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RESEARCH ARTICLE

Seasonal contrast among bacterial, trophic, and biotic indicators in a multi-use coastal protected area

Pelin Saliha Çiftçi Türetken[1] · Menekşe Didem Demircan Akyasan[2] · Eylül Balcıoğlu[3] · Onur Gönülal[1] · Deniz Devrim Tosun[4] · Bayram Öztürk[1]

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Abstract

Monitoring programs in coastal ecosystems commonly combine microbial, trophic, and biotic indicators under the assumption that they provide a consistent picture of environmental status. Yet these indicators reflect processes operating at different temporal scales and may therefore respond differently to human pressures. In this study, we evaluated the responses of bacterial, trophic, and benthic assessment tools in a multi-use coastal protected area in the Eastern Aegean Sea (Karaburun–Ildır Special Environmental Protection Area (SEPA), Türkiye), where tourism and aquaculture coexist. Surface and bottom waters, together with benthic sediments, were sampled seasonally (May and September, 2022–2023) at stations representing aquaculture, tourism, and reference conditions. Bacterial indicators (fecal coliforms and intestinal enterococci) showed significant seasonal and activity-related variation ($p < 0.001$), reflecting periods of intensified human use. The detection of *Salmonella* increased during peak activity, pointing to episodic sanitary risks that were not captured by trophic or benthic metrics. By contrast, trophic index (TRIX) values were mainly shaped by seasonal dynamics and did not differ significantly among activity types. Similarly, benthic indices (BEnthic IndeX [BENTIX], MEDiterranean COastal Classifier [MEDOCC], Turkish Biotic Index [TUBI], Shannon diversity) remained relatively stable across space and time. These results indicate a divergence in seasonal responses between rapidly responding microbial indicators and more stable trophic and benthic assessments. The divergence does not imply methodological inconsistency; rather, it reflects the different ecological time scales represented by each indicator group. Our findings highlight the need for monitoring approaches that integrate short-term sanitary signals with longer-term ecological assessments in multi-use coastal systems.

✉ Pelin Saliha Çiftçi Türetken
pciftci@istanbul.edu.tr

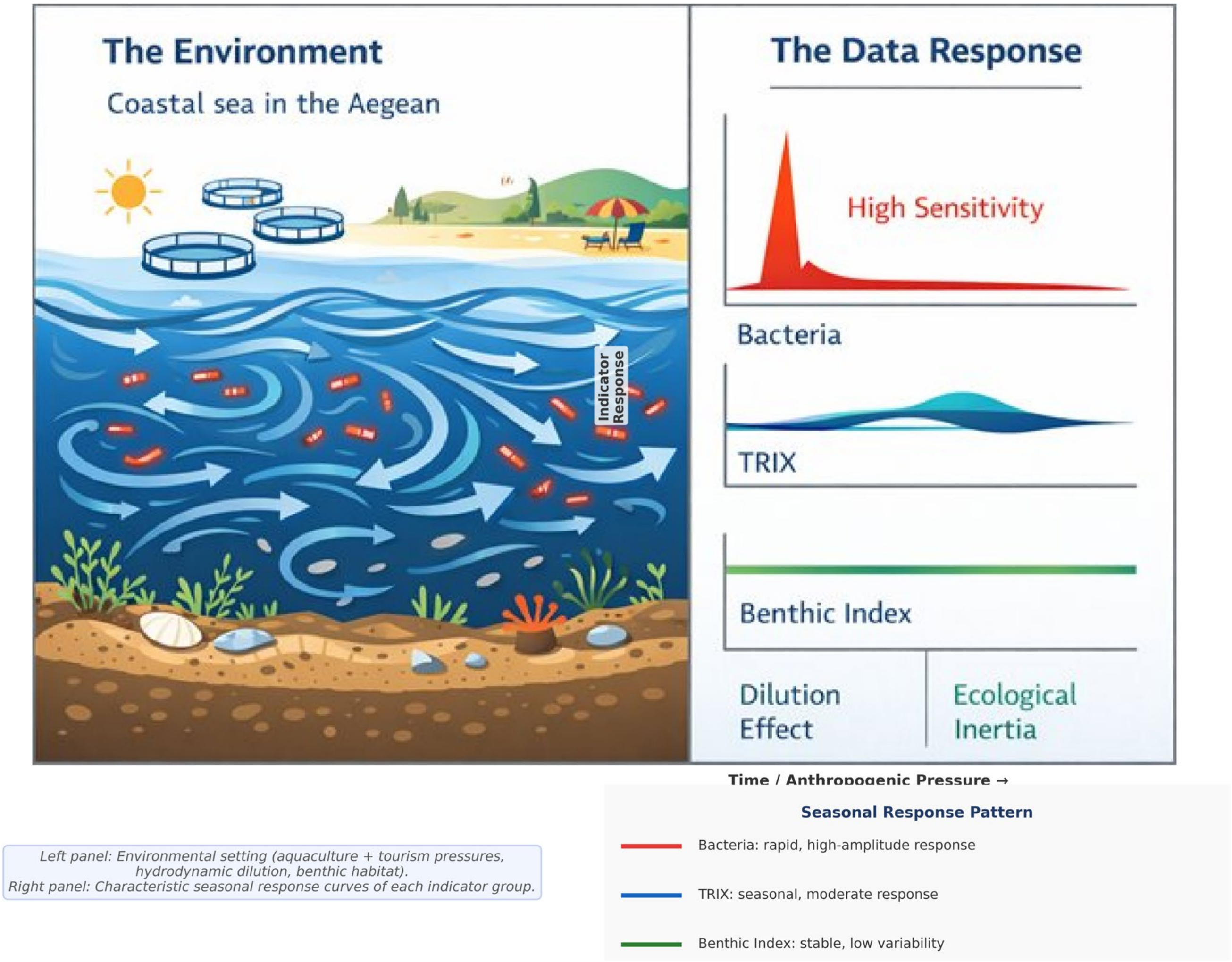
[1] Department of Marine Biology, Faculty of Aquatic Sciences, Istanbul University, Istanbul, Türkiye

[2] Department of Fish Disease, Faculty of Aquatic Sciences, Istanbul University, Istanbul, Türkiye

[3] Fish Diseases Programme, Institute of Graduate Studies in Sciences, Istanbul University, Istanbul, Türkiye

[4] Department of Aquaculture, Faculty of Aquatic Sciences, Istanbul University, Istanbul, Türkiye

Graphical abstract



Highlights

- Bacterial indicators exhibit high sensitivity to pulse pressures from tourism and aquaculture.
- Trophic (TRIX) and biotic indices show ecological inertia, masking short-term anthropogenic stress.
- Hydrodynamic dilution buffers nutrient loads, limiting the utility of standard trophic indices.
- *Salmonella* emergence during peak seasons suggests potential concerns for the Blue Economy.
- Multi-purpose Special Environmental Protection Areas (SEPAs) require a tiered monitoring strategy integrating different time-scales.

Keywords Bacterial water quality · *Salmonella* · Fecal indicator bacteria · Trophic status (TRIX) · Biotic indices · Multi-use SEPA · Coastal management

Introduction

The designation of coastal ecosystems as protected areas seeks a delicate equilibrium between the preservation of biodiversity and the accommodation of regulated human activities, such as tourism and aquaculture (Banarsyadhimi et al. 2022). This dual mandate necessitates not only a robust conservation framework but also a sophisticated understanding of how diverse anthropogenic pressures intersect with ecosystem functions (Sala et al. 2021).

Consequently, monitoring and assessment protocols have emerged as the primary instruments for determining both the efficacy of conservation efforts and the thresholds for sustainable use (Claudet et al. 2020). Traditionally, the ecological integrity of coastal protected areas—particularly in the nutrient-poor, oligotrophic waters of the Mediterranean—has been gauged through nutrient-based trophic indices and benthic ecological indicators (Karydis and Kitsiou 2013). These frameworks have proven effective in documenting eutrophication trends and long-term structural shifts in macroinvertebrate communities (Torriente et al. 2019; Tugrul et al. 2019; Oprandi et al. 2023).

However, a significant gap remains in our understanding of multipurpose coastal systems where tourism and aquaculture are seasonally intensified. In these dynamic environments, ecosystem stressors are not confined to gradual, long-term processes; rather, they often manifest as short-term, use-dependent, and episodic disturbances that traditional frameworks may fail to capture (Dauvin 2007). Bacterial water quality indicators, for instance, are highly sensitive to wastewater discharges, localized organic enrichment, and direct human presence, offering a rapid response to anthropogenic stressors. Such indicators can illuminate pressure patterns—including the emergence of opportunistic pathogens—that remain obscured in nutrient-based or benthic assessments. This mismatch between rapid bacterial responses and more inert ecological indicators introduces a source of uncertainty in environmental risk assessment for protected coastal systems under multi-use pressure (Dauvin 2007; Orel et al. 2022).

Such integrated, multi-indicator approaches have been applied in selected coastal systems globally. Oprandi et al. (2023) demonstrated that multiple ecological indices applied to different habitats within a Mediterranean Marine Protected Areas (MPA) provided largely congruent assessments of environmental quality, suggesting convergence among indicator groups under stable conditions. Similarly, Kucuksezgin et al. (2019) combined fecal indicator bacteria with trophic assessments in the Eastern Aegean, revealing that bacterial and trophic metrics capture different dimensions of coastal water quality. However, studies that simultaneously integrate microbial, trophic, and benthic indicators across a gradient of overlapping anthropogenic pressures—including both tourism and aquaculture—within a formally designated protected area remain scarce, particularly in the Eastern Mediterranean context (Orel et al. 2022; Ben-Haddad et al. 2023).

