Cultivation protocols the rhodophytes, *Devaleraea mollis* and *Palmaria hecatensis* from Alaska

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Abstract

Species diversification is crucial for the long-term viability, competitiveness, and sustainability of the seaweed farming industry in the Pacific Northwest. This study varied temperature, photoperiod, and irradiance conditions to grow *Devalaraea mollis* indoors and developed the first on-land cultivation protocols for *Palmaria hecatensis.* Both rhodophytes from Alaskahave ecotypes adapted to environmental conditions that differ from those at lower latitudes. The study also explored the use of different nutrient sources to potentially increase growth rates without compromising biomass quality or raising production costs. Results showed that specific growth rates of *D. mollis* and *P. hecatensis* significantly differed depending on the level of factors tested, indicating distinct ecological and physiological adaptations. *D. mollis* exhibited higher growth rates in warmer temperatures and responded to higher irradiance levels with spore release but showed no clear preference between neutral and long-day photoperiods. In contrast, *P. hecatensis* demonstrated higher growth rates in cooler environments with a long-day photoperiod promoting the most growth without spore release. Nutrient supplementation revealed that growth in *D. mollis* was affected by nutrient formulation, while *P. hecatensis* showed no significant growth variation. These findings underscore the importance of species- and geography-specific protocols for seaweed farming. Further research is needed to optimize the potential cultivation protocols provided here. Cultivation protocols would also benefit from exploring the ecological and physiological nuances of both species.

Keywords: Physiological adaptations; indoor cultivation; species diversification; specific growth rate

Introduction

The seaweed industry, with its vast array of species and applications, is valued at around US $5.5 - 6 billion (FAO 2021). Production for human food consumption accounts for approximately 95% of this total value, highlighting its significant role in global nutrition (FAO 2021). Hydrocolloids extracted from seaweeds are the second most valuable segment, while animal feed additives and fertilizers comprise the remainder (FAO 2016; Holdt & Kraan, 2011). Beyond these, the industry targets new markets for high-value seaweed products, including cosmetic, pharmaceutical, biomedical, and nutraceutical applications (Winberg et al., 2011; FAO 2018; Edwards & Dring 2011). Seaweed is also of interest as a viable protein source with lower cultivation requirements compared to terrestrial sources (Angell et al. 2016; Mendez & Kwon 2021; Garcia-Vaquero et al. 2017; Tibbetts et al. 2016; Wells et al. 2017). The demand for seaweed biomass has increased, driven by trends in sustainable food and holistic health, as it is a crucial component in functional foods and feeds (Wu et al. 2023). It is estimated that seaweed markets have the potential to grow by an additional USD 11.8 billion by 2030 (World Bank, 2023).

Farming of red seaweed holds significant commercial interest due to multiple species being rich in hydrocolloids (e.g., agar and carrageenan), secondary metabolites, and protein (FAO 2014; FAO 2016; Hafting et al. 2012). Red seaweeds are also rich in phycobiliproteins, specifically phycoerythrin and phycocyanin; which are pigment-protein complexes (Freitas et al., 2022). These pigments are widely utilized in scientific, medical, and commercial applications due to their bright fluorescence and beneficial properties. Phycoerythrin is commonly used in flow cytometry, fluorescence microscopy, and immunoassays such as ELISAs for enhanced sensitivity and accuracy in antigen or antibody detection (Smith et al., 2019; Jones & Wang 2020). Phycocyanin is a fluorescent marker in various bioassays and imaging techniques for detecting and analyzing biological molecules and cells (Johnson & Wilson 2018). Beyond biotechnology, phycocyanin is used in the food and cosmetic industries as a natural colorant and antioxidant, offering anti-inflammatory and immune-boosting effects (Garcia et al., 2020). These versatile and high-end applications underscore the need to increase and improve access to red seaweed biomass to satisfy demands for research, clinical diagnostics, and commercial products.

To date, red seaweeds account for approximately 53% of the total cultivated seaweed globally (FAO 2021). Most biomass is produced in Asian countries, although efforts are underway to increase

production in Africa, America, and Europe (FAO 2021). In the United States, experimental farming of red seaweeds started in the early 1990s (Mumford 1990; Yarish 1998), with successful long-term and small-scale commercial operations beginning approximately 15 years later (Kim et al., 2019). Among the species of interest, *Gracilaria tikvahiae*, *Palmaria palmata* on the East Coast, and *Devaleraea mollis* (formerly known as *Palmaria mollis*) and *Palmaria hecatensis* on the West Coast stand out as top candidates for human consumption due to their high protein content, which can range from 8-35% of dry weight (at least in *P. palmata* and *D. mollis*). Both species are also wild-harvested for personal consumption, increasing the potential for consumer acceptance.

D. mollis (commonly referred to as dulse outside of Alaska) has been successfully grown in outdoor land-based systems in Oregon and California. Extensive work has examined the relationship between biomass production and environmental parameters (Demetropoulos et al., 2004a; Demetropoulos et al., 2004b; Rosen et al., 2000). Cultivation of the species has gained traction, given it is often described as the "bacon seaweed" and is currently used as a vegan alternative to animal-based bacon (search for Umaro Foods). No cultivation efforts have been described for *P. hecatensis*. Significant financial investment in the last decade has enabled the Pacific Northwest to emerge as a seaweed farming hub, particularly Alaska. Alaska is now second in production, following Maine (Alaska Mariculture Cluster 2022). The State aims to develop a $100 million mariculture industry in the next 10-12 years (Alaska Mariculture Task Force, 2018). The focus has been primarily on kelp and oyster farming at sea. However, the cultivation of red seaweed on-land could offer new market opportunities and help Alaska achieve its industry goal within the next decade.

In Alaska, *Devaleraea mollis* and *Palmaria hecatensis* (locally known as red ribbon and red stiff seaweed, respectively) hold cultural significance among Tlingit communities. These species are an integral part of coastal Indigenous communities' diet and a trading good (Garza et al., 1989; Johnson et al., 2019). Interestingly, *D. mollis* can naturally be found year-round in specific locations such as Cook Inlet, Southcentral Alaska, where it colonizes mariculture gear (W. Bates, pers. comm.). This year-round availability differs from the seasonal life cycle usually described for the species, which spans from early spring to summer (Hawkes 1985). There is limited information related to the ecology of *P. hecatensis*. However, studies in British Columbia, Canada, describe the potential for perennation of older blades (Hawkes 1985), though this has yet to be confirmed for Alaska and other regions. While *D. mollis* has a geographical distribution that expands from Southern California to the Aleutian Islands, Alaska, *P.*

hecatensis has a more limited presence with patches originally described for Southeast Alaska, British Columbia, Canada, and the eastern coast of Russia.

Early attempts to grow Alaskan ecotypes of *D. mollis* using cultivation protocols from California and Oregon (Oregon Seaweed & Monterey Bay Seaweed) were unsuccessful. The failures were mainly due to unsuitable temperature and light conditions. Natural coastal conditions in Alaska differ significantly from those in California and Oregon, where these protocols were developed. Therefore, cultivation protocols adjusted to conditions naturally experienced in Alaska must be developed as a first step to start the commercial on-land and indoor cultivation of *D. mollis*. Conversely, *P. hecatensis* is a potential novel mariculture species. It shares similarities with *D. mollis* in terms of life cycle, habitable range, traditional consumption history, potential for use as feed in abalone farming, satisfying protein demands, and other biomass supply for high-end markets.

