

Macroalgae cultivation protocol

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1. Industrial production of seedlings

1.1 Basic infrastructure elements

For the cultivation of seedlings, you need a license and a discharge permit. The license is applied for at the Directorate of Fisheries (Fiskeridirektoratet: <https://www.fiskeridir.no/>)

Cultivation of seedlings requires temperature control, either of the air temperature of the whole unit or of the water in the cultures. The air temperature can be controlled with a cooling unit, fan oven, air conditioner (large scale), incubators or refrigerator (small scale). The water temperature can be controlled with electric water coolers.

The seawater must at least have very good, better excellent, quality for both, small and large scale cultivation work; filtration, down to 0.2 - 5 µm, significantly increases water quality and is essential for large-scale production to avoid contamination.

In addition, the kelp needs air supply. It is important to use oil-free compressors or pumps, because the oil can destroy the kelp cultures.

Adjustable light sources, where you can change both, day length and light intensity, are also necessary. Ordinary fluorescent lamps (T5 and T8, cold / warm white) combined with a timer are suitable. We do not recommend the use of LED lights, because the light quality of many LED systems is poorly suited for kelp cultivation and systematic long-term testing is required.

In addition, we recommend the following equipment and consumables:

- Autoclave or steam pressure pot for sterilization
- Stove
- Large pot (> 10 l)
- Microscope
- Stereo magnifier
- Balance
- Light meter
- Dissection tools (spatula, scalpel, knives, tweezers, coarse and fine scissors, etc.)
- Plastic bags
- Styrofoam boxes and cooling elements
- Glassware (cups, bottles, etc.)
- Marker pen
- Rope (kuralon, eventually polypropylene, nylon or other materials)
- Collector for ropes
- Air tubes
- Air filter
- Pipettes
- Nutrients
- Disinfectant

There are big differences when it comes to seedling production of different species, because the life cycle can be different. In any case, the most popular algae in Norway, sugar kelp and winged kelp, have quite similar life cycles, as well as tangle and oarweed: macroscopic sporophytes produce zoospores in so-called sori, which are released and grow into microscopic gametophytes. The gametophytes develop gametes, and eventually fertilized zygotes that grow again into sporophytes (see Figure 1 below).

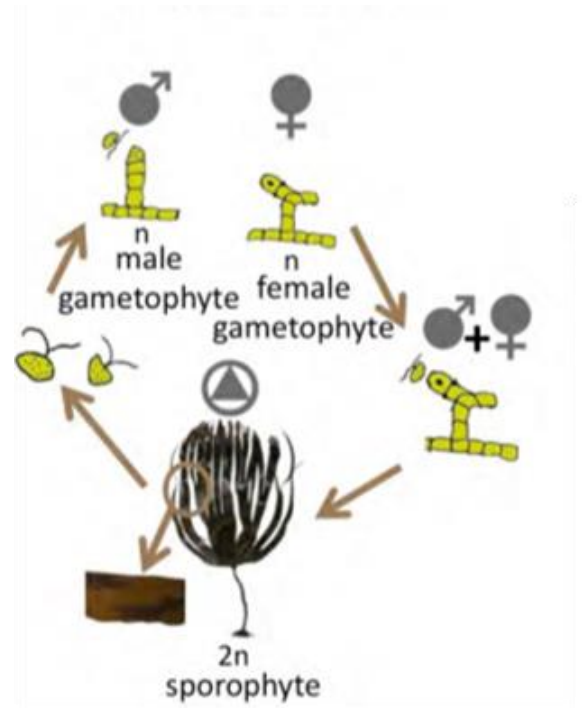


Figure 1: Life cycle of kelp. Source: Edwards et al. 2012

1.2 Collection of mother plants and induction of spore release

We now describe seedling production for the species named above, which begins with the collection of mother plants. We recommend that the collection of mother plants takes place during the sorus season.

If you do not collect mother plants in the sorus season, you have to induce spore production in artificial conditions that mimic temperature and light conditions of the sorus season. Protocols for artificial sorus production are described for sugar kelp in Forbord et al., 2012, but are resource- and time-consuming.

The following table shows the sorus season for the most important kelp species in Northern Norway.

