Deakin Marine
RESEARCH AND INNOVATION CENTRE

Cultivation handbook for *Ecklonia radiata*

Port Phillip Bay

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Summary

This Handbook was produced as part of The Port Phillip Bay Golden Kelp Restoration Project (the Project). The Project is a collaboration between The University of Melbourne, The Nature Conservancy, Parks Victoria and Deakin University, with guidance from Blue Conservation Services, and funded by the Victorian Government's Department of Energy, Environment and Climate Action. The Project trialled and developed techniques to address the loss of Golden Kelp (*Ecklonia radiata*) in Port Phillip Bay (the Bay). Spanning across two years from 2023 to 2024, researchers at Deakin University led the kelp cultivation for the Project, with findings informing this Handbook.

Reproductive material from healthy *Ecklonia radiata* populations in the Bay were collected, fertilised, and cultivated in laboratory settings. Juvenile sporophytes grown on twine and gravel were then outplanted into urchin barrens across three locations - Williamstown, Jawbone Marine Sanctuary, and Ricketts Point Marine Sanctuary – over two phases (experimental and restoration scale), covering an area of 0.5 hectares. Twelve months post the initial experimental phase of outplanting, the cultivated kelp were found with their holdfasts naturally attaching to the reef, standing at approximately 20 cm tall with reproductive tissue starting to form.

This Handbook integrates existing knowledge with new insights gained from pilot studies and experimentation in both laboratory and field settings. It is designed as a practical guide to assist individuals and organisations in cultivating *E. radiata* for research, restoration and/or aquaculture purposes.

For any words that may be unfamiliar, please refer to the Glossary on page 32.

Image: Scott Breschkin, The Nature Conservancy

Acknowledgements

We acknowledge Aboriginal people as Australia's First Peoples and as the Traditional Owners and custodians of the land and water on which we rely. In particular, the Wadawurrung and Bunurong people, custodians of the land and waters of Port Phillip Bay, and pay respect to their Elders past, present, and emerging. We acknowledge that Aboriginal and Torres Strait Islander peoples hold extensive traditional knowledge about seaweeds and their use, which has been practiced for thousands of generations.

This handbook is being delivered as part of The Port Phillip Bay Golden Kelp Restoration Project (the Project). Funding was provided by the Victorian Government Department of Energy, Environment and Climate Action (DEECA). We express our thanks to our collaborators on the project, including The Nature Conservancy (TNC), Parks Victoria, and the University of Melbourne. We also thank Kaylah Gawne, Caitlin Aedy, Sam Wines, Tess Hoinville, Lisa Grubb, Josh McIntyre, Natasha Tropeano and Paul Tinkler who assisted in the laboratory and/or field. We thank Professor Stephen Swearer and Michael Sams for their time reviewing this publication.

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Introduction

The Great Southern Reef (GSR) is a system of interconnected temperate reefs that span the southern coasts of Australia for more than 8,000 km, from as far north as Kalbarri in Western Australia, along the southern coastline to Tasmania and up to the subtropical waters of northern New South Wales (Bennett et al., 2015). The GSR is home to an abundance of endemic species and in the shallower waters is characterised by kelp forests (Bennett et al., 2015). The most common and abundant kelp species across the entirety of the GSR is the Golden Kelp, *Ecklonia radiata*. As a foundational species, it supports high biodiversity, providing critical habitat and food sources for numerous marine species, from fish to invertebrates. In addition to being important for biodiversity, kelp forests enhance and contribute to coastal fisheries, play a critical role in coastal carbon cycling, help reduce nutrient pollution in coastal waters and release oxygen, providing an estimated $11 billion per annum in ecosystem services along the GSR (Eger et al., 2023).

Ecklonia radiata grows natively on rocky reefs in Port Phillip Bay, Victoria and across Australia at depths up to 60 m. It is also found in New Zealand and in several regions around Africa. It has a wide thermal tolerance and occurs in waters ranging from 7° C to 26° C (Britton et al., 2024), with a recorded optimal temperature range for growth and reproduction between 12° C and 20° C (Veenhof et al., 2023).

Ecklonia radiata is a Laminarian kelp, which has a diplohaplontic life cycle, with macroscopic sporophytes followed by a microscopic gametophyte phase with an oogamous mode of sexual reproduction (Baweja et al., 2016). The mature sporophyte produces sorus tissue, which is the reproductive material that releases zoospores. The zoospores then divide asexually and become gametophytes. The microscopic gametophytes are male and female and reproduce sexually via sperm and egg. The fertilised egg then germinates into a juvenile sporophyte, where the life cycle continues, as shown in Figure 1 below.

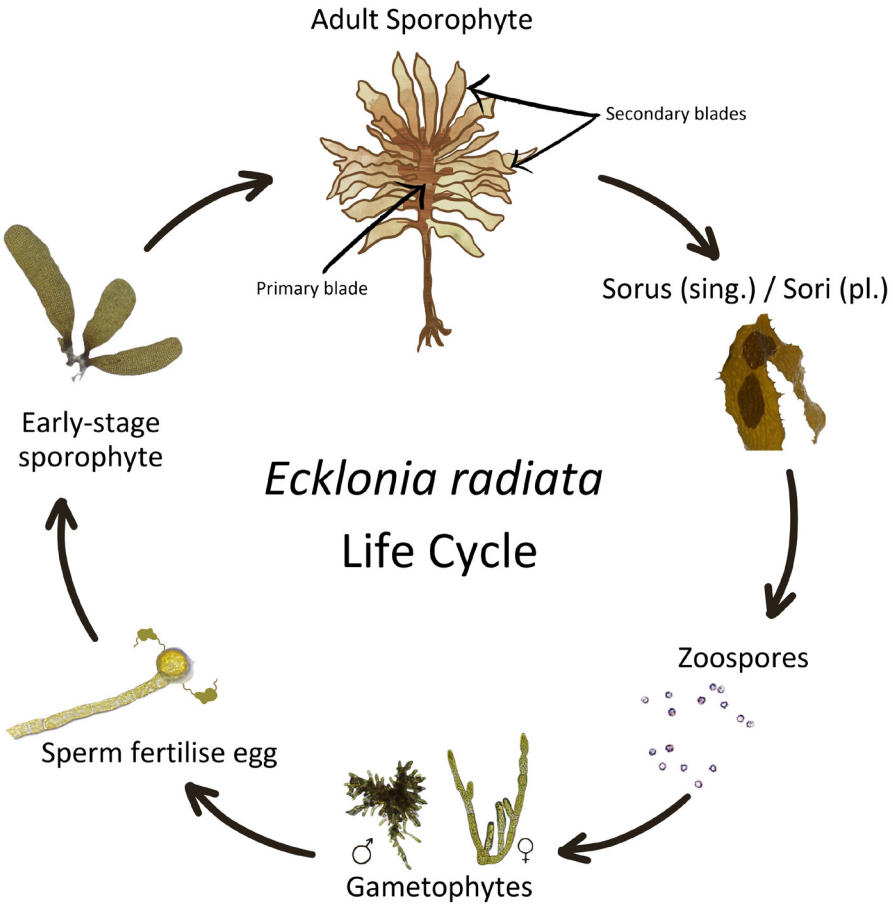

Figure 1. Life cycle of *Ecklonia radiata* (Credit: Kaylah Gawne).

Ecklonia radiata is declining across many areas within its range due to multiple, location-specific stressors. In its northern range in Western Australia, a decline occurred due to a marine heatwave and increasing water temperatures influenced by climate change, such that reefs over ~100 km experienced the complete loss of *E. radiata* (Wernberg et al., 2016). In Port Phillip Bay, there has been significant decline in multiple reefs and marine sanctuaries due to an overabundance of a native sea urchin, *Heliocidaris erthyrogramma* (Short-spined sea urchin or purple sea urchin, (Carnell & Keough, 2019; Ling et al., 2019). The overabundance of these sea urchins sparked changes in their usual feeding behaviours, where they changed from feeding on drift algae to actively feeding on live kelp and many other seaweeds (Kriegisch et al., 2019). Under healthy conditions, these urchins usually occur at densities of 2-3 urchins/m^2. Since 2012, however, certain areas of the Bay have experienced a surge, with densities now exceeding 60 urchins/m^2 (Ling et al., 2019), the highest record of this species in Australia. In high numbers these urchins can completely clear patches of reef resulting in barren conditions (see Figure 2) where biodiversity is severely affected, and without intervention these barrens often persist (Carnell & Keough, 2016).

With the decline of kelp forests in Australia, there is a growing focus on kelp forest restoration, including sea urchin removal where relevant (Layton et al., 2020; Tracey et al., 2015). Reducing sea urchin densities can often lead to successful reef/kelp restoration on its own (Carnell & Keough, 2016; Ling, 2008; Ling et al., 2010), however active kelp restoration actions are sometimes needed to effectively restore reefs (Layton et al., 2020). Common active restoration methods include transplanting adult plants (Campbell et al., 2014; Layton et al., 2021) or seeding kelp gametophytes or sporophytes onto rope or gravel for outplanting in areas requiring restoration (Wood et al., 2024). While these approaches have shown success (Layton et al., 2020), kelp cultivation methods are often tailored to specific species and regional conditions. The cultivation of Laminarian kelps, like sugar kelp (*Saccharina latissimi*) and wakame (*Undaria pinnatifida*), is well-documented in many regions, yet comparatively few studies focus on *E. radiata* (some examples include Praegar et al., 2022; Schwoerbel et al., 2022; Suebsanguan et al., 2021; Tatsumi et al., 2022; Visch et al., 2024). We have adapted methods from these studies as well as cultivation techniques for other Laminarian species in the context of the Project.

