Received: 7 July 2020 | Revised: 28 October 2020 | Accepted: 15 December 2020

DOI: 10.1111/jwas.12765

APPLIED STUDIES

Mariculture research of *Macrocystis pyrifera* and *Saccharina latissima* in Southeast Alaska

Michael S. Stekoll[1,2] | Tamsen N. Peeples[2] | Ann E. T. Raymond[2]

[1]Department of Natural Sciences, University of Alaska Southeast, Juneau, Alaska

[2]College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Juneau, Alaska

Correspondence
Michael S. Stekoll, Juneau Center, College of Fisheries and Ocean Sciences, 17101 Point Lena Loop Road, Juneau, AK 99801.
Email: msstekoll@alaska.edu

Funding information
Advanced Research Projects Agency - Energy, Grant/Award Number: DE-AR0000911; Alaska Department of Commerce and Economic Development, Grant/Award Number: RSA EN 890012; Alaska Sea Grant, University of Alaska Fairbanks, Grant/Award Number: RR/86-01; Japan Overseas Fisheries Cooperation Foundation; National Coastal Resources Research and Development Institute (NCRI), Grant/Award Number: 2-5168-13; National Sea Grant, Grant/Award Number: NA18OAR4170078; Premium Oceanic

Abstract

There has been increasing interest in Alaska regarding the commercial mariculture of kelp. Kelp farming can be an economic engine for coastal communities of Alaska. Other benefits include ecosystem services, including carbon sequestration and mitigation of eutrophication. In support of this interest, several kelp species have been examined for commercial potential. In the 1980s–1990s, experiments were performed on the giant kelp *Macrocystis pyrifera*. Female gametophytes were exposed to varying levels of chelated iron. Relatively low levels of chelated iron (1–5 μM) stimulated the onset of oogenesis. In contrast, higher iron concentrations inhibited egg production. Outplant experiments with *Macrocystis* in Sitka, AK showed growth in the winter and spring, slowing down to zero growth by the end of summer. Fertilizing outplants in August allowed plants to survive and grow during the ensuing winter. Mariculture experiments with *Saccharina latissima* carried out near Juneau, Alaska showed exponential growth for seeded lines set out from September to March. Optimal growth occurred for outplants in October–November, with growth rates of up to 5% per day. The best growth occurred when lines were 2–3 m below the surface. Growth rates declined in May–June corresponding to a decrease in inorganic nitrogen in the water. Slower growth also resulted in severe fouling.

KEYWORDS
Alaska, aquaculture, gametogenesis, gametophyte, iron, kelp

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *Journal of the World Aquaculture Society* published by Wiley Periodicals LLC on behalf of World Aquaculture Society.

1 | INTRODUCTION

Although global seaweed mariculture has a long history with a more or less steady increase in value over the last few decades, with production in 2018 over 30 million tons worth about 13 billion USD (FAO, 2020), the commercial culture of seaweeds in the United States has only recently been active (Kim, Stekoll, & Yarish, 2019). In Alaska, for several years, there have been commercial harvests of natural beds of seaweeds, mostly for the herring spawn-on-kelp market but also for other products such as plant fertilizer supplements and various bull kelp (*Nereocystis luetkeana* [K.Mertens] Postels & Ruprecht) products for human consumption (Stekoll, 2019). It is only in the last few years that any commercial mariculture production of seaweeds in Alaska has occurred (ADF&G, 2020). To date, production in Alaska has focused on the species *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders; *Alaria marginata* Postels & Ruprecht; and *Nereocystis luetkeana*.

The first published research on the mariculture of kelps in Alaska was carried out with *Laminaria groenlandica* (*Saccharina groenlandica*) by placing ropes along the bottom of the ocean in Auke Bay, AK, with plants naturally recruited from nearby beds (Ellis & Calvin, 1981). Subsequent research on kelps was conducted on the mariculture of the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh (as *Macrocystis integrifolia*) for use in the herring spawn-on-kelp fishery in Alaska (Stekoll, 1989; Stekoll & Else, 1990) and the research that is reported here.

Currently, the lack of understanding of the optimization of kelp cultivation is one bottleneck that impedes industry growth in Alaska, partially because of the unique species present and also because the oceanography is different from other geographic areas where kelp is already produced through mariculture practices. Gaps in knowledge exist in both the hatchery production of seed and also in the cultivation of sporophytes on the ocean farm. Here, we present applied mariculture research conducted on the kelps *Macrocystis pyrifera* in the 1990s and *Saccharina latissima* in 2015–2018. The aim of this research is to provide key information to help optimize the developing kelp mariculture industry, focusing on hatchery practices utilizing iron and optimizing outplant depth and outplant dates of sporophytes in the ocean farm setting.

In the 1980s and 1990s, a series of experiments was conducted on *Macrocystis pyrifera* to examine iron as a tool for hatchery seeding and to examine optimal sporophyte-growing conditions in an ocean farm setting. An important aspect of kelp mariculture is the ability to control the timing of the development of the organism. In kelp mariculture, one way to do this is to control when the gametophytes become fertile and produce young sporelings. It has been reported that growing Laminariaceae gametophytes in red light (Lüning, 1980) or without iron in the media (Motomura & Sakai, 1981) will inhibit gametogenesis. Here, we report on how iron affects oogenesis in *Macrocystis*.

Some of this research has been previously reported (Stekoll, 1989; Stekoll & Else, 1990), along with work on other species (Raymond & Stekoll, 2021). Here, we give the results of additional research on *Macrocystis* that was performed in the 1990s. Later, in 2015–2019, experiments were conducted on *Saccharina latissima* to examine the optimization of sporophyte growth in an ocean farm setting. We present the results from both species to provide a framework for industry members to optimize hatchery operations and ocean farm practices with the aim of expediting economic prosperity for the mariculture industry of Alaska and providing additional ecosystem services to coastal communities associated with kelp mariculture.

2 | MATERIALS AND METHODS

2.1 | Hatchery optimization: Iron and oogenesis in *Macrocystis pyrifera* gametophytes

To evaluate the effect of iron on gametogenesis in female gametophytes, we carried out experiments on a single female clone of *Macrocystis pyrifera* in a medium with and without added iron. *Macrocystis* spores were obtained from sporophylls collected from Sitka, Alaska in 1983 and sent by air to the University of California at Santa Barbara. Single spores were isolated, and a single female gametophyte strain (designated as Mi-T-19) was selected for long-term culture. Culture media were either modified Provasoli's enriched seawater (Provasoli, 1968) or enriched artificial seawater (EASW) (Harrison, Waters, & Taylor, 1980). Provasoli enrichment solution was modified by the addition of

