Received: 21 September 2023 | Accepted: 7 November 2023

DOI: 10.1111/jpy.13413

METHODS ARTICLE

Kelps on demand: Closed-system protocols for culturing large bull kelp sporophytes for research and restoration

Varoon P. Supratya | **Patrick T. Martone**

Department of Botany and Beaty Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

Correspondence
Varoon P. Supratya, Department of Botany and Beaty Biodiversity Research Centre, University of British Columbia, 6270 University Blvd, Vancouver, British Columbia V6T1Z4, Canada.
Email: varoonp@student.ubc.ca

Funding information
Canada Foundation for Innovation, Grant/Award Number: #27431; Natural Sciences and Engineering Research Council of Canada, Grant/Award Number: #6563 and #RGPIN-2019-06240

Editor: C. Camus

Abstract

Culturing kelps for commercial, conservation, and scientific purposes is becoming increasingly widespread. However, kelp aquaculture methods are typically designed for ocean-based farms, and these methods may not be applicable for smaller scale cultivation efforts common in research and restoration. Growing kelps in closed, recirculating culture systems may address many of these constraints, yet closed system approaches have remained largely undescribed. Extensive declines of the bull kelp (*Nereocystis luetkeana*), an ecologically important canopy species in the Northeast Pacific, have received widespread attention and prompted numerous research and conservation initiatives. Here, we detail two approaches for cultivating *N. luetkeana* sporophytes in closed recirculating systems. *Nereocystis luetkeana* were reared as attached thalli in custom seaweed growth flumes and also free-floating in tumble culture tanks. Careful control of stocking density, water motion, aeration, and nutrient levels allowed for rapid growth and normal morphogenesis of laboratory-grown kelp. Culture systems reached up to 3 kg·m^{-3}, and individual thalli attained lengths of up to 6 m before the trials were terminated. Our results demonstrate the potential of recirculating, closed culture systems to overcome limitations associated with traditional culture methods. Recirculating systems enable the precise control of culture conditions, improving biosecurity and facilitating cultivar development and other research. Kelps can be grown away from the ocean or outside their native ranges, and seasonal or annual species can be produced year-round without seasonal constraints.

KEYWORDS
aquaculture, conservation, growth, kelp, land-based aquaculture, macroalgae, methodology, Nereocystis, *Phaeophyceae*, restoration

INTRODUCTION

Kelps (order Laminariales) are a major component of nearshore, temperate marine communities (Steneck et al., 2002), where they provide habitat for other organisms (Teagle et al., 2017), modify their surrounding environment (Kennelly, 1989; Pfister et al., 2019), and increase coastal primary productivity (Pfister et al., 2019). Kelps are economically valuable, being farmed and harvested for food, livestock feed, and industrial materials (Ferdouse et al., 2018) and providing ecosystem services such as supporting other commercially valuable species (Bennett et al., 2016). At the same time, kelps are increasingly

Abbreviation:: RGR relative growth rate

threatened by anthropogenic activities (Filbee-Dexter & Wernberg, 2018), notably rising ocean temperatures associated with climate change (Smale, 2020). Extensive, anthropogenically driven kelp declines have been documented in many areas of the world (Krumhansl et al., 2016), with severe ecological and socioeconomic consequences for human and ecological communities (Norderhaug et al., 2020; Rogers-Bennett & Catton, 2019).

With increasing global awareness of the ecological value, economic potential, and anthropogenic threats facing kelps, kelp aquaculture for commercial, conservation, and scientific purposes is becoming increasingly widespread (Ebbing et al., 2022; Eger et al., 2022; Kim et al., 2017; Morris et al., 2020). Typically, kelps are first reared in land-based "hatcheries" before being outplanted at a small size (<2 cm) to nearshore ocean farms for "growing out" (Azevedo et al., 2016; Flavin et al., 2013). However, this approach has several important limitations. Ocean-based seaweed farms are subject to the vagaries of the natural environment, including pathogens, herbivores, adverse environmental conditions, or the inherent seasonality of many cultivated species (Hafting et al., 2011; Kim et al., 2017). Furthermore, farms sometimes operate at large scales, which may lead to conflicts with other marine commercial or recreational activities (Flavin et al., 2013; Hafting et al., 2011) as well as environmental impacts on sensitive coastal habitats (Grebe et al., 2019). Finally, the limited biosecurity of ocean-based farms presents a persistent concern over the potential for cultivated kelp to impact natural populations (Cottier-Cook et al., 2016; Goecke et al., 2020; Grebe et al., 2019).

To mitigate the problems of ocean-based farming, attempts have been made to move the cultivation of kelp and other seaweeds onto land (Gadberry et al., 2018; Sato et al., 2017; Swanson & Fox, 2007). Land-based cultivation systems generally rely on the continuous "flow-through" circulation of seawater pumped in from the ocean to replenish nutrients and remove waste products (Schmitz & Kraft, 2022). Although land-based "flow through" kelp cultivation may allow for greater control over culture conditions and reduce the impact of seaweed farming on marine habitats, they require specialized, expensive infrastructure to draw seawater on shore (Hafting et al., 2011). Because of their reliance on a continuous supply of natural seawater, they must be situated on shorefront land, which is expensive and may result in land-use conflicts (Hafting et al., 2011). These limitations are particularly relevant for smaller scale efforts such as kelp research, cultivar development, or restoration, which may require the cultivation of kelp under highly controlled conditions, close monitoring, and limited resources (Hafting et al., 2011).

Growing kelps in closed, recirculating systems may address many issues associated with traditional ocean-based and land-based flow-through approaches (Schmitz & Kraft, 2022; Sebök et al., 2017). Recirculating culture systems have been used to cultivate many microalgae and macroalgae (e.g., Borowitzka, 1999; Friedlander & Levy, 1995), but the cultivation of kelp sporophytes in recirculating systems has remained rare for thalli larger than a few centimeters (Boderskov et al., 2016; Peteiro et al., 2014; Redmond et al., 2014; Westermeier et al., 2006). In recirculating cultures, maintaining the quality of the culture medium without continuously replenishing seawater can be a major challenge (Schmitz & Kraft, 2022). Moreover, the large size and specific environmental demands of kelps also require special consideration (Charters & Neushul, 1979; Winberg et al., 2011). However, if these obstacles can be overcome, recirculating culture of kelp sporophytes could enable even greater control over cultural conditions, permit year-round cultivation of seasonal species, and expand research possibilities at institutions without direct ocean access.