Table 1: Sorus season for the most important kelp species in northern Norway

Species	Sorus season
<i>S. latissima</i>	November - January
<i>A. esculents</i>	December - March
<i>L. digitata</i>	June - August
<i>L. hyperborea</i>	December - February

The kelp plants are picked up with a pole or something similar from a boat, on the shore at low tide (possibly with waders), by diving or from a floating dock in a small-boat harbor. Seaweed plants should have sorus (see figure 2 for sugar kelp below). If possible, choose kelp plants with clean leaves, without visible growth of other organisms. Then you cut all of the kelp leaf that is not sorus. Sori should be thoroughly cleaned with unscented toilet paper (see Figure 2).



Figure 2: Cleaning of sugar kelp sorus;
Photo: Sapine Popp, University of Bergen

It is important that the kelp plants are not exposed to temperatures below 0 °C or above 15 °C or dry out. Use of polystyrene boxes, cooling elements in the summer (or through transport in a warm car) and plastic boxes or plastic bags is recommended.

Cleaned sori are packed in a plastic bag or plastic box and stored overnight at 4 - 10 °C, e.g. in a refrigerator. The next day, sori are mixed with sterile saltwater (10 °C).

The saltwater is sterilized by autoclaving (heating to 123 °C for 1 hour) in a steam pressure pot or an autoclave (highest level or appropriate autoclave programs), double pasteurization (heating to 60 °C for 1 hour, repeated after cooling on the next day), or by 0.2 µm filtration. Autoclaved or pasteurized saltwater has low oxygen concentrations and must be re-oxygenated before use.

Depending on the production method, you continue in different ways:

- 1.3 Production of microsporophytes on ropes in basins or plastic boxes
- 1.4 Direct sowing of gametophytes on cultivation ropes
- 1.5 Production of (micro) sporophytes and targeted fixation on ropes

1.3 Production of microsporophytes on ropes in basins or plastic boxes

Companies: PolarAlge, Eukaryo AS, SES

This method is well suited if you have large amounts of sorus available, and is thus used mainly for the production of sugar kelp seedlings.

Following this method, sori (spore discharge induced) are mixed with sterile saltwater (10 °C) in plastic boxes or basins (10 - 200 l) to trigger sporulation. You stir the sori every 5 - 10 minutes. The spores are released after 30 - 60 minutes, and the water becomes eventually turbid. If spore release occurs (microscopic control!), add the seedling ropes bundled around collectors, and incubate them for 10 - 15 min (see Figure 3).

You can use 1-2 mm thin ropes as seedling ropes, which provide greater production capacity in a basin or plastic box than for example 10 mm rope, but connection to a thick production rope is required when released into the sea. If you want to use seedling ropes directly as production ropes, we recommend diameters of 6 - 8 mm or thicker. All types of ropes should be thoroughly cleaned before use, e.g. via 1 hour boiling in freshwater.

After the spores have become attached, the ropes are transferred to cultivation basins with filtered saltwater (see Figure 3). We recommend the use of at least 30 cm² sorus per 100 m of rope. Sori should be from at least 5 different kelp individuals, to ensure good genetic starting material.



Figure 3: Left: Settling of zoospores on ropes; Right: cultivation of microsporophytes in basins;
Photo: Sabine Popp, University of Bergen

Especially for *A. esculenta*, *L. digitata* and *L. hyperborea*, which have small sori compared to sugar kelp, you can get better results if you grow larger amounts of gametophytes as a start-up culture.

Therefore, sori (spore discharge induced) are mixed with sterile saltwater in glasses or small plastic boxes (1 - 5 l, see figure 3). You stir the sori every 5 - 10 minutes. The spores are released after 30 - 60 minutes, and the water eventually becomes turbid. Then remove the sori with a filter (e.g. cotton cloth) and grow the gametophyte cultures at 10 °C and 10 - 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (equivalent to about 500 - 1000 lux). Gametophyte cultures stored in this way consist of many individuals with different genetic material, but one can also purify male and female gametophytes and use them as starting material (breeding and variety development). Sugar kelp, *A. esculenta* and *L. hyperborea*, are incubated with 10 hours of light per day, *L. digitata* with 24 hours of light. The cultures must be supplied with air via a pump (see figure 4). Once a week you stop the air supply and wait until the

gametophytes gather on the bottom. Then remove as much water as possible and top up with new saltwater.

