

Article

Physiological Responses and Functional Performance of Eucheumatoid Seaweeds Under Prolonged Low-Salinity Stress

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Abstract

Low-salinity events caused by heavy rainfall and freshwater runoff pose significant environmental constraints on eucheumatoid seaweed mariculture in coastal areas. In this study, five eucheumatoid seaweeds, including three strains of *Kappaphycus alvarezii* (Hn-R, Hn-G, and Ma-R), *Kappaphycus striatum*, and *Eucheuma denticulatum*, were exposed to 22, 26, and 30 psu for 36 days. Growth performance, photosynthetic pigments, chlorophyll fluorescence parameters, oxidative damage, and antioxidant responses were determined. To compare the actual physiological performance of different seaweeds under each salinity condition, a functional performance score was calculated using membership function analysis based on relative growth rate, chlorophyll a, carotenoids, photosynthesis, and malondialdehyde. Low salinity generally reduced growth, photosynthetic pigment content, and photosystem II performance, while increasing malondialdehyde content and antioxidant-related parameters, indicating that prolonged low-salinity exposure induced both physiological inhibition and oxidative stress responses. At 22 psu, *K. alvarezii* Ma-R showed the strongest functional performance, followed by *K. striatum* and *E. denticulatum*. At 26 psu, *E. denticulatum* showed the strongest functional performance, followed by *K. alvarezii* Ma-R and *K. striatum*. These results indicate that eucheumatoid seaweeds differ in their functional performance under mild and more severe low-salinity tolerance, providing physiological evidence for selecting candidate eucheumatoid seaweeds for salinity-fluctuating coastal mariculture.

Keywords: eucheumatoid seaweeds; low-salinity stress; coastal mariculture; marine aquaculture; chlorophyll fluorescence; oxidative stress; functional performance score



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1. Introduction

Eucheumatoid seaweeds of the genera *Kappaphycus* and *Eucheuma* belong to Rhodophyta, Florideophyceae, Gigartinales and Solieriaceae [1]. They are widely distributed and cultivated in tropical regions such as Malaysia, the Philippines and Indonesia, and occur in southern China, including Hainan Island, the Xisha Islands, the Nansha Islands and Taiwan. Their cell walls and intercellular matrix are rich in carrageenan, making them important raw materials for carrageenan extraction [2,3]. Carrageenan and carrageenan-derived products are widely used in food, cosmetics and pharmaceutical industries [4–8]. In addition, eucheumatoid seaweed cultivation can contribute to nutrient removal and improved water quality in integrated aquaculture systems [9,10].

Commercial cultivation of *Kappaphycus* and *Eucheuma* commonly uses rope, net and tubular-net systems in natural coastal waters [11]. Among environmental variables, salinity is a key factor affecting seaweed growth and physiological status. Fluctuations in salinity can affect algal growth, biomass accumulation, quality, and metabolic composition [12]. During the rainy season, intense precipitation and freshwater runoff can markedly reduce coastal seawater salinity. Low salinity may disturb osmotic and ionic balance, promote the production and accumulation of reactive oxygen species (ROS), induce oxidative stress and impair photosynthesis [13,14]. For example, *Kappaphycus alvarezii* was reported to bleach and die after three days at 15 psu, whereas higher growth was observed at 30–35 psu [15]. In *Betaphycus gelatinus*, 20 psu reduced growth, photosynthetic performance and pigment contents, while increasing superoxide dismutase (SOD), malondialdehyde (MDA), soluble sugars and glutathione (GSH) [16]. Similar salinity-dependent changes in growth and photosynthetic pigments have also been reported in other macroalgae [17,18]. These findings suggest that seaweed responses to low salinity are species-specific and involve multiple physiological processes.

Although salinity stress has been examined in several macroalgae, studies on eucheumatoid seaweeds have mainly focused on single species or a limited number of traits [16,19,20]. Comparative information on the long-term low-salinity responses of different *Kappaphycus* strains and related eucheumatoid species remains limited. Such information is needed for selecting cultivation materials suitable for coastal areas exposed to seasonal rainfall or freshwater intrusion. Therefore, the present study compared the growth, photosynthetic characteristics, oxidative damage, and antioxidant responses of five eucheumatoid seaweeds after 36 days of exposure to different salinity levels. To avoid over-reliance on a single indicator, a functional performance score based on membership function analysis was used to compare the actual physiological performance of different seaweeds under each salinity condition. The results provide a physiological basis for selecting eucheumatoid seaweed materials suitable for marine aquaculture areas affected by episodic salinity decline.

2. Materials and Methods

2.1. Seaweed Materials and Experimental Design

Three strains of *Kappaphycus alvarezii* (*K. alvarezii* Hn-R, *K. alvarezii* Hn-G and *K. alvarezii* Ma-R), *Kappaphycus striatum* and *Eucheuma denticulatum* were obtained from the Qionghai research base of the Hainan Academy of Ocean and Fisheries Sciences. Healthy and intact thalli of similar size were selected, and epiphytes and surface attachments were gently removed with a soft brush before the experiment. The thalli were acclimated for five days in natural seawater in culture tanks (60 cm × 30 cm × 39 cm; 30 L seawater) at 30 psu, water temperature 28 °C and light intensity 90–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The initial fresh weight of each experimental unit was 3.5 g, and the culture density was approximately 2 g L⁻¹. The species names, strain information and abbreviations of the five eucheumatoid seaweeds used in this study are listed in Table 1.