Contrary to cultivation at sea, on-land systems allow precise control of environmental factors such as light, temperature, salinity, pH, water turbulence, and nutrient concentrations (Redmond et al. 2014). These controls facilitate customizing biomass production to ensure biosafety and meet high-quality standards tailored to market demands (Mendoza et al., 2018). This study adjusted temperature, photoperiod, and light conditions to grow Alaskan *D. mollis* in indoor on-land settings. The study also presents the first on-land cultivation protocols for *P. hecatensis*. Once suitable growing conditions were established, we explored the effect of different nutrient sources on growth performance for both species. The goal of testing different nutrient sources was to potentially increase specific growth rates without compromising biomass quality while keeping costs low. Overall, we aim to diversify the mariculture portfolio in the Pacific Northwest of the United States by increasing species options for on-land cultivation, improving growth and quality without raising costs.

Methods

Collection and acclimation of stocking biomass

Stocking biomass for *Devaleraea mollis* and *Palmaria hecatensis* was collected from Middle Bay Kalsin Bay and Pasagshak Bay in Kodiak Island, Alaska, in March 2022 and 2023 when biomass abounds (Fig. 1). Approximately 25 kg of healthy thalli were detached from the intertidal zone at low tide (- 0.5 m MLLW). Thalli with proliferations, minimal herbivory, and no signs of decay or bleaching were selected (Fig. 2). Biomass was wrapped in seawater-damped paper towels, packed into sealed plastic tubs with gel

ice packs, and transported in coolers to the University of Alaska Fairbanks in Juneau. Transit time did not exceed 24 hours.

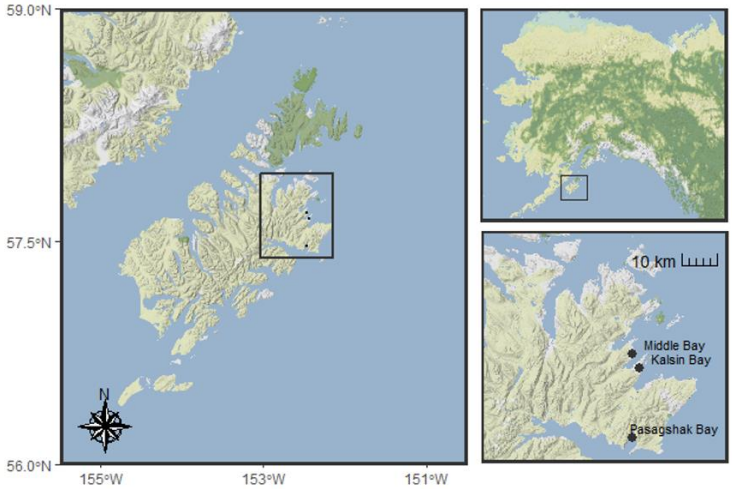

Figure 1. Collection sites (Middle, Kalsin, and Pasagshak Bays) on the east side of Kodiak Island in Southcentral Alaska.

Upon arrival, thalli were placed in independent 1000L recirculating tanks (30 ± 1 ppt salinity, 4 ± 0.5 °C, 50 ± 10 µmol photons m^{-2} s^{-1}, 12L:12D) for 14 days to acclimate. Tank conditions were similar to those at the collection sites. Tanks were cleaned and refilled with filtered (1 µm) and UV-treated seawater every seven days. A portion of the biomass was used for experiments, while the rest was maintained under the same conditions as stock for future experiments.

Figure 2. (A) *Devaleraea mollis* and (B) *Palmaria hecatensis* showing multiple proliferations growing from the edge and middle section of their thalli.

Preparation of experimental samples

After the 14-day acclimation, 90 proliferations per species were detached from at least ten parental thalli. Proliferations were rinsed with sterile seawater (SSW), rubbed with a Kimwipe to remove surface contaminants, and grouped in groups of three (0.01 g ± 0.006 g). Each set was placed into 250 ml Erlenmeyer flasks filled with SSW (30 ± 1 ppt) enriched with F/2 and germanium dioxide (250mg/l stock 2 ml per liter) to control diatoms. Flasks were placed in Conviron Growth Chambers (Conviron GE1000) set to 4 °C, 40 µmol photons m^{-2} s^{-1}, 12L:12D, with experimental conditions adjusted as needed. Flasks were aerated throughout the experiment. See Figure 3 for a diagram of the experimental workflow.

Experimental Design

The experiments exposed *D. mollis* (n = 5) and *P. hecatensis* (n = 5) to various combinations of temperature (4 °C, 8 °C, 12 °C), photoperiod (16L:8D, 12L:12D, 8L:16D), and irradiance (20, 40, 100 µmol photons m^{-2} s^{-1}) to identify grow conditions in indoor settings. Growth was measured using wet weight as the metric. Temperature and photoperiod were tested on all possible combinations. However, given the availability of only three incubators, culture conditions were adjusted such that only one experimental factor was manipulated at a time, using a stepwise approach rather than a full factorial setup. Irradiance was evaluated individually. Nonetheless, this facilitated a clearer understanding of the direct effects and differences per factor on a per-species basis. Each experiment lasted 28 days. Fresh proliferations were used for each factor tested. Experiments were conducted in 2022 and repeated in 2023 for replicability.

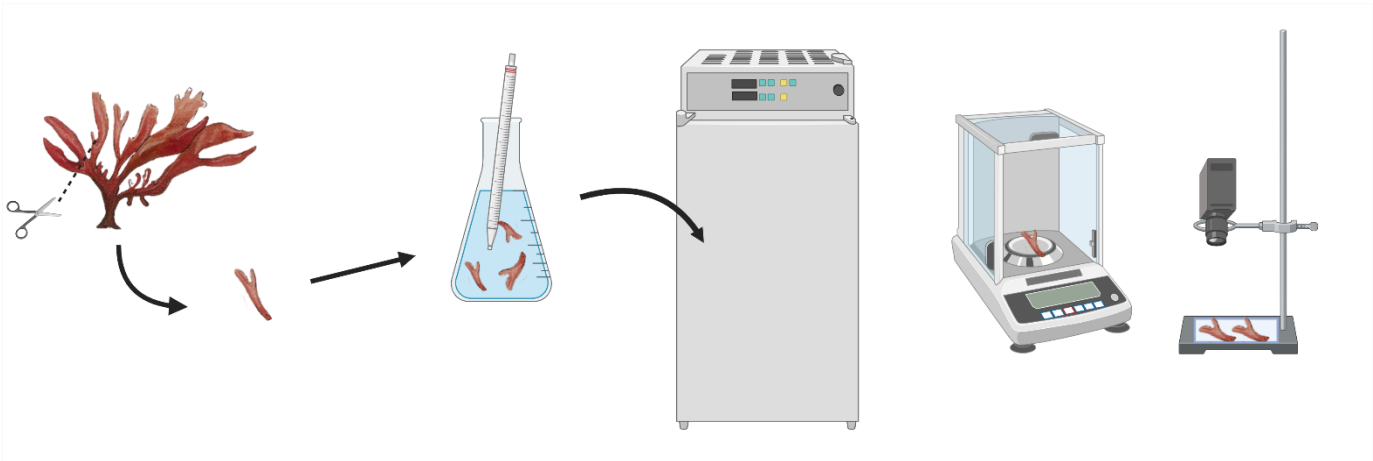

Figure 3. Experimental workflow including selection of proliferations (samples), allocation into 250mL Erlenmeyer flasks (n=5), exposures to treatments inside growth chambers, weighing, and photographing. Diagram built using biorender.

