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Review of Temperature Pre-Treatment Methods in The Preparation of *Eucheuma Cottonii* Seedlings for Transportation Engineering

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ABSTRACT

This study aimed to evaluate various temperature pre-treatment strategies for preparing *E. cottonii* seedlings within a transportation engineering framework. The experiment was conducted from June - September 2025 at the Marine and Terrestrial Bioindustry Research Center, National Research and Innovation Agency (BRIN), Indonesia. A factorial Completely Randomized Design (CRD) was employed, involving two factors: temperature treatment (S) and transportation duration (W). The temperature factor consisted of five levels based on the number of ice packs applied: 0 (S1), 1 (S2), 2 (S3), 3 (S4), and 4 (S5). Transportation duration comprised three levels: 12 hours (W1), 24 hours (W2), and 36 hours (W3). The initial weight of the seedlings was standardized at 120 g. Observed parameters included weight loss, storage temperature, seedling color characteristics, and salinity content. Data were analyzed using analysis of variance (ANOVA) in SPSS, followed by Duncan's multiple range test. The results demonstrated that single-factor temperature treatments (S), transportation duration (W), and their interaction (S×W) significantly affected absolute weight loss, storage temperature, and all color parameters of *E. cottonii* seedlings. However, none of these treatments exerted a significant effect on seedling salinity. The interaction between four ice packs and a 12 h transportation period (S5W1) was identified as the optimal treatment, as it best preserved seedling color performance, with lightness (L*) of 23.3±0.01, redness (a*) of −0.2±0.12, and yellowness (b*) of 7.5±0.07. Despite these advantages, this treatment also resulted in a relatively high weight loss (25.4±0.5 g), likely due to cold shock induced by a substantial reduction in styrofoam container temperature to 24.2±0.09°C. In contrast, seedling salinity remained statistically unchanged across all treatment combinations, ranging from 3.75±0.2% to 3.76±0.28%

KEYWORDS: *E. cottonii*, transportation engineering, storage temperature, seedling transport

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1. INTRODUCTION

The aquaculture sector plays a critical role in Indonesia's national economy, with seaweed emerging as one of the country's most valuable and strategic commodities. Among cultivated seaweeds, *Eucheuma cottonii* is the dominant species contributing to national production and serves as a major source of income for coastal communities. Strong market demand, both domestically and internationally, has driven the intensification of seaweed farming activities across the country (Hidayat & Safitri 2019). Indonesia possesses exceptional potential for seaweed aquaculture, as evidenced by the presence of thousands of seaweed species, positioning the country as a strategic global hub for seaweed cultivation and an important contributor to the international seaweed supply (Prita *et al*., 2021)

Eucheuma cottonii is a commonly cultivated red macroalga, favored for its comparatively high resistance to disease and its capacity to thrive under stable environmental conditions. Despite its considerable production potential, the cultivation of *E. cottonii* faces significant challenges, particularly during the early stages of farming, namely seedling preparation and distribution. One of the most critical links in the seaweed aquaculture supply chain is the transportation of seedlings from nursery facilities to grow-out sites. Transportation has been recognized as a decisive factor influencing cultivation success and the final quality of harvested seaweed (Tamonob *et al*., 2025). Long transportation distances and extended travel durations frequently expose seedlings to multiple stressors, including physical stress (e.g., vibration and friction) and thermal stress from temperature fluctuations or excessive heat. In practice, seaweed seedlings are commonly transported in insulated containers using either dry or wet packaging systems, often supplemented with ice to maintain temperature and preserve seedling quality. The application of ice media and adequate ventilation has been shown to help stabilize internal temperatures within the optimal range of 20–30°C (Nafanu *et al*., 2025). However, excessive temperature fluctuations can induce physiological stress, thallus damage, and, in severe cases, seedling mortality. Stress experienced during transportation directly compromises the physiological and morphological quality of seedlings, leading to increased mortality rates and reduced growth performance after planting. Such deterioration ultimately results in substantial economic losses for seaweed farmers. Consequently, developing effective and efficient strategies to mitigate transportation-induced stress is essential for improving seedling survival and ensuring sustainable seaweed cultivation.

Based on the considerations outlined above, it is necessary to investigate the optimal temperature conditions for transporting *Eucheuma cottonii* seedlings using an ice-assisted packaging technique. The addition of ice packs within insulated box packaging is intended to maintain a stable internal temperature during transport, thereby preserving seedling freshness and improving overall quality. Ice has been widely used as a cooling medium during biological material transport due to its ability to stabilize container temperature and reduce metabolic stress, allowing seaweed seedlings to remain in a fresh condition throughout the transportation process (Yohanes et al., 2020).

2. METHODOLOGY

2.1. Materials and Equipment

The materials used in this study included styrofoam boxes, ice packs, bottled water, tissue paper, and *E. cottonii* seedlings. The equipment utilized consisted of a refractometer, thermometer, digital balance, colorimeter, smartphone camera, paranet sacks, and standard stationery.

2.2. Experimental Design

The study was conducted from June to September 2025 at the Marine and Terrestrial Bioindustry Research Center, National Research and Innovation Agency (BRIN), Pemenang District, North Lombok Regency, West Nusa Tenggara, Indonesia. An experimental approach was employed using a factorial Completely Randomized Design (CRD) with two factors: temperature treatment (S) and transportation duration (W). The temperature factor consisted of five levels based on the number of ice packs applied, while the transportation duration factor consisted of three levels. Each treatment combination was replicated three times, resulting in a total of 45 experimental units. The treatment structure was as follows:

I. Factor I: Temperature treatment (S), based on the number of ice packs

a) S1: No ice packs
b) S2: One ice pack
c) S3: Two ice packs
d) S4: Three ice packs
e) S5: Four ice packs

II. Factor II: Transportation duration (W)

a) W1: 12 h
b) W2: 24 h
c) W3: 36 h

The initial weight of the seedlings used in each experimental unit was standardized at 120 g. All experimental units were randomly assigned to treatments to ensure unbiased distribution. The overall experimental layout is illustrated in Figure 1.

