Distribution of free-living nematodes in a tidal flat of Banate Bay, Iloilo, Philippines

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ABSTRACT

Meiobenthos samples and related environmental parameters were examined along a transect with six stations extending 4 km from the shoreline in Banate Bay, Iloilo, Philippines in April (dry), July (onset of SW monsoon rain) and October (end of rainy season) in 2014. Free-living nematodes constituted 90% of the total meiofauna and showed a mean density of 51.4 ind. cm^{-2}. A total of 89 nematode genera from 23 families were identified, with *Paracomesoma, Viscosia, Daptonema, Paramonohystera,* and *Cyatholaimus* as the top five genera (35.6% of overall frequency). Nematode densities (ln-transformed) showed significant differences (p=0.03) temporally but not spatially. Higher densities were observed during the rainy season than dry season. A stepwise multiple linear regression analysis indicated that nematode density was negatively correlated with bottom salinity, while genus richness was negatively correlated with surface turbidity and bottom salinity. The nematode assemblage was dominated by epigrowth feeders, with a shift to non-selective deposit feeders as sediment organic matter had accumulated in October.

INTRODUCTION

Free-living nematodes represent a highly diverse group of taxa (Bhadury et al. 2006) and are considered the most abundant metazoans in marine sediments, with typical densities of 10^6 individuals per square meter (Heip et al. 1985). They are numerically the most important components of the meiofauna, thus representing a significant portion of the energy flow in benthic systems (Vranken & Heip 1986; Tahseen 2012). Besides serving as prey for higher trophic levels (Vincx 1996; Coull 1999), they stimulate bacterial productivity in the sediments through bioturbation and feeding on microbes, and enhancing mineralization rates of organic matter (Gerlach 1978; Nascimento 2010) in the process. The updated global inventory of marine species diversity (Appeltans et al. 2012) currently includes 11,400 marine nematode species, of which 6,900 are free-living, and perhaps another 50,000 more still undiscovered species. This makes the group the fourth among marine taxa with the largest number of undiscovered species.

Despite their ecological and taxonomic importance, there are still very limited studies about their distribution and diversity. Most studies are confined to temperate regions and only a few have been published for tropical areas, mostly from various coastal areas of India (e.g. Ansari & Parulekar 1998; Ansari et al. 2001; Sajan & Damodaran 2007). In the Indo-Pacific, tropical intertidal habitats are subjected to distinct monsoon-driven wet and dry seasons which determine the physical and chemical conditions of the environment. How these seasons affect the meiobenthic community is poorly documented.

This study forms part of a larger investigation on how the benthic infauna are affected by run-off driven by rainfall and substrate disturbance due to typhoons during the Southwest monsoon season. It focuses on the nematode assemblage and addresses the paucity of such studies in tropical waters, specifically in the Philippines.

MATERIALS AND METHODS

Banate Bay (10°58'26" N, 122°48'11" E) is located on the eastern coast of Panay Island (Fig. 1) and, together with Barotac Bay, covers a total area of 22,000 ha with a coastline length of 20 km. It has an extensive shallow tidal flat extending up to 1 km from the shore, and receives runoff from several watersheds on Panay Island (Fortes & Nadaoka 2015).

Sediment core samples were collected at six stations (Stations 12-17) along a transect extending from the mouth of Anilao River to about 4 km from the shore (Fig. 2) on 24 April 2014 (dry; Summer), 4 July 2014 (right after a 3-5 day period of heavy rain at onset of the SW monsoon) and 14 October 2014 (1

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day after passing of a strong typhoon at the tail-end of the SW monsoon). Meiobenthos were sampled using SCUBA by pushing a 2.5 cm diameter PVC tube (area = 4.9 cm^2) 5 cm deep into the sediment. Station depths ranged from 2.5 to 7 m. The core samples were immediately fixed in borax-buffered 10% formalin-seawater solution and stained with rose bengal dye for analysis in the laboratory. Additional parallel core samples were also taken for sediment size analysis and total organic matter (TOM) content determination. In each sample, 100 nematodes (all if <100, see Gourbault et al.1995; Armenteros 2009; Gambi & Danovaro 2016) were randomly picked under a stereo-microscope, mounted and identified to the genus level under a compound microscope.

