

Journal home page: <http://ajarcde-safe-network.org> ISSN 2581-0405

Environmental Growth Response of *Gracilaria* sp. under Two Water-Medium Ratios for Aquatic Biofiltration

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ARTICLE INFO

Article History:

Received: 02 August 2026

Final Revision: 10 August 2026

Accepted: 11 August 2026

Online Publication: 13 August 2026

KEYWORDS

Gracilaria, environmental conditions, water quality, biofilter, bioremediation, specific growth rate

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A B S T R A C T

Environmental conditions regulate macroalgal growth and determine whether living biomass can function as an aquatic biofilter. This preliminary study described changes between day 0 (D0) and day 7 (D7) in one best final-weight culture unit selected from each of two water-medium ratios: P1 (1:0) and P2 (1:1). Wet weight, pH, temperature, salinity, dissolved oxygen (DO), and nitrate concentration were evaluated. Wet weight increased from 100.0 to 105.0 g in the 1:0 unit (+5.0%; specific growth rate 0.70% day⁻¹) and to 105.1 g in the 1:1 unit (+5.1%; 0.71% day⁻¹). In the 1:0 unit, pH rose from 7.14 to 7.44, temperature from 26.4 to 28.9 °C, salinity from 40.9 to 42.0 ppt, and DO remained at 5 mg L⁻¹. In the 1:1 unit, pH rose from 7.22 to 7.43 and temperature from 26.4 to 29.1 °C, while salinity declined from 42.0 to 38.3 ppt and DO from 6 to 5 mg L⁻¹. Nitrate concentration decreased from 9.62 to 0.00 mg L⁻¹ in the 1:0 unit and from 8.58 mg L⁻¹ to not detected (ND) in the 1:1 unit. Both selected units maintained positive short-term growth under different environmental trajectories, supporting the preliminary feasibility of *Gracilaria* sp. as a biofilter component. Because deliberately selected single units were analyzed and nitrate was the only nutrient parameter quantified, the findings do not establish ratio superiority or comprehensive biofiltration efficiency. Replicated trials with direct nutrient mass balances are required.

Contribution to Sustainable Development Goals (SDGs):

SDG 6: Clean Water and Sanitation

SDG 12: Responsible Consumption and Production

SDG 14: Life Below Water

1. INTRODUCTION

1.1. Research Background

Nutrient enrichment and organic loading from aquaculture and coastal activities can accelerate eutrophication and destabilise oxygen dynamics. Algal treatment is attractive because dissolved nutrients can be converted into harvestable biomass rather than transferred to another waste stream. In integrated multi-trophic aquaculture, macroalgae occupy the dissolved-extractive level and recover nitrogen, phosphorus, and carbon [1], [2]. Global

evidence indicates that nutrient-removal capacity varies among taxa, cultivation systems, and environmental settings [3].

Gracilaria is promising for environmental biofiltration because it combines commercial value, established cultivation methods, and physiological plasticity [4]. Growth is regulated by temperature, salinity, pH, irradiance, dissolved oxygen, hydrodynamics, depth, and stocking density [5], [6], [7], [8], [9], [10]. These controls are operationally important: a biofilter can retain nutrients only while its biomass remains metabolically active and is harvested before senescence.

Direct studies have reported nutrient recovery by *Gracilaria* in aquaculture wastewater and co-culture systems [11], [12], [13], [14], [15]. However, positive biomass growth alone demonstrates



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biological viability, not remediation efficiency. A defensible treatment claim requires direct measurements of the target dissolved constituents and a mass balance.

1.2. Literature Review

This manuscript is framed as a preliminary methodological study rather than a definitive long-term trial. It aimed to describe D0-to-D7 changes in wet biomass, pH, temperature, salinity, and dissolved oxygen (DO) in a single unit for each water-medium ratio and to evaluate their implications for *Gracilaria*-based aquatic biofiltration. The analysis was descriptive because the unit with the highest final wet weight from each ratio was deliberately selected and no independent biological replicates were available. Therefore, the findings are intended to provide preliminary observations and should not be interpreted as evidence of superiority of the ratio.

