Original Article

Assessing the potential for red seaweed *Asparagopsis taxiformis* aquaculture in Seychelles

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Abstract

Asparagopsis taxiformis macroalgae can potentially reduce methane emissions when fed to ruminants, thus reducing the emission of greenhouse gasses into the atmosphere. Literature indicates water temperatures above 24 °C limit *A. taxiformes* growth rates during culture, however, it grows wild in Seychelles where water temperatures range from 25 to 31.5 °C. The feasibility of cultivating the local strain of *A. taxiformis* in Seychelles was investigated, focusing on growth rates at higher temperatures and bromoform content, the compound responsible for methane reduction. Two tetrasporophyte growth trials were conducted in photobioreactors at 24 °C, 26 °C, 29 °C and 32 °C. In the first trial, unacclimated specimens showed poor growth, with no significant differences between temperatures. In the second trial, daily growth rates of acclimated specimens were 3.98 % across all temperatures, including 32 °C, where temperature did not significantly affect growth. Bromoform concentrations were lower than expected, likely due to storage conditions prior to sample processing. The local strain of *A. taxiformis* in Seychelles may therefore be adapted to higher temperatures, with potential for aquaculture. Further research to optimize cultivation techniques and ensure adequate bromoform content is required.

Keywords: *Asparagopsis taxiformis*, seaweed aquaculture, Seychelles, bromoform, marine climate solutions, climate change mitigation

Introduction

Asparagopsis taxiformis, a species of red seaweed, has garnered significant attention due to its ability to reduce methane production in ruminants, such as cattle and sheep, by up to 99 % when supplemented in their diet (Camer-Pesci *et al*., 2023, Roque *et al*., 2021). This discovery holds immense promise for climate change mitigation, as methane ranks among the most harmful greenhouse gases, exhibiting an insulating effect approximately 10 times greater than that of carbon dioxide. Notably, ruminants contribute a substantial 15 % to global gas emissions (FAO, 2013). The integration of *A. taxiformis* into the diets of a significant portion of the global ruminant herd has great potential in mitigating climate change. Furthermore, *A. taxiformis* may have various other applications from cosmetics to a climate-smart fish feed additive (Pereira *et al*., 2024). Recognizing this, research institutions, governmental bodies, and private enterprises have mobilized substantial resources and efforts towards the production of *A. taxiformis*. For instance, a US-based *A. taxiformis* startup recently concluded a funding round, securing USD 7 million (The Fish Site, 2022).

The life cycle of *A. taxiformis* comprises two distinct phases: the gametophyte and tetrasporophyte phases (Batista, 2020). Mature tetrasporophytes, resembling pink pom-pom balls or candyfloss, release spores that germinate on rocky substrates, initiating the gametophyte phase. Here, male and female gametangia produce gametes, leading to fertilization and zygote formation. These zygotes develop into new tetrasporophytes. Cultivating *A. taxiformis* is possible in either phase (Batista, 2020; Goldman, 2021; Mata *et al.*, 2017; Torres *et al.*, 2023). Tetrasporophytes can be cultured in land-based tumble systems like raceways, tanks, or ponds, while gametophytes are typically cultured off ropes at sea. Each method has its advantages and drawbacks. Land-based tetrasporophyte production relies on vegetative reproduction, making it less technically demanding. However, it may incur higher costs due to energy, infrastructure, and land requirements. In contrast, sea-based gametophyte production can be conducted directly in the ocean, potentially offering a more economical alternative. Nonetheless, it presents significant technical challenges, especially at scale. These challenges include inducing tetrasporophyte sporulation and ensuring the successful settlement of spores onto ropes deployed at sea for gametophyte growth.

Asparagopsis taxiformis is indigenous to Seychelles and grows naturally around Mahe Island (personal observation, 2024). In Seychelles, there may be potential for both sea-based and land-based production. Seychelles presents suitable conditions for sea-based aquaculture, given its stable tropical climate devoid of cyclones, unlike many other tropical areas. The region boasts extensive shallow and sheltered sea spaces, ideal for deploying seaweed cultivation systems like floating rafts or longlines. While land-based aquaculture of tetrasporophytes may face constraints on the inner islands, such as Mahe, due to land area limitations, other Seychelles islands, such as Coëtivity, offer potential. Coëtivity Island possesses approximately 96 hectares of ponds, previously utilized for prawn farming and now partly abandoned, besides recent efforts which have reactivated four hectares for prawn production (Lesperance, 2011). This land could serve as a viable site for *A. taxiformis* cultivation. Additionally, exploring the setup of an integrated multi-trophic system holds promise, where *A. taxiformis* could bioremediate the inorganic waste from prawn farming, showcasing the potential for synergistic aquaculture practices in Seychelles. Furthermore, the political and economic stability of Seychelles ensures a conducive regulatory environment and reliable infrastructure for seaweed farming ventures.

