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Discriminating zooplankton assemblages through multivariate methods: A case for a tropical polluted harbour and Bar-built estuary

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A comparative study (June–July 2001) on zooplankton community structure amid polluted conditions in a stagnant harbour and relatively unaffected tidal estuary near Visakhapatnam, on the east coast of India, revealed a marked disparity in species composition and abundance. While the harbour supported a rich population of calanoids (46.4%), the estuary sustained mostly cyclopoids (55.2%). Univariate and multivariate techniques (species diversity, clustering, non-metric multi-dimensional scaling and one-way ANOSIM) revealed the existence of two differing zooplankton assemblages and associated water quality (similarity 50.6%). While the estuary is typified by high amounts of dissolved silica ($67.4 \pm 17.7 \mu\text{mol l}^{-1}$) linked with monsoon influx, the harbour waters revealed abnormal levels of phosphate ($40.9 \pm 9.2 \mu\text{mol l}^{-1}$) and nitrate ($15.3 \pm 5.41 \mu\text{mol l}^{-1}$) suggestive of intense eutrophication, caused by the discharge of fertilizer-factory waste and domestic sewage. On the basis of routines (e.g. BVSTEP, SIMPER) implemented in Plymouth Routines in Multivariate Ecological Research, it was possible to demonstrate that while species such as *Oithona rigida*, *Oithona brevicornis*, crustacean nauplii, gastropod veligers, *Acartia spinicauda*, and *Acartia centrura* played a key role in discriminating the zooplankton assemblage in the estuary, *Acrocalanus* spp. (mainly *Acrocalanus gracilis*) played a key role in harbour waters. Canonical Correspondence Analysis revealed species–environment relationships; for example, while the distribution of *Oithona* spp. and its associates in the estuary corresponded intimately with high silicate, temperature, and low salinity, it was high salinity, phosphate, and nitrate in the harbour channel that supported a different assemblage of copepods dominated by calanoids.

Keywords: Zooplankton; Harbour; Estuary; East coast of India; Multivariate analysis; CCA

1. Introduction

In the marine environment, effects through urbanization and industrialization are more in evidence in coastal waters than anywhere else, and one basic approach to quantifying pollution-induced impacts on sea life is through measurements based on community structure [1]. Owing

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to their importance both as regulators of phytoplankton production and as a source of nourishment for an assortment of marine organisms at superior trophic levels, there is currently considerable information on pollution effects [2–7] as well as the community structure of zooplankton from tropical waters [8–14]. Monitoring pollution effects in recipient waters through studies on zooplankton proved useful since, in some cases, species composition changed as a result of eutrophication [15]. There is an almost unanimous agreement that for zooplankton, the effects of pollution are best detected by changes in their qualitative composition [16].

In recent years, Visakhapatnam Harbour (latitude 17°41'34" N and longitude 83°17'45" E), located halfway between Chennai and Kolkata on the east coast of India, came to prominence serving both commercial and defence navigation. After the construction of an outer harbour in 1976, the water quality in the inner harbour had deteriorated as a result of poor tidal flushing, accentuating stagnation [17]. While considerable work has been carried out on phytoplankton and planktonic ciliates [18, 19], there is practically no information on the zooplankton community structure in the harbour vis-à-vis environmental conditions where the effect of pollution is rather intense [17]. It is not known whether the harbour, which is often subjected to considerable storm-water discharge, supports different zooplankton assemblages relative to a nearby (Gosthani) estuary where the hydrographical conditions are controlled by summer stratification and monsoon flows, and water quality is largely unaffected, with no major industries (except minor sewage outfalls) in the immediate vicinity [20]. Comparing zooplankton assemblages between polluted and non-polluted conditions could prove useful, since some 'discriminating species' may eventually emerge as a measure of prevailing water quality. Such (comparative) accounts, based on rigorous statistical analysis, e.g. Plymouth Routines in Multivariate Ecological Research (PRIMER) and Canonical Correspondence Analysis (CANOCO), are rare from Indian waters, and the present study held at the time of summer monsoon in India (June–July 2001) was aimed at introducing a comparison between the land-locked (stagnant) harbour under grossly polluted conditions with an estuary typified by a relatively clean environment in the immediate proximity of the open sea. Despite its short-term nature, findings made during the study (as a part of US Minority International Research and Training) are considered noteworthy, given that methods used for comparing populations are based on statistical procedures, known for their extreme usefulness (regardless of sample size), simplicity, conceptual straightforwardness being amenable to simple explanation, and transparent interpretation [21–23].

