

Beyond Seagrass Density: Holothuroid Occurrence across Fine-scale Habitat Mosaics in Reef Flats of Tomini Bay, Indonesia

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ABSTRACT

Seagrass-associated reef flats are spatially heterogeneous, yet holothuroid occurrence is often interpreted primarily in relation to seagrass density. We examined fine-scale seagrass–holothuroid co-occurrence in Tomini Bay, Indonesia, using three stations, three 1×1 m quadrats per station, and 25 microplots per quadrat. The reconstructed sampling frame comprised 225 microplots and 450 plot-time records. Holothuroids were recorded in 37 co-presence microplots, while 33 microplots contained seagrass only and no holothuroid-only microplots were recorded. Among seagrass-present microplots, seagrass shoot-density distributions provided no evidence of a difference between seagrass-only and co-presence classes (Mann–Whitney $U = 664$, $p = 0.533$; rank-biserial effect size = 0.088). Pooled holothuroid observations were numerically greatest at ST1, whereas seagrass shoot density, seagrass richness, and holothuroid richness were numerically greatest at ST3. Field-observed substrate-class frequencies showed descriptive variation among stations. Exploratory Spearman correlations and PCA across nine quadrats summarized combined biological, environmental, and substrate-class gradients; PC1 and PC2 accounted for 35.13% and 20.50% of variation, respectively (55.63% cumulative). These patterns are consistent with a fine-scale habitat-mosaic interpretation rather than a shoot-density-only interpretation, but they do not isolate causal habitat effects. The study provides baseline ecological information for habitat assessment in Tomini Bay.

Keywords: Habitat mosaic, Holothuroid occurrence, Reef flat, Seagrass shoot density, Substrate heterogeneity, Tomini Bay

INTRODUCTION

Seagrass meadows are among the most productive coastal habitats and provide essential ecological functions, including sediment stabilization, organic-matter retention, nursery habitat, and support for diverse benthic communities. Their ecological value is often interpreted through structural attributes such as shoot density, canopy cover, species richness, and meadow continuity. However, seagrass habitats are rarely homogeneous. At small spatial scales, seagrass patches are embedded within mosaics of sand, rubble, coral fragments, macroalgae, and

other benthic substrates, creating heterogeneous microhabitats that may influence the distribution of associated fauna beyond vegetation density alone. This perspective is consistent with seascape ecology, which emphasizes that ecological patterns in coastal biogenic habitats are shaped not only by habitat presence but also by spatial configuration, patch structure, and habitat adjacency (Boström *et al.*, 2006; Pittman *et al.*, 2022). Reviews of seagrass ecosystem services also highlight that biodiversity support depends on habitat structure and landscape context, not merely on meadow area or vegetation abundance (do Amaral Camara Lima *et al.*, 2023).

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Holothuroids, or sea cucumbers, are important benthic organisms in tropical reef-flat and seagrass-associated ecosystems. Many species function as deposit feeders that ingest surface sediments, process organic particles, and contribute to bioturbation, nutrient recycling, and sediment quality. Because of these ecological functions, changes in holothuroid abundance and distribution may affect benthic ecosystem processes, especially in shallow coastal habitats where organic matter accumulates and sediment conditions vary over short distances. Previous studies and reviews have shown that sea cucumbers are commonly associated with seagrass beds, sandy substrates, rubble fields, and reef-flat habitats, but their local distribution may be associated with multiple interacting factors, including sediment characteristics, organic matter availability, shelter, hydrodynamic exposure, and detectability during different observation periods (Purcell *et al.*, 2016; Floren *et al.*, 2021; Pierrat *et al.*, 2022).

Despite the frequent association between seagrasses and holothuroids, the factors underlying this relationship remain incompletely resolved. A common assumption is that denser seagrass provides better habitat because it offers more organic matter, surface complexity, and protection. Yet this assumption may oversimplify reef-flat systems, where holothuroids do not respond only to seagrass structure but also to the surrounding benthic matrix. A seagrass patch with high shoot density may not necessarily provide suitable feeding or resting conditions if substrate texture, detrital accumulation, or adjacent open sediment differs from those of a patch with lower vegetation density. Conversely, a less dense seagrass patch may support holothuroids when embedded within a favorable combination of sand, rubble, mixed substrates, and sheltering structures. Thus, seagrass density may represent only one component of a broader habitat mosaic.

This issue is particularly important for tropical coastal systems such as Tomini Bay, Indonesia, where seagrass-associated reef flats support local biodiversity and benthic resources (Kasim *et al.*, 2024), but remain poorly documented at fine spatial scales. Many field assessments still summarize seagrass and holothuroid data at the station or transect level. While useful for broad

habitat description, such aggregation can obscure microhabitat-level patterns, including whether holothuroids occur across all vegetated areas or only within a subset of seagrass-present patches. Fine-scale sampling is therefore needed to distinguish simple habitat co-occurrence from more specific microhabitat association. This distinction is ecologically important because management decisions based only on seagrass density may overlook substrate heterogeneity and fine-scale habitat context associated with where holothuroids are recorded.

The present study addresses this gap by examining seagrass–holothuroid co-occurrence at the microplot scale in reef-flat habitats of Tomini Bay. Rather than treating seagrass beds as uniform habitat units, each 1×1 m quadrat was subdivided into 25 microplots to evaluate whether holothuroid occurrence was associated only with seagrass density or with a finer-scale habitat mosaic. This design allowed comparison between seagrass-only microplots and seagrass–holothuroid co-presence microplots, while also considering station-level variation in seagrass structure, holothuroid observations, and field-observed substrate classes. The approach does not attempt to prove habitat selection or causal preference, but it provides a more resolved assessment of whether vegetation density alone adequately characterizes the observed holothuroid occurrence pattern.

