



A comparison of physical disturbance and pollution stressors in sandy beaches using nematode functional biological traits

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Received: 8 September 2020 / Revised: 26 July 2022 / Accepted: 27 July 2022 / Published online: 10 August 2022
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Abstract

Nematode functional traits and community structure approaches are receiving attention and being debated regarding the similarity in gained information. This work applied two nematode methods (i.e. functional traits, community structure) and sex ratios to investigate sandy beaches experiencing physical disturbances and anthropogenic stressors along the Alexandria coast of Egypt. It is the first study to identify the Egyptian marine benthic nematodes to genus level yielding fourteen genera. This study addressed the following questions. (A) Which analyses of functional traits, community structure, or sex ratio could distinguish beaches experiencing natural disturbances from anthropogenic stressors? (B) What are the driving forces that govern nematode assemblages? Designated beaches were high pollution and energy (HPHE), high pollution but low energy (HPLE), and clean high energy (CHE). The study was conducted in March 2014, applying a stratified random sampling design and advanced multivariate analyses. Results revealed significant discrimination among and within beaches based on functional traits, community structure, and sex ratio. The functional traits (FBM) grouped data into two main groupings, predators and scavengers. Predators and cylindrical shape-tail types were found in high salinity moderately-sorted fine-grained sand dominant at HPHE and CHE. Scavengers and a variety of tail-shape types inhabited low saline water with well-sorted coarse sediment at HPLE. The community structure (CSM) revealed three groupings, as each beach had a specific assemblage based on its response to the measured physiochemical factors (salinity, pH, and temperature). Three genera were responsible for the high contribution across each beach *Graphonema sp.* (HPHE), *Enoplolaimus sp.* (HPLE), and *Oncholaimus sp.* (CHE), as well as the co-sharing of *Axnolaimus sp.* at HPLE. However, *Axnolaimus sp.* and *Oncholaimus sp.* showed the highest dissimilarity among beaches. The contribution of the measured factors was 53.8% (FBM) and 35.7% (CSM). Trophic guilds were anthropogenic stressors dependent and tail-shape type physically disturbing-related. However, nematode genera were species-specific to habitat type. The sex ratio was male-biased, and Juvenile-stages seemed to be temperature-dependent. The dissimilarity in the functional trait and community structure data recommend their application in biomonitoring and coastal restoration programs.

Keywords Meiofauna · Nematodes · Egyptian Mediterranean coast · Functional trait · Community structure · Sex ratio · Biomonitoring · Restoration

Introduction

Assessing ecosystem health is a tool for protecting coastal environments (Boesch and Paul 2001). Benthic community structure was used in the past, but it requires taxonomic expertise, is time-consuming (Losi et al. 2013), and has

many drawbacks due to the differential species-specific ecological adaptations within the same community (Salmasso et al. 2015). Researchers hypothesize other ecological indicators including taxonomic distinction (Bevilacqua et al. 2012), higher taxa (Semprucci et al. 2015a), Alpha and Beta diversity components (Flach et al. 2012), faunal biomass, allometric attributes (Losi et al. 2013), functional guilds and trophic food (Bhusal et al. 2014), and functional biological traits (Schratzberger et al. 2007; Alves et al. 2014; Semprucci et al. 2018) as surrogates for taxonomic identification.

Nematodes are the most abundant and diverse meiofaunal taxa among marine habitats (Kreuzinger-Janik et al.

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2019), survive under extreme conditions (Coull and Chandler 1992), are easy to collect in large numbers providing a highly reliable statistical database regardless of sample size volume (Platt and Warwick 1980) and can be classified by a number of biological traits (Schratzberger et al. 2007). Buccal morphology is a proxy for trophic relations (Jensen 1987; Vanaverbeke et al. 2004). Tail-shape type (Thistle et al. 1995; Fleeger et al. 2006) is another descriptor important in locomotion, reproduction, and retention in sediments. Each biological trait responds differently to the surrounding environmental condition (Soetaert et al. 2009; Fleeger et al. 2010; Mitwally and Fleeger 2015). Recently, these traits have been used to reflect the human impact on the ecosystems (Alves et al. 2013; Kalogeropoulou et al. 2015; Mitwally and Fleeger 2016).

Under natural conditions, the nematode sex ratio is 1:1 (Tietjen and Lee 1972), or it may be higher in favor of females (Sahraeian et al. 2020). The life cycle comprises six stages (Boufahja et al. 2011). Different juvenile stages and gravid females indicate continuous reproduction and sufficient food for developmental rates (Hodda and Nicholas 1990).

A quantitative study of meiofauna, which ranges from ~44 to 63 μm up to 500 up to 1000 μm (Giere 2009), started in the last century on Egyptian Mediterranean shores (Mitwally et al. 2004). However, biomonitoring and coastal restoration programs in Egypt do not account for meiofauna. Many studies summarize the advantages and disadvantages of using meiofauna in monitoring programs (Danovaro et al. 1995; Moreno et al. 2011; Semprucci et al. 2015a, b). Mitwally and Hamdan (2021) assess the ecological status of Alexandrian sandy beaches, Egypt. They found that meiofaunal natural variability was driven by salinity and sand grain sizes.

Egyptian Mediterranean coastal areas are threatened due to increasing natural disturbances and anthropogenic stressors (Frihy 2001). The Alexandrian coast is highly vulnerable to beach erosion, sea-level rise, rip currents, and anthropogenic activities (Soliman et al. 2014; Masria et al. 2015). The Egyptian Environmental Affairs Agency (EEAA 2015) declares two marine pollution hotspots, Abo-Qir Bay and El-Mex Bay, along the northern coast of Egypt due to their receiving mixtures of variable pollutants (Shreadah et al. 2014, 2019). In these bays, the high levels of chlorinated organic compounds, hydrocarbon oil, and heavy metal pollution can be found (Khairy et al. 2012; El Nemr et al. 2013).

This study aims to examine nematode assemblages and gives the first taxonomic identification of meiobenthic nematode taxa to genus levels at the Alexandrian coast of Egypt to address the following questions. (A) Which analyses of functional traits, community structure, or sex ratio can be used to distinguish beaches experiencing natural disturbances from anthropogenic stressors? (B) What are the driving forces that govern community structure of nematode assemblages?

Our null hypothesis is that examined approaches will not reveal significant variations between beaches under natural disturbances or anthropogenic stressors. The data from each bay will mirror each other.

Materials and methods

Sampling design

The current study applied the same stratified random sampling design as Mitwally and Hamdan (2021) to examine three sandy beaches; Abo-Qir Bay, highly polluted with high energy (HPHE), El-Mex Bay, highly polluted beach with low energy (HPLE), and the North West Coast, a clean beach with high energy (CHE). Our design is three sandy beaches, each composed of four profiles and five stations nested in with two replicates equal to 120 observations during March 2014.

Study area (Fig. 1)

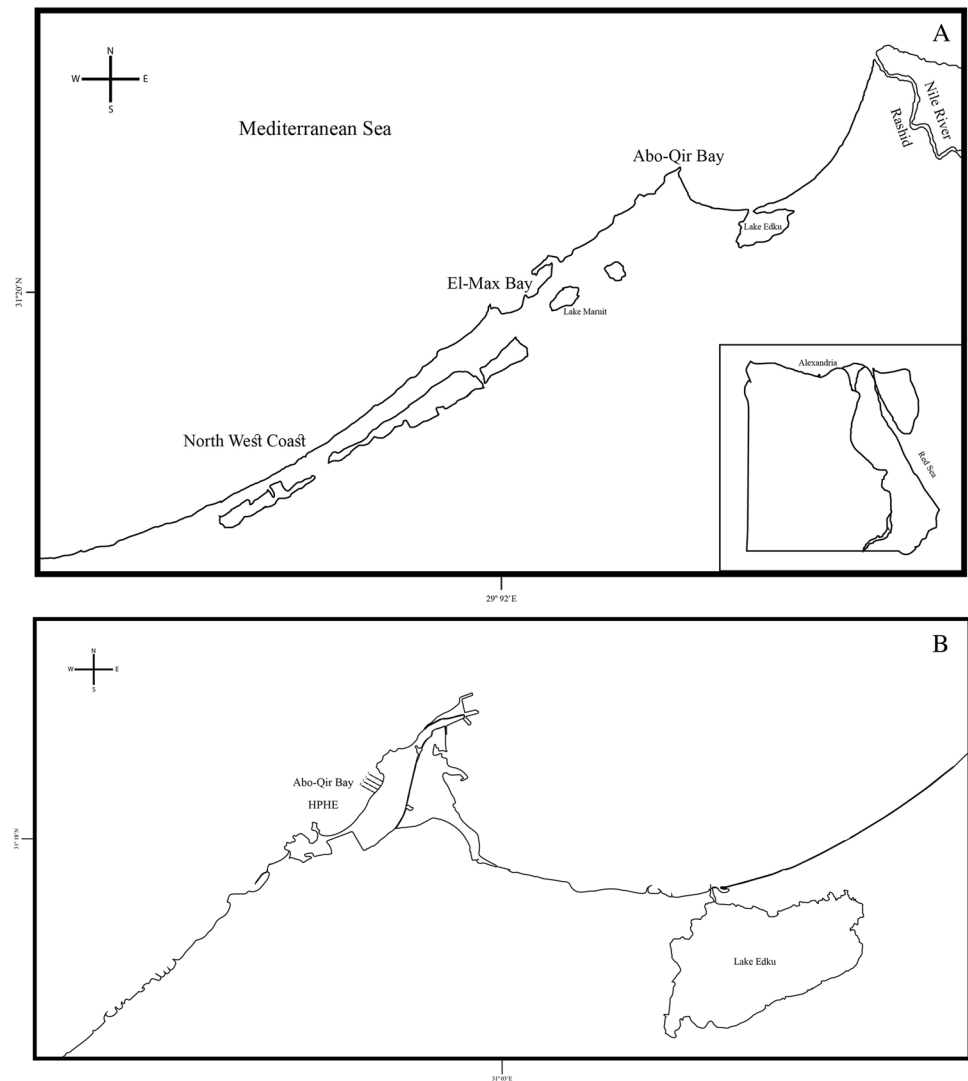
Abo-Qir bay (HPHE)

