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RESEARCH

Growth rate comparison of three *Kappaphycus alvarezii* colour strains cultivated using tubular netting and tie-tie methods in the waters of Ecuador

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Abstract

Kappaphycus alvarezii is widely cultivated in tropical regions, with its growth influenced by cultivation techniques. Despite extensive global studies, there is a knowledge gap in Ecuador regarding the performance of different strains and systems under local specific conditions. The growth performance of *K. alvarezii* strains (green, brown, and red) cultivated in Ecuador using "tubular nets" and "tie-tie systems" was evaluated. Fifteen thalli from each strain were grown for 70 days, with daily measurements of water temperature, salinity, dissolved oxygen, turbidity, and nutrient levels. No significant differences were detected between the systems, and their DGR growth rates were high for algae culture development ($4.0 \pm 0.92\%$ day^{-1} in the tie-tie and $3.8 \pm 0.81\%$ day^{-1} in the tubular net) demonstrating their suitability for commercial cultivation. All strains exhibited DGR > 3%, with the green strain particularly excelling in the tubular system ($6.1 \pm 5.54\%$ day^{-1}). The local environmental conditions were found to be favourable for macroalgal growth, with a particularly strong positive correlation with temperature. The results show that both cultivation systems are viable for the commercial production of *K. alvarezii*, establishing a foundation for future applications in local aquaculture and highlighting the adaptability of the evaluated strains to Ecuador's environmental conditions.

Keywords Seaweed · Rhodophyta · Biomass · Mariculture · Environmental parameters · Productivity

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Introduction

Kappaphycus alvarezii, commonly known as elkhorn seaweed, is a commercially important macroalga cultivated in more than 20 countries worldwide (FAO 2024). The cultivation of this species has expanded significantly in tropical regions from Asia to Oceania, including the Philippines, Malaysia, the Maldives, and Tuvalu (Ask and Azanza 2002), Cambodia, Vietnam, Myanmar (Hurtado et al. 2019) and India (Mantri et al. 2022); as well as in Tanzania, Africa (Tanaka 1990). In the Americas, *K. alvarezii* has been successfully established in Cuba (Areces 1995), Brazil (de Paula et al. 1999), Venezuela (Rincones and Rubio 1999), Mexico (Muñoz et al. 2004), the Lesser Antilles, Belize (Smith and Rincones 2006), Colombia (Rincones and Moreno 2011), and more recently in Ecuador (Montúfar-Romero et al. 2023).

The success of *K. alvarezii* in aquaculture is attributed to its rapid growth and its ability to tolerate a wide range of environmental factors. These factors include cultivation depth (Sulistijo 2002), water current speed (Indriani and Sumiarsih 1999), sunlight intensity (Kune 2007), temperature ranges (Rahim 2009), water transparency (Pong-Masak et al. 2011), and additional variables such as pH, salinity (Yong et al. 2013), nitrite concentration, and phosphate levels (Aslan 1998; Montúfar-Romero et al. 2023). Moreover, internal factors such as thallus age, morphological structure, and genetic variability also influence its growth (Budiyanto et al. 2019). Temperature plays a critical role by regulating chemical reaction rates within cells, and extreme temperatures can lead to protein denaturation (Lobban and Harrison 1994). Previous studies have shown that *K. alvarezii* exhibits high tolerance to fluctuations in temperature and salinity, depending on strain colour and genetics (de Paula et al. 1999, 2002; Reis et al. 2015). In Brazil, for example, 12 new colour strains of *K. alvarezii* were generated from brown and pale brown strains; however, only 8 of these strains survived (Gelli et al. 2024). Notably, the development of new strains with brown coloration was not observed and temperature was found to play a significant role in the generation of new colour strains of *K. alvarezii*. The emergence of novel strains holds potential for the selection and development of new cultivars suited to local mariculture conditions (Gelli et al. 2024). In Mexico, three morphotypic varieties have been reported: green, brown, and red, each of which displays different productivity performances across regions and cultivation systems (Muñoz et al. 2004).

Culture methods can be classified into surface culture, suspended culture in water column, bottom culture and mixed systems (surface and water column combined). Among the surface culture methods, the raft or flotation method stands out, which includes the floating cage, basket net, longline, mooring, tubular net, monoline tubular method and net bag method (Hurtado et al. 2013; Kasim and Mustafa 2017; Periyasamy and Rao 2017; Yahya et al. 2024). Previous studies have reported the cultivation of *K. alvarezii* using mooring and tubular net techniques (de Góes and Reis 2011; Hurtado et al. 2013, 2019; Kasim & Mustafa 2017). In Brazil, the tubular net system has emerged as a promising alternative in regions with adverse environmental conditions, such as muddy bottoms and strong sea currents (Reis et al. 2015). In contrast, the tie-tie method is more commonly used in areas with milder currents (de Góes and Reis 2011).

Physiological differences between the colour variants have been consistently documented in Brazilian literature, suggesting the existence of specific adaptations to environmental conditions (de Paula et al. 1999, 2002; Reis et al. 2011). Furthermore, the three-colour strains (brown, green, and red) showed similar growth rates, carrageenan yields, sulphate, and gel strength, although the 3,6-anhydrogalactose content was variable among them (Hayashi et al. 2007). However, the green strain is the one that has shown greater plasticity to temperature and salinity fluctuations (Araújo et al. 2014). These findings suggest that combined cultivation methods could be beneficial for seaweed farmers facing difficulties with the use of seeds of a single variety during cultivation in different climatic seasons (Muñoz et al. 2004; Hung et al. 2009; Abdillah and Triastuti 2021).

Due to technological limitations for the extraction of carrageenans, seaweed aquaculture in Ecuador has shifted towards the development of alternative value-added applications, such as the production of biofertilizers and bio-stimulants for agricultural use. An alternative that presents great potential since bio-stimulants of marine origin, derived from macroalgae such as *K. alvarezii*, offer a sustainable alternative to synthetic fertilizers (Paul et al. 2024). This takes advantage of the composition rich in bioactive compounds, including polysaccharides, phytohormones and polyphenols, which can improve plant growth, nutrient absorption and stress tolerance (Trivedi et al. 2023).

In Ecuador the productivity and economic feasibility of floating raft cultivation have recently been reported (Montúfar-Romero et al. 2023). However, no comparative studies have yet been conducted on the productivity of tie-tie versus tubular net systems, nor on the growth differences between *K. alvarezii* strains under these conditions. Therefore, this study aimed to compare the growth of three *K. alvarezii* strains cultivated using tie-tie and tubular netting systems, and to determine the environmental factors influencing their cultivation for the exclusive conditions in Bahía Las Conchas, Santa Elena Province, Ecuador.

