

Epiphytic Biomass And Chlorophyll-a Concentration – Relations In Seagrass Leaves *Enhalus acoroides* In Sanur Waters, Bali

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ABSTRACT: Seagrass beds are important coastal ecosystems that functioned as primary producers and habitats for various marine biota; however, their persistence was vulnerable to environmental pressures. One of these pressures was the increase in epiphytic biomass on seagrass leaves, which had the potential to inhibit photosynthesis. This study aimed to assess the condition of *Enhalus acoroides* seagrass cover and to analyze the relationship between epiphyte biomass and chlorophyll-a concentration in seagrass leaves in the waters of Sanur, Bali. This study was based on seagrass ecological theory, the role of epiphytes, and chlorophyll-a as an indicator of seagrass physiology and productivity. The research methods included field observations using line transects and quadrats, measurement of seagrass cover, analysis of chlorophyll-a in seagrass leaves using spectrophotometric methods, and calculation of epiphyte biomass using gravimetric methods. The results showed that *Enhalus acoroides* seagrass cover in Sanur waters was classified as moderate to dense, with water quality conditions that still supported seagrass growth. The relationship between epiphyte biomass and chlorophyll-a concentration indicated a weak negative correlation, in which an increase in epiphyte biomass tended to be followed by a decrease in chlorophyll-a. The findings of this study indicated that epiphytes had the potential to reduce the photosynthetic efficiency of seagrass, although their influence was also affected by local environmental factors.

KEYWORDS: Seagrass; *Enhalus acoroides*; Epiphytes; Chlorophyll-a; Phyllosphere

1. Introduction

Seagrasses are marine angiosperms that have fully adapted to high-salinity environments and play a fundamental role in coastal ecosystems worldwide by functioning as primary producers, supporting coastal productivity, and providing critical habitats for a wide range of marine organisms [1, 2]. Structurally complex seagrass meadows act as feeding, spawning, and nursery grounds for fish and megafauna such as green turtles and dugongs, while also contributing to shoreline protection through sediment trapping, wave attenuation, and seabed stabilization [3].

The ecological performance and persistence of seagrass meadows are strongly linked to their photosynthetic capacity, which is governed by chlorophyll-a as the primary light-harvesting pigment in seagrass leaves, enabling the conversion of light energy into chemical energy required for growth and survival [4]. Consequently, chlorophyll-a concentration in seagrass leaves is widely used as a physiological indicator of seagrass health and environmental quality.

In their natural habitat, seagrasses coexist with epiphytic organisms that colonize leaf surfaces and form an integral component of seagrass ecosystems. Epiphytes may provide ecological benefits by enhancing nutrient availability, particularly inorganic nitrogen, through microbial activity on leaf surfaces, and by reducing harmful ultraviolet radiation exposure [5]. However, excessive epiphytic cover can negatively affect seagrass performance by reducing light availability at the leaf surface, impairing photosynthetic efficiency, and inducing physiological stress and cellular damage in seagrass tissues. The balance between the beneficial and detrimental effects of epiphytes is highly sensitive to environmental conditions, especially nutrient enrichment, water clarity, and hydrodynamic regimes [6].

Globally, seagrass meadows have experienced rapid declines, with estimated losses of approximately 7% per year since the 1990s, largely driven by increasing anthropogenic pressures in coastal zones [7]. The coastal waters of Sanur, Bali, represent a region where seagrass meadows, dominated by *Enhalus acoroides*, coexist with intensive marine tourism and increasing anthropogenic stressors. Previous studies have reported variable seagrass density and coverage in this area, suggesting potential vulnerability to environmental changes driven by human activities [8].

Despite the ecological importance of *Enhalus acoroides* and its dominance in Sanur waters, quantitative assessments linking epiphyte biomass to chlorophyll-a concentration in seagrass leaves remain limited. Understanding this relationship is essential for elucidating how epiphyte proliferation influences seagrass physiological condition and productivity under conditions of elevated nutrient input. Therefore, this study aims to analyze the relationship between epiphyte biomass and chlorophyll-a concentration, particularly in relation to reduced light availability and diminished photosynthetic efficiency. Such findings are expected to provide valuable insights for seagrass monitoring, management, and conservation strategies in heavily utilized coastal environments.

2. Materials and Methods

2.1. Description of the study area.

The study was conducted in the coastal waters of Sanur, Bali, Indonesia, located at 08°38'00"–08°42'30" S and 115°14'30"–115°16'30" E, covering an area of approximately 1,548.27 ha along an eastern coastal tourism destination in Denpasar City. This area is characterized by intensive human activities, including hotel developments, fishing vessels, and tourist boats that utilize the area as a harbor, which are considered potential sources of marine pollution and may impact coastal organisms [9]. Increasing anthropogenic and tourism-related pressures in the Sanur coastal waters have the potential to degrade seagrass communities beyond the environmental carrying capacity, leading to a decline in ecosystem services such as carbon sequestration and marine natural resources if seagrass meadows are degraded [8, 10].

