Summary of Culturing Conditions

		Temp	Light
Sorus	Induction	10-12°C	White (400-700nm), 8:16 L:D, 100 µmol
	Release	10-14°C	Dark
Gametophytes	Active growth	10-12°C	Red (600-700nm), 12:12 L:D, ≤30 µmol
	Low growth	5-10°C	Red (600-700nm), 12:12 L:D, ≤10 µmol
Sporophytes/crosses	1-7 days post-cross	12°C	White (400-700nm), 12:12 L:D, ~20 µmol
	7-14 days post-cross	12°C	White (400-700nm) or blue (450-495nm), light cycle varies, ~30-50 µmol
	Nursery	12°C	White (400-700nm), 12:12 L:D, start 50 µmol and increase slowly to 130 µmol

Benefits of Gametophyte Culturing

- Enables gene banking and storing "seed" for farming and restoration
- Doesn't depend on ripe wild seed timing or scarce supply
- Limits pressure on natural populations
- Enables experimentation and selection of superior and resilient strains for farming

About Us

The Lindell Lab at the Woods Hole Oceanographic Institution researches and advances sustainable marine aquaculture to meet society's needs for low carbon alternative food, feed, fuel, and other bio-products. Our lab works at the intersection of technology, genomics and policy to advance the feasibility, economics and acceptance of aquaculture. Current projects funded by the US Department of Energy's ARPA-E MARINER program and the WWF aim to develop scalable technology for open ocean farming (temperate and tropical) and selectively breed sugar kelp, all with the potential to generate feedstocks for bio-fuel production.

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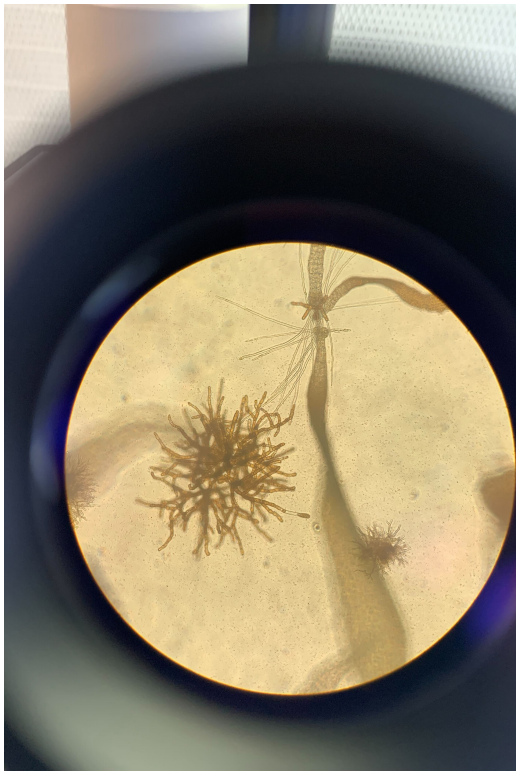

Gametophyte Culturing Process for Sugar Kelp (*Saccharina latissima*)

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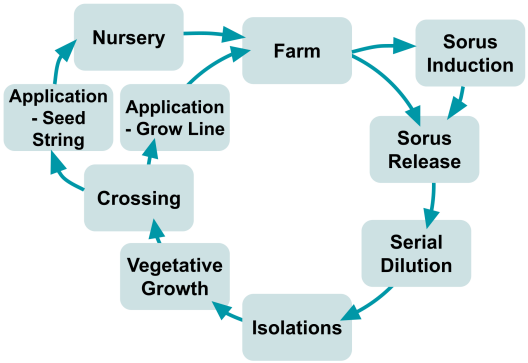
The Process



Sorus Induction

Blades collected without mature sorus tissue can be held in tanks under white light to induce sorus formation. Sorus may develop in 1-8 weeks.

Sorus Release

A spore release can be performed on blades with mature sorus tissue. Sorus tissue is cut from the blade and scraped with a blade, dipped in iodine and then filtered seawater. Disinfected sorus is put through a period of desiccation at 5-12°C in the dark. To promote spore release, the tissue is put in cold, filtered seawater and brought up to no more than 14°C. A sample of seawater is checked under a microscope for the presence of spores.

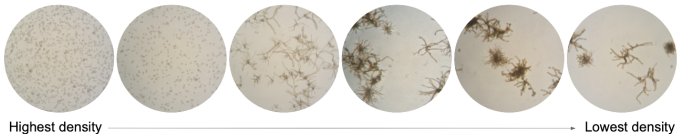
Serial Dilution

Spores from the release are counted on a hemocytometer to estimate total density. A serial dilution is then performed for:

1. *Optimal growth* – gametophyte tufts at lower densities have more available space and nutrients
2. *Easy isolating* – a less dense well allows individual tufts to be easily separated
3. *Reduced contamination* – contaminants are diluted out and are less likely to impact the health of the culture

Serial Dilution continued...

The starting concentration of spores in the first well is ~10,000 spores/mL. Subsequent wells are diluted to create a concentration of ~123 spores in the final well.



Isolations

Individual spores develop into male or female gametophytes, which can be isolated from the least dense wells of the serial dilution. Males typically have a smaller cell size and are less branching than females.

Growth

Cultures are kept in conditions optimal for gametophyte growth and are held in various size containers based on biomass (seen below, left). As biomass increases, cultures are moved to a bigger container. To prevent clumping and encourage growth, gametophytes are fragmented. Smaller cultures are chopped with a razor blade and larger cultures are blended. Water changes are performed as needed to replenish nutrients. A small portion of each culture should be kept as a backup in low growth conditions.

Crossing

Gametophytes are mixed in a 2:1 female to male ratio (10mg wet weight of female plus 5mg wet weight of male per 1 meter of line) and moved into white light to induce fertility. A sample of the cross can be checked under the microscope for the presence of eggs and juvenile sporophytes to ensure the cross was successful before application. Selective breeding can be used to select for desirable traits, such as biomass yield.

Application

Seed, a successful cross, can be applied to seed string by painting or spraying and then incubated in a nursery. Seed can also be applied directly to grow lines and put out on the farm following a period of drying, skipping the nursery stage.

Nursery

Seed string is held in the nursery until sporophytes are ~2-5mm in length. Light conditions are set for optimal sporophyte growth, containers are aerated and water is changed as needed to replenish nutrients.

Farm

Gametophyte seed string is transported to the farm and outplanted following traditional methods. Directly seeded grow lines are transported in larger containers and installed on the farm. Reproductive material is collected at harvest.