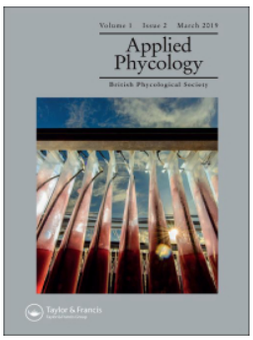

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Reproduction, hatchery and culture applications for the giant kelp (*Macrocystis pyrifera*): a methodological appraisal

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ABSTRACT

Although much is known regarding the physiology, ecology and life history of *Macrocystis pyrifera*, there is little accessible information for establishing robust and reliable culturing practices to support aquaculture and habitat restoration. Naturally occurring kelp forests formed by *M. pyrifera* support productive coastal ecosystems, and because it is one of the fastest growing macroalgal species, its high biomass production, and high affinity for nutrients and significant polysaccharide content makes it a species of considerable interest for aquaculture. This species has undergone substantial decline throughout its biogeographic range and is threatened by local and global stressors. Here, we synthesize the current knowledge on culturing of *M. pyrifera* and discuss approaches to stock collection, preservation of diversity and applications for experimental studies. It is crucial to preserve the current genetic diversity of this species immediately and long-term culture storage approaches such as germplasm banking and cryopreservation provide the tools to allow this. A concerted effort is also needed to better understand the physiological attributes of *M. pyrifera* in order to select strains for aquaculture and restorative applications that may provide resilience to future environmental stressors. Finally, attention must be given to developing effective *in situ* restoration approaches whereby large-scale stock production can be optimized and out-planting strategies developed to ensure restoration success.

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Introduction

Farming of marine macroalgae has a long history but has seen a significant increase in scale and advancement in research and infrastructure since the middle of the twentieth century. In 2018, the global production of macroalgae reached more than 32.4 million tonnes per annum (FAO, 2020a), with 97.1% coming from farmed systems (Chopin & Tacon, 2021). 45.3% of that production came from cultivated kelp species of the order Laminariales (FAO, 2020a). The main focus of macroalgal production is on polysaccharide products followed by direct human consumption (Naylor et al., 2021). However, in recent years, and in response to environmental challenges, there has been a shift in research focus to better understand culturing practices for other uses, such as multitrophic aquaculture, preserving and buffering ecosystem function, maintaining biodiversity and restoration (Layton et al., 2020; Ling, Johnson, Frusher, & Ridgway, 2009; Terawaki, Hasegawa, Arai, & Ohno, 2001).

Of all macroalgae, kelps (Laminariales) are generally the largest and exhibit some of the highest productive yields (Bolton, Anderson, Smit, & Rothman, 2012; Reed, Rassweiler, & Arkema, 2008). They are found throughout temperate regions and provide key services to nearshore and coastal systems through their role as ecosystem engineers (Bolton et al., 2012; Graham, Vásquez, & Buschmann, 2007). Globally, 38% of ecoregions have exhibited kelp forest decline over the past 50 years (Krumhansl et al., 2016). Multiple drivers, such as increased ocean temperature (Filbee-Dexter, Feehan, & Scheibling, 2016; Johnson et al., 2011; Wernberg et al., 2013), sedimentation (Connell, 2003; Filbee-Dexter & Wernberg, 2018), eutrophication (Filbee-Dexter & Wernberg, 2018), overgrazing (Kriegisch, Reeves, Johnson, & Ling, 2019; Ling et al., 2009), invasion (Blamey & Branch, 2012) and overfishing (Andrew & O'Neill, 2000; Ling et al., 2009; Mabin, Johnson, & Wright, 2019) are to blame for continued decline.

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The giant kelp, *Macrocystis pyrifera*, forms and maintains the largest ecosystems of all macroalgae, creating forests that modify the physical and chemical environment, providing key habitat, food resources and services to a plethora of species (Graham et al., 2007). *M. pyrifera* holds significant value as a direct harvest resource, predominantly for the extraction of the polysaccharide alginate (McHugh, 2003; Ortiz et al., 2009), abalone feed (Correa et al., 2016), as an additive for liquid fertilizer production (Gutierrez et al., 2006) and for its role in supporting cultural, recreational and commercially important fisheries (Jones, Lawron, & Shachak, 1997; Wernberg, Krumhansl, Filbee-Dexter, & Pedersen, 2019). Historically, the majority of harvests have been taken from wild populations (Buschmann et al., 2004; Purcell-Meyerink, Packer, Wheeler, & Hayes, 2021), with Chile and Peru being the main producers (*i.e.*, 33 979 and 32 794 tonnes, respectively, in 2019; Cai et al.,). Like many other kelp species, wild *M. pyrifera* forests have undergone significant global decline (Hay, 1990; Johnson et al., 2011; Krumhansl et al., 2016; Filbee-Dexter & Wernberg, 2018; Tait et al., 2021) and as a result, many of the services they provide have declined or been lost.

As attention shifts more towards the culturing of *M. pyrifera* for both harvest and bioremediation and restoration purposes, it has become clear that there is a lack of comprehensive information regarding culturing practices for this important species. Many studies have investigated the life history of *M. pyrifera* and by contrasting these bodies of work it is apparent that the conditions needed for successful growth are variable from one region to another (Gutierrez et al., 2006; Leal, Roleda, Fernández, Nitschke, & Hurd, 2021; Lüning, 1981; Macchiavello, Araya, & Bulboa, 2010; Neushul, 1963; Schiel & Foster, 2015; Westermeier, Patiño, Piel, Maier, & Mueller, 2006), making it difficult to establish robust and reliable culturing practices. Approaches to culturing a particular species are also highly dependent on the purpose of culturing. This critical assessment aims to synthesize the current methods and conditions used when culturing *M. pyrifera*, with a specific focus on the needs and application of the cultured product. This assessment will discuss methods for stock collection, storage and grow-out, with reference to experimental, aquacultural and restorative applications.

