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Protozoan colonization on artificial substrates in relation to water quality in a tropical Indian harbour

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Abstract: A field study was conducted to evaluate the protozoan colonization patterns on artificial substrates in relation to organic pollution within a tropical harbour. The composition of protozoans and their succession rates on artificial substrates (polyurethane foam units) were compared between two field stations (A and B), and their presence were considered with regards to the prevailing water quality conditions at the study sites. Altogether 44 genera of flagellates and ciliates were documented. The common genera of flagellates encountered included *Monas*, *Polytoma*, and *Chromalina*. Among the ciliates, the predominant genera were *Tetrahymena*, *Vorticella*, *Lagynophyra*, and *Heloiphyra*. These groups exhibited characteristic successional patterns in relation to ambient water quality. At Station A, located close to the sewage outfall, the water quality parameters included poor Secchi-disc transparency (0.48m), dissolved oxygen of 1.93 mg/ml, salinity of 18 psu, and temperature 31.3 ℃. Here, the nanoflagellates (*spumella*) colonized first, followed by microcilliate (*Tetrahymena*) and sessile form (*Vorticella*). Station B, located on the seaward side, was characterized by relatively less-stressed environmental conditions with transparency 1.85m and dissolved oxygen value of 6.04 mg/ml. Salinity of 27.27 psu, and mean temperature of 30 ℃ were recorded at "B". At this station, the nanoflagellate *Polytoma* was first documented to colonize on the substrates, followed by microcilliate (*Lagynophrya*) and suctorid (*Heliophyra*). These findings support the use of protozoans as indicator species for evaluating the hazards posed by organic pollution to natural estuarine communities.

Introduction

In aquatic ecosystems biological communities are remarkably complex in both their operation and response to anthropogenic activities. This has led many investigators to conduct laboratory and field studies for evaluating the ecological risk posed by humans to ecosystem (Cairns, 1983; 1987). Any disturbance or stress due to anthropogenic activities may result in a change in the community structure, thereby affecting the entire aquatic ecosystems (Hall, 1997; Blay, 1996).

Protozoans are among the major life forms that occupy a wide range of habitats in marine ecosystems. These protists play significant roles in the overall food web by mediating energy flow and materials cycling (Pratt, 1985; Sherr, 1984). Since protozoans are unicellular and have high reproductive rates, they respond rapidly to environmental changes and can reflect ecosystem stress within a short amount of time. These protists have been used to monitor ecosystem health, including organic pollution (Abraham, 1997; Panswad, 1997).

Aquatic habitats subjected to organic pollution show characteristic successions of microbes over several days and weeks on various substrate (Fenchel, 1987). An initial bloom of bacteria is immediately followed by the appearance of heterotrophic nanoflagellates and shortly thereafter by ciliates. Other ciliates-feeding organisms appear and eventually are followed by metazoan colonization. Since protist communities are complex and vary with substrates, attempts have been made to standardize pollution monitoring by using glass slides (Jax, 1996) or blocks of polyurethane foam; the latter are preferred and their properties have been well described (Cairns, 1982). Artificial substrates such as PFS are widely used in protistan sampling (McCormick, 1997; Cairns, 1983). Such procedures have shown promise in providing basic information on ecosystem health in relation to changing aquatic conditions.

The succession of bacteria, heterotrophic flagellates, and ciliates following phytoplankton blooms was first observed and interpreted in upweling areas in the open sea by Sorokin (Sorokin, 1977). Since then, numerous studies have used protists in water quality assessment, but mostly in freshwater environments (Katiyar, 1997; Madoni, 1993). However, little is known of the effects of organic pollution on the community structure of marine protozoans within a tropical harbour setting.

The Visakhapatman harbour holds great commercial importance and is considered to be one of the most anthropogenically-impacted harbours on the east coast of India. In recent times, the water quality in the harbour has

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significantly deteriorated as a number of industries(e.g., oil refinery, fertilizer factory, polymer producer) routinely discharge their wastes into the harbour. Additionally, appreciable quantities of untreated domestic sewage from an estimated 1.5 million population of this township are emptied into the already polluted harbour, further accelerating the decrease in water quality(Raman, 1995).