While Seychelles hosts natural populations of *A. taxiformis* (Seychelles National Herbarium, 2023) and demonstrates significant potential for its cultivation, a key concern lies in temperature compatibility. Sea temperatures in Seychelles typically range from 25.5 to 31.5 °C (Zinke *et al.*, 2014; personal observation, 2023). However, the literature indicates minimal growth occurs beyond 24 °C (Mata *et al.*, 2017) and cultures will crash above 25 °C (Goldman, 2021). This poses a paradox given the absence of temperatures below 25.5 °C in Seychelles waters. This suggests a potential adaptation of the Seychellois strain to higher temperatures. However, no research had been conducted on growth of *A. taxiformis* grown in this region specifically. Moreover, evidence suggests phenotypic adaptability among different strains of *A. taxiformis* depending on their origin, indicating the possibility of local strains being well-suited for cultivation in the warmer Seychelles climate (Mata *et al.*, 2017). Further investigation into the growth patterns and adaptability of Seychellois *A. taxiformis* could shed light on its suitability for aquaculture in the region.

Bromoform is the compound responsible for inhibiting methanogenesis in the digestive system of ruminants. However, the bromoform content in *A. taxiformis* can vary widely, with some strains exhibiting low levels, while others have significantly higher concentrations (Mata *et al.*, 2017). This variability in bromoform content strongly influences the efficacy and value of the end product, making it a crucial factor to consider. Despite the importance of bromoform content, there has been no assessment of its levels in the Seychelles' strain of *A. taxiformis*. Understanding the bromoform content of Seychellois strains is essential for evaluating the feasibility and potential value of this strain for methane mitigation in ruminants.

The objective of this study was to investigate the feasibility of *Asparagopsis taxiformis* aquaculture in Seychelles. Given that temperature may be the primary limiting factor, evaluating growth rates at temperatures relevant to the Seychelles oceanic climate is crucial for assessing feasibility. If growth cannot be achieved at observed Seychellois temperatures, large-scale aquaculture may not be economically viable due to the high cost of cooling water. However, if *A. taxiformis* can exhibit high growth rates at higher temperatures, aquaculture may indeed be feasible. Furthermore, assessing the bromoform content of Seychellois *A. taxiformis* is essential to ensure the resulting biomass possesses high value. Ultimately, these assessments will provide valuable insights into the potential for *A. taxiformis* aquaculture in Seychelles.

Methods

Collection

The gametophyte phase (Fig. 1a) was observed in various locations, primarily in shallow rocky reefs ranging from 0.5 to 2 metres deep, sheltered by the outer reef around Mahe. However, it was noted to decline during the North-West monsoon season (December to March) when water temperatures exceed 31 °C (personal observation, 2023). The tetrasporophyte (Fig. 1b) was exclusively found in the Saint Anne Marine National Park (4°37'52.10"S, 55°30'28.62"E) located on Mahe growing on the base of *Sargassum spp*. Collection took place in October 2023 with a license granted from the Seychelles Parks and Gardens Authority. Subsequently, specimens were transferred to 1-litre bottles and transported to the Seychelles Fishing Authorities' Broodstock Acclimatization and Quarantine Facility at Providence, Mahe (4°39'10.06"S, 55°29'17.52"E). Upon arrival, epiphytes, sand, *Sargassum* spp. pieces, and other foreign objects were removed manually and by washing with seawater against a 100 µm sieve.

Growth Trials

Conducted in twelve 10-litre photobioreactors equipped with bottom aeration to ensure constant biomass movement, the trials utilized LED strip daylights providing 35 µmol photons m^{-2} s^{-1} of light for 24 hours a day at the centre point of each tank. Temperatures were maintained at 24, 26, 29, and 32 °C, with the latter three temperatures regulated by 50 W aquarium rod heaters. There were three photobioreactors for each temperature treatment (n = 3). Although a lower temperature of 22 °C would have been preferable for better comparability with Torres *et al*. (2023), this was unattainable due to limitations of the culture facility, where the air conditioning could not lower the room temperature below 24 °C. Daily temperature checks were conducted at 9 am, with an average standard deviation of 0.586 °C across all treatments. The growth trials were designed to be as similar as possible to Torres *et al*. (2023), so some provisional comparisons between *A. taxiformis* strains can be deduced.