Abo-Qir Bay is a shallow semicircular bay with ~360 km^2 surface area and a maximum depth of ~16 m. The slope of the beach is gentle, backed by dunes. The Bay is bordered from the west by Abo-Qir Peninsula and from the east by the Rosetta Peninsula, where the Rosetta branch of the River Nile flows into the sea (Hamouda et al. 2015). Medium sandy sediment is dominant (Frihy 2003) except for the eastern side composed of muddy sediment due to discharge associated with the Rosetta estuary. The northwestern part has many rocky ridges, which cause some limitations in water exchange with the open sea (Elshanawany 2010). Abo-Qir Bay receives brackish and polluted waters via the El-Madyia outlet and El-Tabia pump station, respectively (Shreadah et al. 2019). High levels of chlorinated organic compounds and heavy metals enrichment were recorded at Abo-Qir Bay (Khairy et al. 2012; Abdel Ghani et al. 2013). It is a fully dissipative, eroding, beach that experiences dramatic erosion and sea-level rise (Frihy et al. 1996).

El-Mex bay (HPLE)

This elliptical-shaped bay has an area of ~20 Km^2 and an average depth of 10 m, a rocky shoreline with narrow sandy beaches (Aboul Ezz et al. 2014) covered by coarse-grain particles, characterized by eddy currents, classified as a microtidal estuary, and water masses move eastward (Shreadah et al. 2014). It has two different water salinity masses: a surface water mass with salinity values less than 10 PSU and a bottom mass with salinity higher than 25 PSU (Nessim

Fig. 1 The Egyptian Mediterranean Coast of Alexandria (A). Abbreviations: HPHE (B)= High Polluted High Energy beach, Abo-Qir Bay, HPLE (C)= High Polluted Low Energy beach, El-Mex Bay, CHE (D)= Clean High Energy beach, North West Coast beach. The numbers 1 to 4 indicate the sampling area at each beach



et al. 2010). It is highly eutrophic (Aboul Ezz et al. 2014), receiving polluted effluents via Lake Maruit and the El-Mex Pump station. Other pollutants include high concentrations of heavy metals and trace elements (Abdallah 2007), and different concentrations of polychlorinated biphenyl (PCBs) and organochlorine pesticides (Said et al. 2017).

The North West Coast (CHE)

This beach lies 100 km west of Alexandria, covered by white fine-carbonate oolitic sand grains (Nafaa and Frihy 1993; Iskander et al. 2007). This area is highly dynamic (Zaki et al. 2009), lacks civilization, has high water clarity, a moderately wide dissipative beach, a steep-slope shore, and hazardous rip currents (Frihy 2009). Coastal engineering projects such as sea walls, touristic villages, and others are dominant in this area of the northwestern coast of Alexandria (Iskander et al. 2007).

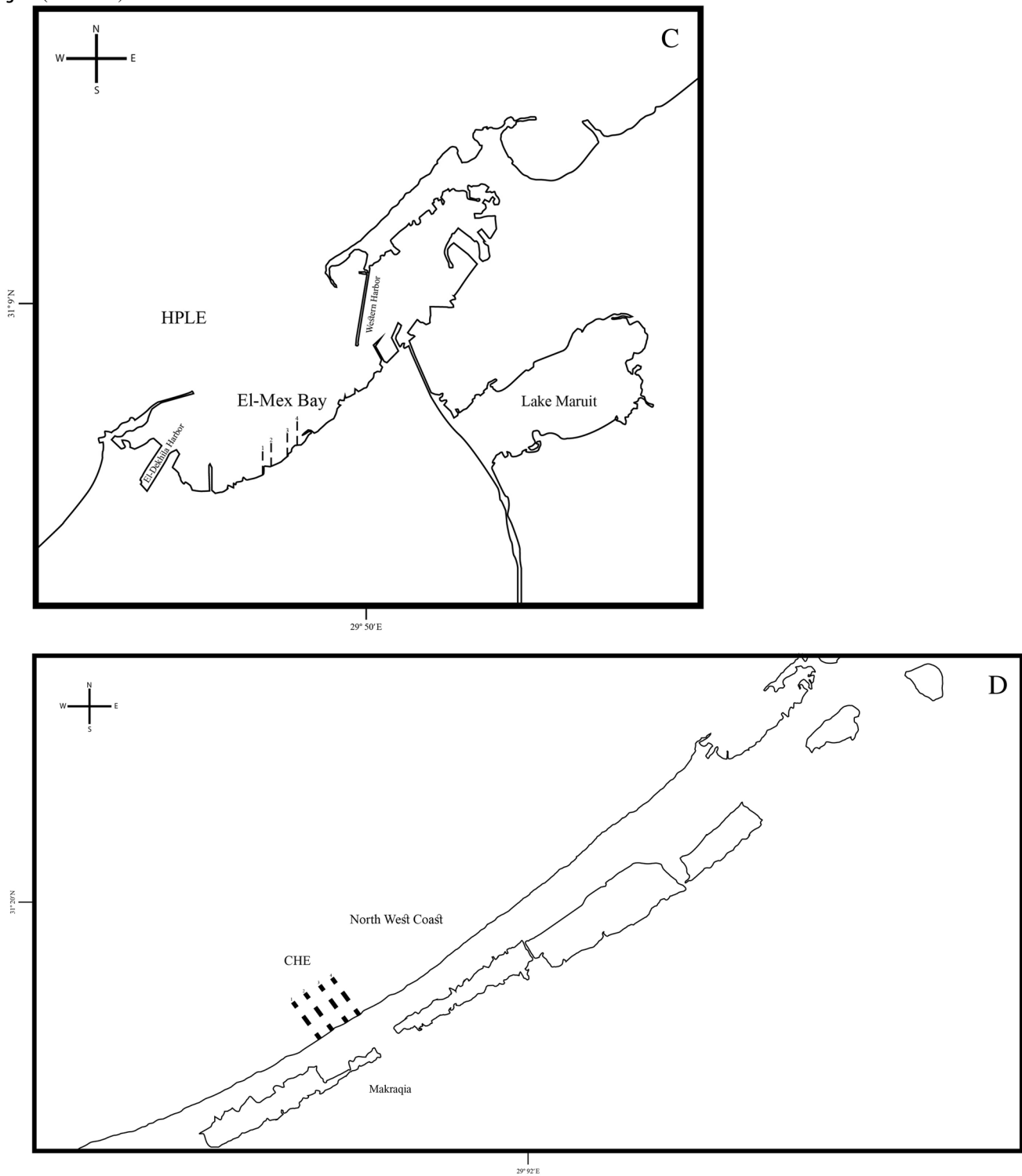
Field sampling

At each station, two replicate sediment samples were collected to a depth of 11 cm with a hand-held corer (4.8 cm² surface area) for meiofaunal analysis. Sediment samples were preserved with 4% formalin containing Rose Bengal dye. Water temperature (T°C) was measured in situ using a 0.1 graduated mercury thermometer, water salinity samples were collected using standard glass salinity bottles, and the pH was measured by a pH meter (HANNA HI98107). For sedimentological analysis, sediment was scooped to a depth of 11 cm at each station.

Laboratory analysis

A Huys et al. (1996) technique was applied to extract meiofauna from sediment using a sieve of 63 µm mesh size, organisms were sorted, counted under a dissecting stereomicroscope, and abundance was standardized as the number of

Fig. 1 (continued)



individuals per unit area of sediment (individuals 10 cm^{-2}). Nematode specimens were picked out, processed with pure glycerin, and mounted on permanent slides. Jensen (1987) buccal cavity identification was used to describe the mouth-parts of nematodes in general and that of predators and

scavengers individually. Four trophic groups were assigned, deposit and epistrate feeders, predators and scavengers, using oil immersion (100X). The tail-shape type of each nematode was identified, according to Thistle and Sherman (1985), and each nematode was assigned to one of four

tail-shape types; round shape (RT), elongate shape (ET), clavate conico-cylindrical shape (CCT), and conical shape (CT) types, using 20X and 40X compound microscope. The relative abundance of each feeding group and tail-shape type was calculated for each sample. Nematode taxonomic identification to genus level using the pictorial key of Platt and Warwick (1983), and life-history stage (i.e., male, female, and juvenile), were made and counted. Salinity (PSU) was determined, according to Strickland and Parsons (1972). We analyzed the organic carbon according to El Wakeel and Riley (1957), then the TOM percentage (%TOM) was calculated using Olausson (1975). Mean grain size and sediment sorting coefficient (sorting) were analyzed and calculated using Folk and Ward (1957).

Data analysis

All multivariate analyses were done using the Bray–Curtis similarity measure of the square root transformed data. PERMANOVA analysis (Anderson 2005) was conducted to test for variations in response variables among and within categorical factors (i.e., beaches, profiles, and stations) and was run (A) on the relative abundance functional biological matrix (FBM) of four feeding groups and four tail-shape types, and (B) on nematode community structure matrix (CSM) that consisted of 14 nematode taxa identified to genus. The sample design consisted of two fixed factors (beach and profile) and a random factor station. The beach factor has three levels (HPHE, HPLE, and CHE), and the factor profile is nested in the beach and has four levels (profiles 1 to 4). The station factor has five levels (stations 1 to 5) and is nested in beach and profile factors. Pair-wise comparisons were performed to detect posterior differences among/within all pairs of categorical factor levels. The permutation number of residuals is 9999 under a reduced model. The Monte Carlo p -values were calculated when a few unique values of permutation statistical (unique-perm) tests were obtained (Anderson et al. 2008), and all statistical results were described based on a significant α level ≤ 0.05 .

