environmental microbiology 82 (8):2494-2505. doi:10.1128/AEM.03965-15

Biancacci C, Sanderson J, Evans B, Callahan D, Francis D, Skrzypczyk V, Cumming E, Bellgrove A (2022a) Nutritional composition and heavy metal profiling of Australian kelps cultured in proximity to salmon and mussel farms. Algal Research 64:102672. doi:10.1016/j.algal.2022.102672

Biancacci C, Sanderson JC, Evans B, Callahan DL, Francis DS, Skrzypczyk VM, Cumming EE, Bellgrove A (2022b) Variation in biochemical composition of wild-harvested *Macrocystis pyrifera* (Ochrophyta) from sites proximal and distal to salmon farms in Tasmania, Australia. Algal Research 65:102745. doi:10.1016/j.algal.2022.102745

Biancacci C, Visch W, Callahan DL, Farrington G, Francis DS, Lamb P, McVilly A, Nardelli A, Sanderson JC, Schwoerbel J, Hurd CL, Evans B, Macleod C, Bellgrove A (2022c) Optimisation of at-sea culture and harvest conditions for cultivated *Macrocystis pyrifera*: yield, biofouling and biochemical composition of cultured biomass. Frontiers in Marine Science 9:1-19. doi:10.3389/fmars.2022.951538

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28 (1):25-30. doi:10.1016/S0023-6438(95)80008-5

Briggs C, Hemer M, Howard P, Langdon R, Marsh P, Teske S, Carrascosa D (2021) Offshore Wind Energy in Australia: Blue Economy Cooperative Research Centre. Launceston, TAS, Australia

Buschmann AH, Varela DA, Hernández-González MC, Huovinen P Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of *Macrocystis* and *Gracilaria* as biofilters. In: Nineteenth International Seaweed Symposium, 2007. Springer, pp 121-127. doi:10.1007/978-1-4020-5670-3_5

Camus C, Buschmann AH (2017) *Macrocystis pyrifera* aquafarming: Production optimization of rope-seeded juvenile sporophytes. Aquaculture 468 (1):107-114. doi:10.1016/j.aquaculture.2016.10. 010

Charrier B, Abreu MH, Araujo R, Bruhn A, Coates JC, De Clerck O, Katsaros C, Robaina RR, Wichard T (2017) Furthering knowledge of seaweed growth and development to facilitate sustainable aquaculture. New Phytologist 216 (4):967-975. doi:10.1111/nph.14728

Chopin T, Tacon AGJ (2021) Importance of seaweeds and extractive species in global aquaculture production. Reviews in Fisheries Science & Aquaculture 29 (2):139-148. doi:10.1080/ 23308249.2020.1810626

Cumming EE, Matthews TG, Bellgrove A (2020a) Developing seaweed production techniques for *Phyllospora comosa*. Deaking University, Warrnambool, VIC, Australia

Cumming EE, Matthews TG, Sanderson CJ, Ingram BA, Bellgrove A (2019) Optimal spawning conditions of *Phyllospora comosa* (Phaeophyceae, Fucales) for mariculture. Journal of Applied Phycology 31 (5):3041-3050. doi:10.1007/s10811-019-01788-8

Cumming EE, Matthews TG, Sanderson JC, Ingram BA, Bellgrove A (2020b) Growth and survivorship of *Phyllospora comosa* (Phaeophyceae, Fucales) on different mariculture seeding twines in a hatchery setting. Aquaculture 523:735216. doi:10.1016/j.aquaculture.2020.735216

Dagostin JLA (2017) Use of Blanching to Reduce Antinutrients, Pesticides, and Microorganisms. In: Richter Reis F (ed) New Perspectives on Food Blanching. Springer International Publishing, Cham, pp 61-94. doi:10.1007/978-3-319-48665-9_4

Dang TT, Van Vuong Q, Schreider MJ, Bowyer MC, Van Altena IA, Scarlett CJ (2017) Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant activities of the alga *Hormosira banksii* using response surface methodology. Journal of Applied Phycology 29 (6):3161-3173. doi:10.1007/s10811-017-1162-y

de Bettignies T, Wernberg T, Gurgel CFD (2018) Exploring the Influence of Temperature on Aspects of the Reproductive Phenology of Temperate Seaweeds. Frontiers in Marine Science 5. doi:10.3389/fmars.2018.00218

Durrant HM, Barrett NS, Edgar GJ, Coleman MA, Burridge CP (2015) Shallow phylogeographic histories of key species in a biodiversity hotspot. Phycologia 54 (6):556-565. doi:10.2216/15-24.1

Durrant HMS, Barrett NS, Edgar GJ, Coleman MA, Burridge CP (2018) Seascape habitat patchiness and hydrodynamics explain genetic structuring of kelp populations. Marine Ecology Progress Series 587:81-92

Ebbing APJ, Fivash GS, Pierik R, Bouma TJ, Kromkamp JC, Timmermans K (2022) The SeaCoRe system for large scale kelp aquaculture: a plug-and-play, compatible, open-source system for the propagation and transport of clonal gametophyte cultures. Journal of Applied Phycology 34 (1):517-527. doi:10.1007/s10811-021-02638-2

Edwards M, O'Mahony F, Connellan I, Dring M, Werner A (2011) Aquaculture explained: cultivating *Laminaria digitata*. https://bim.ie/wp-content/uploads/2021/02/BIM,Aquaculture,Explained, Issue,26,-,Cultivating,Laminaria,digitata.pdf. doi:10.13140/RG.2.1.2153.3283

Elisabeth B, Rayen F, Behnam T (2021) Microalgae culture quality indicators: a review. Critical Reviews in Biotechnology 41 (4):457-473. doi:10.1080/07388551.2020.1854672

FAO (2023) Global aquaculture production. FishStatJ: Universal software for fishery statistical time series (v4.03.00).