Image: Jasmine Bursic, Deakin University

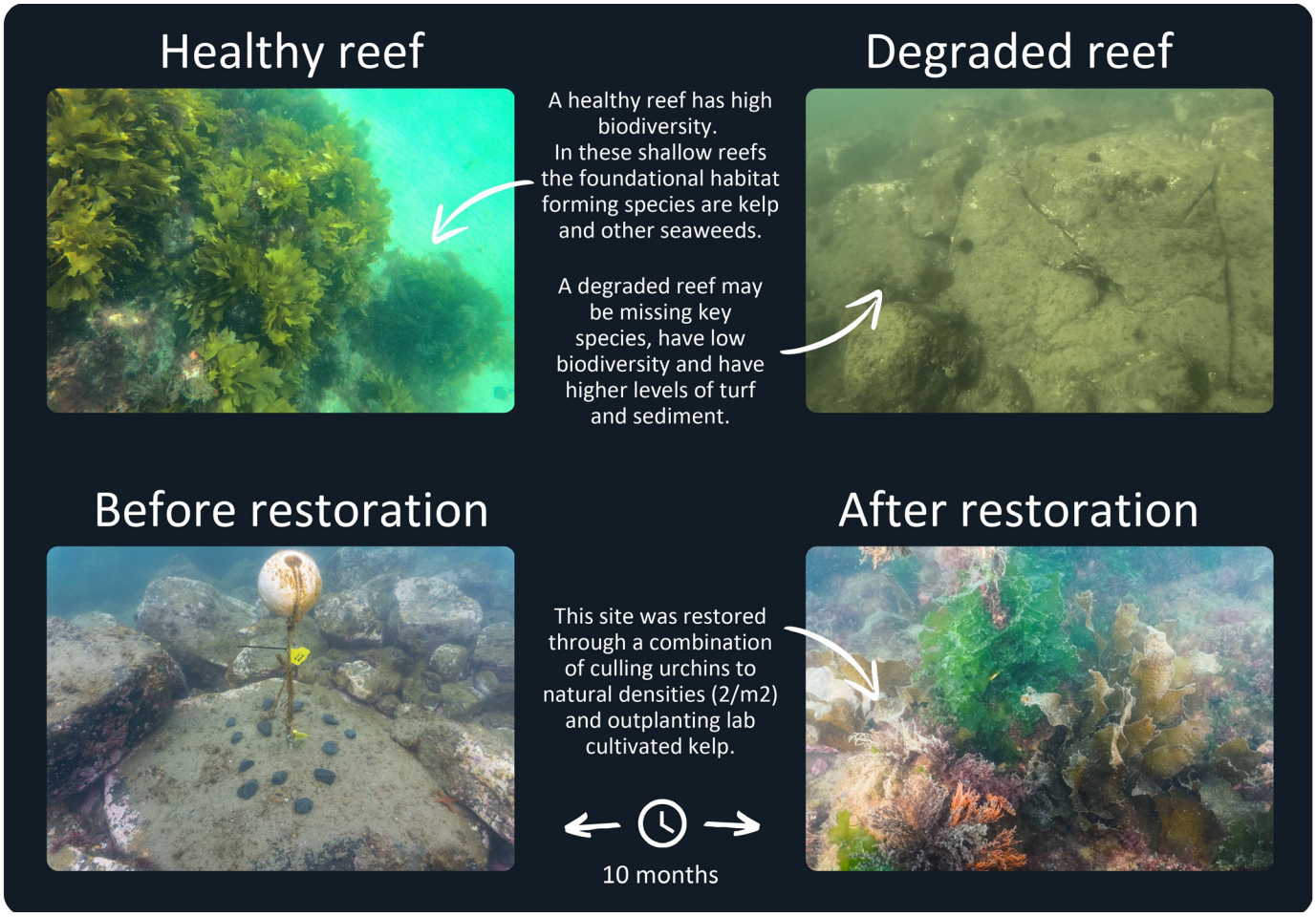

Figure 2. **Images of reefs in Port Phillip Bay. Top row depicting healthy reef (St Leonards) vs. degraded reef (Jawbone Marine Sanctuary) (Credit: Jasmine Bursic, Deakin University). Bottom row showing effects of restoration trials for The Port Phillip Bay Golden Kelp Restoration Project, both images at Jawbone Marine Sanctuary (Credit: Scott Breschkin, TNC) taken 10 months apart.**

The Port Phillip Bay Golden Kelp Restoration Project (the Project) aimed to trial and develop techniques to address the loss of Golden Kelp (*Ecklonia radiata*) and other macroalgae that form kelp forest habitats in Port Phillip Bay. Restoration methods were piloted, and ongoing monitoring of the success will determine the approach for future restoration attempts. Initially, urchin culling was employed as a restoration method to reduce numbers to healthy levels, alleviating grazing pressure and allowing natural recruitment of kelp and seaweed to regenerate on the reefs (Layton et al., 2020). The Project also trialled active restoration methods, including transplanting adult kelp from healthy donor sites to degraded areas, and cultivating juvenile sporophytes under laboratory conditions for outplanting on the reef. Juvenile sporophytes were cultivated on biodegradable twine and gravel (green gravel method, see Fredriksen et al., 2020), though cultivated kelp can grow on various substrates depending on site-specific conditions; further details on substrate selection are provided in the Seeding subsection in the Methods.

Methods

This Cultivation Handbook of *Ecklonia radiata* for Port Phillip Bay serves as a guide for the process and method of cultivating *E. radiata* under laboratory conditions for either restoration or aquaculture applications. Additionally, this handbook can assist future efforts in developing kelp hatcheries, providing a foundational resource for scalable kelp cultivation and contributing to sustainable marine practices. We recognise there are a myriad of ways and methods of cultivation, which may provide similar or very different results, and therefore we do not provide a specific recipe but a range to which you can experiment and adapt to suit your setup, facilities and budget.

Seaweed cultivation in Australia is still in its infancy, with each species requiring different conditions for optimal growth (Visch et al., 2023). Methods for cultivating kelp, including *E. radiata*, have largely been adapted from overseas, where other Laminarian kelp species have been farmed at large scales for many years. While various trials and experiments of the cultivation process of *E. radiata* have been published in Australia (see References and Further Reading), there remain knowledge gaps in best-practice methods, as not all stages of the process have undergone rigorous experimental testing. Our methods below combine existing knowledge with our own trialled techniques developed specifically for this Project. We recommend trialling different methods on a small scale to identify what works best for your setup and kelp seedstock. The following guidelines outline variables that can be adjusted to suit your resources and budget.

Setup & Equipment

Equipment & Consumables

Equipment can vary depending on the size and scale of your cultivation setup. In Table 1, we have listed our recommended equipment along with some alternatives. Additional general laboratory equipment and a list of laboratory consumables can be found in Table 2. While not exhaustive, these lists cover most of the specialised equipment essential or beneficial for successful cultivation.

A list of chemicals is provided in the Troubleshooting tips section to help address any contamination of the kelp cultures – a common challenge in kelp cultivation.

Table 1. A list of recommended equipment for the cultivation of *Ecklonia radiata*. For some equipment there may be alternative options, which are listed with a key, *equal alternative, **reasonable alternative, ***less favourable.

Recommended Equipment	Use	Stage of cultivation	Pros	Cons	Alternative
Laminar flow cabinet with UV light	A clean working space	All stages	Can eliminate airborne contamination when working with zoospores, cultures, and seeding. Can use to dry decontaminated equipment.	Cost, space	Biosafety or PCR cabinet*, cleanroom***
Incubator	House cultures, and cultivate on small scale	All stages	Temperature controlled, light programmed, stable environment	Cost	Temperature controlled room*, fridge**
Lights	Kelp requires specific light for growth	All stages. Red LED lights for gametophyte cultures. Broad spectrum/ white lights for cultivation.	Necessity. Light type can vary and will depend on your budget. From cheap LED to purpose specific aquarium lights.	More costly lights may produce better results due to spectrum emitted	Cheap LED lights with socket timer**

Autoclave	Sterilises equipment and water	All stages	Highly effective at eliminating possible contaminants	Cost, space	For equipment - decontaminate with chemicals** For water - use filters for water**, UV**, tyndallisation*, pasteurisation*
Seawater filtration unit	Physical filtration of particles and organisms from seawater supply. Level of filtration depends on filters (≤1 µm pore size recommended).	All stages	Cleans water, reduces contamination	Slows water flow	If no natural seawater supply, can create artificial seawater with sterilised freshwater and salt and chemicals**. Preferable to still do some physical filtration.
UV water steriliser	To UV sterilise water after filtration	All stages	Kills most bacteria, viruses and algae that pass through the filter. Combination of high-quality physical filtration and UV sterilisation can be enough - eliminates need to autoclave seawater for higher volumes.	Not 100% effective, and effectiveness depends on having appropriate size for flow of water	Autoclave or other method for cleaning water*
Distillation device	To distil tap water. Used to make nutrient media and chemical treatment concentrates. Used to 'top-up' cultures to adjust for evaporation and/ or reduce salinity in cultures.	All stages	Clean, does not have minerals. We recommend sterilising this water before use.	None	Purchase distilled water*. There are a range of devices that can be used with varying price.
Temperature controlled room (12-20° C)	To grow kelp, important to keep temperature stable	Cultivation stage or all stages	Keeps temperature stable, if room is controlled have more space to work with to grow larger quantities	May be limited by space or building capabilities	Aquarium style set up with water baths and chillers for temperature control*
Quantum light sensor	To help set lights on appropriate light intensity	All stages	Can accurately set light intensity to optimal range for growth	Meter can be costly depending on type/brand. But relatively wide range of brands/types with varying prices.	None
Air pumps with filters	To provide air to growing kelp and cultures	All stages	Provides air supply and flow to growing kelp	Can contaminate water - to limit this use 0.22 µm and/or 0.4 µm hydrophobic syringe filters	Air compressor**

Compound microscope (ideally with camera)	To count densities of zoospores and gametophytes for seeding. To assess contamination and growth.	All stages, but particularly prior to seeding	Can see zoospores, gametophytes, and contamination. Allows for density counts.	Cost	None
Finnpipettes (2-20 µl, 20-100 µl, 100-1000 µl) and tips	For accurate quantities of nutrients, chemicals, spore solution and gametophytes	All stages	Tips can be sterilised to limit contamination. Ensures correct dosage is always provided.	Cost	Pasteur pipette***
Analytical balance (0.001 g)	For weighing nutrient media and chemical powders	All stages	Accurate quantities for correct dosing	Higher cost for more precision	Lower precision balance (0.01 g)**
Refractometer	To measure salinity in seawater	All stages, check salinity in seawater and flasks	Can maintain optimal salinity level for kelp	None	None
Sedgwick rafter	To calculate approximate densities of zoospores and gametophytes	Spore release and seeding	Easy to calculate	None	Haemocytometer*
Beakers (borosilicate)	For measuring liquids	Spore release, seeding, culture tending	Can be sterilised with autoclave and UV. Can be sterilised before use to minimise contamination.	Breakable	Plastic***
Chemical fridge (4° C)	Long term storage of gametophytes (seedbank)	Gametophyte	Can keep gametophytes indefinitely for future use	None. But not needed if not keeping a seedbank.	Cool room*. Temperature must be stable and have very low red light on 12:12 cycle.
Flat bottom round boiling flasks with rubber stoppers and glass tubing	For gametophyte cultures	Bulking of gametophytes	Reduces need to continually collect reproductive material from field sites. Preferred shape.	Cost	Round bottom boiling flasks with stand*, Erlenmeyer flasks***. Parafilm instead of stoppers***

Table 2. A list of extra equipment and consumables for the cultivation of *Ecklonia radiata,* including extras for transporting kelp to site for deployment/outplanting.

Item	Use	Stage of cultivation
Dissection kit (scissors, forceps)	Cutting reproductive material, cleaning sori	Zoospore release
Jugs, large autoclavable bottles, Schott bottles	For decanting, transporting, sterilising and storing liquids including culture medium, nutrient media, seawater and distilled/deionised water	All stages
Funnels	To assist with decanting liquids where necessary	All stages
Hose, silicone airline	For airline	All stages
Plastic tubs with lids / tanks	Used for growing kelp, size dependent on substrate	Cultivation, cultures
Esky, ice blocks / Nally bins	To keep kelp cool during transport to site	Outplanting
Rags, hessian	Wet rags can be placed onto green gravel during transport to site, and wet hessian can be placed over tubs to keep cool during transport to site	Outplanting
Paper towel & lint-free tissues	Cleaning sori, cleaning lab surfaces, cleaning microscope, air outflow 'filter'	All stages
Sterile syringe filters, hydrophobic PTFE, 0.22-0.45 µm, 25 mm width	To filter air going into cultivation systems and cultures	Cultivation, cultures
Luer Lock syringes, 10 cc & 50 cc	Used for dosing nutrients, seeding gravel, and as air outflow 'filter'	Cultivation, cultures
Parafilm	Sealing flasks	Cultures
Foil	Autoclaving	All stages
Gloves	PPE	All stages

Cultivation systems

There are a variety of different systems that can be used to cultivate kelp including closed systems, recirculation and flow-through systems, all of which have their advantages and disadvantages, see Table 3. The choice of the system will depend on facilities and budget. We recommend growing kelp in contained, closed systems to minimise external contamination, and prevent cross-contamination that could jeopardise the entire batch. The closed system method was used for the Project, cultivating kelp in enclosed plastic containers immersed in temperature-controlled water baths with aeration added for flow (see Figure 4), at the final stage before outplanting some of the kelp was moved to flow-through system due to delays in outplanting. Regular changes of water are required for the closed system, as detailed in the Cultivation method section below.

