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On the recurrence of *Noctiluca scintillans* bloom in Minnie Bay, Port Blair: Impact on water quality and bioactivity of extracts

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A *Noctiluca scintillans* bloom in the coastal waters of Minnie Bay, Port Blair was studied. Physico-chemical and biological properties of bloom-infested waters were monitored during the bloom period lasting five days. The bloom appeared as a green streak along the entire coastline of Minnie Bay, with cell counts of 17×10^3 cells/l. The bloom appeared as a sudden spurt in cell number and persisted for a period of 48 h. The antibacterial properties of extracts from this algal species were also investigated. Conspicuously, the bloom inhibited the common resident phytoplankton species. Total suspended solids showed a marked increase during day-one of bloom compared to ambient levels. The bloom appeared to be limited by dissolved inorganic nitrogen species availability. The differential growth of phytoplankton reveals the involvement of specific trigger factors for such blooms. From the present viewpoint, micro-scale studies on hydrobiological factors preceding the onset of bloom would reveal what cycle of events lead to a bloom and the causal factors of such blooms. However, prediction of occurrence of such blooms and *in situ* measurements are practical difficulties to be addressed. Since a similar bloom was reported earlier in 2001, it is worthwhile to keep a watch and investigate as to whether there is any anthropogenic or environmental cause for the recurrence of the bloom.

NOCTILUCA represents a substantial fraction of bloom-forming marine dinoflagellate population of the world's oceans. This bioluminescent dinoflagellate is known to light the wakes of boats and the breaking waves on beaches. Occurrences of *Noctiluca* bloom in coastal waters of the Indian subcontinent are not uncommon phenomena. However, the causes for the occurrence of *Noctiluca* bloom in Indian coastal waters are not well understood. The increased frequency of appearance of blooms throughout the world in the recent past has led some scientists to believe that a change in the marine planktonic ecosystems on a global scale is caused by human alterations to the coastal zone. Algologists have long grappled with the fundamental question of what factors determine the differential growth

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of species in phytoplankton communities, which may result in the dominance, or bloom, of one particular species at a particular time[1].

Earlier reports on *Noctiluca* bloom in the Indian subcontinent date back to the late eighties and recent reports on phytoplankton bloom on the west coast[2–5] and east coast[1,6,7] are noteworthy. Though numerous incidences of phytoplankton blooms have been reported from the east and west coasts of India, reports on such blooms from Andaman coastal waters are limited except for the report of *Noctiluca* blooms in Port Blair Bay[8].

Our investigation attempted to answer the following questions: What types of nutrient dynamics occurs in bloom-infested ecosystems? Was there any relationship between the occurrence of dinoflagellate bloom and mortality of existing bacterial and planktonic assemblages? If so, the source, nature and bioactivity of any toxic metabolites produced by such dinoflagellate blooms is of primary importance.

Port Blair Bay extends as a narrow stretch in a north-east to southwest direction and opens to Andaman Sea on the eastern side of the South Andaman Island (Figure 1). Minnie Bay is situated in the head end of the Port Blair Bay; it is about 1.5 km^2 in area with an average depth of 3.0 m. Dense mangrove vegetation comprising *Rhizophora* sp. and *Avecenia* sp. inhabit its embankments. A sudden intense bloom of *Noctiluca scintillans* was observed on 20 December 2002 in Minnie Bay (11°38.705′N; 92°42.513′E). The bloom persisted for three days and disappeared suddenly; the event of bloom and its impact on the sea water quality was monitored continuously for a period of five days from 20 to 24 December 2002 during high and low tide period. Surface sea water samples were collected with acid-cleaned polyethylene bottles and immediately transported to the laboratory in an ice-box, where they were filtered through Whatman (0.45 μm) GF/C filters, (under vacuum pressure lower than 75 mm Hg) and frozen at – 20°C for later determination of nutrient content. The water samples were analysed for pH, salinity, dissolved oxygen, total suspended solids and nutrients (nitrite nitrogen, nitrate nitrogen, ammonia nitrogen, inorganic reactive phosphate, total phosphate, inorganic reactive silicate) and phytoplankton pigments, using SHIMADZU UV-1201V spectrophotometer and following the standard methods[9]. Phytoplankton density was estimated following net and standard sedimentation technique. Zooplankton population density was estimated by filtering a known quantity of surface sea water in 198 μm mesh. All the samples were collected in triplicate and the mean values were given.

