PACIFIC SALMON FOUNDATION

HANDBOOK FOR BIOBANKING OF KELP IN BRITISH COLUMBIA

Liam J. M. Coleman, PhD
Pacific Salmon Foundation
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Illustration:Delaney Cox of Drawing it Out

PREFACE

Estuaries and nearshore ecosystems provide vital support to juvenile and adult Pacific salmon, as well as the larger food web they depend upon. There is increasing interest in protecting and restoring the interconnected nearshore habitats of kelp, salt marshes, and eelgrass habitats within these critical salmon systems.

However, the success of nearshore recovery projects is hampered by a number of factors: a paucity of open-access information about nearshore habitat restoration and monitoring methodologies, a lack of knowledge about priority areas and suitable site selections for restoration, and a need for knowledge based approaches to conservation strategies under worsening climate change scenarios.

With funding from Fisheries and Oceans Canada's Aquatic Ecosystem Research Fund (AERF), the Pacific Salmon Foundation has created a Restoration Resource Hub of open-access informative resources and decision-support tools. The purpose is to guide adaptive nearshore habitat restoration and monitoring approaches to kelp, salt marsh, and eelgrass habitats.

This Kelp Biobanking Handbook is one of the components of this Hub.
Other documents can be found through this LINK.

Photos, unless otherwise stated: Liam J. M. Coleman. Cover photo: Eiko Jones

EXECUTIVE SUMMARY

Biobanking, the practice of storing biological material for later use, represents an invaluable tool for research, conservation, and food security. While it is a common practice for many biological systems, including human cells and tissues and agriculturally important plants, there are currently few biobank collections for kelp. These large brown algae, widely recognized for their ecological, economic, and cultural significance, are common in nearshore marine ecosystems at temperate latitudes, including in British Columbia. However, they are currently showing losses in parts of their ranges, largely driven by the effects of climate change. In light of this, the development of biobanks as part of a kelp conservation strategy would be immensely valuable and could prevent irreversible loss of biodiversity in the future.

The purpose of this handbook is to facilitate kelp biobanking in BC. We first provide relevant background information on kelp biology, with a focus on the anatomy and life cycle. We then discuss and compare kelp biobanking methodologies, including both traditional culture-based protocols and more novel cryopreservation techniques. We then provide detailed step-by-step instructions for all necessary procedures for developing a kelp biobank, including building culture environments, collecting kelp from the wild, establishing and maintaining kelp gametophyte cultures, introducing and removing kelp from cryopreservation, and producing kelp sporophytes from biobanked gametophytes for use in lab and field settings. We also provide additional resources to support the execution of these procedures and make recommendations for the development of a kelp biobanking strategy in BC.

Photo: Ryan Miller

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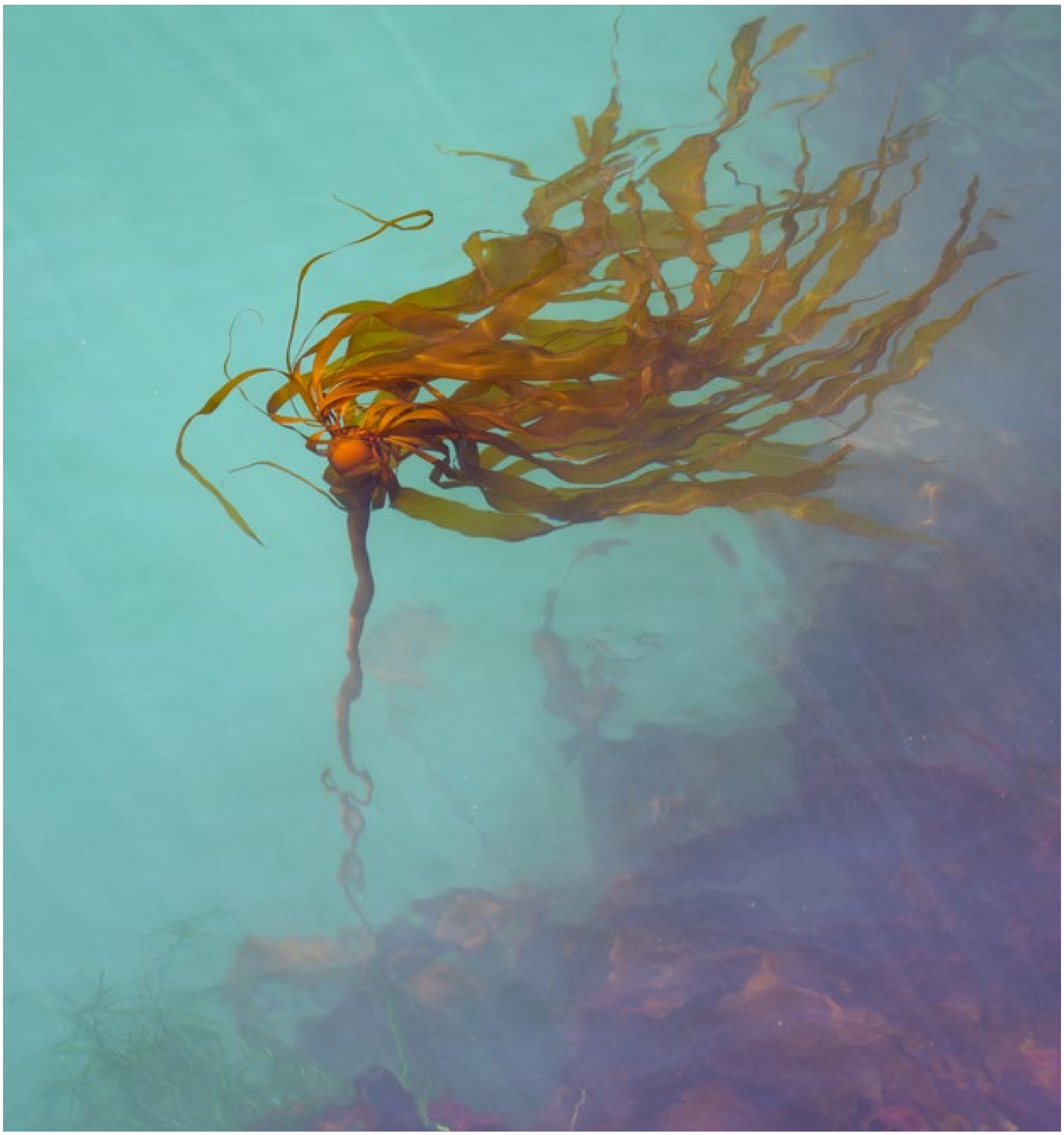

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Photo: Nicole Christiansen

ABOUT THE AUTHOR

Dr. Liam Coleman is a marine biologist and an expert in kelp biology. He holds a BSc in biology from the University of Victoria and a PhD in botany from the University of British Columbia. His PhD work, conducted under the supervision of Dr. Patrick Martone, examined developmental mechanisms underlying phenotypic plasticity in bull kelp, *Nereocystis luetkeana*. After completing his graduate studies, he worked as postdoctoral researcher at Simon Fraser University under the supervision of Dr. Sherryl Bisgrove. His postdoc work focused on developing a cryopreservation protocol for biobanking of bull kelp. He has since continued to work with Dr. Bisgrove and other collaborators to help develop kelp biobanking in BC. He has also taught undergraduate courses in phycology and nonvascular plant biology at UBC and the Bamfield Marine Sciences Centre.

1. INTRODUCTION

1.1 BIOBANKING AND ITS IMPORTANCE AS A TOOL FOR KELP CONSERVATION

Biobanking[1] is the practice of storing collections of biological material for later use (Day & Stacey, 2008). This is common practice for many organisms, such as agriculturally important plants (Tanksley & McCouch, 1997) and even human cells and tissues (Coppola et al., 2019). As repositories of biodiversity, biobanks are valuable resources and have applications for scientific research, conservation, and food security (Hofmann et al., 2025; Wade et al., 2020). However, at present, few **kelp** biobank collections exist worldwide (Hofmann et al., 2025; Wade et al., 2020). Kelps are a group of large **brown algae** that are common in nearshore marine ecosystems at temperate latitudes, including in British Columbia. These conspicuous **seaweeds** (a.k.a. **macroalgae**) are not only important **primary producers** (Duggins et al., 1989; Pessarrodona et al., 2022), but also form complex three-dimensional **kelp forests** (Figure 1) that provide habitat for diverse communities of marine organisms (Dayton, 1985; Shaffer et al., 2023; Steneck et al., 2002; Teagle et al., 2017). Kelps are also hugely valuable to humans as sources of food and industrial products (García-Poza et al., 2020; Heidkamp et al., 2022; Kawashima, 1984) and through the provision of varied **ecosystem services** (Bennett et al., 2016; Eger et al., 2023; Filbee-Dexter & Wernberg, 2020; Shaffer et al., 2023). In spite of the importance of kelps, they are currently in decline throughout much of the world, including in British Columbia and Washington (Berry et al., 2021; Krumhansl et al., 2016; Mora-Soto et al., 2024; Starko et al., 2022, 2024). These declines are largely driven by the effects of climate change, particularly ocean warming and marine heatwaves (Berry et al., 2021; Mora-Soto et al., 2024; Starko et al., 2022; Wernberg et al., 2016). Given these observations, calls to develop kelp biobanking strategies as part of a larger kelp conservation effort have been becoming increasingly frequent (e.g. Barrento et al., 2016; Coleman et al., 2025; Hofmann et al., 2025; Lang-Wong et al., 2022; Wade et al., 2020). For more information on the current state of kelp in BC, see the 2026 Pacific Salmon Foundation (PSF) Kelp State of Knowledge report.

Figure 1. A kelp forest near Victoria, British Columbia.

1. Terms in bold are defined in the glossary at the end of this document.

The development and maintenance of biobank collections would have many valuable utilities for kelp conservation, research, and industry in BC and worldwide. Perhaps most importantly, having a large amount of kelp **biodiversity** safely stored in biobank collections can act as biological "insurance," preventing the extinction of **genotypes** or even species should they be lost from the wild (Barrento et al., 2016), and perhaps even create opportunities to reintroduce lost biodiversity into nature. Furthermore, biobanked collections can facilitate genetic research and breeding programs, potentially leading to the development of novel organismal strains with strategically useful traits (Liu et al., 2014). For example, stored **germplasm** could be used to develop kelp strains that are tolerant of high temperatures, which could enable reforestation of kelp habitats that have become too warm for wild type strains. This may prove essential for conserving kelp forests and the ecological and economic functions they perform as the world continues to warm. Biobank collections could also be similarly leveraged to develop kelp strains with desirable traits for BC's burgeoning kelp industry, such as high growth rates, larger sizes, or even the inability to produce spores (Liu et al., 2014; Vissers et al., 2024). Given the declines already observed and the expectation that climate change-driven warming will worsen the future (IPCC, 2023), it is important that kelp biobanking strategies be developed quickly to avoid loss of irrecoverable biodiversity.

1.2 PURPOSE OF THIS HANDBOOK

The aim of this document is to facilitate the development of kelp biobanking in British Columbia. To this end, we first provide brief introduction to kelp biology, with a focus on anatomy and life history. We then provide detailed instructions for establishing and maintaining a kelp biobank, considering both traditional culture-based protocols and more novel cryopreservation methods. Finally, we make a series of recommendations for the continued development of a larger kelp biobanking strategy for the province. We assume minimal preexisting subject expertise of on the part of the reader throughout this document in the hope that the information within can be useful to diverse practitioners from a variety of backgrounds.

2. BACKGROUND

2.1 KELP BIODIVERSITY IN BRITISH COLUMBIA

BC is a biodiversity hotspot for kelp and is home to at least 30 species (Druehl, 1970). The most conspicuous of these are the large buoyant species that span the breadth of the water column and make up the **canopies** of kelp forests. In BC, there are two such species: bull kelp (*Nereocystis luetkeana*; Figure 2A) and giant kelp (*Macrocystis pyrifera*; Figure 2B). Bull kelp is common on the west coast of Vancouver Island, the BC central coast, and throughout the Salish Sea, especially in wave- or current-swept areas. Giant kelp is also common on the west coast of Vancouver Island and along BC's central coast but is absent from most of the Salish Sea, making bull kelp the most dominant canopy-forming kelp in the Salish Sea. The many other BC kelp species can be found in intertidal zones or can make up the **understories** of subtidal kelp forests. Sugar kelp (*Saccharina latissima*; Figure 2D) is one such species that is also commonly cultivated and has potential to become an economically important kelp in BC (reviewed in Sæther et al., 2024). From a conservation perspective, it would be beneficial for as many local kelp species to be biobanked as possible and we would encourage practitioners undertaking kelp biobanking to consider as many species in their plans as is feasible. However, for purposes of this handbook, we present bull kelp as a priority species because it is the dominant canopy-forming kelp found in the Salish Sea and recent data indicates it is showing losses in parts of its range (Berry et al., 2021; Mora-Soto et al., 2024; Starko et al., 2022, 2024). Furthermore, in some regions, such as the inner areas of Puget Sound, bull kelp shows very low genetic diversity, which makes these populations vulnerable to loss and in need of swift conservation action (Gierke et al., 2023).

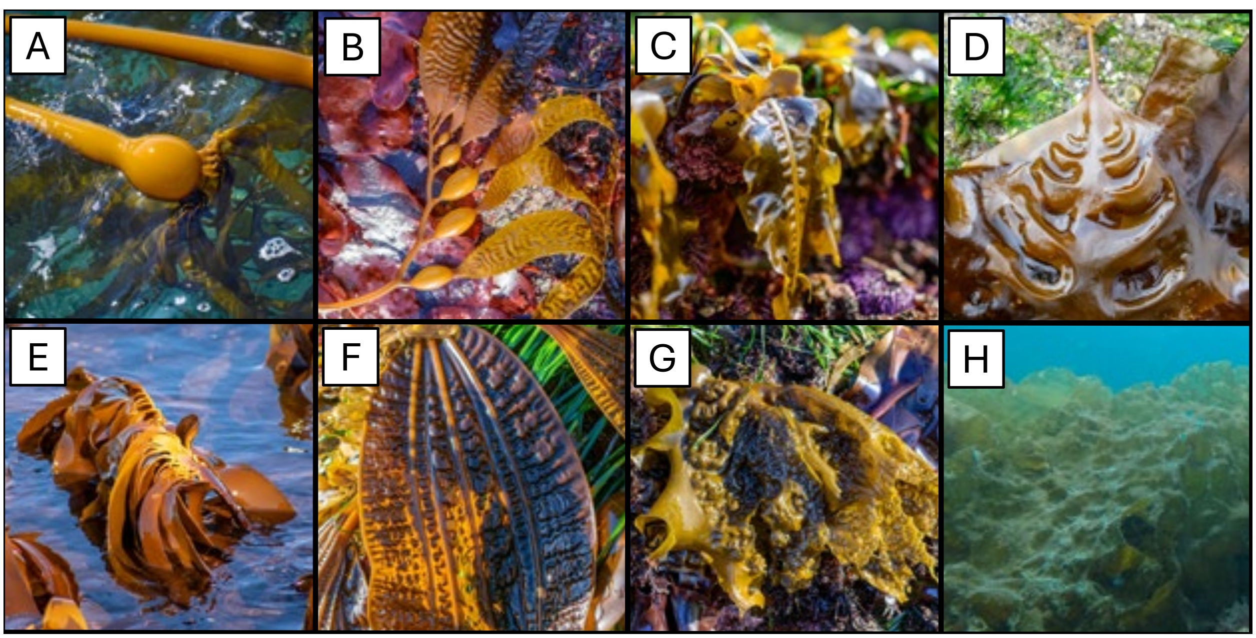


Figure 2. Common kelp species found in British Columbia.
A) Bull kelp (*Nereocystis luetkeana*).
B) Giant kelp (*Macrocystis pyrifera*).
C) Winged kelp (*Alaria marginata*).
D) Sugar kelp (*Saccharina latissima*).
E) Walking kelp (*Pterygophora californica*).
F) Seersucker kelp (*Costaria costata*).
G) Sea cabbage (*Hedophyllum sessile*).
H) Sea colander (*Neoagarum fimbriatum*).

2.2 THE LIFE HISTORY AND BIOLOGY OF KELPS

Biobanking of kelps requires a basic understanding of kelp life cycles and anatomy. All kelps have a complex life history involving alternation between a macroscopic phase called a **sporophyte**, which is the form of the kelp that people most commonly observe and interact with, and a microscopic phase called a **gametophyte**, which grows inconspicuously on the ocean bottom (Graham et al., 2017; Figure 3). Sporophytes are **diploid** (2N), which means each sporophytic cell has a nucleus containing two copies of each of its chromosomes, like most human cells do. Gametophytes, conversely, are **haploid** (1N), which means that their cells have nuclei containing only one copy of each of their chromosomes.

