

Article

Characterization of Marine Fauna Assemblages in the Presence of Upside-Down Jellyfish (Genus *Cassiopea*) at Jobos Bay National Estuarine Research Reserve (JBNERR), Puerto Rico

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Abstract

Estuaries are highly productive systems that sustain diverse assemblages of fauna. In tropical estuaries such as the Jobos Bay National Estuarine Research Reserve (JBNERR), faunal composition plays a critical role in maintaining ecosystem services. Organisms such as fishes and macroinvertebrates provide essential ecosystem services, including trophic balance, nutrient recycling, bioturbation, and support for local economies. Despite their importance, faunal inventories and their ecological interactions within Jobos Bay NERR remain limited. In this study, we characterized the faunal community present in the estuary, focusing on fishes and macroinvertebrates. Sampling was conducted between October 2022 and April 2024 using bimonthly video transects (five 10 m transects per site) across three sites. Results showed that *Cassiopea* spp. were the most abundant macroinvertebrate and present at all sites. The faunal composition and *Cassiopea* abundance exhibited spatial variability, with bony fishes dominating assemblages. PERMANOVA results indicated that while community composition differed significantly among sites, *Cassiopea* abundance was not a significant driver of these assemblages ($p = 0.635$). Additionally, burrows were observed at all sites, showing an inverse relationship with *Cassiopea* abundance. Our findings suggest a potential relationship between the presence of *Cassiopea* and other fauna in Jobos Bay and highlight the need for a better understanding of the potential effects that high *Cassiopea* densities may have on the structure of faunal communities.



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1. Introduction

Estuaries are among the most productive ecosystems in the world, supporting high population densities of many Invertebrate and fish species [1]. Marine faunal assemblages have an important role in maintaining healthy estuaries, including the services they provide [1,2]. For example, some macroinvertebrates, such as crustaceans, mollusks, cnidarians, and echinoderms, support processes including nutrient recycling and bioturbation, which directly influence carbon sequestration and productivity [1,3,4]. Due to their abundance and diversity, estuarine detritivores may also help recycle organic matter, and

grazers like gastropods control phytoplankton or bacteria biofilms [5,6] while influencing nitrification and denitrification activity in the sediment [7]. Additionally, the estuary serves as a nursery, hosting a wide range of migratory and stationary fish species that can not only determine the composition and distribution of other organisms [8], but also contribute to the economy through recreational and commercial fishing, generating an economic benefit for local communities [3,9].

Despite the importance of estuaries and the faunal communities that inhabit them, estuaries are increasingly under threat by anthropogenic activities, including nutrient loading, sedimentation, and rising sea levels from global climate change [10]. As such, it is increasingly important to document estuarine community and ecosystem dynamics to better understand how these critical ecosystems are changing. This is particularly necessary in the Caribbean, where estuarine evaluations remain scarce, despite the growing threat of human activities.

One of them is the Jobos Bay National Estuarine Research Reserve (JBNERR), which is a natural reserve located on the south-central coast of Puerto Rico (17° 56' N, 66° 13' W) between the municipalities of Salinas and Guayama. Jobos Bay is the second-largest estuary of Puerto Rico and is classified as a coastal plain estuary, where groundwater inputs are the main source of freshwater [11]. An assessment was conducted in 2011 at Jobos Bay [12], which provided valuable information about the system's biological resources, including the fish communities present in the bay. However, more than a decade has passed, and subsequent work in the bay does not include faunal characterization data. Since estuarine systems are very dynamic and animals are sensitive to environmental changes, it is important to keep the census updated.

Despite its ecological importance, updated information on the composition and diversity of fauna communities within the reserve remains limited. Therefore, the main objectives of this study were (1) to quantify and identify the marine fauna present at different sites within Jobos Bay and (2) to describe possible interactions that may be occurring between the fauna present using video surveys. The data obtained in this study contribute to a better understanding of the structure of the faunal community in Jobos Bay. In addition, these data will help support future studies, as well as provide relevant information for the management of the reserve.

2. Materials and Methods

2.1. Site Location and Field Sampling

This study took place on the Jobos Bay National Estuarine Research Reserve (JBNERR), where nine sampling events were carried out on board the R/V *Pelicano* from October 2022 to April 2024 (except for April 2023, which could not be documented) at three locations: Cayo Caribe, Cayo Puerca, and Cayo Puerca Lulú (Figure 1). The three sites had similar traits: surrounded by red mangroves (*Rhizophora mangle*), low water movement, and similar seagrass species (dominated by *Halophila stipulacea*) and coverage—approximately 75–80%.

2.2. Environmental Parameters

In situ water parameters, including temperature (°C), salinity, dissolved oxygen (mg/L), and pH, were collected from both the surface and bottom upon arrival at each station using a calibrated multiparameter sonde (YSI®-556 MPS, USA). Sampling time varied throughout the study depending upon season and the weather, including wind direction.

2.3. Faunal Surveys

Five fixed 10 m transects were established that ran perpendicularly to the mangroves at each site. Transects were established by placing two 1.5 m PVC pipes at the start and end

of each transect, securing the transect tape underwater. Once the transects were in place, we conducted photogrammetric surveys using two GoPro Hero 10 cameras affixed to a dual-camera mount (Figure 2A). This setup surveyed a 2 m swath from 0 m to 10 m of the transect covering an area of 20 m². The GoPro Hero 10 had the following parameters: resolution: 4k, FPS: 30, lens: wide, HyperSmooth: standard, scheduled capture: off, duration: no limit, hindsight: off, bit rate: standard, zoom 1.0×, shutter: auto, EV comp: 0, white balance: auto, ISO min 100, ISO max 1600, sharpness: medium, color: natural, raw audio: off, wind: auto, SD card: 512 GB. The surveyor snorkeled at the surface from one end of the transect tape to the other (Figure 2B) while holding the camera mount pointed towards the transect and using a flotation device for stability. This procedure was repeated for each transect. Once the sampling was completed, video transects were uploaded to the BIIGLE platform, where they were manually annotated [13].



Figure 1. Sampling locations at Jobos Bay National Estuarine Research Reserve monitored bimonthly from October 2022 to April 2024.

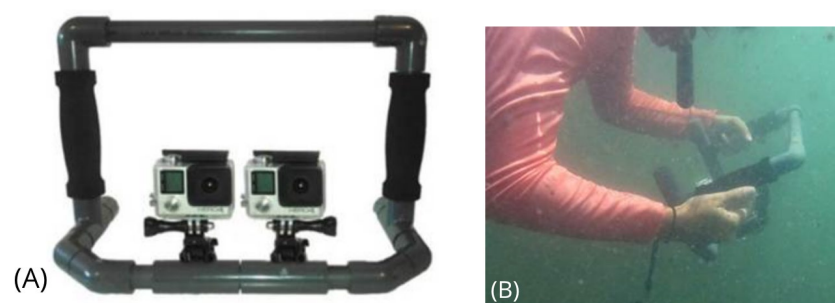


Figure 2. (A) Custom two-camera GoPro mount for photogrammetric surveys [14], (B) Vega-Pérez, J. conducting a field survey, demonstrating the use of the mount.

2.4. Data Analysis

Videos were annotated using the BIIGLE platform [13], an open-source cloud-based platform for image annotation. Species identification was performed using taxonomic guides from Neumann and DeLoach [15] and the WoRMS online marine species registry [16]. Identification of *Cassiopea* to species level was not possible using morphology alone, as the genus exhibits high phenotypic plasticity that makes visual identification unreliable [17]. Although DNA samples were collected for molecular confirmation, they

were lost following power failures caused by hurricanes Milton and Helene in 2024. Individuals were therefore identified to genus level and referred to throughout this study as *Cassiopea* spp. Fauna quantity, diversity, richness, and evenness analyses were performed using RStudio (Version 4.0.2).