Experiments

Three growth chambers were used to test the effects of temperature, photoperiod, and irradiance. Initial trials determined upper-temperature limits, showing thalli bleached at 15 °C but growing at 12 °C, though pale. Thus, temperature levels were set at 4, 8, and 12 °C, mirroring seasonal temperatures in Southeast and Southcentral Alaska. Samples were conditioned to their experimental temperatures by increasing 2 °C every 24 hours. Growth data were recorded every seven days from pat-dried biomass placed on an analytical balance (Metter Toledo ME204E). Once weighed, biomass was photographed to record potential changes in color that could be indicative of nutrient limitation light and morphology, or texture potentially indicating fertility or asexual reproduction. After that, biomass returned to their flask, where they continued to grow until the completion of the experimental cycle. The same data collection process was repeated for photoperiod and irradiance using fresh proliferations for every experiment. Biomass was normalized to obtain specific growth rates (SGR), the equation provided by Shin et al:

$$SGR\ (\%\ day^{-1}) = \frac{(lnWt_2 - lnWt_1)}{(t_2 - t_1)} \times 100$$

Photoperiod levels resembled natural light-to-dark ratios (8L:16D, 12L:12D, 16L:8D) during the seaweed natural growing period from late February to July. Irradiance levels (20, 40, 100 µmol photons m^{-2} s^{-1}) were selected based on existing growing protocols for *D. mollis* (Demetropoulos and Langdon 2004a). Irradiance was gradually adjusted by changing photon fluence 10 µmol photons m^{-2} s^{-1} every 6 hours until desired setups were reached. Subsequent evaluations tested nutrient sources (VSE, F/2, JS) to potentially improve growth while minimizing costs without compromising biomass quality. These nutrient sources were selected based on accessibility, price, and formulation, mainly focusing on the molarity of available nitrogen (Table 1).

Von Stosch enrichment media (VSE) is a reagent-grade nutrition formulation specifically recommended for cultivating red seaweed. This enriched media was prepared following Redmond et al. (2014), who provided a generic formulation for red seaweeds, and Werner & Dring (2011) with a tailored recipe for *Palmaria palmata*. F/2 is a liquid pre-mix mainly advertised for microalgae cultivation following Guillard & Ryther (1962). It requires no further preparation but a dilution of Part A and B (130 µL) per liter of seawater. Lastly, Jack's Special 25-5-15 (JS) is a commercial pellet-based plant fertilizer (Mendoza et al. 2018) requiring seawater dissolution before utilization. In this experiment, we utilized 100 g of JS, dissolved in 1 L of MilliQ water. Once dissolved, the solution was filtered (0.2 µm) to remove any undissolved particles before using it as an experimental treatment.

Table 1. Chemical composition of Von Stosch enrichment, (VSE) F/2, and Jack's Special (JS) used as experimental nutrient sources. Modified from Jung et al. 2024, *submitted*.

Nutrient (µM)	**Von Stosch Enrichment Medium (VSE)**	**Guillard's F/2 Medium (F/2)**	**Jack's Special (N-P-K, 25-5-15) (JS)**
Nitrogen	500 (100% NO_3-)	500 (100% NO_3-)	500 (57% NO_3- and 43% NH_4^+)
Phosphorus	30	15	45
Iron	1	11.7	0.6
Manganese	10	0.9	0.3
EDTA	10	11.7	-

Vitamins	Vitamin B_1, Biotin, Vitamin B_{12}	Vitamin B_1, Biotin, Vitamin B_{12}	-
Potassium	-	-	107
Boron	-	-	0.6
Copper	-	0.04	0.05
Molybdenum	-	0.03	0.03
Zinc	-	0.05	0.004
Magnesium	-	-	1.15

A biomass threshold of 1 g per L was set to prevent carbon, nutrient, light, or flow limitations. Thalli were transferred from 250 ml flasks to 500 ml or 1 L flasks when this threshold was met (Redmond et al., 2014). Water changes, conducted immediately after weighing and photographing, involved replacing the flask with filtered, UV-treated, and autoclaved seawater pre-adjusted to the desired temperature. After establishing suitable cultivation protocols, the experimental biomass was gradually scaled from 250 ml flasks to a 150 L cylindrical fiberglass tank

Data analysis

Differences in SGR between days 0 and 28 for temperature, photoperiod, irradiance, and nutrient sources were analyzed using one-way ANOVAs. Analyses were conducted after confirmation of all assumptions. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was checked with Bartlett's test. Welch's one-way ANOVA was employed when assumptions were not met. Significance was set at $\alpha = 0.05$, and post-hoc analyses included Tukey's HSD, Games – Howell test, and pairwise t-tests with Bonferroni corrections to minimize type I error. Line graphs were generated to visualize changes in weekly SGR per factor and species. All statistical analyses were conducted using R Studio (version 4.1.3)

Results

D. mollis

The effect of temperature on SGRs for *D. mollis* differed depending on the photoperiod (Table S1 and S2; Figure 4). For this species, SGRs were positive at all temperatures tested, with

significantly higher SGRs of approximately 9.8% per day on average at 8 and 12 °C on 16L:08D (Tukey's HSD $p < 0.05$; Figure 4, Table S2). No significant differences were found at 12L:12D, where SGRs held at approximately 11% daily (Tukey's HSD $p < 0.05$; Figure 4, Table S1). SGRs for all temperatures were below 4% when thalli were grown on 8L:16D (Figure 4).

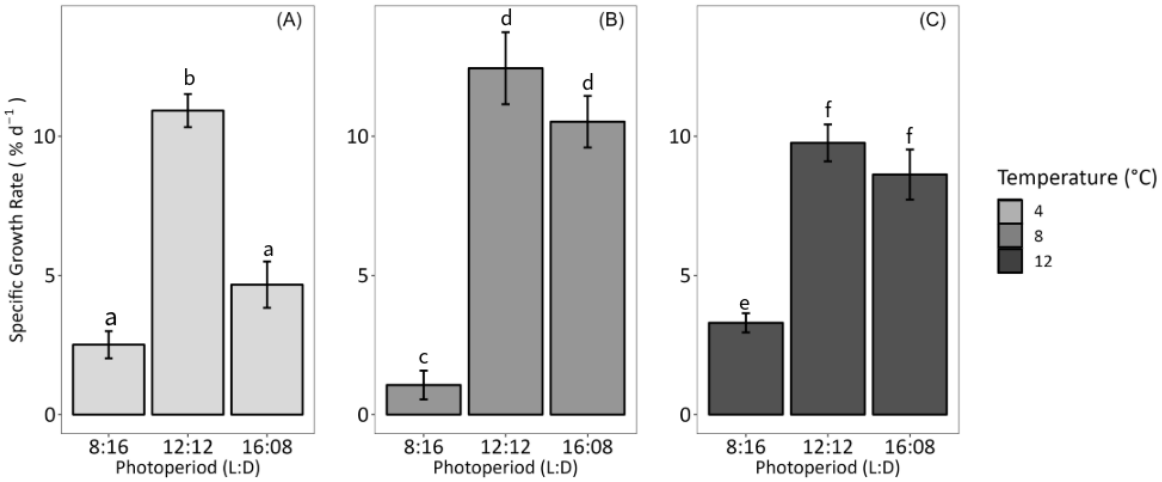

Figure 4. (A) Specific growth rate of *Devaleraea mollis* as a function of temperature (4, 8, and 12 °C) and photoperiod (16:8, 12:12, and 8:16) grown with 40 µmol photons m^{-2} s^{-1} and F/2 as the nutrient source. (B) Irradiance (20, 40, and 100 µmol photons m^{-2} s^{-1} grown at a temperature of 8 °C, 16L:8D, with F/2. (C) Nutrient source (VSE, F/2 and JS) grown at 8 °C, 16L:8D, and 40 µmol photons m^{-2} s^{-1}, with F/2. Seaweeds were acclimated for two weeks, followed by a 28-day data collection. Data shows mean values ± SE. Significance is denoted by letters.