Zooplankton samples were collected weekly from the Entrance Channel of Visakhapatnam Harbour and Gosthani estuary, Bheemunipatnam, about 30 km north of Visakhapatnam (figure 1) on seven different occasions during June–July 2001. Topographically, Visakhapatnam harbour can be divided into two major regions, namely the inner harbour (with a central basin and four radiating arms) and the outer harbour. The entrance to the sea is by a narrow channel called the Entrance Channel. Besides its naval prominence, the industrial development in Visakhapatnam (e.g. oil refinery, fertilizer factory, zinc smelter unit, polymer industry, ore handling, shipbuilding) has had considerable influence on harbour water quality, since these establishments are located in its immediate vicinity, discharging their wastes into harbour waters. In addition, the harbour also receives appreciable amounts of (partially treated) domestic sewage. There is considerable freshwater drainage into the harbour, especially during the rainy season. The average depth of the harbour channel is 20 m.

River Gosthani is a monsoon-fed stream and opens into the sea (Bay of Bengal) at Bheemunipatnam (17°54'423" E; 83°27'624" N). The tidal portion of the river is about 6 km, which is occasionally dispelled by the formation of a sand-spit. Currents at the bar mouth are swift and strong, and determine the flow regime in the estuary, where the tidal range varies from about 0.45 to 1.37 m, depending on the season. The average depth of the estuary is 1.5 m. There are no major industries in the immediate vicinity of the estuary and no apparent discharges except inflows from surrounding marshes and used water from the nearby establishments.

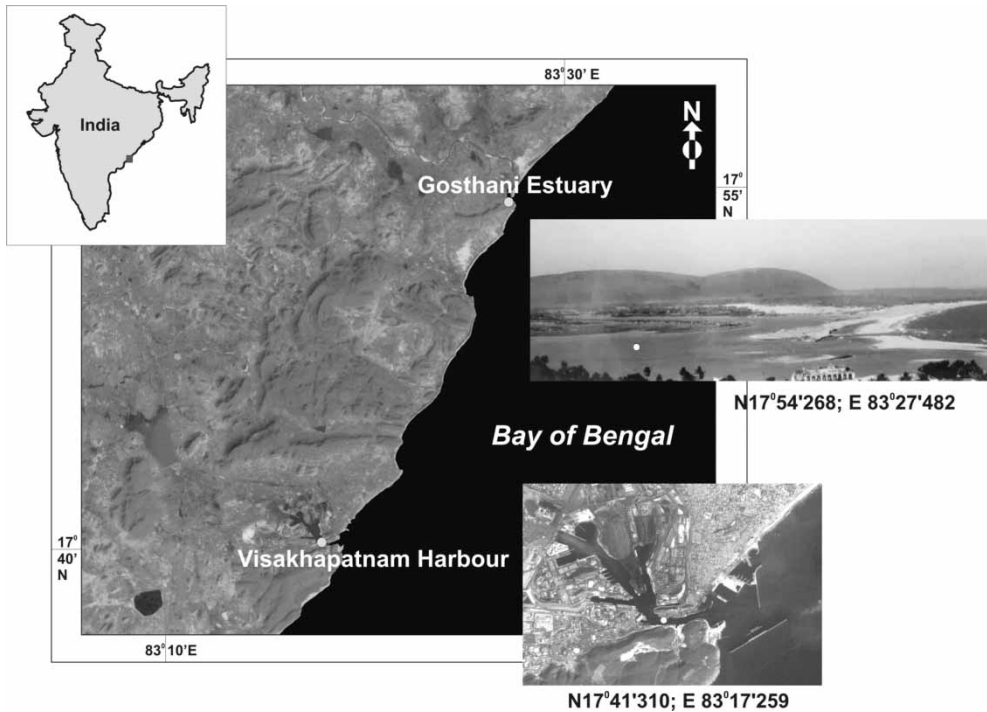


Figure 1. Map showing sampling locations.

During the summer months (April–May), the sand-spit across the estuary mouth hampers tidal action, leading to stagnation. Salinity during this period is relatively high (32 PSU). During the monsoon season (July–October), large volumes of freshwater enter the estuary, causing considerable dilution (salinity 5 PSU).

2. Materials and methods

Near-surface zooplankton samples were collected using a net (mouth area 0.13 m^2 , mesh size $200\ \mu\text{m}$) towed horizontally behind a mechanized boat for 10 min. A digital flow meter (Hydrobios Model 438 110) was used to determine the volume of water filtered. Zooplankton samples were preserved in 5% buffered formaldehyde. All large organisms were removed prior to taking aliquots [24, 25]. Each time, two or three aliquots (10 ml) were counted using a binocular stereomicroscope (OLYMPUS SZ40) and zooplankton abundance (including large forms) expressed as ind m^{-3} . Copepods were identified up to genus/species level and others up to the lowest possible taxon.

Surface-water quality estimations consisted of a suite of environmental variables, namely temperature ($^{\circ}\text{C}$), secchi-disc transparency (m), (Harvey) salinity (practical salinity units), (Winkler) dissolved oxygen (mg l^{-1}), inorganic nitrogen (nitrite, nitrate), soluble phosphate, and reactive silicate ($\mu\text{mol l}^{-1}$) examined according to standard protocols [26, 27]. A UV (single beam) spectrophotometer (Chemito) with a 1 cm light path proved useful for all photometric measurements.

