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A Reliability Assessment of Nematode Copepod Ratio from the Unpolluted Sandy Beaches of Palk Bay, India

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Abstract
The nematode copepod ratio (N/C ratio) a meiofaunal attribute used in biomonitoring studies was tested for its efficacy in three unpolluted sandy beaches of the Palk Bay, India. Grain size composition showed the existence of fine to coarse sand (mean grain size = 0.207 mm to 0.669 mm). Temperature varied between 26.2 ˚C and 39.2 ˚C. Dissolved oxygen ranged between 3.08 mg/l and 6.81 mg/l. Salinity range was from 29.04 PSU to 33.96 PSU. The N/C ratio values recorded are between 0 – 52. Alternate meiofaunal parameters like the density of nematodes, copepods and total density also considered as monitoring tools. The results of the N/C ratio as well as any other parameters showed that the stations are unpolluted. We conclude that the N/C ratio can be used as an indicator for testing the health of Palk bay beaches.

Keywords
Meiofauna, Nematode Copepod ratio, Biomonitoring, Palk Bay, Sandy Beach.

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INTRODUCTION
Macrofauna and meiofauna represent two major components of benthos; both are used as indicators to understand the health of marine habitats. As a bioindicator meiofauna has several advantages over the macrofauna [1, 2, 3]. Studies have demonstrated that meiofauna can be considered a suitable tool to monitor the environmental impacts of intertidal, subtidal and other soft-bottom habitats.
Several major taxa of meiofauna have been used in monitoring studies. Opisthobranchs [4], nematode species abundance [5], harpacticoid copepods [6, 7], nematode copepod ratio (N/C ratio) [8], bacteria and meiofauna ratio [9, 10, 11], meiofauna diversity [12], main meiofauna taxa [13] are few to mention.
Though the use of N/C ratio as a tool for biomonitoring was first proposed by [8], [14] popularized it as a fast, easy and reliable method to monitor the effects of organic pollution of sandy beaches. They reported a high N/C ratio where sewage pollution is strong. The index is based on habitat requirements of nematodes and copepods, their ability of each to exhibit a monotonic response and the degree of organic enrichment examined along a gradient [15]. This is based on the fact that a pattern of the dominance varies in polluted and unpolluted environments [16].
Despite the several attributes proposed the N/C ratio is till now widely used in various studies. However, the use of this index for ecological monitoring has been under scrutiny because of its simplicity and the difficulties in interpreting the data [17, 18]. Although such indices have obvious limitations [15], their simplicity remain attractive and gain general

acceptance by most of intertidal [19, 20], subtidal [21] and laboratory studies [22]. Modern works have extended the application of this index to habitats like fish farms [23, 24, 25] and to pollution types e.g. heavy metals [7].

The present study was undertaken in three sandy beaches of Palk Bay, Bay of Bengal. The Palk Bay is characterized by shallow sandy and muddy intertidal zones with seagrass and mangroves in its subtidal zones. Meiofaunal studies on Palk Bay are limited [26] and the works on the nematode and copepod ratio in an unpolluted condition of sandy beaches are rather scanty. Hence the present study was undertaken to understand the physicochemical features of seawater (temperature, salinity and dissolved oxygen) and sand grain size composition and the role of N/C ratio in unpolluted beaches. Other biological factors (the density of nematodes, the density of copepods and the total meiofaunal density) also considered as monitoring tools.

2. MATERIALS AND METHODS

Study area

The present study was conducted in three sandy beaches of Palk Bay, Bay of Bengal, India. The Palk Bay is surrounded by the by the state of Tamil Nadu in North and West and Sri Lanka in East.

Sampling stations

The studies were conducted in three selected sandy beach stations of Point Calimere (10.27′ N - 79.81′E), Manamelkudi (10.04′ N – 79.26′ E) and Thondi (9.73′ N – 79.01′ E), of Palk Bay (Fig. 1). The sampling of meiofauna and physicochemical analysis was conducted for a period of 24 months (from January 2016 to December 2017). Separate samples were collected for grain size and meiofaunal studies using PVC corer (2.5 cm inner diameter).

Sampling of physicochemical parameters

Physicochemical factors such as grain size, temperature, dissolved oxygen (DO), salinity was studied. Grain size composition was analyzed following the methods of [27] and [28]. The temperature was recorded using a digital thermometer, DO in seawater was estimated by modified Winkler's method [29]. Salinity was estimated by Mohr's titrimetric method.

Meiofauna sampling

The sediment samples were collected with a PVC Corer by pushing up to 15 cm depth. Sampling was made during low tide, at the mid-tide level of the intertidal region. Collected samples were immediately fixed with Rose Bengal formalin (0.5 g/l) and transported to the laboratory for extraction.

