



First report of *Leptocylindrus* sp. bloom in the coastal waters of Kerala, southeast Arabian Sea

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Original Article

Abstract

Leptocylindrus spp. are chain-forming diatoms found in estuaries and coastal waters. Although commonly encountered in the coastal phytoplankton community, massive blooming of this centric diatom is very rare. This is the first report of a bloom of *Leptocylindrus* sp. in the coastal waters of Kerala, Southwest coast of India. The cell densities varied from 1.5×10^5 to 1.8×10^5 no. l⁻¹ from shallower to deeper waters (10 m to 30 m depth). Unlike the common blooming phytoplankters, *Leptocylindrus* filaments formed closely interwoven tufts to gain the appearance of woolen ball like aggregates in the surface waters off Kollam (N 09° 56'50"- 09° 55'13"; E 076° 10'43"- 076° 03'34") on 11th November, 2014. contributed 99.9% of the total phytoplankton standing crop consisting of species belonging to 18 genera. High concentration of silicate triggered the bloom, and low N:P ratio probably acted as a major stress factor forcing the algal cells to flocculate and sink into the water column, resulting in dissipation of the bloom. Formation of aggregates and their sinking can be viewed as the culmination of the blooming process probably controlled by inherent growth characteristics of the diatom (Alldredge and Gotschalk, 1989). Sinking of the clumps of this species reduced the turbidity of the blooming area and helped in retaining the Redfield ratio. No toxic effects were recorded during the bloom.

Keywords: *Leptocylindrus*, flocculation, algal sinking, phytoplankton community, southeast Arabian Sea.

Introduction

Algal blooms are formed in marine and limnetic ecosystems by phytoplankton belonging to dinoflagellates, diatoms and cyanobacteria, resulting in the discoloration of water from red to green hue depending on the species involved (Sellner *et al.*, 2003). The formation of blooms is influenced by a variety of reasons which include combination of light and upwelling, increased nutrient conditions (eutrophication) (Anderson *et al.*, 2002; Smayda, 2006), and biological processes like competition, grazing and allelopathy (Granéli and Johansson 2003).

The majority of the algae blooms reported along the west coast of India are caused by dinoflagellates whereas diatom blooms dominate the east coast (D'Silva *et al.*, 2012). Excluding the toxic diatom *Pseudo-nitzschia* whose toxin, domoic acid, causes Amnesic Shellfish Poisoning and mortality of birds and marine mammals, diatoms usually are not included among harmful phytoplankton groups. However, diatom blooms cause mortality and physiological impairment to fish, most often due to their morphology or non-toxicological stressors (Smayda, 2006). Hornell and Nayudu (1923) reported the first diatom bloom from Indian waters along the Malabar coast. Since then several diatom blooms were reported from the South West coast of India. Blooming of *Fragillaria oceanica*, (Devassy,

1974); mixed bloom of *Skeletonema costatum* and *Nitzschia sigma* (Devassy and Bhattathiri, 1974); *Skeletonema* (Tiwari and Nair, 1998); *Coscinodiscus asteromphalus* (Padmakumar *et al.*, 2007); *Proboscia alata* (Anit *et al.*, 2014) are a few among them.

This paper reports *Leptocylindrus* bloom, for the first time from the coastal waters of southwest India and also describes the subsurface clumping of cells due to the changes in environmental conditions. According to Jackson and Lochmann (1992) coagulation of algal cells is an important mechanism to remove biological material from eutrophic regions. This phenomenon of phytoplankton coagulation followed by sinking has been reported from the marine environment by few authors (Jackson, 1990; Ullal *et al.*, 2001).