2. MATERIALS AND METHODS

2.1. Study design and case selection

The study was conducted in a semi-outdoor experimental area in Malaka Village, Pemenang District, North Lombok Regency, West Nusa Tenggara, Indonesia, from 26 May to 2 June 2026. No independent taxonomic identification was performed. Therefore, the experimental macroalga is conservatively reported at the genus level as *Gracilaria* sp. The study focused on the ecophysiological responses by analysing environmental parameters and biomass growth.

Two water-medium ratios consisting of shrimp-culture wastewater and seawater were evaluated. The ratios were expressed as wastewater: seawater. The 1:0 treatment consisted of 12 L of shrimp-culture wastewater without the addition of seawater, whereas the 1:1 treatment consisted of 6 L of shrimp-culture wastewater and 6 L of seawater. Thus, each experimental unit contained a total water volume of 12 L. The source dataset contained daily observations during the seven-day culture period. In accordance with the predefined analytical scope, only D0 and D7 data were used. The unit with the highest D7 wet weight within each ratio was retained: P1 for the 1:0 ratio (105.0 g) and P2 for the 1:1 ratio (105.1 g). These codes were used only as experimental-unit identifiers.

The experiment was conducted under semi-outdoor conditions with natural daylight. Light intensity was not measured, and the cultures were exposed to an approximately 12 h light:12 h dark natural photoperiod. Continuous subsurface aeration was provided in each culture vessel for 24 h day⁻¹. No water exchange or makeup water was applied during the seven-day culture period; therefore, the experiment was operated as a batch system. No external nutrients were added. Nitrate was naturally present in the initial water medium and was the only nutrient parameter measured in this study.

2.2. Analytical methods

Water-quality measurements were conducted at D0 and D7 between 16:00 and 17:00 Central Indonesian Time (WITA). Water temperature, pH, and salinity were measured using an EZ-9909 5-in-1 pen-type water-quality tester. The meter has a pH resolution of 0.01, a temperature resolution of 0.1 °C, and a salinity resolution of 0.1 ppt within the measurement range used in this study. According to the instrument specifications, the pH

function can be calibrated using standard buffer solutions at pH 4.00, 6.86, and 9.18, whereas the conductivity-based salinity function can be calibrated using standards at 1,413 µS cm⁻¹, 12.88 mS cm⁻¹, and 111.8 mS cm⁻¹. However, the field calibration records were not retained; therefore, the specific calibration points applied during the experiment could not be verified. Before each measurement, the sensor was immersed directly in the culture water, and the pH, temperature, and salinity values were recorded after the displayed readings stabilized.

Dissolved oxygen (DO) was measured using a commercial DO test kit. Water samples were collected without vigorous agitation to minimize atmospheric oxygen exchange, reacted according to the test-kit instructions, and read using the concentration scale supplied with the kit. Because the manufacturer and model of the DO kit were not recorded, these details could not be reported.

Wet biomass was measured using a Yanhui USB Charging Edition digital scale (Model 5 kg/0.1 g) with a readability of 0.1 g. *Gracilaria* sp. was removed from the culture vessel and drained using a sieve for approximately 30 s to remove excess surface water before weighing. The same draining and weighing procedure was applied at D0 and D7.

Water samples for nitrate analysis were transported to the laboratory and analyzed using the cadmium-reduction method with a Shimadzu UV-1800 UV-Vis spectrophotometer. In this method, nitrate is reduced to nitrite, followed by color development. The absorbance of the resulting colored solution was measured at 543 nm and converted to nitrate concentration using a calibration curve prepared from standard nitrate solutions. The results were reported as nitrate in mg L⁻¹. For the D7 sample from the 1:1 unit, the analytical result was displayed as a dash and was therefore recorded as not detected (ND). Because a numerical detection limit was unavailable, the ND result was not treated as zero.

2.3. Calculations and statistical analysis

Wet weight, pH, temperature (°C), salinity (ppt), dissolved oxygen (DO; mg L⁻¹), and nitrate concentration (mg L⁻¹) were recorded at D0 and D7. When the nitrate-measuring instrument displayed a dash at D7, the result was recorded as not detected (ND). Because a numerical detection limit was unavailable, ND was not treated as zero, and its absolute or percentage change was not calculated. Relative wet-weight change was $((W_T - W_0)/W_0) \times 100\%$. Specific growth rate (SGR) was $(\ln W_T - \ln W_0)/7 \times 100\%$ day⁻¹. Because no independent biological replicates were available and each ratio was represented by one deliberately selected unit ($n = 1$), the data were analyzed descriptively. Standard deviations, error margins, and inferential statistical tests were not calculated.