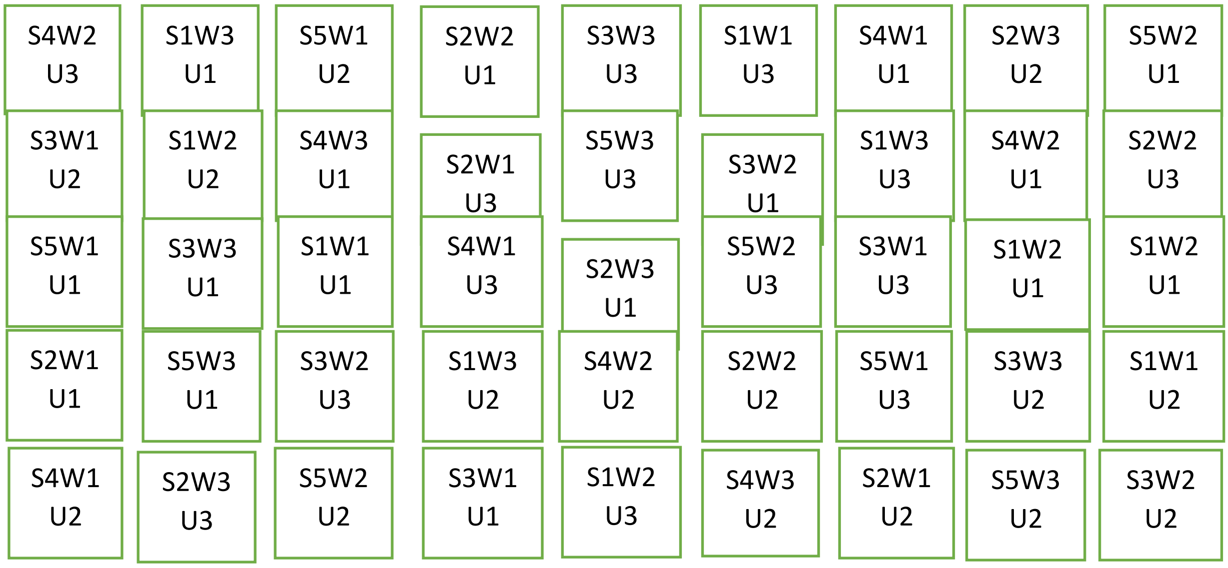

Figure 1. Experimental Design Layout

2.3. Preparation of Packaging and Ice Packs

This study employed styrofoam boxes measuring 25 cm in length, 20 cm in width, and 31.5 cm in height, with a capacity equivalent to 2.5 kg. A total of 45 boxes were used. Ice packs were prepared using 220 mL plastic cups filled with bottled drinking water and frozen prior to use. The frozen ice cups were placed inside the styrofoam boxes according to the assigned treatments. It was estimated that each frozen cup reduced the internal box temperature by approximately 1.5–2.0°C.

2.4. Seedling Preparation and Collection

The *E. cottonii* seedlings used in this study were collected from Seriwe Bay, East Lombok Regency, Indonesia. Seedlings were selected based on uniform size and quality criteria to ensure experimental consistency. Collection was conducted in the morning using a dry transport method, whereby seedlings were placed inside insulated boxes lined with paper on all inner surfaces to minimize evaporation and prevent dehydration that could compromise seedling quality. Upon arrival at the research facility, the seedlings underwent an acclimatization period to homogenize physiological condition prior to transportation simulation. After completing a 7-day acclimatization and immersion period, the seedlings were immediately prepared for packaging.

2.5. Transportation Simulation

Transportation engineering was simulated using 45 styrofoam boxes corresponding to the experimental treatments. Acclimatized seedlings were weighed to an initial mass of 120 g for each replicate and placed inside the styrofoam boxes according to treatment assignment. In the control treatment, seedlings were packaged without ice packs. For the remaining treatments, ice packs were placed at the bottom layer of the box, with the seaweed seedlings positioned above the ice layer. The boxes were then sealed and subjected to transportation simulation for the designated durations. The transportation simulation was conducted in a semi-outdoor environment, allowing the internal box temperature to be partially influenced by ambient external conditions, thereby reflecting realistic transportation scenarios.

2.6. Monitoring and Seedling Assessment

Throughout the experimental period, routine monitoring was conducted three times daily to ensure that all boxes remained intact and undamaged. Measurements of seedling weight, internal styrofoam box temperature, seedling color, and salinity were recorded at the beginning and at the end of each transportation simulation period according to the assigned treatments. Seedling weight was measured using a digital balance, box temperature using a thermometer, salinity using a refractometer, and color parameters using a colorimeter.

2.7. Research Parameters

Weight Loss

Seedling weight loss was calculated using the following formula (Damayanti & Setyono, 2019):

$$G = Wt - Wo$$

Where :

G = absolute weight loss (g)

Wt = final weight (g)

Wo = initial weight (g)

Styrofoam Box Temperature

Temperature measurements were obtained by creating a small opening at the top of each styrofoam box and inserting a thermometer into the interior. The thermometer was left in place for 3–5 minutes to allow temperature stabilization, after which the temperature reading was recorded.

Color Measurement

Seedling color was measured using a colorimeter. Prior to measurement, the instrument was calibrated using a standard white reference plate. The sensor was then placed directly on the surface of the seaweed thallus, and color readings were recorded by activating the sensor button. Color parameters were expressed as L* (lightness), a* (redness), and b* (yellowness). The L*, a*, and b* values represent standardized color scale intervals indicating the intensity of the measured color attributes (Rahayu & Sari, 2022).

Salinity Content

Salinity content of the seaweed seedlings was measured by first rinsing the samples with bottled mineral water to remove surface impurities. The seedlings were then crushed to extract cellular fluid. A drop of the extracted liquid was placed onto the refractometer prism, and salinity readings were recorded (Apriantini *et al*., 2022). Salinity values measured in ppt were converted to percentage (%) using the following equation:

$$Percentage\ (\%) = \frac{ppt}{10}$$

2.8. Statistical Analysis

All experimental data were analyzed using analysis of variance (ANOVA). When significant differences were detected, Duncan's multiple range test was applied to determine statistical differences among treatment means.

3. RESULTS AND DISCUSSION

3.1. Weight Loss of *E. cottonii* Seedlings

The results indicated that the average weight loss of *E. cottonii* seedlings across single-factor ice treatments (S), transportation duration treatments (W), and their interactions (S×W) ranged from 3.2±0.6 g to 39.4±1.2 g. Overall, seedling weight loss increased with both the number of ice packs applied and the length of the transportation simulation period. The lowest weight loss was observed in the control treatment without ice packs and a transportation duration of 12 hours (S1W1). In contrast, the highest weight loss occurred under the temperature treatment involving four ice packs combined with a transportation duration of 36 hours (S5W3), as illustrated in Figure 2.

