PUGET SOUND
RESTORATION FUND

Kelp Cultivation Handbook

This handbook is a compilation of helpful resources and the step-by-step process staff at Puget Sound Restoration Fund (PSRF) have used (with success!) to cultivate native Puget Sound kelp in a nursery for the purpose of outplanting. This process has been primarily used for sugar kelp (*Saccharina latissima)*, particularly in New England and Europe, but PSRF has used it extensively for bull kelp (*Nereocystis luetkeana)*, and it can be applied to most kelp species of interest.

Table of Contents

Appendices/Useful Resources

READ THIS FIRST

Major Considerations and questions to ask yourself at the onset of kelp cultivation:

1. Are you familiar with the rules, regulations and permits required by Washington state to cultivate kelp/seaweed, including sourcing seed?
2. What are you setting this nursery up for? What will be the scale of cultivation?
3. In setting up your cultivation space/nursery how do you plan to maintain the following?
    a. Temperature control (cold room or chilled water via recirculation or heat exchange)
    b. Light supply & control (irradiance, wavelength, photoperiod)
    c. Aeration (maintain pH, nutrient cycling)
    d. Sterile seawater production and storage
4. When selecting aquaria, first build a spool for your farm application, determine the number of spools you will be seeding (think back to question 2) and then source aquaria to accommodate those spool dimensions.

Lessons learned by PSRF (aka where are the 'tricky' spots in the process?):

- Bull kelp sorus can be collected from rocky shore habitats (ex. Strait of Juan de Fuca) year-round in most years where the adults can overwinter.
- February is the best time to transfer cultivated bull kelp seed, if making benthic deployments.
- Sorus collected from 1 plant is sufficient to produce spore stock, however collecting sorus from 10-20 plants is recommended to attempt to capture the genetic diversity within wild populations.
- In designing the layout of your cultivation space, ensure proper and efficient workflow via easy access for cleaning tanks and moving materials/equipment around space (ex. inserting/removing spools).
- Spool preparation is a crucial step - see page 5.
- Maximize spool number to media volume (little extra space) in cultivation aquaria, to be efficient with media.
- See Table 2.1 in Ocean Approved 'Nursery Recommendations' in Appendices. Note: PSRF uses a combination of filtration and UV treatment to produce the large volumes of sterile water.
- Buy nutrient media kits (as opposed to making your own, though to do so check out 'Nutrient Media Suggestions and Supplies Checklist' from Connecticut Sea Grant and Ocean Approved). PSRF recommends PES or Guillard's F/2.
    - Change media every 10-14 days unless contamination is occurring
    - Provide nutrient media with 10 mL media stock /L sterile seawater
- Batch sporulation - see the note in Step 9 of 'Sorus prep & desiccation.'

- We do not recommend sporophyte cultures for seed transfer to be grown in the lab past 5 weeks; there is no benefit to lab culture beyond the initial observations of germling sporophytes, which occurs around day 21. Naked eye evaluations of germling seed distribution are possible around day 25-30.
- Sources of contamination - spore stock, people, media and aeration system.
    - Use chlorine bath outside of cultivation space for materials
    - Use Vortexx (disinfectant), microwave, autoclave, steam, filtration, UV light inside cultivation space
    - Minimize people traffic and time spent in the cultivation space!

Nursery Cultivation Calendar

Before Starting: Purchase and set up nursery equipment, sterilize and wind spools, collect and filter seawater						
Day 0 or 1: Collect Sorus Material just prior to the new or full moon - Bull Kelp (Jul-Jan), Sugar Kelp (Oct/Nov).						
1	2	3	4	5	6	7
Prepare and Dessicate Sorus Material	Sporulation and Substrate inoculation (to step 7)	Sporophyte Culture (steps 8-11)	Growth assessment & Daily nursery maintenance*	Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance
8	9	10	11	12	13	14
Daily nursery maintenance	Growth assessment & Daily nursery maintenance	Media Change (*if needed*)	Daily nursery maintenance	Daily nursery maintenance	Growth assessment & Media Change (*if needed*)	Daily nursery maintenance
15	16	17	18	19	20	21
Daily nursery maintenance	Daily nursery maintenance	Growth assessment & Media Change	Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance
22	23	24	25	26	27	28
Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance	Growth assessment & Media Change (*if needed*)	Daily nursery maintenance
29	30	31	32	33	34	35
Daily nursery maintenance	Daily nursery maintenance	Growth assessment & Media Change	Daily nursery maintenance	Daily nursery maintenance	Daily nursery maintenance	**Transfer to Ocean Site**
* Daily nursery maintenance = check that everything is working (water levels, temperature, air flow, etc.)						

PSRF Nursery Cultivation Supplies/Materials

Preparing and Sterilizing Spools

- 3-inch Sch-40 PVC cut 13 inches high (round/notch edges for twine)
- Pillowcases (large, natural fiber with high thread count)
- Warm freshwater
- Biodegradable/castile soap & sponge
- Surgical/exam gloves
- Chlorine solution (100-200 ppm)
- #15 of #18 nylon twine
- Na_2CO_3 solution (15g/gallon)
- Buckets or other containers w/ known volume

Preparing and Desiccating Sorus Material (Day 0 or 1) -

- Cooler w/ ice packs or chilled seawater
- Working surface - cleaned with 90% isopropyl alcohol
- Surgical/exam gloves
- Cutting board
- Knife/Razors
- Clean paper towels (at least six rolls)
- 4 to 5 sterilized 2 L wide mouth Nalgene containers filled with chilled sterile seawater (50°F/10°C)
- 1 sterilized 2 L wide mouth Nalgene filled with 1 L solution of diluted betadine (5 mL iodine / L seawater)
- Tupperware or large plastic bag
- Refrigerator

Sporulation, Substrate inoculation and Cultivation (Day 2 - on) -

- Surgical/exam gloves
- 4 sterile 1 L glass beakers
- Sterile seawater
- Aluminum/tin foil
- Tongs/tweezers
- Cheese cloth
- Rubberbands
- Clean Pasteur or plastic micropipettes
- Plastic culture well plate
- Lugol's solution
- Microscope
- Hemocytometer
- Calculator
- Cultivation room w/ environmental controls
- Slide stand & 8-10 test slides
- Clean 20 L/5-gal buckets with lids or settling aquaria
- Deionized or distilled freshwater
- Cultivation aquaria (ex. 60 L acrylic tanks) w/ environmental controls (i.e. cold room)
- Clean air stones w/ tubing
- Vortexx
- Clean prepared nursery spools
- Air pump
- Mechanical timer
- Culture nutrients: Provasoli-Enriched Seawater (PES) or Guillard's F/2, Vitamins, Germanium Dioxide (Ge02)
- Grow lights (ex. cool white T8) and mechanical timer

Media change (as needed)

- Vortexx solution
- 20 L/5 gal buckets (2)
- Sponges
- Nutrient media (10-20 ml/L Guillard's F/2 Part A and B)
- Clean pipettes
- Sterile seawater
- Distilled freshwater
- Handpump
- Germanium Dioxide saturated solution (0.02 to 0.2 mL/L cultivation tank volume
- Isopropyl alcohol
- Clean air stones

Spool Preparation

1. Clean spools and pillowcases
    a. 1 hour soak in warm freshwater & biodegradable/castile soap
    b. Scrub with sponge or scour pad as needed to remove visible dirt/fouling
    c. Rinse 3x with freshwater
2. Sanitize spools and pillowcases (*gloves on*)
    a. Place in chlorine solution (100-200 ppm) for 12 hrs.
    b. Rinse with freshwater 1x
    c. Air dry spools and pillowcases for 10-12 hours (minimum)
        i. Cover with cardboard, plastic, or paper towel to keep dust out
3. Build twine spool
    a. #15 twine = 300 Ft./spool; #18 twine = 230 Ft./spool
    b. Store wrapped/ built spools in dust-free container
    c. Use datasheet (next page) to track lot, label containers with lot information
4. Remove hydrophilic contaminants and dyes
    a. Place twine spools in a distilled freshwater bath for 72 hours
        i. Rinse 1 x with tap water
    b. Repeat distilled bath for 48 hours (recommended)
        i. Rinse 1x with tap water
5. Remove oils (*gloves on*)
    a. Prepare $NaCO_3$ solution (15g/gallon) in container(s)
        i. Dissolve detergent in warm water, then add water to achieve total volume
    b. Add spools, ensure all are sufficiently submerged
        i. Soak spools 5-10 hrs. then rotate/agitate
        ii. Soak spools and another 5-10 hrs.
    c. Rinse clean spools 3x in freshwater
    d. Soak in freshwater for 10-12 hrs.
    e. Rinse 1x
6. Load clean (and still wet) spools into clean, dry pillow cases (*gloves on*)
    a. Air dry 24-48 hours (rotate every 12 hrs); fan optional
    b. Load clean & dry spools (in pillowcases) into clean & dry tote with lid for storage.
        i. Option: store in freezer – but will need to re-dry after remove

TWINE SPOOL PREPARATION LOG

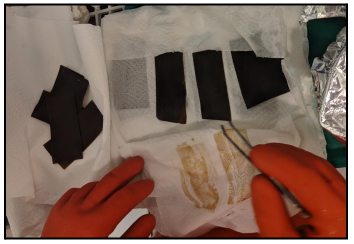
	STEP 1: WARM WATER & SOAP CLEANING		STEP 2: SANITATION BATH (100 PPM HYPOCHLORITE)			STEP 3: BUILD SPOOLS	STEP 4: DISTILLED WATER BATH	STEP 5: SODIUM CARBONATE BATH					STEP 6: PREPARED SPOOL ARE DRIED AND STORED FOR USE		
SPOOL BATCH	WASH	RINSE 3X	START TIME	END TIME	AIR DRY 10-12 HRS	WRAP PREPARED SPOOL WITH TWINE	72 HRS	PHASE 1 START	PHASE 2 START	RINSE (3X)	FRESHWATER SOAK (10-12 HRS)	FINAL RINSE 1X	LOAD PILLOW-CASE	AIR DRY	STORE SPOOLS

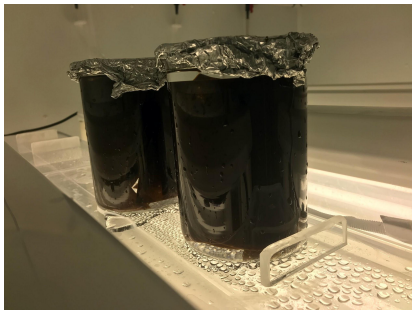
Step-by-Step: PSRF's Nursery Cultivation of Kelp

Step	Procedure	Materials	Time	Notes
Sorus Collection (Day 0 or 1)	Collect ripe sorus and place in cooler	• Cooler with ice packs or chilled seawater		Dark chocolate brown and thickness indicates ripeness  When collecting wild sorus, look for healthy plant material with minimal grazing/fouling
Sorus prep & desiccation (Day 0 or 1) *Step 1*	Trim sorus tissue to a size that can fit comfortably on a section of paper towel	• Working surface - cleaned with 90% isopropyl alcohol • Gloves • Knife • Cutting Board		Remove any heavily fouled or grazed sections of the sorus 

Sorus prep & desiccation (Day 0 or 1) *Step 2*	Mechanically clean each trimmed sorus tissue with clean paper towel	• Paper towels		Use light pressure to gently wipe surface 
Sorus prep & desiccation (Day 0 or 1) *Step 3*	Place sori in 2L wide-mouth Nalgene containing sterile seawater	• 2L sterile wide-mouth Nalgene • Sterile seawater		Use multiple containers if necessary
Sorus prep & desiccation (Day 0 or 1) *Step 4*	Move sori to new Nalgene containing a 1L diluted betadine solution, cap and shake vigorously	• 2L sterile wide-mouth Nalgene • 5mL Betadine (10% iodine) in 5mL/L of sterile seawater	***30 sec.***	Rinse no longer than 30 seconds
Sorus prep & desiccation (Day 0 or 1) *Step 5*	Drain Betadine solution from Nalgene, replace with sterile seawater and shake. Do 3 rounds of this rinse.	• Sterile seawater	***30 sec. bath***	You can also remove the sorus material from the betadine Nalgene and rinse in a new Nalgene with sterile seawater

Sorus prep & desiccation (Day 0 or 1) *Step 6*	Place sori in sterile seawater Nalgene	• 2L sterile wide-mouth Nalgene • Sterile seawater		Cap Nalgene and sterilize the exterior of the bottle by quickly pouring boiling hot water around it.
*** At this stage, sori will be sterilized, and care should be taken to keep hands, tools, and surfaces sterilized ***				
Sorus prep & desiccation (Day 0 or 1) *Step 7*	Remove the sorus from seawater one at a time and blot dry gently before alternatively layering paper towel and sorus tissue.	• Paper towels • Gloves		Build a sorus 'lasagna". See note in step 10
Sorus prep & desiccation (Day 0 or 1) *Step 8*	Place layers of sori and paper towels in tupperware or large plastic bag with one to two pieces of paper towels dampened with sterile seawater	• Sterile tupperware or large plastic bag		Goal is to create conditions for mild desiccation 
Sorus prep & desiccation (Day 0 or 1) *Step 9*	Place tupperware/bag in refrigerator/room (temperature around 4-5°C)	• Refrigerator • Gloves	1-24 hrs.	Length of desiccation required is dependent on ripeness of sori, less ripe (lighter in color) will take longer to release spores Option to "batch" sporulation by desiccating ripest for ~4 hours, attempting sporulation, leaving less

				ripe sori longer and sporulating after ~24 hours (sugar kelp might take 48 hrs.)
Sporulation (Day 2) *Step 1*	Remove tupperware/bag from desiccation, check if ready to release spores	• Gloves		Peel back covering layer of paper towel If ready to release spores, sorus will leave a brown stain on paper towel 
Sporulation (Day 2) *Step 2*	Split sorus between two large beakers of sterile seawater	• 2 1 L sterile glass beakers • Sterile seawater		Group A: most ripe sorus Group B: less ripe
Sporulation (Day 2) *Step 3*	Place clean tinfoil over beakers and place in cultivation room (set to 10°-15°C)	• Tinfoil • Temperature controlled room	1-3 hrs.	Room should be well illuminated
Sporulation (Day 2) *Step 4*	Visually assess spore release			Good release will leave the water cloudy

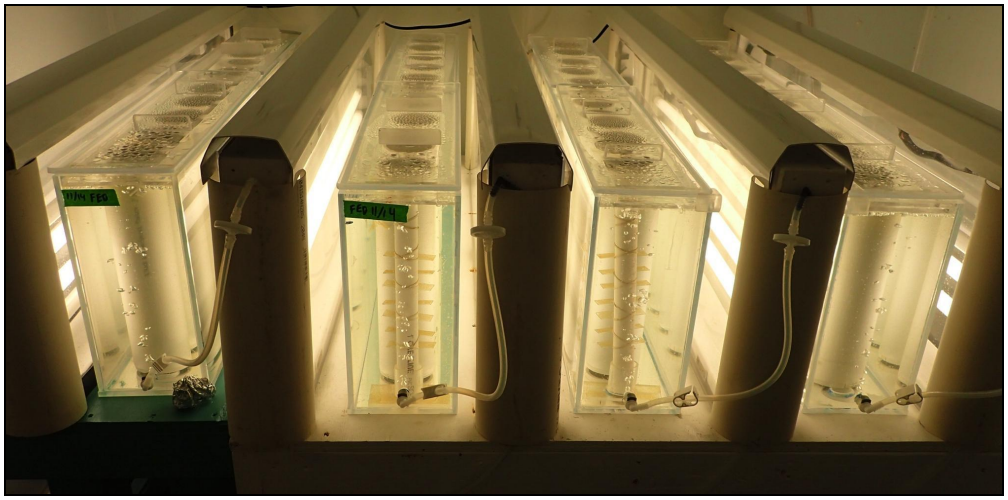
				
Sporulation (Day 2) *Step 5*	Remove sorus tissue from beakers using sterile forceps and allow mucilage to settle	• Gloves • Forceps	30 min	You can discard sori at this time and should fully remove it from the cultivation space
Sporulation (Day 2) *Step 6*	Place new cheesecloth over the tops of 2 new clean beakers.	• 2 1 L sterile glass beakers • Cheesecloth • Rubberbands		Secure around edges using rubber bands. Make sure to leave them loose over the top to allow for filtration of spore solution
Sporulation (Day 2) *Step 7*	Filter spore stock by decanting through cheesecloth into fresh beakers			Wet cheese cloth slightly before pouring solution 

Sporulation (Day 2) *Step 8*	Using a sterile pipette, collect two small volume samples of spore stock from each spore batch in a culture well plate for use in spore density assessments.	● Pipette ● Culture well plate ● Lugol's solution		Move quickly to density and motility assessment as the longer the stock is in the well, the more spores will die from exposure. Use lugol's solution to kill one sample from each spore group.
Sporulation (Day 2) *Step 9*	Using a sterile pipette, place a drop of spore stock in the hemocytometer well and use a microscope to assess densities. *See notes (right) or the Ocean Approved 'Counting Zoospores and Calculating Stocking Density' in the Appendices*	● Pipette ● Hemocytometer ● Microscope		Hemocytometer density assessment - done on <u>lugols-killed</u> sample 1. Count number of spores per square in hemocytometer a. Count 5 squares in each of 3 columns b. Average these counts together c. Multiply by 25 (total number of squares in hemocytometer) d. Multiply this by 10,000 to get an estimate of spores per mL Visually assess spore motility in <u>non-lugols sample</u> 2. Estimate percent motility a. More motile spores = better b. Nonmoving spores may be dead but can germinate

				Multiply calculated spores per mL (number in 1.d.) by percent motility to get final estimate of spores per mL
Sporulation (Day 2) *Step 10*	Using spore density, calculate total volume of spore solution necessary to inoculate substrate with a range of **1-5k spores/mL**			
The next step should take place in a cold room that allows inoculation to occur at 10-12 °C				
Substrate inoculation (Day 2) *Step 1*	Clean test slide stand and 8-10 slides using standard glassware cleaning procedure (detergent, water rinse, distilled water rinse) secure slides to stand via rubber bands.	• Slide stand • 8-10 slides • Rubber bands		 Slides and stand can also be sterilized after cleaning with steam, Vortexx or chlorine bath (Vortexx 1mL/ or 100 ppm chlorine for 12 hours, air dry)
Substrate inoculation (Day 2) *Step 2*	Clean inoculation buckets by sponge with Vortexx, let stand for 2 minutes, then rinse with freshwater (x3), and then sterile seawater (x3), before filling with sterile seawater	• Buckets (20 L/5 gal) or Aquaria • Vortexx (2mL/L) • Freshwater • Sterile seawater • Sponge		
Substrate inoculation (Day 2)	Set up air stones - Connect tubing and place one stone in each bucket	• Air stones • Tubing and bucket lids		

Step 3		• Cultivation room		
Substrate inoculation (Day 2) *Step 4*	Add desired spore stock volume to the settling vessels. Let air stones agitate and aerate spore stock for several minutes before dialing the air flow down to a trickle.		5-10 min	See sporulation steps 9 and 10
Substrate inoculation (Day 2) *Step 5*	Shut off air and hook up air pump to outlet with mechanical timer	• Air pump • Mechanical timer		Set timer to turn aeration on for 30 minutes at midnight
Substrate inoculation (Day 2) *Step 6*	Place cleaned and dry spools in buckets or aquaria. Top off the volume with sterile seawater so that spools are covered.	• Clean prepared nursery spools • Sterile seawater		Place slide stand(s) with spools.
Substrate inoculation (Day 2) *Step 7*	Secure lids on settling buckets and/or shut off all lights and allow spores to settle		24 hrs.	
Sporophyte Culture (Day 3) *Step 8*	Fill cultivation aquaria with sterile seawater and add nutrient media to achieve 10 ml/L	• Aquaria • Sterile seawater • Nutrient media (e.g. F2 algae food)		Clean aquaria with the same process as in Day 2, step 2. Allow water in aquaria to reach around 10 C and allow nutrients to mix in before transferring spools (~15 min)

Sporophyte Culture (Day 3) *Step 9*	<u>Gently</u> transfer spools and slides to cultivation aquaria			
Sporophyte Culture (Day 3) *Step 10*	Plug lights into a mechanical timer. Set timer for 16:8 light:dark cycle	• Grow lights • Mechanical timer		For non bull kelp cultivation consider using a 12:12 light:dark cycle, as recommended by other manuals. Irradiance recommendations: 25-50 µmoles m^{-1} s^{-1} during weeks 1-2 80-100 µmoles m^{-1} s^{-1} during weeks 3-4
Sporophyte Culture (Day 3) *Step 11*	Set air stones at either end of aquaria, gently bubbling to circulate nutrients and water	• Air stones	__ days	Air stones and tubing should NOT be making contact/bubbling directly on spools Air is left ON at all times during cultivation



Once a week (or more) remove a slide from the slide stand, wipe top surface clean, and examine the underside under a microscope to assess growth and culture cleanliness. ***If diatoms and ciliates are observed, steps can be taken to mitigate them - see media change section below***