Materials and methods

Location and cultivation system

The study was conducted during the summer between July and September 2016 within the concession area of the Santa Rosa Artisan Fisheries Cooperative, located in Santa Elena, Ecuador, South America (2°12′48.31"S, 80°56′27.28"W) (Supplemental Fig. 1S). This area is characterized by a sandy marine substrate, with a bathymetry between 8–10 m. The experimental setup involved a floating raft system, consisting of modules 5 m in length and 3 m in width, constructed using PVC tubes sealed at each end to ensure buoyancy, anchored with 250 kg cement blocks (Fig. 1). The raft was positioned perpendicular to the current to optimize water flow through the system. Seedlings were randomly removed from *K. alvarezii* vegetative apical portion of the plants commercially cultivated at this site and they were placed at 30 cm below the water surface.

Experimental design

The study employed a design with three replicates assigned to each cultivation method. Three different colour strains of *K. alvarezii* were used for the experiment: green, brown, and red (Fig. 1a, b and c).

The tie-tie method consisted of ten long lines, each 5 m long, per replicate (Fig. 1d). Each line was equipped with 20 loop knots to hold seedlings and was suspended vertically from a floating raft. A nylon net with a mesh size of 60 mm was placed below the raft to mitigate herbivory and reduce seedling loss. Seedlings were attached to the long lines at 20 cm intervals using polyethylene lines. Moreover, for the tie-tie technique, 200 propagules were introduced in each experimental unit with initial wet biomass of 164.0 ± 1.69 g (95% confidence intervals in all average dispersion index) for the green strain, 165.8 ± 1.90 g for the brown strain, and 165.6 ± 2.02 g for the red strain. The corresponding initial dry biomass values were 15.8 ± 0.73 g for the red strain, 15.4 ± 0.48 g for the brown strain and 15.6 ± 0.78 g for the green strain. A total of 1800 propagules were used (200 × 3 treatments × 3 strains).

In the tubular net method, modifications were made to improve the ease of handling seedlings during sampling, which consisted of omitting a standardized 15 cm spacing between seedlings, as described in previous studies (Fig. 1e). Ten long lines, each 5 m long, were used per replicate. Each line supported 18 seedlings, wrapped in a tubular net. The tubular net method did not use a protective net below the raft. The seedlings wrapped in the tubular net were held vertically at 20 cm intervals along the long lines using polyethylene lines. Furthermore, in the tubular net technique, each experimental unit consisted of 180 propagules. The initial wet biomass was 164.8 ± 1.30 g for the green strain, 165.0 ± 1.64 g for the brown strain, and 165.0 ± 1.39 g for the red strain. The initial dry biomass of these strains was 15.6 ± 0.48 g (green), 15.4 ± 0.78 g (brown), and 15.4 ± 0.48 g (red). A total of 1620 propagules were used (180 × 3 treatments × 3 strain).

Data collection

There is no consensus and after a large literature review, we concluded that the sampling frequency for *K. alvarezii* cultivation varies significantly across geographic regions, influenced by local environmental conditions and research objectives. Studies in India have used intervals of 15–45 days (Subba et al. 2008; Kumar et al. 2016), Indonesia 42–45 days (Luxton 1993; Aslan et al. 2019), the Philippines 60 days (Hurtado et al. 2001) and Brazil 36, 42, 45, 47, and 97 days (Hayashi et al. 2007, 2011; de Góes & Reis 2011), reflecting diverse cultivation practices. Considering this variability and the emerging nature of seaweed farming in Ecuador, this study implemented a multi-phase sampling strategy (Montúfar-Romero et al. 2023). Sampling was conducted at 19 days (Period I), 33 days (Period II), and 70 days (Period III) post-sowing to capture critical growth stages and enable a thorough analysis of cultivation dynamics (Supplemental Table 1S).

Wet biomass growth was measured by randomly sampling 15 samples of propagules per period, weighed in situ with a digital portable scale (0.01 g precision). From this sample, three tissues were randomly selected for dry biomass evaluation, washed with freshwater, and dried in an oven at 105 °C for 3 h, following Ohno et al. (1996). The remaining tissue was returned to the respective experimental unit (Supplemental Fig. 2S).

For the growth curves, the final absolute dry weight was used in each of the cultivation periods and strains. For comparative use with other *K. alvarezii* cultures, we utilized the daily growth rate equation in percentage (% DGR) based on wet mass as proposed by Yong et al. (2013) with Eq. (1) as follows:

$$\%DGR = \left[\left(W_f - W_o\right)^{1/t} - 1\right] * 100 \quad (1)$$

where W_o is the initial weight (g), W_t is the final weight (g), and t is the number of days of culture. The data are presented as mean daily growth rates for periods I, II and III.