Cleanliness, temperature, and light (in that order) remain the most important variables for successful cultivation. To support the growth of *E. radiata*, and avoid contamination, maintaining the cleanest possible environment within the available scope and budget is recommended. Contamination from microbes, microalgae, and/or protozoans poses the greatest impediment to successful cultivation; limiting these contaminants can improve outcomes. Below are various options to help establish a clean cultivation environment, which can be adjusted according to budget and space availability.

Maintaining a stable temperature is also essential, as described in Table 1. This can be achieved through a temperature-controlled space, such as a laboratory, shipping container, or airconditioned room. If you are unable to control the temperature in an entire room, you can use water baths with the aid of a chiller or heater to maintain desired temperature. Incubators are also useful for small scale cultivation, experimental trials and cultures.

Table 3. A list of types of cultivation systems for seeded kelp onto gravel and twine (spools).

System	Setup/requirements	Pros	Cons
Closed system	Individual tanks/tubs of chosen size. Incubator or temperature-controlled room or water baths with chiller/heater for temperature control.	Highly controlled system, resulting in more consistent results due to optimisation. Reduces risk of contamination. Almost eliminates risk of cross-contamination, can isolate contaminated batches and treat individually or remove without having to treat whole system or lose all kelp. Have greater control over nutrient dosing.	Requires some manual labour for regular water changes. Slightly larger footprint of space required than other systems, or less kelp in same footprint. Low flow, with waterflow coming from air supply, however wave makers can be used if requiring more flow. Kelp can have limited life span (~10-12 weeks) in closed system, must be outplanted before die-off.
Recirculating system	Water recirculating throughout system. In-line UV recommended to minimise contamination. Temperature-controlled room or chiller/heater needed for temperature control of water.	Can grow more kelp in same space. Slightly less labour intensive for water change.	Likely to become contaminated. And when contaminated, entire batch will be contaminated. Risk of losing entire batch. If needing to treat contamination will be more costly, as larger volume of water to be treated. Still requires water change and nutrient dosing, harder to control nutrient levels. Will require more nutrients due to increased water volume.
Flow-through system	Need access to flowing natural seawater. Filtration system and in-line UV to clean incoming water to limit contamination.	Can grow more kelp in same space. Reduced labour time in water changes and preparation of clean water. If kelp do grow successfully, they may grow much larger due to increased flow rates.	No control over temperature. Very low control over nutrients. Highly likely to become very contaminated, and all kelp can be outcompeted which can result in stunted growth, low overall output or even kelp death. Loss of entire batch highly possible. Not recommended due to many variables being uncontrollable which may result in low output.
Mixed system	Using closed system from seeding to early sporophyte stage, then once sporophytes have developed to approximately 2-3 mm they can be moved into flowthrough system before outplanting.	This can reduce labour due to less water changes. Can improve growth of kelp due to more space and waterflow, if conditions are suitable (temperature, light, nutrients).	High risk of contamination, however kelp may be large enough to withstand and outcompete. No control over temperature and nutrients once in flowthrough, depending on ambient conditions this may affect the growth/health of the kelp.

Lighting

Having appropriate, effective, and reliable lighting is also important in successful cultivation. There are two components to cultivating kelp which require different light regimes. One component is the maintenance of gametophyte cultures which are housed in red light, and the other component is growing sporophytes, which is primarily achieved with white light. Artificial light from LED lighting is recommended over natural light, as you have greater control over the light intensity and light period.

Ecklonia radiata can be successfully cultivated under affordable LED lighting; however, investing in higher-quality lighting that mimics the natural marine spectra may achieve improved results. In the absence of marine spectrum lights, white light has shown effective results for growing sporophytes in other species (e.g. *Undaria pinnatifida* (Takahide et al., 2017), *Saccharina latissima* (Ebbing et al., 2020); *Sargassum fusiforme* (Huang et al., 2021)); as it emits the full range of wavelengths simultaneously.

Maintaining the correct light intensity is essential for any lighting setup. We recommend using a quantum light meter to measure and adjust the intensity as needed. For growing sporophytes from

seeded zoospores, it's optimal to start with a low light intensity of around 10 µmol m^{-2} s^{-1} for the first week; after this time, light can be increased to approximately 40-60 µmol m^{-2} s^{-1}. Kelp cultivation can be adapted to various light cycles as noted by Praegar et al., (2022). We used a 12:12 hour day:night cycle, however a 16:8 day:night cycle is also commonly used. Light positioning is important and can influence growth; overhead lighting is ideal for gravel setups, whereas vertical or multidirectional lighting suits spool setups. Combining vertical and overhead lights along with regular spool rotation can improve growth outcomes. Using reflective materials such as white corflute or foil helps to diffuse light and reduce shadowing. Regular repositioning of substrates is also beneficial for achieving uniform growth.

Red light is appropriate for housing gametophyte cultures, as the energy output is very low which prevents transitioning into the sporophyte life-stage, and thus also allowing you to maintain long term cultures of vegetatively growing gametophytes. Light intensity should be approximately 15-20 µmol m^{-2} s^{-1} to enable vegetative growth, on a 12:12 hour day:night cycle. We recommend using multidirectional light positioning for gametophyte cultures.

Nutrient media & Culture medium

Kelp requires nutrients for growth, and while natural seawater contains some essential nutrients, supplementing with additional nutrients can promote optimal reproduction and growth. Different species have different nutrient requirements, with *E. radiata* having a wide tolerance of nutrient levels (Blain & Shears, 2020). Therefore, nutrient levels can be adjusted to suit specific setups; for instance, in very large water volumes, it may be cost-prohibited to use the standard aquaculture dosage, so reduced amounts may be preferred. In flow-through systems, additional nutrients might be unnecessary depending on the nutrient levels in the ambient seawater supplied. As we recommend using closed systems, we suggest using the standard aquaculture dosage of F/2 strength, based on Guillard F/2 medium (Guillard & Ryther, 1962). Nutrients are typically supplied as a highly concentrated powder, which can be mixed into a concentrated liquid nutrient solution according manufacturer instructions. This solution is then added to clean seawater to create the culture medium.

Culture medium is the term used for the nutrient dosed seawater that is added to your cultivation system to grow kelp. It is recommended to clean seawater as much as practical (e.g. through physical filtration, UV treatment and/or autoclaving) according to the setup and volume required before adding nutrients. Once the culture medium is dosed with nutrients, it should be used immediately; storing nutrient-enriched seawater is not advised, as it may promote the growth of unwanted species if contaminated. Additionally, nutrients can settle during storage, which may lead to inaccurate or inconsistent dosages.

Image: Stefan Andrews, Great Southern Reef Foundation

Cultivation method

Spore release

To begin spore release, reproductive material from wild adult *E. radiata* must be collected. Local authority permits may be required for this process (e.g. Victorian Fisheries Authority, DEECA &/or Parks Victoria for Port Phillip Bay region). When selecting a site to collect reproductive tissue, we recommend liaising with relevant stakeholders first (e.g. local research organisations, Traditional Owners, community environmental groups, Councils), as factors such as environmental conditions, biodiversity, genetic diversity, cultural importance and futureproofing against climate change pressures may need to be considered. Only small 'leaf clippings' containing reproductive tissue are needed; collect only this tissue, not the entire kelp, to minimise disturbance to wild populations. The reproductive tissue, called sorus (sing.) or sori (pl.), can generally be found on mature *E. radiata* on the primary blade (central laminar) and/or on the secondary blades (laterals) closest to the primary blade (see Figure 1 for diagram). Collection of sori is best achieved while snorkelling or scuba diving, in accordance with relevant organisational diving safety and training policies. Sori may sometimes be found in beach wrack on freshly washed-up adult *E. radiata* (i.e. ideally only a few hours old and still moist and healthy), which can be an alternative if underwater collection is not possible – though sori in good condition are harder to find from beach-cast kelp. Once located on adult kelp, sori tissue can be cut using a dive knife or scissors and placed in a catch-bag. Avoid collecting any sori with fouling where possible, though minor fouling can be removed in the laboratory during the cleaning process (see below). Figure 3 provides a visual guide for identifying mature, healthy, and reproductive sori.

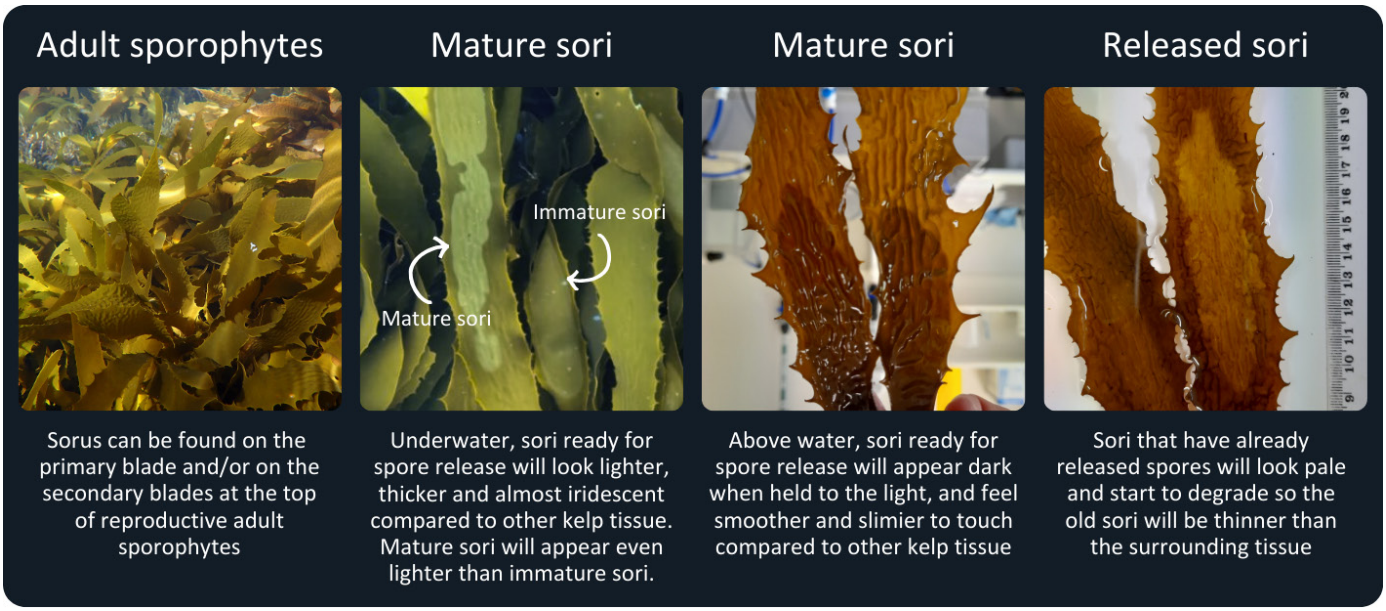

Figure 3. Images showing how to identify reproductive material of *Ecklonia radiata* (Image credit: left to right, 1. Jacqui Pocklington, 2-4. Jasmine Bursic).