The bull kelp, *Nereocystis luetkeana*, is an ecologically important canopy forming species in the Northeast Pacific (Berry et al., 2021; Schiltroth, 2021). Extensive, climate-driven declines of *N. luetkeana* have received widespread attention and prompted numerous research and conservation initiatives (Beas-Luna et al., 2020; Berry et al., 2021; Carney et al., 2005; Oster et al., 2020; Pfister et al., 2018; Rogers-Bennett & Catton, 2019; Schroeder et al., 2020; Springer et al., 2006). The ability to cultivate *N. luetkeana* in land-based, recirculating systems could facilitate research and conservation efforts, but until now has yet to be successfully accomplished, with previous land-based cultivation trials relying upon flow-through seawater systems (Amsler & Neushul, 1989; Swanson & Fox, 2007). Within the framework of a wider study on *N. luetkeana* ecophysiology, we trialed the cultivation of large (>2 cm) *N. luetkeana* sporophytes in recirculating culture. Here, we describe two successful approaches: (1) attached cultures on ceramic tiles in custom "growth flumes" and (2) freely floating tumble cultures driven by aeration.

MATERIALS AND METHODS

Gametophyte culture

Mature *Nereocystis luetkeana* sori were collected from wild sporophytes and transported to the University of British Columbia (UBC), where the sori were induced to release spores following published protocols (Flavin et al., 2013). In brief, sori were gently scraped with a dull knife to remove macroscopic epibionts, cleaned with a 30-s dip in commercially available 3% povidone-iodine solution, rinsed with chilled autoclaved natural seawater, and then layered between dry paper towels for 24 h in an incubator at 10°C. Cleaned sori were

then induced to release spores by being immersed in autoclaved natural seawater kept at 10°C. The resultant spore solution was diluted to a spore density of 3000 spores · mL^{-1} to accelerate gametogenesis and reduce sporophyte competition (Ebbing et al., 2020; Reed et al., 1991; Tatsumi et al., 2022). The spore solution was poured into 10-L glass aquaria containing autoclaved ceramic tiles and glass cover slips as settlement substrates. Glass cover slips were chosen as a settlement substrate as their transparency facilitated the monitoring of gametophyte development, and their smoothness allowed sporophytes to be scraped off for subsequent tumble culture. On the other hand, ceramic tiles were easy to handle due to their large size and negative buoyancy, and they more closely mimicked a natural substrate conducive to growing larger sporophytes. Additional ceramic tiles and glass cover slips were inoculated separately with gametophyte fragments from 1- to 2-year-old stock cultures maintained under red light to inhibit gametogenesis when sori were not available (e.g., during the winter). Gametophyte biomass from stock cultures was gathered in a 1.5-mL microcentrifuge tube and fragmented with a pellet pestle. The fragments were diluted with autoclaved seawater and distributed over the settlement substrates in the same manner as with fresh spores.

After inoculation, gametophyte fragments and spores were placed in a dark incubator and allowed to settle and germinate on the substrates for 24 h at 10°C, before being transferred to 10-L glass aquaria inside a climate-controlled growth chamber (Conviron). In the aquaria, the gametophytes were cultured under white LED light (16:8 day:night photoperiod) at an irradiance of 15 μmol photons · m^{-2} · s^{-1} for the first 2 days to avoid photoinhibition, at 30 μmol · m^{-2} · s^{-1} for days 2–7, and at 50 μmol · m^{-2} · s^{-1} thereafter (Flavin et al., 2013). The temperature was maintained at 12 ± 2°C based on published *Nereocystis luetkeana* thermal tolerance curves (Supratya et al., 2020). The culture medium initially was autoclaved natural seawater (salinity 32, pH 7.9–8.1) enriched to f/2 medium (Guillard, 1975), but the nutrient concentration was subsequently halved (=f/4) to reduce the growth of competing microorganisms in the culture. The culture medium was fully replaced every 2 weeks, a compromise between promoting rapid gametophyte development and managing the increased risk of culture contamination during media replacement. Under these culture conditions, growing gametophytes were visible to the naked eye as a brown film on the settlement substrates within 1 week, and microscopic sporophytes were detected after 2–3 weeks.

Sporophyte culture

While *Nereocystis luetkeana* gametophytes and young sporophytes up to ~0.5 cm thrived under established kelp culture protocols, further sporophyte development required careful control of specific cultural conditions. Initial attempts to culture sporophytes based on published methods for other kelp species (e.g., Redmond et al., 2014) experienced severe mortality due to thallus necrosis when sporophytes reached ~1 cm in length. As similar patterns of tissue necrosis in open water and flow-through seaweed cultures are known to be caused by opportunistic bacterial infections under stressful conditions (Friedlander & Levy, 1995; Ward et al., 2020), we hypothesized that the tissue degradation observed in our cultures was also due to the overgrowth of opportunistic bacteria. We experimented with published strategies to reduce microbial loading in recirculating algae cultures (Friedlander & Levy, 1995) and identified conditions necessary to maintain *N. luetkeana* in recirculating culture. Nutrient levels were reduced to a quarter (=f/8) or an eighth (=f/16) of the initial full-strength f/2. To minimize the accumulation of particulate matter, metabolic wastes and the depletion of nutrients, the culture medium was fully replaced at least once per week. As high stocking densities appeared to greatly increase thallus necrosis, a low stocking density of ≤3 kg · m^{-3} was accomplished by inspecting and thinning the cultures at least weekly, removing up to half of the culture biomass.

Tumble culture

To produce free-floating sporophytes, cover slips previously settled with spores or gametophyte fragments were scraped with a razor blade when sporophytes became visible. Detached sporophytes and gametophytes were then transferred to non-sterile 26.5-L tumble culture vessels (FerMonster™ homebrewing fermenter; Figure 1a) in which water motion and aeration were provided by custom-made weighted aquarium bubblers. All sporophyte culture vessels were filled with natural seawater (salinity 28–33, pH 7.9–8.1), which was transported to UBC via tanker truck. The irradiance was maintained at 60–80 μmol photons · m^{-2} · s^{-1} (16:8 day:night photoperiod), while the temperature was maintained at 12 ± 0.5°C. Each week, the culture was strained through a quarter-inch (0.64 cm) mesh, and retained thalli (generally 2–5 cm long) were transferred to 600-L cylindrical flat-bottomed fiberglass tanks (Figure 1b). The design of the culture tanks was loosely based on published tank designs for land-based flow through culture (Sato et al., 2017), with a ring-shaped air bubbler surrounding a central drain pipe providing water motion and aeration (Figure 1c; Video S1 in the Supporting Information). The tanks were maintained at 12 ± 1°C by commercially available chillers (Ecoplus 1/4HP), which received water through the central drainpipe before recirculating the water back into the tanks. The irradiance in the large tanks ranged between 30