iodine at a level of 1.4 mg/L in the stock solution, by the elimination of iron and vitamins, and—in some cases—by the omission of the Tris buffer. The artificial seawater was enriched with the same enrichment solution used in the enriched natural seawater. Fe-EDTA solutions were made using ferric (iron III) ammonium sulfate and disodium ethylenediaminetetraacetic acid (EDTA) in a 1:1 M ratio. Stock solutions of Fe-EDTA were made at 0.1 mg Fe/ml (1.8 mM). All iron concentrations are those used in the initial formulation. For each set of experiments, the following general procedure was used. Bulk cultures of *Macrocystis* gametophytes were grown in EASW with no added iron at 15°C under cool white fluorescent lighting (16:8 L:D photoperiod) at an irradiance of 40–60 μmol m^{-2} s^{-1}. Every 2–3 weeks, the cultures were harvested and subcultured into fresh media. At the beginning of each of the experiments, a growing culture of female *M. pyrifera* was filtered through a 160-μ mesh Nitex screen. The filtered plant tufts were rinsed several times with sterile artificial seawater. Portions of the washed tufts were placed in a sterile, Dounce-type, all-glass hand homogenizer with 15 ml of cold, artificial seawater. The suspension was fragmented and filtered through a 95-μ screen onto a 43-μ Nitex screen. The residue was rinsed and resuspended in artificial seawater and adjusted to a concentration of about 10,000 fragments/ml using a hemocytometer. The plant fragments consisted of only three to five cells each. About 30 ml of the plant fragment suspension were poured over a precleaned 10 cm × 10 cm × 15 mm deep, square plastic Petri dish containing nine plastic cover slips (22 mm). All plastic ware were cleaned by soaking for at least 24 hr in 6 N HCl, followed by several rinses in sterile artificial seawater. The plant fragments in suspension were allowed to settle on the plastic cover slips under cool white fluorescent lighting (40–60 μmol m^{-2} s^{-1}, 16:8 hr (L:D)) at 15°C for 48 hr. After the fragments had settled and adhered to the cover slips, they were removed with sterile, plastic forceps and rinsed with sterile artificial seawater to remove unattached plants. Each cover slip was then placed face up in a sterile 15 × 60-mm round plastic Petri dish containing 12 ml of enriched seawater or ESAW, with or without the addition of varying amounts of Fe-EDTA. The experimental dishes with cover slips were incubated under the same conditions as above but were placed on a moving platform, which oscillated at 32 cycles per minute. The growth and egg production of plant fragments were measured by examining the cover slips in the experimental dishes using a Unitron inverted phase-contrast microscope. Egg production was measured by counting the proportion of plant fragments with extruded eggs. Gametophyte growth was measured by counting the increase in the number of cells per plant fragment over the experimental time period. For growth measurements, all plant fragments were taken initially from the same batch of *Macrocystis* (Mi-T-19) and averaged 3.65 (SD = 2.0, *N* = 100) cells/plant fragment.

2.2 | Optimization of *Macrocystis pyrifera* sporophyte growth on an ocean farm

To examine the effect of depth and timing on the growth of *Macrocystis pyrifera*, several outplantings were set out in Whiting Harbor, Sitka, AK (57.051697°N, 135.373653°W) in 1988–1990. Detailed methods for this study have been published previously (Stekoll & Else, 1990). The study reported here lasted 2 years longer than that of Stekoll and Else (1990). The cool, dried sporophylls collected from a natural bed in Whiting Harbor were immersed in seawater at temperatures of 10–13°C and placed near an artificial light source at 100 μmoles m^{-2} s^{-1}. The resulting spore solution was filtered through fine nylon mesh (30 μ in diameter) to remove mucus. The spore density was determined by use of a hemocytometer and was diluted to a concentration of about 5,000–10,000 spores/ml. The solution was then poured over a polyvinyl chloride (PVC) frame wrapped with previously boiled *Kuremona* string. Each PVC frame held about 20–30 m of *Kuremona*. The wrapped PVC frame was maintained in the spore solution in the dark at about 10–13°C for 24 hr. About 5 L of enriched sterile seawater were added to transparent containers holding the PVC frames. The enrichment solution was designated as PESI and was modified from that of Provasoli (1968). Modifications included the omission of all vitamins and the addition of iodine (see above). The seeded string was incubated for 6 weeks at 10–13°C, under 70–80 μmol m^{-2} s^{-1}, with a photoperiod of 16:8 hr (L:D). Media changes were performed every 7–9 days.

Outplants were deployed in Whiting Harbor on vertical lines and one longline. The 50-m longline was deployed only once in February 1989 to simulate normal farming practices. It was set at 3 m below mean of the lower low

waters (MLLW). Seeded strings 4–6 cm in length were inserted into the weave of the longline at 1-m intervals. For timing of outplants, each vertical line structure was anchored with a concrete block and suspended with a submerged float. To place kelp plants upon the vertical structures, 4–6-cm sections of seeded *Kuremona* string were inserted into the 1 cm-diameter braid of a 50-cm length of rope cable tied into a ring and attached just above the float at 3 m below MLLW. Thirty of these rings were deployed each month. Vertical lines were randomly placed on a grid layout set on the ocean floor. For the depth studies, we used dropper lines with seeded *Kuremona* strings attached at 0.5-m intervals from 0.5 to 9.5 m below MLLW. For all of the outplantings, growth was followed by measuring the length of the plants underwater. Initially, the outplanted sporelings were about 0.3–0.5 cm long. Final biomass yield (kg/m) of the *Macrocystis* plants was not assessed because the goal of this project was to grow large bladed plants for the herring spawn-on-kelp fishery that was occurring at the time in Prince William Sound.

2.3 | Fertilizing *Macrocystis* sporophytes on an ocean farm

Results of the outplantings of *Macrocystis* throughout the year showed that most outplants did not successfully overwinter. An experiment was devised to determine if nutrient limitation in late summer was a possible cause. A longline, seeded with sporelings obtained from sporophylls gathered from plants in Whiting Harbor, was set at 3 m below MLLW with healthy plants of about 225–270 cm frond lengths. For this trial, about 200 g of a slow-release solid fertilizer (Osmocote N-P-K, 19-6-12, the Scott's Company) was placed in nylon sacks tied to one half of the longline and placed between plant sections. The other half of the longline served as an unfertilized control. Plant numbers and frond lengths were monitored over the fall and winter.

2.4 | Optimal time of outplanting *Saccharina latissima* seed on an ocean farm

For the experiments involving *Saccharina latissima*, spores were obtained from parent plants collected nearshore near Juneau, AK. Spores were released from cleaned, dried, fertile sorus sections by immersion in sterile seawater at 12°C as described previously (Redmond, Green, Yarish, Kim, & Neefus, 2014; Stekoll & Else, 1990). All solutions were made using reagent-grade chemicals dissolved in freshly made Q water (Milli-Q water system).

To examine the optimal outplanting month of *Saccharina latissima*, seeded *Kuremona* strings of *Saccharina* were wound around rope longlines 15 m in length. Outplants were placed on a monthly basis from October 2015 to March 2016 and from October to December 2016. Lines were set at 2 m below the surface parallel to the shore on the northwest coast of Coghlan Island (58.356600°N, 134.705363°W) near Juneau, AK. Approximately every 2 weeks, growth was assessed by measuring the longest frond/blade at every meter along the lines, starting at a random point near one end of the line. Plants were also assessed for condition by noting fouling or bleaching. A HOBO® temperature and light logger was placed at 2 m below the surface. Oceanographic samples for light irradiance, temperature, and salinity were taken from the surface to 10 m deep. Water samples for NOx (nitrate + nitrite) and phosphate were collected with a Niskin-type bottle at depths of 0, 2, and 5 m. Water samples were kept on ice until filtered through 0.22-μ filters and were frozen for later analysis. Phosphate was assessed by the Determination of phosphate procedure in Parsons, Maita, and Lalli (1984). Total NOx was assessed either by the cadmium reduction procedure (Parsons et al., 1984) or by using a commercial nitrate reductase assay (Nitrate Reductase [AtNaR], NECi Superior Enzymes).

2.5 | Optimal depth of outplanting *Saccharina latissima* seed on an ocean farm

To assess the optimal depth for outplanting *Saccharina latissima*, vertical dropper lines were assembled with a length of line anchored to the bottom and suspended with a surface buoy. Seeded strings of *Saccharina* were attached to

the vertical line every 0.5 m from the surface to a depth of 6 m. A weight was attached to the line below the 6-m mark to keep the line vertical in the water column. The dropper lines were placed along the shore at the Coghlan Island site. Dropper lines were outplanted each month from September 2016 to April 2017. Periodic measurements of the plant growth were made by measuring the length of the three longest plants at each depth.

