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SEASONAL OBSERVATIONS ON MASS AGGREGATION OF CYCLOPOID COPEPOD (*OITHONA* SP.) WITH DIATOM BLOOM FROM COCHIN ESTUARY KERALA, INDIA

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Abstract: The present communication deals with the occurrence of cyclopoid copepod (*Oithona* sp.) associated with diatom bloom along the Cochin estuary, southwest coast of India, during the late monsoon season (August 2016). Total diatom cell density of the bloom area was $13x10^5$ cells L⁻¹. The distribution of chlorophyll a pigments, which is known as a reliable measure of phytoplankton biomass of the bloom area contributed 14.50 ± 3.95 mg m⁻³. Average nutrient concentration of the bloom area for nitrate- nitrogen was $13.22\pm3.74\mu$ mol L⁻¹, $4.28\pm0.92\mu$ mol L⁻¹ for nitrite-nitrogen, $7.45\pm2.96\mu$ mol L⁻¹ for ammonia-nitrogen, $6.70\pm2.77\mu$ mol L⁻¹ for phosphate- phosphorus and $41.23\pm6.27\mu$ mol L⁻¹ for silicate-silicon. *Coscinodiscus* sp. (98%) dominated followed by *Skeletonema* sp. (1%) and *Pleurosigma* sp. (1%) in the collections. Mesozooplankton density on an average in the estuary was very less (463ind.m⁻³), having cyclopoid copepod s alone contributing, 75% of the zooplankton biomass. Unusual occurrence of elevated cyclopoid copepod over calanoid copepod in relation with diatom bloom was a unique observation during the study. Presence of such bloom mainly indicates nutrient enrichment and eutrophication issues associated with habitat shifts along the coastal environment. Statistical analysis also revealed that diatom bloom had a significant relation (p<0.05) with cyclopoid copepod; other environmental parameters such as silicate, chlorophyll a, temperature, pH and salinity were also significant during the study.

Keywords: Diatom, bloom, cyclopoids, swarm, Cochin estuary

INTRODUCTION

Diatoms plays a key role in the primary production of pelagic food chains that dominate the trophic food webs consisting of suspension feeding planktonic copepods eventually leading to top consumers and many other important fisheries. Although considerable works on the occurrences of single or multi-species of phytoplankton blooms have been reported by Padmakumar et al. (2016, 2017) from open waters of southwest coast of India, reports from estuaries are scarce. Increasing nitrogen (N) and phosphorus (P) caused by anthropogenic activities influences severe eutrophication resulting in enrichment of primary production (Khan and Mohammad, 2013; Wang and Wang, 2009; Howrath and Marino, 2006). Coastal waters, such as bays, estuaries and lagoons are diverse system where both biology and physical dynamics are strongly influencing fresh water runoff from land as well as the exchange of seawater. An elevated nutrient supply from inland to seaward through rainfall run off coupled with turbulence is causing proliferation of diatoms and dinoflagellates. Diatoms dominated the phytoplankton blooms reported in neritic seas that are usually terminated by aggregation. Devassy *et al.* (1979) reported the succession of phytoplankton following *Trichodesmium* bloom in the coastal waters of Goa.

Copepods are known as primary consumers of phytoplankton; the most diversified and abundant metazoans on earth accounting 71% of planktonic population comprising more than11, 500 species. Most of the cyclopoid copepods are primarily benthic; however few species thrive in pelagic zones of lakes, seas and oceans. Their diversity and abundance are significant in shallower bodies of water, such as wetlands and temporary ponds. Cyclopoid copepod genus, *Oithona* is the most abundant

mesozooplankton in a wide variety of oligo- trophic and eutrophic marine water habitats from open oceanic to shallow coastal regimes (Paffenhofer, 1993). Mass swarming of calanoid copepods belonging to Family Acartiidae was reported from Cochin estuary (Santu et al., 2016), but information on aggregation of cyclopoid copepods in estuarine regions have been limited. Experiments on Oithona species, revealed a multitude of feeding behavior patterns including carnivorous, herbivorous or omnivorous and detritus mode of habits which could be the reason for dominance of cyclopoid copepods over calanoid species (Gauld, 1966; Turner, 1986; Paffenhofer, 1993). Therefore, this paper discusses certain observations on the association of cyclopoids with diatoms in Cochin estuary.