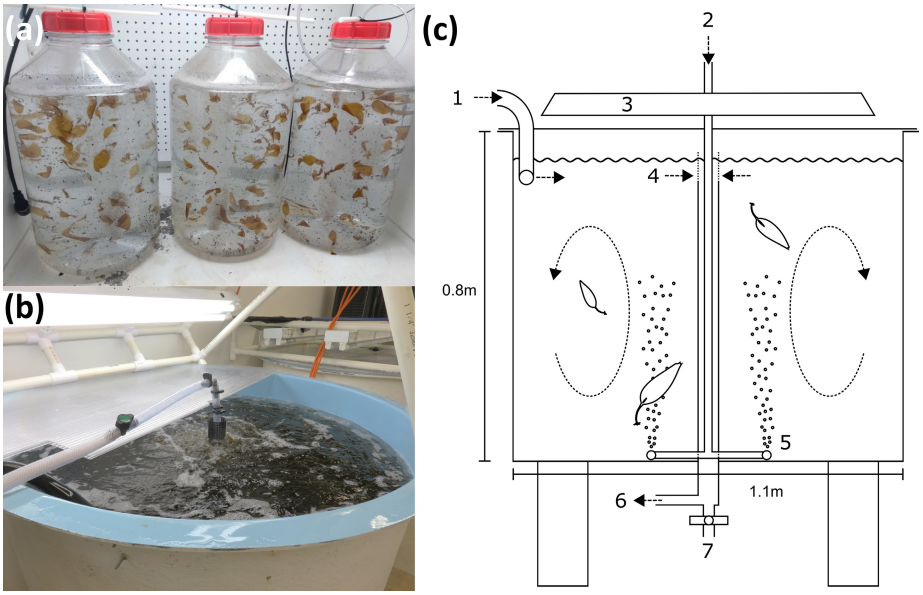

FIGURE 1 Cultivation of *Nereocystis luetkeana* in closed tumble culture. (a) Initial 26.5-L tumble culture vessels with juvenile sporophytes. (b) Six-hundred-L cylindrical tumble culture tank for growing out sporophytes. (c) Schematic view of the 600-L tumble culture tank, including (1) incoming water from chiller unit, (2) pressurized air driving bubblers, (3) light source, (4) central standpipe with mesh drain, (5) air bubbler ring providing aeration and water motion, (6) outflow to chiller unit, and (7) drain valve. Arrows indicate water motion. [Color figure can be viewed at wileyonlinelibrary.com]

and 50 μmol photons · m^{-2} · s^{-1} (16:8 day:night photoperiod), and transparent plastic lids reduced evaporation.

Each week, the length and developmental stage of the 5–10 largest individual sporophytes were recorded. Additionally, the aggregate live biomass in the 600-L tumble tanks was weighed before and after culture maintenance and thinning to calculate the maximum stocking density and daily relative growth rate (RGR). To measure live mass, the culture was netted out and allowed to drip-dry for 20 min in a climate-controlled incubator. The daily RGR was then calculated with the following formula (Gao et al., 2017):

$$\mathrm{RGR}\left(\%\,\mathrm{day}^{-1}\right) = \ln\left(\frac{W_\mathrm{f}}{W_\mathrm{i}}\right) \times \frac{100}{t}$$

where W_i is the initial wet mass, W_f is the final wet mass and t is the number of days between W_i and W_f (in this case, $t = 7$ d).

Attached culture

Ceramic tiles previously settled with gametophytes were left in the original 10-L aquaria to continue growing. When sporophytes became visible to the naked eye, the irradiance was increased to 60–80 μmol photons · m^{-2} · s^{-1} (16:8 day:night photoperiod), and water motion and aeration were provided with an aquarium bubbler from this point onwards. When sporophytes reached 5–10 mm length, the tiles were transferred into custom, programmable "growth flumes" capable of reproducing complex bidirectional water motion, temperature, and light, to emulate natural marine environments (COANDA; Figure 2). The flumes had a capacity of 200 L and were fully recirculating (i.e., not connected to a flow-through seawater system). As we observed that irregular (i.e., non-unidirectional) water flow appeared to be crucial for normal morphogenesis, juvenile sporophytes were grown in a symmetrical oscillating flow regime with a periodicity of ~30 s and a peak flow of 0.2 m · s^{-1}, increased to 0.4 m · s^{-1} when the sporophytes reached 10 cm length and 0.6 m · s^{-1} as the sporophytes reached 0.5 m length (Video S2; Figure S1 in the Supporting Information). Flume cultures were sometimes supplemented with free-floating sporophytes from tumble cultures, which were attached to various substrates using zip ties, by braiding their holdfasts into rope (Westermeier et al., 2006) or by blotting holdfasts dry and attaching them onto tiles with cyanoacrylate glue (Krazy glue® gel). All methods of attachment were successful, and new haptera formed rapidly and naturally attached to the substrate. The irradiance in the growth flumes was initially maintained at 60–80 μmol photons · m^{-2} · s^{-1}, but the irradiance was increased to 120–150 μmol · m^{-2} · s^{-1} when the sporophytes reached 0.5 m length and 250–300 μmol · m^{-2} · s^{-1} when the sporophytes started exceeding 1 m length. As the sporophytes exceeded 0.5 m and outgrew the length of the flume working section, the pneumatocysts were harnessed to one end of the tank to allow the blades and stipe to continue elongating without abrading against the tank walls (Figure S2; Video S2 in the Supporting Information). The harnesses were made of commercially available airline tubing and did not damage the kelp. To accommodate the attachment of the

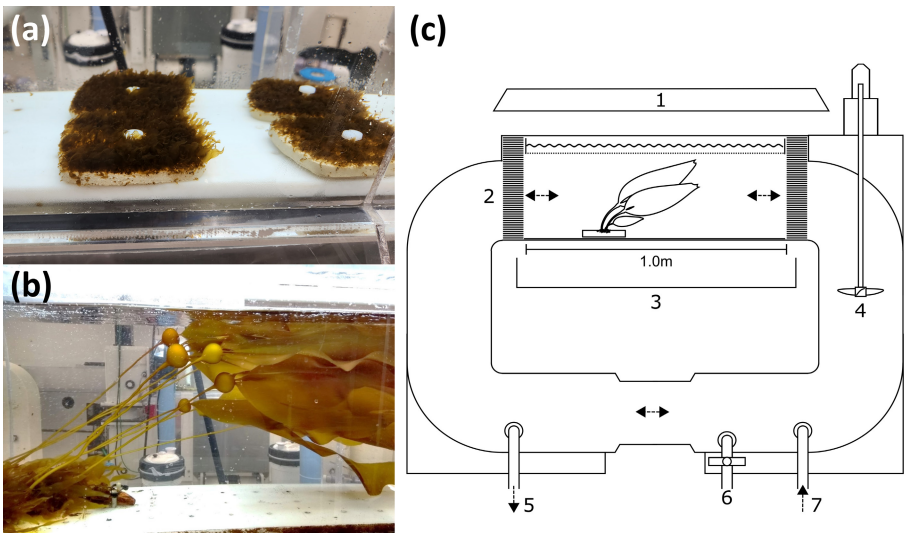

FIGURE 2 Cultivation of *Nereocystis luetkeana* on ceramic tiles in custom flumes. (a) Square ceramic tiles seeded with *N. luetkeana* spores or gametophyte fragments, now densely covered in young sporophytes. (b) Tiles in a flume with larger sporophytes. Note the continuous recruitment of younger sporophytes. (c) Schematic view of a 200-L recirculating flume, including (1) light source, (2) flow straightening baffles, (3) working section, (4) propeller driving water motion, (5) outflow to chiller, (6) drain valve, and (7) inflow from chiller. Arrows indicate water motion. [Color figure can be viewed at wileyonlinelibrary.com]

kelp thalli to one side of the tank while maintaining oscillating flow, an asymmetric flow regime was programmed. Water flow was mostly unidirectional from the side of the flume that had the attached thalli to the side without attached thalli. Approximately every 15 s however, the flow briefly reversed for just long enough to fold and bend the kelp blades without pressing them against the end of the flumes (Video S2; Figure S1). To avoid the blades of the anchored thalli from growing into the opposite end of the flumes, the flumes were inspected daily, and the tips of the rapidly growing blades were trimmed as needed. To prevent the elongating stipe from entangling the blades, it was tightly coiled and tied to the bottom of the flume working section. The length and developmental stage of the 5–10 largest individual sporophytes were recorded weekly, and the daily RGR was calculated as described above.

