



Identifying the thermal optima for physiological performance and bromoform production of the red alga *Asparagopsis taxiformis*

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Abstract

The red alga *Asparagopsis taxiformis* has potential for methane mitigation in ruminant animals due to its high concentration of halogenated methane analogs. However, large scale production of *A. taxiformis* is constrained by intensive cultivation requirements. Additionally, the broad geographic distribution and several genetically distinct lineages of this seaweed require that cultivation strategies be tailored to a particular region. To address these limitations, we conducted two sequential experiments to investigate the thermal optima of a Lineage 2 strain of *A. taxiformis*. In the first experiment, cultures from Santa Catalina Island (Channel Islands, CA, USA) and La Jolla (San Diego, CA, USA) were exposed to temperatures from 13–30 °C to compare physiological responses (growth rates, maximum quantum yield of photosystem II (PSII), and bromoform production) across geographic locations. No significant differences between locations were observed, leading us to conduct a second experiment to test whether pre-acclimation to environmental variability improved thermal tolerance, comparing indoors versus outdoor cultivated cultures across an expanded temperature range of 8–33 °C. The outdoor strain of *A. taxiformis* was able to survive across a wide thermal range, with the highest growth rates, PSII photosynthetic yields, and bromoform concentrations observed at 23 °C. Previous exposure to environmental variability showed potential for acclimation to elevated temperatures, with biomass increasing by 11% per day, accompanied by bromoform concentrations reaching roughly 3% of total dry weight (30 mg g⁻¹). These findings provide critical insight into optimizing culture conditions and advancing the commercialization of *A. taxiformis* as a methane mitigation strategy in ruminants.

Keywords *Asparagopsis taxiformis* · Rhodophyta · Aquaculture · Livestock · Methane · Seaweed

Introduction

Methane (CH₄) is one of the most potent greenhouse gases (GHG) and is a significant contributor to global warming. While CH₄ accounts for a smaller fraction of total anthropogenic GHG emissions compared to carbon dioxide (CO₂), its global warming potential (GWP) is approximately 27 times greater than that of CO₂ over a 100-year time period (Forster et al. 2021). Additionally, CH₄ has a relatively short atmospheric lifespan of twelve years compared to the century-scale persistence of CO₂ (Forster et al. 2021). This combination of high radiative efficiency and short atmospheric residence time indicates that CH₄ emission reductions could be an

immediate and measurable climate mitigation strategy (Mar et al. 2022). A substantial portion of anthropogenic CH₄ emissions originates from the agricultural sector, particularly from ruminant livestock which accounts for approximately 32% of global anthropogenic CH₄ emissions (Ravishankara et al. 2021). In fact, the agricultural sector contributes more to GHG emissions than the global transportation industry (cars, trucks, trains, planes and ships) (Steinfeld et al. 2006). This is primarily due to the ruminants' digestive process, enteric fermentation, in which plant carbohydrates (C₆H₁₂O₆) are anaerobically fermented by a microbial community in the rumen. During this process hydrogen (H₂), CO₂, and volatile fatty acids (VFAs) are produced via the following reaction: C₆H₁₂O₆ → CO₂ + H₂ + VFAs (Morgavi et al. 2010). VFAs are readily absorbed into the bloodstream and serve as the primary energy source for ruminants. H₂ and CO₂ are produced as byproducts and can accumulate inside the rumen. If concentrations exceed a critical threshold, they can inhibit key microbial enzymes essential for

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fermentation, particularly those involved in electron transfer reactions (Moss et al. 2000; Morgavi et al. 2010). To mitigate this, a microbial interspecies H_2 transfer occurs as a regulatory mechanism to maintain low partial pressure of H_2 within the rumen environment (Moss et al. 2000; Morgavi et al. 2010). The primary H_2 -consumption pathway is hydrogenotrophic methanogenesis in which methanogens (methanogenic archaea) utilize H_2 to reduce CO_2 into CH_4 and water (H_2O) (Greening et al. 2019). This results in the release of CH_4 into the atmosphere via ruminant eructation (e.g., burps), creating one of the largest anthropogenic CH_4 sources globally.

Asparagopsis taxiformis is a red alga of the order Bonnemaisoniales (Guiry and Guiry 2025). Phylogenetic analyses of the *cox2–3* marker have identified six genetically distinct lineages distributed across tropical and subtropical regions around the world (Andreakis et al. 2007, 2016; Sherwood 2008; Dijou et al. 2014). Our study focuses on lineage 2, a subtropical lineage primarily known to have the broadest geographic distribution, found throughout the North Atlantic, Indo-Pacific, Mediterranean Sea, and South Africa (Kurihara et al. 2016). Ruminant dietary supplementation with *A. taxiformis* has been shown to reduce enteric methane emissions by up to 99% in vitro using rumen microbiota populations (Kinley et al. 2016). Further, Roque et al. (2021) found that adding *A. taxiformis* to the diet of live steers had up to an 80% reduction in CH_4 production while also reducing dry matter intake and increasing feed conversion efficiency. Comparative studies by Machado et al. (2014) screened the CH_4 reduction potential of over 25 species of macroalgae and identified that *A. taxiformis* was the most effective methane-reducing taxon far surpassing other red, brown, and green freshwater and marine algae.

The methane-mitigating potential of *A. taxiformis* is attributed to its production of secondary metabolites, including halogenated compounds (Machado et al. 2016). These compounds are commonly found in marine macroalgae and are hypothesized to serve as chemical defenses against herbivory and microbial colonization due to their toxicity (Paul et al. 2006). Within *A. taxiformis* these secondary metabolites are stored in specialized inclusions commonly referred to as “gland cells” that exist inside the pericentral cells which help the alga avoid autotoxicity. The gland cells are structurally connected to the walls of the pericentral cells, facilitating the controlled release of metabolites into the surrounding environment (Paul et al. 2006).

Among these compounds, bromoform ($CHBr_3$) is the most abundant and is hypothesized to be one of the primary compounds responsible for methane inhibition in ruminants. Bromoform concentrations in *A. taxiformis* can exceed 5% of the total algal dry weight, which is significantly higher than in other marine algae (Thapa et al. 2020). Once ingested, bromoform is released into the rumen where it inhibits the

Wolfe Cycle: the enzymatic pathway during methanogenesis where methanogens convert CO_2 and H_2 into CH_4 in the following reaction: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (Belanche et al. 2025). Bromoform disrupts this pathway by acting as a competitive inhibitor of methyl-coenzyme M reductase (MCR), the key enzyme catalyzing the final step of methane formation (Morgavi et al. 2010). By disrupting MCR activity, bromoform prevents the reduction of CO_2 to CH_4 , thereby lowering enteric methane production.

While other methane mitigation strategies in livestock exist, such as supplementation of lipids, essential oils, and probiotics, each face notable limitations including toxicity and cost-effectiveness (Bačėninaitė et al. 2022; Króliczewska et al. 2023). Other strategies, such as selective breeding of livestock and manure management, also show potential but present challenges related to the effectiveness across varying farm systems (Peterson et al. 2013; Worku 2024). *A. taxiformis* presents a promising and sustainable alternative, but large-scale application of the algae also faces challenges that require further investigation. Therefore, there is an urgent need to develop cultivation strategies that not only optimize growth rates and the production of the bioactive compound bromoform but also enhance the overall feasibility of *A. taxiformis* production. *A. taxiformis* has a triphasic heteromorphic life cycle (Rojas et al. 1982), with the majority of land-based cultivation focused on the tetrasporophyte stage. To maintain high-quality cultures, intensive cultivation techniques and sterilization protocols are required to ensure optimal growth and prevent microbial contamination (Satton 2024; Rule et al. 2025). Beyond maximizing biomass production, optimizing bromoform concentration is a key priority. Higher bromoform concentrations in seaweed tissue would reduce the amount of biomass required for methane mitigation applications. The biosynthesis and accumulation of bromoform, like other secondary metabolites, are influenced by abiotic factors such as light, nutrient availability, and temperature (Mata et al. 2011; Tan 2023; Hargrave et al. 2024; Resetarits et al. 2024; Torres et al. 2024). Identifying the optimal conditions at which both growth and bromoform production are enhanced is critical for improving cultivation efficiency.

Temperature is among the most important factors that influence enzymatic activity, metabolic rates and ultimately growth and photophysiological performance in algae (Hurd et al. 2014). High temperatures can cause enzyme denaturation, impair photosynthesis and metabolite synthesis, while low temperatures can slow enzymatic activity, reducing overall metabolic output. Thermal optima can vary between species, strains, and life stages, shaped by both algal physiology and acclimation or adaptation to local conditions (Hurd et al. 2014). Populations from habitats with frequent temperature fluctuations may develop broader thermal tolerance than those from more stable environments (Hurd et al. 2014). The lineage 2 of *A. taxiformis* spans a wide

geographical range (Kurihara et al. 2016) and has shown potential of thermal plasticity (Zanolla et al. 2014), suggesting an ability to withstand a wide temperature distribution. Understanding how temperature affects growth, photophysiology and bromoform production in *A. taxiformis* is essential for the land-based cultivation strategies of tetrasporophytes, which currently represent the primary focus of the commercial industry. Additionally, identifying the thermal breadth of each lineage can help to inform site selection for potential offshore aquaculture operations. This study investigates the effects of temperature on *A. taxiformis* growth, photophysiology and bromoform production and examines whether these responses vary based on geographic origin and cultivation conditions.