To evaluate whether the spatial association between *Cassiopea* spp. and faunal burrows differed from random expectation, we applied the probabilistic model of co-occurrence [18], as implemented in the *cooccur* package [19]. This approach calculates the probability of the observed number of co-occurrences given the individual occurrence frequencies, providing a framework to detect significant attraction or avoidance patterns. This analysis was appropriate for our data, as it accounts for the frequency of occurrence independently of density fluctuations that may be driven by separate environmental factors.

Non-metric multidimensional scaling (nMDS) ordination was performed using Bray–Curtis dissimilarity to visualize faunal community patterns across sites [20,21]. To address zero-inflated samples, a constant value of 0.01 was added to all abundance values before analysis, a standard approach in community ecology [22]. The nMDS was conducted with $k = 2$ dimensions, 50 random starts, and 100 maximum iterations. Stress values were interpreted following Clarke [23]: <0.05 = excellent, $0.05–0.10$ = good, $0.10–0.20$ = acceptable. Convex hulls delineated site groupings in ordination space. Compositional differences among sites were tested using permutational multivariate analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM), both based on Bray–Curtis dissimilarity with 999 permutations [23]. To address whether *Cassiopea* abundance was a primary driver of community structure, an additional PERMANOVA was performed using its abundance as a predictor variable, excluding *Cassiopea* from the community matrix to avoid circularity. PERMANOVA quantifies the proportion of variance explained by grouping factors (R^2), while ANOSIM provides a measure of group separation (R statistic, ranging from 0–1). Statistical significance was assessed at $\alpha = 0.05$.

3. Results

3.1. Spatial Variability: Environmental Parameters

Sampling times varied during the field expeditions due to differences in environmental conditions and site accessibility. For instance, priority was given in the morning to Cayo Caribe as winds were not as strong and the mangroves partially shaded the transects, which could result in lower temperatures. Water conditions were similar across sites, with temperatures ranging from $28.8\text{ }^{\circ}\text{C}$ to $29.3\text{ }^{\circ}\text{C}$, yet with a consistent salinity of 35 (Table 1). Dissolved oxygen varied more among sites, with the highest mean at Cayo Puerca ($7.8\text{ mg/L} \pm \text{SE: } 0.37$) and lowest at Cayo Caribe ($5.6\text{ mg/L} \pm \text{SE: } 0.47$). Variation within sites was moderate for temperature and salinity, while dissolved oxygen and pH showed greater variability within sites.

3.2. Fauna Spatial and Temporal Abundance

Marine fauna were categorized into two major groups: macroinvertebrates and bony fishes. For macroinvertebrates, we identified four phyla, five classes, and seven species ($n = 7$), with *Cassiopea* spp. being the most abundant group (see Supplemental Table S1). For bony fishes, we identified nine families and ten species. Cayo Puerca had the highest total species for bony fishes ($n = 10$), whereas for macroinvertebrates both Cayo Puerca and Cayo Puerca Lulú had four species identified. Overall, Cayo Caribe had the lowest abundance and species presence during the sampling period, with five bony fishes and three macroinvertebrates. Of macroinvertebrates, upside-down jellies (Genus *Cassiopea*) were the most abundant within this group and present at all sites. As such, we separated them into an additional category to compare changes in their abundance versus faunal

assemblages (Figure 3). The mean abundance ($\pm$ SE) of *Cassiopea* and other faunal groups, calculated across space and time, was highly variable (Supplemental Table S1). The most bony fishes were recorded at Cayo Puerca Lulú in August 2023, followed by Cayo Puerca in October 2023 and lastly Cayo Caribe in October 2022.

Table 1. Descriptive statistics of water parameters measured for all sites.

Site	Mean	SE
Cayo Caribe		
Temperature	28.4	0.65
Salinity	35.5	0.50
Dissolved oxygen (mg/L)	5.9	0.47
pH	8.2	0.12
Cayo Puerca		
Temperature	29.4	0.65
Salinity	35.5	0.47
Dissolved oxygen (mg/L)	7.9	0.37
pH	8.4	0.11
Cayo Puerca Lulú		
Temperature	29.1	0.61
Salinity	35.7	0.55
Dissolved oxygen (mg/L)	6.6	0.24
pH	7.9	0.22

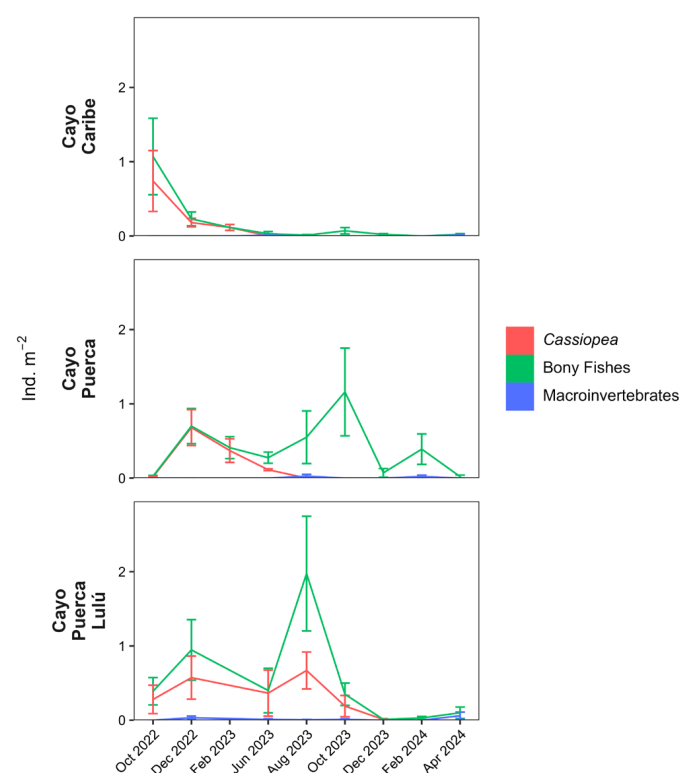


Figure 3. Mean ($\pm$ SE) abundance of *Cassiopea* spp. and main macrofaunal groups extracted from the underwater survey footage.

Subsequently, the abundance of bony fishes at Cayo Caribe decreased dramatically over the following months (Figure 3). Peak mean abundance of *Cassiopea* was less than one individual per square meter (ind. m^{-2}), and were always present at Cayo Puerca Lulú throughout the sampling months. In October 2022 at Cayo Caribe, few bony fishes ($1.07 \pm 1.15 \text{ ind. m}^{-2}$) and *Cassiopea* ($0.74 \pm 0.92 \text{ ind. m}^{-2}$) were recorded. Over the

sampling period, bony fish and *Cassiopea* abundance were dynamic both spatially and temporally in Cayo Caribe. Relative abundance (%) of faunal groups varied across sites and months, revealing strong temporal turnover in community composition (Figure 4). *Cassiopea* were recorded at all sites during the study period and contributed a large proportion of the assemblage during the early sampling periods, particularly from October 2022 to February 2023. Among the bony fishes, *Anchoa* spp. (bay anchovy), *Gerres cinereus* (yellowfin mojarra), and *Scarus iseri* (striped parrotfish) were the most recurrent across sites. Community composition became more heterogeneous later in the study, with *G. cinereus*, *Anchoa* spp., *S. iseri*, and *Haemulon flavolineatum* (French grunt) contributing substantially to relative abundance in several months. In contrast, macroinvertebrates showed more restricted spatial and temporal occurrence. For example, *Isostichopus badionotus* (three-rowed sea cucumber) was observed only at Cayo Caribe in June 2023, whereas *Tripneustes ventricosus* (white sea urchin) was recorded only at Cayo Puerca Lulú in April 2024. Similarly, *Elysia crispata* (lettuce sea slug) and *Dondice* spp. were observed only in Cayo Caribe and Cayo Puerca Lulú, and only during specific sampling months.