You can cultivate kelp from one individual reproductive adult, but for genetic diversity we recommend choosing sori from at least 10 different individuals, growing at a minimum ~5 m apart, with one piece of mature sori per individual. Once sori has been collected, it can be transported in an esky to the laboratory for cleaning (alternative transporting options discussed in Suebsanguan et al., 2021). The process used, from preparing sori to calculating spore release densities, is detailed below and shown in Figure 4.

Steps for spore release:

1. In advance, prepare by sterilising seawater and all equipment needed (scissors, forceps, beakers, pipette tips, flasks etc).
2. Cut around and trim excess tissue (non-reproductive tissue) from samples, keeping only the healthy sori tissue. If there is any visible fouling (e.g. bryozoans, filamentous algae) use a razor blade or spatula, to gently scrape the sample and remove.
3. Dip each sori individually using forceps into a series of three large beakers filled with; 1. Autoclaved filtered seawater, 2. Iodine solution (5% liquid betadine, autoclaved filtered seawater), 3. Autoclaved filtered seawater.
4. After dipping, dry sori with clean paper towel, rubbing gently to ensure any remaining fouling or contaminants are removed as much as possible.
5. Once dried, repeat the dipping and drying process two more times (totalling three times).
6. Wrap each dried sorus tissue in a piece of clean and dry paper towel.
7. Once all sori have been cleaned, dried, and wrapped, place them in an incubator or refrigerator at a temperature of 12-17° C for two to 10 hours to ensure gentle drying. Typically, samples are stored in this manner for about two hours before proceeding to the next step.
8. After a few hours of gentle drying, work in a laminar flow cabinet to place the dried sori into a medium-sized beaker containing approximately 400 ml of autoclaved filtered seawater, ensuring the sori are fully submerged. Leave the sori in the seawater for about 30 minutes to allow for the release of zoospores; however, the majority of spores are typically released within the first few minutes of submersion.
9. After your designated time has passed, remove the sori tissue from the beaker using forceps to stop further spore release. Alternatively, the liquid can be poured into another sterilised beaker to separate the sori.
10. Use a Finnpipette to sample 1000 µl of the zoospore solution for measuring zoospore density in a Sedgwick rafter. Note that this only provides an estimate of spore density within the specific spore solution. To calculate a properly standardised measure of zoospore density for comparisons across different projects, sites or time periods, it is necessary to adjust the initial density estimate based on both the amount of sori tissue (mm^2) used and the volume of seawater into which the spores were released in Step 8.
11. Count the spores using the Sedgwick rafter and calculate the zoospore density. This density will be used to determine the desired seeding density (see the Seeding substrates subsection).

Image: Jasmine Bursic, Deakin University

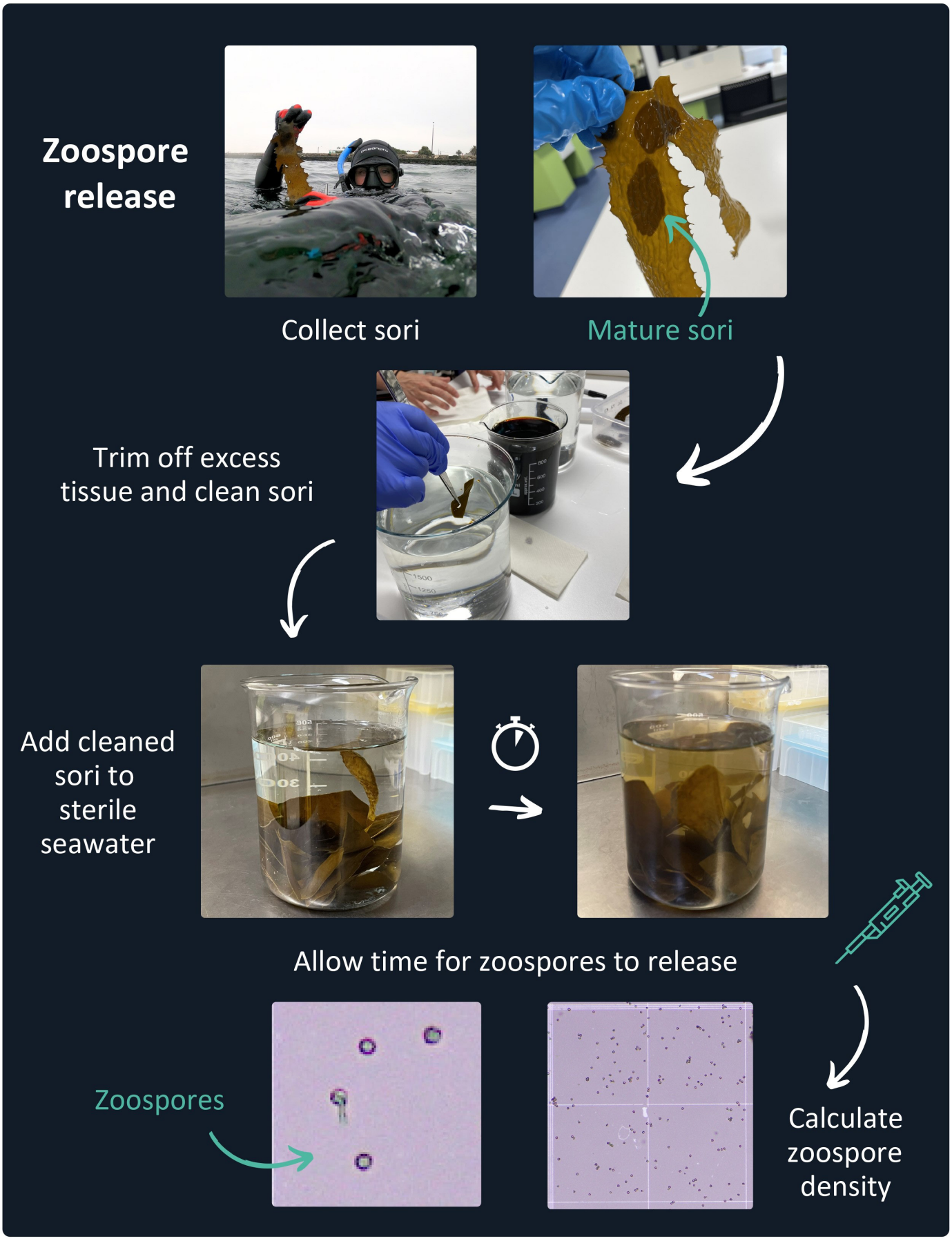

Figure 4. Infographic showing process of zoospore release for *Ecklonia radiata*.

Gametophyte cultures

Gametophyte cultures can be stored for long-term use but require regular maintenance. Utilising gametophyte cultures allows for scaling up or conducting seeding or cultivation at any time without the need to collect sori from the wild, which saves time and money while reducing potential environmental impacts on local populations. To create a gametophyte culture, add the zoospores to a flask of autoclaved filtered seawater with nutrients (we recommend standard aquaculture dilution of F/2; see Nutrient media and Culture medium subsection for more details), then add an airline and seal the flask. It is important to label the culture with the date and the location of adult donors.

In the wild, the gametophytes (<0.5 mm) are typically hidden in the macroalgal understory and can remain there alive for extended amounts of time (weeks, to maybe even years). Under low light conditions, they grow vegetatively and reproduce asexually. However, when conditions are right, and light levels are optimal, females produce eggs while males release sperm, leading to sexual fertilisation, where the fertilised egg develops into a sporophyte. These biological processes can be mimicked to maintain the cultures at the gametophyte stage and prevent sexual reproduction and fertilisation. To maintain this, cultures must be kept under low light, ideally housed in red light with an intensity of approximately 15-20 µmol m^{-2} s^{-1} on a 12:12 hour day:night cycle. A stable temperature is also crucial; successful results have been achieved by storing cultures sourced from Port Phillip Bay in an incubator at 16° C, though a temperature-controlled room can be effective as well.

Maintaining and bulking the cultures requires regular maintenance, including periodic changes of the culture medium. First, check the salinity of the culture medium and adjust it with deionised water to achieve a concentration of approximately 35 ppt. Allow the gametophytes to settle to the bottom of the flask before decanting approximately 50-70% of the culture medium from the top. Replace this with fresh culture medium dosed with nutrients at F/2 strength. This process should be repeated at least every few months, and for bulking, water changes can be increased to a monthly frequency.

To bulk the cultures and increase vegetative growth of the gametophytes, it is essential to allow room for growth. This can be achieved by periodically scraping the sides of the flasks with a sterilised silicone spatula, as the gametophytes tend to adhere to the sides, and/or by blending the cultures using a sterilised household kitchen stick blender for a maximum of 30 seconds. For more details on this method, refer to Visch et. al., (2024). This process can be performed during water change cycles to limit the time spent opening the culture flasks, which could lead to contamination. Once the cultures become dense, they can be split into additional flasks to provide more space for vegetative growth.

Maintaining clean cultures is crucial, as contamination can compromise the cultures, and the subsequent cultivation of kelp. All work with cultures should be conducted within a clean environment, such as a laminar flow cabinet, using only sterile or decontaminated equipment.

Additionally, a small sample of the cultures can be stored long-term under red light at very low intensity of ~5 µmol m^{-2} s^{-1}, on a 12:12 hour day:night cycle at 4° C. This sample can serve as a seedbank that can be bulked up again in future when needed (Layton & Johnson, 2021). This long-term storage method minimises the space required to house cultures.

Seeding

Once a zoospore solution is prepared, it can be seeded directly onto substrates (e.g., twine, gravel) (Figure 5). For large or repeated restoration planting or aquaculture efforts, it is highly recommended to invest time in creating a supply of high-quality gametophyte cultures that are contamination free (or low contamination at least) and have high biomass. Although bulking gametophyte cultures may take several months to produce enough material for seeding a substantial quantity of substrate, these cultures can be used continuously for seeding with some maintenance (see Gametophyte cultures subsection above).

Substrate selection remains an emerging area of research and should be tailored to the specific environmental conditions, setup, and budget. Two techniques trialled for *E. radiata* in the Project involved seeding onto twine-wrapped spools (using PVC pipe for the spool) or onto gravel using the green gravel method (Fredriksen et al., 2020). *Ecklonia radiata* can grow on a variety of surfaces, so innovative methods can be explored (see also differences in attachment between gravel surfaces in Alsuwaiyan et al., 2022).

Synthetic twines specifically designed for seaweed cultivation (e.g., Algatwine, Kuralon) offer increased resistance to contamination and enhanced durability in seawater. While synthetic twines can be beneficial in aquaculture for their durability and ease of removal during harvest, general-purpose synthetic options like braided nylon are used internationally as a more cost-effective alternative. However, these options are made from synthetic materials, such as polyvinyl, polyester, and nylon, which are not biodegradable and can contribute to marine debris. To minimise waste and reduce environmental impact, various natural and biodegradable twines are available that can be used for growing kelp. For the Project, unbleached cotton twine (3 mm) was utilised, yielding successful growth in laboratory and field trials. The use of cotton twine, which is biodegradable, aligns with efforts to reduce debris in the marine environment while still being durable enough to last at least 12 months, making it suitable for kelp attachment to the reef. However, it may be more susceptible to bacterial contamination, necessitating strict cleanliness protocols when used as a substrate.

The green gravel method (Fredriksen et al., 2020) was developed to streamline large-scale restoration compared to the twine method. This technique aims to reduce the need for in-water divers during deployment, since the green gravel can be deployed remotely from a boat. However, this method is still undergoing trials at scale (Wood et al., 2024). Remote deployment can be unpredictable and complicates post-deployment monitoring. Given the Project was trialling different methods, careful handling of gravel was prioritised, and deployment was performed using SCUBA or surface-supplied divers to ensure accurate placement on the reef. Direct placement ensured the sporophyte-facing gravel was placed upright, minimising damage to sporophytes during deployment.