At the start of each experiment, each photobioreactor was filled with 10 litres of water which had been filtered to 5 µm using a sand filter. The water was then sterilized with 7.5 ml of bleach (4.5 % Sodium hypochlorite). After 10 minutes, 4.5 ml of sodium thiosulfate (10 %) was added to deactivate the bleach thus likely preventing it from influencing *A. taxiformis* growth. Each tank was fertilized with 10 ml of Varicon F2 stock media. Weekly measurements of *A. taxiformis* biomass involved draining the entire photobioreactor through a 100 µm sieve, where the seaweed was retrieved, flattened into thin disks, and patted dry with tissue until no moisture was evident. The seaweed was then weighed and returned to the bioreactor with water treated and fertilized as described above.

Two growth trials were conducted, each over the course of a month. The first trial utilized "un-acclimated" *A. taxiformis*, harvested directly from the wild. It had an initial stocked density of 0.14 g.l^{-1} in each bioreactor. This initial density was utilised based on the maximum availability of that which could be located and harvested from the wild population. The subsequent "acclimated" trial used *A. taxiformis* from the previous trial, with biomass mixed from each replicate and stocked at a density of 0.4 g.l^{-1} in each bioreactor. The same *A. taxiformis* biomass used in

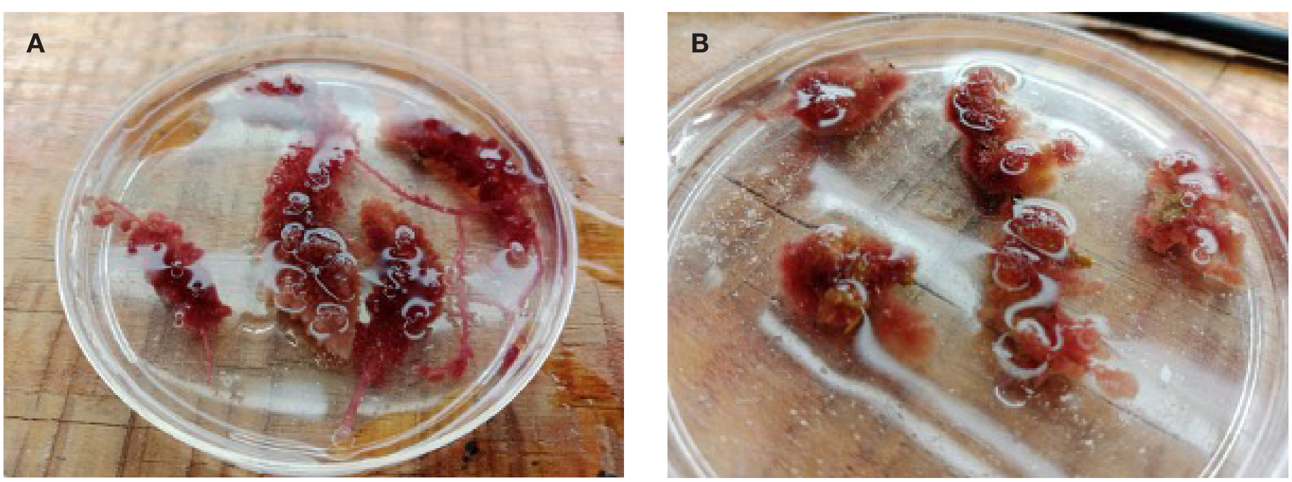

Figure 1 A. *Asparagopsis taxiformis* gametophyte samples collected at Anse Forbans, Mahe, Seychelles in October 2023; **B.** *Asparagopsis taxiformis* tetrasporophyte used for the growth trials conducted in this study. Samples were collected in the St Anne Marine Park near Mahe, Seychelles in October 2023.

specific treatments in the initial “un-acclimated” trial was reused in corresponding treatments for the subsequent acclimated trial, with mixing between replicates. The exception was the biomass from the 32 °C treatment, where some biomass from the 29 °C treatment was incorporated to achieve consistent starting masses.

The daily growth rate (DGR) expressed as % day^{-1} (Equation 1)

$$DGR = \left[\left(\frac{W_t}{W_0} \right)^{1/d} - 1 \right] \times 100 \quad (1)$$

Where W_t and W_0 are the final and initial FW mass, and d is the time in days.