3 | RESULTS

3.1 | Hatchery optimization: Iron and oogenesis in *Macrocystis pyrifera* gametophytes

A test comparison of female *Macrocystis pyrifera* gametophytes grown in enriched seawater and in EASW showed no differences in growth rates or gross morphology. All experiments described in this section were performed using EASW as the growth medium. Fe-EDTA added to the growth medium had definite effects on the rate at which the female *Macrocystis* gametophyte strain became fertile. The female plant fragments averaged about 3.2 cells per fragment at the beginning of the experiments. No fragments were found to be fertile for at least 4 days after the addition of Fe-EDTA to the growth media. After the fourth day, there was an approximately linear increase in the percentage of plant fragments that extruded eggs (Figure 1). The rate at which the fragments became fertile was much faster in the treatment receiving Fe-EDTA at 9 μM than in the other two treatments (0 and 35 μM). Treatments receiving either no added Fe-EDTA or 35 μM of Fe-EDTA showed increases in fertility in the order of 3–4% per day after Day 4, whereas the 9-μM treatment had increases of better than 16% per day in fertile plant fragments. Statistical analysis of the slopes of the regression lines indicates no differences between the 0- and 35-μM treatments, but the slopes of both treatments are significantly lower than the slope of the 9-μM treatment ($Z > 11$, $p << .001$).

The stimulation–inhibition of the formation of fertile plant fragments by Fe-EDTA was investigated further by measuring the proportion of plant fragments that were fertile after 7 days of exposure to varying concentrations of Fe-EDTA. It is clear from the data presented (Figure 2) that relatively low concentrations of added Fe-EDTA

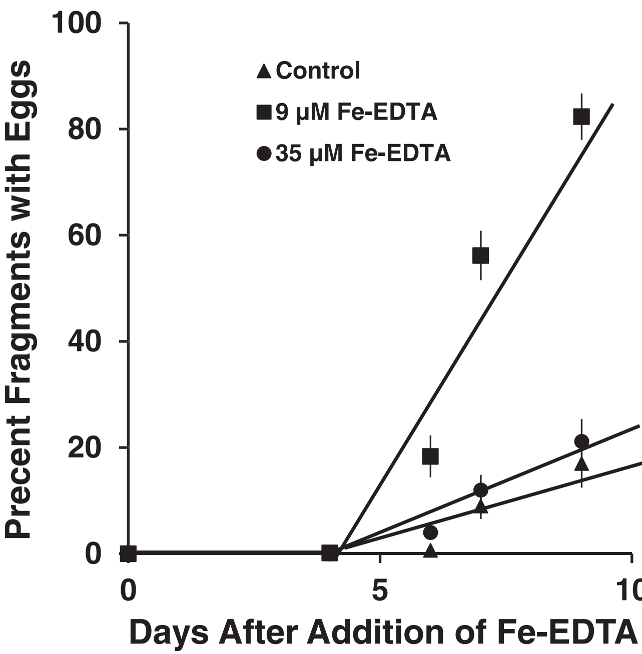


FIGURE 1 Rate of formation of oogonia in a *Macrocystis pyrifera* gametophyte exposed to varying levels of Fe-EDTA. Fe-EDTA was added at Day 0. All plant fragments are clones of strain Mi-T-19. Each point represents the mean of six datasets. Each dataset contains 100 counted plant fragments. Error bars indicate one standard deviation

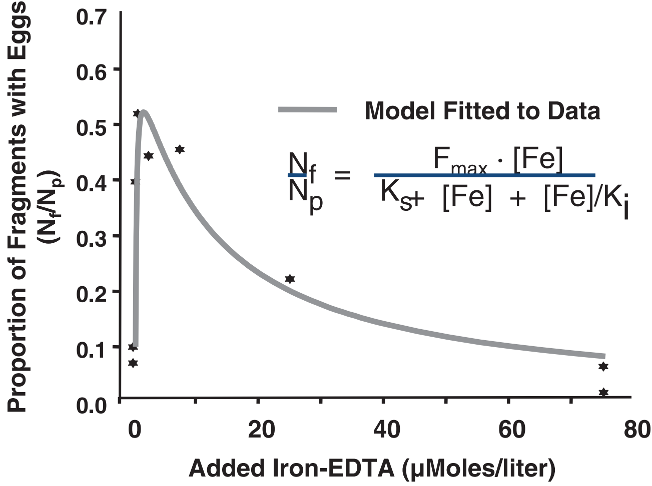


FIGURE 2 Effect of 7 days of exposure to varying concentrations of Fe-EDTA on the proportion of *Macrocystis pyrifera* gametophyte fragments forming eggs. The solid line represents the model fitted to the data. The lines were fitted to the data points using nonlinear least-square analysis. Each data point represents counts on all viable plants on a cover slip. Actual numbers of plants vary from 94 to 153 plant fragments per point. The amount of iron found in the usual formulation of Provasoli's enrichment is 9 μM. A concentration of 35 μM was the amount found to be optimal in the inducement of Laminarian gametophytes (Motomura & Sakai, 1984)

stimulated egg production but that higher levels inhibited egg production. An iron-binding model based on an enzyme kinetic model of substrate inhibition was the best fit for the data:

$$\frac{N_f}{N_p} = \frac{F \cdot [Fe]}{K_s + [Fe] + [Fe]^2/K_1}$$

where N_f is the number of plants in the fertile state, and N_p is the number of plants in the prefertile state or the number of plants at the beginning of the experiment. The quantity N_f/N_p is the proportion of fertile plants at any one time. F is related to the maximum proportion of plants that could be fertile. K_s is a combined constant. K_I is the equilibrium constant for iron inhibition.

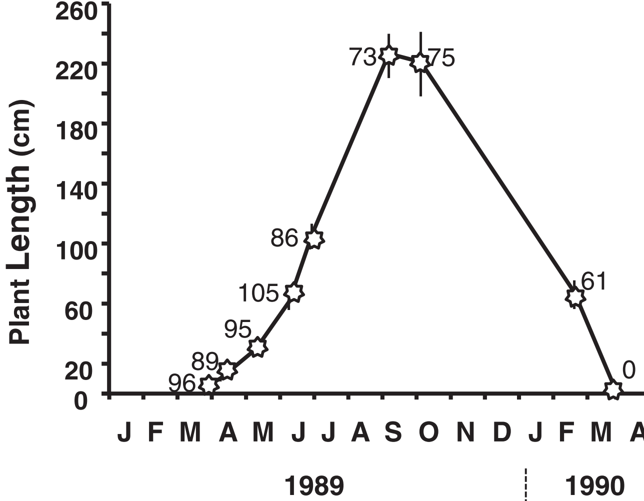
Growth rates of the plant fragments were similar in all of the treatments (data not shown). Combining all of the data from all treatments gave a linear growth model for the increase in cell number per plant fragment of 0.68 cells/day. At the end of the 9 days, the average plant fragment had increased its size by about threefold to 9.4 cells.

3.2 | Optimization of *Macrocystis pyrifera* sporophyte growth on an ocean farm

Juvenile cultured *Macrocystis pyrifera* plants were outplanted approximately every month from November 1988 to March 1990 adjacent to a natural bed located near Sitka, Alaska. There was no or very little growth on the longlines outplanted in November, December, and January. Growth on the longline outplanted in February reached a length of over 2 m by the middle of September but declined over the winter to a point where all plants were lost by the following March (Figure 3).