Illustration: Cassandra Buunk

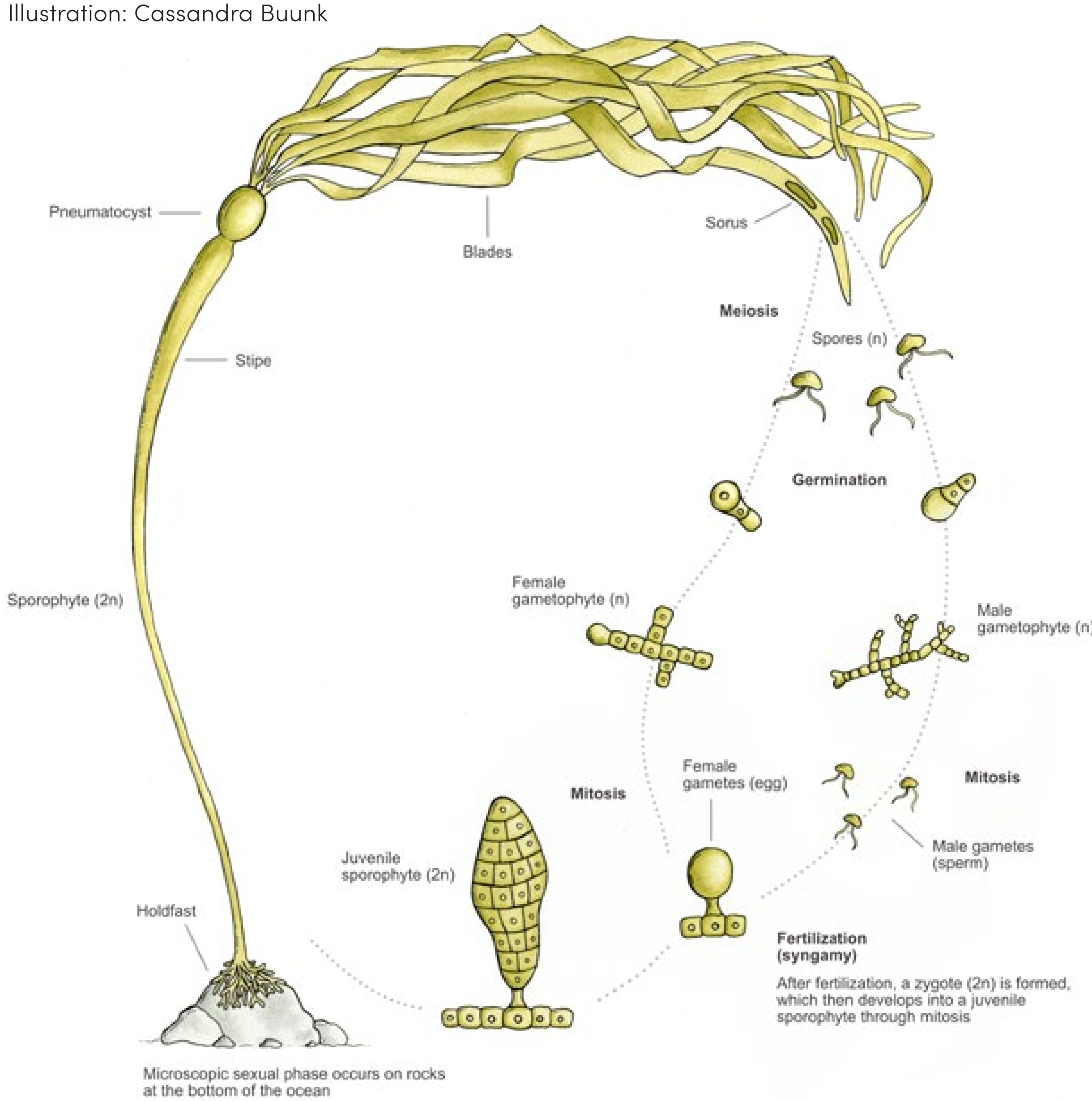


Figure 3. Kelp morphology and life history, using bull kelp (*Nereocystis luetkeana*) as an example.

Sporophytes of different kelp species generally have variations on the same basic morphology, which consists of a root-like attachment structure called a **holdfast**, one or more stem-like **stipes**, and one or more leaf-like **blades**; the whole body of a kelp (either a sporophyte or a gametophyte) is termed a **thallus** (Figure 4). Blades of some kelp species, such as *Alaria* and *Costaria*, have one or more stiff midribs. Some kelp sporophytes, such as those of bull kelp, have gas-filled bladders called **pneumatocysts** that provide buoyancy. Mature, reproductive sporophytes produce spores in specialized regions of blade tissue called **sori**. Some kelp species, including bull kelp and sugar kelp, can develop sori on any blade, while others, including winged kelp and giant kelp, only develop sori on specialized blades called **sporophylls**, which are generally located near the holdfast. The surface of sorus tissue is covered with many spore-producing structures called **sporangia**, which produce haploid spores through the process of **meiosis** before releasing them into the environment. Kelp spores are motile and use structures called **flagella** to actively swim around the environment before ultimately settling onto a surface upon which they will germinate and develop into gametophytes.

Illustration: Cassandra Buunk

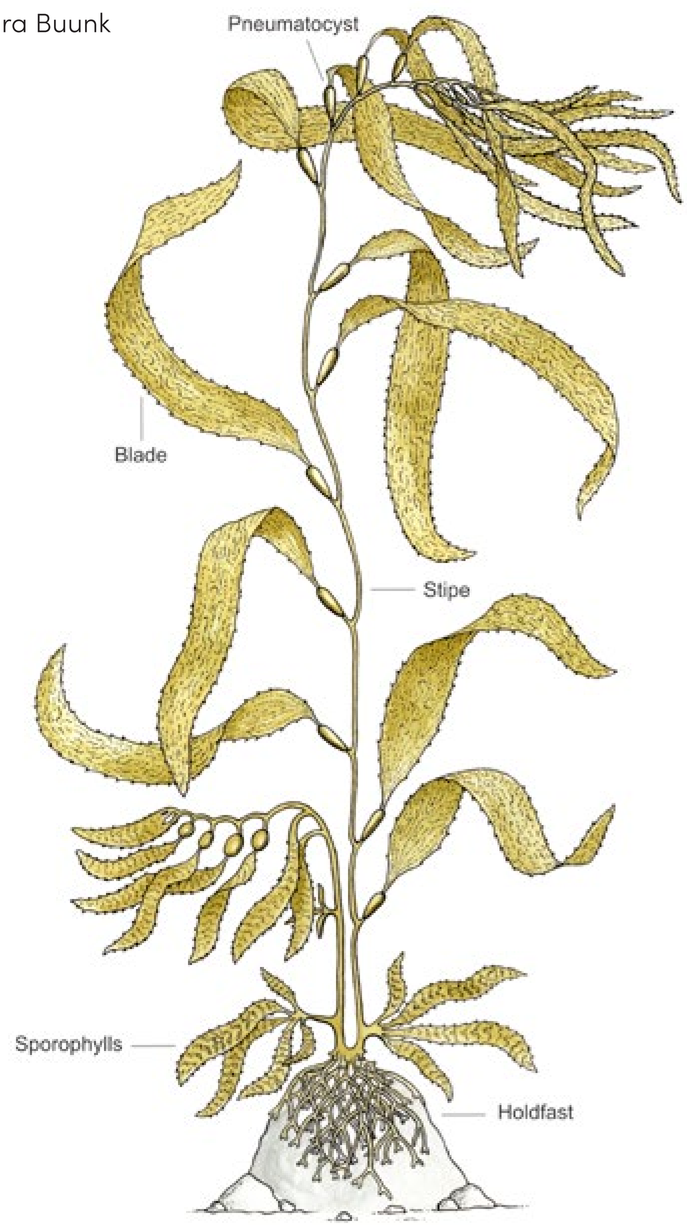


Figure 4. Morphology of the sporophyte of giant kelp (*Macrocystis pyrifera*).

Kelp gametophytes are morphologically simple compared to sporophytes, being composed of branched filaments made up of single-file rows of cells, though they do show variability in their growth forms between species. While sporophytes are sexless, gametophytes have separate male and female individuals. Cells of female gametophytes tend to be larger than those of male gametophytes. Both male and female gametophytes produce **gametes** through **mitosis** in a process called **gametogenesis**. Similarly to kelp spores, once produced, male gametes are released into the environment, where they then use flagella to swim around. Unlike spores, however, instead of seeking a surface to settle on, male gametes seek female gametes. Female gametes remain attached to the female gametophyte and release a pheromone which male gametes are attracted to. When a male gamete comes into contact with a female gamete, the two cells fuse in a process called **syngamy**, forming a diploid **zygote** that ultimately grows into a new sporophyte. Some kelp species, such as bull kelp, follow an annual life cycle, with sporophytes mostly persisting for one growing season (Rigg, 1912), while others, such as giant kelp and walking kelp, are perennial, with individual sporophytes lasting potentially many growing seasons (Hymanson et al., 1990). Comparatively little is known about the ecology of kelp gametophytes, but research indicates that individuals can persist on the seabed for months or even years after spore germination and are capable of delaying gametogenesis until environmental conditions are more favourable for sporophyte growth (Carney, 2011; Carney et al., 2013; Carney & Edwards, 2006).

2.3 INTRODUCTION TO KELP BIOBANKING METHODS

There are two main methodological approaches to biobanking live kelp samples: (1) culture-based methods and (2) cryopreservation. Each approach presents with its own advantages and challenges. In the following sections we describe each approach and the factors to consider when deciding on which methods to use for kelp biobanking. See sections 3.5 and 3.6 for more information on culture-based biobanking and cryopreservation respectively.

2.3.1 CULTURE-BASED BIOBANKING

Culture-based approaches are the most used and best-established methods for kelp biobanking. These methods are generally methodologically simple, reliable, inexpensive, and accessible; it is logistically straightforward for a new practitioner to acquire and use the resources needed to set up a biobank using these methods (Wade et al., 2020). They can also be used with any kelp species with minimal or no protocol changes. If well maintained, kelp gametophyte cultures can be kept for many years, possibly even indefinitely.

While there may be slight variations in protocols, culture-based storage of kelp germplasm generally involves the establishment and maintenance of live cultures of the gametophyte stage under minimal conditions for growth. Gametophytes are used because they are much easier to work with than the sporophyte stages on account of the large sizes that sporophytes can reach, as well as the demanding culture requirements for keeping sporophytes healthy, such as high amounts of water movement (Supratya & Martone, 2023). The small sizes of gametophytes, conversely, allow them to be cultured in small, readily available culture environments, such as petri dishes, and the culture conditions required for gametophytes are much easier to achieve than those of sporophytes. Furthermore, kelp gametophytes may naturally lend themselves to being stored for extended periods of time on account of their ability to delay reproduction (Carney, 2011; Carney et al., 2013; Carney & Edwards, 2006).

While culture-based biobanking has many advantages and will likely be the best approach for most practitioners, it does have limitations. Firstly, maintaining live cultures necessitates regular media changes, which can represent a significant amount of time and labour, especially when a collection is very large. However, this task only needs to be performed once every six months if the biobank is maintained under proper environmental conditions for long term storage. Live cultures are also vulnerable to contamination by pest organisms, which can outcompete and eventually kill kelp gametophytes if they are introduced into a culture (see section 3.5.1 for more information). To avoid this, multiple levels of contamination mitigation, including the use of sterile technique and appropriate facilities, are critical when establishing and accessing kelp gametophyte cultures. Additionally, because live cultures require constant, strict control of light and temperature conditions to remain healthy, unintended loss of electrical power to the environmental control systems can result in catastrophic culture loss (Yang et al., 2021). Also, because cultured cells continue to grow and divide, they may be susceptible to accumulation of **mutations** or **epigenetic** changes over time (Lakeman et al., 2009). Finally, some practitioners report that individual gametophytes can become difficult to induce to reproduce after being stored for multiple years, which has potential to limit the usefulness of these individuals for purposes such as restoration. This phenomenon is not yet well understood and research is needed to learn what causes it and whether it can be prevented.

2.3.2 CRYOPRESERVATION

An alternative to culture-based approaches to kelp biobanking is **cryopreservation**, the storage of living cells or tissues at ultracold temperatures (Day, 2018). This is a common practice in many fields, including in animal research and in medicine (Coppola et al., 2019). It is also used for biobanking of some microscopic **algae** (Andersen, 2005; Day & Harding, 2008). However, at the time of writing, it has not been widely adopted for biobanking of kelps. We would encourage more practitioners to consider the use of cryopreservation for biobanking kelp, as we believe the method presents numerous, largely untapped advantages.

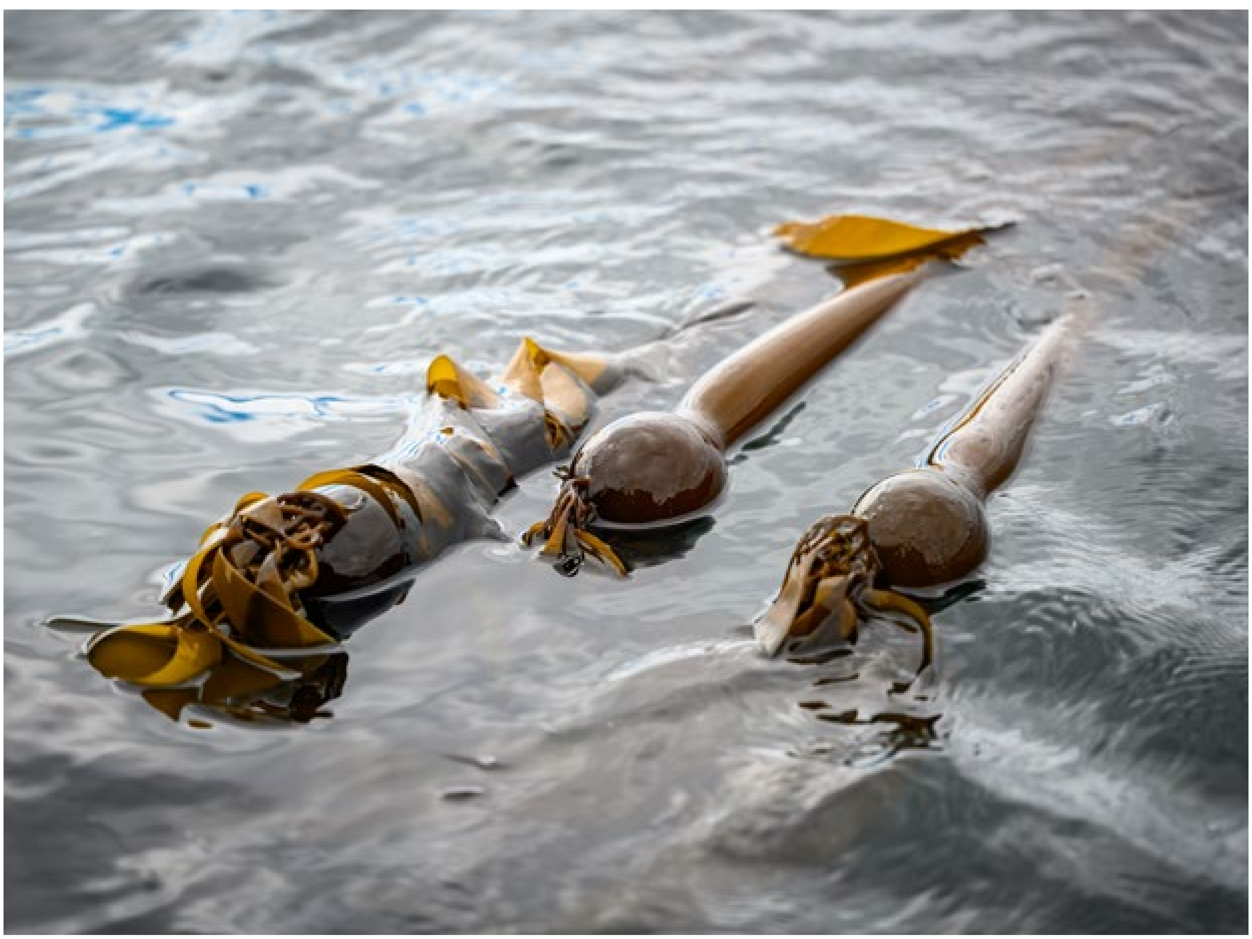
Kelp cryopreservation protocols generally involve storing gametophytes in liquid nitrogen at a temperature of -196°C (e.g. Coleman et al., 2025; Kuwano et al., 2004; Visch et al., 2019; Zhang et al., 2007). These conditions would normally be lethal, as the process of freezing damages cells, but cryopreservation protocols include measures to mitigate cryoinjury. The two main methodological approaches to cryopreservation are (1) slow-cooling and (2) **vitrification** (Day, 2018; Jaiswal & Vagga, 2022). In the slow-cooling approach, cells are reduced to the target temperature over a period of several hours in the presence of chemicals called **cryoprotective agents** (CPAs). These chemicals mitigate the effects of cryoinjury and allow living cells to reach extremely cold temperatures without being fatally damaged. (Day, 2018; Jaiswal & Vagga, 2022). Examples of common CPAs include dimethyl sulfoxide (DMSO), ethylene glycol, and glycerol (Jaiswal & Vagga, 2022). Vitrification, alternatively, involves dehydrating cells to convert the liquid components of a culture to a stable glass (i.e. a solid state in which no crystals form) and then quickly introducing the samples into cryogenic conditions (Day, 2018; Jaiswal & Vagga, 2022). Unlike in a slow-cooling approach, vitrification does not necessarily utilize CPAs (Day, 2018). Cryopreservation protocols of both types are specific to individual species and tissue types and the exact set of methods that is effective for the intended subject must be experimentally determined; what works for one species/tissue combination will not necessarily be effective for another (Taylor & Fletcher, 1999; Yang et al., 2021). Cryopreservation protocols have been developed for several species of kelps (Table 1). Almost all utilize a slow-cooling approach (e.g. Coleman et al., 2025; Kuwano et al., 2004; Visch et al., 2019), although vitrification has been successfully employed in at least one instance (Wang et al., 2011). Several studies have found that male gametophytes yield better survivorship than females of the same species when subjected to the same cryopreservation protocols (e.g. Coleman et al., 2025; Visch et al., 2019). Almost all published kelp cryopreservation protocols use gametophytes, except for one, which has successfully documented cryopreserving spores (Zhang et al., 2007).