MATERIALS AND METHODS

Aggregation of Oithona species coupled with diatom bloom was observed in Cochin estuary during late monsoon season, August 2016. Being a dynamic estuary, Cochin estuary is a complex, shallow estuarine network (250km²) running parallel to the coastline of Kerala extending 9° 40' and 10° 12'N and 76° 10' and 76° 30'E with its northern boundary at Azheekode and southern boundary at Thanneermukkam bund. The estuary forms a major part of the Vembanad backwater system, a Ramsar site on the south west coast of India. Six rivers namely Periyar in the north; Pampa, Achankovil, Manimala, and Meenachil in the south; Muvattupuzha, midway between the two discharge freshwater into this estuarine system and connected to the Arabian Sea at Cochin gut and another at Azheekode. Sampling locations were fixed by Global Positioning System (GPS) (Magellan ® Triton 200/300) (Fig.1). Nine stations were selected for sampling on a seasonal basis, of which three stations including Barmouth (Stn.3), Bolgatty (Stn.4) and Marine Science jetty (Stn.5) depicted extensive diatom blooming in association with cyclopoid copepods. The bloom affected sites are characterized by sewage discharge, strong variation in tidal influx, oil- ballast water discharges and extensive reclamation in combination with construction activities. The data collected for water quality and zooplankton assessment during the monsoon season (August 2016) formed the basis of this paper.



Fig. 1. Map of Cochin estuary showing study locations

Micro phytoplankton net with a mesh size of 20 µm was used for the collection of phytoplankton and a zooplankton net with a mouth area of 0.28 m^2 (mesh size of 200 μ m) was used for the collection of mesozooplankton samples. A calibrated flow meter (General Oceanics model number-2030®, 2012) was attached to the plankton net and was towed horizontally just below the surface at a fixed speed of approximately 1 knot for 10-15 min. Both phytoplankton and mesozooplankton samples were preserved in 3% and 4% buffered formalin (0.5g sodium borate and 6.5g disodium hydrogen phosphate/L) respectively. Standing crop of phytoplankton was represented by Chlorophyll measurements (APHA, 2005) and that of zooplankton was estimated by displacement volume method and expressed as ml m⁻³ (Harris et al., 2000; Johnson and Allen, 2005). Dissolved oxygen concentration was estimated by modified Winkler's method and Mohr-Knudsen method was adopted for measuring salinity (Strickland and Parsons, 1972; Grasshoff et al., 1983). The inorganic nutrients, nitrate-nitrogen, nitrite-nitrogen, ammonia-nitrogen, phosphate-phosphorus and silicate-silicon samples were acidified with conc. HNO₂ and analyzed based on standard methods (Grasshoff *et al.*, 1983). Samples were sorted for major zooplankton taxa (Omori and Ikeda, 1984; Tait, 1981; Todd and Laverack, 1991) and enumerated and density was expressed in ind. m⁻³. Phytoplankton was enumerated; density was expressed as cells L⁻¹ and identified up to genus level using standard keys (Allen and Cupp, 1935; Subrahmanyan, 1946, 1959). Data obtained were subjected to statistical analysis for t-test and ANOVA, using SPSS 17.0 software, to determine the significance of the results.