For the vertical lines, growth of outplants was found to be dependent on both the depth and the time of outplanting. Most outplants except a February outplant grew until the late summer, and then, growth slowed or stopped. The best growth occurred between February and August (Figures 4 and 5). Some of the February 1989

FIGURE 3 Growth (±95% CI) of *Macrocystis pyrifera* outplanted on a 50-m longline in Sitka, Alaska. Cultured germlings were outplanted in March 1989 and maintained at a constant 3 m below MLLW throughout the year. The graph shows the mean overall plant lengths. The numbers near each point are the number of plants measured at that time. By March of 1990, all of the plants had disappeared from this longline



outplants at 3 m beneath MLLW reached sizes of approximately 3 m and 5-kg wet weight by August 1989, but only two of these plants initiated new growth after the subsequent winter. The maximum growth rate of 3.8% per day achieved by this outplanting in the first 2 months of their growth was less than the maximum of 5–7% per day achieved by the April to July outplants (Figure 5). However, the latter outplantings were unable to attain nearly the size of the earliest outplants (Figure 4). Except for the February outplant, growth dropped rapidly for all outplantings after August or September (Figure 4). As with the plants on the longline, mortality was very high during the winter. Significant growth did not occur again until April 1990. This growth persisted until October 1990 for most plants when plants exhibited a decline through the winter, similar to that of 1989.

The optimum depth for initial growth occurred near the surface, especially when outplanted in April and March. As the plants grew later in the year, growth was better at deeper depths (Figure 6).

3.3 | Fertilizing *Macrocystis* sporophytes on an ocean farm

Fertilization experiments conducted in the late summer of 1990 suggested that the application of fertilizer to juvenile plants during this season promoted growth and survival (Figure 7). All of the unfertilized plants disappeared by the end of winter. In contrast, 40% of the fertilized plants survived over the winter, and those that did showed increased growth of nearly 300%.

3.4 | Optimal time of outplanting *Saccharina latissima* seed on an ocean farm

Growth of *Saccharina* was exponential during the first months (Figure 8). Growth rates of 3–5%/d were attained. Even so, the final "harvestable" length varied with the outplant month (Figure 9). The final length of the plants was longest with outplants in October–November (Figure 9). Unlike *Macrocystis* in Sitka, *Saccharina* in Juneau grew well through the winter when outplanted in the fall or the winter. All outplants showed slowed growth in May–June, with blades becoming fouled with organisms such as bryozoans and diatoms.

Fresh weight yields of *Saccharina latissima* were determined in late April 2016 and varied with the time of outplant. The maximum biomass of 10.4 kg/m occurred with the November 2015 outplant. The lowest yield was 1.8 kg/m from the outplanting in January 2016. The mean of the yields from four monthly outplantings of October 2015 to January 2016 was 4.74 ± 1.95 kg/m.

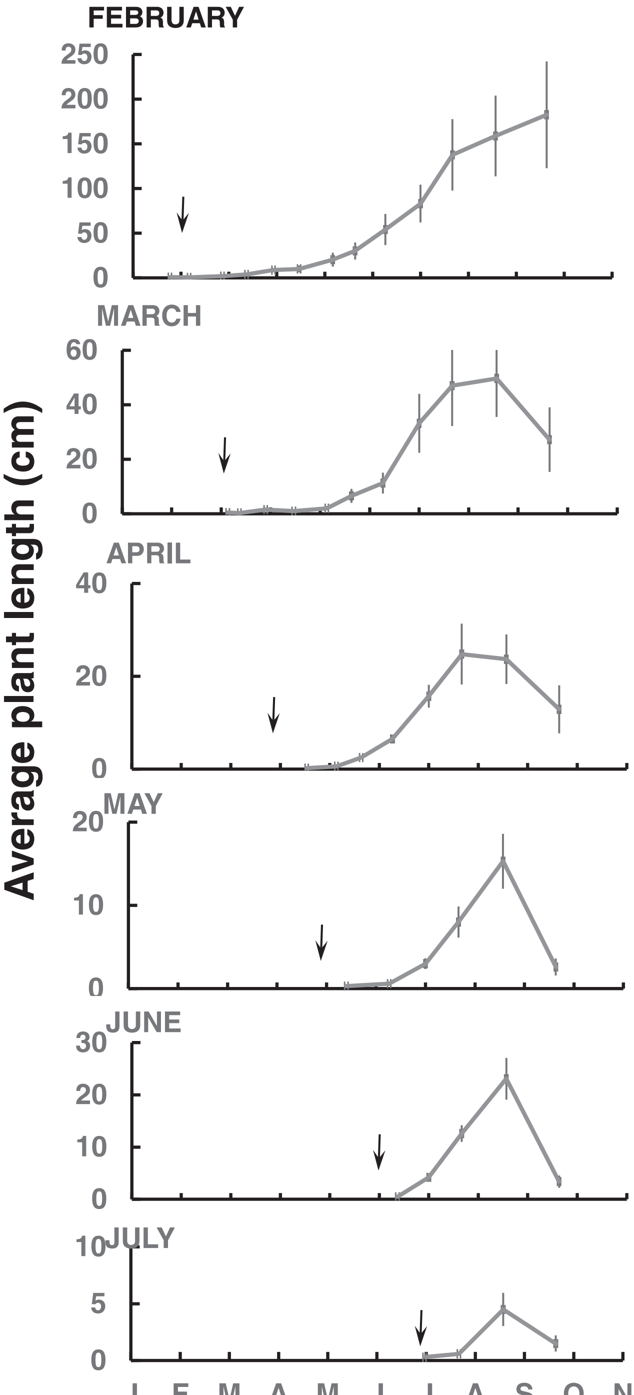


FIGURE 4 Average growth (±95% CI) of outplanted *Macrocystis pyrifera* on vertical lines set at 3 m below MLLW in Whiting harbor, Sitka, AK in 1989. The month of the outplanting is shown in the upper left hand corner of each graph. The arrows indicate the outplant date

Water temperatures at the 2-m depth ranged from 4°C in the late winter to 15°C in late summer (data not shown). NOx and phosphate were elevated in the late fall and winter and dropped quickly during the plankton bloom in April (Figure 10). Salinity at the 2-m depth peaked at 31 psu in the winter, dropping to less than 17 psu in September (data not shown).

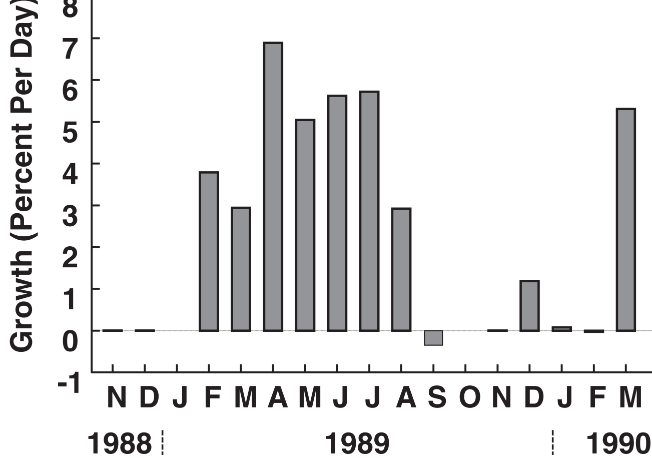
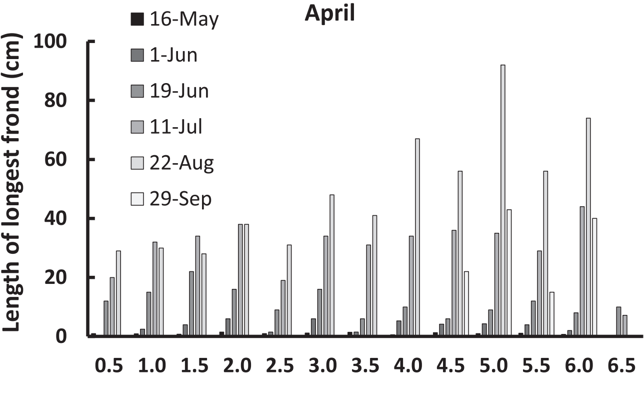


FIGURE 5 Initial growth rates of *Macrocystis pyrifera* as a function of the time of outplanting. These growth rates were determined from the slope of the log-transformed growth curves for each outplanting. Outplantings were not performed in January or October of 1989



