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Monitoring of Harmful Algal Bloom (HAB) of *Noctiluca scintillans* (Macartney) along the Gulf of Mannar, India using in-situ and satellite observations and its impact on wild and maricultured finfishes

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ABSTRACT

In the Gulf of Mannar, *Noctiluca scintillans* blooms have been observed three times in September 2019, September and October 2020, and October 2021. It was determined and measured how the bloom period affects ichthyodiversity. *Noctiluca* cell density varied slightly from year to year, ranging from1.8433 $\times$ 10^3 cells/L to 1.3824 x 10^6cells/L. In surface and sea bottom waters, high ammonia levels and low dissolved oxygen levels were noted. During the bloom period a significant increase in chlorophyll concentration was found. The amount of chlorophyll in GOM was extremely high, according to remote sensing photos made using MODIS-Aqua 4 km data. Acute hypoxia caused the death of wild fish near coral reefs and also in fish reared in sea cages. The decay of the bloom resulted in significant ammonia production, a dramatic drop in the amount of dissolved oxygen in the water, and ultimately stress, shock, and mass mortality of fishes.

1. Introduction

The Physico-chemical characteristics and meteorological conditions determine the seasonal occurrence of Harmful Algal Blooms (HABs), which occur naturally around the planet (Ibrahim, 2007). According to D'Silva et al. (2012), there were 101 incidents of HAB episodes on India's east and west coasts between 1908 and 2009. Algal blooms eventually have detrimental repercussions that have a huge negative influence on the environment and human health as well as causing severe financial losses to aquaculture and fisheries (Al-Ghelani et al., 2005).

In India, harmful algal bloom occurs during pre- and post- south-west monsoon periods along the west coast and east coast. Reports of HABs in Indian waters are aplenty (Raghu Prasad, 1953; Gopakumar et al., 2009; Anantharaman et al., 2010; D'Silva et al., 2012; Padmakumar et al., 2012; Sulochanan et al., 2014; Tholkapiyan et al., 2014; Baliarsingh et al., 2016; Shaju et al., 2018; Oyeku and Mandal, 2020). It has been reported that on Indian coasts, mass mortality of fishes due to harmful algal bloom is generally associated with the species such as *Cochlodinium polykrikoides*, *Karenia brevis*, *Karenia mikimotoi*, *N. scintillans*, *Trichodesmium erythraeum*, *T.thiebautii* and *Chattonella marina* (D'Silva et al., 2012). HABs affect the feeding response and respiratory mechanisms due to toxin transfer which cause the mortality of fish and other organisms in the marine ecosystem. The bloom was the source for hypoxia or oxygen depletion in the area (Karlson et al., 2021).

There were two types of *Noctiluca* found in Indian waters, viz., red *Noctiluca* and green *Noctiluca*, in the temperatures ranging from 10 to 25 °C and 25 to 30 °C respectively (Turkoglu, 2013). The eastern, western, and northern Arabian Seas are the main locations where Green *Noctiluca* is found (Harrison et al., 2011). Despite being regarded as non-toxic, the *N. scintillans* bloom is linked to the anoxic mass extinction of fish and marine invertebrates (Gopakumar et al., 2009; Shaju et al.,

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Fig. 1. Three HAB incidences, all by *Noctiluca scintillans* have been recorded in the Gulf of Mannar, viz. during 2019 September (B1), 2020 September and October (B2) and 2021 October (B3).

2018). Since *N. scintillans* are known to produce ichthyotoxic chemicals (NH3), their dense blooms may deplete oxygen, harming aquatic life and perhaps causing mass mortality (Smayda, 1997).

The first report of *Noctiluca* bloom in the Palk Bay, east coast of India, was in April–June 1952 by *N. miliaris* and the first report of widespread fish mortality due to the presence of *Noctiluca* was recorded by Aiyar (1935) (Raghu Prasad, 1953). *Noctiluca scintillans* bloom has also been reported from the Gulf of Mannar (GoM), which started near Appa, Thalaiyari Island, Valai Islands which subsequently intensified into a dense bloom in Muthupettai area and spread from Kizhakarai to Pamban (Gopakumar et al., 2009).

Satellite remote sensing of ocean colour provides an inexpensive and viable alternative to monitor HABs over a large area and monitor their progress. The distribution and abundance of phytoplankton in the ocean's upper layers changes the ocean's colour due to selective absorption of blue and red light of the visible spectrum by the dominant photosynthetic chlorophyll-a (Chl-a) pigment with very less absorption in the green part of the spectrum. As a result, the intensity and spectral composition of light emerging from the sea surface gets modified (Morel and Prieur, 1977). As the chlorophyll-bearing phytoplankton concentrations increase, the upwelled or backscattered radiation progressively changes from blue and blue-green to various shades of green. The property of colour change due to variations in Chl-a concentration is measured by a remote sensor in the various spectral bands and is shown to be quantitatively related to the ratios of upwelled light in blue-green wave lengths (Clarke et al., 1970; Hovis and Leung, 1977; Yentsch, 1960). This fundamental premise allows phytoplankton abundance to be indexed as Chl-a concentrations to provide synoptic images of phytoplankton biomass and distribution. Subsequently, bio-optical algorithms were developed using radiance ratios for estimating Chl-a concentrations and mapping phytoplankton abundance from the first proof of concept mission called Coastal Zone Colour Scanner (CZCS) launched by NASA in November 1978 (Gordon and Clark, 1981). Some of the ocean colour sensors that provided or currently providing data are Sea WiFS, MODIS, NPP-VIIRS of NASA, U.S.A., OCTS and G.L.I. of NASDA Japan, Sentinel OLCI 3, 3A of E.S.A., European UnionandOceansat1 & 2 O.C.M. India. The spatial extent and temporal dynamics of surface algal blooms can be detected in near real-time using remote sensing techniques, thus providing a quick and effective means to monitor the bloom (Chauhan and Raman, 2017).

The current study documents the HAB occurrences that have been happening frequently over the past three years in fin fish cage culture sites in Mandapam, on the southeast coast of Tamil Nadu, in the Gulf of Mannar, India, as well as their effects on both wild and cultured finfishes.

2. Methods

2.1. In-situ observations

The seasonal occurrence of *Noctiluca sp* bloom during three consecutive years viz., 2019, 2020 and 2021 were recorded. The latitude and longitude of the sampling stations were recorded using a portable Global Positioning System (Garmin GPS map 76, USA). Twenty litres of surface sea water was collected onboard fishing vessel from the surrounding waters off Mandapam and filtered using a bolting silk of mesh size 40 µm. The collected phytoplankton were preserved in 10 % neutralised formalin till taxonomic identification (Tomas,1997) and quantitative estimation using Sedgewick-Rafter counting chamber. *Noctiluca* sp. that caused the HAB in all the three cases were photographed using Oxion camera equipped with Image focus 4English version software. The water samples were collected and analysed following standard procedure

Fig. 2. Occurrence of *N. scintillans* bloom at Mandapam on 15th September 2019.

(APHA, 1995). The water samples were collected in separate polythene bottles for nutrients, chlorophyll-a and in glass stopper bottles for the estimation of dissolved oxygen which were then stored in refrigerator at 4 °C. Chlorophyll a was extracted by adding 90%v/v acetone and analysed by spectrophotometric method (Jeffrey and Humphrey, 1975) using UV-VIS spectrophotometer (Biotek-EPOCH 2). During cruises, discrete water samples form surface and bottom were collected and temperature was measured in situ using digital thermometer (Hanna instrument), pH and salinity by WTW Germany-series Multi parameter 720 water analyzer. The surface and bottom water samples in triplicates were collected in 125 mL transparent DO bottles individually was measured by Winkler's titration method using a digital burette. Nutrients-nitrate, silicate, phosphate, and ammonia were measured as per Strickland and Parsons (1984) using UV-VIS spectrophotometer (Biotek-EPOCH 2). Light transparency in water was measured by Secchi disc having white with black sectors and with a diameter of 24 cm. Atmospheric temperature and wind speed data were obtained from Indian Meteorological Station, Pamban.