Material and methods

Sampling was done on 11th November 2014 in the coastal waters off Kollam (southwest coast of India) from locations where water depth ranged between 10 m (N 09° 56' 50"; E 076° 10' 43") and 30 m (N 09° 55' 13"; E 076° 03' 34") (Fig. 1). The surface waters were discoloured in the blooming regions. Surface water samples were collected using Hydrobios water sampler of 5 l capacity. Water column transparency and sea surface temperature (SST) of the sampling sites were measured

using Secchi disc and bucket thermometer, respectively. Dissolved oxygen (DO) was estimated following Winkler method (Grasshoff *et al.*, 1983), salinity by ERMA hand refractometer with an accuracy of ± 0.1. Dissolved nutrients such as nitrate, silicate, and phosphate were estimated following standard procedures (Grasshoff *et al.*, 1983; Parsons *et al.*, 1984). Phytoplankton collection was done by filtering water samples onboard using a net of 20µm mesh size. During the water sample collection, the net was clogged by mucilaginous aggregates of the filamentous diatom *Leptocylindrus* sp. Some of them formed woolen ball like clumps below surface layer. Phytoplankton was counted using Sedgwick Rafter counting chamber and identified with the aid of Nikon Eclipse microscope attached to a Nikon DN 100 series digital camera. Phytoplankton was identified using taxonomic monograph of Tomas (1997).

Results and discussion

Microscopic analysis revealed a mono specific bloom belonging to the genus *Leptocylindrus* with cell densities of 1.5×10^5 and 1.8×10^5 no.l⁻¹ at the sampling stations distributed in the 10 m to 30 m deep continental shelf (Table 2). *Leptocylindrus* is a radial centric diatom genus containing two species, viz., *L. danicus* and *L. minimus*, distributed in the coastal waters worldwide (Horner, 2002). The genus has tiny cylindrical yellow, brown