We hypothesized that holothuroid occurrence in Tomini Bay reef flats would not be fully characterized by seagrass shoot density. Specifically, we expected holothuroids to occur within seagrass-present microplots but not necessarily in the densest seagrass patches, indicating that fine-scale habitat context may provide a more informative ecological framework than seagrass density alone. Accordingly, this study aims to: 1) describe seagrass and holothuroid patterns across reef-flat stations; 2) compare seagrass-only and seagrass–holothuroid co-presence microplots; and 3) evaluate whether observed holothuroid occurrence is consistent with a fine-scale habitat-mosaic framework. By doing so, this study examines seagrass-associated holothuroid habitat from a fine-scale habitat-context perspective that is consistent with the heterogeneous nature of tropical reef flats.

MATERIALS AND METHODS

Study area and sampling design

The study was conducted in a shallow reef-flat seagrass system in Tomini Bay, Gorontalo Province, Indonesia. The study area represents a heterogeneous tropical coastal habitat where seagrass patches occur together with unvegetated sand, coral rubble, gravel, and mixed substrate classes. Three sampling stations were selected within the same reef-flat setting to represent local variation in seagrass structure and field-observed substrate classes. Station selection was intended to capture local habitat heterogeneity rather than represent a broad environmental gradient. The sampling stations were located at ST1 (122°53'29.7"E, 0°29'16.69"N), ST2 (122°53'44.94"E, 0°29'24.05"N), and ST3 (122°54'28.61"E, 0°29'25.57"N). The locations of the sampling stations are shown in Figure 1.

An observational field design was applied using a nested spatial framework. At each station, three 1×1 m quadrats were established, resulting in nine quadrat-level sampling units. Each quadrat was subdivided into 25 microplots measuring 20×20 cm arranged in a 5×5 grid. This design produced a complete sampling frame of 225 possible microplots. Each microplot was assigned a unique spatial code based on station, quadrat, and microplot number. This nested design enabled fine-scale biological occurrence to be evaluated while retaining the quadrat as the independent unit for broader habitat-association analyses.

In this study, a fine-scale habitat mosaic refers to the combination of seagrass occurrence, seagrass shoot density, field-observed substrate classes, and local environmental conditions recorded within and among quadrats. Microplot-level data were used to characterize occurrence, co-occurrence, and fine-scale density variation, whereas quadrat-

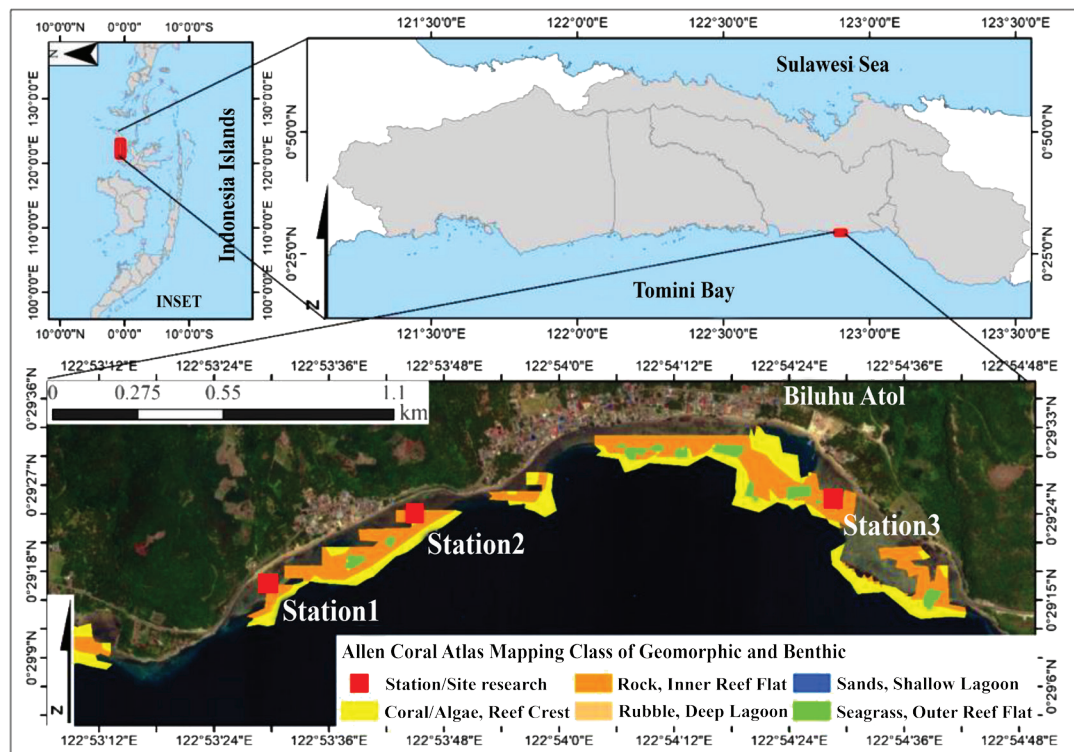


Figure 1. Map of the study area in Tomini Bay, Gorontalo Province, Indonesia, showing the locations of the three sampling stations within the reef-flat seagrass system.

level data were used to evaluate broader exploratory associations among biological, substrate-class, and environmental variables. The full methodological sequence from field sampling to statistical analysis is summarized in Figure 2.

Biological observations

Seagrass and holothuroid observations were recorded using the same quadrat–microplot framework. All seagrass species encountered within each 20×20 cm microplot were identified in situ, and shoot counts were recorded for each species. The Shoots field contained raw shoot counts recorded within each 20×20 cm (0.04 m²) microplot. Microplot-level seagrass shoot density was calculated as raw count/0.04 m². For quadrat-level summaries, shoot counts from the 25 microplots within each 1×1 m quadrat were aggregated and expressed as shoots·m⁻².

