

Effects of low salinity on the morphology and survival of the seaweed *Kappaphycus alvarezii* (Rhodophyta) cultivated in Ubatuba Bay, southeastern Brazil

Efectos de la baja salinidad sobre la morfología y supervivencia del alga *Kappaphycus alvarezii* (Rhodophyta) cultivada en la Bahía de Ubatuba, Brasil suroriental

Valéria C. Gelli¹ , Nair S. Yokoya² , Estela M. Plastino³ 

¹Instituto de Pesca, Agência Paulista de Tecnologia dos Agronegócios, Secretaria da Agricultura e Abastecimento do Estado de São Paulo. Estrada do Professor Joaquim Lauro Monte Claro, 2275, Ubatuba, SP, 11688-102, Brazil

²Instituto de Pesquisas Ambientais, Av. Miguel Estéfano 3687, São Paulo, SP, 04301-902, Brazil

³Instituto de Biociências, Universidade de São Paulo, Rua do Matão 277, 05508-090, São Paulo, SP, Brazil

Correspondencia: Valeria Cress Gelli **E-mail:** valeria.gelli@sp.gov.br

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ABSTRACT | The cultivation of *Kappaphycus alvarezii* (Rhodophyta) provides renewable raw materials and contributes to ecosystem services, such as carbon sequestration, nutrient recycling, and climate change mitigation. However, extreme climatic events, including abrupt fluctuations in salinity, significantly affect its growth and survival. This study evaluated the impact of a heavy rainfall event in March 2024 on the morphology, survival, and recovery of 12 *K. alvarezii* strains cultivated at the Experimental Marine Farm of the Fisheries Institute, in Ubatuba Bay, São Paulo, Brazil. During the event, salinity decreased from 35 to 10 ‰, accompanied by reductions in water transparency and temperature. These changes caused necrosis of apical branches, loss of pigmentation, and abnormalities in thallus morphology. Mortality rates varied among the 12 color strains of *K. alvarezii*, with the light green gametophytic 'Edison de Paula' strain, the brown and emerald green tetrasporophytic strain, and the red and green tetrasporophytic strain exhibiting the highest losses. Strains of *K. alvarezii* cultured in the biobank of the Fisheries Institute enabled replanting the different strains in the sea, though recovery outcomes varied in accordance with each strain. This study highlights the critical importance of biobanks in mitigating the impacts of extreme climatic events on seaweed aquaculture, ensuring the *ex situ* conservation of genetic diversity and production sustainability. Cultivation strategies and the development of more tolerant strains are essential to enhance the resilience of *K. alvarezii* farming. The findings also underscore the need for public policies and investment in biobanks to safeguard aquaculture against environmental adversities and support sustainable development. Future research should focus on strain-specific responses to salinity levels below 15 ‰ and provide practical guidelines for cultivation under extreme environmental conditions.

Palabras clave

cambio climático
biobanco
cepas
hiposalinidad

RESUMEN | El cultivo de *Kappaphycus alvarezii* (Rhodophyta) proporciona materias primas renovables y contribuye a servicios ecosistémicos, tales como la captura de carbono, el reciclaje de nutrientes y la mitigación del cambio climático. Sin embargo, eventos climáticos extremos, incluyendo fluctuaciones abruptas en la salinidad, afectan significativamente su crecimiento y supervivencia. Este estudio evaluó el impacto de un evento de fuertes lluvias en marzo de 2024 en la morfología, supervivencia y recuperación de 12 cepas de *K. alvarezii* cultivadas en la Estación Experimental de Cultivo Marino del Instituto de Pesca, en la Bahía de Ubatuba, São Paulo, Brasil. Durante el evento, la salinidad disminuyó de 35 a 10 ‰, acompañada de reducciones en la transparencia y temperatura del agua. Estos cambios causaron necrosis de las ramas apicales, pérdida de pigmentación y anomalías en la morfología del talo. Las tasas de mortalidad variaron entre las 12 cepas de color de *K. alvarezii*. La cepa gametofítica verde clara 'Edison de Paula', la cepa tetrasporofítica marrón y verde esmeralda, y la cepa tetrasporofítica roja y verde presentaron las mayores pérdidas. Las cepas de *K. alvarezii* cultivadas en el biobanco del Instituto de Pesca permitieron la resiembra de las diferentes cepas en el mar, aunque los resultados de recuperación variaron según cada cepa. Este estudio destaca la importancia crucial de los biobancos para mitigar los impactos de los fenómenos climáticos extremos en la acuicultura de algas marinas, garantizando la conservación *ex situ* de la diversidad genética y la sostenibilidad de la producción. Las estrategias de cultivo y el desarrollo de cepas más tolerantes son esenciales para mejorar la resiliencia del cultivo de *K. alvarezii*. Los hallazgos también subrayan la necesidad de políticas públicas e inversión en biobancos para proteger la acuicultura frente a las adversidades ambientales y apoyar el desarrollo sostenible. Las investigaciones futuras deberían centrarse en las respuestas específicas de cada cepa a niveles de salinidad inferiores a 15 ‰ y proporcionar directrices prácticas para el cultivo en condiciones ambientales extremas.