The n MDS analysis (Clark and Warwick 2001) was applied using FBM and CSM matrices. The categorical factors were used as samples to test which matrix could discriminate among sandy beaches. The ANOSIM (Anderson et al. 2008) was run to test the null hypothesis that data within the same grouping are not clumped together as those among different groups. The ANOSIM is more sensitive to heterogeneity and dispersion within samples of the same group (Anderson and Walsh 2013) than PERMANOVA. The two-way partially hierarchical design was applied using the matrices of FBM and CSM. The sample statistic (R) and the significant statistic at the 0.1% level were calculated at all possible permutations 9999. To test for the contribution (%) of each functional trait and nematode genus to the average

similarity within each site and average dissimilarity between every beach pairs, we applied the simple percentage of contribution (SIMPER) analysis using dissimilarity matrices of FBM and CSM at a cut of 70% of the low impact (Clarke and Gorley 2015).

Principal Coordinates analysis (PCO), an unconstrained ordination, was conducted to visualize the percentage of the total variation in the original resemblance data matrix of FBM and CSM alone without any specific model or hypothesis (Anderson et al. 2008) to test for clear separation among data from beaches. Distance-based redundancy analysis (dbRDA) (Legendre and Anderson 1999), a constrained model seeking linear combinations of response criterion and predictor variables, was conducted to visualize the percentage of variability in the original resemblance data matrix of FBM and CSM fitted the model and explained by dbRDA axes. The relative contribution, strength, and direction of each explanatory predictor variable (temperature, salinity, pH, TOM%, grain size, and sorting) in driving the variation along dbRDA axes were examined with one vector per predictor variable. The same response matrices of other analyses were used. All response data were square-root transformed based on Bray–Curtis similarity, whereas the environmental resemblance matrix was built on Euclidean distance after data normalizing. Ordination plots were visualized among beaches. All statistical analyses were performed by PRIMER 7 with PERMANOVA +.

Results

Nematode abundance

Nematode abundance was higher at HPLE beach (346 ± 360 individuals per 10cm^2) than at HPHE (106 ± 264 individuals per 10cm^2) and CHE (148 ± 198 individuals per 10cm^2 , Fig. 2A). Mean data was higher at profiles 2, 3, and 4 at HPLE than at the opposite profiles, at HPHE and CHE (Fig. 2B), and increased seaward direction towards station 5 (Fig. 2C).

Functional biological analysis

The relative abundance results of feeding groups (FTM %) revealed that the predator was the dominant group at HPHE and CHE, and scavenger was the dominant group at HPLE (Fig. 3A). The CT nematodes dominated the three beaches, followed by the CCT shape-type (Fig. 3B). The relative abundance of ET nematodes was relatively high at HPLE, whereas RT nematodes ranked third at HPHE. PERMANOVA (Table 1) revealed significant variations in the mean FBM among beaches and within profiles (Table 1). The highest significant differences in FBM were detected

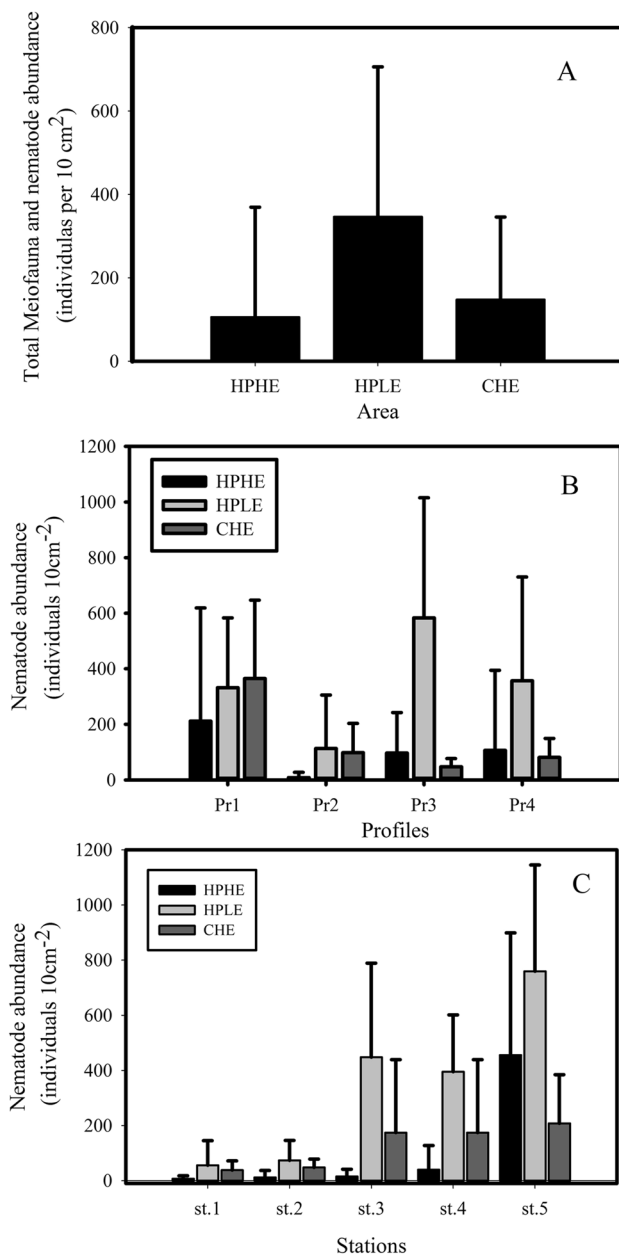


Fig. 2 A Spatial distribution of nematode and total meiofaunal abundance (individuals 10 cm^{-2}) among HPHE, HPLE, and CHE beaches, (B) at four profiles and (C) at five stations. Abbreviations: Pr. = profile, St. = station

between HPLE and CHE (Table 2), whereas the lowest variation was between HPHE and CHE (Table 2). Analysis within profile levels showed significant variations in mean FBM at HPHE profile 1 compared to HPHE profiles 2, 3, and 4 (Table 2). Significant variation was also found between profiles 1 and 4 at HPLE. At CHE, variations within each profile couple were ranked descendingly based on the t-test values (Table 2). Pair-wise comparisons within nested

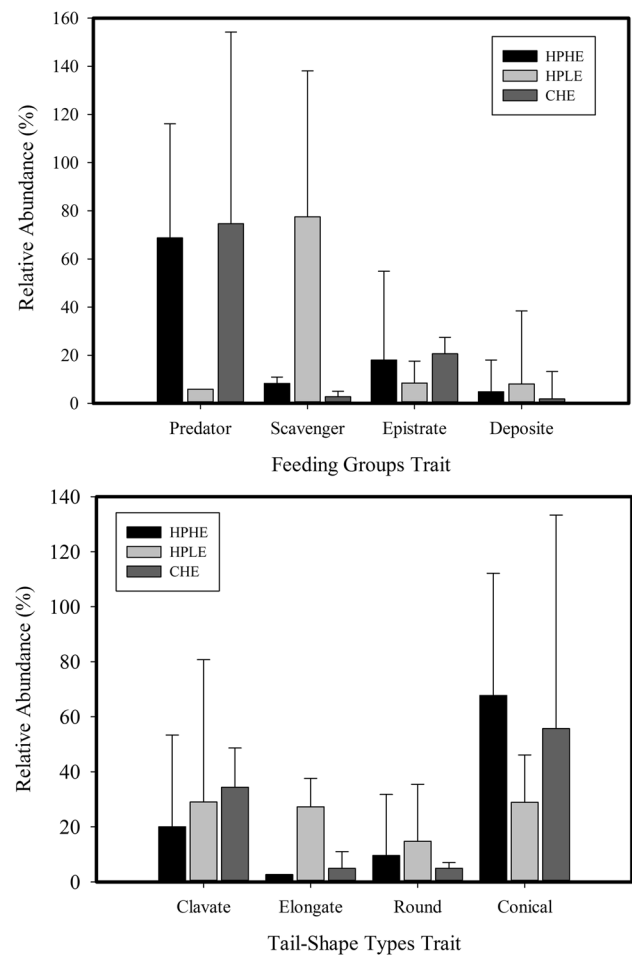


Fig. 3 The relative abundance (%) of functional biological traits at HPHE, HPLE, and CHE. (A) Feeding groups Trait, (B) Tail-shape types Trait

stations in profiles and beaches revealed non-significant variations. Therefore, we showed these results in Appendix 1.