2.2. Identification and quantification of fishes affected in the wild

The samples of affected fishes along seashore were collected and brought to the Fishery Biology laboratory of ICAR-CMFRI, Mandapam Regional Centre. The species was identified (Froese and Pauly, 2000; Nair and Kuriakos, 2014) and sorted to different families for counting total fish groups affected. Body weight and stomach weight of the fish recorded to an accuracy of 0.01 g using an electronic balance. Later, the gut of each fish was emptied into a petridish and examined under binocular microscope (Nikon SMZ1270) at 8× magnification. Gut contents were identified up to species level for phytoplankton (Tomas, 1997). The weight of each species collected from a specified area (1 km) is raised to the total affected coastal stretch, where dead fishes beached. Total weight of fish affected during bloom period was estimated by adding the raised weights. Personal enquiry with fishers and village people was also conducted to quantify the fish death.

2.3. Microscopic (wet smear) examinations

A drop of preserved bloom water sample was smeared over the clean glass slide, examined under microscope, (Oxion) photographed in Euromex camera and morphometry details were recorded with the Image focus 4 English version software. To demonstrate the prominent tentacle and nucleus Giemsa and methylene blue staining were done for 5–10 min and washed twice in Phosphate-Buffered Saline (PBS) and examined.

2.4. Gross pathology and histology

Samples of dead fishes from the seashore and those floating on the sea surface (10 nautical miles) were collected during the bloom event, and the species were identified by standard methods (Nair and Kuriakos, 2014). The gut of each fish was emptied into a Petri dish and examined under a binocular microscope (Nikon SMZ1270) at 8× magnification. The dead fishes from wild and sea cages were examined both externally and internally. The affected fish organs and gills were preserved in 10 % neutral buffered formalin for histopathology. Paraffin-embedded tissues were sectioned at 5 μm thickness and stained with haematoxylin and eosin (H&E) for histopathological examination (Roberts and Agius, 2008).

2.5. Satellite data processing

Daily images of MODIS Aqua standard Level-2 Chl-a product at 1 km pixel resolution from the NASA website (http://oceancolor.gsfc.nasa.

Fig. 3. The occurrence of *Noctiluca* bloom (B2) started along the coast of the Gulf of Mannar from Mandapam (9.2770° N, 79.1252° E) to Vedalai (9.2723° N, 79.1040° E) (about 5 km during 27th September 2020.

gov) were scanned during the bloom period in the Gulf of Mannar for the years 2019, 2020 and 2021. There were no cloud-free dates available during September 2019, when the bloom occurred near the cage culture area. Therefore, daily chlorophyll images for cloud-free dates over the cage site during 2020 and 2021 were considered for this study.

Standard Chl-a product from MODIS is the operational satellite based remote sensing global product distributed to users for various marine applications. Chl-a product is generated by implementing a default bio-optical algorithm which employs an empirical Band Ratio (OCx) algorithm (O'Reilly et al., 2000) merged with the Colour Index (CI) algorithm, where x represents the number of bands used in the algorithm. The OCx algorithm exploits the maximum band ratio (MBR) of remote sensing reflectances (Rrs (λ) in blue and green wave lengths and is applied for waters with Chl-a values >0.2 mgm^{-3}. The algorithm depicts a fourth order polynomial relationship between a ratio of Rrs and Chl-a derived from a large set of in-situ measurements of chlorophyll-a concentrations and remote sensing reflectances in the blue-green wavelengths. The functional form OCx is given as

$$Chl = 10^{(a_0+a_1*R+a_2*R2+a_3*R3+a_4*R4)}$$ where,

R = log10(Rrs (λblue) /Rrs (λgreen)) and a0-a4 are sensor specific coefficients.

In case of MODIS Aqua the x of OCx algorithm is 3 using two blue bands 443 nm and 488 nm and one green band at 547 nm. The R then takes the form;

$$R = log_{10}((R_{rs}443>R_{rs}488)/R_{rs}547) \text{ and } a_0=0.2424, a_1=-2.7423, a_2=1.8017, a_3=0.0015, a_4=-1.2280$$

The Colour Index algorithm is applied for oligotrophic waters having Chl-a concentrations ≤0.25mgm^{-3}and is the difference between remote-sensing reflectance at the green band and baseline formed linearly with blue and red bands (Hu et al., 2012). Daily images were obtained from the website as mentioned earlier, and GoM and the region surrounding the cage site were extracted and analysed using the image processing tools provided by Sea DAS 7.5.3 software. Weekly averaged and spatially binned Level 3Chl-a images at 4 km resolution (Aqua/MODIS Level-3 Mapped Chlorophyll Data Version 2018: DOI https://doi.org/10.5067/AQUA/MODIS/L3M/CHL/2018) were also analysed using the online web application tool Giovanni developed and maintained by NASA GES DISC (http://giovanni.gsfc.nasa.gov/giovanni/).

3. Results

3.1. Chronology of bloom events

This study examined the prevalence of toxic algal blooms and their effects on wild fish as well as those raised in sea cages. In the Gulf of Mannar, three *N. scintillans*-related HAB incidences—during September 2019 (B1), September and October 2020 (B2), and October 2021—have been noted (B3) (Fig. 1).

On September 10, 2019, the deep green bloom (B1) was spotted along the Gulf of Mannar's coast between Kunthukkal (9.2723° N, 79.1040° E) and Vedalai (9.2723° N, 79.1040° E), over a distance of about 10 km. By the 14th and 15th of that month, it started to crash in that region (Fig. 2), resulting in mass mortality of about 20 fish species, mostly those connected to coral reefs. It has been determined and measured how the icthyo-diversity was impacted during the bloom period. The bloom persisted till September 28, 2019. It had also caused mass mortality of cobia fish which were being raised in cages by local fisherman in the Gulf of Mannar.

The second incidence of *Noctiluca* bloom (B2) began on September 27, 2020, along the Gulf of Mannar coast from Mandapam (9.2770° N,

Fig. 4. The green *N. scintillans* bloom(B3) was observed along the coast of the Gulf of Mannar stretching from Thonithurai to Seeniyappa Dharga (about 15 km) with the crashing of the bloom occurring around 20th and 21st October 2021. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

79.1252° E) to Vedalai (9.2723° N, 79.1040° E), over a distance of about 5 km. On September 29, 2020, the algae started crashing (Fig. 3). However, on October 5, 2020, the bloom reappeared once more. By the 6th of October 2020, it began to fade away, and by 7th October 2020, it had fully disappeared.

The third occurrence of green *Noctiluca scintillans* bloom (B3) was noticed in 2021 from 8th to 22nd October along the Gulf of Mannar coast from Thonithurai to Seeniyappa Dharga (spread over a distance of approximately 15 km), with the bloom collapsing on October 20 and 21 (Fig. 4) from Mandapam (9.2770° N, 79.1252° E) to Vedalai (9.2723° N, 79.1040° E) (about 5 km).

The initial days of the bloom from October 11 to 19 were marked by persistently strong winds with velocities ranging from 6 to 25 km/h. As a result, the sea became very turbulent and water was turbid due to the harmful algal bloom of *Noctiluca scintillans*. After the heavy rains, the current direction was observed to be from north to south. The bloom disappeared when the northeast monsoon intensified, and the sea returned to normal the very next day.

3.2. Physico-chemical parameters

A detailed study of the mandapam water during the *Noctiluca* sp. bloom was carried out, and the Physico-chemical parameters are documented in Table 1.

Surface water temperature (SWT), as measured in-situ, was consistently higher than 30 °C during the bloom period in 2019, 2020, and 2021. SWT ranged from 31.20 °C to 32.1 °C in 2019, but very barely fluctuated (0.3 °C) between the minimum and maximum temperatures in 2020. (31.1 °C to 31.4 °C). However, in 2021, a 0.6 °C difference between the minimum (31.0 °C) and the maximum SWT temperature was noted (31.6 °C). In the Mandapam area, the recorded atmospheric temperature ranged from 31.4 °C to 34.4 °C in 2019, 31.9 °C to 33.5 °C in 2020, and 31.8 °C to 33.4 °C in 2022.