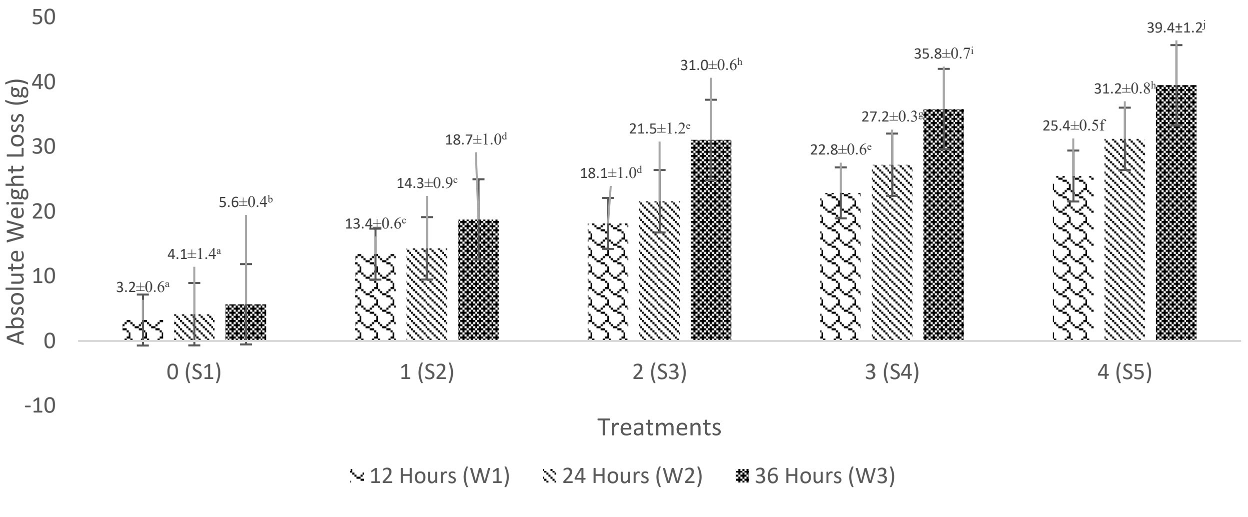

Figure 2. Weight Loss of *E. cottonii* Seedlings

Analysis of variance (ANOVA) revealed that the interaction between the number of ice packs and transportation duration (S×W) had a highly significant effect ($P < 0.05$, 95% confidence level) on the weight loss of *E. cottonii* seedlings. Similarly, the main effects of ice pack number (S), independent of transportation duration, and transportation duration (W), independent of ice pack number, also significantly influenced seedling weight loss. Duncan's multiple range test indicated that the interaction between the control treatment without ice packs and a transportation duration of 12 h (S1W1) resulted in the lowest weight loss of *E. cottonii* seedlings and did not differ significantly from the control treatment without ice packs at 24 h (S1W2). However, both interaction treatments differed significantly from all other treatment combinations.

3.2. Final Temperature of the Styrofoam Packaging Medium

The results showed that the initial temperature of the styrofoam packaging medium was uniform across all treatments, 28 °C. Following the application of experimental treatments, the final temperature of the styrofoam boxes varied across treatments, depending on the duration of the transportation simulation and the number of ice packs applied. The mean final temperature of the styrofoam medium across single-factor ice treatments (S), transportation duration treatments (W), and their interactions (S×W) ranged from 24.2±0.09°C to 28.25±0.20°C. The lowest final temperature was observed in the treatment involving four ice packs and a 12-hour transportation duration (S5W1). In contrast, the highest final temperature occurred in the control treatment without ice packs and a transportation duration of 24 hours (S1W2), as shown in Figure 3.

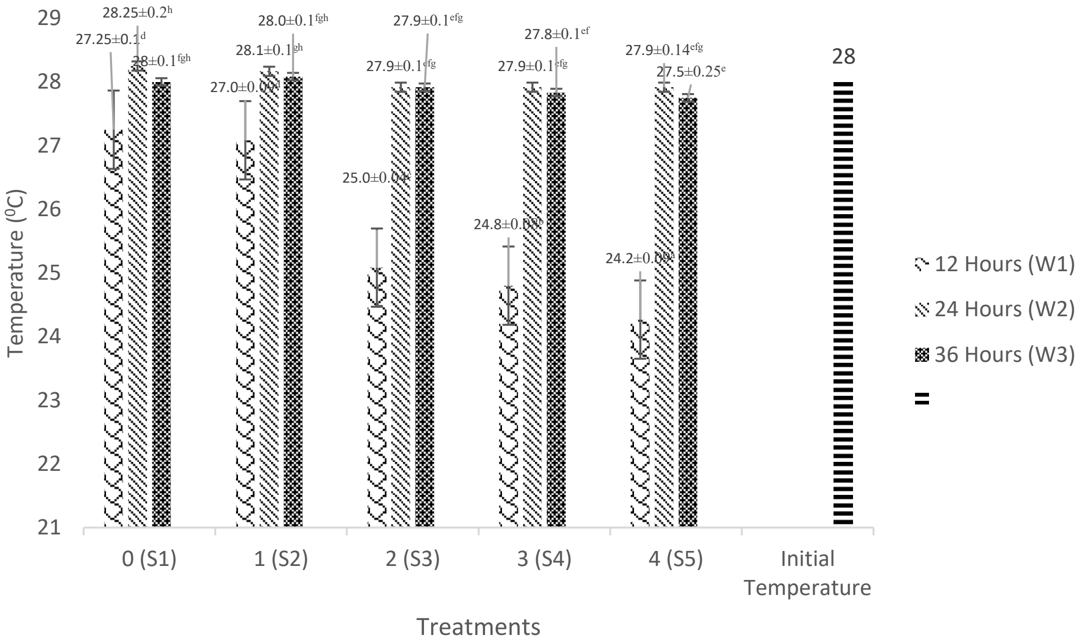

Figure 3. Final Temperature of the Styrofoam Packaging Medium

Analysis of variance (ANOVA) demonstrated that the interaction between the number of ice packs and transportation duration (S×W) had a highly significant effect ($P < 0.05$, 95% confidence level) on the final temperature of the styrofoam packaging medium. In addition, the main effects of ice pack number (S), independent of transportation duration, and transportation duration (W), independent of ice pack number, also significantly influenced the final styrofoam temperature. Duncan's multiple range test revealed that the interaction treatment involving four ice packs and a transportation duration of 12 hours (S5W1) resulted in the lowest final styrofoam temperature and differed significantly from all other treatment combinations.

