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Seasonal Distribution of the Various Diarrhetic Shellfish Poisoning (DSP) Causing *Dinophysis* species in the Tidal Channel of Manori Creek at North Mumbai and the Environmental Parameters Influencing Their Presence

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Abstract

The Coastal water of Mumbai in the west coast of India is well known for its diverse fishery resources. This locality is at present under stress due to treated and untreated urban wastewater discharge through storm water drains. The present study deals with the species of *Dinophysis* group present in the tidal channel of Manori creek and the various environmental parameters, which influence their presence. A total of five species of *Dinophysis* i.e. *Dinophysis caudata, D. norvegica, D. dens, D.acuminata* and *D.miles* were recorded during the one and a half years of study from July 2005 - December 2006. These groups were present below 100 cells L^{-1} and constituted a very minor fraction of the total phytoplankton community. They were sporadically present during the monsoon months when salinity was low. In the study the environmental parameters, trace metals (Fe, Cu, Co, Mo, Zn, Ni and Cd), pH, atmospheric temperature, NH_3 - N, NO_3 – N, NO_2-N, PO_4-P and dissolve oxygen were not found to be influencing the *Dinophysis* presence. It was only correlated with low salinity. The result also indicated the eutrophic condition of this creek caused by anthropogenic activities. This is the first detailed study of *Dinophysis* species in correlation to their environmental parameters from this region.

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Introduction

Dinophysis are the microscopic planktonic algae found in brackish and marine waters of both tropical and temperate regions (Larsen and Moestrup 1992). Some of the *Dinophysis* members are known to produce toxins. The shellfishes filter-feed on the algae and accumulate the toxins produced by them. The toxins are known to be the cause of Diarrhetic Shellfish Poisoning (DSP) in humans. The poisoning is characterized by diarrhea, vomiting, nausea and gastrointestinal distress (Yasumoto et al. 1980; Kat 1985) and is non-fatal. However, the toxins can promote tumour formation in the digestive tract and thus produce chronic health problems among shellfish consumers (Suganuma et al. 1988). *Dinophysis* members produce okadaic acid (OA), dinophysis toxins (DTX), pectenotoxins (PTX), and yessotoxins (YTX), which are fat-soluble polyether compounds and are responsible for poisoning effects.

About 200 species of *Dinophysis* have been described (Sournia 1986). Some of them are known to cause shellfish toxicity even at very low cell concentration of 200 cells L^{-1} (Yasumoto et al. 1980). In India, several *Dinophysis* species were reported from both east and west coasts (Subrahmanyan 1958; Kannan and Vasantha 1992; Godhe et al. 2001; Rajasekar 2005). Reports of their bloom formation are relatively rare. However, their presence may indicate a potential threat of bloom formation and diarrhetic shellfish poisoning outbreaks under favorable environment.

The aim of the present study is to record the species of *Dinophysis* present in the northernmost tidal creek of Mumbai (Lat 18°55’N Long 72°49’E) and to study the hydrological and other environmental parameters influencing their presence. We specifically looked for *Dinophysis* keeping an eye on the natural clam (*Meretrix* sp.) beds of this creek, which form the fishery throughout the year except during the monsoon months. To our knowledge, prior to this study there had been no detailed investigations on the *Dinophysis* species in this area.

Materials and Methods

Study site

Manori creek (19°11’45.05”N 72°47’42.89”E) is a tidal channel (Figure 1**)** situated in north Mumbai. The bottom of the creek is muddy with high organic load. Patches of mangrove border its bank. This creek supports the bivalve fishery of *Meretrix* sp. throughout the year except during the south-west monsoon. A few recreational parks and many beach resorts are situated along its bank.