Salinity levels during the blooms in (2019) ranged from 36 to 37.2 psu. Salinity ranged from 35.2 to 35.5 psu between 2020 and 2021, with no discernible difference. The typical wind speed (Km/h) in Mandapam area during the bloom period ranged from 5.6 to 10.6 km/h. Wind speeds during peak bloom days ranged from 6 to 12 km/h in 2019, 4 to 12 in 2020, and 1 to 11 in 2021.

The mandapam water's ammonia level fluctuated from 0.78 to 6.21 (g-at/L) in 2019; from 0.69 to 6.53 (g-at/L) in 2020; and from 1.21 to 18.73 (g-at/L) in 2021. In 2019, the mandapam water's nitrate concentration ranged from 0.41 to 2.29 g-at L-1; in 2020, it ranged from 0.48 to 2.86 g-at L-1; and in 2021, it ranged from 0.62 to 2.38 g-at L-1. During the bloom time, the silicate range in the mandapam water varied from 1.92 to 5.78 g-at L-1, 1.43–5.61 g-at L-1 and 2.15–7.36 g-at L-1, in 2019, 2020 and 2021 respectively. In 2019, phosphorus levels ranged from 0.41 to 2.12 g/L, while in 2020 it ranged from 0.28 to 1.86 g/L and in 2021 it ranged from 0.42 to 2.68 g/L. Compared to 2020 and 2019, the water's phosphorus content was high in 2021. The dissolved oxygen levels range from 3.24 to 4.35 mL/L in2019, 4.41 to 5.18 mL/L] in 2020 and in 2021 it ranged from 2.4 to 5.23 mL/L. During 2021, a minimum value of 2.40 mL/L was seen.

3.3. Cell density and chlorophyll concentration

At the cage location in 2019, Noctiluca cell density ranged from 1.84 $\times 10^3$ to 8.9 $\times 10^5$ cells/L. During the bloom phase, the in-situ chlorophyll concentration ranged from 1.25 to 9.78 mg/m^3, and during the non-bloom period, it was recorded at 0.085 mg/m^3. At the cage location

Table 1
The Physico-chemical parameters of the mandapam water during the bloom period.

Sl. No	Parameter		2019	2020	2021
1	Atmospheric Temperature (°C)	Min.	31.4	31.9	31.8
		Max.	34.4	33.5	33.4
		Mean	32.74	32.87	32.73
		SD (±)	±0.79	±0.54	±0.62
2	SST (°C)	Min.	31.2	31.1	31
		Max.	32.1	31.4	31.6
		Mean	31.5	31.28	31.35
		SD (±)	±0.27	±0.11	±0.24
3	Salinity (psu)	Min.	36	35.3	35.1
		Max.	37.2	35.6	35.5
		Mean	36.66	35.48	35.33
		SD (±)	±0.41	±0.11	±0.15
4	pH	Min.	7.49	8.12	7.88
		Max.	8.05	8.2	8.14
		Mean	7.84	8.16	8.04
		SD (±)	±0.14	±0.02	±0.1
5	Dissolved oxygen (mL/L)	Min.	3.24	4.41	2.4
		Max.	4.35	5.18	5.53
		Mean	3.96	4.92	4.3
		SD (±)	±0.38	±0.29	±1.18
6	Light transparency (m)	Min.	0.88	1.28	0.76
		Max.	2.33	2.45	2.53
		Mean	1.98	1.91	1.97
		SD (±)	±0.42	±0.49	±0.63
7	Wind speed (kmph)	Min.	6	4	1
		Max.	12	12	11
		Mean	10.6	6.18	5.61
		SD (±)	±1.76	±2.4	±2.97
8	Bloom Cell density (cells/L)	Min.	1.84×10^3	2.38×10^4	2.93×10^4
		Max.	8.9×10^5	2.9×10^5	1.38×10^6
		Mean	3.8×10^5	1.85×10^5	8.62×10^5
		SD (±)	$\pm 2.64 \times 10^5$	$\pm 8.37 \times 10^4$	$\pm 3.87 \times 10^5$
9	Ammonia (μg-at/L)	Min.	0.78	0.69	1.21
		Max.	6.21	6.53	18.73
		Mean	2.87	3.19	5.94
		SD (±)	±1.88	±1.83	±5.37
10	Silicate(μg-at/L)	Min.	1.92	1.43	2.15
		Max.	5.78	5.61	7.36
		Mean	4.02	4.29	5.23
		SD (±)	±1.16	±1.21	±1.45
11	Phosphate(μg-at/L)	Min.	0.41	0.28	0.42
		Max.	2.12	1.86	2.68
		Mean	1.34	1.19	1.63
		SD (±)	±0.51	±0.45	±0.65
12	Nitrate(μg-at/L)	Min.	0.41	0.48	0.62
		Max.	2.29	2.86	2.38
		Mean	1.45	1.78	1.61
		SD (±)	0.6	0.76	0.58
13	Chlorophyll a (mg/m^3)	Min.	1.25	1.14	1.61
		Max.	9.78	11.62	12.85
		Mean	7.23	7.05	8.15
		SD (±)	2.43	3.01	3.12

in 2020, Noctiluca cell density ranged from 2.38×10^4 to 2.9×10^5cells/L. The content of chlorophyll during the bloom period ranged from 1.14 to 11.62 mg/m^3, whereas it was 0.076 mg/m^3 during the non-bloom period. There were 2.93×10^4 to 1.38×10^6 cells/L at the cage site during 2021. The range of chlorophyll values in 2021 was 1.61 to 12.85 mg/m. It has been noted that annual bloom occurrences take place between September and October. In the current study, in-situ measurements showed an upward trend in chlorophyll-a concentration at sea surface beginning on October 8, 2021, and rising to a peak concentration between October 10 and October 12, 2021 (12.85 mg/m3). Between October 13 and October 16, 2021, chlorophyll concentrations began to decline (Fig. 5).

3.4. *Microscopic examination of the bloom*

Based on morphological characteristics, the phytoplankton that caused the bloom was identified through microscopic investigation, and it was later proven to be *Noctiluca scintillans*. The individual cells varied in size from 400 to 900 μm. Large, round, unarmed Noctiluca cells with a single flagellum could be seen (Fig. 6).

They are widely distributed in tropical and arctic environments and highly bioluminescent species with a global range. Green defined the HAB. *Pedinomonas noctilucae*, a photosynthetic symbiont, is found inside the Noctiluca species that creates green tide. This species is a non-photosynthetic heterotroph because its cytoplasm lacks colour and has no chloroplasts. The phytoplankton Noctiluca feeds on other phytoplankton, primarily diatoms, and is phagotrophic. Different diatom species have been found in multiple independent food vacuoles Fig. 7.

These characters confirmed that this dinoflagellate is *N. scintillans*. The plankton abundance during bloom period consisted of 98 % *N. scintillans* and remaining with *Thalassiothrix* sp. 0.3 %, *Navicula* sp. 0.5 %, *Cosinodiscus* sp. 0.5 %, *Rhizosolenia* sp. 0.4 % and *Dinophysis* sp. 0.3 %.

3.4.1. *Fish mortality*

Fish species belonging to 38 genera under 20 families were affected by *Noctiluca* bloom happened in September 2019. They were mostly minor perches and other reef associated fishes (Fig. 8a). The most affected species were from genera such as, *Siganus* (Rabbitfishes), *Epinephelus* (Groupers), *Scarus* (Parrotfishes), *Arothron* (Pufferfishes), *Gymnothorax* (Moray eels), *Saurida* (Lizard fishes), *Lethrinus* (Emperors), *Lutjanus* (Snappers), *Acanthurus* (Surgeon fish), *Sillago* (Sand whiting), *Terapon* (Terapons), *Pelates* (Terapons), *Liza* (Mullets), *Etroplus* (Pearlspot)etc. The algae was noticed in the stomach of surgeonfish in huge quantity, little in pearlspot and parrotfishes whereas in other groups it was not observed in the stomach.