Nematode community structure

The nematode community structure consisted of eleven families; according to their dominance Oncholaimidae, Thraucostomospidae, Axonolaimidae, Chromadoridae, Xyalidae, Enoplidae, Monhysteridae, Epsilonematidae, Cyatholaimidae, Cephalobidae, and Ironidae (Fig. 4). Fourteen nematode taxa belonging to nine families were identified at the genus level. *Axonolaimus* sp., *Graphonema* sp., *Oncholaimus* sp.1, *Oncholaimus* sp.2, *Theristus* sp., *Enoploides* sp., *Enoplus* sp., *Enoplolaimus* sp.1, *Enoplolaimus* sp. 2, *Epsilonema* sp., *Halomonhystera* sp., *Daptonema* sp., *Paracanthochus* sp., and *Prochromadorella* sp. Specimens of families Cephalobidae and Ironidae are assigned as unknown genera. The community structure consisted of 13 (HPHE), 14 (HPLE), and 9 (CHE) species (Fig. 4). PERMANOVA analysis results

Table 1 Permutation Multivariate analysis (PERMANOVA) results based on Bray–Curtis similarity resemblance of square-root transformed the relative abundance data of four feeding groups and four tail-shape types among-within categorical factors at actual probability

Effect	df	SS	MS	Pseudo-F	P-perm	u-per
Beach	2	71,162	35,581	46.87	0.0001	4738
Profile (Beach)	9	18,057	2006.3	2.64	0.0003	9920
Station (profile (Beach))	48	36,437	759.11	0.72	0.9545	9835
Residuals	60	63,019	1050.3			
Total	119	1.8867E+05				

Bold values indicate the results ≤ 0.05

Abbreviations: *df* degree of freedom, *SS* sum squares, *MS* mean square, *Pseudo-F* Pseudo-F statistic, *P-perm* probability, *u-per* unique-perms

Table 2 Results of Pair-wise a posteriori comparisons using PERMANOVA analysis based on Bray–Curtis similarity resemblance of square root transformed the relative abundance data of four feeding groups and four tail-shape types among-within categorical factors at actual probability

Area	t-test	P-perm	u-per	P(MC)						
HPHE, HPLE	6.961	0.0001	9956	0.0001						
HPHE, CHE	0.499	0.4842	9960	0.4687						
HPLE, CHE	10.47	0.0001	9956	0.0001						
Profiles										
		HPHE	HPLE	CHE						
u-per	Pro	t-test	P-perm	P(MC)	t-test	P-perm	P(MC)	t-test	P-perm	P(MC)
126	1, 2	2.50	0.0153	0.0190	0.88	0.5378	0.4923	0.78	0.5413	0.5687
126	1, 3	1.85	0.0494	0.0565	0.79	0.5980	0.5503	3.30	0.0155	0.0020
126	1, 4	2.31	0.0644	0.0302	2.23	0.0150	0.0264	1.45	0.1860	0.1391
126	2, 3	1.17	0.3204	0.2828	0.49	0.7798	0.7855	3.51	0.0078	0.0021
126	2, 4	1.14	0.3182	0.3017	1.06	0.2968	0.3367	2.05	0.0290	0.0342
126	3, 4	0.95	0.4547	0.4331	1.64	0.0861	0.1083	3.37	0.0083	0.0003

Abbreviations: *HPHE* high polluted high energy, *HPLE* high polluted low energy, *CHE* clean high energy. The rest of the abbreviations are listed in Table (1)

(Table 3) revealed significant variations among and within beaches (Table 3). The variation in community structure between HPHE and CHE was relatively higher than between HPLE and CHE, whereas the lowest variation was between HPHE and HPLE (Table 4). Analysis of profile levels (Pr.) revealed the most significant variations confined to Prs. 1 and 4 against each other, Prs. 1 and 2 (HPHE), Pr. 1 and 3, and Pr. 2 vs. Pr. 4 at CHE (Table 4). The station-level analysis detected scattered variations within the stations, but their permutations were quite a few, and data is shown in Appendix 2.

Environmental predictor parameters

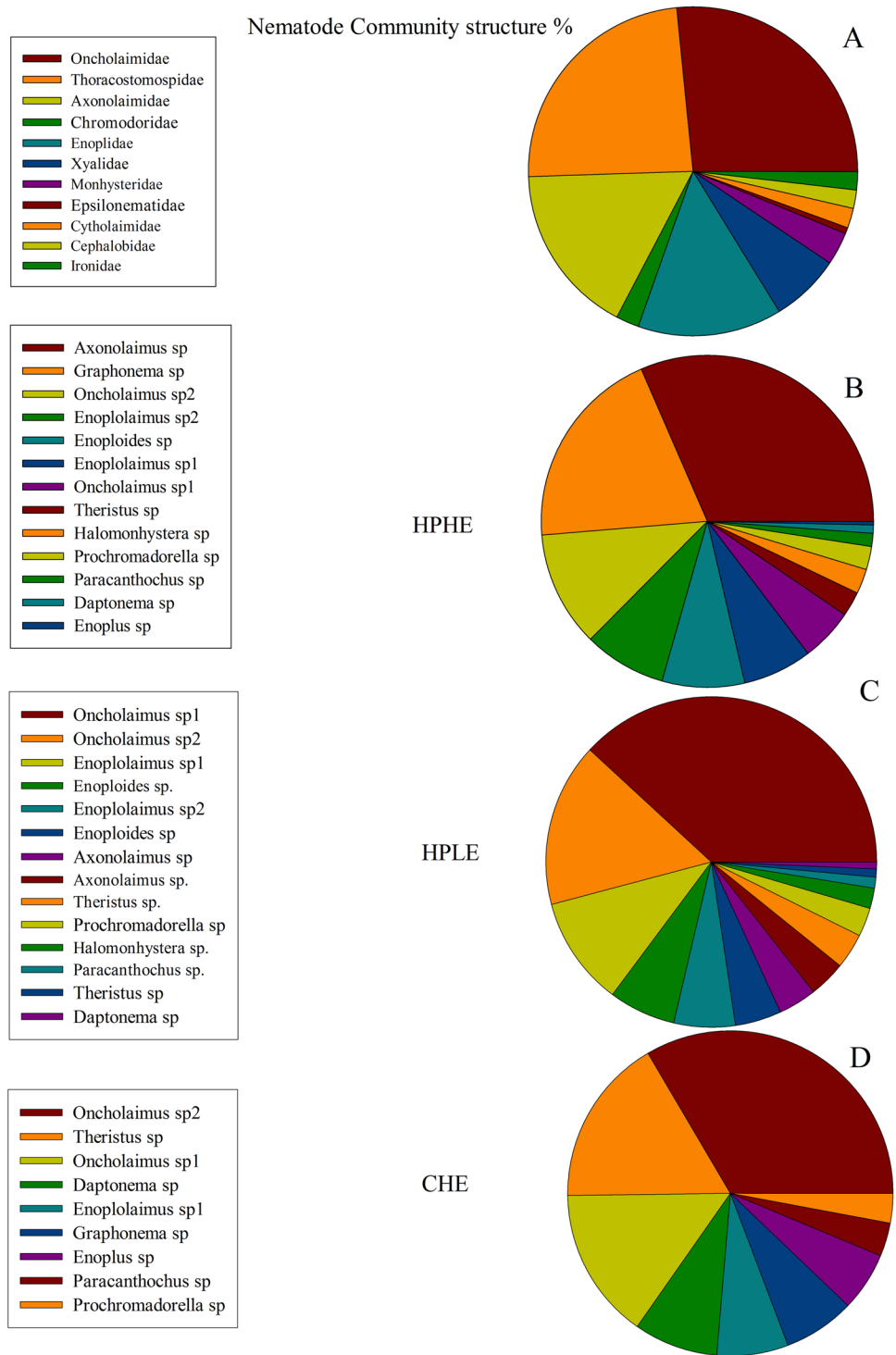
The Temperature variation range was tight among beaches; the lowest salinity and pH were recorded at HPLE and HPHE, respectively (Table 5). Moderately-sorted, fine-grained sand dominated HPHE and CHE versus well-sorted coarse sand particles at HPLE. The highest values of mean sedimentary % TOM were detected at HPHE and the lowest at HPLE.

Analysis of criterion biota data

Ordinations of *nMDS* (Fig. 5) revealed variation among-within categorical factors following PERMANOVA analyses. The FBM ordinations revealed two main data groupings HPLE data vs. HPHE and CHE grouping (Fig. 5A). Ordination *nMDS* based on CSM (Fig. 5B) revealed three groupings, a left-hand side that consisted of most HPLE data and a few data of HPHE, a right-hand side grouping composed of CHE data, and the third grouping of HPHE data and the remaining data, at HPLE. The R statistics were 0.66 (FBM) and 0.81 (CSM), respectively. The highest significant differences for FBM were between HPHE vs. HPLE and HPLE vs. CHE. The ANOSIM analysis of CSM revealed significant differences between each pair of beaches (Table 6). A relatively small and modest global R statistic between profile groupings across all beaches was 0.07 and 0.26, respectively, for FBM and CSM ($P=0.007$ and 0.0001).

The average similarity of FBM within each beach (Table 7) was the lowest at HPHE (52.14%) and the highest at CHE (65.63%). Predatory and CT nematodes contributed 75.32% of the similarity across the HPHE, whereas the contribution of the same traits (predators and CT) at

Fig. 4 Nematode community structure (%); (A) Nematode family data combined among-within beaches, the community structure (%) at (B) HPHE, (C) HPLE, and (D) CHE



CHE was 74.60%, and the CCT type shared the similarity with 15%. Scavengers were responsible for the highest contribution of similarity across HPLE (~50%), followed by CT and ET nematodes. The average dissimilarity between each pair of beaches ranked descending: HPHE vs. HPLE (68.68%), HPLE vs. CHE (65.45%), and HPHE vs. CHE (41.75%, Table 7). Five functional groups were responsible

for the dissimilarity between each pair, and their cumulative contribution ranged from 78.08% (HPHE vs. HPLE) to 79.28% (HPLE vs. CHE). Scavengers and predators were responsible for the dissimilarity between HPHE vs. HPLE and HPLE vs. CHE, and cumulative contribution accounted for 40.52% and 43.17%, respectively (Table 7). The CCT and CT nematodes led the distinction between HPHE vs.