3.3. L* (Lightness) Values of *E. cottonii* Seedlings

The results indicated that the mean L* (lightness) values of *E. cottonii* seedlings across single-factor ice treatments (S), transportation duration treatments (W), and their interactions (S×W) ranged from 16.5±0.32 to 26.3±0.10. The initial L* value of the seedlings prior to treatment was 24. Following the application of experimental treatments, L* values varied across treatments with respect to transportation duration. The lowest L* value was observed in the treatment involving three ice packs and a transportation duration of 36 hours (S4W3), whereas the highest L* value occurred in the control treatment without ice packs and a transportation duration of 24 hours (S1W2), as shown in Figure 4.

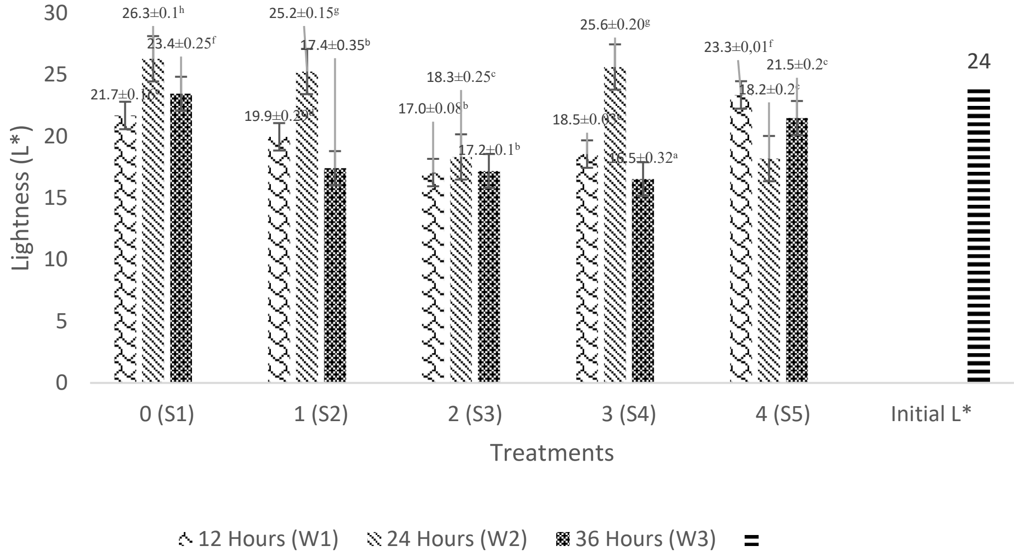

Figure 4. L* (Lightness) Values of *E. cottonii* Seedlings

Analysis of variance (ANOVA) howed that the interaction between the number of ice packs and transportation duration (S×W) had a highly significant effect ($P < 0.05$, 95% confidence level) on the L* (lightness) values of *E. cottonii* seedlings. Similarly, the main effects of ice pack number (S), regardless of transportation duration, and transportation duration (W), regardless of ice pack number, also significantly influenced L* values. Duncan's multiple range test indicated that the interaction treatment without ice packs and a transportation duration of 36 hours (S1W3) was able to maintain L* (lightness) values closer to the initial seedling condition prior to treatment and did not differ significantly from the interaction treatment involving four ice packs and a transportation duration of 12 hours (S5W1). However, both interaction treatments differed significantly from all other treatment combinations.

3.4. a* (Redness) Values of *E. cottonii* Seedlings

The results demonstrated that the mean a* (redness) values of *E. cottonii* seedlings across single-factor ice treatments (S), transportation duration treatments (W), and their interactions (S×W) ranged from −10.4±0.25 to 1.6±0.10. The initial a* value of the seedlings prior to treatment was 1. Following the application of experimental treatments, a* values varied among treatments in relation to transportation duration. The lowest a* value was observed in the treatment involving one ice pack and a transportation duration of 36 hours (S2W3), whereas the highest a* value occurred in the treatment involving four ice packs and a transportation duration of 24 hours (S5W2), as illustrated in Figure 5.

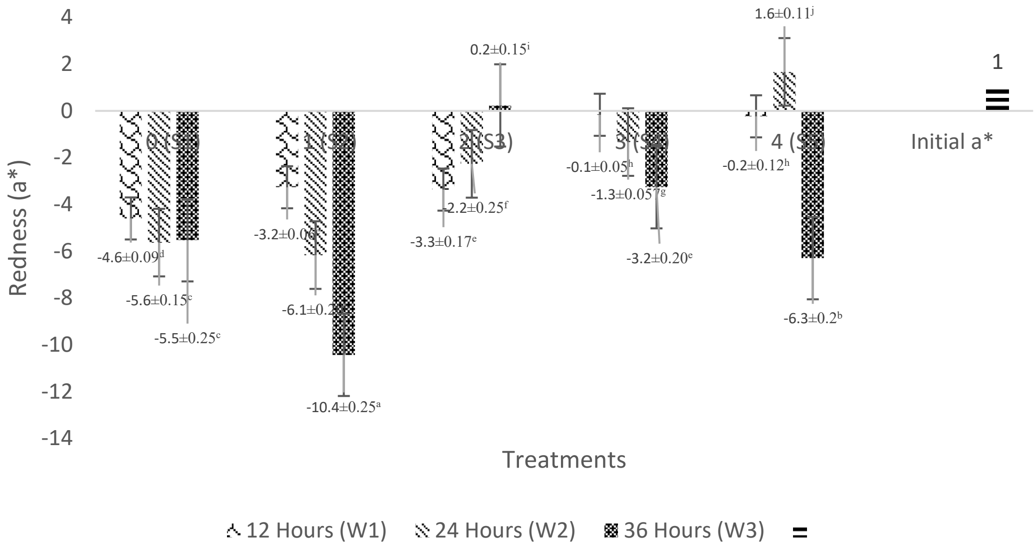

Figure 5. a* (Redness) Values of *E. cottonii* Seedlings

Analysis of variance (ANOVA) revealed that the interaction between the number of ice packs and transportation duration (S×W) had a highly significant effect ($P < 0.05$, 95% confidence level) on the a* (redness) values of *E. cottonii* seedlings. In addition, the main effects of ice pack number (S), independent of transportation duration, and transportation duration (W), independent of ice pack number, also significantly influenced the reduction in a* values. Duncan's multiple range test showed that the interaction treatment involving four ice packs and a transportation duration of 24 hours (S5W2) most effectively maintained a* (redness) value close to the initial condition prior to treatment and differed significantly from all other interaction treatments.