RESULTS AND DISCUSSION

Microscopic sporophytes were observed during routine culture inspection 2–3 weeks after inoculation with spores or gametophyte fragments (T_0; see Figure 3a). In tumble culture, young sporophytes became visible to the naked eye (~1–2 mm) 2 weeks after T_0, reached 0.5 cm length after 4 weeks, and 10 cm length after 6 weeks (Figure 3a). On ceramic tiles, sporophytes became visible to the naked eye 4 weeks after T_0, reached 0.5 cm length after 6 weeks, and 10 cm length after 8 weeks (Figure 3a). A distinct stipe became visible by around 5 cm length. At approximately 10 cm length, rootlike haptera began developing above the initial discoid holdfast in both attached and freely floating thalli. The nascent pneumatocyst also became visible as a small bump at the junction between the blade base and the stipe, subsequently forming a gas-filled cavity at 10–15 cm length. The blade splitting process was first observed after 8 weeks in tumble culture and 10 weeks on tiles at 15–20 cm length, though lines of dehiscence were often already visible on much smaller thalli.

Nereocystis luetkeana sporophytes grown in tumble culture were culled after they became too buoyant to submerge and tumble at approximately 10 weeks of age, a pneumatocyst diameter of around 2 cm, and a thallus length of around 40 cm. This size was more than large enough for the intended ecophysiological and developmental research, and we did not attempt to overcome this physical limitation. However, removing the stipe and a portion of the pneumatocyst could have allowed for even further growth by eliminating buoyancy and entanglement (Graham et al., 2023). Unlike the tumble culture sporophytes, the sporophytes on tiles in the flumes were allowed to develop further, ultimately attaining lengths of 3–6 m within 4 months, vastly outgrowing our initial objective of producing >2 cm thalli in recirculating culture (Figure 3b,c).

In larger (>1 m) flume-grown sporophytes, individual thalli exhibited growth rates of 2.0% ± 0.9% · d^{-1} (mean ± SD, $n = 14$) or, in absolute terms, a daily length increase of 4.3 ± 2.0 cm. These growth rates are similar to those measured in comparably sized wild *Nereocystis luetkeana* (Duncan, 1973; Kain (Jones, 1987; Nicholson, 1970), as well as *N. luetkeana* cultivated in flow-through systems (Swanson & Fox, 2007). However, as this length does not account for the daily trimming of blade tips to avoid abrasion (up to 5 cm · d^{-1}), it is a potentially substantial underestimate of *N. luetkeana* growth performance in recirculating systems. In the 600-L tumble cultures, the mean

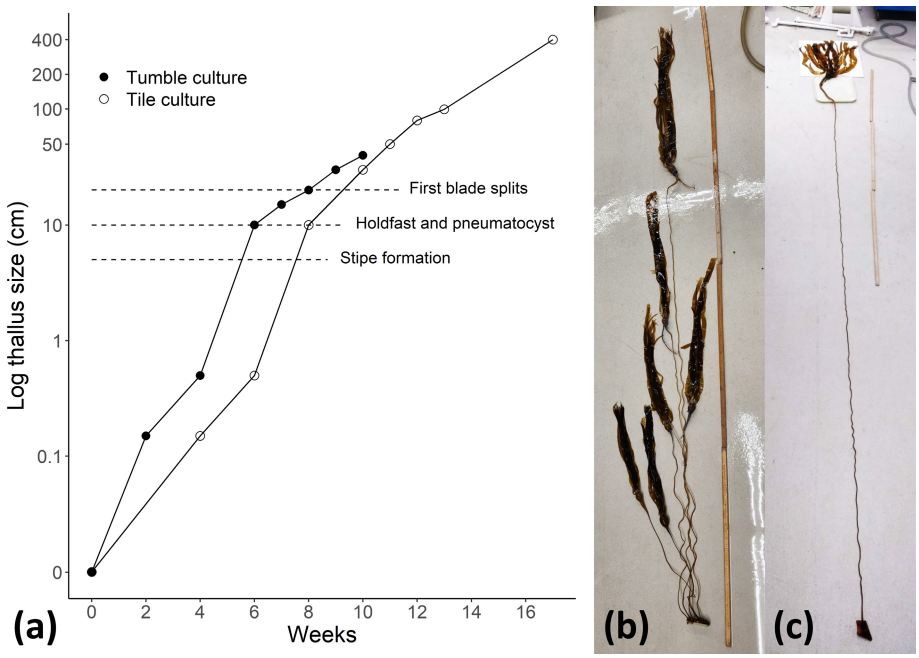

FIGURE 3 (a) Approximate growth and developmental timeline of cultivated *Nereocystis luetkeana* under two different culture approaches. Week 0 represents the time at which microscopic sporophytes were first observed, not the time of culture inoculation. Note that the y-axis is on a logarithmic scale. (b, c) Large kelp sporophytes produced in attached culture. Note the relatively small holdfasts of the lab-grown harnessed kelp. The rulers next to the kelps are 1 m in length. [Color figure can be viewed at wileyonlinelibrary.com]

daily RGR of aggregate biomass was 11.9% ± 4.3% (mean ± SD, $n = 10$). In other words, overall biomass increased by a factor of 2.4 ± 0.9 each week. The mean stocking density immediately prior to weekly thinning was 1.85 ± 1.09 kg · m^{-3} ($n = 10$), though a stocking density of up to 3 kg · m^{-3} could be attained without apparent detriment to the health and growth of the sporophytes. However, sporophytes exhibited rapid degradation followed by a complete collapse of the culture when it once exceeded 4 kg · m^{-3}. Both tumble and tile cultures responded to the removal of large sporophytes by producing repeated flushes of new sporophytes without further inoculation; inoculated ceramic tiles and 26.5-L "seed" tumble cultures produced a continuous supply of sporophytes for at least 6 months until the cultures were terminated for unrelated reasons.