Salinity (surface and bottom; psu), dissolved oxygen (water column; mg/L), turbidity (surface and bottom; NTU), and bottom temperature (^{o}C) were measured at every station with the use of a Horiba multi-parameter water quality meter.

For each sample, density (ind. cm^{-2}), number of genera (*S*), and evenness at the genus level (*J'* = Pielou index) were calculated. Stepwise multiple linear regression analysis was performed to determine the relationship of the abiotic variables with the nematode assemblage. Two-way ANOVA was done on nematode densities and number of genera, and on each of the abiotic variables to examine variation between stations and months. F-tests and T-tests were also done to determine variation in densities and number of genera. The feeding type of nematodes was also identified according to the classification of Wieser (1953) based on the structure of the buccal cavity. The four categories include selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), and omnivores/ predators (2B). The shape and presence of teeth or mandibles of the buccal cavities of each specimen were examined under the compound microscope. The identified feeding types were confirmed using information from the literature, such as Heip et al. (1985), Trebukhova & Pavlyuk (2006), and Gourbault et al. (1995).

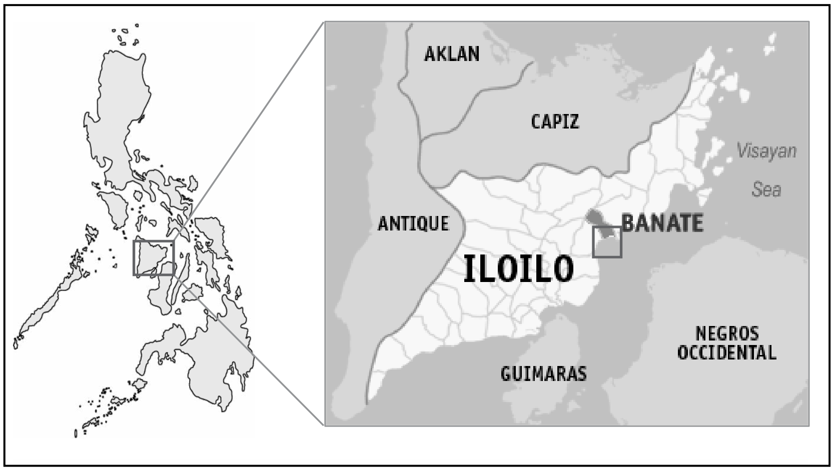

Figure 1. Study area in Banate Bay, Iloilo, Philippines.

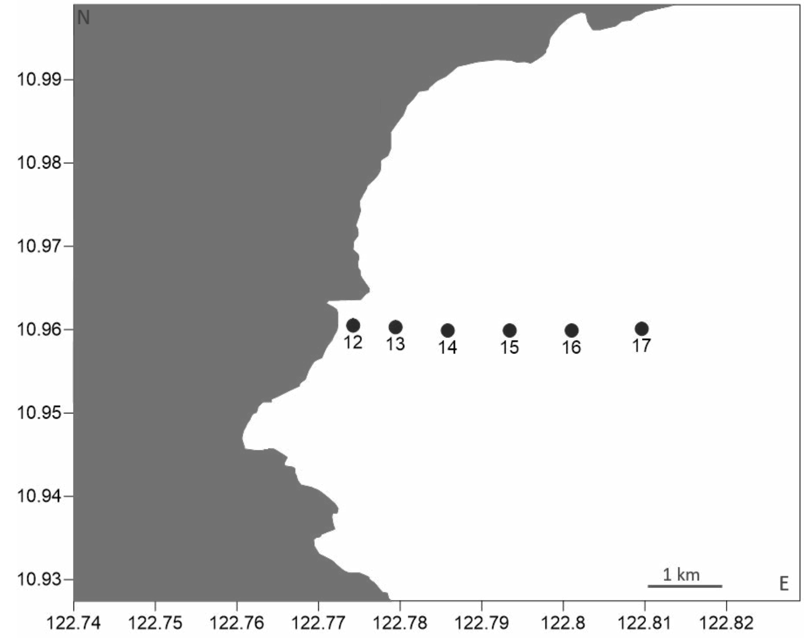

Figure 2. Map of sampling sites in Banate Bay showing the transect with six stations.