INTRODUCTION

The cultivation of *Kappaphycus alvarezii* Doty L. M. Liao (Rhodophyta) can be carried out commercially not only to provide renewable raw materials, but also to play an important role in carbon sequestration (Bhushan *et al.* 2023). Encouraging seaweed farming activities can provide several ecosystem services, many of which fall within the scope of the United Nations Sustainable Development Goals (Troell *et al.* 2023). Among them, oxygen production, nutrient recycling, especially nitrogen and phosphorus, reducing eutrophication of coastal environments, biofuel production (renewable energy), absorption of heavy metals, job creation, food and animal feed production, climate change mitigation, improvement of human and animal health, and poverty reduction.

In 2022, global seaweed farming produced 36.5 million tons, and the red alga *K. alvarezii* (Ekthorn Sea Moss) contributed approximately 5% of this production in fresh weight (FAO, 2024). In Brazil, *K. alvarezii* was introduced into the Marine Experimental Farm of the Fisheries Institute in 1995 for research and evaluation of its bioinvasion potential, carrageenan content, and biofuel (ethanol and hydrogen), and currently, 12 different color strains are being cultivated (Gelli *et al.* 2023, 2024). However, extreme weather events, such as abrupt fluctuations in water salinity, temperature, and turbidity, can negatively affect the cultivation of this species.

Among the various important factors for the growth and survival of *K. alvarezii* in commercial cultivation, salinity is a parameter subject to fluctuations, especially in areas such as bays and inlets, and can significantly affect the growth, metabolite composition, and concentrations of seaweed biomass (Khatri *et al.*, 2023). Furthermore, salinity fluctuation is an uncontrolled phenomenon (Siddiqui *et al.* 2022). To survive under such conditions, the species reorganizes its physiological functioning and synthesizes a wide range of compounds, such as amino acids, lipids, proteins, polysaccharides, and phenolic compounds. Hayashi *et al.* (2011) found that the green tetrasporophyte of *K. alvarezii* tolerated salinity variations from 25 to 45 ‰, suggesting that this strain can be cultivated in sites with salinities around 25 ‰. Araújo *et al.* (2014) corroborated these data, showing that temperature and salinity affect the growth, survival, and dispersal of *K. alvarezii*, and in extreme conditions (15 and 55 ‰) led to growth inhibition, necrosis, and loss of pigments.

The knowledge of the physiological and morphological features of different *K. alvarezii* strains in response to salinity variations is essential to support seaweed farmers in managing them under conditions of hyposaline stress, especially in areas with high river influence. Therefore, the objective of this study was to evaluate the effects of low salinity, resulting from an intense rainfall event in March 2024, on the morphology, survival, and recovery of *K. alvarezii* strains cultivated at the Experimental Marine Farm of the Fisheries Institute (Ubatuba, São Paulo State, Brazil).

MATERIAL AND METHODS

Kappaphycus alvarezii

The Fisheries Institute in Ubatuba, São Paulo (23°27'7" S; 45°2'49" W), maintains 12 strains of the red seaweed *K. alvarezii* under two cultivation systems: (i) *in situ*, at the Experimental Marine Farm, and (ii) *ex situ*, in the biobank cultivation system of the "Ricardo Toledo Lima Pereira" Marine Macroalgae Laboratory (Gelli *et al.* 2023, 2024).