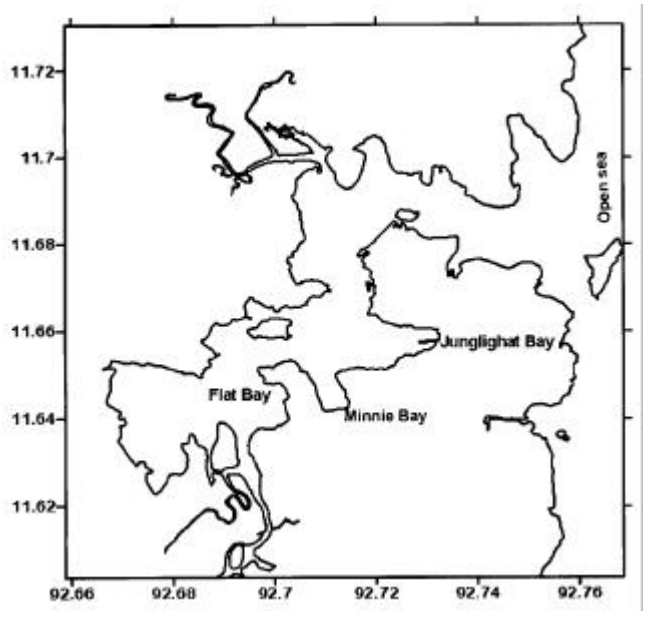

Figure 1. Map showing Port Blair Bay and the study area.

N. scintillans was collected in a clean glass beaker and concentrated in 198 μm screen. The concentrated soup was rinsed with filtered sea water and blotted on a blotting paper. Ten gram wet weight of *N. scintillans* was homogenized in glass pestle and mortar with 50 ml of the following solvents, namely ethanol, methanol, acetone and chloroform, separately. The homogenate was collected in 100 ml separating funnel and left undisturbed overnight after thorough agitation. The supernatant/extract was carefully separated and filtered through Whatman GF/C 0.45 μm filter paper and stored in a pre-weighed 50 ml beaker. The beaker was evaporated to dryness in a water bath maintained at 40–45°C. The absorption spectrum from 400 to 750 nm of the crude extract was measured on a SHIMADZU UV-1201 V spectrophotometer and absorption and wavelength values were plotted on a graph sheet.

Five species of pathogenic bacteria, *Escherichia coli*, *Vibrio cholerae* (clinical isolate), *Serratia marcescens*, *Pseudomonas aeruginosa* and *Streptococcus faecalis* were isolated from the coastal waters of Port Blair and identified according to standard methods[10]. Pure strains of these bacteria were cultured in sterile nutrient broth up to one optical density and overnight active exponential phase cultures were seeded in Muller–Hinton agar medium. Antibacterial assay was carried out by standard disc diffusion method[11]. The dried crude extract was reconstituted in 1ml of the respective solvents and impregnated to the sterilized disc in four different concentrations (20, 40, 80 and 100 mg for chloroform extract and 25, 50, 100 and 200 mg for acetone, ethanol and methanol extracts) and the discs were dried with the help of an air-drier. The dried discs were incorporated in the culture plates and incubated at 37°C. The antibacterial activity was studied based on the zone of inhibition measurement after 48 h.

Generally phytoplankton bloom occurs as patches, lanes, lines and streaks and exists as massive accumulation (10^4 to 10^6 cells/ml) of single or, less often, two coexisting phytoplankton species. In the present study, a single species of *N. scintillans* with thick soup-like consistency was observed as a broad streak about 2 m in width, throughout the entire coastal margin of Minnie Bay and adjacent area,

imparting green colouration to the coastal margins. In addition, the entire water column recorded dense homogenous population of *N. scintillans* (80 to 12,880 cells/l during low tide and 17 to 17,111 cells/l during high tide). This constituted as much as 98 to 99% of the resident phytoplankton on first and second days of bloom and was marginally at higher density than that of the earlier report from Port Blair Bay[8]. *N. scintillans* cells were circular in shape and measured 400 µm in diameter with a twisted flagellum and harboured innumerable motile green flagellates, *Pedinomonas noctilucae*, which were responsible for imparting a deep green colouration to the coastal waters.