SGR responses to photoperiod varied with temperature ($p < 0.05$; Table S3). SGRs were always significantly higher at 16L:8D and 12L:12D compared to 8L:16D regardless of the temperature (Tukey's HSD $p < 0.001$, Table S4). The photoperiod of 12L:12D and 16L:8D only differed at 4 °C, with significantly higher SGRs measured at 12L:12D (Tukey's HSD $p < 0.001$, Table S4). Overall, SGRs at 12L:12D were above 9 % per day across all temperatures. SGRs at 16L:8D showed a similar trend at 8 and 12 °C but reduced to ~5 % at 4 °C, while SGRs at 8L:16D never exceeded 4% regardless of the temperature.

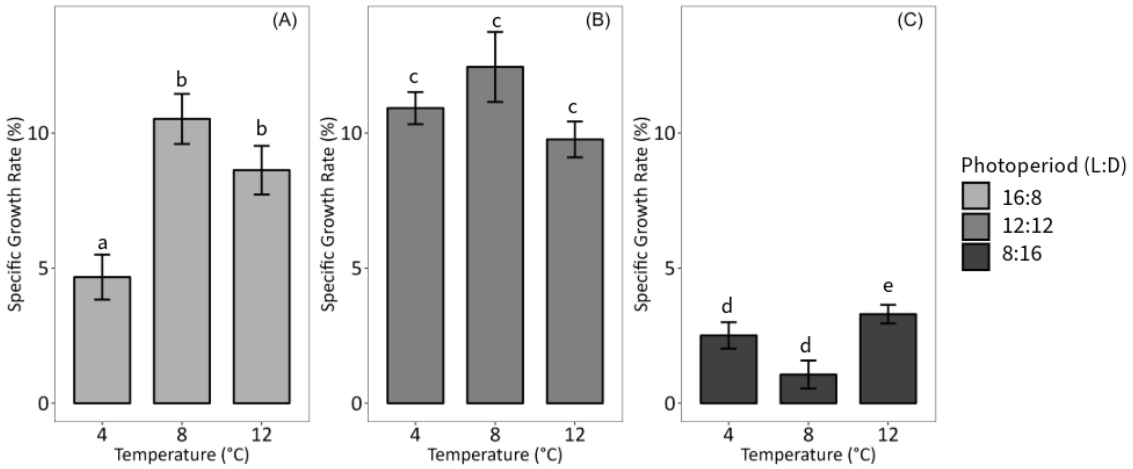

Figure 5. Specific growth rate of *Devaleraea mollis* as a function of photoperiod (16:8, 12:12, and 8:16) and temperature (4, 8, and 12 °C) grown with 40 µmol photons m^{-2} s^{-1} and F/2 as the nutrient source. Seaweeds were acclimated for two weeks, followed by a 28-day data collection. Data shows mean values ± SE. Significance is denoted by letters.

As with temperature and photoperiod, growth significantly differed based on the irradiance level utilized in combination with 8 °C and 16L:8D ($p < 0.001$; Figure 6A; Table S10). SGR was higher on samples exposed to an irradiance of 100 µmol photons m^{-2} s^{-1} (Tukey's HSD $p < 0.0001$ Table S10), followed by 40 µmol photons m^{-2} s^{-1} and 20 µmol photons m^{-2} s^{-1}, which were also significantly different between them (Tukey's HSD $p < 0.01$; Table S10). Biomass exposed to 100 µmol photons m^{-2} s^{-1} showed signs of fertility (i.e., change in thalli color and texture) in week 2, followed by a spore release in week 3, which led to a 9 % reduction of SGR on the week (see Figure S1).

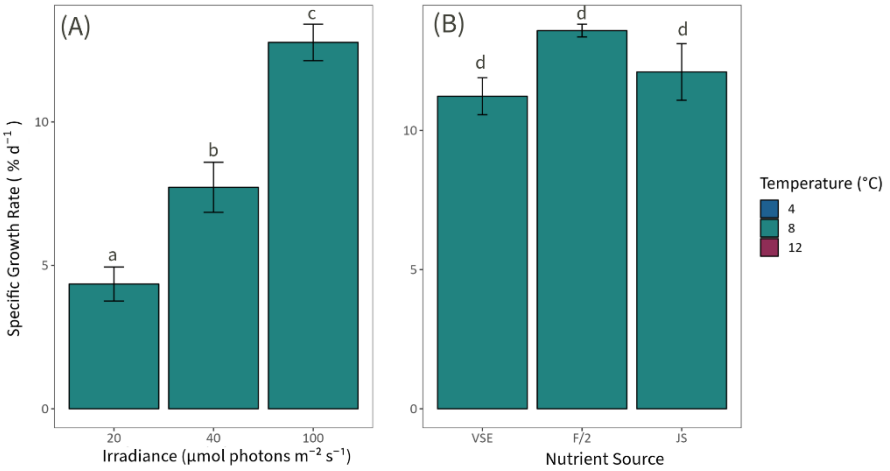

Figure 6. Specific growth rate of *Devaleraea mollis* as a function of (A) irradiance (20, 40, and 100 µmol photons m-2 s-1) with 16L:8D, 8 °C, and F/2 as the nutrient source. (B) SGR as a function of nutrient source, with 8 °C, 16L:8D and 40 µmol photons m^{-2} s^{-1}. Seaweeds were acclimated for two weeks, followed by a 28-day data collection. Data shows mean values ± SE. Significance is denoted by letters.

Biomass SGRs were close to significance based on nutrient sources (F = 5.726, df = 2; p = 0.03; Figure 6B; Table S11), with SGRs slightly higher for thalli exposed to F/2 than JS and VSE. In contrast with growth metrics, qualitative data showed clear coloration differences depending on the nutrient source utilized (Figure 6B). Biomass nutrified with VSE appeared pale, while those grown with JS and F/2 showed a dark red color (Figure 6B).

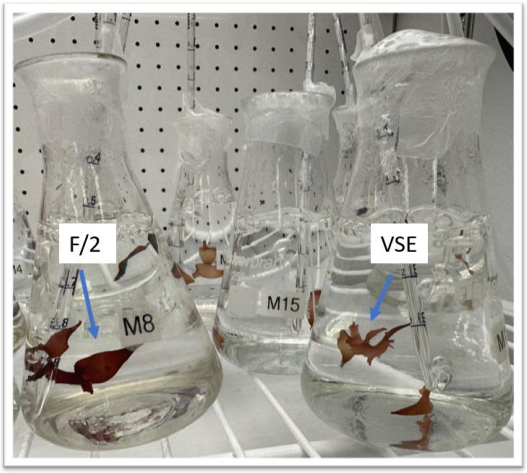

Figure 7. Experimental *Devaleraea mollis* nutrified with F/2 (left flask) and VSE (right flask) showing differences in biomass coloration.

3.1.2 *P. hecatensis*

Growth was observed at all temperatures tested (Figure 8). However, its suitability to sustain positive SGRs varied depending on the photoperiod set (Figure 8; Table S5). Specifically, thalli exposed to 4 and 8 °C showed the most growth compared to 12 °C at a photoperiod of 16L:8D (HSD $p < 0.05$; Figure 8 A & B). A temperature 8 °C promoted significantly higher SGRs than 4 and 12 °C in 12L:12D photoperiod (Tukey's HSD $p < 0.05$; Figure 8A & B). No significant differences were observed in 8L:16D (Figure 8C). Generally, thalli grown at 12 °C showed signs of contamination more often than any other temperature treatment, regardless of the photoperiod

used. SGRs of thalli at 8 °C ranged from approximately 9 to 3.9 % per day on average in long and neutral day-length scenarios (Figure 8A). While 4 °C was conducive for growth at photoperiods, it only sustained SGRs comparable to 8 °C (i.e., 10% per day) when combined with a long-day photoperiod (Figure 8A). At a neutral and short-day photoperiod, SGRs of thalli exposed to 4 °C dropped to approximately 0.3-1.5 % per day (Figure 9B and C).

