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BIOLOGICAL INDICES TO CHARACTERIZE THE ENVIRONMENTAL STATE OF A MARINE ECOSYSTEM SUBJECTED TO ANTHROPOGENIC PRESSURE

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ABSTRACT

The present study characterised the environmental status of Tortugas Bay (Casma, Peru) by integrating biological indices with physicochemical variables, drawing on analyses of subtidal macrobenthic assemblages and water-column conditions. Sampling was undertaken at six stations spanning a gradient of anthropogenic pressure across three contrasting seasonal periods in 2023; sediments and benthic macrofauna were collected using a Van Veen grab and processed according to standardised protocols. Biodiversity metrics (species richness and abundance, together with Shannon, Simpson, Pielou and Margalef indices) were computed, and multivariate approaches (PCA, nMDS and PERMANOVA) were employed. The results indicated a pronounced spatial gradient of sediment organic enrichment, peaking at station E-01 ($5.20 \pm 0.32\%$) and reaching a minimum at E-06 ($1.70 \pm 0.50\%$), with an inverse association to water transparency (1.77 ± 0.23 m at E-01 versus 3.10 ± 0.27 m at E-04). Principal component analysis accounted for 57% of the overall environmental variability and identified sediment organic matter as the dominant structuring driver. Stations subject to greater organic enrichment displayed elevated abundances (up to 512 individuals) alongside reduced diversity ($H' = 2.00$) and evenness ($J' = 0.67$), whereas comparatively open and better-flushed stations supported assemblages that were both more diverse and more evenly distributed (H' up to 2.50). Collectively, the integrated use of biological indices and multivariate analyses yielded a robust appraisal of the bay's environmental condition and established a sound baseline for long-term monitoring and sustainable management.

KEYWORDS: Anthropogenic Pressure; Subtidal Macrobenthic Communities; Sediment Organic Enrichment; Biological Indices; Tortugas Bay.

1. INTRODUCTION

Marine ecosystems constitute some of the most productive natural systems and play a pivotal role in regulating biogeochemical processes, sustaining biodiversity, underpinning food security, and supporting the socio-economic development of coastal regions (Braulik et al., 2023). Nevertheless, they are highly susceptible to disturbance driven by anthropogenic activities (Abraham & Aswathy Prakash, 2025). The intensification of fisheries, aquaculture and tourism, together with the expansion of coastal infrastructure, has brought about substantial alterations in the physicochemical and biological conditions of many shallow marine environments, thereby disrupting key ecological processes (Sanz-Lazaro et al., 2021).

Environmental assessment of marine ecosystems has consequently become a priority for both environmental management and scientific research (Braulik et al., 2023). Traditionally, monitoring programmes have relied on physicochemical parameters to diagnose the condition of the marine environment (Hounyèmè et al., 2023); however, such indicators provide only a partial view of the underlying ecological processes and may fail to capture the synergistic and cumulative effects of anthropogenic pressures on ecosystems (Fuller et al., 2024).

Within this context, subtidal macrobenthic communities have been extensively studied and validated as highly sensitive and robust biological indicators of the environmental status of marine ecosystems (Wang et al., 2025); (Velasco-Charpentier et al., 2022). Their close association with sediments, limited dispersal capacity and differential responses to gradients of organic enrichment and contamination render them particularly informative (Espinoza Tongo et al., 2022). In parallel, the application of biological indices derived from benthic community structure including metrics of diversity and dominance, alongside ecological diagnostic tools has become a central approach in marine monitoring and environmental management programmes (Ballesteros Navia et al., 2023).

Along the south-eastern Pacific coast, numerous semi-enclosed bays are exposed to complex anthropogenic pressures (Tan, 2021). Tortugas Bay (Casma, Áncash) represents a coastal system emblematic of this challenge, being influenced by artisanal fisheries, benthic resource extraction, mariculture, tourism and the expansion of human settlements (Hurtado-Gutierrez et al., 2025). These pressures generate variable inputs of organic matter and nutrients, modify water-column

physicochemical characteristics including dissolved oxygen, pH, salinity and transparency and alter sediment structure, thereby directly shaping the composition and functioning of macrobenthic communities (Fuller et al., 2024); (Zhou et al., 2025).