The type and size of gravel should be determined by the environment in which the outplanting occurs. Ideally, gravel matching the natural substrate at the site should be chosen; for example, at Jawbone Marine Sanctuary, where the natural bedrock and boulders are basalt, basalt gravel were used for seeding and deployment. However, matching the existing reef can be challenging, as not all gravel types are suitable for cultivation. Many reefs in Port Phillip Bay consist of sandstone, which is too friable for effective use and difficult to source from suppliers. Limestone, another common reef substrate in Australia, is reactive, meaning it can alter the water chemistry. Trials in cultivation conditions have shown it to have harmful effects on juvenile kelp due to pH changes (Alsuwaiyan et al., 2022). Inert gravel types, such as basalt, granite, and quartz, are best suited for cultivation, as they do not alter the water chemistry. Sourcing gravel material can be challenging, as landscape suppliers often categorise gravel types by aesthetics rather than specifying their composition.

The size of the gravel should be aligned with the overall energy of the site; smaller gravel are suitable for low-energy environments (e.g., enclosed bays), while larger gravel/rocks may be necessary in high-energy areas (e.g., open coast) to prevent displacement. However, larger rocks have only been used with limited success and do detract from some of the potential benefits of small rock sizes, such as easier deployment (Earp et al., 2024). For this Project, basalt gravel approximately 40-60 mm in size were used. Finding the ideal size gravel or rocks involves balancing the risk of displacement by surge (too small) against the difficulty of handling larger rocks and the potential challenges of kelp growing their haptera (holdfast) onto the natural reef (too large). Trialling the green gravel method on a small-scale with a range of gravel sizes will be the best way to ensure success at the site. Further information regarding global initiatives and projects using the green gravel method can be found via the Green Gravel Action

Group website; www.greengravel.org (Wood et al., 2024).

Regardless of chosen seeding substrate, proper cleaning and decontamination are essential. Gravel should be rinsed and scrubbed as necessary, with each piece inspected to ensure no mud or debris remains. The gravel should then be soaked overnight in a Decon 90 solution (see Table 2) according to label instructions, and rinsed and soaked in freshwater for one hour before drying in a laminar flow cabinet. PVC pipes (used as spools for twine; see Figure 5) should be cleaned with soapy water then dried before wrapping with twine, ensuring that the twine is wrapped tightly with no overlaps, and ends secured with knots. Gloves should be worn during wrapping to avoid transferring oils from hands onto the twine. The wrapped spools should then be soaked overnight in a Decon 90 solution according to label instructions, then rinsed and soaked in freshwater for one hour before drying in a laminar flow cabinet. Once cleaned and dried, gravel and/or spools should be stored in a laminar flow hood before seeding to prevent recontamination. If a laminar flow hood is unavailable, they should be placed in sealed and covered containers (e.g., plastic tubs). To minimise recontamination, avoid handling substrates where possible. When handling spools for seeding, this should be done only inside a laminar flow hood, using clean gloves or clean hands. If ultraviolet light (UV) is available (e.g., in a laminar flow hood), it is recommended to UV sterilise the substrates directly before seeding. Additionally, cultivation tanks/tubs, and airlines should be decontaminated using the same method described above.

Once the substrate is prepared, seeding can occur either directly with zoospores or with gametophytes (Figure 5). Density of seeding is important and will influence the successful growth of sporophytes (Schwoerbel et al., 2022). In this trial, a seeding density of 10/mm^2 for zoospores on both substrates, and 100/mm^2 of gametophytes on gravel and 400/mm^2 for twine spools, was successful. We suggest trialling different densities to see what produces the best outcome for the specific context. A Sedgwick rafter can be used to calculate densities, providing an approximate density/ml. Blending gametophyte cultures for up to 30 seconds with a kitchen stick blender before counting and seeding is recommended. Blending facilitates the fragmentation of gametophytes, increasing the accuracy of density counts and reducing clumping, leading to more even distribution of sporophytes on the substrates.

Seeding spools (twine) is best achieved spraying seedstock (zoospores or gametophytes in autoclaved filtered seawater) directly onto dry spools (see Figure 4) using a household spray bottle. After application, allow around 15 minutes for the seedstock to settle before adding them to the culture medium. To allow time for the kelp to attach to the twine, leave the spools stagnant for 48 hours before introducing air.

Seeding green gravel requires adding the culture medium first to the gravel and then evenly pouring the seedstock into the growing container (see Figure 5). Note that if seeding with zoospores, they will attach to the gravel quickly (within ~30 minutes), and air can be added after 48 hours. If using gametophytes, they will not attach immediately and should not be disturbed for seven days to ensure attachment to the gravel. Premature disturbance may result in the loss of the gametophytes. Therefore we recommend waiting seven days before adding airflow, starting with gentle aeration (e.g., a flow rate sufficient to create small, consistent bubbles without dislodging gametophytes) and increasing after two weeks or when sporophytes are visible. The rate of airflow at this stage should be more than the initial flow rate, but not too excessive that sporophytes are dislodging from the substrate.

Growing containers/tanks

When selecting growing containers or tanks for kelp cultivation, consider the availability of supplies, setup specifics, restoration scale, and substrate type. Containers should ideally be clear plastic to ensure adequate light penetration, and appropriately sized for the setup and chosen substrate. For airtightness and to reduce contamination, drill holes in the lids for airline entry, ensuring a tight fit.

Gravel can be grown in shallow containers but

allow at least 1 cm of water above the gravel. To minimise handling, we recommend housing the gravel in a perforated tray (e.g., landscaping tray) inside the container, allowing for easy movement during water changes.

Twine spools can be cut to size to suit the container. We recommend cutting the spool as straight as possible and drilling two holes at each end of the pipe to hang the spools in the container. Alternatively, spools can be suspended in the water column by drilling holes into the containers and using 316 stainless steel rods or plastic rods with cable ties to hold the spools. This allows water to flow in and around the spools to avoid stagnation of water. Be mindful of light coverage, as overcrowding spools can lead to shading and lower growth. Regular rotation of spools can help to mitigate this; however, adjusting the spool density may be required to optimise light exposure.

Always ensure the gravel and spools are fully submerged in the culture medium, as the kelp will desiccate and die quickly. Airlines are used for providing oxygen however it also provides flow within the growing containers. Adjust airflow based on the age and stage of kelp, as described in the Seeding Section above. Airflow can be increased when sporophytes are visible to provide better water circulation and encourage stronger attachment.

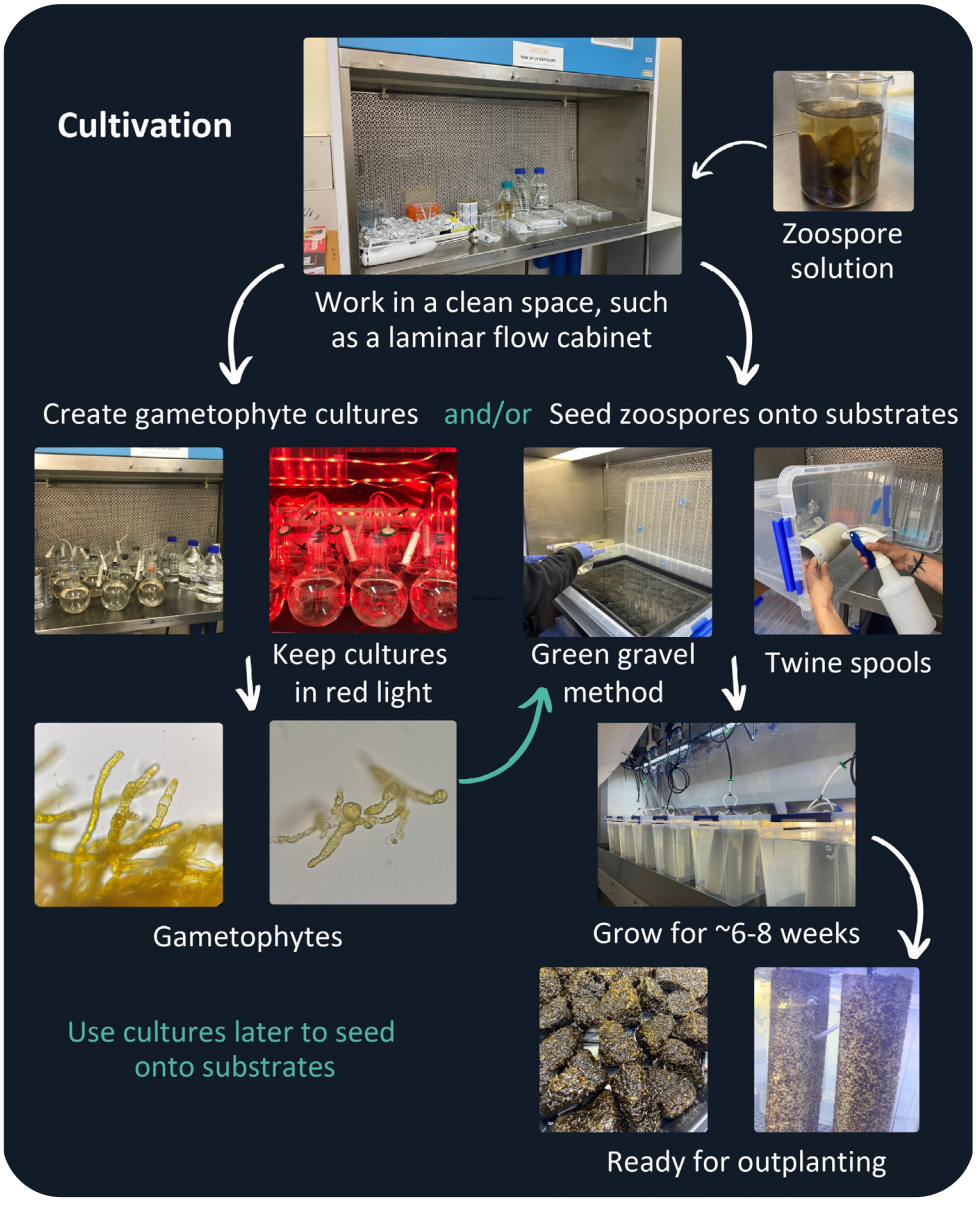

Figure 5. Infographic showing process of cultivation for *Ecklonia radiata.*

Maintenance of cultivars

Ecklonia radiata cultivation requires controlled conditions, with a carefully selected, stable temperature and a consistent light schedule. While they can grow under a variety of light schedules, the result may differ. Light is critical in kelp growth and getting it right will impact success. We grew our kelp under marine spectrum lights on a 12:12 hour day:night cycle, starting at ~10 µmol m^{-2} s^{-1} light intensity for one week, then increasing to ~40-60 µmol m^{-2} s^{-1} light intensity thereafter (see Praegar et al., 2022).

Temperature can be selected based on the natural range of the kelp but is best to match an average temperature for the local conditions (of both donor kelp and/or planned deployment area). We cultivated our kelp at 16° C with regular maintenance and monitoring. More information around temperature ranges can be found in Mabin et al., (2013).