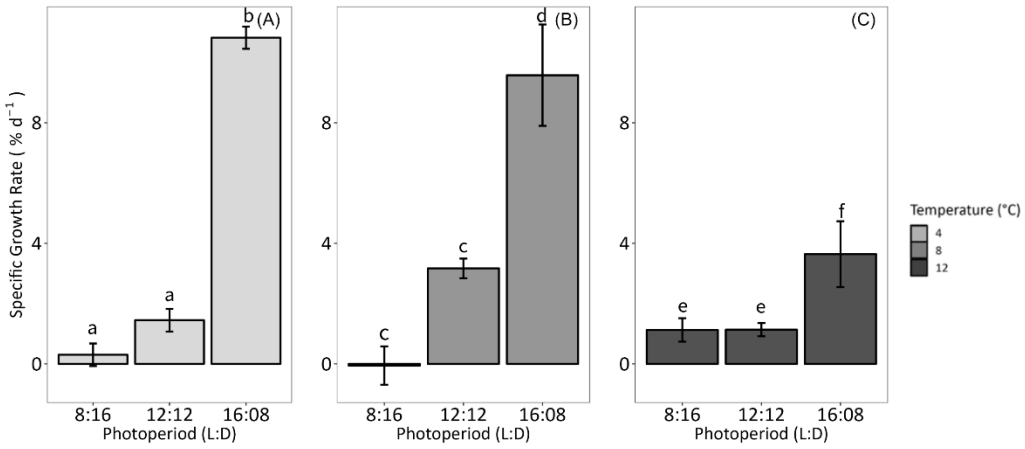

Figure 8. Specific growth rate of *Palmaria hecatensis* as a function of temperature (4, 8, and 12 °C) and photoperiod (16:8, 12:12, and 8:16) grown with 40 µmol photons m^{-2} s^{-1} and F/2 as the nutrient source. Seaweeds were acclimated for two weeks, followed by a 28-day data collection. Data shows mean values ± SE. Significance is denoted by letters.

Similarly, SGR responses to photoperiods varied with temperature ($p < 0.05$; Table S7). Overall, 16L:8D promoted significantly more growth than any other photoperiod (Tukey's HSD $p < 0.001$; Figure 9A; Table S8). However, growth decreased from approximately 9 % per day on average at 4 and 8 °C (Figure 9) to about 4 % at 12 °C. Growth at the short and neutral photoperiods did not exceed 3.9 % per day, regardless of the temperature.

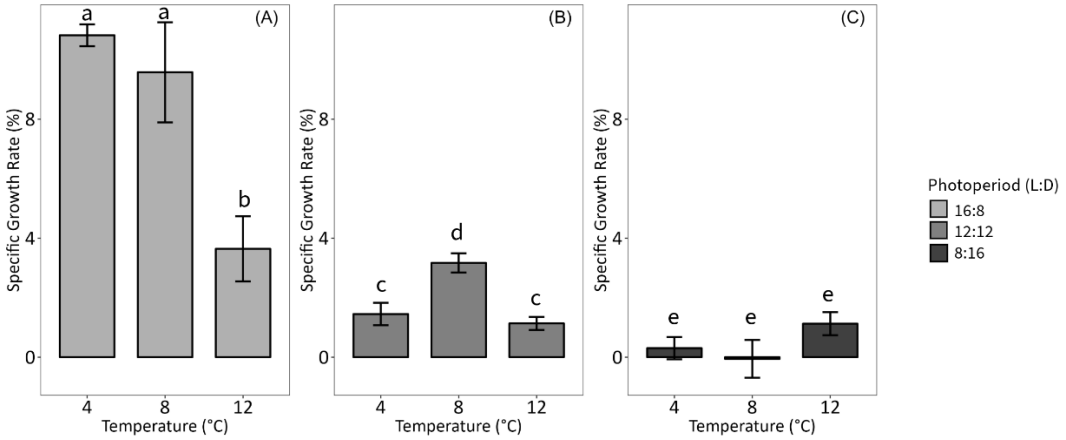

Figure 9. Specific growth rate of *Palmaria hecatensis* as a function of photoperiod (16:8, 12:12, and 8:16) and temperature (4, 8, and 12 °C) grown with 40 µmol photons m^{-2} s^{-1} and F/2 as the nutrient source. Seaweeds were acclimated for two weeks, followed by a 28-day data collection. Data shows mean values ± SE. Significance is denoted by letters.

Similar to *D. mollis*, irradiance showed significant differences in SGRs ($F = 22.28$, $df = 2.00$, $p < 0.001$; Figure 10A; Table S12). SGR was higher on samples exposed to 100 µmol photons m^{-2} s^{-1} (Tukey's HSD $p < 0.001$; Table S12), followed by 40 µmol photons m^{-2} s^{-1} (Tukey HSD $p < 0.05$; Table S12) which was significantly higher than 20 µmol photons m^{-2} s^{-1} (Tukey HSD $p < 0.001$; Table S12). No signs of fertility or spore release were observed for any level tested.

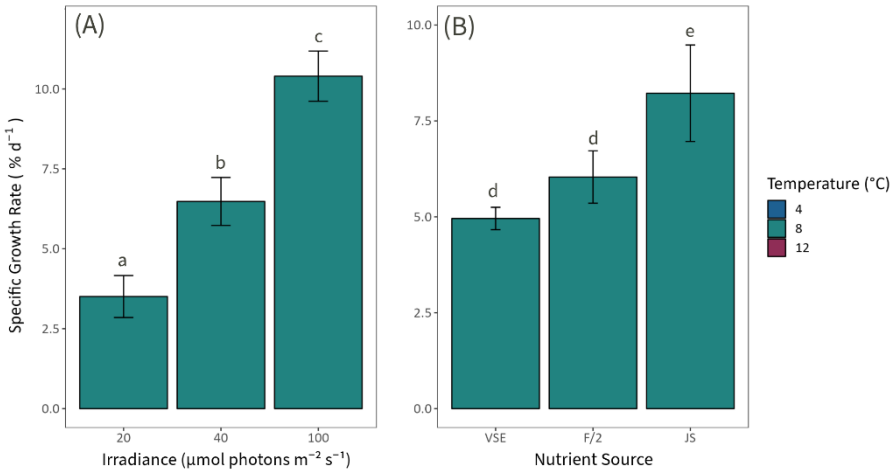

Figure 10. Specific growth rate of *Devaleraea mollis* as a function of irradiance (20, 40, and 100 µmol photons m^{-2} s^{-1}) with 40 µmol photons m^{-2} s^{-1} and F/2 as the nutrient source. Seaweeds were acclimated

for two weeks, followed by a 28-day data collection. Data shows mean values ± SE. Significance is denoted by letters.

No differences were observed based on nutrient sources (Figure 10). However, SGRs were slightly higher on biomass exposed to JS, with values close to significance (Bonferroni's p = 0.054; Table 12). In contrast with *D. mollis*, biomass did not show clear differences in their coloration. All samples remained dark red throughout the experiment.

Discussion

Species diversification is essential to enhance the long-term viability, competitiveness, and sustainability of the seaweed farming industry in the Pacific Northwest of the United States. By farming various species besides kelp and employing different practices at sea and on land, the industry can possibly reduce its potential vulnerability to disease (Loureiro et al., 2015), market fluctuations (Bindu et al., 2010), and environmental changes (Redmond et al., 2014). This approach also increases opportunities to mitigate risks, enhance economic stability, and promote environmental sustainability by reducing current pressure on wild stocks used as seed sources (Huang et al., 2022).

