

## Occurrence of red tide caused by *Karenia mikimotoi* (toxic dinoflagellate) in the Southwest coast of India

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Intense brownish discoloration was observed in the surface waters of Cochin barmouth on 21<sup>st</sup> October 2009, which found to extend a few kilometers in the coastal waters. Continuous monitoring was carried out in the region for a period of 5 days (21<sup>st</sup> to 25<sup>th</sup> October 2009) and generated data on changes in water quality and phytoplankton community associated with the bloom. Relatively high level of inorganic nutrients (ammonia 0.5-9.7  $\mu\text{M}$ , nitrate 1.3-2.9  $\mu\text{M}$ , phosphate 1-2.3  $\mu\text{M}$ , silicate 23.5-39.9  $\mu\text{M}$ ) and chlorophyll *a* (av.  $56.8 \pm 23.7 \text{ mg m}^{-3}$ ) concentration were observed during the bloom period. Microscopic analysis revealed that the discoloration was caused by an unarmored toxic dinoflagellate, *Karenia mikimotoi* Miyake & Kominami ex Oda (*Karenia*, Gymnodiniaceae) belongs to Gymnodiniales, which cause massive fish mortality by releasing hemolytic compounds.

**[Keywords:** - Dinoflagellate bloom; *Karenia mikimotoi*, Cochin estuary]

### Introduction

Incidences of red tides are common, especially in the coastal waters that threaten marine living resources and local economy<sup>1</sup>. At times, red tides are dominated by harmful algal bloom (HAB) species that adversely affect aquatic ecosystem by releasing toxic substances. So far, several phytoplankton blooms were frequently reported from the west coast of India<sup>2</sup>. Among them, blooms of *Noctiluca miliaris*<sup>3</sup> (dinoflagellate) and *Trichodesmium erythraeum*<sup>4-5</sup> (blue green algae) are frequently occurring red tides. Even though these two blooms are recurrent and sometimes cause fish kill due to the oxygen depletion, both of them are not accounted to be toxic yet. Few years back (September-October 2004) an unusual stench event observed in the coastal waters off Trivandrum and Kollam had created some health problems (nausea, chest pain and breathlessness) to the local people in the region<sup>6</sup>.

### Materials and Methods

National Institute of Oceanography, Regional Centre, Kochi, during its regular monitoring in the Cochin estuary and adjoining neritic waters, observed a brownish discoloration in the surface waters of

Cochin inlet (Figure 1) on 21<sup>st</sup> October 2009 and it was found to extend towards the coastal waters up to a few kilometers. The region was monitored continuously for a period of 5 days from 21<sup>st</sup> to 25<sup>th</sup> October 2009. Water samples from the inlet were collected in a cleaned polythene carbuoys (10 L capacity) between 10 to 11 am on everyday for assessing the physico-chemical changes during the bloom event. Water temperature, salinity, pH were estimated immediately and dissolved inorganic nutrients (Ammonia-NH<sub>4</sub><sup>+</sup>, Nitrite-NO<sub>2</sub><sup>-</sup>, Nitrate-NO<sub>3</sub><sup>-</sup>, Phosphate - PO<sub>4</sub><sup>3-</sup> and Silicate - SiO<sub>4</sub><sup>4-</sup>) were estimated according to the standard protocols<sup>7</sup>. Chlorophyll *a* was determined by fluorometrically (Turner designs, Model 7200) using 90% acetone<sup>8</sup>. Phytoplankton (>5  $\mu\text{m}$ ) identification and counting was carried out using a binocular inverted microscope (Olympus) following the standard protocols<sup>9,10</sup>.

### Results and Discussion

From 21<sup>st</sup> to 25<sup>th</sup> October, the atmosphere over the region was sunny with weak winds (<3  $\text{ms}^{-1}$ ). Water temperature was >30°C throughout the 5 days during the time of collection (Table 1). Salinity and pH did not show much variation, with an averages of 26.5 ( $\pm 2.9$ ) and 7.4 ( $\pm 0.6$ ), respectively. Concentration of SiO<sub>4</sub><sup>4-</sup> was high (av.  $32.3 \pm 6.4 \mu\text{M}$ ) as compared to NH<sub>4</sub><sup>+</sup> (av.  $6 \pm 3.6 \mu\text{M}$ ), NO<sub>3</sub><sup>-</sup> (av.  $2 \pm 0.6 \mu\text{M}$ ) and