Differences between sites (harbour/estuary) were examined based on (zooplankton) species abundance (square-root-transformed) data using the Bray–Curtis similarity index

[28], i.e. hierarchical clustering through group average linking and multidimensional scaling (MDS). Student's *t*-test was used to determine differences in zooplankton abundance between the two sites. Species diversity indices, i.e. species richness (d'), were calculated after Margalef [29], species diversity (H') according to Shannon and Weaver [30], and evenness (J') after Pielou [31]. Combinations of species considered ultimately responsible for patterns (in biotic assemblages) noticed were investigated using the BVSTEP protocols implemented in PRIMER. Species characterizing zooplankton communities at each location were determined using the similarity percentage (SIMPER) procedure. A one-way analysis of similarity (ANOSIM) test was performed to measure the level of significance (Global R) for differences in water quality. These procedures were followed according to PRIMER v.5 [21, 22]. Environmental variables affecting zooplankton species composition could be documented using CANOCO version 4.53 [23]. Along with species-area data (square root transformed), the CCA triplots (for each environmental variable) yielded invaluable information. Variables increased in value along the vector from the origin [32]. The significance of the species–environment relations was tested using Monte Carlo permutation tests.

3. Results and discussion

In the harbour channel, there were 24 taxa represented by 16 diverse groups of zooplankton. Calanoids, cyclopoids, bivalve veligers, tintinnids, and crustacean nauplii were numerically important (figure 2). Zooplankton abundance was rather high (2128 ± 1325 ind m^{-3} , mean ± 1 SD), and copepods were the most abundant group, contributing 38–97% of the total zooplankton population. They were represented by calanoids (six species), cyclopoids (three species) and harpacticoids (two species). Among the calanoids (46.4%) *Acrocalanus* spp. (mainly *A. gracilis*) outweighed all others. Next in abundance were cyclopoids (30.3%) dominated by *Oithona* spp. (mainly *O. rigida* followed by *O. nana*, and *O. brevicornis*), and

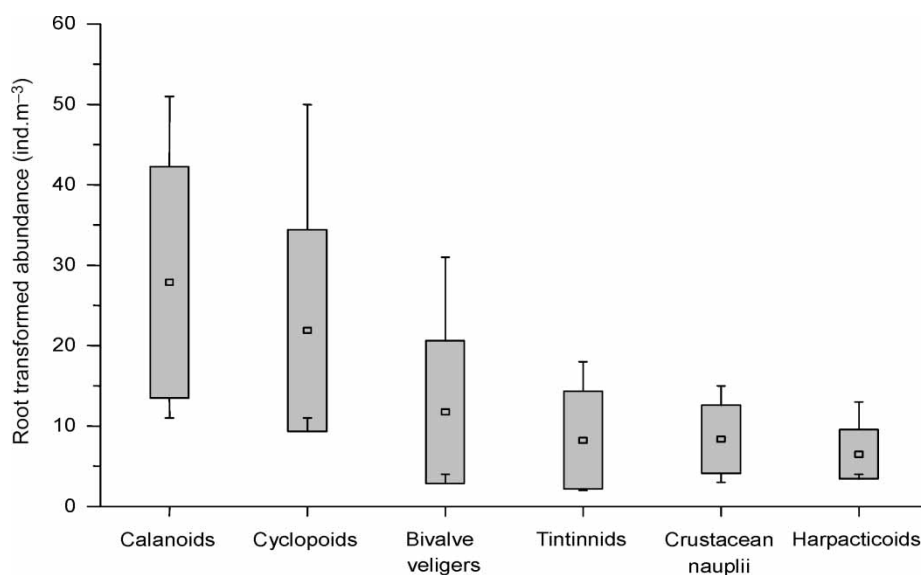


Figure 2. Dominant zooplankton taxa of the harbour channel, Visakhapatnam. A box denotes mean ± 1 SD; whiskers, min–max.

harpacticoids dominated by *Euterpina acutifrons*. Overall, the holoplankton made up 84.3%. Bivalve veligers (10.2%) and crustacean nauplii (4.2%) were the major meroplanktonic forms.

In Gosthani estuary, there were 27 taxa represented by 17 groups, of which cyclopoids, crustacean nauplii, calanoids, gastropod, and bivalve veligers dominated, in that order (figure 3). The zooplankton abundance was higher ($6730 \pm 4704 \text{ ind m}^{-3}$) here than in the harbour. Even here, copepods outnumbered all others, contributing up to 92% (mean 70%) of the total zooplankton. There were calanoids (six species), cyclopoids (three species), and harpacticoids (three species). Cyclopoids contributed greatly (55.2%), followed by calanoids (13.9%) and harpacticoids (1.1%). *Oithona* sp. (mainly *O. rigida* and *O. brevicornis*) (99.9%) represented almost exclusively the cyclopoid population: *Paracalanus* spp. and *Acartia* spp. (mainly *A. spinicauda* followed by *A. centrura*), the calanoids, and *Longipedia* sp. and *Euterpina acutifrons*, the harpacticoids. Holoplankton constituted more than two-thirds of the population. Larval forms consisted of crustacean nauplii (18.3%) and gastropod (8.92%) and bivalve veligers (1.1%).