Media change *Step 1*	Prepare sterile water 24 hours prior to media change			Allows water to achieve temperature - PSRF uses cold room to regulate seawater and media temperature
Media change *Step 2*	Prep materials - 1. Prep 10 L of Vortexx solution in a clean 20 L bucket 2. Place sponges in Vortexx solution bucket and allow to soak 3. Gather nutrient media, pipet and handpump	• Vortexx solution (2 ml/L) • 20 L bucket (2) • Sponges • Nutrient media • Pipet • Handpump		Prep materials in lab before entering cultivation room

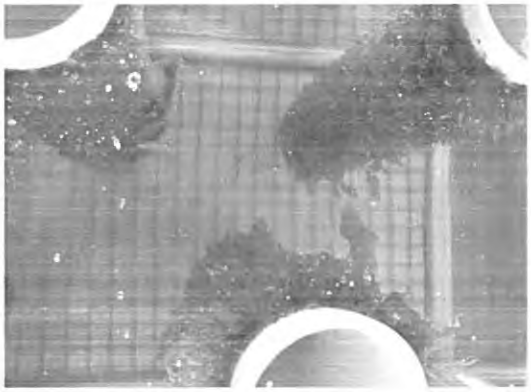
	4. Prepare a clean 20 L bucket to be filled with sterile seawater (as needed for gentle spool rinse)			
Media change *Step 3*	Rinse temporary holding vessels (clean aquaria or buckets) thoroughly – Freshwater rinse (3x), Sterile seawater rinse (3x) – before filling	● Aquaria or buckets ● Freshwater ● Sterile seawater		Fill container with sterile seawater sufficient to cover spools
Media change *Step 4*	Remove spools from a cultivation tank #1 and place in temporary holding container			
Media change *Step 5*	Drain cultivation tank #1 completely			
Media change *Step 6*	Thorough freshwater rinse 3x			Take care to remove any/all debris
Media change *Step 7*	Scrub all cultivation tank surfaces with Vortexx-soaked sponge			Make sure to scrub all corners, seams and sides as well as lids Wait a minimum of 2 minutes before rinsing cultivation tank #1
Media change *Step 8*	Rinse cultivation tank #1 with freshwater 3x			

Media change *Step 9*	Begin filling cultivation tank #1 with sterile seawater for 30 seconds with drain open to rinse out any residual freshwater		30 sec	
Media change *Step 10*	Close drain and fill cultivation tank #1 with sterile seawater to 1-inch below minimum level, including 1. Germanium Dioxide saturated solution (0.02 to 0.2 mL/L cultivation tank volume) 2. Nutrient media (0.13 mL/L, Guillard's F/2 Part A and B)	● Sterile seawater ● Germanium Dioxide saturated solution (0.02 to 0.2 mL/L cultivation tank volume) ● Nutrient media (such as 0.13 mL/L, Guillard's F/2 Part A and B		Germanium solution is used *as needed* to mitigate diatom growth - see Shea & Chopin (2007) J. Appl Phycol 19:27-32
Media change *Step 11*	Sterilize air tubing (wipe exterior with isopropyl alcohol) and replace air stones with fresh ones before setting aeration back up	● Isopropyl alcohol ● Air stones		Clean stones by soaking in 200 ppm chlorine bath and allowing to air dry for 24 hours
Media change *Step 12*	Move spools from holding tanks into the prepared (cleaned & media change) cultivation tank #1. Reverse spools orientation and order (end/edge vs. middle) and invert when replacing in cultivation tank #1			Hygiene option (can mitigate ciliates): Rinse each spool in 20 L bucket of sterile seawater by ***gently*** plunging and rotating.

Media change *Step 13*	Repeat steps 4 to 12 as needed until all cultivation tanks are clean and prepared			Can also use a prepared cultivation tank #1 to move tank #2 spools, then drain, clean, and change media in tank #2. Repeat until done, then move tank #1 spools into the last tank.

Kelp Farming Manual

A Guide *to the* Processes, Techniques, and Equipment for Farming Kelp in New England Waters



Katie Flavin
Nick Flavin
Bill Flahive, PhD

Ocean APPROVED
FARMING THE NORTH ATLANTIC

Copyright © 2013 by Ocean Approved

All rights reserved.

This manual contains general information about farming kelp in New England waters. The information is based on Ocean Approved's experiences with learning how to farm and is not to be considered the definitive resource. You must not rely solely on the information contained in this manual as an alternative to advice and direction from your State or Federal regulating agencies. If you have any specific questions about any regulatory matter you should consult the appropriate regulating agency. The technical information in this manual is provided "as is" without any representations or warranties, express or implied. Ocean Approved, LLC makes no representations or warranties in relation to the technical information in this manual.

Acknowledgments

The three year process of learning how to farm kelp in New England waters was a collaborative effort led by Ocean Approved, the University of Connecticut and the Bridgeport Regional Aquaculture Science and Technology Education Center. We thank the following organizations and individuals that generously supported this research:

Funding Support

The U.S. Department of Commerce/National Oceanic and Atmospheric Agency
The Maine Technology Institute
Coastal Enterprises Inc.

Research Support

Organizations

The University of Connecticut
The Gulf of Maine Research Institute
Kennebec River Biosciences
Northeast Laboratories
The Bigelow Laboratory for Ocean Sciences
Woods Hole Oceanographic Institute
The Maine Department of Marine Resources
BioArchitecture Laboratories
Dr. Hyung Geun, Gangneung-Wonju, National University of South Korea
Bridgeport Regional Aquaculture Science and Technology Education Center (BRASTEC)
Maine Sea Grant
The University of New Hampshire
The Aquaculture Research Institute at the University of Maine, Orono
The Maine Aquaculture Association
Bodega Algae, LLC

Individuals

Gary Arnold
Seth Barker
Chris Barlett
Michael Chambers, PhD
John Curtis
Paul Fischer
Bill Flahive, PhD
Katie Flavin
Nick FlavinLucia Flavin
Elin Haugen
Chris Higgins
Jang Kim, PhD
Jon Lewis
Scott Lindsey, PhD
Art Mathieson, PhD
Dana Morse
Sarah Redmond
Diantha Robinson
Eric Tamigneaux, PhD
Holly Turner
Robert Vadas, PhD
Charlie Yarish, PhD

Finally, we would like to thank John Forster, who encouraged Ocean Approved and introduced us to Charlie Yarish. Without John's continued encouragement and Charlie's encyclopedic knowledge of all things kelp, we would still be picking kelp off of rocks.

Chapter 2
Nursery

Overview

The nursery or laboratory is an area that is used for isolating kelp spores and supporting the early growth of young kelp plants (e.g., sporophytes) for later out-placement to sea. Regardless of the size or goals of the nursery, each nursery designed to grow kelp must aim to replicate the essential environmental conditions (water temperature, light, salinity, nutrient levels) found in their native habitat. One key advantage of a nursery setup is the ability to control these conditions for optimal growth and increased survival. As such, the function of every nursery is threefold: 1) support the growth of kelp from spores through sporophytes, 2) replicate the environmental conditions found naturally, and 3) control contamination. The purpose of this section is to provide a general overview of the essential capabilities a nursery must support, a comparison of water sterilization techniques, and a suggested list of equipment.

Operation of the nursery requires the understanding and use of basic laboratory equipment, attention to detail, and the ability to monitor and control the environmental conditions to support growth of the kelp. A variety of nursery designs and procedures are available for private and commercial seaweed growers; however, each of these are specifically tailored for the goals and capabilities of that particular

nursery. For instance, factors such as cost can vary dramatically depending on the nursery design and equipment purchased to meet these requirements.

The definitions, equipment, and procedures described in this chapter are those found to be successful and were utilized by Ocean Approved (OA) following more than four years of experimentation and trials. They do not represent the most inexpensive or costly setup that may be used. Depending on one's background, fisherman or scientist, these nursery processes may be considered a relatively easy stage or difficult stage in the farming process. However, if an interested kelp grower, no matter what background he or she has, follows these procedures and maintains the described conditions, the spores from reproductive kelp plants may be isolated and grown in the nursery. In a matter of four to six weeks' time the young sporophytes will be ready for out-placement to ocean farm sites.

Essential Capabilities

Temperature

Kelp grows in water between 41 and 59°F (5 and 15°C, respectively). The nursery must have the capability of controlling water temperature within this range through either the use of cold rooms or water chillers.

Light

Light intensity, wavelength, and light hours per day (photoperiod) must also be controlled. This may be accomplished by the use of environmentally controlled growth chambers or by external light fixtures. A photoperiod of 12 hours "lights on"/12 hours "lights off" was used in the OA lab.

Fresh Water

Distilled or deionized water must be readily available for cleaning glassware and equipment and mixing reagents. The volume needed will depend on the scale of the operation.

Seawater

A reliable source of seawater will be necessary for isolation and grow-out of the kelp spores and plants. Depending on location, this may be directly piped in or carried in. Culture nutrients are added to the seawater for optimal kelp growth. Contaminants must be removed from the seawater (i.e., the seawater must be disinfected or sterilized) prior to use. Synthetic seawater may be used, but was found by OA to be less productive. Table 2.1 (on page 40) describes and compares various methods for cleaning seawater.

Aquaria

(Also referred to as production aquaria or growth tanks.) A great deal of flexibility is possible here. Aquaria ranging in size from 5 to 50 gallons (or larger) may be used, but the environmental parameters mentioned here must be maintained.

pH

The acidity/alkalinity range must be monitored and controlled. Kelp spores and plants grow best between a pH of 7.0 and 9.0. The pH must be measured regularly and adjusted with the addition of carbon dioxide (CO_2) gas as needed.

Microscope

A compound microscope and counting chamber is required. The reproductive spore cells that are released and captured for culture are very small and can only be seen with the use of a microscope. A counting chamber is necessary to determine the number of spores released so that the proper density of spores can be established for each culture.

Aeration

Adding air, or "bubbling air," into the production aquaria is also essential to maintaining pH. This is typically done by the use of small aquaria or laboratory air pumps with the use of air filters to remove airborne contaminants. OA recommends using HEPA air filters to eliminate potential contaminants.

Sterilization and Contamination Control

A process for sterilization or removing contaminants is mandatory. As shown on page 40 in Table 2.1, standard sterilization techniques include autoclaving, tyndallization, pasteurization, filtration, and ultraviolet (UV) irradiation. There are numerous considerations (e.g., cost, time, effectiveness) to be aware of when choosing the most effective technique that meets the needs of the nursery. The pros and cons of each of these processes are listed in Table 2.1.

After experimenting with most of the procedures described in Table 2.1, OA adopted the protocol illustrated in Figure 2.1 This protocol was able to remove almost all contaminants at a cost and time much less than transporting autoclaved seawater prior to use.

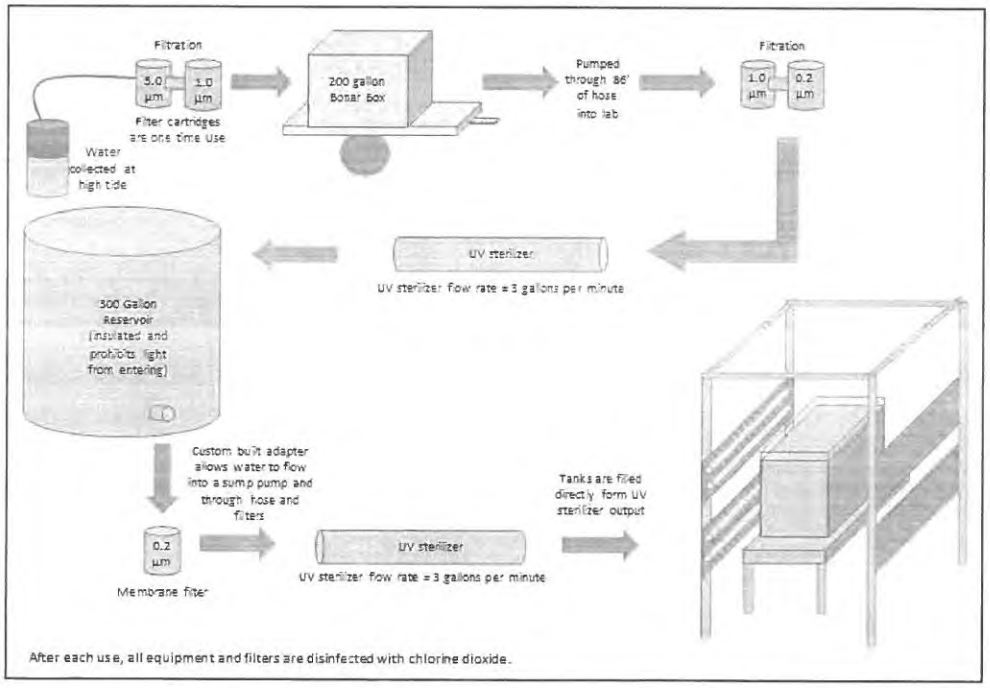


Figure 2.1 Process used by OA to collect seawater from the ocean and sterilize it for use in the nursery aquaria

Advanced Capabilities

Large universities, research institutes, or aquaculture facilities may have the resources, space, and capabilities to have flowing treated seawater, large walk-in autoclaves, and large-scale environmentally controlled growth chambers. While

these facilities are expensive to build and maintain, they do provide opportunities for much greater control of all phases of nursery operations. These facilities also allow for more advanced nursery operations, such as the cloning and growth of gametophytes, to allow for year round inoculation of spools.

Table 2.1 Seawater Treatment Methods to Control Contaminants

Technique	Definition	Example Duration	Equipment Needed	Pros/Cons	Comments
Autoclaving	Sterilization using steam (water) under high pressure.	15 minutes at 121°C	Autoclave	**Pros:** • 100% effective • Minimum manpower **Cons:** • Expensive • Time consuming with large volumes	Autoclaving is very expensive for large volumes of seawater and may alter the chemistry of the water. All living organisms are destroyed. It takes only a few hours with little supervision.
Pasteurization	Partial decontamination of a substance at a specific temperature and a duration that kills most organisms without major chemical alteration of the substance.	30 minutes at 61.7°C	Standard stove	**Pros:** • 80% effective **Cons:** • Labor intensive	This is relatively inexpensive, can be done quite quickly, but only partially destroys living organisms in the liquid. Pasteurization has been used effectively to control contaminants in some algal nurseries.
Tyndallization	Sterilization by heating a substance (seawater) for several minutes on three or four successive occasions.	5 minutes at 90°C-100°C Once per day for three days (24 hours between heating)	Standard stove	**Pros:** • >99% effective **Cons:** • Moderate manpower • Labor intensive with large volumes	This is effective in destroying most living organisms but requires 24 to 72 hours of time and is more labor intensive.
Filtration	The process of passing the seawater through filters.	Varies depending on filter pore or membrane size. Also depends on number of uses.	Polypropylene and Membrane Filters 5.0, 1.0, 0.2 µm	**Pros:** • 80% effective • Filter large amounts of water in a short time **Cons:** • Filtration begins fast, but increased amounts of debris will cause the filter to clog and water flow will decrease.	Relatively inexpensive, takes little time and removes most living organism in the seawater. Effectiveness and cost may vary extensively depending on the quality and type of filter used. For example, 0.2 µm membrane filter cartridges cost more and take more time to filter than a 5.0µm filter.
Ultra-violet (UV) Radiation/ Sterilization	The process of passing the seawater past a closed UV light system.	3 gallons per minute	UV apparatus	**Pros:** • >99% effective **Cons:** • Moderately expensive	Relatively inexpensive, takes little time, but requires management of flow rates and exposure times to maximize effectiveness.
Chlorine Dioxide (ClO_2)	Sterilization by adding (ClO_2) into the seawater.	3-20 parts per million & 18-24 hours for ClO_2 dissipate into the air.	ClO_2; Personal protective equipment (respirator); Refrigerator & approved containers (storage)	**Pros:** • >99% effective • Inexpensive • Minimum effort **Cons:** • Toxic substance, protective equipment required	Relatively inexpensive but requires contact time in the range of 18-24 hours for complete dissipation into air. Also requires safe handling and storage practices and measurement equipment to monitor concentrations and residuals.

Equipment and Supplies

Aquaria

(Also referred to as production aquaria or growth tanks.) Aquaria ranging from 5 to 50 gallons (or larger) may be used depending on the scale of the nursery. The aquaria are used to hold and grow the spools of kelp in the nursery. OA used 20-gallon production aquaria that hosted between 8-10 nursery spools at a time.

Nursery Spools

Made of 2-inch PVC pipe, the nursery spools are cut to fit the height of the aquaria being used. Nylon twine is wrapped around the PVC and fastened with rubber bands. The spools provide the surface area needed for the spores to attach to and grow in the nursery.

Settling Tubes

Made from 4-inch PVC pipe, settling tubes are cut to fit the height of the spools and capped at the bottom to prevent water from leaking out the bottom. Settling tubes are used during inoculation of spools with spores and for transporting the spools with young sporophytes to the ocean site.

Water Chiller

A refrigeration unit used to cool individual aquaria or can be plumbed to cool numerous aquaria simultaneously. It is essential throughout the kelp culturing process to have water temperatures regulated at approximately 50°F (10°C).

Fluorescent Lighting

Light bulbs, fixtures, and timers are required to provide the 12 hours of lights ("lights on") and 12 hours of darkness ("lights off") that the growing sporophytes require. Many different configurations are available for purchase. OA used 4-foot T12 fluorescent lights in a standard light bank fixture. A series of mesh screens were used to regulate the amount of light penetrating the production aquaria.

Air Pump

Air is administered by use of air pumps, tubing, sterile pipette, or air stone, all of which can be found in a local pet supply store. Aeration is essential for proper sporophyte growth in the production aquaria. Air filters can be placed in line with the air tubing to reduce the risk of introducing airborne contaminants.

Water-Filtering Device

Any configuration of pumps, filters, and tubing used to purify the seawater for use in the nursery.

Water Filters

Various size mesh filter cartridges are used in line with the pumping system to purify the seawater. Filters can be purchased in a wide range of pore and membrane sizes. 5.0, 1.0, and 0.2 micron (μm) filters can be used together to obtain relatively low bacterial counts in the water. Some varieties are meant for single use, while others can be cleaned or autoclaved for multiple uses.

0.1 micron at PSRF

Refrigerator

An appliance that is used to cool its contents. The refrigerator is necessary for storing culture nutrients and sorus tissue, as well as chilling small quantities of seawater. OA recommends storing several gallons of filtered seawater for releases and as emergency backup water if a water chiller fails. Stored seawater, however, should be used within one week of collection to prevent growth of bacteria.

Nutrient Media

(Also referred to as culture nutrients or culture media.) Provasoli's Enriched Seawater (PES) and vitamins are the components added to the seawater to accelerate kelp growth. Germanium dioxide (GeO_2) is added to suppress diatom growth. See Appendix B for nutrient media composition and preparation that OA found most effective.

Microscope

An instrument used to magnify the reproductive spore cells, or "zoospores," during a release. Zoospores that are released and captured for culture can only be seen with the use of a microscope. A compound microscope or similar having a 40x and 100x magnification is recommended to observe and count these spores to properly calculate the stocking density.

Cell-Counting Chamber

(Also referred to as a hemocytometer.) A microscope slide that is specifically used for counting the zoospores during release. A counting chamber is necessary to determine both the number of spores released, and to calculate the proper density of spores to add to the settling tubes. Grids are etched into the glass to allow for easier counting.

Laboratory Glassware

Beakers, graduated cylinders, and flasks are used most often during spore release, measuring culture media, preparing aquaria, and establishing gametophyte cultures.

Thermometer

An instrument that is used for measuring the temperature of both the air and water. Digital thermometers are used during the spore releases and inside production aquaria to monitor the water temperature. Thermometers are also useful to monitor the air temperature in the nursery and refrigerator.

pH Meter

An instrument that measures the acidity or alkalinity of the water. Many different models are on the market that allow for easy monitoring of seawater pH level. A digital interface makes readings more precise.

Light-Measuring Meter

An instrument used to measure and monitor the intensity of light the nursery spools receive. The light wavelengths are measured in micromoles per square meter per second (μmol $m^{-2} s^{-1}$). The measuring probe can be submerged in the aquaria to gain an accurate reading. It is important to use a meter that is submersible and measures in micromoles.

Ultraviolet (UV) Radiation Sterilization Device

An instrument used to sterilize filtered seawater by exposing it to UV radiation, which is used in many aspects of nursery production. OA's unit recommended a flow rate of three gallons per minute to eliminate most contaminants in the seawater. If necessary, small individual units can be added to the aquaria to reduce or prevent bacterial contamination.

Carbon Dioxide (CO_2) Supply

An air tank containing carbon dioxide gas may be used to lower the pH inside the production aquaria. As the kelp plants grow in the nursery, the pH may slowly rise to 9.0 or higher. Slowly introducing CO_2 through tubing and an air stone will lower the pH to the more preferred range of 7.0–9.0.

Nursery Setup

Temperature

The air temperature in the nursery was maintained at 65°F–70°F (18°C–21°C, respectively). The temperature of the seawater in the aquaria was maintained at 50°F (10°C) through the use of external chillers (Figure 2.2). Seawater was continuously circulated through ½-inch silicon hoses that were custom fit to each individual system. Gray PVC pipes, elbows, and adapters were inserted into the intake and outflow hose ends and fastened using hose clamps to prevent leaks.