Experimental salinities were prepared by mixing natural seawater collected from the coastal area near the Qionghai research base (approximately 19.37° N, 110.67° E) with distilled water. The pH of the undiluted natural seawater before dilution ranged from 8.16 to 8.28 during the experimental period. Three salinity treatments were established: 22 psu as the stronger low-salinity treatment, 26 psu as the mild low-salinity treatment and 30 psu as the normal-salinity reference treatment. Each strain/species had at least three biological replicates per salinity treatment. The culture medium was renewed every three days. Water temperature and light conditions were maintained as during acclimation. Salinity was measured using a handheld digital salinity meter (AZ8373, Hengxin Industrial Co., Ltd.,

Taichung City, Taiwan, China). Salinity was expressed in practical salinity units (psu). The 36-day cultivation period was selected to represent prolonged, cultivation-relevant low-salinity exposure, allowing cumulative growth responses and sustained physiological changes to be assessed while maintaining sufficient comparability among treatments for physiological measurements. After 36 days of cultivation, samples were collected for growth and physiological analyses.

Table 1. Seaweed materials used in the present study.

Strain/Species	Abbreviation	Source
<i>Kappaphycus alvarezii</i> Hainan red strain	<i>K. alvarezii</i> Hn-R	Qionghai, Hainan, China
<i>Kappaphycus alvarezii</i> Hainan green strain	<i>K. alvarezii</i> Hn-G	Qionghai, Hainan, China
<i>Kappaphycus alvarezii</i> Malaysia red strain	<i>K. alvarezii</i> Ma-R	Qionghai, Hainan, China
<i>Kappaphycus striatum</i>	<i>K. striatum</i>	Qionghai, Hainan, China
<i>Eucheuma denticulatum</i>	<i>E. denticulatum</i>	Qionghai, Hainan, China

Note: All seaweed materials were provided by the Qionghai research base of Hainan Academy of Ocean and Fisheries Sciences, Hainan, China.

2.2. Relative Growth Rate

After 36 days of cultivation, thalli were removed from the culture medium, and surface moisture was gently absorbed using tissue paper. To minimize physiological disturbance, the period of air exposure was kept as short as possible. Relative growth rate (RGR) was calculated as:

$$\text{RGR (\% d}^{-1}\text{)} = \frac{100 \times [\ln(W_t) - \ln(W_0)]}{t}$$

where W_0 is the initial fresh weight (g), W_t is the final fresh weight (g) after cultivation, and t is the culture duration in days (36 days in this study).

2.3. Photosynthetic Pigments and Chlorophyll Fluorescence

Chlorophyll a and carotenoids were extracted with anhydrous methanol. Fresh seaweed tissue (0.1 g) was ground with 5 mL anhydrous methanol and extracted in darkness at 4 °C for 24 h. The extract was centrifuged at 5000 r min^{−1} for 10 min at 4 °C, and absorbance values were measured using a UV-visible spectrophotometer (UV-3200, Shanghai Mapada Instruments Co., Ltd., Shanghai, China). Chlorophyll a and carotenoid contents were determined using established spectrophotometric equations [21–23].

At day 36, thalli were dark-adapted for 30 min, and chlorophyll fluorescence parameters were measured using a dual-channel pulse-amplitude modulation fluorometer (DUAL-PAM-100, WALZ, Effeltrich, Germany). The maximum quantum efficiency of photosystem II (Fv/Fm) and the effective quantum yield of photosystem II [Y(II)] were recorded.

2.4. Oxidative Damage and Antioxidant Responses

Fresh tissue (0.1 g) was ground in liquid nitrogen and homogenized with 0.9 mL 0.1 mol L^{−1} phosphate buffer (pH 7.0). The homogenate was centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was used for biochemical assays. MDA, SOD, catalase (CAT) and peroxidase (POD) were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; kit numbers A003-1, A001-1, A007-1-1 and A084-3-1, respectively). Absorbance was measured using a microplate reader (BioTek Synergy H1, Agilent Technologies, Santa Clara, CA, USA). Reduced GSH was determined using a GSH assay kit (A061-2-1, Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. The principles, detection wavelengths and evaluation methods of the biochemical assays are summarized in Table 2.

Table 2. Principles, wavelengths and evaluation methods of biochemical assays used in this study.

Parameter	Principle and Wavelength	Evaluation Method
MDA	TBA method; red chromogen measured at 532 nm.	TBA method; red chromogen measured at 532 nm.
SOD	Xanthine oxidase–hydroxylamine method; colored product measured at 550 nm.	Xanthine oxidase–hydroxylamine method; colored product measured at 550 nm.
CAT	Ammonium molybdate method; residual H ₂ O ₂ complex measured at 405 nm.	Ammonium molybdate method; residual H ₂ O ₂ complex measured at 405 nm.
POD	H ₂ O ₂ -dependent colorimetric method; reaction product measured at 420 nm.	H ₂ O ₂ -dependent colorimetric method; reaction product measured at 420 nm.
GSH	DTNB recycling method; T-GSH and GSSG measured at 412 nm.	DTNB recycling method; T-GSH and GSSG measured at 412 nm.

Note: TBA, thiobarbituric acid; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); T-GSH, total glutathione; GSSG, oxidized glutathione. All values were calculated according to the corresponding kit formulas.

2.5. Functional Performance Score Under Low Salinity

To compare the actual physiological performance of different seaweeds under each salinity condition, a functional performance score (D_{FP}) was calculated using membership function analysis. Six core traits were included: RGR, chlorophyll a, carotenoids, Fv/Fm, Y(II) and MDA. RGR, chlorophyll a, carotenoids, Fv/Fm and Y(II) were treated as positive indicators, whereas MDA was treated as a negative indicator because it is a lipid peroxidation product that reflects oxidative membrane damage; therefore, higher MDA content indicates greater oxidative injury rather than better low-salinity performance. CAT, SOD, POD and GSH were analyzed separately as antioxidant response indicators and were not included in the main D_{FP} calculation.