RESULTS

Habitat and Environmental Variables

The coastal environment of Banate Bay is a complex habitat affected by many factors. The overall sediment type of the study area was fine sand (mean median phi: 1.85) and was classified as poorly sorted with a sorting index ranging from 1.25 to 1.78 (mean: 1.48) (Table 1). Sand comprised the dominant sediment fraction (mean: 94.1%). Coarse and fine sand showed complementary distribution along the transect in which coarse sand was decreasing and

Table 1. Measurements of physico-chemical parameters in Banate Bay, Iloilo, during the study period in 2014.

Month/ Station	Depth (m)	% Coarse Sand	%Fine Sand	%Silt & Clay	% TOM	Bottom salinity (ppt)	Surface Salinity (ppt)	Temp (°C)	DO (mg/L)	Bottom NTU	Surface NTU
Apr/12	2.5	34.2	65.2	0.6	23.8	30.8	30.6	29.2	4.60	15.80	15.80
Apr/13	2.5	21.2	78.5	0.3	27.8	30.7	29.9	29.8	4.80	15.50	12.40
Apr/14	3.0	4.1	95.3	0.5	22.7	30.6	29.1	30.4	5.00	15.20	9.00
Apr/15	4.0	16.0	83.9	0.1	22.0	30.5	28.6	30.8	5.10	15.00	6.69
Apr/16	5.0	6.5	93.4	0.1	19.3	30.8	27.5	30.4	5.50	9.10	5.10
Apr/17	7.0	30.3	69.7	0.0	25.0	31.1	26.4	30.1	5.90	3.19	3.44
Jul/12	2.5	26.0	73.6	0.4	21.3	27.8	24	30.4	9.10	50.40	47.60
Jul/13	2.5	21.1	78.6	0.3	29.4	27.8	25.5	30.0	8.20	32.50	47.60
Jul/14	3.0	20.3	79.3	0.4	33.2	27.8	27.0	29.7	7.30	14.60	47.60
Jul/15	4.0	4.3	95.6	0.1	28.1	27.8	28	29.5	6.70	2.63	47.60
Jul/16	5.0	31.0	69.0	0.1	22.4	28.1	28.2	29.8	6.10	2.10	27.70
Jul/17	7.0	10.0	89.1	1.0	26.2	28.3	28.4	30.2	5.40	1.50	7.80
Oct/12	2.5	6.4	87.6	6.1	32.6	22.8	22.40	33.1	6.42	606.00	326.00
Oct/13	2.5	4.7	93.8	1.5	22.2	23.8	23.04	32.3	5.94	381.05	206.99
Oct/14	3.0	15.3	70.0	14.7	33.4	24.8	23.68	31.5	5.46	156.09	87.98
Oct/15	4.0	9.5	88.0	2.4	23.9	25.5	24.10	30.9	5.14	6.12	8.64
Oct/16	5.0	32.4	67.3	0.3	22.5	25.8	23.80	30.4	5.21	6.78	6.30
Oct/17	7.0	4.2	88.1	7.7	33.2	26.0	23.50	29.8	5.27	7.44	3.95

fine sand was increasing at least for stations 12 to 15 (Fig. 3). This is consistent with settlement of sediment particles brought by run-off from the river mouth. There was a change in trend for the deeper stations 16 and 17, similar to the change in trend for stations 15 to 17 for silt and clay and TOM (Fig. 3), suggesting that the deeper stations are influenced by other factors. The portion of silt and clay was found to be significantly different between months (*p*=0.021), with higher values in Oct ($\bar{x}$ = 5.5%) compared to April ($\bar{x}$ = 0.3%) and July ($\bar{x}$ = 0.4%).