3.5. b* (Yellowness) Values of *E. cottonii* Seedlings

The results indicated that the mean b* (yellowness) values of *E. cottonii* seedlings across single-factor ice treatments (S), transportation duration treatments (W), and their interactions (S×W) ranged from 7.5±0.07 to 25.4±0.25. The initial b* value of the seedlings prior to treatment was 5. Following the application of experimental treatments, b* values varied among treatments in relation to transportation duration. The lowest b* value was observed in the treatment involving four ice packs and a transportation duration of 12 hours (S5W1), whereas the highest b* value occurred in the treatment involving four ice packs and a transportation duration of 36 hours (S5W3), as illustrated in Figure 6.

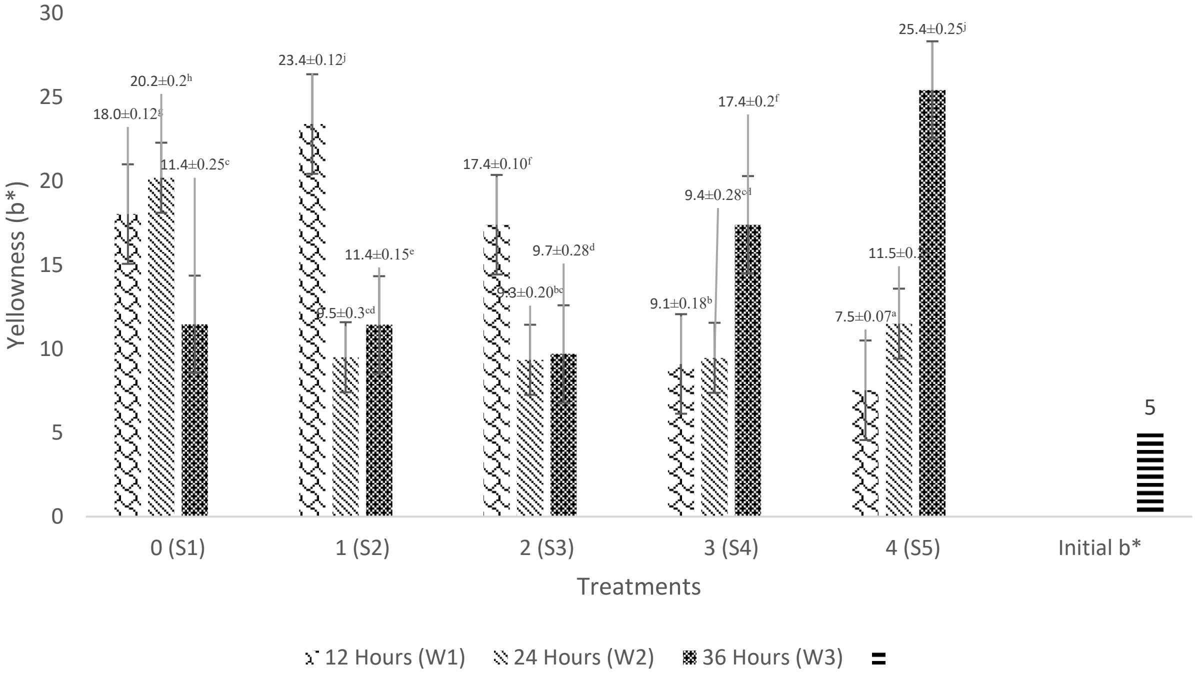

Figure 6. b* (Yellowness) Values of *E. cottonii* Seedlings

Analysis of variance (ANOVA) indicated that the interaction between the number of ice packs and transportation duration (S×W) had a highly significant effect ($P < 0.05$, 95% confidence level) on the b* (yellowness) values of *E. cottonii* seedlings. Likewise, the main effects of ice pack number (S), regardless of transportation duration, and transportation duration (W), regardless of ice pack number, also significantly influenced the reduction in b* values. Duncan's multiple range test showed that the interaction treatment involving four ice packs and a transportation duration of 12 hours (S5W1) most effectively maintained b* (yellowness) values close to the initial condition prior to treatment and differed significantly from all other interaction treatments.

3.6. Salinity Content of *E. cottonii* Seedlings

The mean salinity (%) of *E. cottonii* seedlings across single-factor ice treatments (S), transportation duration treatments (W), and their interactions (S×W) ranged from 3.75±0.20% to 3.76±0.28%. The initial salinity of the seedlings prior to treatment was 3.8%. Following the experimental treatments, slight variations in salinity were observed among treatments in relation to transportation duration. All treatments resulted in a minor reduction in salinity compared to the initial values. The lowest salinity was observed in the treatment with three ice packs and a transportation duration of 12 hours (S4W1), as shown in Figure 7.