For positive indicators, including RGR, chlorophyll a, carotenoids, Fv/Fm and Y(II), the membership function value was calculated as:

$$U_{ij} = \frac{X_{ij} - X_{j,\min}}{X_{j,\max} - X_{j,\min}}$$

For MDA, which was treated as a negative indicator of oxidative damage, the reverse membership function was used:

$$U_{ij} = \frac{X_{j,\max} - X_{ij}}{X_{j,\max} - X_{j,\min}}$$

where X_{ij} is the value of indicator j for strain i , and $X_{j,\min}$ and $X_{j,\max}$ are the minimum and maximum values of indicator j among the five seaweeds under the same salinity treatment. The functional performance score D_{FP} was calculated as the mean of the six membership function values:

$$D_{FP,i} = \frac{1}{6} \sum U_{ij}$$

For the supplementary sensitivity analysis, an all-trait score (D_{all}) was calculated using the same membership function procedure. RGR, chlorophyll a, carotenoids, Fv/Fm, Y(II), CAT, SOD, POD and GSH were treated as positive indicators, whereas MDA was treated as a negative indicator. D_{all} was calculated as the mean of the ten membership function values:

$$D_{all,i} = \frac{1}{10} \sum U_{ij}$$

This calculation used the actual treatment means within each salinity level rather than tolerance coefficients relative to 30 psu, because different species or strains may have different optimal salinity ranges. This approach allows the score to reflect actual functional performance under a given salinity condition. The calculation followed the general principle of multi-trait stress evaluation using membership function analysis [24–26].

To further describe the relative magnitude of antioxidant responses, tolerance coefficients (TCs) for CAT, SOD, POD and GSH were calculated as the ratio of the value under low salinity to the value under the normal-salinity reference treatment at 30 psu. These coefficients were used only to support the interpretation of antioxidant response intensity and were not included in the main D_{FP} ranking.

2.6. Statistical Analysis

Data processing was performed using IBM SPSS Statistics 27.0 and GraphPad Prism 10. Data are presented as mean $\pm$ standard deviation (SD) where replicate statistics are shown in figures. Treatment means, standard deviations and replicate numbers for all measured traits are provided in Supplementary Table S1. All measurements included at least three biological replicates, and analyses were performed using the available valid replicates for each measurement. Two-way analysis of variance (ANOVA) was used to test the effects of salinity, seaweed taxon and their interaction. Differences were considered significant at $p < 0.05$. D_{FP} and D_{all} were calculated from treatment means using membership function analysis.

3. Results

3.1. Growth Responses Under Prolonged Low-Salinity Stress

After 36 days of salinity acclimation, the five eucheumatoid seaweeds exhibited species- and strain-specific growth patterns across treatments (Figure 1). *Eucheuma denticulatum* displayed a unique growth response, achieving its maximum relative growth rate (RGR) at 26 psu ($1.30 \pm 0.10\% \text{ d}^{-1}$), whereas it decreased at 22 psu but remained relatively high ($1.04 \pm 0.03\% \text{ d}^{-1}$). *K. alvarezii* Ma-R maintained an RGR at 26 psu ($0.87 \pm 0.10\% \text{ d}^{-1}$), similar to that at 30 psu ($0.89 \pm 0.03\% \text{ d}^{-1}$), but its growth declined at 22 psu ($0.70 \pm 0.06\% \text{ d}^{-1}$). In contrast, *K. alvarezii* Hn-R, *K. alvarezii* Hn-G and *K. striatum* showed lower RGR values under both low-salinity treatments than at 30 psu. Under 22 psu, RGR was lowest in *K. alvarezii* Hn-R ($0.32 \pm 0.03\% \text{ d}^{-1}$), whereas *E. denticulatum* and *K. striatum* maintained comparatively higher growth levels (1.04 ± 0.03 and $0.95 \pm 0.10\% \text{ d}^{-1}$, respectively). These results indicate that prolonged low-salinity exposure inhibited growth to different extents among the tested species and strains, notably, *E. denticulatum* and *K. alvarezii* Ma-R retained relatively strong growth performance under mild low salinity.

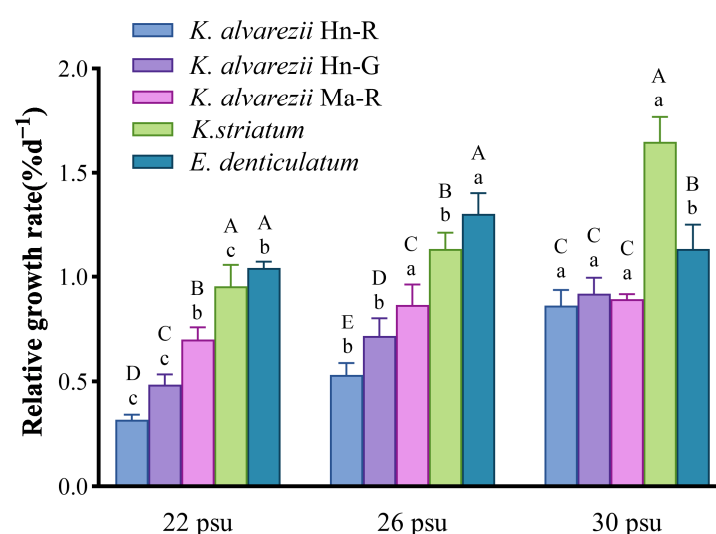


Figure 1. Relative growth rate of five eucheumatoid seaweeds under three salinity treatments (22, 26 and 30 psu). Uppercase letters indicate differences among taxa/strains within the same salinity treatment; lowercase letters indicate differences among salinity treatments within the same taxon/strain ($p < 0.05$).

