ANSF
APPLIED AND NATURAL SCIENCE FOUNDATION
Boundless Science

Journal of Applied and Natural Science
18(1), 232 - 249 (2026)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Benthic biodiversity assessment in epipelic and epissamic habitats of Tapi Estuary, Surat, Gujarat

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Article Info
https://doi.org/10.31018/jans.v18i1.7165
Received: September 19, 2025
Revised: February 09, 2026
Accepted: February 21, 2026

How to Cite

Menon, A. K. M. *et al.* (2026). Benthic biodiversity assessment in epipelic and epissamic habitats of Tapi Estuary, Surat, Gujarat. *Journal of Applied and Natural Science*, 18(1), 232 - 249. https://doi.org/10.31018/jans.v18i1.7165

Abstract

Epipelic and epipsammic benthic habitats play a critical role in estuarine ecosystem functioning by regulating nutrient cycling, stabilizing sediments, and supporting primary production. However, these habitats remain poorly studied in Indian estuaries, particularly in the Tapi Estuary, despite increasing anthropogenic pressures. The present study aimed to evaluate the benthic biodiversity of epipelic and epipsammic habitats in the Tapi Estuary and to assess their ecological significance in relation to environmental and seasonal variations. Quarterly sampling was conducted at selected reference and impacted locations within the Tapi Estuary during post-monsoon, winter, and summer seasons. Sediment samples were analyzed for biological parameters, focusing on algal communities and meio- to macrofaunal assemblages. Habitat characteristics and species–environment relationships were examined to identify key regulatory factors influencing community structure. The study revealed distinct spatial and seasonal variations in benthic assemblages. Periphyton density ranged from [89–6336 units/m^2 in Epipelic region and 96 – 2458 units/m^2 in Epissamic region, while meiofaunal (Zoobenthic) abundance varied between 52 – 550 Number/m^2 in Epipelic region and 25 – 284 Number/m^2 in Epissamic region across seasons. Impacted locations exhibited reduced diversity and dominance of pollution-tolerant taxa, indicating stress from industrial and domestic effluents. This study represents one of the first integrated assessments of epipelic and epipsammic biodiversity in the Tapi Estuary. The findings highlight the potential of benthic communities as sensitive indicators of water quality and ecosystem health, providing valuable baseline data for estuarine monitoring, management, and conservation under escalating anthropogenic influence.

Keywords: Benthic biodiversity, Epipelic, Epissammic, Inter-tidal sediments

INTRODUCTION

The sustainable coexistence of humans and the environment is a reminder of a critical concern water environments, such as rivers and lakes, are being particularly affected by anthropogenic activities. (Yoshioka and Hamagami, 2025). Examples of such activities include industrial water pollution (Aklilu *et al*., 2025), hydropeaking in dam-downstream regions, which causes fish stranding (Insulaire *et al*., 2024), contraction of lake area due to irrigation water use (Wine, 2022) and the introduction and spread of invasive species (Finnsson, 2025). Benthic algae, also known as attached algae or periphyton, are crucial species in aquatic ecosystems, as they function as primary producers that drive food webs and ensure nutrient cycling (Zhao *et al*., 2023). Consequently, the study of benthic algal population dynamics is paramount for evaluating the sustainability of human-environment interactions (Yoshioka and Hamagami, 2025). Herein, benthic populations were studied in the Epipelic and Epissamic habitats. They refer to environments inhabited by benthic algae, specifically diatoms, in intertidal zones. These habitats are essential for understanding aquatic ecological dynamics. Epipelic habitats pertain to organisms residing on or within the upper sediment layer, particularly mud or fine substrates. These habitats contain microorganisms, mainly diatoms, bacteria, and algae, that move through or adhere to sediment. These organisms are crucial for primary production, nutrient cycling, and food sources in aquatic ecosystems. Environmental factors such as light, temperature, and water flow can significantly influence community structure. The term "Epissammic" refers to organisms inhabiting sandy substrates in environments such as stream beds, lake shores, beaches, and the seabed. These habitats have distinct conditions, including increased porosity and water flow, affecting organism types. Epissammic communities include diatoms, cyanobacteria, microalgae, and microscopic fauna, which attach to sand grains or live between them, contributing to ecosystem productivity. Epipelic algae tolerate darkness but not prolonged anaerobic conditions, requiring movement to reach the sediment surface, while Epissammic algae have the ability to withstand both darkness and lack of oxygen, maintaining photosynthetic potential without rapid movement (Moss, 1977).

Epipelic and Epissamic habitats support distinct communities due to differences in substrate composition and environmental factors. These habitats are crucial in aquatic ecosystems, supporting biodiversity, food chains, and nutrient circulation. Studying these habitats is important for assessing ecosystem health, understanding environmental changes, and preserving biodiversity. Monitoring these microenvironments provides insights into ecosystem processes and ecological conditions. Epipelic and Epissamic algae stabilize sediments, regulate nutrient cycling between benthic and pelagic environments, and contribute to primary production. Understanding their ecological roles is crucial attributable to changes in sediment loading from land-use modifications and management (Hašler *et al*., 2008).

Benthic communities play a vital role in the health and functioning of riverine ecosystems, as demonstrated by ecological research across diverse locations. These communities serve as important secondary consumers, contributing to trophic interactions and nutrient circulation. They maintain biodiversity and connect primary production to higher trophic levels, particularly for economically important fish species (Frithsen and Holland, 1992). Benthic macroinvertebrates serve as indicators for biomonitoring and conservation planning, reflecting the health of river habitats. Their composition and contribution vary across river habitats, such as riffles and riverine wetlands (Curry *et al*., 2012).

Benthic communities are important to riverine ecosystem structure and function, influencing biodiversity and ecosystem processes through their roles in food webs, nutrient cycling, and environmental monitoring (Curry *et al*., 2012; Frithsen and Holland, 1992). Understanding these dynamics is crucial for effective conservation of riverine ecosystems, highlighting the importance of protecting benthic habitats for ecosystem integrity. Understanding these dynamics is vital for riverine ecosystem management. Benthic communities play multiple roles in rivers, including bioindication, biodiversity support, and interactions with the environment. Research demonstrates the importance of protecting benthic habitats to preserve the functionality of riverine ecosystems.

Water quality monitoring traditionally focused on chemical and physical measures, comparing results to established guidelines to provide status snapshots. A modern river health assessment examines the physical, chemical, and biological interactions within a river. Biological monitoring complements traditional approaches by measuring the effects of stressors on biota and detecting impacts such as changes in water quantity and habitat degradation that chemical monitoring may miss. Aquatic biomonitoring can indicate river conditions weeks or months before collection, as damage to aquatic biota remains detectable long after events like chemical spills that periodic water sampling might miss (Envirothon Aquatic Ecology Committee, 2025).

Epipelic and Epissamic organisms perform a range of ecosystem functions, including bio stabilization of sediments, regulation of benthic–pelagic nutrient cycling, and primary production. There is a growing need to understand their ecological role in light of current and future alterations in sediment loading resulting from land-use change and anthropogenic activities. The present study aimed to assess the benthic biodiversity of epipelic and epipsammic habitats in the Tapi Estuary and to assess their ecological significance in relation to environmental and seasonal variations.

MATERIALS AND METHODS

Study area

Tapi estuary

Estuaries comprise one of the world's most productive and extensive ecosystems which deliver vital ecosystem services used by humans (Costanza, 2014; Levin, 2001). An estuary is a semi-enclosed coastal body of water that has a free connection to the open sea and to fresh water from land drainage. Estuaries are high dynamic and efficient productive ecosystems (Pillay *et al*., 2003). Estuarine processes vary in an organized manner within the tidal cycle (semidiurnal, diurnal) and weather cycles (seasonal and interannual cycles) (Dyer, 1986). The Tapi Estuary, located in the western state of Gujarat, India, is a significant riverine estuary system where the Tapi River meets the Arabian Sea. Its length from source to mouth is about 720 Km, with a catchment area of approximately 1,650,000 km^2 (Ranjana *et al*., 2007). This estuary plays a crucial role in supporting a diverse range of flora and fauna, contributing to the region's biodiversity. It is an important

LOCATION 1 – DUMAS SULTANABAD OWARA

LOCATION 2 - SEWAGE OUTFALL LOCATED NEAR GINGER HOTEL NEAR PIPLOD

LOCATION- 3 CABLE STAY BRIDGE

LOCATION – 4 SEWAGE OUTFALL LOCATED NEAR NAWADI OWARA

Fig. 1. *Sampling locations of Tapi estuarine area*

habitat for various fish and bird species, making it a vital ecological zone. However, like many estuarine ecosystems, the Tapi Estuary faces environmental challenges, including pollution, habitat degradation, and the impacts of climate change. Efforts to preserve its ecological integrity are critical for the sustainability of local communities and the preservation of its unique biodiversity.

Methodology

Sampling protocol

To assess the Epipelic and Epissamic habitats along the Tapi estuary, sampling was conducted at four locations (Fig.1) spanning from Dumas to Navadi Owara. This sampling took place in 2024 and encompassed three distinct seasons: post-monsoon, summer, and winter. Sediment samples were collected from the intertidal zone of the Tapi estuary. Prior to sampling, tidal timing was considered, as sampling was conducted exclusively during low tide when the intertidal sediment area was exposed. Sediment samples were collected and preserved with formalin for biological analysis. Photographs and sampling coordinates were documented at the time of sampling.