Life history and distribution

M. pyrifera is found in temperate waters along the coasts of Southern Africa, east and west South America, Tasmania and South Australia, central and southern New Zealand (including the sub Antarctic Islands) and the west coast of North America and Canada (Figure 1). It requires rocky substrata for attachment and can be found in both sheltered and exposed environments (Graham et al., 2007; Schiel & Foster, 2015).

Figure 1. Global distribution of *Macrocystis pyrifera* (modified from Graham et al., 2007).

Reproduction of *M. pyrifera* can occur year round (Brown, Nyman, Keogh, & Chin, 1997; Reed, Ebeling, Anderson, & Anghera, 1996) but the abundance of reproductive tissue (sorus) and density of zoospores (hereafter spores) within sori varies seasonally (Buschmann et al., 2004; Leal et al., 2021; Neushul, 1963; Reed et al., 1996). Like other Laminariales, *M. pyrifera* has a biphasic life cycle, alternating between a microscopic haploid stage and a macroscopic diploid stage (Schiel & Foster, 2015). The main reproductive tissue is located on the sporophyll at the base of the sporophyte, above the holdfast, although spore bearing tissue can also be found in the blades and the apical scimitar of some individuals (Leal, Hurd, & Roleda, 2014; Leal et al., 2021; Neushul, 1963).

In the wild, spore reproduction occurs throughout the year, with the highest production coinciding with low-temperature months, such as early winter and late spring/early summer (Anderson & North, 1967; Reed et al., 1996), and storms (Reed et al., 2006). Mature sori release spores that swim freely in the water column and are transported by currents with a typical dispersal range of <1 km. Spore settlement occurs within hours to a few days post release (Devinny & Volse, 1978; Reed, Amsler, & Ebeling, 1992) and is triggered by the available nutrients (*i.e.*, by following a concentration gradient) (Amsler & Neushul, 1989), presence of other biota (Reed et al., 1996), and light condition (Reed et al., 1992). Germination can occur immediately after spore release and is typically complete within 48 hrs under laboratory conditions (Anderson & Hunt, 1988; Garman, Pillai, Goff, & Cherr, 1994), or a few days post release in the field (Devinny & Volse, 1978; Reed et al., 1992; Santelices, 1990). After germination, germlings start to grow by increasing cell number and size, forming male and female gametophytes (Devinny & Volse, 1978; Reed et al., 1992; Schiel & Foster, 2015). In optimal laboratory conditions, gametophytes of both sexes can become fertile in approximately two weeks (Lüning & Neushul, 1978). The fertilization process takes place when eggs of female gametophytes produce a pheromone called lamoxirene, which attracts the sperm of male gametophytes (Maier, Hertweck, & Boland, 2001). The effective distance for hormone attraction is reported to be within 1 mm (Boland, Marner, Jaenicke, Muller, & Folster, 1983). If successful fertilization occurs, then female gametophytes turn into embryonic sporophytes.

Once established, the growth of sporophytes can be rapid. Sporophyte growth can result in a doubling of the length every month from an initial length of 1–2 cm (Neushul, 1963). Growth rates are variable across season and by region (North, 1976a; Wheeler & North, 1981; González-Fragoso, Ibarra-Obando, & North, 1991; Hernández-Carmona, 1996; Brown et al., 1997; Graham et al., 2007; Macchiavello et al., 2010), for example, greatest growth rates occur during the summer in Alaska and British Columbia, while populations in California and New Zealand displayed greatest growth during the winter and spring (North, 1976a; Wheeler & North, 1981; Brown et al., 1997; Graham et al., 2007). This variation in growth is driven by resource

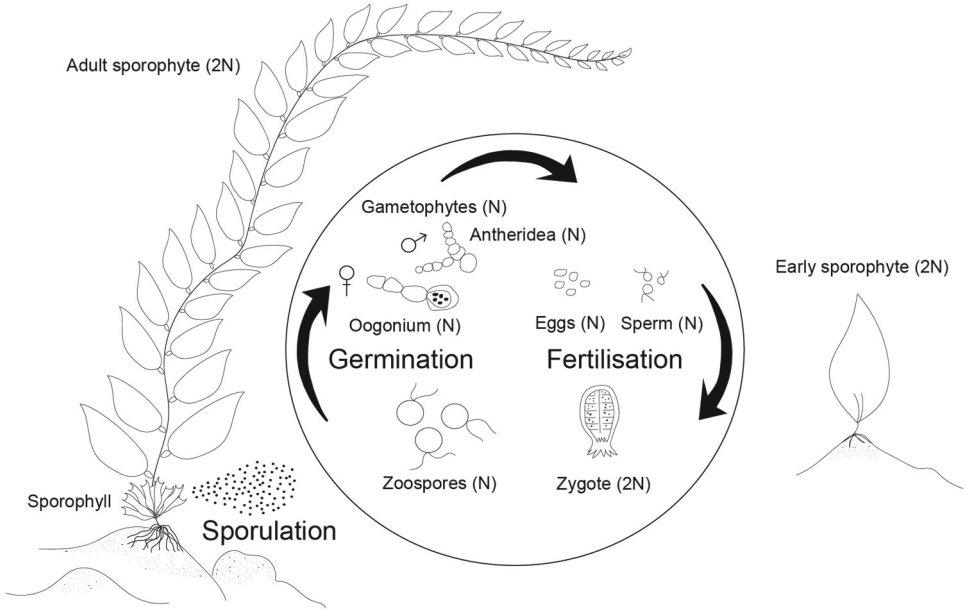

Figure 2. Lifecycle of *Macrocystis pyrifera*.