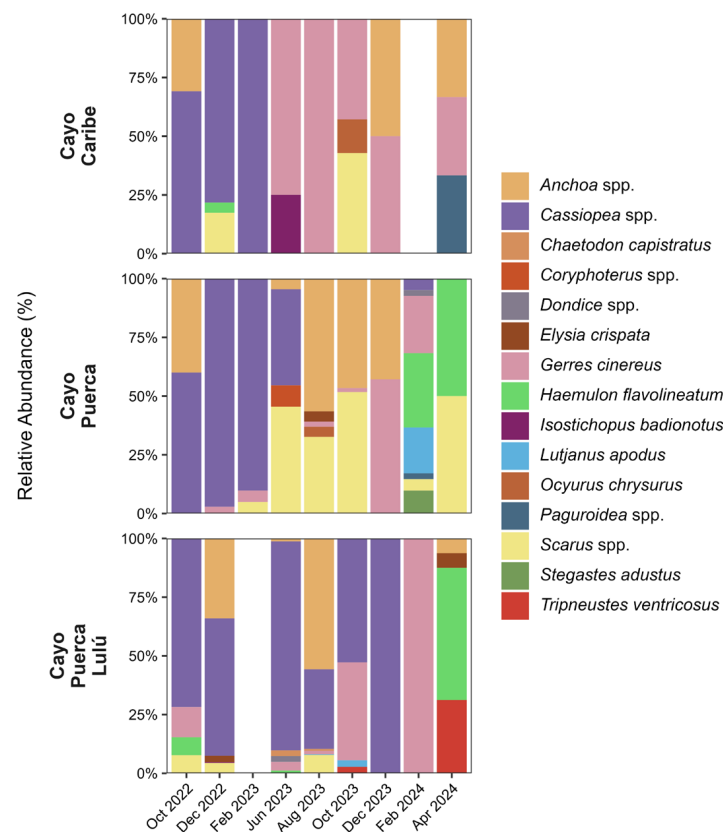


Figure 4. Relative abundance of bony fishes and macroinvertebrates recorded per site and month.

3.3. Community Structure

Shannon diversity (H') and taxonomic richness showed strong spatiotemporal variation across sites and months (Figure 5). Cayo Caribe showed the lowest values of both metrics throughout the study, reaching zero in subsequent sampling months. Conversely, Cayos Puerca and Puerca Lulú showed higher and more variable diversity and taxonomic richness, with peaks in mid-2023. The peak diversity was observed in June 2023 at Cayo Puerca, while the peak richness was observed in August 2023 at Cayo Puerca Lulú. Hence, the patterns presented in Figure 5 indicate strong spatiotemporal fluctuations in diversity and richness over the study period.

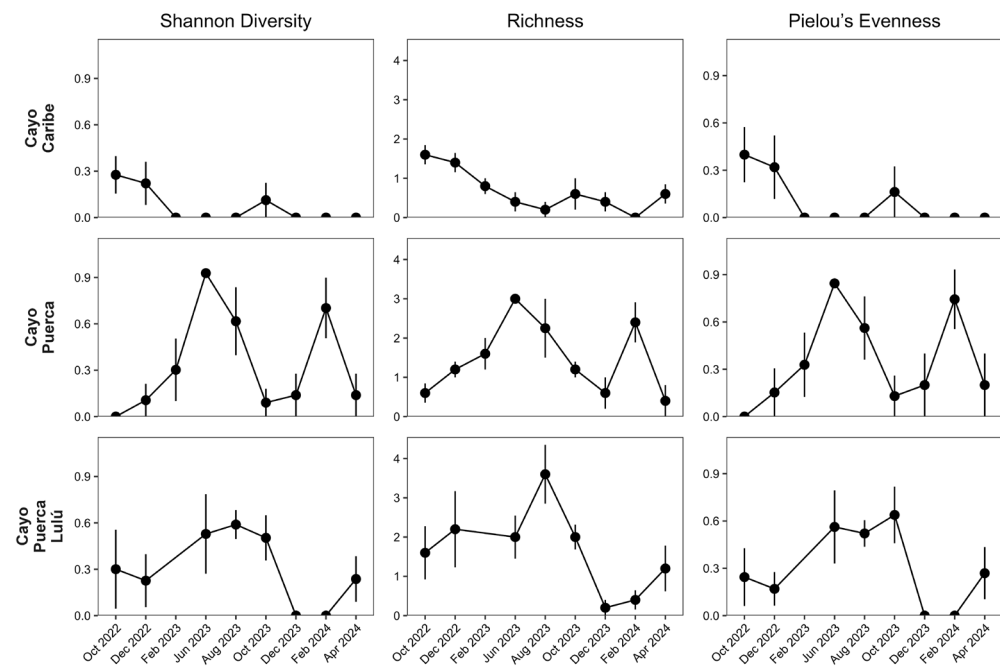


Figure 5. Spatiotemporal variation in mean ($\pm$ SE) Shannon's diversity, species richness, and Pielou's evenness measured in video surveys.

Pielou's evenness varied across sites and sampling periods, indicating changes in how evenly individuals were distributed among taxa. In general, periods with higher Shannon diversity tended to coincide with higher evenness, particularly at Cayo Puerca and Cayo Puerca Lulú, suggesting that increases in diversity were driven not only by the presence of more taxa, but also by a more balanced relative abundance among them. In contrast, low evenness values at Cayo Caribe throughout most of the study indicate strong dominance by one or few taxa, which corresponded with consistently low Shannon diversity despite occasional variation in richness. Richness and evenness did not always follow the same temporal pattern, showing that the number of taxa present alone did not determine diversity. Rather, the relative distribution of abundance among taxa also contributed substantially to community structure.

nMDS ordination successfully represented community dissimilarity in two dimensions (stress = 0.176, Figure 6), indicating an acceptable fit based on stress criteria. Visual inspection revealed an overlap between the sites. Faunal communities differed significantly among sites. PERMANOVA detected significant compositional heterogeneity (pseudo- $F_{2,113} = 2.47$, $R^2 = 0.042$, $p = 0.01$), with site identity explaining 4.2% of total community variance. PERMANOVA models indicated that community composition differed significantly among sites (pseudo- $F_{2,113} = 2.47$, $R^2 = 0.042$, $p = 0.01$), while *Cassiopea* abundance alone did not significantly explain community structure (pseudo- $F_{1,114} = 0.70$, $R^2 = 0.006$, $p = 0.63$). When both factors were included in a marginal-effect model to control for site-specific differences, site identity remained the only significant predictor ($p = 0.006$), whereas *Cassiopea* abundance remained non-significant ($p = 0.367$). ANOSIM corroborated these findings ($R = 0.047$, $p = 0.004$), indicating weak, but statistically significant separation among site assemblages.