2.1.1. Sample and data collection.

Field sampling was conducted in October 2025 in the coastal waters of Sanur, Bali, to minimize the effects of the rainy season on water clarity. Seagrass data were collected using a line transect method, with three 100m transects established per station and five sampling points at 20m intervals along each transect, oriented from the shoreline toward offshore areas starting from the first occurrence of seagrass, more details location can seen at Figure 1. At each sampling point, a 50 × 50 cm quadrat subdivided into four subplots was used to assess seagrass cover, identify *Enhalus acoroides*, and record environmental parameters (temperature, salinity, pH, and substrate type) following standart seagrass monitoring protocols [11,12]. Five epiphyte-covered leaves of *Enhalus acoroides* were collected per sampling point by carefully uprooting the plants and measuring growth depth. All samples were stored in ziplock bags, kept in a cooler box, and transported to the Marine Science Laboratory, Faculty of Marine and Fisheries, Udayana University. In the laboratory, epiphytes were carefully scraped from seagrass leaves and their biomass was determined using a gravimetric method after rinsing with filtered seawater and deionized water and oven-drying at 70°C to constant weight [13]. While chlorophyll-a concentration in cleaned seagrass leaves was quantified using a spectrophotometric method following DMSO extraction and absorbance measurements at 663 and 645 [14].

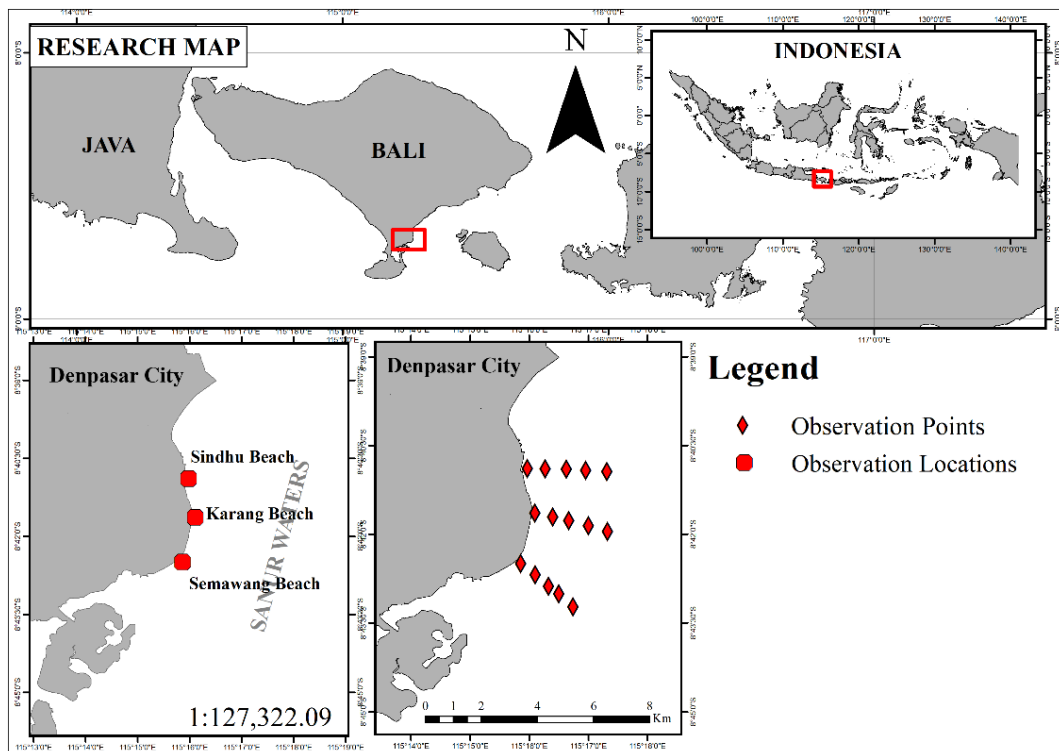


Figure 1. Field data collection research location along the Sanur Waters, Bali.

2.2. Data analysis.

2.2.1. Water quality parameters.

Water quality parameters were measured at each sampling point along the transects and included temperature, salinity, pH, and substrate type. Temperature was measured in situ using

a water thermometer, while salinity was determined using a refractometer and pH using a portable pH meter. Substrate type was identified through direct visual observation. Measurements from all sampling points were averaged and expressed as mean \pm standard deviation for each station [11].