Kalavati *et al*.(Kalavati, 1997) investigated the ciliate composition in relation to water quality from this harbour and reported that *Aspidisca* was most abundant under eutrophic conditions and *Euplotes* dominated in areas with improved water quality conditions. Subsequent sampling revealed a preponderance of flagellates from this study area. Thus, biomonitoring of the harbour's water quality through additional protozoan sampling seemed necessary to understand the impact of organic pollution on the harbour ecosystem.

Our study examined trends in the colonization rates of protozoans in and around the vicinity of the sewage discharge and their taxonomic composition was discussed in relation to water quality. Specifically, the differences in temporal successional patterns among the protozoans were compared between two stations, one hypertrophic and the other relatively mesotrophic, and the species tolerant of the prevailing physico-chemical conditions were taxonomically categorized and correlated.

1 Materials and methods

1.1 Study site

Visakhapatnam harbour, located on the east coast of India(17°42′N, 83°25′E), is halfway between Calcutta and Madras and considered to be one of the major ports in India. Two stations were chosen: Station A was located near the inner harbour and was in close proximity to the sewage outfall. Station B was situated about 6 km downstream in the entrance channel towards the open sea of the Bay of Bengal.

1.2 Field experimental design

Polyurethane foam substrate (PFS) were used to characterize the protistan assemblage at different time periods from our study sites. All experiments were performed in replicates of two. Sets of rectangular PFS, each measuring 7 × 5 × 10 cm, were placed at a depth of 1m at the two selected stations. The substrates were kept submerged by tying them to metallic weights. Preliminary studies had shown the approximate time for the densities of protozoans to reach equilibrium by 6 days; nematodes started appearing beyond this time period(Kalavati, unpublished data). Thus, one PFS sample from each site was harvested at 24 h intervals for a period of 6 days during the present study.

At the time of PFS collection, surface water samples were collected to quantify for several water quality parameters. Turbidity was measured by Secchi disc, temperature by thermometer, salinity by refractometer, dissolved oxygen concentration by Winkler method, and pH by portable digital pH meter.

1.3 Laboratory analysis

Following collection, the PFS were transported to the laboratory and squeezed carefully into a wide mouthed glass jar. Samples were processed within 5h of collection following the protistological guidelines by McCormick *et al*. (McCormick, 1997) and Kalavati *et al*.(Kalavati, 1996). Protozoans were allowed to settle for half an hour prior to analyzing their assemblage. For taxonomic identification, the samples were stained with methyl green in 1% acetic acid and 1% aqueous neutral red solution. Dry silver impregnation and nigrosin techniques were used to determine ciliature structure. To enumerate numerical abundance, samples were fixed using acidified lugol's iodine in a measuring jar containing one liter of seawater. Samples were concentrated by gravity sedimentation and the residue was made up to 50 ml in a volumetric flask. For final counts, 1 ml aliquots in replicate were pipetted into a Sedgewick-Rafter counting chamber and the numerical counts were made with Nicon microphot microscope at 800× magnification. The number of cells for each protistan group was tallied in order to determine their abundance on a given day. Identification of genera was conducted according to Lee *et al*.(Lee, 1985). Three replicate subsamples were processed before drawing any conclusions.

1.4 Data analysis

Physico-chemical parameters at the two fields station were compared using the Chi-square test(Brower, 1997). Based on cell size and structure, the protozoan were categorized into 6 major groups: nanoflagellates(< 20 μm), microflagellates(20 − 200 μm), microciliates(20 − 50 μm), macrociliates(50 − 200 μm), sessile ciliates(> 50 μm) and suctorids (50 μm). Species richness, species composition, and protozoan diversity indices were calculated; the latter included the Margalef's index(D), the Shannon-Wiener index (H), and the equitability index(J), following guidelines by Brower *et al*.(Brower, 1997). Chi-square test was used to analyze the differences in genera abundance and diversity between the two stations. The Spearman rank correlation test was used to determine similarities between the relative abundance of the 6 major groups between the two stations. A value of $p < 0.05$ was accepted as statistically significant for all statistical analyses.