Seagrass assemblages were described using species richness, total shoot density, Shannon diversity, and Pielou evenness where appropriate. Species-specific shoot counts were retained in the reconstructed matrix, but the main habitat-association analyses emphasized total seagrass density and richness to avoid including too many predictors given the limited number of independent quadrat-level units.

Holothuroids were surveyed by visual census, identified to the lowest possible taxonomic level in the field, and counted without intentional disturbance. Daytime and nighttime observations

were retained separately. Holothuroid records were retained as individual observations per microplot for plot-time summaries. Daytime and nighttime records were also summed within each quadrat as pooled holothuroid observations (observations·quadrat⁻¹). Because the same individual may have been recorded in both periods, pooled values were not interpreted as abundance or density. Because visual detection of holothuroids may vary with exposure, shelter use, and activity, diel patterns were interpreted as variation in recorded observations and detectability rather than as direct evidence of behavioural rhythm.

Substrate composition and environmental variables

Substrate was assessed visually within the quadrat–microplot framework. Field-observed substrate classes were Sand, Coral rubble, Gravel mix sand, Coral rubble mix sand, Sand mix coral rubble, and Sand mix gravel. Mixed-substrate classes were retained as field observations rather than recast as continuous cover estimates.

Within substrate-recorded microplots, unrecorded components of a field-observed substrate class were coded as zero. However, substrate data were valid only for microplots in which biological observations were recorded. Therefore, substrate-related analyses were restricted to biologically recorded microplots and expressed as relative frequency among recorded microplots, not as continuous substrate cover, substrate availability, substrate preference, or sediment-property effects across the full 225-microplot sampling frame.

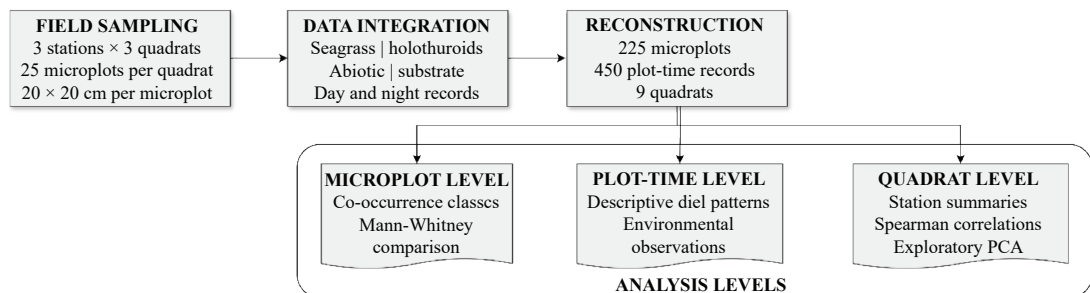


Figure 2. Sampling design, data reconstruction, and analytical levels used in the study.

Environmental variables, including water temperature, salinity, and pH were measured during both daytime and nighttime observations within the same spatial framework using a multiparameter waterproof meter (HI98194; pH/ORP/EC/TDS/Salinity/DO/Pressure/ Temperature). Environmental data were treated as measured physicochemical values. Therefore, unmeasured values were retained as missing data rather than replaced with zero. For plot-time-level analyses, environmental measurements were matched to the corresponding microplot and observation period. For quadrat-level analyses, environmental variables were aggregated within each quadrat to match the spatial resolution used for exploratory habitat-association analyses.

Data standardization and reconstructed sampling frame

All raw datasets were standardized prior to analysis. Plot identity was harmonized into a consistent coding structure containing station, quadrat, microplot number, and, where relevant, observation period. Four standardized datasets were generated: seagrass, holothuroid, environmental, and substrate datasets.

Because the original biological field records followed a presence-based recording structure, not all possible microplots appeared explicitly in the raw datasets. A complete sampling frame of 225 possible microplots was therefore reconstructed based on the field design: 3 stations $\times$ 3 quadrats $\times$ 25 microplots = 225 microplots.

For biological data, unrecorded microplot–species combinations were treated as zero abundance within the reconstructed sampling frame. Thus, zero values in the seagrass and holothuroid matrices represented biological absence, not missing data. For holothuroids, the reconstruction was extended to a plot-time structure because observations were conducted during daytime and nighttime periods: 225 microplots $\times$ 2 observation periods = 450 plot-time records.

Environmental data were also aligned to the plot-time structure, but unmeasured environmental values were retained as missing

values rather than converted to zero. Substrate data were attached to the 225-microplot frame for traceability, but valid substrate values were retained only for biologically recorded microplots. For microplots without substrate observations, substrate fields were retained as missing values rather than interpreted as zero cover.

This distinction was maintained throughout the analysis: biological absence = 0; environmental or substrate non-measurement = missing value.

Three main analytical datasets were generated. The plot-level matrix was used for microplot-level occurrence, co-occurrence, seagrass-density comparison, and fine-scale density variation. The plot-time-level matrix was used for diel comparisons and environmental summaries. The quadrat-level matrix, consisting of nine quadrats, was used for station summaries and exploratory associations among biological, substrate-class, and environmental variables. This three-level structure aligned each analysis with the appropriate spatial and temporal resolution of the data.

Statistical analysis

All statistical analyses were conducted using Python. The analytical workflow followed the nested sampling design and explicitly separated descriptive microplot-level analyses from exploratory quadrat-level analyses.

Seagrass and holothuroid assemblages were summarized using species richness, seagrass shoot density, pooled holothuroid observations, Shannon diversity, and Pielou evenness where appropriate. Distributional form was evaluated using the Shapiro–Wilk test as a diagnostic procedure. Because several biological variables were zero-inflated and the number of independent quadrat-level units was small, normality tests were not used as the sole basis for inference. Normality diagnostics are provided in Figure S5 and were used diagnostically rather than as confirmatory tests. Non-parametric analyses were prioritized where parametric assumptions were not supported. All statistical tests were evaluated at a significance threshold of $\alpha = 0.05$.