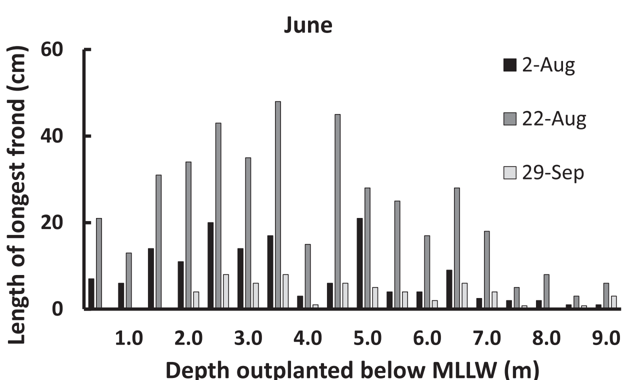


FIGURE 6 Growth of cultured *Macrocystis pyrifera* outplanted April 28 and June 22, 1989 on dropper lines at 0.5–9.5 m below MLLW. Lengths are means of the longest fronds at each outplanting site. Dates are dates of measurements

3.5 | Optimal depth of outplanting *Saccharina latissima* seed on an ocean farm

In most cases, the best growth on the dropper lines was initially near the surface (Figure 11), but by the end of the growing season, the best depth for outplanting seeded strings of *Saccharina latissima* was 2–3 m below the surface

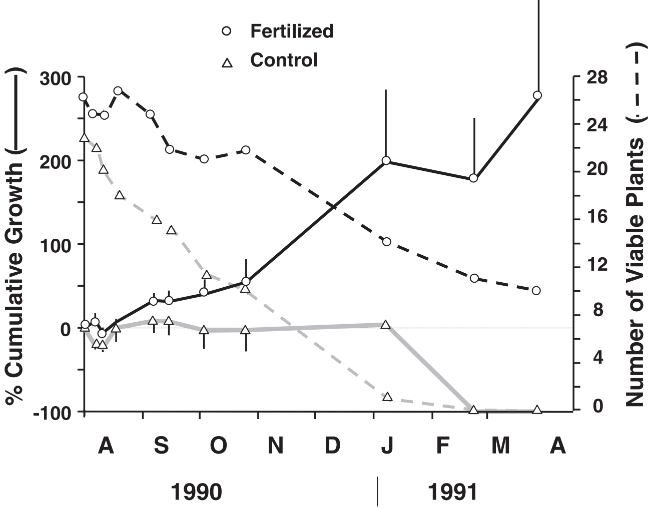


FIGURE 7 Effect of fertilization on the growth of *Macrocystis pyrifera*. Growth was significantly enhanced for outplants fertilized in August of 1990. These plants had been outplanted on longlines in the spring and were about 60 cm in mean length at the time of fertilizer application. Vertical bars represent one-half of the 95% CI. Δ: no added fertilizer; ◦: fertilizer added

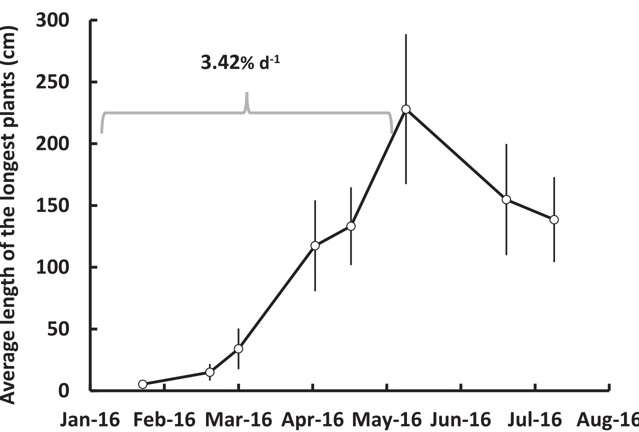


FIGURE 8 Growth of *Saccharina latissima* (±95% CI) outplanted in November 2015. Bracket shows the period of exponential growth. After May, growth slowed or stopped, and blades became fouled with epiphytes

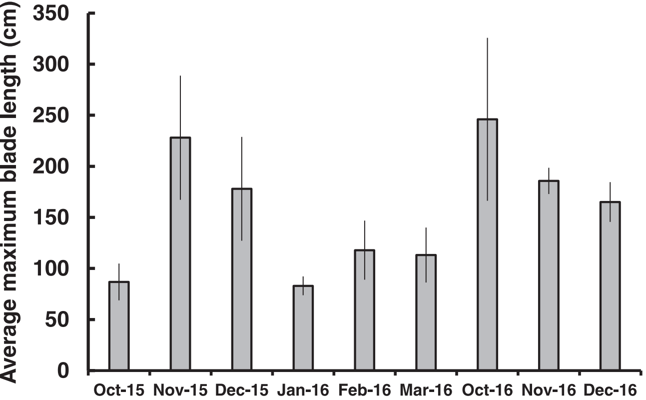


FIGURE 9 Growth of *Saccharina latissima* (±SE) outplanted near Coghlan Island at the indicated month—measured in the following May at the peak of growth prior to fouling

(Figure 12). The dropper lines also reinforced the timing of outplants. Outplant months in the fall yielded the longest plants at the end of the growing season. Dropper lines outplanted in late spring and summer showed little or no growth at depths from near the surface to 6 m below.

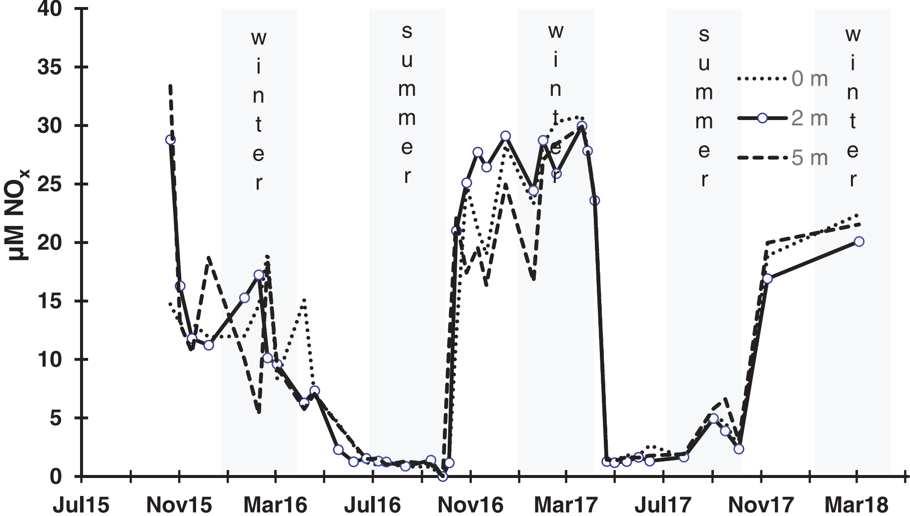
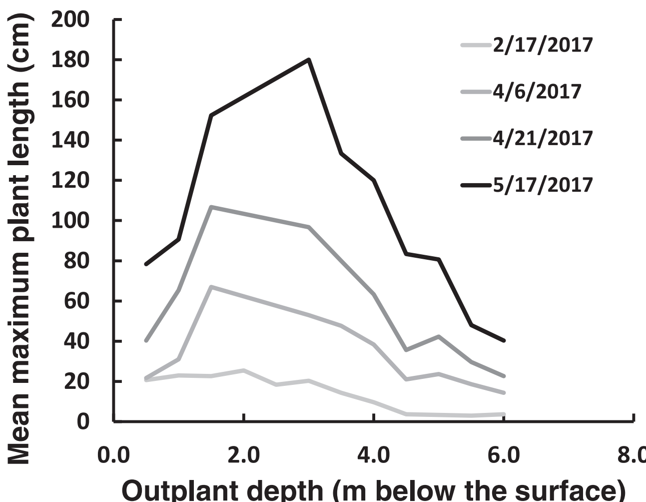