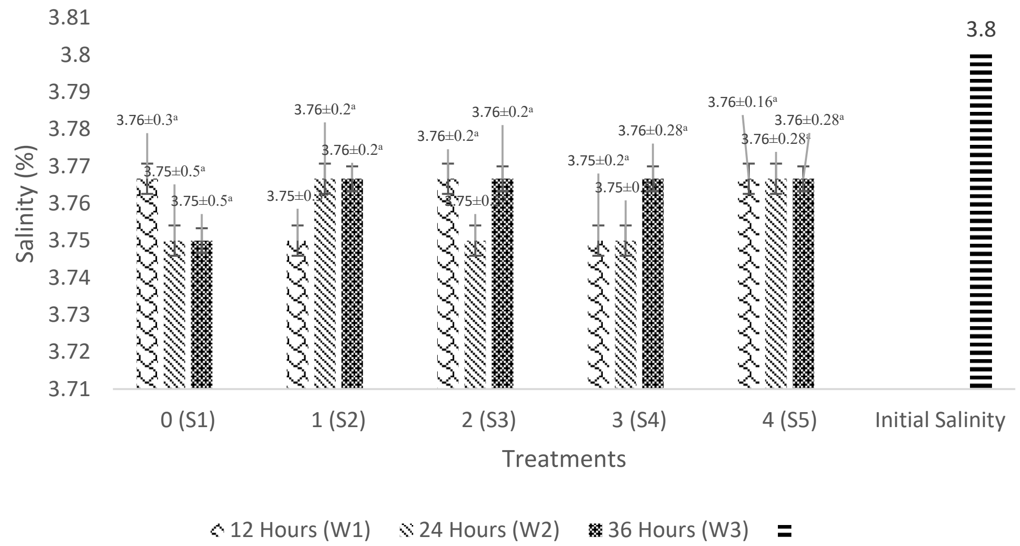

Figure 7. Salinity of *E. cottonii* Seedlings

Analysis of variance (ANOVA) showed that the interaction between ice pack number and transportation duration (S×W) had no significant effect ($P > 0.05$, 95% confidence level) on the salinity of *E. cottonii* seedlings. Similarly, the main effects of ice pack number (S) and transportation duration (W), when considered individually, did not significantly affect salinity reduction.

3.7. Discussion

Weight Loss of E. cottonii Seedlings

Weight loss during transportation is a physiological response to stress. This study demonstrated that seedling weight loss increased with both the number of ice packs and the duration of transport, ranging from 3.2±0.6 g to 39.4±1.2 g. Interestingly, the addition of ice, intended to maintain freshness, significantly increased weight loss. This response can be attributed to two main mechanisms. First, low temperatures can induce cold shock in tropical seaweed. Kumar *et al.* (2020) reported that deviations from the optimal temperature range (28–32°C) reduce growth rates and cause degradation of photosynthetic pigments in *E. cottonii*. Eucheumatoid seaweeds are particularly sensitive to extreme temperature fluctuations, whether hot or cold (Handajani et al., 2025). Additionally, melting ice reduces the salinity of the surrounding medium, creating osmotic imbalances that drive water out of cells, causing tissue dehydration and biomass loss.

The duration of transport significantly contributed to seedling weight loss, likely because longer transport times expose the seedlings to prolonged stress conditions, including limited light, restricted gas exchange, and the accumulation of respiratory metabolites. Under low-light conditions, photosynthesis is suppressed, causing respiration to dominate, which draws on internal energy reserves and leads to biomass reduction. Tan *et al.* (2024) highlighted that optimal cultivation methods and environmental conditions are critical for the survival of eucheumatoid seaweeds; however, minimizing stress during transport is equally essential to maintain seedling quality.

The practical implication of this study is the need to balance maintaining seedling freshness with avoiding excessive stress caused by low temperatures and prolonged transport durations (Iswahyuddin *et al*., 2024). For short-distance transport lasting up to 24 hours, the use of ice is recommended. Alternative transport methods—such as aeration systems, packaging media that maintain optimal temperatures without ice, or controlled cooling technologies—should be considered to minimize biomass loss and preserve the quality of *E. cottonii* seedlings during transport.

Final Styrofoam Temperature

Temperature control during transport is a critical factor determining the successful distribution of *E. cottonii* seedlings. The results of this study showed that final temperatures inside the styrofoam boxes ranged from 24.2±0.09°C to 28.25±0.20°C. The treatment with four ice cubes over 12 hours of transport (S5W1) produced the lowest temperature, whereas the control without ice over 24 hours (S1W2) resulted in the highest temperature. Styrofoam proved highly effective as an insulating material, limiting heat transfer through conduction, convection, and radiation. The expanded polystyrene (EPS) structure, with its air pockets, minimizes conductive heat transfer, while the trapped air reduces convective heat exchange. n Kumar *et al. (*2020) reported that *E. cottonii* exhibits optimal growth at 27–30°C in tropical and subtropical waters, highlighting the importance of maintaining temperatures within this range to preserve seedling quality. However, excessive ice addition can lower temperatures below the seaweed's physiological tolerance, inducing thermal stress and negatively affecting seedling viability (Tarmizi et al., 2022).

Statistical analysis revealed that both the individual factors—ice quantity and transport duration—as well as their interaction had a highly significant effect ($P < 0.05$) on the final temperature reduction in styrofoam containers. This finding confirms that the two factors act synergistically in influencing temperature dynamics during transport. Although low temperatures can slow the metabolic rate and degradation of seedlings, excessive cooling may induce cold shock in tropical seaweed (Yohanes et al., 2020). Furthermore, Datangmanis et al. (2020) reported that maintaining temperatures within an optimal range preserves seedling freshness and mitigates spoilage during transport. *Eucheuma cottonii* exhibits greater tolerance to low temperatures compared with other species; however, exposure to 17°C results in a significant decline, indicating damage to the photosynthetic apparatus. Therefore, while the addition of ice effectively reduces temperature, it is crucial to consider the lower thermal limit that seedlings can tolerate to prevent physiological damage.

The practical implications of these findings underscore the importance of optimizing both ice quantity and transport duration to achieve a balance between temperature control and prevention of thermal stress during transit. Temperature increases resulting from exposure to sunlight or ambient heat can accelerate ice melting (Tamonob et al., 2025). The addition of 1–2 ice cubes is sufficient to maintain temperatures within the optimal range without causing excessive cooling (Datangmanis et al., 2020). Further research on long-term acclimatization to temperature fluctuations and the identification of *E. cottonii* strains with greater tolerance to thermal variation is also necessary to support the sustainability of the seaweed cultivation industry.

L* (lightness) Values of E. cottonii Seedlings

The L* value (lightness) is a key color parameter used to describe the brightness or darkness of an object within a color space, ranging from 0 (black) to 100 (white). In this study, the L* values of *E. cottonii* seedlings ranged from 16.5±0.32 to 26.3±0.1, with the initial seedling L* value prior to treatment recorded at 24. The control treatment without ice during 24-hour transport (S1W2) produced the highest L* value (26.3), indicating bleaching of the seedlings (thallus appearing lighter or paler). Conversely, the treatment with three ice cubes over 36 hours (S4W3) resulted in the lowest L* value (16.5), suggesting darkening of the seedlings. Changes in L* values are closely associated with the dynamics of photosynthetic pigments, particularly phycoerythrin, which imparts the characteristic red color of red seaweeds. Recent studies on *E. perplexum* demonstrated that green morphotypes contain lower phycoerythrin levels compared with red morphotypes, directly contributing to observed color differences (Handajani et al., 2025). Phycoerythrin is an accessory pigment highly sensitive to environmental stress, and its degradation during transport can significantly influence the L* value of seedlings.