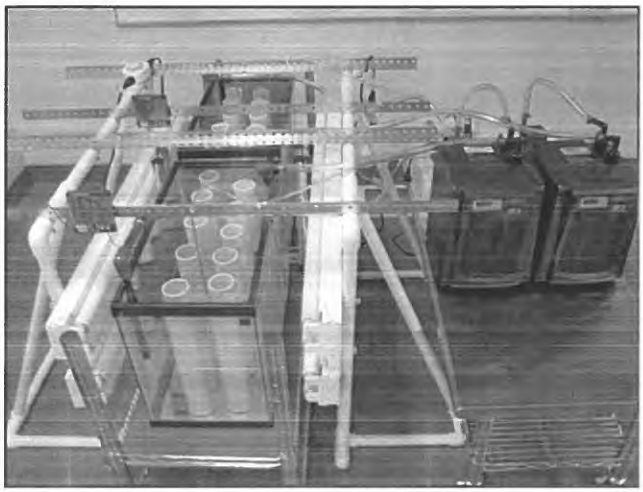


Figure 2.2 A two-aquaria setup including light bank, two chillers (one for each aquaria), and one air pump with separate tubing for each aquaria

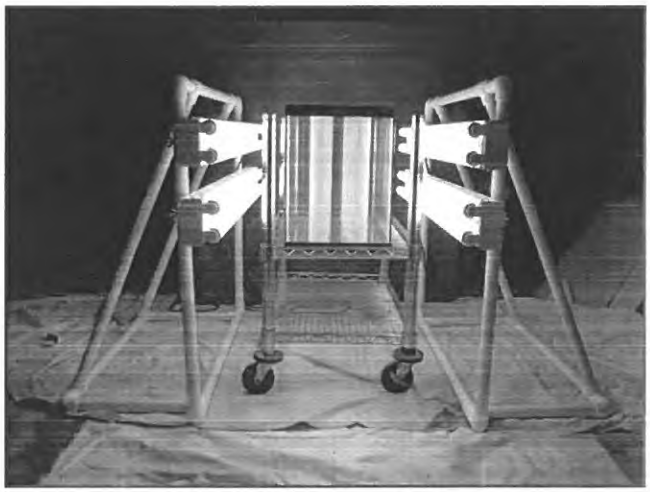


Figure 2.3 The light bank setup fabricated by OA to administer light to the growing spools

Light

The light intensity and photoperiod must be controlled and monitored. This may be accomplished through the use of external light banks, screens, and timers. This lighting system was designed to allow for flexibility in light levels. As seen in Figure 2.3, light banks were created out of PVC, nuts, bolts, and counterweights (placed on opposite side of lights to counteract the weight of the lights). The light banks stood alone and could be modified to fit side by side with another light bank or next to a wall.

The 4-foot T12 fluorescent lighting fixtures were attached directly to the light banks. Mesh screens were fabricated using PVC pipe, pet screen (for fine mesh), plastic hardware net (for wide openings), and zip-ties. All supplies were purchased from a local hardware store. Plans for building the light banks can be found in Appendix C.

A light meter was used to measure the distance needed between the aquaria and light bank to achieve the 100 micromoles per square meter per second intensity (μmol m^{-2} s^{-1}) that the spools would receive at maximum intensity. Fine mesh screens (Figure 2.4) allowed light levels in the aquaria of approximately 20 μmol m^{-2} s^{-1}, which is suitable for the beginning growth. These fine mesh screens were used for the first two weeks of growth in the nursery. The wide mesh screens (Figure 2.5) created light levels in the aquaria of approximately 55 μmol m^{-2} s^{-1}. These screens were used between weeks 2 and 4. The screens were easily hung between the tank and the light source with zip-ties, to create the appropriate light levels. The screens diffuse the light and spread it evenly across the surface of the aquaria. By week 4 (or sometimes earlier), the screens can be removed, providing full light from the light banks (approximately 100 μmol m^{-2} s^{-1}). Table 2.2 shows the light intensity, photoperiod and duration that have been successfully used by OA.

Figure 2.4 Fine mesh screen used to create light levels of 20 µmol m^{-2} s^{-1}

Figure 2.5 Wide mesh screen used to create light levels of 55 µmol m^{-2} s^{-1}

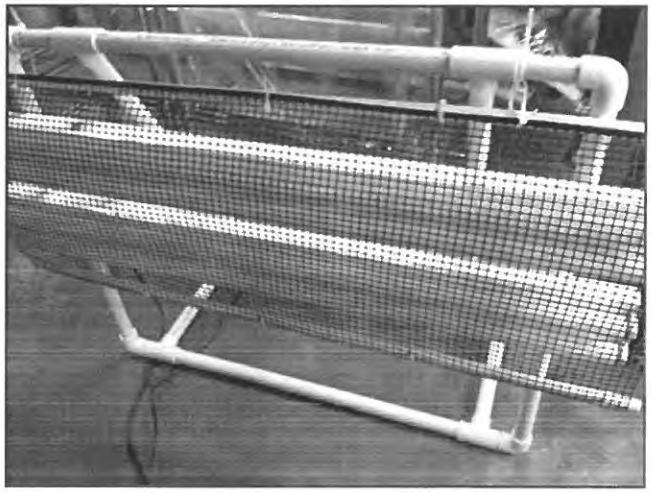


Table 2.2 Light Level Measurements in Ocean Approved Production Aquaria

Zoospore Release Date (Fine Mesh Screen)[a]	Number of Days Using Fine Mesh Screen[a]	Increase Light Intensity (Wide Mesh Screen)[b]	Number of Days Using Wide Mesh Screen[b]	Increase Light Intensity (No Screen)[c]	Number of Days Using No Screen[c]	Out-planting to Farm Site	Total Days in Nursery
9/26/10	19	10/15/10	19	11/3/10	3	11/6/10	41
9/26/10	19	10/15/10	19	11/3/10	9	11/12/10	47
11/16/10	14	11/30/10	14	12/14/10	16	12/30/10	44
11/16/10	14	11/30/10	14	12/14/10	17	12/31/10	45
11/16/10	14	11/30/10	14	12/14/10	24	1/7/11	52
11/17/10	13	11/30/10	14	12/14/10	31	1/14/11	58
11/17/10	13	11/30/10	14	12/14/10	15	12/29/10	42
12/9/10	12	12/21/10	17	1/7/11	7	1/14/11	36
12/9/10	12	12/21/10	17	1/7/11	8	1/15/11	37

[a]Fine mesh screen: 20 µmol m^{-2} s^{-1} [b]Wide mesh screen: 55 µmol m^{-2} s^{-1} [c]No screen: 100 µmol m^{-2} s^{-1}

Seawater

Seawater was collected at high tide from open-ocean sites and stored in the nursery until utilized. Water in each aquaria was changed every seven days. This was accomplished by transferring the spools to another aquarium with fresh, filtered, and sterilized seawater and culture nutrients.

The sterilization or the removal of contaminants (e.g., unwanted algae, protozoa, and bacteria) from the collected seawater is one of the most important and challenging activities in the growth process. As outlined earlier in Table 2.1 there are a number of ways this may be accomplished. The availability of running seawater, the volume of seawater being used, and the equipment on site all play a role in deciding which process may be utilized.

The protocol for the collection and storage of seawater was dependent upon the amount of seawater that was needed for the nursery. For instance, for smaller operations (<10 aquaria), OA transferred seawater to the nursery in 5-gallon plastic jugs (Figure 2.6) However, for a larger-scale operation (≥10 aquaria), OA transferred seawater to the nursery using a 200-gallon Bonar Box on a trailer. The seawater was then placed in a sterile, 300-gallon, insulated storage tank (Figure 2.7). until needed. Regardless of the amount of seawater brought into the lab, all seawater was filtered (5.0, 1.0, 0.2 μm filters) and exposed to UV sterilization to remove contaminants prior to use.

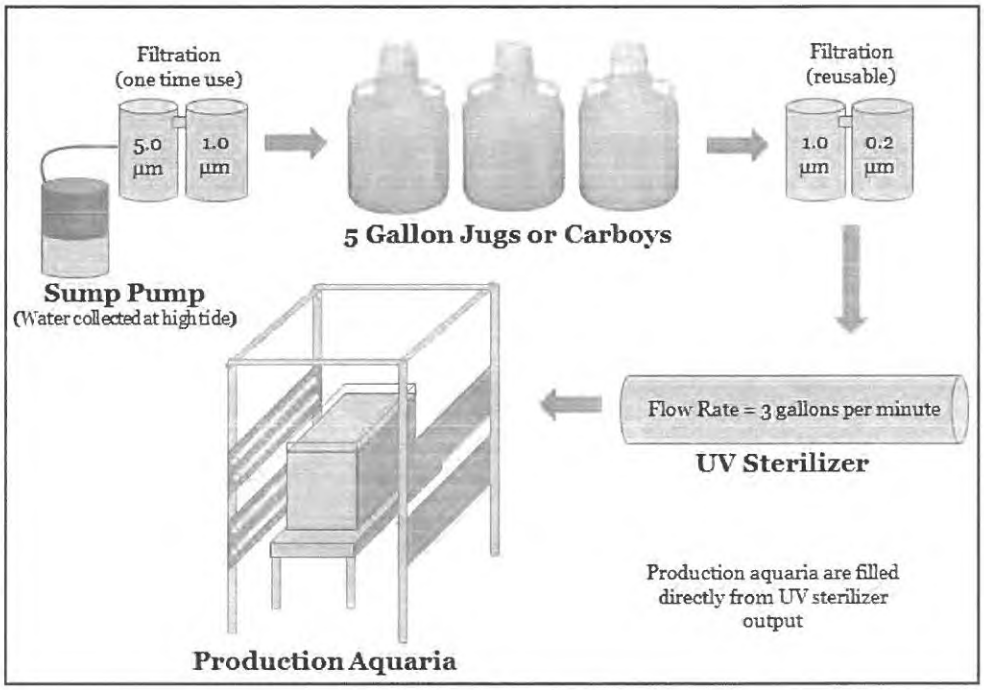


Figure 2.6 Water filtration process used by OA when less than 10 aquaria in the nursery.

Figure 2.7 300-gallon holding tank and filtration system used in the nursery to fill the aquaria

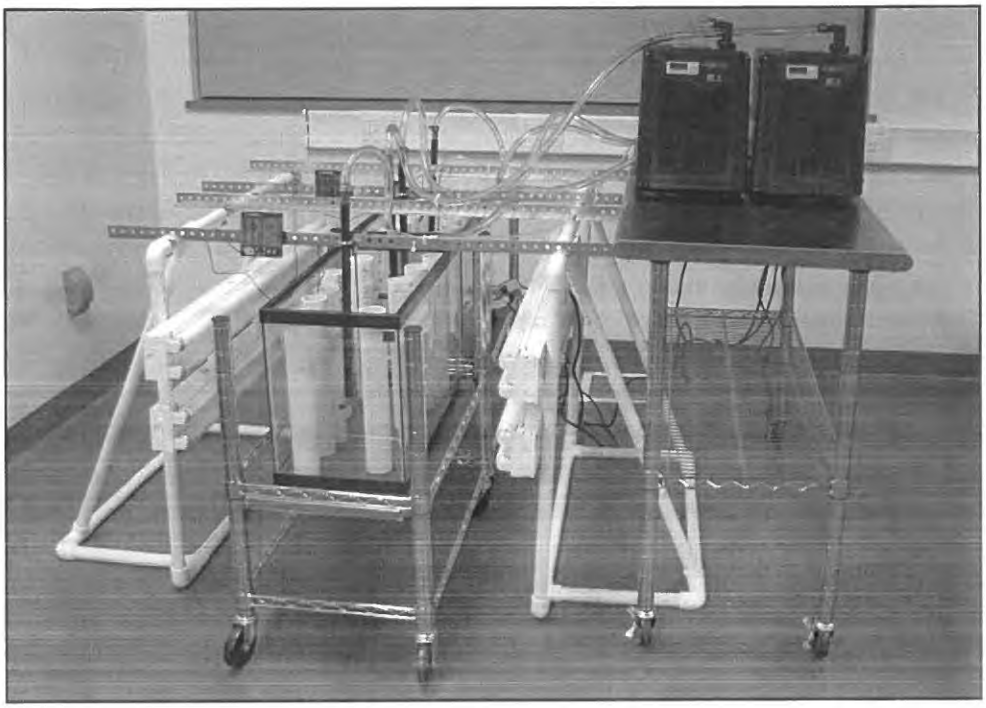


Figure 2.8 Aquaria setup with lighting, chilling, and aeration system in place

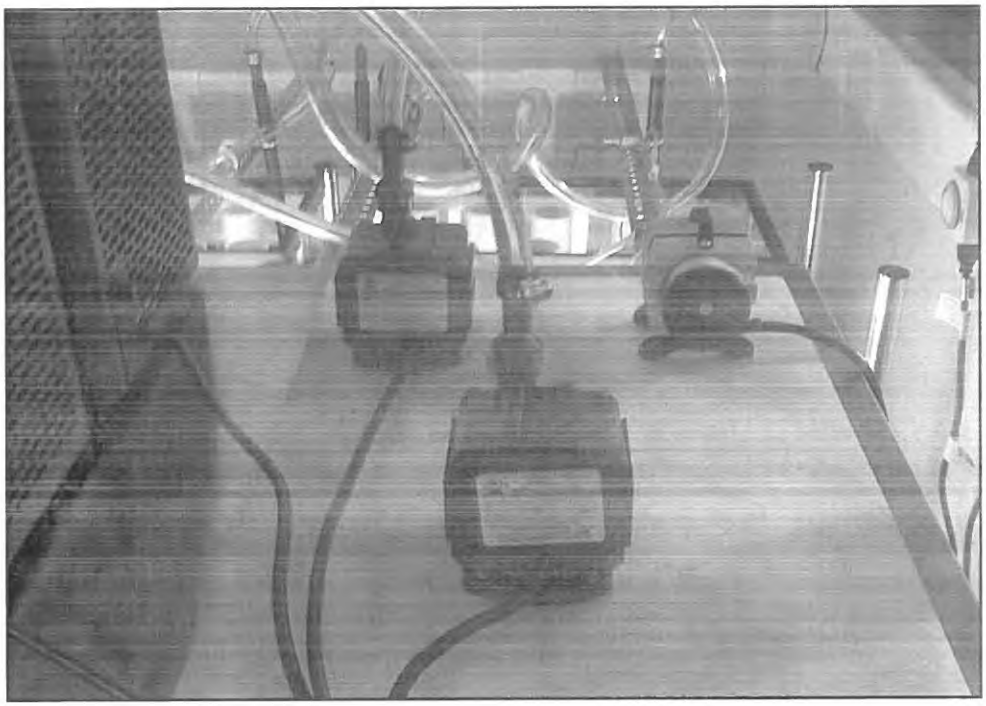


Figure 2.9 Large cutting board used to anchor the chiller and air pumps

Production Aquaria

OA used standard 20-gallon glass tanks or aquaria to hold the spools. Each aquarium was fitted with a plexiglass lid to minimize evaporation and reduce contamination. Light banks were constructed to allow two aquaria to fit end to end(Figure 2.8). Each aquarium had a separate pump that circulated water through the chiller. Two aquaria shared an air pump. The air pump and two pumps that fed the chillers were fastened to a large cutting board and placed on the cart to provide stability (Figure 2.9).

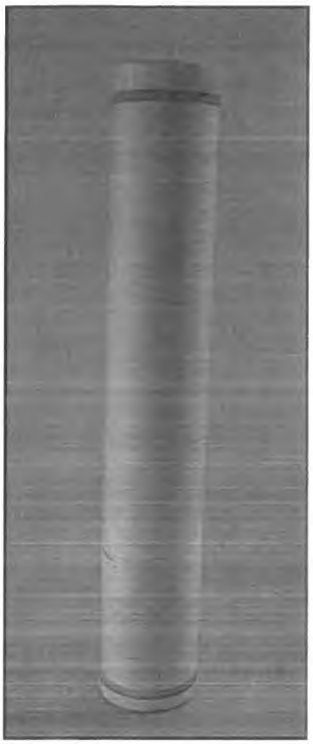


Figure 2.10 Nursery spool used as substrate for spores to attach and grow

Spools and Settling Tubes

Various techniques and materials are used successfully worldwide to hold the 1-mm twine. OA adopted a method that was effective in the nursery as well as when transferring to the lines at the farm site. The OA method used 2-inch PVC pipe as the base of the nursery spools (Figure 2.10). The PVC pipe may be cut to the appropriate length for the size of the aquaria or growth tank being used. Since the OA nursery uses 20-gallon aquaria, the PVC pipe was cut to 15.25 inches in length. These dimensions allowed for approximately 200 feet of the 1-mm twine to be wound on each section of pipe.

Once the pipe is cut, the next step is to wind the twine onto the pipe. Winding the twine on the PVC pipe may be done manually, which is very time consuming, or mechanically. OA created a jig to spin the twine onto the pipe using a sewing machine motor (Figure 2.11). The advantage of this jig is that speed can be controlled with the sewing machine motor foot pedal, allowing the use of both hands to guide the twine onto the PVC pipe.

Regardless of the technique used, it is important that the seed twine does not overlap itself throughout the winding process and that the sides of the twine are tightly touching. Exam gloves are recommended while handling twine to minimize exposing the twine to oils and dirt that may be on one's hands.

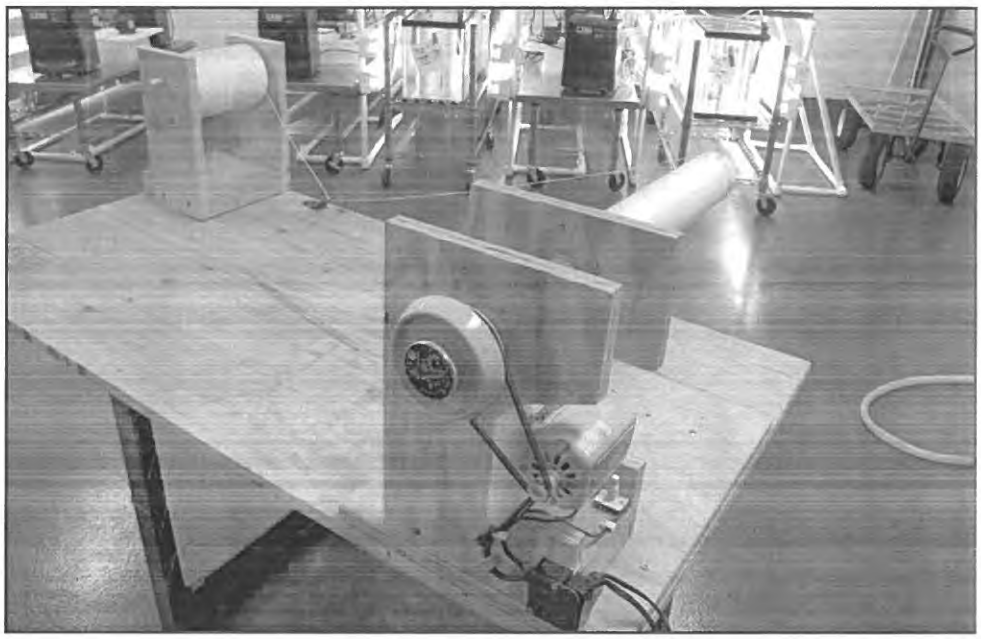


Figure 2.11 A handmade spool winder, using a sewing machine motor as the power source

Process to Construct Spools

Step 1. Cut the PVC pipe to the desired length. Ensure that the cuts are at 90 degrees. A cut that is not 90 degrees may result in the spool leaning or falling over in the aquaria.

Step 2. With a fine file and sandpaper, round the outside edge of each end of the pipe. This minimizes sporophyte damage when seed twine is deployed from the tube to the long line at the farm site.

Step 3. Boil pipes to remove deposits, wash with soap (e.g., gentle dish detergent) and water, rinse, and then soak in deionized water for 72 hours to remove any other contaminants.

Step 4. Clean, dry pipes are then stored in clean plastic bags.