FIGURE 10 NOx (nitrate + nitrite) concentrations at Coghlan Is, Juneau, AK measured at 0, 2, and 5 m below the surface. The drop in NOx begins to occur in mid-April, coinciding with the spring phytoplankton bloom

FIGURE 11 *Saccharina latissima* growth on a dropper line outplanted in September 2016. Points are the average of the three longest blades at each depth. Dates are the days measurements were taken



4 | DISCUSSION

4.1 | Hatchery optimization: Iron and oogenesis in *Macrocystis pyrifera* gametophytes

Added chelated iron, the most common form of iron used to culture marine algae, had a stimulatory effect on egg production in *Macrocystis pyrifera* gametophytes. Plants exposed to higher levels of iron were inhibited in their ability to produce eggs but were still able to grow by means of cell division. These results replicate the published work of others on kelp gametophytes and added iron (Kuwabara & North, 1980; Lewis, Green, & Afzal, 2013; Motomura & Sakai, 1981, 1984; Suzuki, Kuma, & Matsunaga, 1994). Our model for the iron effect assumes saturation uptake kinetics, leading to a pattern indicative of substrate inhibition (Cornish-Bowden, 1979). The model predicts that the optimal iron concentration for egg production after 7 days' exposure to Fe-EDTA is about 1.3 μM. In addition the

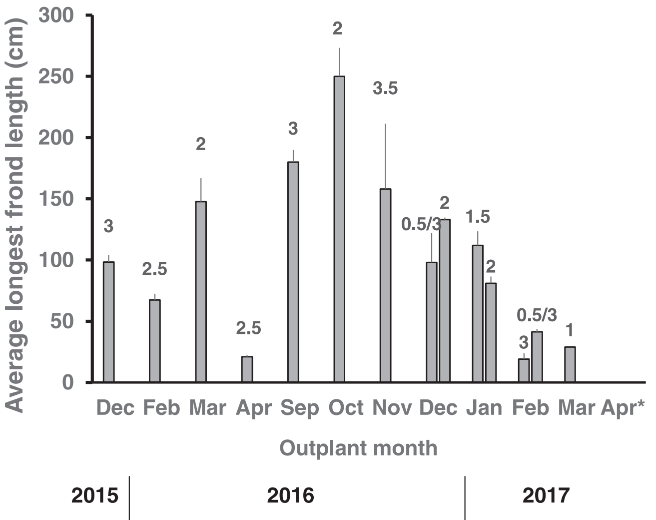


FIGURE 12 *Saccharina latissima* growth on dropper lines by month of outplanting (2015–2017). Averages (+SE) are of the three longest plants at each depth. The numbers above the bars are the depth in meters where growth was maximal at the end of the growing season (May–June). Two numbers above a bar indicate that maximal growth occurred at two depths. There were duplicate droppers in December 2016 and January, and February 2017. The data for the March 2017 dropper is from one plant. *Outplants in April and later did not have any measurable growth at any depth

model predicts that the iron concentration in the treatment with no added iron is 0.027 μM. These numbers are similar to those reported for *Laminaria japonica* in Suzuki et al. (1994) but are about 3.7 times higher than those reported for *Macrocystis* using Aquil as the growth medium (Kuwabara & North, 1980). Although Kuwabara (1980) did not investigate inhibitory levels, Motomura and Sakai (1984) found that Fe-EDTA at about 88 μM (5.0 mg/L) inhibited egg formation by about 20%. Similar levels of Fe-EDTA in the present study caused almost complete inhibition of egg formation.

Lüning (1980) proposed that two developmental pathways are available for germinating spores in *Laminaria* (*Saccharina*) species. In this scheme, the total quanta of blue light is the trigger that controls gametogenesis or vegetative development. The results presented here and by others (Lewis et al., 2013; Motomura & Sakai, 1981, 1984; Suzuki et al., 1994) modify this scheme by incorporating iron concentration into the control of development. A gametophyte will develop gametes only if sufficient blue light and the correct concentration of iron are available. If the light quanta received are below a certain threshold, or if the iron concentration is too low or too high, the spores will develop into vegetative, multicellular gametophytes. When environmental conditions are favorable at a later time, gametogenesis will be triggered.

These results have practical implications for kelp mariculture operations. If natural spore release occurs too early relative to the optimal time of outplanting, it may be necessary to delay gametogenesis so that sporelings will be ready at the desired time. Another reason to delay gametogenesis or to keep gametophytes for long periods of time is an alternative way to produce young juvenile kelp blades. Gametophyte clones whose genes promote faster growth, larger plants, or better-quality plants for the commercial markets can be selected. The ability to store these clones will not only allow for breeding of select blades but will also ensure a continuous supply of seed and will provide for a seed stock free of contamination (Redmond et al., 2014). When one is ready to produce the desired sporophytes, selected male and female gametophytes can be combined in media with iron or under white light conditions to induce gametogenesis. These results have been replicated in the lab for other kelp species, including *Nereocystis luetkeana*, *Alaria marginata*, and *Agarum clathratum* Dumortier. Removing iron from the gametophyte culture media is an easy and cost-effective method for long-term storage of kelp gametophytes.

4.2 | Optimization of *Macrocystis pyrifera* sporophyte growth on an ocean farm

Timing of outplanting of kelp species is an important consideration for any commercial mariculture enterprise. The end goal is usually the production of optimal biomass per unit line length and/or optimal quality for the intended

uses. Because *Macrocystis* in the Sitka, AK area is fertile almost every month of the year, farmers have the ability to outplant at any time. The results from our Sitka outplantings would indicate that the best time for outplanting *Macrocystis* in this area would be when the water temperature is 4–5°C in February, which—although it will not have the fastest initial growth rate—will achieve the longest length by the end of the first growing season in late summer, giving the plants a better chance to survive through the winter.

There are many factors that can determine the growth of *Macrocystis* in an aquaculture setting. Variability in site location, latitude, oceanographic parameters, presence/absence of herbivory, and season are some of the considerations necessary for optimal timing and depth of outplants. This has been found for *Macrocystis* aquaculture attempts in Chile (Camus, Infante, & Buschmann, 2018), China (Liu et al., 1984), and the United States (Kim et al., 2019).

4.3 | Fertilizing *Macrocystis* sporophytes on an ocean farm

The light compensation point for *Macrocystis pyrifera* in California has been reported to be between 0.4 and 0.7 mol m^{-2} d^{-1} (Dean & Jacobsen, 1984). In Sitka, AK, during November through January, there are, on average, about 7 hr of daylight each day. An irradiance of 0.7 mol m^{-2} d^{-1} would be, on average, about 28 μmol m^{-2} s^{-1}. At a depth of 2 m, over 90% of the light is absorbed by the water column in Sitka in the winter (data not shown). Although we did not measure irradiance on a continual basis, our data show that light levels were often below 30 μmol m^{-2} s^{-1} measured at 2–5 m below the surface at mid-day during the winter. We conclude that if the light compensation point for *Macrocystis pyrifera* in Sitka is similar to that in California, then plants in Sitka may be light limited during the shortest days in the winter. During these times, the plants would need to rely on stored reserves to stay alive. If the low nutrients combined with high water temperatures and lower salinity in the late summer are too stressful, the plants will not have enough reserves to survive the dark winter. This would explain why adding fertilizer to the plants in August might increase the viability of the fronds enough to get through until light levels increase later in the winter.