2.2.2. Analysis Leaf Area Index.

Leaf Area Index (LAI) of *Enhalus acoroides* was calculated to quantify leaf surface availability for epiphyte attachment. Morphometric measurements of leaf length and width were conducted for all collected samples using a ruler, and individual leaf area was calculated accordingly. Total leaf area per sampling unit was summed and divided by the observation area to obtain LAI values, which were subsequently used to support analyses of epiphytic biomass and seagrass chlorophyll-a concentration. Analyze Leaf Area Index (LAI) calculated using the following equation [15]:

$$LAI = D \times \bar{a}$$

where LAI represents the Leaf Area Index, D is the shoot density (shoots/m²), and \bar{a} is the average leaf area per shoot (m²).

2.2.3. Seagrass cover.

Seagrass cover was assessed using a 50 \times 50 cm quadrat subdivided into four subplots placed at each sampling point along the transects. Percent cover of *Enhalus acoroides* was estimated using the seagrass checklist method following the COREMAP-LIPI (2017) protocol and categorized into sparse, moderate, dense, and very dense cover classes at Table 1.

$$\text{Seagrass Cover (\%)} = \frac{\text{Total value of seagrass cover}}{4}$$

Table 1. Percentage Cover Categories

No	Percentage Cover (%)	Category
1	0–25	Sparse
2	26–50	Moderate
3	51–75	Dense
4	76–100	Very Dense

2.2.4. Seagrass Chlorophyll-a concentration.

Chlorophyll-a concentration in *Enhalus acoroides* leaves was determined in the laboratory using a spectrophotometric method. Cleaned seagrass leaves (10-20 g) were extracted using dimethyl sulfoxide (DMSO) and incubated at 70°C for 24 h to ensure complete pigment extraction. Absorbance was measured at wavelengths of 663 and 645 nm, and chlorophyll-a concentration was calculated using established equations [14, 16].

$$\text{chlorophyll-a} \left(\frac{\text{mg}}{\text{g}} \right) = (12.7 \cdot A_{663} - 2.69 \cdot A_{645}) \times \frac{V}{1000W}$$

where A₆₆₃ and A₆₄₅ represent absorbance values at 663 nm and 645 nm, respectively, V is the volume of extract (mL), and W is the fresh weight of the sample (g).

2.2.5. Epiphytic Biomass.

Epiphytic biomass was quantified using a gravimetric method. Epiphytes were carefully scraped from seagrass leaves, rinsed with filtered seawater followed by deionized water to remove salts, and oven-dried at 70°C until a constant weight was achieved. The dry epiphyte mass was then normalized to leaf surface area to express epiphytic biomass per unit leaf area [13].

$$\text{Epiphytic Biomass} \left(\frac{\text{mg}}{\text{cm}^2} \right) = \left(\frac{A^1 - A^0}{L} \right)$$

where A^1 is the weight of the cup containing dry epiphytes (mg), A^0 is the weight of the empty cup (mg), and L is the seagrass leaf area (cm^2).

3. Results and Discussion

Water quality parameters measured in the coastal waters of Sanur generally indicated environmental conditions that remained supportive of the growth of *Enhalus acoroides* (Table 2). Water temperature across the three stations ranged from 31 to 31.5°C, slightly exceeding the optimal range for tropical seagrass growth (28–32°C), yet still within the tolerance limits of *Enhalus acoroides*, which is known for its adaptability to temperature fluctuations in shallow tropical waters. The pH values recorded at Sindhu Beach (8.15 ± 0.09), Karang Beach (8.23 ± 0.07), and Semawang Beach (8.28 ± 0.03) were relatively homogeneous and fell within the acceptable range for marine waters (7.0–8.5). Salinity ranged from 30 to 31 ppt, with the highest value observed at Semawang Beach and the lowest at Karang Beach. Although these values were slightly lower than the optimal salinity range for seagrass (33–34 ppt), they remained within tolerable limits for *Enhalus acoroides*. Substrate composition across all stations was dominated by sand, a substrate type favorable for seagrass establishment due to good light penetration and effective anchoring of roots and rhizomes.

Table 2. Water parameter matrix in Sanur Waters (Mean \pm SD).

Parameter	Standard Value	Sindhu Beach	Karang Beach	Semawang Beach
Temperature (°C)	28–32	31.5 ± 0.00	31.0 ± 0.24	31.0 ± 0.24
pH	7.0–8.5	8.15 ± 0.09	8.23 ± 0.07	8.28 ± 0.03
Salinity (ppt)	33–34	30.5 ± 0.20	30.0 ± 0.00	31.0 ± 0.08
Substrate Type	—	Sand	Sand	Sand

Table 3 presents morphological observations showing that *Enhalus acoroides* at Sindhu Beach, Karang Beach, and Semawang Beach exhibited similar overall plant structures, with variations occurring primarily in leaf dimensions. Mean leaf length ranged from 25.12 ± 3.5 cm at Karang Beach to 28.08 ± 2.6 cm at Semawang Beach, while leaf width was relatively uniform across all stations, ranging from 1.02 ± 0.1 cm to 1.06 ± 0.1 cm. The low standard deviation values for leaf width indicate relatively homogeneous leaf morphology throughout the study area, whereas greater variability in leaf length suggests site-specific influences on leaf growth.

