their effects on migration and entrapment of hydrocarbons within the syn-rift plays are critical. Detailed studies of the same are presently underway.

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Proboscia alata (Brightwell) Sandström bloom in the coastal waters off Bekal, southwest India

The habitual increase in the phenomenon of harmful algal blooms (HABs) in the coastal waters and its consequences on the economy, impact on the environment and human health are particularly being noticed worldwide[1]. The seasonal reversal of monsoon, upwelling and eutrophication make the west coast of India highly productive, leaving the Malabar coast highly vulnerable to HABs[2].

Since 2006, as part of the HAB monitoring programme of the Ministry of Earth Sciences, Government of India, regular monitoring has been carried out in the coastal and estuarine waters along the Kerala coast. A bloom of *Proboscia alata* (Brightwell) Sandström was observed (Figure 1) from 10 to 12 October 2009 in the coastal sea off Bekal (12°38.02′N, 75°04.31′E) with pale brown discolouration of water. The information was provided by the local inhabitants/fishermen of the area, which has been substantiated with satellite imagery of the MODIS 'AQUA' (courtesy: INCOIS, Hyderabad). For this, MODIS AQUA level-2 ocean colour imageries (1 km × 1 km resolution) during the period 10–20 October 2009 were downloaded from OceanColor website (http://oceancolor.gsfc.nasa.gov/cgi/browse.pl?sen=am). The images were further processed and analysed using SeaWiFS data analysis system (SeaDAS), ver. 5.4. No classification of the image was done. The exercise was just to find out the spatial extent of the high chlorophyll area in the imagery. The present correspondence discusses the dynamics of the bloom along with the probable role of bacteria associated with the event.

Proboscia is a cosmopolitan centric diatom (order: Biddulphiales) species[3–6]. Diatom–diazotrophic cyanobacterial association and its episodic, monospecific bloom formation, particularly with the diatom genus *Proboscia* is quite frequent. This association thereby contributes to high rate of carbon and nitrogen fixation in the marine ecosystem. Even though *Proboscia* is a dominant genus in highly productive areas, its occurrence as a bloom along the Indian coast is quite uncommon[7].

During the present bloom event, 50 litres of discoloured surface water was filtered through 20 µm bolting silk and transferred into 250 ml clean polyethylene bottles, preserved in 1–3% neutralized formalin and Lugol's iodine solution. For a comparative analysis, sampling was also done from two reference stations, off Thykadapuram (St. 1; 12°22.84′N, 75°10.94′E) and off Puthur (St. 2; 12°55.18′N, 74°95.18′E), one before and one after the bloom station in the same latitude. Quantitative analysis of phytoplankton was done using a Sedgwick–Rafter counting cell and identification of the microalgae was done using standard taxonomic keys[3–6,8–10].

In situ measurements of hydrographic variables like temperature, salinity and pH were done using standard instruments. Inorganic nutrients like nitrate, nitrite, silicate and phosphate, and pigments were also analysed[11] with Hitachi U-3900 spectrophotometer. Dissolved oxygen and primary productivity were measured by Winkler's method[12]. Leica DM 2000 phase contrast microscope with DFC 295 attached digital camera was used for taking photomicrographs. The environmental scanning electron microscopic (ESEM) images were taken using Carl Zeiss EVO-18. In order to understand the probable role of culturable bacteria associated in the bloom dynamics, their isolation, characterization and screening for hydrolytic enzyme production were carried out.

On 10 October 2009, a pale brown discolouration of the surface water, seemingly a monospecific bloom, which extended around 3 nautical miles along the coastal area, was observed. The cell abundance

of *P. alata* was 8×10^4 cells l^{-1} and chlorophyll *a* concentration was 10.80 µg l^{-1}. No foam production and fish mortality were noticed. No salient differences between chemical and biological variables were observed between the first and the second day of observation. However, on the third day (12 October 2009), the surface water discolouration nearly faded. Though *P. alata* was recorded as the dominant flora with a cell abundance of 2.8×10^3 cells l^{-1}, a number of other diatom and dinoflagellate species were also enumerated from the sample on the third day, which apparently indicated that the bloom was in a stage of decline. The constituent species comprised of *Chaetoceros decipiens* Cleve (115 cells l^{-1}), *Coscinodiscus asteromphalus* Ehrenberg (79 cells l^{-1}), *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin (29 cells l^{-1}), *Ceratium fusus* (Ehrenberg) Dujardin (119 cells l^{-1}), *Ceratium trichoceros* (Ehrenberg) Kofoid (72 cells l^{-1}) and *Pyrophacus steinii* (Schiller) Wall & Dale (360 cells l^{-1}). The chlorophyll *a* value (6.48 µg l^{-1}) was also reduced on the third day.