4.4 | Optimal time of outplanting *Saccharina latissima* seed on an ocean farm

Unlike the outplanting results for *Macrocystis pyrifera*, outplants of *Saccharina latissima* near Juneau had measurable growth when placed in the water in the fall, and they grew well through the winter, reaching maximal length in May/June. However, growth stopped at that point, and the blades became fouled with epiphytes such as diatoms and bryozoans. We had no success in getting outplants to last over the summer. This was a bit different than the growth of *Macrocystis*, whose growth peaked in mid-summer. *Saccharina latissima* also did well when outplanted in the winter and through to March. Initial growth was similar to other outplant times, but the final length was shorter because, no matter when the plants were set out, growth ceased at about the same time. The plants slowed or ceased growing about a month or so after the spring bloom.

The average biomass yield reported here of about 5 kg/m is somewhat low when compared to other studies. The published fresh weight yields of *Saccharina latissima* from mariculture operations have ranged from 0.5 kg/m (Bruhn et al., 2016) to over 24 kg/m (Kim et al., 2019), with about 10–15 kg/m being a rough average. Yields from farms in the Faroe Islands varied from 5.2 ± 0.4 kg/m to 9.9 ± 1.5 kg/m depending on the wave exposure of the sites (Mols-Mortensen, Ortind, Jacobsen, & Holdt, 2017). Yields from farms in Spain situated at exposed and sheltered sites were 16 and 12 kg/m, respectively (Peteiro & Freire, 2013). High yields of 24 kg/m were reported for *S. latissima* after 6 months (December to May) of growth in Long Island Sound and New York estuaries (Kim et al., 2019).

Work with *Laminaria longicruris* (*Saccharina longicruris)* found that the nitrogen pool in the blades was depleted following the disappearance of the external NOx with a lag period of up to 2 months (Chapman & Craigie, 1977; Egan & Yarish, 1990). *Saccharina latissima* (as *Laminaria saccharina)* under conditions of low external nitrogen relied

on internal reserves of NO_3 and organic compounds, but the nitrogen reserves were depleted rapidly with reduced growth after 3 months (Korb & Gerard, 2000). In the northern section of southeastern Alaska, the spring bloom of phytoplankton occurs around the middle of April concomitant with a rapid drop in NOx. Thus, we would expect that *S. latissima* would deplete its internal reserves of nitrogen and slow or cease growth in May–June, depending on the state of its nitrogen reserves. The conclusion from a mariculture standpoint is to outplant *S. latissima* in the fall to coincide with the increase in nutrients in the water that usually occurs in late October to early November. Earlier outplantings can be successful, but we have encountered more opportunistic recruits of unwanted kelps plus massive settlements of invertebrates with these early outplantings. We recommend harvest in May in order to have the largest plants with the best quality, that is, fewest epiphytes and/or grazing by snails.

4.5 | Optimal depth of outplanting *Saccharina latissima* seed on an ocean farm

Most of the experiments with the dropper lines showed that depths of outplants work best at 2–3 m below the surface, and that is the suggestion we make for the mariculture of *Saccharina latissima* in the area of the Gulf of Alaska. That is the range that current farmers in Alaska have used. Others have recommended outplanting depths of 1–2 m (Flavin, Flavin, & Flahive, 2013; Kim, Kraemer, & Yarish, 2015; Redmond et al., 2014), but good growth can occur as deep as 8 m depending on the water quality and season (Handa et al., 2013). At our site, initial growth was the best near the surface (0.5 m), but later, the plants at depths of 2–3 m outgrew the near-surface plants. A strategy to obtain bigger plants would be to outplant near the surface and drop the plants to 2–3 m in February as daylight increases. In our experience, *Saccharina latissima* in Alaska does not develop a hollow stipe and, therefore, remains negatively buoyant throughout the growing season. In fact, to keep the lines at the desired depth requires maintaining the tension on the longlines or using buoys attached to the longlines. Because oceanic conditions vary from site to site, it is recommended that dropper lines be deployed to determine the optimal timing and depth for outplanting *Saccharina latissima* and other kelp species.

4.6 | Recent research in Alaska

In the late 1970s, as a result of the so-called energy crises, there was a concerted effort to grow *Macrocystis* in large-scale offshore structures in southern California (e.g., Flowers and Bird 1984). The idea was to produce biogas or other energy from a renewable resource. For various reasons, this endeavor ended with no commercial production (Kim et al., 2019). Recently, there has been renewed interest in generating energy from offshore kelp farms. The United States Department of Energy has a program, Macroalgae Research Inspiring Novel Energy Resources (MARINER), under their ARPA-E division (https://arpa-e.energy.gov/?q=arpa-e-programs/mariner). The University of Alaska under MARINER is currently conducting research with the title "Scalable Coastal and Offshore Macroalgal Farming" (https://arpa-e.energy.gov/?q=slick-sheet-project/scalable-coastal-and-offshore-macroalgal-farming), looking at the potential for large-scale, offshore farming of *Saccharina latissima* for generating biomass for energy production. The goal is to design and test systems that will produce biomass at a cost that would be competitive with fossil fuels. This project, which includes the University of Connecticut, Woods Hole Oceanographic Institute, the Marine Biological Laboratory, the Alaska Fisheries Development Foundation, Goudey & Associates, GreenWave, and Blue Evolution, deployed its first farm array in Kodiak, AK in the fall of 2019 with harvest in the following June (2020).

5 | CONCLUSIONS

Withholding iron from the culture medium has been a successful method to maintain kelp gametophytes from several species for several years without them becoming fertile. In most cases, refragmenting the gametophytes and

using a normal culture medium restores gamete formation, allowing farmers to both time outplants to their needs and to have a route for trait selection.

Predicting the success of kelp mariculture in Alaska or elsewhere is problematical because of the natural variability of parameters in the ocean, by location, by season, by year, etc. Of the greatest importance are the availability of nutrients, especially nitrogen, and the range of water temperatures. Site selection and farm deployment should be augmented by using dropper lines to assess seasonality and optimal depths. Critical oceanographic measurements will aid in the consideration of site suitability. Moving kelp farming to offshore locations will present a host of new issues for kelp mariculture. Studies such as those funded by ARPA-E will help to determine the success of such operations.

ACKNOWLEDGMENTS

This work was supported in part by sabbatical funds from the University of Alaska and by the Alaska Sea Grant College Program (RR/86-01); by grants from Premium Oceanic (dba Blue Evolution); by research sponsored by Alaska Sea Grant with funds from the National Oceanic and Atmospheric Administration Office of Sea Grant, Department of Commerce, under Grant No. NA18OAR4170078 (Project No. R/40-09); and from the University of Alaska with funds appropriated by the state, the Japan Overseas Fisheries Cooperation Foundation, the Alaska Department of Commerce and Economic Development (RSA EN 890012), and the National Coastal Resources Research and Development Institute (Contract No. 2-5168-13). Additional funding was provided by the ARPA-E MARINER Program (Macroalgae Research Inspiring Novel Energy Resources) under Grant No. DE-AR0000911.

ORCID

Michael S. Stekoll https://orcid.org/0000-0001-9089-7162
Tamsen N. Peeples https://orcid.org/0000-0001-6474-1201
Ann E. T. Raymond https://orcid.org/0000-0002-1354-0976

REFERENCES

ADF&G (Alaska Department of Fish and Game). (2020). Aquatic farming. Aquatic plants production data. Retrieved October 6, 2020 from https://www.adfg.alaska.gov/index.cfm?adfg=fishingaquaticfarming.aquaticfarminfo_aquaticplants.

Bruhn, A., Tørring, D. B., Thomsen, M., Canal-Vergés, P., Nielsen, M. M., Rasmussen, M. B., … Petersen, J. K. (2016). Impact of environmental conditions on biomass yield, quality, and bio-mitigation capacity of *Saccharina latissima*. *Aquaculture Environment Interactions*, *8*, 619–636. https://doi.org/10.3354/aei00200

Camus, C. I., Infante, J., & Buschmann, A. H. (2018). Overview of 3 year precommercial seafarming of *Macrocystis pyrifera* along the Chilean coast. *Reviews in Aquaculture*, *10*, 543–559. https://doi.org/10.1111/raq.12185

Chapman, A. R. O., & Craigie, J. S. (1977). Seasonal growth in *Laminaria longicruris*: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology*, *40*, 197–205. https://doi.org/10.1007/BF00390875

Cornish-Bowden, A. (1979). *Fundamentals of enzyme kinetics*. London: Butterworth & Co (Publishers) Ltd.