The water quality of the bay was significantly different between months for bottom (*p*=0.0) and surface salinity (*p*=0.0007), dissolved oxygen (*p*=0.009), and temperature (*p*=0.049), but no significant spatial differences were found. Yet looking at the plots of standard deviations, it can be seen that inshore stations (12 to 14) have more variable water quality parameters (bottom and surface salinity, temperature, and turbidity) compared to the deeper stations (15-17) (Fig. 3). A prominent difference was also observed for the surface and bottom turbidity (NTU) in which both were much higher in October (up to 330 and 600 at the surface and bottom, respectively). This trend was particularly evident in the inshore stations (12 to 14) and decreased towards the offshore stations (15 to 17) (Table 1).

Nematode Assemblage

Nematodes were the dominant meiofaunal taxon in all but one sample, comprising an average of 90% (Table 2) of all meiofauna. Their overall density was 51.4±20.5 ind. cm^{-2} (mean±1SD; n=18). There was a significant difference in densities between months (ANOVA on Ln-transformed data; *p*=0.03). Significantly higher densities were observed in the rainy months of July (72.6 ± 60.5 ind. cm^{-2}; *p*=0.04) and October (56.8 ± 16.8 ind. cm^{-2}; *p*=0.03) than in the dry summer of April (24.9 ± 27.6 ind. cm^{-2}) (Figs. 4 and 5A). There were no significant spatial differences in densities, but offshore stations showed more variable densities (*p*=0.047) compared to inshore stations (Fig. 5A).

On the other hand, genus richness showed no significant temporal or spatial differences (Figs. 4 and 5B). The highest genus richness combined across all stations was recorded in October (77 taxa) and the lowest was in April (57 taxa) (Fig. 4). Evenness showed a somewhat opposite trend with significantly

Table 2. Meiofauna composition and list of the top ten genera of nematodes, their abundance, density, and feeding type.

GENUS	Mean Density (ind. cm^{-2})	Relative Abundance	Feeding Type
Nematodes	**51.4**	**89.8**	
Paracomesoma	5.18	10.1	2A
Viscosia	4.23	8.2	2B
Daptonema	3.57	7.0	1B
Enoploides	2.94	5.7	2B
Cyatholaimus	2.36	4.6	2A
Desmodora	2.02	3.9	2A
Eleutherolaimus	1.94	3.8	1B
Paramonohystera	1.69	3.3	1B
Paracanthonchus	1.42	2.8	2A
Anticomidae	1.35	2.6	1A
Other nematodes	24.71	48.1	
Other meiofauna	**6.16**	**10.20**	
TOTAL	57.56	100.00	