Fitton JH, Stringer DN, Karpiniec SS (2015) Therapies from Fucoidan: An Update. Marine Drugs 13 (9):5920-5946. doi:10.3390/md13095920

Flavin K, Flavin N, Flahive B (2013) Kelp farming manual. A guide to the processes, techniques and equipment for farming kelp in New England waters. Ocean Approved LLC, https://maineaqua.org/wp-content/uploads/2020/06/OceanApproved_KelpManualLowRez.pdf

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226 (1):497-509

Forbord S, Steinhovden KB, Rød KK, Handå A, Skjermo J (2018) Cultivation protocol for *Saccharina latissima*. In: Charrier B, Wichard T, Reddy CRK (eds) Protocols for Macroalgae Research. CRC Press, New York, NY, USA, pp 37-59

Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J (2020) Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case study. Journal of Applied Phycology 32 (4):2201-2212. doi:10.1007/s10811-019-01936-0

Førde H, Forbord S, Handå A, Fossberg J, Arff J, Johnsen G, Reitan K (2015) Development of bryozoan fouling on cultivated kelp (*Saccharina latissima*) in Norway. Journal of Applied Phycology 28 (2):1225-1234. doi:10.1007/s10811-015-0606-5

FRDC FRaDC (2023) The Australian Aquatic Plant Names Standard AS 5301-2020 https://www.frdc.com.au/knowledge-hub/standards/aquatic-plant-names-standard.

Fukumoto LR, Mazza G (2000) Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds. Journal of Agricultural and Food Chemistry 48 (8):3597-3604. doi:10.1021/jf000220w

Guillard RR (2005) Purification methods for microalgae. In: Andersen RA (ed) Algal culturing techniques. Elsevier Academic Press, San Diego, CA, USA, pp 117-132

Guillard RR, Ryther JH (1962a) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Canadian Journal of Microbiology 8 (2):229-239. doi:10.1139/m62-029

Guillard RRL, Ryther JH (1962b) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian Journal of Microbiology 8:229-239. doi:10.1139/m62-029

Gutierrez A, Correa T, Munoz V, Santibanez A, Marcos R, Cáceres C, Buschmann AH (2006) Farming of the giant kelp *Macrocystis pyrifera* in southern Chile for development of novel food products. Journal of Applied Phycology 18:259-267. doi:10.1007/978-1-4020-5670-3_5

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R (2008) A Global Map of Human Impact on Marine Ecosystems. Science 319 (5865):948-952. doi:10.1126/science.1149345

Holdt S, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology 23 (3):543-597. doi:10.1007/s10811-010-9632-5

Hu ZM, Shan TF, Zhang J, Zhang QS, Critchley AT, Choi HG, Yotsukura N, Liu FL, Duan DL (2021) Kelp aquaculture in China: a retrospective and future prospects. Reviews in Aquaculture 13 (3):1324-1351. doi:10.1111/raq.12524

Hurd CL, Wright JT, Layton C, Strain EMA, Britton D, Visch W, Barrett N, Bennett S, Chang KJL, Edgar G, Fitton JH, Greeno D, Jameson I, Johnson CR, Karpiniec SS, Kraft GT, Ling SD, Macleod CM, Paine ER, Park A, Sanderson JC, Schmid M, Scott FJ, Shelamoff V, Stringer DN, Tatsumi M, White CA, Willis A (2023) From Tasmania to the world: long and strong traditions in seaweed use, research, and development. Botanica Marina 66 (1):1-36. doi:10.1515/bot-2022-0061

Kadam SU, Álvarez C, Tiwari BK, O'Donnell CP (2015) Chapter 4 - Processing of seaweeds. In: Tiwari BK, Troy DJ (eds) Seaweed Sustainability. Academic Press, San Diego, pp 61-78. doi: 10.1016/B978-0-12-418697-2.00004-0

Kazamia E, Aldridge DC, Smith AG (2012) Synthetic ecology–a way forward for sustainable algal biofuel production? Journal of Biotechnology 162 (1):163-169. doi:10.1016/j.jbiotec.2012.03.022

Keesing JK, Liu D, Shi Y, Wang Y (2016) Abiotic factors influencing biomass accumulation of green tide causing *Ulva* spp. on *Pyropia* culture rafts in the Yellow Sea, China. Marine Pollution Bulletin 105 (1):88-97. doi:10.1016/j.marpolbul.2016.02.051

Kelly J (2020) Australian seaweed industry blueprint: A blueprint for growth. https://www.agrifutures.com.au/wp-content/uploads/2020/09/20-072.pdf, Publication No. 20-072. AgriFutures, Australia

Kelly J (2022) Australian seaweed industry blueprint implementation plan: A way forward to grow the Australian seaweed industry. Australian Seaweed Institute, AgriFutures Australia, Wagga Wagga, NSW, Australia