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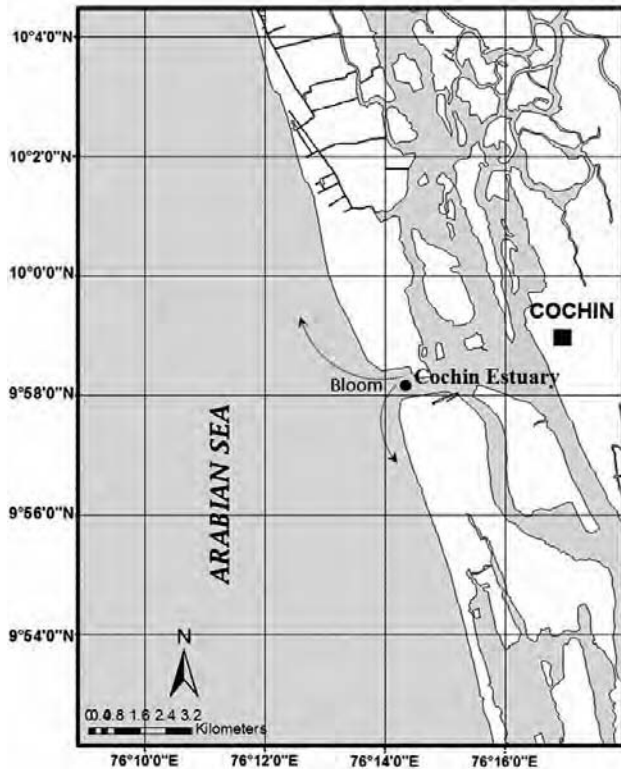


Fig. 1—Station location (black circle shows the sampling station; black arrows indicates the bloom affected area)

Table 1—Major environmental and water quality parameters

Parameters	Days (October 2009)				
	21 <sup>st</sup>	22 <sup>nd</sup>	23 <sup>rd</sup>	24 <sup>th</sup>	25 <sup>th</sup>
Temperature (°C)	30.5	31.2	30.9	31.7	30.7
pH	8.1	7.6	6.5	7.8	7.2
Salinity	26.8	24.9	22.5	29.8	28.6
Nitrite (µM)	0.3	0.1	0.1	0.2	0.2
Nitrate (µM)	2.9	1.9	1.7	1.3	2.1
Ammonia (µM)	5.5	9.7	8.9	0.5	1.2
Phosphate (µM)	2.3	1.2	1.0	1.8	1.0
Silicate (µM)	29.3	23.5	39.9	36.7	32.1
N/P	3.7	9.8	10.9	1.1	3.7
N/S	0.3	0.5	0.3	0.1	0.1
S/P	12.5	19.6	40.7	20.3	33.8
Chlorophyll <i>a</i> (mg m <sup>-3</sup> )	46.3	56.4	71.5	85.8	24.0

PO<sub>4</sub><sup>3-</sup> (av. 1.5 ± 0.6 µM). Ammonia was the major component (25.6 - 83.4%) of dissolved inorganic nitrogen (DIN) and varied from 0.5 to 9.7 µM. For the initial 3 days, concentration of NH<sub>4</sub><sup>+</sup> was high (>8 µM), which dropped to <1.5 µM on 4<sup>th</sup> and 5<sup>th</sup> day, whereas the NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> remained consistently low (<3 and <0.3 µM, respectively). Fluctuations in the concentration of NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup>,

lead to inconsistent nutrient-ratios in the bloom water, as evident from the N/P (av. 5.8 ± 4.3) and S/P (av. 25.4 ± 11.5) ratios (Table 1). Very high chlorophyll *a* concentration (av. 56.8 ± 23.7 mgm<sup>-3</sup>) was observed in the bloom waters with a maximum of 85.8 mgm<sup>-3</sup> on 24<sup>th</sup> October. Throughout the observation, the phytoplankton density varied from 336.1 × 10<sup>4</sup> cells L<sup>-1</sup> to 1568.5 × 10<sup>4</sup> cells L<sup>-1</sup>, with a maximum density on 21<sup>st</sup> October (Table 2). Among these, dinoflagellates were numerically abundant group (72.2-99.2%) followed by diatoms (0.8-27.8%). Microscopic analysis revealed that the discoloration was caused by an unarmored dinoflagellate, *Karenia mikimotoi* Miyake & Kominami ex Oda 1935 (*Karenia*, Gymnodiniaceae, Gymnodinales), mostly occurring in the temperate coastal environment<sup>11</sup> and formerly known as *Gymnodinium mikimotoi*. Cells appeared oval in shape and dorsoventrally compressed with 20-30 µm long and 18-25 µm wide (Plate 2a & b). Epitheca seemed to be broadly rounded and smaller than the notched, slightly bilobed hypotheca. Nucleus was not clearly visible as it was surrounded by many shapeless yellowish-brown colored chloroplasts. Other morphological characteristic of this species matched completely with the description of Tomas<sup>10</sup>. Observation of live sample under the microscope showed that the navigation of cells similar to falling a leaf, turning over and over through waters as mentioned by Takayama and Adachi<sup>12</sup>.