3.4. Habitat Structure

Non-living biological components are commonly recorded during benthic surveys and include remains or biogenic structures associated with organisms [24]. In this study, these components included egg cases, empty shells, and burrows (Supplemental Table S2). Burrows were encountered at all sites in large quantities (Figure 7), and because the

burrowers themselves were not recorded, we classified burrow structures as “non-living” (Supplemental Table S2). Burrow density differed across sites. Therefore, we decided to quantify them to evaluate possible interactions between the burrowers and *Cassiopea*. Burrows were consistently higher in Cayo Caribe, increasing from 24.4 ind. m⁻² from the first time of sampling (October 2022) to 217.0 ind. m⁻² after the field sampling (April 2024; Figure 7). Conversely, Cayo Puerca Lulú showed moderate burrow densities, and Cayo Puerca had the lowest densities reported (2.3–68.1 ind. m⁻²).

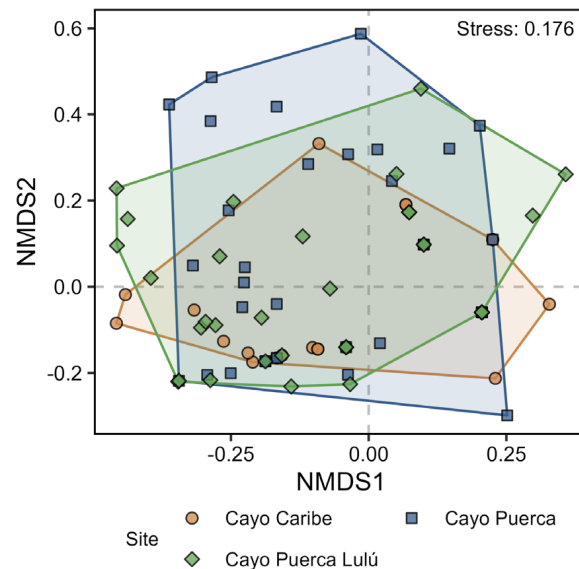


Figure 6. Non-metric multidimensional scaling (nMDS) ordination of faunal community composition across three sampling sites in mangrove habitats. Each point represents a single sampling event ($n = 126$ total samples: Cayo Caribe = 45, Cayo Puerca = 41, Cayo Puerca Lulú = 40). Ordination is based on the zero-adjusted Bray–Curtis dissimilarity matrix of species abundance data. Colored polygons represent convex hulls enclosing all samples from each site. The two-dimensional nMDS solution achieved convergence with stress = 0.18, indicating an acceptable representation of community structure. Community composition differed significantly among sites (PERMANOVA: $R^2 = 0.042$, $F = 2.47$, $p = 0.01$; ANOSIM: $R = 0.047$, $p = 0.004$), but was not significantly influenced by *Cassiopea* abundance ($R^2 = 0.006$, $p = 0.63$).

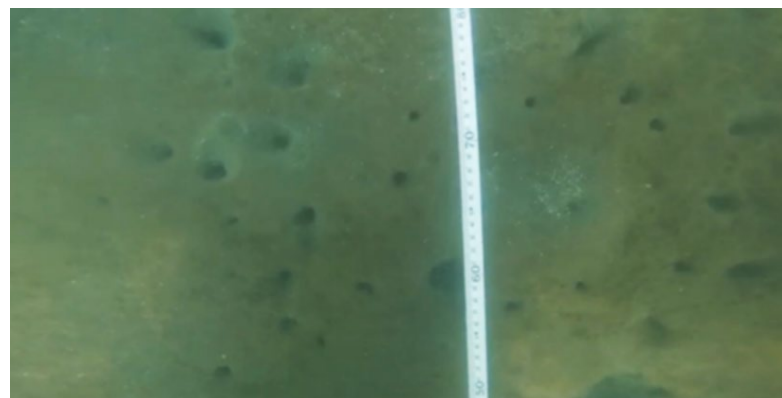


Figure 7. Burrows in the sediment in Cayo Caribe, April 2024.

We suspected that *Cassiopea* and burrow densities exhibited opposite temporal dynamics, and therefore tested their relationship in the data. *Cassiopea* peaked at the beginning of the sampling period and declined progressively thereafter: at Cayo Caribe, abundance became absent after February 2023; at Cayo Puerca, counts approached zero after June 2023 (<0.2 ind. m⁻²); and at Cayo Puerca Lulú, densities dropped from 3.0–6.9 to <0.1 ind. m⁻²

by December 2023. Burrow densities, in contrast, were consistently high and increased over time. At Cayo Caribe, burrow counts exceeded 20 m^{-2} by April 2024, while at Cayo Puerca. Numbers reached near zero during the peak *Cassiopea* period (December 2022–February 2023) before recovering (Figure 8). Despite these apparent inverse trends, the probabilistic co-occurrence analysis [18] showed that the association between *Cassiopea* spp. and faunal burrows was purely random (observed co-occurrences = 58, expected = 59.77, $p = 0.869$), with no significant patterns of attraction or avoidance detected at the transect scale.

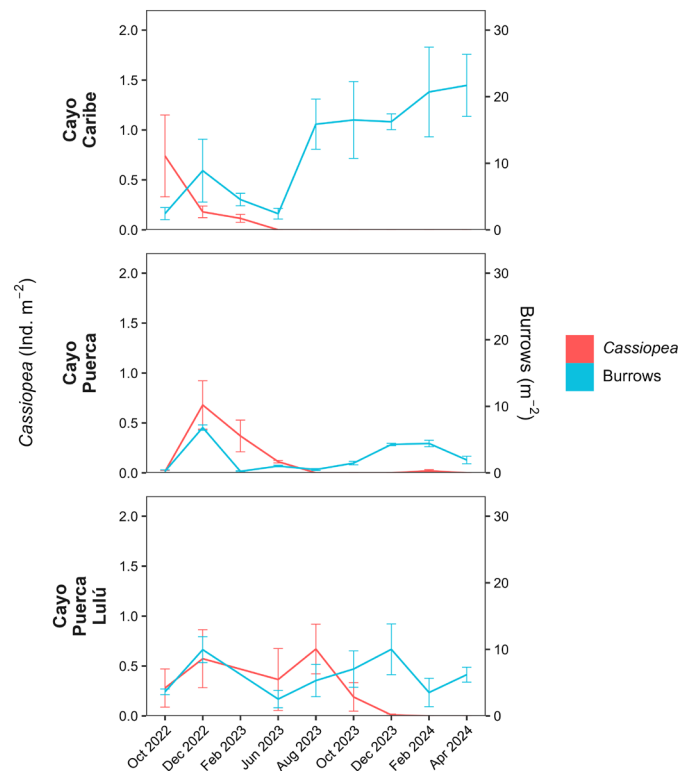


Figure 8. Mean ($\pm$ SE) abundance of *Cassiopea* and burrows. As *Cassiopea* abundance decreased, burrows increased over time, suggesting a potential relationship between the two populations in Jobos Bay.

4. Discussion

This study aimed to characterize the composition of faunal assemblages in different sites of the Jobos Bay estuary and to assess potential interactions between local faunal communities. In this context, the observed associations between fauna assemblages not only advance our understanding of estuarine species interactions but also establish an updated baseline for long-term monitoring and management within the reserve.