Kerrison PD, Stanley MS, Black KD, Hughes AD (2017) Assessing the suitability of twelve polymer substrates for the cultivation of macroalgae *Laminaria digitata* and *Saccharina latissima* (Laminariales). Algal Research 22:127-134. doi:10.1016/j.algal.2016.10.001

Kerrison PD, Stanley MS, Hughes AD (2018) Textile substrate seeding of *Saccharina latissima* sporophytes using a binder: An effective method for the aquaculture of kelp. Algal research 33:352-357. doi:10.1016/j.algal.2018.06.005

Le DM, Desmond MJ, Buschmann AH, Pritchard DW, Camus C, Hurd CL, Hepburn CD (2022) Reproduction, hatchery and culture applications for the giant kelp (*Macrocystis pyrifera*): a methodological appraisal. Applied Phycology 3 (1):368-382. doi:10.1080/26388081.2022. 2086823

Li J, Li Q, Su L, Pang S (2021) Effects of light intensity on the epiphytic bacterial community of sporelings of *Saccharina japonica*. Journal of Applied Phycology 33 (3):1759-1764. doi:10.1007/s10811-021-02406-2

Li J, Pang S, Shan T, Su L (2020) Changes of microbial community structures associated with seedlings of *Saccharina japonica* at early stage of outbreak of green rotten disease. Journal of Applied Phycology 32 (2):1323-1327. doi:10.1007/s10811-019-01975-7

Lincoln EP, Hall TW, Koopman B (1983) Zooplankton control in mass algal cultures. Aquaculture 32 (3):331-337. doi:10.1016/0044-8486(83)90230-2

Liot F, Colin A, Mabeau S (1993) Microbiology and storage life of fresh edible seaweeds. Journal of Applied Phycology 5 (2):243-247. doi:10.1007/BF00004025

Lüning K (1988) Photoperiodic control of sorus formation in the brown alga *Laminaria saccharina*. Maring Ecology Progress Series 45:137-144. doi:10.3354/meps045137

Mabin CJ, Gribben PE, Fischer A, Wright JT (2013) Variation in the morphology, reproduction and development of the habitat-forming kelp *Ecklonia radiata* with changing temperature and nutrients. Marine Ecology Progress Series 483:117-131. doi:10.3354/meps10261

Mabin CJ, Johnson CR, Wright JT (2019) Physiological response to temperature, light, and nitrates in the giant kelp *Macrocystis pyrifera* from Tasmania, Australia. Marine Ecology Progress Series 614:1-19. doi:10.3354/meps12900

Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I (2015) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. Journal of Applied Phycology 27 (5):1963-1973. doi:10.1007/s10811-014-0519-8

Mock TS, Francis DS, Drumm DW, Versace VL, Glencross BD, Smullen RP, Jago MK, Turchini GM (2020) A systematic review and analysis of long-term growth trials on the effect of diet on omega-3 fatty acid levels in the fillet tissue of post-smolt Atlantic salmon. Aquaculture 516:734643. doi:10.1016/j.aquaculture.2019.734643

Mohring MB, Wernberg T, Wright JT, Connell SD, Russell BD (2014) Biogeographic variation in temperature drives performance of kelp gametophytes during warming. Marine Ecology Progress Series 513:85-96. doi:10.3354/meps10916

Nardelli AE, Visch W, Farrington G, Sanderson JC, Bellgrove A, Wright JT, MacLeod C, Hurd CL (2023a) A new nursery approach enhances at-sea performance in the kelp *Lessonia corrugata*. Journal of Applied Phycology. doi:10.1007/s10811-023-03061-5

Nardelli AE, Visch W, Farrington G, Sanderson JC, Bellgrove A, Wright JT, Macleod C, Hurd CL (2023b) Primary production of the kelp *Lessonia corrugata* varies with season and water motion: Implications for coastal carbon cycling. Journal of Phycology. doi:10.1111/jpy.13408

Nardelli AE, Visch W, Wright JT, Hurd CL (2023c) Concise review of genus *Lessonia* Bory. Journal of Applied Phycology. doi:10.1007/s10811-023-02968-3

Naylor RL, Hardy RW, Buschmann AH, Bush SR, Cao L, Klinger DH, Little DC, Lubchenco J, Shumway SE, Troell M (2021) A 20-year retrospective review of global aquaculture. Nature 591 (7851):551-563. doi:10.1038/s41586-021-03308-6

Nielsen CW, Holdt SL, Sloth JJ, Marinho GS, Sæther M, Funderud J, Rustad T (2020) Reducing the high iodine content of *Saccharina latissima* and improving the profile of other valuable compounds by water blanching. Foods 9 (5):569. doi:10.3390/foods9050569

Nimura K, Mizuta H (2002) Inducible effects of abscisic acid on sporophyte discs from Laminaria japonica Areschoug (Laminariales, Phaeophyceae). Journal of applied phycology 14 (3):159-163. doi:10.1023/A:1019929712633

Paine ER, Schmid M, Gaitán-Espitia JD, Castle J, Jameson I, Sanderson JC, Hurd CL (2021) Narrow range of temperature and irradiance supports optimal development of *Lessonia corrugata* (Ochrophyta) gametophytes: implications for kelp aquaculture and responses to climate change. Journal of Applied Phycology 33 (3):1721-1730. doi:10.1007/s10811-021-02382-7