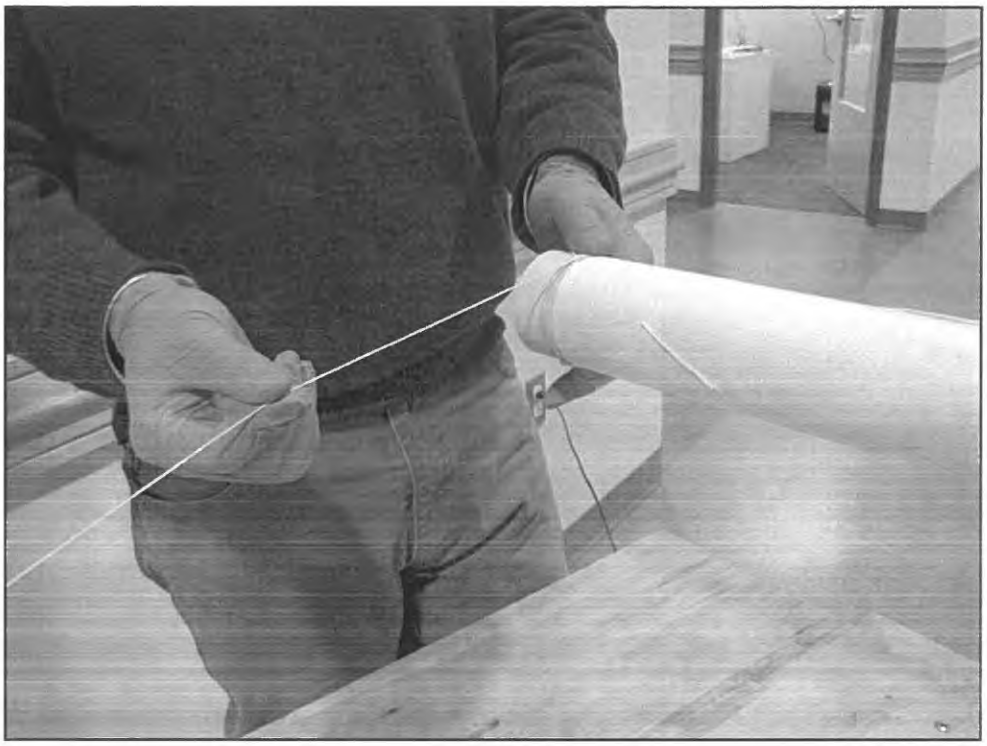


Figure 2.12 Wrapping twine around PVC pipe to create spools

Process to Attach Seed Twine to PVC Pipe

Step 1. Wearing exam gloves, wrap a rubber band around one end of the pipe about ¼ inch from the end.

Step 2. Insert the end of the seed twine under the rubber band, so that the end of the twine is pointing to the end of the pipe.

Step 3. Tie an overhand knot at the end of the seed twine to ensure that the twine does not pull under the rubber band.

Step 4. Wind the seed twine tightly around the pipe away from the rubber band either manually or using an automated device like OA's "jig." Wrap the twine tightly and do not overlap (Figure 2.12).

Step 5. After wrapping approximately 3 inches away from the rubber band, a separate 2-inch piece of twine should be inserted under one of the winds, sticking out away from the tube. This 2-inch piece of twine will allow for the monitoring of early sporophyte growth under the microscope (Figure 2.13). Small sections of these pieces can be cut off the spool throughout nursery production, without disturbing the entire length of twine.

Step 6. Continue winding until within 3 inches of the end of the pipe and then insert another piece of 2-inch sample twine.

Step 7. Continue winding until there is approximately ⅜ inch of exposed PVC pipe remaining. Place a rubber band over the PVC pipe and the end of the seed twine. As before, tie an overhand knot to keep the twine from pulling under the end of the rubber band.

Step 8. Nursery spools may be kept in a clean plastic bag in the freezer until ready to use (Figure 2.14).

Figure 2.13 Sample twine viewed under the microscope used to monitor growth of sporophytes

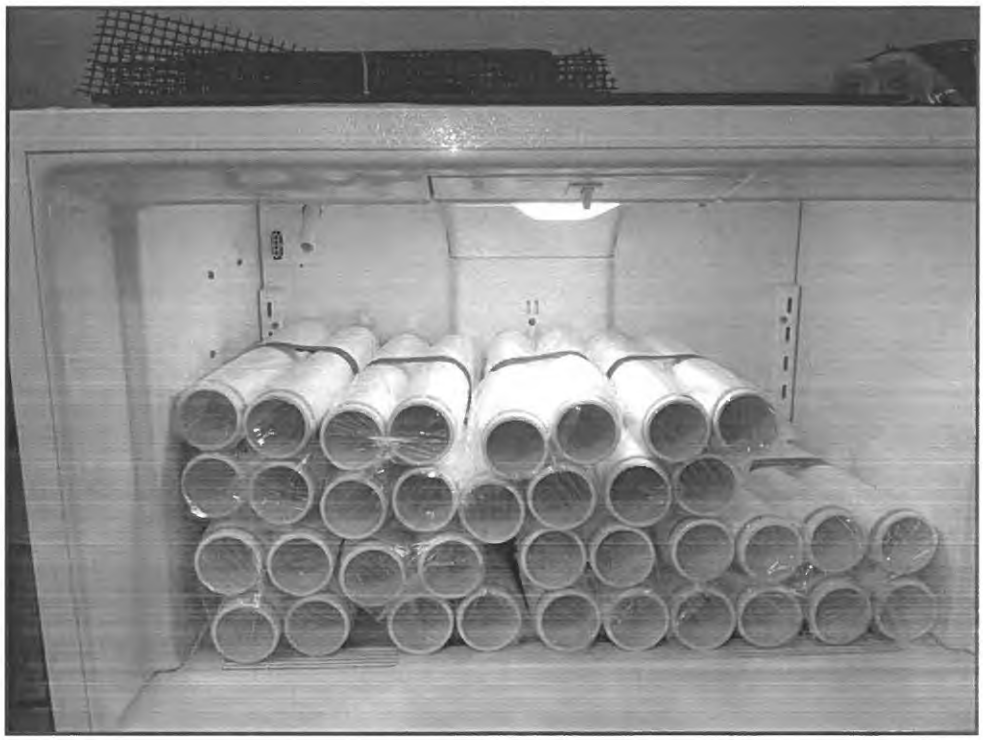
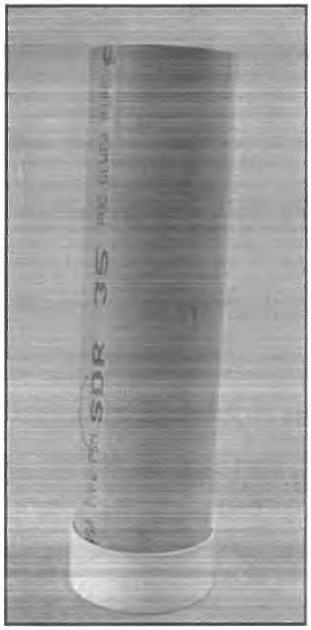


Figure 2.14 Nursery spools are stored in plastic bags in the freezer until needed.



Process To Construct Settling Tubes

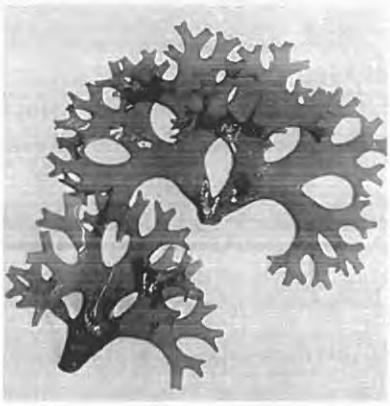
The steps used to construct and clean the 4-inch-wide PVC pipe used for settling tubes (Figure 2.15) are the same as the steps used to construct the 2-inch PVC spools that hold the twine, with the exception of the need to sand and file the ends. The PVC pipe is cut to 15.75 inches in length in order to hold the 15.25 inch spools. One end must have a cap glued on so that it is watertight. These tubes must be washed and leached using the same process employed for the nursery spools. In addition to being used to hold the spools with twine for spore settling, these same tubes are used to transfer the spools to the farm sites as described in Chapter 5, page 94.

Figure 2.15 Settling tube used when inoculating spools and transferring spools out to farm sites

New England
Seaweed Culture Handbook

Nursery Systems






Sarah Redmond, Lindsay Green
Charles Yarish, Jang Kim, Christopher Neefus
University of Connecticut & University of New Hampshire

To cite this publication:

Redmond, S., L. Green, C. Yarish, , J. Kim, and C. Neefus. 2014.
New England Seaweed Culture Handbook-Nursery Systems. Connecticut Sea Grant CTSG-14-01. 92 pp. PDF file. URL: http://seagrant.uconn.edu/publications/aquaculture/handbook.pdf. 92 pp.

Contacts: Dr. Charles Yarish, University of Connecticut. charles.yarish@uconn.edu
Dr. Christopher D. Neefus, University of New Hampshire. chris.neefus@unh.edu

For companion video series on YouTube, see: http://s.uconn.edu/seaweedplaylist

Sea Grant Connecticut

Sea Grant New Hampshire

Connecticut Sea Grant
University of Connecticut
1080 Shennecossett Road
Groton, CT 06340-6048
http://seagrant.uconn.edu

CTSG-14-01 ©2014

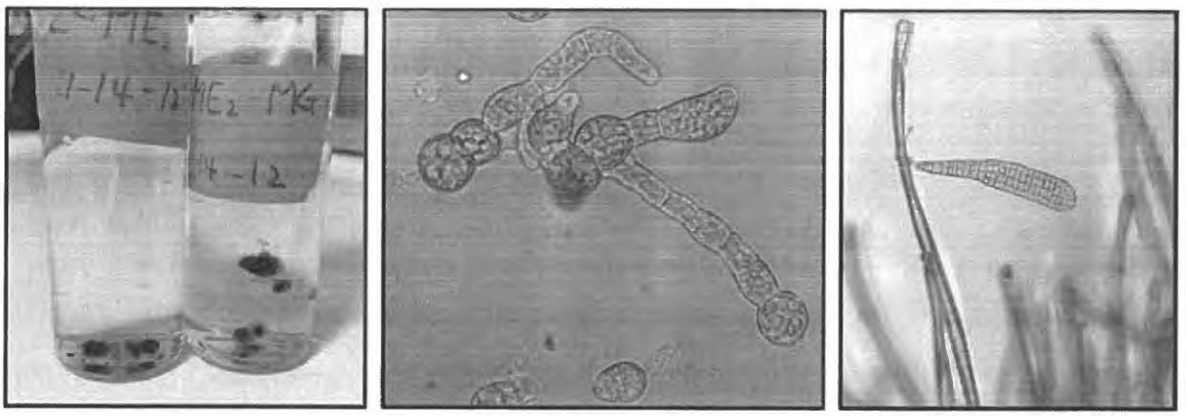
UCONN UNIVERSITY OF CONNECTICUT

NOAA NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION U.S. DEPARTMENT OF COMMERCE

General Culture System Materials and Estimated Costs		
Item	Base	
Seawater System		
Seawater filters—3-step cartridge system, down to 1 micron		Online, hardware stores
Filter cartridge housings	40	Pentek Blue
Filter cartridges (20, 5, 1 micron size)	20	Pentek
Seawater holding tank system		
500-gallon plastic holding tank	400	Ace Roto-mold
UV light for seawater sterilization	400	Smart UV Sterilizers/Emperor Aquatics
External water pump to circulate water	200	http://marinedepot.com
Round polyethylene or fiberglas tanks, various sizes	200-2000	Pentair Aquatic Eco-Systems Inc.
2 20-gallon high (24x12x16") fish tanks	45	
Aquarium Chiller	500-800	Ecoplus Water Chiller: Sunlight Supply; Seachill Aquarium Refrigeration Unit: Pentair Aquatic Eco-Systems Inc.; aquarium suppliers
MD3 pump, mag drive pump, 350 gph, 35 W	62	Pentair Aquatic Eco-Systems Inc.
Water tubing 50' coil	130	http://www.PentairAES.com
Laboratory		
Polycarbonate or glass 10L clear autoclavable carboys	50-200	Laboratory, homebrewing suppliers
Flasks	30-100	Laboratory suppliers
Petri dishes	100	
Forceps	50	
Microscope	100-600	
Pasteur pipettes	20 (box of 200)	http://www.sigmaaldrich.com
Digital Scale	60-200	General lab supply
Temperature and power alarm and auto dialer	330	Omegaphone. http://www.omega.com
Lights		
CW-HO fluorescent lamps - high output - cool white		Greenhouse growers supply
Light bulbs & fixtures	200	Lighting Suppliers
Photoperiodic timers	30	Aquarium, Hardware supply
Neutral density screening for light adjustment (roll of window screen)	15	Hardware stores
Aeration		
Aquarium aeration pumps	30	Aquarium Supply
Aeration tubing, small diameter, 25'	6	Aquarium Supply
Rigid aeration tubing for large tanks, large diameter	30	Aquarium, Aquaculture Supply
Hepa-vent inline air filters (pack of 10)	80	General lab supply
Glass tubing for aeration	20	General lab supply

Table 3.1. Kelp culture conditions for gametophyte and sporophyte culture systems.

Optimal Environmental Conditions:	Gametophyte Phase		Sporophyte Phase	Culture Equipment
In general, for all months	**Release & Settlement of Meiospores**	**Optimal Growth & Sporophyte Formation**	**Juvenile Sporophytes**	Individual rectangular glass aquaria fitted with 2" PVC seed string (cotton/nylon blend)
• Temperature (°C)	10-20	10-15	10-15	Recirculating saltwater aquarium chiller units
• Light fluence (μmol photons m-1 s-1)	0-25	5-60	25-150 W1 25 W3 W2 W4	High output cool white fluorescent lights 24-hour programmable light timers
• Photoperiod	12:12 L:D	12:12 L:D	12:12 L:D	
• Seawater salinity • Seawater source	30-32 Natural	30-32 Natural	30-32 Natural	Sterilized seawater: autoclaved, Micro filtered, or inline UV light
• Nutrient media	Provasoli's Enrichment Media (PES), ½ strength	PES ½ strength	PES ½ strength	Available for order from algal culture centers, or made up in the laboratory
• Diatom Control	Germanium Dioxide	Germanium Dioxide	Not needed if culture is clean 0.5mL/L	Available for order from laboratory supply companies
•Aeration levels	Very low	Medium	Medium	Air should be filtered to avoid contamination



Vegetative gametophyte cultures | **Vegetative female gametophytes** | **Juvenile sporophyte on seed string**

Figure 3.16. Seeding with vegetative cultures.

Nutrient Media

PROVASOLI ENRICHED SEAWATER MEDIA (PES)			
To the base solution (I), add the following amounts of prepared solutions II, III, & IV			
Enrichment Stock Solution	Quantity (ml) 1000 (final)	X2 (ml) 2000 (final)	X3 (ml) 3000 (final)
Solution I: Base Solution	599 (final)	1198 (final)	1797 (final)
Solution II: Fe (as EDTA complex; 1:1molar)	200	400	600
Solution III: P II metals	200	400	600
Solution IV: Vitamins Vitamin B_{12}, Biotin	1	2	3
*Adjust pH of final solution to 7.8 using HCl			
Solutions I, II, III & IV			
Ingredients			
Solution I: Base Solution	Quantity	Quantity (X2)	Quantity (X3)
Deionized water	599	1198	1797
Tris Buffer	4 g	8 g	12 g
$NaNO_3$	2.8 g	5.6 g	8.4 g
Na_2 glycerophosphate	0.4 g	0.8 g	1.2 g
Thiamine-HCl (Vit. B_1)	0.004 g	0.008 g	0.012 g
Solution II: Fe (as EDTA complex; 1:1 molar)	Quantity	Quantity (X2)	Quantity (X3)
Deionized water	1L (total)	2L (total)	3L (total)
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.700 g	1.4 g	2.1 g
Na_2EDTA	0.600 g	1.2 g	1.8 g
Solution III: P II metals	Quantity	Quantity (X2)	Quantity (X3)
Deionized water	1L (total)	2L (total)	3L (total)
Na_2EDTA (Disodium ethylenediamine tetraacetate)	1 g	2 g	3 g
H_3BO_3 (Boric Acid)	1.140 g	2.28 g	3.42 g
$FeCl_3 \cdot 6H_2O$ (Ferric Chloride)	0.049 g	0.098 g	0.147 g
$MnSO_4 \cdot H_2O$ (Manganese sulfate monohydrate)	0.130 g	0.26 g	0.39 g
$CoSO_4 \cdot 7H_2O$ (Cobaltous sulfate heptahydrate)	0.005 g	0.01 g	0.015 g
$ZnSO_4 \cdot 7H_2O$ (Zinc sulfate, 7-hydrate)	0.022 g	0.044 g	0.066 g
Solution IV: Vitamins	Quantity		
Deionized water	25 mL (total)		
Vitamin B_{12}	0.002 g		
Biotin	0.001 g		

Notes on PES preparation

- Solutions II, III, & IV should be made up as separate solutions to be added to base solution I. Prepare the base solution by dissolving ingredients in about half of the total volume of water, and then add solutions II, III, and VI before adding the remaining water to bring the final volume up to 1, 2 or 3 liters.
- If enriching natural seawater, boric acid can be left out of solution III.
- Prepare solutions separately using clean sterilized volumetric flasks, clean pipettes, and digital balance. Mix with magnetic stirring bars.
- pH should be adjusted with HCl after media is prepared.
- Media can be filter sterilized or pasteurized. Vitamins (in solutions I and IV) should not be heat sterilized.
- Media solutions and vitamin components should be stored in the refrigerator.
- All chemicals should be dated when received and when opened on the bottle.
- Clearly label and date all solutions and chemicals.
- Use aseptic technique in preparation of solutions. Use only clean, sterilized glassware and clean working environments.
- Add stock solution at 20ml/L of seawater for full strength. Add 10ml/L for half-strength, which has been found to be suitable for kelp.
- Germanium dioxide is another, separate component that is added to cultures to prevent growth of diatoms. The solution can be prepared in advance and refrigerated. This solution is added at 2ml/L of water.
- For Kelp Culture: 20 gallon tank ~ 75 Liters. ½ strength PES = 750 ml/week per tank

PES Ordering InformaÀon

All chemicals are listed with Fisher Scientific (Thermo Fisher Scientific Inc., (http://www.fishersci.com) ordering number, but other chemical companies are available with competitive pricing, including Sigma-Aldrich. Order information is intended as a guide to assist in finding chemicals.

Solution I: Base Solution	Ordering info	FISHER SCIENTIFIC	SIGMA-ALDRICH
$NaNO_3$	Sodium nitrate (granular)	#S342-3 (3kg)	S8170-250G
Na_2 glycerophosphate	Sodium glycerophosphate	#21655-100G (10g)	G9422-10G
Thiamine-HCl (Vit. B1)	Thiamine hydrochloride	#AC14899-0100 (10g)	T1270-25G
Tris Buffer ($CH_4H_{11}NO_3$)	Tris hydroxymethyl aminomethane	BP152-500 (500g)	T1503-500G
Solution II: Fe (as EDTA complex; 1:1 molar)			
Fe $(NH_4)2$ $(SO_4)2 \cdot 6H_2O$	Ferrous ammonium sulfate	I77-500 (500g)	F1543-500G
Na2EDTA	Disodium ethylenediamine tetraacetate	BP S311-100 (100g)	E6635-100G
*1 ml of this solution = 0.1 mg Fe. We have recently found that we can substitute 403 mg FeNaEDTA (=$C_{10}H_{12}FeN_2NaO_8$; molecular weight 367.05)			
Solution III: P II metals			
H_3BO_3 (Boric Acid)	Boric acid H_3BO_3	A74-500 (500g)	B6768-500G
$FeCl_3 \cdot 6H_2O$	Ferric chloride hexahydrate	ICN19404580 (100g)	236489-100G
$MnSO_4 \cdot H_2O$*	Manganese sulfate monohydrate	M113-500 (500g)	M7899-500G
Na_2EDTA	Disodium ethylenediamine tetraacetate	S311-100 (100g)	E6635-100G
$CoSO_4 \cdot 7H_20$	Cobaltous sulfate heptahydrate	C386-500 (500g)	C6768-100G
$ZnSO_4 \cdot 7H_20$	Zinc sulfate, 7-hydrate, crystal	AC42460-5000 or JT Baker Chemical Co. #4382-01 (500g)	Z0251-100G
Solution IV: Vitamins			
Vitamin B_{12}		IC-N10327101 (1g)	V2006-1G
Biotin		AAA1420703 (1g)	B3399-100MG

*Can substitute $MnSO_4 \cdot 4H_2O$ (164mg per 1000mL) *OR* $MnSO_4 \cdot 7H_2O$ (187mg per 1000mL)
Sigma-Aldrich offers smaller-volume bottles online, for a lower total start-up cost, but the online prices of both Fisher and Sigma are comparable, at an estimated $2.85 per Liter PES (Sigma) to $2.95 per Liter PES (Fisher).

PES nutrient media is added at 10mL/L seawater, so 1 liter of PES will provide for 100 liters of seawater. This works out to an estimated 3 cents ($0.03) cost for every 1 liter of seawater used for kelp culture.