2 Results

The water quality parameters are listed in Table 1. There were appreciable differences in these parameters between the two selected stations. Station A was characterized by high temperature (31.3 ℃), poor Secchi disc transparency(48 cm), low dissolved oxygen(1.93 mg/ml) and moderate salinity(17.92 psu). Station B showed significantly different water quality conditions in terms of water transparency(185 cm), oxygen content(6.04 mg/ml), and higher salinity(27.7 ppt; χ^2 test, $P < 0.05$). Slightly cooler temperature (30 ℃) and more alkaline conditions(pH 7.18) were recorded at "B" but were not significantly different compared to Station A.

Table 1 Mean values of physico-chemical parameters of harbour water at the two sampling stations

Parameters tested	Station A	Station B	Statistical decision
Temperature, ℃	31.3	30.2	NS
Salinity, psu	17.92	27.67	*
pH	7.18	7.76	NS
Dissolved oxygen, mg/ml	1.93	6.04	*
Transparency, cm	48	185	*

Notes: Results of χ^2 test included for statistical comparison; NS= not significant; *. significant at 0.05 level

During the study, a total of 44 genera of protozoans were identified. Overall the nanoflagellates formed the bulk of colonizers(50%), followed by microciliates(18%), sessile ciliates(11.5%), suctorids(9.6%), macrociliates (8.45), and microflagellates(1.7%). Selected representatives of nanoflagellates included *Monas*, *Polytoma*, *Bodo*, and *Dinematomonas*, microflagellate *Chromulina*; microciliates *Prorodon*, *Lagynophyra*, *Strombidium*, and *Tetrahymena*; macrociliates *Aspidisca*, *Plagiopyla*, and *Euplotes*; sessile forms like *Vorticella* and *Carchesium*; and suctorids included *Sphaerophyra*, *Heliophyra*, and *Podophyra*. Abundances of these groups and association with their respective stations are listed in Table 2. Of all the groups sampled, highly significant differences were documented with regards to the presence of sessile ciliates and microciliates between the sites(χ^2 test, $P<0.05$), the flagellates were more or less equally distributed between the sampling stations. Selected representatives from each group from both field sites are also listed in Table 2.

Faunal mean density and the species diversity indices were different between the two stations and are listed in Table 3. Thirty-three different genera of protozoans were identified at Station A and 31 were indicated at Station B. Station A supported significantly higher faunal density compared to Station B(χ^2 test, $P < 0.05$). Relative abundances of the six major taxonomic groups from Stations A and B for the entire sampling period were statistically correlated and r_s values are listed in Table 4.

Table 2 Group abundance (% composition) at two sampling sites (Percentages of dominant genera representatives are also listed for both stations)

Groups sampled	Station A	Station B
Nanoflagellates, <20 m	50.30	49.70
Microflagellates, 20 - 200 m	1.69	1.70
Microciliates, 20 - 50 m	11.50	26.20*
Macrocilliates, >50 m	7.70	9.10*
Sessile ciliates, stalked	20.60	2.30*
Suctorids, tentacles	8.20	11.00
Dominant genera	*Monas*(31.7)	*Polytoma*(19.64)
	Polytoma(14.2)	*Chromulina*(16.3)
	Vorticella(11.97)	*Lagynophyra*(9.3)
	Carchesium(8.62)	*Heliophyra*(8.22)
	Tetrahymena(6.21)	*Dinematomonas*(7)

Notes: * indicates significant differences between stations based on χ^2 analysis($P<0.05$)

Table 3 Comparison of the protozoan composition and biological indices of the two study stations