3. RESULT AND DISCUSSION

3.1. Growth, environmental, and nitrate trajectories

Wet biomass increased by 5.0 g in the 1:0 unit and 5.1 g in the 1:1 unit, corresponding to SGR values of 0.70 and 0.71% day⁻¹. The 0.1-g difference is negligible and cannot support a conclusion that either ratio was superior. Both cases exhibited higher pH and temperature at D7, whereas their salinity and DO trajectories differed (Table 1).

Table 1. D0-to-D7 changes in biomass, environmental variables, and nitrate concentration

Unit	Ratio	Variable	D0	D7	Δ
P1	1:0	Wet weight (g)	100.0	105.0	+5.0
		pH	7.14	7.44	+0.30
		Temperature (°C)	26.4	28.9	+2.5
		Salinity (ppt)	40.9	42.0	+1.1
		DO (mg L ⁻¹)	5	5	0
		Nitrate (mg L ⁻¹)	9.62	0.00	-9.62
P2	1:1	Wet weight (g)	100.0	105.1	+5.1
		pH	7.22	7.43	+0.21
		Temperature (°C)	26.4	29.1	+2.7
		Salinity (ppt)	42.0	38.3	-3.7
		DO (mg L ⁻¹)	6	5	-1
		Nitrate (mg L ⁻¹)	8.58	ND	NC

Note: Values represent one deliberately selected culture unit for each ratio ($n = 1$); therefore, standard deviations and error margins could not be calculated. SGR was 0.70% day⁻¹ for the 1:0 unit and 0.71% day⁻¹ for the 1:1 unit. ND: not detected; NC: not calculated because the instrument did not provide a numerical detection limit.

Nitrate concentration in the 1:0 unit decreased from 9.62 mg L⁻¹ at D0 to 0.00 mg L⁻¹ at D7, corresponding to an absolute decrease of 9.62 mg L⁻¹ and a concentration-based reduction of 100%. In the 1:1 unit, nitrate concentration decreased from 8.58 mg L⁻¹ at D0 to not detected (ND) at D7. Because the instrument displayed only a dash and no numerical detection limit was available, the exact D7 concentration and percentage reduction for the 1:1 unit could not be calculated.

The D0-to-D7 decreases indicate substantial nitrate depletion in both selected culture units. However, because each ratio was represented by only one selected unit and no untreated control was included, the observed decrease cannot be attributed exclusively to nitrate uptake by *Gracilaria* sp. Other processes within the batch system may also have contributed. Therefore, these results represent preliminary nitrate-concentration dynamics rather than definitive evidence of biofiltration efficiency.

3.2. Environmental regulation of growth

The approximately 5% biomass gain indicates short-term net growth in both selected units. Comparable studies show that *Gracilaria* productivity depends on cultivation method, propagule condition, density, depth, and hydrodynamic exchange [5], [6], [7], [8], [9]. The final temperatures of 28.9–29.1 °C are compatible with several tropical *Gracilaria* systems, but longer exposure near a thermal optimum can increase respiration. Seasonal work also demonstrates that biomass production and remediation vary with nutrients, turbidity, and climate [11].

The increase in pH from 7.14 to 7.44 in the 1:0 unit and from 7.22 to 7.43 in the 1:1 unit may be associated with photosynthetic inorganic-carbon uptake by *Gracilaria* sp. During photosynthesis, the uptake of dissolved CO₂ and potentially bicarbonate (HCO₃⁻) can reduce the concentration of inorganic carbon in the culture medium and shift the carbonate equilibrium toward a higher pH. Because measurements were conducted at the same time of day, between 16:00 and 17:00 WITA, differences caused by measurement time were partially controlled. Continuous subsurface aeration may also have promoted gas exchange between the culture water and the atmosphere. Nevertheless, alkalinity, dissolved inorganic carbon, irradiance, and diel pH

fluctuations were not measured. Therefore, the observed pH increase is consistent with photosynthetic carbon uptake but cannot be interpreted as direct evidence of inorganic-carbon depletion [12]. DO remained 5 mg L⁻¹ at D7 in both cases. The 1 mg L⁻¹ decline in the 1:1 unit may be attributable to respiration, microbial oxygen demand, temperature-dependent solubility, or sampling time; it cannot be attributed to a single process based on endpoint observations alone.