The budget and setup may influence how water changes are conducted, so adapt the following guidelines as needed. Regular water changes, ideally once per week, are recommended with a 100% water change and nutrient dosing into clean seawater. A nutrient concentration of F/2 strength is recommended (see Nutrient media & culture medium subsection). Seawater should be as clean as possible, with the cleanest water filtered to 0.1 µm, autoclaved, and UV sterilised. If large volumes cannot be autoclaved, ensure that water filters are replaced according to the manufacturer's guidelines and that the UV system is suitable for the water flow rate. We also recommend when conducting water changes, that the cultivation container is changed to a freshly decontaminated container. Where possible conduct water changes within a laminar flow hood; if containers are too large, create a workflow that minimises the time spent with the containers opened. Clean working procedures should be maintained to reduce contamination risks. An efficient way to conduct a water change is to move the kelp (whether in a tray of gravel or on a rod of spools) into a clean and decontaminated container, then add the new culture medium. When placing spools into new tubs we also recommend rotating and flipping to ensure even distribution of light during the cultivation period. The kelp should not be left out of the water for longer than five minutes, as they will start to desiccate. Dispose of the old culture medium and decontaminate the used containers for the next water change. This method requires having double the quantity of containers for growing kelp for easy switching. In our experience, sporophytes will also settle on the culturing containers, so weekly container changes help reduce intraspecific competition for the kelp on the remaining substrates.

Kelp growth may initially seem slow since it cannot be observed with the naked eye while in the microscopic stage. Progress can be monitored by wrapping extra twine on the spools, which can be cut off to observe under a microscope. If seeding with zoospores, it may take three weeks before visible growth can be observed without the need of a microscope. Once the zoospores develop into gametophytes, they start to vegetatively reproduce into clusters, which become visible to the naked eye (Figure 6 & 7). At this stage, clusters may not be seen on submerged gravel, but during water changes, small lumps on the rock surface may be visible when removed from the water (Figure 7). A sample rock can be taken for close observation using a dissection microscope if desired. Sporophyte development generally begins around four to six weeks post-seeding, depending on the seeding method - approximately six weeks for zoospore seeding and around four weeks for gametophyte seeding.

Regularly check the airline filters are still functioning, if they become wet or blocked, airflow will be limited or halted, replace as necessary. We recommend using a minimum 0.4 µm PTFE hydrophobic syringe filter, but for extra precaution we run a series of two filters on a line, with 0.4 µm first, then 0.22 µm. This precaution minimises the risk of bacterial contamination from the air supply.

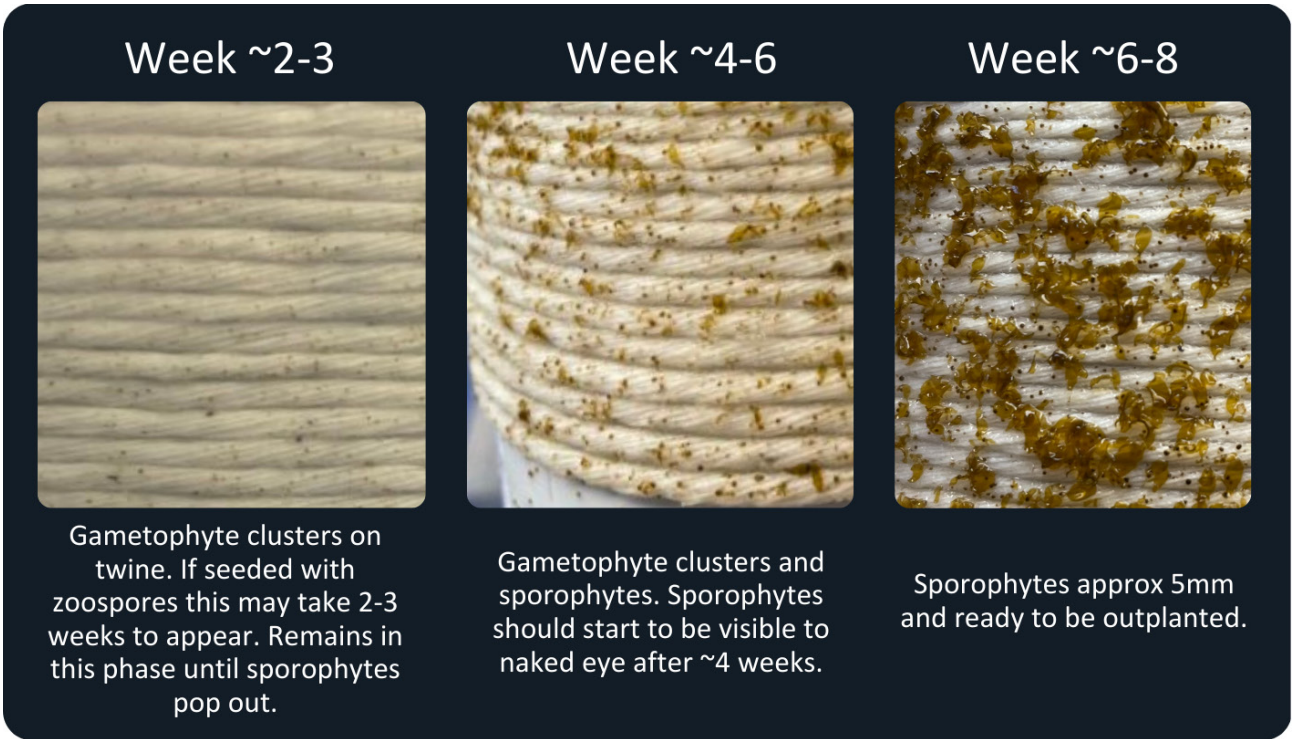

Figure 6. Images of twine seeded with kelp showing different stages of development during cultivation.

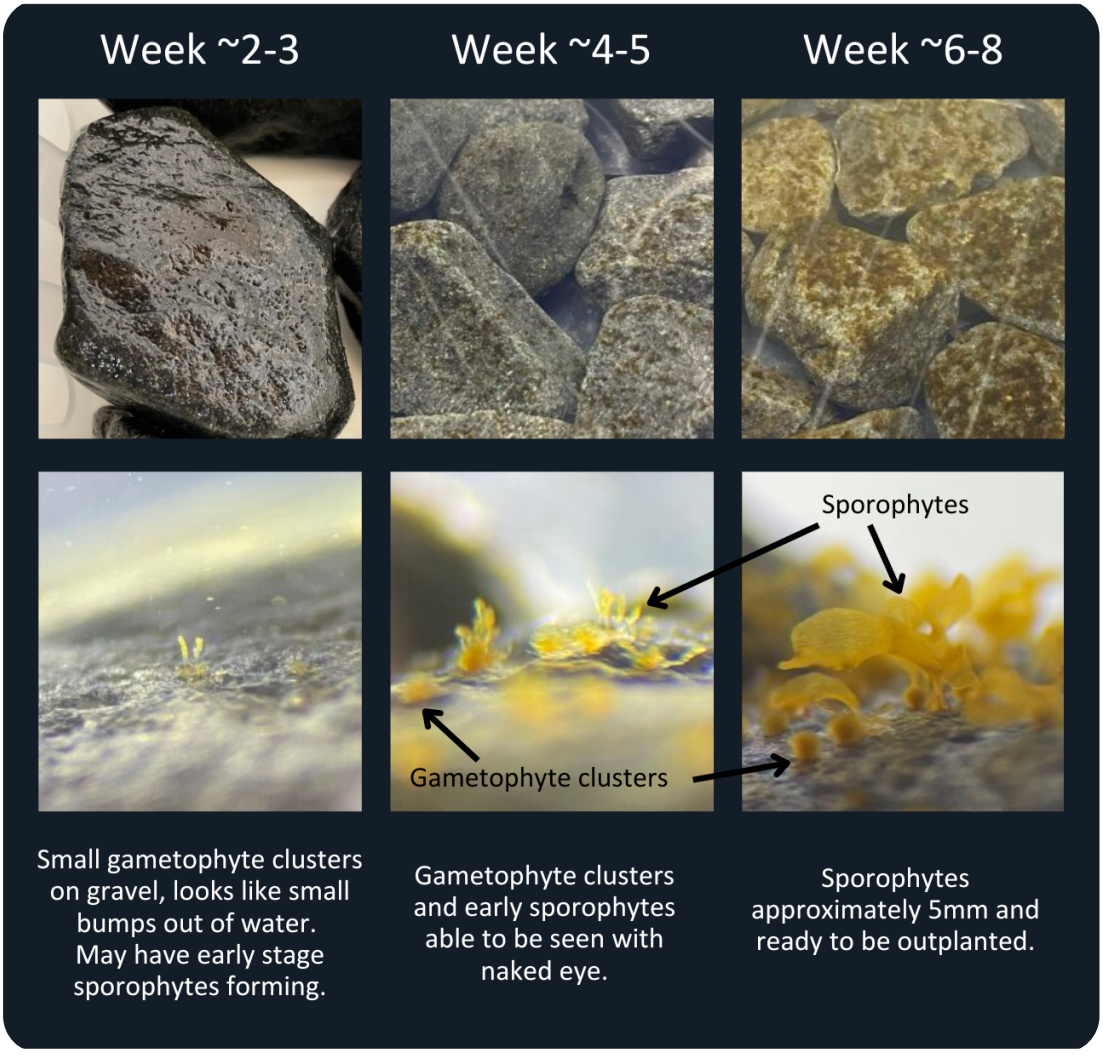

Figure 7. Images of green gravel seeded with kelp showing development during cultivation.

Outplanting

Sporophytes can be deployed into the field at any size; however we believe around 3-5 mm length is optimal (see Figures 6 & 7 for visual examples). Outplanting sporophytes that are too small, patchy, or early may limit their ability to compete for space in the field against faster-growing algae that could also colonise the substrate. Conversely, keeping sporophytes in laboratory conditions for extended periods, can also be detrimental to their success due to crowding, nutrient limitations, increased maintenance costs, and extended time requirements. As the sporophytes grow larger, they require more flow, space, and frequent nutrient dosing; if these needs are not met, they will start to deteriorate in health, often initially visible through pale spots or patches, eventually leading to die-off. Once they reach approximately 5 mm in size they can be outplanted; if delays arise, they can be placed into a flowthrough system, allowing for continued growth for a few weeks. Extended culture can weaken holdfast development and attachment strength, as laboratory conditions do not mimic the natural environment fully, which may lead to a large number of kelp detaching from the substrate during transport and outplanting.

In the Project it was shown that *Ecklonia radiata* sporophytes around 3-5 mm are sufficiently sized to outcompete other algae in the field, whilst remaining young enough to still be healthy for outplanting. Time taken to grow to this size will depend on variables such as light, temperature, nutrients, seeding density, and culture cleanliness. Generally, this requires approximately eight weeks when seeding from zoospores or six weeks if seeding from gametophytes.

Outplanting requires careful transport to the field (see Figures 8 and 9). During travel, temperature needs to be controlled to avoid the kelp becoming too warm (avoid temperatures above 20° C). We recommend using ice bricks and wet cloth/hessian and aligning outplanting in the cooler seasons. If outplanting during warm weather, either eskies (ice boxes), or temperature-controlled vehicles should be used for transport. If possible, keep the kelp at a similar temperature they were cultured at and/or will be outplanted to avoid temperature shock. A period of acclimatisation may be required. Spools can be transported inside larger PVC pipes with lids, filled with clean seawater inside an esky (see Figure 8). Transporting gravel is typically easier on trays without seawater, due to the size and weight of seawater-filled containers. Seawater-soaked rags can be placed over the kelp to keep wet and cool (see Figure 9). Directly before outplanting, remove rags gently to limit sporophyte damage and loss. It is best to transport gravel in trays and tubs, however for boat transport, adding wet hessian over trays or tubs helps keep them cool and limits sun exposure.