3.2. Photosynthetic Pigment and Chlorophyll Fluorescence Responses

Low-salinity stress induced taxon-specific alterations in photosynthetic pigment concentrations across the five eucheumatoid seaweeds (Figure 2). Chlorophyll a content generally decreased relative to 30 psu, but the magnitude of reduction differed among taxa. At 22 psu, *K. alvarezii* Hn-R and *K. alvarezii* Ma-R showed clear decreases in chlorophyll a content, with values of $(9.83 \pm 1.06 \mu\text{g g}^{-1})$ and $(8.64 \pm 1.01 \mu\text{g g}^{-1})$, respectively, compared with $(15.21 \pm 2.01 \mu\text{g g}^{-1})$ and $(11.99 \pm 0.77 \mu\text{g g}^{-1})$ at 30 psu. In contrast, *K. alvarezii* Hn-G and *K. striatum* showed relatively smaller fluctuations in chlorophyll a content under low salinity. *E. denticulatum* maintained the highest chlorophyll a content among the five seaweeds under both low-salinity treatments, reaching $(26.36 \pm 2.24 \mu\text{g g}^{-1})$ at 26 psu and $(17.46 \pm 1.93 \mu\text{g g}^{-1})$ at 22 psu. Carotenoid content also varied markedly among taxa. *E. denticulatum* showed significantly higher carotenoid levels than the other four taxa across salinity treatments and reached its maximum at 26 psu $(21.91 \pm 1.42 \mu\text{g g}^{-1})$, whereas the value decreased at 22 psu $(9.80 \pm 1.60 \mu\text{g g}^{-1})$. These results suggest that pigment accumulation in *E. denticulatum* was favored under mild low-salinity conditions, while stronger low-salinity exposure suppressed carotenoid accumulation.

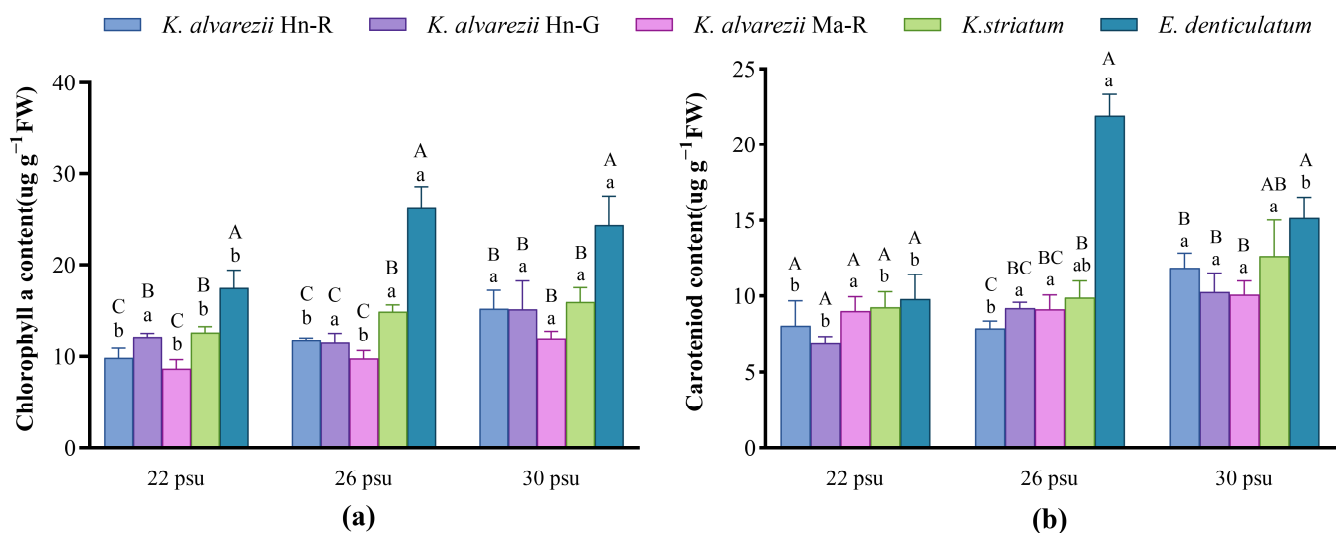


Figure 2. Photosynthetic pigment contents of five eucheumatoid seaweeds under three salinity treatments (22, 26 and 30 psu): (a) chlorophyll a and (b) carotenoids. Uppercase letters indicate differences among taxa/strains within the same salinity treatment; lowercase letters indicate differences among salinity treatments within the same taxon/strain ($p < 0.05$).

Chlorophyll fluorescence parameters indicated that low salinity markedly impaired photosystem II performance (Figure 3). The maximum quantum efficiency of PSII (F_v/F_m) declined universally at 22 psu relative to the 30 psu control, indicating that stronger low salinity reduced the maximum quantum efficiency of PSII. *K. alvarezii* Ma-R maintained comparatively high fluorescence performance at 22 psu, with F_v/F_m and $Y(II)$ values of (0.44 ± 0.03) and (0.43 ± 0.03) , respectively. $Y(II)$ values at 22 psu were significantly lower than those at 30 psu in most taxa, except for *K. striatum* and *E. denticulatum*, which maintained comparatively stable effective quantum yield. *E. denticulatum* showed the highest F_v/F_m and $Y(II)$ at 26 psu (both 0.47 ± 0.02), consistent with its strong growth and pigment performance under mild low-salinity conditions. Overall, low salinity inhibited PSII function, but the degree of inhibition showed significant differences among species and strains.