Materials and methods

Two sets of experiments were conducted to assess 1) the variability and optimal thermal performance from *A. taxiformis* collected from two different locations in southern California and, 2) how preacclimation to highly variable temperatures affected the ability of one isolate to withstand thermal extremes.

Algal cultures

Experiment 1 used wild harvested *A. taxiformis* tetrasporophytes collected from La Jolla (i.e. “LJ”; 32.8663°N, 117.2546°W) and Santa Catalina Island, California, USA (i.e. “SCI”; 33.444173°N, 118.48869°W) in 2021. Both cultures are Lineage 2, as determined previously (Hargrave et al. 2024). These locations were selected due to their distinct oceanographic conditions within the Southern California Bight (SCB) of the California Current System (CCS). La Jolla is influenced by coastal upwelling, resulting in cooler, variable waters while Santa Catalina Island, located centrally in the SCB, experiences reduced upwelling resulting in warmer, stable conditions. These differences are also reflected in higher pH values at Santa Catalina Island compared to La Jolla (Gelpi 2023). The contrasting environmental conditions at these sites provided a basis for examining potential differences in thermal responses.

Upon collection the biomass from both sites underwent a multistage process of isolating and purifying the algal cultures. This process involved several rounds of initiating vegetative propagation via tip cutting and the removal of fouled biomass (Rule et al. 2025). Cultures were then upscaled into progressively larger vessels and were subsequently cultured under standardized laboratory conditions at Scripps Institution of Oceanography (SIO) in La Jolla, California, USA at ~22 °C and a light intensity of 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

under a 12:12-h light: dark cycle, and nutrient supplementation with Proline F/2 Algae Food at 75 $\mu\text{L L}^{-1}$.

Lineage 2 tetrasporophytes used in Experiment 2 were initially wild harvested from La Jolla, California, USA in 2021 where half of the biomass went through the same protocol outlined above and the other half was grown in a flow-through aquarium system. The latter was transferred to an outdoor aquaculture system at SIO and was exposed to seasonal and diel natural fluctuations in light, temperature, and nutrient availability. Outdoor water temperatures were continuously monitored using HOBO data loggers (Onset). Seawater was supplied from the SIO Pier via a filtered and UV-sterilized flow-through system and nutrient concentrations fluctuated naturally. The outdoor cultivated seaweed was provided with a 2 day period to acclimatize to the indoor common garden conditions before the experiment, ensuring that differences in thermal performance were measured under standardized light and nutrient levels. Experiment 2 compared the thermal performance of seaweeds that were acclimated to indoor (low variability) versus outdoor (high variability) conditions.

Experimental conditions

In experiment 1 we exposed *A. taxiformis* cultures collected from two locations in southern California (LJ and SCI) to six temperature treatments (13, 15, 21, 24, 27, and 30 °C). The 15 °C and 21 °C treatments were used to represent the range of natural seasonal fluctuations experienced at both collection sites (Shore Stations Program 2023). In 2023, seawater temperatures at 5 m depth averaged 19.6 ± 1.3 °C in summer, 17.4 ± 0.7 °C in fall, and 15.6 ± 0.6 °C in winter at SCI (Villanueva 2024). LJ had slightly cooler temperatures at 18.7 ± 1.4 °C in the summer, 16.9 ± 0.2 °C in the fall, and 14.2 ± 0.5 °C in the winter (Shore Stations Program 2023). The 24 °C and 27 °C treatments evaluated physiological responses under conditions in which temperature was elevated above what was observed in the field during summer months. 13 °C and 30 °C assessed stress-induced limitations on physiological performance and bromoform production at thermal extremes.

A wider range of temperature treatments were selected for Experiment 2 to represent both natural seasonal fluctuations and extreme conditions beyond typical field temperatures. During Experiment 2, the cultures were exposed to six temperature treatments (8, 13, 18, 23, 28, and 33 °C). The 13 °C, 18 °C, 23 °C, and 28 °C treatments reflect temperatures commonly observed in the outdoor aquaculture system at SIO, where tank temperatures ranged from an average of 15.3 ± 2.48 °C in winter to an average of 22.8 ± 3.9 °C in summer. The 8 °C and 33 °C treatments were included as extreme conditions to evaluate thermal tolerance limits

and assess potential differences in physiological plasticity between indoor and outdoor populations.

Experimental design

Both experiments included six independent water baths, each maintained at a specific temperature. Each bath contained six 1-L flasks, with three replicates per source population ($n=3$ samples per location per temperature). In Experiment 1, three flasks were inoculated with biomass from the LJ culture and three from the SCI culture. In Experiment 2, three flasks contained biomass from the indoor LJ culture (I-LJ), and three from the outdoor LJ culture (O-LJ). Flasks were randomly placed within each bath to minimize positional effects, and continuous aeration of the flasks ensured oxygenation and uniform movement of cultures. Biweekly water changes with F/2 media-enriched sterilized autoclaved seawater ensured cultures would not become nutrient limited. Light intensity was standardized at $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ across all flasks on a 12:12-h light: dark cycle. Each treatment was achieved using a series of water baths with varying temperature control systems (flow-through seawater lines, aquarium heaters, and chillers). Experiment 1 was run for four weeks, while Experiment 2 ran for three weeks as increased contamination was observed in the 33°C treatment biomass during week 4. HOBO data loggers were placed in each water bath and continuously recorded temperature every five minutes.

Response metrics

Relative growth rates

Relative growth rates (RGR) were calculated based on the daily change in fresh weight biomass. At the start of the experiment, each flask was inoculated with a standardized density of 1 g L^{-1} of fresh weight algae. Every two weeks the biomass in each flask was reset to 1 g to eliminate variability in growth due to changing densities. To remove excess water from the algae, the algae samples were placed in fine mesh bags which were inserted into falcon tubes and centrifuged at 5000 rpm for 3 min , ensuring consistent measurements. RGR was calculated using Eq. 1.

$$RGR(\Delta\text{day}^{-1}) = \left(\frac{W_f(g) - W_i(g)}{W_i(g)} \right) \times \frac{1}{t(\text{days})} \times 100 \quad (1)$$

where W_f is the final fresh weight, W_i is the initial fresh weight, and t is the time interval in days. The RGR value is expressed as a percent change in wet weight (gram) per day ($\% \Delta \text{day}^{-1}$).

Maximum quantum yield of Photosystem II

As a proxy for photosynthesis and overall physiological status we measured the maximum quantum yield of Photosystem II (PSII). A Diving Pulse-Amplitude Modulation (PAM) fluorometer (Walz) was used to measure F_v/F_m prior to the start (T_0) and end of the experiment (T_4 for Experiment 1; T_3 for Experiment 2). 100 mg of biomass from each flask was dark-adapted for 15 min to allow all reaction centers to return to their open state. Measurements were conducted in the dark to ensure minimal ambient light interference. F_i readings were maintained between $150\text{--}300$ before initiating a saturation pulse by changing the gain value or the measuring pulse intensity. The saturation pulse intensity was set to 8 with a duration of 0.8 s .

Bromoform concentration and production

To quantify the concentration of bromoform in *A. taxiformis* tissue, a modified protocol based on Thapa et al. (2020) and Dishon et al. (2023) was used. 100 mg of fresh weight biomass were collected at T_0 and the end of experimentation (T_4 Experiment 1; T_3 Experiment 2). Samples were stored at -80°C until lyophilized to remove all water content. The samples were incubated in methanol for 36 h in the dark at room temperature. Following incubation, the supernatant was stored in the dark at 4°C until analysis. A gas chromatograph-mass spectrometer (GC-MS) was used to quantify bromoform concentrations in the extracts (Agilent 7820 A, Agilent 5977B). The GC oven program was as follows: initial temperature of 40°C held for 3 min , ramped to 200°C at $10^\circ\text{C min}^{-1}$, each temperature held for 1 min . The split ratio was $50:1$ with a split flow rate of 60 mL min^{-1} . The MS source temperature was set to 230°C , and the quadrupole temperature was maintained at 150°C . The MS operated in selected ion monitoring (SIM) mode, with a scan speed of 1.562 u s^{-1} . The quantifier ion was 172.8 m/z , while the qualifier ions were 170.8 , 174.8 , 251.8 , and 253.8 m/z .

