The Effect of Salinity Variation on the Growth Performance, Physiological Response, and Nutrient Utilization of Seaweed Chaetomorpha linum as a Bioremediation Candidate

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ABSTRACT

This study evaluated the effect of salinity variations on the physiological response, growth performance, chlorophyll content, and survival rate of the seaweed Chaetomorpha linum over a 30-day experimental duration under controlled laboratory conditions. A Completely Randomized Design (CRD) was employed, consisting of nine salinity treatments, namely P1 (40 ppt), P2 (35 ppt), P3/control (30 ppt), P4 (25 ppt), P5 (20 ppt), P6 (15 ppt), P7 (10 ppt), P8 (5 ppt), and P9 (0 ppt), with five replications each (initial fresh biomass: 5 gper unit). The observed parameters included absolute weight, Specific Growth Rate (SGR), Survival Rate (SR), dry biomass yield, thallus diameter, total chlorophyll, and water quality. Data were subjected to One-Way ANOVA followed by Duncan's Multiple Range Test ($\alpha = 0.05$). The results showed that salinity significantly affected ($P < 0.05$) all growth and physiological parameters. The hyper-saline treatment of 40 ppt (P1) yielded the highest growth performance with an absolute weight of 5.23 ± 3.61 g, SGR of 2.39 ± 1.10%/day, dry yield of 8.89 ± 1.07%, and total chlorophyll content of 273 mg/100g, accompanied by maximum survival (Score 4). Conversely Hypo-saline conditions (0-10 ppt) induced severe osmotic shock, leading to cell lysis, tissue bleaching, and negative growth rates. Nutrient analysis revealed substantial uptake of nitrate and ammonia in marine-salinity treatments (30--40 ppt), distinguishing growth vigor from nutrient removal capacity. In conclusion, C. linum exhibits optimal physiological tolerance and growth in the 30--40 ppt range, validating its potential application as a biofilter in high-salinity aquaculture systems and coastal environments

Keywords: Bioremediation; C. linum; Chlorophyll; Growth Rate; Salinity.

INTRODUCTION

Coastal ecosystems face increasing environmental pressure due to anthropogenic activities, municipal waste disposal, and the rapid expansion of intensive aquaculture. Excess loads of inorganic nutrients, particularly nitrogen (N) and phosphorus (P), frequently result in eutrophication, algal blooms, and oxygen depletion. Macroalgae function as primary producers and natural biofilters capable of assimilating excess inorganic nutrients while synthesizing valuable biomass, making integrated multi-trophic aquaculture (IMTA) and bioremediation strategies highly appealing [1]. Macroalgae can efficiently absorb excess nutrients while converting them into economically valuable biomass [2].

Among green macroalgae (Chlorophyta), Chaetomorpha linum has emerged as a promising biofilter candidate due to its high growth rate, dense biomass formation, and broad ecological tolerance [3]. Previous studies suggest that C. linum is euryhaline and can survive across salinities from 3.4to 90 ppt, with growth rates reaching up to 60%/dayunder optimum conditions [4]. Its capacity to absorb inorganic nitrogen and orthophosphate from wastewater has been reported in controlled aquaculture trials [5,6].

However, salinity variations represent a crucial environmental factor influencing cellular osmotic balance, photosynthetic efficiency, ionic transport, and nutrient uptake rates in macroalgae [7]. Under extreme hyposalinity, hypo-osmotic shock causes water influx, triggering cell swelling, membrane lysis, and pigment degradation. Under hyper-osmotic conditions, algae must expend metabolic energy to accumulate organic osmolytes (such as proline and polyols) and regulate internal ion concentrations (K^+/Cl^-) [1]. While earlier literature highlights its broad tolerance, the distinction between mere survival, maximum growth performance, and actual nutrient bioremediation efficiency across a detailed salinity gradient (0-40 ppt) remains insufficiently characterized

Therefore, this study aims to systematically evaluate the effect of salinity variations (0-40 ppt) on the physiological response, structural cell changes, growth performance, and nutrient removal dynamics of C. linum under controlled laboratory conditions. The findings provide quantitative thresholds necessary for optimizing its operational deployment as a bioremediation agent in brackish, marine, and hypersaline aquaculture systems

RESEARCH METHODS

Experimental Setup and Culturing Conditions

The experiment was conducted from February to March 2026 at the Marine Aquaculture Research Center Laboratory, National Research and Innovation Agency (BRIN), North Lombok, Indonesia. Seawater was collected from Kodek Bay, filtered (1µm filter bag), chlorinated with 30 ppmNaOCl for 24 hours, neutralized using $Na_2S_2O_3$, and continuously aerated.