Among these strains, three were identified as gametophytes: Original Edison de Paula (G1), Yellow Edison de Paula (G2), and Light Green Edison de Paula (G3). The remaining nine strains were identified as tetrasporophytes: Original Brown (T1), Original Red (T2), Original Green (T3), Sugarcane Green (T4), Flag Green (T5), Olive Green (T6) Dark Green (T7), Red and Green (T8), and Brown and Emerald Green (T9).

Experimental design

Twelve strains of *K. alvarezii* were cultivated using a floating raft system, with 10 plants per cultivar, totaling 120 plants. The tie-tie planting method was employed, with individual plants attached at 20-cm intervals to 2.0-m ropes submerged at a 50-cm depth. The ropes were secured to a raft constructed from 10-cm-diameter PVC pipes, each 3.0 m long, spaced 2.5 m apart, and anchored with 70-kg weights. Plants were harvested and replanted every 30 days under standard management conditions. This raft-based cultivation system is illustrated in Figure 1.



Figure 1. Cultivation of *Kappaphycus alvarezii* using the tie-tie planting method in a floating raft system in March 2024. (A) Tie-tie planting process. (B) Floating raft system.

Figura 1. Cultivo de *Kappaphycus alvarezii* mediante el método de plantación con amarres en un sistema de balsas flotantes en marzo de 2024. (A) Proceso de plantación con amarres. (B) Sistema de balsas flotantes.

Strains were monitored weekly from March to July 2024, covering the periods of the low salinity event. During this monitoring period, changes in thallus structure, plant detachment, mortality rates, epibiont attachment, and morphological anomalies were systematically recorded. Harvesting was performed immediately upon observing that the plants were detaching from the ropes, and they were replanted on the same day using the same system. Some strains preserved at the Fisheries Institute's biobank were used for replanting.

Surface seawater temperature was recorded daily at a depth of 0.5 m at the Fisheries Institute using a calibrated alcohol thermometer. Salinity measurements were performed with a handheld refractometer ($\pm 1\text{‰}$ accuracy). Water transparency

was assessed using a Secchi disk; the maximum depth at the cultivation site was 4 m. Daily rainfall data were collected by the National Center for Monitoring and Alerts for Natural Disasters (CEMADEN) (Brazil 2024); data from March 2024 were considered in the present study.

RESULTS

The physical oceanographic changes observed during the period of intense rainfall from March 6 to 8, 2024, along the northern coast of São Paulo State (Brazil) caused physiological and morphological damage to the 12 color strains of *K. alvarezii* cultivated at the Experimental Marine Farm of the Fisheries Institute. According to data from the Civil Defense in Ubatuba (Cruz 2024, March 8th), rainfall reached 347 mm in 72 h from March 5th to 8th, 2024. There was an impact in the region, including the watershed draining into Ubatuba Bay, which resulted in a significant influx of freshwater. This was primarily driven by the discharge of four major rivers: Acaraú, Tavares, Grande, and Indaiá (Fig. 2).