The observed differences in SGRs due to temperature and photoperiod, as well as responses to irradiance between D. mollis and P. hecatensis, suggest distinct ecological and physiological adaptations despite their similar life histories and habitats. *D. mollis* showed higher SGRs in warmer temperatures, indicating that it may be more thermally tolerant or even thrive in warmer conditions. This supports its large geographical distribution compared to *P. hecatensis* (Skripsova et al., 2023). *P. hecatensis* does not show the same level of increased growth at higher temperatures, suggesting it may have a different thermal optimum to be considered when optimizing the protocols presented here. This could mean *P. hecatensis* is better adapted to cooler environments or has a narrower thermal tolerance range, preventing its dispersion to lower latitudes exposed to warmer temperatures. Moreover, higher irradiance levels triggered spore release in *D. mollis* but not in *P. hecatensis* at the combination tested. This may indicate a different reproductive strategy or sensitivity to light. The lack of spore release in *P. hecatensis*

suggests that it may have a different trigger for reproduction or require even higher light levels than *D. mollis.*

The overall SGRs reported for *D. mollis* here are consistent with those described for *D. mollis* produced in Hawaii and Oregon utilizing flow-through and tumbling setups (Demetropoulos et al., 2004a; Demetropoulos et al., 2004b). In those studies, growth was observed between 12 °C and 17 °C, with SGRs peaking at 14 °C and achieving an average daily increase of 10% (± 0.5). these results, which highlight the striking difference between the suitable temperature ranges of Alaskan D. mollis and those from lower latitudes. While Alaskan *D. mollis* grew at 12 °C, filamentous contaminants frequently proliferated. It is presumed that these contaminants were introduced to the system as endophytes and, therefore, are unlikely to be avoided.

Interestingly, a 12L:12D photoperiod regime is recommended for culture storage for *D. mollis* (Evans, 2000). However, our results support that this photoperiod can be used for biomass production, as SGRs were similar between 16L:8D and 12L:12D. This outcome contrasts with other studies on *P. palmata* and *D. mollis,* showing a long-day photoperiod promoting the most growth (Demetropoulos et al., 2004b; Pang et al., 2004). A neutral photoperiod at any of the temperatures tested may be used to store cultures of *P. hecatensis.*

D. mollis exhibited a daily SGR of 12% ± 1 on average when exposed to 100 µmol photons m^{-2} s^{-1}, combined with a temperature of 8 °C and a photoperiod of 16L:8D. Such SGRs are consistent with studies conducted on ecotypes from lower latitudes (Demetropoulos et al., 2004a; Demetropoulos et al., 2004b; Parjikolaei, 2013). However, continuous exposure to such irradiance may be unsuitable for cultures with relatively low initial biomass, as spore releases followed by tissue degradation may decrease the efficiency of biomass production. While spore release is well-documented for *P. palmata*, it is relatively understudied for *D. mollis*. It is likely that higher stocking densities of 3-4 kg per m^{-2}, as described for on-land cultures in Hawaii, could mitigate the impact of high irradiance on the reproductive cycle of *D. mollis* (Demetropoulos et al., 2004b). Until that is tested, and full factorial experiments are completed for the three factors tested here, 40 µmol photons m^{-2} s^{-1} seem to be the most suitable irradiance to promote continuous growth while supporting healthy biomass. An alternative to using lower

irradiance levels could be utilizing opaque tanks with high light levels only permeating the surface of the tank (Demetropoulos et al., 2004b).

In contrast with *D. mollis*, *P. hecatensis* showed limited growth when exposed to a 8L:16D regime, particularly at lower temperatures. With only 8 hours of light, *P. hecatensis* individuals did not have enough energy to sustain growth throughout the 24-hour cycle, leading to an energy deficit. At lower temperatures, metabolic rates typically decrease, reducing the efficiency of photosynthesis and other physiological processes (Harrison & Hurd, 2001). This means that to compensate for lower metabolic rates *P. hecatensis* would require more light to achieve the same growth as it would at higher temperatures. Thalli of *P. hecatensis* have a leathery texture that resembles that of kelp, contrasting the more leafy texture of *D. mollis* (per.obs.). The relatively lower SGR observed on the short-day photoperiod compared to *D. mollis* may be linked to the thickness of the thalli requiring more energy to grow (Hawke, 1986). However, to our knowledge, this aspect has not been explored.

Previous studies on *D. mollis* co-cultured with abalone showed that supplementation with nitrate promotes more growth in the long term (i.e., nine weeks). In comparison, ammonium is more effective in 2-5 weeks (Demetropoulos *et al.,* 2004b). Such results partially align with our finding where the addition of VSE (with nitrate as the source of nitrogen) showed suboptimal biomass performance compared to those supplemented with F/2 (also with nitrate as a source of nitrogen) and JS (with ammonium and nitrate as a source of nitrogen). Although differences in SGR seem marginally related to the nitrogen source, protein content was higher on thalli supplemented with JS. Up-taking and utilizing nitrate is costly, as energy is needed to assimilate nitrate via ammonium (Jin *et al.,* 1998; Cohen and Fong, 2004). The addition of ammonium (NH_4^+) likely could contribute to higher protein content by direct incorporation into amino acids, potentially facilitated by ammonium transporters increasing rapid uptake into cells (Lobban and Harrison, 1994; Kim *et al.,* 2012; Jung *et al., submitted*).

Furthermore, the observed differences in blade coloration over time show the direct effect of nutrient supplementation on seaweed assimilation and storage capacity. *D. mollis* blades were darker in coloration, potentially from a higher concentration of pigments at the end of the

experiment when grown with F/2 and JS. Jung et al. (submitted) experimented further, finding that increased pigment concentration was present when *D. mollis* was exposed to F/2 and JS. Still, this trend did not remain valid for VSE. Seaweed can store excess nitrogen in pigments and other compounds during nutrient-replete events for future growth (McGlathery et *al.*, 1996; Naldi and Wheeler, 1999). Jung et al. (submitted) outputs indicate that the differences observed due to nutrient supplementation cannot be explained based on coloration and nitrate alone. Another contributing factor could be the presence of trace metals such as zinc and copper. Both metals are essential for photosynthesis, cellular metabolism, and growth and, therefore, merit further exploration into their effect on biomass performance (Jung et al., *submitted*; Howarth and Cole 1985; Demetropoulos and Langdon 2004b).

Finally, *P. hecatensis* might likely have different nutrient uptake mechanisms or efficiencies than *D. mollis* (Harrison & Hurd, 2001). This is a topic worth exploring further. Similar to *D. mollis*, phycobiliproteins in thalli exposed to F/2 and JS have shown significantly higher concentrations compared to VSE after 14 days of exposure (Jung et al. 2024). Such outcomes suggest that F/2 and JS formulations likely provide a more enriched nutrient supply, perhaps with a more favorable balance of micro and macronutrients, aside from nitrogen, compared to VSE. This could be responsible for enhancing overall metabolic activity, including synthesizing phycobiliproteins, which are key for light capture and photosynthesis, and hence, growth.

Conclusions

Outputs from this study allow the creation of a discrete list of suitable cultivation protocols that can be refined further to obtain optimal conditions to maximize the growth of *D. mollis* and *P. hecatensis* grown in on-land indoor systems. Although a pairwise approach was used here, optimizing these cultivation protocols would require a full factorial approach. Such an approach would allow the investigation of the interaction effects between the multiple factors tested. In turn, this would provide a deeper understanding of how different variables influence each other and the outcome. It would also provide broader insight into the experimental conditions by simultaneously examining all possible combinations of factors.