Dean, T. A., & Jacobsen, F. R. (1984). Growth of juvenile *Macrocystis pyrifera* (Laminariales) in relation to environmental factors. *Marine Biology*, *83*, 301–311. https://doi.org/10.1007/BF00397463

Egan, B., & Yarish, C. (1990). Productivity and life history of *Laminaria longicruris* de la Pyl. at its southern limit in the Western Atlantic Ocean. *Marine Ecology Progress Series*, *67*, 263–273.

Ellis, R. J., & Calvin, N. I. (1981). Rope culture of the kelp *Laminaria groenlandica* in Alaska. *Marine Fisheries Review*, *43*(2), 19–21.

FAO. (2020). Fishery statistical collections. Global Aquaculture Production. Food and Agriculture Organization of the United Nations. Retrieved June 15, 2020 from http://www.fao.org/fishery/statistics/global-aquaculture-production/en.

Flavin, K., Flavin, N., & Flahive, B. (2013). Kelp farming manual a guide to the processes, techniques, and equipment for farming Kelp in New England Waters. Ocean Approved, Portland, ME. Retrieved from https://static1.squarespace.com/static/52f23e95e4b0a96c7b53ad7c/t/52f78b0de4b0374e6a0a4da8/1391954701750/OceanApproved_KelpManualLowRez.pdf.

Handa, A., Forbord, S., Wang, X., Broch, O. J., Dahle, S. W., Størseth, T. R., … Skjermo, J. (2013). Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. *Aquaculture*, *414–415*, 191–201. https://doi.org/10.1016/j.aquaculture.2013.08.006

Harrison, P. J., Waters, R. E., & Taylor, F. J. R. (1980). A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *Journal of Phycology*, *16*, 26–35. https://doi.org/10.1111/j.1529-8817.1980.tb00724.x

Kim, J. K., Kraemer, G. P., & Yarish, C. (2015). Sugar kelp aquaculture in Long Island sound and the Bronx River estuary for nutrient bioextraction and ecosystem services. *Marine Ecology Progress Series*, *531*, 155–166. https://doi.org/10.3354/meps11331

Kim, J. K., Stekoll, M. S., & Yarish, C. (2019). Opportunities, challenges and future directions of open-water seaweed aquaculture in the United States. *Phycologia*, *58*, 446–461. https://doi.org/10.1080/00318884.2019.1625611

Korb, R. E., & Gerard, V. A. (2000). Effects of concurrent low temperature and low nitrogen supply on polar and temperate seaweeds. *Marine Ecology Progress Series*, *198*, 73–82. https://doi.org/10.3354/meps198073

Kuwabara, J. S. (1980). Micronutrient requirements for *Macrocystis pyrifera* (L.) C. A. Agardh (giant kelp) gametophytes determined by means of a chemically defined medium, Aquil. Ph.D. Thesis, California Institute of Technology. Pasadena, CA. 185 pp. https://doi.org/10.7907/ECSY-FZ64. https://resolver.caltech.edu/CaltechETD:etd-12122006-091318

Kuwabara, J. S., & North, W. J. (1980). Culturing microscopic stages of *Macrocystis pyrifera* (Phaeophyta) in Aquil. A chemically defined medium. *Journal of Phycology*, *16*, 546–549. https://doi.org/10.1111/j.1529-8817.1980.tb03072.x

Lewis, R. J., Green, M. K., & Afzal, M. E. (2013). Effects of chelated iron on oogenesis and vegetative growth of kelp gametophytes (Phaeophyceae). *Phycological Research*, *61*, 46–51. https://doi.org/10.1111/j.1440-1835.2012.00667.x

Liu, T., Suo, R., Liu, X., Hu, D., Shi, Z., Liu, G., … Wang, F. (1984). Studies on the artificial cultivation and propagation of giant kelp (*Macrocystís pyrífera*). *Hydrobiologia*, *116*(117), 259–262.

Lüning, K. (1980). Critical levels of light and temperature regulating the gametogenesis of three *Laminaria* species (Phaeophyceae). *Journal of Phycology*, *16*, 1–15. https://doi.org/10.1111/j.1529-8817.1980.tb02992.x

Mols-Mortensen, A., Ortind, E. G., Jacobsen, C., & Holdt, S. L. (2017). Variation in growth, yield and protein concentration in *Saccharina latissima* Laminariales, (Phaeophyceae) cultivated with different wave and current exposures in The Faroe Islands. *Journal of Applied Phycology*, *29*, 2277–2286. https://doi.org/10.1007/s10811-017-1169-4

Motomura, T., & Sakai, Y. (1981). Effect of chelated iron in culture media on oogenesis in *Laminaria angustata*. *Bulletin of the Japanese Society of Scientific Fisheries*, *47*(12), 1535–1540.

Motomura, T., & Sakai, Y. (1984). Regulation of gametogenesis of *Laminaria* and *Desmarestia* (Phaeophyta) by iron and boron. *Japanese Journal of Phycology*, *32*, 209–215.

Parsons, T. R., Maita, Y., & Lalli, C. M. (1984). *A manual of chemical and biological methods for seawater analysis*, Oxford: Pergamon Press.

Peteiro, C., & Freire, O. (2013). Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in a coastal bay in the Atlantic coast of Spain. *Journal of Applied Phycology*, *25*, 205–213. https://doi.org/10.1007/s10811-012-9854-9

Provasoli, L. (1968). Media and prospects for the cultivation of marine algae. In A. Watanabe & A. Hattori (Eds.), *Cultures and collections of algae. Proc. U.S. - Japan Conf. Hakone, September 1966* (pp. 63–75). Hakone: Jap. Soc. Plant Physiol.

Raymond, A. E. T., & Stekoll, M. S. (2021). Mariculture of *Saccharina latissima* and *Alaria marginata*: A method for staggering and delaying kelp outplantings by modifying nursery conditions. *Journal of the World Aquaculture Society (this Publication).*

Redmond, S., Green, L., Yarish, C., Kim, J., & Neefus, C. (2014). New England Seaweed Culture Handbook-Nursery Systems. Connecticut Sea Grant CTSG-14-01. 92 p. Retrived from https://seagrant.uconn.edu/2014/01/01/new-england-seaweed-culture-handbook-nursery-systems/

Stekoll, M. S. (1989). Mariculture of the kelp, *Macrocystis*, in Southeast Alaska. *The Northwest Environmental Journal*, *5*, 141–143.

Stekoll, M. S. (2019). Seaweed resources of Alaska. *Botanica Marina*, *62*, 227–235. https://doi.org/10.1515/bot-2018-0064

Stekoll, M. S., & Else, P. V. (1990). Cultivation of *Macrocystis integrifolia* (Laminariales, Phaeophyta) in southeastern Alaskan waters. *Hydrobiologia*, *204*(205), 445–451. https://doi.org/10.1007/BF00040269

Suzuki, Y., Kuma, K., & Matsunaga, K. (1994). Effect of iron on oogonium formation, growth rate and pigment synthesis of *Laminaria japonica* (Phaeophyta). *Fisheries Science*, *60*, 373–378. https://doi.org/10.2331/fishsci.60.373

How to cite this article: Stekoll MS, Peeples TN, Raymond AET. Mariculture research of *Macrocystis pyrifera* and *Saccharina latissima* in Southeast Alaska. *J World Aquac Soc*. 2021;52:1031–1046. https://doi.org/10.1111/jwas.12765