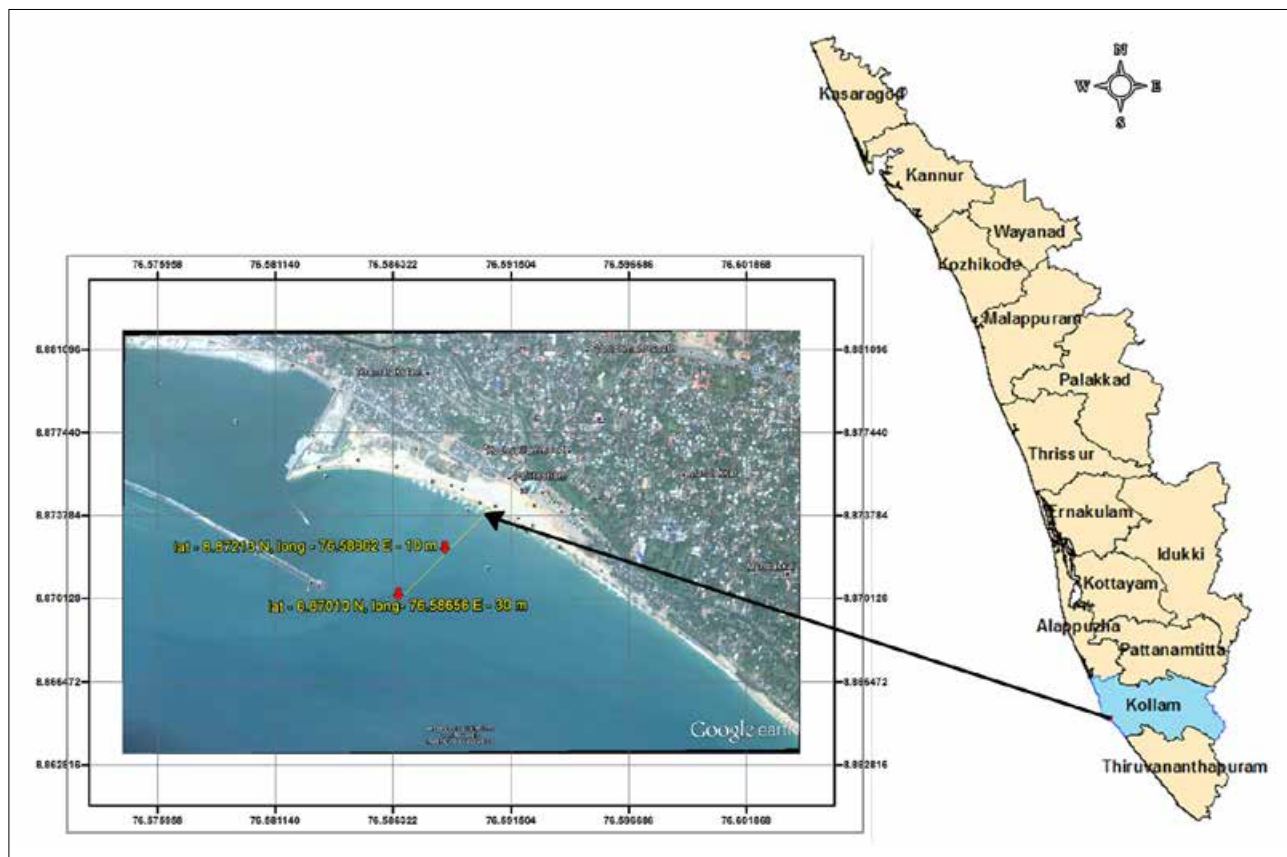


Fig. 1. Location map off Kollam showing the three sampling sites where the water depth of the stations were 10 m, 20 m and 30 m respectively

Table 2. Qualitative and quantitative composition of phytoplankton (No. of cells / l) at the 3 sampling depths during the survey

	10 m	20 m	30 m
<i>Asterionella</i> sp.	45	58	102
<i>Biddulphia</i> spp.	12	20	14
<i>Chaetoceros</i> spp.	89	258	240
<i>Corethron</i> sp.	0	1	0
<i>Coscinodiscus</i> sp.	8	12	6
<i>Ditylum brightwellii</i>	28	8	10
<i>Eucampia</i> sp.	0	0	3
<i>Gyrosigma</i> sp.	8	6	2
<i>Leptocylindrus</i> sp.	15,42,530	10,42,585	18,50,540
<i>Melosira</i> sp.	60	15	32
<i>Navicula</i> sp.	0	10	0
<i>Rhizosolenia</i> sp.	85	108	58
<i>Skeletonema</i> sp.	25	40	25
<i>Thalassiosira</i> sp.	8	14	10
<i>Ceratiumfurca</i>	48	42	38
<i>Prorocentrum</i> sp.	2	18	14
<i>Dinophysis caudata</i>	65	47	24
<i>Protoperidinium</i> sp.	12	24	10
Total	15,43,025	10,43,266	18,51,128

cells, united in long, straight or gently undulating chains with flat valve faces devoid of spines (Fig. 2). Cells have one to two elongated chloroplasts near the center of each cell (Kraberg *et al.*, 2010). Cosmopolitan in distribution, these species coexist with other major phytoplankton genera like *Coscinodiscus*, *Chaetoceros*, *Asterionellopsis* and *Skeletonema* along the coast of Kerala.

The bloom had a characteristic appearance with *Leptocylindrus* filaments forming small woollen flocs below the surface (Fig 3a and 3b). Similar aggregations were reported in chain forming diatoms like *Chaetoceros* and *Nitzschia* (Alldredge and Gotschalk,

Fig. 2. Chain of *Leptocylindrus* sp. under light microscope (40 x).

1989). Southwest coast of India faces an increase in the frequency of large scale algae blooms during the past decade (D'Silva *et al.*, 2012; Padmakumar *et al.*, 2012). There exists a relationship between phytoplankton bloom and nutrient concentration. Often a nutrient from a suite of nutrients found in the coastal area can become a limiting factor. Smayda (1990) points out that phytoplankton growth in the sea is often nutrient limited and that the uptake of nutrients such as N, P and Si follow the Redfield Ratio. Redfield ratio is the atomic ratio of carbon, nitrogen and phosphorus found in phytoplankton and throughout the deep oceans. It is found to be C:N:P=106:16:1. This ratio plays an important role as regulators, in terms of species selection. Hodgkiss and Ho (1997) reviewed the role of nutrient concentration and phytoplankton bloom and concluded that a drop in N:P ratio led to increase in the occurrence of red tide. Smayda (1990) has indicated how long term declines in the Si:N and Si:P ratios in response to nitrification cause corresponding increase in the non siliceous phytoplankton groups.