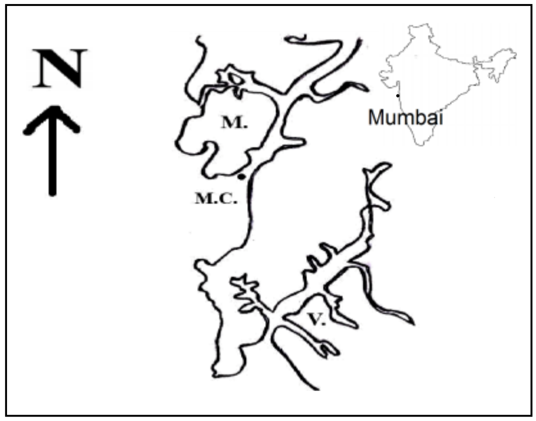

Fig. 1. Manori creek of Mumbai coast from where phytoplankton and water samples were collected during our study. The dot at the mouth of creek is the exact location of sampling.

Sampling and field procedure

Sampling was done from July 2005 to December 2006 with a minimum of one sample per month. A total of 24 samplings were done. Surface water samples were collected from the mouth of the creek during high tide. Sampling could not be undertaken during August 2006 due to heavy rains and stormy weather. Using a 5L capacity plastic container, a total of 50 L was filtered through a plankton net of 10μ mesh size attached to a 50mL collection bottle at the end. Collected plankton concentrate (50 ml) was then divided into approximately three equal parts. One part was brought to the laboratory as live sample while the other two were immediately fixed in lugol's iodine and 3% neutralized formaldehyde as suggested by Throndsen (1978).

Nutrient Analysis

Surface water samples were collected in acid rinsed glass bottles and analysis commenced within two hours of collection. The NH_3-N (ammonia–nitrogen) was estimated by Phenate method (Strickland and Parsons 1968), NO_2-N (nitrite-nitrogen) was estimated by diazotization of nitrite with sulphanilamide hydrochloride (Strickland and Parsons 1968). The NO_3-N (nitrate-nitrogen) was estimated by cadmium copper reduction column for reactive nitrate (Strickland and Parsons 1968), while PO_4-P (phosphate-phosphorus) was estimated folllowing the automated ascorbic acid method (Murphy and Riley 1962).

Hydrological parameter analysis

Salinity was estimated using hand refractometer (Erma, Tokyo), pH was estimated through digital pH meter (Expo HiTech) with an accuracy of 0.1. Sea surface temperature (SST) and Atmospheric temperature (AT) were recorded using a standard mercury thermometer of ±0.5 °C accuracy. For determination of BOD, direct method was employed for samples having BOD < 4 mg L^{-1} and for samples having higher BOD, unseeded dilution method was employed (Martin 1972). The samples were incubated for five days at 20±1°C in a BOD incubator. The DO (Dissolved oxygen) was estimated following Winkler's method. Heavy metals were estimated in seawater by atomic absorption spectrophotometer (Perkin-Elmer model 3000) after concentrating and digesting using a microwave digester (Perkin Elmer Anton Paar multiwave 3000).

Phytoplankton analysis

The 1 ml aliquot of collected phytoplankton concentrate was transferred to gridded Sedgwick rafter cell (Wildlife supply company, USA). Planktons were examined under light microscope (Carl Zeiss, Axioscop 40) at X200 and X400. For each sample, counting was done three times and the mean was taken (expressed as cells L^{-1}) as the final value. Phytoplanktons were identified based on their external morphology (Subrahmanyan 1971; Dodge 1982; Taylor et al. 1995; Steidinger and Tangen 1997). *Dinophysis* species were identified by morphology of the left sulcal list and ribs supporting it, shape and size of the cingular lists, size of epicone and other external characteristics (Taylor et al. 1995; Steidinger and Tangen 1997).

Data analysis

Spearman's rho correlation was estimated for the magnitude and direction of the association between 17 physico-chemical **v**ariables and *Dinophysis* by SPSS version 15.0 for windows.