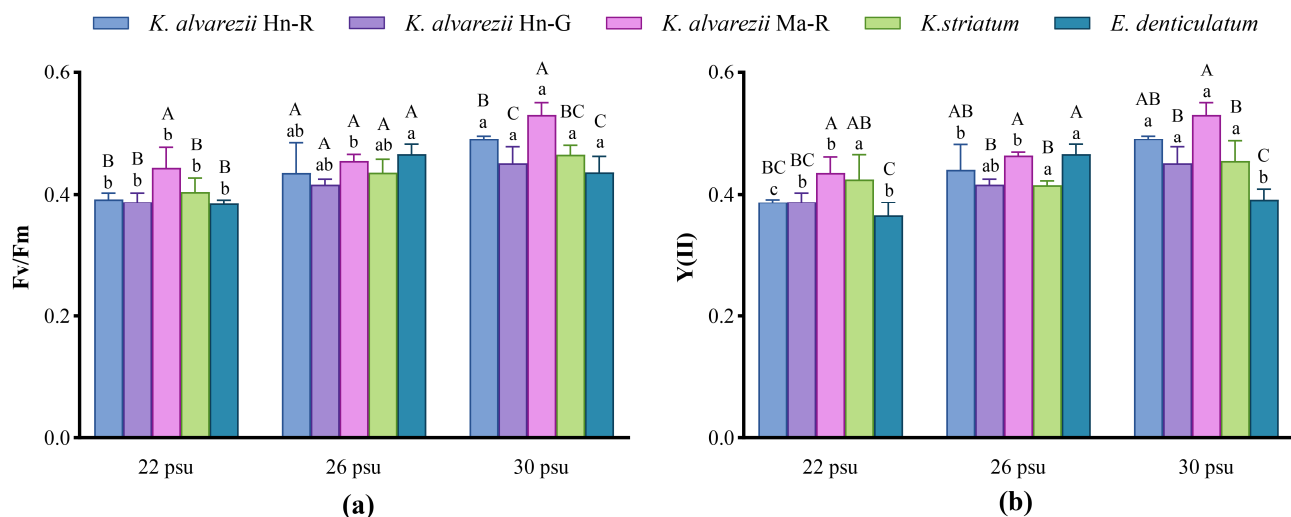


Figure 3. Chlorophyll fluorescence parameters of five eucheumatoid seaweeds under three salinity treatments (22, 26 and 30 psu): (a) Fv/Fm and (b) Y(II). Uppercase letters indicate differences among taxa/strains within the same salinity treatment; lowercase letters indicate differences among salinity treatments within the same taxon/strain ($p < 0.05$).

3.3. Oxidative Damage and Antioxidant Responses

Low-salinity stress elevated MDA concentrations across all examined seaweeds, indicating aggravated membrane lipid peroxidation under prolonged salinity limitation (Figure 4). At 22 psu, MDA content was significantly higher than that at 30 psu in all five seaweeds. *K. alvarezii* Hn-R and *K. alvarezii* Hn-G increased from (7.42 ± 0.19) and (6.36 ± 1.16) at 30 psu to (16.06 ± 1.16) and (13.03 ± 1.53) at 22 psu, respectively, suggesting strong membrane lipid peroxidation under stronger low-salinity stress. *E. denticulatum* showed the highest MDA content at 22 psu (22.35 ± 0.80), whereas *K. alvarezii* Ma-R showed a comparatively lower MDA level (10.82 ± 0.11). At 26 psu, MDA changes were weaker than those observed at 22 psu, confirming that 22 psu imposed stronger oxidative pressure. These results show that the tested eucheumatoid seaweeds differed markedly in oxidative damage under prolonged low-salinity exposure.

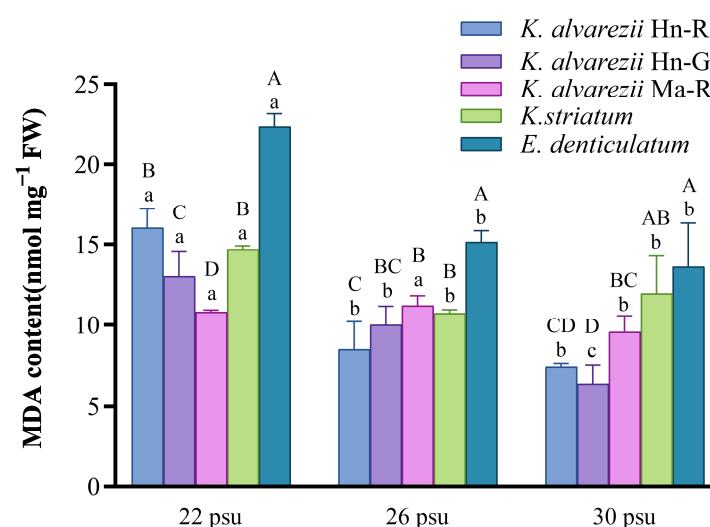


Figure 4. MDA content of five eucheumatoid seaweeds under three salinity treatments (22, 26 and 30 psu). Uppercase letters indicate differences among taxa/strains within the same salinity treatment; lowercase letters indicate differences among salinity treatments within the same taxon/strain ($p < 0.05$).