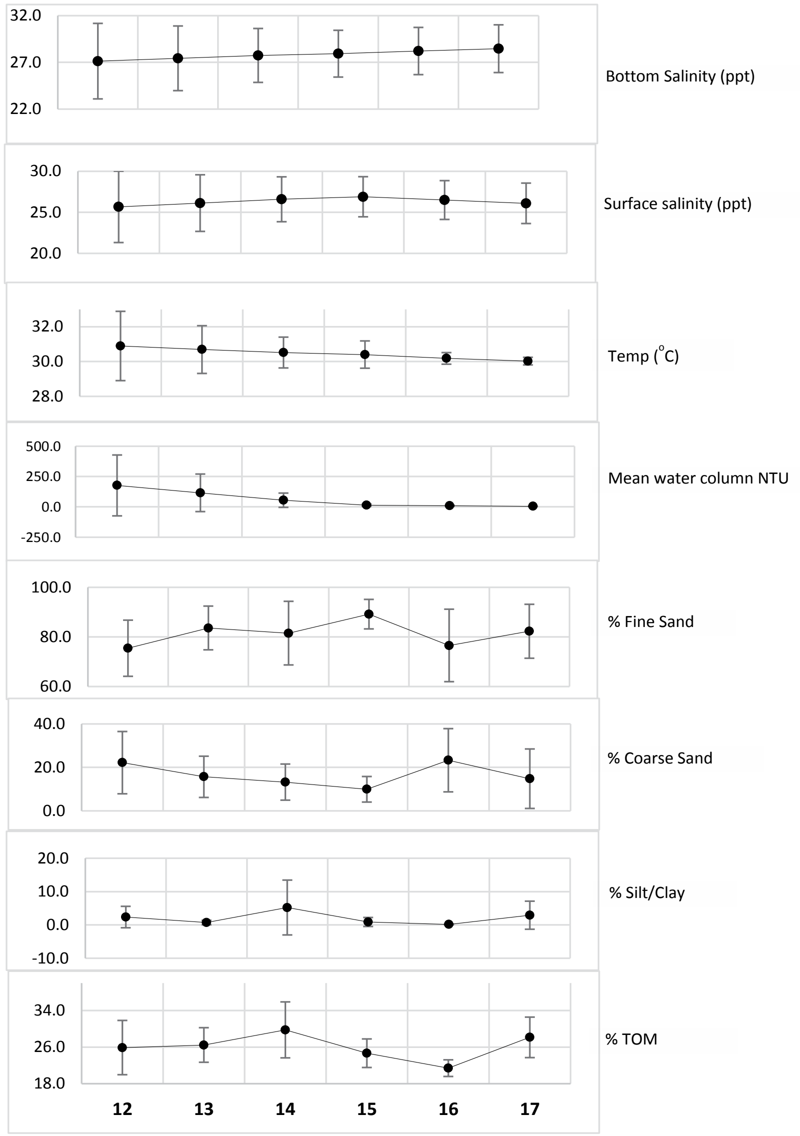

Figure 3 . Plots of abiotic factors along the stations in Banate Bay. Vertical bars denote SD.

higher variability in July (p=0.007) compared to October.

A total of 23 families and 89 nematode genera were identified from 1,575 individuals examined. The top ten genera, listed in Table 2, together comprised 52% of all nematodes recorded.

The results of stepwise multiple regression analysis showed that nematode density (ND) correlated negatively with bottom salinity (SAL), and positively with depth (D), % coarse sand (CS) and TOM contents (TC): ND = 2.49 – 0.08SAL + 0.08D + 0.01CS + 0.03TC. The model was statistically significant (p = 0.02) and accounted for around 58% of the variance (R^2 = 0.577).

Genus richness (GR), on the other hand, was negatively correlated with bottom salinity (BS) and surface NTU (SN), and to a lesser extent with depth and DO, while it was positively correlated with the amount of fine sand (FS): GR = 98.98 – 2.48BS – 0.08SN – 1.08D – 1.34DO + 0.13FS). This model was statistically significant (p = 0.03) and accounted for around 60% of the variance in genus richness (R^2 = 0.604).

Feeding Type Composition

Epigrowth feeders (2A) dominated the assemblage and comprised 42% of all nematodes, these included the genera *Paracomesoma*, *Desmodora* and *Dichromadora* (Table 2, Fig. 6).

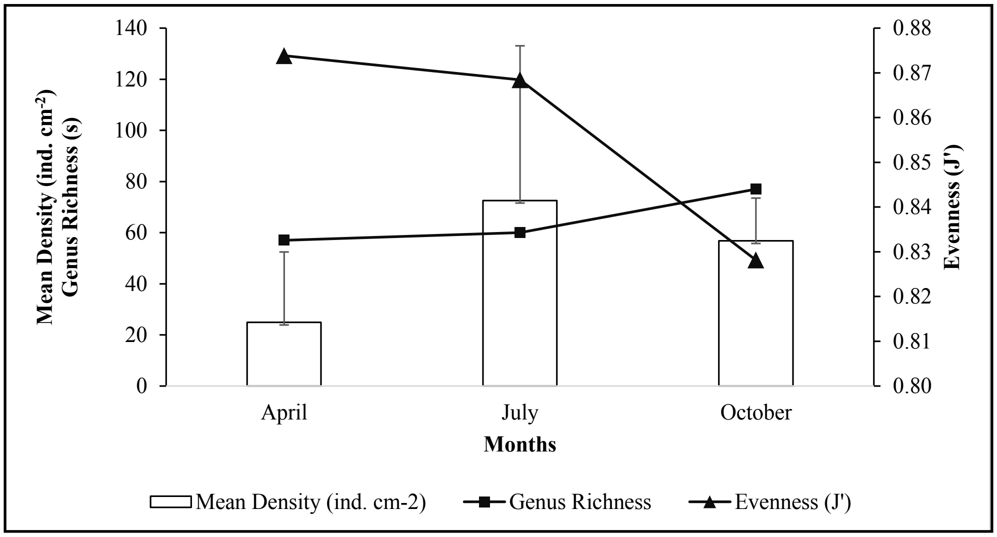

Figure 4. Mean density (ind. cm^{-2}), genus richness and evenness of nematodes per month in