4.1. Spatiotemporal Patterns and Community Structure

The pronounced spatial and temporal variation in faunal communities documented across the study sites reflects the dynamic in shallow tropical estuaries (Figure 3). Peak bony fish abundance varied both seasonally and spatially, with Cayo Caribe reaching its maximum in October 2022 before declining dramatically. Cayo Puerca Lulú showed maximum fish densities during August 2023 (nearly two individuals/ m^2), while Cayo Puerca peaked in October 2023 (Figure 3). The observed summer peaks in species richness at both Cayo Puerca and Cayo Puerca Lulú (Figures 3 and 5), contrasting with winter declines, suggest temperature-mediated recruitment patterns consistent with tropical estuarine fish assemblages [25]. However, the dramatic decline at Cayo Caribe following its early peak

indicates site-specific factors may override regional seasonal patterns, potentially reflecting differences in habitat quality, predation pressure, or environmental stressors [26].

Cayo Caribe consistently showed low diversity indices and species richness compared to other sites (Figure 5). Cayo Puerca and Cayo Caribe exhibited the greatest exposure to physical disturbance (e.g., wind-driven currents). However, Cayo Puerca is located within the mangrove channels of Mar Negro, which can dissipate the wave energy, whereas Cayo Caribe, located in the lower keys, is more directly influenced by hydrodynamic forces (Figure 1). This suggests that Cayo Caribe may support fewer faunal assemblages due to the hydrodynamics of Jobos Bay.

Although site identity explained a small portion of the total variance (4.2%), it was a more consistent predictor than *Cassiopea* abundance, which did not significantly influence the overall community composition ($R^2 = 0.006$, $p = 0.63$). This suggests that while *Cassiopea* is a dominant component of the marine fauna in this study, its density does not dictate the broader assembly patterns of fish or other invertebrates in Jobos Bay. The low variance explained by both factors indicates that other unmeasured environmental variables or stochastic recruitment processes likely play a larger role in shaping these estuarine assemblages.

4.2. Natural History Observations

Jobos Bay had several fish species consistently represented across space and time. While the most abundant fish on the surface were *Anchoa* spp., we documented that the most abundant species recorded on the bottom near *Cassiopea* were the *G. cinereus* and the juvenile *S. iseri* (Figure 4). During the surveys, it was possible to identify that most of the fish were in their adult stage, while *S. iseri* (Figure 9), and the *H. flavolineatum* were always observed in their juvenile stage, indicating that the estuary plays a role of nursery habitat for juvenile animals [27,28].



Figure 9. An aggregation of juvenile *Scarus iseri* around *Cassiopea* spp.

A total of six macroinvertebrate species were identified, with *Cassiopea* representing the most abundant and consistently observed group across all sampling sites and months (Figures 4 and 10; Supplemental Table S1). The presence of *Cassiopea* is an important component of the faunal community within the estuarine system. Estuaries like Jobos Bay represent a suitable habitat for these upside-down jellyfish, which are commonly found in shallow tropical and subtropical waters with low movement [29]. Recent studies indicate that under favorable environmental conditions, particularly when natural predation is limited, *Cassiopea* populations can increase very rapidly. Stoner et al. [30] found that *Cassiopea* presence in seagrass beds decreased in epifauna densities, as well as a decrease in grazers. Hence, *Cassiopea* can cause physical changes to the environments they inhabit, which can lead to cascading effects on benthic ecosystem dynamics such as declines in benthic fauna [31].



Figure 10. Macroinvertebrates observed in the video surveys. (A) *Elysia crispata*, (B) *Tripneustes ventricosus*, (C) *Isostichopus badionotus*, (D) *Pagurus* sp.

However, *Cassiopea* do have some predators [32–35]. One of them is a nudibranch that occurs on the southwest coast of Puerto Rico: *Dondice parquerensis*, which according to previous studies [32,36] has only been found within *Cassiopea*, not outside them. The interaction between *D. parquerensis* and *Cassiopea* is more consistent with parasitism or a predation–parasitism continuum, as the nudibranch can persist on the host, feed on oral arm tissues, and reproduce without immediately killing the *Cassiopea*. In this study, we were able to identify a nudibranch from the genus *Dondice*, but species-level identification was not possible. The *Dondice* spp. individuals were recorded on seagrass *Halophila stipulacea* or bare sediment (Figure 11). However, there is evidence that other species of *Dondice* are not exclusive to these jellyfish, but can consume them, like *Dondice arianeae* [37]. In addition, this sea slug had not been documented before in Jobos Bay [12,38], updating the inventory of macroinvertebrates in the reserve.



Figure 11. *Dondice* sp. and polychaete burrows adjacent to a juvenile *Cassiopea* sp.

4.3. Potential Interactions

The second objective of this study was to document possible interactions between the fauna present in the estuary. While we have no direct interactions on camera (e.g., predation), we used the photogrammetric survey data to explore potential interactions due to the proximity of certain taxa.

4.3.1. Bony Fish–*Cassiopea* Interactions

Bony fishes are commonly found in estuarine environments where *Cassiopea* are abundant, though the literature on their potential interactions remains scarce (see Stoner et al. [30]). One of the fish that attracted attention due to their behavior around *Cassiopea* was *Chaetodon capistratus* (four-eyed butterflyfish, Figure 12), which was observed in the field actively biting *Cassiopea* oral arms. This represents a remarkable example of trophic plasticity in coral-reef fish, which has also been documented in the Bahamas [39]. *C. capistratus* are typically specialized corallivores with highly specific dietary preferences [40,41], making this apparent medusivory behavior noteworthy. In coral-poor environments, these fishes have been documented expanding their diets to include alternative prey items, including soft corals, hydroids [42,43], and jellyfish like *Aurelia aurita* [44], *Aequorea* spp. [45], and *Chrysaora lactea* [46]. The nutritional quality of *Cassiopea* tissues, enhanced by their symbiotic zooxanthellae, along with their high abundance and ease of predation may make them an attractive alternative prey for butterflyfish when facing resource limitations [29], leading to opportunistic feeding behavior [46].

A field observation that stood out in this study was the persistent presence of juvenile *Sphyraena barracuda* (great barracuda) in proximity to *Cassiopea* (Figure 13A). Although predation activity was not directly observed or recorded, at sites where *S. barracuda* were present, *Cassiopea* exhibited missing complete oral arms and/or pieces of tissue surrounding the benthos, indicating an interesting ecological interaction. The documented tissue damage, including missing oral arms and torn bell margins (Figure 13B), is consistent with the shearing bite mechanics of barracuda [47]. While barracudas are not typically considered medusivores, their high-speed pursuit predation strategy targeting teleost prey around *Cassiopea* aggregations could result in collateral damage to the jellyfish themselves [48]. Similar observations of barracudas persistently present in areas of *Cassiopea* blooms have been noted in the Bahamas, Bonaire, and Cuba [authors' anecdotal remarks]. We suggest that future studies could involve metabarcoding of gut content of *S. barracuda* to identify

if they are consuming the jellies, or deployment of cameras to record the behavior of the barracudas with *Cassiopea*.



Figure 12. *Chaetodon capistratus* in the study site.