C. linum specimens were sourced from Buleleng, Bali, and acclimated for 7 days at 30 pptsalinity. Healthy filaments (bright green, free of epiphytes) were selected for testing. Salinity levels were adjusted using distilled water (to decrease salinity) or food-grade NaCl (to increase salinity) and verified using a calibrated digital refractometer ($\pm$0.1 ppt). Micro-nutrients were provided using KW21 commercial liquid fertilizer (1 mL/L) containing essential nitrogen, phosphorus, and potassium compounds.

The maintenance room was maintained at a controlled temperature (24.17-25.23°C), under continuous continuous artificial light provided by 30-watt LED units suspended $\pm$40 cmabove the containers (418-577 lux). Experimental jars (1 L capacity) were placed randomly with a 10 cm spatial gap to avoid shading and cross-contamination. Water changes (100%) were conducted every 7 days.

Experimental Design

This study was an experimental study using a Completely Randomized Design (CRD) was implemented with 9 salinity treatments and 5 replications (45 experimental units in total):

- P1: 40 ppt (Hyper-saline)
- P2: 35 ppt
- P3: 30 ppt (Control / Ambient Salinity)
- P4: 25 ppt
- P5: 20 ppt
- P6: 15 ppt
- P7: 10 ppt
- P8: 5 ppt
- P9: 0 ppt (Freshwater)

Each experimental jar contained an initial uniform wet biomass weight (W_0) of 5.00 ± 0.01 gmaintained over a 30-day culture period

Parameter Measurements

1. Growth Performance

- **Absolute Weight Gain (**BM**,** g**):**

$BM = W_t - W_0$…………..[8]

- **Specific Growth Rate (**SGR**,** %/day**):**

$SGR = \left(\frac{\ln W_t - \ln W_0}{t}\right) \times 100\%$.............[9]

(where $W_t$is final wet weight at day $t = 30$ days).

Survival Rate (SR)

Assessed qualitatively on a 0 to 4 point scale based on thallus physical integrity [10] (4: Very fresh/dark green; 3: Moderately fresh; 2: Partially pale/softened; 1: Highly bleached/fragmented; 0: Completely dead/lysed

Dry Biomass Yield (%)

Samples were oven-dried at $45°C$until constant mass was reached:

$$\text{Yield (\%)} = \left(\frac{DW}{WW}\right) \times 100\% \dots\dots\dots\dots\dots\dots\dots [11]$$

(where $DW$is dry weight and $WW$is wet weight).

Total Chlorophyll Content (mg/100g**):**

Extracted in 90%acetone and measured spectrophotometrically at 649 nm and 665 nm on days 1 and 30 using Jeffrey & Humphrey equations [12] :

Chlorophyll a (mg/L) = (13.7 × OD665) − (5.76 × OD649)

Chlorophyll b (mg/L) = (25.8 × OD649) − (7.7 × OD665)

Total Chlorophyll (mg/L) = 20 (OD649) + 6.1 (OD665)

where OD (Optical Density) is the chlorophyll absorbance value.

Morphometric Characteristics (Thallus Diameter) (µm**)**

Measured at three random points per sample using a biophotomicroscope with a digital micrometer [13] :

NSO = 10 × (SO / SOk)

NSO : ocular scale unit value (µm)

SO : number of overlapping objective scale divisions

Sok : number of overlapping ocular scale divisions

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Water Quality

Physical-chemical parameters (pH, temperature, dissolved oxygen, salinity, light intensity) were recorded weekly using calibrated multi-parameter meters. Dissolved inorganic nutrients—Nitrate (NO_3^-), Phosphate (PO_4^{3-}), and Ammonia (NH_3/NH_4^+)—were measured every 3 days using standard spectrophotometric methods to monitor nutrient uptake dynamics as an operational proxy for bioremediation [14].