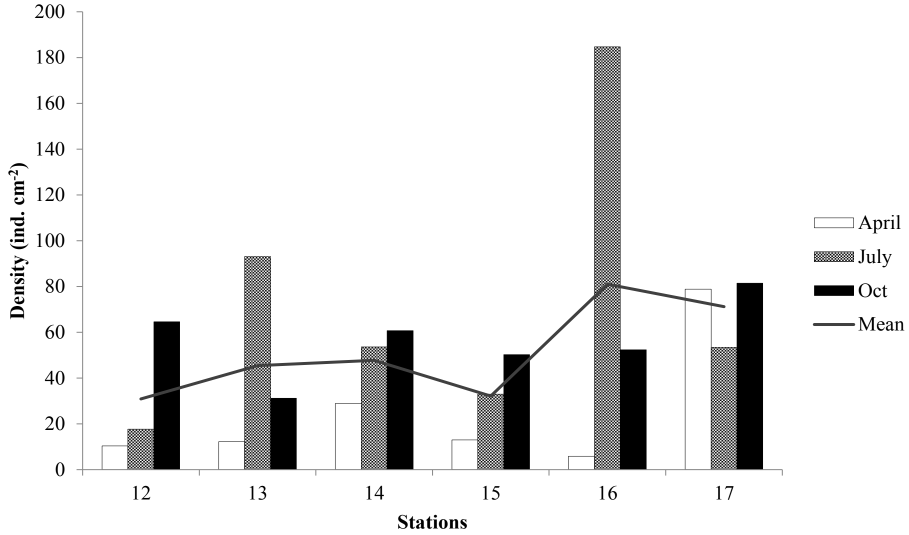

Figure 5. (A) Nematode densities in each station per month

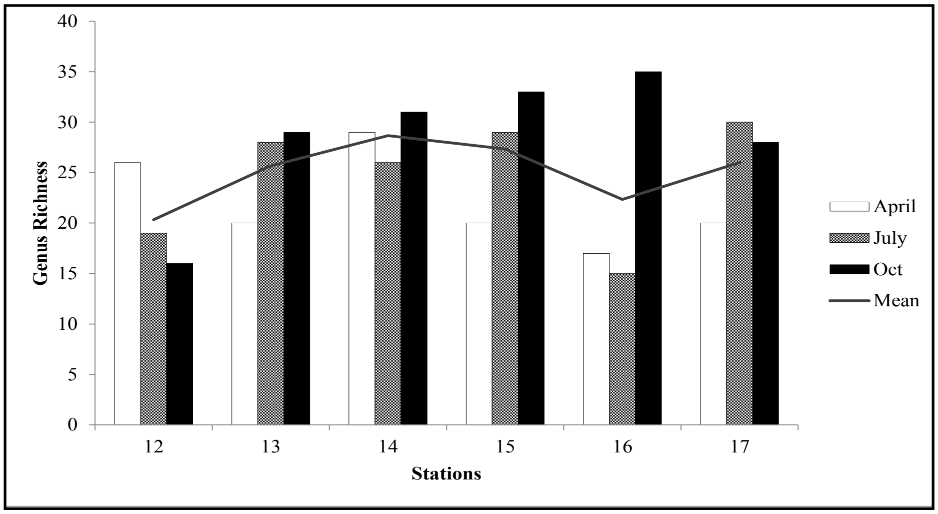

Figure 5. (B) Genus richness of nematodes in each station per month.

The non-selective deposit feeders (1B) comprised 30% of the total, represented mainly by *Daptonema*, *Paramonohystera*, *Eleutherolaimus*, and *Metadesmolaimus.* The omnivores/predators (2B) and selective deposit feeders (1A) comprised 21% and 7%, respectively. In April (40%) and July (48%), epigrowth feeders (2A) were dominant while non-selective deposit feeders (1B) were dominant in October (40%) (Fig. 6).