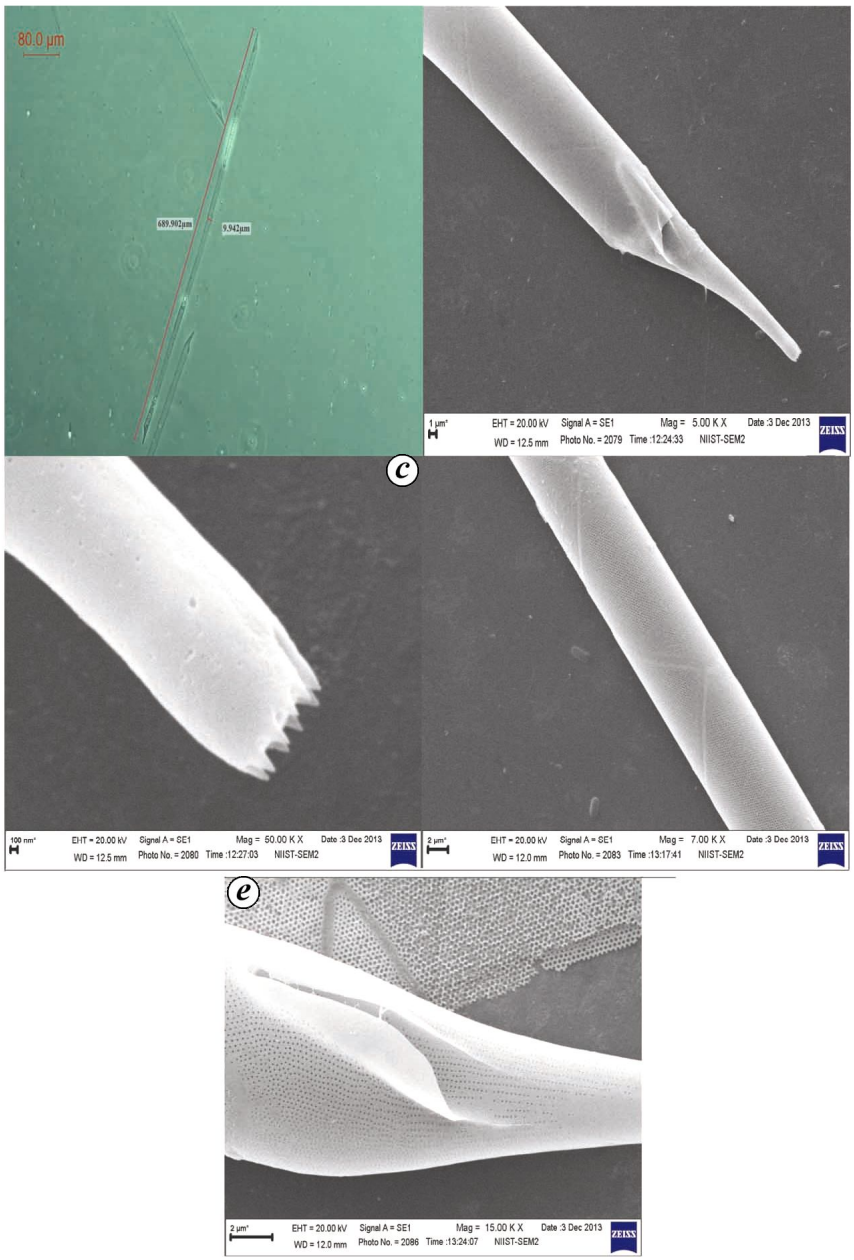

Figure 1. *Proboscia alata*. ***a***, Complete cell (phase contrast microscopy). ***b***, Apical part of valve (ESEM). ***c***, Proboscis structure, varied spinules (ESEM). ***d***, Girdle segments (ESEM). ***e***, Details of clasper and contiguous area (ESEM).