DATA ANALYSIS

All quantitative data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. One-Way Analysis of Variance (ANOVA) was applied at a confidence level of 95%($\alpha = 0.05$). Upon identifying significant effects ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was conducted for post-hoc pair-wise mean comparisons. Qualitative data and water quality ranges were summarized descriptively.

RESULTS

Growth Performance and Dry Biomass Yield

Salinity variation significantly impacted ($P < 0.05$) the absolute weight gain, SGR, and dry biomass yield of C. linum over the 30-day maintenance period (Table 1).

Table 1. Growth performance parameters and dry biomass yield of C. linum under varying salinities (Mean $\pm$SD, n = 5).

Treatment	Salinity (ppt)	Absolute Weight (g)	SGR (% day^{-1})	Dry Yield (%)
P1	40 ppt	5.23 ± 3.61 c	2.39 ± 1.10 d	8.89 ± 1.07 f
P2	35 ppt	0.84 ± 0.79 b	0.52 ± 0.45 c	6.20 ± 0.59 e
P3 (Control)	30 ppt	0.24 ± 0.21 ab	0.16 ± 0.13 bc	5.76 ± 0.37 e
P4	25 ppt	0.50 ± 0.43 ab	0.32 ± 0.27 bc	4.91 ± 0.39 d
P5	20 ppt	0.00 ± 0.62 ab	0.00 ± 0.43 abc	4.48 ± 0.15 d
P6	15 ppt	0.06 ± 0.48 ab	0.04 ± 0.32 bc	3.64 ± 0.47 c
P7	10 ppt	-0.86 ± 0.73 a	-0.63 ± 0.61 a	2.80 ± 0.35 b
P8	5 ppt	-0.40 ± 0.48 ab	-0.28 ± 0.36 ab	2.83 ± 0.37 b
P9	0 ppt	-0.40 ± 0.45 ab	-0.28 ± 0.34 ab	2.04 ± 0.33 a

Note: Different superscript letters within the same column indicate statistically significant differences ($P < 0.05$) based on Duncan's test

The highest growth vigor was recorded in the hypersaline treatment P1 (40 ppt), reaching an absolute weight of 5.23 ± 3.61 gand SGR of 2.39 ± 1.10%/day($P < 0.05$). Reducing salinity below 15 ppt(P7–P9) resulted in negative SGR values, indicating progressive biomass loss. Dry biomass yield exhibited a linear positive correlation with salinity, peaking at 8.89 ± 1.07%in P1 and dropping to 2.04 ± 0.33%in freshwater (P9).

Survival Rate Score

Qualitative evaluation revealed that treatments P1–P3 (30-40 ppt) retained maximum vitality (Score 4) with dark glossy green pigmentation and rigid filament structures. Under hyposalinity (0-10 ppt), structural integrity disintegrated, resulting in severe tissue bleaching and score reductions down to 1 (critical) in P9.

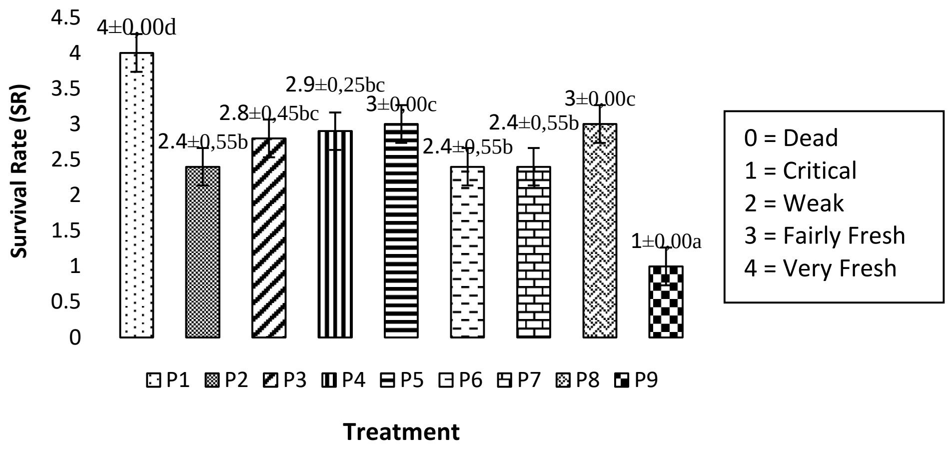

Fig 1. Survival Rate of C. linum

Structural Morphometrics (Thallus Diameter)