Chromatograms were extracted and analyzed using Qualitative Analysis software. Peaks corresponding to bromoform were identified at a retention time of 6.8 min . Bromoform concentrations were determined using a standard curve generated from the bromoform standards and calculated in terms of percent of algal dry weight ($\% \text{ dry weight}$) using Eq. 2:

$$\text{Bromoform Concentration (Weight)} = \left(\frac{[\text{Bromoform } \mu\text{g uL}^{-1}] \times \text{Extraction Volume (uL)}}{1000 \times \text{Mass (mg)}} \right) \times 100 \quad (2)$$

where bromoform is the concentration value determined via GC–MS analysis, extraction volume is the amount of methanol used to extract bromoform from the biomass, and mass is the amount of biomass utilized during the extraction process.

Bromoform content was then standardized by growth rates, allowing it to be expressed as bromoform production and calculated via Eq. 3:

$$\text{Bromoform Production (mg L}^{-1} \text{ day}^{-1}) = [\text{Bromoform (mg g}^{-1})] \times \text{RGR (g L}^{-1} \text{ day}^{-1}) \quad (3)$$

where bromoform is the concentration value determined via GC–MS analysis and RGR is the growth rate of the algae ($\text{g L}^{-1} \text{ day}^{-1}$).

Statistical analysis

To analyze the effects of temperature, and geographic origin or cultivation background on growth, photosynthetic yield and bromoform concentration/production of *A. taxiformis* over time, two- and three-way factorial ANOVA tests were used. Temperature, origin/cultivation background, and time were treated as fixed effects while individual flasks were treated as a random effect. This approach accounted for the repeated measures structure of the data, as some measurements were taken across multiple time points throughout both experiments. Residuals were assessed for normality using the Shapiro-Wilks test and for homogeneity of variance we used Levene's test. The assumptions were further

confirmed visually using Q-Q plots and histograms. Post hoc pairwise comparisons of estimated marginal means (emmeans) were performed to determine which treatments differed significantly for each main effect and interaction. Statistical significance was set at $p < 0.05$. All analyses were performed using R Studio.

Results

Experiment 1

Relative growth rates varied significantly across temperature treatments (Fig. 1 a and b; ANOVA: $F_{(5,24)} = 51.43$, $p < 0.01$), with post hoc comparisons indicating significant differences between multiple temperature groups (0.75–7.66%). The highest growth rates were observed at 21 °C ($7.56 \pm 0.44\% \Delta \text{ day}^{-1}$) and 24 °C ($7.66 \pm 0.41\% \Delta \text{ day}^{-1}$). Growth was significantly reduced in all other treatments with the largest reduction occurring at 13 °C ($0.75 \pm 0.21\% \Delta \text{ day}^{-1}$), followed by the 15 °C treatment ($2.92 \pm 0.31\% \Delta \text{ day}^{-1}$). Growth rates at 27 °C ($5.32 \pm 0.30\% \Delta \text{ day}^{-1}$) and 30 °C ($5.64 \pm 0.68\% \Delta \text{ day}^{-1}$) were at intermediate levels but were significantly lower than those observed at 21 °C and 24 °C.

There were no significant effects of location on growth rate (ANOVA: $F_{(1,24)} = 1.73$, $p = 0.20$) but there was a significant effect of time (ANOVA: $F_{(1,24)} = 5.02$, $p = 0.03$). Overall, the algae from both locations had higher growth rates during week 2 ($5.37 \pm 0.45\% \Delta \text{ day}^{-1}$) than during week 4 ($4.59 \pm 0.50\% \Delta \text{ day}^{-1}$). There were no significant

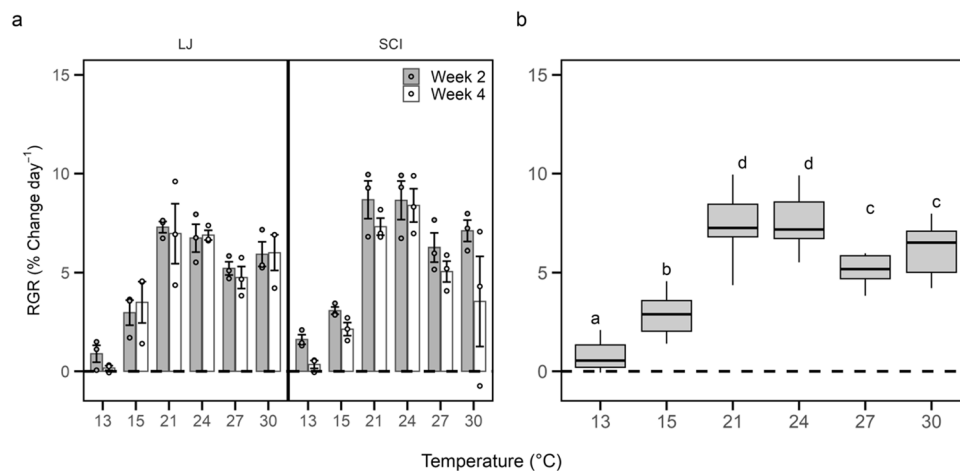


Fig. 1 Relative growth rates of two southern California strains of *Asparagopsis taxiformis* cultivated under a range of temperature treatments. **a** Percent change in daily growth (mean $\pm$ standard error) in cultures from two locations: Santa Catalina Island (SCI; left panel) and La Jolla (LJ; right panel) at Week 2 (grey bars) and Week 4 (white bars) of the experimental period ($n=3$, biological replicates). **b** Boxplot highlighting the results of statistical analysis, with location and time point combined. Letters above the boxes indicate

statistical groups ($p < 0.05$) ($n=6$, biological replicates). Boxes represent the interquartile range (IQR), with horizontal lines inside the boxes indicating the median, the bottom and top lines of each box representing the 25th and 75th percentile respectively, and whiskers highlighting maximum and minimum. Dashed lines indicate 0, showing the threshold in which the cultures grew (positive growth rates) or experienced mortality (negative growth rates).

interactions detected. The significance of the temperature and time main effects but not the interactions imply that the effect of temperature on growth was consistent across time points.

Bromoform concentrations, as expressed by percent dry weight, varied significantly across temperature treatments (Fig. 2a; ANOVA: $F_{(5,24)} = 15.56$, $p < 0.01$). There was no significant effect of location (ANOVA: $F_{(1,24)} = 2.51$, $p = 0.13$) and no significant interaction effects. Overall Bromoform concentrations were lowest at 13 °C ($0.32 \pm 0.02\%$ DW) and 15 °C ($0.74 \pm 0.17\%$ DW). In general concentrations were higher in the warmer treatments: 21 ($2.37 \pm 0.3\%$ DW), 24 ($3.5 \pm 0.25\%$ DW), 27 ($2.43 \pm 0.42\%$ DW), and 30 °C ($2.47 \pm 0.5\%$ DW) with over an order of magnitude reduction in bromoform concentration between 24 °C and 13 °C.

Bromoform production, as standardized to growth rates showed similar results (Fig. 2b) with a significant main effect of temperature (ANOVA: $F_{(5,24)} = 14.44$, $p < 0.01$) and no effect of location (ANOVA: $F_{(1,24)} = 0.95$, $p = 0.34$). Production was highest in the 21 °C ($0.87 \pm 0.18 \text{ mg L}^{-1} \text{ day}^{-1}$) and 24 °C ($1.3 \pm 0.08 \text{ mg L}^{-1} \text{ day}^{-1}$) treatments, which were significantly higher than the 27 °C ($0.67 \pm 0.14 \text{ mg L}^{-1} \text{ day}^{-1}$) and 30 °C ($0.75 \pm 0.18 \text{ mg L}^{-1} \text{ day}^{-1}$). The lowest values were observed in the 13 °C ($0.03 \pm 0.00 \text{ mg L}^{-1} \text{ day}^{-1}$) and 15 °C ($0.155 \pm 0.05 \text{ mg L}^{-1} \text{ day}^{-1}$) treatments.

There was a significant effect of temperature on the maximum quantum yield of PSII (ANOVA: $F_{(5,24)} = 13.57$, $p < 0.01$) with the highest values correlating with the treatments where the highest growth rates were recorded (Fig. 3a and b). Post hoc comparisons revealed that F_v/F_m was lowest at 13 °C (0.12 ± 0.02) and 27 °C (0.15 ± 0.05). While notably higher values were observed at 21 °C (0.36 ± 0.03) and 24 °C (0.43 ± 0.04). F_v/F_m values at 15 °C (0.24 ± 0.03) and 30 °C (0.21 ± 0.04) were intermediate, with no significant differences from the highest or the lowest groups. There was no significant effect of location (ANOVA: $F_{(1,24)} = 3.29$, $p = 0.08$) or the interaction between temperature and location indicating that temperature effects were consistent across locations (Fig. 3a).