Characteristics	Station A	Station B
Number of species	33	31
Faunal density mean(No./ml)	17690	14509
Diversity indices:		
Margalef's index(D)	3.27	3.1
Shannon-weiner index(H)	3.4	3.8
Equitability index(J)	0.22	0.28

Table 4 Results of the Spearman rank correlation tests (r_s values) for the six major protozoan groups between sampling stations A and B for days 1 - 6

Days	1	2	3	4	5	6
r_s values	0.956*	0.820*	0.714	0.232	0.429	0.829*

Note: * $P<0.05$

With regards of the generalized colonization pattern, the flagellates were documented of colonize first followed by microciliates, sessalines, and suctorids. However, the percentage composition of the functional groups at different time intervals varied considerably between the two study sites. At Station A, the nanoflagellates(83%) represented by *Monas* and *Polytoma* were the pioneering colonizers. Within 48h, an increase in the numerical abundance of microciliates (*Tetrahymena* and *Aspidisca*) in the order of approximately 30% was evident. By 72h, there was a general increase in the ciliate

population; consequently the flagellates deceased, and the served ratio between flagellates and ciliates was about 1:2. Sessile ciliates such as *Vorticella* and *Carchesium* were the dominant forms by day 4(Fig. 1).

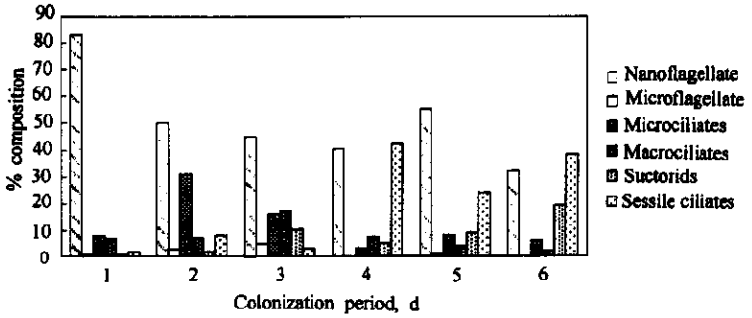

Fig. 1 Temporal patterns of protozoan colonization(succession) at Station A

The pioneer colonizers at station B were similar in composition compared to Station A, among the microciliates, the oligotrich *Strombidium* and *Lagynophyra* were the next to follow(Fig. 2). However, *Euplotes* and *Heliophyra* dominated the macrociliate population on day 3 at "B". Nematodes started appearing by day 6. There was a gradual decrease of flagellates and ciliates by this time period at both sampling stations.

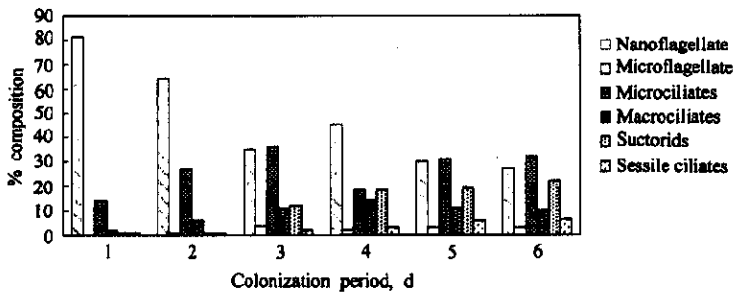

Fig. 2 Temporal patterns of protozoan colonization (succession) at Station B

3 Discussion

The diversity and abundance of protozoans in aquatic ecosystems are usually a function of the amount of available organic matter (Fenchel, 1987). Addition of organic material to an aquatic environment changes the system's physico-chemical parameters and populations of protozoans tolerant of low dissolved oxygen amounts and high organic content develops. Thus, investigators have used protozoans as indicators of aquatic pollution. Curds(Curds, 1973) showed that the presence of protozoans substantially reduce the amount of organic material, the viable count of bacteria, and the turbidity of the system.