Location characteristics

All four Locations were selected within the intertidal region, characterized by largely undisturbed sediments devoid of human or animal footprints. This was due to their remote locations, which allowed the sediment layers to maintain their natural state between sampling periods, thereby providing a suitable habitat for epipelic and Epissamic organisms. Sampling station - 1, located in Dumas Owrara, served as a reference Location due to its predictable minimal disturbance and substantial mangrove cover. It is situated near the estuary mouth and experiences submergence during all tidal cycles (21°06'32.64" N, 72°42'23.39" E). Sampling station - 2 is positioned near a sewage outfall adjacent to the Ginger Hotel in Piplod (21°09'36.56" N, 72° 45'59.02" E). Sampling station - 3 is located beneath the cable-stayed bridge, situated between the sewage pipelines of Locations 2 and 4 (21°10'49.72" N, 72° 47'48.02" E). Sampling station - 4 is in Nawadi Owrara, close to a sewage pipeline (21°11'22.84" N, 72° 48'38.22" E). Despite its proximity to a temple and several fishermen's boats, this Location has a presence of deeply deposited sediments.

Biological parameters: Biological parameters selected for this current research include Periphyton and Zoobenthic abundance and diversity study in the Epipelic and Epissamic habitats. Estuarine stretchwere considered in the current research, as they are dynamic and highly productive.

Field and laboratory procedures for biological parameters

Field method

At the designated sampling Location, a quadrat measuring 0.5 meters by 0.5 meters was established. Sediment was subsequently collected in layers, beginning with the uppermost epipelic layer and inner Epissamic layer using a scraper. The sediment samples collected from the 0.25 m^2 area at various locations along the riverbed and from different layers were stored separately and preserved in 5% formalin. Upon arrival at the laboratory, the collected sediment samples were sieved through meshes of varying sizes to isolate benthic algae and zoobenthos.

Laboratory method

Upon arrival at the laboratory, sediment samples were classified into epipelic, epipsammic benthic fractions. For periphyton and Zoobenthic analysis in the epipelic and epipsammic sediments, preserved samples were processed using a modified sequential sedimentation technique. The sediments were thoroughly washed with double-distilled water and passed through a filtration assembly fitted with a 20 µm Nylobolt mesh. This procedure facilitated the separation of organisms from sediment particles. The retained sediment–water matrix was repeatedly rinsed with double-distilled water to ensure maximum recovery of organisms. The washing was performed by centrifugation at 1000 rpm for 5 minutes to settle the sediment particles, and the concentrated organisms were collected in the supernatant. This laboratory devised method was developed by refering (Whitelam *et al*., 1983). The concentrated supernatant was collected in sterile sample containers for further analysis. An aliquot of 2 mL from the concentrated sample was transferred onto a glass slide, and periphytic organisms were enumerated using Lackey's drop method. The specific biological parameters analyzed following completion of the above procedures included qualitative and quantitative assessments of benthic communities. These comprised periphyton species composition, abundance, and community structure, as well as zoobenthic species composition, density, and assemblage patterns. Identification and documentation were performed under a Labovision binocular microscope, and representative microphotographs were captured for taxonomic confirmation and record-keeping.

Statistical analysis

The results were computed using the STAMP (Statistical Analysis of Metagenomic Profiles) software package to analyze metagenomic profiles. The results were reported as a comparison between seasons (i.e., Summer, Winter, Post-monsoon) and depths (i.e., Epipelic [upper layer] and Epissammic [inner layer]) of the sediment strata. Genus-level comparisons of proportional abundance were performed using STAMP (Statistical Analysis of Metagenomic Profiles). Differences in mean proportions between groups (seasons, depths, or locations) were calculated using Welch's t-test, which is implemented in STAMP and is appropriate for datasets with unequal variances and small sample sizes. Confidence intervals (95%) were computed using STAMP's default Welch's inverted method, which estimates uncertainty around the difference in mean proportions without assuming equal variances. Although the data represent proportions, STAMP applies normalization and variance-correcting procedures that enable robust comparisons of proportional datasets, particularly when sample sizes are limited. This approach has been widely used in microbial and ecological community studies for relative abundance comparisons. For genus-level comparisons shown in Figs. 2–5, 'n' represents the number of independent sampling units, where each unit corresponds to a unique combination of location and season. Specifically, samples were collected from four locations across three seasons (winter, summer, and post-monsoon), resulting in independent biological replicates per group. Each replicate reflects pooled counts obtained from standardized quadrat sampling within a given location and season. These independent replicates were treated as separate observations in STAMP, and mean proportions were calculated across replicates within each comparison group (e.g., summer vs. winter, epipelic vs. epipsammic). The latest version of the software -PRIMER V 6 (Plymouth Routines in Multivariate Ecological Research) developed by The PRIMER E-Ltd (Plymouth Routines in Multivariate Ecological Research) software by Dr. Bob Clarke and Dr. Ray Gorley of Plymouth Marine Laboratory, UK. was used for the diversity indices calculation. This software can perform analysis of ecological data entered in spread sheets. It was used for calculating various univariate biodiversity measures such as Margalef's index, Shannon-Weiner index and Simpson diversity index

RESULTS

The outcomes of the study are depicted in Figs. 1 – 6 and Tables 1 and 2. The results shown below illustrate the Periphyton and Zoobenthic population dynamics at different depths (Epipelic and Epissamic) and Seasons (Winter, Summer, and Post-monsoon), with statistical data on abundance and Diversity indices.

Seasonal comparison

[A] Genus level seasonal (summer and winter) comparison:

The seasonal comparison of Periphyton communities revealed distinct shifts in genus-level abundance be-

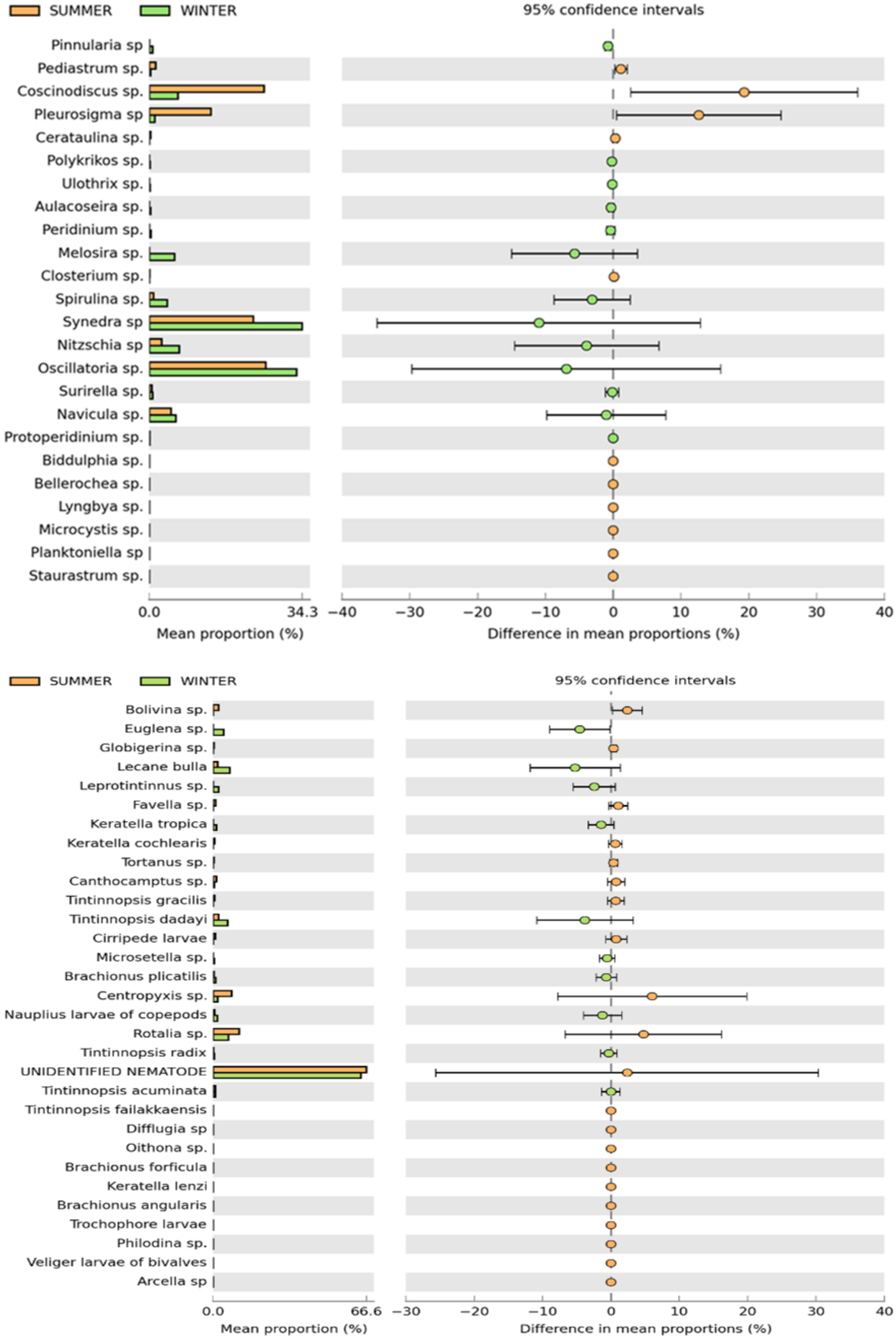


Fig. 2. *Seasonal comparisons of Periphyton and Zoobenthic genus composition between Summer (orange) and winter (green). Left panel shows mean proportion (%) of each genus; right panel displays the difference in mean proportions with 95% confidence intervals*