Morphometric tracking demonstrated cell enlargement under low salinities. Filament diameter was stable under ambient marine conditions (38.12--42.15µm at 30--40 ppt), but swelled significantly to 65.60 ± 88.66µmin freshwater (P9), reflecting membrane swelling and localized cell wall rupture caused by hypo-osmotic influx.

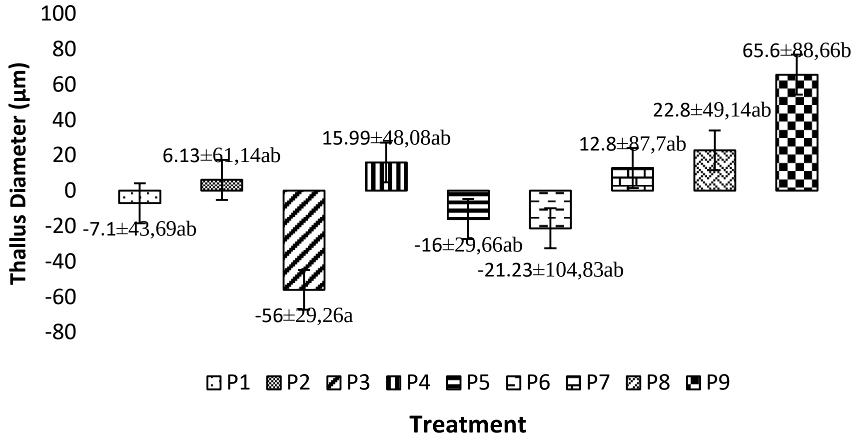

Fig 2. Thallus Diameter of C. linum

Pigment Concentration (Chlorophyll)

Total chlorophyll content was significantly influenced by salinity ($P < 0.05$). Maximum chlorophyll content occurred in P1 (40 ppt) at 273 mg/100g. A secondary minor peak appeared at 10 ppt(167 mg/100g), likely reflecting a short-term physiological stress response, before pigment concentrations collapsed in 0 ppt due to chloroplast destruction.

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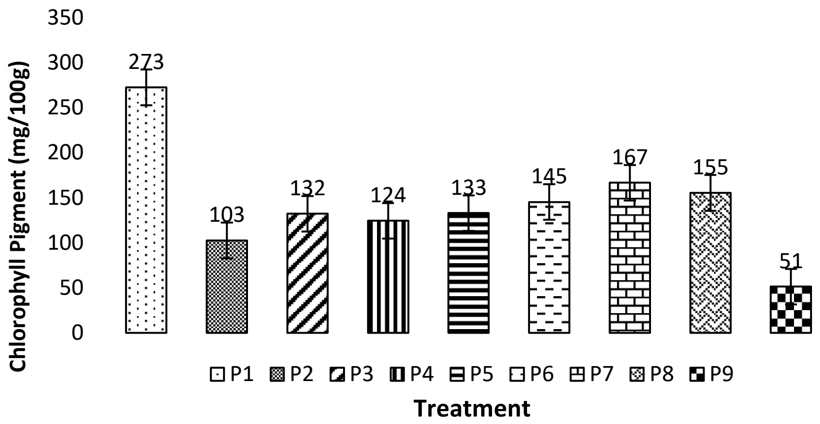

Fig 3. Chlorophyll Pigment of C. linum

Water Quality

Water quality metrics remained controlled across treatments (Table 2).

Table 2. Measured water quality metrics relative to Indonesian National Standards (SNI 7579.2:2010).

Parameter	**Recorded Value Range**	**Optimal Reference Standard**
pH	6.09-7.98	7.0-8.5
Temperature (°C)	24.17-25.23	26-32
DO (mg/L)	19.58-36.64	>3
Light Intensity (lux)	418-577	100
Nitrate (mg/L)	10-50	<1
Phosphate (mg/L)	0	<0.5
Ammonia (mg/L)	0.5	<0.1

Nutrient uptake monitoring showed rapid assimilation of nitrate and ammonia in active growing treatments (30--40 ppt), whereas nutrient removal halted in hyposaline treatments (0--10 ppt) due to cellular mortality.