Laboratory process

Sediment containing meiofauna was separated by decantation method following [30], animals passing through sieves of 1,000 µm mesh size and retained in 63 µm mesh size were considered as meiofauna. The process of decantation was repeated for five times to extract maximum fauna from the sediment. Separated meiofaunal components were preserved in 5% Rose Bengal buffered formalin.

Quantitative and qualitative analysis of meiofauna

All the meiofauna retained on the 63 µm sieve were identified and counted to the major taxa level under a stereo zoom research microscope. For quantitative study, meiofauna was enumerated up to major taxa level using Sedgwick-Rafter counting chamber and density was expressed as the number of individuals/10cm^2 (ind./10cm^2). For the qualitative study, meiofauna was identified up to major taxa level by preparing temporary and permanent mounts.

Nematode Copepod ratio

N/C ratio was calculated by dividing the number of nematodes by copepods. When the copepods or nematodes were 0, the ratio was considered as 0. For the calculation of the index, juveniles of copepods (copepodids) were also considered as adults.

Statistical Analysis

To understand the relationship between the N/C ratio and relevant physicochemical parameters, Spearman rank correlation was performed using SPSS 16.0.

RESULTS AND DISCUSSION

The physicochemical parameters studied are presented in Table – 1. Temperature was high during summer months and low values are recorded during winter and monsoon seasons. The Dissolved Oxygen (DO) was ranged from 3.08 mg/l and 6.81 mg/l, usually high in Point Calimere. While salinity was low in Point Calimere. The mean grain size varied between 0.207 mm to 0.669 mm. The physicochemical conditions recorded are comparable and similar to the previous reports of East coast [31, 32, 33].