Table 1. Kelp species with published cryopreservation protocols.

Species	Publication
Ecklonia cava subsp. stolonifera	Kuwano et al., 2004
Ecklonia cava subsp. kurome	Kuwano et al., 2004
Eisenia bicyclis	Kono et al., 1998
Kjellmaniella crassifolia	Kuwano et al., 2004
Laminaria digitata	Vigneron et al., 1997
Macrocystis pyrifera	Piel et al., 2015
Nereocystis luetkeana	Coleman et al., 2025
Saccharina japonica	Kuwano et al., 2004; Zhang et al., 2007a; Zhang et al., 2007b; Zhang et al., 2008
Saccharina japonica var. diabolica	Sakanishi & Saga, 1994
Saccharina latissima	Visch et al., 2019
Saccharina longissima	Kuwano et al., 2004
Undaria pinnatifida	Arbault et al., 1990; Ginsburger-Vogel et al., 1992; Renard et al., 1992; Kuwano et al., 2004; Nanba et al., 2009; Wang et al., 2011

Cryopreservation presents several advantages for purposes of biobanking kelps when compared with culture-based approaches. Perhaps most notably, cryopreserved cells stored in liquid nitrogen require very little maintenance; media never needs to be changed and culture conditions do not need to be maintained. However, it is essential that liquid nitrogen be replenished whenever it runs low. The relative low maintenance requirements of cryopreservation have potential to reduce the amount of time, labour, and expense required to keep large germplasm collections. Additionally, the fact that stored samples are not being kept in culture means that risk of contamination and catastrophic samples loss to electrical failure are significantly reduced. Finally, because the metabolism of cryopreserved samples is almost complete halted, risk of accumulation of mutations and epigenetic changes may be reduced when compared to culture-based biobanking.

Cryopreservation, though potentially advantageous in a number of ways, does come with its own set of challenges. Culture protocols for kelp biobanking are very well established, simple to implement, and known to be reliable for any kelp species. Cryopreservation protocols, however, are comparatively complex and require a higher level of technical skill in laboratory settings to conduct effectively. They also require resources that may not be available to some practitioners, notably continuous access to liquid nitrogen, and are species specific, which may necessitate novel research to develop methods. Additionally, because cryopreservation is a relatively new method of biobanking kelps, questions remain about the specifics of its use, such as how long individual samples can be stored for and whether there are any unintended side effects of the process on kelp biology. More research is needed to improve understanding of kelp cryopreservation and thereby build trust in the method among would-be users.



2.3.3 CHOOSING WHICH METHODS TO USE

When first setting out to establish a kelp biobank, an important question to consider early on is what methods you will use (Table 2). Your situation may dictate which methods you can make use of. We recommend most operations at least begin with a culture-based kelp biobank, as this is the simplest and most reliable way of establishing a collection. This is especially true if you represent a small operation with limited resources or if you are in a remote location that cannot be reliably supplied with liquid nitrogen. However, if you represent a more established operation and are based at a facility like a university that would have consistent access to liquid nitrogen and -80° freezers, then we would recommend at least considering integrating cryopreservation into your strategy.

Table 2. Relative advantages and disadvantages of culture-based methods vs. cryopreservation for kelp biobanking.

	Culture-based methods	**Cryopreservation**
Ease of use	Simple, accessible.	More complex, higher technical skill required.
Cost	Low up front, may increase with size of collection.	Higher up front, but may be less expensive for larger collections compared to culture-based methods.
Reliability	Reliable, well understood protocols.	Novel protocols, uncertainty remains in aspects of use.
Labour required	Maintenance can be labour intensive, especially for larger collections.	Little labour required for maintenance.
Contamination risk	Present, but can be mitigated through specific practices and good technique.	Reduced compared to culture-based approaches.
Vulnerability to power loss	Vulnerable. Use of generators and backup storage locations essential.	Not vulnerable.
Additional considerations	Metabolically active cultures may change genetically or epigenetically over long periods of time; some report that gametophytes become difficult to make reproduce after multiple years spent in culture.	Consistent access to liquid nitrogen is critical; long-term effects of cryopreservation on kelp biology not known.

3. INSTRUCTIONS FOR ESTABLISHMENT AND MAINTENANCE OF KELP BIOBANKS

3.1 SETTING UP A CULTURE ENVIRONMENT FOR KELP GAMETOPHYTES

Required materials

- [x] Growth chambers or other temperature-controlled environment that can be maintained at temperatures ranging from 8-15°C
- [x] Fluorescent or LED growth lights (white and red); red cellophane can be used in combination with white light as an alternative to red lights
- [x] Light timer(s)
- [x] Thermometer
- [x] Light meter capable of measuring irradiance in µmol/m2/s
- [x] Coarse mesh

Regardless of what methods you are using for kelp biobanking, the first step is to establish culture environments for housing live kelp gametophytes. If you are using culture-based methods for biobanking, you will need at least two of these environments: a *growth environment* for encouraging gametophytic growth and a *storage environment* for maintaining gametophytes long-term; the latter will be your primary biobanking environment (Table 3). If you are using cryopreservation, you will only need a growth environment in which to maintain gametophytes for several weeks after culture establishment before you freeze them.

An appropriate culture environment consists of a physical space that can be maintained at a cool internal temperature, is equipped with lighting, and has enough room for hundreds if not thousands of kelp samples. A storage environment may need to be larger and will need to able to hold a cooler temperature than a growth environment (Table 3). Examples of spaces that can make suitable culture environments include, in increasing order of cost and complexity, a wine fridge, a commercial plant growth chamber, and a temperature-controlled room. Growing kelp requires full spectrum growth lights like those used to grow plants on land. These can be fluorescent or LED. If lighting is not already built into your growth space, any fixtures you acquire must be of a size that can fit into the space and be secured above the area where the cultures will be. We recommend acquiring a light meter capable of measuring irradiance in µmol/m^2/s (as opposed to lux, lumens etc.) to help set up and monitor your lighting environment. Note that irradiance is sensitive to the distance between the light source and the point at which it is measured and you can increase or reduce it by moving your lights down or up respectively. You can also reduce irradiance by placing a coarse mesh over your cultures to block some of the incoming light.

Table 3. Environmental conditions to be maintained in gametophyte growth and storage environments.

Environmental factor	Growth environment	Storage environment
Temperature (°C)	10-15	8
Irradiance (µmol/m^2/s)	30-45	5
Photoperiod (hours of light:hours of dark)	12:12	4:20
Light quality	Red by default, situationally changing to white	Red

While your lighting should be capable of producing white full spectrum light, most of the time you will want the light reaching your gametophyte cultures to be red. Growing kelp gametophytes under red light inhibits gametogenesis, which means that no sporophytes will develop in your culture (Lüning & Dring, 1972). This is generally a useful state to hold your cultures in for purposes of biobanking. You can always opt to trigger production of sporophytes when they are required by exposing the cultures to white light. You can use red bulbs or LED lights to produce red light or cover white light sources or the cultures with red cellophane (Figure 5).

In addition to light intensity and quality, it is also important to be able to control photoperiod in your culture environments to simulate a day/night cycle. Some apparatuses, including commercial growth chambers, can control photoperiod intrinsically. If you are not using such an apparatus, we highly recommend connecting your lighting to a light timer so the lights will switch on and off automatically at set times.

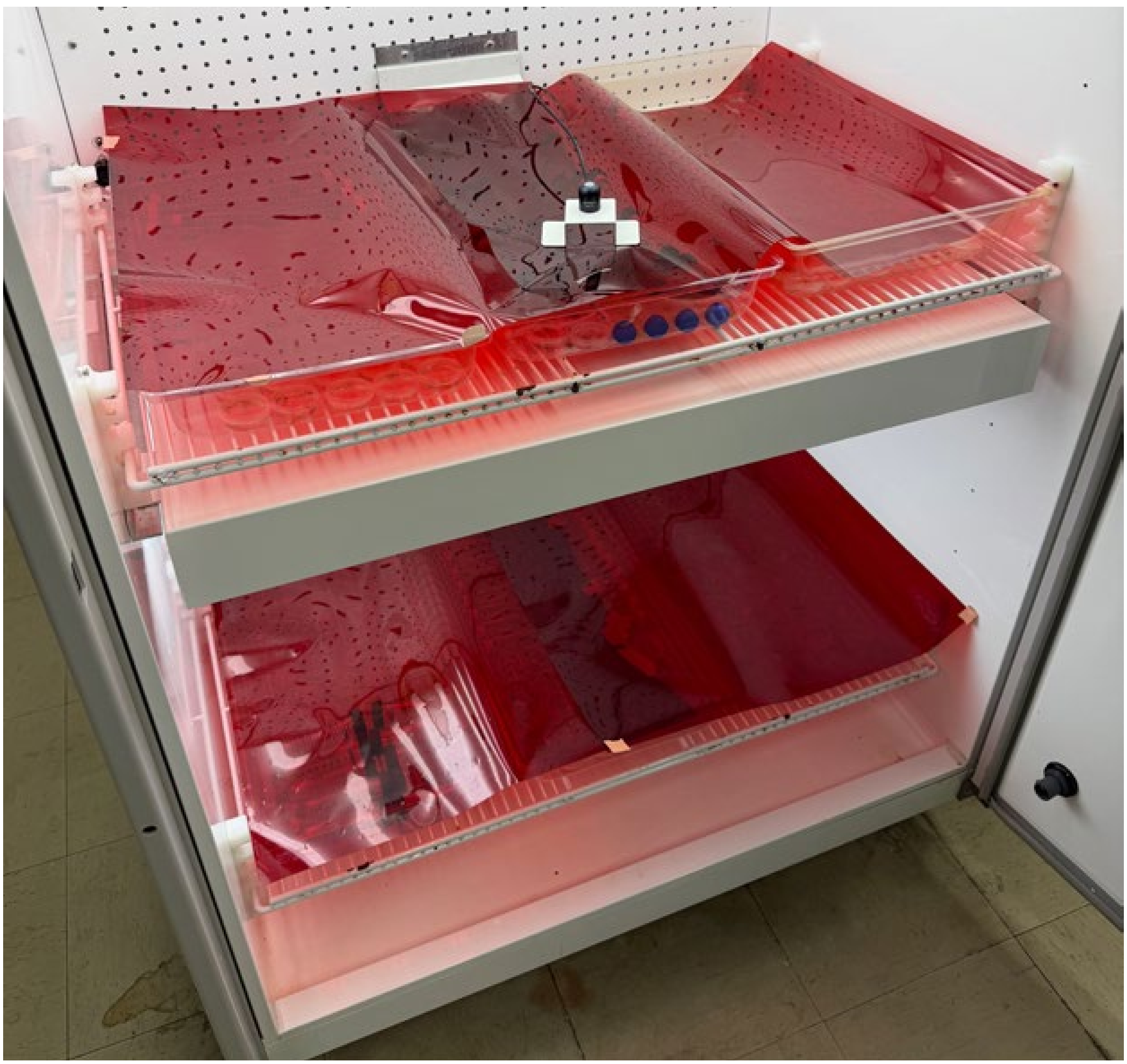


Figure 5. A modest collection of kelp gametophytes stored in a commercial growth chamber under red cellophane.

3.1.1 LABORATORY WORKSPACE

In addition to culture environments for housing kelp samples, kelp biobanking requires regular use of a lab space for actively working with your collection. Essentially all tasks described after this point in this handbook other than field work will take place in your lab. The main purposes of having a dedicated lab space for conducting your biobanking tasks are safety and cleanliness. Labs have features that help mitigate safety hazards that come with conducting some of the tasks described later in this handbook. They are also to be kept very clean and come with fixtures that will help you protect your cultures from contamination.

If possible, we recommend finding a lab at a university, government facility, or similar space that you can work in. It is also advantageous if your culture environments can be kept close to your lab space. Once you find a lab, ensure you get a proper safety orientation from appropriate personnel familiar with the space before you begin using it. The lab should have all typical safety features, including a fire extinguisher, eye wash station, safety shower, fumehood, chemical spill kit, sharps and glass disposal, and appropriate storage spaces for chemicals. Always follow all safety guidelines associated with the space.

In addition to basic safety equipment, we recommend that your lab have a biosafety cabinet or laminar flow hood (Figure 6). These are apparatuses that use air currents to create a space within which you can expose sensitive materials to the air without contaminants getting into them. We recommend conducting all work involving sterile liquids and as much work involving kelp cultures as is feasible within one of these spaces. Ensure you are properly trained in the use of this equipment before using it. We also advise keeping the lab space in general as clean as possible. Wipe down all work surfaces with 70% ethanol before beginning and after finishing your work.

Figure 6. A biosafety cabinet.

3.2 SORUS COLLECTION

Required materials

- ✓ Field knife
- ✓ Cooler(s) with sufficient volume for amount of kelp tissue required
- ✓ Paper towel
- ✓ Ice packs
- ✓ Rubber boots and waders, if collecting from the beach
- ✓ Data sheet

Once you have your culture environments prepared, you can begin the process of establishing kelp gametophyte cultures. This process will generally begin with the collection of sori from mature sporophytes of the target species in the field (Figure 7). In bull kelp, sori can often be found as early as June, but are most numerous between September and October (Foreman, 1984). *Saccharina latissima* shows peak sorus formation between October and December (Flavin et al., 2013). The sori can be recognized as darkened patches on the kelp blades (Figure 8) and should be collected when they are as mature as possible. In bull kelp, mature sori will be very dark and have a noticeable light edge around them, whereas less developed sori will be lighter in colour. Bull kelp also develops sori in rows along the lengths of its blades; the most mature sorus on a given blade will always be the one furthest from the pneumatocyst. Sorus-bearing blades of many species can be accessed from the beach during low tide, or by boat. However, for some species, such as *Macrocystis*, the use of scuba divers may be required to access the sori, as the sorus-bearing sporophylls will only be found deep underwater near the kelp holdfasts. Note that only trained scientific or commercial divers should be enlisted to collect sori underwater. In British Columbia, collection of wild kelp tissue, including sori, is regulated at the provincial level by the BC Aquatic Plant Program. It is important to review these regulations before you harvest any wild kelp to ensure you are obeying local laws.

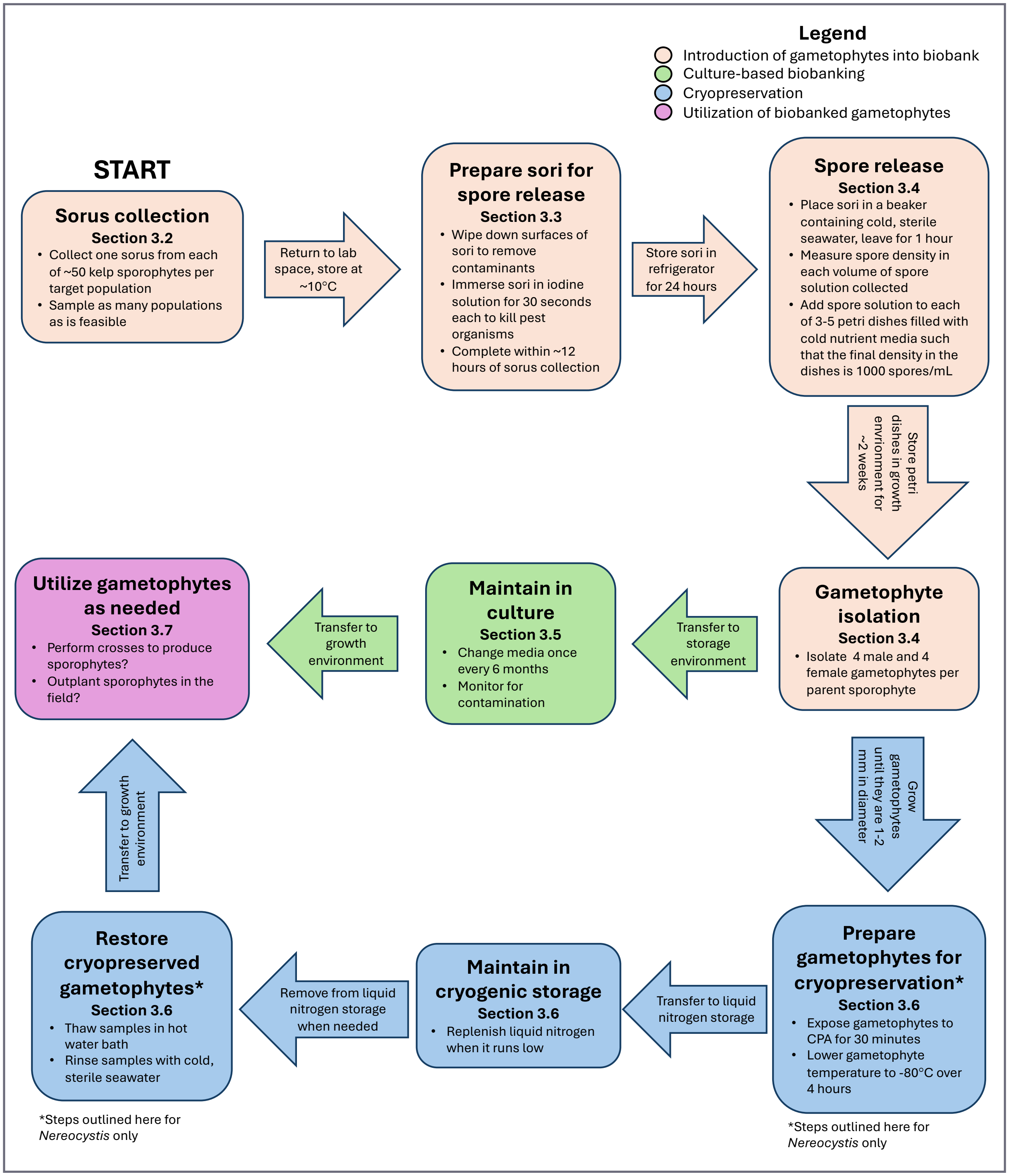


Figure 7. The kelp biobanking workflow.