20 mL/L for gametophytes

B. Nutrient Formulations

Provasoli's Enriched Seawater (PES) Culture Media

PES Culture Media

Solution I: Base Solution	**1000 mL quantity**
Deionized water	1,000 mL (total) (1 L)
$NaNO_3$	2,800 mg (2.8g)
Na_2glycerophosphate	400 mg (0.4 g)
Thiamine-HCl (Vit. B1)	4 mg (.004g)
Tris Buffer	4,000 mg (4g)
(tris(hydroxymethyl)amino-methane; 2-amino-2[hydroxymethyl]1,3-propanediol)	

Specialty solutions normally made up in 2000 mL volumes:

Solution II: Fe (as EDTA complex; 1:1 molar)

Deionized water	250 mL (total)
$Fe(NH_4)_2(SO4)_2 \cdot 6H_2O$	175 mg
Na_2EDTA	150 mg

1 mL of this solution = 0.1 mg Fe. We have recently found that we can substitute 403 mg FeNaEDTA (=$C10H12FeN2NaO_8$; molecular weight 367.05)

Solution III: P II metals P II metals as one solution which should be kept refrigerated

Deionized water	200 mL (total)
H_3BO_3 (Boric Acid)	228 mg
$FeCl_3 \cdot 6H_2O$ (Ferric Chloride)	9.8 mg
$MnSO_4$ $4H_2O$	32.8 mg

OR

$MnSO_4$ H_2O (Manganese sulfate monohydrate) 26.0 mg

OR

$MnSO_4$ $7H_2O$	37.4 mg
Na_2EDTA	200 mg
(Disodium Ethylenediamine Tetraacetate)	
$CoSO_4$ $7H_2O$	1.0 mg
(Cobaltous sulfate heptahydrate)	
$ZnSO_4 \cdot 7H_2O$	4.4 mg
(Zinc sulfate, 7-Hydrate)	

Solution IV: Vitamins

Vitamin B12 and Biotin solution should be made up as follows in 25 ml volumetric flasks to give final concentrations of 80 µg and 40 µg, respectively, per 1000 of original stock solution:

vitamin B12	2.0 mg
biotin	1.0 mg

To the base solution (I), add the following amounts of prepared solutions II, III, & IV.

Solution I: Base Solution	1000 mL quantity
Solution II: Fe (as EDTA complex; 1:1 molar)	20 mg = 200 mL
Solution III: P II metals	200 mL
Solution IV: Vitamins Vitamin B12, Biotin	1 mL

Notes:

Enrichment media need to be sterilized. Since pH needs to be adjusted after the media are prepared, it is easiest to mix the solutions, adjust pH with HCl, then filter sterilize the finished media, though solutions II and III can be autoclaved separately after they are made up. Vitamins (in solutions I and IV) should not be heat sterilized. The media should be refrigerated, and all vitamins should be stored in the refrigerator.

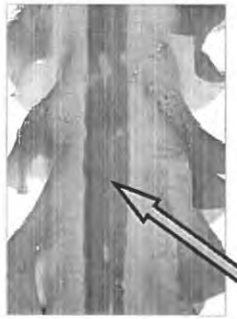
Nutrient Concentrations Table

Nutrient concentrations used in the OA nursery		
Release Beakers	**Settling Tubes**	**Aquaria**
1000 mL Seawater	2300 mL Seawater	20 gallons/ 75,700 mL Seawater
9 mL PES	21 mL PES	700 mL PES
.9 mL Vitamins	2 mL Vitamins	70 mL Vitamins
.8 mL GeO_2	2 mL GeO_2	60 mL GeO_2

Sorus Preparation

Materials

Freshly Collected Mature Sorus Tissue
Cooler
Ice Packs
Several Containers & Trays
Cutting Board
Clean Razors or Scalpels
Chilled Filtered Seawater (50°F/10°C)
Squirt Bottle
Refrigerator
Deionized Water
Production Aquaria with Chiller
3% Iodine
Tongs/Tweezers
Paper Towels
Exam Gloves
Clorox® Regular-Bleach
70% Isopropyl Alcohol
Aluminum Foil
Settling Tubes



1 Collect Healthy Kelp

Collect mature sorus tissue with minimally attached algae and organisms (biofouling). Transport to the nursery.

2 Identify & Isolate Sorus Tissue

Cut out healthy sorus tissue and discard tissue that has biofouling or blemishes.

3 Remove Excess Biofouling

ONLY if the sorus has excessive biofouling, gently scrape the surface with a razor blade.

4 Clean & Remove Mucilage

Firmly wipe the front and back 3-4 times with a paper towel. Discard paper towels after each use.

5 Disinfect Tissue

Dip the sorus in 3% iodine solution for 30 seconds.

6 Rinse

Rinse the sorus with chilled filtered seawater until iodine is removed and water drips clear.

7 Dry

Dry the sorus by gently rubbing front and back with a paper towel. Discard paper towels after each use.

8 Prepare for Overnight Storage

Place pieces of sorus on dry paper towels and cover with additional sheets of paper towel.

9 Refrigerate

Set prepped sorus in dark refrigerator that is set at 50°F/10°C for 14 – 24 hours.

10 Prepare Settling Tubes

Chill 2300 mL chilled filtered seawater in each settling tube. Place the tank lid on top of the settling tubes to prevent contamination.

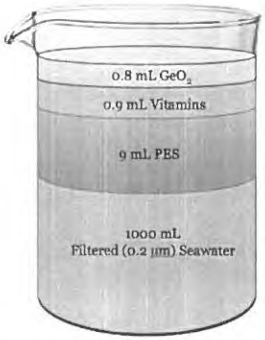
Release of Zoospores

Materials

Prepped Sori Refrigerated for 14-24 hours
Submersible Thermometer
1000 mL (1 L) Beakers
Chilled & Filtered Seawater (50°F/10°C)
Culture Nutrients: PES, Vitamins, GeO_2
Graduated Cylinder
Pipettes
Spatula
Paper Towels
Exam Gloves
Clorox® Regular-Bleach
70% Isopropyl Alcohol

1 Begin Setup

Remove spools from freezer and make sure settling tubes are prepared with chilled filtered seawater.



2 Prepare the Release Beaker(s)

Fill 1000 mL (1L) beakers with: 1000 mL (1 L) chilled filtered seawater, 9 mL PES, 0.9 mL vitamins, 0.8 mL GeO_2 .

3 Monitor Water Temperature

Use a digital thermometer to monitor water temperature. Starting water temperature should be approximately 50°F/10°C, or the temperature sorus was stored in.

4 Add Sori to Beaker(s)

Add sorus pieces to the beakers. Cut sorus into smaller pieces (if needed) to submerge everything.

5 Monitor Beaker(s)

Occasionally stir beakers. Monitor for initial signs of the spore release. Record data on worksheet.

6 Count & Assess Viability of Spores

As sori begin to release spores the beaker water will become cloudy. Start counting the zoospores and calculating the stocking density.

Counting Zoospores and Calculating Stocking Density

Materials

Hemocytometer Cell Counting Chamber
Lens Paper
Micropipettes
Microscope (40x & 100x)
Calculator
Pen & Paper
Paper Towels
Exam Gloves
Clorox® Regular-Bleach
70% Isopropyl Alcohol

1 Prepare Workstation

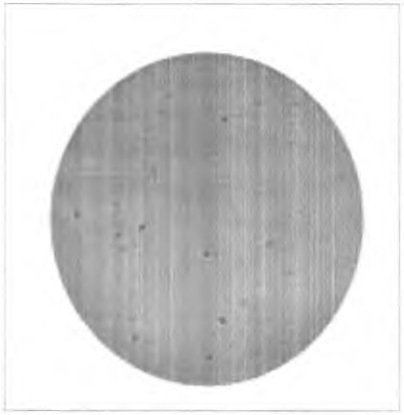
Set up the microscope and focus on the empty hemocytometer counting chamber slide.

2 Prepare the Hemocytometer

Clean hemocytometer and cover slip with lens paper and 70% isopropyl alcohol.

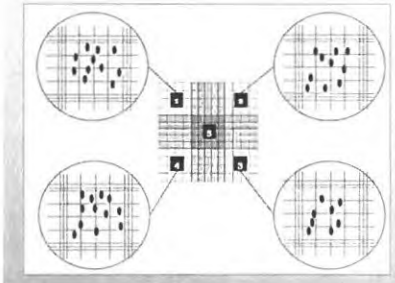
3 Add Release Water into Hemocytometer

Using the micropipette, add the appropriate volume of water from the release beaker into the hemocytometer filling chamber.*



4 Preliminary Evaluation

Place the hemocytometer under the microscope under 40X or 100X and assess the overall health and number of spores.



5 Counting Spores

Method 1: Count the number of cells in the four corner squares, divide the sum of the four corners by four, and multiply by 10,000.

Method 2. If spore density is high, then only the large center square is counted, and the total number of spores counted in the center square is multiplied by 10,000 to determine the total number of spores per mL.

See text for additional information.

6 Calculating Stocking Density

There are three components that you will need to calculate the stocking density:

1) Desired stocking density (recommended=5,000 to 10,000 spores, or an average of 7,500 spores)
2) Volume of seawater (mL) in the settling tubes
3) Concentration of spores in the release beaker (spores per mL)

Stocking densities are calculated using the equation below. See manual for additional information.

$$\text{Volume of Release Water (mL)} = \left(\frac{\text{Desired Stocking Density (Spores/mL)}}{\frac{\text{Release Water (Spores/mL)}}{\text{Seawater in Settling Tubes (mL)}}} \right)$$

*It should be noted that each respective cell counting chamber has procedures for the filling and counting of cells and those should be followed. See the manufacturer's guidelines for the recommended filling procedures.

Inoculating Spools in Settling Tubes

Materials

1000 mL (1 L) Beakers
Chilled & Filtered Seawater (50°F/10°C)
Culture Nutrients: PES, Vitamins, GeO_2
Graduated Cylinders
Pipettes
Spatula
Cheese Cloth or Canning Mesh
Tweezers
Prepared Spools (nylon wound, thawed)
Aluminum Foil
Production Aquaria Equipment & Supplies
Paper Towels
Exam Gloves
Clorox® Regular-Bleach
70% Isopropyl Alcohol

1 Add Nutrients to Settling Tubes

Add culture nutrients to the settling tubes being inoculated with spores: 21 mL PES, 2 mL vitamins, 2 mL GeO_2 (based on 2300 mL of filtered seawater in each settling tube).

2 Place Spools in Settling Tubes

Place spool into the center of the settling tube making sure that it is not touching the sides of the tube.

3 Remove Sori from the Beakers

Discard sorus tissue from the release beaker. Sorus tissue that did not release the first time can be dehydrated more and saved for a second release attempt.

4 Gently Stir & Decant

Gently stir release water to ensure spores are suspended in the water column. Decant the number of mL (previously calculated in Section 4) needed to stock one set tube into a clean measuring container.

5 Add Spores to Settling Tubes

Pour the water containing spores water into the settling tube around the outside of the spool.

6 Adjust Water Level in Settling Tubes

Either remove or add chilled seawater so the level is just above twine.

7 Cover Settling Tubes

Cover each settling tube with aluminum foil and place Plexiglass lid on top.

8 Ensure System is Working Properly

Water chillers should be set to 50°F/10°C. Water should be circulating around the outside of the settling tubes. The spools will remain in settling tubes for 24 hours.

9 Set up Production Aquaria

Fill a 20 gallon production aquaria with filtered seawater. Turn on the water chiller and set it for 50°F/10°C. Cover with the Plexiglass lid. Hang fine mesh screens in front of the light banks. Make sure all light banks (bulbs and timers) are working properly.

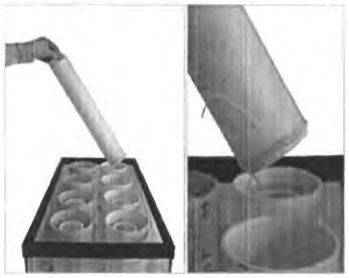
Transfer Spools to Aquarium

Materials

Culture Nutrients: PES, Vitamins, GeO_2
Pipettes
Chilled Production Aquaria (50°F/ 10°C)
Production Aquaria Equipment & Supplies
Aluminum Foil
Paper Towels
Exam Gloves
Clorox® Regular-Bleach
70% Isopropyl Alcohol

1 Add Nutrients to Production Aquaria

Add culture nutrients to the production aquaria: 700 mL PES, 70 mL vitamins, 60 mL GeO_2 (based on 20 gallons of filtered seawater in production aquaria). Allow culture nutrients and seawater to mix for at least 15 minutes in the production aquaria before adding spools.



2 Remove Spools from Settling Tubes

Gently pick up the spool by the PVC top and hold spool at an angle for a few seconds to allow the water to run off.

3 Place Spools into Production Aquaria

Quickly transfer spools into the prepped production aquaria. Position spools as seen in the picture to the left. Do not allow the spools to touch each other or the walls of the production aquaria.

4 Adjust Aquaria Environmental Conditions & Check System

Attach a new sterile pipette to air tubing, turn on the air, and adjust the air flow rate to aerate the production aquaria accordingly. Cover with Plexiglass lid. Set the light timers for a 12 hours on/12 hours off photoperiod. Check all components to ensure system is working properly.

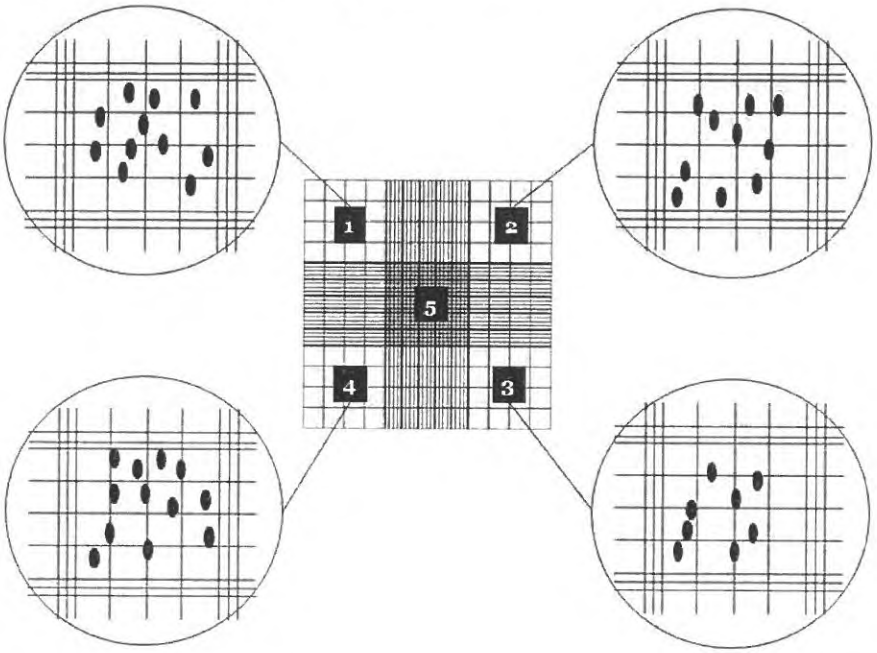
5 Label Release Details

Label the production aquaria with the following release details: release date, species, source of sorus, number of spools in production aquaria, and the dates of water changes. Other important identifying information should also be added.

6 Clean Equipment & Store Settling Tubes

Discard remaining water in the settling tubes. Clean the settling tubes and allow them to air dry. Once dry, cover the opening with aluminum foil. This will help keep them clean until the next use.

Method 1: Count the number of spores in the four corner squares, divide the sum of the four corners by four, and multiply by 10,000.



Example 1. Count the number of spores in the four corner squares, divide by four and multiply by 10,000.

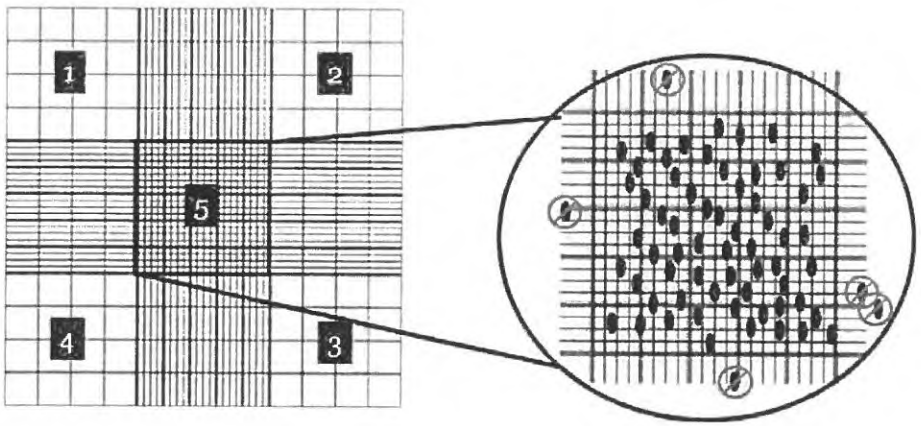
Square 1 = 11 spores
Square 2 = 10 spores
Square 3 = 8 spores
Square 4 = 12 spores

Total Counted = 41 spores

Spores per mL = [(41/4) x 10,000]
Spores per mL = 102,500

Method 2. If spore density is high, then only the large center square may be counted, and the total number of spores counted in the center square is multiplied by 10,000 to determine the total number of spores per mL.

By applying these two counting techniques, OA was able to calculate the stocking densities necessary for successful inoculations. Use the Counting Zoospores and Calculating Stocking Density Worksheet, found on page 69 and in Appendix G, as an aid when counting spores.



Example 2. Count the number of spores in the large center square and multiply by 10,000.

Square 5 = 62 spores
Total Counted = 62 spores
Spores per mL = [62 x 10,000]

Spores per mL = 620,000

Step 6. Calculate Stocking Density

Depending on the species, the health of the sorus tissue, the time of year (different species release during different months), and the nursery environment, the concentration or density of spores that are released will vary. For example, OA observed spore densities in release beakers that ranged from 55,000 to 960,000 spores per mL (see Table 3.3).

Research has demonstrated that a stocking density in the range of 5,000 to 10,000 (average=7,500) spores per mL should be used to inoculate the settling tubes.

The formula that OA used to calculate the volume (mL) of water from the release beaker to inoculate the settling tubes is:

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{\text{Desired Stocking Density (Spores/mL in Settling Tubes}}{\left(\frac{\text{Number of Spores/mL in Release Water}}{\text{Volume of Seawater (mL) in Settling Tubes}}\right)}$$

For example, the three known components are:

1) Recommended stocking density = 5,000 to 10,000 (average = 7,500) Spores/mL
2) Settling tubes = 2,300 mL seawater (Volume OA filled settling tubes)
3) Number of spores per mL in the release beaker = 102,500 spores (based on count from Example 1 above)

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{\text{7,500 Spores/mL}}{\left(\frac{\text{102,500 Spores/mL}}{\text{2,300 ml Seawater}}\right)} = \text{168.3mL}$$

Based on these calculations, **168 mL from the release beaker** would be added to each settling tube.

In a second example, the three components are:

1) Desired stocking density = 10,000 spores/mL
2) Settling tubes = 2,300 mL seawater
3) Number of spores per mL in the release beaker = 620,000 spores

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{\text{10,000 Spores/mL}}{\left(\frac{\text{620,000 Spores/mL}}{\text{2,300 ml Seawater}}\right)} = \text{37.09mL}$$

Table 3.3 Summary of Release Beaker Spore Densities (Spores per mL) Observed in Ocean Approved's Nursery.

Release Date	Water Temperature at First Sign of Release (°F)	Observed Spore Concentration (spores/mL)	Volume of Release Water Inoculated in Settling Tubes (mL)[a]
		Laminaria digitata (Horsetail kelp)	
6/05/12	57	450,000	38
11/13/12	53	200,000	86
11/14/12	56	100,000	173
		Saccharina latissima (Sugar kelp)	
9/20/12	56	115,000	150
9/26/12	59	60,000	287
9/27/12	51	115,000	150
9/28/12	54	55,000	314
10/01/12	51	200,000	86
10/01/12	51	500,000	35
11/13/12	53	960,000	18
11/21/12	51	250,000	69

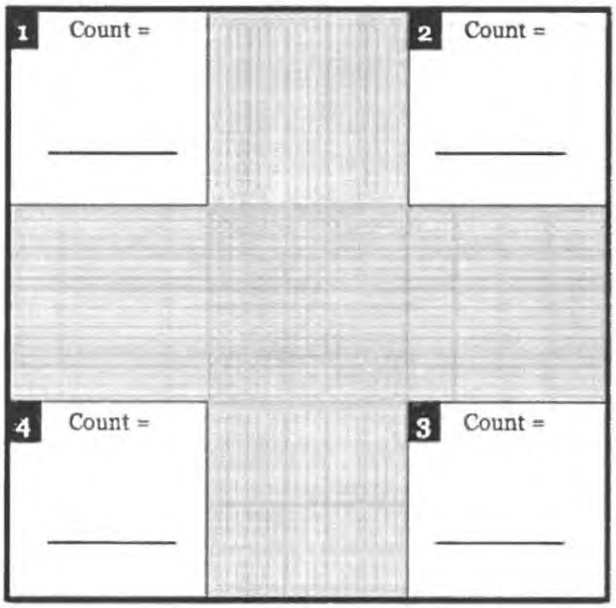
[a]Volume was calculated using the equation explained below with the following parameters: 1) Desired stocking density of spores for settling tubes=7,500; 2) volume of seawater in settling tubes=2,300 mL.

G. Counting Zoospores & Calculating Stocking Density Worksheet

Counting Zoospores & Calculating Stocking Density Worksheet

Date: _____ Species: _____

Method 1.