GSH, CAT, SOD and POD generally increased under reduced salinity, indicating activation of antioxidant responses (Figure 5). GSH content increased in all taxa at 22 psu relative to 30 psu, ranging from (64.67 ± 12.56) in *K. alvarezii* Ma-R to (86.71 ± 2.33) in *K. alvarezii* Hn-G. SOD activity also increased under low-salinity treatments in all tested seaweeds, and *E. denticulatum* showed the highest SOD activity at 22 psu (38.65 ± 0.17). CAT activity increased with decreasing salinity, especially in *E. denticulatum*, where CAT increased from (11.33 ± 1.98) at 30 psu to (28.47 ± 1.53) at 22 psu. POD activity also responded strongly to low salinity. In *K. alvarezii* Ma-R, POD increased from (4.22 ± 1.38) at 30 psu to (21.33 ± 0.36) at 22 psu, indicating a pronounced POD-mediated antioxidant response. Taken together, the antioxidant response patterns suggest that *E. denticulatum* maintained high SOD, CAT and POD activities, whereas *K. alvarezii* Ma-R showed a particularly strong POD response. Because increased antioxidant activity may reflect both defense activation and stronger stress perception, these indicators were interpreted as antioxidant response traits rather than being used alone to determine the final performance ranking.

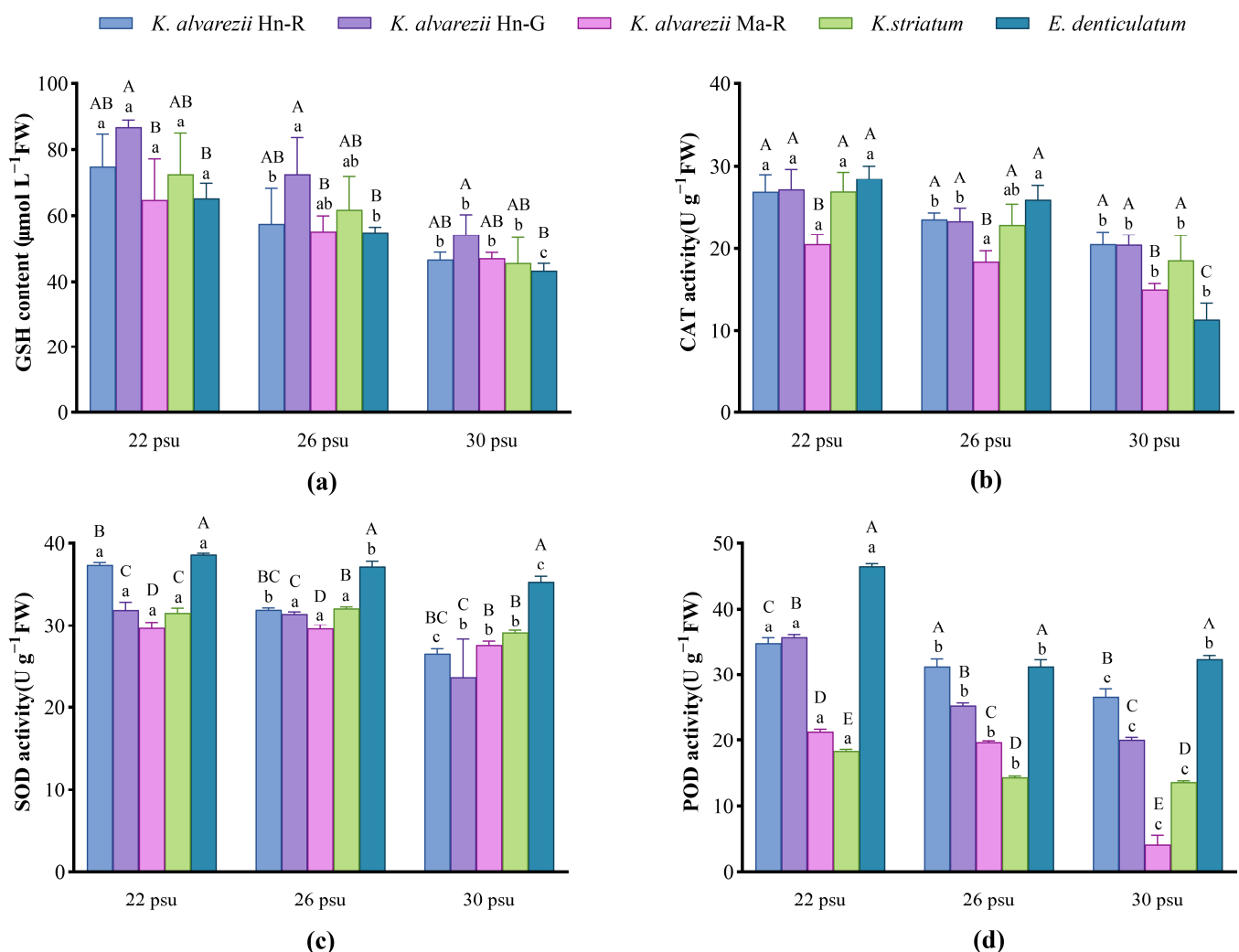


Figure 5. Antioxidant response indicators of five eucheumatoid seaweeds under three salinity treatments (22, 26 and 30 psu): (a) GSH, (b) CAT activity, (c) SOD activity, and (d) POD activity. Uppercase letters indicate differences among taxa/strains within the same salinity treatment; lowercase letters indicate differences among salinity treatments within the same taxon/strain ($p < 0.05$).

The relative magnitude of CAT, SOD, POD and GSH responses was further summarized as tolerance coefficients relative to 30 psu and is provided in Supplementary Table S2.

These coefficients showed that low salinity generally enhanced antioxidant responses, although the magnitude of antioxidant activation differed among taxa and strains.