Results

Physico-chemical parameters

The descriptive statistics of the physico- chemical parameters estimated from July 2005 to December 2006 is given in Table 1. The SST and AT showed a narrow annual range with the highest and lowest values during summer and winter respectively. Salinity showed considerable fluctuations. Low salinity values (range 19 ppt to 37 ppt) were observed during the monsoon months (July-October) due to fresh water influx and showed truly marine conditions during other months of the year. The pH showed the alkaline condition of the water in this creek with an annual gradient of 1.1. Dissolved oxygen (DO) values were fluctuating widely reaching its minimum during winter and maximum during pre-monsoon. Highest BOD was recorded during autumn and at the start of winter and lowest during monsoon.

Table 1. Descriptive statistics of the physico-chemical parameters from Manori creek

Parameters	**Minimum**	**Maximum**	**Mean**
NH_3-N (μg L^{-1})	16.96	588.57	158.5941
NO_3-N (μg L^{-1})	5.97	100.24	32.8059
NO_2-N (μg L^{-1})	2.81	149.41	64.17
PO_4-P (μg L^{-1})	0.012	1211.37	337.5447
Fe (mg L^{-1})	0.3	20.7	2.8876
Cu (mg L^{-1})	0.02	0.53	0.0894
Co (mg L^{-1})	0.01	0.15	0.0724
Mo (mg L^{-1})	0.01	0.09	0.0494
Zn (mg L^{-1})	0.02	0.13	0.0688
Ni (mg L^{-1})	0.01	0.27	0.0853
Cd (mg L^{-1})	0.001	0.1	0.02
SST (°C)	24.7	29.65	27.6894
AT (°C)	27	32.5	30.4
DO (mg L^{-1})	0.5	7.1	3.2235
BOD (mg L^{-1})	0.1	10.4	3.9588
Sal (ppt)	19	37	32.4882
pH	7.1	8.1	7.5765

All the four nutrients viz., NH_3-N, NO_3-N, NO_2-N and PO_4-P showed conspicuous seasonal variation with a very wide range (Table 1). Level of reactive phosphorus in seawater was abnormally high (average 337.5 μg L^{-1}). The concentration of NH_3-N, NO_3-N and NO_2-N was also high in this creek.

Similar to the nutrients, trace metals also showed conspicuous seasonal variations with a very wide gradient. Among the studied trace metals, Fe was the most abundant (average 2.88 mg L^{-1}). All the heavy metals show higher values at this site, which clearly indicate the influence of human activity on this creek.

Dinophysis species observed in the waters of Manori creek

The phytoplankton count in this creek ranged from 598 cells L^{-1} in September 2005 to 157140 cells L^{-1} in June 2006 (Data not shown). The centric diatoms were the most common groups (44%) followed by pennate diatoms (21%) and dinoflagellate (29%) in decreasing order of their presence (unpublished data). Marine microscopic flagellates occupied a very small fraction (6%).

During the study period in this tidal channel, five species of *Dinophysis* species *i e*. *D. caudata, D. norvegica, D. miles, D. dens* and *D. Acuminata* were observed (Table 2). In all the samples collected except one, only one species of *Dinophysis* was present at a time. The occurrence of *Dinophysis* groups ranged between 19 cells L^{-1} and 93 cells L^{-1}. In October 2006, *D. acuminata* (19 cells L^{-1}) and *D. den* (29 cells L^{-1}) were present together. Observed twice during the study were *D. acuminata* and *D. caudata* whereas *D. dens*_and *D. norvegica* were recorded thrice each, while *D. miles* were present once in November 2006 (22 cells L^{-1}). Maximum cell numbers were found for *D. norvegica* in November 2006 (93 cell L-1). On the contrary, the lowest cell number was found for *D. acuminata* in October 2005 (19 cells L^{-1}). These groups were not seen in the samples collected on autumn, winter and summer. They were present during the onset of the southwest monsoon when weather was cloudy and humid, till the end of the monsoon months. Though the *Dinophysis* species did not constitute a very major fraction (0.12%-10.6%) of the total phytoplankton concentration, they constituted a significant portion (0.47%-63.15%) of the dinoflagellate concentration occasionally. Bloom was never observed throughout the study. In 2005, the *Dinophysis* cells were recorded from the month of July till November and were again recorded in 2006 during the months of July, September and October. The presence of small size cells of *Dinophysis* was never observed in the samples.