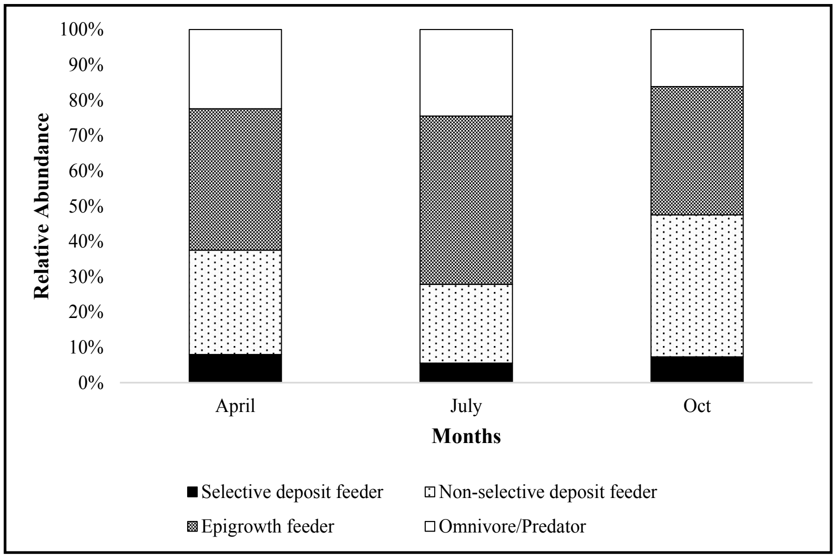

Figure 6. Feeding type composition (based on Wieser 1953) of nematodes in each month in Banate Bay, Iloilo in 2014.

DISCUSSION

The intertidal flat in Banate Bay is a fairly homogeneous sedimentary habitat of fine sand (185 to 330 μm) based on the sediment analysis. However, the present study showed that rainfall and run-off during the wet season from July to October brought significant changes in the physical and hydrographical conditions of the bay. This was shown by lower surface and bottom salinities, increased amounts of silt and clay, increased surface and bottom turbidity, and an accumulation of organic matter in the sediment.

One of the major environmental issues in Banate Bay is water quality and habitat degradation due to coastal turbidity brought by terrestrial inputs from the watershed (Fortes & Nadaoka 2015). Based on a coastal ocean simulation model, Yamamoto and Nadaoka (2018) present the concept of "stress connectivity matrix" which explains that water quality in Guimaras Strait is governed by terrestrial loads not just from adjacent watersheds but also from distant ones by means of coastal currents. In Banate Bay, this influence on water quality is by means of tributaries, particularly the Jalaur River located further south of the study area. During the Southwest monsoon, the terrestrial load is transported towards the northeast, with stronger currents at the outer edges of the tidal flat. Thus shallower stations (12-15) near the coast are more affected by inputs of the nearby tributaries, while deeper stations (16-17) have additional influence from watershed inputs from the south (Jalaur River).

The observed changes in nematode densities are consistent with the abovementioned dynamics of the bay. A clear seasonal pattern of higher densities in rainy season and lower densities in dry season was observed in this study. This, however, is opposite to the trends observed from Indian coasts where lowest densities were recorded during the monsoon (rainy) season due to drastic reductions in salinity (32.8 to 0.2 ppt in Ansari & Parulekar 1998; 34 to 8.3 ppt in Ingole & Parulekar 1998; 35 to 18 in Ansari et al. 2001), and resuspension of the sediments which causes high mortality of the meiobenthos. Salinity often acts as a community regulator (Ingole & Parulekar 1998), with fluctuations causing osmotic stress which negatively impacts nematode assemblages (Forster 1998). Accompanying factors like turbidity may have parallel effects. The higher densities during the rainy season in this study, indicate that salinity fluctuations (22.8 to 31.1 ppt) are not that drastic to negatively impact nematode populations. Instead, along with higher run-off, there might be factors that have a stronger positive influence on the nematode assemblage, such as food (Nascimento 2010).