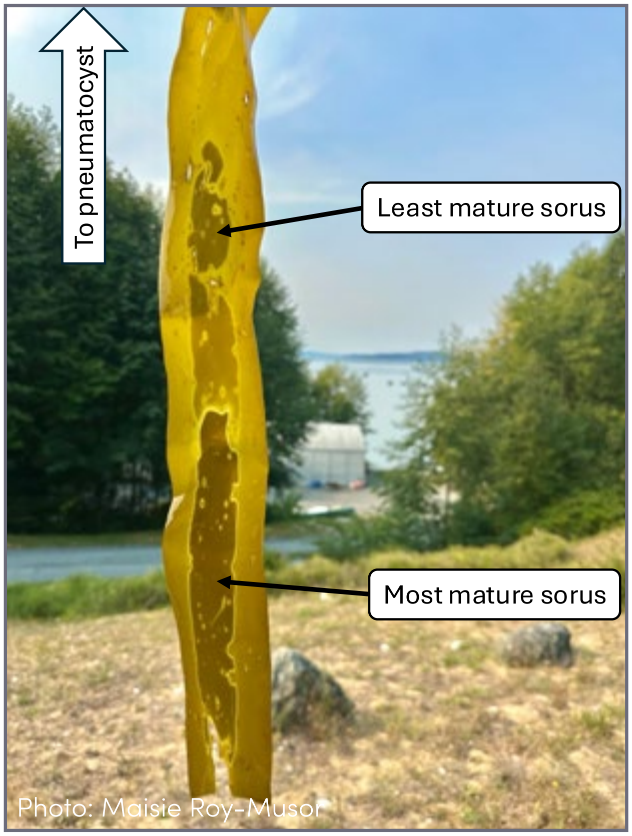


Figure 8. Sori of bull kelp (*Nereocystis luetkeana*). In this species, sori develop in rows along the length of blades and the most mature sori will be found furthest from the pneumatocyst.

To gather sori, cut the blade bearing the sorus off the kelp thallus with a knife, leaving the rest of the thallus intact and still attached to the substratum. Excess non-reproductive blade tissue can be discarded, preferably back into the ocean. We recommend sampling a single sorus from each of approximately 50 individuals to capture most of the genetic diversity present in the population (Alberto, 2023; Gierke et al., 2023). Note that this guideline is an approximation and does not guarantee that all genetic diversity will be captured for all species and populations. What constitutes a population can only be truly determined through genetic data (e.g. Bemmels et al., 2024; Gierke, 2019), but in the absence of specific population information, we suggest treating the recommended 50 samples as being representative of all individuals of the target species found within a 50 km radius.

As you collect the sorus-bearing blades, place them in a cooler and lay paper towel dampened with seawater between each blade collected to retain moisture; do not fill the collection cooler with water. It is critical to keep the samples cool as you work, as kelp tissue begins to experience stress and eventually dies as temperatures near about 20°C. To this end, we recommend including cold packs in the cooler with the collected samples and maintaining the samples at a temperature of as close to 10°C as is feasible. Care must also be taken to not freeze the samples, as that would also damage the sorus tissue.

Once you have collected the required sori, transport them to a secure storage location, such as a refrigerator, as quickly as possible such that they do not overheat or dry out. Be sure to record as much information about your sampling as possible, including exactly where and when you sampled the sori, how many sori were collected, what species were collected, and other details before concluding your fieldwork (see Appendix 3 for a sample data sheet). We recommend printing the data sheet found in Appendix 3 on waterproof paper and bringing it into the field with you when you venture out to collect.

3.2.1 GENETIC DIVERSITY CONSIDERATIONS

When planning sampling for biobanking, population genetics of the target species should be considered. A biobank will be most useful when it has as much genetic diversity safely stored in it as possible. While kelp population genetics in BC is not fully understood and requires more research, studies on bull kelp and giant kelp have shown that there are several distinct genetic populations of both species in different regions of BC's coast (Bemmels et al., 2024; Gierke et al., 2023). In bull kelp, (1) the west coast of Vancouver Island, (2) the Strait of Juan the Fuca and southern Strait of Georgia, (3) the northern Strait of Georgia, (4) Puget Sound, (5) the Broughton Archipelago, and (6) the central and north coast of mainland BC and Haida Gwaii form distinct populations (Bemmels et al., 2024). In giant kelp, distinct populations have been identified in (1) west coast of Vancouver Island, (2) the Broughton Archipelago, (3) the central and north coast of mainland BC, and (4) Haida Gwaii (Bemmels et al., 2024). We recommend ensuring that as many distinct populations and as much genetic diversity as possible are represented in biobanked collections of these species. Research is needed on population genetics of BC's other kelp species.


Photo: Nicole Christiansen

3.3 PREPARING SORI FOR SPORE RELEASE

Required materials

- [x] Cooler(s) containing kelp sorus tissue
- [x] Razor blades
- [x] Cutting board
- [x] Several rolls of paper towel
- [x] Nitrile or latex gloves
- [x] Chilled sterilized seawater (10°C)
- [x] Several large (~1 L) beakers or similar containers
- [x] 5 mL/L solution of Betadine® diluted in sterilized seawater
- [x] Several pairs of tongs or tweezers
- [x] Squirt bottle
- [x] Refrigerator
- [x] Ice bucket

Once kelp sori are collected, the next step in preparing kelp gametophyte cultures is to induce the release of spores from sori. This process takes at least two days, possibly more if the initial attempt at spore release fails. On the first day, the sori are prepared for release, while actual spore release takes place on the second day. The task of preparing sori for spore release can be laborious and we strongly suggest working in a team of at least two to conduct this work. Sori should be prepared for spore release as soon as possible, ideally within a matter of hours, after collection.

When you are ready to prepare sori for spore release, begin by assembling the required supplies and setting up your work area; you will want to have everything ready to go before you begin processing tissue so the work can proceed quickly. We suggest doing this work on lab benches on account of how much space it requires — it is not necessary to do this in a biosafety cabinet or laminar flow hood.

> ★ **Remember, it is important to prevent sorus tissue from getting too warm when preparing it for spore release; keep all tissue in the collection cooler with ice packs or in a refrigerator as much as possible and limit the time spent processing an individual sorus to no more than 5-10 minutes.**

It is also important to keep sorus tissue as clean as possible throughout the process to minimize the chances of contaminating the spore solution that will be produced with unwanted pest organisms. Start the process with a clean work bench, wipe down the workspace with ethanol before beginning, and wear gloves throughout the sorus prep procedure.

*** Establishing and maintaining kelp gametophyte cultures frequently calls for the use of sterile seawater. Where possible, we recommend the use of natural seawater. However, if this is not an option for you, artificial seawater made with Instant Ocean® can be used as a substitute. To sterilize the seawater, the use of an autoclave is recommended where possible. These apparatuses are commonly available in universities or similar facilities. However, if autoclaves are not an option for you, pasteurization, filtration, or UV sterilization can be viable alternatives (Flavin et al., 2013). It is important to use some form of sterilization on your seawater, as contamination of a kelp culture with pest organisms can ruin the culture. Once you have sterilized your seawater one way or another, it is important to keep it sealed and not exposed to the air to maintain sterility. All lab work involving sterile seawater should take place in a biosafety cabinet or laminar flow hood whenever possible.**

To prepare a sorus for spore release, follow the steps below:

1. Remove sorus from cooler and use a razor blade to cut off all vegetative tissue, leaving only sorus tissue. Use the cutting board for this.
2. If the sorus is fouled by **bryozoans**, sessile animals that form white, ornate-looking crusts on kelp (Figure 9), use the razor blade to scrape them off.
3. Use a dry paper towel to firmly wipe both sides of the sorus 3-4 times each to remove mucilage, debris, and possible contaminants. Do not re-use paper towels for multiple sori to avoid cross contamination.
4. Use tweezers to pick up the sorus and dip it into the 5 mL/L Betadine® solution for about 30 seconds. This will kill bacteria and other possible contaminants while inflicting minimal damage to the sorus tissue. We recommend keeping the Betadine® solution in an ice bucket throughout the sorus prep procedure to avoid warming the tissue.
5. Remove the sorus from the Betadine® solution and use a squirt bottle filled with chilled sterilized seawater to rinse as much of the residual Betadine® from the tissue as possible. Continue until water dripping off the tissue is clear and no longer orange.
6. Use dry, clean paper towel to firmly wipe both sides of the sorus until it is dry. Do not re-use paper towel.
7. Lay the sorus down on a dry, clean sheet of paper towel and cover it with another clean sheet. Use the squirt bottle to slightly dampen the sorus and the sheets of paper towel with seawater. Move the sorus tissue and paper towel to a cool location, such as a refrigerator or your growth environment, for storage while you prepare the remaining sori.
8. Repeat steps 1-7 for all remaining sori. Sori sandwiched between sheets of paper towel can be stacked while being held in the refrigerator.
9. Leave all sori in the cool environment for about 24 hours. If there is any ambient light in the space, the sori should be covered such that they receive no light.

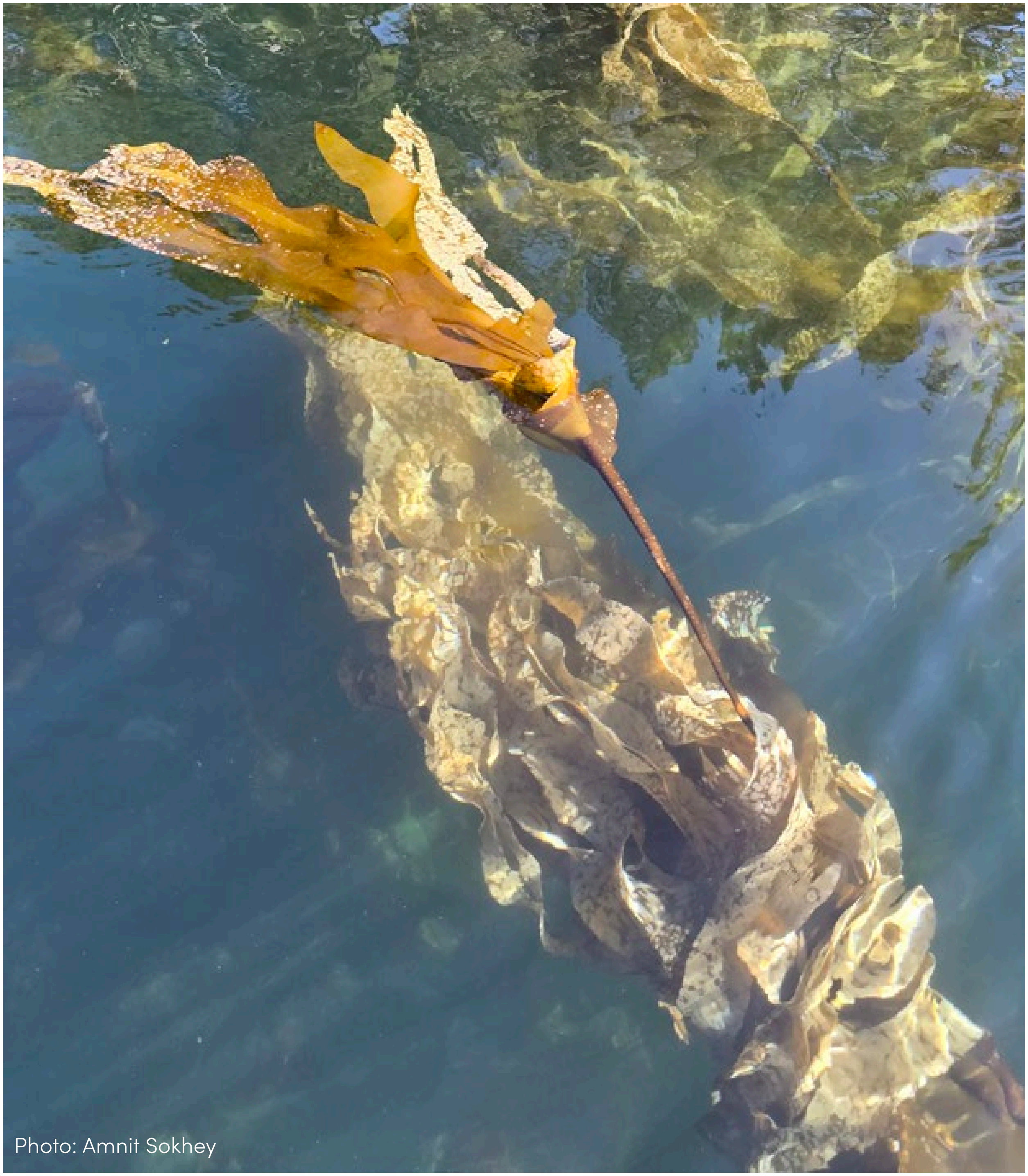


Figure 9. The encrusting bryozoan *Membranipora membranacea* on kelp blades.

3.4 SPORE RELEASE AND CULTURE INOCULATION

Required materials

- ☑ Prepped kelp sorus tissue
- ☑ Clean ~1 L beakers
- ☑ Chilled sterilized seawater (~10°C)
- ☑ Nutrient media
- ☑ 20 µL micropipette and tips
- ☑ 1000 µL micropipette and tips
- ☑ Serological pipettes and pump
- ☑ 30-60 mm petri dishes
- ☑ Fine-tipped permanent marker
- ☑ Parafilm® wax
- ☑ Latex or nitrile gloves
- ☑ Tongs or tweezers
- ☑ Spatulas
- ☑ 70% ethanol
- ☑ Growth chamber or refrigerator
- ☑ Submersible thermometer
- ☑ Compound microscope
- ☑ Dissecting microscope
- ☑ Hemocytometer and paired coverslip
- ☑ Calculator
- ☑ Tally counter
- ☑ Lens paper
- ☑ Lens cleaner or 70% ethanol solution
- ☑ Refractometer
- ☑ Pasteur pipettes and bulbs
- ☑ 24-well plates

Before attempting to release spores from prepared sori, you should prepare culture vessels for your gametophyte cultures. We recommend using 30-60 mm petri dishes at this stage, as this will make later steps of the culture establishment procedure simpler. You will need a minimum of one dish for each unique spore solution you make, but we suggest preparing three to five dishes for each solution to maximize the probability of getting a good inoculation with no contamination. Begin by using a permanent marker to label all dishes with the species of kelp gametophyte they will house, the density of the final spore solution (we recommend 1000 spores/mL), the date you inoculate the dish with spores, the location you collected the sori from, and the individual kelp the spores were derived from. Then, use serological pipettes to fill each dish with sterile culture media.

★ Media for culturing kelp is seawater enriched with nutrients to promote growth. Many media formulations have been designed for different purposes, but we recommend either half strength Guillard's solution (f/2 medium; Guillard, 1975; Guillard & Ryther, 1962) or Provasoli Enriched Seawater (PES; Provasoli, 1968) for kelp culture — both will work well for kelp. Stock solutions of these media can be purchased, or they can be made from scratch if the ingredients are available to you. Recipes can be found in Andersen (2005). Purchasing stock solutions is convenient and saves labour, but it is generally more cost effective to make media from scratch, especially when large amounts are required. If you opt to use Guillard's solution, we advise excluding silicate from the formulation. Whether you are making media from a purchased stock solution or from scratch, you will be required to add chemicals to sterile seawater. As such, we advise always mixing your media in a biosafety cabinet or laminar flow hood to maintain sterility. The salinity of all culture media should be between 30 and 35 ppt; this can be measured with a refractometer.

After filling your dishes, keep them chilled until you are ready to inoculate them with spores.

Kelp sori that have been prepped for spore release should be ready to release spores after being held in a refrigerator at about 10°C for about 24 hours. At this point, remove the sori from the fridge and inspect them visually. Sori that are ready to release spores will likely have left brown stains on the paper towel within which they are sandwiched and may have taken on a patchy appearance (Figure 10). These are signs that the sori have already begun releasing spores and are therefore in a good state to continue the procedure. If there is no evidence of this staining, place the sori back in the fridge for another 24 hours. Continue to check sori that do not appear ready to release about every 24 hours until they either look ready or appear to be decomposing — at this point they should be discarded. Dead kelp tissue often appears green. It is entirely possible that some sori may not be able to be induced to be released; this may indicate that they were not developed enough when collected.

Figure 10. Brown stains on paper towel like this indicate that kelp sori are ready to release spores.