Table 3. Length and width of seagrass leaves *Enhalus acoroides* at the research station (Mean \pm SD).

Parameter	Sindhu Beach	Karang Beach	Semawang Beach
Leaf Length (cm)	26.04 ± 3.7	25.12 ± 3.5	28.08 ± 2.6
Leaf Width (cm)	1.06 ± 0.1	1.03 ± 0.1	1.02 ± 0.1

Figure 2 shows that the Leaf Area Index (LAI) at Sindhu Beach ranged from 0.05 to 0.18, with the highest values recorded at distances of 40 m (0.18) and 80 m (0.17), and the lowest at 100 m (0.05). Karang Beach exhibited relatively lower and more stable LAI values, ranging from 0.07 to 0.11 across all observation distances. Meanwhile, Semawang Beach showed the most consistent LAI values, ranging from 0.10 to 0.11 at distances between 20 m and 100 m. The highest overall LAI values were observed at Sindhu Beach, while Karang Beach had lower average values compared to the other two locations.

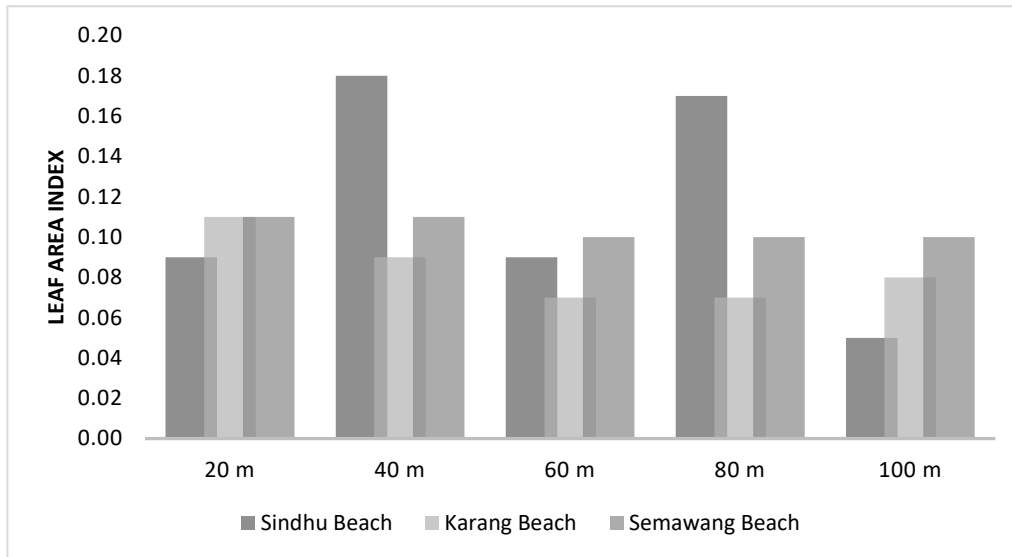


Figure 2. Leaf Area Index (LAI) *Enhalus acoroides* at the entire observation distance

Figure 3 illustrates that Sindhu Beach exhibited the highest seagrass cover in the mid-zone (40–80 m), reaching a maximum of 81.25%, followed by a sharp decline at 100 m. In contrast, Semawang Beach showed an increasing trend in seagrass cover with distance from the shore, with the highest cover (56.25%) recorded at 100 m. Karang Beach displayed a more fluctuating pattern, with peak cover values (50%) at 20 m and 60 m and a marked decrease at 0 m. These results indicate heterogeneous distribution patterns of *Enhalus acoroides* related to site-specific environmental conditions and spatial gradients.