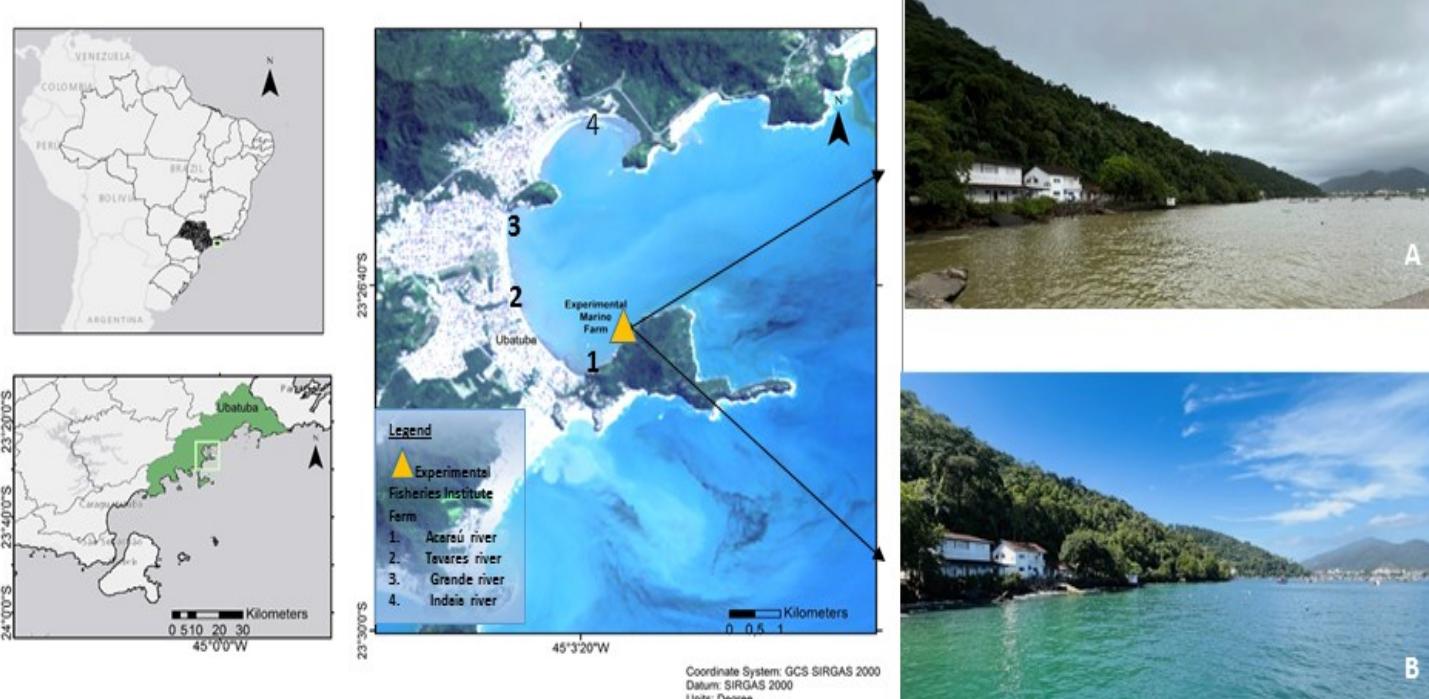


Figure 2. Geographical representation of Ubatuba Bay and its hydrographic system: 1. Acaraú, 2. Tavares, 3. Grande, and 4. Indaiá Rivers. Changes in the seawater transparency at the Experimental Marine Farm of the Fisheries Institute on March 7, 2024 (A) compared to normal days (B).

Figura 2. Representación geográfica de la Bahía de Ubatuba y su sistema hidrográfico: Ríos 1. Acaraú, 2. Tavares, 3. Grande y 4. Indaiá. Cambios en la transparencia del agua de mar en la Granja Marina Experimental del Instituto de Pesca el 7 de marzo de 2024 (A) en comparación con días normales (B).

This climatic event triggered a sharp yet temporary decline in salinity, temperature, and water transparency. These abrupt environmental fluctuations are illustrated in Figure 3. The 12 strains of *K. alvarezii* were subjected to acute variations over a brief period of approximately five days. During this time frame, salinity levels decreased from 35 to 18 and 10 ‰, seawater temperature dropped from 27 to 24 and 23°C, and water transparency declined drastically from 3.5 m to 0.4 m (Fig. 3).

Strains were monitored weekly from March to July 2024, covering the period of the low salinity event. The first morphological changes observed in all strains of *K. alvarezii* were in the plant apices after five days of the climatic event (Fig. 4). The apical portions became whitish, and necrosis was subsequently observed. Furthermore, there was a change in the consistency of the thalli of the twelve strains, making them more fragile (Fig. 5).