Dinophysis and abiotic factors

No significant relationship between the presence of *Dinophysis* and trace metals was found (Table 3). The correlation test showed a negative relationship ($r = -0.588$) between *Dinophysis* presence and salinity at the significance of $P \leq 0.01$ (Table 3). The BOD and *Dinophysis* presence were not significantly negatively correlated. Any other physico-chemical parameters were not found to be influencing their presence. *Dinophysis* were observed mostly during monsoon and showed an inverse relation with the salinity of the creek water.

Table 2. Percentage composition of different phytoplankton groups and the *Dinophysis* species in Manori creek on the samplings when *Dinophysis* species were seen.

Sample no.	Dinoflagellates (%)	Diatoms	% of *Dinophysis*		Species
			In phytoplankton	In dinoflagellates	
8.7.2005	2.688	97.311	0.483	17.968	*D.acuminata*
16.7.2005	18.437	76.649	1.69	9.169	*D.dens*
5.9.2005	16.851	80.266	10.643	63.157	*D. norvegica*
29.9.2005	23.991	71.07	1.112	4.637	*D.caudata*
5.10.2005	56.25	43.75	6.054	10.763	*D.dens*
13.10.2005	33.268	63.914	1.165	3.503	*D.acuminata, D.dens*
13.11.2005	27.145	71.587	0.129	0.476	*D. norvegica*
18.7.2006	8.316	91.683	3.081	37.051	*D. norvegica*
30.8.2006	23.566	76.433	1.63	6.919	*D.caudata*
18.11.2006	26.926	11.839	0.246	0.915	*D.miles*

Relationship between physico-chemical parameters

Most of the trace metals were significantly positively correlated to each other (Table 3). The NH_3-N was negatively correlated to NO_2-N (r = -0.494) and PO_4-P (r = -0.417) at $P \leq 0.05$. Sea surface temperature was inversely related to NO_3-N (r = -0.448) and NO_2-N(r = -0.506) at $P \leq 0.05$. The DO and BOD were negatively correlated (r = -0.488) at $P \leq 0.05$ and BOD was also negatively correlated with sea surface temperature and atmospheric temperature.

Table 3. Spearman's rho correlation for the *Dinophysis* and abiotic factors

	Dinophysis	Fe	Cu	Co	Mo	Zn	Ni	Cd	NH3-N	NO3-N	NO2-N	PO4-P	DO	BOD	Salinity	pH	SST
Dinophysis	1																
Fe	-0.039	1															
Cu	0.162	0.366	1														
Co	-0.002	0.285	0.045	1													
Mo	-0.1	-0.015	.484(*)	-0.024	1												
Zn	0.347	0.022	0.115	.494(*)	-0.139	1											
Ni	0.128	.475(*)	.516(**)	0.359	.423(*)	0.269	1										
Cd	0.26	-0.141	0.074	0.303	-0.118	0.169	-0.087	1									
NH_3-N	-0.261	0.325	0.027	0.334	0.132	0.158	0.244	-0.27	1								
NO_3-N	0.018	-0.235	-0.388	-0.085	-0.181	0.095	0.001	-0.31	-0.103	1							
NO_2-N	-0.058	-0.227	-0.113	-0.154	-0.323	-0.243	-0.169	0.095	-.494(*)	0.389	1						
PO_4-P	0.262	-0.275	0.141	-0.25	0.097	-0.06	0.047	0.181	-.417(*)	0.143	0.225	1					
DO	0.11	0.319	0.36	-0.125	0.209	-0.131	-0.03	-0.31	0.213	-0.136	-0.107	0.21	1				
BOD	-0.288	0.008	-0.158	-0.057	-0.103	-0.073	-0.092	0.015	-0.145	0.045	0.267	-0.29	-.488(*)	1			
Salinity	-.588(**)	-0.261	-.489(*)	0.062	-0.245	-0.122	-.454(*)	0.297	-0.063	0.213	0.333	0.17	-0.188	0.3	1		
pH	0.022	-0.15	0.012	-0.318	0.01	0.012	-0.251	-0.14	-0.135	0.033	-0.215	0.09	-0.037	0.259	0.015	1	
SST	0.186	-0.095	0.185	-0.129	0.399	-0.084	0.084	0.162	0.047	-.448(*)	-.506(*)	0.12	0.331	-.475(*)	-.423(*)	0.12	1
AT	0.129	-0.093	-0.112	0.141	-0.095	0.158	-0.188	0.12	0.34	-0.33	-0.382	0.1	0.275	-.446(*)	-0.106	-0.2	0.39