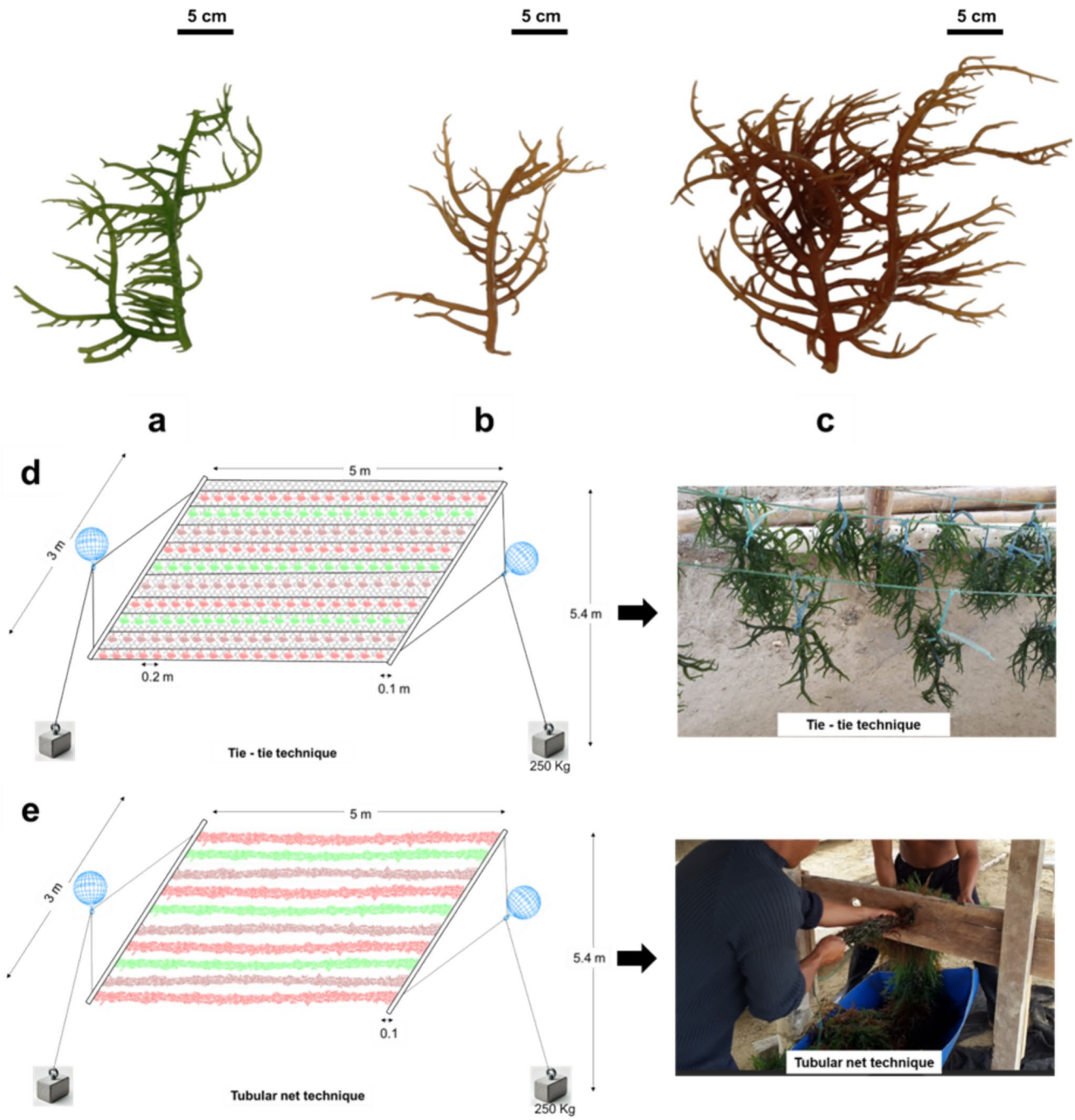


Fig. 1 Cultivation techniques and morphological characteristics of *K. alvarezii* strains. (**a**) green strain, (**b**) red strain, and (**c**) brown strain, (**d**) Schematic representation and field photograph depicting the tie-tie cultivation technique for *K. alvarezii*. The schematic illustrates the arrangement and spacing between thalli (0.2 m), as well as the use of anchoring weights (250 kg) and buoys for maintaining stability and positioning in the marine environment. (**e**) Schematic representation and field photograph of the tubular net cultivation system, highlighting the structural configuration of the tubular nets and their application to *K. alvarezii* cultivation. This technique employs anchor weights and buoys similar to those used in the tie-tie method to ensure structural integrity

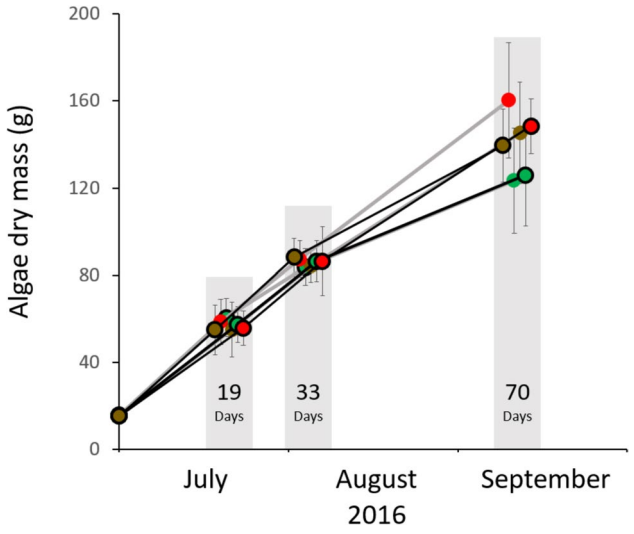


Fig. 2 Changes in the dry biomass of *Kappaphycus alvarezii* strains (green, brown, and red) across three cultivation periods in Bahía Las Conchas, Ecuador. The grey lines correspond to the Tie-Tie technique, while the black lines represent the Tubular Netting technique. Vertical lines indicate the 95% confidence intervals for the mean dry biomass increase during each period

Environmental factors monitoring

To evaluate the abiotic factors that influence the growth and physiological responses of *K. alvarezii* sea surface temperature, salinity, pH, dissolved oxygen and dissolved oxygen saturation were monitored daily with previously calibrated equipment using a YSI Professional Plus (Pro Plus) multiparameter probe. Water turbidity was assessed using a Secchi disk to determine water clarity, a factor that influences light penetration. The nutrient profile was determined using the Hanna Instruments HI 83200 device with an accuracy of 0.01 ppm, which quantifies the following parameters: ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}).

Sowing and harvesting times

To quantify the labour and time requirements associated with each culture method, the time spent on operations in the water and on the beach was assessed. The time required to deploy the long lines from the boat to the water and to retrieve them from the raft to the boat was systematically recorded. The time taken to prepare the long-lines on the beach and at sea was recorded, which involved tying 20 lasso knots to attach the seedlings to each 5-m-long line. A total of 18 long-lines were used for time measurements: 9 long-lines for the tube net method (three for each strain colour) and 9 long-lines for the tying-tying method (three for each strain colour).

Statistical analysis

Differences between cropping systems and periods, as well as sowing and harvesting times were determined according to the recommendation of Hsu (1996). Independent variables (systems, strains, and periods) were converted into categorical factors while dependent variables (weight dry and weight wet) were logarithmically transformed to stabilize variances and improve data normality. To determine differences in sowing and harvesting times at beach and sea, Levene's test was applied to verify homogeneity of variances between groups. Data normality was assessed using the Shapiro–Wilk test. A Welch three-way ANOVA was used with a Tukey's Honest Significant Difference (HSD) to unequal variances (Games-Howell test) was applied for post hoc comparison (Zar 2010). To determine the correlation, a Principal Component Analysis (PCA) was used between environmental parameters (temperature, dissolved oxygen, dissolved oxygen saturation, turbidity, nitrate, nitrite and ammonium) and the growth rates of three *K. alvarezii* strains (red, green and brown) in tie-tie and tubular culture systems. The data was standardized by z-score to ensure equality in the scales of the variables. The principal components were selected according to the Kaiser-Guttman criterion (eigenvalues > 1), evaluating the loadings to identify the contributions of the variables. A biplot was generated to visually interpret the underlying patterns and correlations in the data. In addition, a correlation analysis was carried out using a heat map with a dendrogram where a data matrix was constructed where the rows represented the samples and the columns the variables. Subsequently, the Pearson correlation matrix was calculated to quantify the linear relationships between the variables. Using the complete linkage method, a hierarchical cluster analysis was performed to group variables with similar correlation patterns. Statistical analyses were performed using RStudio (version 2024.09.1).