tween summer and winter. *Coscinodiscus sp.* and *Pleurosigma sp.* exhibited significantly higher mean proportions during the summer, indicating their preference for warmer conditions. In contrast, *Synedra sp.*, *Nitzschia sp.*, and *Oscillatoria sp.* showed marked increases in abundance during the winter, suggesting a seasonal dominance in cooler months. Moderate winter-associated increases were also observed for *Melosira sp.* and *Spirulina sp.*, whereas genera such as *Peridinium sp.*, *Closterium sp.*, and *Surirella sp.* showed minimal seasonal variation. Several Periphyton genera, including *Protoperidinium sp.*, *Biddulphia sp.*, *Bellerochea sp.*, *Lyngbya sp.*, *Microcystis sp.*, *Planktoniella sp.*, and *Staurastrum sp.*, had very low mean proportions in both seasons and did not exhibit any notable seasonal trend. Overall, the results indicate a clear seasonal structuring of the Periphyton community, with diatoms such as Synedra and Nitzschia dominating in winter, while centric diatoms, such as Coscinodiscus sp., were more prevalent in summer. The Zoobenthic community structure exhibited clear seasonal variation between summer and winter. Several Zoobenthic taxa, including *Euglena sp.*, *Lecane bulla*, *Leprotintinnus sp.*, *Keratella tropica*, *Tintinnopsis dadayi*, and *Brachionus plicatilis*, showed significantly higher abundance during the *winter* season. These taxa had positive differences in mean proportions, with confidence intervals indicating consistent winter dominance. In contrast, taxa such as *Centropyxis sp.*, *Rotalia sp.*, *copepod nauplii,* and particularly *Unidentified Nematode* were more abundant in summer than in winter, suggesting favourable conditions for their growth during warmer months. Many other taxa exhibited relatively low abundance and showed no significant seasonal differences, as indicated by overlapping confidence intervals.

[B] Genus level seasonal (summer and post monsoon) comparison

The comparative analysis of Periphyton composition between summer and post-monsoon seasons revealed notable shifts in abundance among several genera. *Pleurosigma sp. Navicula sp.* and *Synedra sp.* showed significantly higher mean proportions during the summer, indicating a seasonal preference for warmer conditions. In contrast, *Coscinodiscus sp.* and *Oscillatoria sp.* were more abundant in the post-monsoon period, with *Coscinodiscus sp.* showing the largest positive shift. Moderate increases in the post-monsoon season were also seen for *Planktoniella sp.*, *Aulacoseira sp.*, and *Pediastrum sp.*, suggesting these genera favour post-rainfall conditions. Genera like *Nitzschia sp.*, and *Lyngbya sp.* showed slight increases during the post-monsoon, though with relatively smaller effect sizes. Several taxa, including *Protoperidinium sp.*, *Pinnularia sp.*, *Closterium sp.*, *Biddulphia sp.*, *Bellerochea sp.*, *Surirella sp.*, and *Spirulina sp.*, exhibited minimal seasonal change. Overall, the results highlight a clear seasonal restructuring in the Periphyton community, with post-monsoon conditions favouring the growth of centric diatoms and filamentous cyanobacteria, while summer favoured benthic and epipelic forms such as Pleurosigma, Navicula sp., and *Synedra*. The Zoobenthic community structure varied notably between the summer and post-monsoon seasons, with several taxa exhibiting clear seasonal preferences. The Unidentified Nematode group was more prevalent in the summer samples than in the post-monsoon samples, with a significantly higher mean proportion and a wide confidence interval that clearly excludes zero, indicating strong seasonal prevalence. In contrast, several taxa—including *Arcella sp.*, *Tintinnopsis gracilis*, *Keratella tropica*, *Lecane bulla*, *Bolivina sp.* and *Trochophore larvae*—were more abundant in the *post-monsoon* season, with positive differences in mean proportions and non-overlapping confidence intervals. Other species such as *Rotaria sp.* showed moderately higher representation in the post-monsoon samples, although with variable confidence intervals. Several taxa including *Tintinnopsis dadayi*, *Brachionus plicatilis*, *Tortanus sp.*, *Centropyxis sp., Canthocamptus sp., Keratella cochlearis* and *Oithona sp.*—showed little to no difference between seasons, with overlapping confidence intervals suggesting stable distribution. Low-abundance species such as *Euglena sp.*, *Philodina sp.*, *Globigerina sp.*, and *Microsetella sp.* were observed with minimal seasonal variation.

[C] Genus level seasonal (winter and post monsoon) comparison

The comparison of Periphyton communities between winter and post-monsoon seasons revealed distinct seasonal preferences among several genera. *Oscillatoria sp.* was dominant in both seasons but showed higher abundance in the post-monsoon period. *Coscinodiscus sp.* also increased notably in post-monsoon, indicating its preference for conditions following rainfall. In contrast, *Synedra sp. Navicula sp., Melosira sp., Spirulina sp.* and *Nitzschia sp.* were more abundant during winter, suggesting their adaptation to colder conditions. Minor seasonal variations were observed in genera such as *Peridinium sp. and Pleurosigma sp., which showed slightly higher abund*ance in winter. Whereas species such as *Pinnularia sp.*, *Biddulphia sp.*, *Bellerochea sp., Pediastrum sp., Planktoniella sp.,* and *Aulacoseira sp.* showed a slight increase during the post-monsoon period. Several other genera displayed low abundance and minimal seasonal change. Overall, the Periphyton community structure showed clear seasonal shifts, with winter favouring diatoms and post-monsoon supporting the growth of centric diatoms and cyanobac-

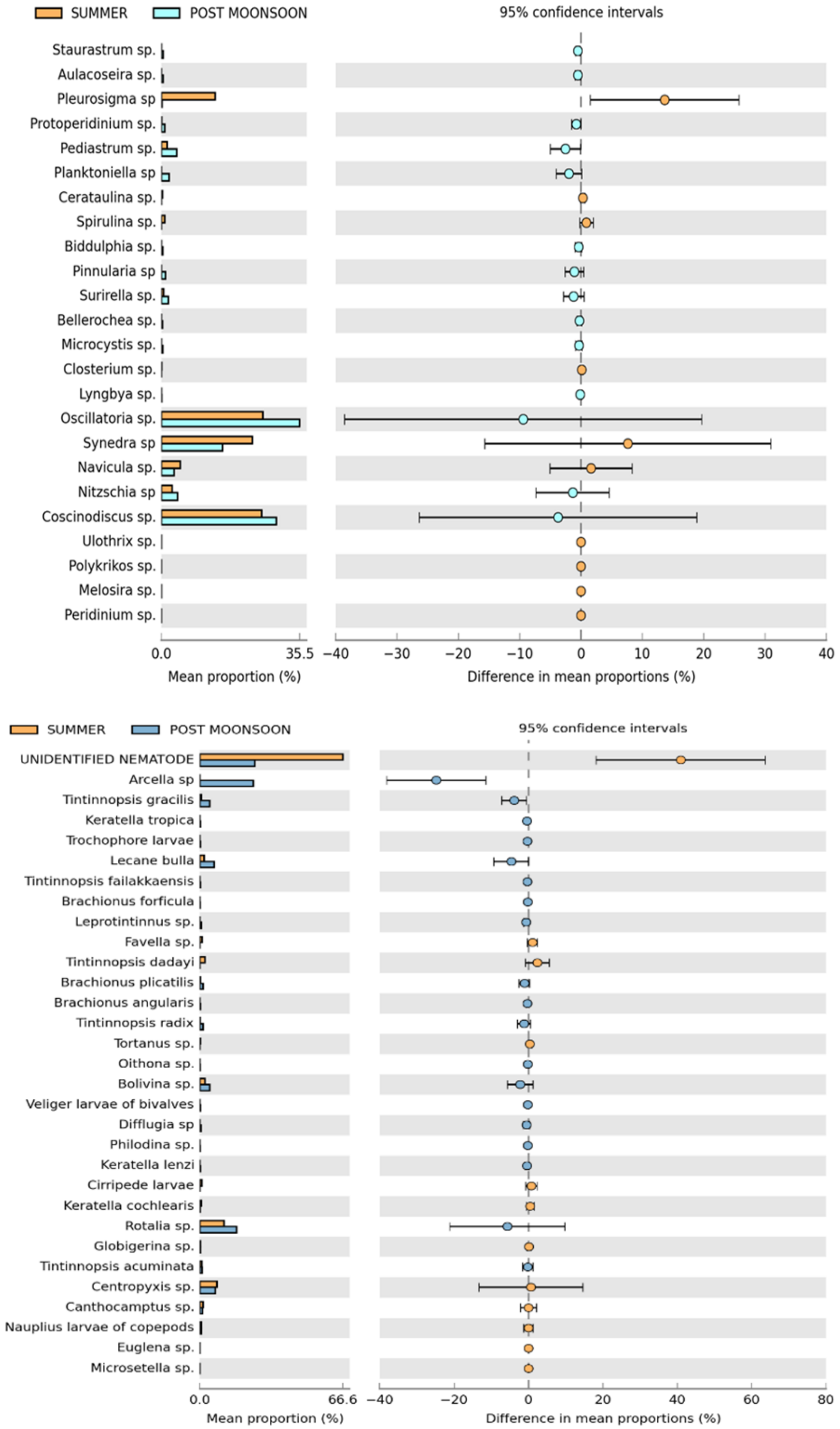


Fig. *3.* *Seasonal comparisons of periphyton and zoobenthic genus composition between summer (orange) and post-monsoon (blue). Left panel shows mean proportion (%) of each genus; right panel displays the difference in mean proportions with 95% confidence intervals*