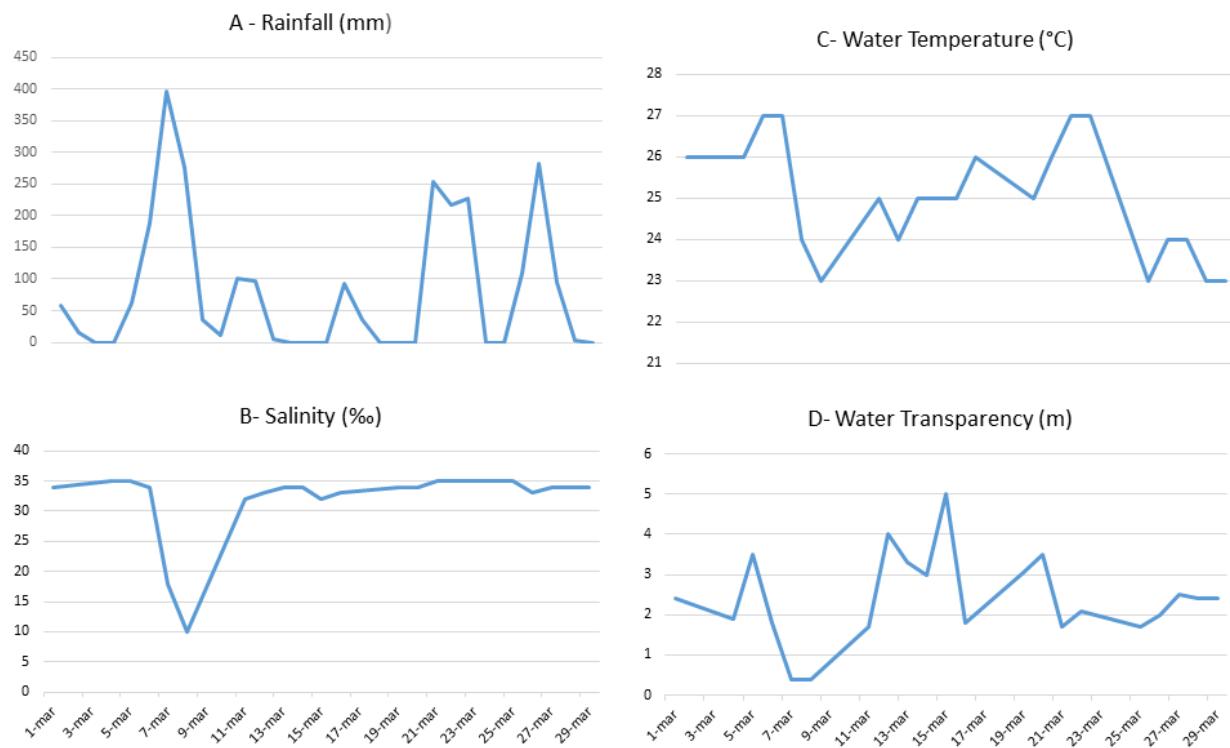


Figure 3. Daily variation of abiotic factors: (A) Rainfall (mm), (B) Salinity (‰), (C) Water Temperature (°C), and (D) water transparency (m) in the Experimental Marine Farm of Fisheries Institute, Ubatuba Bay, northern of São Paulo State, Brazil, during March 2024.

Figura 3. Variación diaria de los factores abióticos: (A) Precipitación (mm), (B) Salinidad (‰), (C) Temperatura del agua (°C) y (D) Transparencia del agua (m) en la Granja Marina Experimental del Instituto de Pesca, Bahía de Ubatuba, norte del estado de São Paulo, Brasil, durante marzo de 2024.



Figure 4 Apical branch bleaching in 12 strains of *Kappaphycus alvarezii* following heavy rainfall on March 11, 2024: Original Edison de Paula (G1), Yellow Edison de Paula (G2), Light Green Edison de Paula (G3), Original Brown (T1), Original Red (T2), Original Green (T3), Sugarcane Green (T4), Flag Green (T5), Olive Green (T6), Dark Green (T7), Red and Green (T8), and Brown and Emerald Green (T9).

Figura 4. Blanqueamiento de ramas apicales en 12 cepas de *Kappaphycus alvarezii* luego de fuertes lluvias el 11 de marzo de 2024: Edison de Paula original (G1), Edison de Paula amarilla (G2), Edison de Paula verde claro (G3), Marrón original (T1), Rojo original (T2), Verde original (T3), Verde caña de azúcar (T4), Verde bandera (T5), Verde oliva (T6), Verde oscuro (T7), Rojo y verde (T8), y Marrón y verde esmeralda (T9).



Figure 5. Brown strain (T1) of *Kappaphycus alvarezii*: Loss of thallus consistency and bleaching (March 11, 2024).

Figura 5. Cepa marrón (T1) de *Kappaphycus alvarezii*: Pérdida de consistencia del talo y blanqueamiento (11 de marzo de 2024).

After 22 days following the climatic event, the mortality of several *K. alvarezii* strains was observed. The seaweed was harvested and replanted. Among strains, the light green Edison de Paula (G3), the brown emerald-green strain (T8), and red and green strain (T9) were more severely damaged when compared to the others (Table 1). At this time, the most resilient strains were replanted (Fig. 6).

Table 1. Mortality percentage and number of seedling loss of *Kappaphycus alvarezii* strains due to the abrupt climatic events in Ubatuba Bay, Ubatuba, Brazil (March 28, 2024).