Results

No natural mortality or grazing damage was observed in the *K. alvarezii* cultivation during the study. The final wet biomass increased up to tenfold from the initial biomass, with the red strain exhibiting the highest biomass accumulation in both cultivation systems: 1773.0 ± 292.28 g in the tubular netting system and 1642.0 ± 139.37 g in the tie-tie system (Fig. 2S). The most significant biomass increases were observed during periods II and III. Dry biomass growth, in absolute terms, showed no significant difference ($p > 0.05$) between both cultivation systems. However, significant differences ($p < 0.05$) were found between the propagules of the red strain (160.3 ± 26.42 g) and the green strain (126.0 ± 23.36 g) across both cultivation systems (Fig. 2).

The average DGR was was higher in tie-tie than the tubular system (4.0 ± 0.92 and $3.8 \pm 0.81\%$ day^{-1}) although not significantly ($p > 0.05$). In the tie-tie system the green strain showed the highest growth rate during period III measured in % DGR ($6.1 \pm 5.54\%$ day^{-1}), while the brown strain peaked in period II ($6.1 \pm 4.53\%$ day^{-1}). The red strain exhibited the lowest growth rates, particularly in period III ($1.7 \pm 0.57\%$ day^{-1}). In the tubular netting system, the green strain maintained consistently high growth rates across all periods, with a maximum of $6.0 \pm 5.50\%$ day^{-1} during period III. The brown strain peaked in period II ($5.1 \pm 3.43\%$ day^{-1}), and the red strain exhibited the lowest %DGR in period III ($1.9 \pm 0.55\%$ day^{-1}) (Table 1S). Significant variations in DGR were observed between periods and strains, which did not allow significant differences to be established between the DGRs of the strains in the different growth periods, except for the lowest DGRs in the red strain at 70 days (Fig. 3).

Principal component analysis (PCA) shows that within environmental parameters, phosphate (PO_4^{3-}) and nitrite (NO_2^-) exhibit strong positive contributions along Dim2, suggesting their influence on specific macroalgal growth patterns. Turbidity and dissolved oxygen are located closer to the negative side of Dim1, indicating a weaker and potentially inverse association with green macroalgae growth rates. Red macroalgae (tie-tie and tubular network) growth rates cluster on the positive axis of Dim1, indicating their sensitivity to parameters such as ammonium (NH_4^+) and temperature. In contrast, these growth rates show an opposite relationship with PO_4^{3-}. Growth rates of brown macroalgae (both tie-tie and tubular network) also align positively along Dim1, reflecting their sensitivity to NH_4^+ and temperature, and similarly show an inverse relationship with PO_4^{3-} (Fig. 4). This PCA visualization underlines the differential influence of nutrient concentrations and physicochemical parameters on macroalgal growth, revealing specific environmental drivers for each algal group.

Nutrient parameters (NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}) formed a distinct cluster with moderate to strong intercorrelations, particularly with PO_4^{3-} and NH_4^+ sharing a negative association. Furthermore, NO_3^- and NO_2^- shared a negative association. Growth rate metrics showed pronounced positive intragroup correlations, particularly between red and brown macroalgal formations in the tie-tie and tubular netting growth forms. Temperature and NH_4^+ showed moderate correlations with macroalgal growth, predominantly positive for brown and red macroalgal structures, but variable in green strains. Dissolved oxygen and turbidity showed weak correlations with macroalgal growth rates but showed

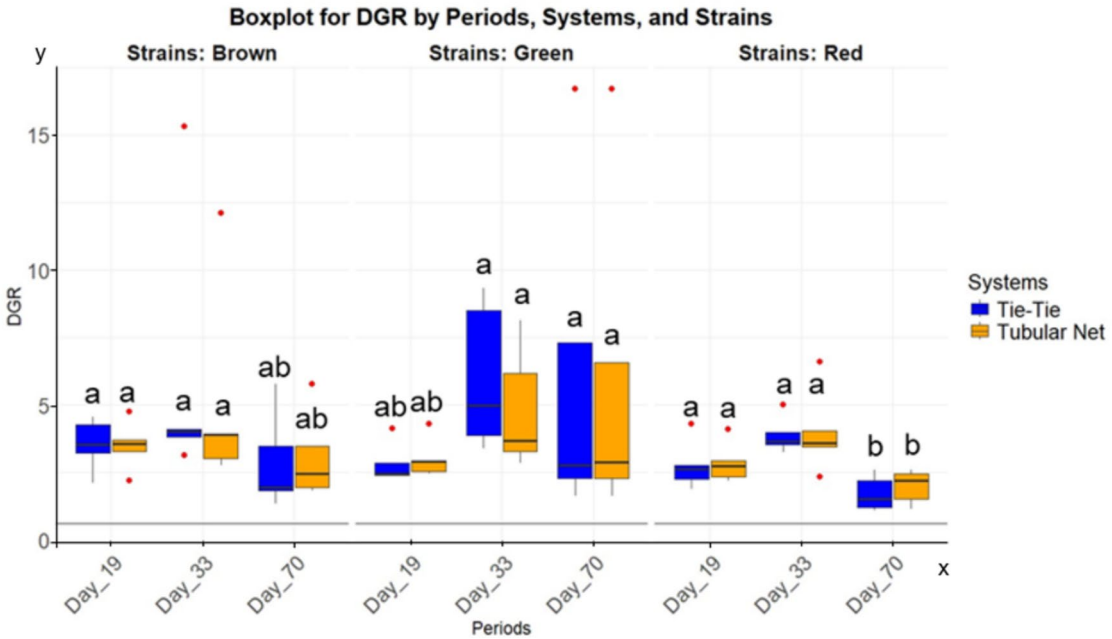


Fig. 3 Daily growth rate of *K. alvarezii* across time, system, and strain. This figure presents box plots illustrating the distribution of daily growth rate (DGR) for *K. alvarezii* across three cultivation periods (Days 19, 33, and 70), two cultivation systems (tie-tie and tubular net), and three strains (brown, green, and red). The box plots depict the median, interquartile range (IQR), and potential outliers (red points) for each treatment combination. A three-way Welch ANOVA revealed significant effects of the factors "Period" and "Strain:Period" on DGR. Tukey's post hoc tests further identified significant differences ($p < 0.05$) among several groups, notably between Brown Day_19, Green Day_19, Brown Day_33, Green Day_33, Red Day_19, and Red Day_33 when compared to Red Day_70, indicating substantial variability in growth response depending on cultivation duration and strain characteristics