The major fish species affected during October 2021 was remarkably different from previous incident, where in fishes from 43 genera under 25 families were beached due to mass bloom. The most affected genera were *Sardinella* (Lesser sardines), *Nematalosa* (Gizzard shad), *Karalla* (Ponyfishes), *Gazza* (Toothed ponies), *Gerres* (Mojarras), *Terapon* (Terapons), *Pelates* (Terapons), *Alepes* (Scads), *Hemiramphus* (Halfbeaks), *Tylosurus* (Needlefish), *Cypselurus* (Flyingfish), *Arothron* (Pufferfishes), *Gymnothorax* (Moray eels), *Siganus* (Rabbitfishes), *Epinephelus* (Groupers), *Scarus* (Parrotfishes), *Triacanthus* (Triggerfishes), *Netuma* (Giant catfish), *Sepia* (cuttlefish) and *Syngnathoides* (Pipefish) (Fig. 8b). In addition to it few sea turtles also died and washed ashore. Except *Sepia* sp., no other molluscs or crustaceans were affected by the bloom. Similarly, elasmobranchs were also not found along with dead fishes indicating their resilience to the bloom. Altogether 6 t of fish died and washed ashore of 15 km stretch from Mandapam to Rochma Nagar along Gulf of Mannar during 2021. Reef associated eels, groupers, rabbitfishes and parrotfishes also died and washed ashore in Krusadai Island.

3.4.2. *Mortality of cage cultured fishes*

During 2019, a total of 49 brood fishes of cobia (35 males and 14

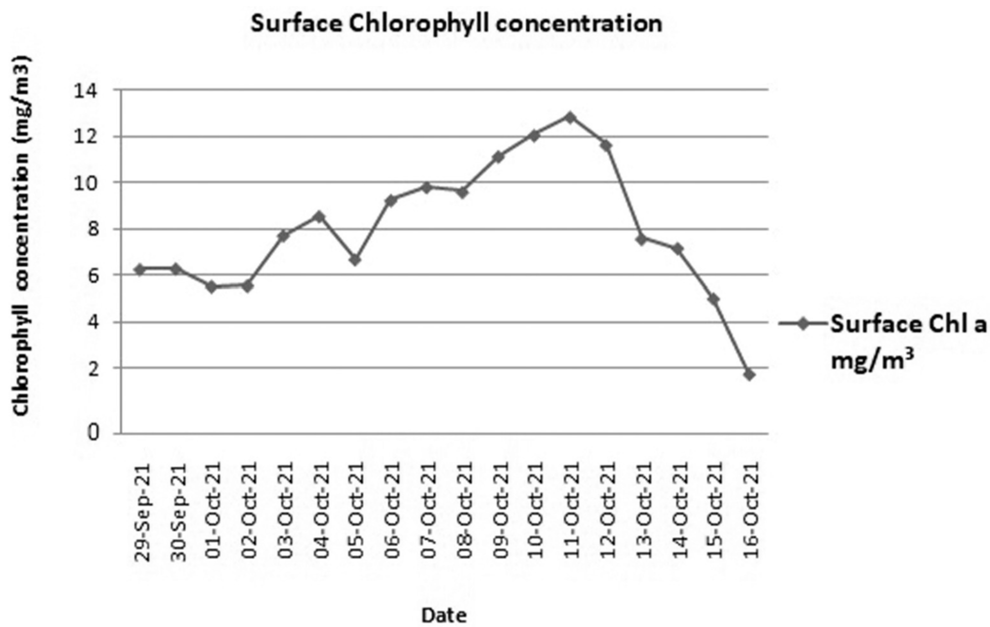


Fig. 5. Chlorophyll concentrations during bloom period 2021.

Fig. 6. Microscopic picture of *Noctiluca scintillans* large and spherical in shape with a single flagellum.

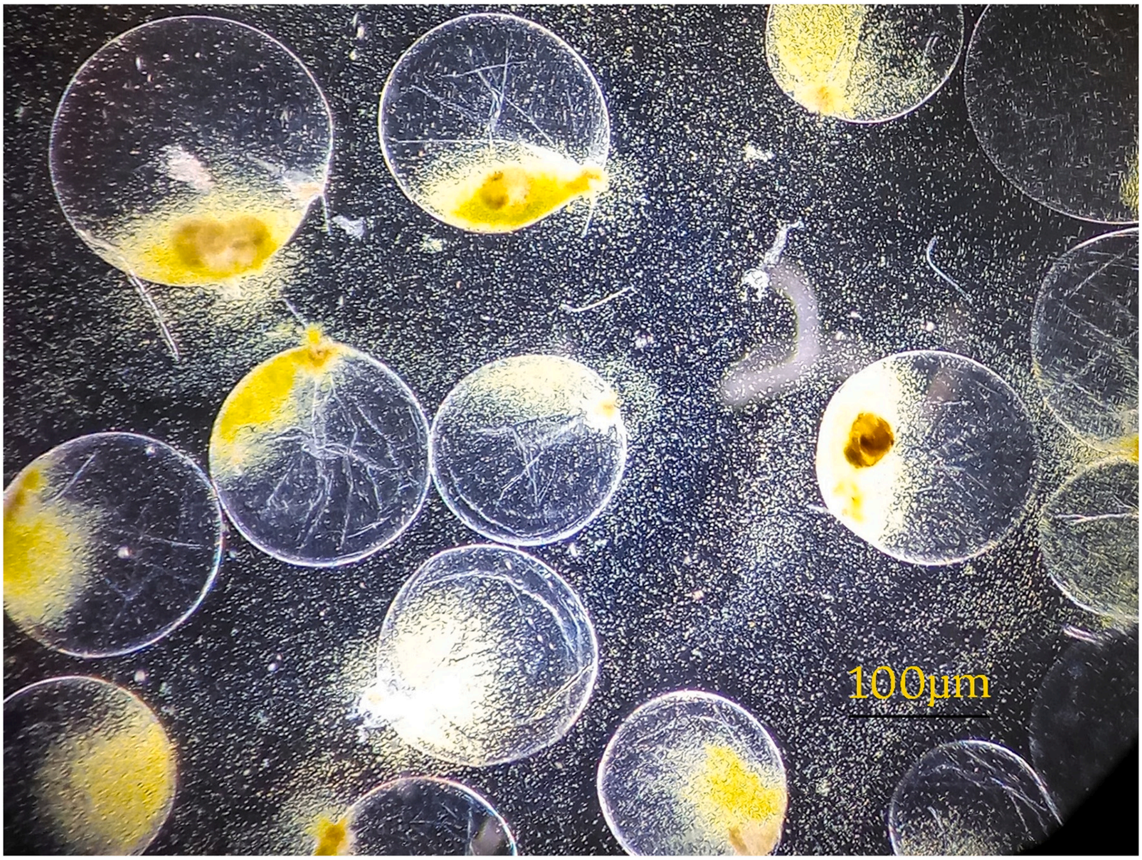


Fig. 7. Microscopic picture of *Noctiluca scintillans* with the presence of several separate food vacuoles containing different diatom species has been observed.

females) were maintained in sea cages for breeding and seed production. A total of 32 cobia brooders (24 males and 8 females) succumbed to death due to choking of the gills and hypoxia within two days of the harmful algal bloom event Fig. 9.

Following this unusual death of fish, they were examined *post-mortem,* and tissue samples were taken for further examination. Efforts were taken on a war footing basis to save the surviving brood fishes from the sea cages and bring them to the indoor recirculation facility of the Centre.