Experiment 2

There was a significant three-way interaction between temperature, culture background, and time (ANOVA: $F_{(5,24)} = 3.69$, $p = 0.01$) on relative growth rate, indicating that temperature effects varied depending on both cultivation background and time (Fig. 4b). However, in general both cultures showed optimal growth rates at 23 °C ($9.69 \pm 0.78\% \Delta \text{ day}^{-1}$) and 28 °C ($8.88 \pm 0.78\% \Delta \text{ day}^{-1}$) regardless of time (Fig. 4a). Further, there was some evidence to suggest

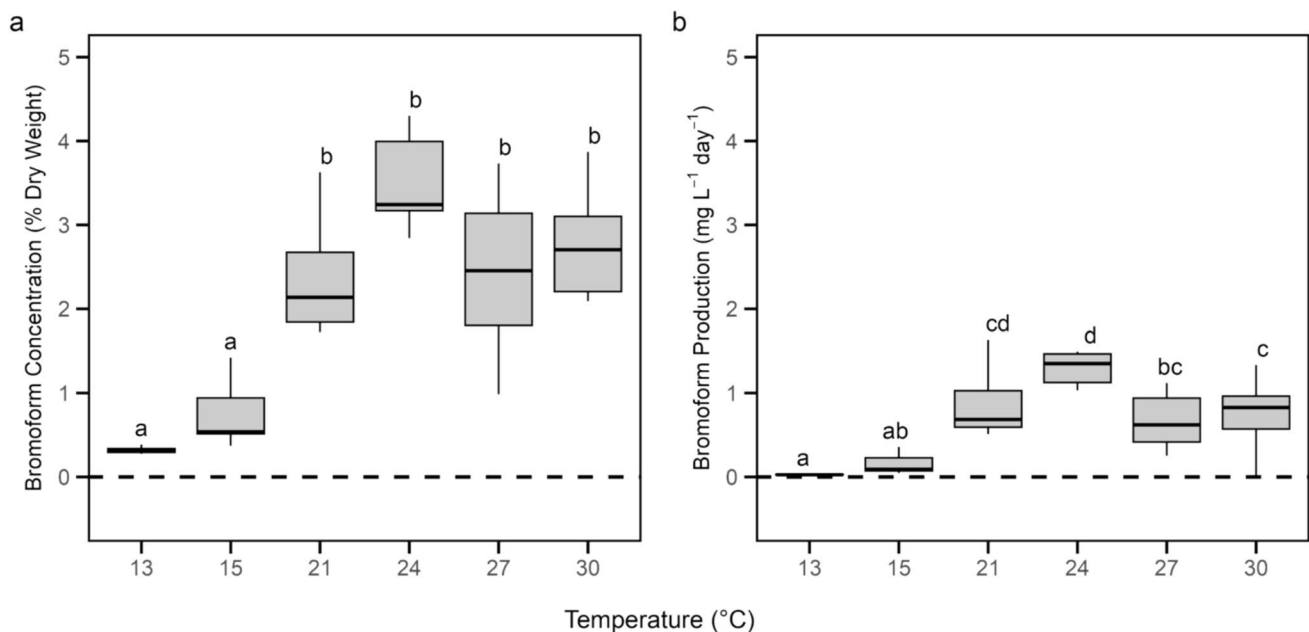


Fig. 2 Bromoform concentrations in *Asparagopsis taxiformis* under various temperature treatments ($n=3$, biological replicates). **a** Bromoform concentrations (% dry weight of algal biomass). Data was pooled between locations; letters indicate significance groups ($p < 0.05$). **b** Bromoform production ($\text{mg L}^{-1} \text{ day}^{-1}$), as standardized by growth rates, letters indicate significance groups ($p < 0.05$) ($n=6$, biological replicates). Boxes reflect the IQR with horizontal lines

inside the boxes indicating the median, the bottom and tops lines of each box representing the 25th and 75th percentile respectively, and whiskers highlighting maximum and minimum values. Dashed lines indicate 0, showing the threshold in which the cultures produced bromoform (positive concentrations) or released bromoform (negative concentrations).

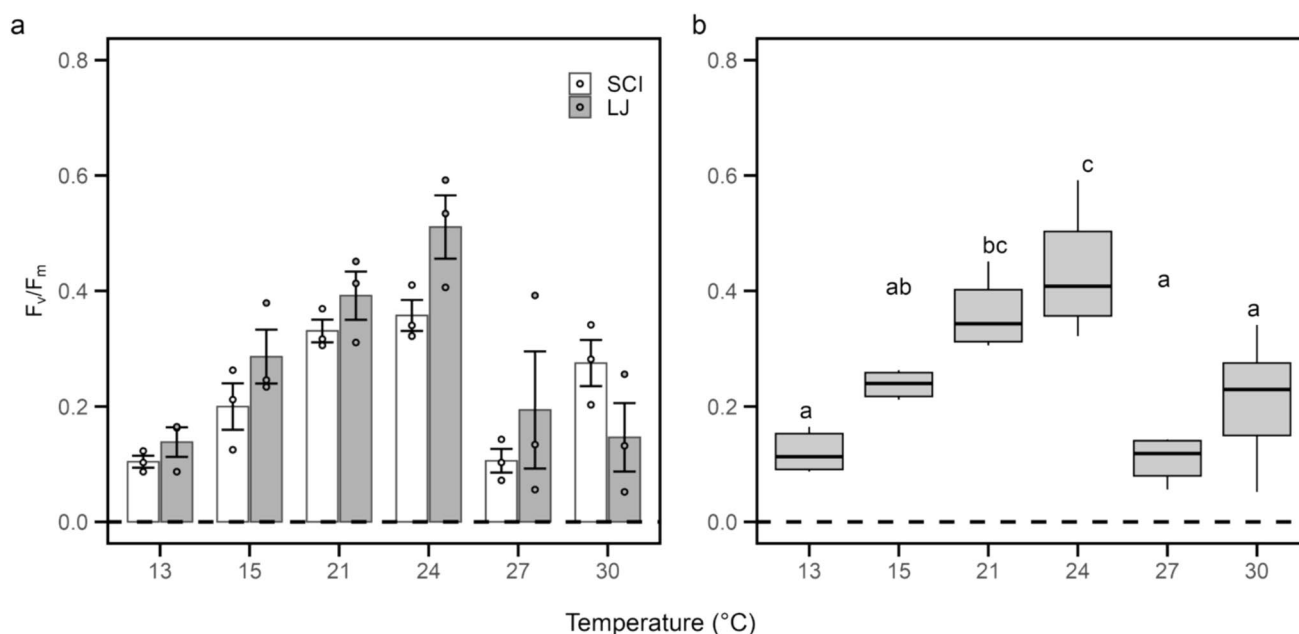


Fig. 3 Maximum quantum yield of PSII (F_v/F_m), of *Asparagopsis taxiformis* cultures sourced from Santa Catalina Island, CA (SCI) and La Jolla, CA (LJ). **a** F_v/F_m (mean $\pm$ standard error) for each culture location. Grey bars represent SCI cultures and white bars represent LJ cultures. **b** Boxplot representing the mean F_v/F_m values of the culture locations combined. Letters represent statistical significance groups

using estimated marginal means (emmeans) test ($p < 0.05$) ($n=6$, biological replicates). Boxes reflect the IQR with horizontal lines inside the boxes indicating the median, the bottom and tops lines of each box represent the 25th and 75th percentile respectively, and whiskers highlight maximum and minimum values.