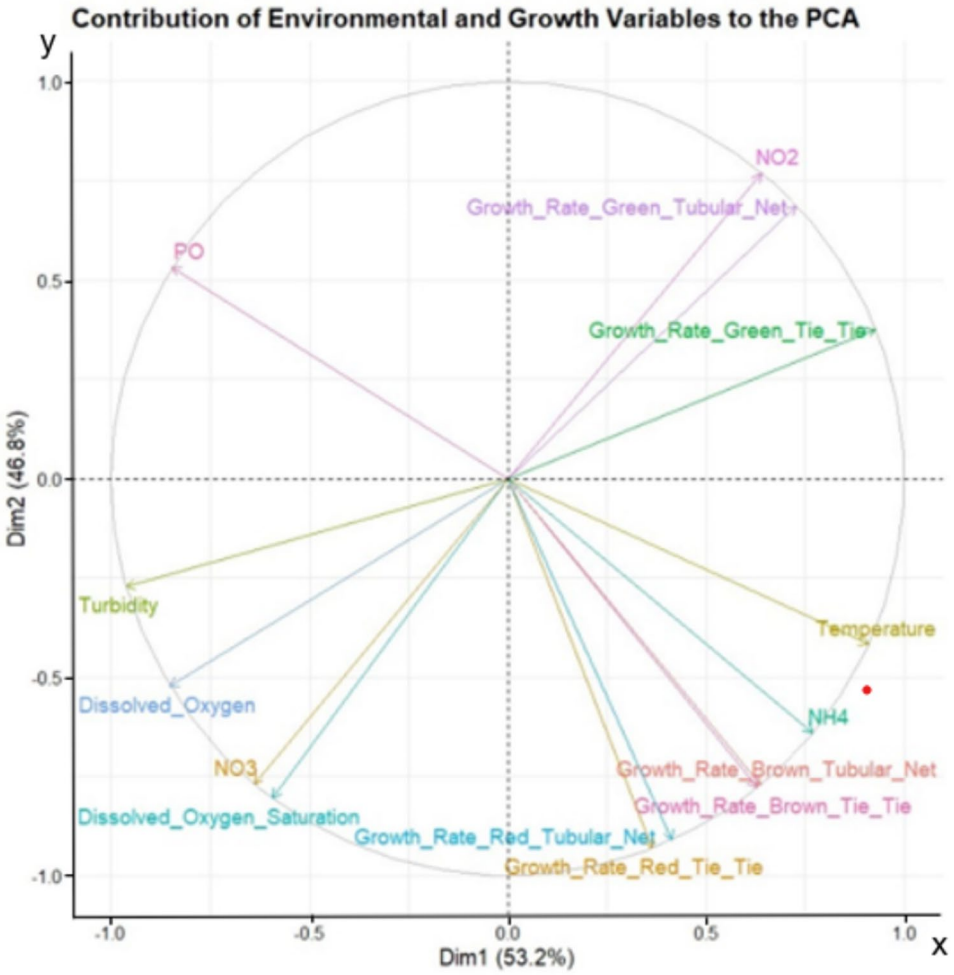


Fig. 4 Contribution of environmental and growth variables to the principal component analysis (PCA). This biplot represents the contribution of various environmental factors (e.g., temperature, dissolved oxygen, nutrient concentrations) and growth rates of *K. alvarezii* strains (red, green, and brown) cultivated under tie-tie and tubular net systems to the first two principal components (Dim1 and Dim2). The axes, Dim1 (53.2%) and Dim2 (46.8%), indicate the percentage of variance explained by each principal component. The vectors represent the direction and strength of each variable's contribution to the overall variation within the dataset. Longer vectors indicate variables with a stronger influence on the respective principal components. The angles between vectors provide insight into the correlation between variables, with smaller angles suggesting stronger positive correlations

moderate positive correlations with nutrient concentrations (Fig. 5).

The tie-tie technique required an average of 1.7 ± 0.58 min to attach the long line to the raft, while the time needed to tie the seaweed onto the long line was 4.2 ± 0.11 min. In comparison, the tubular netting technique took 1.7 ± 0.65 min to secure the long line to the raft and 9.7 ± 0.85 min to tie the seaweed onto the long line. Additionally, the time required for harvesting seedlings onto the long line using the tie-tie technique was 1.4 ± 0.12 min, whereas the tubular netting technique required 5.3 ± 0.27 min. For harvesting seedlings in the raft, the tie-tie technique took 0.7 ± 0.60 min, and the tubular netting technique required 0.6 ± 0.70 min. The times for both techniques were statistically comparable for attaching the long line to the raft and for harvesting seedlings in the raft. However, significant differences were observed when tying the seaweed onto the long line and harvesting seedlings onto the long line, with the tubular netting technique being markedly slower than the tie-tie technique ($p < 0.05$) (Fig. 6).

Discussion

The cultivation of *K. alvarezii*, a commercially valuable macroalga due to its carrageenan content, has been extensively studied worldwide owing to its adaptability to various environmental conditions and its fast growth rate (Nunes et al. 2024; Tabacof et al. 2024). Previous research had primarily focused on assessing the growth performance of different coloured strains and the efficiency of cultivation systems such as tubular nets and tie-tie systems (Muñoz et al. 2004; de Góes and Reis 2011; Reis et al. 2015; Tan et al. 2017; Mantri et al. 2022; Capacio et al. 2024; Gelli et al. 2024). Although Capacio et al. (2024) established the existence of growth relationships with temperature, salinity and pH, most studies have largely overlooked the interactions between