availability, namely, light and nutrients (Desmond, Pritchard, & Hepburn, 2015; Graham et al., 2007; Schiel & Foster, 2015; Stephens & Hepburn, 2014). *M. pyrifera* populations have a relatively high turnover rate due to their large size, which makes them vulnerable to physical forces, but individuals have been shown to persist for up to 7 years (Graham et al., 2007).

Stock collection

In the following section stock refers to the culture of *M. pyrifera* from a known source that may be used for multiple applications as outlined below.

Seasonality and timing

M. pyrifera can be reproductive year-round, but there appear to be seasonal trends when spore production is highest, and depending on location, there may be periods where no sorus tissue is present (Brown et al., 1997; Buschmann et al., 2004; Hay, 1990; Leal et al., 2021; Neushul, 1963; Reed et al., 1996). To ensure the greatest chance of successful spore release, collection should be carried out when spore production is highest. Highest sporophyll counts and spore densities are typically observed during the austral summer and autumn period in the Southern Hemisphere (Buschmann, Moreno, Vásquez, & Hernández-González, 2006; Hay, 1990; Henríquez et al., 2011), while populations along the west coast of the United States show highest spore densities during the winter and spring (Reed et al., 1996).

Collection and transportation

Mature sorus tissue can be removed by cutting it away from the sporophyll without removing the entire individual. If possible, immediate sporulation in the field is the best method to obtain the highest spore density (Suebsanguan, Strain, Morris, & Swearer, 2021) but often this is not practical. If it is not possible to sporulate immediately, then spore tissue can be transported either semi-dry, packed in damp paper cloth which maintains higher spore densities when compared to wet transportation (Bak, Mols-Mortensen, & Gregersen, 2018; Kim, Yarish, Hwang, Park, & Kim, 2017; Suebsanguan et al., 2021), or in a plastic zip-lock bag or similar sealed container filled with seawater and kept in the dark at ~4°C (Barrento, Camus, Sousa-Pinto, & Buschmann, 2016; Gutierrez et al., 2006; Westermeier et al., 2006). Generally, longer transportation time (>24 hrs) leads to lower spore yield so facilities and equipment should be well prepared and sterilized to be ready to receive reproductive material (North, 1976a; Devinny & Leventhal, 1979; Gutierrez et al., 2006; Kim et al., 2017; Suebsanguan et al., 2021) .

Sporulation

Pre-treatment

After transporting the fresh mature sorus to the laboratory or processing facility, it is important to clean the tissue to reduce the likelihood of culture contamination. Typically, cleaning is performed by gently rinsing the sorus tissue with filtered (0.2 µm pore diameter) and/or sterilized (autoclaved) seawater and wiping off any visible fouling. Tissue can also be rinsed using freshwater, freshwater with chlorine (5 ml of chlorine l^{-1} water) or iodine without any noted negative effects on spore quality (Alsuwaiyan et al., 2019; Camus & Buschmann, 2017; Plá & Alveal, 2012). After cleaning, the sorus tissue should undergo a desiccation period to enhance spore release (Leal et al., 2014). Most studies store sorus tissue in damp paper cloth for between 1 and 24 hrs in the dark at temperatures between 4°C and 15°C (Camus & Buschmann, 2017; Gutierrez et al., 2006; Leal et al., 2014; Plá & Alveal, 2012). After the desiccation period, sporulation can take place. It should be noted that sporulation can take place without a desiccation period; however, this approach is more time consuming, has a greater risk of contamination and produces more mucilage (Neushul, 1963; Sanbonsuga & Neushul, 1978).

Medium

For sporulation, a range of culture media are reported, the most common being filtered, sterilized and/or nutrient enriched seawater (*i.e.*, Provasoli, Alsuwaiyan et al., 2019). Artificial seawater is sometimes used; however, the advantages or disadvantages of this approach are not clear (Amsler & Neushul, 1989, 1990). A general guide should be that the medium used should minimize the chance of infection or contamination and therefore both filtration and sterilization either using UV or autoclave are recommended. Very few studies report using germanium oxide or antibiotics to control contamination at the time of sporulation, and it is advised that if used this should be precise in concentration and duration to reduce negative effect on growth (Kawai, Motomura, & Okuda, 2005; Markham & Hagmeier, 1982; Shea & Chopin, 2007).

Sporulation time, temperature, and light intensity

The desiccated sorus tissue should be submerged in the medium and left for a period of time ("sporulation time") to allow release. Of the studies that report sporulation time, most range between 15 minutes to 1 hr (Camus & Buschmann, 2017; Gutierrez et al., 2006; Leal et al., 2014; Macchiavello et al., 2010; Neushul, 1963; Plá & Alveal, 2012). Many studies do not state the temperature used for sporulation, of those that do, most range between 10°C and 18°C (Alsuwaiyan et al., 2019; Camus & Buschmann, 2017; Gutierrez et al., 2006; Leal et al., 2014; Macchiavello et al., 2010; Neushul, 1963; Plá & Alveal, 2012). The lowest temperature reported was 0°C (James, Stull, & North, 1990) and the highest 19.8°C (Hollarsmith, Buschmann, Camus, & Grosholz, 2020). Little information is available regarding the potential effects of sporulation temperature on developmental parameters, and this is an area that requires further research given its potential ecological importance. Light intensity is variable across studies, ranging from complete darkness to dim irradiance (Alsuwaiyan et al., 2019). Most studies did not mention irradiance levels during sporulation (Alsuwaiyan et al., 2019). Care must, however, be taken to avoid potential photo damage during this time (Graham, 1996), and it is therefore recommended that sporulation be conducted under dim light conditions.