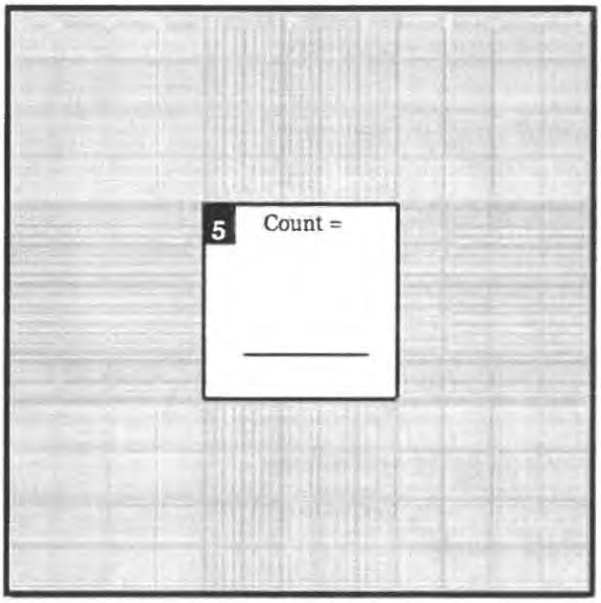


Zoospore Density (Spores/mL) = ((Sq. 1 + Sq. 2+ Sq. 3 + Sq. 4) / 4) X 10,000

Zoospore Density (Spores/mL) = (_____ / 4) X 10,000

Zoospore Density (Spores/mL) = _____

Method 2.



Zoospore Density (Spores/mL) = Square 5 X 10,000

Zoospore Density (Spores/mL) = _____

Calculating Stocking Density

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{\text{Desired Stocking Density (Spores/mL) in Settling Tubes}}{\left(\frac{\text{Number of Spores/mL Release Water}}{\text{Volume of Seawater (mL) in Settling Tubes}}\right)}$$

$$\text{Volume of Release Water (mL) to Inoculate Settling Tubes} = \frac{\text{Spores/mL}}{\left(\frac{\text{Spores/mL}}{\text{mL/Seawater}}\right)}$$

Volume of Release Water (mL) to Inoculate Settling Tubes = _____ mL

H. Nursery Daily Maintenance Checklist

Nursery Daily Maintenance Checklist							
Task	**Sun**	**Mon**	**Tues**	**Wed**	**Thurs**	**Fri**	**Sat**
Check nursery air temperature							
Check aquaria water temperature							
Overall sound inspection							
Overall smell inspection							
Check for leaks in plumbing							
Visual health inspection of spools							
Visual inspection of aquaria water visibility							
Check all lights and timers							
Rotate spools							
pH readings							
Clean/disinfect nursery equipment and aquaria							
Clean plexi glass aquaria lids							
Notes:							

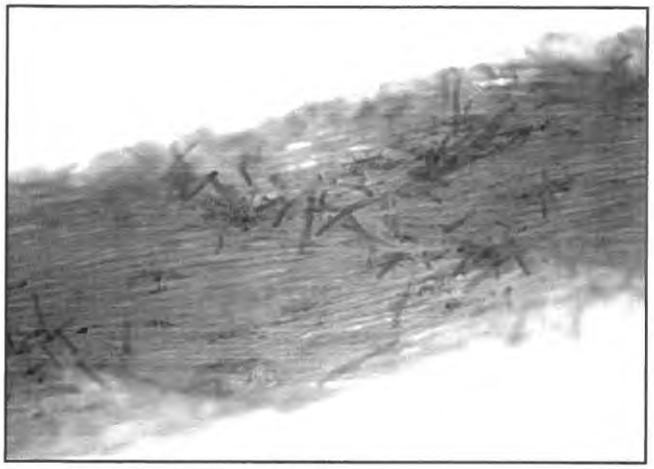


Figure 4.7 Day 26 sporophytes (40x)

Figure 4.8 Day 31 sporophytes (40x)

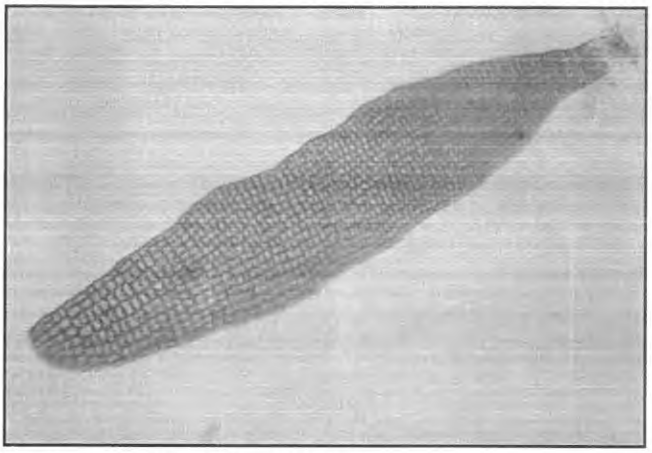


Figure 4.9 Day 37 sporophytes (40x)

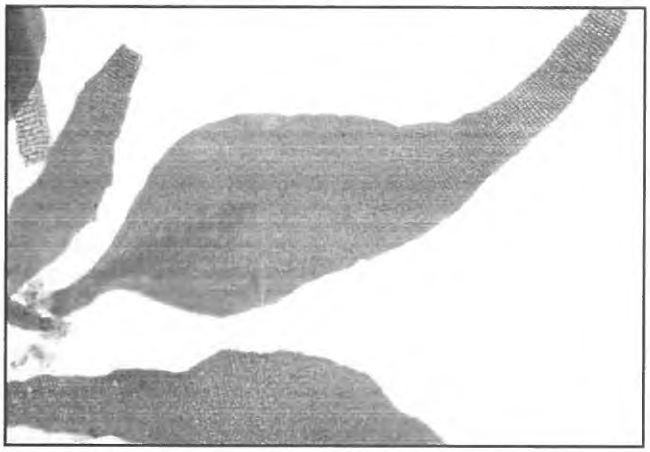


Figure 4.10 Day 40 sporophytes (40x)

Management of Environmental Parameters

Similar to a garden needing specific soil, light, and nutrients for a particular plant, the kelp nursery also needs several key environmental factors to grow. By paying close attention to specific environmental conditions, OA has successfully grown kelp in a nursery for the last four years. Table 4.2 summarizes the environmental parameters: temperature, salinity, pH, and light intensity, among others maintained in the OA nursery. These conditions are essential for the optimal growth, health, and survival of the kelp culture. Kelp can withstand some fluctuation in salinity, but are more sensitive to slight changes in the water temperature and pH.

Seawater

OA changed the seawater in each aquarium on a weekly basis. The replacement seawater must be filtered and sterilized prior to use. It is critical to remove unwanted contaminants by one or more of the techniques (i.e., autoclaving, filtration, and/or UV sterilization) discussed in Table 2.1, page 40. Salinity does not need to be tested on a daily basis if collected from a clean and relatively stable ocean source.

Table 4.2 Parameter Ranges in OA Nursery

Parameter	Recommended Value or Range	
Water Temperature	50°F/10°C	
Salinity	28-34 ppt	
pH	7.0-9.0	
Light		
Days 1-14	20 $\mu mol\text{-}m^{-2}s^{-1}$	(Fine mesh screen)
Days 15-28	55 $\mu mol\text{-}m^{-2}s^{-1}$	(Wide mesh screen)
Days 29+	100 $\mu mol\text{-}m^{-2}s^{-1}$	(No screen)

Nutrients

At the time of each weekly water change, the culture nutrients, vitamins and germanium dioxide (GeO_2) were replenished. Formulas for the media concentrations used in the OA lab, as well as sources for purchasing premade and sterilized media are listed in Appendix B and E.

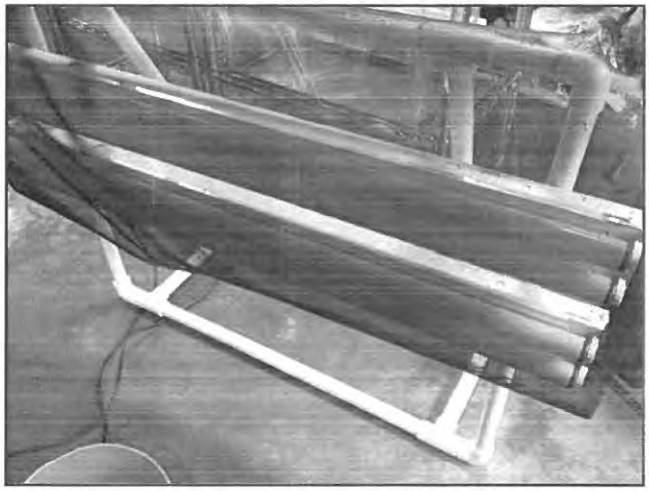
Light

The light intensity and the photoperiod (12 hours "lights on"/12 hours "lights off") were regulated through the use of timers on the lights and fine mesh and wide mesh screens. The screens should be hung between the aquarium and the light source to create the appropriate light levels. OA used plastic zip-ties to secure the screens in front of the light banks. The screens diffuse the light and spread it evenly across the surface of the aquarium. The fine mesh screens (Figure 4.11) created light levels in the aquaria of 20 micromoles per square meter per second (μmol m^{-2} s^{-1}), which is suitable for the beginning growth. The wide mesh screens (Figure 4.12) created light levels in the aquaria of 55 μmol m^{-2} s^{-1}.

Table 4.3 contains data from the 2010 growth season where light levels and

Figure 4.11 (Below) Fine mesh screen used to create light levels of 20 μmol m^{-2} s^{-1}

Figure 4.12 (Right) Wide mesh screen used to create light levels of 55 μmol m^{-2} s^{-1}



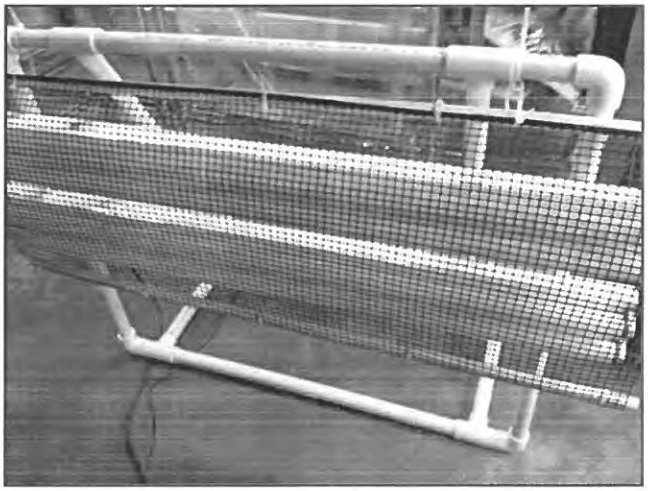


Table 4.3 Light Level Measurements in Ocean Approved's Production Aquaria

Zoospore Release Date (Fine Mesh Screen)[a]	Number of Days Using Fine Mesh Screen[a]	Increase Light Intensity—(Wide Mesh Screen)[b]	Number of Days Using Wide Mesh Screen[b]	Increase Light Intensity — (No Screen)[c]	Number of Days Using No Screen[c]	Out-planting to Farm Site	Total Days in Nursery
9/26/10	19	10/15/10	19	11/3/10	3	11/6/10	41
9/26/10	19	10/15/10	19	11/3/10	9	11/12/10	47
11/16/10	14	11/30/10	14	12/14/10	16	12/30/10	44
11/16/10	14	11/30/10	14	12/14/10	17	12/31/10	45
11/16/10	14	11/30/10	14	12/14/10	24	1/7/11	52
11/17/10	13	11/30/10	14	12/14/10	31	1/14/11	58
11/17/10	13	11/30/10	14	12/14/10	15	12/29/10	42
12/9/10	12	12/21/10	17	1/7/11	7	1/14/11	36
12/9/10	12	12/21/10	17	1/7/11	8	1/15/11	37

[a]Fine mesh screen: 20 micromoles per square meter per second (μmol m -2 s -1) [b]Wide mesh screen: 55 micromoles per square meter per second (μmol m -2 s -1)
[c]No screen: 100 micromoles per square meter per second (μmol m -2 s -1)

time spent in the nursery were varied by design. Based on these experiments, and additional work performed during the 2011 and 2012 seasons, OA adopted the protocol of using the fine mesh screens for days 2 through 14 in the nursery., and the wide mesh screens from day 15 through day 28. By week 4 (or sometimes earlier), the screens can be removed, providing full light of 100 μmol m^{-2} s^{-1} It should be noted that these are general guidelines. Decisions on when to change from fine mesh to wide mesh should be based on the overall appearance and growth of the sporophytes.

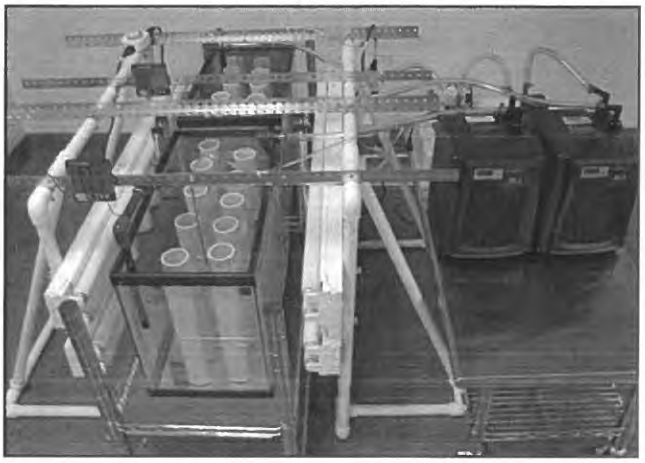
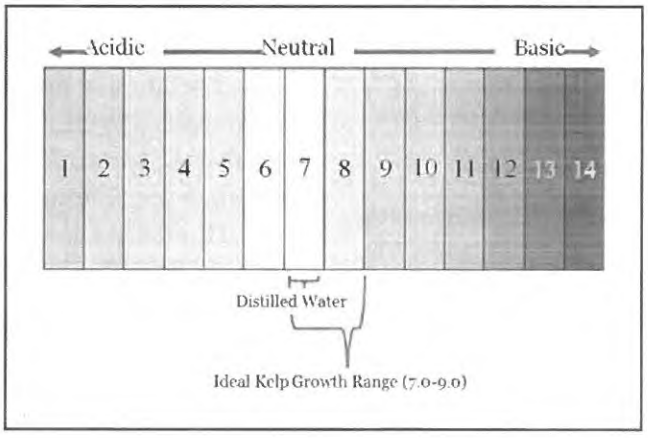


Figure 4.13 Nursery setup with the ability to sustain two production aquaria

Temperature

Kelp plants grow best in water that remains at or near 50°F (or 10°C). This temperature was most frequently used in the nursery using commercially available water chillers for each aquarium. Nurseries that have a constant supply of natural running seawater may not have to cool the water, but still must prevent unwanted contaminants from entering the aquaria. Figure 4.13 shows the setup of aquaria and chillers that were used by OA to maintain a constant tank temperature of 50°F (10°C) in a room maintained at a temperature of 68°F (20°C).

Figure 4.14 On the pH scale of 0–14, distilled water is neutral with a pH of 7.0, and kelp grows at a pH between 7.0–9.0



pH

The acidity/alkalinity index must be monitored and maintained. Kelp grows best in the pH range of 7.0–9.0 on the pH scale of 0–14 (Figure 4.14). The pH may be measured using aquarium or pool test kit "dip sticks" or electronic handheld meters. The pH of natural seawater is in the range of 7.8 to 8.2. As the kelp plants grow in the aquaria, the pH will slowly increase. This is normal. The pH may be controlled and lowered by bubbling CO_2 gas through the water for short periods of time to maintain a pH close to 8.0.

Aeration

Air is introduced into the aquaria using air pumps, tubing, sterile pipettes, and air filters. An air pump provides the seawater with dissolved oxygen and water movement. It is important to provide an ample supply of air bubbles to the growing sporophytes for healthy growth and for promoting strong holdfasts. The air flow rate should be high enough that it circulates the water in the aquaria, but not so high that it moves the spools around (Figure 4.15).

Figure 4.15 Adjust air flow so it does not move spools around

Contamination

For this manual, a contaminant refers to any organism (e.g., bacteria, protozoans, other species of algae) that negatively affects the growth of the kelp. Contaminants typically compete with the kelp for light, nutrients or space. It is very important to remove as many of these contaminants as early as possible. Techniques for reducing contamination in the seawater are discussed in Chapter 2. Additionally, frequent water changes are also effective in minimizing competition from these organisms. It is possible to see contamination with regular visual observations of the aquaria. Oftentimes, if contamination is

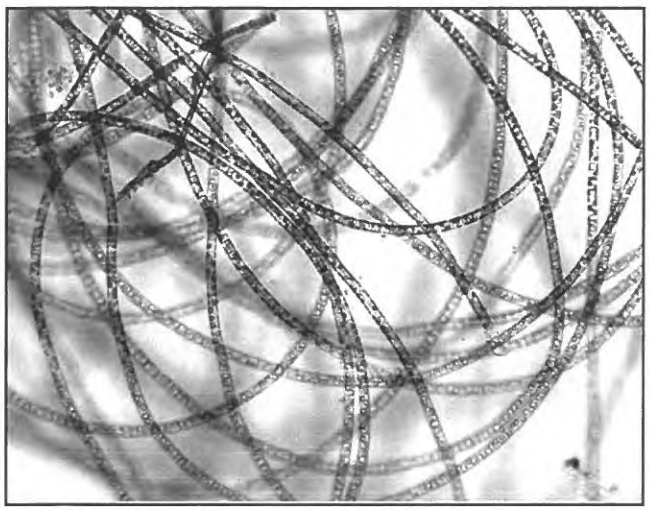


Figure 4.16 Green filamentous algae

present, the visibility in the aquaria will rapidly deteriorate and the aquaria will become cloudy. Figure 4.16 shows a microscopic view of a green filamentous algae observed in a production aquarium. Possible contamination may also be evaluated by observing in a small water sample under the microscope. For a more accurate analysis, samples can be sent to a laboratory specializing in the counting and identification of these types of organisms. Completely eliminating contaminants in the aquaria may be difficult. When needed, OA used a small ultraviolet (UV) sterilization device on individual aquaria. These systems are relatively inexpensive, easy to install, and continually treat the seawater with UV radiation as it circulates through the system.

Daily Nursery Tasks

Daily checklists are used for both efficiency and consistency in the OA nursery (Figure 4.17). This checklist can also be found in Appendix H. The checklist helps with recognizing problems when they first arise. OA recommends being proactive about maintaining the nursery and its systems.

Checking Nursery Air and Aquaria Water Temperatures.

The temperature of the seawater in the aquaria is a vital part of culture success. It is recommended to check the temperature of the aquaria frequently. It is important to note that the warmer the room temperature in the nursery, the harder chillers have to work to keep the aquaria at temperature.

If the temperature in the aquaria rises by a few degrees for a short period of time, it most likely will not affect the sporophytes, but if the temperature rises over 60°F (or 15.5°C), this could be damaging. In the OA nursery, production took place throughout the year to support our research. During the summer months, with increased outside temperatures, poor air circulation in the nursery, and increased number of production aquaria, the chillers at times could not keep the seawater chilled to 50°F (10°C). A large temperature difference between air and seawater, may also cause condensation to build up on the outside of the aquaria. Heavy amounts of condensation reduce the light that the spools receive. At one point it was necessary to install a small air conditioning unit in the nursery to lower the nursery's air temperature, reduce the strain on the chillers, and reduce condensation. Taking steps to correct this situation early will prevent chillers and equipment from burning out and failing in the future.

Figure 4.17 Checklist used by OA to assist with maintaining daily tasks in the nursery

Nursery Daily Maintenance Checklist							
Task	Sun	Mon	Tues	Wed	Thurs	Fri	Sat
Check nursery air temperature							
Check aquaria water temperature							
Overall sound inspection							
Overall smell inspection							
Check for leaks in plumbing							
Visual health inspection of spools							
Visual inspection of aquaria water visibility							
Check all lights and timers							
Rotate spools							
pH readings							
Clean/disinfect nursery equipment and aquaria							
Clean plexi glass aquaria lids							
Notes:							

Overall Sound and Smell Inspection

Getting into the habit of recognizing what a properly functioning nursery sounds and smells like will

help prevent a number of malfunctions from occurring. For instance, knowing how loud the nursery is with the systems and pumps running may help to diagnose any faulty equipment. Before a pump burns out, a high-pitched hum or a rattling may be heard that was not there before. The smell of the nursery is key to preventing burnout as well. If the room has a "hot" smell, this could signify a pump is overheating, or if there is a slight scent of melting plastic, it is critical to look into the problem right away.

Checking for Leaks in Plumbing

The OA nursery experienced leaks in pipes and pumps infrequently, but they could have caused problems if left unfixed. Most often leaks were small drips caused by a hose clamp that was too loose or a pump hose incorrectly positioned. Puddles on the floor or under pumps are easily recognized with a quick walk through the nursery or when performing everyday maintenance on the aquaria. In the OA nursery, power strips and cords were hung from the ceiling to prevent any water from coming in contact with electricity sources.

Visual Health Inspection of Spools

When the spools are placed into the aquaria, they are white in color (or the color of the twine used). As the weeks progress, a light brown mottled coloration is noticeable, followed by a more uniform light brown. The spools continue to get darker and more "fuzzy-looking" as the sporophytes grow. If the darkening of the brown coloration stops, or if other colors such as green are noticed, it is recommended to examine a piece of the sample twine. Look for signs of contamination by other organisms or algae. During the summer months, numerous species of green and brown algae (e.g., *Enteromorpha* and *Ectocarpus*) grow prolifically in the Gulf of Maine, and can make their way into the production aquaria when performing water changes. Figure 4.18 shows dots of green algae, which have contaminated the twine.