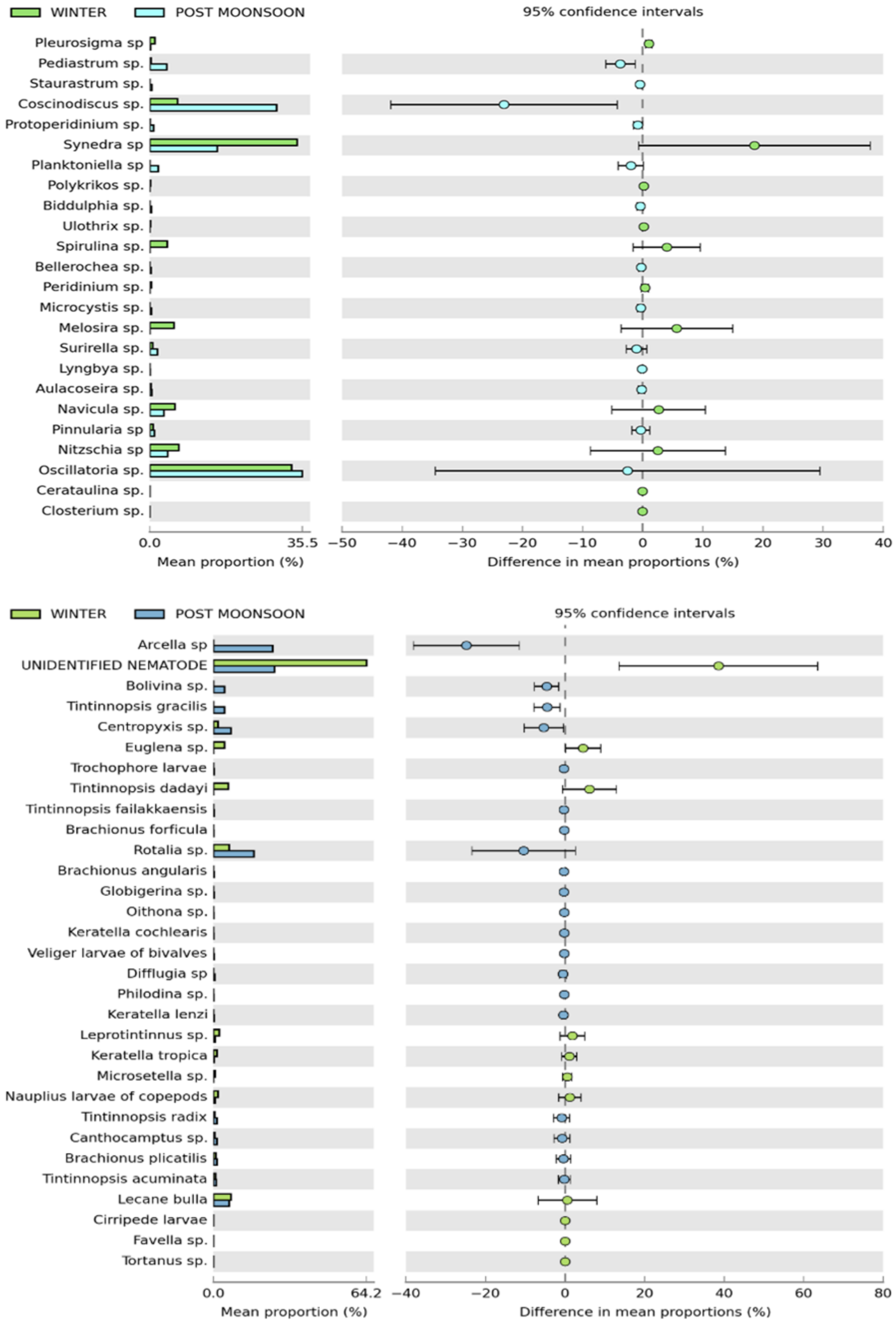


Fig. 4. *Seasonal comparisons of periphyton and zoobenthic composition between winter (green) and post-monsoon (blue). The left panel shows the mean proportion of each genus, while the right panel displays the difference in mean proportions with 95% confidence intervals*

teria. Zoobenthic community composition showed notable differences between the winter and post-monsoon seasons, with several species exhibiting distinct seasonal preferences. The Unidentified Nematode group was significantly more abundant in the *winter* season than in the post-monsoon season, as indicated by a large positive difference in mean proportion and a non-overlapping confidence interval. This group dominated the winter samples. In contrast, a variety of species showed higher abundance during the *post-monsoon* season. Notable among these were *Arcella sp.*, *Rotalia sp Bolivina sp.*, *Tintinnopsis gracilis*, *Centropyxis sp.*, *Trochophore larvae*, and *Brachionus forficula*. These species exhibited negative mean differences with confidence intervals excluding zero, suggesting consistent post-monsoon enrichment. Species such as., *Euglena sp.*, *Tintinnopsis dadayi*, *Keratella tropica* and *Lecane bulla* were more abundant in the *winter*, though the differences were relatively smaller. Many other taxa, including *Oithona sp.*, *Philodina sp.*, *Globigerina sp.*, *Brachionus plicatilis*, and *Tintinnopsis acuminata*, showed low or comparable proportions across both seasons, with overlapping confidence intervals suggesting minimal seasonal variation.

Depth comparison

[D] Genus level depth comparison

The comparison between epipelic and Epissamic habitats revealed distinct differences in the periphyton community structure. *Oscillatoria sp.* showed clear dominance in the epipelic habitat, with substantially higher proportions than in the Epissamic zone. Melosira sp. and *Pleurosigma sp.* also exhibited higher abundance in epipelic samples. Conversely, *Coscinodiscus sp. and Synedra sp. were more dominant in the Epissamic habitat, indicating a* preference for sandy sediment-associated environments. Genera such as *Nitzschia sp.*, *Ulothrix sp., Microcystis sp.*, *Spirulina sp.*, *Protoperidinium sp.*, *Planktoniella sp.*, and *Polykrikos sp.* were also equally present at both depths; however, they showed slightly higher representation in Epissamic conditions. Meanwhile, *Surirella sp., Navicula sp.*, *Pinnularia sp.*, *Closterium sp.*, and *Lyngbya sp.* were more prevalent in epipelic sediments. Genera, including *Cerataulina sp.*, *Aulacoseira sp.* and displayed low abundance and minimal variation between the two habitats. The genus-level composition of Zoobenthic varied notably between epipelic and Epissamic habitats, indicating distinct microhabitat preferences. The Unidentified Nematode group showed clear dominance in *epipelic* samples, with a substantially higher mean proportion and a confidence interval that did not overlap zero. This suggests a strong affinity of nematodes for fine sediment environments. Other genera such as *Nauplius larvae of copepods* and *Trochophore larvae* were slightly more abundant in the epipelic zone, though with varying levels of statistical confidence. *Tintinnopsis acuminata* showed higher representation in epipelic samples with clear positive mean differences. In contrast, *Lecane bulla*, *Leprotintinnus sp., Keratella cochlearis, Keratella tropica, Arcella sp.*, *Favella sp.*, *Centropyxis sp.*, *Tortanus sp.*, and *Brachionus angularis* were more frequently observed in the *Epissamic* habitat, with negative differences and non-overlapping confidence intervals, suggesting they are better adapted to coarser or sandy sediments. Several genera including *Oithona sp., Philodina sp., Keratella lenzi,* and *Euglena sp.,* exhibited relatively minor differences across habitats, with overlapping confidence intervals, indicating a more generalist distribution. Low-abundance genera, such as *Bolivina sp., Globigerina sp.,* and *Microsetella sp.,* did not show any meaningful habitat-specific trends.