This discrepancy raises an important question for coastal governance: do bacterial, trophic, and biotic indicators respond coherently to overlapping anthropogenic pressures in multi-use coastal protected areas, or do their divergent temporal response scales introduce uncertainty into integrated environmental assessments? Failure to resolve this question may contribute to an underestimation of microbial risks, with potential implications for both ecosystem resilience and public health. Ignoring these transient but significant bacterial pressures, particularly within areas of high conservation value, may widen the disconnect between statutory conservation goals and the socioeconomic reality of environmental use. This is especially pertinent given that microbial contamination can jeopardize the "Blue Economy" by impacting recreational safety and the sanitary quality of seafood production (Vikas and Dwarakish 2015). From a risk-based management perspective, the absence of short-term sanitary signals in conventional assessment outcomes may generate a false sense of environmental security.

Among anthropogenic drivers, tourism and aquaculture represent two of the most widespread yet distinct pressures in many coastal environments. Seasonal tourism exerts a sudden burden on local wastewater infrastructure, often leading to elevated fecal pollution through discharge and recreational contact (Moschino et al. 2017; Ben-Haddad et al. 2023). Conversely, aquaculture introduces a more localized and persistent organic load, where metabolic waste can trigger localized shifts in trophic dynamics (Sarà 2007). In the Karaburun–Ildır Special Environmental Protected Area (SEPA), aquaculture represents a structurally well-defined and continuous activity, with multiple licensed fish farms operating within the bay, as documented by recent technical assessments of farm distribution, capacity, and operational characteristics (Tosun et al. 2024). While both stressors have been studied in isolation, their synergistic impact on protected coastal components—and the capacity of different monitoring tools to resolve these impacts—remains largely unexplored.

The Karaburun–Ildır Special Environmental Protection Area (SEPA) serves as a unique "natural laboratory" to address these uncertainties. As a region where strict protection status coexists with heterogeneous patterns of tourism and intensive aquaculture, it offers an ideal setting for a comparative evaluation of assessment tools. The ecological relevance of the Karaburun–Ildır SEPA is further supported by recent macrofaunal assessments reporting a high species richness, including 121 fish and 58 invertebrate taxa, indicating a biologically diverse system of conservation concern (Keskin et al. 2023). The primary aim of this study was to assess the responses of bacterial, trophic, and biotic indicators to spatial and seasonal variability across a gradient of anthropogenic pressure—ranging from intensive aquaculture and tourism zones to offshore reference conditions—within the Karaburun–Ildır SEPA. Specifically, we sought to determine whether these indicator groups respond coherently to the same pressures, or whether their divergent temporal response scales affect the interpretation of environmental status in multi-use protected areas.

Ultimately, we hypothesize that bacterial water quality indicators will exhibit a higher sensitivity to immediate anthropogenic inputs compared with the more buffered responses of trophic and biotic metrics. Distinguishing these response patterns may support the development of more integrated and risk-aware monitoring approaches for multipurpose coastal protected areas.

Materials and methods

Study area and sampling strategy

The study was conducted along the Karaburun Peninsula in the eastern Aegean Sea, a region characterized by complex hydrography positioned between İzmir Bay and the Çeşme Channel. The focal point of this research, the Karaburun–Ildır SEPA, was designated in 2019 and encompasses a marine domain of approximately 503 km^2 with depths extending to 95 m.

To capture the spatial heterogeneity of anthropogenic pressures, a network of 20 sampling stations was established across the SEPA (Table 1; Fig. 1). The station network was stratified into three impact categories based on predominant human activities. Aquaculture-impacted zones (five stations) were located in the immediate vicinity of active fish farms (S1, S17, S18, S19, and S20). Tourism-impacted zones (nine stations) were situated in nearshore areas subject to seasonal recreational pressure and discharge (S2, S3, S4, S5, S7, S8, S14, S15, and S16). Reference zones (six stations) were subdivided into coastal reference sites (S11, S12, and S13) with minimal land-based influence and offshore reference sites (S6, S9, and S10) representing open-water conditions.

Sampling campaigns were conducted seasonally during May (onset of tourism) and September (peak/end of tourism and harvest season) of 2022 and 2023. This temporal design was chosen to capture the contrasting conditions between the onset (May) and peak/end (September) of human activity, hereafter referred to as seasonal contrasts. We acknowledge that this biannual sampling scheme represents a limited temporal resolution and does not permit robust inference about high-frequency variability or episodic contamination events; these constraints are discussed as limitations in Sect. 4.

Sample collection and physicochemical measurements

Water samples were collected from surface (0–30 cm) and bottom layers using Nansen sampling bottles (Hydro-Bios, Germany) onboard the R/V Yunus-S. Vertical profiles of physicochemical parameters—including temperature, salinity, dissolved oxygen (DO), and pH—were recorded in situ using a calibrated CTD profiler (Sea-Bird SBE 19plus V2).

Table 1 Geographic coordinates, depths, and classification of sampling stations in the Karaburun–Ildır Special Environmental Protection Area (SEPA)

Station No	Latitude (N)	Longitude (E)	Depth (m)	Activity Type
S1	38° 27565	26° 37407	27	Aquaculture
S2	38° 29906	26° 38331	12.5	Tourism Activity
S3	38° 32044	26° 37235	15	Tourism Activity
S4	38° 33750	26° 34494	20	Tourism Activity
S5	38° 36295	26° 33835	29	Tourism Activity
S6	38° 37212	26° 38348	70	Reference (offshore)
S7	38° 37632	26° 32297	45	Tourism Activity
S8	38° 39278	26° 31456	44	Tourism Activity
S9	38° 43972	26° 38829	75	Reference (offshore)
S10	38° 42661	26° 31354	95	Reference (offshore)
S11	38° 40707	26° 26095	48	Reference (coastal)
S12	38° 40481	26° 23551	70	Reference (coastal)
S13	38° 38079	26° 20708	53	Reference (coastal)
S14	38° 36067	26° 21023	58	Tourism Activity
S15	38° 31359	26° 22072	40	Tourism Activity
S16	38° 29145	26° 23880	75	Tourism Activity
S17	38° 28987	26° 22553	80	Aquaculture
S18	38° 26738	26° 23075	74	Aquaculture
S19	38° 24709	26° 22354	65	Aquaculture
S20	38° 27066	26° 26710	56	Aquaculture

Each station was sampled four times: May 2022, September 2022, May 2023, and September 2023

For bacteriological analysis, water samples were drawn into sterile amber glass bottles under strict aseptic conditions and processed within 4–6 h of collection. Samples for nutrient and chlorophyll-*a* (Chl-*a*) analyses were collected in acid-cleaned high-density polyethylene (HDPE) bottles. Chl-*a* samples were immediately filtered on board through Whatman GF/C glass fiber filters. All chemical samples were stored at −20 °C in the dark until laboratory analysis.

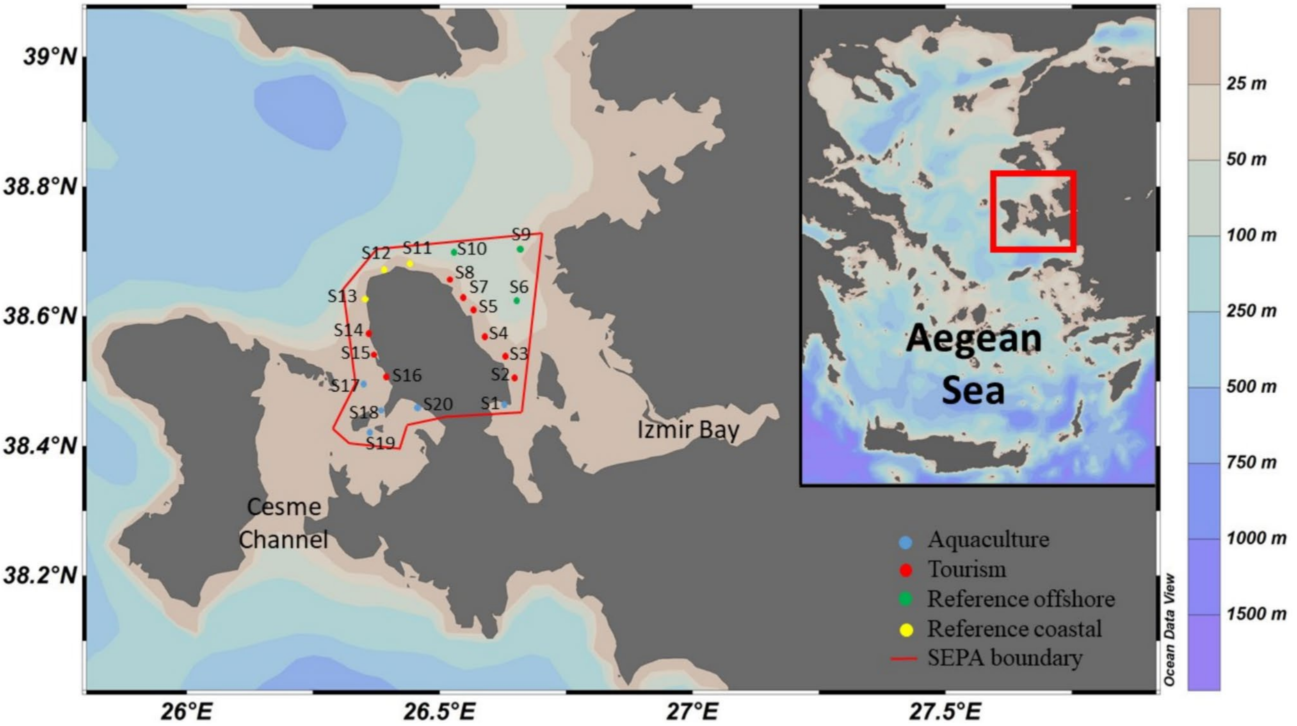


Fig. 1 Sampling stations in the Karaburun–Ildır Special Environmental Protection Area (SEPA). Symbols are color-coded by activity type: blue = aquaculture; red = tourism; green = reference offshore; yellow = reference coastal. The red polygon indicates the SEPA boundary (Schlitzer, Reiner, Ocean Data View, https://odv.awi.de, 2023). For station coordinates and depths, see Table 1

Benthic surveys were performed at a subset of 10 representative stations using a Petersen grab (0.1 m^2 surface area). Three replicate grabs were taken at each station from soft-bottom substrates (depth range: 15–75 m). Sediment samples were sieved through a 1 mm mesh sieve, and the retained material was fixed in situ with 4% buffered formaldehyde solution, following standard benthic sampling protocols (Eleftheriou and Moore 2013).