Park CS, Hwang EK (2012) Seasonality of epiphytic development of the hydroid *Obelia geniculata* on cultivated *Saccharina japonica* (Laminariaceae, Phaeophyta) in Korea. Journal of Applied Phycology 24 (3):433-439. doi:10.1007/s10811-011-9755-3

Peteiro C, Bidegain G, Sánchez N (2019) Experimental evaluation of the effect of water velocity on the development of string-attached kelp seedlings (Laminariales) with implications for hatchery and nursery production. Algal Research 44:101678. doi:10.1016/j.algal.2019.101678

Philippsen A, Wild P, Rowe A (2014) Energy input, carbon intensity and cost for ethanol produced from farmed seaweed. Renewable and Sustainable Energy Reviews 38:609-623. doi:10.1016/j.rser.2014.06.010

Pradana GB, Prabowo KB, Hastuti RP, Djaeni M, Prasetyaningrum A (2019) Seaweed drying process using tray dryer with dehumidified air system to increase efficiency of energy and quality product. IOP Conference Series: Earth and Environmental Science 292 (1):012070. doi:10.1088/1755-1315/292/1/012070

Praeger C, Magnusson M, Lawton R (2022) Optimising the zoospore release, germination, development of gametophytes and formation of sporophytes of *Ecklonia radiata*. Journal of Applied Phycology 34:2535–2549. doi:10.1007/s10811-022-02806-y

Redmond S, Green L, Yarish C, Kim J, Neefus C (2014) New England seaweed culture handbook-nursery systems. http://seagrant.uconn.edu/publications/aquaculture/handbook.pdf, Connecticut Sea Grant CTSG-14-01

Richmond A (2004) Biological principles of mass cultivation. Handbook of microalgal culture: Biotechnology and applied phycology. Blackwell Publishing. doi:10.1002/9780470995280

Rød KK (2012) Sori disinfection in cultivation of *Saccharina latissima*: Evaluation of chemical treatments against diatom contamination. NTNU, Department of Biology, https://ntnuopen.ntnu.no/ ntnu-xmlui/handle/11250/245060, Trondheim, Norway

Rolin C, Inkster R, Laing J, Hedges J, McEvoy L (2016) Seaweed Cultivation Manual. Shetland Seaweed Growers Project 2014-16. NAFC Marine Centre, https://oceansalaska.org/wp-content/uploads/2021/06/Seaweed-Cultivation-Manual.pdf

Sanderson J, Di Benedetto R (1988) Tasmanian seaweeds for the edible market. Technical report, Marine Laboratories Department of Sea Fisheries, Hobart, Tasmania, Australia, https://eprints.utas.edu.au/14537/

Schiel DR, Foster MS (2015) The biology and ecology of giant kelp forests. University of California Press, Oakland, CA, USA

Schmid M, Kraft LGK, van der Loos LM, Kraft GT, Virtue P, Nichols PD, Hurd CL (2018) Southern Australian seaweeds: A promising resource for omega-3 fatty acids. Food Chemistry 265:70-77. doi:https://doi.org/10.1016/j.foodchem.2018.05.060

Schwoerbel J, Visch W, Wright JT, Bellgrove A, Sanderson JC, Hurd CL (2023) Thermal performance curves identify seasonal and site-specific variation in the development of *Ecklonia radiata* (Phaeophyceae) gametophytes and sporophytes. Journal of Phycology. doi:10.1111/jpy.13406

Scott F (2017) Marine Plants of Tasmania. Tasmanian Herbarium, Tasmanian Museum and Art Gallery, Hobart, TAS, Australia

Scott F, Sanderson JC (1994) Pilot study to assess the provision of seaweed suitable for abalone feed., vol 18. International report, Marine Laboratories Department of Sea Fisheries, Hobart, Tasmania, Australia

Seely GR, Duncan MJ, Vidaver WE (1972) Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. Marine Biology 12 (2):184-188. doi:10.1007/BF00350754

Segovia NI, Vásquez JA, Faugeron S, Haye PA (2015) On the advantage of sharing a holdfast: effects of density and occurrence of kin aggregation in the kelp *Lessonia berteroana*. Marine Ecology 36 (4):1107-1117. doi:10.1111/maec.12206

Shea R, Chopin T (2007) Effects of germanium dioxide, an inhibitor of diatom growth, on the microscopic laboratory cultivation stage of the kelp, *Laminaria saccharina*. Journal of Applied Phycology 19 (1):27-32. doi:10.1007/s10811-006-9107-x

Shurin JB, Abbott RL, Deal MS, Kwan GT, Litchman E, McBride RC, Mandal S, Smith VH (2013) Industrial-strength ecology: trade-offs and opportunities in algal biofuel production. Ecology letters 16 (11):1393-1404. doi:10.1111/ele.12176

Sioen Industries N (2013) Drager voor het cultiveren van macro algen. Belgium Patent, 23/06/2015

Skrzypczyk VM, Callahan DL, Francis DS, Bellgrove A (2023) Australian brown seaweeds as a source of essential dietary minerals. Journal of Applied Phycology. doi:10.1007/s10811-023-03074-0