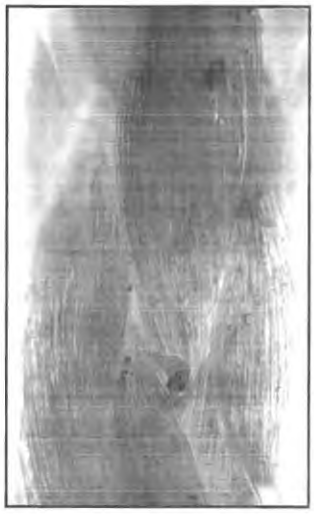


Figure 4.18 Green algae contamination present on the twine

Visual Inspection of Aquaria Water Visibility

Looking at the visibility of the seawater in the aquaria is also a very important task. If contamination does take hold in the aquaria, the visibility will very rapidly decline and clear water will turn cloudy. Over the course of the week, a small amount of cloudiness may occur as the time to change the water approaches. In this case, slight cloudiness is normal. If an entire aquaria becomes cloudy overnight, significant contamination (usually bacteria) may be present. OA occasionally placed small UV sterilizers in aquaria to control growth of potential contaminants. These individual units can clear cloudy aquaria in less than a day and can be purchased at pet stores or online.

Checking all Lights and Timers

Another vital component of kelp growth in the nursery is providing the correct intensity and duration of light. Power outages and turning a power strip off (that has a timer plugged into it), may disrupt the time on the timers, resulting in incorrect light/dark cycles. It is recommended to check the timers frequently.

Rotating Spools

The one very essential task in the nursery to complete every day is "rotating the spools" or turning each spool 180 degrees in the aquaria. This process ensures that

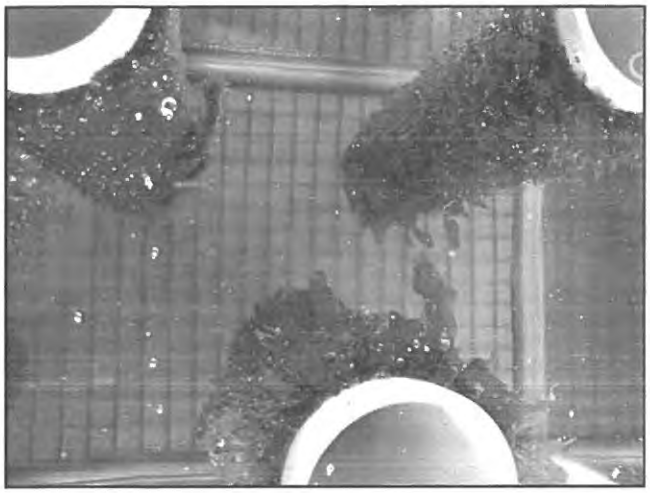


Figure 4.19 Oxygen bubbles rising from maturing sporophytes in week 6 in the nursery

Figure 4.20 Equipment soaking overnight in bleach and deionized water solution

every part of the spool is exposed to light. It is important to wear gloves while rotating the spools to prevent contamination from hands entering the aquaria and coming into contact with the culture media added to the seawater. Gloves should always be worn when handling any part of the aquaria and their systems.

pH Readings

As the sporophytes grow and photosynthesize, they utilize more carbon dioxide dissolved in the water and release more oxygen into the aquaria. In the last week(s) of growth in the nursery, oxygen bubbles can be seen coming off the sporophytes and rising to the surface (Figure 4.19). At this stage, testing the pH of the seawater in the aquaria is an important task, as the pH may begin to approach 9.0 or higher. In the OA nursery, the pH was monitored once a week for the first two weeks, followed by every three days, and then almost every day in the last week of nursery culture. To bring the pH back down to approximately 8.0, CO_2 was slowly bubbled into the aquaria that exhibited high pH readings.

Cleaning and Disinfecting Nursery Equipment and Aquaria

Figure 4.21 Glassware with an aluminum foil cover to prevent contamination

If the space and equipment being used for culture is kept clean, then there is less risk of introducing contamination into the systems. Cleanliness is a key attribute of a successful kelp nursery. Prepare sori and clean coolers outside of the nursery production area. Clean glassware, containers, and equipment used for sorus preparation after use. Remove the trash from the nursery often. The cleaning method that OA used in the nursery started with soaking the equipment in a bleach solution overnight (Figure 4.20). Use the bleach concentration recommended on the manufacturer's label. After soaking, rinse thoroughly until there is no bleach smell left on the equipment and it no longer has a slippery feel. Then wash with gentle dish soap and thoroughly rinse again. Glassware, cutting tools, plexiglass aquarium lids, etc., can be sprayed with 70% isopropyl alcohol and wiped dry with a paper towel. When completely dry, aluminum foil (Figure 4.21) or parafilm can be used as a cover to prevent contamination from reattaching to the surfaces. Counter spaces can be wiped down with 70% isopropyl alcohol after sorus preparation and spore release and throughout the grow-out period in the nursery.

Cleaning Plexiglass Aquarium Lids

Plexiglass aquarium lids will frequently need to be cleaned of salt buildup. While this does not need to be done daily, it is good practice to clean the lids every couple of days and soak them in bleach during weekly water changes.

Weekly Maintenance

Four tasks were completed on a weekly basis in the OA nursery. These included: 1) filling the holding tank or jugs with seawater, 2) water changes, 3) cleaning the aquaria and systems after water changes, and 4) observing growth under the microscope and taking photos.

Filling Holding Tank or Jugs with Seawater.

Depending on how many aquaria are in production, collecting water may need to be done more or less frequently. It is recommended to replenish the seawater supply at least once a week to prevent any contamination in the jugs. Disinfecting the holding container weekly or biweekly with bleach or chlorine dioxide (ClO_2) reduces the chance of contamination.

Water Changes

The weekly water change is a 100% change of the seawater in the aquaria. Spools are removed from each aquarium and transferred into aquaria that were previously prepared to receive the spools. OA found that having extra aquaria set up with active systems (i.e., chillers, air, light banks, etc.) allowed for a quick transfer of spools from the old seawater to the new. OA accomplished this by filling the new aquaria with fresh filtered seawater the day before the water change and covering with a plexiglass lid. The chiller was turned on to allow the water to cool ahead of the transfer of spools. Nutrients were added either the night before or a few hours earlier to allow for thorough mixing. At the time of water change, the new water temperature must be cooled to 50°F (or 10°C). Use the same guidelines for transferring spools into the aquaria from Chapter 3, page 74. Transfer the data record, or clipboard shown in Figure 4.22, to the new aquaria after moving the spools.

Figure 4.22 Release details on a movable clipboard follow the spools with every water change

Cleaning Aquaria and Systems After Water Changes

After the spools are transferred, the old aquaria and systems, now without spools, should be disinfected and cleaned. Depending on the amount of contamination, a specific amount of stock solution of 3,000 ppm commercial grade ClO_2 was added to each 20 gallon aquarium. Final concentrations of 3 to 10 ppm CLO_2 were most frequently used. Follow manufacturers guide lines for use and dilution of ClO_2. The 3 to 10ppm ClO_2 solution was circulated through pumps and chillers for 24 hours. Aquaria were then drained using an inexpensive siphon (similar to one used for siphoning fuel), rinsed with deionized water, scrubbed thoroughly with dish soap, and rinsed thoroughly with deionized water. It is important to rinse away all traces of soap residue from the aquaria before using for culture again. Aquaria were set upside down to dry and then covered until next use. If plastic tubing appeared to be cloudy or if residue started to build up, they were cleaned or replaced.

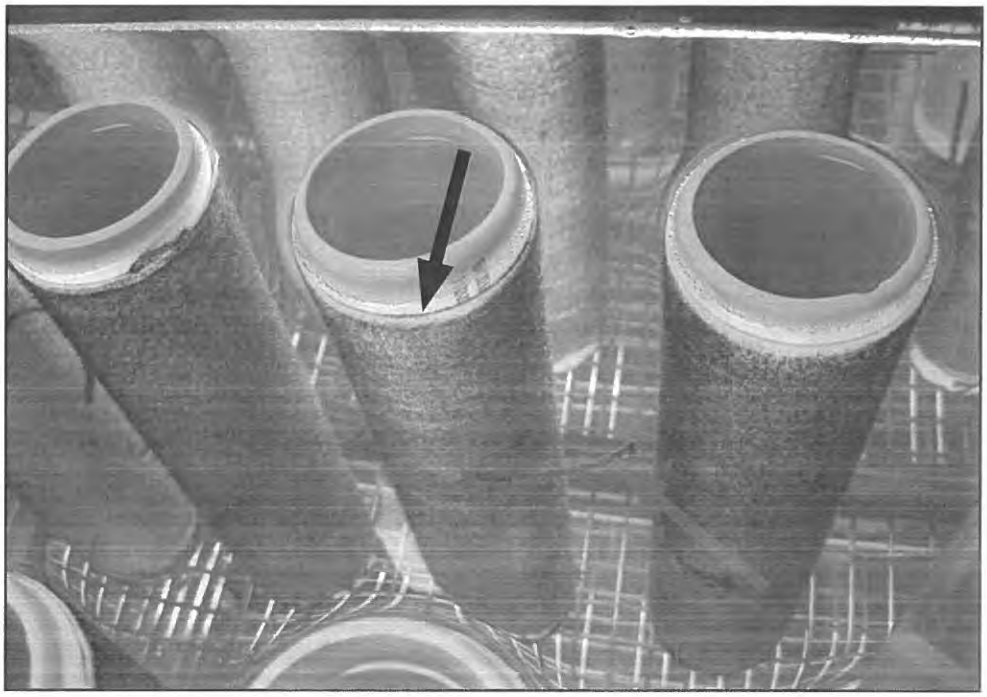
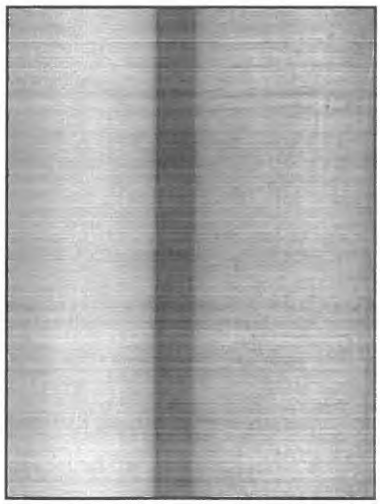
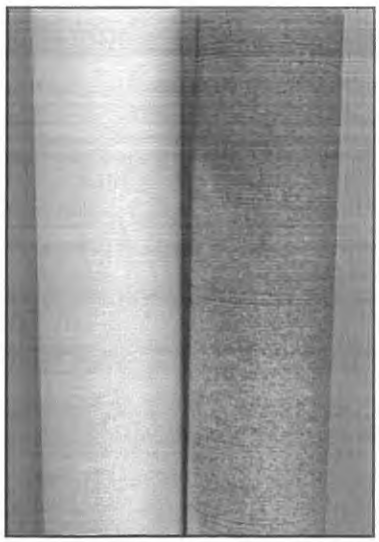


Figure 4.23 The red arrow is pointing to the piece of sample twine that can be used to observe growth under the microscope. The blue arrow points to the rubber band holding the twine in place.

Figure 4.24 Spool at week 1 (left); spool at the end of week 2 (right)





Observe growth under the microscope and take photos

OA cut small pieces of sample twine (Figure 4.23) off the spools as they were lifted out of the aquaria during water changes. The kelp's growth on the twine was observed under 40x and 100x magnification of the microscope. When looking at the spools during the first two weeks in the nursery, there is little visual evidence that any growth is occurring. Looking closer at the sample twine under the microscope is a reassurance that the proper growth is taking place. Observing growth on a weekly basis also makes it possible to track the progression from the zoospore to the gametophyte stage and then to the sporophyte stage. If the microscope is equipped with a camera, taking photos will allow for comparing growth at a later date. Taking photos of the spools in the aquaria at regular intervals is another way to ensure that the kelp is growing, as it is possible to see the spools becoming darker and the growth more dense. Figures 4.24–4.26 show spool growth and color change over time in the nursery. These photos were captured out of the water during a water change.

Figure 4.25 Spool at week 1 (left); spool at the end of week 3 (right)

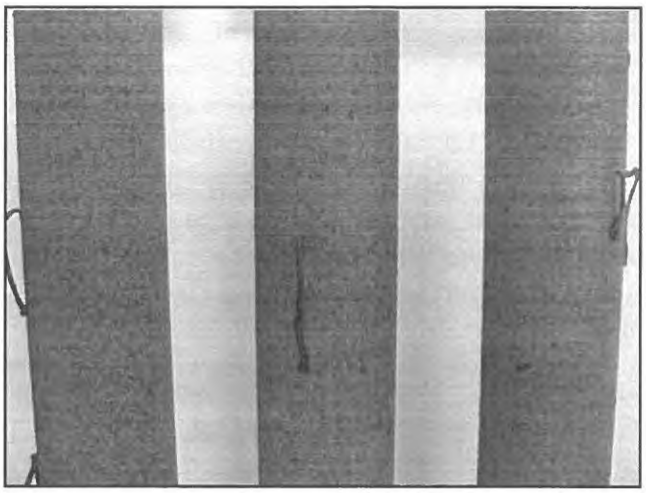


Figure 4.26 Spools at week 4 before transport to the ocean farm site

Appendices

A. Glossary

Aeration: a process where air is circulated through, mixed with, or dissolved in a liquid or substance.

Alaria: *Alaria esculenta* is a species of kelp plant commonly known as "winged kelp" that is found in temperate coastal water growing below mean low water. It has been successfully grown in the nursery and at the farm site of OA.

Alternation of generations: a term used to describe the life cycle of algae (and other plants) that alternate between two phases of growth: a multicellular gametophyte (1n) and a multicellular sporophyte (2n) plant. In the kelp grown in OA's nursery and farm sites the sporophyte and gametophyte are of different size and appearance.

Aquaculture: farming of aquatic organisms such as fish, mollusks, and algae.

Autoclaving: a process of sterilizing utensils or liquids by subjecting them to high-pressure saturated steam at 121°C for 15 to 20 minutes.

Bacteria: a large group of microorganisms, typically a few microns in size that have various shapes and exist in water, soil, and air. An mL of water may have a bacterial count of over one million individuals.

Biofouling: accumulation of microorganisms, plants, algae, or animals on wetted surfaces such as boat hulls, rope, pilings, or other organisms.

Blade: the part of the kelp plant that is flattened and expanded.

Bryozoans: a group of aquatic invertebrate animals typically about 0.5 mm in size that live in colonies in marine waters and may form large deposits of colonies on marine plants such as kelp.

Carbon dioxide (CO_2): a chemical compound naturally occurring in water and air and utilized by plants in photosynthesis. CO_2 is also the byproduct of respiration (breakdown of sugars) by plants and animals. Concentrated CO_2 gas may be

introduced into aquaria or other culture tanks to adjust and regulate the acidity/alkalinity (pH) of the seawater.

Cell-counting chambers: glass or plastic slides that contain a chamber designed to hold an exact volume of liquid. Grid patterns are etched on the slides to allow for accurately counting the number of cells or zoospores in the liquid.

Chiller: a refrigeration unit that may be used to control the temperature of water or other liquids that is circulated through it. Chillers have been used in the OA nursery to maintain the aquaria at desired temperatures.

Chlorine dioxide (ClO_2): a chemical compound that is a useful and potent disinfecting agent. It may be used to disinfect water and other materials. One should follow the manufacturer's instructions for the proper concentrations and handling in the various uses.

Clorox: a commercially available cleaning and disinfecting product containing approximately 5.5% Sodium hypochlorite (NaOCl) as the active ingredient. Clorox may be used to treat water, equipment, and surfaces. One should follow the manufacturer's instructions for the proper concentrations and handling in the various uses.

Decant: a process that can be used to separate a solid from a liquid when the solid material does not stay in solution and settles to the bottom. The solid is allowed to settle to the bottom of the beaker or container and then the liquid is carefully poured off without disturbing the solid on the bottom.

Deionized water: water that has been "softened" by passing it through cylinders with resins that exchange the "hard ions" of magnesium, calcium, and iron with "soft" sodium ions.

Diatoms: a large and diverse group of microscopic algae (phytoplankton) found in abundance in many aquatic habitats. Although mostly microscopic they may grow in colonies to a large size.

Dropper (depth control system): depth maintenance buoys used on long lines at the farm sites to keep the lines at a desired depth throughout the kelp growth period.

Ectocarpus: a genus of small filamentous brown algae that frequently grow attached to kelp.

Enteromorpha: a genus of filamentous and tubular green algae that are very prevalent in the intertidal zone during the warmer months. Occasionally species of enteromorpha may be introduced into the nursery aquaria and outcompete the young kelp.

Exam gloves: disposable gloves made of latex, vinyl, or other materials and are widely used in medical, scientific, and other industries to protect the hands from chemicals or contaminants and to eliminate the transfer of materials from one's hands to the materials being worked with.

Filtration: the process of separating solids from liquids. Filtration in this manual describes the passing of seawater through a polypropylene or membrane filter to remove potential contaminants such as bacteria, protozoa, and plankton.

Fish boxes: watertight boxes frequently used in the marine industries to transport fish, water, and other substances. A Bonar box is one type of fish box.

Fixative: a solution used to preserve or stabilize cells for observation under the microscope.

Flagella: a thin, whip-like structure extruding from some microscopic algae cells which quickly sway back and forth providing motility to the cells.

Gametophyte: the phase in the kelp life cycle that is microscopic and produces male and female sex cells (gametes) that merge to produce the macroscopic sporophyte phase.

Germanium dioxide (GeO_2): a chemical compound that is added in small concentrations to culture media to prevent the growth of diatoms.

Graduated cylinder: laboratory and nursery glassware used to measure liquids in milliliters (1/1000 liter).

Hemocytometer: a cell-counting chamber holding a known volume of liquid and used under the microscope to count and/or observe very small cells and zoospores.

Heteromorphic: having two different phases in the life cycle, each having a different appearance.

Iodine: weak solutions of elemental iodine that are frequently used as antiseptics. OA frequently used over-the-counter providone iodine diluted to 3% to destroy organisms attached to the kelp blades and sori prior to attempting to induce the release of spores.

Kelp: the common name of a group of brown algae of the order laminariales that are common in temperate marine environments and are grown in many aquaculture operations.

Laminaria: *Laminaria digitata* is a species of kelp known as "horsetail kelp" and is commonly found in temperate coastal waters growing below mean low water. It has been successfully grown in the nursery and farm sites of OA.

Lease site: a farm location leased from the state.

Long line: rope used at the farm sites for the attachment and growth of kelp. OA's sites used 7/16 inch line.

Lugol's solution: a solution of elemental iodine and potassium iodide in water often used as an antiseptic and disinfectant or as a fixative for cells in laboratory procedures.

Macroscopic: the scale of objects which are large enough to be seen without magnification.

Mean low water: the average level of low tides over a period of time—usually many years.

Meristem: a region of cells at the base of the kelp blade where cells are actively dividing, causing the blade to grow.

Micropipettes: laboratory and nursery glassware used for accurately measuring small volumes (milliliters) of liquid.

Microscope: an instrument used to magnify organisms that are too small to be seen with the naked eye.

Microscopic: the scale of objects smaller than those that can be seen with the naked eye and that therefore require a lens or microscope to see them clearly.

Milliliter (mL): one thousandth of a liter (1/1000). One quart of liquid contains 946 milliliters.

MSDS (Material Safety Data Sheets): Intended to provide worker and emergency personnel with procedures for handling or working with a potentially hazardous substance in a safe manner.

Mucilage: a term used to describe the slimy carbohydrate complex that is found on the surface and cell walls of many algae, especially kelp.

Nursery spools: two-inch diameter PVC pipe used to hold the twine on which the young kelp spores set and grow.

OSHA (Occupational Safety and Health Administration): the federal agency that regulates workplace safety and health.

Pasteurization: the process of heating a food or liquid to a specific temperature for a predetermined time (61.7°C for 30 minutes) and then cooling it quickly. Unlike sterilization, it does not kill all microorganisms but significantly reduces their numbers and does not cause major chemical alteration of the substance.

PES: Provasoli's Enriched Seawater. A defined culture media containing a complex of compounds found to be beneficial to the growth of many marine organisms.

pH: the measure of the acidity or basicity of a solution. Solutions with a pH less than 7.0 are acidic and solutions with a pH greater than 7.0 are basic (or alkaline). A pH of 7.0 is neutral. Pure water has a pH of approximately 7.0. Kelp grows best at a pH of 7.0 to 9.0.

Phytoplankton: photosynthesizing microscopic organisms that inhabit the upper,

sunlit layer of oceans and bodies of fresh water.

Protozoans: a diverse group of unicellular animal-like microorganisms, many being motile, found in almost all environments.

Saccharina: *Saccharina latissima* is a species of kelp known as "sugar kelp" and is commonly found in temperate coastal waters growing below mean low water. It has been successfully grown in the nursery and farm sites of OA and is one of the most frequently grown varieties of kelp in aquaculture around the world.