Overall, the required adjustments on the environmental parameters to grow *D. mollis* in on-land indoor settings and the differences measured between the *D. mollis* and *P. hecatensis* highlight the relevance of tailoring cultivation protocols based on seedstock origin. Our findings also highlight the importance of considering targeted seaweed species' specific nutritional and storage needs to optimize productivity and quality.

In brief, suitable combinations of temperature, photoperiod, and irradiance to grow *D. mollis* include 8 °C with a photoperiod of 16L:8D or 12L:12D. While a photoperiod of 8L: 16D at 4, 8 or 12 °C and irradiance of 20 and 40 µmol photons m^{-2} s^{-1} could be used for storage. Further optimization should explore 12L:12D at any of the temperatures tested and 16L:8D at 8 °C and 12 °C. On the other hand, priority should be placed on 4 and 8 °C with a 16L:8D photoperiod to grow P. hecatensis, with further protocol optimization comparing these combinations with multiple irradiance levels.

Moreover, further research is essential to understand the nitrogen storage mechanisms and protein compositions of *P. hecatensis*. This understanding could lead to a more efficient use of nutrient sources to increase biomass production. In addition, understanding species-specific responses to nutrient supplementation is also valuable for ecological studies in the context of changing environmental conditions, particularly in rapid warming environments such as those experienced in the Gulf of Alaska, where both species thrive. Finally, the recent range expansion of *P. hecatensis* along the east coast of Russia and Japan may increase the potential for the commercial cultivation of the species (Skriptsova et al., 2023). Therefore, additional research on its ecology and reproductive phenology could greatly improve cultivation practices.

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Supplementary material

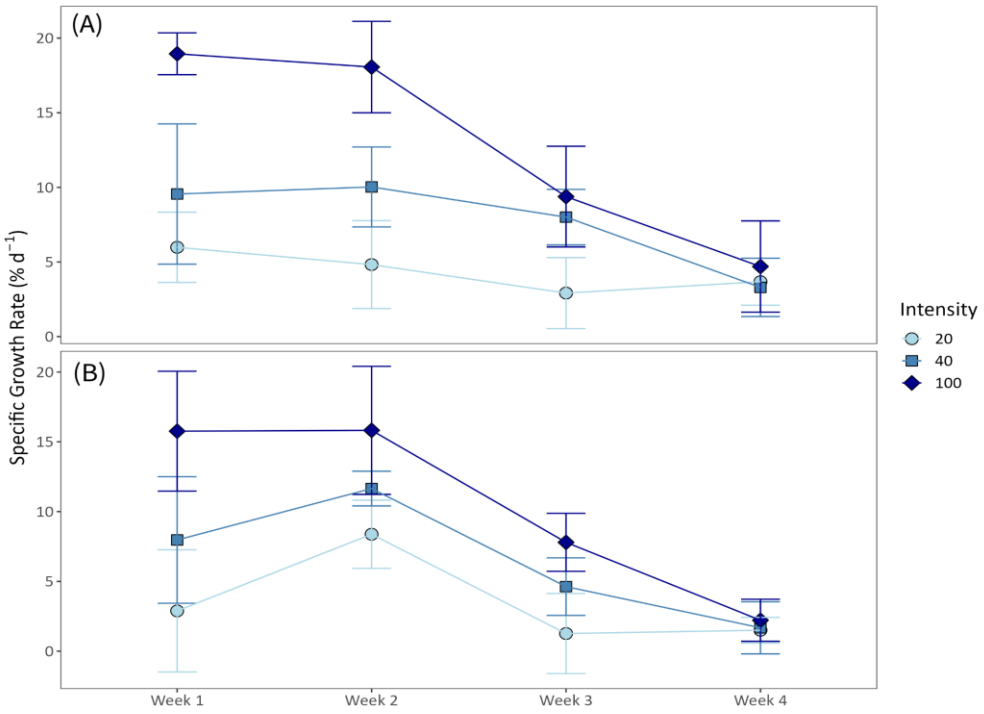

Figure S1: Weekly specific growth rates as a function of irradiance in µmol photons m^{-2} s^{-1} for (A) *Devaleraea mollis* and (B) *Palmaria hecatensis*. Cultures were grown at 8 °C with a 16L:8D, with F/2 for nutrients. Data shows mean values ± w SD.

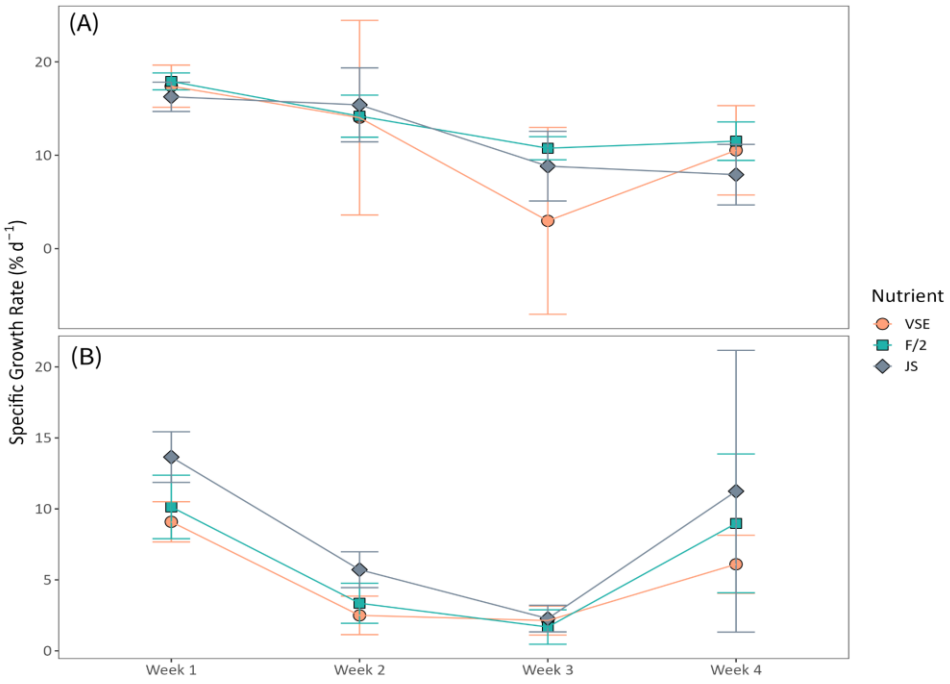

Figure S2: Weekly specific growth rates as a function of nutrient source for (A) *Devaleraea mollis* and (B) *Palmaria hecatensis*. Cultures were grown at 8 °C, 16L:8D, and 40 µmol photons m^{-2} s^{-1} for *Devaleraea mollis* and 100 µmol photons m^{-2} s^{-1} for *Palmaria hecatensis*. Data shows mean values ± w SD.

Table S1. One-way ANOVA for the effect photoperiod (8L:16D, 12L:12D, and 16L:8D) on specific growth rates of *Devaleraea mollis* at three different temperatures (4, 6, and 12 °C).

Temperature: 4 °C					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Photoperiod	2	190.97	95.48	44.56	2.79e-06
Residuals	12	25.71	2.14		
Temperature: 8 °C					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Photoperiod	2	371.3	185.67	39.71	5.11e-06
Residuals	12	56.1	4.68		
Temperature: 12 °C					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Photoperiod	2	119.19	59.59	26.12	4.25e-05
Residuals	12	27.38	2.28		

Table S2. Tukey's HSD examining differences in the specific growth rates of *Devaleraea mollis* as a function of photoperiod (8L:16D, 12L:12D, and 16L:8D) at three different temperatures (4, 6, and 12 °C).