Skrzypczyk VM, Hermon KM, Norambuena F, Turchini GM, Keast R, Bellgrove A (2019) Is Australian seaweed worth eating? Nutritional and sensorial properties of wild-harvested Australian versus commercially available seaweeds. Journal of Applied Phycology 31 (1):709-724. doi:10.1007/s10811-018-1530-2

Smale DA, Burrows MT, Moore P, O'Connor N, Hawkins SJ (2013) Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. Ecology and evolution 3 (11):4016-4038. doi:10.1002/ece3.774

Smart JN, Schmid M, Paine ER, Britton D, Revill A, Hurd CL (2022) Seasonal ammonium uptake kinetics of four brown macroalgae: Implications for use in integrated multi-trophic aquaculture. Journal of Applied Phycology 34 (3):1693-1708. doi:10.1007/s10811-022-02743-w

Solvang T, Bale ES, Broch OJ, Handå A, Alver MO (2021) Automation Concepts for Industrial-Scale Production of Seaweed. Frontiers in Marine Science 8. doi:10.3389/fmars.2021.613093

Spillias S, Cottrell RS, Kelly R, O’Brien KR, Adams J, Bellgrove A, Kelly B, Kilpatrick C, Layton C, Macleod C, Roberts S, Stringer D, McDonald-Madden E (2022) Expert perceptions of seaweed farming for sustainable development. Journal of Cleaner Production 368:133052. doi:10.1016/j.jclepro.2022.133052

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. In: Environmental Conservation, vol 29. vol 04. Cambridge University Press, Cambridge, UK, pp 436-459. doi:10.1017/S0376892902000322

Stévant P, Marfaing H, Duinker A, Fleurence J, Rustad T, Sandbakken I, Chapman A (2018) Biomass soaking treatments to reduce potentially undesirable compounds in the edible seaweeds sugar kelp (*Saccharina latissima*) and winged kelp (*Alaria esculenta*) and health risk estimation for human consumption. Journal of Applied Phycology 30 (3):2047-2060. doi:10.1007/s10811-017-1343-8

Su L, Pang SJ, Shan TF, Li X (2017) Large-scale hatchery of the kelp *Saccharina japonica*: a case study experience at Lvshun in northern China. Journal of Applied Phycology 29 (6):3003-3013. doi:10.1007/s10811-017-1154-y

tom Dieck I (1993) Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. Marine Ecology Progress Series 100:253-253

Torres MD, Kraan S, Domínguez H (2019) Seaweed biorefinery. Reviews in Environmental Science and Bio/Technology 18 (2):335-388. doi:10.1007/s11157-019-09496-y

Valero M, Guillemin M-L, Destombe C, Jacquemin B, Gachon CM, Badis Y, Buschmann AH, Camus C, Faugeron S (2017) Perspectives on domestication research for sustainable seaweed aquaculture. Perspectives in Phycology 4 (1):33-46. doi:10.1127/pip/2017/0066

Visch W (2019) Sustainable kelp aquaculture in Sweden. Department of Marine Sciences, Gothenburg University, Gothenburg, Sweden

Visch W, Bergström P, Nylund GM, Peterson M, Pavia H, Lindegarth M (2020a) Spatial differences in growth rate and nutrient mitigation of two co-cultivated, extractive species: The blue mussel (*Mytilus edulis*) and the kelp (*Saccharina latissima*). Estuarine, Coastal and Shelf Science 246:107019. doi:10.1016/j.ecss.2020.107019

Visch W, Larsson AI, Åberg P, Toth GB (2023a) Adherence of kelp (*Saccharina latissima*) gametophytes on ropes with different binder treatments and flow regimes. Journal of Applied Phycology 35 (1):195-200. doi:10.1007/s10811-022-02860-6

Visch W, Layton C, Hurd CL, Macleod C, Wright JT (2023b) A strategic review and research roadmap for offshore seaweed aquaculture—A case study from southern Australia. Reviews in Aquaculture:1-13. doi:10.1111/raq.12788

Visch W, Lush H, Schwoerbel J, Hurd CL (2023c) Nursery optimization for kelp aquaculture in the Southern Hemisphere: the interactive effects of temperature and light on growth and contaminants. Applied Phycology 4 (1):44-53. doi:10.1080/26388081.2023.2174903

Visch W, Nylund GM, Pavia H (2020b) Growth and biofouling in kelp aquaculture (*Saccharina latissima*): the effect of location and wave exposure. Journal of Applied Phycology 32 (5):3199-3209. doi:10.1007/s10811-020-02201-5

Visch W, Rad-Menéndez C, Nylund GM, Pavia H, Ryan MJ, Day J (2019) Underpinning the development of seaweed biotechnology: cryopreservation of brown algae (*Saccharina latissima*) gametophytes. Biopreservation and Biobanking 17 (5):378-386. doi:10.1089/bio.2018.0147

Wang C-Y, Chen Y-C (2016) Extraction and characterization of fucoidan from six brown macroalgae. Journal of Marine Science and Technology 24 (2):26. doi:10.6119/JMST-015-0521-3

Ways P, Hanahan DJ (1964) Characterization and quantification of red cell lipids in normal man. Journal of Lipid Research 5 (3):318-328. doi:10.1016/S0022-2275(20)40200-7