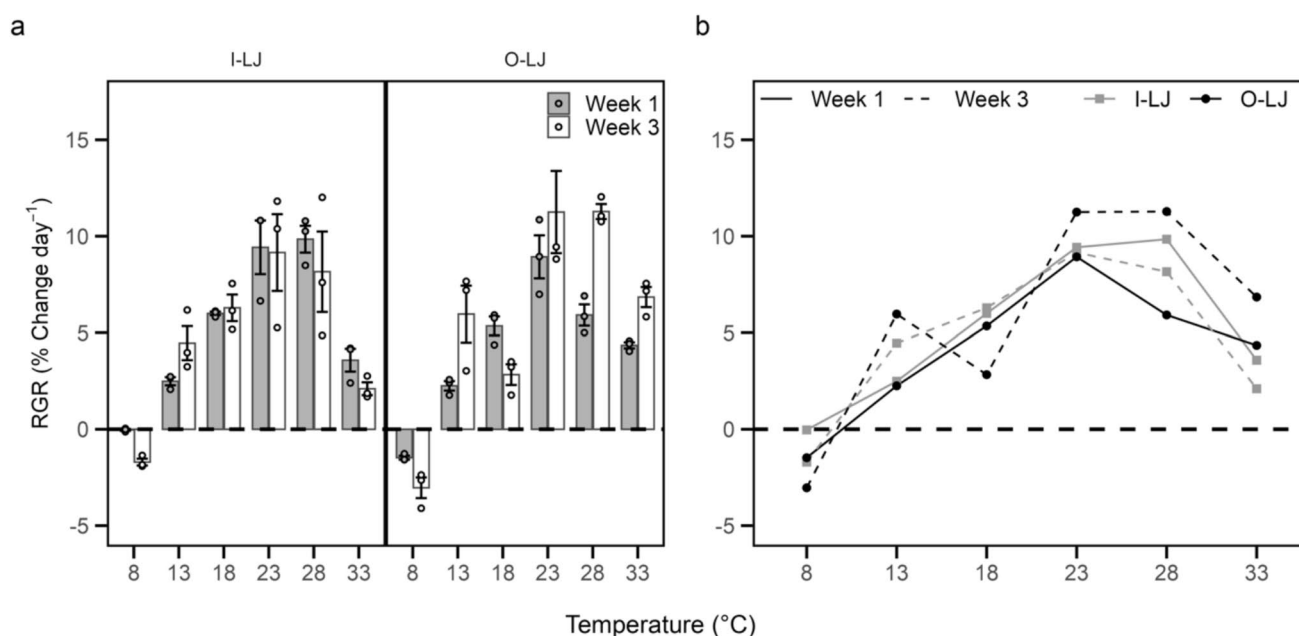


Fig. 4 Relative growth rates of La Jolla, CA strain of *Asparagopsis taxiformis* from different cultivation backgrounds exposed to multiple temperature treatments. **a** Percent change in daily growth (mean $\pm$ standard error) in cultures from cultivation backgrounds: Indoor- La Jolla (I-LJ; left panel) and Outdoor- La Jolla (O-LJ; right panel) at Week 1 (grey bars) and Week 3 (white bars) of the experimental period ($n=3$, biological replicates). **b** Interaction plot highlighting the different RGR responses to temperature treatments

between cultivation backgrounds over time. Black solid line represents the O-LJ culture during Week 1 and the black dashed line represents the O-LJ culture during Week 3. Grey solid line indicates the I-LJ culture during Week 1 and the grey dashed line indicates I-LJ during Week 3. Dashed lines indicate 0, showing the threshold in which the cultures grew (positive growth rates) or experienced mortality (negative growth rates).

that the outdoor acclimated cultures tolerated the 33 °C treatments better than the indoor acclimated cultures.

At 8 °C, growth was negative in both O-LJ and I-LJ cultures, with pronounced declines in O-LJ cultures over time ($-1.48 \pm 0.10\% \Delta \text{day}^{-1}$ at Week 1 to $-3.04 \pm 0.54\% \Delta \text{day}^{-1}$ at Week 3) and in I-LJ cultures ($-0.03 \pm 0.05\% \Delta \text{day}^{-1}$ at Week 1 to $-1.70 \pm 0.18\% \Delta \text{day}^{-1}$ at Week 3). During Week 1, growth peaked at 23 °C ($9.42 \pm 1.39\% \Delta \text{day}^{-1}$) and 28 °C ($9.84 \pm 0.70\% \Delta \text{day}^{-1}$) in I-LJ cultures, while O-LJ cultures showed peak growth at 23 °C ($8.93 \pm 1.12\% \Delta \text{day}^{-1}$). Beyond 23 °C during Week 1, O-LJ cultures exhibited a steep decline, with growth dropping by $5.91 \pm 0.55\% \Delta \text{day}^{-1}$ at 28 °C and $4.35 \pm 0.55\% \Delta \text{day}^{-1}$ at 33 °C. I-LJ cultures maintained elevated growth at 28 °C but showed a significant decline at 33 °C ($3.58 \pm 0.60\% \Delta \text{day}^{-1}$).

By Week 3, the growth pattern shifted, with I-LJ cultures showing the highest growth at 23 °C ($9.15 \pm 2.00\% \Delta \text{day}^{-1}$), and a decline at 28 °C to $8.16 \pm 2.08\% \Delta \text{day}^{-1}$. In contrast, O-LJ cultures exhibited increased growth at 23 °C ($11.3 \pm 2.13\% \Delta \text{day}^{-1}$), 28 °C ($11.3 \pm 0.40\% \Delta \text{day}^{-1}$), and 33 °C ($6.84 \pm 0.52\% \Delta \text{day}^{-1}$) in comparison to Week 1 measurements.

Bromoform concentration was strongly influenced by temperature (ANOVA: $F_{(5,24)} = 22.11$, $p < 0.001$) but was not affected by cultivation background (ANOVA: $F_{(1,24)} = 1.89$, $p = 0.18$) and there were no significant interactions (Fig. 5a).

The highest bromoform concentration was recorded at 23 °C ($2.33 \pm 0.30\% \text{DW}$), which was significantly higher than all other treatments. At 28 °C ($1.23 \pm 0.23\% \text{DW}$), bromoform concentrations remained elevated but were not significantly different from 18 °C ($0.66 \pm 0.17\% \text{DW}$), suggesting a decline from the peak concentration observed at 23 °C. The lowest bromoform concentrations were observed under 8 °C ($0.10 \pm 0.02\% \text{DW}$), 13 °C ($0.40 \pm 0.13\% \text{DW}$), and 33 °C ($0.34 \pm 0.14\% \text{DW}$) treatments, which did not significantly differ from each other. Bromoform concentrations increased slightly at 18 °C, but this treatment was not significantly different from the 8 °C, 13 °C, or 33 °C treatments.

Bromoform production showed a similar temperature dependent pattern to bromoform concentrations (Fig. 5b), where temperature had a significant impact on production (ANOVA: $F_{(5,24)} = 18.20$, $p < 0.01$). There was no significant effect of cultivation background (ANOVA: $F_{(1,24)} = 0.01$, $p = 0.93$) or interaction effects. Bromoform production was significantly higher in the 23 °C treatment ($1.14 \pm 0.20 \text{ mg L}^{-1} \text{ day}^{-1}$) than all other treatments. Although growth rates in outdoor cultures increased over time in the 23 °C, 28 °C, and 33 °C treatments, this trend did not result in significant differences in bromoform production between cultivation backgrounds.

Temperature significantly affected photosynthetic yield (ANOVA: $F_{(5,24)} = 81.10$, $p < 0.01$) in *A. taxiformis* (Fig. 6a) and

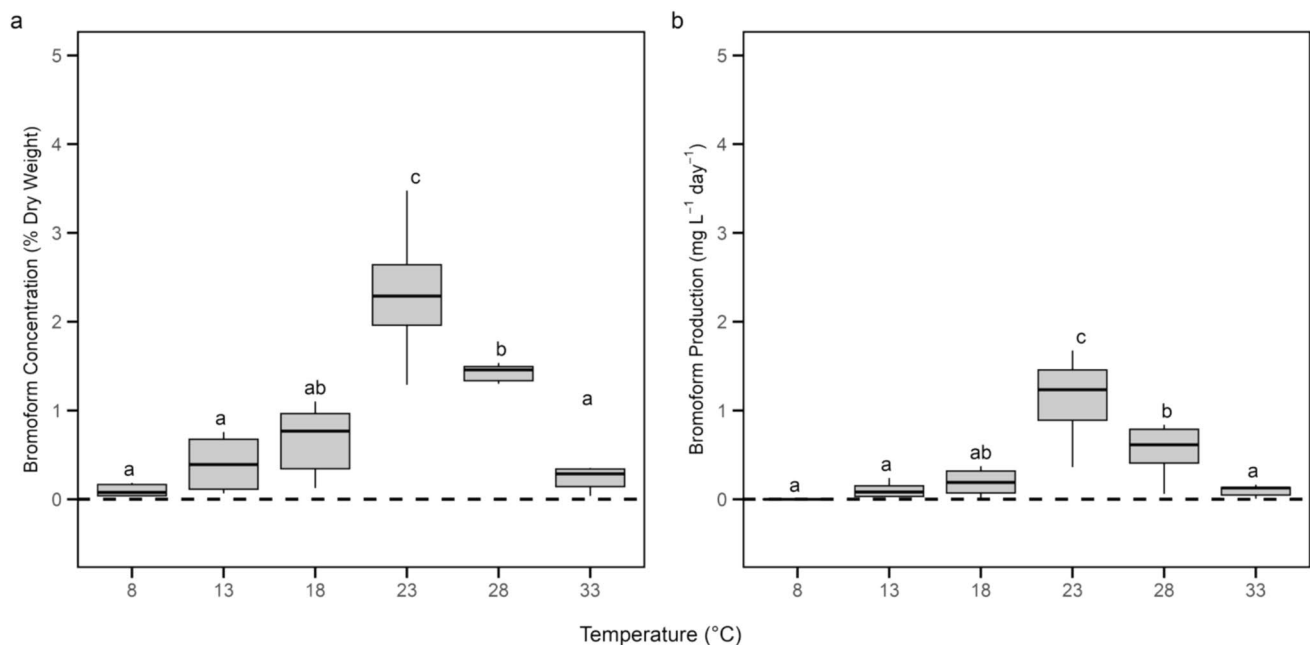


Fig. 5 Bromoform concentrations in *Asparagopsis taxiformis* across various temperature treatments ($n=6$, biological replicates). **a** Bromoform concentrations (% dry weight of algal biomass). Data was pooled between locations; letters indicate significance ($p < 0.05$). **b** Bromoform production ($\text{mg L}^{-1} \text{ day}^{-1}$), as standardized by growth rates, letters indicate significance groups ($p < 0.05$). Boxes reflect the

IQR with horizontal lines inside the boxes indicating the median, the bottom and tops lines of each box representing the 25th and 75th percentile respectively, and whiskers highlighting maximum and minimum values. Dashed lines indicate 0, showing the threshold in which the cultures produced bromoform (positive concentrations) or released bromoform (negative concentrations).