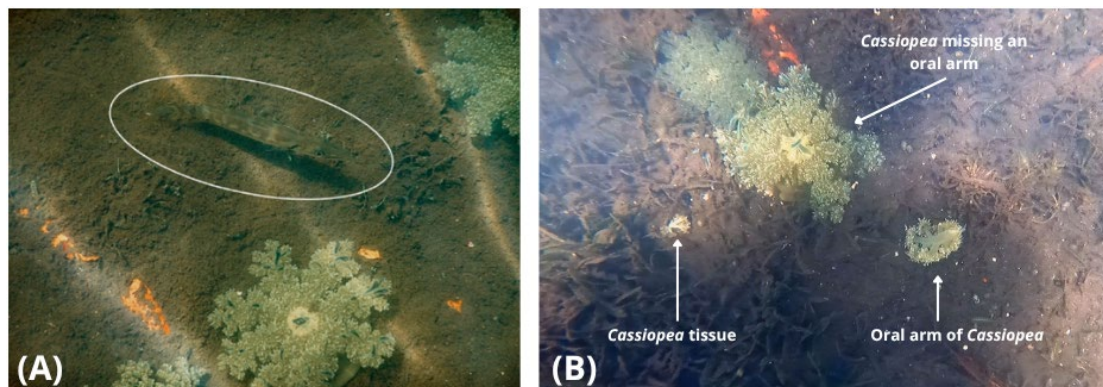


Figure 13. (A) Juvenile *S. barracuda* near *Cassiopea* spp. at Cayo Puerca Lulú. (B) *Cassiopea* with missing oral arms.

4.3.2. Cassiopea–Burrow Interactions

Our temporal observations initially suggested a potential decoupling between *Cassiopea* density and burrow abundance (Figure 14). However, the probabilistic co-occurrence analysis revealed that the association between these two components is purely random ($p = 0.869$). This indicates that at the scale of our survey, there is no significant spatial attraction or exclusionary pressure (e.g., competitive exclusion) between the jellyfish and the burrowing macrofauna.

At Cayo Caribe, the decline in *Cassiopea* from October 2022 to June 2023 coincided with an increase in burrow density, which reached over 20 burrows m^{-2} by April 2024 (Figure 8). While this could be interpreted as a recovery from competitive pressure, the statistical evidence of random co-occurrence suggests that these shifts are more likely independent responses to separate environmental drivers. For instance, the increase in burrows might be

related to seasonal sediment stabilization or organic matter accumulation, while *Cassiopea* declines are typically driven by temperature and light availability.

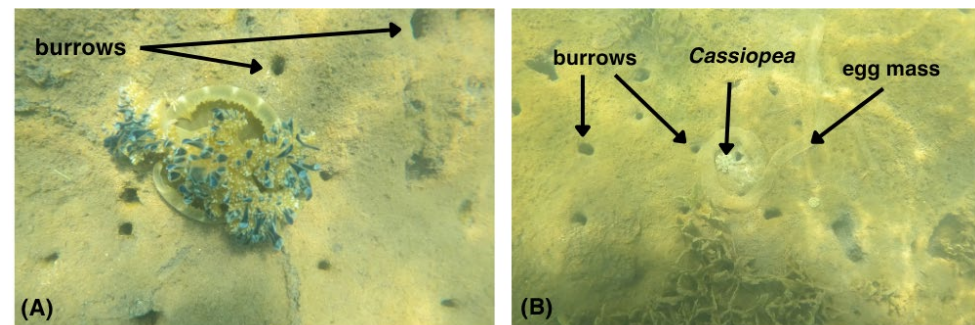


Figure 14. (A) Adult *Cassiopea* missing oral arms surrounded by burrows. (B) Polychaete egg mass and burrows surrounding a juvenile *Cassiopea* sp.

Similarly, the inverse patterns observed at Cayo Puerca, where peak *Cassiopea* abundance coincided with a decline in burrows, do not necessarily reflect a direct threshold effect or physical exclusion. Instead, the random association ($p > 0.05$) suggests that environmental conditions favoring high *Cassiopea* recruitment at this site might simultaneously be less optimal for the specific bioturbating fauna present there.

Although *Cassiopea* settles on the benthos and could theoretically obstruct burrow openings, our findings suggest that this physical presence does not significantly dictate the overall distribution or persistence of burrowing organisms across the study area. The variability observed at Cayo Puerca Lulú, where both groups exhibited parallel trends at certain times, further supports the interpretation that site-specific environmental factors, rather than direct interspecific interactions, drive the observed abundance patterns.

4.4. Study Limitations and Future Research Directions

While this study provides valuable insights into fauna interactions, several limitations should be acknowledged. First, the video transect survey method, while minimally invasive, likely underestimated the abundance of mobile species. Fish, due to their motile behavior, may have affected the count by exhibiting avoidance responses [49–52]. Bony fish may also have been double-counted by passing more than once in front of the camera. Hence, video transects can provide a permanent record, but camera movement and the presence of equipment can also cause avoidance in mobile species. Method choice should therefore take account of species' sensitivity to disturbance [53,54]. Turbidity conditions should also be considered during video analysis, since reduced visibility can limit the ability to identify organisms.

Second, the suggestions of causal mechanisms for observed patterns rely primarily on correlative evidence. While the negative correlation between *Cassiopea* and burrows is robust, experimental manipulation would strengthen causal inferences. Future research should include controlled field experiments, such as jellyfish removal treatments or artificial coverage experiments, to directly test competitive exclusion hypotheses. In addition, methods should also include collection techniques to identify and have accurate counts of burrowing organisms, since organisms such as polychaetes tend to make U-shaped burrows, having two holes for the same individual. However, for this study, each hole was counted as a single organism. This may have resulted in an overestimation.

In addition, future research should include molecular techniques, including DNA barcoding and environmental DNA (eDNA) analysis, to resolve taxonomic ambiguities and provide accurate species identifications [55,56]. Such approaches are particularly important

in tropical systems where cryptic species diversity is high and morphological identification is challenging [57].

5. Conclusions

Our findings show characterization of faunal communities in the Jobos Bay NERR, and spatiotemporal dynamics with bony fish *Cassiopea* and macroinvertebrate communities. The bony fish and macroinvertebrates found add valuable knowledge to the managers of the Jobos Bay National Estuarine Research Reserve (JBNERR). Organisms such as *Dondice* spp. had not been previously reported in studies or reports for the estuary, which may be a possible predator of *Cassiopea* [37]. The interactions between *Cassiopea* and burrowers represent a novel finding for Jobos Bay with broad implications for understanding competitive interactions in the estuary. Observations of *Chaetodon capistratus* actively making contact with *Cassiopea* oral arms should be studied in the future, both in terms of behavior and physiology. Future research should focus on experimental validation of competitive mechanisms, long-term monitoring of ecosystems, and development of predictive models for faunal interactions. Understanding and managing potential faunal interactions is critical for predicting ecosystem responses.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/d18050306/s1>, Supplemental Table S1: Mean ($\pm$ SE) of marine fauna density (ind. m⁻²) recorded per site and month. Supplemental Table S2: Categories of non-living macrofauna components across sites.