Wernberg T, Coleman MA, Babcock RC, Bell SY, Bolton JJ, Connell SD, Hurd CL, Johnson CR, Marzinelli EM, Shears NT, Steinberg PD, Thomsen MS, Vanderklift MA, Verges A, Wright JT (2019) Biology and ecology of the globally significant kelp *Ecklonia radiata*. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) Oceanography and Marine Biology: An Annual Review. vol 57. CRC Press Taylor & Francis, pp 265-324

Westermeier R, Patiño D, Piel M, Maier I, Mueller DG (2006) A new approach to kelp mariculture in Chile: production of free-floating sporophyte seedlings from gametophyte cultures of

Lessonia trabeculata and *Macrocystis pyrifera*. Aquaculture Research 37 (2):164-171. doi:10.1111/j.1365-2109.2005.01414.x

Wichard T From model organism to application: Bacteria-induced growth and development of the green seaweed *Ulva* and the potential of microbe leveraging in algal aquaculture. In: Seminars in Cell & Developmental Biology, 2022. Elsevier. doi:10.1016/j.semcdb.2022.04.007

Zhao Y, Zheng Y, Wang J, Ma S, Yu Y, White WL, Yang S, Yang F, Lu J (2018) Fucoidan Extracted from *Undaria pinnatifida*: Source for Nutraceuticals/Functional Foods. Marine Drugs 16 (9):321. doi:10.3390/md16090321

Zhu X, Zhang Z, Hinds LM, Sun D-W, Tiwari BK (2021) Applications of ultrasound to enhance fluidized bed drying of *Ascophyllum Nodosum*: Drying kinetics and product quality assessment. Ultrasonics Sonochemistry 70:105298. doi:10.1016/j.ultsonch.2020.105298

Appendix 1 – List of equipment for culturing gametophytes

Table 1.1 List of equipment required for the laboratory culture of Laminarian kelp gametophytes. Some of the equipment may not need to be purchased if locally available at the culturing facility. Equipment should be ordered depending on the scale of the gametophyte culture and extra equipment may be required as production increases. The supplier and price estimate should be taken as indicative and provided in an Australian or where possible Tasmanian context in November 2023. In Hobart, Tasmania, the local marine and laboratory science supplier is IMBROS (https://imbros.com.au).

Item	Details	Supplier	Price estimate
Air pump	Minimum 2 outlets	Aquarium shops (e.g., Aquasonic Pty. Ltd.)	Depending on size ranging from AU$15 to 250+
Antibiotics	Antibiotic Antimycotic Solution (100x)	Merck or Sigma-Aldrich	AU$78 for 100mL
Autoclave	Not necessary to buy if there is local access to one at the facility and vary in size – from small portable 8L up to 1000+ L laboratory autoclaves.	Merck, Thermo-Fisher, or eBay/Amazon.	Starting at around AU$200 up to 50,000+
Betadine	Antiseptic iodine solution for cleaning seaweed tissue prior to spore release.	Local chemist, pharmacist, or supermarket	AU$15 for 100mL
Beakers	Various sizes 1 L, 2 L, 500 mL	IMBROS, Merck, Livingstone, or eBay/Amazon	Depending on size, starting at AU$20 for a 7Pcs set (5ml-250mL)
Cellophane	Red	Supermarket or eBay/Amazon	AU$1.5 for 2 sheets 50x70cm
Chemicals for F2 nutrient medium	See Guillard and Ryther (1962b) for preparation of F2 stock solution or the full nutrient-mix can be purchased (see Appendix 2 and 3).	The individual chemicals at Merck, or Thermo-fisher. Pre-made F/2-nutrient mix from Fresh-by-design or Variconaqua	The pre-made F/2-nutrient mix is AU$35 for 1kg (excl. shipping).
Cool box	Esky and ice blocks	Local camping / outdoor / hardware store	Varying on size and quality, ranging

			from AU$50 to 300+.
Dissection kit	Scalpel, blades, tweezers, scissors etc.	Merck, or eBay/Amazon	Ranging from AU$30 to 100+.
Ethanol	75% v/v	Merck or LabChem	Depending on volume and purity, ranging AU$10 to 25 for 1L.
Filters	Sterile air syringe filters, hydrophobic PTFE, 0.22 μm, 25 mm width	IMBROS, Livingstone, Merck, MedCart, or eBay/Amazon	Ranging from AU$80 to 200, for a 100 pack.
Flasks	Glass conical flasks (500 mL) and flat-bottomed round culture flasks (1 L and 3 L)	IMBROS, Merck, Livingstone, or eBay/Amazon	Depending on size, starting at AU$12 for a single 500mL
Flask stoppers	Conical, rubber 2-hole stoppers (appropriate width and height to fit neck of glass flasks)	IMBROS, RS Components Pty, Westlab, or eBay/Amazon	Depending on size, starting at AU$50 for a 10 pack
Fluorescent or LED lights	Ideally, outdoor weatherproof that can be covered with red cellophane	Hardware store (e.g., Bunnings)	Ranging from AU$50 to 150, depending on size, power, and irradiance level
Foil	Aluminium	Supermarket	AU$5 to 10 for 50m.
Glass Schott bottles	Various volumes (0.25, 0.5, 2 L)	Merck, IMBROS, general laboratory supply store, eBay/Amazon	AU$10-50, depending on size
Glass tubing	Walls 6 mm x 1 mm cut to appropriate lengths for air bubbling	IMBROS, glasstubing.com.au, eBay/Amazon	AU$60 for 1kg (750 mm length)
Gloves	Disposable, PPE	Merck, IMBROS, eBay/Amazon, or ppesupplier.com.au	AU$15-25 box of 200
Laboratory tissue	Kim wipes® or another low lint tissue	Merck, IMBROS, general laboratory supply store, eBay/Amazon	AU$8-15 box of 280 sheets