The method for outplanting will vary depending on substrate type, site variables, permit requirements, and budget. Substrate attachment may involve securing spools, or placing gravel directly on the seabed. Equipment deployed for outplanting, such as temporary anchors or supports, should be removed as soon as they are no longer required to reduce debris and waste in the marine environment. Where possible, natural (e.g., matching gravel to natural rock type) or biodegradable materials (e.g., cotton twine) are preferred to minimise environmental impact. For reference, see Figures 8 and 9 for transport and outplanting method.

Monitoring the success of outplanting is essential to assess survival, growth, and broader ecosystem benefits. Where possible, use photographs or video to document progress and compare changes over time. Monitoring should continue for at least 12 months or longer, depending on project objectives and funding. A general guideline for monitoring may include:

1. Initial assessment (after ~2 weeks): check for signs of survival and stress.
2. Growth monitoring (monthly if possible): measure sporophyte length, biomass, or density to track growth rates over time. Look for visible signs of holdfast attachment and reproductive sorus tissue development indicating maturity.
3. Long-term monitoring (quarterly): track kelp canopy development and expansion and assess broader ecosystem recovery, including associated biodiversity.

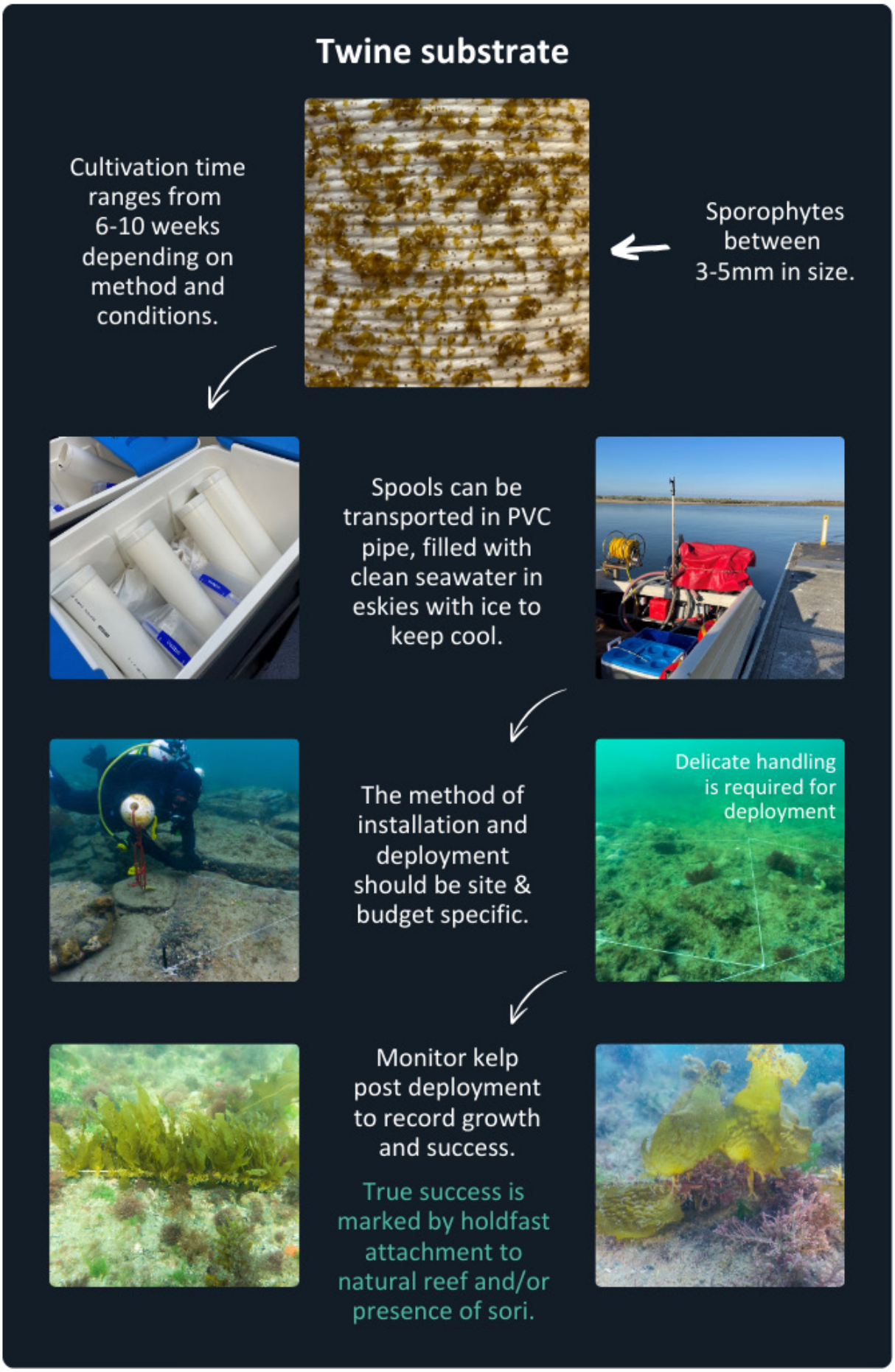

Figure 8. Infographic showing process of outplanting twine-seeded kelp for restoration purposes.

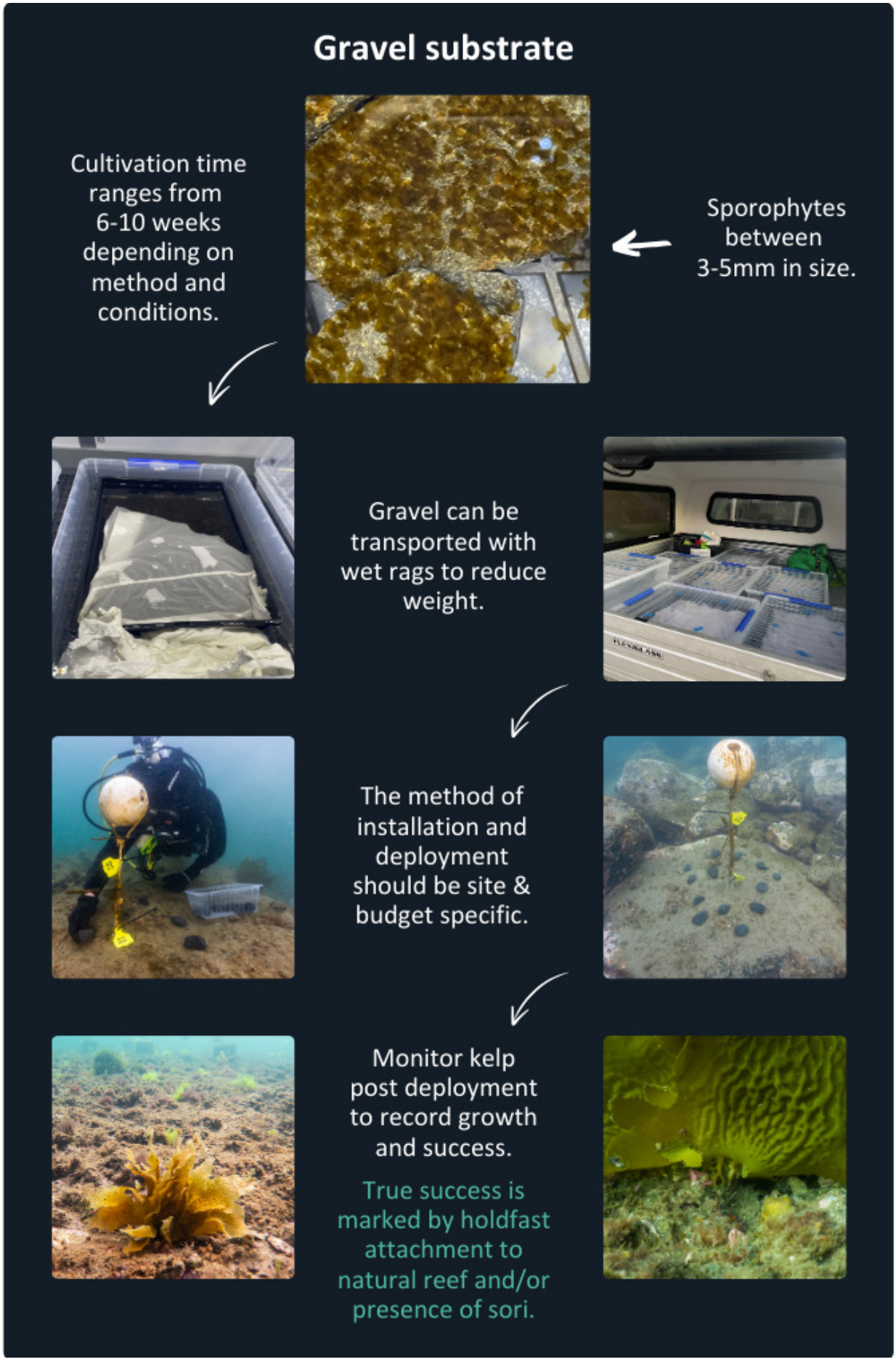

Figure 9. Infographic showing process of outplanting gravel-seeded kelp for restoration purposes.

Troubleshooting tips

Cultivating kelp presents numerous challenges and often requires ongoing troubleshooting. Although it may seem straightforward, achieving healthy sporophyte growth can be both difficult and demanding. Aside from technical issues related to cultivation systems and setup, contamination is the biggest and most common challenge.

If you notice contamination in your cultures or kelp, it is best to determine the type of contamination (see Figure 10 for examples of common contaminants). Contamination types and prevalence can vary depending on factors such as kelp source, water source, and laboratory conditions (e.g., cleanliness, presence of other potentially contaminating work). Taking a sample and examining it under a compound microscope can help identify the contaminate broadly (e.g., diatoms, bacteria, algae), which assists in selecting an appropriate control method.

Some of the most common contaminants with our *E. radiata* cultures were diatoms. While most diatom species do not directly affect the kelp, they can impede growth by competing for space, nutrients, or light at high densities. Germanium dioxide (also known as Germania, GeO2; see Table 3) is effective in controlling diatoms by interfering with their silica-based cell walls. The recommended dosage is 0.1-0.5 mL of GeO2 per litre of seawater (see Shea & Chopin (2007) for the method). Germanium dioxide can be used both reactively, when contamination occurs, or proactively to prevent contamination (refer to Visch et al., 2024 for more detail). Germanium dioxide may slow the development of sporophytes, and therefore we recommend only using when diatom contamination is present.

Bacterial contamination is also common, with colonies varying in colour and appearance depending on species. With the microscope it can look more indistinct – as a standard compound microscope will not usually have high enough magnification to see the small bacterial cells individually. Generally, however, bacterial contamination looks more like a fine slime or film, unlike algal contamination, which tends to look more 'hairy' or filamentous. Depending on the type, bacteria can be very detrimental if left untreated. Typical species are from the cyanobacteria group. Controlling bacterial growth includes rinsing with clean seawater, physical removal and, as a last resort, antibiotic treatment (see Table 4).

If the water supply is not highly filtered and sterilised, or if cross-contamination occurs, additional algal species (typically filamentous algae) may grow with the kelp (see Figure 8). We have found these to be very detrimental, as they can outcompete the kelp quickly. The best method here is prevention, by maintaining clean cultures and ensuring nutrient media and seawater are filtered to the finest level possible and sterilised by any available method. If contamination does occur, the next step is physical removal. This may involve wiping away the intruder species, rinsing with clean seawater, or, if contamination is severe, remove the highly contaminated substrates to prevent further spread.