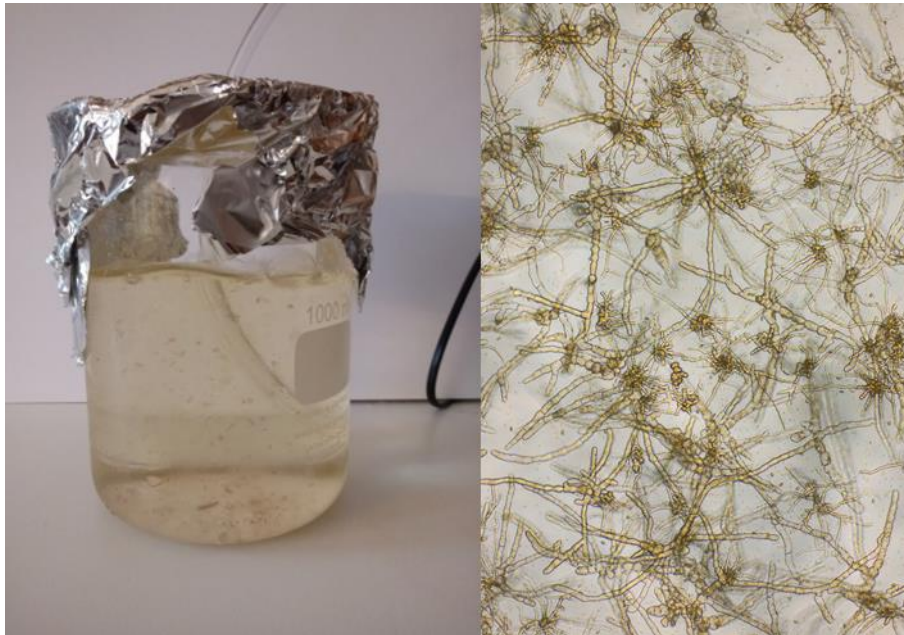


Figure 4: Gametophyte culture;

Photo: Christian Bruckner, Salten Havbrukspark, PolarAlge

If the natural nutrient concentration in the salt water is low, we recommend the use of growth medium, for example Provasoli Enriched Seawater (680 μM N, 32 μM P) (Andersen, 2005). If you have enough biomass (after 3 to 12 weeks), change the light conditions to 12 hours of light per day to induce gamete and zygote development.

After a week with a new light regime, the gametophytes are gently crushed with a mixer. The gametophyte suspension is sprayed on culture ropes. After half an hour of drying time, the ropes are transferred to a culture basin with filtered saltwater.

Cultures are grown at 10 °C and 10 - 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (equivalent to 500 - 3500 lux). At high light intensity and use of nutrient-rich growth medium, the microsporophytes are ready for deployment at sea after 10 - 14 days. At low nutrient salt concentrations, low light and lower temperatures (5 °C) you can keep the sporophytes in the basin for up to 3 months. The ropes are ready for deployment, if they are dense with gametophytes, and if you see zygotes or microsporophytes on the ropes (control with a stereo magnifier!) We recommend putting out the ropes before the sporophytes reach 2 mm in size. Large sporophytes are more vulnerable and sensitive, and handling during deployment at sea is difficult.

The method is well suited for both, large-scale and small-scale production (Figure 5). For example, PolarAlge AS can produce 36 000 m of seeded rope (2 mm) within 10 days in 40 m² basins with a total volume of 20 000 l. Nevertheless, the method is also suitable for small-scale production. Here you use plastic boxes in an incubator or refrigerator instead of a basins. When using a refrigerator, insert fluorescent lamps into the refrigerator. It is important to choose refrigerator models that do not emit explosive gases. In a 200 l refrigerator you can produce 500 - 1,000 m of 2mm seeded rope within 10 days.