There was no direct measurement or estimates of food in the bay, although some inferences can be drawn from the observed sediment particle distribution and TOM content. The observed dominance of epigrowth feeders (2B) in the study area reflects the type of sediment and food available. Epigrowth feeders are known to feed on microalgae or diatoms and other microphytobenthos (Platt & Warwick, 1983). In coarse sandy sediments, the internal surfaces of the sediment particles serve as an area for the establishment of biofilms in which microalgae, diatoms, bacteria, fungi and mucus secretions (Giere, 1993) serve as an important food sources especially for epigrowth feeders. Thus the combination of sandy sediment plus high availability of microphytobenthos favors high abundance of epigrowth feeders (Netto & Fonseca, 2006). This is consistent with observations in April and July. The dominant feeding type shifted during the rainy season so that non-selective deposit feeders were more abundant in October, perhaps in response to a shift in available food sources. Non-selective deposit feeders (1B) are known to dominate silty habitats (Vanreusel et al. 1985; Trebukhova & Pavlyuk 2006; Muresan 2012) which are associated with higher amounts of organic particles (Heip et al. 1985; Ansari & Parulekar, 1998).There was no significant difference in the TOM content (19.3 to 33.4%) between months but silt and clay showed a significant 5 to 30-fold increase in October. Since this sediment type is closely associated with higher TOM content, the increase in such fine particles in the area is likely accompanied by an increase in TOM (food) towards the end of the rainy season (October), resulting in higher nematode density in the rainy season.

Spatially, nematode densities were not significantly different but offshore stations showed more variable densities compared to inshore stations. This can be explained by the stronger influence of coastal currents at the seaward edge of the tidal flat during the Southwest monsoon (Yamamoto and Nadaoka 2018), especially for the month of July. Station 16 showed the most variable densities with both the highest (184.73 ind. cm^{-2}) and lowest (5.91 ind. cm^{-2}) values, and both the highest (35) and lowest (15) numbers of genera. In this outer portion of the transect, in addition to the influence of the nearby tributary (Anilao River), other inputs from further south (Jalaur River) add to the complexity of habitat conditions in these areas.

Although mean density was generally low, genus richness (89 genera from 23 families) is much higher than most reported values in studies conducted in tropical habitats with comparable sampling regimes (e.g., 76 species in Gourbault et al. 1995; 40 genera in Calles 2005; 78 species in Armenteros 2009; 50 species in Nanajkar & Ingole 2010; and 51 species in Chen et al. 2012). These results indicate that Banate Bay supports high meiobenthic diversity even if densities are low. This follows the typical trend of diversity and density in nematode populations (Heip et al. 1985; Giere 1993).

There were no significant differences in the genus richness between stations and months, but similar to density, a higher number of genera was also recorded in the rainy season. The overall highest number of genera was recorded in October (77) immediately after a strong typhoon.

The estimated overall mean nematode density of 51.4±20.5 ind. cm^{-2} is one to two orders of magnitude lower than densities reported in other tropical intertidal and coastal habitats, e.g. 115.7 ± 62.6 ind. cm^{-2} in India (Ingole & Parulekar 1998) and a range of 376 to 2,388 ind. cm^{-2} in Ecuador (Calles 2005). It is also generally lower compared with nematode densities in some temperate areas (e.g. Steyaert et al. 2003; Gheskiere et al. 2004 & 2005).

This may be attributed to siltation caused by the high sediment load of run-off from the watershed during the SW monsoon season (Fortes & Nadaoka 2015). This sediment load settles and accumulates in the bay causing the substrate to be unstable. During periods of strong wind and waves (SW monsoon) the resuspension of fine sediment results to disturbance of the meiobenthic community (Kumary 2016) through frequent smothering of organisms in the sediment and disruption of their life cycles. While such conditions lead to reduced densities, they appear to promote diversity, with genus richness in the area increasing towards October. It thus appears that in Banate Bay, the intensity and frequency of disturbances are sufficiently moderate to limit overall densities while promoting diversity. This is consistent with Connell's (1978) intermediate disturbance hypothesis.

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