Microplot-level co-occurrence between seagrass and holothuroids was evaluated using the reconstructed 225-microplot matrix. Each microplot was classified into one of four categories: co-presence, seagrass only, holothuroid only, or co-absence. Because holothuroids occurred only within seagrass-present microplots in the reconstructed dataset, an additional conditional comparison was conducted among seagrass-present microplots only. Seagrass shoot density was compared between seagrass-only and seagrass–holothuroid co-presence microplots using a two-sided Mann–Whitney U test. The two-sided Mann–Whitney U test was retained as the primary formal comparison. Because microplots were nested within quadrats and stations rather than fully independent, the result was interpreted with this structural non-independence in mind.

Daytime and nighttime holothuroid observations and measured environmental variables were summarized descriptively. Paired Wilcoxon signed-rank tests were conducted only as post hoc sensitivity analyses and were not treated as primary inferential evidence. Diel patterns were interpreted as variation in recorded observations and detectability rather than as direct evidence of behavioural rhythm.

At the quadrat level, Spearman rank correlations were used to explore monotonic associations among seagrass shoot density, seagrass richness, pooled holothuroid observations, holothuroid richness, temperature, salinity, and pH. Principal component analysis was used to summarize multivariate gradients in the habitat mosaic. Spearman correlations and PCA were exploratory because only nine quadrats were available; no multiplicity-adjusted confirmatory inference was made. PCA used 13 standardized quadrat-level variables, with no zero imputation. PCA results were interpreted descriptively and were not used for causal inference.

Analytical scope

The analytical scope of this study was limited to evaluating whether observed holothuroid occurrence was more consistently interpreted through a fine-scale habitat-mosaic framework than through seagrass shoot density alone. The analyses therefore combined microplot-level co-occurrence,

conditional comparison of seagrass shoot density between seagrass-only and seagrass–holothuroid co-presence microplots, and exploratory quadrat-level associations among biological, substrate-class, and environmental variables. The study did not infer causal habitat selection, sediment-property effects, or anthropogenic disturbance gradients.

RESULTS

Biological assemblage structure and microplot-level occurrence patterns

Seagrass and holothuroid assemblages showed descriptive variation among stations (Table 1; Figure 3a–3b). Seagrass shoot density and species richness were numerically greatest at ST3, with mean values of $4,875.67 \pm 2,553.98$ shoots·m⁻² and 7.00 ± 1.00 species, respectively. Mean pooled holothuroid observations were numerically greatest at ST1 (57.33 ± 34.00 observations·quadrat⁻¹). Holothuroid richness was numerically greatest at ST3 (3.33 ± 1.15 species). Salinity and pH were numerically greatest at ST2, whereas temperature was numerically smallest at ST1. Among biologically recorded microplots, Coral rubble mix sand was the modal quadrat-level substrate class at ST1 and ST2, whereas Sand was modal at ST3. These station-level patterns were descriptive. Detailed biological summaries are provided in Figure S1.

Microplot-level occurrence analysis based on the reconstructed 225-microplot sampling frame identified three observed occurrence classes (Figure 3c). Seagrass–holothuroid co-presence occurred in 37 microplots, seagrass-only occurrence in 33 microplots, and co-absence in 155 microplots. No holothuroid-only microplots were recorded. Thus, within the reconstructed sampling frame, holothuroids were observed only within seagrass-present microplots, occurring in 37 of the 70 vegetated microplots.

Among seagrass-present microplots, seagrass shoot-density distributions provided no evidence of a difference between seagrass-only ($n = 33$) and co-presence microplots ($n = 37$) (two-sided Mann–Whitney U = 664, $p = 0.533$; rank-biserial effect size = 0.088; Figure 3d).

Table 1. Station-level summary of seagrass assemblages, holothuroid observations, environmental variables, and modal quadrat-level substrate class. Values are mean $\pm$ SD across three quadrats per station, except for substrate, which is reported as the modal quadrat-level field-observed class. H' denotes Shannon diversity and J' denotes Pielou evenness. Pooled holothuroid observations are sums of daytime and nighttime records and may include repeated observations of the same individuals.

Variable	ST1	ST2	ST3
Seagrass richness (S)	5.67 $\pm$ 1.53	4.33 $\pm$ 1.53	7.00 $\pm$ 1.00
Seagrass shoot density (shoots $\cdot$ m $^{-2}$)	3,628.33 $\pm$ 2,331.31	3,359.33 $\pm$ 693.19	4,875.67 $\pm$ 2,553.98
Seagrass Shannon diversity (H')	1.255 $\pm$ 0.163	1.098 $\pm$ 0.324	1.354 $\pm$ 0.075
Seagrass evenness (J')	0.755 $\pm$ 0.190	0.771 $\pm$ 0.116	0.699 $\pm$ 0.025
Holothuroid richness (S)	1.67 $\pm$ 0.58	1.67 $\pm$ 0.58	3.33 $\pm$ 1.15
Pooled holothuroid observations, day + night (observations $\cdot$ quadrat $^{-1}$)	57.33 $\pm$ 34.00	8.33 $\pm$ 9.45	7.00 $\pm$ 3.61
Daytime holothuroid observations (observations $\cdot$ quadrat $^{-1}$)	44.33 $\pm$ 23.18	3.00 $\pm$ 5.20	2.67 $\pm$ 2.52
Nighttime holothuroid observations (observations $\cdot$ quadrat $^{-1}$)	13.00 $\pm$ 11.53	5.33 $\pm$ 4.51	4.33 $\pm$ 2.31
Temperature ($^{\circ}$ C)	29.81 $\pm$ 0.51	30.73 $\pm$ 0.55	30.55 $\pm$ 0.52
Salinity (psu)	30.79 $\pm$ 0.07	32.57 $\pm$ 0.35	30.29 $\pm$ 0.39
pH	7.55 $\pm$ 0.17	8.02 $\pm$ 0.05	7.75 $\pm$ 0.09
Modal quadrat-level substrate class	Coral rubble mix sand	Coral rubble mix sand	Sand