Laboratory analyses

Nutrient and chlorophyll-*a* analysis

Dissolved inorganic nutrient concentrations were determined spectrophotometrically following standard methods (APHA 2017). Nitrate nitrogen (NO_3^--N) was analyzed via the Cadmium Reduction Method (APHA 4500-NO_3^- E), nitrite nitrogen (NO_2^--N) via the Colorimetric Method (APHA 4500-NO_2^- B), and ammonium nitrogen (NH_4^+-N) via the Phenate Method (APHA 4500-NH_3 F). Total phosphorus (TP) underwent persulfate digestion (APHA 4500-P B) prior to quantification using the Ascorbic Acid Method (APHA 4500-P E). Chlorophyll-*a* extraction and quantification were performed according to APHA Method 10,200-H.

Bacteriological analysis

Microbial indicators were quantified using membrane filtration techniques. For Total Heterotrophic Aerobic Bacteria (THAB), serial dilutions were plated on Marine Agar (Difco) and incubated at 22 ± 1 °C for 72–96 h. Fecal pollution indicators—Total Coliform (TC), Fecal Coliform (FC), and Intestinal Enterococci (IE)—were isolated by filtering 100 mL aliquots through sterile 0.45 µm membrane filters. Filters were placed on specific nutrient pads: Endo-NKS for TC (37 ± 1 °C), m-FC-NKS for FC (44.5 ± 1 °C), and Azide-NKS for IE (44.5 ± 1 °C), using Sartorius media types. Colonies were enumerated after 24–48 h incubation. The presence of *Salmonella* spp. was assessed by filtration onto Bismuth Sulfite Agar (Sartorius), incubated at 37 ± 1 °C for 48 h, with presumptive positive colonies confirmed following standard biochemical protocols (APHA 2022). *Salmonella* results are reported as presence/absence per 100 mL; quantitative enumeration and microbial source tracking (MST) were not performed, which represents a limitation in attributing contamination to specific sources.

Presumptive coliform colonies were confirmed using the cytochrome oxidase test (bioMérieux); oxidase-negative colonies were included in the enumeration. Presumptive fecal coliform colonies were subjected to both cytochrome oxidase (bioMérieux) and indole (HiMedia) tests; colonies yielding oxidase-negative and indole-positive results were confirmed as fecal coliforms. All bacteriological analyses were performed in triplicate, and results are reported as the mean of three replicates (CFU/100 mL). Sterile negative controls (membrane-filtered autoclaved seawater) were processed alongside each sample batch to ensure the absence of contamination.

Benthic Taxonomy

In the laboratory, macrozoobenthic organisms were sorted, counted, and identified to species level in collaboration with specialists using stereomicroscopes. Taxonomy was verified against the World Register of Marine Species (WoRMS). Biotic indices were calculated using established formulae: Shannon–Wiener diversity index (Shannon and Weaver 1949), BENTIX (Simboura and Zenetos 2002), and MEDOCC (Pinedo et al. 2012). The national database developed within the DEKOS (2014) project was employed to resolve discrepancies in species-to-ecological-group assignments between index classifications.

Data analysis and indices

To account for vertical stratification, surface and bottom water datasets were analyzed separately. The Shapiro–Wilk test was used to assess data normality; bacterial counts were $\log_{10}(x+1)$ transformed to satisfy parametric assumptions.

Ecological Indices: ecological status was assessed using a combination of trophic and biotic indicators selected to capture different temporal dimensions of environmental response.

Trophic Status was evaluated using the TRIX index (Vollenweider et al. 1998), which integrates Chl-*a*, oxygen saturation, dissolved inorganic nitrogen, and total phosphorus into a single trophic quality score. TRIX was selected as it is specifically validated for Mediterranean coastal waters and provides a basin-scale assessment of eutrophication. The index ranges from 0 to 10, where values below 4 indicate oligotrophic to mesotrophic conditions (good–moderate quality), values between 4 and 6 indicate eutrophic conditions (poor quality), and values above 6 indicate hypereutrophic conditions (bad quality) (Vollenweider et al. 1998).

Biotic quality was assessed using the Shannon–Wiener diversity index (H'), alongside the biotic indices BENTIX (Simboura and Zenetos 2002), MEDOCC, and TUBI (Çınar et al. 2015). These indices were selected because they are specifically validated for Mediterranean soft-bottom benthic communities and are sensitive to organic enrichment over longer time scales. BENTIX ranges from 0 to 6, where values above 4.5 indicate good ecological status and values below 2 indicate poor status (Simboura and Zenetos 2002). MEDOCC and TUBI range from 1 to 5, where lower values indicate better ecological conditions (Çınar et al. 2015). The Shannon–Wiener index (H') was calculated as a measure of species diversity; values above 3 generally indicate high diversity, while values below 1 suggest degraded community structure.

Statistical testing: prior to analysis, the Shapiro–Wilk test was applied to assess normality within each group × season combination. Since most variables—particularly bacterial counts—violated normality assumptions, nonparametric approaches were prioritized. Differences across activity groups and between seasons were tested using the Kruskal–Wallis test, followed by Dunn's post-hoc test with Bonferroni correction. Where normality was confirmed, one-way ANOVA with Tukey's HSD post-hoc test was additionally applied. To evaluate the simultaneous effects of season and activity group on bacterial community structure, PERMANOVA (Anderson 2001) was applied based on Bray–Curtis dissimilarities computed on $\log(x+1)$-transformed bacterial counts, with 999 permutations. Principal component analysis (PCA) was performed on all standardized variables to explore multivariate gradients across indicator groups. Variance partitioning was applied to decompose the unique and shared contributions of season and activity group to bacterial community variation. Spearman rank correlations (ρ) were calculated between log-transformed bacterial indicators (THAB, TC, FC, IE) and environmental variables (DO, pH, Temp, NO_2, NO_3, NH_4, TP, Chl-*a*) to explore cross-indicator relationships. *Salmonella* occurrence, being a binary presence/absence variable, was not included in the correlation analysis. All analyses were performed in R (v.4.3.2) using the vegan, ggplot2, and scikit-posthocs packages (Wickham 2016; Oksanen et al. 2022).

Results

Physicochemical environment

The physicochemical characteristics of the study area exhibited typical seasonal dynamics characteristic of the Eastern Aegean Sea (Table 2). While salinity remained remarkably homogeneous across all stations and depths (39.23–39.47 psu), water temperature reflected distinct seasonal variation across all activity groups, with September surface waters significantly warmer (24.33 ± 1.16 °C) than May surface waters (18.53 ± 1.16 °C) (Kruskal–Wallis: $H = 51.83$, $p < 0.001$). Thermal stratification between

Table 2 Values represent means ± SD (standard deviation) pooled across both sampling years (2022 and 2023) and both seasons (May and September) for each activity group and depth

Group	Depth	Temp (°C) mean ± SD	Sal (psu) mean ± SD	DO (mg/L) mean ± SD	pH mean ± SD
Tourism	Surface	21.47 ± 3.22	39.42 ± 0.15	4.58 ± 0.58	8.26 ± 0.19
Tourism	Bottom	18.68 ± 2.53	39.30 ± 0.14	5.56 ± 0.74	8.23 ± 0.12
Aquaculture	Surface	21.50 ± 3.00	39.47 ± 0.25	4.60 ± 0.69	8.22 ± 0.14
Aquaculture	Bottom	17.50 ± 1.40	39.26 ± 0.04	5.39 ± 0.55	8.23 ± 0.22
Reference offshore	Surface	21.40 ± 3.48	39.46 ± 0.21	4.57 ± 0.57	8.24 ± 0.13
Reference offshore	Bottom	17.22 ± 1.35	39.24 ± 0.05	5.74 ± 0.74	8.31 ± 0.26
Reference coastal	Surface	21.22 ± 3.05	39.37 ± 0.07	4.82 ± 0.79	8.2 ± 0.14
Reference coastal	Bottom	17.37 ± 0.79	39.23 ± 0.06	5.92 ± 0.70	8.18 ± 0.14

Seasonal differences in surface water temperature between May and September were statistically significant (Kruskal–Wallis: $H = 51.83$, $p < 0.001$). Surface–bottom thermal stratification was significant in both seasons ($p < 0.001$)

Table 3 Mean values ± SD (standard deviation) of nutrient and chlorophyll-*a* concentrations in surface and bottom waters across activity groups (aquaculture, tourism, reference offshore, reference coastal) in the Karaburun–Ildır SEPA