Several investigators have examined different assemblages of colonizing species in relation to organic enrichment but mostly from freshwater habitats(Abraham, 1997; Madoni, 1981). It is possible that protozoan occurrence may also be influenced by other water quality parameters. Although significant salinity differences were documented between our study station, it is unlikely that this difference played any role in terms of their differing colonization patterns at the two study sits. According to Fenchel(Fenchel, 1987), lemnic and marine ciliates tend to fill mostly identical niches in comparable habitats.

Our station A is routinely subjected to considerable organic input, Raman and Phaniprakash(Raman, 1989) noticed high levels of inorganic nitrogen(median ammonia 0.47 mg/ml, nitrate 0.12 mg/ml, nitrate 0.01 mg/ml) and phosphate (1.77 mg/ml) and often undetectable level of dissolved oxygen. As "A" was inclose proximity to the sewage input point, intense bacterial activity led to high utilization of oxygen, thereby severely reducing the amount of dissolved oxygen and increasing turbidity. Slightly lower pH conditions were probably due to higher amounts of carbon dioxide from bacterial respiration activities.

Further downstream, towards the sea at Station B, organic matter was oxidized and less oxygen was consumed by the becteria which lead to increased dissolved oxygen levels, more alkaline conditions and less turbidity. Being seaward, high salinity and slightly cooler temperatures were also encountered. In general, the physico-chemical parameters indicated improved water quality conditions(oligosaprobic) at this station compared to "A".

Our findings on protozoan colonization patterns in an estuarine setting suggest a strong resemblance with freshwater systems. Given that environments are not homogeneours, it was possible to find a few representations of any part of the succession at any one time. The heterotrophic nanoflagellates, which are the main predators of

planktonic bacteria, were the first to colonize at both stations and in great abundances (Gasol, 1995). This was followed by microciliates. The flagellates and ciliates together reduced the number of bacteria and allowed for colonization by hypotrich ciliates and suctorids. When other metazoans started to invade, protozoan populations declined.

Station A supported bacterivorous groups like *Monas*, *Polytoma*, *Vorticella*, *Carchesium*, and *Tetrahymena*, all believed to withstand low amounts of dissolved oxygen, and contribute to the beginning of mineralization process of organic matter. The gradual decrease of flagellates and ciliates by day 5 was probably due to predation by sessile ciliates and nematodes, the latter appeared by day 6.

On the contrary, Station B with improved water quality conditions showed presence of genera such as *Chromulina*, *Lagynophyra* and *Heliophyra*, all believed to be oligosaprobic in nature. Xia and Kuang(Xia, 1992) also reported that *Chromulina* was abundant in mesotrophic water body in China that had undergone slight natural eutrophication. Both *Euplotes* and Heliophyra are characteristic of unpolluted water and dominated the microcilliate population on day 3 at Station B(Kalavati, 1997).

Sleigh(Sleigh, 1989) reported that largest number of protozoan individuals occurs in polysaprobic conditions and this agrees with our finding that Station A supported a significantly greater number of species than "B". Heterotrophic nanoflagellates are considered to be the main predators of planktonic bacteria and our finding agree with the fact that the nanoflagellates were the pioneer colonizers at both stations, regardless of their location. The relative dominance of polysaprobic, bactrivorous species (*Monas*, *Vorticella*, *Tetrahymena*) at Station A were strongly indicative of aquatic-stressed conditions. Principally bacterivorous forms tolerate low oxygen levels, they include the flagellates *Monas* and *Bodo*, and the ciliates like *Vorticella* and *Tetrahymena*. Abraham *et al*. (Abraham, 1997) reported *Vorticella* and *Aspidisca* from activated sludge plant in the U. K. Forms like Polytoma can depends on organic matter for sustenance. Possession of effective filtration apparatus enables the peritrichs(*Vorticella*, *Carchesium*) to fulfil important tasks in the biological purification of sewage.

In addition to flagellates and ciliates, a variety of other types of protozoans are found associated with surfaces in the brackish water environments. They include euglenoids and other phytoplanktonic forms as well as various sarcodines. Further related studies should consider these protistan groups and establish how environmental conditions may affect their presence in any aquatic ecosystem.

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