Despite the ecological and socio-economic significance of this system, comprehensive assessments that explicitly link benthic community responses to environmental variability remain limited (Hsiao et al., 2021); (Nuttall et al., 2025). The integration of univariate diversity metrics, multivariate community analyses and standardised diagnostic tools such as the AZTI Marine Biotic Index (AMBI) and Abundance-Biomass Comparison (ABC) analysis enables more precise delineation of disturbance gradients and ecological quality classes, thereby strengthening the scientific basis for decision-making in marine ecosystem management (Santibañez-Aguascalientes et al., 2018); (Planque & Arneberg, 2018).

Accordingly, the present study aimed to characterise the environmental status of Tortugas Bay by integrating biological indices and physicochemical variables, based on analyses of subtidal macrobenthic communities and water-column conditions across six sampling stations during three contrasting seasonal periods in 2023. Within this framework, the study was guided by the following research question: what was the environmental condition of Tortugas Bay in 2023, as inferred from an integrated assessment of biological indicators and physicochemical parameters? This approach provided a detailed ecological diagnosis and contributed to establishing a scientific baseline to support monitoring and the sustainable management of anthropogenically pressured marine-coastal ecosystems.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was conducted in Tortugas Bay, located in the province of Casma, Áncash Region, Peru. The bay is characterised by a high capacity to retain fine sediments and by limited water renewal, conditions that promote the accumulation of organic matter on the seabed. Accordingly, six subtidal sampling stations were established throughout the bay, selected to represent a spatial gradient of anthropogenic pressure associated with local human activities. The location and spatial distribution of the stations are shown in Figure 1, which summarises the sampling design applied within the study area. The geographical coordinates of each station were

recorded using Google Earth Pro, following methodological recommendations to ensure adequate spatial accuracy in marine ecological studies (Savoska et al., 2025).

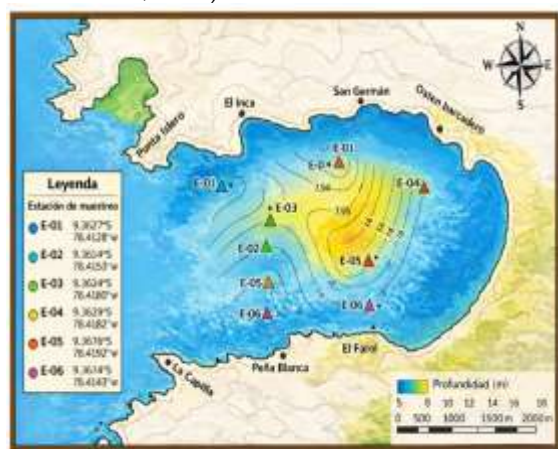


Figure 1: Study area and spatial distribution of sampling stations in Tortugas Bay (Peru).

2.2. Sampling Design

Six subtidal stations (E1-E6) were selected and distributed along gradients of environmental impact. Sampling was undertaken during three representative seasonal periods of the year (summer, autumn and spring) in order to capture temporal variability in community structure, in accordance with international benthic monitoring protocols (Ji et al., 2025). At each station, three independent replicates were collected to ensure statistical robustness and to enable assessments of spatial and temporal variability, consistent with established best practice in ecological sampling design (Aubry et al., 2024).

2.3. Sediment Collection And Processing

Surface sediments were collected using a 0.05 m² Van Veen grab, a standard device for quantitative sampling of macrobenthos in soft-bottom habitats (Van & Gümüş, 2021). Each sample was sieved in situ through a 500 µm mesh, a widely adopted threshold for characterising marine benthic macrofauna, following the methodology described by (Hernández-Miranda et al., 2021). The retained material was preserved in 70% ethanol and transported to the laboratory for analysis.

2.4 TAXONOMIC IDENTIFICATION AND BIOMASS ESTIMATION

In the laboratory, organisms were sorted, counted and identified under a stereomicroscope, and assigned to the lowest practicable taxonomic level. Taxonomic nomenclature was verified using the

(ISO 16665, 2014). Biomass was estimated by gently blotting individuals to remove excess water, followed by weighing on an analytical balance with a precision of ±0.001 g. These procedures followed the guidance of (ISO 5667-1, 2023), which provides standardised protocols for the processing, identification and quantitative analysis of marine benthic macrofauna, and were consistent with approaches commonly applied in community-structure studies.

2.5. Physicochemical Parameters And Organic Matter

Sampling and measurement of water physicochemical parameters followed the guidance set out in (Anđelković et al., 2025), which provides recommendations for sampling programme design and the application of standardised techniques in coastal and marine water-quality studies. Measurements were made in situ using a previously calibrated portable multiparameter probe, thereby ensuring sample representativeness and minimising artefacts associated with transport and storage. Total organic matter (TOM) in sediments was determined in accordance with the general principles of sampling and handling described in the same standard, using the loss-on-ignition method (450 °C for 4 h), a widely used technique for estimating organic content in marine sediments.