Analysis of the physico-chemical parameters indicates that *Leptocylindrus* sp. bloom was triggered by the abundance of silicates, nitrates and phosphates. According to Hecky and Kilham (1988) silica is required for the cell wall of diatoms and

Fig. 3a. Sub surface coagulates of *Leptocylindrus* sp.Fig. 3b. Sinking of *Leptocylindrus* chain *in situ*.

Table 1. Physico chemical parameters of waters off Kollam during the bloom as well as during a non-bloom phase in 2014 for each of the three stations with bottom depths respectively 10, 20 and 30 meters

Month	Station details	Nitrate $\mu\text{mol/L}$	Phosphate $\mu\text{mol/L}$	N:P ratio	Silicate $\mu\text{mol/L}$	Temp $^{\circ}\text{C}$	Salinity psu	DO ml/L
Sept.		9.52	0.95	10.02	15.23	26.5	29	6
Nov	10 mS	2.31	2.107	1.09	11.363	29.1	29	5.6
Sept.		9.13	1.04	8.78	18.65	26.5	29.5	5.8
Nov	10 mSS	2.24	2.113	1.95	10.335	29.1	29.2	4.4
Sept.		11.35	0.86	13.2	18.55	27.2	29	6.2
Nov	20 mS	1.73	1.149	1.246	10.234	29.3	29.5	6.6
Sept.		11.26	0.98	11.49	19.82	27.3	29	6.2
Nov	20 mSS	3.53	1.389	2.632	9.856	29.3	29.5	6.4
Sept.		10.45	1.14	9.167	19.44	27.5	29	6.9
Nov	30 m S	2.82	1.341	1.13	8.382	29.5	29.5	7.2
Sept.		11.56	1.26	9.175	19.52	27.5	29.5	6.6
Nov	30 mSS	3.06	2.496	1.226	8.806	29.5	29.5	6.6

S - surface waters, SS - subsurface waters

is also essential for channelizing nitrogen and phosphorus into them. Similarly high values was observed during *Proboscia alata* bloom ($38.13\mu\text{mol/L}$) by Anit *et al.* (2014) and *Coscinodiscus asteromphalus* bloom by Padmakumar *et al.* (2007). The present sampling was done at a time when the bloom was not in its log phase of growth, hence the silicate concentration in the water was high ($8.382 - 11.369\mu\text{mol/L}$). The sampling areas have a high N:P ratio during the late monsoon and early post monsoon season (September, October). However, the post bloom (crashing phase) recorded relatively low N:P ratio and high concentrations of Si (Table 1). Increase in the transparency of the surface waters was noticed towards the off shore sampling area (Table 3).

Table 3. Transparency of waters off Kollam during *Leptocylindrus* bloom

Station details	Transparency (m)
10 m	2.8
20 m	3.2
30 m	3.8

The *Leptocylindrus* sp. bloom led to depletion of nitrates, which acted as limiting factor for further proliferation of the diatoms. Rapid utilisation of nitrates from the waters reduced the levels of nitrate to $1.73\mu\text{mol L}^{-1}$, upsetting the N:P ratio of the region when the bloom was at its peak. Nixon and Pilson (1983) states that nitrogen is more limiting in phytoplankton blooms than phosphorus. Further, phosphorus is quickly regenerated from dying phytoplankton, which helps to maintain the high concentration of phosphates ($1.341 - 2.496\mu\text{mol L}^{-1}$) even during the declining phase of the bloom (Ryther and Dunston, 1971). Partial correlation coefficient (r) between phytoplankton and nutrients can provide interesting information regarding the order of utilization of nutrients by the phytoplankton. In the case of