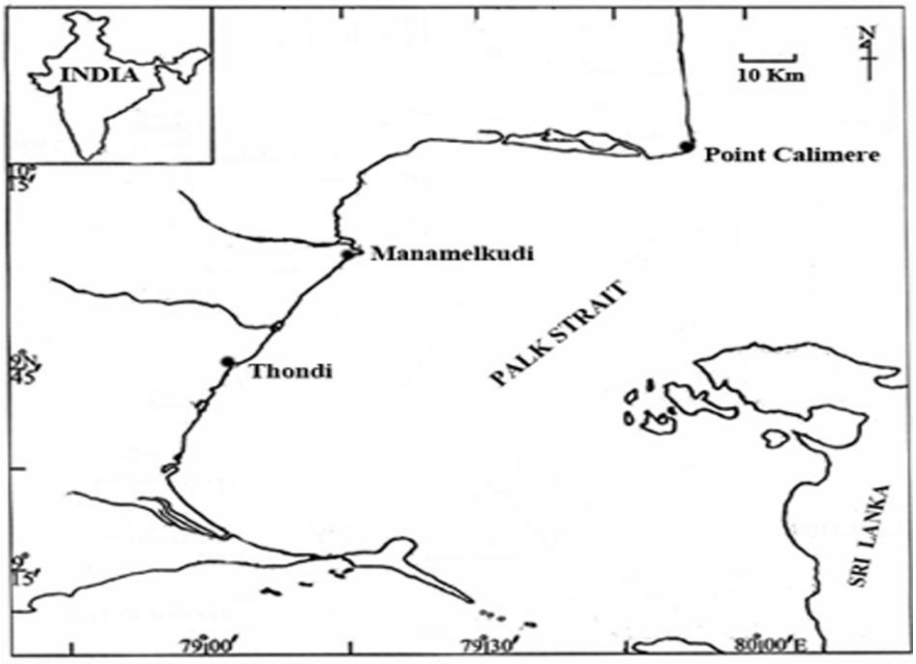

Fig. 1. Map of showing the location of sampling stations

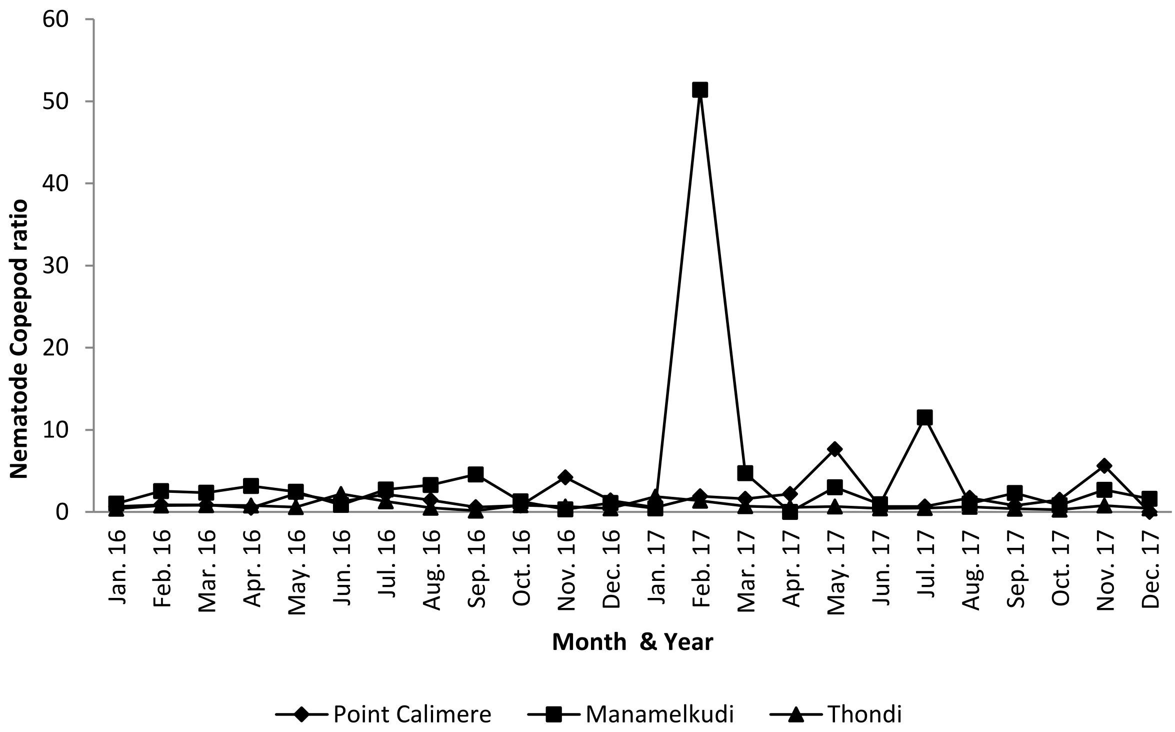

Fig. 2. Nematode copepod ratio recorded from three stations of Palk Bay

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Table 1 Physicochemical parameters recorded from three stations of Palk Bay

Month	Temperature (ºC)			Dissolved Oxygen (Mg/l)			Salinity (PSU)			Mean Grain Size (mm)		
	Point Calimere	Manamelkudi	Thondi	Point Calimere	Manamelkudi	Thondi	Point Calimere	Manamelkudi	Thondi	Point Calimere	Manamelkudi	Thondi
Jan. 16	26.8	27.4	26.2	5.74	5.66	5.68	30.8	32.78	32.52	0.357	0.300	0.334
Feb. 16	26.6	27.6	27	6.23	6.52	5.15	31.86	32.86	31.48	0.548	0.207	0.467
Mar. 16	30	28.8	29.6	5.45	6.59	6.81	32.3	32.68	32.94	0.522	0.264	0.239
Apr. 16	30.4	29.6	29.4	6.4	5.62	5.92	32.14	33.58	32.86	0.515	0.314	0.285
May. 16	30.6	30	31	6.18	5.66	6.22	33	31.78	31.48	0.515	0.425	0.312
Jun. 16	29.8	28.6	30.2	6.81	6.52	6.01	32.3	32.29	33.22	0.522	0.441	0.525
Jul. 16	28	29.6	29.2	6.43	6.22	5.77	33.88	32.83	32.47	0.494	0.293	0.315
Aug. 16	29	29.4	30	5.14	4.94	6.39	32.91	33.11	32.01	0.548	0.293	0.300
Sep. 16	28.2	29.8	29.2	3.6	3.7	4.32	32.47	32.01	33.26	0.525	0.344	0.261
Oct. 16	28.6	29.6	28.8	3.49	3.29	3.29	32.6	31.81	31.29	0.405	0.310	0.221
Nov. 16	28	29	28	6.2	3.49	3.7	31.78	30.76	29.09	0.464	0.312	0.351
Dec. 16	26.4	27.4	28.2	3.7	3.49	3.7	30.3	30.48	30.53	0.437	0.408	0.330
Jan. 17	26.6	27.6	26.5	5.72	4.63	6.22	32.4	32.58	33.14	0.669	0.592	0.541
Feb. 17	28.2	28	28.4	6.01	3.3	6.72	32.76	31.32	33.22	0.439	0.388	0.528
Mar. 17	29.8	28.6	29.8	4.94	3.3	4.74	32.84	32.86	33.14	0.326	0.370	0.522
Apr. 17	30	29.2	29.6	3.9	3.91	4.94	33.6	33.5	33.42	0.467	0.344	0.351
May. 17	30.4	30.2	29.8	4.74	5.98	5.15	33.88	32.42	31.61	0.473	0.338	0.485
Jun. 17	30	30	31.2	3.87	3.47	4.12	33.78	33.96	32.32	0.473	0.463	0.367
Jul. 17	29.8	30.8	32	4.97	4.87	3.87	32.12	31.14	33.78	0.450	0.425	0.437
Aug. 17	28.6	29.6	31	4.87	3.89	3.49	32.5	32.88	32.87	0.447	0.350	0.398
Sep. 17	29.2	29	29.6	4.32	4.49	3.91	32.27	32.17	33.91	0.500	0.452	0.340
Oct. 17	29.2	39.2	28.2	3.7	4.94	4.57	32.86	31.6	31.6	0.500	0.366	0.386
Nov. 17	28.8	29.2	29	3.08	3.87	4.26	31.86	29.09	30.09	0.408	0.329	0.371
Dec. 17	28.2	29	28.4	4.35	4.27	3.87	30.27	30.53	29.04	0.515	0.359	0.362