Table 3 Permutation Multivariate analysis (PERMANOVA) table of results based on Bray–Curtis similarity resemblance of square root transformed abundance data of 14 nematode species among and within categorical factors at actual probability. Abbreviations are listed at Table (1)

Effect	df	SS	MS	Pseudo-F	P-Perm	u-per
Beach	2	62,510	31,255	25.45	0.0001	4719
Profile (Beach)	9	27,999	3111	2.53	0.0001	9874
Station (profile (Beach))	48	58,942	1228	3.35	0.0001	9789
Residuals	60	21,986	366.44			
Total	119	1.7144E+05				

Table 4 Results of Pair-wise a posteriori comparisons using PERMANOVA analysis based on Bray–Curtis similarity resemblance of square-root transformed abundance data of 14 species among and within categorical factors at actual probability. Abbreviations are listed at Tables (1&2)

Area	t-test		P-perm		u-per		P(MC)			
HPHE, HPLE	3.71		0.0001		9945		0.0001			
HPHE, CHE	6.17		0.0001		9947		0.0001			
HPLE, CHE	5.39		0.0001		9951		0.0001			
Profiles										
		HPHE			HPLE			CHE		
u-perm	Pr	t-test	P-perm	P(MC)	t-test	P-perm	P(MC)	t-test	P-perm	P(MC)
126	1, 2	2.39	0.0074	0.0042	1.35	0.1236	0.1612	1.43	0.1438	0.1537
126	1, 3	1.75	0.0179	0.0382	1.55	0.0868	0.0894	1.88	0.0255	0.0224
126	1, 4	2.12	0.0067	0.0095	2.08	0.0151	0.0116	2.93	0.0081	0.0006
126	2, 3	1.26	0.1791	0.2009	1.44	0.1352	0.1283	1.11	0.3128	0.3145
126	2, 4	1.14	0.3323	0.2902	1.24	0.1899	0.2133	1.82	0.0380	0.0395
126	3, 4	0.997	0.4388	0.4085	1.50	0.1033	0.1077	1.41	0.1145	0.1230

Table 5 Environmental parameters values (reported in mean ± standard deviation): Temperature (T°C), salinity (PSU), Hydrogen ions (pH), the percentage of total organic matter (%TOM), mean grain size (φ), and sediment sorting coefficient (sorting) among sandy beaches

Parameter	HPHE	HPLE	CHE
	Abo-Qir Bay	El-Mex Bay	North West Coast
Temperature (T°C)	21.0±0.1	22.0±0.4	21.5±0.3
Salinity (PSU)	37.3±0.3	27.6±0.4	37.8±0.0
Hydrogen Ions (pH)	7.9±0.1	8.0±0.1	8.1±0.0
% TOM	1.6±1.1	1.2±0.6	1.4±1.0
Mean grain size (φ)	1.3±0.2	0.6±0.8	1.7±0.4
Sediment sorting coefficient	0.55±0.1	0.45±0.2	0.52±0.05

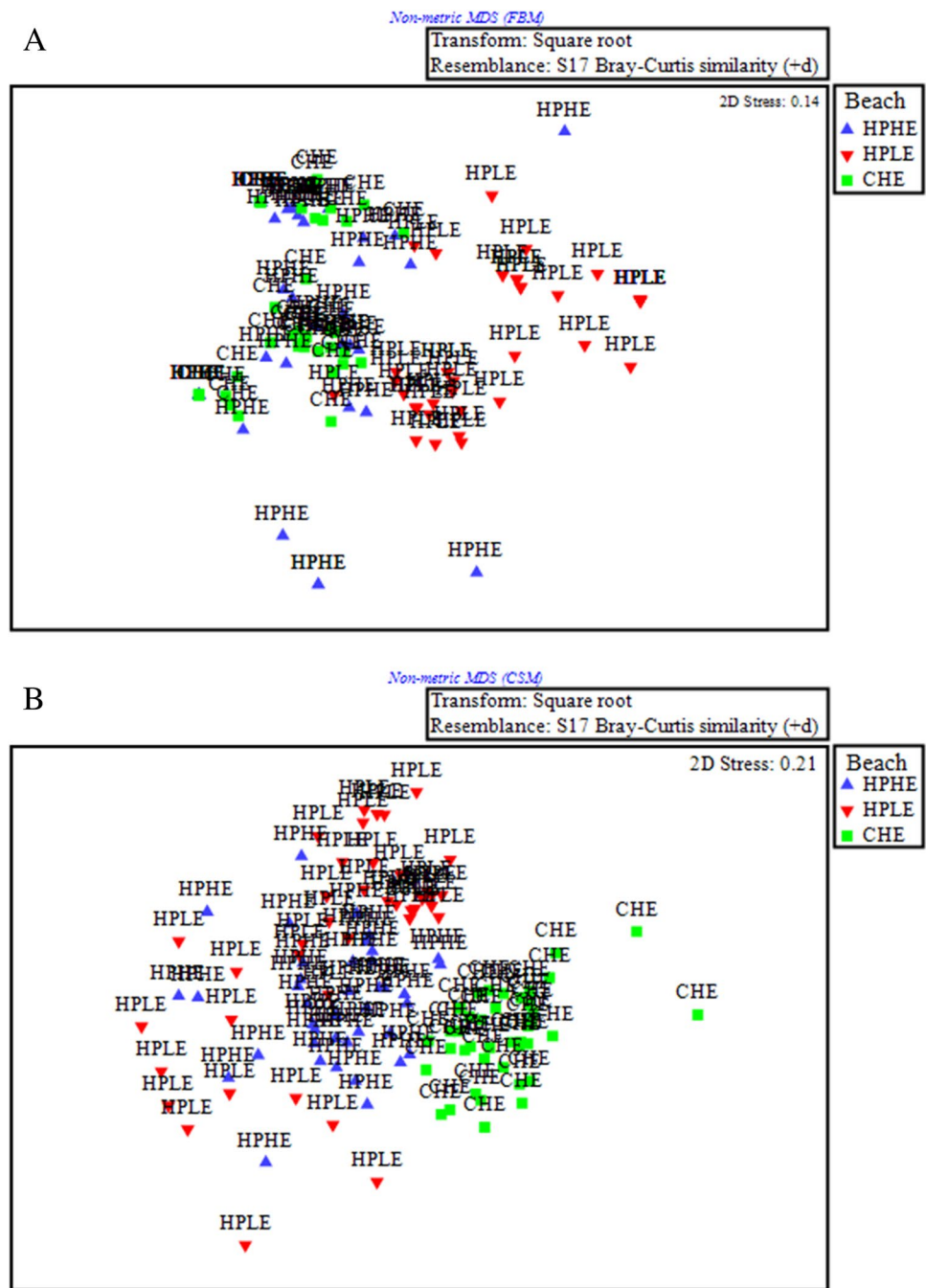
CHE (41.40%), and Epistrate feeders accounted for 16.75% of the average dissimilarity between beaches. The contributions (%) of tail types were relatively equal between HPHE vs. HPLE and HPLE vs. CHE. However, they have different rankings (Table 7).

The average similarity of the CSM matrix at HPHE and CHE consisted of two data groupings each comprising five nematode species (Table 8). *Graphonema sp.* and one species of *Oncholaimus sp.* contributions to the average similarity were the highest at HPHE and CHE, respectively. At the HPLE, four species were responsible for the similarity across the beach *Enoplolaimus sp.2* (23.45%) and *Ax nolaimus sp.* (21.62%) were dominant. The highest average dissimilarity was between HPLE vs. CHE (64.86%), HPHE vs. CHE (59.95%), and HPHE vs. HPLE (58.13% Table 8). Six nematode species were responsible for

the dissimilarity between HPHE vs. HPLE, and *Ax nolaimus sp.* was the key species (16.54%). Two groupings consisted of seven nematode species, each differentiated between HPHE vs. CHE and HPLE vs. CHE. The *Oncholaimus sp.1* (15.70%) was responsible for the dissimilarity between HPHE vs. CHE, whereas *Ax nolaimus sp.* and *Oncholaimus sp. 1* co-shared the contribution (~15%) to differentiate between HPLE vs. CHE.

The percentage of variation of the original unconstrained FBM resemblance matrix was explained by the first and second PCOs ordinations accounted for 81.4% among beaches (Fig. 6A). PCOs grouped data into two main groupings. Most of the HPLE data were located on the left-hand side of the plot, whereas HPHE and CHE data were grouped on the right-hand side. The first and second PCO coordinates accounted for 52.7% of the

Fig. 5 Plots of *n*MDS analysis based on Bray–Curtis similarity of square-root transformed data of (A) Functional Biological Matrix (FBM) and (B) Nematode Community Structure Matrix (CSM)



total variations, CSM matrix, among beaches (Fig. 6B). PCO analysis revealed two main groupings of CSM data; HPHE and HPLE data were gathered against CHE data.

Environmental parameters versus biota data

The FBM analysis revealed that the first *db*RDA axis was responsible for 92.0% of the fitted model, 51.6% of the total variation, and was strongly related to salinity and grain size

(Fig. 7A). The second axis was responsible for 3.9% of the fitted model, 2.2% of the total variation, and linked to pH. The *db*RDA ordinations visualized two main groupings. The first grouping consisted of HPHE and CHE data clustered within the vector overlay circumference on the left-hand side and associated with salinity, grain size, and sorting coefficient vectors. The second grouping consisted of HPLE data and was located on the right-hand side away from the vector overlay circumference. The CSM model explained that the 1st

Table 6 Analysis of Similarity (ANOSIM) based on the Bray–Curtis similarity index of square-root transformed the relative abundance data of eight functional groups and fourteen nematode species, revealing the higher significant differences among groups than that within groups at actual p values

	Functional Groupings		Community Groupings	
	Among beach Groupings (using profile as samples)			
Global Test	R	P-value	R	P-value
	0.66	0.03	0.81	0.0002
Groups	Pair-wise tests			
	R	P-value	R	P-value
HPHE, HPLE	1	0.029	0.65	0.029
HPHE, CHE	0.05	0.343	0.99	0.029
HPLE, CHE	1	0.029	0.83	0.029
	Between Profile Groupings (across all beaches)			
Global Test	R	P-value	R	P-value
	0.07	0.007	0.26	0.0001

Abbreviations: R R statistic

*db*RDA was responsible for 48.5% and 20.4% of variations in the fitted model and the total variation, respectively (Fig. 7B), and was related strongly to salinity, pH, and temperature. The 2nd axis explained 36.4% of variations in the fitted model and 15.3% of the total variation. It was related to pH. The *db*RDA ordination diagrams visualized three clear groupings, one grouping for each beach. The HPHE and CHE groupings were associated with salinity, grain size, sorting, and %TOM vectors, whereas HPLE was closely associated with temperature, and data groupings of CHE and HPLE were linked to the pH vector.