Zooplankton diversity (Margalef, d ; Shannon-Wiener, H' and Evenness J' values) was low for both harbour (mean d 1.16, H' 1.52, J' 0.61) and estuary (mean d 1.55, H' 1.42, J' 0.55) (table 1), albeit the population was rich in the estuary where *Oithona* sp. occurred almost singularly, implying the species, preference to this area relative to the polluted conditions in the harbour.

On the basis of Bray–Curtis similarities using zooplankton (root-transformed) abundance data, it was possible to categorize the Harbour/Estuary samples into two groups (similarity, 50.6% figure 4). Cluster I samples (GE1–GE7) corresponded to Gosthani estuary (lower dendrogram), and Cluster II (with two sub-clusters) i.e. EC1–EC7 (upper dendrogram) represented the Harbour environment. The dendrogram provided a sequence of fairly convincing groups of samples confirmed by the MDS plot (stress 0.06) (figure 5). Student's t -test showed

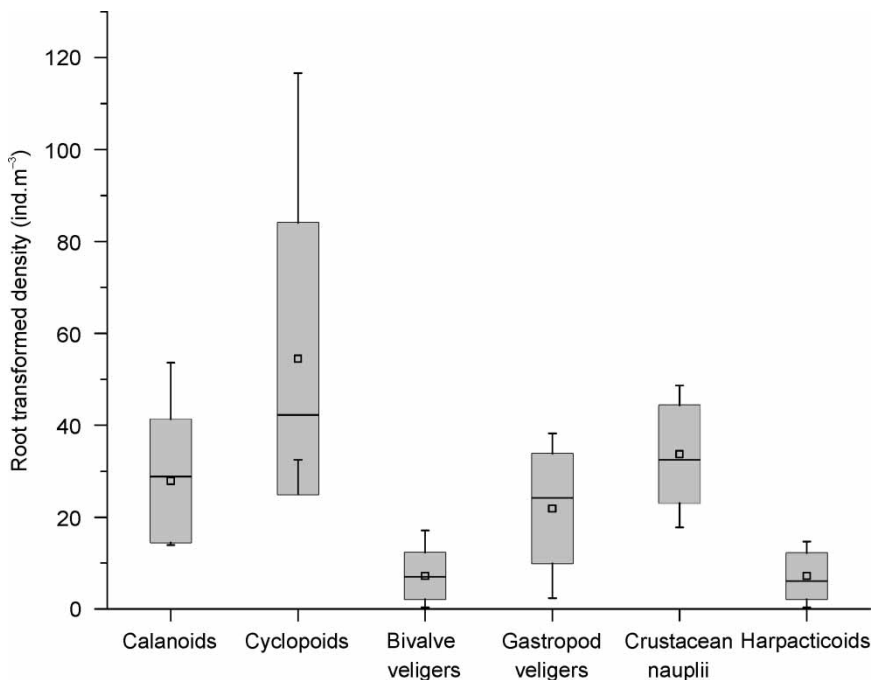


Figure 3. Dominant zooplankton taxa of Gosthani estuary, Bheemunipatnam. A box denotes mean \pm 1 SD; whiskers, min–max.

Table 1. Zooplankton abundance and diversity.

Diversity index	Harbour channel Range (mean \pm 1 SD)	Gosthani estuary Range (mean \pm 1 SD)
No. of species	10–15 (13 \pm 2)	6–20 (14 \pm 5)
Numerical abundance (ind m ⁻³)	580–4312 (2128 \pm 1325)	2220–15666 (6730 \pm 4704)
Margalef (d')	1.16–1.81 (1.55 \pm 0.23)	0.65–2.21 (1.55 \pm 0.51)
Evenness (J')	0.47–0.77 (0.61 \pm 0.11)	0.21–0.66 (0.55 \pm 0.15)
Shannon–Weaver (H')	1.24–1.85 (1.52 \pm 0.22)	0.64–1.82 (1.42 \pm 0.44)