To induce spore release in ripe sori, follow the following steps:

1. Use tongs or tweezers to place a sorus in a container (e.g. a clean ~1 L beaker) filled with chilled sterilized seawater. If a single sorus is too large to be completely submerged in its container, it is ok to cut it into smaller pieces. For purposes of biobanking, we recommend allocating each sorus to its own container.

*** Releasing spores from sori into separate volumes of water will ultimately allow you to make cultures of isolated individual gametophytes, termed "isolates", with known parentage. This approach is recommended because it will allow for maximal preservation of the biodiversity in your samples and can also help limit contamination. However, releasing spores from many sori into separate volumes of water is a very labour-intensive process and the number of isolate cultures the approach will ultimately yield (up to 400 per population if all guidelines presented here are followed) can be challenging to maintain, especially if you have a smaller operation with more limited resources. If the amount of labour presented by the gametophyte isolate approach is infeasible, an alternative method is to release spores from all sori into a single large volume of water. This will yield what we term a "mixed culture" of gametophytes that contains individuals from many parents. If using this approach, the method of releasing spores described here is the same except you may wish to prepare more than five petri dishes before beginning and step 11 of the protocol is omitted. Note that while the mixed culture approach can be easier than the isolate approach, it also presents issues that can limit its usefulness for purposes of biobanking. Firstly, it will be impossible to know exactly how much biodiversity is present in a mixed culture since each gametophyte will not be able to be traced back to a parent. Secondly, it is almost certain that the full breadth of biodiversity present in your original sorus samples will not ultimately make it into your plated cultures. Thirdly, anecdotal reports suggest that when mixed cultures are maintained for longer periods of time (ie. multiple years), selection can occur between individual gametophytes, potentially eliminating some of the biodiversity that was originally present (research is needed to better understand how this occurs). Finally, but importantly, if there is any contamination present when first releasing spores for a mixed culture, it will likely make its way into all the plates that are made from that spore release. For these reasons, we advise making gametophyte isolates as opposed to mixed cultures whenever feasible, but making a mixed cultures is still preferable to not biobanking any kelp at all.**

2. Transfer the sori to a growth chamber (at an ambient temperature of approximately 10°C) or refrigerator and leave them there for one hour. Stir the sori every 5-10 minutes with a spatula throughout this period; do use the same spatula to stir multiple beakers without rinsing it with fresh water and wiping it down with 70% ethanol first. If the sori are releasing spores as intended, within minutes of being immersed in the seawater, you should start to see the water begin to turn cloudy or brown. This indicates that spores have been released and are now suspended in the water. Even if you do see evidence of spores being released quickly, we recommend leaving the sori in the water for the full hour to capture as many spores as possible.

3. After one hour has elapsed, inspect the containers containing sori. If water appears very cloudy or brown, the spore release has succeeded, and you can proceed to step 4 (Figure 11). If water does not look at all discolored, it is likely the spore release has failed. At this point, you may be able to retrieve the sori from the water, rinse them with fresh sterile seawater, dry them with paper towel, and return them to the refrigerator to await a second attempt at spore release in 24 hours. If sorus tissue appears green or is disintegrating, it should be discarded.

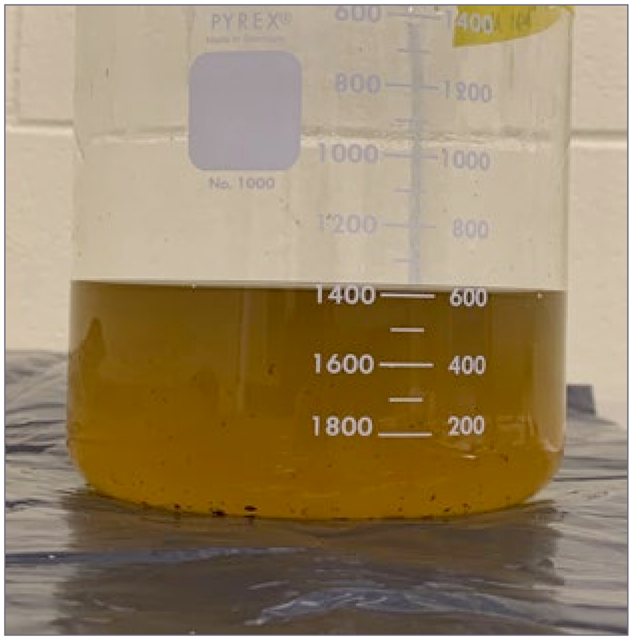


Figure 11. Heavily brown-stained water indicates that kelp spores have been successfully released.

4. Remove all sori from the water and discard the tissue. Retain the raw spore solutions and return them to the growth chamber or refrigerator to maintain them at a cool temperature.

5. Use a **hemocytometer** to measure the density of spores in the seawater for each solution produced (see Appendix 4 for further instructions).

6. Once spore density is measured, use the following equation to solve for V_1

$$V_1 = \frac{C_2 V_2}{C_1} \qquad \textit{Equation 1}$$

where V1 is the volume of raw spore solution needed to inoculate the culture dish (in mL), C2 is the intended final spore concentration of the culture solution, V2 is the final volume of the intended culture dish (in mL), and C1 is the measured spore density of the raw spore solution (in spores/mL). A final concentration of 1000 spores/mL is a good target to get a strong gametophyte culture while minimizing the chance of contamination. If you have a spore density of under 1000 spores/mL, you can proceed, but you may yield fewer or even no gametophytes.

7. Use a 1000 µL micropipette to add the calculated volume of spore solution from step 6 to each of your now media-filled culture dish(es) designated for that solution.

8. Seal your culture dishes with Parafilm® wax and transfer them to your gametophyte growth environment. At this stage, the cultures should not be exposed to light while spores are settling. Turn off the culture lighting or completely cover the dishes with something opaque, such as aluminum foil, for the first 24 hours after inoculating them.

9. After 24 hours have elapsed, uncover the cultures. At this point, they should be exposed to 30-45 µmol/m^2/s red light on a 12:12 hr photoperiod.

10. Leave the inoculated dishes in the growth environment for approximately two weeks.

11. After about two weeks of growth, young individual gametophytes should be visible under a dissecting or compound microscope (if you do not yet see gametophytes at this point, return the cultures to the growth environment and give them another one to two weeks to grow). At this time, use a Pasteur pipette and a microscope to carefully draw up individual gametophytes from culture dishes and transfer each to its own well in a new media-filled, chilled, labeled 24-well plate. You will need to isolate at least two male and two female gametophytes derived from each of your originally sampled kelp sporophytes to capture the breadth of genetic diversity present in your cultures. However, we suggest isolating four males and four females to account for the possibility of culture failures. While gametophyte morphology varies in different kelp species, female gametophytes of a given species generally have noticeably larger cells than males of the same species (Figure 12).

12. Once all gametophytes are transferred to well plates, seal the plates with Parafilm®. Then, if you are using culture-based biobanking, transfer filled plates to your storage environment. If you are intending to cryopreserve the gametophyte isolates, instead transfer them to the growth environment. Once all required gametophytes are isolated, the original culture plates are no longer needed and can be discarded or used for other purposes. If you are in contact with other kelp practitioners, we would encourage you to reach out to them and see if they can use surplus gametophytes before discarding them.

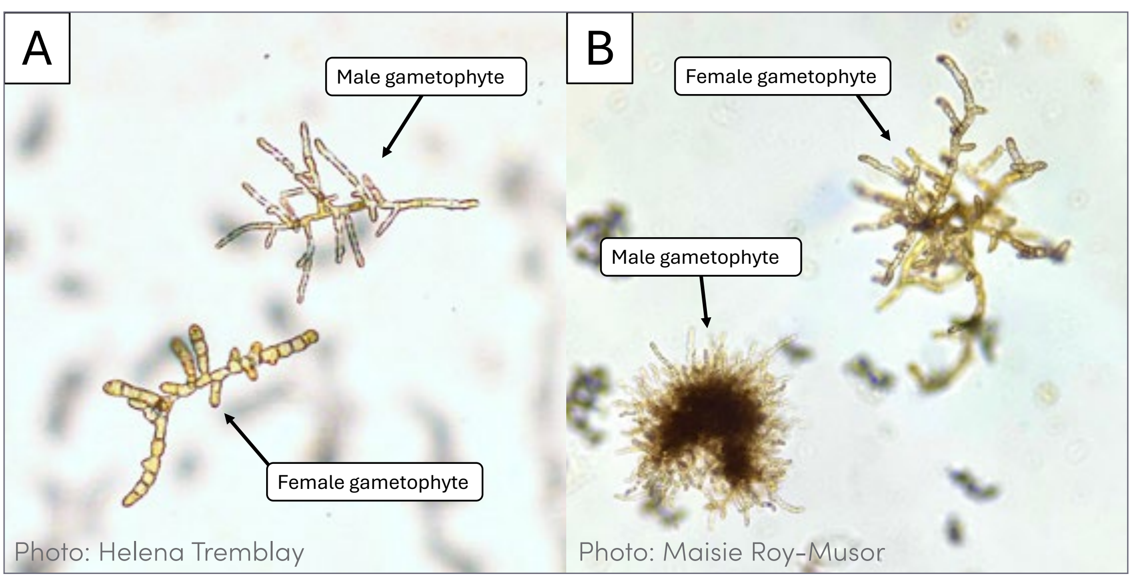


Figure 12. Male and female gametophytes of (A) bull kelp and (B) giant kelp.

3.5 BIOBANKING OF GAMETOPHYTE CULTURES

If you have been following the steps described throughout section 3.2 of this handbook and opted to transfer your gametophyte isolates to a storage environment in step 12 of section 3.4, you should now have a collection of individual kelp gametophytes, each housed in their own wells in 24-well plates, securely held in a culture environment optimized for long-term storage. Congratulations – you now have a culture-based kelp biobank! Your cultures can be kept in these conditions indefinitely. Since you have individual gametophytes isolated, you have single known genotypes accessible, which is very useful for genetic research and breeding. You can also repeat the steps described in section 3.2 of this handbook to add new genotypes or species to your culture collection as needed.

3.5.1 CULTURE MAINTENANCE

Now that you have your biobank started, it will require regular maintenance, the most important form of which is media changes. Assuming you are maintaining your storage environment under the conditions indicated in Table 3, you should only have to change media once every six months. Note that kelp gametophyte cultures maintained at higher temperatures, higher irradiance, or longer day lengths will need to have media changed more frequently. To perform a media change, you will need to remove and discard as much of the old media as possible, then replace it with fresh media. As you work, you must avoid cross-contaminating media from different cultures. We recommend performing media changes in a biosafety cabinet or laminar flow hood if one is available to you and utilize sterile technique when working.

Over a period of weeks to months after starting your biobank, your gametophyte cultures will visibly grow. Before long, they will likely take on the appearance of discrete dark brown balls or "pom poms" of up to several mm in diameter (Figure 13). They may attach to a surface of their wells, or they may tumble freely. Healthy kelp gametophytes are dark brown in colour; if you see any becoming pale or green, it is a sign that they are not healthy and there may be something wrong in their culture environment. As you observe your cultures, it is also important to watch for signs of contamination.

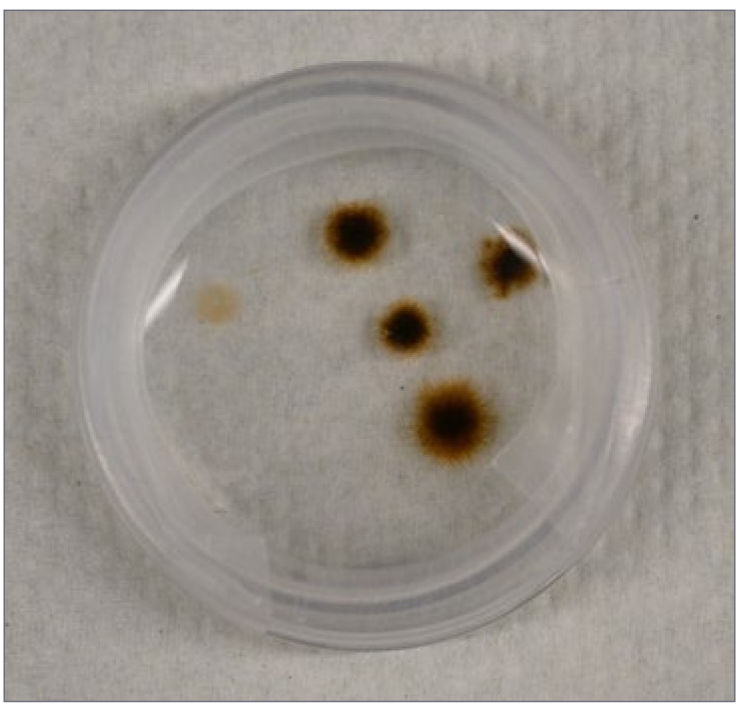


Figure 13. Healthy, well-developed bull kelp gametophytes.

Pest organisms in your culture can quickly overtake your gametophytes and can ultimately kill them by smothering them or outcompeting them for resources. The most common and insidious pests in kelp culture are **diatoms**, unicellular algae that are naturally abundant in the water where kelp grow. These **microalgae** can live suspended in the water or on the surfaces of rocks and seaweeds. Diatoms and kelp share some photosynthetic pigments, notably **fucoxanthin**, the pigment that gives kelp their distinct brown colour. As such, diatoms and kelps can be about the same shade of brown, which can sometimes make it difficult to tell whether you are looking at kelp gametophytes or an abundance of diatoms when observed with a naked eye (Figure 14A). When observed under a microscope, however, diatoms have distinctive, highly geometric appearances that can be easily differentiated from the filamentous forms of kelp gametophytes (Figure 14B). If you experience diatom contamination, one possible way of eliminating them is to add germanium dioxide (GeO^2) to your culture media. This is a chemical that inhibits the growth of diatoms by interfering with the synthesis of their silica cell walls. If you do opt to use it, we recommend including it at a concentration 0.1 mL of saturated GeO^2 stock solution per L of media; we don't advise exceeding this concentration, as this may start to have negative effects on the kelp (Shea & Chopin, 2007). Other common pests that can be found in kelp cultures include **cyanobacteria** and **green algae** of the genus *Ulva*; these can both be easily recognized by their cyan or green colour.

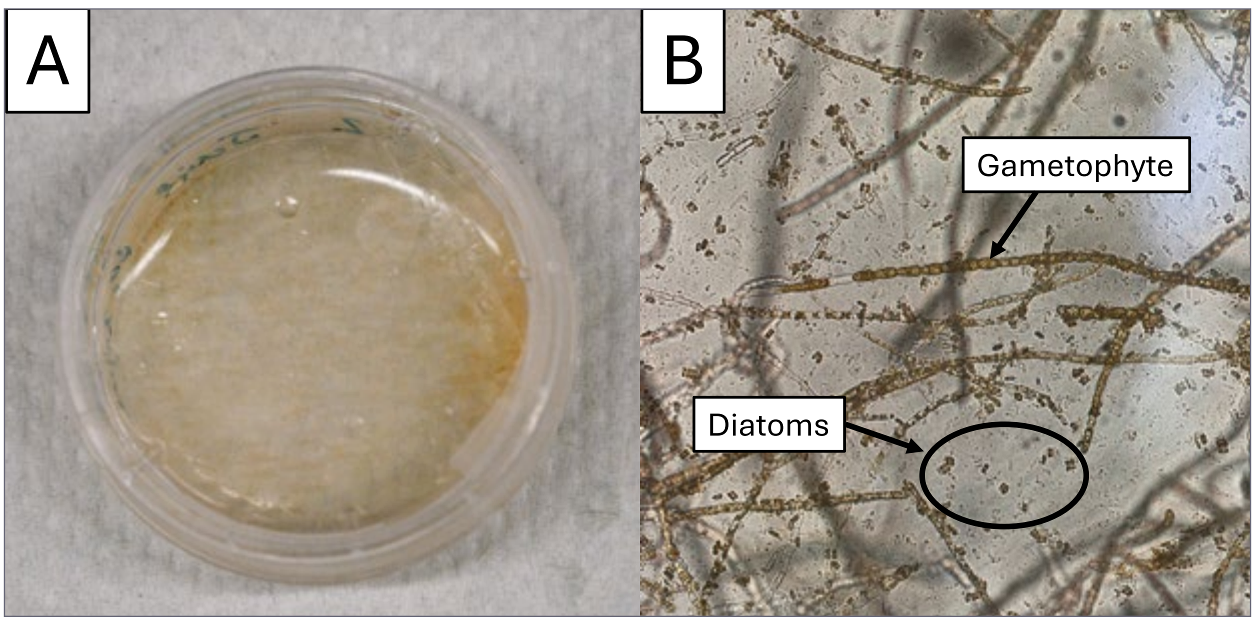


Figure 14. (A) Macroscopic and (B) microscopic view of a kelp gametophyte culture heavily contaminated with diatoms.