[E] Genus level location (Reference vs all other test sites) comparison:

The comparative analysis of algal community structure between Location 1 and the combined group of Locations 2, 3, and 4 revealed several genera with significant differences in abundance. Notably, *Coscinodiscus sp.* and *Synedra sp.* were significantly more abundant in Location 1, as indicated by their higher mean proportions and 95% confidence intervals not overlapping zero. *Pediastrum sp.* and *Protopteridinium sp.* also showed elevated levels in Location 1, though with wider variability. In contrast, genera such as *Oscillatoria sp., Pleurosigma sp., Spirulina sp., Navicula sp.,* and *Nitzschia sp.* were significantly more abundant in the combined group of other locations, suggesting a distinct algal assemblage in Location 1 compared to the others. *Oscillatoria sp.* in particular exhibited a notably lower proportion in Location 1, as evidenced by a large negative difference in mean proportion with a narrow confidence interval. Other taxa, including *Microcystis sp., Lyngbya sp., Pinnularia sp., Planktoniella sp.,* and *Ulothrix sp.,* were present at low levels and were relatively similar across locations, contributing little to community-level differences. The genus-level comparison between Location 1 and all other locations (Locations 2, 3, and 4) reveals marked variation in the composition and relative abundance of zooplankton genera. *Unidentified* nematode emerged as the most dominant genus in Location 1, with a mean proportion exceeding 60%, significantly higher than the combined average from all other sites. The wide confidence interval, however, indicates high variability across replicates. Genera such as *Rotalia sp.*, *Arcella sp.*, and *Keratella cochlearis* also showed distinctly higher abundance in Location 1 compared to the pooled samples from other locations, with positive differences in mean proportions and confidence intervals that do not overlap zero, suggesting

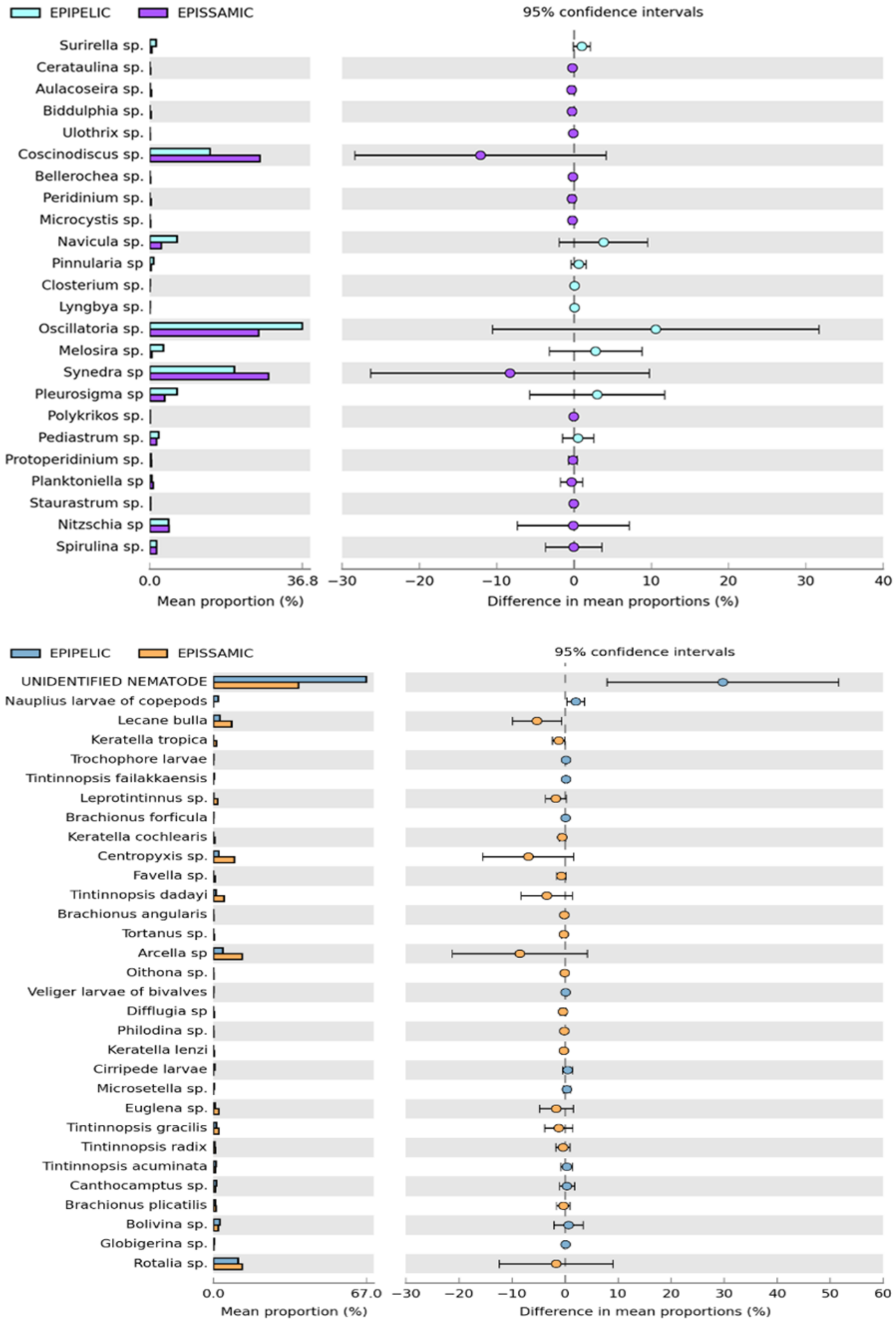


Fig. 5. *Comparison of periphyton genus composition between epipelic (blue) and Epissamic (purple) habitats. It also describes the Comparison of zoobenthic genus composition between epipelic (blue) and Epissamic (orange) habitats. The left panel shows the mean proportion of each genus; the right panel displays the difference in mean proportions with 95% confidence intervals*

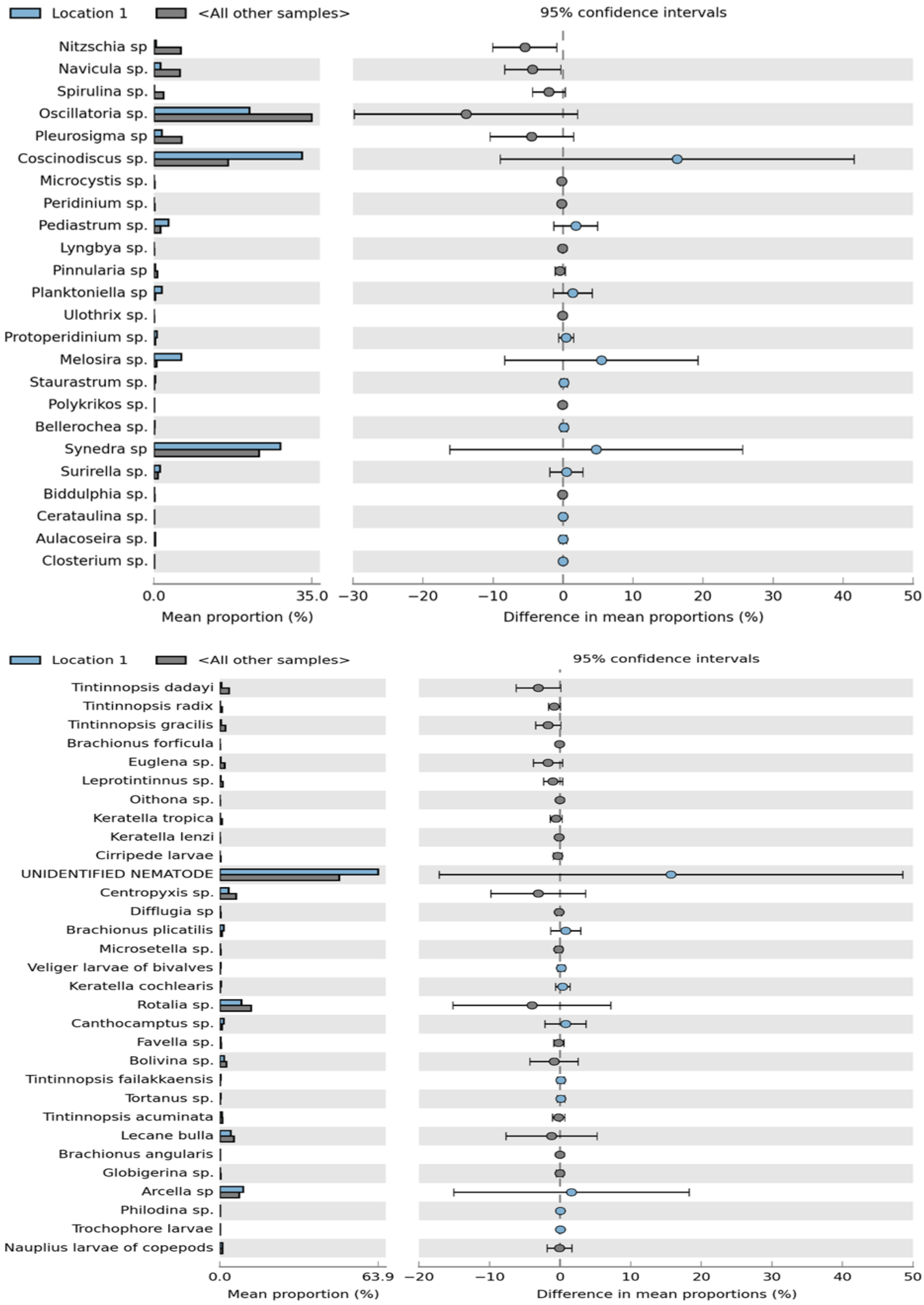


Fig. 6. *Differential abundance of Periphyton genera and Zoobenthic genera between Location 1 and all other locations (Locations 2, 3, and 4 combined). The left panel shows the mean proportion (%) for each genus in Location 1 (blue) compared with the combined mean across all other locations (grey). The right panel shows the difference in mean proportions with corresponding 95% confidence intervals*

statistically significant enrichment. *Veliger larvae of bivalves* and *Brachionus plicatilis* likewise exhibited higher proportions in Location 1. In contrast, several genera, including *Tintinnopsis dadayi*, *Tintinnopsis gracilis*, *Tintinnopsis radix*, *Brachionus forficula*, *Euglena sp.*, and *Leprotintinnus sp.* were relatively more abundant in the other locations than in Location 1. This is evident from the negative differences in mean proportions and non-overlapping or marginally overlapping confidence intervals. These patterns suggest that these genera are more characteristic of environments prevailing outside Location 1. A subset of genera, including *Microsetella sp., Tortanus sp.,* and *Globigerina sp.,* showed minimal differences between Location 1 and the pooled group, as reflected by overlapping confidence intervals, suggesting a more uniform distribution.