Phytoplankton blooms have often been sudden and dramatic; however, their development and persistence are brought by multiple interactions of physical, chemical and biological parameters in proper combination. Stable temperature and muggy weather without heavy rain are considered to be favourable for *Noctiluca* blooms[1]. The present observation also corroborates this statement, as the surface sea water temperature remained fairly stable during the bloom period, except for a marginal drop in temperature during the peak bloom period. There were no marked differences in pH and salinity (Table 1), except for the slightly lower pH during the peak bloom period. The minor reduction in pH during the peak bloom period may be due to the generation of carbon dioxide by the respiratory activity of reproducing *N. scintillans*.

The concentration of total suspended solids (TSS) was substantially high during the peak bloom period compared to the normal value (unpublished report), and gradually declined towards the waning period during both low and high tides (Table 1), which may be due to the contribution of *Noctiluca* cells to TSS. Higher concentration of TSS during low tide and low concentration of TSS during high tide may be due to the dilution of the bay waters by open sea water, indicating thereby that it is of local origin.

The dissolved oxygen (DO) concentration was high during the peak bloom period and gradually declined during the waning period (Table 1). The higher concentration of DO during the peak bloom period may be due to increased photosynthetic activity, while reduction in DO concentration during disappearance of bloom could be attributed to decomposition of allochthonous organic matter, which raises oxygen consumption and increases oxygen demand of the rapidly reproducing asexual spores, nonmotile spores and homogenous of the algae and the bacteria that thrive in the putrefying algal bloom. All the data indicate that in the present study the bloom was recorded during the peak period and the observation essentially covered the peak and wane periods.

The availability of different forms of nitrogen and their relative rates of utilization are important factors contributing to the relative success of different phytoplankton. Typically, fast-growing diatoms have been found to be

Table 1. Physico-chemical parameters of surface sea water during *Noctiluca* bloom

Parameter	Tide	20 Dec. 2002	21 Dec. 2002	22 Dec. 2002	23 Dec. 2002	24 Dec. 2002
Temperature (°C)	HT	29.30	29.47	29.40	29.80	29.80
	LT	30.10	30.30	30.10	30.20	30.00
Salinity (psu)	HT	33.22	33.32	33.33	33.32	33.32
	LT	33.2	33.33	33.33	33.33	33.33
pH	HT	7.60	7.91	7.91	7.89	7.90
	LT	7.40	7.81	7.90	7.91	7.96
DO (mg/l)	HT	7.59	6.49	6.53	5.94	6.02
	LT	7.38	7.31	6.57	6.57	5.71
TSS (mg/l)	HT	38.80	38.40	38.10	30.70	26.80
	LT	74.60	69.20	63.20	36.90	24.30
Nitrite (µmol/l)	HT	0.02	0.02	0.02	0.02	0.02
	LT	0.06	0.03	0.02	0.02	0.03
Nitrate (µmol/l)	HT	0.60	0.36	0.12	0.14	0.23
	LT	1.06	0.96	0.12	0.16	0.49
Phosphate (µmol/l)	HT	0.08	0.00	0.03	0.01	0.02
	LT	0.10	0.00	0.07	0.03	0.03
Silicate (µmol/l)	HT	12.38	13.63	12.74	12.84	14.89
	LT	18.30	15.79	14.35	12.74	19.55
Ammonia (µmol/l)	HT	2.76	0.46	0.00	0.95	0.03
	LT	2.19	0.76	0.05	1.81	0.16
Total phosphorus (µmol/l)	HT	0.30	0.30	0.48	0.30	0.36
	LT	0.48	0.67	0.24	0.55	0.30

HT, Hight tide; LT, Low tide.

highly correlated with large and frequent additions of NO_3. In contrast, micro-flagellates and dinoflagellates have been correlated with high rates of NH_4 and dissolved inorganic nitrogen (DIN). It is suggested that the overall nitrogen availability may contribute disproportionately to the alteration of phytoplankton succession and to the triggering of harmful algal bloom, which was corroborated by the elevated level of nitrogen species (ammonia and nitrate) during the peak bloom period of the present study.