Spore density

The number of spores per unit area of sorus tissue is variable between populations (Buschmann et al., 2004), season (Buschmann et al., 2004; Neushul, 1963) and tissue types (Leal et al., 2014, 2021; Neushul, 1963); therefore, the number of spores produced during sporulation will also vary. Spore density can be assessed post sporulation by taking an aliquot of the medium and quantifying spore density under magnification using a haemocytometer. Studies report densities in the range of 40,000–5,000,000 spores ml^{-1} (Barrento et al., 2016; Buschmann et al., 2006, 2004; Camus & Buschmann, 2017; Cie & Edwards, 2008; Deysher & Dean, 1984; Leal et al., 2014; Macchiavello et al., 2010; Muñoz, Hernández-González, Buschmann, Graham, & Vásquez, 2004; Westermeier et al., 2006). Spore density can be diluted by adding an additional medium to the sporulation vessel in order to achieve the desired concentration, which will be highly dependent on the application of the cultured stock and will be discussed further below.

Stock management

It is important to have a clear understanding of the intended application of cultured *M. pyrifera* as this will dictate how much tissue should be collected, how sporulation should be undertaken and most importantly what happens to stock post sporulation. In this section, we discuss long-term storage techniques that allow for the preservation of genetic diversity and culture stocks, short-term storage and culturing methods for experimental applications, and finally grow-out approaches for aquacultural and restoration purposes.

Preserving diversity

The need for long-term storage of *M. pyrifera* is multifaceted. Given the decline of *M. pyrifera* forests worldwide, a loss of genetic diversity has more than likely occurred. Continued pressure on these systems means that a concerted effort is needed to preserve current genetic strains for future uses (Barrento et al., 2016), whatever they may be. *In situ* protection measures, such as marine protected areas, do not offer the insurance needed to preserve genetic diversity as these areas remain vulnerable to the effects of global stressors (Williams, 2001). *Ex situ* preservation methods are therefore the only way to safeguard and preserve diversity (Coleman et al., 2020; Wade et al., 2020). Large-scale *ex situ* preservation of terrestrial plants and crop species has occurred in climate-controlled seed banks since the early 20th century in order to ensure food security and preserve biodiversity (Wade et al., 2020). Long-term storage of macroalgae, however, lags significantly behind terrestrial examples, but two main approaches are slowly becoming utilized.

Germplasm banking

Germplasm banking is defined as the preservation of biological, animal or plant, for the purposes of delayed breeding and propagation. For macroalgae, germplasm banking involves holding male and female gametophytes, often separately, in a state of dormancy by placing them under reduced temperature, light and nutrient conditions (Barrento et al., 2016). For most kelps, including *M. pyrifera*, the gametophytic (haploid) stage responds best to long-term storage conditions (Barrento et al., 2016; Carney & Edwards, 2010; Westermeier et al., 2006). Only a small number of studies have reported storing *M. pyrifera* under

germplasm conditions (Barrento et al., 2016; Lewis & Neushul, 1994; Westermeier et al., 2006; Xu et al., 2015).

The key factors found to affect the survivorship of *M. pyrifera* gametophytes under germplasm conditions are temperature, light, nutrient concentration and cell density. Ideally, germplasm storage temperature should be at the low end of the naturally occurring temperature range for the region where stock originates. Temperature conditions vary between 8°C and 15°C which is considered very close to the optimal growth range for *M. pyrifera* (i.e., 12–17°C, Lüning & Neushul, 1978) and so the temperature for germplasm should be lower than 12°C. Importantly, light levels must be kept low, <5 μmol m^{-2} s^{-1} is most common, and light:dark photoperiods between 10 L:14D – 16 L:8D are recommended (Barrento et al., 2016; Westermeier et al., 2006; Xu et al., 2015). The removal of blue wavelength light from the light source (*e.g.*, using red light LEDs) provides further insurance to avoid gametophytic sexual development (Lüning & Neushul, 1978).

Nutrient availability is particularly important for controlling vegetative growth and maintaining germplasm cultures at manageable densities. Cultures should be held in nutrient enriched, filtered, autoclaved seawater with the preferred medium being Provasoli (Barrento et al., 2016; Carney & Edwards, 2010; Wade et al., 2020; Westermeier et al., 2006). If cultures are maintained at low densities, the medium can be changed approximately twice per year as long as evaporation is kept to a minimum (Barrento et al., 2016; Westermeier et al., 2006). To completely prevent any chance of reproduction, it is advised that male and female gametophytes are held separately once distinguishable. The 96-well plates are commonly used to store gametophytes as they offer highly independent storage replication at low densities. Studies that place *M. pyrifera* gametophytes in long-term storage have shown viability of stock after more than 5 years post preservation (Barrento et al., 2016; Lewis & Neushul, 1994; Westermeier et al., 2006; Xu et al., 2015).