Whenever contamination occurs, address it immediately. Maintain clean practices to prevent cross-contamination, and conduct more frequent water changes to support continued kelp growth. Lowering light intensity or temperature may also help slow the growth of the contaminants, however this may also slow kelp growth.

Image: Stefan Andrews, Great Southern Reef Foundation

Table 4. Recommended chemicals for cultivating *Ecklonia radiata*. Some are necessary for the general cultivation process, such as nutrients, while others are used to limit, minimise, and prevent contamination. Always consult the chemical Safety Data Sheets (SDS) to understand hazards, risks, and safe handling and disposal procedures. Alternative options are indicated with a key: *equally effective alternative, **reasonable alternative.

Chemical name/s	When to use	Pros	Cons	Hazardous?	Alternative
Cell Hi-2 F2P Algae Nutrient	Nutrient media for seawater, used to create culture medium	Designed for algae aquaculture, clean	Dangerous Goods - oxidiser	Y	Provasoli's Enriched Seawater (PES)*
Ethanol >70%	Cleaning	Easy to use, quick way to clean working spaces and equipment	Not 100% effective, flammable	Y	Antibacterial surface spray**
Iodine (liquid Betadine)	Cleaning sori tissue	Easily available and cost effective	Not 100% effective, kills most bacteria but does not eliminate diatoms	Y	NaOCl bath (200 ppm)**
Decon 90	Decontaminating equipment that cannot be autoclaved	Highly effective cleaning agent	Hazardous	Y	Bleach**
Germanium dioxide (GeO_2)	If there is a diatom outbreak	Prohibits diatoms from reproducing	Can affect kelp growth in higher doses, cost	Y	NA
Antibiotic Antimycotic solution (streptomycin sulphate; penicillin G sodium salt)	If there is a bacterial or fungal outbreak	Effective at halting spread and growth of bacteria and fungus	Requires a permit, requires special storage, hazardous, requires special disposal	Y	NA

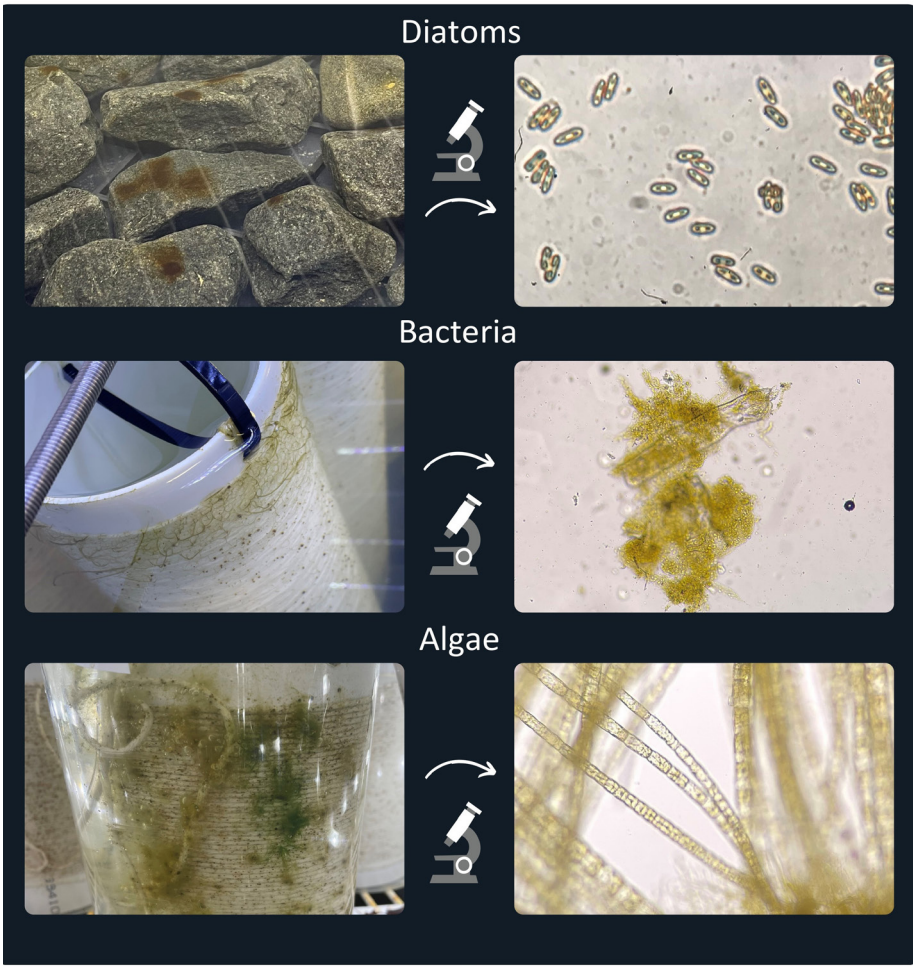

Figure 10. Examples of various types of contamination that may occur during cultivation process of *Ecklonia radiata*.

In addition to contamination, other factors may affect total output, as shown in Figure 11. Patchy coverage is typically influenced by seeding density; trialling different densities may yield varying results. Genetic vigour can sometimes be improved by using a greater number of parent sources or mixing seedstock from multiple sites (if permit regulations allow), potentially increasing the overall number of sporophytes formed.

If sporophytes are very sparse or not forming after four to six weeks (for gametophytes and zoospore seeding, respectively) and gametophyte clusters appear to multiply, resulting in large clusters without sporophytes, this can indicate conditions are not suitable for sporophyte development. This may occur due to low reproductive capacity in the seedstock, though inadequate lighting is often the primary limiting factor. Verify that lighting is appropriately set up, and rotate substrates regularly.

If sporophytes are kept in cultivation for too long, they will start to form translucent patches (dead tissue) and will drop off the substrate. This is likely due to intraspecific competition. Having a slightly lower seeding density can help with this, as well as moving them into a larger body of water and providing higher flow. However, timely outplanting once sporophytes reach approximately 5 mm, is the is the best approach to prevent these issues.

Figure 11. Images of varying results of twine-seeded kelp with potential reasons and suggested solutions.

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Image: Scott Breschkin, The Nature Conservancy

Further Reading

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Glossary

Autoclave	A machine that uses high pressure and high temperature to sterilise equipment and media.
Cultivation	The growing of seaweed within a lab/aquarium in a controlled environment through life stage.
Culture medium	Blend of filtered sterilised seawater with added nutrients at specific dosage.
Nutrient media	Liquid concentrated solution of nutrients based on the Guillard F/2 medium.
Gametophyte	The haploid microscopic life stage of Laminariales species, can be male or female.
Green gravel	Method of using rocks of selected size and type as a substrate for growing kelp to deploy on reefs.
Haptera	Branches of the holdfast that the kelp uses to attach to rocks and substrate.
Kelp	Species of large brown seaweed within the Laminariales family. Although some literature defines kelp as all large brown macroalgae e.g., some Fucoids, however these are not included in the context of this guide.
Outplant	Deploying kelp grown in laboratory/aquaria onto reef via chosen substrate.
Sori, sorus	Sorus is singular reproductive material of kelp, and sori is plural. Smooth and slimy to touch, zoospores are released from the sori.
Spools	PVC pipe with twine wrapped around it, used for seeding and growing kelp.
Sporophyte	Diploid macroscopic life stage of kelp, grows from fertilised egg of female gametophyte, mature adults form sori.
Zoospore	Small, flagellated haploid cell which undergoes mitosis to become gametophyte.

Image: Jasmine Bursic, Deakin University

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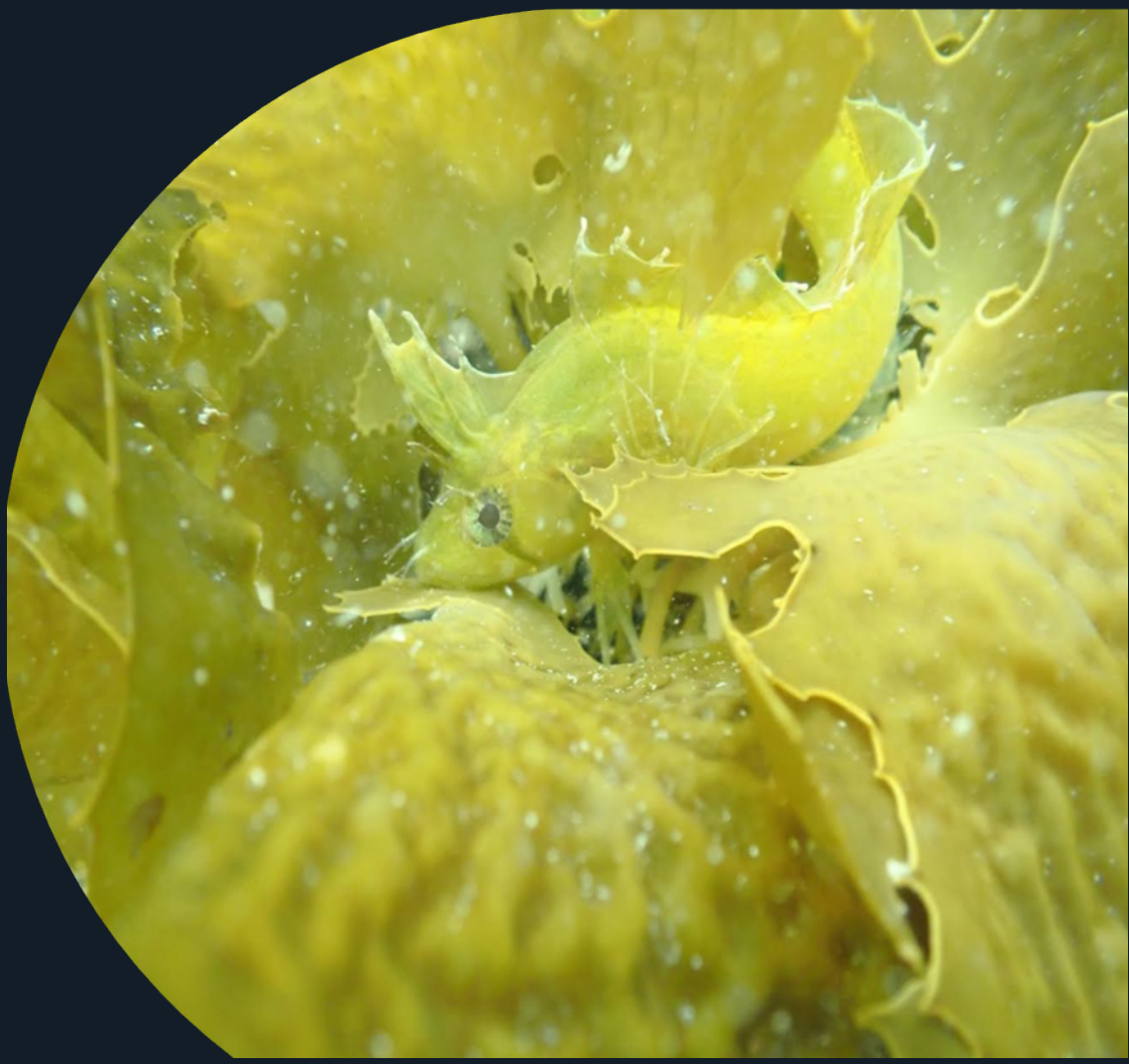

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