RESULTS AND DISCUSSION

During monsoon season, water temperature ranged from 29°C to 30°C. Highest temperature was observed in Stn.1, 2 and 6 (30°C) whereas lowest of 29°C and 29.5°C were observed in the bloom area (Stn.3, 4 and 5). In general, temperature has been recognized as key factor that control phytoplankton blooms in marine ecosystem (Eppley, 1972). The present study also agreed with this observation. pH value ranged from 6.34 to 7.24 and salinity from 2 to 8 ppt. Dissolved oxygen (D.O) concentrations were in a range of 5.21 to 7.24 mg L⁻¹. Highest silicatesilicon was observed in St. 4 (48.12 µmol L⁻¹) followed by St. 5 (46.88 μ mol L⁻¹) and St.3 (41.67 μ mol L⁻¹) with the lowest concentration recorded in St.6 (30.88 µmol L⁻¹). Average silicate-silicon showed $41.23 \pm 6.27 \mu$ mol L⁻¹. Monsoon season indicated that freshwater runoff is the principal source of silicate inputs. One possible explanation for the high values of silicate during August was the discharge of large volumes of silt from freshwater into the estuary by rivers. Average chlorophyll a concentration and diatom cell density of bloom area was 14.50±3.95 mg m⁻³, 13x10⁵ cells L⁻¹ respectively. Seasonally micro-phytoplankton in Stn.3, 4 and 5 composed of diatoms (66%) and dinoflagellates (34%) of which Cylindrotheca closterium (8x 10³ cells L⁻¹), Odontella mobiliensis (8x 10³ cells L⁻¹), Fragilaria oceanica $(8x10^3 \text{ cells } L^{-1})$ were the major diatom followed by Chaetoceros sp. (2x 10³ cells L⁻¹), and Thalassiosira sp. $(2 \times 10^3 \text{ cells } \text{L}^{-1})$. In monsoon season, the highest cell density was observed in Stn.3 (4 x 10³ cells. L⁻¹) followed by Stn.1 (3 x 10^3 cells L⁻¹) and in Stn.5 (2 x 10^3 cells L⁻¹). Among the phytoplankton, the diatom Coscinodiscus sp. dominated contributing 98% of the biomass whereas, Skeletonema sp. (1%) and *Pleurosigma* sp. (1%) were observed in small numbers in the area (Fig.2 A-E). The physico- chemical characteristics of Cochin estuary is presented in Table 1.

The average mesozooplankton biomass recorded in the estuary was 1.64±0.5 ml m⁻³. During the monsoon season zooplankton biomass ranged from 1ml m⁻³in Stn.2 to 2.8 ml m⁻³ Stn.6. The highest density of mesozooplankton was recorded in pre-monsoon (2.04±0.72 ind. m⁻³) followed by post-monsoon (1.6±0.58 ind. m⁻³) and monsoon (1.12±0.41 ind. m⁻ ³) respectively. However, average density of mesozooplankton and cyclopoid copepod of the bloom area in monsoon season was 463±410 ind. m⁻³ and 395±227 ind. m⁻³ (75%) respectively (Fig.3 A-B). Highest density of cyclopoids was observed in St.5 $(1185 \text{ ind.m}^{-3})$ followed by St.4 (408 ind. m⁻³) and in Stn.1 (328 ind. m⁻³). Apart from cyclopoid copepods, fish larvae (7%), cypris larvae (6%), calanoids (2%), harpacticoids (2%) and zoea (2%) were also encountered during the period. Statistical analysis revealed that diatoms had a significant relation (p<0.05) with silicate, cyclopoid copepod, chlorophyll a, temperature, pH and salinity.

Aggregation of zooplankton and blooming of phytoplankton are influenced by several factors. Most of the phytoplankton studies conducted is either from the Arabian Gulf or in the other waters of the world thus indicating that phytoplankton populations are dominated by diatoms (Subba Rao and Al-Yamani, 1998; and Al-Yamani et al., 2004). The results of the present study, also corroborates to this where phytoplankton species composition was clearly dominated by diatom species due to the higher silicate content in Cochin estuary. Even though Cochin estuary is reported to be eutrophic estuary (Qasim, 2003); during monsoon season, an increase in the overall nutrient concentrations was noticed when compared to non-monsoon periods. In an earlier study in Cochin estuary by Gopinathan et al. (1974) observed two major peaks of diatoms during January to February and July to August were reported. He also noticed that the maximum value was obtained for cell counts resulting from the blooming of diatom Skeletonema costatum. The phytoplankton community dominated by diatoms and proliferation of multiple species of diatom S. costatum,

Thalassiosira subtilis and *Nitzschia closterium* at various locations were observed especially during premonsoon season in the same estuary by Madhu *et al.* (2007).