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Table 2 Comparative account of nematode copepod ratio studies

S. No	N/C ratio values		Nature of pollution / field / laboratory	Type of habitat	Conclusion	Author(s)
	Range	Mean				
1	< 15	NA	Oil spill – on field	Sandy beach - fine to medium sand	Fluctuated seasonally than to pollution	Ansari and Ingole, 2002
2	NA	7.26 (control) 81.49 (affected) 17.56 (control) 36.97 (affected)	Organic enrichment	sub tidal - fine and medium sand	Reliable results	Riera et al, 2012
3	NA	18.83	Unpolluted	Intertidal - medium sand	No clear seasonal Trends	Rubal et al, 2009
4	0.65 – 2.49 2.14 – 5.71	NA	sewage pollution	Bay beaches	Casts doubt on NC ratio	Shiells and Anderson, 1985
5	NA	20.0 (dumping area) >10 (reference area)	Heavy metal pollution	Clean Sandy beach	Species composition study needed.	Smol et al., 1991
6	0 – 35	NA	Copper pollution	Sandy beaches	not reliable for metallic pollution	Lee, Correa and Castilla, 2001
7	NA	31 - 57	Sewage pollution	Bay beaches	highest in sewage pollution site	Raffaelli and Mason, 1981
8	1.9 – 14.9	NA	Anthropogenic influences	Polluted harbour - fine sand and silt	useful tool	Moreno et al 2008
9	3 – 914	NA	Storm water / sewage contamination	Bay beaches	Not recommended	Lambshead, 1984
10	17.1 – 4.6	NA	Unpolluted sandy and muddy flat	Sandy and muddy comparison	Suggested modification	Warwick,
11	14 – 59	NA	On field	Sub littoral	Macrofauna must be included	Heip et al 1988
12	72.4-8.0	NA	PAH	Deep sea	Taxonomic interpretation must also include	Baguley et al, 2015
13	622.11 – 0.62	NA	Impact of artificial reefs	Artificial reefs, coastal cliff and river mouth	Is not sensible for artificial reefs	Semprucci et.al., 2016
14	0 - >100	NA	Sulphide concentration	Aquaculture sites	Showed infinity in sulphide concentration	Sutherland et al. 2007
15`	9.00 - 154.13	NA	Organic matter supplies	Mangroves	Better to use with environmental quality	Zhou et al. 2015
16	133 – 720		Organic matters	Sandy beaches	Unreliable as influenced by environmental factors	Wang et al, 2011

The nematode copepod ratio values ranged between 0and 52 (Fig. 2). It is noteworthy to mention that the highest (52) was recorded in only once (February 2017 in Manamelkudi) the remaining are lesser than 8. The 24 months mean value was < 2 in all the stations. The values are lesser than the threshold values proposed by previous workers [14, 19, 23, 35]. In the absence of pollution source our biomonitoring study also confirms the unpolluted nature of all three stations. A comparative account on N/C ratio used in various pollution studies is given in table – 2, which shows organic matter and chemical pollution increased the N/C ratio. According to [14] Index of 100 as a pollution threshold; however, [19] recommended other values, for sand (> 10) and mud (> 40) seabed. [23] Observed that a sharp cut-off for copepod tolerance occurs at N/C > 50. [35] Proposed that values of N/C over 20 in medium sands and values over 70 for fine and sandy silt sediments.