Sex ratio

Results of sex ratio (Fig. 8) revealed that the number of male individuals was approximately 1.5 times higher than that of female and ovigerous female individuals combined among beaches. Three stages of juveniles were accounted for (J2–J4). Juvenile Stage 3 dominated the HPHE and CHE beaches, whereas J4 dominated the HPLE beach.

Discussion

This work detailed a study of free-living benthic nematodes, functional traits, community structure, and sex ratio for the first time at the Alexandrian coast of Egypt. However, earlier studies dealt with nematode abundance,

trophic guilds, and tail types (Mitwally et al. 2004). The aim of applying different approaches to nematode analysis was to discriminate between three beaches experiencing physical disturbances and anthropogenic stressors. Two were high-energy salinity (HPHE, CHE) and consist of moderately fine-grained sand, where the HPHE is highly contaminated, having the lowest pH. The third is energy, salinity low, and the well-sorted coarse sediment was dominant. Multivariate analyses rejected the null hypothesis of two methodologies, differentiated significantly among beaches, revealed different gained information from functional biological (FBM) and community structure (CSM) analyses, and sex-ratios data added some evidence. The FBM analysis revealed two data groupings (HPHE and CHE vs. HPLE), whereas CSM showed three assemblages at beaches. Salinity and sediment structure drove the functional traits among beaches. Evidence that anthropogenic stressors impacted nematodes trophic guilds, whereas tail-shape nematodes' types were physically disturbed. Species responded differently to the environmental variables and were physicochemical dependent. *Graphonema sp.*, *Enoplolaimus sp. 2*, and *Oncholaimus sp.*, respectively, dominated HPHE, HPLE, and CHE. However, two key genera *Axnolaimus*, and *Oncholaimus*, contributed highly to the average dissimilarity among beaches. Results of sex ratios were male-biased. Juvenile stages seemed to be temperature-dependent. A thorough discussion will achieve the aim of the study.

The use of functional traits is promising in the applied analyses of ecosystem assessments (Martini et al. 2020). Trait analysis, herein, discriminated between the high salinity and moderately-sorted fine-grained sand (HPHE and CHE) vs. low water salinity and well-sorted coarse particles (HPLE, Tables 1, 2), revealing two data groupings (Figs. 5A, 6A, 7A), indicating the potential of water salinity and sand particles driving the traits, consistent with Mitwally and Hamdan (2021). Despite the explained proportion (53.8%, Fig. 7A) of the total matrix variation confirming that sand particles and salinity were functional trait drivers, ~46% of the total variation in functional traits data did not account for the measured environmental factors (Fig. 7A), suggesting that other non-quantified variables could affect the response of nematode functional traits.

High-saline water with moderately-sorted fine sediment were predators and CT nematode dominant. The low-saline water with well-sorted coarse sand particles were dominated by scavengers, CT, and ET nematode inhabitants. The potential of sediment grains (Semprucci et al. 2014) and salinity (Hourston et al. 2009; Alves et al. 2014) being principal environmental factors affecting biological traits is well documented. The contribution of predators and CT nematodes across HPHE in conjunction with the lowest pH values (Tables 7 and 5) suggests the

Table 7 Results of similarity percentage analysis (SIMPER) based on Bray Curtis similarity measure of square-root transformed data of the relative abundance of eight functional traits to test for trait contribution across all beaches at a cut off for low contribution (70%)

Average Similarity Across Beaches						
Beach	Species	Av. abundance	Av. Diss	Cont.%	Cum.%	
HPHE (52.14)	Predator	7.58	24.26	46.54	46.54	
	Conical	6.08	15.01	28.79	75.32	
HPLE (58.96)	Scavenger	8.73	29.43	49.91	49.91	
	Conical	4.19	8.70	14.76	64.67	
	Elongated	4.15	7.34	12.45	77.12	
CHE (65.63)	Predator	44.30	37.33	44.69	44.69	
	Conical	27.49	24.98	29.91	74.60	
	Clavate	5.07	10.12	15.43	84.37	
Group HPHE vs. HPLE (Average dissimilarity = 68.68)						
Species	Av. abundance	Av. abundance	Av. Diss	Cont.%	Cum.%	
Scavenger	1.26	8.73	14.84	21.60	21.60	
Predator	7.58	1.30	12.99	18.92	40.52	
Conical	6.08	4.19	8.82	12.84	53.36	
Elongated	0.66	4.15	8.61	12.54	65.90	
Clavate	4.06	3.44	8.37	12.18	78.08	
Group HPHE vs. CHE (Average dissimilarity = 41.75)						
Clavate	4.06	5.07	9.07	21.74	21.74	
Conical	6.08	6.31	8.21	19.67	41.40	
Epistrate	2.51	3.67	6.99	16.75	58.16	
Predator	7.58	8.51	4.91	11.75	69.91	
Round	1.45	1.12	3.49	8.36	78.27	
Group HPLE vs. CHE (Average dissimilarity = 65.45)						
Scavenger	8.73	0.93	14.64	22.37	22.37	
Predator	1.30	8.51	13.61	20.80	43.17	
Clavate	3.44	5.07	8.09	12.36	55.53	
Elongated	4.15	1.18	8.06	12.31	67.84	
Conical	4.19	6.31	7.48	11.44	79.28	

Abbreviations: Av. average, Diss. dissimilarity, Cont. % contribution %, Cum. % Cumulative %, and beach abbreviations at Table (1)

sensitivity of many species to acidified seawater excepting those exhibiting opportunistic behavior. Sarmiento et al. (2015) commented that opportunistic behavior compensates for the density reduction in sensitive nematode species to low pH. The synergetic effect of waves and contaminations could explain predators and CT nematodes' dominance at HPHE (75.32%, Table 7). Most predatory nematodes have opportunistic characteristics (Moens et al. 2013), switch their feeding habit to tolerate the surrounding environmental condition (Mitwally and Fleeger 2015), endure or avoid pollution (Hedfi et al. 2021), and survive at high energy (Mitwally et al. 2004; Kovalenko et al. 2011). Nematodes in the hydrodynamic areas are biased to the CT (Semprucci et al. 2018). The CT tail type provides more efficient use of caudal glands for substratum adherence (Pérez-García et al. 2019). Most predatory nematodes have a CT type (Mitwally and Fleeger 2016). Despite the high contribution of predators and CT nematodes at CHE, the CCT, CT, and epistrate nematodes

contributed more than 50% to the discrimination between the energetic beaches, suggesting their sensitivity to low pH values and contamination. This finding is consistent with Semprucci et al. (2015b), who reviewed the high contribution of epistrate feeders as a healthy ecosystem. A close association between epistrate feeders and CCT in a non-stressful ecosystem was documented (Mitwally and Fleeger 2015, 2016). The high contribution of scavengers (~ 50%) across HPLE could indicate their feeding on dead bodies in a highly polluted wave-less energy beach. Low energy causes pollution retention versus dispersion at high-energy beaches (Defeo et al. 2009). The high eutrophication at HPLE (Aboul Ezz et al. 2014) and pollution retention could inhibit primary production, increase mortality rates, and give a suitable substrate for scavengers to feed on decaying biomass. This interpretation is consistent with other studies that found scavengers and epigrowth feeders will colonize highly polluted environments (Thiermann et al. 1997; Hedfi et al. 2021; Nasri

Table 8 Results of similarity percentage analysis (SIMPER) based on Bray Curtis similarity measure of square-root transformed data of fourteen nematode genera's abundance to test for their contribution across all beaches at a cut off 70% for low contribution. Abbreviations are listed at Table (7)