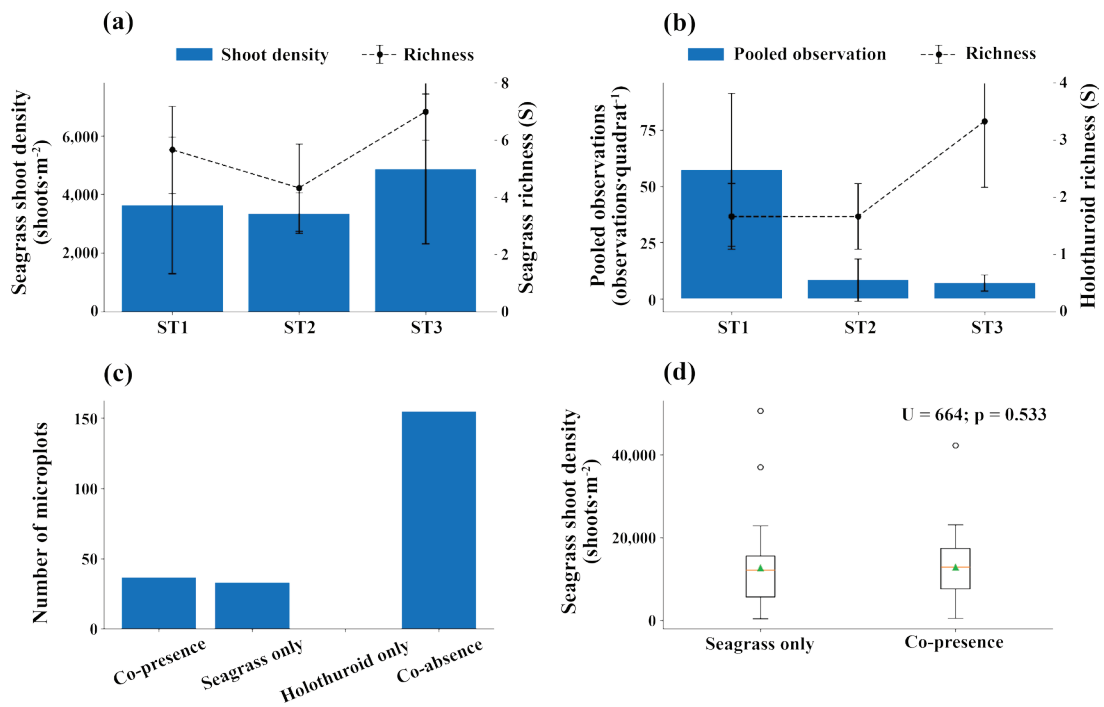


Figure 3. Biological assemblage summaries and microplot-level occurrence patterns. In panels (a) and (b), bars and line symbols show station means and error bars show SD across three quadrats. Pooled holothuroid observations are sums of daytime and nighttime records and may include repeated observations of the same individuals. Panel (c) shows occurrence classes based on the reconstructed 225-microplot matrix. Panel (d) compares microplot-level seagrass shoot density between seagrass-only and co-presence microplots using a two-sided Mann–Whitney U test.

Diel variation in observed holothuroids and environmental conditions

Holothuroid observations were characterized by frequent zeros during both daytime and nighttime surveys (Figure 4a). A paired Wilcoxon analysis was conducted only as a post hoc sensitivity analysis because most paired differences were zero. Full results are provided in Supplementary Table S1 and were not treated as primary inferential evidence. Additional diel summaries restricted to biologically recorded plots are provided in Figure S2.

Environmental variables showed descriptive variation between daytime and nighttime observations (Figure 4b–4d). Temperature was numerically greater during daytime than nighttime, and salinity showed a similar descriptive pattern. In contrast,

pH values showed broader overlap between periods. These environmental comparisons were not evaluated as primary formal comparisons; the full Wilcoxon sensitivity results are reported in Supplementary Table S1. Expanded environmental diel plots are provided in Figure S3.

Quadrat-level habitat associations and substrate composition

Field-observed substrate-class frequencies showed descriptive variation among stations (Figure 5a). At ST1, Sand and Coral rubble mix sand each accounted for 43.75% of recorded microplots. At ST2 Coral rubble mix sand accounted for 50.00%; and at ST3, Sand accounted for 46.88%. Detailed substrate summaries by quadrat and counts of modal substrate classes are provided in Figure S4.