Bromoform extraction

At the end of the second ‘acclimated’ growth trial, the *A. taxiformis* samples were patted dry, packaged in plastic test tubes and frozen at -18 °C. After two months, the samples were analysed for their bromoform content using the methods described in Romanazzi *et al.*, (2021). The two months gap between harvest and storage where the result of limited availability of lab technicians. A bromoform analysis was conducted for each *A. taxiformis* sample (replicate).

Statistical analysis

A two-way repeated-measure analysis of variances (ANOVA) was used to assess the effects of temperature and time on *A. taxiformis* biomass growth, with biomass growth being the independent variable and temperature and time being the dependent variables. These ANOVAs included an error term of the tank and the week at which the biomass was quantified. The assumption of normality was assessed for each dependent variable using Shapiro-Wilks test, and all were found to be insignificant ($p > 0.05$). Outliers were checked via a z-scores analysis where it was found that none of the z-scores exceeded a value of 3. If significant differences were observed in the ANOVA results, the highly conservative Student–Newman–Keuls (SNK) test was applied to determine the presence of significant differences between treatments.

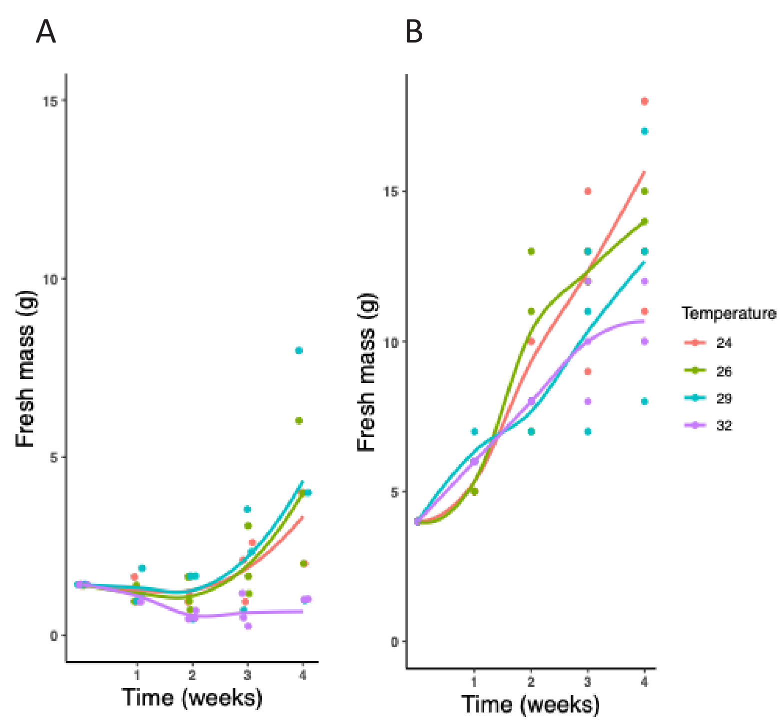

Figure 2 A and B. The graphs depict the growth of *Asparagopsis taxiformis* tetrasporophytes over a one-month period at various temperatures. The data points representing the fresh weights of each *A. taxiformis* replicate (n=3) at each weighing interval (weekly). The lines are generated by a loess model, illustrating the trend in growth over time. Figure A showcases the growth of seaweed harvested directly from the wild, without acclimation to specific temperatures or domesticated conditions, while Figure B reflects the growth of seaweed after acclimatization to both domestication and the specific temperatures it was grown at during the trial.

To analyse the effect of temperature on bromoform content, a one-way ANOVA was conducted. Prior to the analysis, assumptions of normality, homogeneity of variances, and independence of observations were tested. Normality of residuals was confirmed using the Shapiro-Wilk test ($p > 0.05$), homogeneity of variances was validated with Levene's test ($p > 0.05$), independence was found via the Durbin-Watson test ($DW = 2.4543$).

Results

Growth

The un-acclimated *Asparagopsis taxiformis* tetrasporophytes, which were harvested from the wild and then immediately placed into culture bottles at an initial stocking density of 0.14 g FW l^{-1}, exhibited poor growth regardless of the temperature for the first two weeks (Fig. 2a). However, in the final two weeks there was an exponential increase in biomass the 24, 26 and 29 °C temperature treatments. The average daily growth rate (DGR) of the *A. taxiformis* grown at 24, 26 and 29 °C over the month grow-out trial was 2.895 % and the DGR for the same treatments in the last two weeks was 7.559 %. The warmest temperature treatment, 32 °C gradually decreased in biomass over time (Fig. 2a). By the fourth week, one of the three replicates had crashed. The variance of growth between replicates was high. For example, the 29 °C temperature treatment simultaneously observed the highest growth rate (11.079 %) and the second lowest (5.906 %). There is a significant main effect of time on the biomass of *A. taxiformis* ($F_{(4,40)} = 8.838$, $p < 0.001$) but there is no significant evidence that temperature ($F_{(1,10)} = 2.218$, $p = 0.167$) or the interactions between temperature and time ($F_{(4,40)} = 1.909$, $p = 0.128$) influences growth.

