

Bacteriological and physico-chemical factors associated with *Noctiluca miliaris* bloom, along Mangalore, south-west coast of India

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A monospecific bloom of *Noctiluca miliaris* occurred during summer (May 1993), off Mangalore, south-west coast of India. The bloom was in the logarithmic phase of growth when it was first noticed. The bloom persisted for more than a week. Bacteriological and physico-chemical parameters were studied daily till the collapse of the bloom. No significant correlation was observed between *Noctiluca* counts and direct viable bacterial count or total plate count of bacteria. However, percentage incidence of bacteria *Moraxella* during the bloom period was significantly higher compared to non-bloom period.

Incidence of *Noctiluca* blooms and discoloration of waters are regular features in coastal waters¹⁻³. Green water phenomenon due to dense growth of *Noctiluca* along with the innumerable, green flagellates inside them was recorded in Indian waters by many workers^{1,4,5}. Venugopal *et al.*³ observed water discoloration due to dinoflagellate *N. miliaris* along south-west coast of India during summer months of 1976 and 1977. Devassy & Nair⁴, Katti *et al.*⁵ and Shetty *et al.*⁶ reported a green discoloration of water in the same region during 1987 due to the bloom of *N. miliaris*. Blooms of *Noctiluca* sp. have been correlated with different physico-chemical parameters but the bloom formation has never been attributed to any single cause.

A monospecific bloom of *Noctiluca miliaris* (Suriray) occurred during summer 1993, off Mangalore (12°4' N, 74°49' E) on the south-west coast of India extending over 60 km of the coastline. In the present study of *N. miliaris* bloom, quantitative and qualitative estimation of bacteria were made to understand the possible association of bacteria with the bloom formation or its collapse. Physico-chemical parameters were also analysed to compare the influences of biotic and abiotic factors.

Materials and Methods

Hydrological observations were made in Gurpur estuary, one of the estuaries located 10 km north of Mangalore at the lower end before it opens into the sea. Surface water samples were collected during high

tide to get true representative sample of seawater. The occurrence of bloom being in summer months, the freshwater influx was not expected to affect the observations drastically. During high tide the depth of the sampling area remains between 5-7 m. However, only surface water samples were used for the purpose.

Water samples were collected aseptically in sterile bottles for bacteriological analysis. When plankton counts were high, water without filtration was used for plankton count. When the counts came down, plankton net was used for collection of sample. Water temperature was recorded and pH was measured. Dissolved oxygen (DO), biochemical oxygen demand (BOD), nitrite nitrogen (NO₂-N), nitrate nitrogen (NO₃-N), ammonia nitrogen (NH₃-N) and phosphate phosphorus (PO₄-P) were estimated following the methods of Strickland & Parsons⁷.

The sizes of *N. miliaris* and the endosymbiont *Pedinomonas noctilucae* were measured using micrometer. A tentative count of the endosymbiont was done by diluting *N. miliaris* number to single cell using a microtitre plate, suspending the cell in a fixed volume of seawater, rupturing the cell and counting the number of the endosymbiont.

The direct viable count (DVC) of bacteria was taken to be the total bacterial number, which include the culturable and non-culturable portion of bacterial population. The epifluorescent microscopic technique as described by Kogure *et al.*⁸ was used for obtaining DVC. After enriching the water sample with yeast extract (0.025%) and nalidixic acid (0.002%) in the dark for 6 h, the samples were stained with acridine orange and observed under fluorescent microscope (Leitz Wetzlar, Germany). Total culturable bacterial

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count was done by spread plate technique with luminescent agar (LA) media and expressed as total plate count (TPC). About 100 colonies were picked-up everytime from one particular dilution for identification. Bacterial identification was made-up to generic level following the criterion laid down in Bergey's manual⁹. All media and reagents were prepared according to the procedure suggested in the *Compendium*¹⁰.

Results and Discussion

A monospecific bloom of *Noctiluca miliaris* was observed during May 1993, with almost complete exclusion of other phytoplankton and zooplankton. The blooms of *N. miliaris*, however, have previously been observed in August 1984¹¹ and between January to May 1987⁶. The *N. miliaris* count in the present bloom ($1.56 \times 10^6/\text{m}^3$) was found to be low compared to the counts reported by Prasad² ($3.3 \times 10^9/\text{m}^3$) and Shetty *et al.*⁶ ($8.2 \times 10^9/\text{m}^3$). On the other hand, Venugopal *et al.*³ observed a count of 3.01×10^5 - 7.7×10^5 cells/ m^3 during summer 1976 - 77 on the south-west coast of India. Table 1 presents the daily counts of *N. miliaris* from the day it was observed till the disappearance of the bloom. The size of *N. miliaris* varied between 473 μm and 519 μm (Table 2). It is interesting to note that the average size of *N. miliaris* increased as the bloom progressed. The size of the endosymbiont *Pedinomonas noctilucae* was recorded to be 476 - 595 μm . The endosymbiont was present both inside and outside the cell. *Pedinomonas noctilucae* count was found to be between $1.2 \times 10^{12}/\text{m}^3$ and $9.2 \times 10^{12}/\text{m}^3$. A single cell of *N. miliaris* was found to contain about 5×10^4 *P. noctilucae* cells.