Figure 5: Seedling production at PolarAlge AS; left: large-scale production in the pool; right: small-scale production in refrigerators. Photo: Gunnar Skjellvik, PolarAlge, Christian Bruckner, Salten Havbrukspark, PolarAlge

1.4 Direct sowing of gametophytes on cultivation ropes

Companies: Hortimare, Eukaryo

With this method, sori are mixed with sterile saltwater in glasses or small plastic boxes to induce sporulation. You stir the sori every 5 - 10 minutes. The spores are released after 30 - 60 minutes, and the water eventually becomes turbid. Then the gametophyte cultures are grown at 10 °C and 15 - 20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Gametophyte cultures produced in this way consist of many individuals with different genetic material, but one can still purify some male and female gametophytes and use them as starting material (breeding and variety development). Sugar kelp, *A. esculenta* and *L. hyperborea*, are incubated with 10 hours of light per day, *L. digitata* with 24 hours of light. The cultures must be supplied with air by a pump. Once a week you stop the air supply and wait until the gametophytes gather on the bottom. Then remove as much water as possible and top up with new saltwater. If the natural nutrient concentration in the salt water is low, we recommend the use of growth medium, for example Provasoli Enriched Seawater (680 $\mu\text{M N}$, 32 $\mu\text{M P}$) (Andersen, 2005). After a propagation period, if you have enough biomass (after 3 weeks to 12 weeks), change the light regimen to 12 hours of light per day to induce gamete and zygote multiplication. One week later, crush the gametophytes gently with a mixer. The gametophyte suspension (eventually mixed with glue) is either sprayed on culture ropes, or injected it with a specific machine, or the ropes are soaked in the suspension. After a certain drying time the ropes are deployed directly at sea.

1.5 Production of (micro) sporophytes and targeted fixation on ropes

Companies: Seaweed Energy Solutions

With this method, sori are mixed with sterile saltwater in glasses or small plastic boxes. You stir the sori every 5 - 10 minutes. The spores are released after 30 - 60 minutes, and the water eventually becomes turbid. Then the gametophyte cultures are grown at 10° C and 15 - 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Sugar kelp, *A. esculenta* and *L. hyperborea*, are incubated with 10 hours of light per day, *L. digitata* with 24 hours of light. Gametophyte cultures produced in this way consist of many individuals with different genetic material, but one can still purify some male and female gametophytes and use them as starting material (breeding and variety development). The cultures must be supplied with air via a pump. Once a week you stop the air supply and wait until the gametophytes gather on the bottom. Then remove as much water as possible and top up with new saltwater. If the natural nutrient concentration in the saltwater is low, we recommend the use of growth medium, such as Provasoli Enriched Seawater (680 $\mu\text{M N}$, 32 $\mu\text{M P}$). If you have enough biomass (after 3 weeks to 12 weeks), you change the light cycle to 12 hours of light per day to induce gamete and zygote development. Fertilized zygotes grow into sporophytes, which are grown as described for the gametophytes, but with 12 hours of light per day. If they are 0.2 - 2 mm large, carefully distribute the gametophytes on ropes and incubate them in a pool with salt water (10° C and 15 - 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 12 hours per day) until they attach (4 - 12 weeks), before release into the sea.

2. Evaluate farming facilities at sea

If you are considering places to establish sea-based kelp cultivation facilities, you must find out in advance whether those are legally classified as aquaculture area. Aquaculture areas in Norway are usually defined in (inter)municipal coastal zone plans.

A license is required for growing kelp in the sea. The license is applied for at the Directorate of Fisheries <https://www.fiskeridir.no/>.

It is important to choose a cultivation site in the sea that not only ensures good production but also delivers good infrastructure conditions.

The following is a summary of some assessment criteria and questions that require detailed investigation:

- Is there a pier or quayside nearby (access from land)?
- Can you easily access to the water regardless of the tide level?
- Is there anyone available to put boats in and take them out of the water?
- How far away are kelp farms and what does that mean for fuel costs?
- How exposed is the farm?
- Do you manage to work there in most weather conditions?
- Is it easily accessible in all weather conditions?
- What levels of pollution may be present (e.g. untreated sewage, industrial discharges, etc.)?
- What other users are within the area at the cultivation site (potential for conflict with e.g. fishing vessels, shipping and transport, water sports, leisure activities, other aquaculture activities, etc.)?
- Are there other types of aquaculture activity that can create synergies (nutrients from salmon farming, sharing boats, equipment, labor)?

Very important parameters for the establishment of cultivation facilities are depth and seabed type. Tide differences can be 3 m along the coastline and must be taken into account when choosing a minimum depth to install a structure. A proposed absolute minimum depth is 6 m, but a depth of 10 m is advisable. At the other end of the scale, very deep water is also not recommended, due to increased equipment costs, and more difficult conditions for divers to make safety inspections or to perform maintenance.