Zoobenthic diversity index

Epipelic region

In Epipelic region, during the winter season of 2024, Margalef's diversity index (Species Richness) S for Zoobenthic communities at the sampling stations ranged from 1.324 to 2.278, with an average of 1.666. The Shannon-Wiener Index (H) for these communities varied between 0.212 and 0.745 (H'(log10)), averaging 0.368. The Simpson diversity index (1-D) for the winter season was below 0.9, fluctuating from 0.177 to 0.728, with an average of 0.400. In the summer season of 2024, Margalef's diversity index (Species Richness) S for Zoobenthic communities ranged from 1.404 to 1.847, with an average of 1.648. The Shannon-Wiener Index (H) during this period was between 0.190 and 0.3946 (H'(log10)), with an average of 0.284. The Simpson diversity index (1-D) for the summer season remained below 0.9, ranging from 0.181 to 0.377, with an average of 0.277. During the post-monsoon season of 2024, Margalef's diversity index (Species Richness) S for Zoobenthic communities varied from 2.380 to 2.720, averaging 2.573. The Shannon-Wiener Index (H) for this season ranged from 0.646 to 0.854 (H'(log10)), with an average of 0.758. The Simpson diversity index (1-D) during the post-monsoon season was below 0.9, ranging from 0.621 to 0.789, with an average of 0.728.

Epissamic region

In Epissamic region, during the winter season of 2024, Margalef's diversity index (Species Richness) S for Zoobenthic communities at the sampling stations ranged from 1.245 to 2.079, with an average of 1.862. The Shannon-Wiener Index (H) for these communities varied between 0.219 and 0.895 (H'(log10)), averaging 0.635. The Simpson diversity index (1-D) for Zoobenthic communities during winter was below 0.9 at the selected stations, ranging from 0.210 to 0.877, with an average of 0.648. In the summer season of 2024, Margalef's diversity index (Species Richness) S for Zoobenthic communities at the sampling stations ranged from 1.375 to 2.433, with an average of 1.951. The Shannon-Wiener Index (H) for these communities was between 0.609 and 0.675 (H'(log10)), with an average of 0.647. The Simpson diversity index (1-D) for Zoobenthic communities during summer was below 0.9 at the selected stations, ranging from 0.525 to 0.700, with an average of 0.594. During the post-monsoon season of 2024, Margalef's diversity index (Species Richness) S for Zoobenthic communities at the sampling stations ranged from 2.301 to 3.422, with an average of 2.649. The Shannon-Wiener Index (H) for these communities varied from 0.718 to 0.968 (H'(log10)), averaging 0.820. The Simpson diversity index (1-D) for Zoobenthic communities during the post-monsoon season was below 0.9 at the selected stations, ranging from 0.723 to 0.860, with an average of 0.783.

One- way analysis of variance (ANOVA) for interpreting seasonal effects on community structure

One-way ANOVA revealed significant seasonal effects on community structure, particularly for epipelic organisms. One-way ANOVA showed significant seasonal variation in epipelic Margalef's (F = 13.698, p = 0.002), Shannon–Wiener (F = 9.737, p = 0.006), and Simpson's diversity indices (F = 8.931, p = 0.007), with post-monsoon communities differing significantly from winter and summer, while winter and summer remained similar ($p > 0.05$). In contrast, epipsammic organisms exhibited significant seasonal variation only in species richness (Margalef's Index; F = 6.355, p = 0.019), where summer richness was significantly lower than winter and post-monsoon, whereas Shannon–Wiener and Simpson's indices showed no seasonal differences ($p > 0.05$). Overall, these results demonstrate strong seasonal control—especially during the post-monsoon period—on epipelic diversity, while epipsammic communities show seasonal sensitivity primarily in species richness but remain otherwise stable.

DISCUSSION

Benthic algae are among the most successful groups of primary producers in stream and estuarine ecosystems. Their distribution is largely confined to the uppermost sediment layers due to limited light penetration, yet they play a pivotal role in nutrient mineralization and oxygen fluxes at the sediment–water interface. Because environmental conditions tightly regulate their occurrence, benthic algal communities are widely recognized as sensitive indicators of water quality, and their ecological importance as primary producers in littoral and intertidal zones has been emphasized in numerous studies (Aberle and Wiltshire, 2006). Hydrographical dynamics further influence periphyton popula-

Table 1. Periphyton variation in abundance and diversity

SEASON	SAMPLINGsTATION	EPIPELIC oRGANISMS						EPISSAMIC oRGANISMS					
		ABUNDANCE (uNITS/m2)	No of genera/ Or species observed/ Total genera or species	% Diversity	Margalef's diversity index (Species richness S)	Shannon weiner index (H (log10))	(Simpson's Diversity Index 1-D)	ABUNDANCE (uNITS/ m2)	No of genera/Or species observed/ Total genera or species	% Diversity	Margalef's diversity index (Species richness S)	Shannon weiner index (H (log10))	(Simpson's diversity Index 1-D)
WINTER 2024	L - 1	3584	13/13	100	1.466	0.5718	0.6953	2171	15/15	100	1.822	0.4361	0.532
	L – 2	1053	13/13	100	1.724	0.6284	0.667	208	14/15	93.33	2.436	0.6926	0.7006
	L – 3	1165	13/13	100	1.7	0 .7706	0.7859	1954	15/15	100	1.848	0.6127	0.7056
	L - 4	6336	13/13	100	1.371	0.2408	0.2491	1073	14/15	93.33	1.863	0.6812	0.7235
SUMMER 2024	L - 1	368	11/11	100	1.693	0.6775	0.7056	137	7/9	77.78	1.220	0.5658	0.4782
	L – 2	198	9/11	81.82	1.513	0.7479	0.7137	587	8/9	88.89	1.098	0.3375	0.3048
	L – 3	605	11/11	100	1.561	0.5734	0.5971	96	8/9	88.89	1.534	0.746	0.6737
	L - 4	1140	10/11	90.91	1.279	0.7263	0.7263	173	8/9	88.89	1.358	0.5952	0.5375
POST mONSOON 2024	L - 1	89	12/14	85.71	2.451	0.7003	0.6856	193	12/14	85.71	2.09	0.6956	0.7305
	L – 2	3140	12/14	85.71	1.366	0.1515	0.1467	2458	12/14	85.71	1.409	0.2467	0.2466
	L – 3	1201	13/14	92.86	1.692	0.6642	0.6625	173	13/14	92.86	2.329	0.6634	0.643
	L - 4	415	12/14	85.71	1.825	0.8641	0.8459	211	12/14	100	2.429	0.7005	0.711

*L – location.

Table 2. Zoobenthic variation in abundance and diversity

SEASON	SAMPLING sTATION	EPIPELIC oRGANISMS						EPISSAMIC oRGANISMS					
		ABUNDANCE (number/ m^2)	No of genera/Or species observed/Total genera or species	% Diversity	Margalef's diversity index (Species richness S)	Shannon weiner index (H (log10))	(Simpson's diversity index 1-D)	ABUNDANCE (number/ m^2)	No of genera/Or species observed/Total genera or species	% Diversity	Margalef's diversity index (Species richness S)	Shannon weiner index (H (log10))	(Simpson's diversity index 1-D)
WINTER 2024	L-1	386	10/10	100	1.511	0.2116	0.1767	124	7/10	70	1.245	0.2194	0.2101
	L-2	330	10/10	100	1.552	0.228	0.1939	29	8/10	80	2.079	0.7439	0.7956
	L-3	52	10/10	100	2.278	0.7446	0.7278	47	9/10	90	2.078	0.6803	0.7086
	L-4	198	8/10	80	1.324	0.2868	0.2603	50	9/10	90	2.045	0.895	0.8767
SUMMER 2024	L-1	298	9/13	69.23	1.404	0.19	0.1813	81	9/12	75	1.82	0.6092	0.5813
	L-2	76	9/13	69.23	1.847	0.3946	0.3766	38	6/12	50	1.375	0.675	0.5253
	L-3	144	10/13	76.92	1.811	0.2895	0.2895	25	8/12	66.67	2.175	0.6312	0.57
	L-4	358	10/13	76.92	1.53	0.2605	0.2605	61	11/12	91.67	2.433	0.6726	0.7005
POST mONSOON 2024	L-1	172	15/17	88.24	2.72	0.8537	0.7899	284	14/20	70	2.301	0.7709	0.7796
	L-2	283	16/17	94.12	2.657	0.7454	0.7432	258	20/20	100	3.422	0.9681	0.8602
	L-3	550	17/17	100	2.536	0.646	0.621	167	13/20	65	2.345	0.7184	0.7239
	L-4	359	15/17	88.24	2.38	0.7868	0.7581	115	13/20	65	2.529	0.8223	0.7681

*L – location.

tions by modulating light availability, sediment stability, and nutrient exchange. In this context, the present study represents the first systematic investigation of seasonal variations in epipelic and epipsammic benthic algal communities in the intertidal sediments of the Tapi estuary, aiming to elucidate seasonal community patterns and enhance understanding of these ecologically significant assemblages.