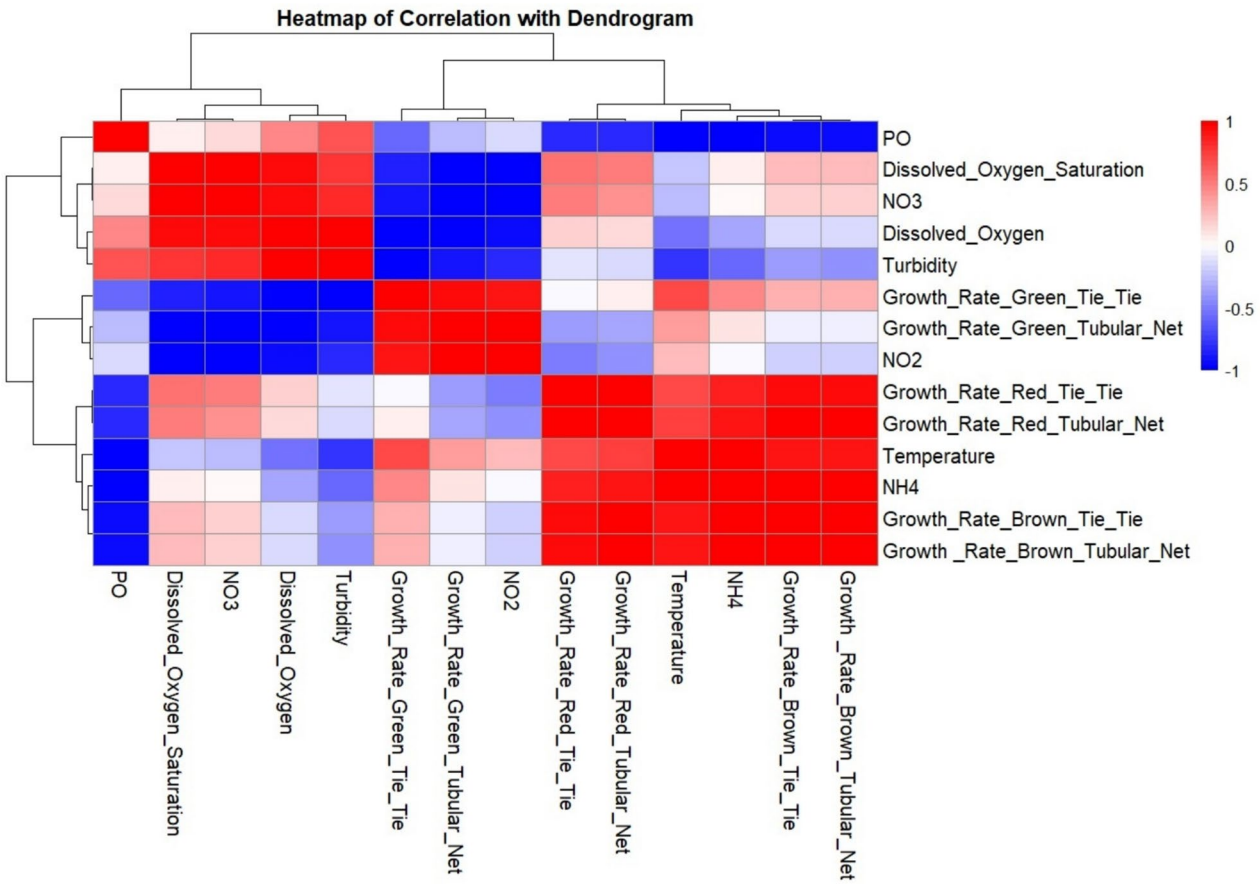


Fig. 5 Heatmap of correlations with dendrogram among environmental and growth variables of *K. alvarezii*. This heatmap illustrates the correlation matrix for environmental variables (e.g., nutrients, temperature, dissolved oxygen) and the growth rates of *K. alvarezii* cultivated in different systems (tie-tie and tubular net) and strains (red, green, and brown). The color gradient represents the strength and direction of correlations, ranging from blue (negative correlation) to red (positive correlation), with white indicating no correlation. The accompanying dendrogram shows the hierarchical clustering of variables based on the similarity of their correlation profiles, providing insights into potential groupings and relationships. Clusters of variables that are closely correlated are visually grouped, which helps in identifying environmental factors that may have a similar impact on growth rates

specific culture systems and coloured strains in different environmental settings. This omission represents a critical knowledge gap that does not allow the development of cultivation strategies in local environmental conditions and aquaculture system. Therefore, our study aimed to address this lack of knowledge by evaluating the differential growth of three *K. alvarezii* strains—green, red, and brown—cultivated in tubular nets and tie-tie systems in Bahía Las Conchas, Ecuador. Additionally, the presence study has determined the viability and potential of each combination to optimize biomass production.

Our results indicate that, although no significant difference in growth rates was found between the two cultivation systems, there was relevant variability among the strains. As such, the green strain showed the highest growth rates in the floating raft system. This variation in performance could be attributed to specific physiological adaptations of each strain and their ability to respond to local environmental factors such as temperature, salinity, and nutrient availability. The stability of these factors in Bahía Las Conchas appears to be a key determinant of the sustained growth of *K. alvarezii*, aligning with previous studies that identified temperature and salinity as critical parameters for the productivity of this species in tropical environments (Hurtado-Ponce 1995; Muñoz et al. 2004; Montúfar-Romero et al. 2023). The observed variability in growth rates suggests that strain selection for different environmental conditions could be an effective strategy to optimize the cultivation performance of this species across various regions.

The environmental conditions observed in Bahía Las Conchas, characterized by an average temperature of 28.9 °C and a salinity of 34 ‰, are in line with the optimal ranges previously established for the cultivation of *K. alvarezii*, between 27–30 °C and 30–35 ‰ (Rahim 2009; Montúfar-Romero et al. 2023). These results reinforce the evidence that *K. alvarezii* thrives in warm climates with low thermal fluctuation, as reported in studies from Japan and Brazil,