Laminar flow hood	To create a sterile environment. They come in various sizes, but needs to be large enough safely work in.	Merck, IMBROS, general laboratory supply store	Varying depending on size and quality, starting at AU$1000 up to 50,000+
Light meter	Licor that measures irradiance in μmol photons m^{-2} s^{-1}	IMBROS, licor.com	Approx. AU$1000
Light timer	To control the light period	Supermarket or hardware store	AU$10-15
Microscope	Normal light microscope, but ideally an inverted light microscope	Laboratory supply store, or online (e.g., microscopes.com.au)	Normal type: AU$300 up to 1000+ Inverted type: AU$4000 up to 15,000+
Paper towel		Supermarket or hardware store	AU$65 for a roll of 150m
Parafilm	To ensure stoppers remain on the flasks and keep the content sterile.	Laboratory supply store or online (e.g., eBay, Labdirect)	AU$15 to 80 depending on length
Pipette	Various sizes (1 mL – 5 mL)	Laboratory supply store (e.g., Merck, IMBROS) or online (e.g., eBay, Labdirect)	AU$100 to 500+ depending on brand and volume
Pipette tips	Various sizes (1 mL – 5 mL)	Laboratory supply store (e.g., Merck, IMBROS) or online (e.g., eBay, Labdirect)	AU$17 for 500pcs 1mL (non-sterile)
Plastic sheeting	White plastic card / corflute (4-5 mm width)	Hardware store (e.g., Bunnings)	AU$19 for 1.2x0.9m sheet
Tubing	Flexible silicone tube 4 x 7 mm (autoclavable)	Laboratory supply store, RS Components Pty., or online (e.g., labdirect.com.au)	AU$150 for 25m

Appendix 2 – F/2-medium recipe

Table 2.1 F/2 Stock Solution Chemical Composition

Components	Stock Solution	Quantity	Concentration (mmol/L)
$NaNO_3$	75 g/L dH2O	1 mL	882
NaH_2PO_4 H_2O	5 g/L dH2O	1 mL	36.2
Na^2CO_3	30 g/L dH2O	1 mL	106
Trace Metal Solution	See table 2	1 mL	---
Vitamin Solution	See Table 3	0.5 mL	---

Table 2.2 Trace metal solution and composition

Components	Stock Solution	Quantity	Concentration (mmol/L)
$FeCl^3$ 6(H_2O)	---	3.15 g	11.7
Na^2(EDTA) 2(H_2O)	---	4.36 g	11.7
$CuSO_4$ 5(H_2O)	9.8 g/L dH_2O	1 mL	0.00393
Na^2MoO_4 2(H_2O)	6.3 g/L dH_2O	1 mL	0.00260
$ZnSO_4$ 7(H_2O)	22.0 g/L dH_2O	1 mL	0.00765
$CoCl^2$ 6(H_2O)	10.0 g/L dH_2O	1 mL	0.00420
$MnCl^2$ 4(H_2O)	180.0 g/L dH_2O	1 mL	0.0910

Table 2.3 Vitamin solution and composition

Components	Stock Solution	Quantity	Concentration (mmol/L)
Thiamine HCl (Vit. B1)	---	200 mg	0.0296
Biotin (Vit. H)	0.1 g/L dH2O	10 mL	0.000205
Cyanocobalamin (Vit. B12)	1 g/L dH2O	1 mL	0.0000369

Appendix 3 – Pre-made F/2-medium

www.variconaqua.com ***Manufacturer and Supplier of:***

Algal nutrient products. Directions for use

There are three formulations for the all-in-one powder, and three for the concentrated liquid

All-in-one powder

Once the pack of Cell-hi has been opened store any that is unused in a sealed container as it is hygroscopic. Once opened, store in a refrigerator to extend the life of the vitamins. Kept in a refrigerator the life of the product will be approximately 6 months once opened. If kept in a cool dark place use within 2 months once opened.

- For direct use, dissolve the Cell-hi powder in sterile culture water with the appropriate salinity desired for the species and purpose of use. Once dissolved adjust the pH to the desired level.
- Alternatively, to prepare a concentrated liquid stock solution, add 1 kg of **Cell-hi F2P** to 10L of sterile distilled water, agitate to dissolve formulation. For long term use, store nutrient solution in a cool dark room or cupboard. If some components dissolve incompletely the addition of small quantities of concentrated HCl will assist dissipation.
- Follow the same procedure for the preparation of **Cell-hi W** however, 1.5 kegs of formulation should be added to 10L of sterile distilled water.
- For typical batch production of algal species use the prepared stock solutions at a rate of 1ml per litre of culture.