Cryopreservation

Cryopreservation is a second approach, which is gaining attention for its potential as a long-term storage option for algal cultures (Choi, Nam, & Kuwano, 2013; Kuwano, Kono, Jo, Shin, & Saga, 2004; Vigneron, Arbault, & Kaas, 1997; Zhang et al., 2007; Zhang, Cong, Qu, Luo, & Yang, 2008). Cryopreservation was first used to preserve human sperm in the 1960′s (Sherman, 1973) and has since been used in both agricultural and horticultural practices to preserve biological material (Taylor & Fletcher, 1998). The process, in general, requires the staged cooling of the subject to very low temperatures where biological cell degradation effectively ceases (Grout, 1995). Typically, the samples are stored under solid carbon dioxide or liquid nitrogen conditions to achieve temperatures of – 80 to –196°C. As with germplasm banking, the gametophytic stage of *M. pyrifera* responds best to cryopreservation (Piel, Avila, & Alcapán, 2015).

The process of preservation requires gametophytes to be suspended in a cryoprotectant solution made up of dimethyl sulphoxide (DMSO), glycerol, sucrose, dextrose and sorbitol (Piel et al., 2015; Zhang et al., 2007, 2008) before it is cooled. The cooling step is separated into two minor steps to avoid the damage to cell walls (Piel et al., 2015; Zhang et al., 2007, 2008). Briefly, gametophyte suspensions are pre-frozen either at –18°C to –20°C for 30 min. After 30 min pre-frozen samples are placed in liquid nitrogen (Piel et al., 2015; Zhang et al., 2007). Only one published study considers the cryopreservation of *M. pyrifera* (Piel et al., 2015). Four weeks after being thawed and returned to optimal growing conditions 29% of female gametophytes were still immature and in a vegetative state, 9% released eggs and 9% developed into embryonic sporophytes. From other species, it appears that survival rate and viability of preserved tissue is species dependent (Ginsburger-Vogel, Arbault, & Pérez, 1992; Kono, Kuwano, & Saga, 1998; Kuwano et al., 2004; Piel et al., 2015; Zhang et al., 2007, 2008) and is significantly influenced by the rate of freezing and thawing process (Zhang et al., 2007). Therefore, more research is needed to optimize this approach to enable the successful long-term storage of *M. pyrifera*.

Both germplasm banking and cryopreservation allow for the long-term storage of *M. pyrifera,* which is essential for preserving the genetic diversity of this species. Government, research agencies and other parties interested in the values surrounding kelp forests should make a concerted effort to establish long-term storage facilities to preserve this species. Not only does storage safeguard genetic diversity but it also forms the basis for breeding programs and allows for timely propagation of stock for experimental, aquacultural and restorative applications.

Farming and applications

Culturing methods for laboratory experimentation

Laboratory experimentation is important to understand the physiological function of *M. pyrifera* and knowledge gained often provides perspective on ecological trends or guide approaches for other applications, such as aquaculture or restoration. Typically, these types of experiments focus on the early life stages from the spore through to the juvenile sporophyte (Barrento et al., 2016; Carney & Edwards, 2010; Hollarsmith et al., 2020; Leal, Hurd, Fernández, & Roleda, 2017; Westermeier et al., 2006). This tends to be because of the importance of these early life stages in future development (Schiel & Foster, 2015) as well as the difficulty of maintaining large adult *M. pyrifera* in culture. This section will specifically assess the culturing of early life stages for experimental purposes.

Experimentation may begin from as early as the sporulation stage when sorus tissue is exposed to different experimental conditions, for these types of experiments, sporulation can take place as advised above while manipulating the factors of interest. After sporulation takes place and before settlement occurs it is important to achieve the desired concentration of spores for any ongoing experiment, this will vary depending on the needs of the experiment. Although a wide range of spore densities have been applied in other studies (Barrento et al., 2016; Buschmann et al., 2006, 2004; Camus & Buschmann, 2017; Cie & Edwards, 2008; Deysher & Dean, 1984; Leal et al., 2014; Macchiavello et al., 2010; Muñoz et al., 2004; Westermeier et al., 2006), the optimal range of settled spores for maximum sporophyte production is roughly 50 spore mm^{-2} (Reed, Neushul, & Ebeling, 1991).

Once the desired concentration of spores is achieved, the spore solution can be seeded to suitable culture vessels depending on experimental purposes. Most studies utilize glass or plastic vessels, usually vials, flasks or Petri dishes (Barrento et al., 2016; Carney & Edwards, 2010; Cie & Edwards, 2008; Deysher & Dean, 1984; Hollarsmith et al., 2020; Morris et al., 2016; Westermeier et al., 2006). When selecting vessels, ensure they are of appropriate size, consider that settlement is greatest on horizontal as opposed to vertical surfaces (Reed et al., 1992) and if the experiment requires observation of development, the vessel has a flat, optically clear and uniform base. Observation is best achieved using an inverted microscope with a magnification range of 4x–80x depending on developmental stage (Carney & Edwards, 2010; Hollarsmith et al., 2020; Leal et al., 2017; Morris et al., 2016; Muñoz et al., 2004).