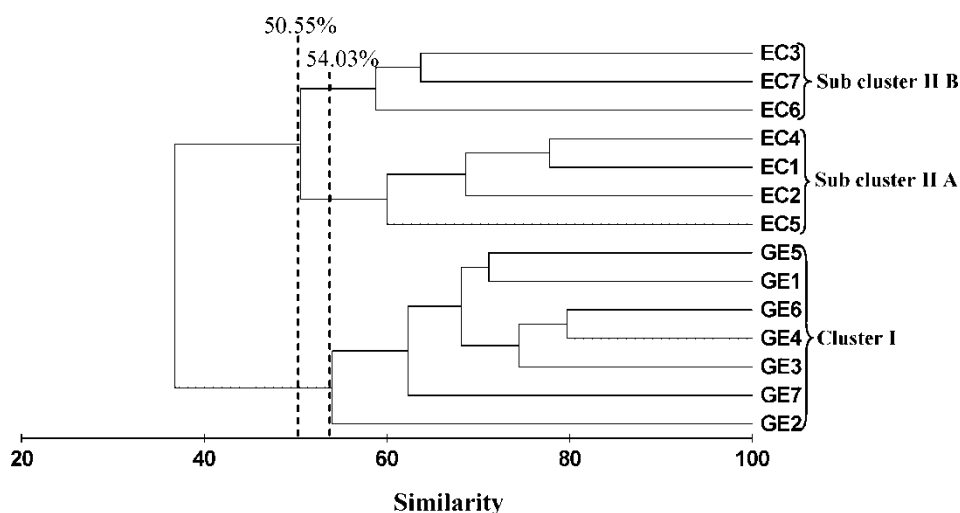


Figure 4. Dendrogram (clustering) of channel and estuary samples showing Bray–Curtis similarities on root transformed zooplankton abundance data.

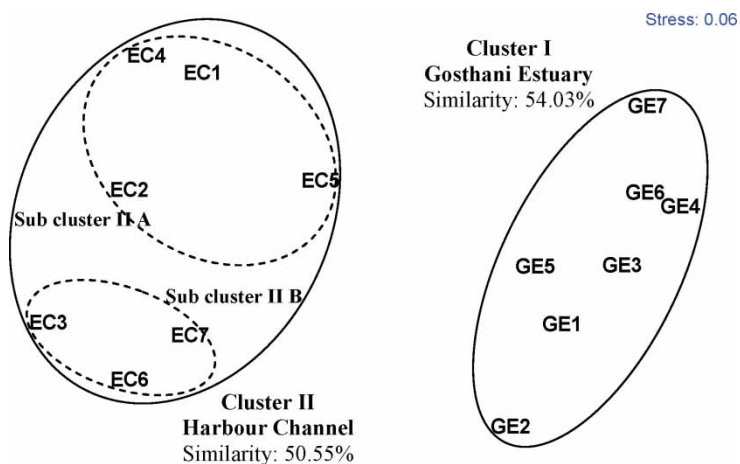


Figure 5. Harbour and estuary Zooplankton: MDS ordination of samples based on root transformed abundance and Bray–Curtis similarity (stress 0.06).

that the zooplankton abundance within the (polluted) harbour channel was significantly lower than the estuary (t -value: 2.307, $P < 0.05$).

Further analysis through the BVSTEP routine (i.e. a stepwise search of combinations of species considered ultimately responsible for patterns observed in biotic assemblages) revealed noteworthy findings. An association of cyclopids *Oithona rigida*, *O. brevicornis*, and *Longipedia* sp., calanoids *Acartia spinicauda*, *A. centrura*, and *Paracalanus* spp., crustacean nauplii, and gastropod veligers was typical of Gosthani estuary, in agreement with the observed biotic pattern with the full set of species (correlation: 0.958). In the Entrance Channel, such a sub-set was formed by *Acrocalanus gracilis* with *Favella* sp., ostracods, *Pseudodiaptomus serricaudatus*, *Oithona rigida*, *O. nana*, and bivalve veligers (correlation 0.956). This is confirmed by the SIMPER (table 2).

There were appreciable differences in the hydrographical conditions between the harbour/estuary samples (table 3). In the harbour channel subjected to considerable pollution, dissolved oxygen ranged between 1.7 and 3.5 mg l⁻¹ (mean 2.78 ± 0.69 mg l⁻¹), secchi-disc transparency 1–3 m (1.71 ± 0.67 m) and water temperature 30–33 °C (30.9 ± 1.03 °C). Nutrient (mean) levels registered high values (inorganic phosphate 40.86 ± 9.2 μmol l⁻¹, nitrite 2.87 ± 0.94 μmol l⁻¹, nitrate 15.26 ± 5.41 μmol l⁻¹). The high levels of nitrate and phosphate in the harbour are a result of discharges of fertilizer-factory waste and partially treated domestic sewage, and their persistence due to inadequate flushing over the years [17, 33]. Findings made during this study revealed a manifold increase in the concentration of both N and P since reported for the same site in 1985–1987 [18]. The Spearman rank correlation ($r=0.713$) and the low N:P ratio (0.5) seemed to signify that the water quality in the harbour had deteriorated further.