Phytoplankton blooms and copepod swarming reported all over the world are caused by several reasons. Phytoplankton is an excellent indicator of marine ecological niches; their dynamics are affected through environmental parameters influencing the plankton abundance, diversity and growth significantly. Also, most of the blooms occurring during monsoon can be result of increased discharge of nutrients by land run off, precipitation and upwelling (Raghu and Anil, 2003; Patil and Anil, 2008). These biotic changes are accompanied by increasing temperature, day length, irradiance and persistent mixing (Pratt, 1966; Smayda, 1973; Hale, 1975). There are numerous studies about the effects of abiotic factors such as temperature, salinity, light intensity and nutrient profiles that play a vital role in diatom growth. Several studies reveal that, the decreased dissolved silicate (Humborg et al., 2006) and eutrophication-associated increase in the nitrogen could be a reason for the increase in diatoms in favour of non-siliceous phytoplankton (Gilpin et al., 2004). In Cochin estuary, Balachandran et al. (2005) reported that, nutrients are not a limiting factor for the optimum phytoplankton growth at any time of the year; transient variations in the water quality play a significant role on phytoplankton behaviour (Madhu et al., 2010). The increased flushing during monsoon resulted in low chlorophyll concentration in surface layers where salinity was low and maximum chlorophyll concentrations were observed in pre monsoon season when the surface salinity was high. However, the increased chlorophyll a concentration during our study was due to diatom bloom during the period. D'Silva et al. (2012) and Sachithanandam et al. (2013) reported around 103 algal blooms have been documented along the west and east coasts of Indian Ocean including Andaman Sea from 1908 to 2012. Moreover, the west coast of India witnessed majority of blooms by dinoflagellates whereas diatom blooms prevail along the east coast only. D'Silva et al. (2012) reported 19 diatom blooms in the west coast of Indian waters resulting from 7 causative agents, while 12 diatom causative

organisms were reported in East coast region, off which *Nitzschia sigma*, *S. costatum* were seldom reported during pre-monsoon (May 1970) and post monsoon (November 1970) in Cochin estuary. Bloom of diatom *Fragilaria oceanica* has also been reported from the coastal waters of Mangalore during the premonsoon season (Devassy, 1974). Attainment of high zooplankton biomass in the coastal waters of central west coast of India during the late monsoon season coincides with the temperature peak (Bhargava *et al.*, 1973). On this scenario; this is the first report of *Coscinodiscus* sp. from west coast of India without any harmful effects.

Mainly zooplankton is subjected to wide range of seasonal fluctuations with major peak during monsoon and minor peak in post monsoon. The imbalance in zooplankton population generally due to fluctuations in environmental conditions leading to poor upwelling, rise in sea surface temperature, under water disturbances, altered monsoon, water currents and by pollution (Sharma and Wilma 2007). Distinct swarms of cyclopoids O. oculata, O. nana from coral reef environments was studied by Emery (1968). However, the reasons for swarming remain unknown; the proposed advantages of swarming could be a less predation, persistence in favorable conditions and proximity to mates. The rise in temperature influencing water column stability, nutrient enrichment and primary production affect abundance, size composition, diversity and trophic efficiency of zooplankton because it plays a single most important physical parameter by structuring the ecosystem.

Usually timing of maximum copepod population densities correlates with the timing of phytoplankton blooms. Phytoplankton biomass is lowest during the spring and summer and copepod food supplies are reduced in these months. However, nanoplankton blooms are common in the spring and summer, providing an alternate food source (Ambler et al., 1985). The spring diatom bloom is considered to initiate and support the cycle of secondary production and growth of fish larvae that depend predominantly on the egg and naupliar stages of planktonic copepods, the dominant constituents of the zooplankton in most oceanic regions, for food (Turner, 1984, Mann, 1993). As copepods mainly

Parameters	Stn.1	Stn.2	Stn.3	Stn.4	Stn.5	Stn.6
Water Temperature (⁰ C)	30	30	29	29.5	29	30
pH	7.24	7.22	7.08	6.86	6.34	6.94
Salinity (ppt)	2	5	8	5	2	5
Dissolved oxygen (mg L ⁻¹)	6.25	6.88	7.24	5.98	7.07	5.21
Nitrate- nitrogen (µmol L ⁻¹)	8.57	18.43	11.35	16.67	10.8	13.54
Nitrite- nitrogen (µmol L ⁻¹)	4.5	4.25	3.27	3.17	5.51	4.98
Ammonia- nitrogen (µmol L-1)	4.67	7.4	6.31	5.79	7.45	13.11
Phosphate-phosphorus (µmol L ⁻¹)	6.65	5.27	11.59	3.2	6.48	7.05
Silicate- silicon (µmol L ⁻¹)	41.76	38.12	41.67	48.12	46.88	30.88
Chlorophyll a (mg m ⁻³)	20.28	10.92	10.21	12.94	17.81	14.87

Table 1. Variations in hydrographic parameters in Cochin estuary during monsoon period in 2016