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Discussion

The southwest monsoon played a major role in the variation of biotic and abiotic factors of Manori creek, which is the Northen most creek of Mumbai. Manori creek was reported as one of the least polluted sites of Mumbai (Zingde et al. 1979; Varghese 2006) compared to other creeks and bays of this city. Despite the absence of a direct sewage discharge here, we observed that the D.O level was alarmingly low (< 1mg L^{-1}) in several occasions, which indicates the further anoxic condition of the water column and bottom water. This could be due to the reducing conditions in the creek and gas effervescence originating from the bottom when disturbed by ferry and fishing activities. Thus, water is highly polluted. However, during those anoxic conditions, *Dinophysis* spp. was not recorded. The D.O was highest during the monsoon, which may be due to the fresh water influx from terrestrial sources. Water in this creek was slightly alkaline in nature (average pH 7.6). Salinity showed wide fluctuation with brackish water condition during southwest monsoon and truly marine environment during summer. Unlike temperate regions, the annual gradient for sea surface temperature and atmospheric temperature was narrow (5.8 ° C and 6 °C respectively). All the four major nutrients viz., NH_3 -N, NO_3-N, NO_2-N and PO_4-P showed prominent seasonal variations. The inorganic phosphate concentration in this water was exceptionally very high (average 337.54 µg L^{-1}). Earlier such high level of phosphorus was also recorded from the coastal waters of Mumbai (Varshney 1985). Trace metals viz., Fe, Co, Cu, Cd, Mo, Zn and Ni concentration in water was high throughout the year. Concentrations of heavy metals in water showed the following order of abundance: Fe> Cu>Ni>Zn>Co>Mo>Cd.

In India, the detailed taxonomic account of dinoflagellates was initially done by Subrahmanyan (1958). Several potentially toxin producing dinoflagellates and their toxins were detected from shellfishes harvested from the Karnataka coast of India in the late 1980s (Segar et al. 1988; Karunasagar et al. 1989a). Subrahmanyan (1958) recorded several *Dinophysis* species i.e. *Dinophysis ovum*, *D. acuminata* and *D. miles*, at very low cell count, whereas *D. caudata, D. caudata f. acutiformis, D. miles f. Indica* were the more common groups. Other *Dinophysis* groups *viz., D. caudata var. pedunculata, D. hastata, D. schuetti, D. nias, D. uracantha* have also been reported from the Indian seas (Gopinathan and Pillai 1975; Rajasekar et al. 2005). Blooms of *Dinophysis* species were reported occasionally (Santhanam and Srinavasan 1996). In our study, we observed the *Dinophysis* count below the toxic level of 200 cells L^{-1}.