As your gametophytes continue to grow, they may eventually become too large to continue to maintain in their original 24-well plates. At this point, we suggest transferring such individuals to larger culture vessels. Which kind of vessel you use is a matter of preference. Petri dishes are useful because they can be stacked, provide good light exposure, and are both inexpensive and readily available (Figure 15A). However, they don't have screw-on lids, which necessitates use of Parafilm® wax to seal them, which can make media changes take longer. Centrifuge tubes, also sometimes called Falcon® tubes, do have screw-on lids, which makes it very quick and easy to change media (Figure 15B). However, they do not stack, which can make it more difficult to pack many of them into a small space, and they are more expensive than petri dishes. Finally, culture flasks combine the best attributes of petri dishes and centrifuge tubes, in that they are both stackable and have screw-on lids, maximising ease of both storage and changing media (Figure 15C). However, they are more expensive than either petri dishes or centrifuge tubes

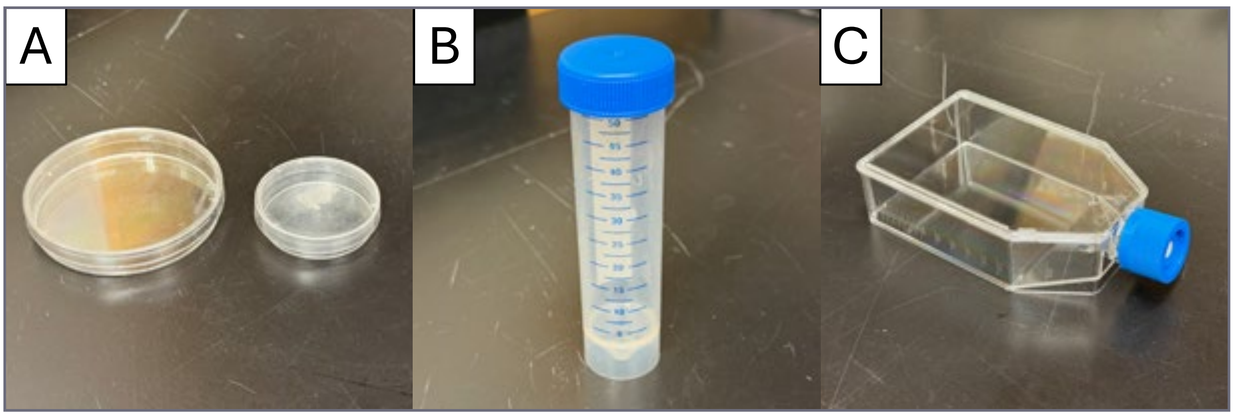


Figure 15. (A) 100 mm (left) and 60 mm (right) petri dishes, (B) a 50 mL centrifuge tube, and a 225 cm^2 culture flask.

3.5.2 POWER AND SYSTEM FAILURES

The survival of your cultures depends on maintaining the specified environmental parameters. As such, it is critical that your lighting and temperature control systems remain functional. We recommend checking on your cultures as regularly as is practical to ensure that your system has not lost power or otherwise malfunctioned. Some culture environments, such as commercial growth chambers, have alarm systems built in to warn users of a system failure such as a temperature spike. Additionally, some practitioners make use of novel systems that will send alerts to a mobile device in the event of a system failure. If you are housing your biobank in a busy setting such as a university, you may be able to enlist other people working near your space to help you monitor your biobank or alert you in the event of a system failure. Additionally, we strongly recommend having at least one if not multiple alternate power sources such as generators that can activate automatically in the event of a power loss, especially if you are working in a remote area where power outages may be more frequent.

If a system failure does occur, it is not necessarily the end of the world. From the point that light and temperature control are lost, you have a window of opportunity within which you can save your cultures. Kelp gametophytes are somewhat resilient to short spikes of high temperature and will generally survive at least a few hours (the exact length of time may vary between species or even populations) exposed to temperatures between 20 and 25°C. Even if some cells die in a temperature spike, if the culture is returned to a cold environment quickly enough, the culture may ultimately rebound. Gametophytes can also survive multiple days with no light if temperatures remain normal (ie. high temperature will kill kelp gametophytes more quickly than a lack of light). If you cannot restore your culture system to working order quickly, we recommend temporarily relocating your cultures. A refrigerator can be a good short-term refuge for kelp cultures, even if the internal temperature is slightly lower than the kelp would normally be maintained at – it just must not be at or below 0°C, as the kelp tissue will die if it freezes. If only the temperature control system in a growth chamber malfunctions but the lights remain on, remove your cultures as quickly as possible, as this will cause the chamber to heat up very rapidly.

Given that system failures do happen and that the consequences can be catastrophic, if you have the resources and ability, we strongly recommend having redundant cultures of as much of your kelp collection as possible, ideally in a different location from your main biobank. Making such cultures simply entails mechanically isolating fragments of your individual gametophytes and transferring them to new dishes or tubes. These can then be taken elsewhere and placed in a new culture environment. A good way of facilitating backup cultures is to collaborate with another practitioner that has their own biobank. You can then share redundant copies of each other's cultures.

3.6 CRYOPRESERVATION

Here, we present a cryopreservation protocol for bull kelp gametophytes, based on that described in Coleman et al. (2025). This protocol has not been tested in other kelp species at time of writing and should not be assumed to be effective for anything other than bull kelp gametophytes. For protocols shown to be effective in other kelp species, refer to Table 1. This protocol should be used with bull kelp gametophytes of at least 1-2 mm in diameter, which should be possible a few weeks after completing up to and including step 12 in section 3.2.4 of this handbook. This is a slow-cooling protocol.

Required materials

- ☑ Individual kelp gametophytes of at least 1-2 mm in diameter
- ☑ Ethylene glycol stock
- ☑ D-sorbitol
- ☑ Sterilized seawater
- ☑ 1000 µL micropipette and tips
- ☑ Latex or nitrile gloves
- ☑ 2 mL internally threaded cryovials
- ☑ Fine-tipped forceps
- ☑ Ice bucket
- ☑ Timer
- ☑ At least one Mr. Frosty®
- ☑ -80°C freezer
- ☑ Freezer boxes
- ☑ Liquid nitrogen storage tank filled with liquid nitrogen
- ☑ Lab coats
- ☑ Safety goggles
- ☑ Face shield
- ☑ Cryogenic gloves
- ☑ Hot water bath
- ☑ Floating tube racks
- ☑ Squirt bottle containing chilled sterilized seawater
- ☑ Funnels
- ☑ Coarse grade filter paper
- ☑ Several Erlenmeyer flasks or beakers
- ☑ Culture vessels containing fresh, chilled media

To introduce bull kelp gametophytes into cryogenic conditions, follow the steps below:

1. Prepare a well-mixed solution of 10% ethylene glycol (v/v) and 9% D-sorbitol (w/v), dissolved in sterilized seawater. This is your cryoprotective agent (CPA) solution. Keep the solution chilled.
2. Allocate up to 1 mL of CPA solution to one 2 mL internally threaded cryovial for every gametophyte you intend to cryopreserve. Store the filled cryovials in an ice bucket. Ensure cryovials are well labeled so that you know which gametophytes will be stored in them.

* **Internally threaded cryovials are recommended over externally threaded ones because externally threaded cryovials have an increased risk of exploding when they are removed from liquid nitrogen, which both compromises your samples and can injure you.**

3. Use fine-tip forceps to manually remove gametophytes you intend to cryopreserve from their culture vessels and gently place them into CPA solution in cryovials. One gametophyte should be allocated to each cryovial. Do not shake or stir the cryovial once the gametophyte is introduced. Close the lids on the cryovials containing gametophytes and carefully place them back on ice.
4. Leave each gametophyte in CPA solution for approximately 30 minutes. Use a timer to keep track of the time.
5. After 30 minutes has elapsed, carefully take the cryovials and transfer them to slots in a Mr. Frosty®. Place the Mr. Frosty® in a -80°C freezer.
6. Leave the Mr. Frosty® in the freezer for four hours.
7. After four hours have elapsed, remove the Mr. Frosty® from the freezer and as quickly as possible transfer the cryovials to a freezer box and place the box in a liquid nitrogen storage tank. Ensure the box is well labeled so you know where to find specific samples. Note: liquid nitrogen is very dangerous and can cause severe burns upon contact with your skin. Wear full personal protective equipment (PPE), including a lab coat, cryogenic gloves, safety goggles, and a face shield whenever working with liquid nitrogen. After introducing the samples into liquid nitrogen, they are in a stable state and can be left here indefinitely.

* **Liquid nitrogen held in a storage tank evaporates over time. For this reason, it is necessary to occasionally refill the tank with fresh liquid nitrogen. The frequency with which this must be done is variable and depends on the volume of the storage tank and how often it is opened. It is essential that the liquid nitrogen in your storage tank is not allowed to fully evaporate, or all samples held in the tank will be lost. We recommend using a storage tank equipped with an automatic monitoring system to make it easier to track when the tank needs to be refilled.**

To remove bull kelp gametophytes from cryogenic conditions, follow the steps below:

1. Fill a hot water bath and raise the temperature in the bath to 40°C.

2. Remove the box containing your samples from liquid nitrogen storage.

3. As quickly as possible, remove the cryovials containing the gametophytes you intend to thaw from the box, insert them into floating tube racks, and place the racks containing the cryovials into the hot water bath.

4. Watch the cryovials in the hot water bath closely. When you can see that almost all the ice in the vials has melted, remove the vials from the water bath and the tube rack and place them in an ice bucket. This should take less than 5 minutes from the point you introduce the cryovials into the water bath.

5. Set up an Erlenmeyer flask or beaker with a funnel in it and a sheet of coarse filter paper in the funnel. Prepare one of these units for each gametophyte you are thawing if possible.

6. Pour the contents of each cryovial through a piece of filter paper, being sure the gametophyte is caught in the filter paper.

7. Use a squirt bottle to rinse each gametophyte with chilled sterilized seawater for about 5 seconds, then let the water drain through the filter paper. Repeat this at least two more times. Use fine tip forceps to transfer the gametophyte back to a culture vessel filled with chilled media. If you do not have enough beakers/flasks and funnels to allocate unique ones to every gametophyte, then rinse the funnel and change the filter paper between gametophytes to prevent cross-contamination.

8. Return culture vessels containing thawed gametophytes to a growth environment. Keep these cultures in darkness for 24 hours after returning them to the environment. We suggest completely covering them with aluminum foil or other opaque sheet so the lights don't need to be turned off, which would affect other cultures in the environment.

9. Uncover the thawed gametophytes after 24 hours. We recommend leaving these cultures undisturbed for a few weeks after removing them from cryogenic conditions to ensure they are fully recovered from the stress of being thawed.

3.7 SPOROPHYTE PRODUCTION

If the intent behind your biobanking effort is simply to store gametophytes, you should now have all the basic information necessary to do that in perpetuity. However, if you plan on using your biobanked kelp for additional applications, which might include research, restoration, or aquaculture, it will eventually become necessary to close the life cycle of your stored gametophytes and produce sporophytes. The specifics of how you go about doing this will depend largely on your use case. For example, research or breeding projects might be able to take place entirely in laboratory conditions, while restoration or aquaculture will likely necessitate outplanting kelp in the field. These different settings lead to variability in the protocols for sporophyte production. Here, we provide example protocols for producing sporophytes from biobanked gametophytes (1) for use in a lab setting and (2) for outplanting. For the outplanting protocol, we will assume the use of a longline setup for growing kelp in the field, comparable to that described in Flavin et al. (2013). The protocols presented below are intended to be accessible and utilize commonly available equipment and materials; your individual use case may require the use of very different methods. Note that use of "bioreactors" manufactured by Industrial Plankton for culturing kelp gametophytes is increasing in popularity and can greatly simplify the processes described below. These devices are, however, very expensive and may not be accessible to all practitioners.

3.7.1 PRODUCING SPOROPHYTES FOR LABORATORY USE

Required materials

- ☑ Male and female gametophyte cultures
- ☑ 1.5 mL microfuge tubes
- ☑ Microfuge pestles or small blender
- ☑ Petri dishes
- ☑ Culture media
- ☑ 1000 µL micropipette and tips
- ☑ Serological pipettes and pump
- ☑ Culture environmen

Additional required materials if growing sporophytes to larger sizes

- ☑ 10-20 L glass carboy
- ☑ Stopper for carboy or Parafilm®
- ☑ Air pump, airline tubing, and air stone

To produce kelp sporophytes from biobanked gametophytes for laboratory use, follow the steps below:

1. Identify male and female gametophytes that you intend to cross. The amounts of biomass needed will depend on your use case, but for research use these amounts may be very small. The number of genotypes you use will also depend on your use case.

2. Isolate target gametophytes and, one individual at a time, remove them from culture and place them in either a 1.5 mL microfuge tube or a small blender with a small amount of chilled sterile seawater (approximately 0.5 mL for a microfuge tube).

3. If using a microfuge tube, use a microfuge pestle to manually fragment the gametophyte tissue. To do this, insert the pestle into the microfuge tube and use a mix of twisting and stirring motions to break up the gametophyte tissue. If using a blender, switch on the blender to fragment the tissue. In both cases, continue fragmenting until the seawater simply appears as a brown solution and no individual tissue particles can be seen (Figure 16).

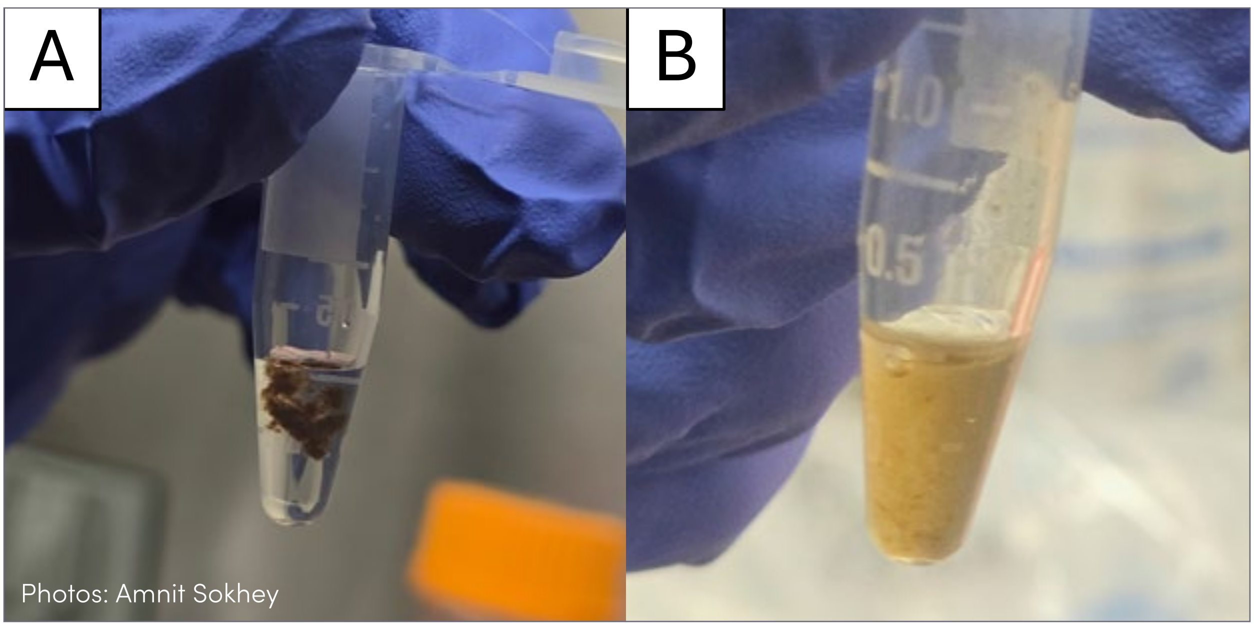


Figure 16. Gametophyte tissue (A) before fragmenting and (B) sufficiently fragmented.

4. Combine the fragmented male and female gametophyte solutions and mix them well.

5. Add the mixed gametophyte solutions to petri dishes filled with chilled sterile culture media and use Parafilm® to seal the dishes. The number of dishes needed will depend on your use case.

6. Introduce the petri dishes into a growth environment. Initially, the culture conditions should be mostly the same as for gametophyte biobanking (see section 3.4), except light should be white instead of red. This will provide the light cue required by the gametophytes to undergo gametogenesis and mate.

7. Gametophytes fragments will adhere to the surface of the culture dishes and begin mating within a few days of fragmentation. Over the next 1-2 weeks, small sporophytes will gradually become visible (Figure 17). Depending on your needs, you may only require sporophytes to grow to a few mm in length before their purpose is fulfilled. If you need sporophytes to last longer and grow to larger than a few mm in length, you will eventually need to move them to a larger culture vessel, likely within approximately two weeks of zygote formation. To keep sporophytes longer than a few millimetres alive, we recommend transferring them to a 10-20 L glass carboy filled with culture media. Sporophytes can be gently scraped off their original petri dishes and dropped into the new vessel. At this stage, introduce a small amount of water movement using an air pump connected to an airline and an air stone. Kelp sporophytes more than a few millimetres in length will die quickly if there is absolutely no water movement in their environment. Additionally, increase irradiance in the environment to 150 $\mu mol/m^2/s$. Ensure the opening of the carboy is closed with a stopper or Parafilm®. As air is pumped into the carboy, the kelp sporophytes will tumble freely, hence the name "tumble culture" for this kind of kelp culture configuration. Media should be changed every 1-2 weeks for this setup. Note that kelp sporophytes can grow very quickly and under favourable conditions it will take only a couple of months for them to grow from zygotes to sizes that will not be feasible to maintain in laboratory environments without very large culture setups (i.e. tanks with volumes measured in hundreds or even thousands of litres).