3.4. Functional Performance-Based Evaluation Under Different Salinities

The functional performance score (D_{FP}) was established based on six core physiological indices, including RGR, chlorophyll a, carotenoids, Fv/Fm, Y(II) and MDA using the mean values of each salinity treatment. D_{FP} was adopted as a study-specific comparative measure of actual functional performance under low-salinity conditions (Table 3). The detailed calculation process, including indicator directions, X_{min}/X_{max} values and membership function values, is provided in Supplementary Table S3. At the severe low-salinity level of 22 psu, *K. alvarezii* Ma-R had the highest D_{FP} value (0.71), followed by *K. striatum* (0.67), *E. denticulatum* (0.50), *K. alvarezii* Hn-G (0.30) and *K. alvarezii* Hn-R (0.26). This ranking indicates that *K. alvarezii* Ma-R maintained the best overall combination of growth, PSII performance and lower relative oxidative damage under stronger low-salinity conditions, whereas *K. striatum* also showed relatively strong functional performance at 22 psu.

Table 3. Functional performance score (D_{FP}) and ranking under low-salinity treatments.

Salinity (psu)	Strain/Species	D_{FP}	Rank
22	<i>K. alvarezii</i> Ma-R	0.71	1
22	<i>K. striatum</i>	0.67	2
22	<i>E. denticulatum</i>	0.50	3
22	<i>K. alvarezii</i> Hn-G	0.30	4
22	<i>K. alvarezii</i> Hn-R	0.26	5
26	<i>E. denticulatum</i>	0.83	1
26	<i>K. alvarezii</i> Ma-R	0.47	2
26	<i>K. striatum</i>	0.38	3
26	<i>K. alvarezii</i> Hn-R	0.33	4
26	<i>K. alvarezii</i> Hn-G	0.20	5

By contrast, under mild low-salinity conditions (26 psu), *E. denticulatum* showed the highest D_{FP} value (0.83), followed by *K. alvarezii* Ma-R (0.47), *K. striatum* (0.38), *K. alvarezii* Hn-R (0.33) and *K. alvarezii* Hn-G (0.20). The contrasting D_{FP} rankings between 22 and 26 psu indicate that the tested eucheumatoid seaweeds differed in their actual physiological performance under mild and stronger low-salinity conditions. Therefore, D_{FP} values at 22 and 26 psu were reported separately rather than being combined into a single overall value.

Compared with the antioxidant response coefficients, the D_{FP} -based ranking was not fully consistent with the magnitude of CAT, SOD, POD and GSH activation. This indicates that antioxidant activation and functional performance reflected different aspects of the low-salinity response. The D_{all} sensitivity analysis including antioxidant response indicators is provided in Supplementary Table S4. This analysis was used to evaluate whether inclusion of antioxidant traits affected the interpretation of low-salinity performance, rather than to replace the main D_{FP} -based ranking.

4. Discussion

4.1. Prolonged Low Salinity Differentially Affects Growth and Photosynthetic Performance

Salinity decline caused by excessive rainfall and freshwater runoff is an important environmental constraint for tropical eucheumatoid seaweed cultivation [27]. In the present study, 36 days of low-salinity exposure reduced growth in most tested seaweeds, although the magnitude of the response differed among species and strains. This confirms that salinity tolerance is not uniform among eucheumatoid seaweeds and that strain-level differences should be considered when selecting cultivation materials. The increased RGR

of *E. denticulatum* at 26 psu suggests that mild salinity reduction may fall within a favorable or tolerable range for this species, whereas 22 psu caused stronger physiological stress. Similar salinity-dependent growth patterns have been observed in other macroalgae [17,18].

Photosynthetic pigments and chlorophyll fluorescence parameters are sensitive indicators of algal stress responses. In this study, low salinity generally reduced chlorophyll *a*, carotenoid content, *Fv/Fm* and *Y(II)*, indicating impaired pigment accumulation and photosystem II performance. These results are consistent with previous reports showing that salinity stress can reduce pigment content and photosynthetic efficiency in macroalgae [28–30]. However, the relationship between fluorescence parameters and overall functional performance was not straightforward. This indicates that chlorophyll fluorescence alone cannot fully reflect long-term low-salinity performance; growth, pigment retention and oxidative damage control should also be considered.

4.2. Oxidative Stress and Antioxidant Activation Under Low-Salinity Stress

Low-salinity stress can induce ROS accumulation and oxidative damage in macroalgae [13,31]. MDA is widely used as an indicator of lipid peroxidation and cellular membrane damage under abiotic stress [32]. In the present study, MDA increased at 22 psu in all seaweeds, indicating that stronger low-salinity stress induced oxidative damage. *K. alvarezii* Hn-R and *K. alvarezii* Hn-G showed large relative increases in MDA, suggesting that their membrane systems were more sensitive to salinity decline. In contrast, *K. alvarezii* Ma-R had relatively lower MDA at 22 psu than several other seaweeds, which partly explains its relatively high *D_{FP}* at 22 psu despite not having the highest growth rate.