Sedgewick-rafter cell (S-R): a glass slide that has a cell to designed to hold 1 mL of liquid. The cell is frequently used with a microscope for counting plankton. It is a relatively inexpensive and accurate method but has been replaced in many applications by automated counters. OA utilized the S-R type of cell on a limited basis because of the small size and abundance of the kelp spores. These small spores were more accurately counted using the cellometer or neubauer type cell, which hold smaller volumes and allow for higher magnification under the microscope.

Sorus: an area of a kelp blade containing a grouping of reproductive cells distinguished in the kelp as a darker and raised area of the blade.

Species: a unit of classification (naming) within a genus.

Sporangium: a cell which produces spores on the kelp blade within the sorus tissue. (Plural: sporangia)

Spore: single-celled reproductive entities that may join with another cell or may grow into a new plant without fusion.

Sporeling: in this manual a sporeling refers to a young kelp plant growing on twine on a nursery spool.

Sporophyte: in this manual a sporophyte refers to a both young kelp plant growing on twine on a nursery spool and adult plant in the ocean this is final stage of the kelp lifecycle.

Stipe: the erect, stem-like portion of kelp that connects the holdfast to the blade.

Stocking density: the number of reproductive spores used to inoculate a settling tube to establish the young gametophyte and sporophyte phase on the twine.

Twine: the 1mm nylon thread that is wound around the nursery spools to provide the substrate for young kelp to grow.

Tyndallization: destruction of microorganisms (contaminants) by heating a substance (seawater) at the boiling point for 15 minutes three days in a row. This process may be used where steam heat (autoclaving) is not possible and will normally sterilize the substance. Heating seawater to 80 degrees centigrade for ten minutes

will eliminate most micro-organisms and has been successfully used in some algal culture operations.

UV light sterilization: the process of passing a liquid (seawater) through a closed UV light system designed to destroy microorganisms.

Vitamins: supplements to nursery culture media to encourage and support the growth of young kelp plants.

Zoospores: motile reproductive cells bearing flagella. In kelp, zoospores normally develop into male or female gametophytes.

Socio-ecological considerations for kelp species native to the Salish Sea.

Scientific Name	Common Name(s)	Distribution (WA State)[1]	Temperature[2]	Nitrate[3]	Salinity/Turbidity[4]	Substrate (O'Clair and Lindstrom 2000)	Depth (O'Clair and Lindstrom 2000)
Alaria marginata	Broad-winged kelp, Ribbon kelp	PS, SJDF, SJI	mortality, no recruitment >18 °C (O'Clair and Lindstrom 2000; Muth et al. 2019)	1-10 µmol/L; optimum 10 µmol/L (Muth et al. 2019)		rocky	mid-low intertidal, 3 m
Costaria costata	Five-ribbed kelp, Seersucker kelp	PS, OC	mortality >20 °C, no recruitment >18°C (O'Clair and Lindstrom 2000; Muth et al. 2019)	1-10 µmol/L; optimum 5 µmol/L at 12°C (Muth et al. 2019)		rocky or woody	low intertidal and upper subtidal, >3 m
Cymathaere triplicata	Three-ribbed kelp, Triple rib, Fold-rib kelp	SJDF, SJI, Whidbey Is (west side)	mortality 15-18 °C (O'Clair and Lindstrom 2000)			rocky	low intertidal and upper subtidal, >3 m
Hedophyllum sessile	Sea cabbage, Sweet konbu	SJDF, SJI (west side)	mortality >15°C (O'Clair and Lindstrom 2000)			rocky	mid to low intertidal, 3 m
Laminaria setchellii	Southern stiff-stiped kelp, Split blade kelp, Wild N. Pacific konbu	outer SJDF, OC on most exposed areas	5-15°C optimal; reproduction inhibited >17 °C;	1-10 µmol/L; optimum 10 µmol/L (Muth et al. 2019)		rocky	extreme low intertidal and upper subtidal, <10 m

			no recruitment >18 °C (O'Clair and Lindstrom 2000; Bartsch et al. 2008; Muth et al. 2019)				
Lessoniopsis littoralis	Flat pompom kelp, Strap kelp, Ocean ribbons	OC, outer SJDF	no recruitment >18 °C (Muth et al. 2019)	1-10 µmol/L; optimum 5 µmol/L (Muth et al. 2019)		rocky, very exposed	low intertidal, 3 m
Macrocystis pyrifera	Giant kelp	SJDF (west of Low Point)	<16.3 °C optimal; recruitment can occur at 18 °C (O'Clair and Lindstrom 2000; Schiel and Foster 2006; Muth et al. 2019)	1-10 µmol/L; optimum 10 µmol/L at 12°C (Luning 1991; Muth et al. 2019)	low salinity poorly tolerated at high temperatures (Mumford 2007)	rocky	lower intertidal to deep subtidal, 5-30+ m (Schiel and Foster 2006; Druehl and Clarkson 2016)
Nereocystis luetkeana	Bull kelp, Bullwhip kelp	PS, OC	10-15 °C optimal; no recruitment >18 °C (Vadas 1972; Maxell and Miller 1996; Muth et al. 2019)	1-10 µmol/L; optimum 10 µmol/L (Muth et al. 2019)	range 26-31 o/00, but 28-29 o/00 ideal; wide salinity tolerance if sedimentation is low (Maxell and Miller 1996; Mumford 2007; Lind 2016)	cobble and rocky	subtidal, 3-17 m (Mumford 2007)
Pleurophycus gardneri	Broad-ribbed kelp, Sea spatula	Straits, SJI, Whidbey Is (west side)	14-15 °C triggers senescence; mortality >15 °C (O'Clair and Lindstrom 2000;	Low NO_3^- leads to blade loss; nitrogen-limited (NO_3^-, NH_4^+) (O'Clair and		rocky	extreme low intertidal and upper subtidal, <15 m

			Germann 2011; Pfister and Betcher 2018)	Lindstrom 2000; Germann 2011)			
Postelsia palmaeformis	Sea palm		no recruitment >18 °C (Muth et al. 2019)	1-10 µmol/L; optimum 10 µmol/L (Muth et al. 2019)		rocky	high to mid intertidal, <3 m (Luning 1991)
Pterygophora californica	Woody-stemmed kelp, Old growth kelp	SJDF, SJI; rare in central and southern PS	no recruitment >18 °C (Muth et al. 2019)	1-10 µmol/L (Muth et al. 2019)		cobble and rocky (Druehl and Clarkson 2016)	low intertidal to subtidal, 2-20 m (typically 10 m) (Dayton 1985; Mumford 2007)
Saccharina latissima	Sugar kelp, Sugar wrack	PS, OC	10-15 °C optimal; mortality >19 °C (O'Clair and Lindstrom 2000; Simonson et al. 2015)	saturation at 10-20 µmol/L (Simonson et al. 2015)	26-31 o/00; tolerates low salinity regardless of temperature (Mumford 2007; Lind 2016)	mixed; rock, shell, debris, and even sand in CA	lower intertidal and upper subtidal, <30 m

(Continued)

Scientific Name	Wave Action	Canopy Form (Gabrielson et al. 2006; Mumford 2007)	Life Cycle (Sporophyte Phenology)	Sporophyte Size	Ecological Adaptations & Vulnerabilities	Human Use(s)[5]

Alaria marginata	fully sheltered to fully exposed (Mumford 2007)	prostrate	annual (Luning 1991)	<3 m (Luning 1991)	fast-growing; mid-canopy species; abundant in pools (Luning 1991; Mumford 2007)	*Food:* wakame substitute *Nutrition:* source of vitamin A, vitamin B6, vitamin K, iodine, calcium, and potassium; contains more than 6% protein (O'Clair and Lindstrom 2000; Mouritsen 2013)
Costaria costata	fully sheltered to fully exposed (Mumford 2007)	prostrate	annual or perennial (O'Clair and Lindstrom 2000)	2 m (O'Clair and Lindstrom 2000)	opportunistic; disturbed areas; phys-iology depends on wave action; may gain warm water tolerance (Dayton 1985; Muth et al. 2019)	*Food:* palatable, but low caloric value *Medicine:* anti-inflammatory; thalassotherapy *Industry:* previous source of potash salts (O'Clair and Lindstrom 2000; Blasco 2012; Mouritsen 2013)
Cymathaere triplicata	moderately exposed (Mumford 2007)	prostrate	annual (Mondragon and Mondragon 2010)	4 m (Mondragon and Mondragon 2010)	tattered by July (Mondragon and Mondragon 2010)	*Food:* generally considered unpalatable (Druehl and Clarkson 2016)
Hedophyllum sessile	moderate to fully exposed (O'Clair and Lindstrom 2000)	prostrate	perennial (2-3 yr.) (O'Clair and Lindstrom 2000)	150 cm (O'Clair and Lindstrom 2000)	competes poorly with other kelps; sea urchins avoid but chiton graze heavily; wave action determines growth form (Mondragon and Mondragon 2010)	*Food:* palatable, but low caloric value *Nutrition:* source of trace minerals, complex carbohydrate and saccharides (O'Clair and Lindstrom 2000)
Laminaria setchellii	moderately sheltered to fully exposed (O'Clair and Lindstrom 2000)	stipitate	perennial (up to 14 yr.) (Germann 1986)	1.5 m (O'Clair and Lindstrom 2000)	possible gain in warm water tolerance (Muth et al. 2019)	*Food:* kombu substitute (Jungwirth 2019)
Lessoniopsis littoralis	extremely exposed habitats, heavy surf (O'Clair and Lindstrom 2000)	stipitate	perennial (O'Clair and Lindstrom 2000; Mondragon and	1-2 m (O'Clair and Lindstrom 2000; Mondragon and	wide holdfast (Mondragon and Mondragon 2010)	*Nutrition:* source of trace minerals and complex carbohydrates (Druehl and Clarkson 2016)

			Mondragon 2010)	Mondragon 2010)		
Macrocystis pyrifera	fully exposed; wave action with 2-4 cm/s current speed enhances N uptake (Schiel and Foster 2006; Mumford 2007)	floating	perennial (O'Clair and Lindstrom 2000)	up to 30 m (Dayton 1985)	adapted for exploitative competition for light/nutrients; susceptible to wave stress and grazing disturbance; possible gain in warm water tolerance (Dayton 1985; Muth et al. 2019)	*Nutrition:* source of many vitamins and minerals; low in tannins *Industry:* major source of alginate (O'Clair and Lindstrom 2000; Mondragon and Mondragon 2010)
Nereocystis luetkeana	fully sheltered to fully exposed; withstands strong currents (Mumford 2007; O'Clair and Lindstrom 2000)	floating	annual (Dayton 1985; O'Clair and Lindstrom 2000)	up to 36 m (Dayton 1985; O'Clair and Lindstrom 2000)	opportunistic; frequent disturbed areas; major source of carbon in inshore intertidal communities; sensitive to grazing; possible loss in warm water tolerance; vulnerable to exposure to petroleum products (Dayton 1985; O'Clair and Lindstrom 2000; Muth et al. 2019)	*Food:* consumed pickled, or as a component of salsas, chips, soups, and other foods *Nutrition:* source of iodine *Medicine:* mucilage may help treat burns *Industry:* feed for mussels, filter feeders (O'Clair and Lindstrom 2000, Mondragon and Mondragon 2010; Mouritsen 2013)
Pleurophycus gardneri	strong currents (O'Clair and Lindstrom 2000)	prostrate	perennial, deciduous (3-6 yr.) (O'Clair and Lindstrom 2000; Pfister and Betcher 2018)	1 m (O'Clair and Lindstrom 2000)	where amphipod infestation occurs, warmer water temperatures may increase resilience to parasitism (Pfister and Betcher 2018)	*Food:* potential kombu substitute; low caloric value (Druehl 1980; O'Clair and Lindstrom 2000)
Postelsia palmaeformis	fully exposed; adapted to extreme wave shock (O'Clair and Lindstrom 2000; Mondragon and Mondragon 2010)	stipitate	annual (O'Clair and Lindstrom 2000)	60 cm (O'Clair and Lindstrom 2000)	forms dense stands; competes for space with mussels; possible loss of warm water tolerance (O'Clair and Lindstrom 2000; Muth et al. 2019)	*Food:* eaten pickled, steamed or fresh *Nutrition:* source of fiber (Mondragon and Mondragon 2010; Mouritsen 2013)

Pterygophora californica	fully sheltered to moderately exposed, strong currents (Mumford 2007)	stipitate	perennial; (13-25 yr.) (Wantanabe et al. 1992; Germann 2011)	<3 m (Mondragon and Mondragon 2010)	long-lived, slow-growing; may form dense stands; adapted to physical stress (wave action, surface exposure) (Dayton 1985; Mondragon and Mondragon 2010)	*Art:* stipes used by basket makers (Druehl and Clarkson 2016)
Saccharina latissima	fully sheltered to fully exposed (O'Clair and Lindstrom 2000)	prostrate	perennial or annual (O'Clair and Lindstrom 2000)	up to 3.5 m (O'Clair and Lindstrom 2000)	Dusky Tegula snail and sea urchins are common grazers; young sporophytes sensitive to high light levels (O'Clair and Lindstrom 2000)	*Food:* commonly used in Asian cuisine; kombu/haidai substitute *Nutrition:* source of vitamin C, iodine, potassium, and calcium *Industry:* potential use for bioremediation (Ahn et al. 1998; O'Clair and Lindstrom 2000; Bruhn et al. 2016)

(Continued)

Scientific Name	Cultivation Method(s)[6]	Sorus Harvest[7]	Outplanting[7]
Alaria marginata	*Source:* wild sorus harvesting, sexual reproduction *Culture:* culture tubes in nursery aquaria *Farming:* sporophytes outplanted to floating long-lines (5 m depth) (Blasco 2012). Blades also have been grown in aerated tanks	fall (Oct.) (Blasco 2012)	
Costaria costata	*Source:* wild sorus harvesting, sexual reproduction *Culture:* culture tubes in nursery aquaria *Farming:* sporophytes outplanted to floating long-lines (5 m depth) or floating raft; co-culture with *N. luetkeana* (Fu et al. 2010; Blasco 2012)	from summer (Jun.) through late fall (Dec.), peak in Sept. (Maxell and Miller 1996; Blasco 2012)	

Cymathaere triplicata			
Hedophyllum sessile			
Laminaria setchellii			
Lessoniopsis littoralis			
Macrocystis pyrifera	*Source:* wild sorus harvesting (parent stock must be adapted to farm site exposure levels), sexual or clonal reproduction *Culture:* inoculation lines in nursery aquaria *Farming:* sporophytes outplanted to floating long-line (1.5-5 m depth, depending on season and epiphyte pressure); direct (free floating inoculation line) or indirect (nylon rope seeding); co-culture with P. californica; avoid siting near high Fe and NH_4^+ levels (O'Clair and Lindstrom 2000; Gutierrez et al. 2006; Schiel and Foster 2006; Westermeier et al. 2006; Macchiavello et al. 2010)	summer (Gutierrez et al. 2006; Macchiavello et al. 2010)	winter outplanting more successful than summer (Gutierrez et al. 2006; Macchiavello et al. 2010)
Nereocystis luetkeana	*Source:* wild sorus harvesting, sexual reproduction *Culture:* culture tubes in nursery aquaria *Farming:* sporophytes directly transplanted to benthic substrate (e.g. rebar stakes); co-culture with C. costata and S. latissima; siting near eelgrass beds may increase habitat benefits (Merrill and Gillingham 1991; Maxell and Miller 1996; O'Clair and Linstrom 2000; Carney et al. 2005; Olson et al. 2019; Calloway 2020)	late spring (May) through fall (Nov.), with peak production in late summer (Sept.) (Maxell and Miller 1996)	winter (Dec.-Feb.) (Calloway 2020)
Pleurophycus gardneri	*Source:* wild sporophyte harvesting *Farming:* sporophytes transplanted to floating long-lines (2.5 m depth) (Germann 2011)	spring (Mar.) through fall (Oct./Nov.) (Germann 2011)	
Postelsia palmaeformis	*Source:* wild sorus harvesting	spores develop in late spring/early summer	spores develop in late spring and summer

	Farming: sori transplanted to cleared rocky substrate bounded by mussels; could be combined with intertidal mussel cultivation (Thompson et al. 2010; Druehl and Clarkson 2016; Calloway 2020)	(O'Clair and Lindstrom 2000; Thompson et al. 2010)	(O'Clair and Lindstrom 2000; Thompson et al. 2010)
Pterygophora californica	*Farming:* co-culture with M. pyrifera, but may struggle to compete for light (Wantanabe et al. 1992)		
Saccharina latissima	*Source:* wild sorus harvesting, sexual reproduction *Culture:* culture tubes in nursery aquaria *Farming:* sporophytes outplanted to floating long-lines (5 m depth); co-culture with N. luetkeana; farm in exposed or semi-exposed areas to minimize biofouling (Blasco 2012; Peteiro and Freire 2013; Bruhn et al. 2016; PSRF 2019)	fall (Oct.) (Blasco 2012)	winter (Jan.) (PSRF 2019)

(Continued)

Scientific Name	Peak Growth[7]	Sporophyte Harvest[7]	(Wild) Harvesting Recommendations[8]
Alaria marginata	no discernable peak growth period (Blasco 2012)	late spring (Jun), to prevent grazing from snails (Blasco 2012)	cut vegetative blades no closer than 4" from the base; leave reproductive sporophylls intact (O'Clair and Lindstrom 2000; Jungwirth 2019)
Costaria costata	spring (Mar.-May) (Maxell and Miller 1996; Blasco 2012)	depending on grazing pressure and tattering, late spring (May) or mid-summer (June/July) (Maxell and Miller 1996; O'Clair and Lindstrom 2000; Blasco 2012)	
Cymathaere triplicata		late spring, after maximal blade development but before tattering (O'Clair and Lindstrom 2000)	
Hedophyllum sessile			cut fronds no closer than 6" from holdfast (Jungwirth 2019)

Laminaria setchellii			cut blades no closer than 2" from the base (Jungwirth 2019)
Lessoniopsis littoralis			harvest no more than 10% of individual plant (Jungwirth 2019)
Macrocystis pyrifera	depends on outplanting time (perennial species) (Gutierrez et al. 2006; Macchiavello et al. 2010)	late spring, before biofouling becomes a problem (Gutierrez et al. 2006)	
Nereocystis luetkeana	peak density and stipe growth in early summer (June); maximum blade growth in late summer (Aug./Sept.) (Maxell and Miller 1996)	summer, to avoid epiphytes and blade erosion; fall (Oct.) to allow for re-growth (Maxell and Miller 1996; O'Clair and Lindstrom 2000; Luning and Mortensen 2015)	only distal ends of blades (>12 from bulb) should be removed if continued growth is desired (O'Clair and Lindstrom 2000; Luning and Mortensen 2015; Jungwirth 2019)
Pleurophycus gardneri	rapid growth in winter (Dec.) until peak growth rate is reached in late spring (May) (Germann 2011)	balding/senescence occurs in summer (June) and fall (Oct.) before regrowth (Germann 2011)	breaks at the frond above the abscission zone are non-lethal; individuals live 3-6 yr (O'Clair and Lindstrom 2000; Pfister and Betcher 2018)
Postelsia palmaeformis	late winter/early spring (Thompson et al. 2010)	spring (Apr.-June) before spore production begins (Thompson et al. 2010)	cut blades at least 2" from base, leaving 1-3" of grooved blade for regrowth (Druehl and Clarkson 2016; Jungwirth 2019)
Pterygophora californica			
Saccharina latissima	winter and spring; highest density reached in late spring (May-June) (Maxell and Miller 1996; Blasco 2012)	spring (Apr.-May), to reduce loss to blade erosion, ephiphytes, and grazers; timing affects the proportion of sugars, proteins, minerals (Blasco 2012; Peterio and Freire 2013; Luning and Mortensen 2015; Bruhn et al. 2016; Sharma et al. 2018)	boiling after harvest can reduce iodine levels, which may be needed for use as food (Luning and Mortensen 2015)

[1] Geographic distribution may need to be updated. Abbreviations: SJDF = Strait of Juan de Fuca, SJI = San Juan Islands, PS = Puget Sound, OC = Outer Coast (Gabrielson et al. 2006; Mumford 2007)

[2] The same species may have different temperature tolerances between regions. Recruitment refers to sporophyte production.

[3] Temperature is more limiting than nutrients (Muth et al 2019). Optimum refers to maximum sporophyte density.
[4] Very little species-specific information is available, but these are still crucial environmental factors to consider for sporophyte success With the lack of numerical values, one might infer this information by observing nearby and use (for the possibility of sediment and freshwater influx), local currents/water flow, and the species' canopy form and depth.
[5] With the exception of a few commonly cultivated species, it is difficult to find species-specific human uses. Generally speaking, most kelp species can be put to the following uses: food, nutrition, medicine, art, and industry (food additive, animal feed, fertilizer, biofuel, etc.) (Tiwari and Troy 2015).
[6] Some species have not yet been commercially cultivated. When available, literature on restoration mariculture and experimental transplants was consulted for species that have commercial potential.
[7] Limited species-specific information, especially for species that are not commercially cultivated.
[8] These are recommendations for wild harvesting, that world apply if and only if a self-perpetuating farm is desired and/or permitted (as might be the case with restorative mariculture). Concerns about preserving wild genetic population diversity might, conversely, require complete adult sporophyte removal prior to sorus/spore production