Bottom sediments constitute a major reservoir of nutrients in estuarine systems, and nutrient cycling within these environments is strongly mediated by microorganisms inhabiting sediment microhabitats (Hašler *et al*., 2008). The four sampling stations examined in this study differed markedly in environmental characteristics, including light availability, grazing pressure, anthropogenic activity, temperature, and nutrient composition. Such variability is ecologically relevant, as microphytobenthos biomass and community structure are governed by complex interactions between abiotic factors—particularly light, nutrient availability, and sediment resuspension—and biotic controls such as grazing pressure (Haro *et al*., 2020).

Sampling Station 1, designated as the reference site, was characterized by extensive mangrove cover. Mangrove ecosystems are globally recognized for their high productivity and capacity to support diverse biological communities while providing critical ecological functions (Neba *et al*., 2021). At this station, mangrove presence likely enhanced sediment stability, organic matter accumulation, and nutrient retention, factors that were reflected in the structure of benthic microalgal assemblages. In contrast, Sampling Stations 2, 3, and 4 were designated as test sites due to their proximity to sewage outfalls. Such outfalls are typically associated with elevated organic loading and nutrient enrichment of adjacent sediments, conditions that often result in discernible shifts in microbenthic community structure. These shifts commonly manifest as increased dominance of pollution-tolerant taxa and altered diversity patterns, reinforcing the utility of microbenthic assemblages as sensitive indicators of anthropogenic disturbance in estuarine environments.

Across all stations, cell densities were consistently highest in the uppermost surface sediment layer (epipelic zone) and declined with increasing sediment depth in the underlying epipsammic layers. This vertical pattern is attributable to greater light availability at the sediment surface, as photosynthetic activity in benthic algae is restricted to a very thin illuminated layer (Aberle and Wiltshire, 2006). Additionally, calm hydrodynamic conditions and prolonged exposure to sunlight during low tide facilitate the development of higher microphytobenthic biomass in intertidal zones (Haro *et al*., 2020). While these patterns may be broadly representative of estuaries with similar tidal regimes and pollution profiles, caution is warranted when extrapolating these findings to systems characterized by different hydrodynamic conditions or anthropogenic pressures.

Over the annual study period encompassing winter, post-monsoon, and summer seasons, a total of 24 microbenthic algal taxa and 31 microfaunal taxa were recorded and their abundance data are given in Supplementary Tables 1 -3. Microphotographs of some organisms are depicted in Fig. 7. Microbenthic algal abundance was highest during the winter season, followed by the post-monsoon period, and lowest during summer. The elevated winter abundance was associated with the dominance of *Oscillatoria* sp., *Synedra* sp., *Nitzschia* sp., and the chain-forming benthopelagic diatom *Melosira* sp., the latter being recorded exclusively during winter as shown in Supplementary table 3. Although *Melosira* is considered relatively pollution tolerant (Palmer, 1969), its seasonal occurrence in the present study is interpreted as an ecological association rather than as evidence of a direct causal relationship with pollution. Similarly, mass occurrences of cyanobacterial colonies are often observed under nutrient-enriched conditions, reflecting associations with environmental enrichment rather than direct causation (Aberle and Wiltshire, 2006).

Light availability emerged as a critical driver of benthic algal growth and survival, as photosynthetic organisms rely on adequate irradiance to convert inorganic compounds into biomass. Variations in light conditions strongly regulate colonization and distribution patterns of benthic microalgal communities (Aberle and Wiltshire, 2006). In the present study, light availability in conjunction with temperature played a dominant role in shaping seasonal abundance patterns. This observation is consistent with previous findings indicating that irradiance and temperature exert the strongest influence on microphytobenthic photosynthetic rates, and that the combined effects of light, temperature, and day length are more influential than any single factor alone (Rhee and Gotham, 1981; Haro *et al*., 2020). Winter conditions, characterized by relatively optimal temperatures, favored higher microphytobenthic abundance, whereas elevated summer temperatures coincided with reduced diversity.

Although grazing pressure is often invoked to explain summer declines in microphytobenthic biomass—as benthic consumers typically reach peak abundance during this period (Colijn and Dijkema, 1981)—this mechanism does not fully explain the patterns observed in the present study. Notably, microfaunal abundance was also lowest during summer, suggesting that reduced diversity during this season cannot be attributed solely to grazing pressure. The decline in *Oscillatoria* sp. during summer may instead be related to its limited ability to attach to sand and silt substrates under elevated temperatures, as previously suggested by Hickman (1974).

Among diatoms, *Synedra* sp. emerged as the dominant taxon, followed by *Oscillatoria* sp. The apically attached *Synedra* forms hemispherical rosettes on substrates and spherical planktonic colonies composed of radially arranged cells (Stevenson *et al*., 1996). In the present study, *Synedra* sp. showed greater abundance in deeper, nutrient-rich epipsammic layers than in the surface epipelic zone. This distribution aligns with the known motility of certain benthic diatoms, which enables vertical migration within sediments to access higher nutrient concentrations under hypoxic or anaerobic conditions (Round, 1981). Other diatoms, including *Coscinodiscus* sp., *Nitzschia* sp., and *Navicula* sp., also contributed substantially to overall algal abundance.

Within the zoobenthic community, nematodes were the most dominant group across seasons and sediment layers. Nematodes are ubiquitous in aquatic sediments and are widely employed as bioindicators of environmental conditions due to their sensitivity to pollution gradients (Nzeako *et al*., 2015). Their highest abundance was recorded in the epipelic layer during winter, likely reflecting favorable environmental conditions and proximity to pollution sources. In addition to nematodes, protozoans and foraminifers contributed significantly to microfaunal diversity, with peak abundance observed during the post-monsoon season.

The patterns observed in the Tapi estuary are consistent with findings from other anthropogenically impacted Indian estuaries. Previous studies have demonstrated that sewage and industrial discharges lead to reduced benthic diversity and a shift toward pollution-tolerant taxa, as reflected by macrobenthic indices such as AMBI in monsoonal estuaries of northwest India (Mulik *et al*., 2020). Similar responses have been documented in the Mahanadi estuary, where anthropogenic loading resulted in decreased benthic diversity and increased dominance of opportunistic species (Dash *et al*., 2022). Studies on benthic foraminifera from the Uppanar and Beypore estuaries further reveal that trace metal and industrial pollution significantly alter community composition and reduce species diversity (Reddy *et al*., 2019; Ganugapenta *et al*., 2025). Moreover, research on microphytobenthos in Indian estuaries indicates that disturbances associated with anthropogenic activity—such as increased sedimentation, runoff, and turbidity—strongly regulate microalgal biomass and assemblage structure (Sanil Kumar *et al*., 2014). Collectively, these studies suggest that the biodiversity responses observed in the present investigation are broadly consistent with trends reported from other polluted tropical estuaries, thereby reinforcing the ecological relevance of the findings.

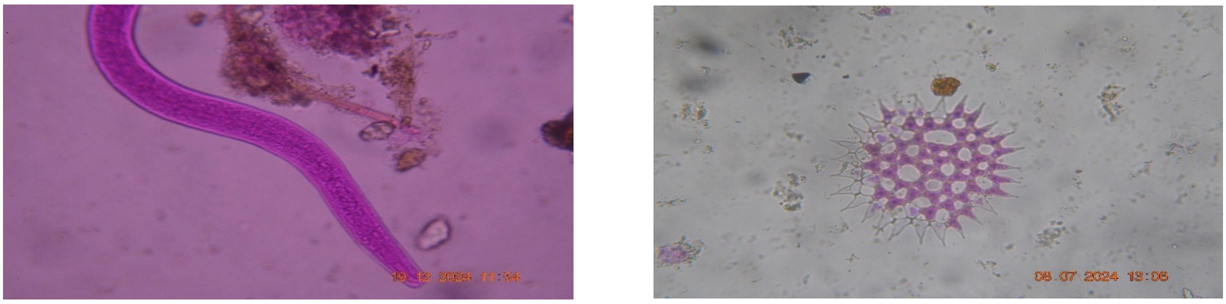
While the ecological drivers influencing benthic microalgal communities—such as light availability, nutrient dynamics, and seasonal temperature variation—are well established, the present study advances understanding by demonstrating how these drivers interact *vertically (epipelic vs. epipsammic strata), seasonally, and under localized sewage influence* in a tropical monsoon-driven estuary. Specifically, the present findings reveal that pollution-associated nutrient enrichment does not uniformly enhance benthic productivity but instead selectively favors pollution-tolerant taxa within surface epipelic layers, while deeper epipsammic communities retain distinct assemblages shaped by nutrient-rich but light-limited conditions. This stratified response highlights a previously underreported decoupling between surface and subsurface benthic functioning in sewage-impacted estuarine sediments. Moreover, the seasonal dominance patterns observed for key taxa such as *Oscillatoria*, *Synedra*, and *Nitzschia* indicate that pollution effects are strongly modulated by hydrological seasonality, suggesting that assessments based on single-season sampling may underestimate ecosystem sensitivity. By integrating vertical sediment structure, seasonal variability, and proximity to anthropogenic inputs, this study provides a more nuanced framework for interpreting benthic periphyton responses to pollution in tropical estuaries, with direct implications for refining habitat-specific biomonitoring tools in Indian estuarine systems.