Tabla 1. Porcentaje de mortalidad y número de plántulas perdidas de cepas de *Kappaphycus alvarezii* debido a los eventos climáticos abruptos en la Bahía de Ubatuba, Ubatuba, Brasil (28 de marzo de 2024).

Strains *	Number of seedling loss	Mortality (%)
G1	2	20
G2	0	0
G3	8	80
T1	0	0
T2	0	0
T3	0	0
T4	0	0
T5	0	0
T6	0	0
T7	0	0
T8	10	100
T9	10	100

*Original Edison de Paula (G1), Yellow Edison de Paula (G2), Light Green Edison de Paula (G3), Original Brown (T1), Original Red (T2), Original Green (T3), Sugarcane Green (T4), Flag Green (T5), Olive Green (T6), Dark Green (T7), Red and Green (T8), and Brown and Emerald-Green (T9).

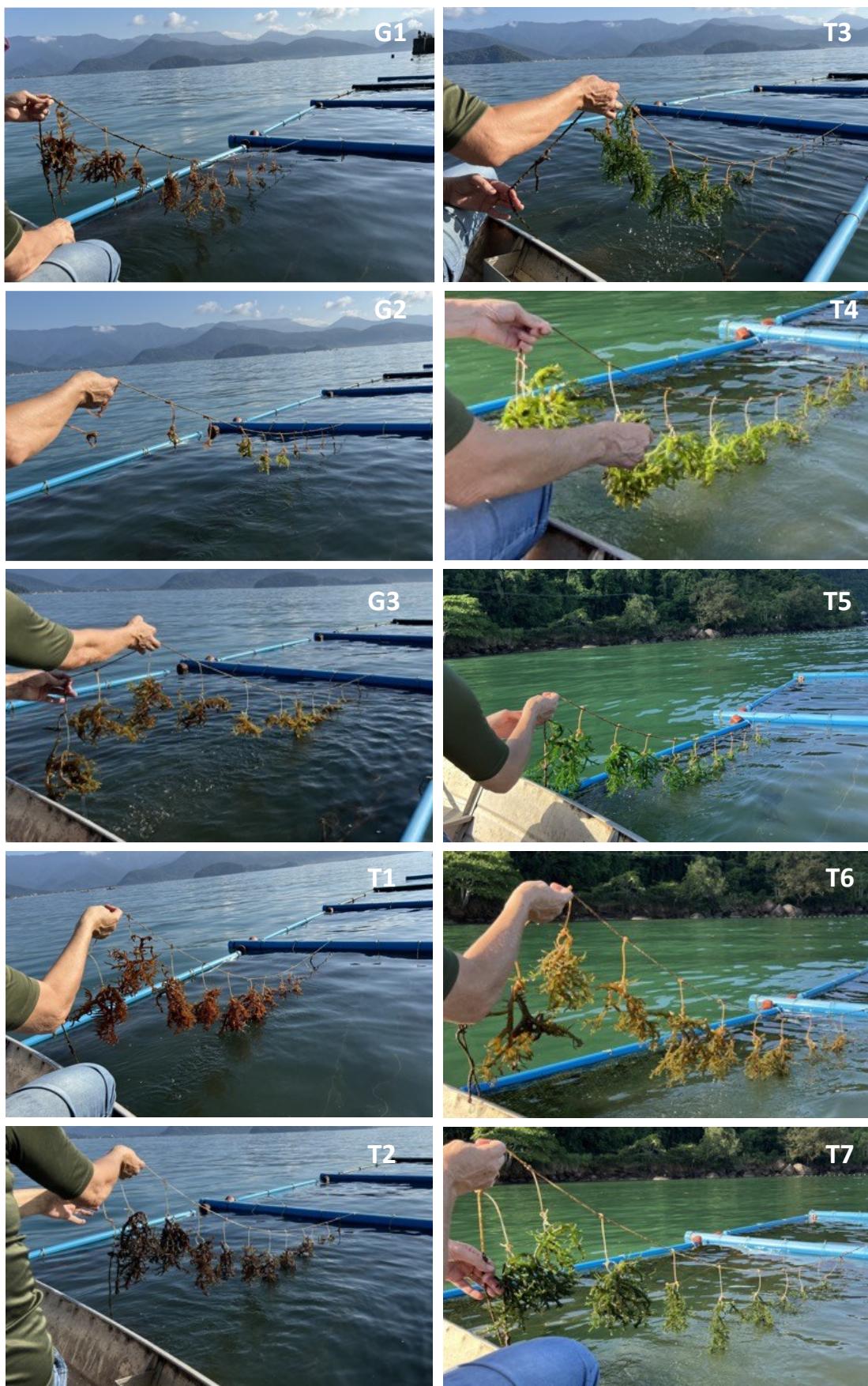


Figure 6. Planting of *Kappaphycus alvarezii* strains using the tie-tie method in a floating raft cultivation system on March 28, 2024. Strains: Original Edison de Paula (G1), Yellow Edison de Paula (G2), Light Green Edison de Paula (G3), Original Brown (T1), Original Red (T2), Original Green (T3), Sugarcane Green (T4), Flag Green (T5), Olive Green (T6), Dark Green (T7).

Figura 6. Plantación de cepas de *Kappaphycus alvarezii* mediante el método tie-tie en un sistema de cultivo en balsas flotantes el 28 de marzo de 2024. Cepas: Edison de Paula Original (G1), Edison de Paula Amarilla (G2), Edison de Paula Verde Claro (G3), Caf

Original (T1), Rojo Original (T2), Verde Original (T3), Verde Caña de Azúcar (T4), Verde Bandera (T5), Verde Oliva (T6), Verde Oscuro (T7).

However, the three most fragile strains could only be replanted using seedlings cultivated in the biobank of the Fisheries Institute, as the original strains did not survive the environmental stressors. The light green Edison de Paula (T3) strain was successfully recovered after two planting cycles, approximately 60 days later, but the brown and emerald green (T8), and the red and green (T9) strains did not survive. A month after the replanting, an intense attachment of epibionts was observed on surviving strains (Fig. 7).



Figure 7. *Kappaphycus alvarezii* seedlings with an intense epibiotic attachment after one month of replanting.