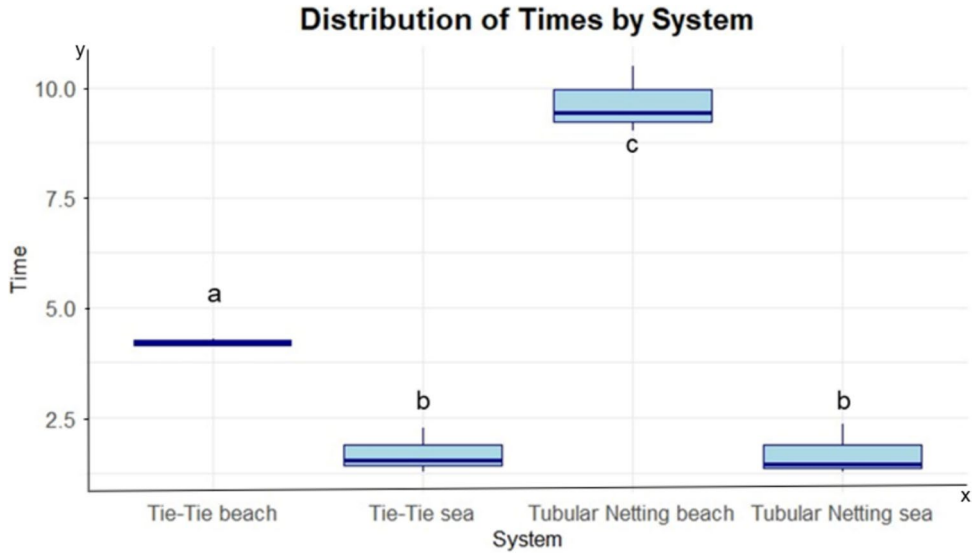


Fig. 6 Harvesting time distribution for *Kappaphycus alvarezii* across systems and locations. This figure presents box plots illustrating the distribution of harvesting times (in minutes) for *K. alvarezii* cultivated using tie-tie and tubular net systems, both in beach and sea environments. The box plots depict the median, interquartile range (IQR), and potential outliers for each combination of system and location. A one-way ANOVA revealed significant differences in harvesting times between several groups, including tie-tie sea vs. tie-tie beach, tubular net beach vs. tie-tie beach, and tubular net sea vs. tubular net beach. No significant difference was found between tubular net sea and tie-tie sea, suggesting comparable harvesting efficiency in these settings. The results indicate that both the cultivation system and the location significantly influence harvesting time, with the tubular net system on the beach exhibiting the greatest variability

which indicated optimal growth rates at temperatures above 20 °C (Ohno et al. 1994; de Paula et al. 2002; Araújo et al. 2014; Mandal et al. 2015). The preference of this species for environments with elevated temperatures and moderate salinity underscores the importance of local environmental conditions for maximizing biomass production, especially in controlled cultivation systems.

The temperature increase observed during the second cultivation period, peaking at 29.7 ± 0.06 °C, coincides with an increase in growth rates documented, suggesting that *K. alvarezii* exhibits better performance in environments with temperatures closer to this value compared to periods with lower temperatures (28.3 ± 0.06 °C). The thermal stability observed in Bahía Las Conchas, with minimal variation of ± 0.72 °C throughout the study, along with stable salinity, provides an optimal environment for the continuous growth of *K. alvarezii* by minimizing osmotic stress and enhancing critical physiological processes such as photosynthesis and carbon fixation, essential for its accelerated growth (Siddiqui et al. 2024).

Furthermore, the availability of nutrients, particularly phosphate concentration, plays a crucial role in the differential growth of the strains evaluated (Rathod et al. 2014). Significant correlations were observed between nitrogenous compounds (NH_4^+, NO_2^- and NO_3^-) and macroalgal growth rates (Fig. 4), the recorded PO_4^{3-} concentrations (0.16 to 0.18 ppm) were considerably higher than the minimum values of 0.0071 to 0.0169 ppm reported as necessary for the optimal development of *K. alvarezii* (Aslan 1998). Nitrate concentrations (1.92 ppm) in the experimental environment exceeded the minimum values of 0.3 to 0.6 ppm reported for adequate macroalgal development (Doty and Norris 1985; Glenn and Doty 1990; Luxton 1993; Areces 1995; Montúfar-Romero et al. 2023). We hypothesise this abundance of NO_3^- in the environment may have contributed to maintaining stable growth rates during the experimental period, especially under irradiance conditions, where nitrogen metabolic demand tends to increase as also showed in previous studies (Satriani et al. 2022). These findings highlight the environmental drivers most strongly associated with the growth performance of specific algal structures. This integrative analysis underscores the potential influence of nutrient concentrations and temperature on algal growth, contributing to a deeper understanding of macroalgal ecology in fluctuating environmental contexts.

The growth of macroalgae is regulated by multiple environmental factors (Terada et al. 2016). This has been documented in various studies; for example, a negative correlation between growth and temperature has been reported in *K. alvarezii* cultivated in Yucatán, Mexico (Muñoz et al. 2004). Periyasamy et al. (2015) found negative correlations between DGR and temperature, as well as a positive correlation with NO_2^- and PO_4^{3-}. In Indonesia, no significant correlations were found with any of the analysed environmental factors (salinity, temperature, dissolved oxygen, pH, NO_2^-, and PO_4^{3-}), possibly due to the limited data available (Simatupang et al. 2021). Our results (Fig. 5) show moderate to strong intercorrelations between growth and nutrients

(NH_4^+, NO_2^-, and NO_3^-) and negative associations with PO_4^{3-}. Additionally, we observed a strong positive association between growth and temperature, a factor that increases metabolism, contrary to previous reports.

The comparative analysis between the cultivation systems, tubular nets and tie-tie, shows that tubular nets offer significant advantages in seedling management and protection against herbivory. This system effectively minimizes biomass dispersal and fragmentation, which are detrimental to crop stability and overall yield. Although no statistically significant differences in final biomass were observed between the two systems, tubular nets are better suited for regions with high herbivory pressure, as evidenced by studies from other regions where herbivore activity can reduce biomass by up to 50% (Russell 1983). Additionally, tubular nets may present logistical benefits, such as more efficient handling during transplantation and harvesting, reducing unintentional biomass loss and enhancing profitability in large-scale of *K. alvarezii* farming systems for the unique environmental conditions of this region in Ecuador. Throughout the study, no natural mortality or grazing damage was observed on the *K. alvarezii* crop, likely due to the protective conditions of the tubular nets. However, in other months, herbivorous species like turtles and herbivorous fish, including *Scarus rubroviolaceus,* were observed grazing on the raft, indicating potential vulnerability without such protection. Thus, the tubular net system is more suitable for year-round cultivation in regions with high herbivore pressure, offering a more reliable and effective solution to prevent grazing damage and ensure crop stability over time.

In other countries with different conditions (e.g., Brazil), the efficiency of the tubular net technique was superior to the tie-tie technique in the cultivation of *K. alvarezii*, obtaining higher daily growth rates and lower cultivation costs, as well as retaining more seedlings on the floating raft, which is better for the environment by preventing invasiveness (Reis et al. 2015). However, more recent studies in India (Megarajan et al. 2024) have also demonstrated the superiority of tubular net systems for commercial-scale seaweed production in exposed coastal waters. These findings align with the results of our study, further validating the effectiveness of tubular nets in optimizing macroalgae cultivation.

This assessment of the differential growth observed among the three strains over the 70-day cultivation period underscores the critical importance of selecting genotypes that are optimally suited to specific local cultivation conditions, at least over the span of one year. The green strain consistently outperformed the red and brown strains in both the tubular net and tie-tie systems. This finding aligns with previous research conducted in Brazil and Mexico, which highlights the adaptability of green strains to environments characterized by high irradiance and elevated temperatures (Hurtado-Ponce 1995; Muñoz et al. 2004; Araújo et al. 2014). The observed growth advantage of the green strain is likely attributable to its enhanced photosynthetic efficiency under high light conditions (Zhang et al. 2024).