DISCUSSION

The results showed that C. linum had the best growth performance, biomass accumulation, and survival rate under hyper-saline to moderate conditions (30–40 ppt), with the optimum peak in the 40 ppt treatment (P1) (absolute weight, specific growth rate, dry biomass yield, and survival rate). This is thought to be because at 30–40 ppt salinity, the osmotic condition of the environment is close to the internal osmotic turgor of C. linum cells, resulting in balanced energy partitioning between cellular biosynthesis processes and osmoregulation. Under salinity conditions close to the ideal osmotic balance, energy from photosynthesis can be directed almost entirely toward cell division and thallus filament elongation, rather than osmoregulatory ion-pumping activity [15]. However, this osmotic optimum point does not appear to be uniform across species or strains within the genus Chaetomorpha. [16] reported that the longest filament growth of C. valida actually occurred at 34 ppt salinity, while [4] reported that the highest specific growth rate of C. linum (60%) occurred at 20–30 ppt, far below the 40 ppt found to be optimum in this study, so differences in strain, culture conditions, and interaction with other environmental factors (temperature, pH) are thought to have shifted the position of this optimum point.

Conversely, when salinity deviates from this optimal point, most metabolic energy is diverted to maintaining internal cellular stability, so that energy available for growth becomes very limited [1,3]. This is seen in treatments experiencing a decrease in media salinity below 20 ppt, particularly in the 0–10 ppt range (P7–P9), which triggered negative growth and a significant decline in survival rate. This phenomenon is thought to occur because in this treatment range C. linum was exposed to hyposaline media, causing hypo-osmotic shock, namely excessive water entry into cells that increases turgor pressure beyond the cell wall's capacity, triggering tissue lysis manifested as weight loss and thallus bleaching [3]. Nevertheless, the negative response to hyposalinity is not a universal pattern across all algal groups. [17] noted that the microalga Coccomyxa parasitica actually showed the highest cell development at 13–18 ppt, indicating a short-term stimulatory effect of hyposalinity. Mechanistically, [18] also noted that algae have an active response to lower internal fluid pressure concentration when exposed to hypo-osmotic conditions in order to avoid lysis, so that the cell damage in C. linum observed in this study likely reflects the limitations of the strain's specific adaptive capacity, rather than an absolute, unavoidable consequence of hypo-osmotic conditions.

This is in line with the survival rate (SR) parameter, where the 40 ppt treatment (P1) produced a better appearance of C. linum, thought to be because the optimal salinity condition was able to maintain cellular structural integrity without causing mechanical stress, in line with the pattern of high photosynthetic efficiency under these conditions [15]. At medium salinity ranges (P2–P8), survival remained maintained despite slowed growth, indicating the euryhaline nature of the genus Chaetomorpha, which is able to sustain life through accumulation of organic osmolytes and internal ion regulation (K^+ and Cl^-) to balance environmental osmotic pressure [19,4]. The most critical condition at 0 ppt salinity (P9) is in line with the lower lethal salinity limit for C. linum, reported to be below 15 to 8 ppt [20], where the very rapid rate of water diffusion into cells causes widespread cell lysis. The assumption that high salinity is always identical with optimal photosynthetic efficiency is not entirely consistent. According to [21], high salinity can actually inhibit at least three points in the photosynthetic mechanism, namely photoactivation of electron flow in photosystem I, electron flow at the water-splitting side of photosystem II, and energy transfer between pigment complexes, so the relationship between high salinity and photosynthetic capacity in C. linum is likely species- or strain-specific.