The impact of the bloom on sea cage farming was less severe in 2019 and in 2020 it caused 36.52 % mortality in cobia fingerlings which were being farmed in sea cages. However, in 2021, the bloom caused mass mortality of cage reared fishes which included grow out as well as brooders of various fish species such as *Rachycentron canadum*, *Trachinotus blochii*, *Caranx ignobilis*, *Gnathanodon speciosus*, *Lethrinus nebulosus*, *Lutjanus rivulatus* etc. (Fig. 10).

3.5. Histology

Since the fatality was due to acute hypoxia, only the gill tissues were taken for the histological examination. The section of gills revealed general congestion and moderate to severe hypertrophy of primary lamellae. The primary lamellar odema and erosion and necrosis of secondary lamellae were the predominant lesions of the all fishes. Due to the congestion, there was extreme hyperaemia and haemorrhage observed in the secondary lamellae. The secondary lamellae of the pompano gills showed degradation and necrosis, and the primary lamellae showed hypertrophy. In all the gill filaments, there was uniform primary lamellae hypertrophy (Fig. 11a). The secondary lamellae additionally showed hyperaemia and haemorrhage (Fig. 11b).

The medial or shaft of the cobia gills as well as the tip of the gills both exhibited medullary hypertrophy and formed spear-shaped synechiae at the tip of the primary lamellae (Fig. 12a). The medullary part also revealed oedema and hypertrophy (Fig. 12b). The secondary lamellae displayed erythrocyte aggregation, hyperaemia erosion, and necrosis. Complete necrosis and sloughing of secondary epithelium with cellular reaction also observed in the secondary lamellae. The dilatation of the primary lamellae and medullary cavity are the main lesions observed in almost all the fish gills. This acute hypertrophy of the medullary region revealed acute respiratory lesions. Histology of the wild fish gills were not carried out due to the extreme autolysis.

3.6. Chlorophyll images from satellite data

Daily data of chlorophyll images had cloud and glint-contaminated pixels for most of the regions in GoM and therefore were masked in the standard product. Only 3 cloud-free days for 2020 and 4 clear sky dates for 2021 near the cage culture sites were obtained during the bloom period. In order to analyse the distribution of chlorophyll concentration near the cage site and in GoM during the bloom period, weekly averages were also considered. On 1st October 2020, most regions of the GoM was cloud-covered except few areas south of the cage site and coastal off-shore waters in the northeast. As can be seen from the figure (Fig. 13a), large patches of very high bloom-like chlorophyll concentration is observed along the coastal areas of Perriyapattinam -Muthupetta-Mandapam where the cages were located and in the adjacent continental shelf waters.

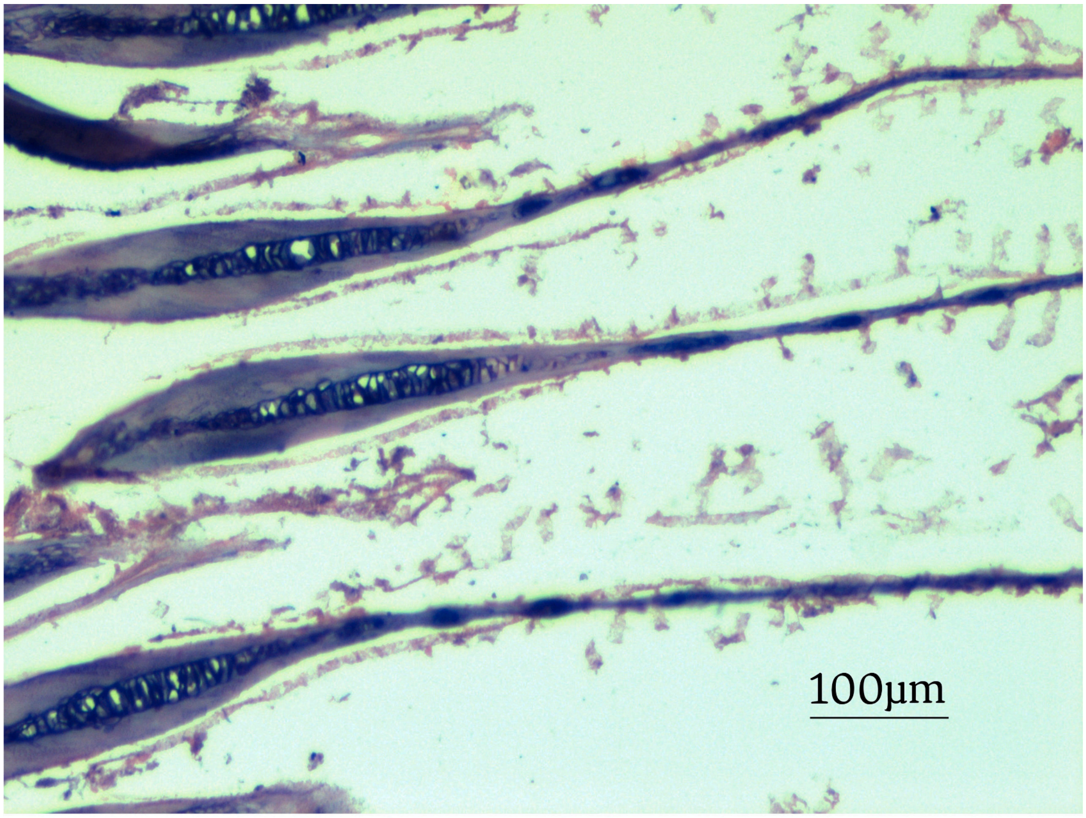
The central GoM is cloud-covered. The chlorophyll values ranged from 8 to 25 mgm^{-3} compared to 0.85–3 mgm^{-3} in the surrounding non-bloom waters of GoM. On 3rd October 2020 chlorophyll image (Fig. 13 b) revealed the bloom to occupy the central-eastern GoM waters in a semi-circular pattern flanking the south-western coast of Sri Lanka. Elevated chlorophyll concentration (15–45 mgm^{-3}) is contained in a ribbon-like pattern. Narrow filaments of high chlorophyll concentration

Fig. 8a. *N. scintillans* bloom caused mass mortality of fishes associated with coral reefs during September 2019.

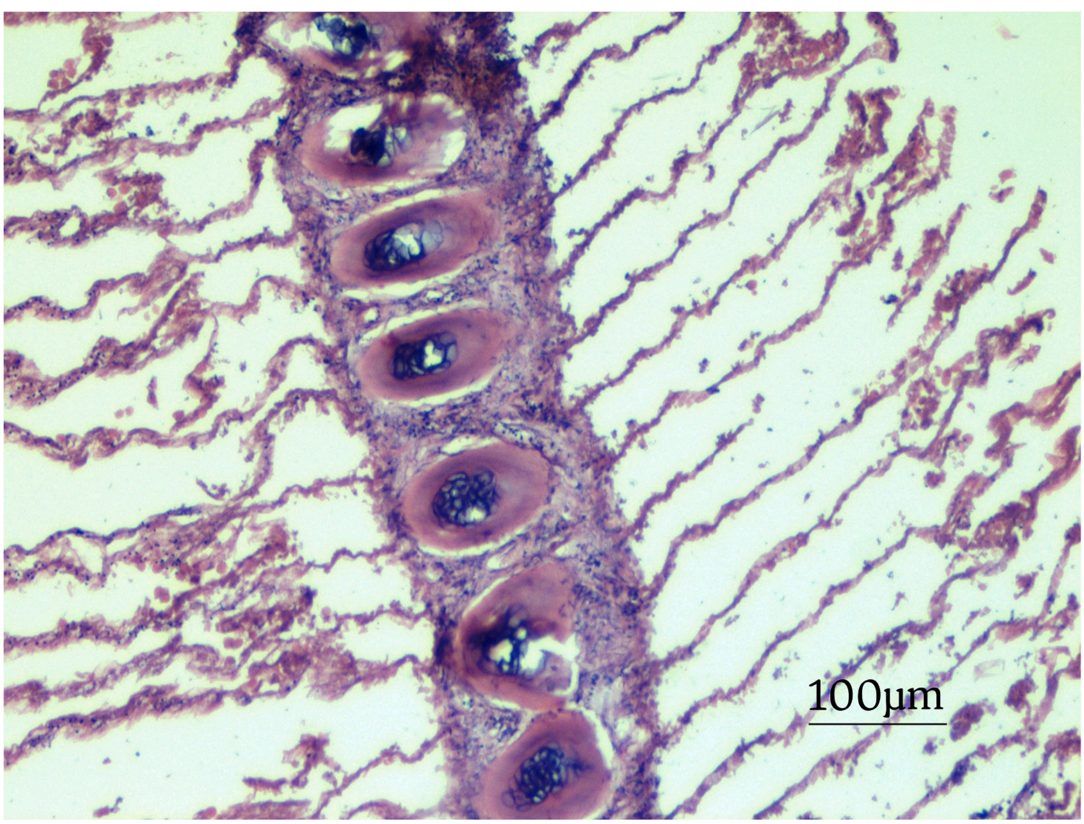
Fig. 8b. Fish death observed along bloom affected area in Mandapam during October 2021.