The increase in L* observed in the control treatment reflects pigment bleaching due to phycoerythrin degradation. Under ice-free conditions, higher temperatures and longer transport durations impose severe stress on seedlings. Kumar et al. (2020) reported that at temperatures above 32°C, *E. cottonii* exhibits a significant reduction in pigment content (chlorophyll-a, carotenoids, and phycobiliproteins), accompanied by decreased photosynthetic efficiency and growth rate. Phycoerythrin degradation is accelerated by the combined effects of elevated temperature, limited light, reactive oxygen species (ROS) accumulation, and disruption of the photosynthetic apparatus during transport, resulting in pigment loss and increased L* values (thalli appearing paler). Tissue bleaching is considered a primary indicator of sublethal stress, with visual discoloration (whitening or greenish hues) reflecting compromised surface integrity, pigment loss, and a corresponding decline in photosynthetic performance. In contrast, the decrease in L* in ice-treated seedlings suggests that although ice slows pigment degradation by lowering temperature, prolonged exposure to low temperatures can also induce cold stress, leading to structural changes in cells or pigment condensation, which darkens the thalli. Gelli et al. (2025) reported that brown strains of *E. cottonii* begin losing pigments at 16°C, while green strains retain more intense coloration throughout the experiment, with pigment loss observed only at 14°C.

Statistical analysis revealed that the single factors of ice quantity and transport duration, as well as their interaction, had a highly significant effect ($P < 0.05$) on changes in the L* values of *E. cottonii* seedlings. Prolonged transport under ice-free conditions was more effective in minimizing extreme color alterations compared with ice addition, which tended to induce excessive darkening associated with cold stress. Nevertheless, excessively high L* values—such as those observed under the S1W2 treatment—also indicate substantial quality degradation, as extreme bleaching reflects severe pigment loss.

The practical implications of these findings underscore the importance of monitoring colorimetric parameters as non-destructive indicators for assessing the physiological condition of seedlings during transport. An optimal L* value close to the initial level (approximately 24) suggests that photosynthetic pigment content remains well preserved. In contrast, large deviations—either increases (bleaching) or decreases (darkening)—signal physiological damage that may negatively affect seedling viability and subsequent cultivation performance (Rohmat *et al*., 2014). Therefore, transport optimization should consider not only temperature and duration but also continuous visual quality assessment through L* measurements to ensure seedlings remain in optimal condition

a* (Redness) Values of E. cottonii Seedlings

The a* value (redness) is a color parameter that represents the red–green axis of an object, where positive values indicate redness and negative values indicate greenness. In this study, the a* values of *E. cottonii* seedlings ranged from −10.4±0.25 to 1.6±0.1. The treatment involving one ice cube with a transport duration of 36 hours (S2W3) resulted in the lowest a* value (−10.4), indicating a pronounced shift in seedling color toward green. Variations in temperature and processing duration have been shown to influence seaweed pigmentation, including brightness, redness, and yellowness (Orilda *et al*., 2022). In contrast, the treatment with four ice cubes over 24 hours (S5W2) produced the highest a* value (1.6), reflecting better retention of red coloration. Positive a* values indicate the dominance of phycoerythrin, which confers the characteristic red color, whereas negative a* values reflect extensive phycoerythrin degradation and a shift in pigment dominance toward green chlorophyll. The marked decline in a* values observed under the one-ice, 36-hour transport condition suggests severe phycoerythrin degradation resulting from the combined effects of prolonged transport and low-temperature stress.

Previous studies have demonstrated that incubation of *K. alvarezii* at temperatures above ambient (32°C, 36°C, and 40°C) leads to significant reductions in pigment content, with inhibition intensity increasing with temperature (Kumar et al., 2020; Ray, 2025). Although these studies focused on high-temperature stress, similar pigment degradation mechanisms also occur under low-temperature conditions. Recent findings indicate that gradual temperature reductions in *K. alvarezii* disrupt daily growth rates in both green and brown strains, with sharp declines occurring below 18°C, and significant strain–treatment interactions observed for phycobiliproteins, including phycoerythrin and phycocyanin (Liu et al., 2019). Excessive degradation of phycoerythrin allows chlorophyll to become the visually dominant pigment, resulting in a color shift from red to green, as reflected by negative a* values. Conversely, the increased a* values observed under the four-ice, 24-hour treatment suggest that, under certain conditions, ice addition can aid in preserving phycoerythrin content. This effect is likely attributable to reduced temperatures slowing respiration and metabolic rates that consume pigments, as well as suppressing the activity of degradative enzymes that compromise the structure of phycoerythrin.

Statistical analysis demonstrated that the main effects of ice quantity and transport duration, as well as their interaction, had a highly significant influence ($P < 0.05$) on changes in the a* values of *E. cottonii* seedlings. Duncan's multiple range test further indicated that the treatment involving four ice cubes over a 36-hour transport period (S5W3) maintained significantly higher a* values compared with all other treatments. These results suggest that during very long transport durations, the application of an adequate amount of ice (four ice cubes) can effectively minimize phycoerythrin degradation and preserve the characteristic red coloration of the seaweed.

The practical implications of these findings highlight the importance of monitoring a* values as a quality indicator for seedlings during transport. Highly negative a* values, reflecting a pronounced shift toward green coloration, indicate extensive phycoerythrin degradation, which may adversely affect photosynthetic capacity and seedling viability. In contrast, a* values that are negative or close to zero indicate better pigment retention and greater physiological stability. Therefore, optimization of transport conditions should consider a balanced combination of temperature, duration, and ice quantity to maintain a* values within an optimal range, thereby preserving seedling quality and performance for subsequent cultivation of *E. cottonii*.

b* (Yellowness) Values of E. cottonii Seedlings*

The b* value (yellowness) represents the yellow–blue axis of color space, where positive values indicate yellow coloration and negative values indicate blue coloration. In this study, b* values of *E. cottonii* seedlings ranged from 7.5±0.07 to 25.4±0.25. The treatment involving four ice cubes with a 12-hour transport duration (S5W1) produced the lowest b* value (7.5), whereas the same ice quantity applied over 36 hours (S5W3) resulted in the highest b* value (25.4). Elevated b* values are associated with the accumulation or increased visibility of carotenoid pigments, particularly xanthophylls such as lutein and zeaxanthin, which impart yellow coloration. Green seaweeds are rich in bioactive compounds, including pigments such as chlorophylls and carotenoids, that contribute substantially to thallus color (Firdaus, 2019). Xanthophylls—primarily zeaxanthin and lutein—are the dominant carotenoids in green seaweeds (Merdekawati et al., 2017), and their coloration becomes more pronounced when their relative concentration increases compared with other pigments, such as phycoerythrin. In *E. cottonii*, carotenoid pigments include β-carotene and several xanthophylls, namely zeaxanthin, violaxanthin, cryptoxanthin, and lutein (Rizky *et al*., 2024).