In this study, the growth rates of the green strain ($6.1 \pm 5.54\%$ day^{-1} in the tie-tie system and $6.0 \pm 5.50\%$ day^{-1} in tubular nets) were somewhat higher than those reported in Mexico, where rates ranged from 2.0 to 3.3% day^{-1} (Muñoz et al. 2004), and those recorded in Brazil, which averaged 1.7% day^{-1} at 32 °C over a 28 day period (Araújo et al. 2014). However, these rates were higher than those recorded in Brazil during winter, which averaged less than 0.54% day^{-1} (Hayashi et al. 2011). These differences may be explained by the thermal stability of Bahía Las Conchas, where the average temperature of 28.9 ± 0.72 °C creates an optimal environment for continuous growth, minimizing physiological stress and promoting higher biomass production. In contrast, the red and brown strains, despite showing higher growth rates in the tie-tie system, demonstrated increased sensitivity to environmental fluctuations, which suggests that their production is less predictable under varying conditions. The green strain, however, displayed consistent growth across both systems, making it a promising candidate for commercial cultivation in areas with high irradiance and stable thermal conditions.

From a commercial perspective, all strains evaluated in our study demonstrated growth rates suitable for large-scale production, consistently surpassing the critical growth threshold of 3.5% day^{-1}, which is essential for the successful aquaculture of *K. alvarezii* (Doty 1987; Glenn and Doty 1990). Montúfar-Romero et al. (2023) suggested that harvesting at 33 days could further optimize yields, especially for the brown strain, which achieved the highest growth rates during this period in both cultivation systems. However, the brown strain exhibited greater variability, indicating less predictable outcomes. This observation aligns with studies from the Philippines, where the brown strain's growth rates ranged from 0.6 ± 0.2 to $2.8 \pm 0.1\%$ day^{-1}, while the green strain's growth rates ranged from 0.5 ± 0.1 to $1.6 \pm 0.1\%$ day^{-1} over a 63-day cultivation period (Dawes et al. 1994). Similarly, Ohno et al. (1994) reported growth rates ranging from 0.13 to 8.12% day^{-1} for the brown strain and 0.23 to 7.3% day^{-1} for the green strain in the subtropical waters of Shikoku, Japan. Additionally, Hurtado-Ponce (2001) noted growth rates of 2.0–7.1% day^{-1} for the brown strain, 2.0–8.1% day^{-1} for the green strain, and 2.6–6.5% day^{-1} for the red strain in Dzilan, Hawaii, reinforcing the variability observed in this study.

The time required for tying the seaweed onto the longline and for harvesting seedlings using the tie-tie technique was significantly shorter compared to the tubular netting technique. This result contrasts with the findings of de Góes and Reis (2011), who reported that the tubular netting technique (21.42 h) was more time-efficient than the tie-tie technique (38.08 h). The discrepancy observed in our study is likely

attributed to the expertise and skill level of the personnel involved in seaweed cultivation in Ecuador, which may have contributed to the increased efficiency of the tie-tie method.

The results of our study not only confirm the suitability of Bahía Las Conchas for *K. alvarezii* cultivation but also provide practical guidelines for optimizing aquaculture operations in the region. The stability of temperature and salinity, combined with adequate phosphate concentrations, could create an optimal environment for year-round cultivation of this species (Hurtado et al. 2015; Roleda and Hurd 2019). The use of tubular nets stands out as a cost-effective and efficient strategy in areas with high herbivory, minimizing fragmentation and facilitating handling and harvesting—key factors for reducing biomass losses and increasing cultivation profitability (de Góes and Reis 2011). Commercially, the strains evaluated in this study showed growth rates suitable for large-scale production, exceeding the optimal thresholds defined for *K. alvarezii* aquaculture development. Among them, the green strain exhibited more stable and higher growth in both cultivation conditions, suggesting its potential to maximize yield in environments with high irradiance and low thermal variability. This supports the use of green strains in commercial cultivation programs, with the potential to diversify seaweed aquaculture in Ecuador and generate new economic opportunities for local communities.

The expansion of *K. alvarezii* cultivation in Ecuador could not only boost economic development and job creation in coastal communities, but also contribute to mitigating environmental impacts through nutrient absorption and carbon sequestration, providing additional benefits for ecological sustainability (Valderrama et al. 2015; Rao and Periyasamy 2019; Behera et al. 2022; Luo et al. 2023). In this context, implementing nutrient management strategies and selecting strains based on local environmental conditions would be key to ensuring the long-term sustainability and profitability of operations. The expansion of this crop in Ecuador could promote employment generation and improve social inclusion, especially in coastal communities that rely on fishing and aquaculture for their livelihood (Poeloengasih et al. 2014; Rebours et al. 2014; Samonte 2017; Rameshkumar and Rajaram 2019; Rimmer et al. 2021; Montúfar-Romero et al. 2023).

Conclusion

This study confirms the environmental viability of cultivating *K. alvarezii* in Bahía Las Conchas, Ecuador, using tubular netting and tie-tie techniques, with no statistically significant differences in daily growth rates between the two systems. These results support the implementation of either system from an artisanal to an industrial scale, meeting the commercial parameters for this species. The identification of the green strain as having the highest growth potential, and the validation of tubular netting technique as an effective alternative in areas with high herbivory, represent key advances for optimizing production. Additionally, the three evaluated strains (green, red, and brown) demonstrated promising growth rates, suggesting that this cultivation can contribute to the diversification of aquaculture in Ecuador. This approach not only creates new economic opportunities for coastal communities but also promotes environmental sustainability by absorbing nutrients and sequestering carbon, thereby benefiting the local ecology.

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Declarations

Ethical statement According to article 104 of the General Regulations of the Organic Law for the Development of Aquaculture and Fisheries published in the Official Registry on February 25, 2022, in Ecuador, the natural or legal person who wishes to obtain authorization to carry out mariculture activities must obtain a concession issued by the governing body. The concession grants individuals the exclusive use and enjoyment of the marine area identified in the corresponding ministerial agreement. In this study the *K. alvarezii* seedlings were imported from the Punta Laurel sector, Bocas del Toro Archipelago, Panama, with a phytosanitary certificate of origin and invoice. The experimental farm in Panama is certified by the Undersecretary of Aquaculture as an exporting establishment of *Kappaphycus* seaweed, certified as free of any pathological agent by Panama's National Government through the National Directorate of Animal Health, Ministry of Agricultural Development – MIDA.

Conflict of interest The authors declare no competing interests.

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