In the Gosthani estuary, which is relatively free from pollution, dissolved oxygen was 2.9–3.6 mg l⁻¹ (mean 3.25 ± 0.27 mg l⁻¹), secchi-disc transparency 0.55–1 m (mean 0.84 ± 0.18 m), and water temperature 30–34 °C (mean 31.7 ± 1.32 °C). Nutrient (mean) concentrations (μmol l⁻¹) were relatively low (inorganic phosphate 1 ± 0.51, nitrite 0.3 ± 0.16 and nitrate 6.1 ± 3.63). It is noteworthy that the estuary recorded a higher amount of dissolved silica (mean 67.37 ± 17.71 μmol l⁻¹) than the harbour (mean 15.4 ± 1.57 μmol l⁻¹). In Gosthani estuary, influenced by freshwater discharge (mean salinity 27.8 psu), the relationship between

Table 2. SIMPER showing percentile contribution by individual zooplankton species to the average similarity within each group/assemblage.

Species	Harbour Channel Average abundance	Estuary Average abundance	Average dissimilarity	Dissimilarity/SD	Contribution (%)	Cumulative (%)
Average dissimilarity = 63.20						
<i>Oithona</i> sp.*	634.96	3714.22	11.07	1.45	17.52	17.52
Crustacean nauplii (except cirripede nauplii)*	43.19	1132.29	9.45	2.71	14.95	32.47
<i>Acrocalanus</i> sp.*	662.56	2.88	7.09	1.61	11.23	43.69
Gastropod veliger*	2.74	600.29	6.43	2.00	10.17	53.86
<i>Acartia</i> sp.*	0.83	405.73	5.20	1.82	8.23	62.09
<i>Paracalanus</i> spp.	302.27	494.54	3.35	1.45	5.30	67.39
Bivalve veliger	217.72	73.70	2.83	0.89	4.47	71.86
<i>Longipedia</i> sp.	0.81	69.40	2.23	1.31	3.53	75.39
<i>Favella</i> sp.	102.70	7.78	2.15	1.03	3.40	78.79
<i>Cirripede nauplii</i>	46.09	100.11	1.96	1.22	3.10	81.89

*Discriminating species.

Table 3. Water quality in the Harbour Channel (EC) and Gosthani estuary (GE) (June–July 2001).

Samples	W.T (°C)	pH	D.O (mg l ⁻¹)	Salinity (PSU)	Secchi- disc (m)	NO ₂ -N (μmol l ⁻¹)	NO ₃ -N (μmol l ⁻¹)	PO ₄ -P (μmol l ⁻¹)	SiO ₄ -S (μmol l ⁻¹)
EC1	33	7.5	2.4	31.9	1.2	4	14.8	48.4	13.4
EC2	30.5	7.4	1.7	33.1	1.9	4.1	15.7	47.9	17.6
EC3	30	7.4	2.2	29.1	1.9	2.2	20.2	25.7	17.4
EC4	30	7.4	3.0	34.7	1.7	2.2	23.7	31.9	14.3
EC5	30.5	7.4	3.5	33.7	3	1.6	9.5	40.4	15.2
EC6	31	7.5	3.3	34.7	1	2.9	8.5	50.4	15.5
EC7	31	7.5	3.4	34.6	1.3	3.1	14.4	41.3	14.6
GE1	32.5	7.3	3.0	31.9	0.55	0.2	4.6	1.7	68.5
GE2	30	7.4	3.6	29.1	0.95	0.5	8.9	1.8	95.5
GE3	34	7.6	3.6	27.7	0.9	0.4	7	0.7	79.9
GE4	31.5	7.6	2.9	27.0	0.85	0.5	12.4	1	75.9
GE5	30.5	7.7	3.2	27.6	1	0.2	2.6	0.7	52.2
GE6	31.5	7.7	3.2	24.6	1	0.1	2.3	0.6	47.4
GE7	32	8	3.3	26.5	0.65	0.4	4.7	0.7	52.2

inorganic nitrogen, phosphate, and silicate was even (r 0.677–0.868, $P < 0.05$) indicating that the estuary revealed conditions different from those in the harbour (ANOSIM test, Global R : 0.98, $P < 0.05$). The N:P ratio (6.7) revealed the mesotrophic nature of the estuary during this time of the year.

Clarke and Warwick [21] explained that in ecosystem research, the biotic data could be matched by a set of environmental variables measured at the same set of sites corresponding to the biota. Based on Bristol Channel–Celtic Sea zooplankton data [34], they were able to show salinity as an important factor determining zooplankton community structure in the Severn Estuary. In an appealing study on Messolongi Lagoon phytoplankton, it was found that the levels of inorganic nitrogen, phosphate, salinity, silicate, and dissolved oxygen correlated well with the diatom distribution [35]. A number of such studies (relating biotic with abiotic factors) were carried out on marine benthos as well [21]. In the context of Visakhapatnam Harbour/Estuary sites, there were essentially two communities of zooplankton characterized by *Oithona rigida*, *O. brevicornis*, crustacean nauplii, gastropod veligers, and *Acartia* spp. (mainly *A. spinicauda*), in the Gosthani estuary and, *Acrocalanus* spp. (mainly *A. gracilis*) for harbour.