Reduction in nitrate and marginal increase in phosphate concentration were recorded during the waning period, as reported by earlier workers[8]. Reduction in nitrate could be due to its fast utilization by the developing *Noctiluca* and marginal increase in phosphate level may be due to sinking of decomposing plankton in sea water, resulting in oxygen consumption and phosphate liberation in addition to the phosphate liberated by bacteria from dead zooplankton[12].

Relatively high concentration of reactive silicate was recorded during the peak bloom period, which is attributed to the process of 'comminution' by *Notiluca* bloom. On other hand, increased concentration of silicate at the end of the bloom period may be the silicate released from dead and decaying *Thalssiothrix* or alternatively silicate excreted from *Noctiluca*, which is known to be a consumer of the diatoms[12].

Chlorophyll *a*, *b*, *c* and phaeophytin *a* concentration was generally higher during low tide compared to high tide, which was mainly due the higher density of *Noctiluca* during low tide compared to high tide. Chlorophyll *a*, *b*, *c*, phaeophytin *a* and carotenoid concentrations were several fold higher than that of the normal concentration during the peak bloom period, which gradually declined towards the waning period (Table 2). Chlorophyll *b* and *c* concentrations surpassed the chlorophyll *a* concentration twofold, which may be attributed to the fact that chlorophyll *b* and *c* are the principal pigments of dinophyceae. Further, it could be of bacteriochlorophyll, as *Noctiluca* is known to ingest bacteria at remarkably high rate.

N. scintillans was found to be the single dominant species, constituting 99% of the resident population during the peak bloom period, while *Thalassiothrix frauenfeldii* dominated the phytoplankton density (75%) on the third day from the peak bloom. The normal phytoplankton diversity was restored on the fifth day from the peak bloom period (Table 3).

Oithona sp., *Corycaceus* sp., copepod nauplii and cirriped nauplii were the only zooplankton recorded during *Noctiluca* bloom. Interestingly, substantial number of cirriped nauplii was recorded during the peak bloom period, which may be due to some chemical stress induced by the *Noctiluca* bloom.

Noctiluca cell density showed a negative relationship with phytoplankton and zooplankton diversity, indicating that this species exhibits a predation pressure and inhibiting effect on the phytoplankton and zooplankton. The higher chlorophyll *a* concentration observed in the present study is due to the presence of flagellates (*P. noctiluca*) associated with *N. scintillans*.

Acetone, ethanol, methanol and chloroform extracts yielded similar spectral pattern, which was represented by two major peaks at 420 and 670 nm and three minor peaks at 455, 545 and 610 nm respectively. The peaks at 610 and 670 nm represent chlorophyll *a* and *c* while those at 455 and 545 nm probably correspond to carotenoid. The spectral signature of the long wavelength major peak at 420 nm remains unclear and does not correlate to phytoplankton pigments (Figure 2).

Methanol extracts did not show any activity against the bacterial strains tested. The ethanol extract showed antibacterial activity only against *E. coli* at maximum concentration and acetone extract showed activity against *S. faecalis* at maximum concentration. The chloroform extract showed antibacterial activity against *E. coli*, *V. cholerae* and *S. faecalis* even at lower concentrations. The antibacterial efficacy assay of the *Noctiluca* extract against the bacteria tested is of a preliminary nature and demands further detailed studies.

Though *Noctiluca* blooms have already been reported, the present observation points to a recurrence, the cause of which is worth detailed investigation. Although the precise cause for the present bloom is unclear, it is suggested that localized enrichment of nutrients by terrigenous and allochthonous inputs to the coastal waters of