The acclimated *Asparagopsis taxiformis* tetrasporophytes all showed sustained and high growth rates throughout the one-month experimental trial (Fig. 2b). By the end of the trial, the subsequent average stocking density across all treatments was 1.325g FW l^{-1}. Where the average DGR was 4.197 % for one month across the 24, 26 and 29 °C temperature treatments and the 32 °C had a DGR of 3.31 %. There was no significant effect of temperature on growth ($F_{(1,10)} = 4.267$, $p = 0.065$). However, there was a significant effect of time ($F_{(4,40)} = 72.223$, $p < 0.001$). There was no evidence of significant differences within stepwise comparisons (Student–Newman–Keuls; $p > 0.517$).

Bromoform content

The bromoform content of *A. taxiformis* from the acclimated growth trial exhibited low variation between the four temperature treatments (Fig. 3). The mean bromoform content across all temperatures was 0.360 mg/g dry weight, with an average standard deviation of 0.015 mg/g dry weight of across all replicates. There was no significant effect of temperature on bromoform content (ANOVA; $F_{(3, 8)} = 1.39$, p > 0.314), with the temperature accounting for only a small portion of the variance (Sum of Squares = 0.0015).

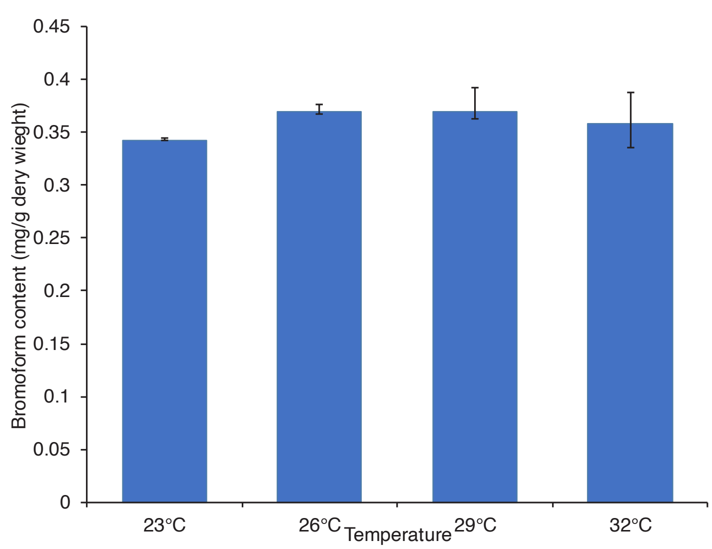

Figure 3. Bromoform content (mg/g dry weight) of *Asparagopsis taxiformis* from the acclimated growth trial, where the seaweed was cultured for four weeks at specific temperatures (23 °C, 26 °C, 29 °C, and 32 °C). Bars represent the mean bromoform content, with error bars indicating the standard deviation (n = 3).

Discussion

This study provides evidence that the Seychelle's strain of *Asparagopsis taxiformis* tetrasporophytes can achieve growth rates similar to those observed in the literature (Torres *et al*., 2023) under artificial conditions at temperatures as high as 32 °C when acclimated. While further research is required, particularly regarding the bromoform content, this initial study implies *A. taxiformis* aquaculture in Seychelles may be plausible.

The marked contrast in growth rates between the two trials likely results from *A. taxiformis* needing to acclimate to culture conditions or temperatures. Given that the specimens were harvested from 29 °C water, culture conditions are probably the main factor. The minimal impact of temperature on growth in the unacclimated trial may be due to high variability between replicates. While 32 °C may be too high for unacclimated *A. taxiformis*, this study found no evidence that this temperature affects production when acclimated. Furthermore, sea temperatures are unlikely to exceed 32 °C in Seychelles (Zinke *et al*., 2014). Interestingly, the lack of significant growth differences from 24 °C to 32 °C suggests that the Seychellois strain of *A. taxiformis* may be inherently adapted to higher temperatures, unlike *A. taxiformis* from other regions which will cease growing or die at temperatures exceeding 25 °C (Mata *et al*., 2017; Goldman, 2021).