Water motion markedly influenced morphogenesis. Young thalli that had experienced low or unidirectional water flow sometimes exhibited a persistent delay in blade splitting that resulted in abnormally broad blades even after strong oscillating flow had been restored (Figure 4a). Kelps growing attached on tiles in the flume tended to develop long stipes, while kelps moving freely in tumble culture tended to develop short stipes with relatively large pneumatocysts (Figure 4c). "Harnessing" kelps, which prevented their holdfasts from experiencing significant tensile forces, appeared to stunt holdfast development (Figure 3b,c). The observed effects of different flow regimes on the morphogenesis of cultured *Nereocystis luetkeana* are consistent with the remarkable phenotypic plasticity kelps are known to exhibit in response to water motion (Coleman & Martone, 2020; Koehl et al., 2008; Supratya et al., 2020).

Thallus degradation was a significant initial challenge to cultivating *Nereocystis luetkeana* in closed systems. Sporophytes degraded rapidly under suboptimal culture conditions, such as stocking densities >3 kg · m^{-3}, excessively high or low nutrient levels, stagnant flow, delayed media changes, and temperature fluctuations (e.g., temperature spikes due to power outages). Thalli developed pale, necrotic lesions generally starting from the distal end of the blade, which progressively spread and could consume the entire thallus as quickly as 24–48 h after the first visible signs. Thallus necrosis was often accompanied by foam on the water's surface and discoloration of the culture medium as culture conditions worsened. For short-term stress events such as pump or chiller failures, necrosis was often not immediately apparent, but manifested within 24–48 h. While sporophytes of all developmental stages could exhibit necrosis, larger sporophytes >20 cm length seemed less sensitive than smaller sporophytes, and often remained healthy even as smaller thalli in mixed-size cultures deteriorated.

Although we did not attempt to isolate or identify any potential pathogens, the observed triggers, symptoms, and solutions were consistent with opportunistic bacterial infections documented in other recirculating macroalgal cultures (Friedlander & Levy, 1995; Ward et al., 2020). Many kelp-associated bacteria are capable of degrading

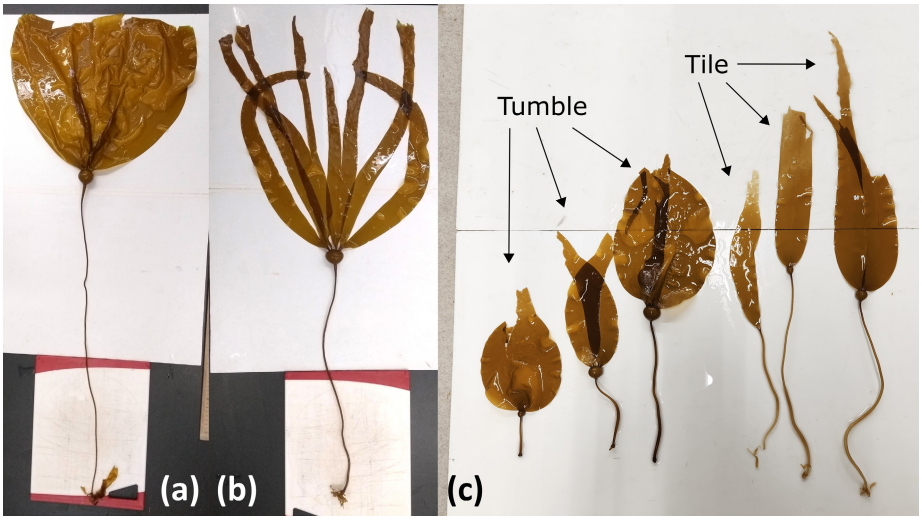

FIGURE 4 Morphological variation in cultivated *Nereocystis luetkeana*. (a) A sporophyte that had experienced insufficient flow exhibiting delayed blade splitting. (b) A similarly sized sporophyte exhibiting the normal blade splitting process. (c) A comparison of representative younger sporophytes from tumble culture (left) and attached tile culture (right). [Color figure can be viewed at wileyonlinelibrary.com]

kelp cell wall constituents (Bengtsson et al., 2011; Lin et al., 2018). In a closed system, a high density of kelp, kelp-derived organic matter, and added nutrients drive the excessive proliferation of cell wall degrading bacteria (Goecke et al., 2010; Weinberger et al., 1994), especially if immune responses are suppressed by stress (Campbell et al., 2011). Alternatively, high levels of compounds derived from microbial degradation of kelp tissue (e.g., oligosaccharides) could trigger strong immune responses with the side effect of accelerating tissue senescence and necrosis (Goecke et al., 2010; Wang et al., 2019). As in closed-system cultures of red algae (Friedlander & Levy, 1995), thallus degradation was avoidable or in mild cases, reversible by removing necrotic tissue or excess biomass and ensuring that the culture medium remained fresh and aerated.

Other kelp species have been raised in recirculating culture systems (Boderskov et al., 2016; Peteiro et al., 2014; Redmond et al., 2014; Westermeier et al., 2006), and *Nereocystis luetkeana* has been grown in land-based flow-through culture (Amsler & Neushul, 1989; Graham et al., 2023; Swanson & Fox, 2007). However, to our knowledge our culture trials represent the first instance of *N. luetkeana* being successfully grown to large sizes (>1 m) in recirculating culture. Furthermore, they demonstrate the potential of recirculating systems to create new opportunities in research and restoration by overcoming some limitations of traditional kelp culture methods (Kim et al., 2017). Conditions can be precisely controlled, permitting year-round "on demand" cultivation independent of the natural environment (Hafting et al., 2011) and facilitating the development of kelp as model systems to further scientific understanding of their morphogenesis, physiology, genetics, life histories, and partnerships with microbes. Due to the physical isolation from the ocean, stronger biosecurity measures can be implemented to avoid introducing pests to the culture or to prevent the escape of non-native culture species (Schmitz & Kraft, 2022). Perhaps most importantly, closed culture systems are not reliant on suitable kelp farming locations, direct proximity to the ocean, or flow-through infrastructure, highlighting their potential for increasing the accessibility of kelp research.

Although our culture trials highlighted key advantages of recirculating cultures, we ultimately remained reliant on a steady supply of natural seawater. Implementing steps to extend the life of the culture medium, such as physical filtration and UV sterilization, could vastly reduce the amount of natural seawater required and the labor involved in culture maintenance (Schmitz & Kraft, 2022; Wold et al., 2014). Using artificial seawater could further reduce or even eliminate reliance on natural seawater (Sebök et al., 2017), although artificial mixes must be carefully chosen to ensure that trace elements required by seaweeds are present in appropriate proportions (Redmond et al., 2014).

AUTHOR CONTRIBUTIONS

Varoon P. Supratya: Conceptualization (lead); funding acquisition (supporting); investigation (lead); methodology (lead); project administration (equal); resources (supporting); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (supporting). **Patrick T. Martone:** Conceptualization (supporting); funding acquisition (lead); investigation (supporting); methodology (supporting); project administration (supporting); resources (lead); supervision (supporting); validation (supporting); visualization

(supporting); writing – original draft (supporting); writing – review and editing (lead).