Group	Depth	Chl-*a* (µg/L) mean ± SD	NH_4^+-N (mg/L) mean ± SD	NO_2^--N (mg/L) mean ± SD	NO_3^--N (mg/L) mean ± SD	TP (mg/L) mean ± SD
Tourism	Surface	1.38 ± 1.89	0.20 ± 0.17	0.05 ± 0.03	5.22 ± 2.20	0.33 ± 0.17
Tourism	Bottom	1.45 ± 2.38	0.29 ± 0.48	0.05 ± 0.05	5.68 ± 4.36	0.32 ± 0.24
Aquaculture	Surface	1.97 ± 3.64	0.30 ± 0.39	0.05 ± 0.02	5.02 ± 1.84	0.56 ± 0.31
Aquaculture	Bottom	1.05 ± 0.96	0.17 ± 0.11	0.03 ± 0.02	4.31 ± 0.81	0.61 ± 0.37
Reference offshore	Surface	1.26 ± 1.24	0.14 ± 0.07	0.04 ± 0.02	4.41 ± 0.54	0.34 ± 0.19
Reference offshore	Bottom	1.20 ± 1.26	0.13 ± 0.07	0.03 ± 0.02	4.43 ± 0.75	0.35 ± 0.26
Reference coastal	Surface	2.69 ± 5.01	0.26 ± 0.22	0.05 ± 0.03	6.03 ± 2.90	0.32 ± 0.16
Reference coastal	Bottom	0.89 ± 0.53	0.45 ± 0.64	0.07 ± 0.07	6.92 ± 5.99	0.30 ± 0.22

Values represent means pooled across both sampling years (2022 and 2023) and both seasons (May and September)

Kruskal–Wallis test results for group comparisons (aquaculture versus tourism versus reference offshore versus reference coastal): Chl-*a* ($H = 0.97$, $p = 0.810$, ns), NH_4^+-N ($H = 9.24$, $p = 0.026$, *), NO_2^--N ($H = 5.05$, $p = 0.168$, ns), NO_3^--N ($H = 6.76$, $p = 0.080$, ns), TP ($H = 14.82$, $p = 0.002$, **). For significant parameters, Dunn's post-hoc test (Bonferroni correction) revealed the following pairwise differences: TP—aquaculture versus reference offshore ($p = 0.022$, *), aquaculture versus reference coastal ($p = 0.004$, *). Significance levels: ns = not significant; *$p < 0.05$; **$p < 0.01$

surface and bottom layers was statistically significant in both seasons (May: $H = 39.31$, $p < 0.001$; September: $H = 45.24$, $p < 0.001$), with surface waters consistently warmer than bottom waters throughout the study period. Dissolved oxygen (DO) levels followed an inverse pattern to temperature; surface waters consistently displayed lower concentrations compared with the well-oxygenated bottom layers across all activity groups. Notably, pH values showed high stability (8.22–8.26) with negligible spatial fluctuation, suggesting a well-buffered system with limited sensitivity to local acidification under the observed anthropogenic conditions.

Nutrient concentrations and chlorophyll-*a*

Inorganic nutrient and chlorophyll-*a* (Chl-*a*) concentrations revealed subtle spatial gradients (Table 3). Chl-*a* values were moderately elevated in surface waters of aquaculture and tourism zones compared with reference sites, though standard deviations indicated high variability within groups. No significant spatial pattern was observed in Chl-*a* concentration ($p = 0.880$). Nitrogen speciation was dominated by nitrate (NO_3^--N) across the water column. Ammonium (NH_4^+-N), a potential indicator of fresh organic input, appeared marginally higher in the surface waters of impacted zones (tourism and aquaculture), whereas nitrite (NO_2^--N) remained at near-background levels throughout the SEPA. TP concentrations appeared to be highest at aquaculture-impacted stations, suggesting

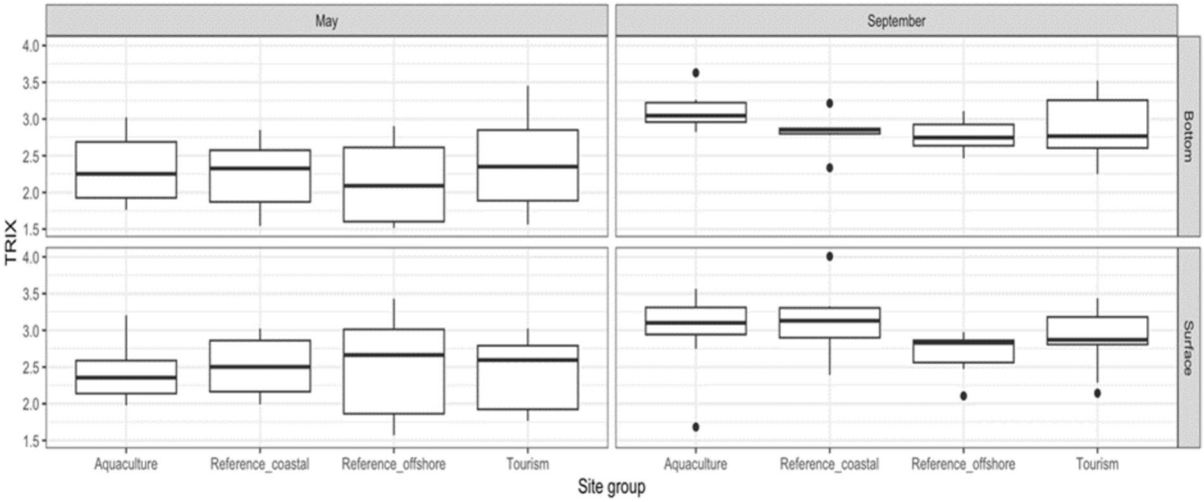


Fig. 2 Distribution of TRIX index values stratified by season (May versus September), depth (Surface versus Bottom), and activity type. Boxplots illustrate the comparative trophic status of aquaculture, tourism, and reference stations within the study area

localized nutrient enrichment associated with fish farming activities.

Trophic status (TRIX)

The TRIX index, integrating dissolved inorganic nutrients and phytoplankton biomass (as Chl-*a*) data, ranged between 1.5 and 4.0, generally characterizing the area as oligotrophic to mesotrophic (Fig. 2). Contrary to the expected pressure gradient, TRIX values displayed a high degree of overlap among aquaculture, tourism, and reference sites. In May, no consistent separation was observed between site groups in either surface or bottom layers. In September, while index values slightly increased in surface waters, the distributions remained broadly similar across activity types. The TRIX index was not designed to resolve point-source pollution gradients at the station level; rather, it integrates nutrient and biomass signals across broader spatial and temporal scales relevant to basin-level eutrophication (Vollenweider et al. 1998). ANOVA revealed that TRIX was strongly driven by seasonal dynamics ($F = 81.46$, $p < 0.001$), but showed no significant differentiation among activity groups ($F = 0.814$, $p = 0.487$), confirming the scale mismatch discussed above. The absence of clear TRIX differentiation among activity zones therefore reflects a scale mismatch between the indicator's design criteria and the localized, episodic nature of the pressures examined, rather than an inherent limitation of the index itself.

Bacterial water quality and *Salmonella* occurrence

In sharp contrast to physicochemical and trophic markers, bacterial indicators revealed a distinct footprint of anthropogenic pressure (Fig. 3). Levels of Fecal Coliforms (FC) and Total Coliforms (TC) were significantly amplified in tourism and aquaculture zones compared with reference sites. This divergence was most pronounced during the September sampling campaign, coinciding with the cumulative peak of the tourist season and aquaculture harvesting. Although median values for Intestinal Enterococci (IE) remained generally low, sporadic outliers (spikes) were detected exclusively at impacted sites, pointing to episodic contamination events. Statistical analysis confirmed these patterns: bacterial indicators showed highly significant effects of both season ($F = 16.25$, $p < 0.001$) and activity type ($F = 7.75$, $p < 0.001$), along with a significant depth-dependent response ($F = 7.32$, $p = 0.015$).

The detection frequency of *Salmonella* spp. showed a striking seasonal amplification (Fig. 4). While distinct in May, the positivity rate surged in September, with impacted zones (tourism/aquaculture) exhibiting substantially higher detection frequencies compared with the pristine reference stations.

Biotic indices

Benthic community integrity across the SEPA was generally characterized by "Good" to "Moderate" ecological status (Table 4), with limited differentiation between impacted and reference zones. This pattern is consistent with the long-term integrative nature of macrobenthic indices, which reflect cumulative rather than episodic disturbances.

Station S14 (tourism) stood out with consistently low Shannon–Wiener diversity ($H' = 1.82$–1.86) and elevated MEDOCC scores (5.10–5.30) across all campaigns, pointing to relatively degraded benthic conditions at this nearshore