The influence of hydrological parameters on the abundance of *Dinophysis* was earlier investigated by some authors (Godhe et al. 2002; Hoshiai et al. 2003; Koukaras and Nikolaidis 2004). Different *Dinophysis* groups are found at different environment conditions and *D.*

acuminata generally occurs in coastal eutrophic areas (Reguera et al. 1995; Hoshiai et al. 2003). Among the *Dinophysis*, *D. caudata* is the most common group reported from the Indian waters. In our study, we have observed this species twice. *Dinophysis* species were more common during the months of July-November (Subrahmanyan 1958; Aune et al. 1996), which matches with our observation. Seasonal distribution of different *Dinophysis* species was reported from the Norwegian and Mediterranean coasts (Dahl et al. 1995; Aubry et al. 2000). Water temperature appears to be the most important factor influencing the *Dinophysis* abundance (Giacobbe et al. 1995). In our study, we did not find any correlation between these two. An inverse relation between salinity and *Dinophysis* (*D. acuminata* and *D. norvegica*) was observed earlier (Giacobbe et al. 1995; Peperzak et al. 1996; Lindahl and Anderson 1996; Soudant et al. 1997; Godhe et al. 2002). We further observed that when the salinity of this tidal channel was lowered by fresh water influx during rainy season, these groups were recorded. Their disappearance was characterized by high salinity during winter and summer. Therefore, an inverse correlation was observed which corroborates the earlier studies mentioned above. We did not find any relation between trace metals (Fe, Cu, Co, Cd, Ni, Zn and Mo) and the presence of *Dinophysis*. Correlation of *Dinophysis* abundance with NO_3 –N, NO_2 –N, PO_4 –P and NH_3 –N was not significant. Several other investigations indicated the absence of correlation between dissolved inorganic nutrients and number of *Dinophysis* cells (Lindahl and Anderson 1996; Blanco et al. 1998; Aubry et al. 2000; Smayda and Reynolds 2001). However, there are also reports of *D. cf. acuminata* growth in the presence of high concentrations of inorganic nitrogen *viz* NO_3 –N and NH_3 –N (Lassus et al. 1993; Chang 1996). *Dinophysis* bloom was reported occasionally from the coastal waters of east as well as west coast of India and DSP toxins were detected from bivalves collected from the west coast (Karnataka) of India (Segar et al.1988; Karunasagar et al. 1989b) but there was no report of outbreak of human shellfish poisoning due to DSP toxins. It is likely that toxins produced by these organisms were overlooked, as no direct shellfish biotoxin tests had been undertaken .So far no success has been achieved in culturing the *Dinophysis* in laboratory condition, since most of the studies were restricted to natural environment. Due to difficulty of establishing culture of *Dinophysis* spp., the importance of dissolve organic matter, as a source of nutrient (Carlsson and Graneli 1988) is not known. Therefore, studies on abundance of these species *vis a vis* the nutrient provide valuable information to understand the biology of organisms better. *Dinophysis* can also adapt to a mixotrophic mode of life (Graneli and Carlsson 1998).

Species of *Dinophysis* are also known to coexist (Reguera 1995). In our study we found *D. acuminata* and *D. dens* were present together once. However, most of the time only one species was present in one sampling.

The cell count of *Dinophysis* species was low in our study. Thus, it does not pose a health risk to humans now. However, several species, which produce toxins, were recorded signifying an alarming sign. In the future, if environmental condition becomes favorable, they may increase in number and can cause toxicity. Since the sampling site is a bivalve fishery area, the risk is higher.

Conclusion

Despite no direct sewage outlet at this creek, the water was found to be severely polluted by the nutrients and trace metals, which were recorded at very high concentration. The phosphorus, which forms one of the major nutrients for eutrophication, was remarkably high at the sampling site. Several *Dinophysis* species were present in this creek intermittently, but at the count below 100 cells L^{-1}. Their presence was correlated with southwest monsoon when the salinity of this creek lowers due to fresh water influx. No other physico-chemical parameters were found to be influencing their presence. Though the *Dinophysis* cells are not present at the count where they can cause toxicity, in the near future the condition may not remain similar due to rising organic pollution of the coastal water by anthropogenic activity and industrial developments.

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