The average daily growth rate (DGR; % day^{-1}) in the 24 °C, 26 °C, and 29 °C of the acclimated growth trial was 4.197 %. The DGR observed in nearly identical culture conditions in terms of light, nutrients and scale but at 22 °C was approximately 9 % (Torres *et al*., 2023). This implies that *A. taxiformis* is more suited to being cultured at lower temperatures. However, it should be acknowledged that the biomass used by Torres *et al* (2023) had been acclimated to domesticated conditions for 30 months prior to the experiment, which may have resulted in these greater growth rates. It should also be noted that there is still substantial room for improvement in general regarding the stocking densities, light quality and nutrient content of the water etc, where Torres *et al* (2023) found that at a greater stocking density (2g/l) the DGR would drop to 4 %.

While validation at scale and optimization for outdoor culture is necessary, preliminary farm-scale modelling approach offers a rudimentary assessment of the potential for large-scale production of *A. taxiformis*, based on the growth rates observed in this study. Assuming a stocking density of 1 g FW/L and a consistent daily growth rate of 4.197 % day^{-1} (as observed in this experiment between temperatures of 24 °C and 29 °C), cultivation within one-hectare, one-metre deep raceways (possible by repurposing two abandoned prawn ponds in Coetivity) could yield a standing stock of 10 tonnes of *A. taxiformis* biomass, with a daily production of 419.7 kilograms of fresh weight (kg FW). The projected annual production would be approximately 22.5 tonnes of dry weight (DW), based on a 15 % FW:DW conversion ratio (Jia *et al*., 2021).

Although the market value of *A. taxiformis* is not definitively established, recent sales indicate a price of $30/kg DW (USD), which is anticipated to decrease to $10 to $20/kg DW as more farms achieve scalability (World Bank, 2023). Thus, if a sales price of $15/kg DW is attainable, the projected annual income could reach approximately $337,500. Furthermore this *A. taxiformis* production could also provide bioremediation services for wastewater from the prawn ponds. It is crucial to emphasize that this estimate warrants cautious interpretation, and rigorous validation at an appropriate scale is imperative to ensure bioeconomic viability.

Initially, the absence of a significant temperature effect on bromoform content might seem promising for the stability of *A. taxiformis* cultivation. However, a more critical observation is the alarmingly low bromoform content detected in this study, which appears to be the lowest recorded to date. The literature reports bromoform concentrations in *A. taxiformis* ranging from 3.4 to 43 mg/g dry weight, with typical values around 10 mg/g dry weight in aquaculture (Burreson *et al*., 1976; Mata *et al*., 2012; Paul *et al*., 2006; Vucko *et al*., 2017). While it is possible that this strain of *A. taxiformis* inherently produces minimal bromoform, a more likely explanation is the potential degradation of bromoform due to the extended storage of samples in a freezer for two months prior to analysis. This was unfortunately unavoidable due to capacity limitations at the lab. The study from which the bromoform extraction protocol was adapted highlights that storage conditions and freeze-drying can significantly impact bromoform retention, emphasizing the need for controlled handling to ensure accurate measurements and reliable downstream processing for potential commercial applications (Romanazzi *et al*., 2021). Given these concerns, further research is imperative to reassess the bromoform content under optimized storage and handling conditions.

Once further research confirms the bromoform content of *A. taxiformis*, additional research that is required includes expanding tetrasporophyte cultivation to evaluate its scalability for commercial production under Seychelles' environmental conditions. Moreover, investigating the feasibility of gametophyte production in the Seychelles' coastal environment is crucial for optimizing seaweed cultivation practices.

This study demonstrates that the Seychellois strain of *A. taxiformis* can achieve significant growth rates, even at temperatures as high as 32 °C if adequately acclimated. A rudimentary bioeconomic calculation suggests a positive outlook for large-scale cultivation, indicating the potential economic viability of *A. taxiformis* production in Seychelles. However, before moving forward, it is crucial to re-assess the bromoform content under more controlled storage and handling conditions to ensure accuracy. This study is an important initial step toward evaluating the full potential of *A. taxiformis* aquaculture in Seychelles, with broader implications for the feasibility of farming this species in warmer waters worldwide.

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For further information, contact Tarah Padayachy or Charles Morel (herbariumsey@gmail.com; charles6422@gmail.com)

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