Spore settlement generally ranges from 30 min to 1 h in high light *i.e.*, <50 μmol m^{-2} s^{-1} or 1–2 hrs in darkness if the medium is not constantly agitated (Amsler & Neushul, 1989, 1990). This will allow for the settlement of the majority of viable spores. After 12–24 hrs, the medium can be gently decanted and replaced to discard any unsettled spores. Experimental treatments can commence from this point if not already initiated from the sporulation stage. If it is desirable that development occurs in a free-floating state, *i.e.*, not attached to the bottom of the vessel, then aeration can be applied through an airstone or through mechanical mixing using a shaker table or stirring device. Depending on experimental conditions, the development from spore to juvenile sporophyte may progress over 2 to 4 weeks (Camus & Buschmann, 2017; Carney & Edwards, 2010; Hollarsmith et al., 2020; Neushul, 1963). If conditions are not suitable for development, then development may cease at a particular life stage and remain in a vegetative state or perish (Lüning & Neushul, 1978; Westermeier et al., 2006).

When performing experiments that seek to quantify population characteristics rather than those at the individual level, it is important to utilize sori from many individual sporophytes (>30) as the response of *M. pyrifera* at an individual level can be highly variable due to plastic responses, genetic diversity and/or local adaptations (*i.e.*, Fernández, Navarro, Camus, Torres, & Buschmann, 2021). It should be noted that each life stage will respond differently to experimental conditions, and this should be considered when designing experiments.

Breeding

The purpose of breeding programs is generally to enhance the performance of the macroalgal culture (*e.g.*, to increase yield, breed stress tolerance and increase reproduction). This can be achieved by 1) cross-breeding males and females that exhibit desirable traits to create a hybrid generation, these are usually identified from physiological experiments, 2) creating a combination between hybridization and simple phenotypic mass selection or 3) more advanced gene editing of particular individuals to promote certain characteristics (Goecke, Klemetsdal, & Ergon, 2020).

For *M. pyrifera*breeding strategies are relatively underdeveloped with most approaches focusing on hybridization across one generation (Camus et al., 2021; Murúa, Patiño, Müller, & Westermeier, 2021; Raimondi, Reed, Gaylord, & Washburn, 2004; Westermeier, Patiño, Müller, & Müller, 2010, 2011). However, for other species that have a longer history

in commercial aquaculture, such as *Saccharina* spp. and *Undaria* spp.,*Pyropia spp*., more advanced breeding programs have been developed and have observed success in increasing productivity, disease resistance, heat tolerance, seasonal duration, and chemical contents (Goecke et al., 2020; Hu et al., 2021; Hwang, Yotsukura, Pang, Su, & Shan, 2019; Wang, Yao, Zhang, & Duan, 2020). It is likely that as *M. pyrifera* is incorporated into greater commercial production, similar breeding programs will be undertaken, but this is currently an area for further research. The selection of particular traits will also benefit restoration efforts, which is discussed in more detail below.

Farming

Macroalgal aquaculture is rapidly growing, driven by the demands of the food, pharmaceutical, agricultural and aquacultural industries (Ferdouse, Holdt, Smith, Murúa, & Yang, 2018; Wernberg et al., 2019; Naylor et al., 2021). *M. pyrifera*, being one of the fastest growing macroalgal species with high biomass production (Reed et al., 2008), high affinity for nutrients (Correa et al., 2016; Purcell-Meyerink et al., 2021) and polysaccharide content (Ortiz et al., 2009), holds significant value as a desirable culture species.

The history of *M. pyrifera* farming provides many examples of the challenges faced by this industry. Over time, reliable protocols for mass production have been developed and are now being implemented (Buschmann et al., 2004; Westermeier et al., 2006; Gutierrez et al., 2006; Macchiavello et al., 2010; Camus et al., 2018b). Typically, culture and production rely on sourcing spores directly either from wild populations or from gametophytes, which are held under controlled culture conditions. The latter approach allows the selection of specific traits (*i.e.*, high-yield sporophytes) through cross-breeding specific gametophytes (Westermeier et al., 2010, 2011; Camus, Faugeron, & Buschmann, 2018a; Buschmann et al., 2020; Camus et al., 2021; Murúa et al., 2021). The set-up and requirement for growing kelp is discussed in two separate phases: hatchery and grow-out.

Hatchery requirements

The purpose of this section is to provide a general overview of the essential capabilities a hatchery must support. Several very detailed guides on the requirements for macroalgal hatcheries have been written (Andersen, 2005; Redmond, Green, Yarish, Kim, & Neefus, 2014). Although we focus on *M. pyrifera*, the requirements will scale well to many other laminarian kelps. Depending on the scale of aquacultural application, hatchery requirements will vary; however, approaches to producing and maintaining stock will be comparable regardless of scale. As with all culturing practices, sterility is essential. All water should be filtered and sterilized to the requirements laid out above, and air sources should also be filtered using a 0.2 μm (polytetrafluoroethylene) inline air filter.

Seeding stock onto seed lines is the most common and by far the most efficient means for large-scale aquaculture production (Camus et al., 2018b). These seeded lines should be maintained in the hatchery until juvenile sporophytes of desirable size are present before being relocated to an aquaculture setup in a natural environment where the grow-out phase will take place. Preferable seed lines are synthetic twines such as nylon and vinylon ~1–2 mm thick (Camus & Buschmann, 2017; Gutierrez et al., 2006); this should be wrapped tightly around a sterilized PVC tube cut to an appropriate length for the application needed and the length should match the depth of the aquaria that will house the seed line as growth progresses (Camus & Buschmann, 2017; Gutierrez et al., 2006). Seed lines can be pre-prepared, wrapped in plastic wrap and stored in a refrigerator for later use (Redmond et al., 2014).