Figure 6 shows the MDS of zooplankton communities described earlier (figure 5) with superimposed circles of increasing size representing abiotic variables. That this biotic pattern correlated with some abiotic factors can be best seen, variable-wise on the MDS configuration. Figure 6 shows the distribution of inorganic, nitrite, nitrate, phosphate, and silicate for the channel and estuary areas by circles of differing diameters placed on the MDS, and the pattern across the seven observations is also shown. When each of the above variables is superimposed in turn on the biotic ordination, several informative patterns emerge. A general examination of these would reveal an appreciable difference in water quality between the harbour channel (large circles corresponding to high nitrite, nitrate, and phosphate levels) and estuary (high silicate levels), which should explain the observed differences (in zooplankton nature) between the two sites, as confirmed through the CCA.

In the CCA, ordination axes are chosen in the light of known environmental variables such that the axes are linear combinations of environmental variables which can be directly related to species variations [32]. Local harbour/estuary findings revealed that the first (canonical) axis alone explained up to 74.1% variance concerning species/environment relationships (figure 7). On the positive side of axes 1 and 2, the variables were associated with pollution indices (inorganic nutrients), suggestive of differing pollution effects on zooplankton community structure.

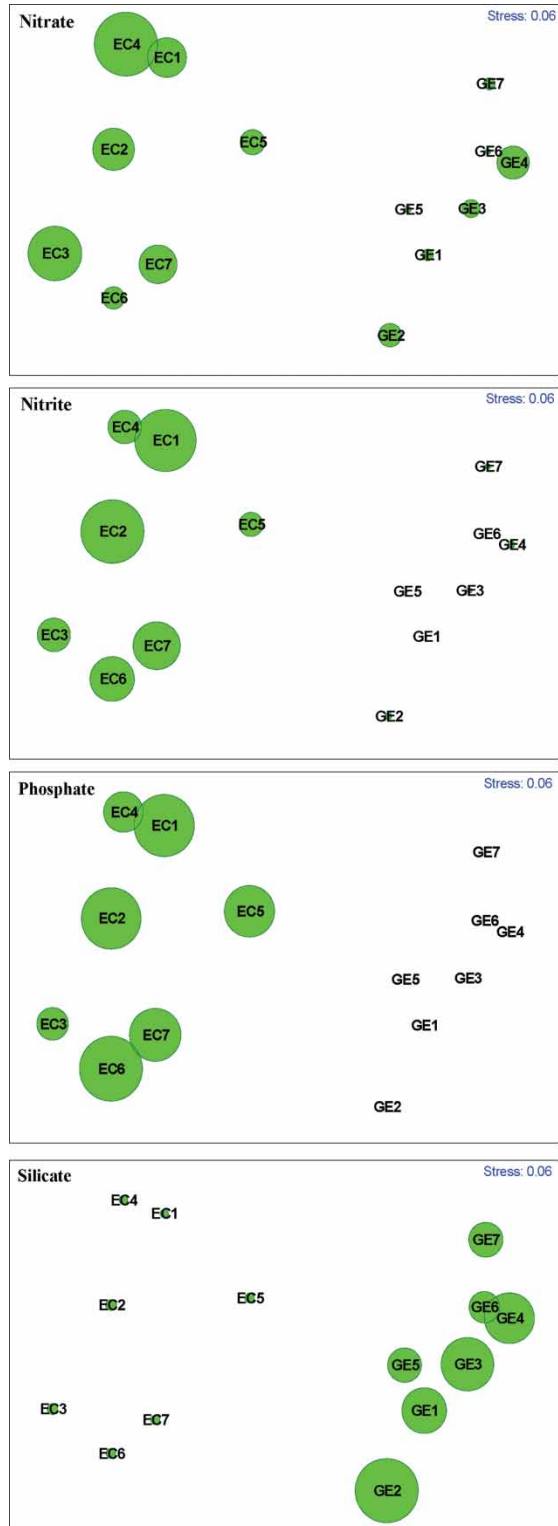


Figure 6. Distribution of inorganic nutrients in the waters of Harbour Channel and Gosthani estuary.