2.6 Statistical Analysis

Statistical analyses were conducted in R (R Core Team, 2024). Environmental variables were standardised using z-score normalisation. Macrobenthic abundance data were square-root transformed to reduce the influence of dominant taxa. Community structure was examined using multivariate analyses based on Bray-Curtis dissimilarity matrices, with non-metric multidimensional scaling (nMDS) employed for ordination. Differences in community composition among stations and seasons were tested using PERMANOVA with random permutations, adopting a significance level of $\alpha = 0.05$. Biological diversity indices (S, N, H', J', 1-D and Margalef) were calculated from station-level aggregated abundance data. Environmental gradients were explored using Principal Component Analysis (PCA), and relationships between environmental variables and biological indices were evaluated using Redundancy Analysis (RDA).

3. RESULTS

The physicochemical parameters of the ecosystem

exhibited moderate spatial variability among sampling stations across Tortugas Bay (Table 1). Temperature was relatively homogeneous at most stations, with mean values close to 22 °C; however, station E-01 stood out by recording the lowest mean temperature and the greatest thermal variability (17.18 ± 8.73 °C). Salinity remained within a narrow range at all stations, with mean values between 34.60 and 35.18 psu, indicating conditions typical of a coastal marine environment with limited continental influence.

Table 1: Descriptive statistics of the ecosystem's physicochemical variables.

Station	Temperature (°C)	Salinity (ups)	Transparency (m)	Dissolved O ₂ (mg L ⁻¹)	pH	Organic matter (%)
E-01	17.18 ± 8.73	35.02 ± 0.32	1.77 ± 0.23	3.32 ± 0.53	8.07 ± 0.04	5.20 ± 0.32
E-02	22.10 ± 1.71	34.88 ± 0.45	2.00 ± 0.18	3.10 ± 0.23	7.98 ± 0.09	3.90 ± 0.54
E-03	22.08 ± 1.54	34.60 ± 0.63	2.47 ± 0.46	3.18 ± 0.55	7.91 ± 0.11	2.67 ± 0.72
E-04	21.97 ± 1.81	35.08 ± 0.13	3.10 ± 0.27	3.17 ± 0.83	8.03 ± 0.08	3.00 ± 0.32
E-05	22.25 ± 1.55	35.18 ± 0.25	2.43 ± 0.31	3.40 ± 0.70	8.01 ± 0.09	2.23 ± 0.19
E-06	22.25 ± 1.32	34.98 ± 0.12	2.10 ± 0.18	3.02 ± 0.79	8.01 ± 0.06	1.70 ± 0.50

Note: Values are expressed as mean $\pm$ standard deviation, averaged across sampling seasons. Stations represent a spatial gradient of anthropogenic pressure within Tortugas Bay.

Water transparency exhibited a clear spatial gradient, with the lowest mean value recorded at E-01 (1.77 ± 0.23 m) and the highest at E-04 (3.10 ± 0.27 m). Dissolved oxygen means ranged from 3.02 to 3.40 mg L⁻¹, with no pronounced differences among stations, although E-05 showed the highest average value. pH varied only slightly across stations, remaining within a near-neutral to slightly alkaline range (7.91–8.07), which indicated relatively stable conditions within the system.

Total organic matter in sediments displayed the most pronounced spatial contrast, with significantly higher concentrations at E-01 ($5.20 \pm 0.32\%$) and a progressive decline towards E-06, where the lowest

value was observed ($1.70 \pm 0.50\%$). This pattern suggested marked spatial heterogeneity in sediment conditions, consistent with differences in organic material accumulation among stations.

Principal Component Analysis explained 57% of the total environmental variability, with the first component (PC1) accounting for 34% and the second (PC2) for 23% of the variance (Figure 2). PC1 was chiefly associated with organic matter content and pH, as well as salinity and dissolved oxygen, defining an environmental gradient that separated observations towards positive scores on the axis. By contrast, PC2 was primarily related to temperature and water transparency, differentiating observations along a vertical gradient.