ACKNOWLEDGMENTS

We thank Camryn Good and Ruby Burns for assisting with culture maintenance, Jennifer Clark and Rachael Wade for providing input on gametophyte and microsporophyte culture conditions, and Catherine Pfister for comments and suggestions. This research was made possible by a Natural Sciences and Engineering Research Council (NSERC) Canada Graduate Scholarship – Masters (CGS-M) to V.P.S. (#6563), a Natural Sciences and Engineering Research Council (NSERC) Discovery grant to P.T.M. (RGPIN-2019-06240), and funding from the Canada Foundation for Innovation awarded to P.T.M (#27431). The authors declare no conflicts of interest.

ORCID

Varoon P. Supratya https://orcid.org/0000-0002-2891-0335
Patrick T. Martone https://orcid.org/0000-0002-6345-1023

REFERENCES

Amsler, C. D., & Neushul, M. (1989). Diel periodicity of spore release from the kelp *Nereocystis luetkeana* (Mertens) Postels et Ruprecht. *Journal of Experimental Marine Biology and Ecology*, *134*(2), 117–127. https://doi.org/10.1016/0022-0981(90)90104-K

Azevedo, I. C., Marinho, G. S., Silva, D. M., & Sousa-Pinto, I. (2016). Pilot scale land-based cultivation of *Saccharina latissima* Linnaeus at southern European climate conditions: Growth and nutrient uptake at high temperatures. *Aquaculture*, *459*, 166–172. https://doi.org/10.1016/J.AQUACULTURE.2016.03.038

Beas-Luna, R., Micheli, F., Woodson, C. B., Carr, M., Malone, D., Torre, J., Boch, C., Caselle, J. E., Edwards, M., Freiwald, J., Hamilton, S. L., Hernandez, A., Konar, B., Kroeker, K. J., Lorda, J., Montaño-Moctezuma, G., & Torres-Moye, G. (2020). Geographic variation in responses of kelp forest communities of the California current to recent climatic changes. *Global Change Biology*, *26*(11), 6457–6473. https://doi.org/10.1111/GCB.15273

Bengtsson, M. M., Sjøtun, K., Storesund, J. E., & Øvreas, L. (2011). Utilization of kelp-derived carbon sources by kelp surface-associated bacteria. *Aquatic Microbial Ecology*, *62*(2), 191–199. https://doi.org/10.3354/AME01477

Bennett, S., Wernberg, T., Connell, S. D., Hobday, A. J., Johnson, C. R., & Poloczanska, E. S. (2016). The "Great Southern Reef": Social, ecological and economic value of Australia's neglected kelp forests. *Marine and Freshwater Research*, *67*(1), 47–56. https://doi.org/10.1071/MF15232

Berry, H. D., Mumford, T. F., Christiaen, B., Dowty, P., Calloway, M., Ferrier, L., Grossman, E. E., & VanArendonk, N. R. (2021). Long-term changes in kelp forests in an inner basin of the Salish Sea. *PLoS ONE*, *16*(2), e0229703. https://doi.org/10.1371/journal.pone.0229703

Boderskov, T., Schmedes, P. S., Bruhn, A., Rasmussen, M. B., Nielsen, M. M., & Pedersen, M. F. (2016). The effect of light and nutrient availability on growth, nitrogen, and pigment contents of *Saccharina latissima* (Phaeophyceae) grown in outdoor tanks, under natural variation of sunlight and temperature, during autumn and early winter in Denmark. *Journal of Applied Phycology*, *28*(2), 1153–1165. https://doi.org/10.1007/s10811-015-0673-7

Borowitzka, M. A. (1999). Commercial production of microalgae: Ponds, tanks, tubes and fermenters. *Journal of Biotechnology*, *70*(1–3), 313–321. https://doi.org/10.1016/S0168-1656(99)00083-8

Campbell, A. H., Harder, T., Nielsen, S., Kjelleberg, S., & Steinberg, P. D. (2011). Climate change and disease: Bleaching of a chemically defended seaweed. *Global Change Biology*, *17*(9), 2958–2970. https://doi.org/10.1111/J.1365-2486.2011.02456.X

Carney, L. T., Waaland, J. R., Klinger, T., & Ewing, K. (2005). Restoration of the bull kelp *Nereocystis luetkeana* in nearshore rocky habitats. *Marine Ecology Progress Series*, *302*, 49–61. https://doi.org/10.3354/meps302049

Charters, A. C., & Neushul, M. (1979). A hydrodynamically defined culture system for benthic seaweeds. *Aquatic Botany*, *6*, 67–78. https://doi.org/10.1016/0304-3770(79)90051-2

Coleman, L. J. M., & Martone, P. T. (2020). Morphological plasticity in the kelp Nereocystis luetkeana (*Phaeophyceae*) is sensitive to the magnitude, direction, and location of mechanical loading. *Journal of Phycology*, *56*(6), 1414–1427. https://doi.org/10.1111/jpy.13043

Cottier-Cook, E. J., Nagabhatla, N., Badis, Y., Campbell, M. L., Chopin, T., Fang, J., He, P., Hewitt, C. L., Kim, G. H., Huo, Y., & Jiang, Z. (2016). *Safeguarding the future of the global seaweed aquaculture industry*. United Nations University (INWEH) and Scottish Association for Marine Science https://inweh.unu.edu/safeguarding-the-future-of-the-global-seaweed-aquaculture-industry/

Duncan, M. J. (1973). In situ studies of growth and pigmentation of the phaeophycean *Nereocystis luetkeana*. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, *24*(1–4), 510–525. https://doi.org/10.1007/BF01609538

Ebbing, A., Pierik, R., Bouma, T., Kromkamp, J. C., & Timmermans, K. (2020). How light and biomass density influence the reproduction of delayed *Saccharina latissima* gametophytes (*Phaeophyceae*). *Journal of Phycology*, *56*(3), 709–718. https://doi.org/10.1111/jpy.12976

Ebbing, A. P. J., Fivash, G. S., Pierik, R., Bouma, T. J., Kromkamp, J. C., & Timmermans, K. (2022). The SeaCoRe system for large scale kelp aquaculture: A plug-and-play, compatible, open-source system for the propagation and transport of clonal gametophyte cultures. *Journal of Applied Phycology*, *34*(1), 517–527. https://doi.org/10.1007/S10811-021-02638-2

Eger, A. M., Marzinelli, E. M., Christie, H., Fagerli, C. W., Fujita, D., Gonzalez, A. P., Hong, S. W., Kim, J. H., Lee, L. C., McHugh, T. A., Nishihara, G. N., Tatsumi, M., Steinberg, P. D., & Vergés, A. (2022). Global kelp forest restoration: Past lessons, present status, and future directions. *Biological Reviews*, *97*(4), 1449–1475. https://doi.org/10.1111/brv.12850

Ferdouse, F., Løvstad Holdt, S., Smith, R., Murúa, P., Yang, Z., FAO, Ferdouse, F., Løvstad Holdt, S., Smith, R., Murúa, P., Yang, Z., & FAO. (2018). *The global status of seaweed production, trade and utilization*. FAO globefish research Programme (Vol. 124).