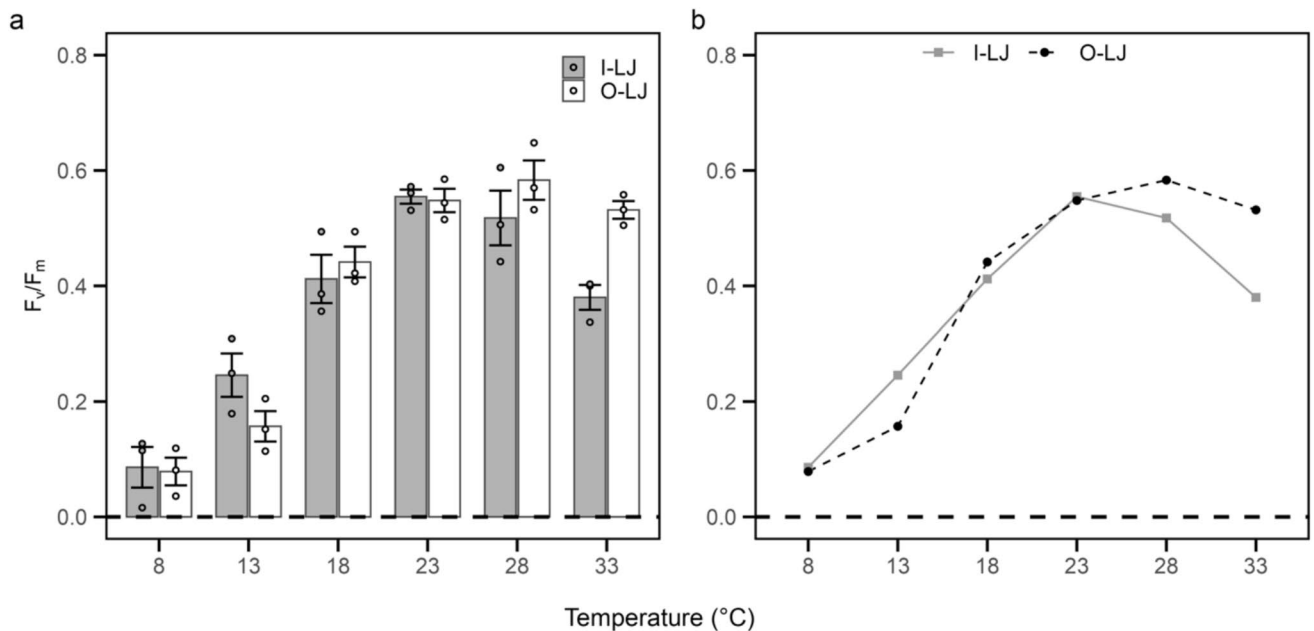


Fig. 6 Maximum quantum yield of PSII (F_v/F_m), of *Asparagopsis taxiformis* cultures sourced from La Jolla, CA (LJ) with different cultivation backgrounds (O-LJ: Outdoor cultivation, I-LJ: Indoor cultivation). **a** F_v/F_m (mean ± standard error) for each cultivation background. Grey bars represent I-LJ cultures and white bars repre-

sent O-LJ cultures. **b** Interaction plot highlighting the different F_v/F_m responses to temperature treatments between cultivation backgrounds. Black dashed line represents the O-LJ culture, and the grey solid line indicates the I-LJ culture. Dashed lines indicate 0.

the response slightly differed between the indoor and outdoor acclimated cultures (ANOVA: $F_{(1,24)}=3.55$, $p=0.02$). Specifically, the outdoor acclimated cultures had higher F_v/F_m values in the 28 °C and 30 °C treatments than the indoor acclimated cultures. However, both cultures followed similar trends with the highest values recorded at 23 and 28 °C and the lowest at 8 °C.

The lowest F_v/F_m values were observed at 8 °C, with no significant difference between I-LJ (0.09 ± 0.04) and O-LJ (0.08 ± 0.02) cultures. F_v/F_m values increased slightly at 13 °C (I-LJ: 0.25 ± 0.04 , O-LJ: 0.16 ± 0.03) but only the I-LJ cultures were significantly higher than those recorded in the 8 °C treatment. The I-LJ cultures had the highest F_v/F_m at 23 °C (0.56 ± 0.01), maintaining high values at 28 °C (0.52 ± 0.05) before decreasing further at 33 °C (0.38 ± 0.02). In contrast, the O-LJ culture peaked at 28 °C (0.58 ± 0.03), marking the highest recorded F_v/F_m across all treatments. O-LJ cultures maintained high F_v/F_m at 23 °C (0.55 ± 0.04) and 33 °C (0.53 ± 0.03), highlighting a more stable response across the warmer temperature treatments compared to I-LJ cultures.

Discussion

The overarching goals of this study were to identify optimal temperatures in which growth, photophysiological performance, and bromoform production of the red alga *Asparagopsis taxiformis* are maximized. Further we sought

to evaluate whether geographic origin (e.g., strain) may influence thermal performance and whether preacclimation to thermal variability may alter responses to temperature especially in extreme conditions. In general, we found that temperature strongly influenced the performance of *A. taxiformis* in terms of physiological health and bromoform production. Geographic origin did not impact temperature response but pre-acclimation to environmental variability proved to increase performance of the seaweed under elevated temperature treatments.

Experiment 1

Temperature had a significant effect on all response variables measured for *A. taxiformis* (growth, maximum quantum yield of PSII, bromoform concentrations, and bromoform production), suggesting that temperature plays a major role in regulating overall health and physiological performance. The highest growth rates, photosynthetic yields, and bromoform production were observed at 21 °C and 24 °C, indicating that these temperatures provide optimal thermal conditions for *A. taxiformis*. This result is consistent with both the typical seawater temperatures within the geographical distribution of Lineage 2 (Shore Stations Program 2023) and the temperature conditions in which these cultures were grown in our indoor laboratory (20–23 °C). Growth and photosynthetic yields peak in this temperature range, suggesting

optimal energy conversion in PSII. Growth rates reached a maximum of $7.66\% \text{ day}^{-1}$, aligning with previously reported rates of $7\text{--}13\% \text{ day}^{-1}$ in tropical and subtropical Australian samples (Luhring and DeLong 2017; Mata et al. 2017).

Bromoform concentrations reached 2.37% DW in the 21 °C treatment and 3.50% in the 24 °C treatment, falling within the known range of 1–5% (Resetarits et al. 2024). These findings are consistent with previous research demonstrating that *A. taxiformis* exhibits optimal growth and metabolic activity within this temperature range (Mata et al. 2017). Studies have shown that temperatures within this range enhance photosynthetic yield and enzymatic activity, thereby promoting increased bromide uptake and bromoform synthesis (Padilla-Gamiño and Carpenter 2007; Resetarits et al. 2024). Values recorded at the 27 °C and 30 °C treatments were statistically similar to 21 °C and 24 °C, ranging between 2.43% and 2.47% DW, respectively.

When considering cultivation of this species with the goal of maximizing the amount of bromoform produced, we must consider both growth rates and bromoform concentration. A given strain may have high growth rates but low bromoform concentrations and vice versa. Ideally strains that are able to grow quickly and produce large amounts of bromoform are the strains that would be selected for commercial scaling. In our data, when considering how much bromoform was produced (by standardizing concentrations with growth rates), a different pattern emerged. Specifically, the amount of bromoform produced was significantly higher at 21 °C and 24 °C compared to the 27 °C and 30 °C treatments. This indicates that although concentrations remained elevated, overall production efficiency declined at higher temperatures due to reduced growth rates. This suggests a trade-off between concentration and production; at warmer temperatures *A. taxiformis* may accumulate bromoform in tissue at similar levels, but slower growth reduces the total amount produced over time. When considering thermal conditions for cultivation it is vital to find the environment where both yield and secondary metabolite production are maximized.