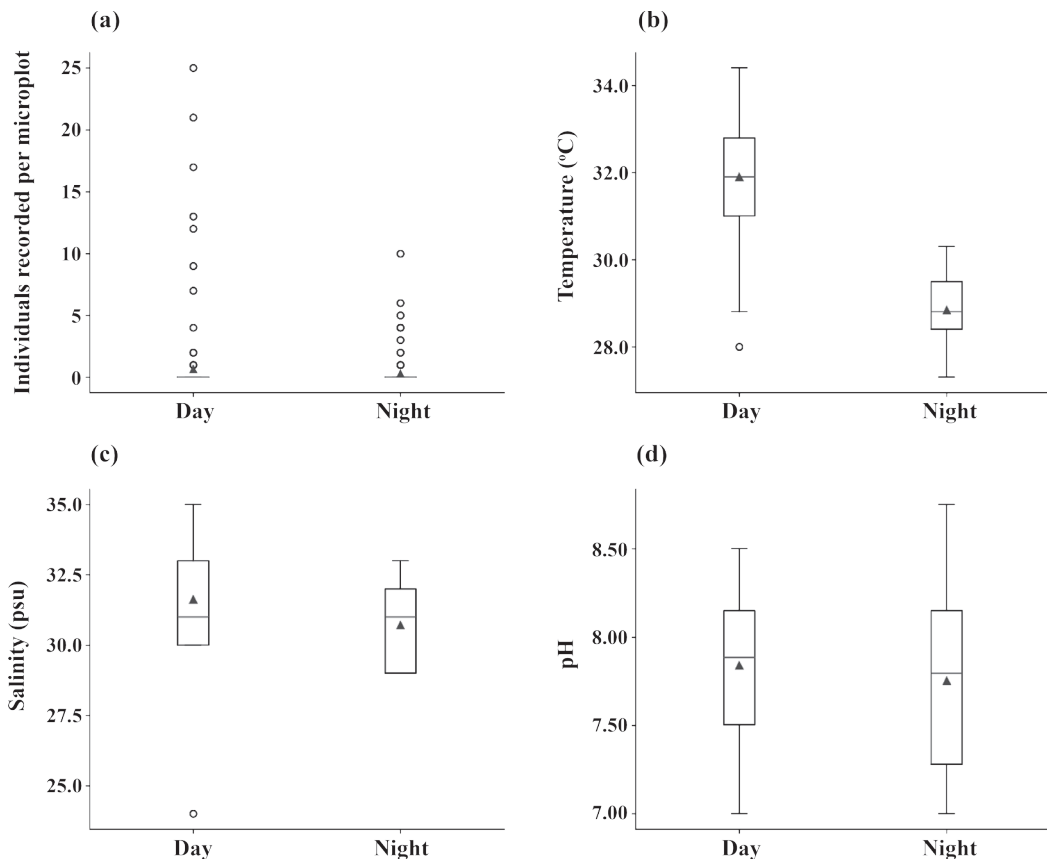


Figure 4. Descriptive daytime and nighttime distributions of holothuroid observations per microplot and measured environmental variables. Boxes represent interquartile ranges, central lines are medians, and mean symbols are shown.

At the quadrat level, Spearman rank correlation analysis was used to explore associations among seagrass shoot density, seagrass richness, pooled holothuroid observations, holothuroid richness, temperature, salinity, and pH. Substrate variables were excluded from this analysis. The full exploratory correlation structure is shown in Figure 5b.

Exploratory PCA of 13 standardized quadrat-level variables ($n = 9$; no zero imputation) summarized 55.63% of the variation on the first two axes ($PC1 = 35.13\%$; $PC2 = 20.50\%$). ST3 quadrats were positioned toward positive $PC1$, aligned with the direction of seagrass shoot density, seagrass richness, holothuroid richness, and selected