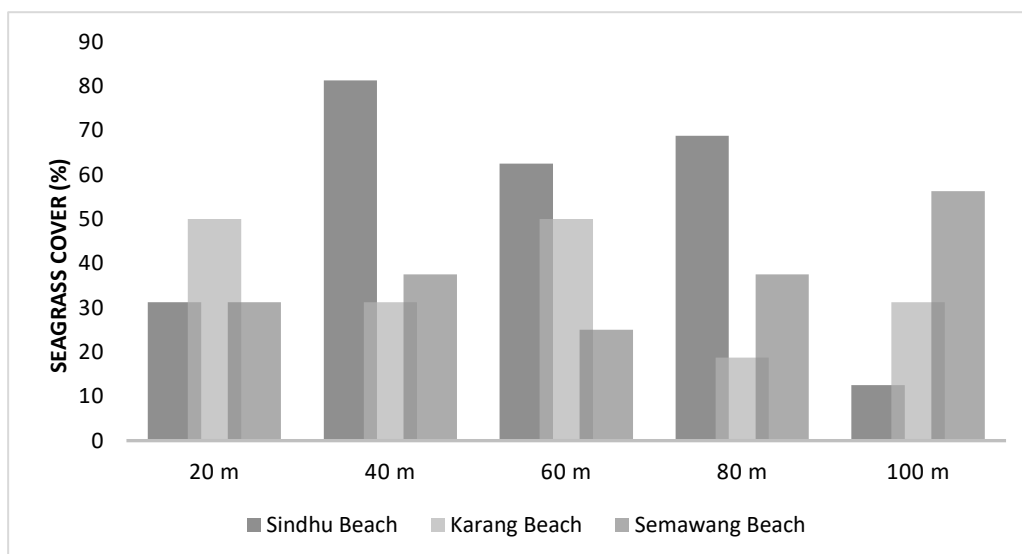


Figure 3. Seagrass cover at each data collection point across all stations.

Figure 4 boxplot analysis showed that epiphytic biomass and chlorophyll-a concentration on *Enhalus acoroides* leaves exhibited site-specific variation across Sindhu, Karang, and Semawang Beaches. Epiphytic biomass had similar median values (~5.0 mg/cm²) but greater variability, especially at Karang and Semawang. In contrast, chlorophyll-a exhibited lower and more consistent values, with a slight increasing trend from Sindhu (~0.55 mg/g) to Karang (~0.58 mg/g) and Semawang (~0.62 mg/g). Sindhu showed the most stable distribution, while Karang and Semawang displayed higher variability and several outliers.

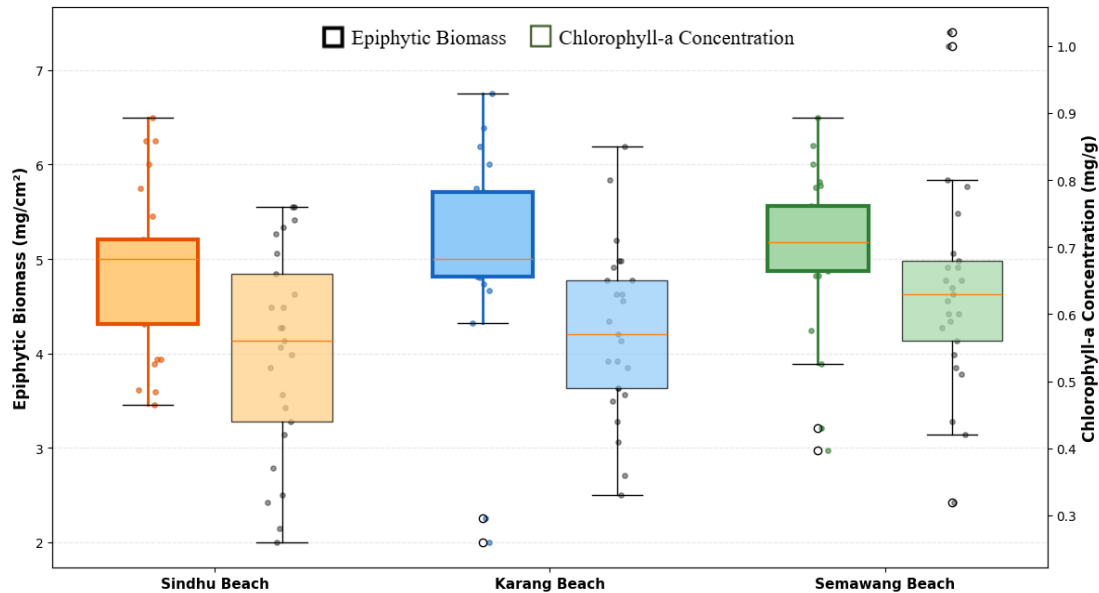


Figure 4. Epiphytic biomass and Chlorophyll-a concentration in seagrass leaves (*Enhalus acoroides*)

Figure 5 analysis illustrates a weak negative relationship between epiphytic biomass and chlorophyll-a concentration in *Enhalus acoroides* leaves, as indicated by a decreasing regression trendline with a coefficient of determination ($R^2 = 0.1916$). This result suggests that increases in epiphytic biomass tend to be associated with reductions in chlorophyll-a concentration, although the strength of the relationship is relatively low.