Figura 7. Plántulas de *Kappaphycus alvarezii* con una intensa adhesión epibiótica después de un mes de replantación.

During the subsequent three-month recovery period of *K. alvarezii* strains, morphological abnormalities were observed, including thallus flattening and the formation of hollow thalli, which were attributed to the degradation or loss of medullary cells (Fig. 8).



Figure 8. Abnormal thallus development of *Kappaphycus alvarezii* strains during the 90 days after the climatic event: (A) Edison de Paula strain (G1) (flattened thalli); (B) olive green strain (T6) (thin, and fan-shaped thalli) and (C and D) sugarcane green strain (T4) and brown original strain (T1) (hollow thalli).

Figura 8. Desarrollo anormal del talo de cepas de *Kappaphycus alvarezii* durante los 90 días posteriores al evento climático: (A) cepa Edison de Paula (G1) (talos aplanados); (B) cepa verde oliva (T6) (talos delgados y en forma de abanico); (C y D) cepa verde caña (T4) y cepa marrón original (T1) (talos huecos).

DISCUSSION

The results obtained after five days of abrupt fluctuations in abiotic factors in the natural environment indicated significant impacts on *K. alvarezii* strains. The observed morphological and physiological changes indicated that short-term salinity fluctuations could have induced osmotic stress in the seaweed strains, compromising their structural and functional integrity. These salinity variations could have directly affected biological processes such as photosynthesis, growth, and development, with potential consequences for survival and productivity in cultivation. Furthermore, the differential responses of the strains to salinity fluctuations suggested variations in resilience, with some strains possibly demonstrating greater acclimation, while others may have shown higher susceptibility to damage. These findings highlighted the importance of considering genetic variability in the management and optimization of seaweed cultivation practices.