Total bacterial counts are presented in Table 1. The DVC ranged from 10^4 to $10^6/\text{ml}$ while TPC was in the range 10^2 - $10^4/\text{ml}$. No definite relation was seen between the rise and fall in *N. miliaris* count and changes in bacterial counts. Neither the TPC nor DVC showed any synchronous fluctuation with the *N. miliaris* counts.

Percentage contributions by different bacterial genera are presented in Table 3. A month before the bloom (27 April 1993), the bacterial flora was diverse with dominance of *Vibrio* and *Moraxella*. During the log phase of the bloom, *Bacillus* was the most dominant species followed by *Moraxella* and *Vibrio*. However, the *Bacillus* declined during later part of bloom and were not detectable during the collapse of

Table 1—Bacterial and *N. miliaris* count before and during the bloom

Date of sampling	DVC (no/ml)	TPC (CFU/ml)	<i>Noctiluca</i> count (No/ m^3)
<i>Before the bloom</i>			
27.4.93	2.200×10^6	5.5×10^2	Nil
<i>During the Bloom</i>			
24.5.93	1.005×10^6	5.80×10^4	8.60×10^5
25.5.93	5.500×10^5	3.33×10^2	1.56×10^6
26.5.93	4.250×10^4	2.75×10^3	1.00×10^6
27.5.93	5.400×10^5	5.50×10^3	1.40×10^6
28.5.93	2.030×10^6	1.50×10^3	6.00×10^5
29.5.93	3.720×10^6	3.65×10^3	5.00×10^5
30.5.93	5.950×10^5	2.58×10^3	1.00×10^5
31.5.93	8.180×10^5	6.50×10^3	Nil

CFU: Colony forming unit; DVC: Direct viable count; TPC: Total plate count

Table 2—Daily record of average size of *Noctiluca* during the bloom

Date	Size
24.5.93	473.35 μm
25.5.93	471.53 μm
26.5.93	495.20 μm
27.5.93	509.15 μm
28.5.93	517.90 μm
29.5.93	519.60 μm
30.5.93	517.69 μm
31.5.93	—

the bloom. *Moraxella*, however, was the most dominant genus contributing between 33 to 55% of the total flora throughout the period. *Vibrio* was another important group found throughout the bloom period. However, luminescent vibrios were detected only during the pre-bloom period and during the early part of the bloom. *Moraxella*-like bacteria increased in significance during later part of the bloom. Regular monitoring studies have shown that the *Moraxella*, *Vibrio* and *Moraxella*-like bacteria were present in the coastal water throughout the year ranging between 0 - 28.57%, 2.15 - 28.82% and 0 - 13.66% respectively. Increase of *Moraxella* up to 33 - 55% during *N. miliaris* bloom is suggestive of a close association between the two. Students' *t* test was used to see if there is any significant difference in the incidence of these bacteria during the bloom and the pre-bloom period. Significant difference ($P = 0.05$) was observed in the percentage incidence of *Moraxella*. Algal blooms may influence bacterial counts and profile.

concept of phycosphere was proposed by Bell & Mitchel¹⁴ to denote the zone surrounding phytoplankton created by their production of extracellular products, which would be an aquatic equivalent of rhizosphere of plants. They demonstrated chemotactic attraction of bacteria to filtrates of old algal cultures. Phycosphere can be inhibitory to some bacteria and stimulatory to others. Bell *et al.*¹⁵ showed that *Pseudomonas* grew well with an actively growing *Skeletonema costatum* culture, whereas *Spirillum* sp. was inhibited by the algae.