Proper placement of cultivation facilities and anchoring of cultivation systems on different types of bottoms is also very important. Bottom structure will e.g. indicate the amount of water flow and the degree of exposure of the site (e.g. deposition of sludge indicates a low water flow). Sludge can also affect the turbidity of the water during stormy weather, and can also affect water temperature. The bottom structure will also determine what kind of anchoring equipment is needed. For example, a large heavy anchoring stone is more suitable for a rocky surface, while CQR plough anchors or something similar can be used effectively in sandy beaches. Combinations of sludge and sand should be avoided as they may not guarantee solid anchoring. In addition, fine sediment will be washed out into the water column and reduce the kelps photosynthetic efficiency and thus development.

Factors that affect the kelp's growth are water flow (and thus access to nutrients), exposure, temperature and salt content.

High water temperatures limit algae growth and development. Ideally, average maximum temperatures should not exceed 15° C in June. We therefore do not recommend places that are exposed to large fluctuations in temperature, e.g. near a large, exposed tidal area that will heat up quickly during the day at low tide. Shallow water over sandy bottoms tends to heat up faster than water over rocky surfaces. Minimum winter temperatures should not be so low that ice develops.

Seaweed cultures require localities with suitable water exchange. Exposure to current as opposed to waves is recommended, because areas with a lot of current are rich in nutrients, while large wave action can damage kelp culture structures. Current at speeds between 5 and 10 cm s⁻¹ is useful for growing kelp.

Places with brackish water should be avoided, such as estuaries or long, sheltered fjord systems. The kelp's productivity drastically decreases on salinity lower than 30 PSU.

Accurate measurement of the various parameters throughout the growing season is time consuming and costs a lot, and some measurements can give incorrect results. We therefore recommend inspecting the area near potential cultivation sites for natural kelp populations. Good kelp population nearby is a good indicator that the place is productive.

3. Protocol for cultivation of sugar kelp and winged kelp at sea

There are many different types of kelp farming systems. Most kelp growers in Norway use square frame moorings, anchored to the bottom, and stretch cultivation ropes between the frame ropes (figures 6 and 7). In very exposed areas, round frame moorings are better suited (figure 8) (Buck & Buchholz, 2004). Others fix the cultivation ropes directly on bottom-anchored bladders (figure 6).

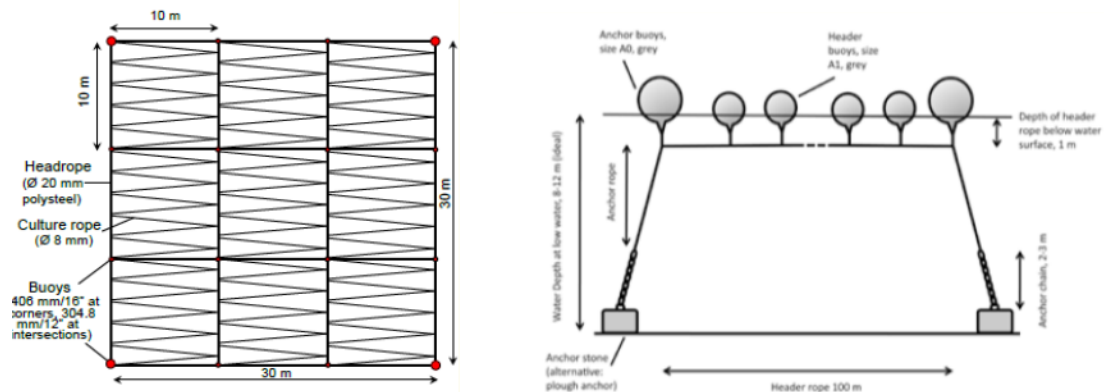


Figure 6: Left: cultivation plant with frame mooring from above Right: single anchored cultivation rope
Source: Edwards & Watson, 2011

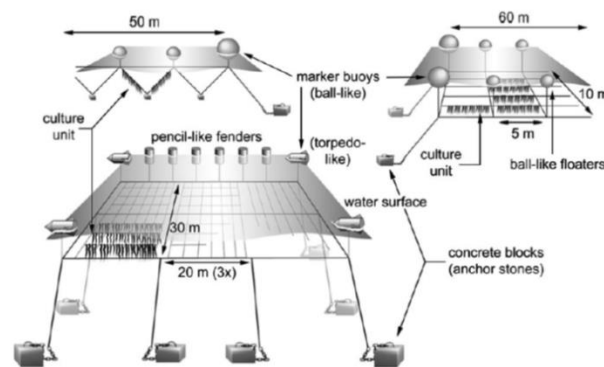


Figure 7: cultivation plant with frame mooring from the front;
Source: Buck & Buchholz, 2004