Growth and photosynthetic efficiency were lowest at the 13 °C and 15 °C treatments, suggesting that these temperatures are approaching the threshold for cold stress in *A. taxiformis* in California. Additionally, bromoform concentrations and production were significantly reduced, likely due to temperature-dependent membrane fluidity. Cellular membranes become more rigid under lower temperatures (Los and Murata 2004), which can disrupt ion transport. As bromide accumulation is essential for the production of bromoform in *A. taxiformis*, a reduction in the cell's ability to uptake bromide ions from the water column would hypothetically lower bromoform production (Beissner et al. 1981). Although the response variables were significantly

reduced at these temperatures, positive values were still observed. This indicates that *A. taxiformis* remains viable and metabolically active even at a reduced capacity in cooler waters. When considering commercial cultivation, if year-round temperature control is not feasible, a seasonal growth strategy may be more effective for maximizing growth and bromoform production.

At 27 °C and 30 °C, growth and photosynthetic yield declined, suggesting the onset of heat stress. While growth in these treatments remained relatively high compared to cold treatments, the decrease in F_v/F_m suggests that prolonged exposure to elevated temperatures may result in stress and ultimately photodamage. PSII is particularly heat-sensitive (Eggert 2012), meaning that under heat stress, damage to the photosystem protein complexes occurs rapidly. Bromoform concentrations and production began to decline at these temperatures but remained relatively high. This trend may be attributed to increased membrane fluidity and partial disintegration of the lipid bilayer (Los and Murata 2004), which could facilitate bromide ion uptake, thereby sustaining bromoform production despite stress conditions.

In our study we did not find evidence that collection location influences thermal tolerance or overall performance of *A. taxiformis*. However, our collection locations were only approximately 160 km apart from one another with only minor variation in seasonal temperature observed between the two collection sites. Further, all specimens used in our study were lineage 2. It is possible that populations originating from more geographically distinct locations with stronger seasonal fluctuations and across different lineages may exhibit greater physiological divergence, particularly in thermal tolerance and metabolic plasticity. Recent studies suggest that species with wide geographic distributions do not represent a homogeneous physiological unit, as local adaptations and phenotypic plasticity contribute to intraspecific differences in thermal niches (Valladares et al. 2014; King et al. 2017). In macroalgae, these differences are shaped by environmental variability, where populations from thermally stable environments tend to have narrower thermal niches, while those from more fluctuating environments develop broader acclimation capacities (Valladares et al. 2014; King et al. 2017). Little effort has focused on characterizing the performance of *A. taxiformis* across its broad geographic distribution or between the different lineages.

Experiment 2

The thermal history of an organism strongly influences its ability to acclimatize to temperatures (Luhring and DeLong 2017). We observed evidence of this for *A. taxiformis* in Experiment 2 where we explicitly tested the

thermal response of statically grown lab cultures compared to cultures previously grown in highly variable outdoor conditions. In this experiment, temperature significantly influenced all measured physiological parameters, including growth, photosynthetic yield (F_v/F_m), bromoform concentrations, and bromoform production. These results reinforce the role of temperature as a primary environmental driver regulating the metabolism and physiological performance of *A. taxiformis* in cultivation systems. The highest growth rates and photosynthetic yield were observed at 23 °C and 28 °C, suggesting that these temperatures provide optimal conditions for maximizing metabolic activity. Growth rates reached 11.3% day⁻¹ at 23 °C, aligning well with previous studies (Clark et al. 2013). Bromoform concentrations reached up to 3.5% DW in the 23 °C treatment, which is towards the higher end of the known range of concentrations of *A. taxiformis* (Paul et al. 2006). This suggests that the enzymatic activity responsible for the production of halogenated compounds is potentially temperature-dependent, with peak performance occurring under moderate thermal conditions (Padilla-Gamiño and Carpenter 2007; Resetarits et al. 2024).

Growth was lowest at 8 °C, with both I-SIO and O-SIO cultures experiencing mortality, indicating that temperatures below 8 °C are unsuitable for sustained cultivation. The decline in photosynthetic yield (F_v/F_m) at 8 °C further suggests that cold stress impairs PSII function, reducing the algae's ability to capture and convert light energy. This aligns with previous studies showing that low temperatures restrict enzyme activity, slow metabolic processes, and limit energy production in marine macroalgae (Tan 2023). At 33 °C, growth and photosynthetic yield showed contrasting trends between I-SIO and O-SIO cultures. While I-SIO cultures experienced a marked decline in growth and F_v/F_m over time, O-SIO cultures maintained relatively high F_v/F_m values. This suggests that acclimation to variable environmental conditions may enhance thermal resilience. However, bromoform production declined at 33 °C in both cultivation backgrounds, indicating that high temperatures disrupt enzymatic stability, limiting metabolic efficiency despite ability to acclimate (Tan 2023). These findings suggest that while *A. taxiformis* exhibits some degree of thermal plasticity, temperatures above 30 °C may impose physiological constraints that ultimately reduce secondary metabolite production.

While no significant differences in bromoform production were detected between cultivation backgrounds, it remains unclear whether long-term exposure to environmental fluctuations could drive adaptive physiological responses over multiple generations. Further research is needed to determine whether continuous outdoor cultivation under varying seasonal

conditions could enhance thermal tolerance in *A. taxiformis* cultures.

Industrial applications

The findings of this study provide valuable insights for optimizing *A. taxiformis* cultivation strategies, particularly for large-scale production in southern California. We found that 23 °C was the optimal temperature for maximizing growth, photosynthetic yield, and bromoform production. While *A. taxiformis* survived across a wide temperature range of 13–33 °C, performance was the highest at 23 °C. Interestingly, all metrics of physiological performance were positively correlated with bromoform production suggesting that factors that optimize growth also support higher production of bromoform which is extremely important for large scale cultivation of this seaweed.

In general, our results suggest that southern California *A. taxiformis* is more sensitive to cold temperatures than warm temperatures. This is not surprising given the temperatures that exist in offshore coastal habitats in the region, which typically only reach an average low of 14 °C (Shore Stations Program 2023). At higher temperatures physiological responses indicate a possible upregulation of metabolic activity before the onset of thermal stress. This may suggest that a short-term exposure to elevated temperatures could stimulate metabolic pathways, potentially enhancing bromoform production prior to any significant protein denaturation or photodamage. However, prolonged exposure to elevated temperatures will likely lead to reduced physiological performance. Interestingly we did find some evidence to suggest that exposure to natural environmental fluctuations may enhance resilience to thermal variation, reducing the need for strict temperature control in large-scale cultivation. Nonetheless, thermal performance should be determined for any population of *A. taxiformis* prior to the development of commercial production to avoid losses in productivity or crop failure. These insights are vital for cost-effective cultivation strategies that improve crop yield and therefore advance the use of *A. taxiformis* as a methane reduction strategy in ruminant livestock.

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Author contributions TR conceived and designed the experiment in collaboration with JES and OBZ. TR cultivated the algal biomass, conducted the experiments, performed data analysis and wrote the manuscript. JES contributed to conceptualization, experimental design, and provided critical feedback on the manuscript. OBZ contributed to experimental design and provided feedback on the manuscript. KC provided experimental support and feedback throughout the study. All authors reviewed and approved the final manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author (Tess Rounds) per request.

Code availability The code of this study is available from the corresponding author (Tess Rounds) per request.

Declarations

Competing interests The authors declare no competing interests.