Bacterial species	Percentage composition					
	April 24	May 24	May 25	May 29	May 30	May 31
<i>Bacillus</i>	—	37.29	3.92	4.55	—	—
<i>Stapylococcus</i>	3.39	—	—	—	—	—
<i>Arthrobacter</i>	15.26	—	1.96	—	16.67	—
<i>Moraxella</i>	18.65	35.59	37.26	54.54	33.33	33.34
<i>Moraxella</i> -like	5.08	—	5.89	13.63	8.33	26.19
<i>Acinetobacter</i>	5.08	—	—	—	—	2.38
<i>Vibrio</i>	28.82	15.26	35.29	9.09	33.33	26.19
<i>Aeromonas</i>	—	—	11.76	9.09	—	2.38
<i>Plesiomonas</i>	1.69	—	—	4.55	4.17	4.76
<i>Alkaligens</i>	1.69	—	—	—	—	2.38
Luminescent vibrio	15.26	11.86	—	—	—	—
<i>Mycoplasma</i>	—	—	1.96	—	—	—
<i>Actinomycetes</i>	3.39	—	—	—	—	—
Unidentified	1.69	—	1.96	4.55	4.17	2.38
Total	100.00	100.00	100.00	100.00	100.00	100.00

Date	Noctiluca count(m ³) (Log No)	Water temperature (°C)	pH	DO	BOD	PO ₄ -P (µg at-P/l)	NO ₂ -N (µg at-N/l)	NO ₃ -N (µg at-N/l)	NH ₃ -N (µg at-N/l)
<i>Before the bloom</i>									
27-4-93	Nil	32	7.90	8.4	1.2	0.54	0.886	0.358	17.60
<i>After the bloom</i>									
24-5-93	2.934	32	8.20	9.6	1.6	0.68	0.609	-	29.35
25-5-93	3.193	32	8.65	5.2	2.4	0.65	0.895	0.15	8.80
26-5-93	3.000	32	8.38	4.4	2.0	2.05	0.895	0.15	23.48
27-5-93	3.146	32	8.20	6.0	2.0	0.55	0.663	0.42	26.42
28-5-93	2.778	32	8.20	4.0	0.8	1.77	1.039	0.33	23.48
29-5-93	2.699	32	8.39	6.4	1.2	1.47	1.093	0.27	44.03
30-5-93	2.000	32	8.23	6.4	1.2	1.53	0.788	BDL	82.19
31-5-93	Nil	33	8.40	6.4	1.2	—	0.609	0.15	23.48
02-6-93	Nil	33	8.23	6.4	1.2	—	—	—	—
— Not determined									
BDL: Below detection limit									

Kogure *et al.*¹⁶ found that *Skeletonema costatum* generally inhibited the growth of a number of *Pseudomonas* sp. and *Vibrio* sp. isolates, whereas the growth of *Flavobacterium* sp. was generally stimulated. In this study, no such inhibition of any specific group was observed but higher percentage incidence of *Moraxella* during the bloom suggests close association between *Moraxella* and *N. miliaris*.

The physico-chemical characters analysed during the bloom are presented in Table 4. Day temperature was constant throughout the bloom period except for the last couple of days when the bloom was collapsing. There was a rise of 1°C coinciding with a nil count of *N. miliaris* in the water. Like temperature, the pH also remained more or less constant. It ranged between 8.20 and 8.65 during the bloom. Both dissolved oxygen and biochemical oxygen demand (BOD) varied widely during the bloom. Neither the DO nor the BOD showed a high significant correlation with *N. miliaris* count. High phosphate (2.4 µg at-P/l), high nitrate (9.4 µg at-N/l) and low nitrite (0.3 µg at-N/l) levels were observed during bloom of *N. miliaris* by Devassy & Nair⁴ who opined that the occurrence of bloom may be due to high phosphate and nitrate. In the present study, the phosphate levels were between 0.55 and 2.05 µg at-P/l, nitrite levels were between 0.609 and 1.093 µg at-N/l and nitrate level varied from undetectable level to 0.4 µg at-N/l. A very high ammonia value was obtained during the last phase or decline of the bloom, which was about 82 µg at-N/l. The high ammonia content may be responsible for the setback to fisheries observed during the bloom. Bhimchar & George¹⁷ reported that abrupt setback to fisheries in Malabar coast in the west coast of India, was due to abundance of *N. miliaris*. Prasad & Jayaraman¹⁸ also concluded that shoaling fish avoid *Noctiluca* bloom. In the present study also, it was observed that fish catch was very low during the bloom period.

"Students' *t*" test was performed to compare the levels of PO₄-P, NO₂-N, NO₃-N, and NH₃-N during bloom period and non-bloom period in the same location. Significant differences were observed in the case of NH₃-N and NO₂-N. This is understandable since ammonia would be produced by bacterial decomposition of dead algal cells and ammonia would be oxidised by bacteria to nitrite and nitrate. However lack of significant difference in the case of nitrate could be due to rapid uptake of nitrate for the growth of *N. miliaris* during the bloom period.

The present study, clearly indicates that the qualitative changes in bacterial flora occur during *N. miliaris* bloom with an increased proportion of *Moraxella*. It also indicates that there is a chance in the increase in ammonia levels during the bloom of *N. miliaris*, which could affect the fish catch in the region.

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