Fig. 9. Mortality of cage reared fishes during harmful algal bloom event in 2019.

Fig. 10. During, 2021 the cage cultured fishes like, Cobia (*R.canadum*), *Caranxignobilis*, Silver pompano (*Trachinotusblochii),* and *Lethrinus* sp. in all the cages were killed completely, which were maintained by M.R.C. of CMFRI.

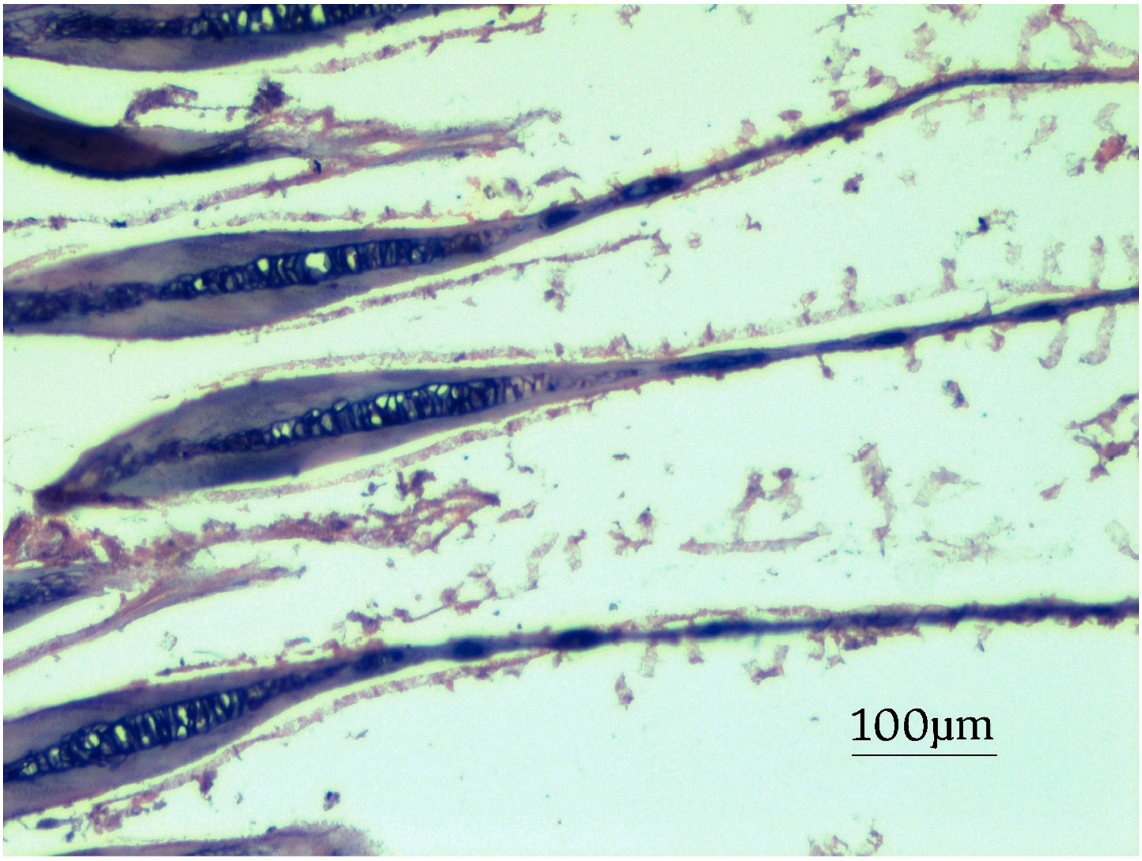


a



b

Fig. 11. a. Pompano gill. Uniform hypertrophy of the primary lamellae was observed in all the gill filaments. H&Ex40x
b. Hyperaemia and haemorrhagic secondary lamellae.H&Ex200x.



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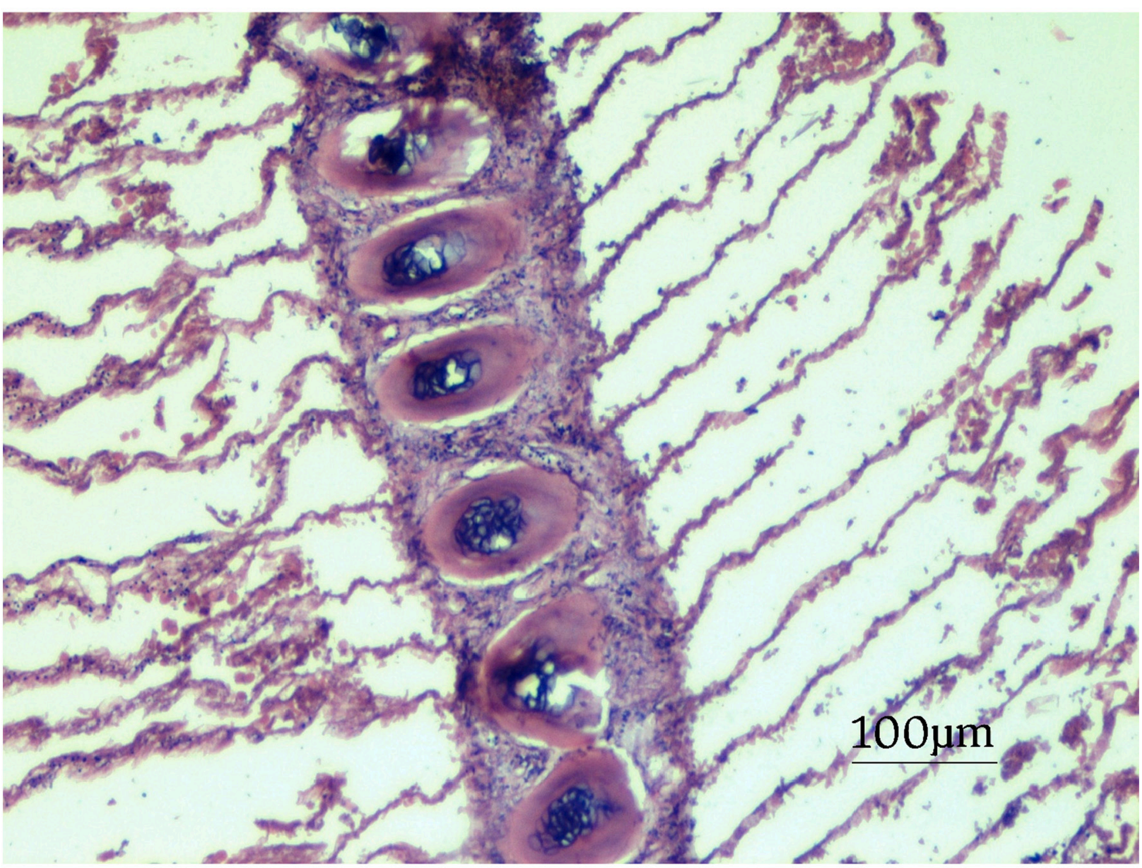


b

(caption on next page)

Fig. 12. a. Synechiae formation was at the tip of the lamellae, and it showed spear-shaped complete necrosis of secondary lamellae and sloughing off of secondary epithelium. H&Ex100x
b. The dilatation of the primary lamellae and medullary cavity. The secondary lamellae revealed aggregation of the erythrocytes, hyperaemia erosion and necrosis. H&Ex100x.

appear to be originating from this feature. The bloom appears to be drifting toward the north according to the flow of currents in GoM, and bloom patches can still be observed stretching along the coastal areas of Perriyapattinam -Muthupetta-Mandapam. The coastal areas and the cage site were cloud-covered in the image on October 19, 2020; however, bloom filaments can be seen in the central-eastern region of GoM, south of the cage site. Chlorophyll concentrations are less intense than 3rd October 2020 (Fig. 13c), with values between 10 and 22 mgm^{-3}.