Taxonomic description of *P. alata*[3–6,8–10,13–18]

Proboscia alata (Brightwell) Sundström 1986

Brightwell, 1858, p. 95, Pl. 5, Fig. 8; Hustedt, 1920, Pl. 317; Cupp, 1943, p. 90, Fig. 52A & B; Subrahmayan, 1946, p. 121, Figs 141, 145 & 146; Okuno, 1960, p. 310, Pl. 1, Fig. 1; Hendey, 1964, p. 146, Pl. 2, Fig. 2; Navarro, 1981, p. 430, Figs 33 & 34 (From Brightwell 1858 to Navarro 1981, *P. alata* was treated as *R. alata*); Sundström, 1986, p. 99, Figs 258–266; Desikachary *et al.*, 1987, p. 6, Pl. 386, Fig. 2 ; Round *et al.*, 1990, p. 320, Figs a–j; Takahashi *et al.*, 1994, p. 413, Figs 2–7; Hasle and Syvertsen, 1997, p. 159, Pl. 30; Yun and Lee, 2011, p. 300–302, Figs 1A–H.

Synonyms. *Rhizosolenia alata* Brightwell 1858, *Rhizosolenia alata f. gracillima*[19] (Cleve) Gran 1905.

Cells are solitary, narrow cylindrical, bilaterally symmetrical, 3.3–13.3 µm in diameter, and up to 1 mm length (4.0–9.9 µm in diameter and 207.6–689.9 µm in length, in the present specimen). Valve is sub-conoidal, the ventral part longer than the dorsal part and 'proboscis' structure is slightly curved, tapering towards the apical part of the valve, 15.0–30.0 µm long. Apical surface of the proboscis is composed of variously sized spinules. Number of spinules is 7–16 (9 in the present specimen), 0.1–0.4 µm long. The valve areolae are rounded, 52–90 in 10 µm (58–62 in the present specimen) arranged in longitudinal striae, converging towards the apex. Girdle segment areolae are loculate, arranged in columns, with the external velum perforated by central pores, and internal circular foramina. The specimen has been deposited at Agharkar Research Institute, Pune, India (accession no: AHMA-DS03).

Distribution. Argentina, Jeju Island and the Korea Strait, Chennai coast, Arabian Sea, Antarctic waters.

Several factors influence algal blooming. The withdrawal of monsoon establishes better conditions for bloom formation, particularly on the west coast of India.

The post-monsoon season offers bright sunlight, nutrient input due to riverine discharge and upwelling, which support the proliferation and blooming of phytoplankton species[20], as opposed to the inefficient nutrient utilization during the monsoons due to low irradiance, as it is an energy-demanding process[21].

Diatoms have an absolute requirement for silicon[22]. In the present study also high silicate concentration was observed on the first day and there was a gradual decrease over the succeeding days. Such a variation was not observed in the reference stations (Table 1). As *P. alata* is weakly silicified, it can adjust its buoyancy and migrate to deeper levels below the euphotic zone to obtain nutrients. In seasonal upwelling regions, this migration often enables it to reach nutrient-rich water layers before the mixing of photic zone, resulting in high contribution to the primary production. Hence, remnants of *P. alata* may serve as biomarkers for upwelling conditions[23].

The southwest coast of India is a known area of upwelling during monsoon period[20]. This might have probably caused the high silicate values to influence the blooming of *Proboscia*, which is a commonly found species in this area. The same species has been recorded in lower cell densities during studies off Vaadi (08°52.01′N, 76°34.26′E, April 2007), off Azheekode (10°11.02′N, 76°09.22′E, January 2008), which lend credence to the view that the concentration of silicate determines the incidence of a bloom.