Seed stock will either be from wild sourced reproductive material or from gametophyte cultures already held in the hatchery under vegetative conditions. If wild sourced stock is used, then sporulation should take place as described above whereby spores are released into a glass vessel after the pretreatment phase, and a density of ~40 000 spores ml^{-1} is desirable (Camus & Buschmann, 2017; Gutierrez et al., 2006). Spore solution is added to aquarium or culture containers, previously filled with filtered seawater, holding the PVC tubes wrapped with seed line (Camus & Buschmann, 2017; Redmond et al., 2014). These should be left for 24 hrs before removing the seed line wrapped tubes and placing them into culture aquaria tanks filled with Provasoli enriched seawater held at a constant temperature. If gametophyte stock cultures are used, then these should be vegetatively propagated in order to obtain a high density of gametophytes. This is done under similar culture conditions described in the germplasm banking section above. This may take weeks to months depending on the initial density of gametophytes held in the culture (Barrento et al., 2016; Westermeier et al., 2006). Once a dense culture is present, it can be transferred to an electric blender or a tissue disruptor and fragmented until a dark brown solution results. This solution can then be sprayed directly onto the seed line using a pressure sprayer, left for 30 min – 1 hr, before being placed in culture aquaria tanks. Seawater changes should be carried out weekly to refresh nutrient

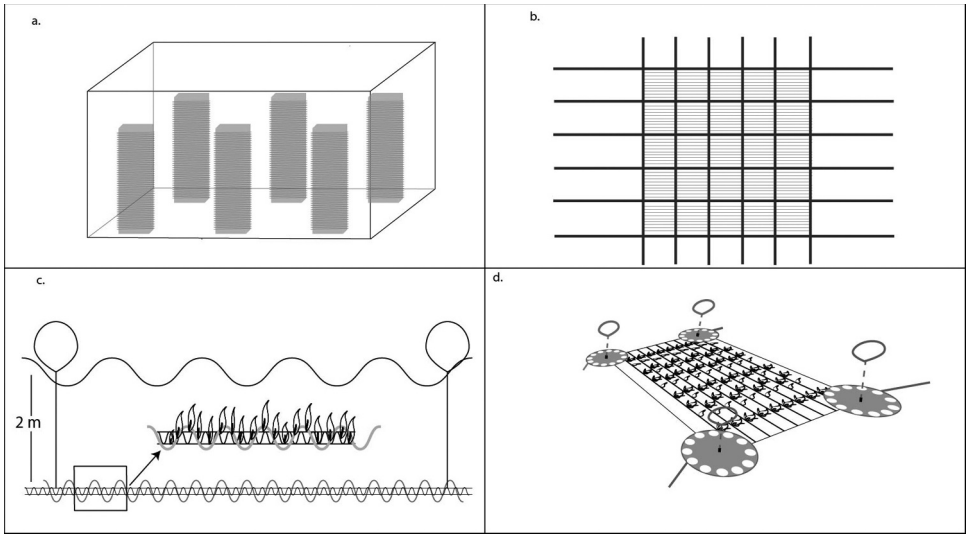

Figure 3. Culture infrastructure for the production of *Macrocystis pyrifera*. Seed line in hatchery aquaria (a), layout of grow-out grid (modified from Camus et al., 2018b) (b), seed line attachment to main backbone line (modified from Gutierrez et al., 2006) (c), low angle view of grid array (modified from Bak et al., 2020) (d).

concentrations (Redmond et al., 2014). Care should be taken to avoid overstocking aquaria with seed line PVC tubes as this can create shading, limit water movement and deplete nutrients, meaning water will need to be changed more frequently. Camus and Buschmann (2017) detail the optimal production conditions for rapid growth at a temperature of 12°C, a photon flux density of 12 μmol m^{-2} s^{-1}, a photo period of 16 L:8D and aeration of 414 l h^{-1}. Under these conditions they were able to produce 4–5 mm long juvenile sporophytes after 45 days post sporulation. During the hatchery phase, environmental conditions should be monitored daily to ensure optimal conditions for growth.

Another applicable seeding approach is to attach free-floating sporophytes directly onto seed lines. This method, which has been described in detail elsewhere (see Camus, Infante, & Buschmann, 2018b; Westermeier et al., 2006), is less common than direct seeding due to its labour-intensive nature (Camus et al., 2018b). Briefly, embryonic sporophytes are produced in floating cultures, by providing constant water motion in the culture vessel, until a desired size of 4–10 cm is reached. These are then extracted from the culture and sown directly onto seed lines either using adhesives or simply by inserting the holdfast between the weaves of the seed lines. Individuals are typically spaced 10–30 cm apart for a grow-out.

Grow-out requirements

The grow-out phase should typically be carried out in a natural environment where nutrient and light availability are not limiting, where water motion is present but not detrimental and where temperature ranges are well within the thermal limits of *M. pyrifera* (Fain & Murray, 1982; Deysher & Dean, 1984; Harrison & Hurd, 2001; Camus & Buschmann, 2017; Camus et al., 2018; North et al., 1986). A wide range of approaches have been trialled using varying infrastructures, ranging from shallow-water grid arrays to offshore deep-water suspension lines (Buschmann et al., 2014; Bak et al., 2018; Bak, Gregersen, & Infante, 2020; Camus et al., 2018b). Grow-out facilities are usually located in shallow coastal environments, <30 m deep with strong current flow, nitrogen levels of no less than 5–8 μMol and high light availability. Site selection is a critical decision as high-temperature pulses (normally not detected by observing average values, salinities and nutrient drops and pest organism (epibionts, grazer and eventually pathogens) can determine the viability of the farming activity (Camus et al., 2018b).