The density of *K.mikimotoi* in the bloom period ranged from 701.3 × 10<sup>4</sup> cells L<sup>-1</sup> to 1550 × 10<sup>4</sup> cells L<sup>-1</sup> which constituted 70.4 to 98.8% of the phytoplankton assemblage (Table 2). Maximum abundance of *K. mikimotoi* was encountered on 21<sup>st</sup> whereas on 25<sup>th</sup> October, the density of *K. mikimotoi* reduced to 0.4 × 10<sup>4</sup> cells L<sup>-1</sup>. The bloom also contained other dinoflagellates (*Prorocentrum micans*, *P. gracile*, *Dinophysis acuta*, *Ceratium furca*) and diatoms (*Skeletonema costatum*, *Nitzschia longissima*, *N.sigma*, *Coscinodiscus radiatus*, *Pleurosigma normanii*) in moderate levels (Table 2). Abundance of *Skeletonema costatum* (11.75 × 10<sup>4</sup> cells L<sup>-1</sup> to 326.5 × 10<sup>4</sup> cells L<sup>-1</sup>) was associated with *K. mikimotoi* bloom throughout the observation and which peaked on 25<sup>th</sup> October.

*K. mikimotoi* is an autotrophic red-tide dinoflagellate, has been linked to massive fish kills including fin fishes as well as benthic fauna of the

Table 2—Phytoplankton composition and abundance

Diatoms	Abundance (No. $\times 10^4$ cells L <sup>-1</sup> )				
	21 <sup>st</sup>	22 <sup>nd</sup>	23 <sup>rd</sup>	24 <sup>th</sup>	25 <sup>th</sup>
<i>Thalassiosira subtilis</i>	0	0	1.95	0.4	0
<i>Skeletonema costatum</i>	11.75	26.45	267.45	323.2	326.5
<i>Coscinodiscus radiatus</i>	0.25	0.15	0	0	0.2
<i>Chaetoceros decipiens</i>	0	0	0.3	0	0
<i>Eucampia zodiacus</i>	0	0	0	0	0
<i>Rhizosolenia setigera</i>	0	0.02	0.3	0	0
<i>Asterionella japonica</i>	0	0.05	0	0	0.2
<i>Thalassionema nitzschioides</i>	0	0	0	0.2	0
<i>Thalassiothrix longissima</i>	0	0.03	0.15	0.1	0.1
<i>Thalassiothrix frauenfeldii</i>	0	0	0	0.2	0
<i>Pleurosigma directum</i>	0	0	0.9	0	0
<i>Pleurosigma normanii</i>	0	0	0.3	0.2	0
<i>Pleurosigma</i> sp.	0	0.04	0.15	0	0
<i>Grammatophora</i> sp.	0	0	0.9	0	0
<i>Haslea</i> sp.	0.25	0.12	0	0.2	0.3
<i>Navicula directa</i>	0	0	0.15	0.8	0
<i>Nitzschia</i> sp.	0	0.001	0.75	0	0.1
<i>Nitzschia longissima</i>	0.25	0	1.8	0.2	0.1
<i>Nitzschia seriata</i>	0	0.04	0.6	0	0
<i>Nitzschia sigma</i>	0.25	0	0.3	0	0
<i>Cylindrotheca closterium</i>	0	0	0.75	0	0
Dinoflagellates					
<i>Karenia mikimotoi</i> *	1550	1200	701.25	960	0.4
<i>Prorocentrum micans</i>	0.25	0.02	2.4	3.2	1.2
<i>Prorocentrum gracile</i>	0.5	0.5	11.25	6.0	5.1
<i>Prorocentrum minimum</i>	0	0	0.15	0	0
<i>Prorocentrum triestinum</i>	0	0	0.15	0	0
<i>Dinophysis acuta</i>	3	0.2	2.0	0.4	1
<i>Dinophysis dens</i>	0	0	0.15	0	0
<i>Ceratium furca</i>	0.75	0	0.45	0.2	0
<i>Noctiluca</i> sp.	0	0.04	0.3	0	0
<i>Gymnodinium</i> sp.	1.00	0	0	0	0
<i>Protoperidinium</i> sp.	0	0	0.3	0.4	0
<i>Protoperidinium divergens</i>	0	0	0.15	0	0
<i>Protoperidinium steinii</i>	0	0	0.15	0	0
<i>Phalacrochroma rotundatum</i>	0	0	0.15	0	0
Others	0.25	0.3	0.7	0.4	0.9
Total	1568.5	1227.97	996.3	1296.1	336.1

\*Bloom species

temperate coastal waters worldwide<sup>11,13</sup>. This bloom was first found in Irish waters, occurring regularly and leads to major kills of marine organisms including fish, shellfish, brittle stars and farmed salmon and cod<sup>14</sup>. In China, bloom of *K. mikimotoi* causes massive fish kills in 1998, resulting economic losses estimated at US\$ 40 million<sup>15</sup>. Toxic blooms of *K. mikimotoi* have become increasingly frequent worldwide and this bloom can produce toxic glycolipids along with numerous other unidentified compounds<sup>13</sup>. Even though *K. mikimotoi* is Ichthiotoxic and hemolysins have been identified as

exotoxins, the exact mechanism of toxic effect to marine organisms remains unclear<sup>11</sup>.