Leptocylindrus sp. the decreasing order of utilization was $\text{NO}_3\text{-N} > \text{SiO}_3\text{-Si} > \text{PO}_4\text{-iP}$ ($r = -0.1724, -0.0577, +0.2063$ respectively), whereas for phytoplanktons other than *Leptocylindrus* sp., the order was $\text{SiO}_3\text{-Si} > \text{PO}_4\text{-P} > \text{NO}_3\text{-N}$ ($r = -0.1769, -0.1700, +0.1786$). Thus *Leptocylindrus* consumed more nitrates than the other phytoplanktons. Due to this reason N:P ratio was lowered during heavy blooming of *Leptocylindrus*. According to Minghui *et al.* (1990) N:P ratios less than 8 indicate that nitrogen acts as a limiting factor and the ratio higher than 30 indicate surplus nitrogen. During the bloom N:P ratios reduced to such low levels as 1.09 in the surface waters and 1.22 in sub surface waters. Reduction in inorganic nitrogen during the heavy bloom created a stressful condition, which affected the sustenance of the bloom eventually leading to flocculation of the cells. Dissolved oxygen levels in the subsurface waters also showed a sharp decline from that of the surface waters. This reduction could be due to the consumption of oxygen during the degradation of the sunken floccs.

The diatom floccs which contained a mucous matrix was proved to be generated from exopolymer excretions (Riebesell, 1991), formed by the aggregation of Transparent Exopolymer Particles (TEP) (Passow and Alldredge, 1994). It is known that TEP are produced when dissolved polysaccharide chains are excreted by phytoplankton align via cation bridging; probably polymerisation of surface active molecules is also involved. Kiorboe *et al.* (1990), found that algal nutritional state affected the stickiness of an algal species. Stickiness increased by over two orders of magnitude to initial condition when the cells were nutrient limited. TEP range from submicron to several hundred microns in size and can occur at abundances up to thousands per millilitre (Passow and Alldredge, 1994). Aggregation rates are governed by the concentration and size of all particles present, and should be higher in the presence of TEP (Jackson, 1990).

A change in cell stickiness, caused by increased mucous production during nutrient limitation has been postulated to trigger mass flocculation (Hoagland *et al.*, 1993). Mostly this happened once the nutrients were exhausted leading to slow growth rate.

Mass flocculation and sinking have been recognized as important stages in the successional sequence of diatom blooms (Smetacek, 1985; Alldredge and Gotschalk, 1989). In the present study, it is clear that the nitrate limitation led the *Leptocylindrus* sp. chains to become coagulated and form woolen ball like structures in the water column. Similar situations are reported earlier also (Jackson and Lochmann, 1992).

Sinking of diatoms has been described to be a complex phenomenon governed by energetics of diatoms. The diatoms sink faster in proportion to their size when under severe energy limiting conditions such as low light, darkness (night) and nutrient deprivation (Waite and Thompson, 1992). This probably explains subsurface sinking of *Leptocylindrus* sp. bloom aggregates. Furthermore, during rapid cell division of diatoms, the shifting of energy away from the mechanism that regulates buoyancy of the cell leads to increased sinking rate (Bienfang, 1981). Other phytoplankton get trapped in the woolen aggregates of *Leptocylindrus* sp. and sink with them, resulting in the removal of cells from surface waters. Inclusion of phytoplankton and other particles into aggregates is dominated by collisions of TEP with other particle types and not by phytoplankton-phytoplankton flocculation. Size of the aggregates increases as they sink in the water column. Similar coagulation during bloom were observed and reported earlier also (Alldredge *et al.*, 1995; Ullal *et al.*, 2001).

Flocculation and sinking of algal cells seem to be a mechanism by which the system regains equilibrium. Intense hydrolytic enzyme activity has been observed on flocs (Smith *et al.*, 1992) and many periphytic bacteria of flocs release enzymes active in breaking down algal acid polysaccharides (Corpe and Winters, 1972). This activity replenishes the nitrates restoring the N:P ratio to Redfield values. The bloom did not cause any perceivable harmful effects like fish kill or foam production which is in agreement with the observations of Pearl (1988) and Ullal *et al.* (2001). No significant changes in water temperature and dissolved oxygen occurred during the *Leptocylindrus* algae bloom.

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