Grid arrays are commonly employed as grow-out structures (Bak et al., 2020) as they provide the greatest area coverage and are rigid and maintain a stable desired depth (Fig 3). These arrays involve a series of mooring blocks or steel anchors that fix the grid to the seabed. From these moorings, ropes are suspended in a cross-linked fashion creating a semirigid grid (Camus et al., 2018b). In each grid, backbone lines can be strung from one side to the other, parallel to one another, and it is on these backbone lines that the seed line will be deployed. Seed line (either direct seeded or free-floating) can be deployed by feeding one end of the backbone line through the PVC seed line tube, tying the end of the seed line to the backbone line, attaching it to the grid and then moving the PVC seed line tube

along the backbone line, letting it coil around the backbone as it moves along, before tying it off once it reaches the other end (Figure 2).

Setting the depth of the grid array is important (Camus et al., 2018b) and should be based on knowledge of light, nutrient, temperature, and water motion characteristics at the chosen site. The depth of the array can be manipulated by attaching surface or subsurface buoys to the grid to provide floatation while altering the length of the mooring ropes that suspend the grid. Manipulation using buoys initially attached to the grid is essential to maintain buoyancy and as pneumatocysts develop, buoys can be removed and weights added to offset the floatation of the developing kelp.

Seasonality dictates growth and for *M. pyrifera* grow-out will typically be most successful if commenced in autumn/winter with a spring/summer harvest (Buschmann et al., 2004, 2014; Camus et al., 2018b). This will, however, depend on local conditions and *in situ* physiological understanding (Gerard, 1982; Varela et al., 2018).

Restoration

Significant declines in kelp forest habitat have been recorded across the globe over the past 50–100 years (Desmond et al., 2015; Friedlander et al., 2018; Krumhansl et al., 2016; Layton et al., 2020; Wernberg et al., 2019), included in that loss have been large swaths of *M. pyrifera* (Hay, 1990; Johnson et al., 2011; Butler, Lucieer, Wotherspoon, & Johnson, 2020; Layton et al., 2020, Tail et al., 2021). The driving factors behind kelp forest decline are relatively well understood and include increasing sea surface temperature (Filbee-Dexter et al., 2016; Johnson et al., 2011; Wernberg et al., 2013), light limitation (Cie & Edwards, 2008; Desmond et al., 2015; Deysher & Dean, 1984; Fain & Murray, 1982; Navarro, Mansilla, & Palacios, 2007), increased storm frequency (Buschmann et al., 2004), sedimentation (Connell, 2003; Filbee-Dexter & Wernberg, 2018), food web alterations through overfishing (Friedlander et al., 2018; Krumhansl et al., 2016), eutrophication (Filbee-Dexter & Wernberg, 2018) and direct harvest (Buschmann et al., 2014). This decade, 2021–2030, is the UN Decade on Ecosystem Restoration with a goal to restore 350 million hectares of degraded ecosystems (FAO, 2020b). Arguably, the revitalization and restoration of vast areas of lost kelp forest would deliver some of the greatest benefits in terms of fisheries productivity, ecosystem services and socio-ecological outcomes of any ecosystem type.

Early kelp forest restoration efforts began sometime before 1970 and have shown mixed results over the last five decades (Campbell, Marzinelli, Coleman, Verge, & Steinberg, 2014; Carney, Waaland, Klinger, & Ewing, 2005; Fredriksen et al., 2020; Mcleod et al., 2018; Mearns, Hanan, & Harris, 1997; North, 1976; Westermeier et al., 2014; Wood et al., 2019). Approaches have included encouraging natural recruitment through the placement of artificial substrate, to directly seeding habitat with microscopic life stages, to transplantation of juvenile and adult individuals. Many of these approaches have been extremely time, labour and financially demanding, often making them prohibitively expensive to maintain. As approaches advance, a move to aquacultural practices (Alleway et al., 2019; Froehlich, Gentry, & Halpern, 2017; Giangrande, Gravina, Rossi, Longo, & Pierri, 2021) is emerging which allows large quantities of stock to be produced under laboratory conditions with known genetic and physiological characteristics. This stock can then be outplanted into the receiving environment when conditions are appropriate. Out-planting approaches vary, and their success is highly dependent on the site conditions. This area of research is currently underdeveloped but promising results have been seen using seed line attached directly to or above the substrate (Giangrande et al., 2021; Kraufvelin & Díaz, 2015), seeded tiles attached to the seafloor (Layton et al., 2020; Shelamoff et al., 2020) and an approach termed "green gravel" which is gravel seeded in the laboratory and distributed directly onto the seafloor (Coleman et al., 2020; Fredriksen et al., 2020). All of these approaches outplant when *M. pyrifera* are at the juvenile sporophyte stage.

Conclusion

This critical analysis summarizes the current state of knowledge regarding the culture of *M. pyrifera* and details the necessary steps for obtaining and preserving stock as well as propagation for experimental, aquacultural and restorative purposes. Current threats to *M. pyrifera* and the value this species holds from an aquacultural perspective mean that a concerted effort is required to standardize preservation and propagation methods. It is essential to safeguard this iconic species from the current local and global stressors and to lock-

in the many benefits it offers for sustainable aquaculture.

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Authors' contribution

CDH and MJD proposed the idea behind this study. DML and MJD wrote most parts of the manuscript as well as produced all the figures. However, all authors contributed critically to the draft with valuable inputs and experience. All authors read and gave final approval for publication.

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