Although most salinity studies have been carried out under controlled laboratory conditions with salinities no lower than 15‰ (Hayashi *et al.* 2011, Araújo *et al.* 2014, Mandal *et al.* 2015, Siddiqui *et al.* 2022, Siddiqui *et al.* 2024), preliminary field observations like those reported in this study may support seaweed farmers in adjusting their cultivation strategies in response to extreme climatic events. The first observed effect on the fifth day after the climatic event was the loss of turgor and apical necrosis of the thallus strains caused by salinity variations (35 to 10‰). Osmotic fluctuations in hypo- and hypersaline environments result in changes in cell volume mediated by water influx (cellular hydration) and efflux (cellular dehydration), which directly influence the growth of seaweeds (Khatri *et al.* 2023). Furthermore, to survive such adverse conditions, *K. alvarezii* reorganizes its metabolism and synthesizes a broad range of compounds, including amino acids, lipids, proteins, polysaccharides, and phenolic compounds (Siddiqui *et al.* 2022). Studies investigating the behavior of this species under hypo- and hypersaline conditions have shown differences in metabolite production at different salinity levels (Rathore *et al.* 2023). Hayashi *et al.* (2011) showed that salinities of 15‰ cause severe cellular modifications, leading to algal death or damage to cellular organization, compromising algal growth. Better performance of the green tetrasporophyte was reported at salinities of 25‰. Araújo *et al.* (2014) also observed that the green strain grows optimally between 25 and 35‰, while salinities of 15, 45, and 55‰ inhibit growth. Deviations from the optimal salinity range lead to the production of reactive oxygen species (ROS) and lipid peroxidation, adversely affecting growth and development. Under low salinity conditions, the efficiency of photosystem II (PSII) is reduced. However, the ability to maintain a balanced K⁺/Na⁺ ratio and increased levels of antioxidants and proline highlights the alga's acclimation strategies under moderate salinity conditions (Siddiqui *et al.* 2024).

Our data show the importance of monitoring and underscore the need for seaweed producers to monitor and manage salinity levels during cultivation. The use of different strategies, such as increasing the depth of the culture or its relocation, the use of protective structures, or selecting strains with higher tolerance to salinity fluctuations, could mitigate the impacts of environmental extremes. In Brazil, studies on these topics are needed to develop the value chain of this seaweed.

We also emphasize that of the three strains of *K. alvarezii* more affected by stress conditions, only one could be recovered, thanks to samples provided by the Fisheries Institute biobank. This underscores the critical importance of a biobank for replenishing *K. alvarezii* strains in commercial cultivation, highlighting its urgent necessity at the national level. Such a resource is essential to ensure the sustainability of production, preserve genetic diversity, and mitigate risks associated with extreme climatic events and other environmental adversities. Establishing efficient protocols for strain management and proper scaling is indispensable to avoid the permanent loss of these valuable genetic resources and to ensure the resilience and competitiveness of Brazil's aquaculture sector. Given the significant economic implications of strain losses, investing in biobanks should be a strategic priority for public and private stakeholders. Biobanks not only safeguard biodiversity but also provide critical protection against economic disruptions caused by adverse climatic conditions in aquaculture (Corrales and Astrin 2023). Implementing national policies to support the creation and maintenance of biobanks, along with funding for research on strain resilience and adaptive management practices, would represent a crucial step toward the sustainable development of aquaculture in Brazil.

Finally, future studies under salinity conditions below 15‰, and strategies to guide seaweed producers in managing different stress levels, would provide valuable and practical insights to optimize cultivation management.

CONCLUSIONS

Abrupt fluctuations in salinity, temperature, and transparency of seawater due to extreme climate changes can significantly impact the morphology and survival of *K. alvarezii* strains.

The recovery capacity of *K. alvarezii* varied among the color strains, suggesting the need to identify and cultivate more resilient strains to ensure the sustainability of the cultivation.

Studies on the effects of salinity variation, including salinity levels below 15 %, should be encouraged due to abrupt climate changes.

In situations of heavy rainfall, it is recommended to: lower the cultivation ropes in the water column to deeper levels where salinity may be higher; allow 7 to 10 days for seaweed recovery, until the thallus becomes more rigid, before replanting; and monitor for associated organisms (epiphytes), replanting if infestation occurs.

The implementation of public policies and the advancement of new studies are essential for establishing and managing official and private biobanks dedicated to the preservation and conservation of genetic strains. Given the significant economic consequences associated with the loss of strain during extreme climate events, investment in biobanks should be prioritized as a strategic initiative by both public and private stakeholders. Biobanks not only safeguard biodiversity but also play a critical role in mitigating economic disruptions caused by adverse climatic conditions.

Conflict of interest statement

The authors declare that they have no conflicts of interest related to this article.

Credit author statement (CrediT)

Valéria C. Gelli: conceptualization, methodology, investigation, funding acquisition, writing – original draft. Estela M. Plastino: conceptualization, writing – review & editing. Nair S. Yokoya: conceptualization, writing – review & editing. All authors contributed to the article and approved the submitted version.

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