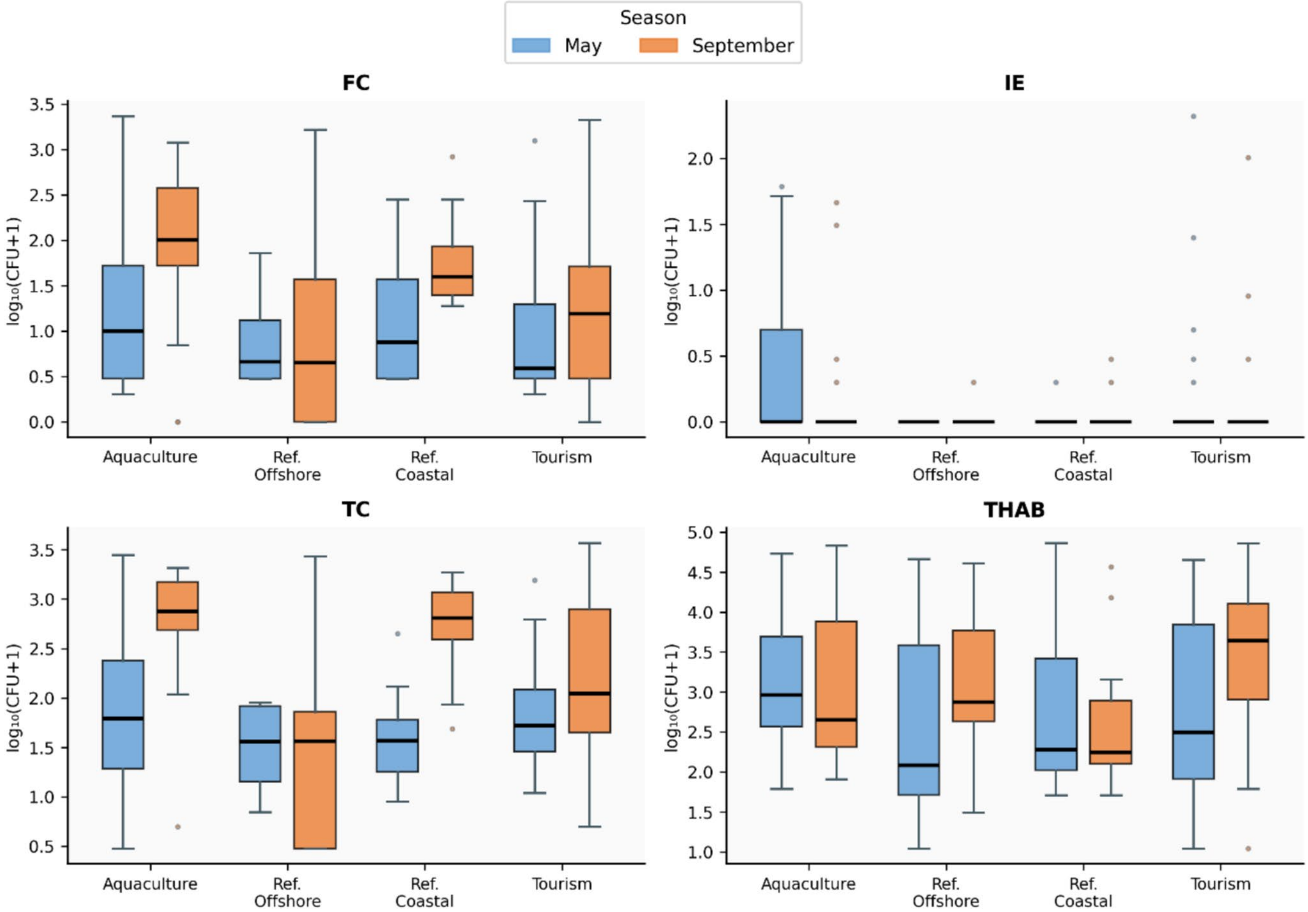


Fig. 3 Variation of bacterial indicators across site groups in the Karaburun–Ildır SEPA. FC: Fecal Coliform; IE: intestinal Enterococci; TC: total coliform; THAB: total heterotrophic aerobic bacteria. Values are $\log_{10}$(CFU + 1) transformed. Blue = May; orange = September

Fig. 4 Percentage of *Salmonella*-positive samples across site groups during May and September in the Karaburun–Ildır Special Environmental Protection Area (SEPA)

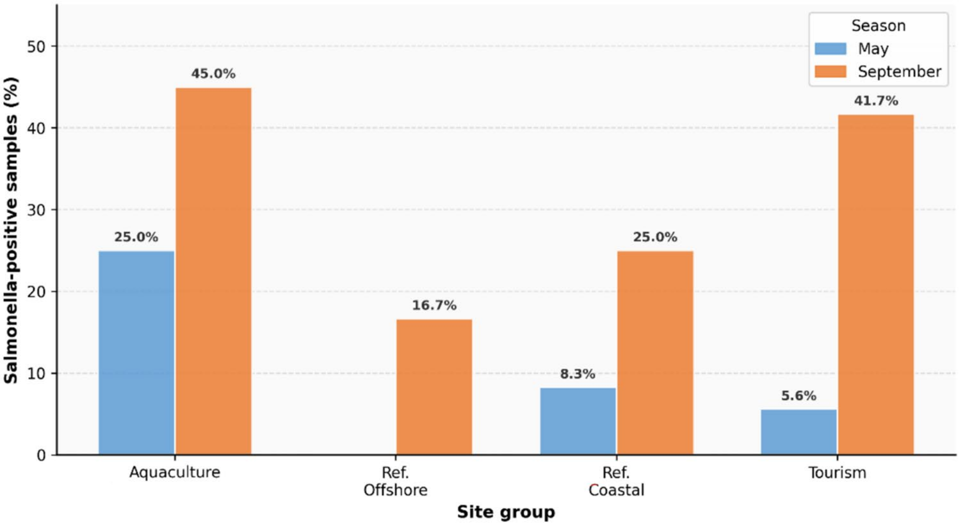
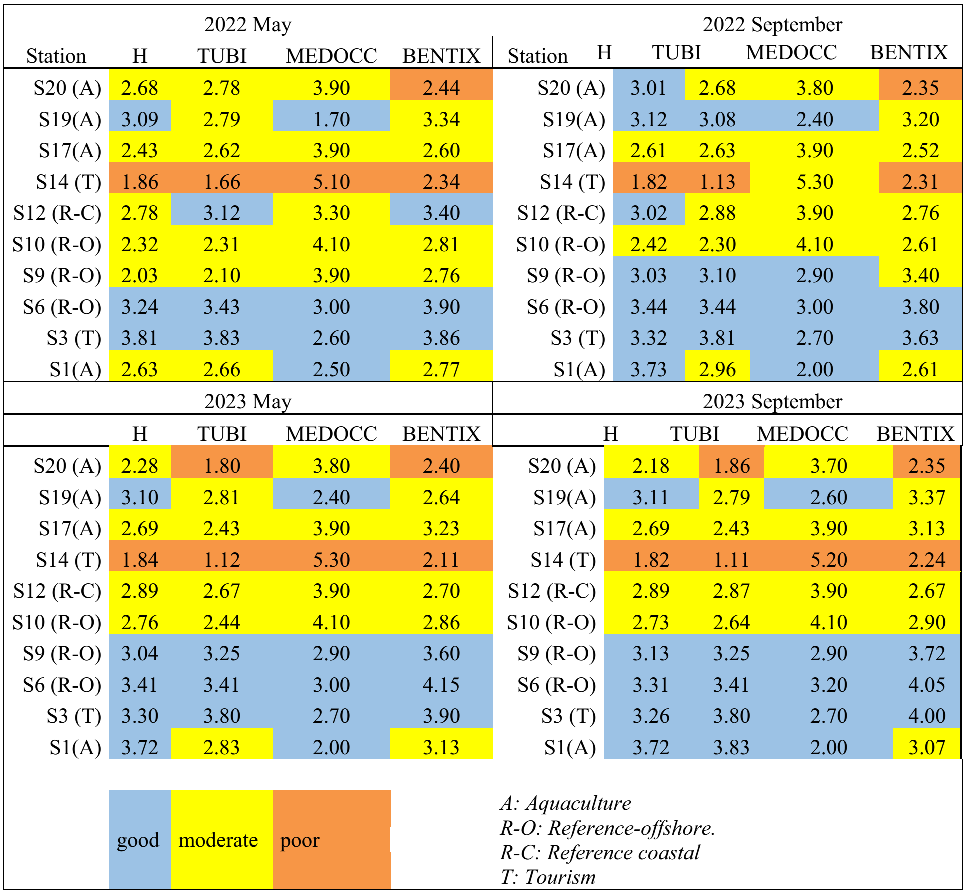


Table 4 Biotic indices (Shannon–Wiener diversity index (H'), BENTIX, MEDOCC, and TUBI) calculated for sampling stations within the Karaburun–Ildır SEPA



	2022 May					2022 September			
Station	H	TUBI	MEDOCC	BENTIX	Station	H	TUBI	MEDOCC	BENTIX
S20 (A)	2.68	2.78	3.90	2.44	S20 (A)	3.01	2.68	3.80	2.35
S19(A)	3.09	2.79	1.70	3.34	S19(A)	3.12	3.08	2.40	3.20
S17(A)	2.43	2.62	3.90	2.60	S17(A)	2.61	2.63	3.90	2.52
S14 (T)	1.86	1.66	5.10	2.34	S14 (T)	1.82	1.13	5.30	2.31
S12 (R-C)	2.78	3.12	3.30	3.40	S12 (R-C)	3.02	2.88	3.90	2.76
S10 (R-O)	2.32	2.31	4.10	2.81	S10 (R-O)	2.42	2.30	4.10	2.61
S9 (R-O)	2.03	2.10	3.90	2.76	S9 (R-O)	3.03	3.10	2.90	3.40
S6 (R-O)	3.24	3.43	3.00	3.90	S6 (R-O)	3.44	3.44	3.00	3.80
S3 (T)	3.81	3.83	2.60	3.86	S3 (T)	3.32	3.81	2.70	3.63
S1(A)	2.63	2.66	2.50	2.77	S1(A)	3.73	2.96	2.00	2.61