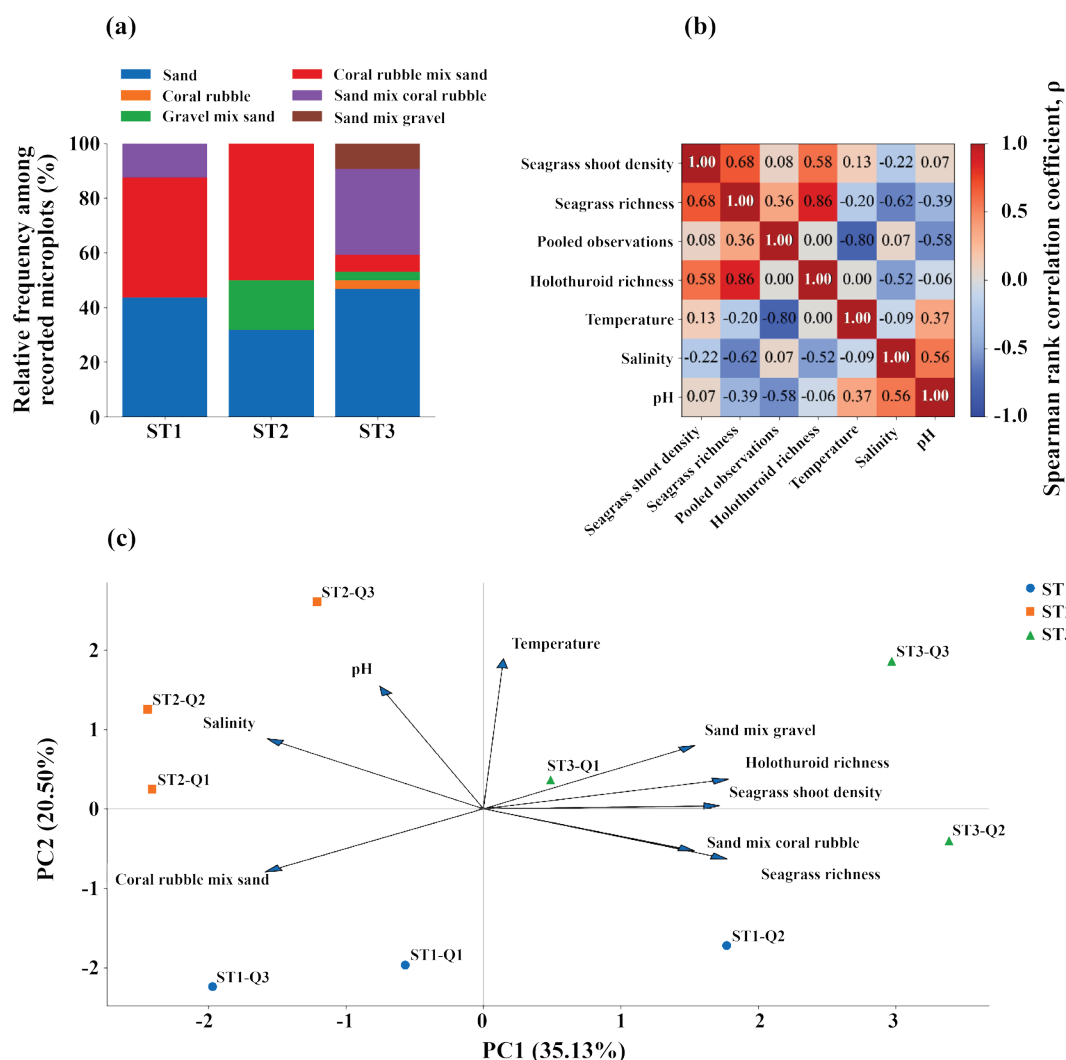


Figure 5. Quadrat-level habitat associations and field-observed substrate classes. (a) Field-observed substrate classes are shown as relative frequency among recorded microplots, not as continuous estimates of sediment cover or availability across the full sampling frame. (b) Spearman rank correlation coefficient, ρ , for seagrass shoot density, seagrass richness, pooled holothuroid observations, holothuroid richness, temperature, salinity, and pH across nine quadrats. Substrate variables are not included. Correlations are exploratory and do not imply influence, drivers, or causal effects. (c) Exploratory PCA biplot of standardized quadrat-level biological, environmental, and substrate-class variables. The nine displayed vectors are a selected subset of variables with large $PC1$ – $PC2$ loading magnitudes; complete loadings are provided in Figure S6. Loading vectors were rescaled for visual clarity.

sand-associated classes. ST2 quadrats were positioned mainly toward positive PC2 and negative PC1, aligned with temperature, pH, and salinity directions. ST1-Q1 and ST1-Q3 were positioned toward negative PC1 and PC2, aligned with Coral rubble mix sand. Pooled holothuroid observations loaded mainly negatively on PC2; this loading is shown in Figure S6 rather than as a vector in Figure 5c.

DISCUSSION

Seagrass presence as habitat context beyond shoot density

Holothuroids were recorded only in seagrass-present microplots, indicating that seagrass presence was a consistent feature of the microplots in which holothuroids were observed. However, among vegetated microplots, seagrass shoot-density distributions provided no evidence of a difference between seagrass-only and seagrass–holothuroid co-presence microplots. These findings indicate that holothuroids were recorded only within seagrass-present microplots, while providing no evidence that shoot density alone distinguished seagrass-only from co-presence microplots. Given the modest sample size and the nested sampling structure, the non-significant result is best interpreted as an absence of evidence for a density effect rather than definitive proof that density plays no role.

This interpretation is consistent with the broader view that seagrass-associated fauna often responds to habitat structure at multiple spatial scales rather than to a single vegetation metric. Seagrass landscapes can influence associated fauna through patch structure, habitat edges, meadow continuity, and spatial configuration (Boström *et al.*, 2006). Macrofaunal assemblages in seagrass seascapes may also vary with combined macrophyte and sedimentary predictors, indicating that vegetation attributes interact with the surrounding benthic environment (Rodil *et al.*, 2021). More generally, habitat-builder complexity can increase the diversity and functional structure of associated fauna, although such effects depend on the spatial scale and biological group examined (Jankowska

and Włodarska-Kowalczyk, 2022). In the present study, this interpretation is consistent with the non-parallel station-level pattern: ST3 had the numerically greatest mean seagrass shoot density and richness, whereas ST1 had the numerically greatest mean pooled holothuroid observations. Thus, local holothuroid occurrence may be better understood in relation to the fine-scale habitat context of the seagrass-associated reef flat than through a simple density gradient.

A broader habitat-context interpretation is also consistent with Kongsap *et al.* (2022), who reported contrasting growth parameters of *Holothuria atra* between seagrass and seaweed habitats. Although their study addressed growth rather than occurrence, it illustrates that holothuroid biological responses may vary among habitat types in ways not represented by vegetation density alone. Kongsap *et al.* further suggested that structural differences between habitats may alter shelter from water movement and low-tide stress. These findings provide useful context for the present habitat-mosaic interpretation but do not directly explain the occurrence patterns observed in Tomini Bay.

Fine-scale habitat mosaic and holothuroid occurrence

The microplot-level occurrence pattern is consistent with the habitat-mosaic interpretation. Within the 225 reconstructed microplots, holothuroids were recorded in 37 seagrass–holothuroid co-presence microplots, 33 microplots contained seagrass without holothuroids, and 155 showed co-absence, with no holothuroid-only microplots recorded. Holothuroids were therefore recorded in only a subset of seagrass-present microhabitats rather than across all vegetated areas, consistent with reviews showing that the seagrass–sea cucumber association reflects multiple interacting factors, including vegetation structure, sediment conditions, food availability, and shelter (Floren *et al.*, 2021). Sea cucumbers are deposit-feeding organisms that process surface sediments, contribute to sediment reworking and nutrient cycling, and interact with benthic surface conditions through feeding, movement, and sheltering behaviour (Purcell *et al.*, 2016; Pierrat *et al.*, 2022). Patchy distributions are common in deposit-feeding

holothuroids and may reflect multi-scale interactions between habitat structure, behaviour, and local conditions (Pierrat *et al.*, 2022). While the present results do not identify a single controlling factor, they show that seagrass shoot density alone did not account for the restricted occurrence of holothuroids among vegetated microplots.