A parameter that further supports this phenomenon is clearly seen in thallus diameter, where the 0 ppt treatment (P9) experienced filament swelling of up to 65.60 µm (+72% compared to the 30 ppt control), while the sharpest diameter shrinkage occurred in the 30 ppt treatment (P3). In low-salinity media, intracellular fluid that is hypertonic relative to the media triggers a massive water influx (osmotic influx) that pushes the plasma membrane outward and swells the filament [3]. This condition also explains why biomass weight decreased significantly (negative SGR). Nevertheless, cell swelling due to osmotic influx does not always result in massive lysis. [22] found that although significant osmotic swelling occurred in Coccomyxa parasitica, the proportion of cells that actually lysed did not exceed 8%, so the decrease in C. linum biomass weight may also be influenced by other mechanisms beyond structural lysis alone, such as metabolite leakage or energy metabolism disruption.

This is further reinforced by the highest dry biomass yield value also being found in the 40 ppt treatment (P1) at 8.89%, indicating dense accumulation of intracellular organic compounds. Dry biomass yield showed a linear decreasing trend as salinity decreased, closely related to the high chlorophyll content and photosynthetic rate at 40 ppt salinity (P1), which promoted the accumulation of carbohydrates and other organic compounds as dry solids. When salinity moves away from the optimal point, carbon utilization efficiency decreases because chemical energy is diverted to maintaining cell turgor pressure instead of forming new tissue [23]. This linear pattern contrasts with findings in the freshwater microalga Acutodesmus dimorphus, where the addition of NaCl to culture media actually inhibited growth and decreased chlorophyll a and b content. [24] show that the positive relationship between salinity and dry biomass yield in C. linum is not a general pattern across all green algae groups, but rather reflects a specific physiological adaptation to the marine-brackish habitat where this species is typically found.

The highest chlorophyll pigment content in the 40 ppt treatment (P1) indicates that C. linum's photosynthetic apparatus was in optimal structural condition at salinity close to isotonic conditions, allowing pigment-protein complex synthesis to proceed without hindrance from cellular degradation [15]. The renewed increase in chlorophyll at medium salinity of 10 ppt (P7) is thought to be a physiological compensation mechanism for decreased inter-molecular chlorophyll energy transfer efficiency due to mild to moderate environmental stress

[25], although this increase was not sufficient to promote structural growth. The drastic decrease in chlorophyll at P9 confirms large-scale chloroplast structural damage due to extreme hyposaline stress. In some other green microalgae, the relationship between salinity and chlorophyll content is actually reversed; limitations in photosynthetic electron transport at high salinity can inhibit pigment synthesis and decrease chlorophyll production (Euchlorocystis marina [1]), so the chlorophyll peak at 40 ppt is likely specific to C. linum, which is naturally adapted to high-salinity environments, rather than a general pattern across green algae species.

Water quality parameters during the study were generally still within the range tolerable by C. linum, although temperature and pH were slightly below the optimal value (SNI, 2010). Dissolved oxygen levels and light intensity far exceeding the minimum value indicate an adequate external energy supply to support photosynthesis, while the high nitrate levels and low phosphate levels (0 mg/L) in all treatments indicate high nutrient uptake activity by C. linum, in line with the luxury uptake mechanism in the genus Chaetomorpha [19]. The ability to absorb inorganic nutrients massively without triggering growth inhibition at 20–40 ppt salinity confirms C. linum's position as an ideal biofilter candidate [6,5]. However, nutrient uptake efficiency in Chaetomorpha appears to be more determined by the media's N:P ratio and biomass density than by salinity alone. [5] noted that the optimal N/P ratio for nutrient uptake by macroalgae varies widely, from 10:1 to 80:1, so the recommendation of a 20–40 ppt salinity range as an ideal biofilter condition needs to be balanced with media nutrient ratio management to keep nitrate-phosphate uptake capacity optimal in the field.

CONCLUSION

Salinity significantly influences the growth, survival, structural integrity, pigment synthesis, and nutrient uptake capacity of Chaetomorpha linum. Optimal performance occurred under hypersaline to marine conditions (30--40 ppt), with peak growth (SGR $= 2.39 \pm 1.10\%$/day), highest chlorophyll content (273 mg/100g), and maximal biomass yield ($8.89 \pm 1.07\%$) recorded at 40 ppt. Hyposalinity below 15 pptcaused lethal osmotic stress, tissue bleaching, filament swelling, and negative growth. These results confirm that C. linum is an effective bioremediation candidate for marine and high-salinity aquaculture systems, provided operational salinities are maintained above 20--30 ppt.

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