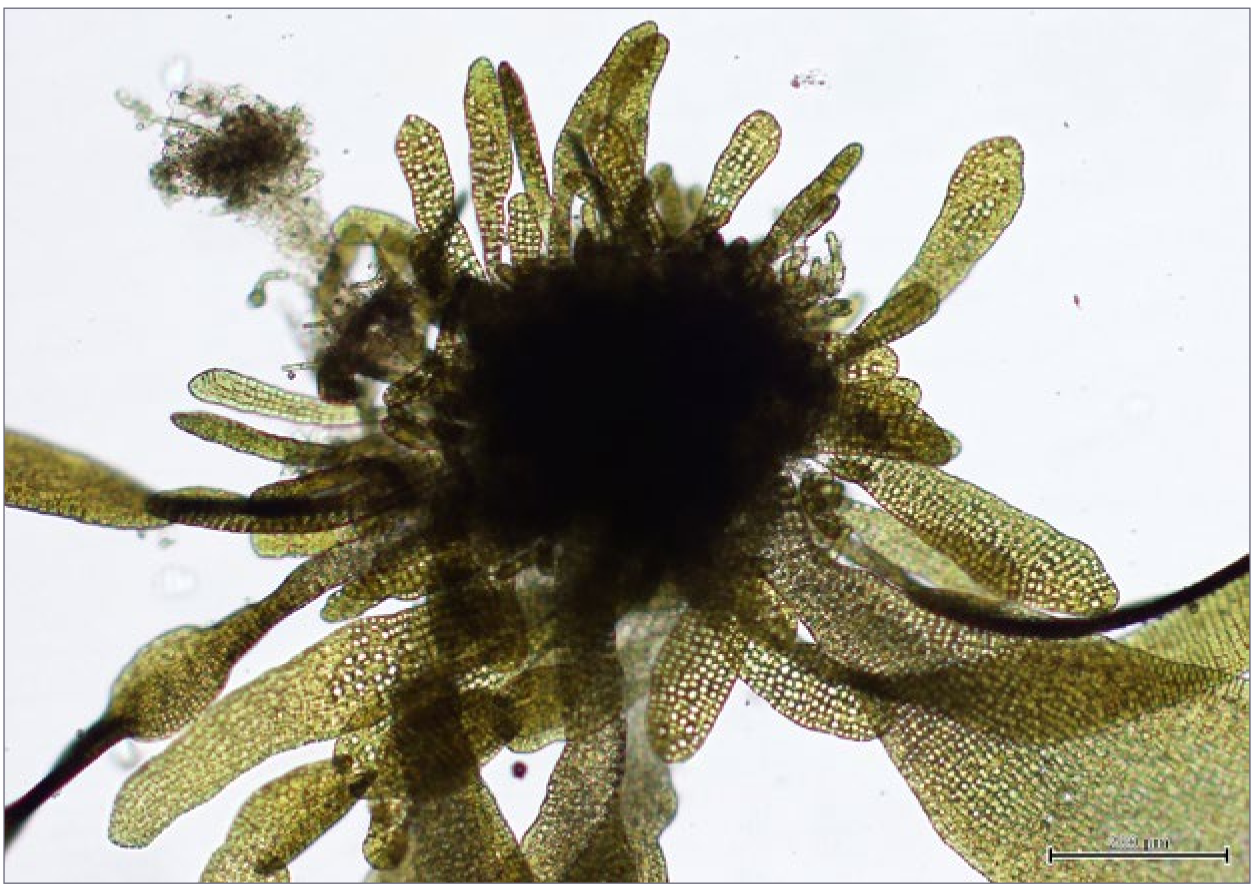


Figure 17. Microscopic view of young kelp sporophytes growing out of a mass of gametophyte tissue. Note that the cells of sporophytes are clearly organized in two dimensional sheets. Gametophyte cells are only ever arranged in single-file rows.

3.7.2 PRODUCING SPOROPHYTES FOR OUTPLANTING IN THE FIELD

Required materials

- ☑ Male and female gametophyte cultures
- ☑ 1.5 mL microfuge tubes
- ☑ Microfuge pestles and/or small blender
- ☑ Petri dishes and/or 50 mL centrifuge tubes
- ☑ Culture media
- ☑ Air pump, airline tubing, and air stone
- ☑ 1000 µL micropipette and tips
- ☑ Serological pipettes and pump
- ☑ Culture environment
- ☑ 30-35 cm lengths of approximately 5 cm-diameter PVC pipe
- ☑ Latex or nitrile gloves
- ☑ PVC cutters
- ☑ Heavy rubber bands
- ☑ Several spools of nylon twine
- ☑ Scissors
- ☑ Standard freezer
- ☑ Large freezer bags
- ☑ 20 US gallon glass aquaria
- ☑ Squirt bottle
- ☑ Aluminum foil
- ☑ Large cooler
- ☑ Ice or cold packs

To produce sporophytes for outplanting in the field, follow the steps below:

1. Identify male and female gametophytes that you intend to cross. If you are outplanting sporophytes, there may be local regulations on the of genotypes you can put in the water. Consult with the BC Aquatic Plant Program to determine how many gametophyte parents you need to use to be allowed to outplant sporophytes derived from those parents.

2. It will generally be necessary to increase the biomass of your gametophytes before attempting to outplant them. To do this, remove them from culture one at a time and place them in either a 1.5 mL microfuge tube (if they are not more than a few millimetres in diameter) or a small blender with a small amount of chilled sterile seawater (approximately 0.5 mL for a microfuge tube).

3. If using a microfuge tube, use a microfuge pestle to manually fragment the gametophyte tissue. If using a blender, switch on the blender to fragment the tissue. In both cases, continue fragmenting until the seawater simply appears as a brown solution and no individual tissue particles can be seen (Figure 16).

4. Introduce the newly ground gametophytes into new, separate petri dishes filled with chilled culture media and place the dishes in a growth environment. Normal growth conditions are ok to use at this stage, but you may be able to increase the growth rates of the newly fragmented gametophytes by increasing irradiance to up to 60 $\mu mol/m^2/s$ red or white light and introducing water motion using an air pump and bubbler system.

5. Leave the ground gametophytes in culture for about 2-4 weeks. During this time, they will grow vegetatively.

6. While your ground gametophytes are incubating for this period, take this opportunity to prepare the settlement spools. These are pieces of PVC pipe wrapped in twine that fragmented gametophytes will be settled onto before outplanting them. To make these spools, cut approximately 5 cm-diameter PVC pipe to lengths of approximately 30-35 cm. Cut as many of these units as you require for your use case; one spool of this size should be able to seed approximately 60 m of longline in the field. Wash these PVC units well with soap and water. Next, while wearing gloves, use a rubber band to secure the end of a spool of nylon twine approximately 1 cm from the end of a piece of pipe. Tightly wrap the twine around the pipe repeatedly such that you gradually cover the length of the pipe. Do not overlap the twine as you proceed. Continue wrapping the pipe until it is completely sheathed in twine except for approximately 1 cm from the opposite end from where you started. At this point, cut the twine and use another rubber band to tightly secure the loose end of twine to the pipe. Store settlement spools in large freezer bags in the freezer until you are ready to use them.

7. Once your gametophytes have shown significant vegetative growth, repeat steps 2-3 with the newly grown tissue. You will likely need to use a blender for fragmentation at this stage to accommodate the increased biomass. If at this point you have an adequate amount of ground gametophyte solution to settle onto your prepared spools, proceed to step 8. If not, you can repeat steps 2-3 again to continue bulking. What constitutes enough biomass to proceed depends on your use case and how many spools you intend to seed, but we would suggest that a combined volume of up to 500 mL of ground male and female gametophyte solution with enough fragment density that the water appears brown in colour should be adequate for a few spools.

8. Combine all fragmented male and female gametophyte solutions into a single volume and mix them well. Transfer the combined gametophyte fragment solution to a squirt bottle.

9. Place a settlement spool vertically in a shallow dish and use the squirt bottle to spray fragmented gametophyte solution all over the surface of the spool. Repeat for all spools you intend to seed at this stage. Some operations utilize specialized adhesives at this stage to improve attachment of the gametophytes to the twine.

10. Once a spool is seeded with gametophyte fragments, immediately place it vertically in a 20 US gallon glass aquarium that has been pre-filled with chilled sterile culture media. This tank should be maintained at 10°C and be exposed to white light at an irradiance of approximately 30 $\mu mol/m^2/s$ on a 12:12 photoperiod. Initially, there should be no water motion. Once all spools are seeded with gametophytes and securely in the culture tanks, leave them undisturbed for two days. During this time, gametophyte fragments will adhere to the twine and begin the processes of gametogenesis and mating.

11. After two days, use an air pump and air stone to introduce a small amount of water movement to the tank.

12. Over the next two weeks, monitor the spools. Keep an eye out for signs of contamination, fluctuations in temperature, system failures, or other potential problems. Change the media in the tank after one week.

13. By approximately two weeks after inoculating the settlement spools, young sporophytes should start to be visible growing on the twine. At this point, they can be outplanted. To outplant the young sporophytes, the twine from the spools will need to be wrapped around kelp culture longlines after they are already deployed in the field. Ensure you already have your field operation in place well before you get to this point. Note that putting kelp and associated infrastructure in the ocean in BC requires permitting through the BC Aquatic Plant Program. This is a separate permitting process than the one required for collecting wild kelp.

14. To transport the spools to the field site, we suggest preparing lengths of PVC pipe that are 10 cm diameter and about 40 cm long. Prepare one of these for each spool. Each transport tube should be capped at one end such that it can still stand vertically on the capped end. Ensure these tubes are clean prior to use.

15. When you are ready to take the spools into the field, fill the transport tubes with chilled sterile seawater, then place one spool in each tube. Use a rubber band to secure a piece of aluminum foil over the open end of each tube. Place each tube upright in a large cooler surrounded with ice or cold packs. Transport the spools to the field site as quickly as possible.

16. At the field site, load the cooler containing the spools onto a boat and proceed to the longline setup.

17. Starting at one end of a longline, detach the line from its mooring and thread the line through a settlement spool. Re-attach the line to its mooring.

18. Remove the rubber band from the end of the spool that is oriented toward the mooring that the longline was just detached from. Tie the now loose end of twine to the longline.

19. While one person holds the spool, a second person should slowly move the boat down the length of the longline towards the mooring on the opposite end of the line such that the twine on the spool gradually unwinds from the spool and coils around the longline.

20. When the twine has completely unwound from the spool, tie the end of the twine to the longline. Briefly detach the end of the longline from its mooring and slide the empty spool off.

21. Repeat steps 17-20 until all seeded twine is deployed in the field.

4. Recommendations for biobanking of kelp in the Salish Sea

We recommend the following steps be taken to develop a kelp biobanking strategy in BC.

1. **Kelp practitioners in BC should biobank as much genetic diversity as possible from as many species of kelp as possible as soon as possible.** These collections will be the "backups" in case genotypes are lost from the wild. Research into kelp population genetics should continue to better understand genetic variation within wild populations, which will inform what sampling needs to take place to ensure that as much biodiversity as possible is captured in biobanks. While canopy-forming species like bull kelp and giant kelp might appear to be the highest priority due to their visibility and ecological importance, the many other kelp species in BC should not be overlooked.
2. **Build a network of biobanking facilities in BC.** Many independent operations are already building their own kelp biobank collections in BC. This momentum should continue. However, we recommend that these operations form a collaborative network so that all parties can know which collections are being held where and by whom. This could be unified by a centralized, accessible database for cataloguing collections held by various network members. This would help streamline efforts to collect as much biodiversity as possible from throughout the region. For example, if it is known that one organization already has bull kelp samples from a specific location, another organization can direct efforts to sampling a different location instead. A network of biobanking facilities could also agree upon and use standardized methods to ensure that sampled germplasm is of high quality and in a predictable state, which would also help facilitate sharing of samples for research purposes. In addition to forming a biobanking network, if resources permit, it may also be useful to also establish a central hub accessible to all parties where samples contributed by as many practitioners as possible can be held. Such a facility may help ensure that germplasm is biobanked in perpetuity and not be lost when one practitioner retires or a grant expires.
3. **Develop a long-term funding solution for biobanking in BC.** Biobanking is by its nature a long-term proposition. There is no sense in going to great effort to grow a germplasm collection only to run out of resources to support it when a single funding source expires. For this reason, it is essential that biobanking efforts are funded long-term. Some possible avenues to consider may include government grants, appealing to private donors/fundraising, establishing or enlisting a nonprofit organization, or finding ways to allow biobanks to generate some of their own funds, such as by charging for services. Multiple staggered funding sources will likely be needed to keep biobanking operations running long-term.
4. **Leverage kelp biobanks for research.** A robust kelp biobank collection containing a large amount of biodiversity represents an extremely valuable resource for kelp research. Having many genotypes of a variety of kelp species easily accessible creates an opportunity to conduct research on kelp genetics and can facilitate breeding programs. More work is needed to understand population genetics in local kelp species, and almost nothing is known about how kelp genetics relates to functional traits. It would be extremely valuable to use the resources afforded by kelp germplasm collections to, for example, identify and breed more thermotolerant kelp strains, or identify the genes governing kelp thermotolerance in order to help wild kelp populations persist in a warming world. Biobanked collections could be similarly used by representatives of BC's burgeoning commercial kelp industry to develop kelp strains with traits advantageous for commercial production.
5. **Continue to develop cryopreservation methods for kelp.** For many cases, building kelp biobank collections using culture-based methods will be the best approach, especially when an operation is new, resources are limited, or the operation is in a remote location. However, we believe that cryopreservation presents largely untapped utility for kelp biobanking and that its use should be considered by practitioners. At time of writing, however, few kelp species found in BC have published cryopreservation protocols, and there remain key unanswered questions about the method, such as how long kelp tissue can be cryopreserved for. We recommend more research be conducted to develop protocols for more species, and to answer outstanding questions that will make cryopreservation more useful and improve trust in the method by kelp practitioners.

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GLOSSARY

Algae	Any member of a large polyphyletic (more than one common ancestor) group of eukaryotes that are usually photosynthetic, aquatic, and structurally and reproductively simple
Biobank	A collection of living biological material
Biodiversity	Variety of life at all biological levels
Blade	A broad, flattened part of a seaweed body that functions primarily as a photosynthetic organ; analogous to the leaf of a plant
Brown algae	Any member of the class Phaeophyceae, a group of multicellular algae in the phylum Ochrophyta that are commonly found in temperate regions and usually have brown pigmentation (fucoxanthin)
Bryozoan	Any member of the phylum Bryozoa ("moss animals"), a group of sessile invertebrates that form colonies and build hard, mineralized exoskeletons
Canopy	The uppermost layer of a forest
Cryogenic	At or relating to extremely low temperatures
Cryopreservation	The practice of preserving biological material at very low temperatures
Cyanobacteria	A group of autotrophic bacteria that derive energy from oxygenic photosynthesis
Diatom	Any member of a group of eukaryotic microalgae in the phylum Ochrophyta that produce cell walls composed of silica
Diploid	Describes a cell or nucleus with two sets of chromosomes, one derived from each parent
Ecosystem service	Goods and services provided to humans by ecosystems
Epigenetic	Pertaining to changes in gene expression that occur without changing the DNA sequence
Flagellum	A hair-like structure possessed by some microorganisms that allows motility
Fucoxanthin	A yellowish-brown carotenoid pigment found in the plastids of brown algae and diatoms
Gamete	A haploid cell that fuses with another haploid cell during sexual reproduction to form a zygote
Gametogenesis	The formation of gametes
Gametophyte	The haploid free-living stage of an organism with a haplodiplontic life history
Genotype	The genetic constitution of an individual organism
Germplasm	Genetic resources of organisms, such as seeds, that are maintained for purposes of breeding
Green algae	Any member of the phylum Chlorophyta, a group of organisms with diverse morphologies and life histories that often have green pigmentation (chlorophyll)
Haploid	Describes a cell or nucleus with one set of chromosomes

Hemocytometer	A specialized microscope slide used to count cells
Holdfast	A structure that anchors a seaweed to the substratum; analogous to the roots of a plant
Kelp	Any member of a group of large brown algae in the order Laminariales
Kelp forest	An underwater area with a high density of kelp
Macroalgae	Macroscopic, multicellular algae
Media	A substance used to support the growth of organisms in culture
Meiosis	A kind of cell division in which a single diploid parent cell yields four haploid daughter cells
Microalgae	Microscopic, usually unicellular algae
Mitosis	A form of cell division in which a parent cell yields two identical daughter cells
Mutation	A change in a genetic sequence
Pneumatocyst	A gas-filled bladder present in some brown algae that provides buoyancy
Population genetics	The genetic makeup of populations of organisms and how that makeup changes over time
Primary producer	Organisms that derive energy from non-living sources, most often through photosynthesis
Seaweed	Marine macroalgae
Sorus	A reproductive region of a kelp blade
Sporangium	A spore-producing structure
Spore	A reproductive cell capable of developing into a new individual without fusing with another reproductive cell
Sporophyll	A specialized blade on which sori develop
Sporophyte	The diploid free-living stage of an organism with a haplodiplontic life history
Stipe	A structure that supports the blade(s) of a seaweed; analogous to the stem of a plant
Syngamy	The fusion of two gametes
Thallus	The vegetative body of a plant-like organism that is not differentiated into true leaves, stems, and roots
Understory	A layer of a forest that grows under the canopy
Vitrification	A process in which a substance is converted into a glass (i.e. a non-crystalline solid), often through rapid cooling
Zygote	A diploid cell formed through the fusion of gametes

ABBREVIATIONS

1N	Haploid
2N	Diploid
CPA	Cryoprotective agent
DMSO	Dimethyl sulfoxide
PES	Provasoli enriched seawater
PPE	Personal protective equipment
PSF	Pacific Salmon Foundation

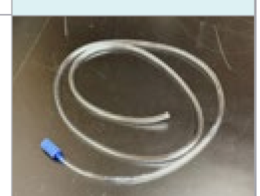
APPENDIX 1 – KELP BIOBANK COLLECTIONS IN PACIFIC NORTH AMERICA

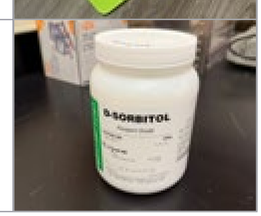
Table A2. Kelp biobank collections in Pacific North America.