	2023 May					2023 September			
	H	TUBI	MEDOCC	BENTIX		H	TUBI	MEDOCC	BENTIX
S20 (A)	2.28	1.80	3.80	2.40	S20 (A)	2.18	1.86	3.70	2.35
S19(A)	3.10	2.81	2.40	2.64	S19(A)	3.11	2.79	2.60	3.37
S17(A)	2.69	2.43	3.90	3.23	S17(A)	2.69	2.43	3.90	3.13
S14 (T)	1.84	1.12	5.30	2.11	S14 (T)	1.82	1.11	5.20	2.24
S12 (R-C)	2.89	2.67	3.90	2.70	S12 (R-C)	2.89	2.87	3.90	2.67
S10 (R-O)	2.76	2.44	4.10	2.86	S10 (R-O)	2.73	2.64	4.10	2.90
S9 (R-O)	3.04	3.25	2.90	3.60	S9 (R-O)	3.13	3.25	2.90	3.72
S6 (R-O)	3.41	3.41	3.00	4.15	S6 (R-O)	3.31	3.41	3.20	4.05
S3 (T)	3.30	3.80	2.70	3.90	S3 (T)	3.26	3.80	2.70	4.00
S1(A)	3.72	2.83	2.00	3.13	S1(A)	3.72	3.83	2.00	3.07

good moderate poor

A: Aquaculture
R-O: Reference-offshore.
R-C: Reference coastal
T: Tourism

Indices are presented separately for 2022 and 2023 to capture potential inter-annual variation, as benthic communities integrate environmental conditions over longer time scales and may reflect cumulative changes between years. For each year, the first column = May, the second column = September. Ecological status color codes are shown in the bottom left corner; activity type is indicated in the bottom right corner of each cell. Color legend: blue = good; yellow = moderate; orange = poor; A: aquaculture; R-O: reference offshore; R–C: reference coastal; T: tourism

site. At the other end of the spectrum, S6 (reference offshore) maintained the highest BENTIX values throughout the study (3.80–4.15). Notably, S3 (tourism) showed diversity and BENTIX scores comparable to reference stations ($H' = 3.26$–3.81; BENTIX = 3.63–4.00), suggesting spatially heterogeneous responses to tourism pressure along the coastline.

Aquaculture stations yielded moderate index values overall. BENTIX scores at S20, S19, and S17 ranged between 2.35 and 3.37, broadly comparable to several reference stations, which may reflect effective organic load dispersal rather than an absence of pressure.

Regarding temporal patterns, ecological status was generally higher in September 2022 than in May 2022 across most stations. This seasonal improvement was less evident in 2023, where inter-station variability increased and some stations showed lower values in September than in the previous year. Such inter-annual variability highlights the value of multiyear monitoring in detecting cumulative benthic change. Statistical analysis confirmed the overall stability of benthic communities: BENTIX, Shannon H', MEDOCC, and TUBI showed no significant response to either season or activity type ($p > 0.05$), with the exception of a marginal, near-significant effect of activity type on MEDOCC ($F = 3.055$, $p = 0.059$).

Multivariate analysis

Principal component analysis (PCA) revealed that the first six components accounted for 77.8% of the total variance in the dataset (PC1: 20.1%, PC2: 16.8%, PC3: 13.0%, PC4: 10.9%, PC5: 9.0%, PC6: 8.0%). PC1 was primarily associated with inorganic nutrient dynamics, with NO_2^--N (loading: 0.586), NH_4^+-N (0.468), DO (0.329), and NO_3^--N (0.346) showing the strongest contributions. PC2 was dominated by fecal indicator bacteria, particularly TC

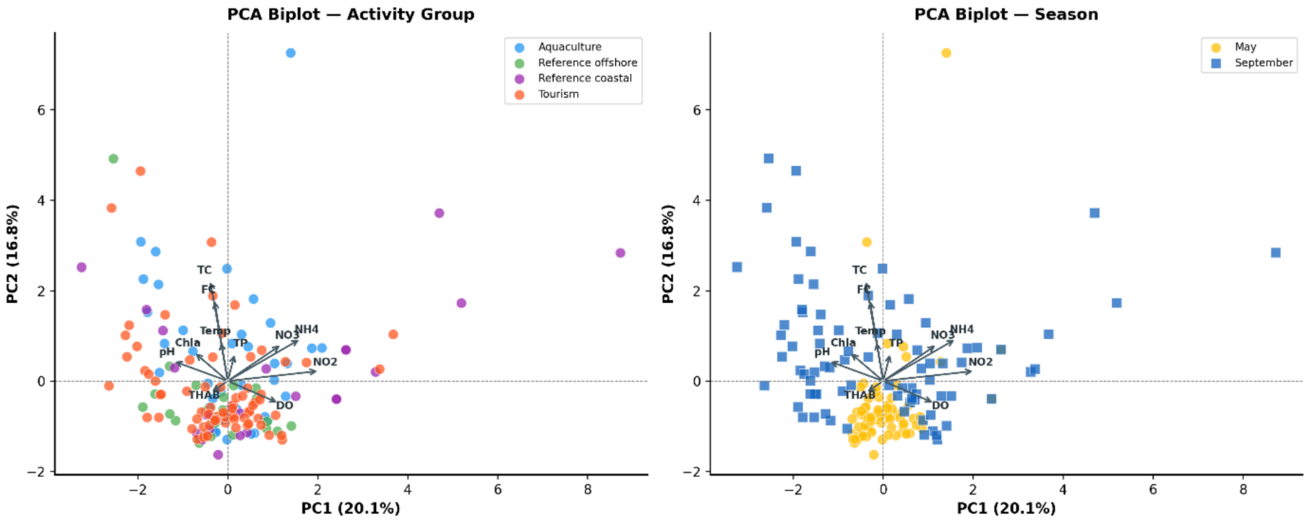
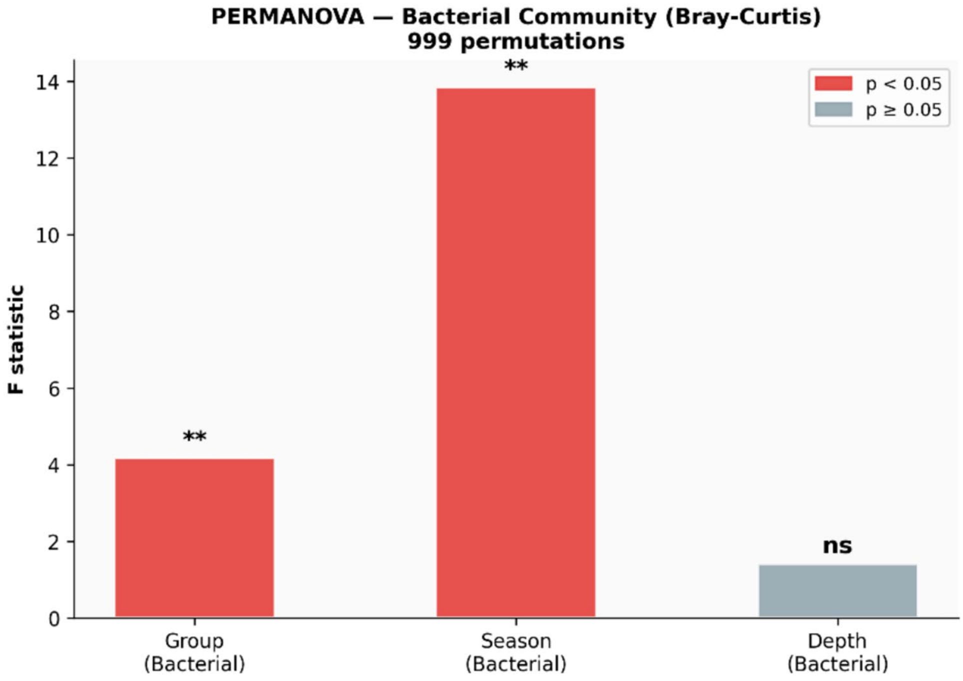


Fig. 5 PCA biplot of physicochemical and bacteriological variables at Karaburun–Ildır SEPA stations, colored by activity group (left) and season (right). Arrows indicate variable loadings. PC1 (20.1%) reflects nutrient dynamics; PC2 (16.8%) reflects bacterial contamination

Fig. 6 PERMANOVA results based on Bray–Curtis dissimilarities of log-transformed bacterial counts (999 permutations). F statistics are shown for each factor. ***$p < 0.001$; ns = not significant



(0.645) and FC (0.523), reflecting the bacterial contamination gradient across activity zones. The separation of the bacterial signal onto a distinct principal component supports the interpretation that microbial indicators capture a different dimension of environmental variability than physicochemical variables (Fig. 5).

PERMANOVA based on Bray–Curtis dissimilarities of log-transformed bacterial counts confirmed significant effects of both season ($F = 13.857$, $p = 0.001$) and activity group ($F = 4.186$, $p = 0.001$) on bacterial community structure (999 permutations). Depth did not show a significant effect ($p = 0.236$) (Fig. 6).

Variance partitioning revealed that season and activity group together explained 19.8% of the total variation in bacterial community composition, with season contributing a unique fraction of 9.5% and activity group contributing 10.3%. The remaining 80.2% of variation was unexplained by these two factors alone, consistent with the limited temporal resolution of the biannual sampling design (Fig. 7).