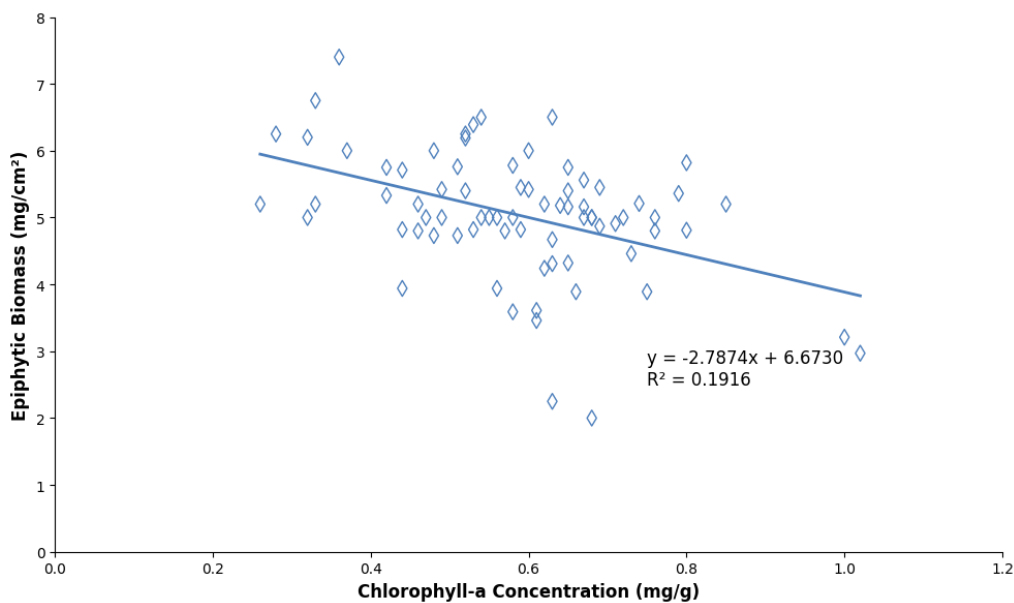
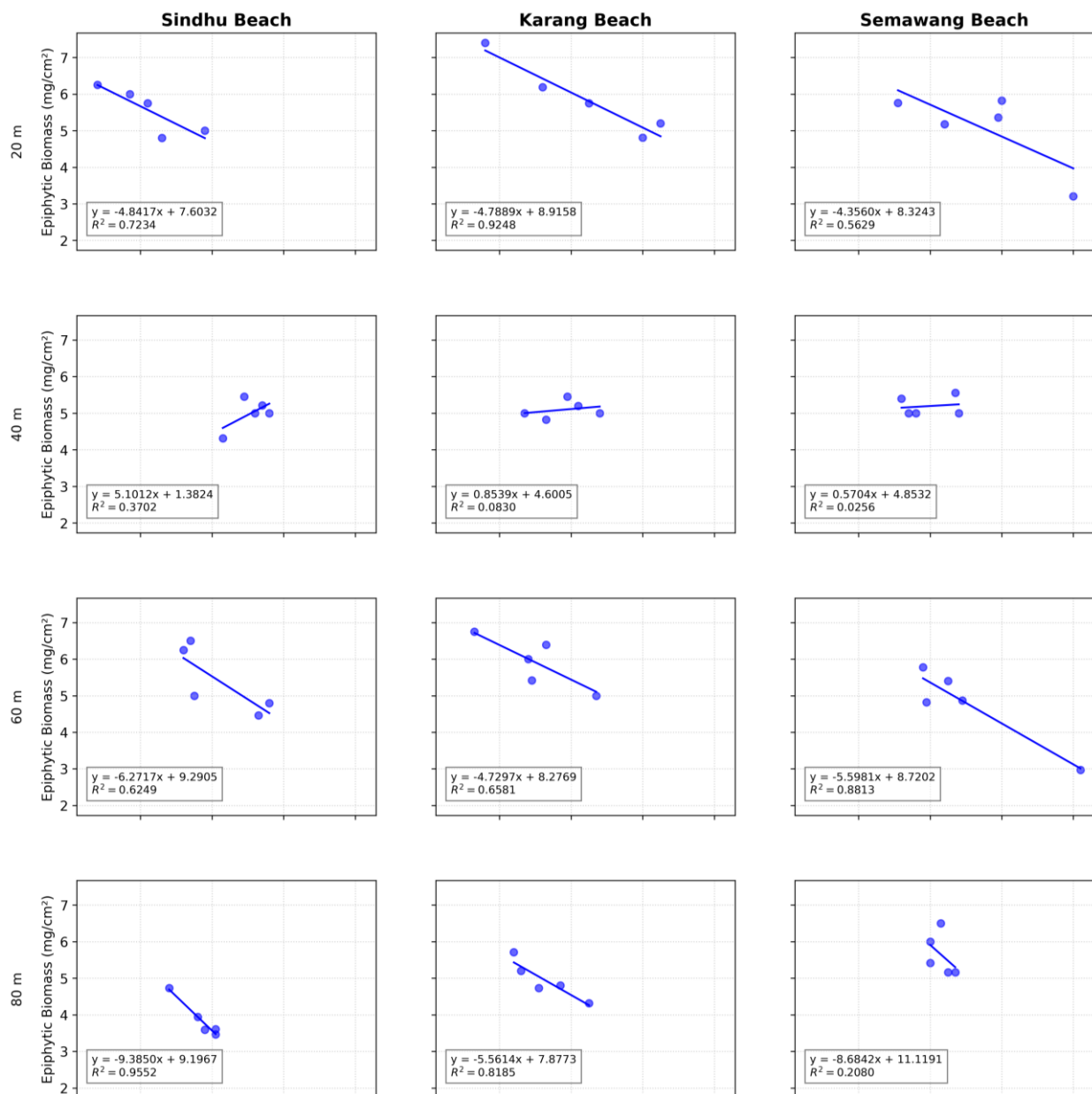