Average Similarity Across Beaches					
Beach	Species	Av. abundance	Av. Diss	Cont.%	Cum.%
HPHE (58.66)	<i>Graphonema sp.</i>	2.71	14.33	24.42	24.42
	<i>Halomonhystera sp.</i>	1.35	9.77	16.65	41.08
	<i>Ax nolaimus sp.</i>	2.00	8.83	15.05	56.13
	<i>Oncholaimus sp.2</i>	1.72	7.89	13.45	69.58
	<i>Prochromadorella sp.</i>	0.99	5.96	10.16	79.74
HPLE (48.88)	<i>Enoplolaimus sp.2</i>	2.44	11.46	23.45	23.45
	<i>Ax nolaimus sp.</i>	3.37	10.57	21.63	45.08
	<i>Theristus sp.</i>	2.39	06.88	14.08	59.16
	<i>Prochromadorella sp.</i>	1.18	05.45	11.15	70.31
CHE (62.39)	<i>Oncholaimus sp.1</i>	3.48	16.38	26.26	26.26
	<i>Graphonema sp.</i>	2.68	10.99	17.62	43.88
	<i>Oncholaimus sp.2</i>	1.90	7.90	12.66	56.53
	<i>Theristus sp.</i>	1.59	6.76	10.84	67.37
	<i>Daptonema sp.</i>	1.69	5.54	8.88	76.25
Species	Group HPHE vs. HPLE (Average dissimilarity = 58.13)				
	Av. abundance	Av. abundance	Av. Diss	Cont.%	Cum.%
<i>Ax nolaimus sp.</i>	2.00	3.37	9.61	16.54	16.54
<i>Graphonema sp.</i>	2.71	1.01	8.05	13.85	30.39
<i>Theristus sp.</i>	0.05	2.39	7.55	12.98	43.37
<i>Enoplolaimus sp.2</i>	1.04	2.44	6.82	11.73	55.11
<i>Oncholaimus sp.2</i>	1.72	1.74	5.79	9.96	65.07
<i>Oncholaimus sp.1</i>	1.55	0.75	5.46	9.40	74.47
	Group HPHE vs. CHE (Average dissimilarity = 59.95)				
<i>Oncholaimus sp.1</i>	1.55	3.48	9.41	15.70	15.70
<i>Ax nolaimus sp.</i>	2.00	0.00	7.09	11.82	27.52
<i>Graphonema sp.</i>	2.71	2.68	6.51	10.86	38.37
<i>Daptonema sp.</i>	0.00	1.69	6.13	10.22	48.60
<i>Theristus sp.</i>	0.05	1.59	5.90	9.85	58.45
<i>Halomonhystera sp.</i>	1.35	0.00	5.01	8.36	66.81
<i>Oncholaimus sp.2</i>	1.72	1.90	4.88	8.14	74.95
	Group HPLE vs. CHE (Average dissimilarity = 64.86)				
<i>Ax nolaimus sp.</i>	3.37	0.00	9.83	15.15	15.15
<i>Oncholaimus sp.1</i>	0.75	3.48	9.82	15.14	30.30
<i>Enoplolaimus sp.2</i>	2.44	0.72	7.10	10.94	41.24
<i>Graphonema sp.</i>	1.01	2.68	6.72	10.36	51.60

et al. 2021). Others found that Epsilonematidae were epigrowth feeders nematodes (Semprucci et al. 2013) which are recorded extensively in coarse-grain sediments (Pérez-García et al. 2019), and found, here, at the high pollution coarse sand particles (HPLE). The contribution of ET nematodes (Table 7, 12.45%) indicate low energy at HPLE. The ET nematodes are typical of a low hydrodynamic environment regime (Armenteros et al. 2009). Our results suggest that functional traits could be used to distinguish between sites experiencing physical disturbances and anthropogenic stressors, where trophic traits could indicate anthropogenic stressors, and the tail-shape type designate sites with physical turbulence. Our results

recommend applying the functional traits methodology in biomonitoring and coastal restoration programs.

Nematode community structure is a traditional analysis applied as a potential biological indicator for monitoring different habitats (Stone et al. 2016). This study is the first identification of marine nematodes to families and genera at Alexandrian beaches. However, Abada et al. (2017) investigated freshwater nematodes, and the *Oncholaimus* genus is common in both studies. Besides, the current results are comparable with North-Western Mediterranean data (Moreno et al. 2006), where seven typical sandy shared genera *Ax nolaimus sp.*, *Oncholaimus sps.*, *Theristus sp.*, *Enoploloides sp.*, *Enoplolaimus sps.*, and *Epsilonema sp.* In contrast

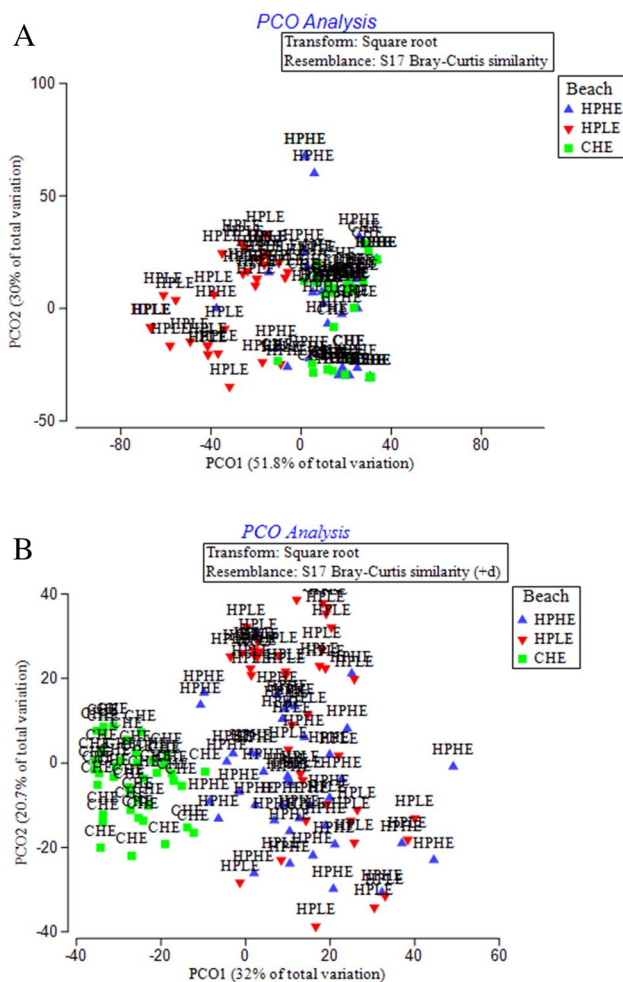


Fig. 6 Principal coordinates (PCO), unconstrained ordination, based on Bray–Curtis similarity of square-root transformed data of (A) Functional Biological Matrix (FBM) and (B) Nematode Community Structure Matrix (CSM)

to Gheskiere et al. (2005), nematode richness herein is low, probably due to long-term beach deterioration (Mitwally and Hamdan 2021) and the low productivity dominating eastern Mediterranean basins in general (Pruska et al. 2021).

The CSM analysis revealed different data groupings at each beach (Tables 3, 4, 5, Figs. 6B, 7B). The contributing genera to the total nematode assemblages were five across HPHE and CHE versus four at HPLE, regardless of the total inhabitants (Fig. 4). The close associations between vector salinity (Fig. 7B) and nematode data at HPHE and CHE indicate that the genera occupied by these beaches are typical marine habitat water (Table 8). Whereas genera that inhabited the HPLE could be more brackish water endurance. Several studies documented a close correlation between nematode abundance, composition, and salinity (Adao et al. 2009; Hourston et al. 2009, 2011; Alves et al. 2013). However, others recorded an inverse relationship

with high saline waters (Baia et al. 2021). Another key is the pH loaded negatively on *dbRDA* axes, indicating the species sensitivity at HPLE and CHE to minor changes in pH, whereas those genera that inhabited HPHE were more pH tolerant (Table 5). Zeppilli et al. (2015) reviewed variable consequences of low pH on meiofaunal assemblages that were study-type dependent. The temperature also had a vital role in affecting the nematode density and composition at HPLE (Fig. 7B). The highest temperature values at HPLE (22.0 ± 0.4 °C, Table 5) were probably due to the shallowness, low hydrodynamic, and sampling timing. However, the T°C variations were 0.5 °C among beaches. Temperature elevation affects reproduction success, population dynamics, species interaction, and community structure (Vafeiadou et al. 2018). Surprisingly, a noticeable shift in the role of sand grains and sorting affecting nematode genera could relate to *Oncholaimus sp* having relatively the same contribution across three beaches (Table 8) and nematodes' species-specific response. Nematode-inhabited coarse sediments differed in abundance and diversity from those occupied by fine-grained sand (Vanaverbeke et al. 2011), consistent with our results (Table 8). Nevertheless, the measured environmental factors accounted for 35.7% of the total variation in the CSM (Fig. 7B), indicating that other non-quantified factors contributed to ~65% of the nematode species-specific masked response to the measured variables. Therefore, nematode community structure is recommended as a potential assessment tool in biomonitoring and coastal restoration programs.

Surprisingly, the *Graphonema sp.* dominance at HPHE (24.42% Table 8) could highlight nematodes' opportunistic behavior and sediment quality at Abo-Qir beach. Members of this genus are typical marine warm-water organisms (Kito 1982; Hourston et al. 2005), and their occurrence was frequent or rare, assigned as epistrate (2A) feeders (Mirtoa et al. 2002; Moreno et al. 2009), classified as colonizers-persisters C-P3, and recorded at high-energy beaches (Hourston et al. 2005). These criteria fit HPHE environmental conditions, but predator nematodes dominated HPHE, and *Graphonema sp* was an epistrate feeder (Mirtoa et al. 2002). Sediment quality at HPHE probably was a good source of phytodetritus for *Graphonema sp* as epistrate feeders due to its proximity to Rosetta Promontory and the Nile River. This genus is tolerant to high-energy levels and could be sensitive to dispersed pollution. It is possible it could switch feeding habits to predators. Meiofauna showed rapid response and resilience to phytodetritus sedimentation after flooding disturbance (Pruska et al. 2021), and the oligotrophic Mediterranean sea is very sensitive to riverine nutrient fluxes (Ait Ballagh et al. 2021). The more resilient the nematode genus to pollution and other disturbance, the more likely to be predatory colonizers (Bongers et al. 1991).