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References

1. Bissoli, L.B.; Bernardino, A.F. Benthic macrofaunal structure and secondary production in tropical estuaries on the Eastern Marine Ecoregion of Brazil. *PeerJ* **2018**, *6*, e4441. [CrossRef]
2. Laurino, I.R.A.; Serafini, T.Z.; Costa, T.M.; Christofoletti, R.A. The role of estuarine macrofaunal patterns for the management of marine protected areas in a changing world. *J. Nat. Conserv.* **2021**, *63*, 126042. [CrossRef]

3. Bhuiyan, M.K.A.; Godoy, O.; González-Ortegon, E.; Billah, M.M.; Rodil, I.F. Salt marsh macrofauna: An overview of functions and services. *Mar. Environ. Res.* **2025**, *205*, 106975. [CrossRef] [PubMed]
4. Ajayi, S.; Najjar, R.; Rivest, E.; Woodland, R.; Friedrichs, M.A.M.; St-Laurent, P.; Davis, S. Benthic macrofaunal carbon fluxes and environmental drivers of spatial variability in a large coastal-plain estuary. *Biogeosciences* **2025**, *22*, 7769–7795. [CrossRef]
5. Herman, P.M.J.; Middelburg, J.J.; Koppel, J.V.D.; Heip, C.H.R. Ecology of Estuarine Macrobenthos. *Adv. Ecol. Res.* **1999**, *29*, 195–240.
6. Cardoso, P.G. Estuaries: Dynamics, Biodiversity, and Impacts. In *Life Below Water. Encyclopedia of the UN Sustainable Development Goals*; Leal Filho, W., Azul, A.M., Brandli, L., Lange Salvia, A., Wall, T., Eds.; Springer: Cham, Switzerland, 2021. [CrossRef]
7. Stief, P. Stimulation of microbial nitrogen cycling in aquatic ecosystems by benthic macrofauna: Mechanisms and environmental implications. *Biogeosciences* **2013**, *10*, 7829–7846. [CrossRef]
8. Sandoval-Huerta, E.R.; Madrigal-Guridi, X.; Escalera-Vázquez, L.H.; Medina-Nava, M.; Domínguez-Domínguez, O. Estructura de la comunidad de peces en cuatro estuarios del Pacífico mexicano central. *Rev. Mex. Biodivers.* **2014**, *85*, 1184–1196. [CrossRef]
9. LeGore, S. *Bait Fisheries Serving the Marine Recreational Fisheries of Puerto Rico*; Department of Natural and Environmental Resources Marine Resources: San Juan, Puerto Rico, 2007; pp. 10–99. Available online: <https://www.drna.pr.gov/wp-content/uploads/2015/05/Baitfish-Final-Report-pdf> (accessed on 1 February 2024).
10. Courrata, A.; Lobryb, J.; Nicolasc, D.; Laffargued, P.; Amarad, R.; Lepagec, M.; Girardinc, M.; Le Papea, O. Anthropogenic disturbance on nursery function of estuarine areas for marine species. *Estuar. Coast. Shelf Sci.* **2009**, *81*, 179–190. [CrossRef]
11. Estudios Técnicos Inc. *Jobs Bay National Estuarine Research Reserve Management Plan 2017–2022*; Departamento de Recursos Naturales y Ambientales de Puerto Rico: San Juan, Puerto Rico, 2017; p. 193. Available online: https://www.drna.pr.gov/wp-content/uploads/2017/08/August-2017_Jobs-Bay-Management-Plan-2.pdf (accessed on 14 May 2026).
12. Whitall, D.R.; Costa, B.M.; Bauer, L.J.; Dieppa, A.; Hile, S.D. (Eds.) *A Baseline Assessment of the Ecological Resources of Jobs Bay, Puerto Rico*; NOAA Technical Memorandum NOS NCCOS 133; Department of Commerce, NOAA: Silver Spring, MD, USA, 2011; 188p. Available online: <https://repository.library.noaa.gov/view/noaa/669> (accessed on 12 December 2025).
13. BIIGLE Team. *BIIGLE 2.0—Browse and Annotate Underwater Imagery*; BIIGLE Team: Bielefeld, Germany, 2019. Available online: <https://biigle.de> (accessed on 14 May 2026).
14. Johnson, S.A.K. *Untapped Potential of Gorgonian Octocorals for Detecting Environmental Change in Biscayne National Park, Florida, USA*; University of South Florida: Tampa, FL, USA, 2019. Available online: <https://www.proquest.com/openview/0b60dddec722256151881641f4d4d9022/1?pq-origsite=gscholar&cbl=18750&diss=y> (accessed on 9 January 2025).
15. Humann, P.; DeLoach, N. *Reef Fish Identification: Florida, Caribbean, Bahamas*; New World Publications: Jacksonville, FL, USA, 2002.
16. WoRMS—World Register of Marine Species. *Marinespecies.org*. Available online: <https://www.marinespecies.org/> (accessed on 27 January 2024).
17. Anthony, C.J.; Heagy, M.; Bentlage, B. Phenotypic plasticity in *Cassiopea ornata* (Cnidaria: Scyphozoa: Rhizostomeae) suggests environmentally driven morphology. *Zoomorphology* **2022**, *141*, 115–131. [CrossRef]
18. Veech, J.A. A probabilistic model for analysing species co-occurrence. *Glob. Ecol. Biogeogr.* **2013**, *22*, 252–260. [CrossRef]
19. Griffith, D.M.; Veech, J.A.; Marsh, C.J. cooccur: Probabilistic Species Co-Occurrence Analysis in R. *J. Stat. Softw. Code Snippets* **2016**, *69*, 1–17. [CrossRef]
20. Bray, J.R.; Curtis, J.T. An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.* **1957**, *27*, 326–349. [CrossRef]
21. Kruskal, J.B. Nonmetric multidimensional scaling: A numerical method. *Psychometrika* **1964**, *29*, 115–129. [CrossRef]
22. Clarke, K.R.; Warwick, R.M. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*; PRIMER-E, Ltd.: Plymouth, UK; Plymouth Marine Laboratory: Plymouth, UK, 2001; Volume 2, pp. 1–168.
23. Clarke, K.R. Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* **1993**, *18*, 117–143. [CrossRef]
24. Di Camillo, C.G.; Luzi, G.; Danial, A.; Di Florio, L.; Calcina, B.; Lo Brutto, S.; de Oliveira, J.L.S.M.; Fumanti, A.; Cerrano, C. Characterization of intertidal macrofaunal communities of two sandy beaches under different anthropogenic pressures. *J. Mar. Sci. Eng.* **2022**, *10*, 1976. [CrossRef]
25. Nagelkerken, I.; Dorenbosch, M.; Verberk, W.C.; de la Moriniere, E.C.; van der Velde, G. Importance of shallow-water biotopes of a Caribbean bay for juvenile coral reef fishes: Patterns in biotope association, community structure and spatial distribution. *Mar. Ecol. Prog. Ser.* **2000**, *202*, 175–192. [CrossRef]
26. Bloomfield, A.L.; Gillanders, B.M. Fish and invertebrate assemblages in seagrass, mangrove, saltmarsh, and nonvegetated habitats. *Estuaries* **2005**, *28*, 63–77. [CrossRef]
27. Nagelkerken, I.; Roberts, C.V.; Van Der Velde, G.; Dorenbosch, M.; Van Riel, M.C.; De La Moriniere, E.C.; Nienhuis, P.H. How important are mangroves and seagrass beds for coral-reef fish? The nursery hypothesis tested on an island scale. *Mar. Ecol. Prog. Ser.* **2002**, *244*, 299–305. [CrossRef]
28. Kraus, R.T.; Secor, D.H. Application of the nursery-role hypothesis to an estuarine fish. *Mar. Ecol. Prog. Ser.* **2005**, *291*, 301–305. [CrossRef]