The pronounced increase in b* values observed under the four-ice, 36-hour transport treatment indicates enhanced xanthophyll accumulation or visibility resulting from the degradation of other dominant pigments. Under transport-induced stress, particularly prolonged exposure to low temperatures, pigment balance shifts as phycoerythrin (red pigment) degrades more rapidly than xanthophylls (yellow pigments), which are relatively more stable or may even accumulate. This shift causes yellow coloration to become increasingly dominant. Rohmat *et al*., (2014) reported that temperature and light conditions strongly influence chlorophyll degradation; once chlorophyll degradation occurs, seaweeds experience a reduced capacity for photosynthesis. The stability of the xanthophyll fucoxanthin in microalgal and seaweed biomass is strongly influenced by storage conditions, with methanolic extracts stored at −20°C and −80°C demonstrating greater effectiveness in preserving xanthophyll pigments than intact algal biomass. In contrast, the relatively low b* value observed under the S5W1 treatment indicates that during short transport durations with ice addition, pigment balance remains largely preserved. Under these conditions, phycoerythrin continues to be the dominant visible pigment, and the yellow coloration associated with xanthophylls has not yet become pronounced.

Statistical analysis showed that the main effects of ice quantity and transport duration, as well as their interaction, had a highly significant influence ($P < 0.05$) on changes in the b* values of *E. cottonii* seedlings. Duncan's multiple range test indicated that the treatment involving four ice cubes over a 12-hour transport period (S5W1) maintained significantly lower b* values compared with the other treatments. These findings suggest that for short transport durations, the addition of an adequate amount of ice can effectively minimize shifts in pigment composition and preserve optimal color balance.

Previous studies examining the effects of biomass processing protocols in *K. alvarezii* have reported substantial spatial and seasonal variability in total carotenoid content, with freeze-drying techniques demonstrating superior carotenoid retention compared with oven-drying methods. The practical implications of the present study emphasize the importance of monitoring b* values as an indicator of pigment composition changes during transport. Excessively high b* values reflect extensive phycoerythrin degradation and increased dominance of xanthophyll pigments. Agustamia *et al*., (2016) reported that chlorophyll loss in plants leads to stunted growth and reduced productivity, highlighting the broader physiological consequences of pigment degradation. Therefore, optimization of transport conditions should not be limited to temperature and duration alone but should also incorporate routine monitoring of b* values to ensure that pigment balance remains within an optimal range. Maintaining stable b* values is essential for preserving seedling quality and sustaining photosynthetic performance of *E. cottonii* for subsequent cultivation.

Salinity Content of E. cottonii Seedlings

The results showed that the salinity content of *E. cottonii* seedlings ranged narrowly from 3.75±0.20% to 3.76±0.28%, indicating very limited variation among treatments. The treatment with three ice cubes for 12 hours (S4W1) resulted in the lowest reduction in salinity, whereas the treatment with four ice cubes for 36 hours (S5W3) produced the greatest decrease. *E. cottonii* grows optimally at salinities ranging from 30 to 37 ppt and can tolerate higher salinity levels; however, it is less tolerant of salinities below its normal range (Moher & Bibin., 2025). Although salinity reduction occurred as a result of ice melting and the introduction of freshwater, the magnitude of change observed in this study was minimal (approximately 0.1%) and remained well within the optimal salinity range for *E. cottonii* growth. This finding indicates that the volume of freshwater released from melting ice during transport was insufficient to cause a substantial alteration of the transport medium's salinity. The stability of salinity is likely attributable to the use of tightly sealed packaging, which limited the direct contact between meltwater and the seaweed seedlings. Yohanes et al. 2020) reported that maintaining dry environmental conditions during transport represents a form of induced hibernation, in which metabolic activity is suppressed to enhance organismal survival.

From a practical perspective, these findings suggest that, in terms of salinity, the use of ice within the tested range (1–4 ice cubes) during transport does not pose a significant risk of osmotic stress to *E. cottonii* seedlings. This is particularly important given that salinity is a critical factor influencing osmoregulation, growth, and overall health in *K. alvarezii*. While salinity itself was not significantly affected, other physiological parameters—such as weight loss, color changes (L*, a*, and b* values), and transport medium temperature—exhibited significant responses to ice treatment, transport duration, and their interaction. Therefore, optimization of transport conditions should adopt a holistic approach that considers multiple physiological and environmental parameters rather than salinity alone. For practical applications, seaweed farmers may use ice to regulate transport temperature without concern for drastic salinity changes, provided that the ice-to-seawater ratio is maintained at reasonable levels and that the Styrofoam containers have sufficient capacity to retain seawater with stable salinity..

4. CONCLUSION

The single effects of temperature treatment (S), transport engineering duration (W), and their interaction (SW) significantly affected absolute weight, transport temperature, and all color parameters of *E. cottonii* seedlings, but had no significant effect on seedling salinity. The interaction between the application of four ice cubes and a 12-hour transport duration (S5W1) was identified as the optimal treatment, as it most effectively preserved seedling color performance, with L* (lightness), a* (redness), and b* (yellowness) values of 23.3±0.01, −0.2±0.12, and 7.5±0.07, respectively. Despite its superiority in maintaining color quality, the S5W1 treatment resulted in a substantial reduction in seedling weight (25.4±0.5 g) during transport. This weight loss is attributed to cold shock induced by a marked decrease in the styrofoam container temperature to 24.2±0.09°C. In contrast, seedling salinity did not differ significantly among treatment interactions, remaining within a narrow range of 37.5±0.20% to 37.6±0.28%.

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