Tests to determine whether the effects could be isolated from one another and whether they are significant (table 4) revealed appealing patterns. While nitrogen and phosphorus, secchi-disc transparency, and salinity (characteristic of harbour) correlated positively with axis I, temperature, dissolved oxygen and silicate (characterizing the estuary) correlated negatively. The Monte Carlo test confirmed the significance of this axis at the 95% level and revealed that $\text{PO}_4\text{-P}$ had a significant relationship ($P < 0.05$) (directly or indirectly) with species distribution. Whereas cyclopoid copepods (e.g. *Oithona rigida* and *O. brevicornis*) characterized the Gosthani estuary along with *Acartia* sp. (mainly *A. spinicauda*) similar to a report in an Indian estuary [36], calanoids (represented by *Acrocalanus gracilis*) contributed to the bulk of zooplankton in the harbour. Lindo [37] made comparable observations on the calanoids from the polluted Kingston Harbour. *Acrocalanus* sp. is known for its distinctive presence in coastal waters in India [38, 39]. The presence of *Acrocalanus* sp. (*A. gracilis*) in large numbers in Visakhapatnam harbour is suggestive of the species' preference to such areas containing rich phytoplankton, where, aided by high nitrogen and phosphorus, the nanoplankton

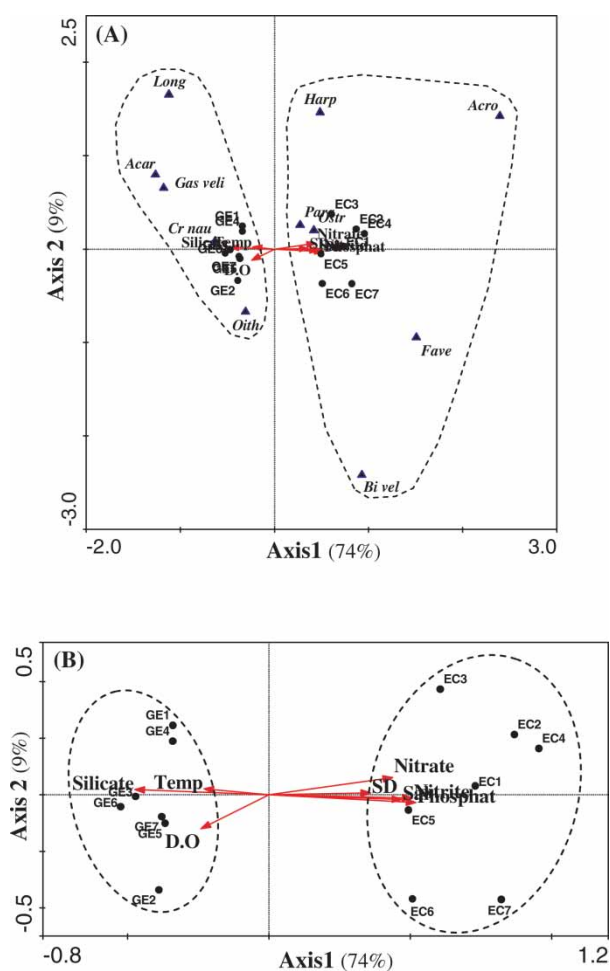


Figure 7. Canonical Correspondence Analysis (CCA) showing the association of environmental parameters with most important species of zooplankton in Harbour Channel and Gosthani Estuary. (A) Species-Area triplot with environmental parameters (B) Environmental-Area biplot. Ostr: Ostracods; Cr nau: Crustacean nauplii; Harp: Harpacticoids; Bi vel: Bivalve veligers; Gas veli: Gastropod veligers; Fave: *Favella* sp.; Acro: *Acrocalanus* sp.; Oith: *Oithona* sp.; Long: *Longipedia* sp.; Para: *Paracalanus* sp.

Table 4. Canonical Correspondence Analysis (CCA) for Harbour Channel and Gosthani Estuary.

Axes	1	2
Eigenvalues	0.303	0.037
Species–environment correlations	0.989	0.641
Cumulative percentage variance of species–environment relation	74.1	83.2
Correlation coefficient		
Temperature	–0.413	0.070
Dissolved oxygen	–0.433	–0.399
Salinity	0.844	–0.056
Secchi-disc transparency	0.647	0.022
NO ₂ -N	0.911	–0.038
NO ₃ -N	0.786	0.203
PO ₄ -P	0.933*	–0.092
SiO ₂ -Si	–0.853	0.061

*Significant at $P < 0.05$.

(<20 μm) could well be an important primary producer contributing up to 70–75% of chlorophyll (unpublished data). The low abundance of crustacean nauplii and gastropod veligers in the harbour waters relative to estuary (t value: 3.537, $P < 0.05$) could be due to the impacts of pollution on meroplanktonic forms.

4. Conclusions

To sum up, there is a magnitude of difference in zooplankton abundance and community structure between harbour/estuary sites evidently caused by a major disparity in the hydrographical conditions between these two waterbodies. The overwhelming dominance of (herbivorous) *Acrocalanus gracilis* in the harbour channel should indicate the species' preference to phytoplankton-rich areas where nanoplankton (<20 μm) contributed (70–75% of Chl *a*) to the total phytoplankton production. In Gosthani estuary, where physical forces (e.g. sand-spit closure during summer) coupled with monsoon regimes (i.e. freshwater influx) seemed important, the zooplankton is structured by species *Oithona rigida*, *O. brevicornis*, and *Acartia spinicauda*, at least for the study made.

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