Temperature 4 °C				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
16:08 - 12:12	-6.25513	-8.724965	-3.785296	0.0000558
8:16 - 12:12	-8.413918	-10.883752	-5.944084	0.0000028
8:16 - 16:08	-2.158787	-4.628621	0.311047	0.0894072
Temperature 8 °C				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
16:08 - 12:12	-1.922669	-5.571009	1.72567	0.3687246
8:16 - 12:12	-11.383925	-15.032265	-7.735586	0.0000069
8:16 - 16:08	-9.461256	-13.109596	-5.812916	0.0000443
Temperature 12 °C				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
16:08 - 12:12	-1.138911	-3.687596	1.409774	0.4798067
8:16 - 12:12	-6.467232	-9.015917	-3.918547	0.0000547
8:16 - 16:08	-5.328321	-7.877006	-2.779636	0.0003276

Table S3. One-way ANOVA for the effect photoperiod (8L:16D, 12L:12D, and 16L:8D) on specific growth rates of *Devaleraea mollis* at three different temperatures (4, 6, and 12 °C).

Photoperiod: 8L:16D					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Temperature	2	12.86	6.428	6.149	0.0145 *
Residuals	12	12.54	1.045		
Photoperiod: 12L:12D					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Temperature	2	18.08	9.042	2.193	0.154
Residuals	12	49.47	4.123		
Photoperiod: 16L:8D					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Temperature	2	89.22	44.61	11.35	0.00171
Residuals	12	47.18	3.93		

Table S4. Tukey's HSD examining differences in the specific growth rates of *Devaleraea mollis* as a function of photoperiod (8L:16D, 12L:12D, and 16L:8D) at three different temperatures (4, 6, and 12 °C).

Photoperiod: 8L:16D				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
8 - 4	-1.4479802	-3.1731693	0.2772089	0.1045146
12 - 4	0.7873909	-0.9377982	2.51258	0.4657695
12 - 8	2.2353711	0.510182	3.9605602	0.0122077
Photoperiod: 12L:12D				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
8 - 4	1.522027	-1.903974	4.9480294	0.4836696
12 - 4	-1.159295	-4.585297	2.2667071	0.6488754
12 - 8	-2.681322	-6.107324	0.7446797	0.1341397
Photoperiod: 16L:8D				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
8 - 4	5.854489	2.5089703	9.200007	0.0014541
12 - 4	3.956924	0.6114061	7.302443	0.0209804
12 - 8	-1.897564	-5.2430826	1.447954	0.3194977

Table S5. One-way ANOVA for the effect of temperature (4, 6, and 12 °C) on specific growth rates of *Palmaria hecatensis* at three different photoperiods (8L:16D, 12L:12D, and 16L:8D).

Photoperiod: 8L:16D					
Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Temperature	2	3.677	1.838	1.585	0.245
Residuals	12	13.917	1.16		
Photoperiod: 12L:12D					
Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Temperature	2	11.987	5.993	12.13	0.00132
Residuals	12	5.931	0.494		
Photoperiod: 16L:8D					
Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Temperature	2	147.25	73.63	10.62	0.00222
Residuals	12	83.21	6.93		

Table S6. Tukey's HSD examining differences on the specific growth rates of *Palmaria hecatensis* as a function of temperature (4, 6, and 12 °C) at three different photoperiods (8L:16D, 12L:12D, and 16L:8D).

Photoperiod: 8L:16D				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
8-4	-0.3584182	-2.1755284	1.458692	0.8601384
12-4	0.8241112	-0.992999	2.641221	0.4699841
12-8	1.1825294	-0.6345808	2.99964	0.2323067
Photoperiod: 12L:12D				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
8-4	1.7187882	0.532608	2.9049685	**0.0058777**
12-4	-0.3154957	-1.501676	0.8706845	0.7625709
12-8	-2.0342839	-3.220464	-0.8481037	**0.0017044**
Photoperiod: 16L:8D				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
8-4	-1.244443	-5.687611	3.198726	0.7409608
12-4	-7.180756	-11.62394	-2.737588	**0.0026846**
12-8	-5.936314	-10.379482	-1.493145	**0.0100648**

Table S7. One-way ANOVA for the effect photoperiod (8L:16D, 12L:12D, and 16L:8D) on specific growth rates of *Palmaria* hecatensis at three different temperatures (4, 6, and 12 °C).

Temperature: 4 °C					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Photoperiod	2	333.1	166.5	239.1	2.15E-10
Residuals	12	8.4	0.7		
Temperature: 8 °C					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Photoperiod	2	240.5	120.25	21.6	0.000105
Residuals	12	66.8	5.57		
Temperature: 12 °C					
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
Photoperiod	2	21.02	10.511	4.52	0.0344
Residuals	12	27.91	2.325		

Table S8. Tukey's HSD examining differences in the specific growth rates of *Palmaria hecatensis* as a function of photoperiod (8L:16D, 12L:12D, and 16L:8D) at three different temperatures (4, 6, and 12 °C).

Temperature 4 °C				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
16:08 - 12:12	9.372095	7.964074	10.7801154	0
8:16 - 12:12	-1.148417	-2.556438	0.2596038	0.1161019
8:16 - 16:08	-10.520512	-11.928532	-9.1124908	0
Temperature 8 °C				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
16:08 - 12:12	6.408864	2.427966	10.3897619	0.0027633
8:16 - 12:12	-3.225623	-7.206521	0.7552747	0.1188724
8:16 - 16:08	-9.634487	-13.615385	-5.6535892	0.000086
Temperature 12 °C				
Comparison	**Difference**	**Lower CI (lwr)**	**Upper CI (upr)**	**Adjusted p-value (p adj)**
16:08 - 12:12	2.506834247	-0.06621531	5.0798838	0.0563951
8:16 - 12:12	-0.008810131	-2.58185969	2.56423943	0.999954
8:16 - 16:08	-2.515644378	-5.08869394	0.05740518	0.0555014

Table S9a and b: One-way ANOVA for irradiance experiments for *D. mollis* (a), with a pairwise comparison post hoc analysis (b).

(a)	df	Sum Sq	Mean Sq	F value	p-value (α < 0.05)*
Irradiance	2	179.64	89.82	35.54	< 0.001*
Residuals	12	30.33	2.53	-	-

(b)	estimate	SE	df	t.ratio	p-value (α < 0.05)*
20 - 40 irradiance	-3.37	1.01	12	-3.53	0.0147*
20 - 100 irradiance	-8.42	1.01	12	-8.37	< 0.0001*
40 - 100 irradiance	-5.05	1.01	12	-5.02	0.0008*

Table S10a and b: One-way ANOVA for irradiance experiments for *P. hecatensis* (a), with a pairwise comparison post hoc analysis (b).

(a)	df	Sum Sq	Mean Sq	F value	p-value (α < 0.05)*
Irradiance	2	119.54	59.77	22.28	< 0.001*
Residuals	12	32.19	2.68	-	-

(b)	estimate	SE	df	t.ratio	p-value (α < 0.05)*
20 - 40 irradiance	-2.97	1.01	12	-2.87	0.0349
20 - 100 irradiance	-6.89	1.01	12	-6.65	< 0.0001*
40 - 100 irradiance	-3.92	1.01	12	-3.78	0.0068*

Table S11: Welch's One-way ANOVA for the nutrient source experiments for *D. mollis* (a), with a pairwise t - test with Bonferroni correction (b).

(a)	df	F value	p-value ($\alpha < 0.05$)*
Nutrient Source	2	5.72	0.039*

(b)	VSE	F/2
F/2	0.11	-
JS	1.00	0.50

Table S12: Welch's One way ANOVA for the nutrient source experiments for *P. hecatensis*, with a pairwise t - test with Bonferroni correction (b).

(a)	df	F value	p-value ($\alpha < 0.05$)*
Nutrient Source	2	3.58	0.09

(b)	VSE	F/2
F/2	1.00	-
JS	0.054	0.276