Filbee-Dexter, K., & Wernberg, T. (2018). Rise of turfs: A new battlefront for globally declining kelp forests. *Bioscience*, *68*(2), 64–76. https://doi.org/10.1093/BIOSCI/BIX147

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 LLC.

Friedlander, M., & Levy, I. (1995). Cultivation of *Gracilaria* in outdoor tanks and ponds. *Journal of Applied Phycology*, *7*, 315.

Gadberry, B. A., Colt, J., Maynard, D., Boratyn, D. C., Webb, K., Johnson, R. B., Saunders, G. W., & Boyer, R. H. (2018). Intensive land-based production of red and green macroalgae for human consumption in the Pacific Northwest: An evaluation of seasonal growth, yield, nutritional composition, and contaminant levels. *Algae*, *33*(1), 109–125. https://doi.org/10.4490/algae.2018.33.2.21

Gao, X., Endo, H., Nagaki, M., & Agatsuma, Y. (2017). Interactive effects of nutrient availability and temperature on growth and survival of different size classes of *Saccharina japonica* (Laminariales, Phaeophyceae). *Phycologia*, *56*(3), 253–260. https://doi.org/10.2216/16-91.1

Goecke, F., Klemetsdal, G., & Ergon, Å. (2020). Cultivar development of kelps for commercial cultivation—Past lessons and future prospects. *Frontiers in Marine Science*, *8*, 110. https://doi.org/10.3389/fmars.2020.00110

Goecke, F., Labes, A., Wiese, J., & Imhoff, J. F. (2010). Review chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, *409*, 267–300. https://doi.org/10.3354/meps08607

Graham, M., Hamilton, S., Bugbee, B., & Kim, A. (2023). *Assessment of practical methods for re-establishment of northern California bull kelp populations at an ecologically relevant scale*. California Sea Grant. https://caseagrant.ucsd.edu/sites/default/files/R_HCEOPC-13_2023_final_report.pdf

Grebe, G. S., Byron, C. J., Gelais, A. S., Kotowicz, D. M., & Olson, T. K. (2019). An ecosystem approach to kelp aquaculture in the Americas and Europe. *Aquaculture Reports*, *15*, 100215. https://doi.org/10.1016/j.aqrep.2019.100215

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. In M. L. Smith & M. H. Chanley (Eds.), *Culture of Marine Invertebrate Animals* (pp. 29–60). Plenum Press. https://doi.org/10.1007/978-1-4615-8714-9_3

Hafting, J. T., Critchley, A. T., Cornish, M. L., Hubley, S. A., & Archibald, A. F. (2011). On-land cultivation of functional seaweed products for human usage. *Journal of Applied Phycology*, *24*(3), 385–392. https://doi.org/10.1007/S10811-011-9720-1

Kain (Jones), J. M. (1987). Patterns of relative growth in *Nereocystis Luetkeana* (Phaeophyta). *Journal of Phycology*, *23*(2), 181–187. https://doi.org/10.1111/j.1529-8817.1987.tb04442.x

Kennelly, S. J. (1989). Effects of kelp canopies on understorey species due to shade and scour. *Marine Ecology Progress Series*, *50*(3), 215–224.

Kim, J. K., Yarish, C., Hwang, E. K., Park, M., & Kim, Y. (2017). Seaweed aquaculture: Cultivation technologies, challenges and its ecosystem services. *Algae*, *32*(1), 1–13. https://doi.org/10.4490/algae.2017.32.3.3

Koehl, M. A. R., Silk, W. K., Liang, H., & Mahadevan, L. (2008). How kelp produce blade shapes suited to different flow regimes: A new wrinkle. *Integrative and Comparative Biology*, *48*(6), 834–851. https://doi.org/10.1093/icb/icn069

Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., Connell, S. D., Johnson, C. R., Konar, B., Ling, S. D., Micheli, F., Norderhaug, K. M., Pérez-Matus, A., Sousa-Pinto, I., Reed, D. C., Salomon, A. K., Shears, N. T., Wernberg, T., Anderson, R. J., … Byrnes, J. E. K. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(48), 13785–13790. https://doi.org/10.1073/pnas.1606102113

Lin, J. D., Lemay, M. A., & Parfrey, L. W. (2018). Diverse bacteria utilize alginate within the microbiome of the giant kelp *Macrocystis pyrifera*. *Frontiers in Microbiology*, *9*, 1914. https://doi.org/10.3389/FMICB.2018.01914

Morris, R. L., Hale, R., Strain, E. M. A., Reeves, S. E., Vergés, A., Marzinelli, E. M., Layton, C., Shelamoff, V., Graham, T. D. J., Chevalier, M., & Swearer, S. E. (2020). Key principles for managing recovery of kelp forests through restoration. *Bioscience*, *70*(8), 688–698. https://doi.org/10.1093/BIOSCI/BIAA058

Nicholson, N. L. (1970). Field studies on the Giant kelp *Nereocystis*. *Journal of Phycology*, *6*(2), 177–182. https://doi.org/10.1111/j.1529-8817.1970.tb02378.x

Norderhaug, K. M., Filbee-Dexter, K., Freitas, C., Birkely, S. R., Christensen, L., Mellerud, I., Thormar, J., Van Son, T., Moy, F., Alonso, M. V., & Steen, H. (2020). Ecosystem-level effects of large-scale disturbance in kelp forests. *Marine Ecology Progress Series*, *656*, 163–180. https://doi.org/10.3354/meps13426

Oster, C., Berry, H., Mumford, T., Naar, N., Peabody, B., Hart, L., Tonnes, D., Copps, S., Selleck, J., Allen, B., & Toft, J. (2020). *Puget Sound Kelp Conservation and Recovery Plan. NOAA-NMFS*. https://www.nwstraits.org/media/2880/pugetsound-kelpconservationandrecoveryplan_public_review_draft_1219.pdf.

Peteiro, C., Sánchez, N., Dueñas-Liaño, C., & Martínez, B. (2014). Open-sea cultivation by transplanting young fronds of the kelp *Saccharina latissima*. *Journal of Applied Phycology*, *26*(1), 519–528. https://doi.org/10.1007/s10811-013-0096-2

Pfister, C. A., Altabet, M. A., & Weigel, B. L. (2019). Kelp beds and their local effects on seawater chemistry, productivity, and microbial communities. *Ecology*, *100*(10), e02798. https://doi.org/10.1002/ecy.2798

Pfister, C. A., Berry, H. D., & Mumford, T. (2018). The dynamics of kelp forests in the Northeast Pacific Ocean and the relationship with environmental drivers. *Journal of Ecology*, *106*(4), 1520–1533. https://doi.org/10.1111/1365-2745.12908

Redmond, S., Green, L., Yarish, C., Kim, J., & Neefus, C. (2014). *New England seaweed culture handbook*. Connecticut Sea Grant. http://seagrant.uconn.edu