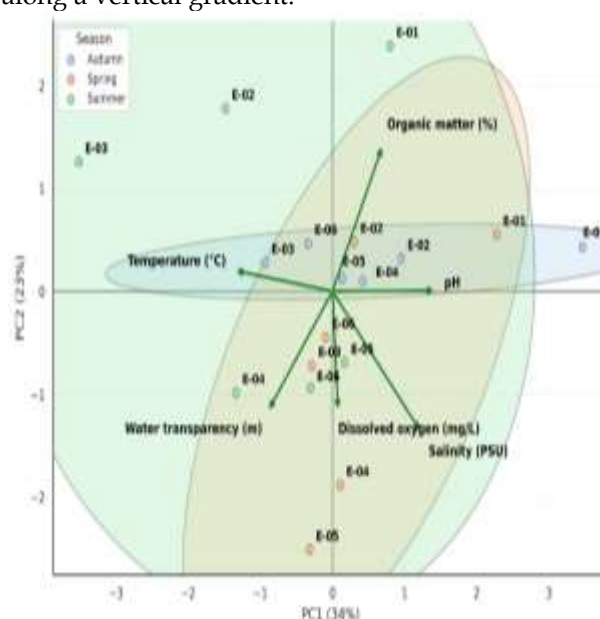


Figure 2. PCA biplot of environmental variables across stations.

The ordination revealed a partial overlap among observations from the different seasons; nevertheless, a moderate degree of spatial and temporal differentiation was evident. Observations associated with station E-01 were located predominantly at positive scores on PC1, indicating a stronger association with variables linked to organic enrichment. By contrast, observations from stations E-03 and E-04 tended to plot towards negative scores on both PC1 and PC2, aligning with higher water transparency and comparatively more stable environmental conditions.

The dispersion ellipses suggested substantial within-season variability, particularly during summer, whereas autumn and spring observations were more tightly clustered within the ordination space. Taken together, the PCA indicated that environmental variability in Tortugas Bay was shaped by

both spatial gradients among stations and a moderate seasonal signal.

Biological diversity indices exhibited contrasting spatial patterns in the structure of the macrobenthic community across the sampling stations (Table 2). Total abundance (N) reached its maximum at station E-01 (512 individuals), while the remaining stations displayed markedly lower values, ranging from 148 to 292 individuals. In contrast, species richness (S) varied more moderately among stations, spanning 16 to 21 species, with station E-04 recording the highest richness.

Table 2: Biological diversity indices by station.

Station	S (richness)	N (abundance)	Shannon (H')	Simpson (1-D)	Pielou (J')	Margalef
E-01	20	512	2.001	0.789	0.668	3.046
E-02	17	292	2.172	0.845	0.767	2.819
E-03	20	148	2.326	0.863	0.776	3.802
E-04	21	182	2.496	0.881	0.820	3.843
E-05	16	164	2.326	0.874	0.839	2.941

Note: S = species richness; N = total abundance. Diversity indices were calculated using pooled abundance data per station across sampling periods.

Shannon diversity (H') ranged from 2.001 to 2.496, with the lowest values observed at E-01 and the highest at E-04, indicating a progressive increase in diversity towards stations supporting more evenly structured assemblages. Consistently, Simpson's index (1-D) was high at all stations and reached its maximum at E-04, reflecting reduced species dominance in these areas.

Pielou's evenness (J') exhibited an increasing pattern from E-01 to E-05, with the lowest value recorded at E-01 and the highest values at E-04 and E-05, indicating communities with a more homogeneous distribution of abundances. Likewise, Margalef's index was higher at stations E-03 and E-04, in agreement with the patterns observed for species richness and overall diversity.

Figure 3 summarises the spatial variation in benthic community structure across the sampling stations, incorporating total abundance (Figure 3a), Shannon diversity (Figure 3b) and Pielou's evenness (Figure 3c). In terms of abundance, the highest value was recorded at E-01, followed by E-02, with a marked decline towards E-03 and broadly comparable values at E-04 and E-05 (Figure 3a). By contrast, both diversity and evenness displayed the opposite response, with minima at E-01 and increasing values towards the intermediate and outer stations, where the highest values were observed particularly at E-04 for Shannon (Figure 3b) and at E-05 for Pielou (Figure 3c).

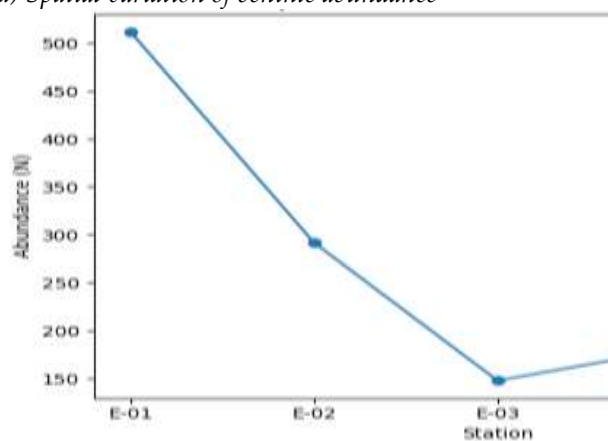
Figure 3. Spatial variation by station (E-01–E-05) of the macrobenthic community in Tortugas Bay: abundance (N), Shannon diversity (H') and Pielou's evenness (J').