The physicochemical parameters DO and grain size has the ability to alter the ratio. Reduced DO will support the nematodes than copepods. However, throughout the study period, the level of DO remained high in all the stations. The correlation coefficient shows that N/C ratio values did not show a clear relationship with either DO or grain size ($p = < 0.05$).

Table 3 Density of nematodes, copepods and total meiofauna (individuals/10cm^2) recorded from Palk Bay

Month & Year	Nematode density			Copepod density			Total meiofaunal density		
	Point Calimere	Manamelkudi	Thondi	Point Calimere	Manamelkudi	Thondi	Point Calimere	Manamelkudi	Thondi
Jan. 16	94	228	83	328	222	211	761	2100	778
Feb. 16	161	667	167	189	261	217	867	3433	822
Mar. 16	311	456	183	272	194	222	1239	1367	944
Apr. 16	194	444	250	400	142	317	2216	1478	1428
May. 16	222	558	144	100	228	250	989	2494	694
Jun. 16	217	253	183	172	297	83	1072	2608	1033
Jul. 16	444	617	150	206	225	117	1367	3772	567
Aug. 16	256	328	78	178	100	150	1161	1664	500
Sep. 16	311	406	22	528	89	150	2067	1211	389
Oct. 16	350	1244	100	456	961	122	2050	4200	533
Nov. 16	328	383	183	78	1181	283	878	6875	772
Dec. 16	47	311	67	33	289	161	323	1011	506
Jan. 17	56	44	83	106	106	44	1111	494	650
Feb. 17	139	417	144	72	8	106	589	1697	522
Mar. 17	267	394	222	167	83	311	1311	1897	1356
Apr. 17	122	167	97	56	0	181	822	3767	450
May. 17	339	100	128	44	33	194	1205	1580	569
Jun. 17	106	403	156	161	431	383	633	2158	944
Jul. 17	117	447	161	178	39	356	1056	2538	1072
Aug. 17	400	78	439	233	77	689	2203	2096	1889
Sep. 17	94	128	111	122	56	283	839	1911	905
Oct. 17	356	267	122	239	333	472	1361	4225	956
Nov. 17	156	311	78	28	117	100	728	1422	350
Dec. 17	139	250	122	0	156	294	517	2006	872

In the alternate way to test, we took the other biological parameters such as density of nematodes, copepods, and total meiofaunal density separately (table - 3). The density of nematodes recorded during the study period ranged from 22 to 1244 (ind./10cm^2). They mostly remained as dominant taxa throughout the study period. Copepods are one of the high prevalent taxa, their density range was between 0 (ind./10cm^2) and 1181 (ind./10cm^2).

Since the 1970s, nematodes have been used as environmental monitors for aquatic systems [43]. Nematodes are one of the numerically dominant communities of meiofauna and exhibit a high tolerance to oxygen deficiency. Several species are able to withstand total anoxia for more than two weeks [44, 45]. In our study, the nematodes are one of the dominant taxa and their density ranged between 22 and 1244 ind./10cm^2 which are similar to the unpolluted sandy beach of Pulicat [32].

The copepods are sensitive to pollution or lower oxygen level [46] reported that 550 µm diameter sand particle was dominated by copepods. In the absence of pollution source and availability of suitable grain size provides ideal interstices for harpacticoids. Being high prevalent taxa, their density was lower than nematodes suggesting unavailability of adequate food and/or interstitial space for their existence.

The total meiofaunal density ranged between 323 and 6875 ind./10cm^2, with high values in Manamelkudi (table - 3). In general, meiobenthic abundance may be impacted by several factors, such as salinity, grain size, organic matter, chlorophyll, temperature, depth, self-propagation [47, 48, 49, 50] organic pollution [51, 52]. The meiofaunal density in the unpolluted beaches was 1730 ± 288 10cm^2 to 7944 ± 436 ind./10cm^2 in Pulicat [32] while [12] reported 1403 ± 658 ind./10cm^2 whereas [53] recorded 66.6 to 122.4 ind./10cm^2. The values of our study are comparable; hence, we suggest that in the absence of pollution the grain size, patchiness (due to food availability) could be the reason for differential density between stations.

5. CONCLUSION

The search of biological parameters to understand pollution is a never-ending process, meiofauna has several potentials characterizes as bioindicator. The N/C ratio, which is in use for more than four decades in various types of pollution, has given a few successful results. The present study undertaken in Palk Bay has tested its use in unpolluted condition. The ratio, as well as the other meiofaunal parameters studied, showed that the study sites come under unpolluted criteria. Having given a clear reflection of pollution the ratio can be used as a bioindicator of Palk Bay biomonitoring studies if not it may be considered as an additional tool to those already available, like macrofauna and diversity indices.

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