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References

- Andreakis N, Costello P, Zanolla M, Saunders GW, Mata L (2016) Endemic or introduced? Phylogeography of *Asparagopsis* (Florideophyceae) in Australia reveals multiple introductions and a new mitochondrial lineage. *J Phycol* 52:141–147
- Andreakis N, Procaccini G, Kooistra WHCF (2004) *Asparagopsis taxiformis* and *Asparagopsis armata* (Bonnemaisoniales, Rhodophyta): genetic and morphological identification of Mediterranean populations. *Eur J Phycol* 39:273–283
- Andreakis N, Procaccini G, Maggs C, Kooistra WHCF (2007) Phylogeography of the invasive seaweed *Asparagopsis taxiformis* (Bonnemaisoniales, Rhodophyta) reveals cryptic diversity. *Mol Ecol* 16:2285–2299
- Bačėninaitė D, Dėrmeikaitė K, Antanaitis R (2022) Global warming and dairy cattle: how to control and reduce methane emission. *Animals* 12:2687
- Beissner RS, Guilford WJ, Coates RM, Hager LP (1981) Synthesis of brominated heptanones and bromoform by a bromoperoxidase of marine origin. *Biochemistry* 20:3724–3731
- Belanche A, Bannink A, Dijkstra J, Durmic Z, Garcia F, Santos FG, Huws S, Jeyanathan J, Lund P, Mackie RI, McAllister TA, Morgavi DP, Muetzel S, Pitta DW, Yáñez-Ruiz DR, Ungerfeld EM (2025) Feed additives for methane mitigation: a guideline to uncover the mode of action of antimethanogenic feed additives for ruminants. *J Dairy Sci* 108:375–394
- Clark JS, Poore AGB, Ralph PJ, Doblin MA (2013) Potential for adaptation in response to thermal stress in an intertidal macroalga. *J Phycol* 49:630–639
- Dishon G, Resetartis HM, Tsai B, Jones AL, Agarwal V, Smith JE (2023) The effect of light intensity, spectrum, and photoperiod on the physiological performance of *Asparagopsis taxiformis* tetrasporophytes. *Algal Res* 76:103304
- Shore Stations program (2023) Data provided by the Shore Stations Program sponsored at Scripps Institution of Oceanography by California Department of Parks and Recreation, Natural Resources Division, Award # C1670003
- Dijou L, Viard F, Payri C (2014) The more we search, the more we find: discovery of a new lineage and a new species complex in the genus *Asparagopsis*. *PLoS One* 9:e103826
- Eggert A (2012) Seaweed responses to Temperature. In: Wiencke C, Bischof K (eds) *Seaweed Biology*. Springer, Berlin, pp 47–66
- Forster P, Storelvmo T, Armour K, Collins W, Dufresne JL, Frame D, Lunt DJ, Mauritsen T, Palmer MD, Watanabe M, Wild M, Zhang H (2021) The Earth's energy budget, climate feedbacks, and climate sensitivity. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, pp 923–1054
- Greening C, Geier R, Wang C, Woods LC, Morales SE, McDonald MJ, Rushton-Green R, Morgan XC, Koike S, Leahy SC, Kelly WJ, Cann I, Attwood GT, Cook GM, Mackie RI (2019) Diverse hydrogen production and consumption pathways influence methane production in ruminants. *ISME* 13:2617–2632
- Guiry MD, Guiry GM (2025) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org/search/species/detail/?species_id=636; accessed 23 October 2025
- Hargrave MS, Islam S, Agarwal V, Smith JE (2024) The influence of light stress on bromoform synthesis and concentration in the red seaweed *Asparagopsis taxiformis*. *J Appl Phycol* 36:321–329
- Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) *Seaweed Ecology and Physiology*, 2nd edn. Cambridge University Press, Cambridge
- Gelpi CG (2023) Dynamics of pH at Santa Catalina Island. *PLOS One* 18:e0290039
- King NG, McKeown NJ, Smale DA, Moore PJ (2017) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography* 41:1469–1484
- Kinley RD, Vucko MJ, Machado L, Nigel TW (2016) In vitro evaluation of the anti-methanogenic potency and effects on fermentation of individual and combinations of marine macroalgae. *Am J Plant Sci* 7:2038–2054
- Króliczewska B, Pecka-Kielb E, Bujok J (2023) Strategies used to reduce methane emissions from ruminants: controversies and issues. *Agriculture* 13:602
- Kurihara A, Horiguchi H, Hanyuda T, Kawai H (2016) Phylogeography of *Asparagopsis taxiformis* revisited: combined mtDNA data provide novel insights into population structure in Japan. *Phycol Res* 64:95–101
- Luhning TM, DeLong JP (2017) Scaling from metabolism to population growth rate to understand how acclimation temperature alters thermal tolerance. *Integr Comp Biol* 57:103–111
- Los DA, Murata N (2004) Membrane fluidity and its roles in the perception of environmental signals. *Biochim Biophys Acta* 1666:142–157
- Machado L, Magnusson M, Paul NA, de Nys R, Tomkins N (2014) Effects of marine and freshwater macroalgae on in vitro total gas and methane production. *PLoS One* 9:e85289
- Machado L, Magnusson M, Paul NA, de Nys R, Tomkins N (2016) Identification of bioactives from the red seaweed *Asparagopsis taxiformis* that promote antimethanogenic activity in vitro. *J Appl Phycol* 28:3117–3216
- Mar KA, Unger C, Walderdorff L, Butler T (2022) Beyond CO₂ equivalence: the impacts of methane on climate, ecosystems, and health. *Environ Sci Policy* 134:127–136
- Mata L, Gaspar H, Santos R (2011) Carbon/nutrient balance in relation to biomass production and halogenated compound content in the red alga *Asparagopsis taxiformis* (Bonnemaisoniaceae). *J Phycol* 48:248–253
- Mata L, Lawton RJ, Magnusson M, Andreakis N, de Nys R, Paul NA (2017) Within-species and temperature-related variation in the

- growth and natural products of the red alga *Asparagopsis taxiformis*. J Appl Phycol 29:1437–1447
- Morgavi DP, Forano E, Martin C, Newbold CJ (2010) Microbial ecosystem and methanogenesis in ruminants. Animal 4:1024–1036
- Moss AR, Juany JP, Newbold J (2000) Methane production by ruminants: its contribution to global warming. Annal De Zootechnie 49:231–253
- Paul NA, de Nys R, Steinberg PD (2006) Chemical defense against bacteria in the red algae *Asparagopsis armata*: linking structure with function. Mar Ecol Prog Ser 306:87–101
- Padilla-Gamiño JL, Carpenter RC (2007) Seasonal acclimatization of *Asparagopsis taxiformis* (Rhodophyta) from different biogeographic regions. Limnol Oceanogr 52:833–842
- Peterson SO, Blanchard M, Chadwich D, Del Prado A, Edouard N, Mosquera J, Sommer SG (2013) Manure management for greenhouse gas mitigation. Animal 7:266–282
- Ravishankara AR, Kuylenstierna JCI, Michalopoulou E, Höglund-Isaksson L, Zhang Y, Seltzer K, Ru M, Castelino R, Faluvegi G, Naik V, Horowitz L, He J, Lamarque JF, Sudo K, Collins WJ, Malley C, Harmsen M, Stark K, Junkin J, Li G, Glick A, Borgford-Parnell N (2021) Global Methane Assessment: benefits and costs of mitigating methane emissions. United Nations Environment Program, Nairobi, 173 p
- Resetarits HM, Dishon G, Agarwal V, Smith JE (2024) The effects of temperature and CO₂ enrichment on the red seaweed *Asparagopsis taxiformis* from Southern California with implications for aquaculture. J Phycol 60:1567–1584
- Rojas JJ, Lemus A, Ganesan EK (1982) El ciclo vital “in vitro” del alga marina roja *Asparagopsis taxiformis* (Delile) Collins & Hervey (Bonnemaisoniales, Rhodophyta) del Mar Caribe. Bol Inst Oceanogr Univ Oriente 21:101–112
- Roque BM, Venegas M, Kinley RD, de Nys R, Duarte TL, Yang X, Kebreab E (2021) Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. PLoS One 16:e0247820
- Rule M, Lane J, Martine AP, Cyrus MD, Nayar S, Hoang T, Mihaila A, Nardelli AE, Tran H, Sjölund MAC, Stålemark COM, Nunn H, Knauer J (2025) Hatchery and cultivation manual for the seaweed *Asparagopsis* spp. AgriFutures Australia, Canberra
- Satton J (2024) *Asparagopsis taxiformis* hatchery and cultivation manual. AgriFutures Australia, Canberra
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10:51–62
- Sherwood AR (2008) Phylogeography of *Asparagopsis taxiformis* (Bonnemaisoniales, Rhodophyta) in the Hawaiian islands: two mtDNA markers support three separate introductions. Phycologia 47:79–88
- Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, de Haan C (2006) Livestock’s long shadow: Environmental issues and options. FAO, Rome
- Tan LT (2023) Impact of marine chemical ecology research on the discovery and development of new pharmaceuticals. Mar Drugs 21:174
- Thapa HR, Lin Z, Yi D, Smith JE, Schmidt EW, Agarwal V (2020) Genetic and biochemical reconstitution of bromoform biosynthesis in *Asparagopsis* lends insight into seaweed ROS enzymology. ACS Chem Biol 15:1662–1670
- Torres R, Campos AM, Goldman J, Barrote I, Mata L, Silva J (2024) Effects of light quality and intensity on growth and bromoform content of the red seaweed *Asparagopsis taxiformis*. J Appl Phycol 36:627–637
- Valladares F, Matesanz S, Guilhaumon F, Araújo MB, Balaguer L, Benito-Garzón M, Cornwell W, Gianoli E, van Kleunen M, Naya DE, Nicotra AB, Poorter H, Zavala MA (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecol Lett 17:1351–1364
- Villanueva AX (2024) Evaluating the effects of temperature on kelp forest persistence. MSc Thesis, California State University, Northridge, USA 59 pp
- Worku D (2024) Unraveling the genetic basis of methane emission in dairy cattle: a comprehensive exploration and breeding approach to lower methane emissions. Anim Biotech 35:2362677
- Zanolla M, Altamirano M, Carmona R, De La Rosa J, Sherwood A, Andreakis N (2014) Photosynthetic plasticity of the genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in response to temperature: implication for invasiveness. Biol Invasions 17:1341–1353

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