Fig. 2. Diatom bloom of *Coscinodiscus* sp. outbreaks in Cochin estuary (A&B). *Coscinodiscus* sp. (10X); (C&F). *Coscinodiscus* sp. (40X); (D) *Skeletonema* sp. (40X); (E) *Pleurosigma* sp. (40X)



Fig. 3.A. *Oithona* sp. assemblage in Cochin estuary associated with diatom bloom (10X).

consume phytoplankton including diatoms, ciliates, and detrital carbon; copepod populations relays on abundance and quality of food sources (Ambler et al., 1985). Santhanam and Perumal (2012) compared the feeding habits of Oithona rigida on various algal feeds comprising of Chorella marina, Cosinodiscus centralis, Chaetoceros affinis and Skeletonema costatum. The study depicted that copepod O. rigida fed efficiently on microalgae C. marina and C. centralis compared to other algal diet mainly due to the restriction of feeding on filamentous and larger microalgae because of their small mouth parts. This limitation in feeding could be the reason for dominance of cyclopoid copepod in the vicinity of diatom bloom over other copepod species. In addition, the highest egg production and hatching success were seen in the larger sized copepod (0.84 mm) at an optimum temperature (26±2°C) and higher algal food concentration (1.5x10³ cells mL⁻¹). One of advantages of swarming may be associated with its reproductive strategy. Breeding would be facilitated in swarms because the swarms of copepods sampled were mostly males and females. The swarm formation provides maximum mating chances and enhance mating success because of potential mates are abundant in swarms (Ambler et al., 1991). Furthermore, the ambient temperature recorded in Coscinodiscus bloom area might be the reason for aggregation of cyclopoid replacing calanoid copepod for breeding purpose during the period. In addition, occupancy of such diatom blooms is also influenced by the grazing



Fig. 3.B. Oithona sp. female bearing pair of egg sac (10X).

structure; copepod predation regulates the phytoplankton predominance in a particular area. However, the importance of diatoms as a dominant and high quality food source for copepod production has recently been questioned by several authors (Kleppel et al., 1991, Kleppel, 1993). Studies by several authors have reported that some diatom species induce copepod egg mortality by blocking embryogenesis (Poulet et al., 1995, Ianora et al., 1996, Uye, 1996). Recent studies showed that mesozooplankton in temperate oceans especially after the spring diatom bloom feed on dinoflagellates and ciliates which help to sustain egg production (Kleppel et al., 1991, Sanders and Wickham 1993, Ohman and Runge 1994). Ban et al. (1997) observed that majority of diatoms cannot act as sole food to support high egg production and hatching rates. According Vehmaa et al (2013) the dominant phytoplankton species in a community, even in a multi-species bloom has major influence on copepod reproductive output. Also, both high and low diatom concentrations can negatively impact copepod reproduction and question the relative roles of diatom blooms and the more complex microbial trophic pathways in supplying energy and materials for copepod growth and reproduction. However, during the study the cyclopoid copepod swarms were observed with adult copepods and female bearing egg sacs. Oithona similis as a key link between small protozooplankton and fish larvae has been particularly emphasized in areas and seasons which are disadvantageous for

calanoid copepods (Nielsen and Sabatini, 1996; Nakamura and Turner, 1997). Copepods are not randomly distributed in their natural habitats but show a patchy distribution. One of the causes for patchy distribution patterns is social aggregation or the performance of swarming behavior (Ueda et al., 1983). There are reports of swarming in the freshwater calanoid Heterocope septentrionalis (Hebert et al., 1980), mass occurrence of Family Acarttidae from Cochin estuary by Santu et al (2016) and in the marine calanoids Acartia, Centropages and in marine cyclopoids belonging to Oithona (Emery 1968; Hamner and Carleton 1979). All these reports points that adaptations that reduce the risk of predation are essential for the survival of these animals in the pelagic environment.

Various studies have highlighted the interaction of diatoms and copepod in controlled experimental conditions, while relevant data from field conditions are scarce due to the lacunae in studies conducted in natural estuarine habitats. However, the shift in environmental parameters such as increase in temperature, elevated silicate and nitrogen combined with fresh water dominance leads to diatom blooms which reduce the abundance of zooplankton eventually ends as substantial amount of unconsumed carbon at primary level of the trophic food web.

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