Figure 5. Scatter plot of epiphytic biomass and Chlorophyll-a concentration in seagrass leaves (*Enhalus acoroides*)

Figure 6 shows that scatter plot analysis across all sampling distances revealed a generally weak negative relationship between epiphytic biomass and chlorophyll-a concentration in *Enhalus acoroides* leaves. At 20 m from the shoreline, increasing epiphytic biomass tended to be associated with lower chlorophyll-a concentrations. At 40 m, the relationship appeared more variable and weak, indicating a reduced influence of epiphytes relative to other environmental factors. From 60 m to 100 m, negative trends became more consistent across all stations, suggesting that higher epiphytic biomass was increasingly associated with lower chlorophyll-a concentrations. Overall, these results indicate that epiphytic biomass may suppress chlorophyll-a content in *Enhalus acoroides*, with the strength of the relationship varying according to distance from the shoreline and local environmental conditions.



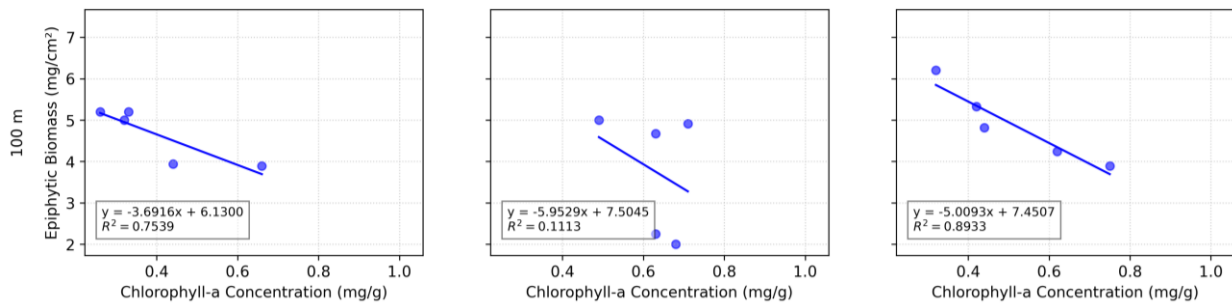


Figure 6. Scatter plot of the relationship between epiphytic biomass and Chlorophyll-a concentration in seagrass leaves (*Enhalus acoroides*) at each observation point.

The water quality conditions in the Sanur coastal waters generally remained within ranges suitable for the growth and persistence of *Enhalus acoroides*. Water temperature, salinity, and pH values were comparable to those reported for tropical seagrass meadows in Indonesia and the broader Indo-Pacific region [15]. The slightly elevated temperature observed during the study period may increase metabolic activity and respiration rates in seagrasses; however, such conditions are still tolerable for *Enhalus acoroides*, which is known for its high physiological plasticity in shallow tropical environments. The dominance of sandy substrates further supports seagrass establishment by enhancing light penetration and facilitating rhizome anchoring. These relatively stable physicochemical conditions suggest that environmental quality alone does not strongly limit seagrass distribution in Sanur, and that other biological or anthropogenic factors may play a more important role in shaping seagrass condition [17,18].

Morphological analysis indicated spatial variation in the Leaf Area Index (LAI) of *Enhalus acoroides*, primarily driven by differences in leaf length, while leaf width remained relatively uniform across stations. Similar patterns of morphological variability have been reported in seagrass populations exposed to heterogeneous environmental conditions, particularly differences in nutrient availability and light regimes. Higher LAI values indicate greater leaf surface area, which can enhance photosynthetic potential and carbon assimilation. However, increased leaf surface area also provides more substrate for epiphytic colonization, potentially increasing shading stress on seagrass leaves. This dual role highlights LAI as an important morphological parameter linking seagrass productivity with vulnerability to epiphytic overgrowth [8].