Table 2. Phytoplankton pigment concentration during *Noctiluca* bloom

Parameter	Tide	20 Dec. 2002	21 Dec. 2002	22 Dec. 2002	23 Dec. 2002	24 Dec. 2002
Chlorophyll *a*	HT	21.11	14.92	2.74	3.41	3.86
(mg/m^3)	LT	32.67	9.61	8.50	4.33	4.09
Chlorophyll *b*	HT	12.08	36.04	0.48	0.56	0.86
(mg/m^3)	LT	39.49	5.51	9.34	0.43	0.65
Chlorophyll *c*	HT	4.75	42.81	0.96	1.44	1.98
(mg/m^3)	LT	46.94	2.18	11.55	1.85	1.92
Phaeophytin *a*	HT	3.42	2.85	0.54	1.73	1.44
(mg/m^3)	LT	4.91	4.09	1.54	1.86	1.08
Carotenoid	HT	0.41	0.29	0.12	0.16	0.15
(mg/m^3)	LT	0.51	0.36	0.20	0.16	0.14

Table 3. Phytoplankton density (No. l) during *Noctiluca* bloom from 20 to 24 Dec. 2002

	High tide					Low tide				
Organism	20	21	22	23	24	20	21	22	23	24
Cosinodisceae										
C. eccentricus	0	0	0	0	640	0	0	0	0	961
Soleniae										
Rhizosolenia alata	0	0	0	0	256	0	0	0	0	384
R. crassispina	0	0	71	384	128	0	0	107	576	192
R.cylindrus	0	0	0	0	256	0	0	0	0	384
L. danicus	0	0	0	256	256	0	0	0	384	384
L. minimus	0	0	0	0	256	0	0	0	0	384
Chaetocereae										
Chaetoceros diversus	0	2	10	17.5	64.7	0	0	2	5	15
C. curvicetus	0	0	0	256	256	0	0	0	384	384
C. dydymus	0	0	2	5	256	0	0	0	0	384
Fragilarioideae										
Thalassiothrix frauenfeldii	0	0	2200	6400	669	0	0	1800	4880	480
T. longissima	0	0	20	128	128	0	0	107	192	192
Naviculoideae										
N. seriata	40	190	210	256	128	111	711	320	384	192
Nitzschia sigma	21	140	140	0	256	67	356	213	0	384
Gyrosigma balticum	10	24	80	0	128	100	119	320	0	192
Dinophysiales										
Dinophysis caudata	0	0	70	210	200	0	0	213	384	961
Peridiniale										
Noctiluca scintillans	12880	5600	2050	280	80	17111	14636	655	87	17
Protoperidinium deprssum	0	0	40	128	256	0	0	213	192	384
P. leonis	0	0	60	0	384	0	0	107	0	576
Ceratium extensum	0	0	0	128	256	0	0	0	192	384
Total	12951	5956	4953	8449	4854	17389	15822	4057	7662	7236

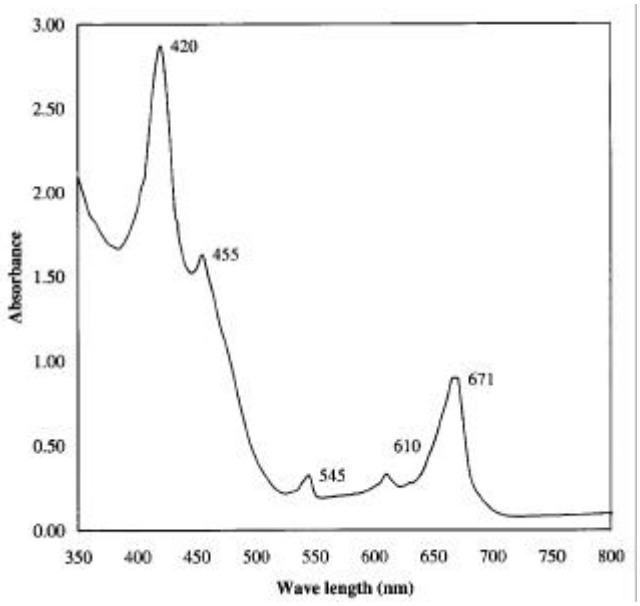

Figure 2. Absorption spectrum of *Noctiluca* crude.

Port Blair Bay may be one of the important causative factors, which was evident by a higher density of *Noctiluca* during low tide and elevated levels of nitrogen and phosphorus species during the peak bloom period. Results of the present study point to a link between quality of coastal water and the bloom. Regular monitoring of the physico-chemical characteristics of the coastal waters in Port Blair Bay on a long-term basis can be a rewarding pursuit to unravel the causative factors for triggering a *Noctiluca* bloom.

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