The final salinities of 42.0 and 38.3 ppt were within the broad tolerance range reported for some *Gracilaria* species, although tolerance does not necessarily indicate optimal growth [10]. Salinity affects osmotic regulation and nutrient transport in macroalgae. In the 1:0 unit, salinity increased from 40.9 to 42.0 ppt, possibly due to evaporation under semi-outdoor conditions. In contrast, salinity in the 1:1 unit decreased from 42.0 to 38.3 ppt, corresponding to a decline of 3.7 ppt. Evaporation cannot explain this decrease because water loss through evaporation would generally concentrate dissolved salts, increasing salinity. No water exchange or makeup water was applied during the experiment; therefore, dilution by intentionally added water can also be excluded. Possible explanations include the entry of rainwater or condensed water under semi-outdoor conditions, incomplete homogenization of the mixed medium, or variation associated with the measurement procedure. Because rainfall, condensation, final water volume, and instrument quality-control data were not recorded, the mechanism responsible for the salinity decrease could not be determined conclusively [10].

3.3. Biofiltration and community-development implications

Positive growth is a prerequisite for living biofiltration because assimilation converts dissolved resources into harvestable biomass. *Gracilaria* species have removed ammonium, nitrate, and phosphate from shrimp wastewater and experimental nutrient solutions [13], [14], and co-culture studies support nutrient recovery in multi-species systems [15], [16]. Integrated fishpond and recirculating systems further show that treatment performance depends on organism combinations, hydraulic design, and microbial processes [17], [18], [19].

For coastal aquaculture communities, a locally managed *Gracilaria* biofilter could couple water-quality improvement with saleable biomass, potentially diversifying income and reducing discharge pressure. Practical deployment requires low-cost culture structures, predictable seed supply, routine harvesting, and locally appropriate biomass markets. Species selection should integrate uptake rate, growth, tolerance, cultivation feasibility, and product safety [20]. Density and salinity must also be optimised because excessive biomass causes self-shading and transfer limitation [21].

Nitrate was the only nutrient parameter measured in this study. Its concentration decreased from D0 to D7 in both selected culture units, indicating nitrate depletion within the batch media. However, because the experiment did not include biological replicates or an untreated control, the observed changes cannot be interpreted as a complete nutrient mass balance or attributed solely to uptake by *Gracilaria* sp. A confirmatory experiment should include independent biological replicates, untreated controls, standardized measurement times, and measurements of nitrate concentration and water volume at D0 and D7.

3.4. Study limitations

Four limitations constrain the interpretation of the findings. First, selecting the unit with the highest final wet weight from each ratio introduced selection bias. Second, because each ratio was represented by only one selected unit ($n = 1$), biological variability could not be estimated and statistical comparisons between the two ratios could not be performed. Third, no independent taxonomic identification was conducted, and the field calibration records and manufacturer information for the DO test kit were unavailable. Fourth, nitrate was the only nutrient parameter measured, while the absence of biological replicates, an untreated control, and a numerical detection limit for the ND result prevented the establishment of a complete nutrient mass balance. Therefore, the results should be interpreted as preliminary observations rather than evidence of ratio superiority or demonstrated biofiltration efficiency.

4. CONCLUSION

The selected 1:0 and 1:1 units increased from 100.0 g to 105.0 g and 105.1 g, respectively, over seven days. Both maintained positive net growth despite different salinity and DO trajectories and ended at pH 7.43–7.44, 28.9–29.1 °C, 38.3–42.0 ppt, and 5 mg L⁻¹ DO. Nitrate concentration decreased from 9.62 to 0.00 mg L⁻¹ in the 1:0 unit and from 8.58 mg L⁻¹ to not detected in the 1:1 unit. Nevertheless, because each ratio was represented by only one selected unit and no untreated control was included, these decreases cannot be interpreted as definitive evidence of nitrate removal efficiency. These findings support the preliminary feasibility of *Gracilaria* sp. as a living aquatic biofilter, but do not establish superiority in ratio or remediation efficiency. Replicated trials with verified species identity, complete culture metadata, controls, and direct nutrient mass balances are required before operational recommendations are made.

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