In the recent years, sporadic events of brownish discoloration and associated fish kills have frequently been reported from the Cochin inlets and coastal waters<sup>4,5</sup>. Nevertheless, there was no fish kills visible from the area during the occurrence of present bloom, probably because the bloom affected waters might be diluted with river water and shifted to the adjacent coastal waters by tidal effect. Even though many reports are available on red tides, the information on the incidence of *K. mikimotoi* bloom in the Indian

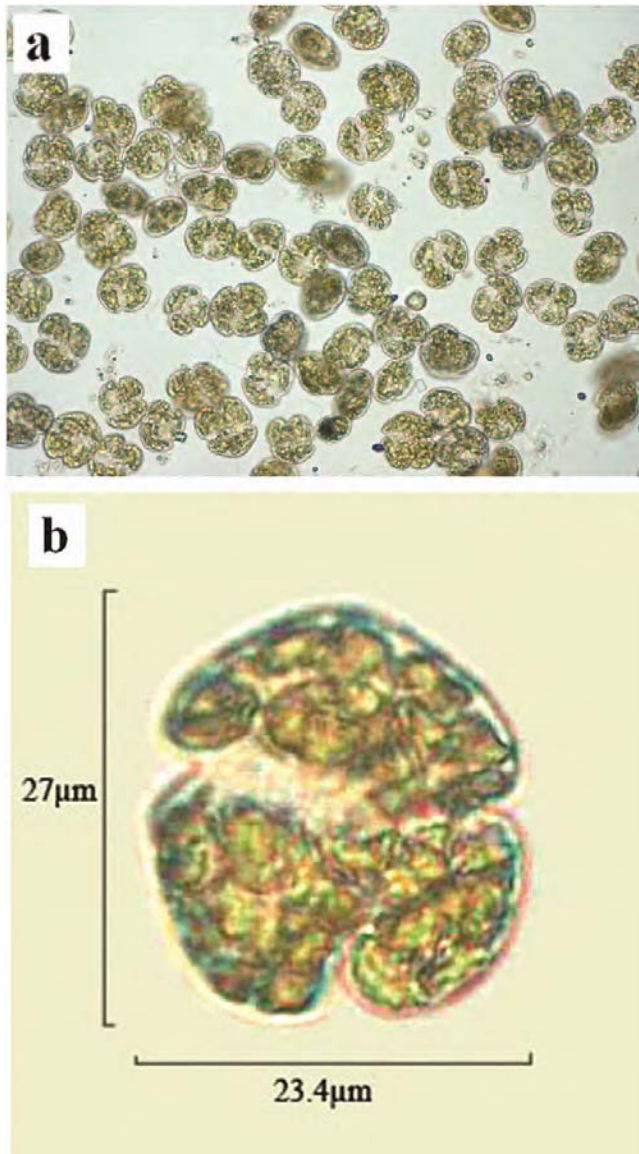


Plate 2a & b—Microscopic view of *Karenia mikimotoi* under inverted stereoscope binocular microscope (OLYMPUS) at magnification of a) 100 × and b) 400 ×; (b)—Enlarged view (400× magnification) of *Karenia mikimotoi* under inverted stereoscope binocular microscope (OLYMPUS)

water is extremely little. The only available report was by Karunasagar and Karunasagar<sup>16</sup> in which they have reported the bloom of *Gymnodinium nagasakiense* (old synonym) and associated fish kills in the coastal waters off Someshwar, ~7 km south of Mangalore port. Besides this, no other information was available on *K. mikimotoi* bloom from Indian waters except the reports of low abundance in the waters around Mangalore port<sup>17,18</sup>. Being mostly a temperate red tide species, the presence and blooming

of *K. mikimotoi* in Indian waters proves the theory of aggravation of harmful algal bloom (HAB) events by ballast waters and ocean currents<sup>1</sup>. Since the Cochin and Mangalore ports are the busiest ports in the west coast of India, the amount of disposal of ballast water is bound to increase, which can introduce alien micro- as well as macro-organisms and disrupt the native food chain. Reports on HABs worldwide have been proved that ballast water is a significant transport vector for global dispersal of toxic microalgae<sup>19</sup>. Therefore, appearance of *K. mikimotoi* in the Cochin barmouth may be an indication of a shift in the phytoplankton community due to increased anthropogenic activity in the Indian port, which needs to be investigated in detail.

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