A similar pattern of bloom–like chlorophyll distribution is observed during 9thSeptember 2021, 13, 18 and 22 October 2021 (Fig. 14 a, b, c&d). Subsequent images for next two weeks were masked due to intense clouds, therefore, the declining phase of the bloom could not be studied.

Weekly averaged images of the GoM, were extracted to analyse the latitude-wise trend in chlorophyll concentration. The extracted region where the bloom was observed to occur covered the central –eastern GoM and coastal waters surrounding the cage sites. As can be seen from the graph (Fig. 15) during September 2019 minimum chlorophyll concentration during the 1–4 weeks in the central-eastern waters of GoM (8°-8.7°) ranged between 0.43 and 0.79 mgm^{-3}. However, the near-coastal regions and near the cage site (9°- 9.3° N) chlorophyll concentrations were observed to be >1 mgm^{-3} (2.9–4.2 mgm^{-3}), indicating presence of bloom.

During the week from 29 September to 5 October, anomalously high chlorophyll concentrations occurred both in central-eastern GoM and in the coastal region. Values ranging from 6 to 18 mgm^{-3} were observed in central-eastern GoM whereas the coastal waters surrounding the cage sites showed a maximum value of 12.6 mgm^{-3}and minimum value of 1.8 mgm^{-3}. Bloom appears to be well established during the first week of September 2020 in the central-eastern GoM region with values as high as 17.8 mgm^{-3}. A maximum value of 25.6 and 20.9 mgm^{-3} chlorophyll was observed during the 2nd and 3rd week of September along 8.95°-8.91° N latitude close to the coastal waters near the cage site(Fig. 16).

Bloom conditions were near-stable during the 4th week of September, with slight decrease in the maximum value of chlorophyll (19.6 mgm^{-3}). Minimum chlorophyll values were maintained at 3.52mgm^{-3}during 3-4th week. High chlorophyll concentrations persisted during the first three weeks of October 2020, with maximum values slightly decreasing from 18 to 16 mgm^{-3}. The last week of October showed a declining trend in the bloom with a maximum value of 8.6 mgm^{-3} and a minimum value of 0.82 mgm^{-3} in the central-eastern GoM. Almost similar conditions prevailed in the coastal waters between 9°- 9.3° N surrounding the cage site. The graph shows elevated chlorophyll concentrations of 16.3, 23.2, 10.01, and 9.9 mgm^{-3} from 1 to 4 weeks of September 2020. Chlorophyll concentrations peaked again during the 1st -2nd week of October 2020 with maximum values reaching 12.6–13.5 mgm^{-3} followed by a sudden decrease in bloom concentration (~2.3 mgm^{-3}) in the subsequent three weeks, indicating the process of degradation of bloom.

4. Discussion

Occurrence of HAB events by the species *Noctiluca scintillans* in Indian waters has increased greatly (Gomes Rosário Do et al., 2008; Griffith and Gobler, 2020). The blooming of *N. scintillans* and associated fish mortality in Gulf of Mannar was first reported during the month of October 2008 along the Appa, Thalaiyari and Valai Islands followed by intensified blooming near Muthpettai, Kilakkarai and Pamban. The bloom persisted for about 12 days before declining (Gopakumar et al., 2009). In the present case, the harmful algal bloom was green colour, and it persisted for nearly two weeks in all the three years.

Warmer temperatures >28 °C and high salinity (>34 psu) seem to favour the proliferation of *N. scintillans species* in GoM. In our present case, SST during 2019, 2020 and 2021 varied from 31.2 °C to 32.1 °C, although minor year-to-year variations was observed. These results are in contradiction to the regular occurrence of *N. scintillans* bloom of the Northern Arabian Sea (N.A.S.) during the winter monsoon period where SST <26 °C favours its growth and spread. (Dwivedi et al.,2008).

The salinity levels above 35 psu showed that Mandapam and the surrounding area did not experience significant freshwater runoff or rainfall, which could have favoured the bloom of *N. scintillans*. Higher salinity is observed during *N. scintillans* bloom in N.A.S. where evaporative cooling and convective mixing brings dense saline waters to the surface. (Dwivedi et al., 2008; Gomes Rosário Do et al., 2008).

Measurement of ammonia during the bloom indicates a very high level in all three years which varied from 0.78 to 18.73 μg-at/L (Table 1) as compared to normal ammonia level in seawater (<0.05–0.1 μg-at/L or nil). *Noctiluca* by virtue of its massive size, is known to accumulate more ammonium ions in its large cell vacuoles, which allows them to be positively buoyant. (Elbrachter and Qi, 1998; Umani et al., 2004). The ammonia is excreted by *N. scintillans* during voracious feeding of other phytoplankton (Okaichi and Nishio, 1976; Fukuyo et al., 1990). During the two weeks of extended bloom in 2019 & 2021, high cell density of the *N. scintillans* led to the accumulation of large concentration of ammonia in surface waters and subsequent decomposition of the bloom, also resulted in high ammonia levels (1.8 mg/L). The dissolved oxygen content at the cage site and other sites in GoM decreased to <5 mg/L as a result of the bloom's breakdown and elevated ammonia (Table 1). This resulted in mass mortality of cage reared and wild fishes, as mentioned in the previous section. When the dissolved oxygen level is below 3.5 mg/L, the fishes are more stressed and in hypoxic conditions, leading to mortality (Hughes et al., 2020; Rabalais et al., 2010). Histological lesions revealed the dilatation of the primary lamellae and medullary cavity with synechiae formation at the tip of the lamellae showed spear-shaped lamellar hypertrophy. The complete erosions and necrosis of secondary lamellae with sloughing of secondary epithelium were main lesions of the suffocated moribund fish gills. The dilatation of the primary lamellae and medullary cavity, aggregation of the erythrocytes, hyperaemia erosion and necrosis were the main gill lesions of all the species. The fish would have to use more oxygen than the amount they could extract from water, thus dying due to oxygen deficiency (Jenkinson, 1993). Mortality of wild fishes and other marine organisms washed ashore along the shores in the Mandapam region and over coral reefs of GoM during *N. scintillans* bloom event in September 2019 was also reported by Gopakumar et al. (2009) and Raj et al. (2020). Sahayak et al. (2005) reported mortality of marine organisms caused due to *N. scintillans* bloom on the southern Kerala coast. However, no mortality in cage reared fish was encountered during 2020 because *Noctiluca* bloom was less dense and patched in 4–5 days intervals leading to less concentration of ammonia with no adverse effect on fishes due to accumulation and decay of bloom.