This integrated pattern indicates that stations characterised by higher total abundance supported assemblages dominated by a limited number of species, whereas stations with lower abundance exhibited more evenly structured and comparatively diverse communities. In particular, stations E-04 and E-05 stood out for their higher evenness and diversity, reflecting a more homogeneous distribution of individuals among the species present.

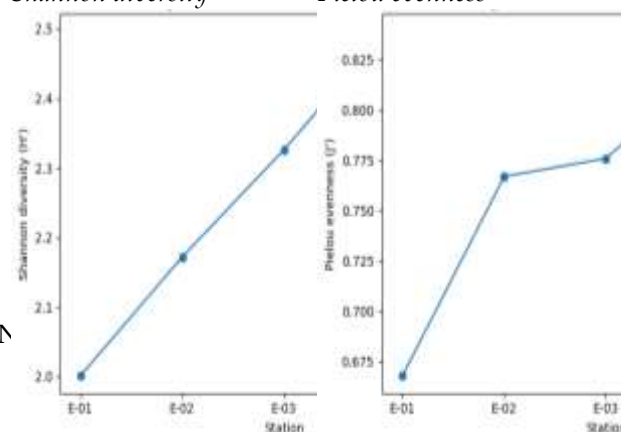
4. DISCUSSION

Table 1 showed significant differences in physicochemical parameters among stations, dominated by a clear gradient of sediment organic enrichment. Organic matter reached its maximum at E-01 ($5.20 \pm 0.32\%$) and declined progressively towards E-06 ($1.70 \pm 0.50\%$), whereas water transparency displayed the opposite pattern, with minimum values at E-01 (1.77 ± 0.23 m). This behaviour is characteristic of coastal systems subjected to anthropogenic pressure, where, as reported by Chen et al., (2025), sustained inputs of particulate and organic material, together with the retention of fine sediments, promote the

a) Spatial variation of benthic abundance



b) Spatial variation of Shannon diversity **c) Spatial variation of Pielou evenness**



accumulation of detritus on the seabed. Consistently, Wu *et al.*, (2022) and Silva *et al.*, (2025) indicated that these processes increase water-column turbidity and reduce clarity, thereby reinforcing the inverse relationship observed here between organic matter and transparency.

Dissolved oxygen remained at moderate and relatively homogeneous levels among stations ($\approx 3.02\text{--}3.40\text{ mg L}^{-1}$), suggesting elevated respiratory demand associated with organic-matter mineralisation, while not indicating pronounced hypoxia in mean values. According to Quintana *et al.*, (2026), this scenario is typical of moderately enriched environments, in which oxygen consumption intensifies at the sediment-water interface without necessarily producing persistent hypoxia in the water column. By contrast, salinity ($34.60\text{--}35.18\text{ psu}$) and pH ($7.91\text{--}8.07$) varied within narrow ranges, indicating that the principal environmental differences were not driven by hydrological variability but rather by local processes of deposition, resuspension and organic-matter degradation, as noted by Freitas *et al.*, (2023). Temperature exhibited high variability at E-01, likely reflecting the averaging of values across seasons; therefore, interpretation required caution, as emphasised by Grenier *et al.*, (2020) and Turner *et al.*, (2021) for studies integrating broad temporal windows.

Figure 2 summarised this environmental gradient via Principal Component Analysis. In the PCA, sediment organic matter and pH were associated with positive PC1 scores, whereas transparency and temperature projected in the opposite direction, indicating an inverse relationship between organic enrichment and water clarity. As Kaberi *et al.*, (2023) reported, this multivariate configuration is common in coastal systems where organic-matter gradients simultaneously structure sediment quality and water-column conditions. In this context, the position of E-01 reinforced its character as the station with the greatest organic accumulation, plausibly linked to higher anthropogenic pressure and/or reduced local hydrodynamic renewal, as described by Son *et al.*, (2025) for semi-confined coastal environments. Conversely, stations plotting towards negative PC1 values were associated with higher transparency, consistent with more open and better-flushed conditions, in line with findings reported by Teubner *et al.*, (2020), Zhou *et al.*, (2021) and Derui *et al.*, (2021). The overlap among seasons suggested temporal variability, albeit without a clear separation among seasonal groups, with the spatial gradient remaining the dominant signal within the

system.