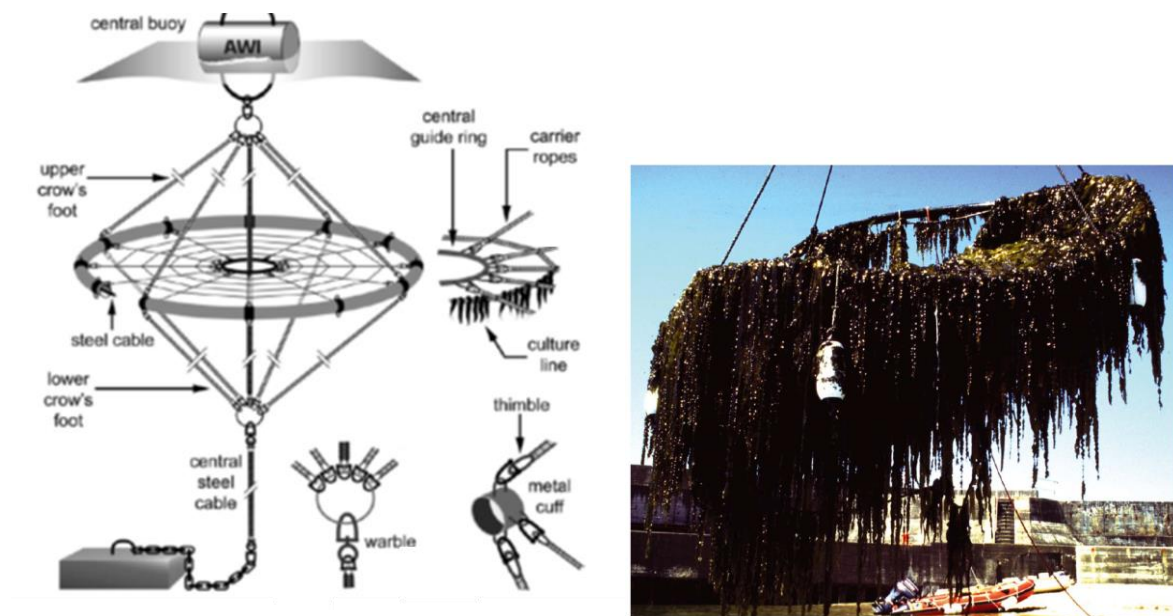


Figure 8: Cultivation plant «the offshore ring» for exposed conditions
Source: Buck & Buchholz, 2004

Subsequently, we describe the most important elements for the establishment of frame moorings and directly anchored cultivation ropes.

Cultivation facilities must be anchored on the seabed. There are many different types of anchoring: concrete anchors, large stones, fluked anchors, etc. Anchor size and weight depend on the bottom type, current, size of cultivation plant and many other parameters. Therefore, one cannot give specific guidance regarding the anchor type and size. We recommend concrete anchors and anchor stones because it is the cheapest. Such anchors shall have an average of 0.5 tons per unit and 1 tons for 100 m of cultivation rope (Figure 6 - 8). Several meters of heavy chain with at least 10 - 20 cm links are required to connect anchors or stones to anchor ropes (Figures 6 and 8). Suitable anchor rope is e.g. 25 mm braided polypropylene rope. The anchor rope must be long enough to compensate for the tidal difference. Top ropes in frame moorings can also be 25 mm thick, while single anchored cultivation ropes can be thinner (8 - 20 mm). Buoys for frame moorings must be large enough to keep entire facilities below the water surface. Buoys for adjusting cultivation ropes do not have to be very large. A larger number of smaller buoys (e.g. 3 - 10 small buoys (1 – 3 l) on 100 m cultivation rope) work well, as long as they are evenly distributed. In this way, you get well-balanced buoyancy, which supports the cultivation ropes, even if one or two buoys are lost over time (Figure 6 - 8). All kelp cultivation facilities are also required to be marked by a standardized marker buoy.