Reed, D. C., Neushul, M., & Ebeling, A. W. (1991). Role of settlement density on gametophyte growth and reproduction in the kelps *Pterygophora californica* and *Macrocystis pyrifera* (Phaeophyceae). *Journal of Phycology*, *27*(3), 361–366. https://doi.org/10.1111/j.0022-3646.1991.00361.x

Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, *9*(1), 15050. https://doi.org/10.1038/s41598-019-51114-y

Sato, Y., Yamaguchi, M., Hirano, T., Fukunishi, N., Abe, T., & Kawano, S. (2017). Effect of water velocity on *Undaria pinnatifida* and *Saccharina japonica* growth in a novel tank system designed for macroalgae cultivation. *Journal of Applied Phycology*, *29*(3), 1429–1436. https://doi.org/10.1007/s10811-016-1013-2

Schiltroth, B. (2021). *Effects of climate change on two species of foundational Brown algae,* Nereocystis luetkeana *and* Fucus gardneri*, within the Salish Sea*. [Master's thesis, Simon Fraser University]. https://summit.sfu.ca/item/34525#:~:text=Home-,Effects%20of%20climate%20change%20on%20two%20species%20of%20foundational%20brown,gardneri%2C%20within%20the%20Salish%20Sea&text=(Thesis)%20M.Sc.&text=Ocean%20acidification%20and%20warming%20have,on%20marine%20organisms%20and%20ecosystems.

Schmitz, T. A., & Kraft, E. (2022). Pilot scale photobioreactor system for land-based macroalgae cultivation. *Journal of Applied Phycology*, *34*(1), 507–516. https://doi.org/10.1007/S10811-021-02617-7/TABLES/3

Schroeder, S. B., Boyer, L., Juanes, F., & Costa, M. (2020). Spatial and temporal persistence of nearshore kelp beds on the west coast of British Columbia, Canada using satellite remote sensing. *Remote Sensing in Ecology and Conservation*, *6*(3), 327–343. https://doi.org/10.1002/rse2.142

Sebök, S., Herppich, W. B., & Hanelt, D. (2017). Development of an innovative ring-shaped cultivation system for a land-based cultivation of marine macroalgae. *Aquacultural Engineering*, *77*, 33–41. https://doi.org/10.1016/J.AQUAENG.2017.01.005

Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytologist*, *225*(4), 1447–1454. https://doi.org/10.1111/nph.16107

Springer, Y., Hays, C., Carr, M., Mackey, M., & Bloeser, J. (2006). *Ecology and management of the bull kelp,* Nereocystis luetkeana*: A synthesis with recommendations for future research* (p. 48). Lenfest Ocean program http://bio.research.ucsc.edu/people/carr/publications/carr/Nereomasterlores.pdf

Steneck, R. S., Graham, M. H., Bourque, B. J., Corbett, D., Erlandson, J. M., Estes, J. A., & Tegner, M. J. (2002). Kelp forest ecosystems: Biodiversity, stability, resilience and future. *Environmental Conservation*, *29*(4), 436–459. https://doi.org/10.1017/S0376892902000322

Supratya, V. P., Coleman, L. J. M., & Martone, P. T. (2020). Elevated temperature affects phenotypic plasticity in the bull kelp (*Nereocystis luetkeana*, Phaeophyceae). *Journal of Phycology*, *56*(6), 1534–1541. https://doi.org/10.1111/jpy.13049

Swanson, A. K., & Fox, C. H. (2007). Altered kelp (Laminariales) phlorotannins and growth under elevated carbon dioxide and ultraviolet-B treatments can influence associated intertidal food webs. *Global Change Biology*, *13*(8), 1696–1709. https://doi.org/10.1111/j.1365-2486.2007.01384.x

Tatsumi, M., Mabin, C. J. T., Layton, C., Shelamoff, V., Cameron, M. J., Johnson, C. R., & Wright, J. T. (2022). Density-dependence and seasonal variation in reproductive output and sporophyte production in the kelp, Ecklonia radiata. *Journal of Phycology*, *58*(1), 92–104. https://doi.org/10.1111/jpy.13214

Teagle, H., Hawkins, S. J., Moore, P. J., & Smale, D. A. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, *492*, 81–98. https://doi.org/10.1016/j.jembe.2017.01.017

Wang, G., Chang, L., Zhang, R., Wang, S., Wei, X., Rickert, E., Krost, P., Xiao, L., & Weinberger, F. (2019). Can targeted defense elicitation improve seaweed aquaculture? *Journal of Applied Phycology*, *31*(3), 1845–1854. https://doi.org/10.1007/s10811-018-1709-6

Ward, G. M., Faisan, J. P., Cottier-Cook, E. J., Gachon, C., Hurtado, A. Q., Lim, P. E., Matoju, I., Msuya, F. E., Bass, D., & Brodie, J. (2020). A review of reported seaweed diseases and pests in aquaculture in Asia. *Journal of the World Aquaculture Society*, *51*(4), 815–828. https://doi.org/10.1111/JWAS.12649

Weinberger, F., Friedlander, M., & Gunkel, W. (1994). A bacterial facultative parasite of *Gracilaria conferta*. *Diseases of Aquatic Organisms*, *18*(2), 135–141. https://doi.org/10.3354/dao018135

Westermeier, R., Patiño, D., Piel, M. I., Maier, I., & Mueller, D. G. (2006). A new approach to kelp mariculture in Chile: Production of free-floating sporophyte seedlings from gametophyte cultures of *Lessonia trabeculata* and *Macrocystis pyrifera*. *Aquaculture Research*, *37*(2), 164–171. https://doi.org/10.1111/j.1365-2109.2005.01414.x

Winberg, P., Skropeta, D., & Ullrich, A. (2011). *Seaweed cultivation pilot trials – towards culture systems and marketable products*. Australian government Rural Industries Research and Development Corporation, RIRDC (Publication No. 10/184. PRJ – 000162). https://ro.uow.edu.au/smfc/2

Wold, P. A., Holan, A. B., Øie, G., Attramadal, K., Bakke, I., Vadstein, O., & Leiknes, T. O. (2014). Effects of membrane filtration on bacterial number and microbial diversity in marine recirculating aquaculture system (RAS) for Atlantic cod (*Gadus morhua* L.) production. *Aquaculture*, *422–423*, 69–77. https://doi.org/10.1016/J.AQUACULTURE.2013.11.019

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Sample oscillating flow profile cycles for cultivating *Nereocystis luetkeana* sporophytes in recirculating flumes. (a) symmetrical oscillation for small sporophytes <0.5 m long. (b) Asymmetrical oscillation for larger sporophytes ≥0.5 m long.

Figure S2. Closeup of kelp harnesses.

Video S1. *Nereocystis luetkeana* tumble culture in a 600-L cylindrical vessel.

Video S2. Large (>1 m) *Nereocystis luetkeana* in a recirculating growth flume.

How to cite this article: Supratya, V. P., & Martone, P. T. (2024). Kelps on demand: Closed-system protocols for culturing large bull kelp sporophytes for research and restoration. *Journal of Phycology*, *60*, 73–82. https://doi.org/10.1111/jpy.13413