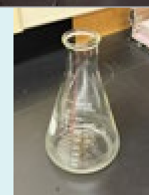
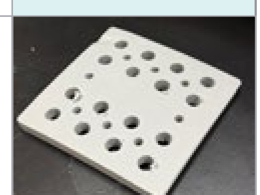
Organization	Location	Contact
Aquarium of the Pacific	Long Beach, CA	
Canadian Kelp Research	Bamfield, BC	CanadianKelpResearch@gmail.com
Cascadia Seaweed	Cedar, BC	jclark@cascadiaseaweed.com
Hakai Institute	Quadra Island, BC	kate.rolheiser@hakai.org; iria.gimenez@hakai.org
Jamestown S'Klallam Tribe & Puget Sound Restoration Fund	Manchester, WA	araymond@jamestowntribe.org
Kelp Ark	San Pedro, CA; Los Angeles, CA	hayden@kelpark.org
Kelp Rescue Initiative	Bamfield, BC	jasmin.schuster@kelprescue.org
North Island College	Campbell River, BC	logan.zeinert@nic.bc.ca
Puget Sound Restoration Fund	Manchester, WA	hilary@restorationfund.org; jessi@restorationfund.org
Simon Fraser University	Burnaby, BC	sbisgrov@sfu.ca
University of British Columbia	Vancouver, BC	patrick.martone@botany.ubc.ca
University of Wisconsin - Milwaukee	Milwaukee, WI	albertof@uwm.edu
Vital Kelp/ BC Conservation Foundation	Pender Harbour, BC; Cannery Bay, BC	vitalkelp@gmail.com

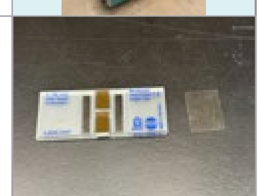
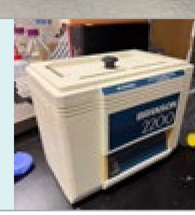
APPENDIX 2 – SOURCING SUPPLIES FOR KELP BIOBANKING

Table A3. Suggested sources of supplies required for kelp biobanking

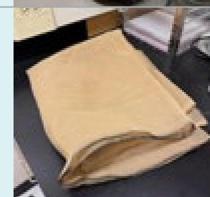
Item	Required for	Where to source	Image
-80°C freezer	Cryopreservation	Science suppliers[1]	
1.5 mL microfuge tubes	Producing sporophytes	Science suppliers[1]	
24-well plates	Culturing gametophytes	Science suppliers[1]	
70% ethanol	Spore release, general lab use	Science suppliers[1]	
Air pump	Producing sporophytes	Aquarium suppliers[2]	
Airline tubing and air stones	Producing sporophytes	Aquarium suppliers[2]	
Aluminum foil	Sorus prep, spore release, culturing gametophytes, outplanting sporophytes	Hardware stores[3], grocery stores[4], science suppliers[1] Uline	
Beakers	Sorus prep, spore release	Science suppliers[1]	

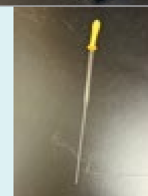
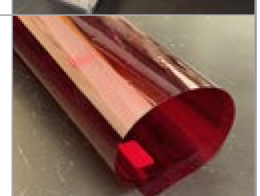
Betadine®	Sorus prep	Pharmacies[5]	
Blender	Producing sporophytes	Science suppliers[1]	
Calculator	Spore release	Phone app, office suppliers[6]	
Compound microscope	Spore release, culturing gametophytes, general lab use	Science suppliers[1], optics specialists[7]	
Cooler	Sorus collection, sorus prep	Outdoor stores[8], hardware stores[3]	
Cryogenic gloves	Cryopreservation	Science suppliers[1]	
Cryovials	Cryopreservation	Science suppliers[1]	
Cutting board	Sorus prep	Hardware stores[3], home suppliers[9]	
D-sorbitol	Cryopreservation	Science suppliers[1]	

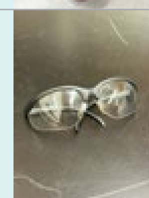
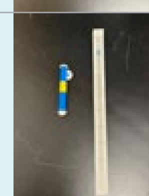
Data sheet	Sorus collection	This handbook	
Dissecting microscope	Spore release, culturing gametophytes, general lab use	Science suppliers[1], optics specialists[7]	
Erlenmeyer flask	Cryopreservation, general lab use	Science suppliers[1]	
Ethylene glycol	Cryopreservation	Science suppliers[1]	
Face shield	Cryopreservation	Science suppliers[1]	
Field knife	Sorus collection	Outdoor stores[8]	
Filter paper	Cryopreservation	Science suppliers[1]	
Floating tube rack	Cryopreservation	Science suppliers[1]	
Freezer	Outplanting sporophytes, general lab use	Appliance stores[10]	

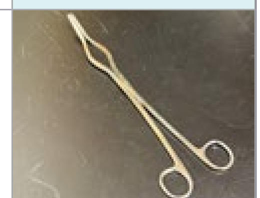
Freezer bags	Outplanting sporophytes,	Hardware stores[3], grocery stores[4], science suppliers[1], Uline	
Freezer boxes	Cryopreservation	Science suppliers[1]	
Funnel	Cryopreservation	Science suppliers[1]	
Glass aquarium	Outplanting sporophytes	Aquarium suppliers[2]	
Glass carboy	Producing sporophytes	Science suppliers[1]	
Grow lighting	Culture environment	Hardware stores[3], aquarium stores[2], lighting suppliers, e.g. Philips	
Growth chamber	Culture environment	Growth chamber suppliers, e.g. Conviron	
Hemocytometer and coverslip	Spore release	Science suppliers[1]	
Hot water bath	Cryopreservation	Science suppliers[1]	

Ice bucket	Sorus prep, spore release, cryopreservation, general lab use	Science suppliers[1]	
Ice packs	Sorus collection, sorus prep, outplanting sporophytes	Hardware stores[3], Uline	
Lab coat	Cryopreservation, general lab use	Science suppliers[1], clothing stores[11]	
Latex or nitrile gloves	All lab work	Science suppliers[1]	
Lens cleaner	Spore release, general lab use	Science suppliers[1], optics specialists[7]	
Lens paper	Spore release, general lab use	Science suppliers[1], optics specialists[7]	
Light meter	Culture environment	Specialty vendors, e.g. Apogee Instruments	
Light timer	Culture environment	Hardware stores[3]	
Liquid nitrogen	Cryopreservation	Gas suppliers, e.g. Linde	

Liquid nitrogen dewar	Cryopreservation	Science suppliers[1]	
Mesh	Culture environment	Hardware stores[3], garden suppliers	
Microfuge pestle	Producing sporophytes	Science suppliers[1]	
Micropipette	General lab use	Science suppliers[1]	
Micropipette tips	General lab use	Science suppliers[1]	
Mr. Frosty®	Cryopreservation	Science suppliers[1]	
Nutrient media	Culturing	Science suppliers[1]	
Nylon twine	Outplanting sporophytes	Hardware stores[3], Uline	
Paper towel	Sorus prep, spore release	Hardware stores[3], Uline	

Parafilm®	Culturing	Science suppliers[1]	
Pasteur pipettes and bulbs	Spore release, general lab use	Science suppliers[1]	
Permanent markers	General lab use	Office suppliers[6]	
Petri dishes	Culturing	Science suppliers[1]	
PVC pipe	Outplanting sporophytes	Hardware stores[3]	
Razor blades	Sorus prep	Hardware stores[3], Uline	
Red cellophane	Culture environment	Hardware stores3, craft supply stores[12], Uline	
Red lights	Culture environment	Lighting suppliers, e.g. Philips	
Refractometer	Culturing	Science suppliers[1], aquarium suppliers[2]	

Refrigerator	Sorus prep, general lab use	Hardware stores[3], appliance stores[10]	
Rubber bands	Outplanting sporophytes	Hardware stores[3], office suppliers[6]	
Rubber boots	Sorus collection	Outdoor stores[8], hardware stores[3], clothing stores[11]	
Safety goggles	Cryopreservation, general lab use	Science suppliers[1]	
Scissors	Outplanting sporophytes, general lab use	Office suppliers6	
Serological pipettes and pump	General lab use	Science suppliers[1]	
Spatula	Spore release, general lab use	Science suppliers[1]	
Squirt bottle	Sorus prep, producing sporophytes for outplanting	Science suppliers[1]	
Submersible thermometer	Culture environment, spore release	Science suppliers[1]	

Tally counter	Spore release	Phone app, science suppliers[1], Uline	
Thermometer	Culture environment	Hardware stores[3]	
Timer	Cryopreservation	Phone app	
Tongs, tweezers	Sorus prep, spore release	Science suppliers[1]	
Waders	Sorus collection	Outdoor stores[8], hardware stores[3], clothing stores[11]	

1. E.g. MilliporeSigma, Thermo Fisher
2. E.g. PetSmart, local businesses
3. E.g. Home Depot, Canadian Tire, Home Hardware
4. E.g. Save-On-Foods, Safeway, Real Canadian Superstore
5. E.g. Shoppers Drug Mart
6. E.g. Staples
7. E.g. Nikon, Leica
8. E.g. Mountain Equipment Company, Cabela's
9. E.g. IKEA
10. E.g. Best Buy
11. E.g. Mark's
12. E.g. Michaels

APPENDIX 3 – SORUS COLLECTION DATASHEET

Date:	Location (lat/long):	Species:	Number of sori sampled:	Notes:

APPENDIX 4 – HOW TO USE A HEMOCYTOMETER TO COUNT SPORES

Required materials

- ☑ Hemocytometer and paired coverslip
- ☑ 20 µL micropipette and tips
- ☑ Compound microscope
- ☑ Calculator
- ☑ Tally counter
- ☑ Lens paper
- ☑ Lens cleaner or 70% ethanol solution

A hemocytometer is a special microscope slide used to count cells. The surface of the slide is engraved with a very precisely engineered, microscopic grid pattern that, when a coverslip is placed over top with a layer of fluid between the two pieces of glass, created units of known volume that can be used to estimate cell density.

To use a hemocytometer to count kelp spore density, follow the steps below:

1. Use lens paper and lens cleaner to gently wipe down the surface of the hemocytometer and the coverslip that pairs with it to ensure you are starting with clean glassware. Do not use generic paper towel or Kimwipes® for this, as they may scratch the glass.

2. Stir your spore solution to homogenize the density and use the 20 µL micropipette to sample 10-20 µL of solution.

3. With the coverslip already placed on top of the hemocytometer, eject the spore solution sample at the edge of the coverslip such that it is drawn underneath.

4. Place the hemocytometer, now bearing a spore solution sample, on the stage of the compound microscope and, using the 10x objective lens initially, focus on the grid pattern on the top surface of the hemocytometer. You should see something similar to Figure A.1.

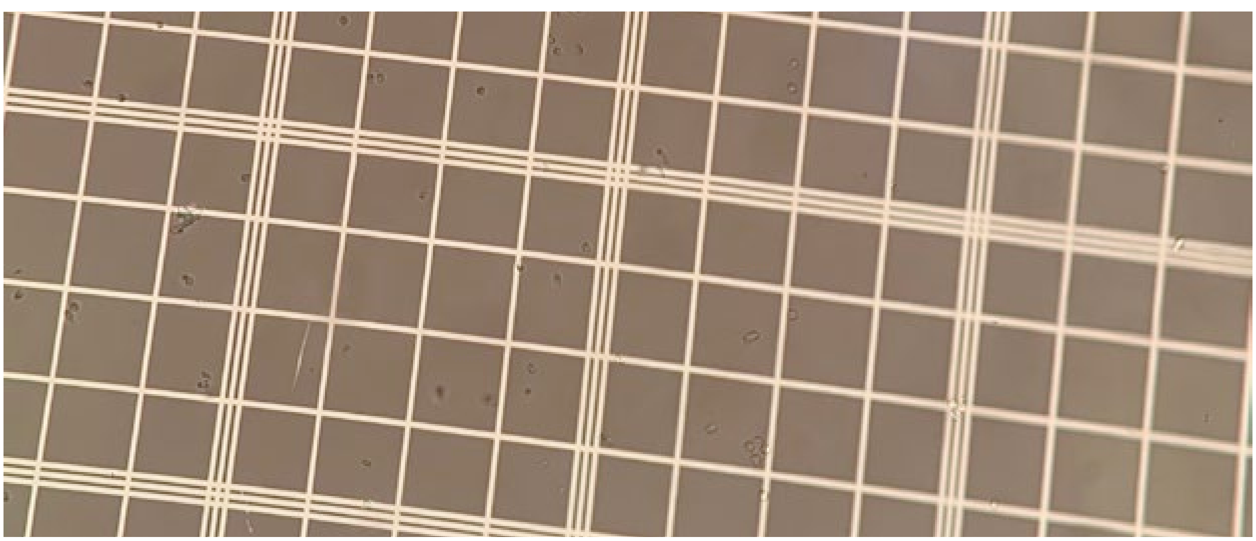


Figure A.1. Kelp spores on a hemocytometer as viewed under a compound microscope.

5. The hemocytometer has a grid pattern engraved in it that looks like Figure A.2A. To estimate your spore density, you will count the cells in specific squares within the pattern. Before you start counting, make a qualitative assessment of the spore density in the sample. If the spore density is low, count all spores within the shaded areas in Figure A.2B. If spore density is high, count all spores in shaded areas in Figure A.2C. A rough indicator of "high" spore density in this instance would be a density such that it would be overwhelming to count all spores in the areas indicated in Figure A.2B. You must also decide at this point whether you count cells that are partially outside the counting area (ie. on the outer perimeter lines). It ultimately does not matter whether you choose to count these cells or not, but whichever choice you make, you must uphold it consistently across all of your counting. For simplicity, we recommend excluding cells that are partially outside the counting area.

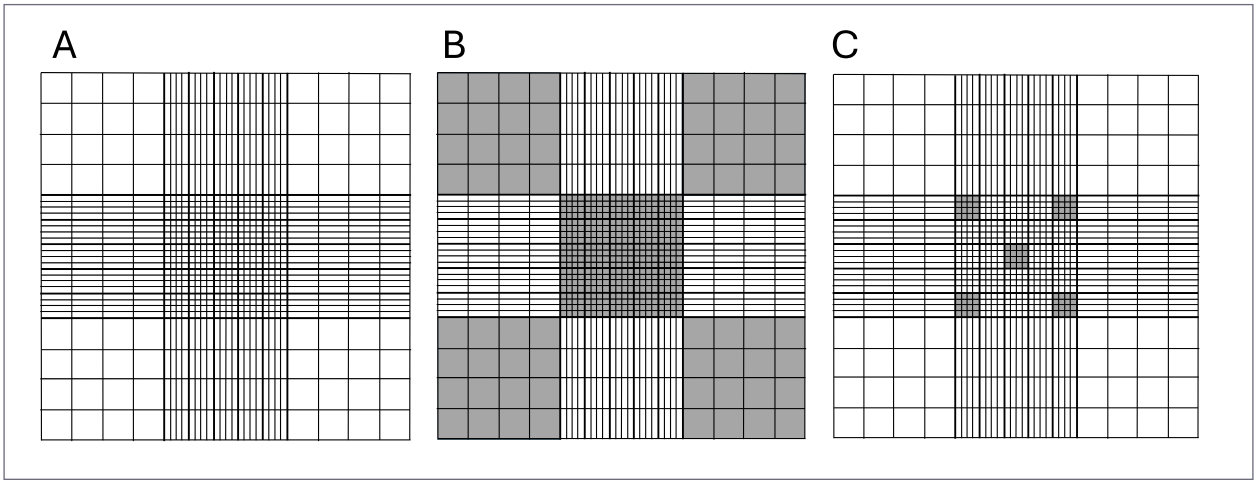


Figure A.2. (A) The grid pattern of a hemocytometer. Count all cells within the shaded areas indicated in (B) if spore density is low and within those in (C) if spore density is high.

6. Use the tally counter to count all spores in the areas chosen in step 5. When you are finished counting, calculate the sum of all spores counted.

7. If you used the low-density area indicated in Figure A2B, use the following equation to calculate your estimated spore density:

$$\text{Spore density}\left(\frac{cells}{mL}\right)=\left(\frac{\text{total cells counted}}{5}\right)X10{,}000 \qquad \textit{Equation A.1}$$

If you used the high-density area indicated in Figure 15C, use the following equation to calculate your estimated spore density:

$$\text{Spore density}\left(\frac{cells}{mL}\right)=\left(\frac{\text{total cells counted}}{5}\right)X250{,}000 \qquad \textit{Equation A.2}$$

PACIFIC SALMON FOUNDATION

1385 West 8th Ave,
Vancouver, BC, V6H 3V9
Tel: 604-664-7664
Email: salmon@psf.ca

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