For deployment, you need a boat, eventually with a crane. Everything must be large and heavy enough to set out anchors and ropes. While you only need a 5 m boat and two people to put out a single cultivation rope (12 mm thick, 100 m long) anchored with two 30 kg dredges, you need a 10 m boat with a crane and crew, possibly also divers, to put out frame mooring.

Deployment of cultivation rope requires two people and a boat. The cultivation ropes must be at least 6 mm thick. Thinner seedling ropes should be spun around thicker ropes when released. We recommend the use of sinking nylon ropes as cultivation ropes. The cultivation ropes are attached either to the frame mooring or directly to the buoys and anchors. You may need weights to keep floating cultivation ropes under water or to stabilize cultivation ropes in heavy currents. At the same time, it is important to adjust the cultivation depth with small buoys. We recommend a cultivation depth of 1 - 3 m. You can test for optimal cultivation depth at your facility with trial cultivation on vertical cultivation ropes. Production on vertical cultivation ropes is not recommended, since the work effort is increased compared to horizontal cultivation ropes.

The season for deploying seedlings at sea in northern Norway is October to February.

Seaweed farms require little care, but regular inspections are necessary to keep plants in good condition. We recommend that facilities are inspected once a week to once a month to replace or insert buoys, remove driftwood, unravel tangled ropes or to fix other minor damage. In addition, we recommend inspection after storms and bad weather.

Harvesting will take place in Northern Norway from May to July, before seawater temperatures increase significantly, and before growth of fouling organisms decrease product quality. Harvesting requires a lot of work, and the use of seasonal workers is recommended.

Large-scale harvesting requires a boat that has enough storage capacity for the biomass until it is delivered at land. At some facilities, you can alternatively store harvested kelp in nets in the sea, and later pull the nets ashore.

For harvesting, the cultivation rope is lifted up from the water (eventually with a crane) and kept at a height that is suitable for processing the kelp. Operators work their way along the rope and remove unwanted growth, before cutting off the kelp and sending it for storage. Harvested cultivation ropes can also be taken in.

One challenge is the stabilization of biomass. Sugar kelp in particular is quite sensitive to warm temperatures, and tightly packed kelp quickly loses quality. After harvesting, the kelp should be processed within 1-2 hours to ensure top quality. Seaweed stored in a basin with flowing water in the boat or in the net on the outside of the boat can, however, last for several days. An alternative to stabilizing the kelp that is popular in Asia is blanching right on the boat or immediately after landing.

Landing works best at ports and quays. If you harvest large quantities, the use of cranes, tractors, trucks, etc. is recommended.

Farming technology used in other countries for kelp cultivation is mainly based on single anchored ropes, not frame mooring. *Undaria* spp. e.g. are cultivated in Asia on single horizontal ropes, either directly or on horizontally attached "droppers". Japanese sugar kelp is also grown on single horizontal ropes, or on connecting ropes between two horizontal ropes. Various seaweeds are also grown on nets, either underwater, floating or semi-floating (Nori spp., *Monostroma* spp.), or the kelp is fixed on the seabed with plastic pipes, ropes or "holders" (Pereira & Yarish, 2008).

4. Overview for the status of other species

As of today, there are cultivation permits for 32 species of seaweed and kelp registered with the Norwegian Directorate of Fisheries. Nevertheless, actual commercial production today focuses on sugar kelp and winged kelp - only one company sells farmed sea lettuce (Daniel Aluwini). The cultivation status of all other species in Norway is at the research level.

However, many other species of seaweed and kelp also have a good potential for cultivation. Almost all seaweed and kelp species are edible and thus a good product (Belghit et al., 2017; Biancarosa et al., 2018), but control of the life cycle and production of seedlings is demanding.

We see a lot of potential in *Palmaria palmata*, a traditional Norwegian food algae, but existing seedling production methods are not very effective for sea-based farming (Werner & Dring, 2011).

Production of other red algae, such as *Chondrus crispus* or *Furcellaria lumbricalis*, requires a lot of working power (Zertuche-González et al., 2001) and is thus poorly suited for production in Norway without automation of the cultivation methods.

Nori algae such as *Porphyra* spp. or *Monostroma* spp. have a complex life cycle; however seedling production has not been established for Norwegian species. Nevertheless we believe that their potential is very large, since Nori sheets are a popular product that is sold all over the world.

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