The growth of diatoms in marine waters is mainly limited by the levels of dissolved silica, when Si : N ratio is less than one[24]. In the present bloom event, Si : N ratio was high (17 : 1) on the first day which receded to 9 : 1 on the third day. These observations suggest that the Si : N ratio has a significant role in the formation and the crash of *P. alata* bloom. The presence of dinoflagellate and other diatom species on the third day indicated the deterioration of the bloom.

The hydrogen ion concentration in the coastal environment is probably altered through nutrient enrichment. Upon the availability of more nutrients the phytoplankton proliferates into bloom condition, which may progressively drive the pH higher[25]. In the present event, the pH was found to be high (8.4) on the first day when the bloom was mono-specific and a gradual decrease in pH was observed by the third day with low intensity of bloom. Hence it may be inferred that on the first day, an increased Si : N ratio with alkaline pH sustained the *P. alata* bloom and lowering of Si : N ratio with decreasing pH value may have caused deterioration of the bloom on the third day. Other physico-chemical parameters did not show much difference from the reference stations. Usually, southwest India is highly vulnerable to algal blooms with physico-chemical factors playing a specific role on the blooming of each species[2]. The marine environment provides different niches that can be exploited by different microalgal species and each species has its own specific combination of necessities to the external environment.

Even though the bacterial communities associated with phytoplankton blooms may be an important microbial regulator of the blooms, their role in the regulation of blooms is still largely unknown[26]. The associated bacterial genera primarily belong to Cytophaga–Flavobacter, gamma-Proteobacteria, alpha-Proteobacteria and beta-Proteobacteria[26]. Specifically, the bacterial assemblage can have inhibitory or stimulatory effects on algal growth during a bloom event and it is also hypothesized that the relative abundance of the bacteria that have an inhibitory effect will increase as the bloom goes through its initiation, development and maintenance phases[27].

The probable algicidal effect of the associated bacteria may be through direct means like protease[28,29] induced lysis of algal cell wall upon direct contact with the algal species or by indirect means like release of dissolved lytic agent(s). Most algicidal bacteria belonged to the Cytophaga/Flavobacterium/Bacteroides (CFB) group and the gamma-Proteobacteria group[30]. In the present study, the associated bacterial composition included the species of *Flavobacterium*, *Acinetobacter*, *Corynebacterium*, *Micrococcus*, *Pseudomonas*, *Bacillus*, *Vibrio* and *Alcaligens*. By the third day, as the bloom almost vanished, the abundance of the *Flavobacterium* sp., *Pseudomonas* sp. and *Vibrio* sp., which belong to the CFB group, and gamma-Proteobacteria was increased considerably.

Hydrolytic enzyme screening of the associated bacteria proved that proteolytic bacteria were more abundant during the *P. alata* bloom and their percentage contribution increased as the bloom entered its decline stage on the third day. *Flavobacterium* sp. and *Vibrio* sp. constituted the major proteolytic bacteria. However, in the reference stations, no such change in the bacterial community and hydrolytic production potential was noticed.

It can be inferred from the results of the bloom event that, in addition to the physico-chemical variables manifested by the Si : N ratio, the gradual increase in the relative abundance of associated bacteria, especially those with high proteolytic potency during the bloom period probably controls the bloom dynamics. Further study about the roles played by the individual bacteria and their specific mechanisms in regulating the bloom is imperative.

Table 1. Comparative surface water characteristics during the bloom event of *Proboscia alata* with reference stations

Variables	10 October 2009	12 October 2009	Ref. St.1	Ref. St. 2
Seawater temperature (°C)	27	28	28	28
Salinity (practical salinity unit)	35	35	35	35
pH	8.4	8.2	7.60	7.80
Nitrate (μmol l^{-1})	2.11	1.40	3.03	2.65
Nitrite (μmol l^{-1})	0.09	0.07	0.21	0.31
Silicate (μmol l^{-1})	38.31	14.20	1.86	2.46
Phosphate (μmol l^{-1})	1.70	1.20	0.13	0.08
Dissolved oxygen (mg l^{-1})	5.42	4.09	6.25	5.64
Chlorophyll *a* (μg l^{-1})	10.80	6.48	4.36	4.32
Primary productivity (gC/m^3/day)	1.87	1.05	0.76	1.41

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