Seagrass cover of *Enhalus acoroides* in the Sanur coastal waters ranged from moderate to dense, indicating that the structural integrity of the seagrass meadow remains relatively intact. Similar cover conditions have been reported in other Indonesian coastal areas experiencing tourism-related pressures, suggesting a degree of resilience at the community level [10, 19]. Nevertheless, chlorophyll-a concentration in seagrass leaves showed spatial variability and tended to be lower at stations (0.28 mg/g at Sindhu Beach, 0.33 mg/g at Karang Beach, and 0.32 mg/g at Semawang Beach) with higher epiphytic accumulation (6.25 mg/cm² at Sindhu Beach, 6.75 mg/cm² at Karang Beach, and 6.20 mg/cm² at Semawang Beach). Chlorophyll-a is widely recognized as a sensitive indicator of seagrass physiological condition, particularly in response to light limitation [20]. Reduced chlorophyll-a concentrations associated with epiphytic shading have been documented in both tropical and temperate seagrass ecosystems, indicating that physiological stress may occur even when seagrass cover remains relatively high [21].

The relationship between Leaf Area Index (LAI), epiphytic biomass, and chlorophyll-a content in *Enhalus acoroides* demonstrates interconnected patterns. A positive correlation is observed between LAI and epiphytic biomass, as higher LAI provides a larger surface area for epiphyte colonization [22]. However, increased epiphytic biomass tends to negatively affect seagrass physiology, as indicated by the negative correlation between epiphytic biomass and chlorophyll-a content. Dense epiphytic biomass can reduce light penetration to the leaf surface, thereby limiting photosynthetic efficiency and lowering chlorophyll-a levels [23, 24]. Nevertheless, the strength of this negative relationship varies across sites, likely influenced by differences in distance from the shore and local environmental conditions [25].

Epiphytic biomass exhibited variability between stations, reflecting spatial differences in environmental conditions and anthropogenic influence. Previous studies in the Sanur waters have shown that anthropogenic pressures such as tourism, fishing, and boat anchoring have contributed to declines in seagrass cover and density, as well as degradation of seagrass meadows. These disturbances may subsequently drive spatial variations in epiphytic biomass across sampling stations [26, 27]. Elevated epiphyte biomass is commonly linked to nutrient enrichment and reduced water clarity in coastal ecosystems, conditions often associated with tourism activities and land-based runoff [17, 28]. Regression analysis revealed a weak negative relationship between epiphytic biomass and chlorophyll-a concentration, indicating that increased epiphyte accumulation tends to suppress chlorophyll-a content in *Enhalus acoroides* leaves [29]. However, the relatively low coefficient of determination suggests that epiphytes alone do not fully explain the observed variability in chlorophyll-a concentration. This finding is consistent with previous studies highlighting the multifactorial nature of seagrass physiological responses, where light availability, nutrient dynamics, turbidity, and hydrodynamic conditions interact to influence seagrass health [13, 30]. Overall, these results emphasize the importance of integrated environmental management to mitigate cumulative stressors affecting seagrass ecosystems in tourism-intensive coastal areas such as Sanur.

4. Conclusions

This study demonstrates that *Enhalus acoroides* meadows in the coastal waters of Sanur exhibit spatial variability in seagrass cover and Leaf Area Index. A weak negative relationship was observed between epiphytic biomass and leaf chlorophyll-a concentration, indicating that increasing epiphyte accumulation tends to reduce the photosynthetic pigment content of seagrass leaves, although the strength of this relationship varies spatially. Overall, epiphytic biomass plays an important role in influencing the physiological condition of *Enhalus acoroides* and may serve as an early indicator of seagrass ecosystem health in the Sanur coastal area.

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Author Contribution

Conceptualization: Janita Devi Adnyana Putri, Gede Astawa Karang, Satya Pratama Atmaja. Methodology: Janita Devi Adnyana Putri, Gede Astawa Karang, Satya Pratama Atmaja. Data Collection: Janita Devi Adnyana Putri. Data Analysis: Janita Devi Adnyana Putri. Writing: Janita Devi Adnyana Putri, Gede Astawa Karang, Satya Pratama Atmaja. Supervision: Gede Astawa Karang, Satya Pratama Atmaja. Funding: Janita Devi Adnyana Putri.

Competing Interest

The authors declare that they have no conflicts of interest related to the publication of this research paper. No financial, personal, or professional relationships influenced this research work.

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