Substrate context and exploratory multivariate gradients

Field-observed substrate classes provided additional context for the observed habitat mosaic. ST1 and ST2 descriptively had larger relative frequencies of Coral rubble mix sand, whereas ST3 had a larger relative frequency of Sand. These patterns indicate that biologically recorded microplots occurred within different benthic surface contexts among stations. Mixed substrates may plausibly modify surface complexity, shelter availability, movement pathways, and sedimentary conditions experienced by holothuroids; substrate conditions have been shown experimentally to affect burying and feeding in *Holothuria scabra* and to contribute to size- or movement-related distribution patterns in shallow coastal habitats (Mercier *et al.*, 2000; Altamirano *et al.*, 2017). However, because substrate data were valid only for biologically recorded microplots and did not represent full substrate availability across the 225-microplot frame, these patterns are interpreted as field-observed microhabitat descriptors rather than evidence that organisms preferred or selected particular substrates.

The quadrat-level analyses were consistent with this cautious habitat-mosaic interpretation. Spearman correlation results (Figure 5b) showed several exploratory associations among biological and environmental variables, without identifying a single relationship that clearly accounted for the observed holothuroid patterns. PCA summarized combined biological, substrate-class, and environmental gradients, with the first two axes accounting for 55.63% of total variation. ST3 quadrats were positioned toward positive PC1, aligned with the direction of seagrass shoot density, seagrass richness, holothuroid richness, and selected sand-associated classes. ST2 quadrats were positioned mainly toward positive PC2 and negative

PC1, aligned with temperature, pH, and salinity directions. ST1-Q1 and ST1-Q3 were positioned toward negative PC1 and PC2, aligned with Coral rubble mix sand. Pooled holothuroid observations loaded mainly negatively on PC2 in the complete loading summary (Figure S6) and were not displayed as a vector in Figure 5c. These exploratory positions are consistent with combined habitat-mosaic gradients rather than a single seagrass-density axis.

This interpretation agrees with recent studies showing that seagrass-associated faunal assemblages can reflect direct and indirect relationships among environmental conditions, vegetation structure, and associated fauna (Milot *et al.*, 2024). Similarly, benthic assemblages associated with eelgrass can be influenced by environmental filtering and biotic interactions acting on different components of diversity (Muller *et al.*, 2023). In the present study, PCA was used only as an exploratory ordination because the quadrat-level dataset contained nine independent units. Therefore, the ordination supports pattern description, not causal inference.

Diel observations and detectability

Diel comparisons showed frequent zero observations of holothuroids during both daytime and nighttime surveys, with some larger values recorded during the daytime. Environmental variables, particularly temperature and salinity, also showed numerically greater daytime values, whereas pH values overlapped more broadly between periods. Because visual census data can be affected by surface exposure, sheltering, burrowing, and species-specific activity, these results are best interpreted as variation in recorded observations and detectability rather than direct evidence of behavioural rhythm. This is consistent with reports of daily burrowing and feeding cycles in *Holothuria scabra* (Mercier *et al.*, 1999), diel burying affecting visual census estimates (Shiell and Knott, 2008), and habitat- and time-dependent movement, sediment turnover, and sheltering behaviour (Gray *et al.*, 2023). Because diel comparisons were not treated as primary inferential tests, and the sensitivity analysis was dominated by zero paired differences, these patterns should not be interpreted as robust evidence of differences between observation periods.

Implications, limitations, and future directions

These findings indicate that seagrass shoot density alone did not adequately characterize fine-scale holothuroid occurrence in the Tomini Bay reef flats. Field-observed substrate heterogeneity and fine-scale habitat context may also be associated with where holothuroids were recorded. Reef-flat monitoring should therefore consider these habitat descriptors in addition to seagrass shoot density. This has practical relevance given that sea cucumbers support small-scale multispecies fisheries across Indonesian intertidal habitats, including seagrass and coral reef systems (Setyastuti *et al.*, 2024), and that habitat assessment is increasingly recognized as a component of sea cucumber management and grow-out site suitability (Ainin *et al.*, 2025).

Several limitations should be recognized. First, the study was observational and cannot infer causal habitat selection. Second, substrate data described biologically recorded microplots and did not represent full substrate availability across all 225 microplots. Third, sediment grain size, organic matter, chlorophyll-*a*, detrital food resources, and hydrodynamic exposure were not directly measured. Fourth, Spearman correlation and PCA were exploratory because the number of independent quadrat-level units was limited. Fifth, station-level descriptive statistics (Table 1) were derived from only three quadrats per station, so the reported standard deviations should be interpreted with appropriate caution given this limited sample size. Future studies should combine full-frame substrate mapping with sediment-property measurements, food-resource indicators, repeated seasonal sampling, and species-specific detectability assessment. Such work would test whether the fine-scale habitat-mosaic patterns observed here remain consistent across broader spatial and temporal scales.

The broader importance of multivariate habitat context is also illustrated by Hempattarasuwan *et al.* (2024), who incorporated wave protection, proximity to existing seagrass beds, shore elevation, seagrass coverage, sediment grain size, and organic matter into seagrass habitat-suitability assessment. Several of these variables, including sediment grain size, organic matter, shore elevation, and wave exposure, were not measured in the present study.

They therefore represent priorities for future investigation rather than demonstrated explanations for the holothuroid patterns reported here.

CONCLUSIONS

Holothuroids were recorded only in seagrass-present microplots, but seagrass shoot density provided no evidence of a difference between seagrass-only and co-presence microplots. Station-level pooled observations and richness showed non-parallel descriptive patterns relative to seagrass shoot density, while field-observed substrate-class frequencies and exploratory quadrat-level ordination indicated heterogeneous habitat contexts. These findings support interpreting holothuroid occurrence within a fine-scale habitat mosaic rather than through seagrass shoot density alone, but they do not isolate causal effects of substrate or environmental variables. Future reef-flat habitat assessments may therefore benefit from considering field-recorded substrate heterogeneity alongside seagrass structure, while future studies should directly measure sediment grain size, organic matter, hydrodynamic exposure, and other potentially relevant habitat variables.

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