The biological diversity indices (Table 2) were coherent with the identified environmental gradient. Station E-01, associated with higher organic-matter content and lower transparency, exhibited high total abundance but comparatively low diversity and evenness, suggesting dominance by a limited set of opportunistic taxa. According to Derui *et al.*, (2021) and (Jiao *et al.*, (2025), this pattern is typical of benthic communities under organic enrichment, in which the proliferation of tolerant species reduces community evenness. In contrast, stations plotting towards negative PC1 values (E-03 and E-04) displayed higher diversity, evenness and standardised richness, consistent with more stable environmental conditions and reduced stress, as described by Tsikopoulou *et al.*, (2019). The remaining stations showed intermediate values, reinforcing the existence of a continuous spatial gradient in which sediment organic enrichment acted as the principal driver structuring the community, in agreement with Xu *et al.*, (2022) and Parent *et al.*, (2021).

Overall, the concordance among physicochemical patterns, the PCA and biological indices confirmed that sediment organic enrichment constituted the primary control on benthic community structure in the study area, while seasonal variability played a secondary role relative to the dominant spatial signal. This pattern was consistent with that described for coastal ecosystems exposed to gradients of anthropogenic pressure and limited hydrodynamic renewal Corinaldesi *et al.*, (2022); Nandi & Pandey, (2025).

5. CONCLUSIONS

It was established that the environmental status of Tortugas Bay was driven primarily by a spatial gradient of sediment organic enrichment, which governed both the variability of physicochemical parameters and the structure of the macrobenthic community, producing stations with high abundance and low diversity associated with greater anthropogenic pressure, alongside stations supporting more diverse and more evenly structured assemblages linked to comparatively stable environmental conditions; the integration of biological indices and multivariate analyses enabled this gradient to be characterised consistently and provided a robust scientific basis for monitoring and environmental management of the system.

Within the physicochemical component, the water column exhibited relative stability, with salinity constrained to a narrow range, pH showing limited

variation (7.91–8.07) and dissolved oxygen remaining at moderate levels ($3.02\text{--}3.40\text{ mg L}^{-1}$); by contrast, transparency and total organic matter provided the most pronounced spatial signal, with minimum transparency at E-01 ($1.77 \pm 0.23\text{ m}$) and maximum clarity at E-04 ($3.10 \pm 0.27\text{ m}$), alongside higher organic concentrations at E-01 ($5.20 \pm 0.32\%$) and a decline towards E-06 ($1.70 \pm 0.50\%$).

The biological response was congruent with this gradient: total abundance peaked at E-01 (512 individuals), yet this station showed the lowest diversity and evenness ($H' = 2.001$; $J' = 0.668$), whereas E-04 and E-05 exhibited more balanced and more diverse assemblages (H' up to 2.496; J' up to 0.839), indicating dominance by a restricted set of species at sites under greater pressure and a more

homogeneous distribution where conditions were comparatively stable.

Taken together, the evidence supported the working hypothesis that anthropo-genic pressure expressed as sediment organic enrichment simultaneously structured the environmental signal and the organisation of the macrobenthic community; on this basis, a baseline suitable for monitoring and management was consolidated, and future research was framed around strengthening multi-year time series, incorporating con-tamination tracers, undertaking functional analyses of the biota, and explicitly inte-grating local hydrodynamic dynamics to identify ecological thresholds and improve the predictive capacity of the diagnosis.

Author Contributions: The authors confirm their contribution to the paper as follows: Conceptualisation: V.G.-N., L.T.-C. and J.M.-M.; Methodology: V.G.-N., L.T.-C. and Y.A.-R.; Validation: L.T.-C., J.M.-M. and E.S.-V.; Formal analysis: V.G.-N., L.T.-C. and S.M.-V.; Investigation: V.G.-N., Y.A.-R., E.S.-V. and M.V.-G.; Resources: L.T.-C. and J.M.-M.; Data curation: V.G.-N., Y.A.-R. and M.V.-G.; Writing – original draft preparation: V.G.-N.; Writing – review and editing: L.T.-C., J.M.-M., E.S.-V. and S.M.-V.; Visualisation: V.G.-N. and E.S.-V.; Supervision: L.T.-C. and J.M.-M.; Project administration: L.T.-C.; Funding acquisition: L.T.-C. and J.M.-M.

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