Cell-hi F2P based on the Guillard F/2 medium and has exactly the same N, P trace element and vitamin content. Most people in aquaculture use this - 1 **kg makes 10,000 litres culture medium at F/2 strength**

Cell-hi WP based on the Walnes medium and has exactly the same N, P trace element and vitamin content. Walnes is recommended by the UK Culture Collection for algae and protozoa. It is particularly good for flagellate algae such as Isochrysis and Tetraselmis - **1.5 kg makes 10,000 litres culture medium at Walnes strength**

For diatoms just add 30 grams of sodium metasilicate for each 1000 litres of culture water after adding the **Cell-hi.**

Appendix 4 – Schematic outline and list of equipment of infrastructure for at-sea cultivation

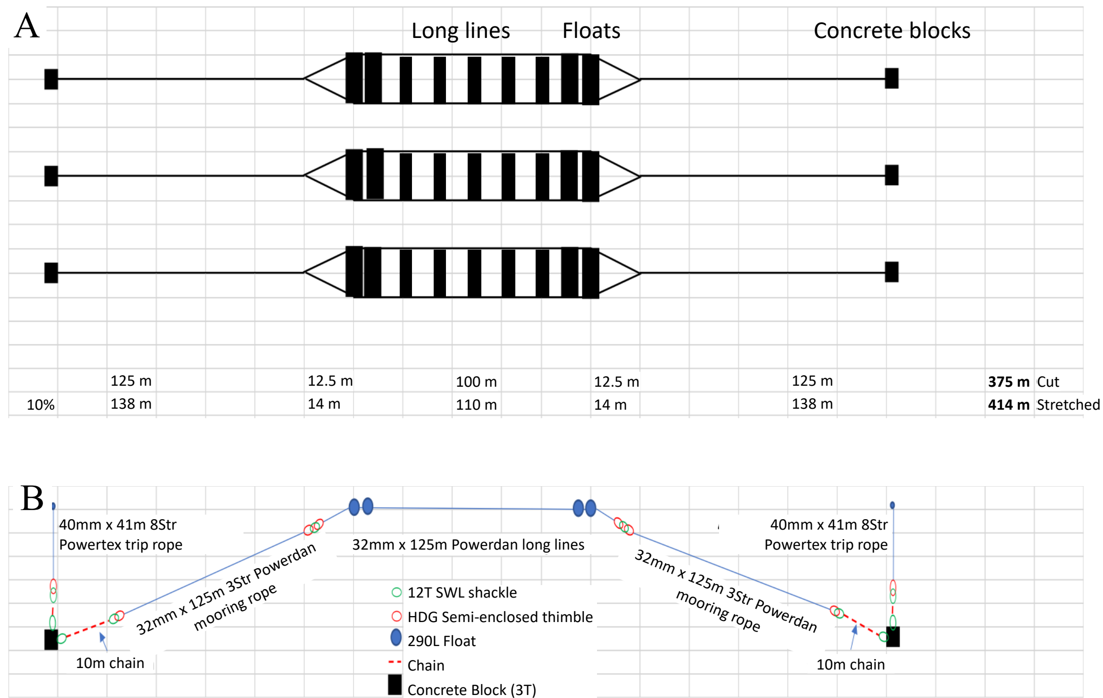

Figure 4.1. Schematic overview of the farm infrastructure using in this project at Tower Bay in association with the items listed in Table 4.1. (A) overhead view with the lengths (cut and stretched) of the long-lines and mooring ropes. (B) Side view of the long-lines and mooring ropes with its associated diameter and length, and the location of the shackles, chain, thimbles, and concrete blocks.

Table 4.1. List of equipment required for the at-sea cultivation of Australian Laminarialean kelps as per schematic in Figure 4.1 in Appendix 4. Equipment should be ordered depending on the scale of the farm, local environmental conditions, and extra equipment may be required as production increases. The costs associated with equipment and installation of this project's farm infrastructure (i.e., three 125-m long-line system with four corner markers) was approximately AU$50k. This price should be taken as indicative and provided in Tasmanian context in June 2020.

Item	Details
Floats (intermediate)	15 x 100L mussel Floats – 5 intermediately spaced in between each long line
Floats (end)	12 x 290L mussel Floats – 2 for each end of each long line 6 x 12" Polystyrene floats for the trip lines
Shackles	12T SWL. All Shackles (30) to be HDG grade "S" screw pin bow shackles
Concrete blocks	10 blocks, approximate ~300 kg each. Two for each long-line (6 in total) and one for each corner marker (4 in total).
Corner markers with lights and splicing	4pcs x 6m x 630mm. Pipe corner markers complete with Carmanah M550 lights and inbuilt radar reflection. Markers are painted yellow and fitted with a 500mm cross
Mooring ropes	125m x 32mm 3 strand Powerdan rope. All mooring ropes (6 in total) have HDG tubular thimbles spliced in each end
Long lines	125m x 32mm 3 strand Powerdan rope. All long lines (3x) are spliced together forming a double back bone with a single thimble attachment point at each. The long lines are joined to the mooring ropes with 12T shackles
Trip roles	35 and 25m x 40mm 8 strand Powerdan rope. All trip ropes (6x) have a thimble one end and heat shrink the other
Chain	For each of the concrete anchor blocks. Long lines: 6 pcs x 10m 36mm. Corner markers: 4 pcs x 1m 36mm.

Seaweed
Solutions
CRC-P