29. Verde, E.A.; McCloskey, L.R. Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: Effect of jellyfish size and season. *Mar. Ecol. Prog. Ser.* **1998**, *168*, 147–162. [\[CrossRef\]](#)
30. Stoner, E.W.; Yeager, L.A.; Layman, C.A. Effects of epibenthic jellyfish, *Cassiopea* spp., on faunal community composition of Bahamian seagrass beds. *Caribb. Nat.* **2014**, *12*, 1–10.
31. Zarnoch, C.B.; Hossain, N.; Fusco, E.; Alldred, M.; Hoellein, T.J.; Perdikaris, S. Size and density of upside-down jellyfish, *Cassiopea* sp., and their impact on benthic fluxes in a Caribbean lagoon. *Mar. Environ. Res.* **2020**, *154*, 104845. [\[CrossRef\]](#)
32. Brandon, M.; Cutress, C.E. A new *Dondice* (Opisthobranchia: Favorinidae), predator of *Cassiopea* in southwest Puerto Rico. *Bull. Mar. Sci.* **1985**, *1*, 139–144.
33. Stoner, E.W.; Layman, C.A. Bristle worms attack: Benthic jellyfish are not trophic dead ends. *Front. Ecol. Environ.* **2015**, *13*, 226–227. [\[CrossRef\]](#)
34. Morejón-Arrojo, R.D.; López-Figueroa, N.B.; Hernández-Albernas, J.I.; Rodríguez-Viera, L.; Stoner, E.W. From sand to bell: Novel predation of Scyphozoans by the Giant Caribbean Sea Anemone *Condylactis gigantea* (Weinland, 1860) from the Western Atlantic. *Diversity* **2025**, *17*, 111. [\[CrossRef\]](#)
35. Hoeksema, B.W.; Tuti, Y.; Becking, L.E. Mixed medusivory by the sea anemone *Entacmaea medusivora* (Anthozoa: Actiniaria) in Kakaban Lake, Indonesia. *Mar. Biodivers.* **2015**, *45*, 141–142. [\[CrossRef\]](#)
36. Mariño, J.; Farfán, E.; Caballer, M. Primer registro de *Dondice parguerensis* (Mollusca: Favorinidae) para Venezuela. *Rev. Mex. Biodivers.* **2011**, *82*, 709–712. [\[CrossRef\]](#)
37. García-Méndez, K.; Padula, V.; Valdés, Á. Integrative systematics of the genus *Dondice* Marcus, 1958 (Gastropoda, Nudibranchia, Myrrhinidae) in the Western Atlantic. *Mar. Biodivers.* **2022**, *52*, 42. [\[CrossRef\]](#)
38. Robles, P.O.; González, C.M.; Laboy, E.N.; Capella, J.; Field, R. (Eds.) *Jobos Bay Estuarine Profile: A National Estuarine Research Reserve*; NOAA: Silver Spring, MD, USA, 2002. Available online: <https://coast.noaa.gov/data/docs/nerrs/Handout-Jobos-Bay.pdf> (accessed on 2 May 2025).
39. Iliff, S.M.; Wilczek, E.R.; Harris, R.J.; Bouldin, R.; Stoner, E.W. Evidence of microplastics from benthic jellyfish (*Cassiopea xamachana*) in Florida estuaries. *Mar. Pollut. Bull.* **2020**, *159*, 111521. [\[CrossRef\]](#)
40. Pratchett, M.S.; Graham, N.A.J.; Cole, A.J. Specialist corallivores dominate butterflyfish assemblages in coral-dominated reef habitats. *J. Fish Biol.* **2013**, *82*, 1177–1191. [\[CrossRef\]](#)
41. Pratchett, M.S. Feeding preferences and dietary specialization among obligate coral-feeding butterflyfishes. *Biol. Butterflyfishes* **2014**, 140–179. [\[CrossRef\]](#)
42. Roberts, C.M.; Ormond, R.F.G. Butterflyfish social behaviour, with special reference to the incidence of territoriality: A review. *Environ. Biol. Fishes* **1992**, *34*, 79–93. [\[CrossRef\]](#)
43. Berumen, M.L.; Pratchett, M.S.; McCormick, M.I. Within-reef differences in diet and body condition of coral-feeding butterflyfishes (Chaetodontidae). *Mar. Ecol. Prog. Ser.* **2005**, *287*, 217–227. [\[CrossRef\]](#)
44. Bos, A.R.; Cruz-Rivera, E.; Sanad, A.M. Herbivorous fishes *Siganus rivulatus* (Siganidae) and *Zebrasoma desjardinii* (Acanthuridae) feed on Ctenophora and Scyphozoa in the Red Sea. *Mar. Biodivers.* **2017**, *47*, 243–246. [\[CrossRef\]](#)
45. Morejón-Arrojo, R.D.; Rodríguez-Viera, L. Opportunistic predation on hydromedusae by the foureye butterflyfish and associated crustaceans. *Discov. Ocean.* **2024**, *1*, 19. [\[CrossRef\]](#)
46. Chaves, L.C.T.; Feitosa, J.L.L.; Pereira, P.H.C. First record of predation on jellyfish by butterflyfish on Brazilian rocky reefs. *Mar. Biodivers. Rec.* **2010**, *3*, e26. [\[CrossRef\]](#)
47. Grubich, J.R.; Rice, A.N.; Westneat, M.W. Functional morphology of bite mechanics in the great barracuda (*Sphyræna barracuda*). *Zoology* **2008**, *111*, 16–29. [\[CrossRef\]](#)
48. Hammerschlag, N.; Heithaus, M.R.; Serafy, J.E. Influence of predation risk and food supply on nocturnal fish foraging distributions along a mangrove–seagrass ecotone. *Mar. Ecol. Prog. Ser.* **2010**, *414*, 223–235. [\[CrossRef\]](#)
49. Schmidt, M.B.; Gassner, H. Influence of scuba divers on the avoidance reaction of a dense vendace (*Coregonus albula* L.) population monitored by hydroacoustics. *Fish. Res.* **2006**, *82*, 131–139. [\[CrossRef\]](#)
50. Patterson, H.M.; Lindsay, M.; Swearer, S.E. Use of sonar transects to improve efficiency and reduce potential bias in visual surveys of reef fishes. *Environ. Biol. Fishes* **2007**, *78*, 291–297. [\[CrossRef\]](#)
51. Dickens, L.C.; Goatley, C.H.R.; Tanner, J.K.; Bellwood, D.R. Quantifying relative diver effects in underwater visual censuses. *PLoS ONE* **2011**, *6*, e18965. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Lindfield, S.J.; Harvey, E.S.; McIlwain, J.L.; Halford, A.R. Silent fish surveys: Bubble-free diving highlights in accuracies associated with SCUBA-based surveys in heavily fished areas. *Methods Ecol. Evol.* **2014**, *5*, 1061–1069. [\[CrossRef\]](#)
53. Emslie, M.J.; Cheal, A.J.; MacNeil, M.A.; Miller, I.R.; Sweatman, H.P.A. Reef fish communities are spooked by scuba surveys and may take hours to recover. *PeerJ* **2018**, *6*, e4886. [\[CrossRef\]](#)
54. Bruneel, S.; Ho, L.; Van Echelpoel, W.; Schoeters, A.; Raat, H.; Moens, T.; Bermudez, R.; Luca, S.; Goethals, P. Sampling errors and variability in video transects for assessment of reef fish assemblage structure and diversity. *PLoS ONE* **2022**, *17*, e0271043. [\[CrossRef\]](#) [\[PubMed\]](#)

55. Thomsen, P.F.; Willerslev, E. Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* **2015**, *183*, 4–18. [[CrossRef](#)]
56. Bohmann, K.; Evans, A.; Gilbert, M.T.P.; Carvalho, G.R.; Creer, S.; Knapp, M.; Yu, D.W.; de Bruyn, M. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* **2014**, *29*, 358–367. [[CrossRef](#)]
57. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological identifications through DNA barcodes. *Proc. Biol. Sci.* **2003**, *270*, 313–321. [[CrossRef](#)] [[PubMed](#)]

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