Diversity indices

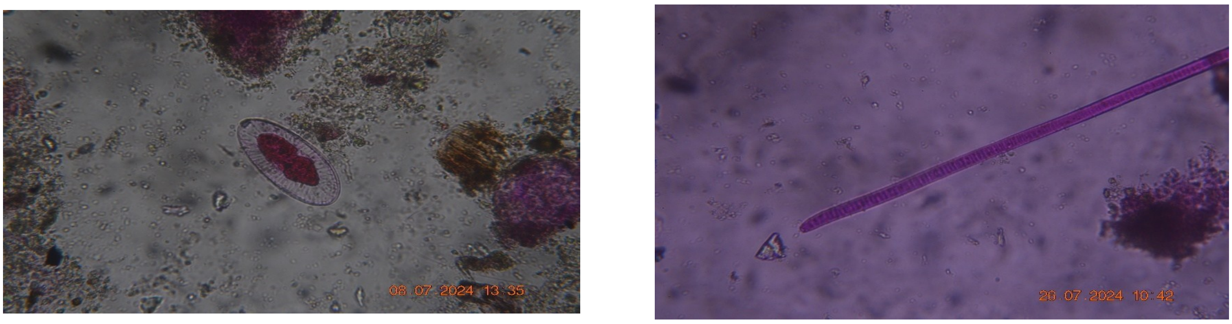
Diversity indices provided additional insights into community structure by integrating species richness and evenness (Magurran, 1988, 2004). Margalef's diversity index, Shannon–Wiener index, and Simpson's index revealed maximum diversity during the post-monsoon season, followed by winter, with minimum values during summer. The elevated post-monsoon diversity likely reflects more even distribution of organisms across sampling stations, driven by favorable ecological conditions and moderated anthropogenic influences during this period. These habitat-specific and seasonal shifts in epipelic and epipsammic assemblages provide a valuable basis for hypothesis-driven future research aimed at strengthening estuarine biomonitoring frameworks. In particular, the consistent dominance of pollution-tolerant taxa during specific seasons highlights their potential utility as indicator species for developing habitat-specific benthic biotic indices tailored to tropical estuaries such as the Tapi.

Limitations

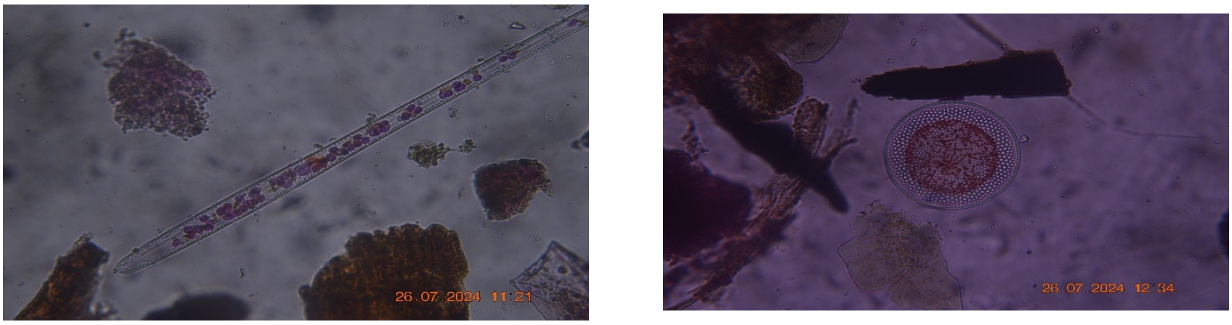
Several limitations of the present study are acknowledged. Multiple genus-level comparisons were conducted across seasons and sediment depths without applying multiple-testing corrections (e.g., Bonferroni or false discovery rate), increasing the potential for Type I errors. Additionally, although sediment chemical anal-



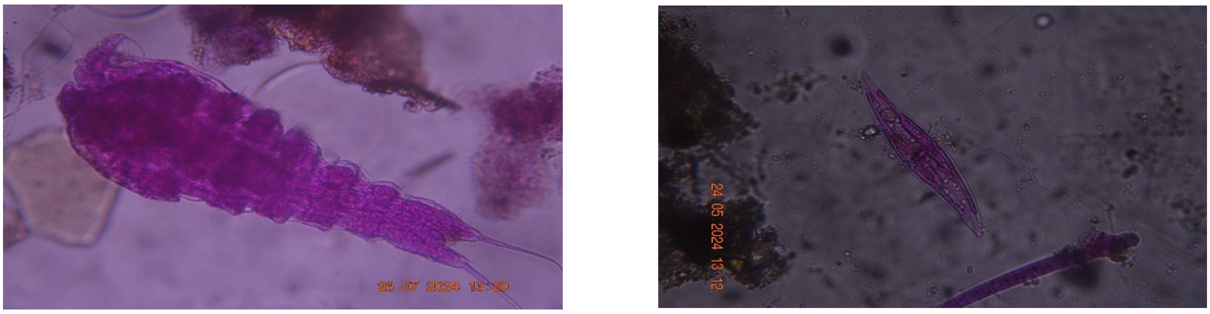
Nematode　*Pediastrum sp.*



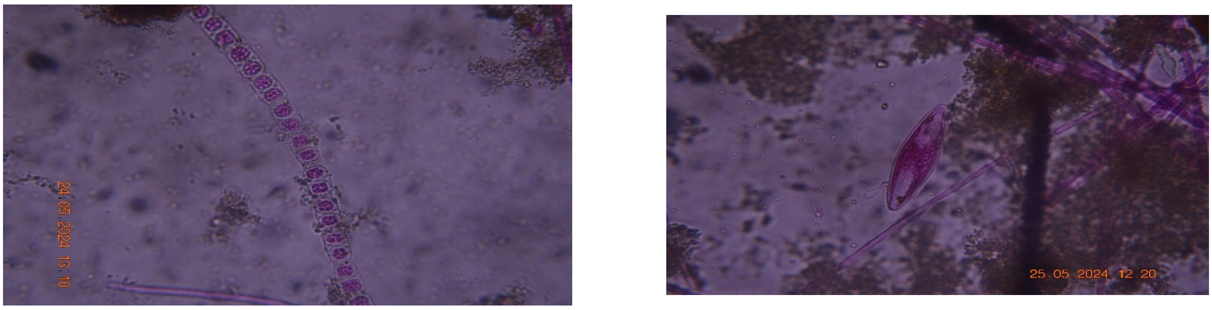
Surirella sp.　*Oscillatoria sp.*



Synedra sp.　*Coscinodiscus sp.*



Canthocamptus sp.　*Pleurosigma sp.*



Melosira sp.　*Navicula sp.*

Fig. 7. *Microphotographs of Micro-Organisms observed during current study*

yses were performed, the results were not included here, limiting the interpretation of pollution impacts. The absence of concurrent measurements of dissolved oxygen, pH, salinity, nutrients, and heavy metals further constrained the ability to establish direct correlations between physicochemical parameters and biological responses. Future studies incorporating these variables will enable a more mechanistic understanding of seasonal dynamics and enhance the use of benthic communities as early-warning bioindicators for estuarine environmental management. Furthermore, limited replication may reduce the power to detect subtle but ecologically meaningful differences, and future studies with higher temporal and spatial replication are recommended.

Conclusion

The present study provides the first integrated assessment of seasonal and vertical variability in epipelic and epipsammic benthic algal communities within the intertidal sediments of the Tapi estuary. The results demonstrate that benthic community structure is jointly regulated by sediment depth, seasonal hydroclimatic conditions, and localized anthropogenic inputs. Consistently higher abundances in surface epipelic layers highlight the overriding role of light availability, while the persistence of distinct epipsammic assemblages underscores the importance of nutrient-rich but light-limited subsurface microhabitats. Seasonal trends revealed winter and post-monsoon periods as phases of enhanced diversity and biomass, whereas summer conditions imposed ecological constraints reflected in reduced abundance and evenness. Importantly, proximity to sewage outfalls did not result in uniform productivity enhancement but instead promoted selective dominance of pollution-tolerant taxa, indicating a non-linear response of benthic communities to nutrient enrichment. This response was further modulated by seasonal hydrodynamics, emphasizing that pollution impacts cannot be reliably inferred from single-season observations. The observed decoupling between surface and subsurface benthic responses highlights the need to incorporate vertical sediment structure into estuarine biomonitoring frameworks. Overall, the study reinforces the sensitivity of benthic microalgal and microfaunal assemblages as indicators of environmental change and provides a foundation for developing habitat- and season-specific biotic indices tailored to tropical monsoon-driven estuaries.

ACKNOWLEDGEMENTS

I want to acknowledge Dr. Ranjhana Benjani from department of Aquatic Biology, VNSGU and Dr. Manoj Godhaniya from Department of Biosciences, VNSGU, for their contribution towards this work

Supplementary information

The author(s) are responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is available for download from the article's webpage and will not be included in the print copy.

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