Antioxidant enzymes and non-enzymatic antioxidants help maintain cellular redox balance under stress. SOD converts superoxide anions into hydrogen peroxide, while CAT and POD participate in hydrogen peroxide detoxification; GSH also contributes to redox homeostasis and free-radical scavenging [33]. Previous studies on macroalgae have reported increases in antioxidant enzyme activity and GSH under salinity or hyposalinity stress [16,19,30,34]. In this study, GSH, SOD, CAT and POD generally increased under low salinity, indicating activation of antioxidant defenses. However, increased antioxidant activity may represent either efficient defense or a stronger stress signal. Therefore, antioxidant traits were discussed as response indicators rather than being directly included in the main *D_{FP}* ranking. Although CAT, SOD, POD and GSH generally increased under reduced salinity, antioxidant activation was not consistently associated with the functional performance ranking obtained from membership function analysis. Environmental stresses, including hyposalinity and hypersalinity, can accelerate ROS production in seaweeds, and antioxidant systems are involved in scavenging ROS and reducing oxidative damage [13]. Therefore, enhanced antioxidant activity may indicate activation of antioxidant defenses in response to ROS production, but in the present study it should not be interpreted as direct evidence of improved low-salinity tolerance. For example, *E. denticulatum* exhibited strong antioxidant responses at 22 psu but also showed the highest MDA content, whereas *K. alvarezii* Ma-R achieved the highest *D_{FP}* value mainly through better growth, photosynthetic performance and lower oxidative damage. Therefore, antioxidant traits were interpreted as response indicators rather than direct indicators of low-salinity tolerance.

The lack of a consistent relationship between antioxidant activity and *D_{FP}* may also reflect the limited antioxidant coverage of this study, as non-enzymatic antioxidants, including polyphenols, ascorbic acid and tocopherols, as well as total antioxidant capacity, were not assessed [35].

4.3. Functional Performance Score and Implications for Cultivation Material Selection

Low-salinity tolerance is a complex trait involving growth, photosynthesis and oxidative damage control. Single indicators may lead to biased evaluation, particularly when different traits respond in contrasting directions. In the present study, D_{FP} was defined as a functional performance score calculated from six traits directly related to growth, photosynthetic performance and oxidative damage control: RGR, chlorophyll a, carotenoids, Fv/Fm, Y(II) and MDA. The use of MDA as a negative indicator allowed oxidative damage to be integrated with growth and photosynthetic performance. This score was calculated using actual values within each salinity level because 30 psu may not represent the optimal condition for all seaweeds.

The D_{FP} results revealed different rankings under mild and stronger low-salinity treatments. Under 26 psu, *E. denticulatum* showed the highest D_{FP} , indicating strong functional performance under mild low-salinity conditions. Under 22 psu, *K. alvarezii* Ma-R ranked first, followed by *K. striatum* and *E. denticulatum*, suggesting that performance under stronger low-salinity conditions differed from that under mild low salinity. These results indicate that cultivation material selection should consider the expected intensity of salinity fluctuation. *E. denticulatum* may be a suitable candidate for areas experiencing mild salinity decline, whereas *K. alvarezii* Ma-R and *K. striatum* may have greater potential under stronger low-salinity risk.

The D_{all} sensitivity analysis further demonstrated that inclusion of antioxidant-response traits altered the ranking of some taxa relative to the main D_{FP} ranking. This suggests that antioxidant activation and functional maintenance represent different aspects of the stress response. Consequently, D_{FP} , which integrates growth, photosynthetic performance and oxidative damage, was retained as the primary indicator of low-salinity performance, whereas D_{all} was used only as a supplementary sensitivity assessment.

Because carrageenan yield and quality were not measured in the present study, the results should be interpreted as physiological evidence of low-salinity tolerance rather than a direct evaluation of commercial carrageenan production performance. Future studies should integrate measurements of carrageenan content, gel quality, and molecular or metabolomic analyses to better link physiological tolerance with production traits.

In addition, the light condition used in this laboratory experiment should be considered when interpreting the results. The experiment was conducted under a controlled irradiance of $90\text{--}100\ \mu\text{mol m}^{-2}\text{ s}^{-1}$, which allowed all salinity treatments and taxa/strains to be compared under the same light regime. This controlled design helped isolate the effects of salinity, but the absolute growth rates, pigment contents, chlorophyll fluorescence parameters and oxidative stress responses may differ from those under natural coastal cultivation conditions, where irradiance can be higher and can fluctuate with season, weather, water depth and turbidity. Lower light intensity may limit photosynthetic carbon assimilation and alter pigment regulation, and it may also affect ROS production and antioxidant responses under salinity stress. Therefore, the present results should be interpreted as comparative physiological responses under controlled light conditions rather than direct predictions of field performance under all coastal cultivation environments. Future studies under field-based conditions are needed to evaluate the combined effects of salinity fluctuation and natural irradiance variation on eucheumatoid seaweed performance.

Nevertheless, the present results provide useful baseline information for selecting eucheumatoid species and strains for cultivation in coastal areas affected by freshwater input and seasonal rainfall.

5. Conclusions

Prolonged low-salinity stress significantly suppressed growth and photosynthetic performance while triggering oxidative stress responses in five eucheumatoid seaweeds. The D_{FP} comprehensive evaluation further uncovered divergent functional adaptation patterns under mild versus severe low-salinity regimes. *E. denticulatum* showed superior functional performance under mild low salinity (26 psu), whereas *K. alvarezii* Ma-R showed the optimal adaptability under severe low-salinity conditions (22 psu). These findings suggested that eucheumatoid species and strains differ in their ability to maintain growth, photosynthetic function and oxidative damage control under low-salinity stress. This study provides physiological insights for the targeted screening and application of salinity-tolerant eucheumatoid germplasms in coastal mariculture regions with frequent salinity fluctuations.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jmse14131193/s1>, Table S1: Treatment means, standard deviations and replicate numbers of physiological traits; Table S2: Tolerance coefficients of antioxidant response indicators relative to 30 psu; Table S3: Calculation details of the functional performance score (D_{FP}); Table S4: D_{all} sensitivity analysis including antioxidant response indicators.

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