Fig. 7 The dbRDA plots, a constrained model based on Bray–Curtis similarity of square-root transformed data of (A) Functional Biological Matrix (FBM) and (B) Nematode Community Structure Matrix (CSM). Abbreviations are listed at Table (5)

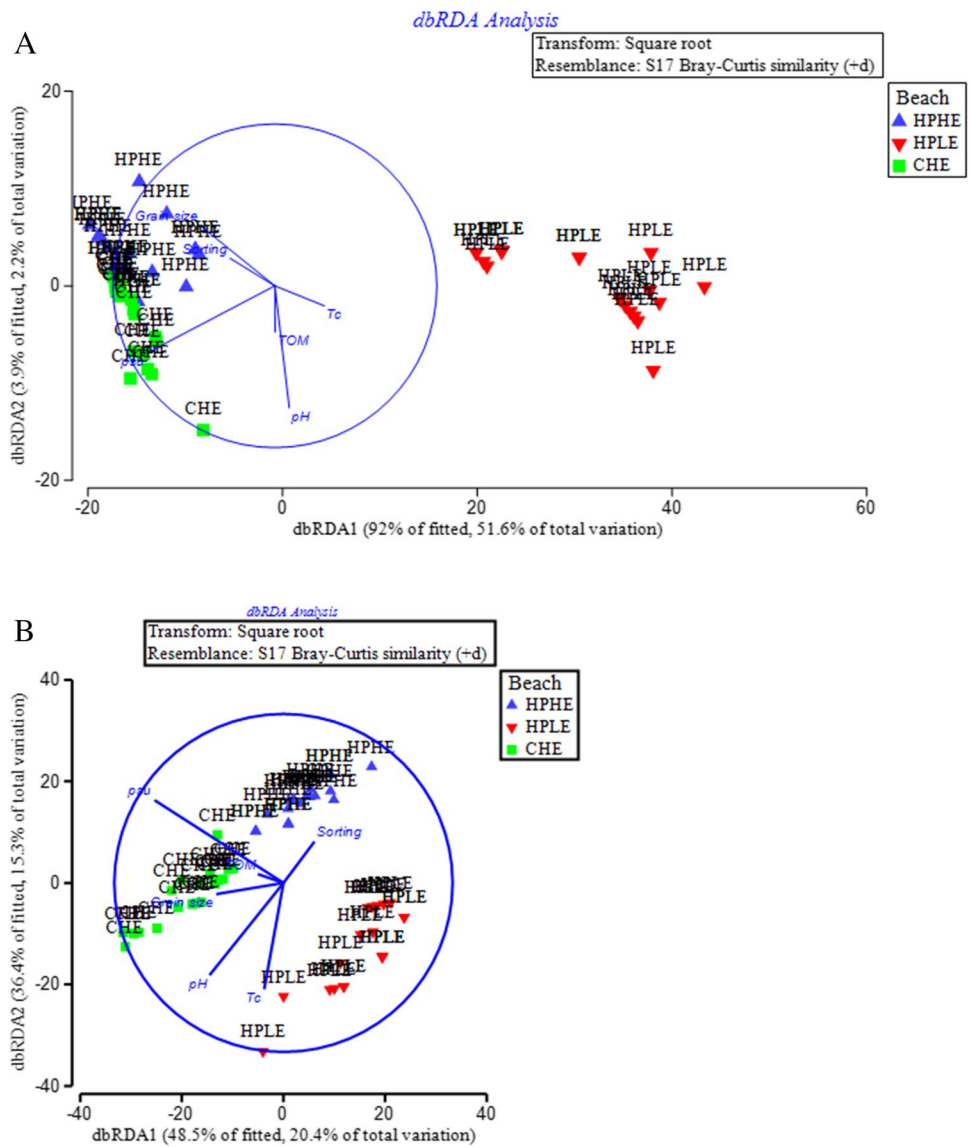
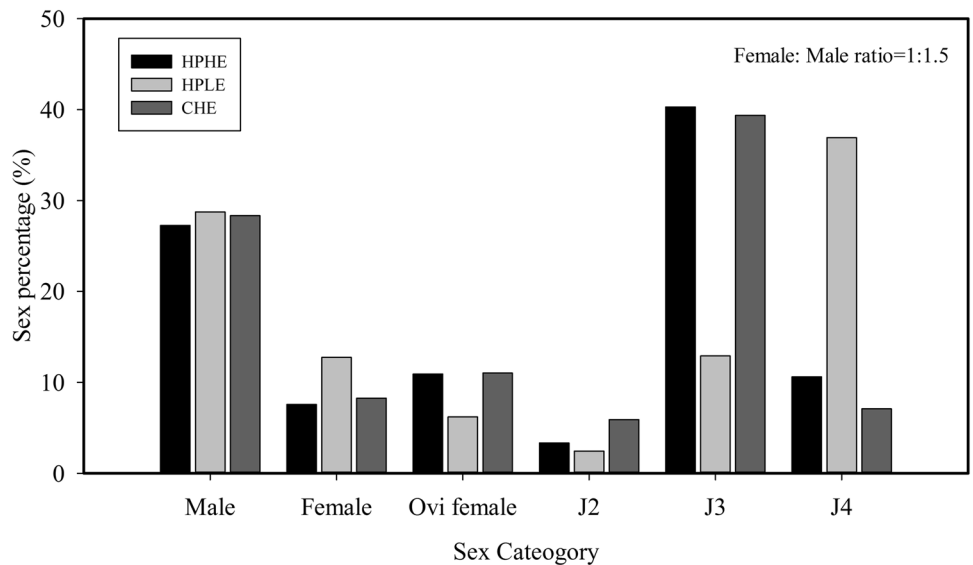


Fig. 8 The percentage composition of sex ratios and juvenile stages among beaches, Abbreviations: Ovi female=Ovigerous female, J2-J4= Juvenile stages 2–4



Conversely, at El-Mex Bay, *Enoplolaimus sp.*, a typical predatory nematode (Baia et al. 2021), dominated at the low salinity coarse-grained sand HPLE beach (Table 8, 23.45%), where scavengers occupied this beach, suggesting this genus could shift feeding habits from predatory to scavengers to feed on decaying biomass, as was explained earlier. *Axnolaimus sp.* can tolerate metal pollution, as Gyedu-Ababio and Baird (2006) stated, and its contribution at HPLE was 21.63%. High levels of heavy metals have been documented along the Alexandrian coast (El Nemr et al. 2007). Besides, *Axnolaimus sp.* are herbivorous nematodes (Moens et al. 2002) and could feed on detached plants at HPLE.

The widespread *Oncholaimus sp.* dominated the clean high-energy beach, the Northwest Coast (CHE, 26.26%, Table 8). Bhadury et al. (2015) and all references therein stated that the *Oncholaimus* genus is dominant in a sandy environment, probably due to its large body size and feeding habits (omnivores/predators). Sahraeian et al. (2020) documented its sensitivity to pollution. As the predators were more resilient to physical disturbances (Table 7), the *Oncholaimus* genus occupied the CHE.

The highest average dissimilarities in the contribution of nematode sets (Table 8) were between HPLE and CHE (~65%), probably due to the influence of *Axnolaimus sp.* (15.15%), which could be more tolerant to prevailing conditions at HPLE. It discriminated against the two highly polluted beaches (Table 8, 16.54%), indicating its sensitivity to high salinity, fine-grained sand, and high energy. The genus *Oncholaimus* distinguished between HPHE and CHE, highlighting the genus's low pH sensitivity at HPHE as a proxy for pollution. The variable response of dominant genera to the measured or non-measured environmental conditions at each beach indicate that nematode is species-specific and agreed with De Meester et al. (2018). The relatively equal nematodes contribution among and within beaches (Table 8) could suggest nematodes' spatial turnover and the tolerant species replacement of the more sensitive individuals, keeping the ecosystem balance (Baselga 2010). However, further studies are necessary for a better understanding of our findings.

Male-biased sex ratios (Fig. 8) is consistent with many studies (Heip et al. 1978; Warwick 1981; Wall et al. 2002; Gambi et al. 2003) and could be an indicator of a physically disturbed environment, resource-limited environment, male higher metabolic index (Kim and Shirayama 2001), and (or) lower expenses to the population than females. However, Sahraeian et al. (2020) found that the ratios were female-biased in anthropogenic-stressed habitats. Our beaches experience physical disturbance and anthropogenic stressors, and the individuals were pooled per genera to obtain the overall sex ratios combined at each beach. Therefore, a further study on the sex per genus

is recommended. The Juveniles stage 3 inhabited HPHE and CHE vs. stage 4 at HPLE could relate to the higher temperature at HPLE (Table 5) that could accelerate the development rates and agreed with Moens and Vincx (2000). However, we assume that a high energy environment could delay the rate of maturation, an assumption that requires further laboratory investigations.

The information gained from the functional analysis is not a simple reflection of the community structure analysis. It captures additional ecological facts and reveals variable spatial patterns (Singh and Ingole 2016; Jansen et al. 2018). The two FBM data groupings (Fig. 5A, 6A) were salinity and sand-grains-dependent (Fig. 7A). Conversely, three gained groupings from CSM analysis (Fig. 5B, 6B) were physicochemical dependent (salinity, pH, and temperature, Fig. 7B) with a species-specific response (Table 8). Functional data fitted the examined matrices better than that of community structure. The stress value (Fig. 5), total variations of PCO analysis (Fig. 6), and *db*RDA (Fig. 7) highlighted the role of measured variables, whereas community structure emphasized the effect of the non-measured factors. The lower the stress value at *n*MDS and the higher the contribution of measured factors to total variations indicate the higher capture of all information into examined models (Anderson et al. 2008). Although our methods and data are different from Semprucci et al. (2018), our study suggests using functional traits, community structure, and sex ratio may be legitimate analyses to determine the disturbance of ecosystems. However, we recommend applying traits analysis in monitoring and coastal restoration programs, giving a generalization about ecosystem challenges but with caution.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11852-022-00884-1>.

Acknowledgements The author is deeply thankful to Prof. Dr. Alexei V. Tchesunov, Department of Invertebrate Zoology, Moscow University, Russia, for nematode species identification. She is also grateful to Prof Dr. John W Fleegeer, Louisiana State University, the USA, for reading the manuscript and for valuable suggestions. She is honored to Dr. Christine Ritter, Rockport, Texas, USA for her help checking English grammar and editing. This research did not have any specific grant from the public, commercial, or not-for-profit sectors.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Declarations

Conflict of interest There is no conflict of interest.

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