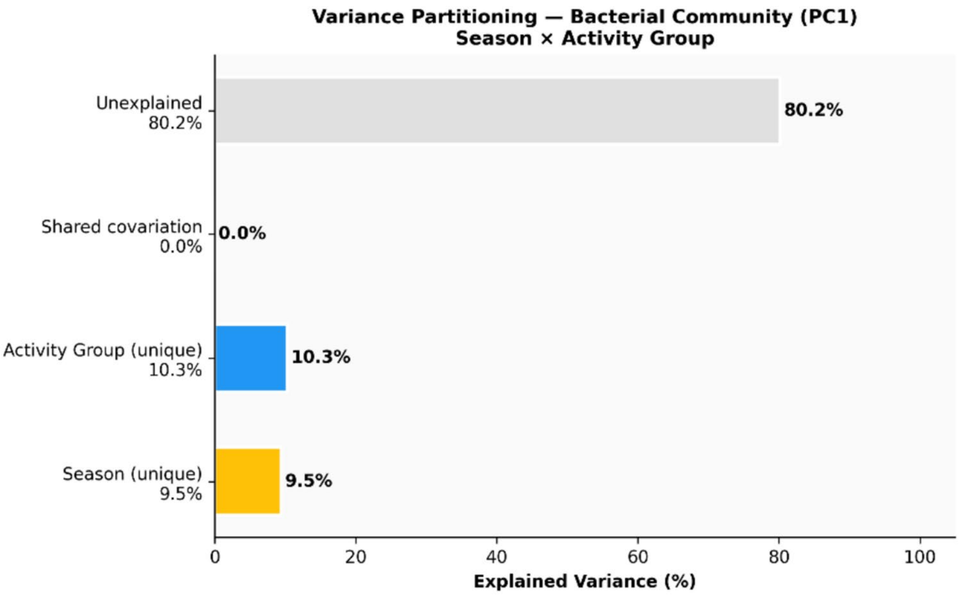


Fig. 7 Variance partitioning of bacterial community variation among season and activity group, based on the first principal component (PC1) of log-transformed bacterial indicators (THAB, TC, FC, IE). Values represent the unique and shared contributions of each factor to total explained variance

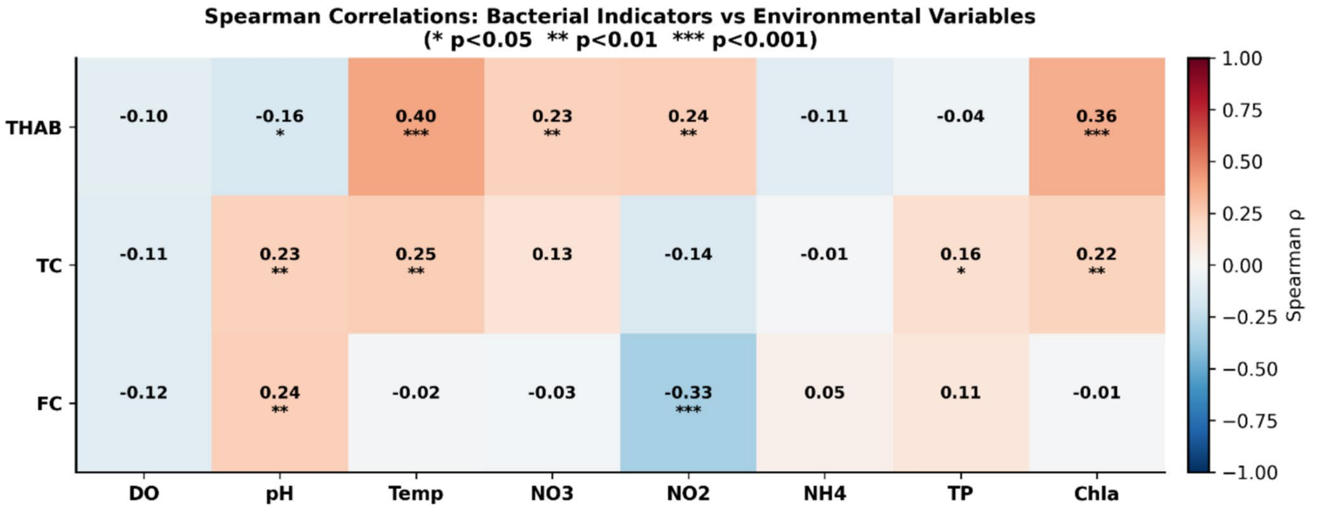


Fig. 8 Spearman rank correlation heatmap between log-transformed bacterial indicators (THAB, TC, FC) and environmental variables. Correlation coefficients (ρ) are shown in each cell. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Spearman rank correlations revealed significant associations between bacterial indicators and environmental variables (Fig. 8). THAB showed positive correlations with water temperature ($\rho = 0.395$, $p < 0.001$), Chl-*a* ($\rho = 0.360$, $p < 0.001$), NO_3^--N ($\rho = 0.228$, $p < 0.01$), and NO_2^--N ($\rho = 0.242$, $p < 0.01$), and a negative correlation with pH ($\rho = -0.161$, $p < 0.05$). TC was positively correlated with temperature ($\rho = 0.246$, $p < 0.01$), Chl-*a* ($\rho = 0.219$, $p < 0.01$), pH ($\rho = 0.227$, $p < 0.01$), and TP ($\rho = 0.159$, $p < 0.05$). FC showed a positive correlation with pH ($\rho = 0.236$, $p < 0.01$) and a negative correlation with NO_2^--N ($\rho = -0.331$, $p < 0.001$). These associations suggest that bacterial proliferation is linked to seasonal warming and organic enrichment, while the negative FC–NO_2^--N relationship may reflect competitive or inhibitory dynamics under elevated inorganic nitrogen conditions.

Discussion

Current coastal monitoring strategies generally rely on the combined use of bacterial, trophic, and benthic indicators to comprehensively assess ecosystem condition. The implicit assumption in this multilayered approach is that these metrics are complementary and will eventually converge to tell a unified story about environmental health. However, the extent to which these parameters respond coherently to overlapping anthropogenic pressures remains highly debated, particularly in multi-use marine protected areas. Our findings from the Karaburun–Ildır SEPA suggest that this assumption of convergence does not hold across all indicator types. Instead of a uniform response to pressure gradients, our results reveal a potential mismatch between the rapid responses of microbial markers and the more gradual integration of trophic and benthic communities, indicating that these indicator groups may reflect different temporal dimensions of ecosystem functioning.

The most immediate and reactive signals in our study emerged from the bacterial water quality indicators. Fecal coliforms and intestinal enterococci exhibited pronounced seasonal variability, with sharp, rapid increases during the summer months. Comparable patterns of fecal indicator bacteria variability associated with environmental conditions and human activities have previously been documented along the eastern Aegean coast (Kacar & Omuzbuken, 2017). This pattern closely mirrors the dynamics reported in other heavily utilized Turkish coastal systems, such as the Gulf of Izmir and the Antalya coastline, where sudden demographic shifts during the tourist season frequently overwhelm local carrying capacities (Gönül et al. 2023; Kucuksezgin et al. 2019). While these broad fluctuations successfully capture use-related pressures, the detection of *Salmonella* spp. introduces an additional management and regulatory challenge. Under the stringent criteria of the "Turkish Regulation on Bathing Water Quality," the permissible level for *Salmonella* in designated bathing waters is defined as absence per 100 mL (Official Gazette No. 29606 available at: https://www.resmigazete.gov.tr); accordingly, any positive detection represents a potential regulatory noncompliance that warrants further investigation. It should be noted that our *Salmonella* results represent only presence/absence data from a single presumptive isolation protocol, without quantitative enumeration, serotyping, or microbial source tracking (MST).

The complexity of managing this risk is compounded by a persistent uncertainty regarding the pathogen's primary origin. It remains entirely unclear whether this contamination stems from seasonal population swells that temporarily overwhelm local wastewater treatment infrastructures, from diffuse land-based agricultural runoff, or from the intensive aquaculture operations in the bay. As Martinez-Urtaza et al. (2004) have previously noted, coastal fish farms and their associated biological outputs can inadvertently serve as environmental reservoirs for opportunistic pathogens. Regardless of its exact source, this ambiguity raises public health and biosecurity concerns, and underscores the need for dedicated MST and quantitative pathogen surveillance in future monitoring programmes. These findings indicate that elevated microbial risks can occur even in water bodies classified as achieving "good" or "moderate" ecological status based on physicochemical or benthic indices.

In stark contrast to these acute bacterial signals, the trophic status of the bay—measured via the TRIX index—painted a picture of relative stability, primarily reflecting natural seasonal productivity dynamics rather than spatial differences in pollution loads. This presents an apparent ecological paradox. The Karaburun-Ildır region is widely recognized as a cornerstone of the regional "Blue Economy". As recently detailed by Tosun et al. (2025), fish farming here is an important driver of local employment and economic output. Structurally, these intensive operations introduce organic metabolic loads and unconsumed feed directly into the water column (Tosun et al. 2024) a process widely recognized as a major driver of nutrient enrichment and ecosystem service trade-offs in coastal aquaculture systems (Haghshenas et al., 2021; Hejazy et al., 2023). The fact that this localized nutrient input does not translate into elevated TRIX values is more accurately interpreted as a scale mismatch between the indicator's design criteria and the nature of the pressures examined. The TRIX index was developed to assess eutrophication at broader basin scales (Vollenweider et al. 1998); it was not designed to resolve point-source pollution gradients at the station level. From a physical oceanographic perspective, the Karaburun–Ildır bay opens into the Eastern Aegean Sea, a highly dynamic system characterized by complex thermohaline circulation and active water exchange driven by prevailing NE and NW winds (Velaoras et al. 2021; Sayın et al. 2011). The Eastern Aegean coastal embayments—including those in proximity to İzmir Bay—are subject to frequent water renewal influenced by the broader Aegean circulation (Eronat and Sayın 2014; Yucel-Gier et al. 2011, 2013). This active hydrodynamic exchange likely facilitates the rapid dispersion of localized nutrient inputs before phytoplankton communities can metabolize them into measurable biomass accumulation, thereby limiting the sensitivity of basin-scale trophic indices such as TRIX to station-level anthropogenic pressures. While direct measurements of residence time and flushing rates were beyond the scope of this study, the broader hydrodynamic context provides a plausible mechanistic explanation for the limited trophic differentiation observed. Future work integrating in situ hydrodynamic measurements or numerical modelling would strengthen this interpretation.

Beyond their regulatory significance, the bacterial indicators measured in this study carry broader functional implications for coastal ecosystem health. Elevated fecal indicator bacteria (FIB) levels during peak tourism periods reflect not only sanitary risk but also increased allochthonous organic loading, which can transiently stimulate heterotrophic microbial activity and alter nutrient cycling dynamics (Azam and Malfatti 2007). The episodic detection of opportunistic pathogens, such as *Salmonella* spp., further suggests that anthropogenic pressures periodically disrupt the natural microbial community structure, with potential consequences for ecosystem services including self-purification capacity and benthic-pelagic coupling (Danovaro et al. 2008). While a formal functional trait analysis was beyond the scope of this study—which relied on culture-based, indicator-focused methods rather than metagenomics or functional gene profiling—these observations underscore the value of integrating functional microbial ecology into future multi-indicator assessments of protected coastal areas.

The divergence in seasonal responses observed among indicator groups in this study is broadly consistent with patterns reported in other coastal systems. Aylagas et al. (2017) demonstrated across 51 estuarine and coastal stations that bacterial assemblages exhibit high sensitivity to pollution and fast response to environmental changes, making them complementary to the slower-responding benthic macroinvertebrate communities in ecological status assessment. Orel et al. (2022) similarly reported in a Slovenian coastal ecosystem that fecal indicator bacteria responded more directly to anthropogenic inputs while the overall microbiome structure was primarily shaped by seasonal dynamics. Conversely, Oprandi et al. (2023) found largely congruent responses among multiple ecological indices in a well-protected Mediterranean MPA with lower anthropogenic pressure, suggesting that indicator convergence may be more likely in less impacted systems. The contrast between these findings highlights that the degree of temporal coherence among indicator groups may itself be a function of pressure intensity. To our knowledge, the simultaneous application of fecal indicator bacteria, TRIX, and macrobenthic biotic indices across a gradient of tourism and aquaculture pressures within a formally designated SEPA has not been previously reported for the Eastern Aegean, underscoring the regional novelty of this approach.

Inherent environmental resilience is further reflected at the seafloor. Benthic ecological indices (BENTIX, MEDOCC, TUBI) maintained a stable trajectory throughout our study, showing no significant structural degradation across different activity zones or seasons. This stability is consistent with the known biological baseline of the region; historical and ongoing surveys have repeatedly documented that the Karaburun-Ildır Bay hosts a remarkably rich biodiversity and a highly complex macrofaunal community (Çınar et al. 2015; Keskin et al. 2023). Our benthic data suggest that this biological integrity remains intact. As Simboura and Zenetos (2002) established, Mediterranean biotic indices are specifically designed to filter out short-term environmental noise and reflect long-term, cumulative disturbances. Macrobenthic assemblages possess a degree of "ecological inertia" or memory, allowing them to tolerate the transient shocks of summer tourism or fluctuating aquaculture outputs without undergoing immediate community collapse. The contrasting temporal response scales of the indicators used in this study are summarized in Fig. 9.

Several limitations of the present study should be acknowledged. First, the biannual sampling design—restricted to May and September across 2 years—provides seasonal contrasts rather than continuous temporal coverage; this low sampling frequency precludes robust inference about high-frequency bacterial variability or episodic contamination events. Second, benthic surveys were conducted at only 10 of the 20 water-column stations, limiting direct spatial comparability between microbial and benthic datasets. Third, sediment organic matter content was not measured, preventing a formal test of the relationship between organic enrichment and benthic community structure. Fourth, *Salmonella* data are restricted to presence/absence without quantitative enumeration or MST. Fifth, the relatively small number of stations per activity group (aquaculture: $n = 5$; reference offshore: $n = 3$; reference coastal: $n = 3$) constrains statistical power. Finally, while hydrodynamic dilution is proposed as a mechanistic explanation for the muted trophic response, no direct current or residence-time measurements were made.

Ultimately, the divergence we recorded among microbial, trophic, and benthic responses should not be viewed as a methodological flaw or a contradiction in the data. Rather, it is an accurate reflection of the varying ecological speeds at which different ecosystem components react to stress. Relying solely on the stable, long-term perspective provided by trophic or benthic assessments creates a potential blind spot, potentially masking acute, short-term microbial risks with implications for human health and food security. Consequently, if the economic benefits of coastal utilization are to be balanced with the conservation mandates of the SEPA, management frameworks must be restructured. We suggest a tiered monitoring strategy that deploys high-frequency bacterial surveillance to actively manage immediate sanitary risks like *Salmonella*, while simultaneously maintaining seasonal ecological audits to ensure that the cumulative footprint of the aquaculture industry does not exceed the bay's natural carrying capacity.

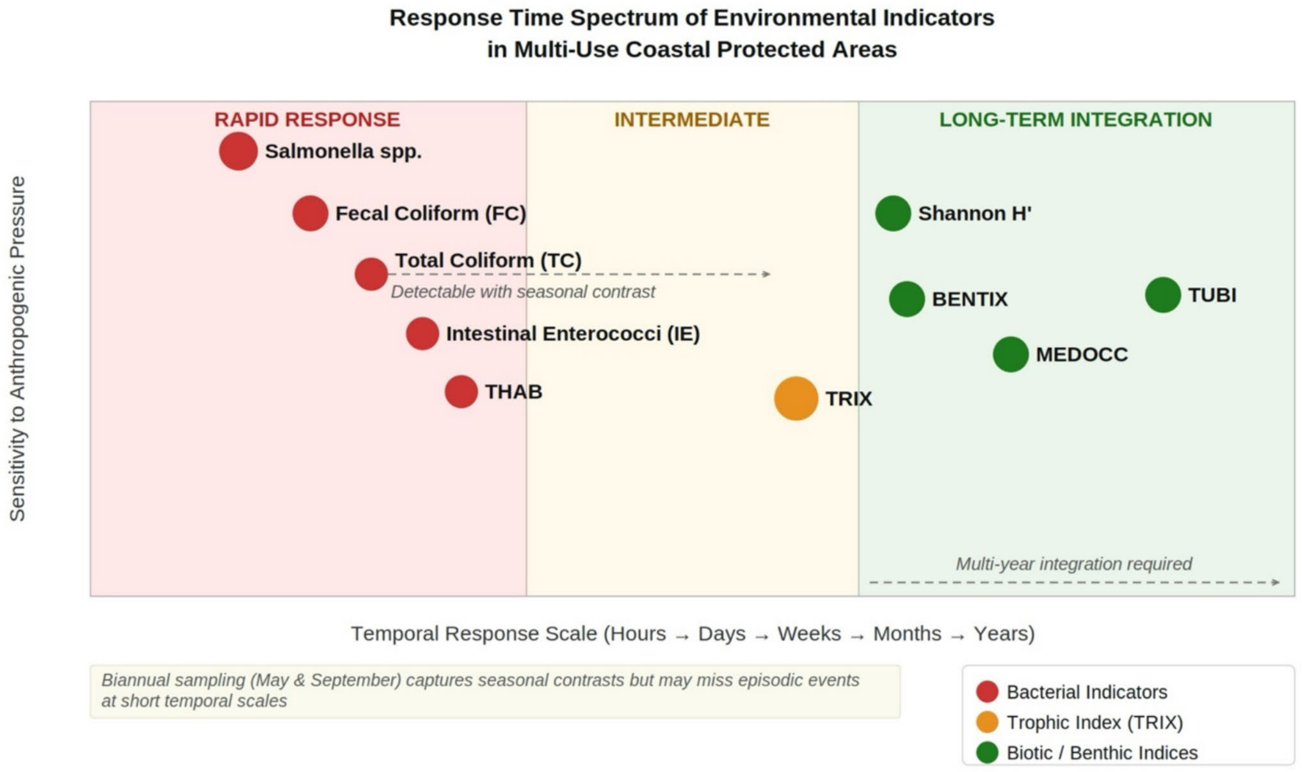


Fig. 9 Response time spectrum of environmental indicators used in this study. Indicators are positioned according to their temporal response scale and sensitivity to anthropogenic pressure. The dashed brackets indicate the temporal windows detectable within the biannual sampling design

Conclusions

Our findings from the Karaburun–Ildır SEPA demonstrate that bacterial, trophic, and benthic indicators do not respond in concert to overlapping anthropogenic pressures. Bacterial indicators proved sensitive to seasonal shifts in tourism and aquaculture activity, capturing acute sanitary risks—including episodic *Salmonella* detection during peak usage periods—that trophic and benthic metrics did not reflect. This divergence carries practical implications: pathogen risks must be managed not only as public health concerns but also as potential vulnerabilities for the region's Blue Economy and aquaculture sector.

By contrast, TRIX values and biotic indices indicated a stable, moderately impacted environment, consistent with the region's active hydrodynamic regime and the long-term integrative nature of these assessment tools. The divergence among indicator groups is not a methodological inconsistency; rather, it reflects the different temporal scales at which ecosystem components respond to stress—a pattern now supported by multivariate statistical analyses (PERMANOVA, PCA, Variance Partitioning) and visualized through the response time spectrum framework introduced in this study.

These results support the adoption of a tiered monitoring strategy for multi-use coastal protected areas: high-frequency microbial surveillance to detect acute sanitary threats, combined with routine seasonal ecological assessments to ensure that long-term ecological limits are not exceeded. Such an integrated approach may better serve both the conservation mandates and the socioeconomic sustainability of multipurpose protected coastal systems.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00027-026-01324-0.

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Author contributions PSCT: conceptualization, data curation, formal analysis, investigation, methodology, and writing—original draft; MDDA: sampling and bacteriological analyses; EB: sampling and bacteriological analyses; OG: benthic sampling and benthic analyses; DDT: provided information regarding the aquaculture activities at the study sites; BÖ: funding acquisition, project administration, and writing—review and editing.

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Data availability Data will be made available on request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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