Detection of monospecific bloom-forming phytoplankton from multi-spectral and hyperspectral ocean colour sensors is still an area of intense research and is based on the bio-optical characteristics of the species. Current approaches to detect and identify the bloom-specific phytoplankton depend on prior knowledge and in-situ data that relate the developed algorithms or methods to field observations. (Ahn and Shanmugam, 2006; Dupouy et al., 2011, Choi et al., 2014; Hu et al., 2005, 2010; Dwivedi et al., 2015; Gokul and Shanmugam, 2016). By virtue of their high chlorophyll concentrations (>1 mg m^{-3}) compared to surrounding waters, blooms can be detected in ocean colour images in

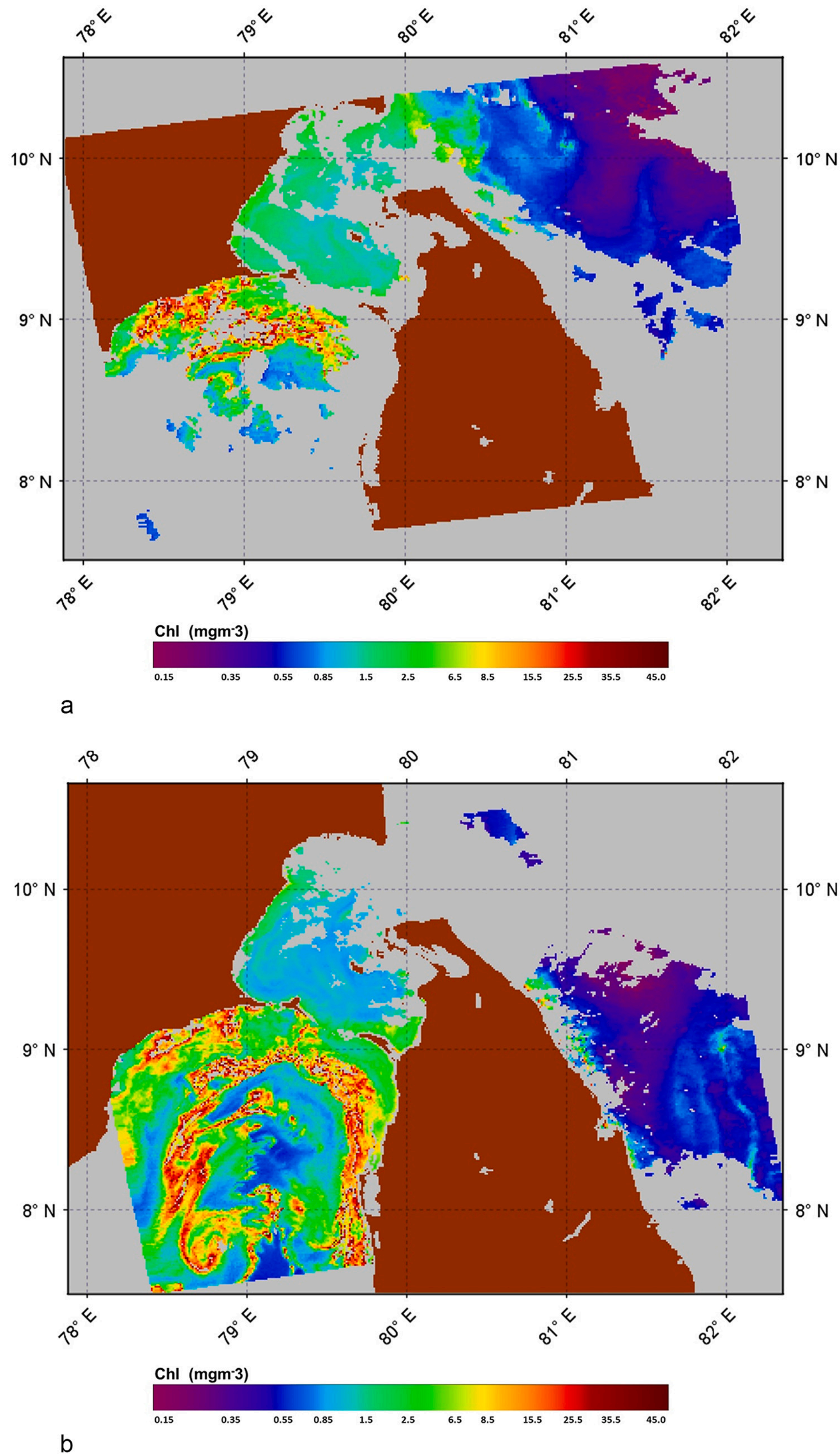


Fig. 13. a. The large patches of very high bloom-like chlorophyll concentration is observed along the coastal areas of Perriyapattinam -Muthupetta-Mandapam where the cages were located and in the adjacent continental shelf waters.

b.The bloom occupies the central-eastern GoM waters in a semi-circular pattern flanking the south western coast of Sri Lanka. Elevated chlorophyll concentration (15–45 mgm^{-3}) is contained in a ribbon –like pattern

c. Chlorophyll concentrations are less intense compared to 3rd October 2020 with values between 10 and 22 mgm^{-3}.

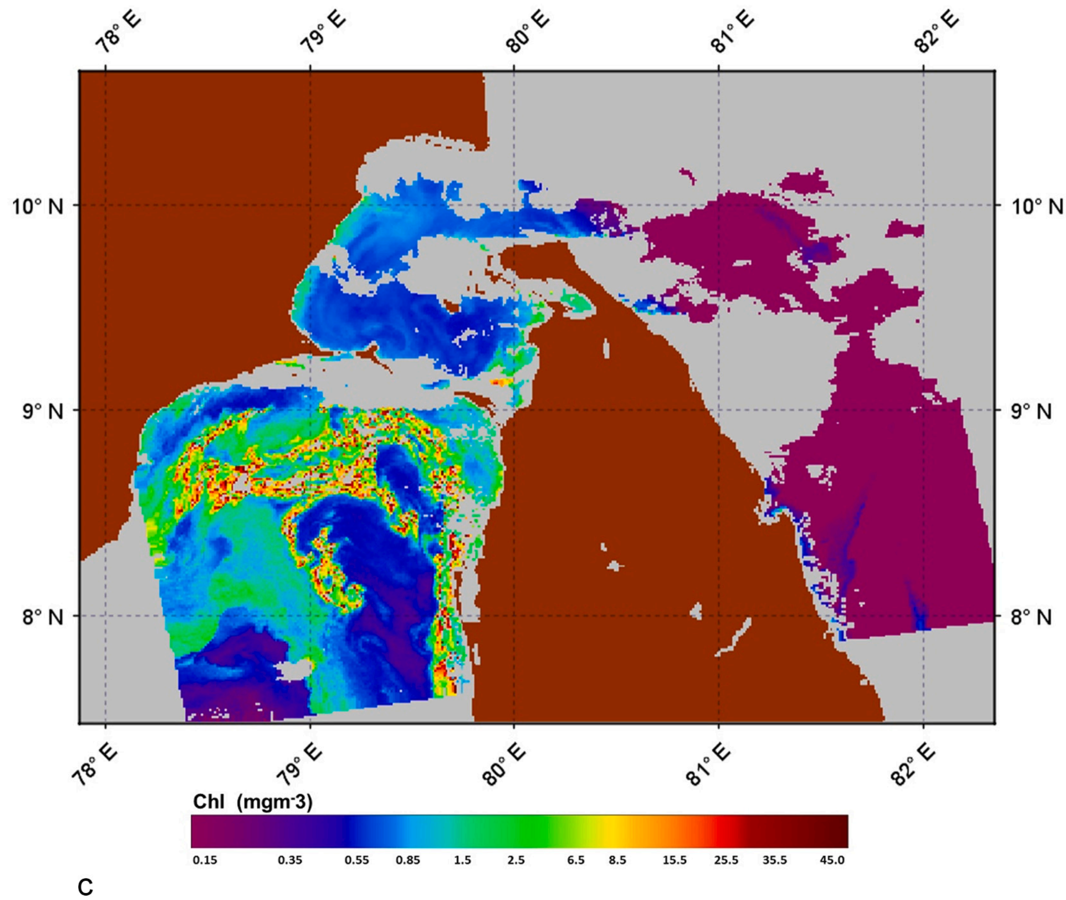


Fig. 13. (*continued*).

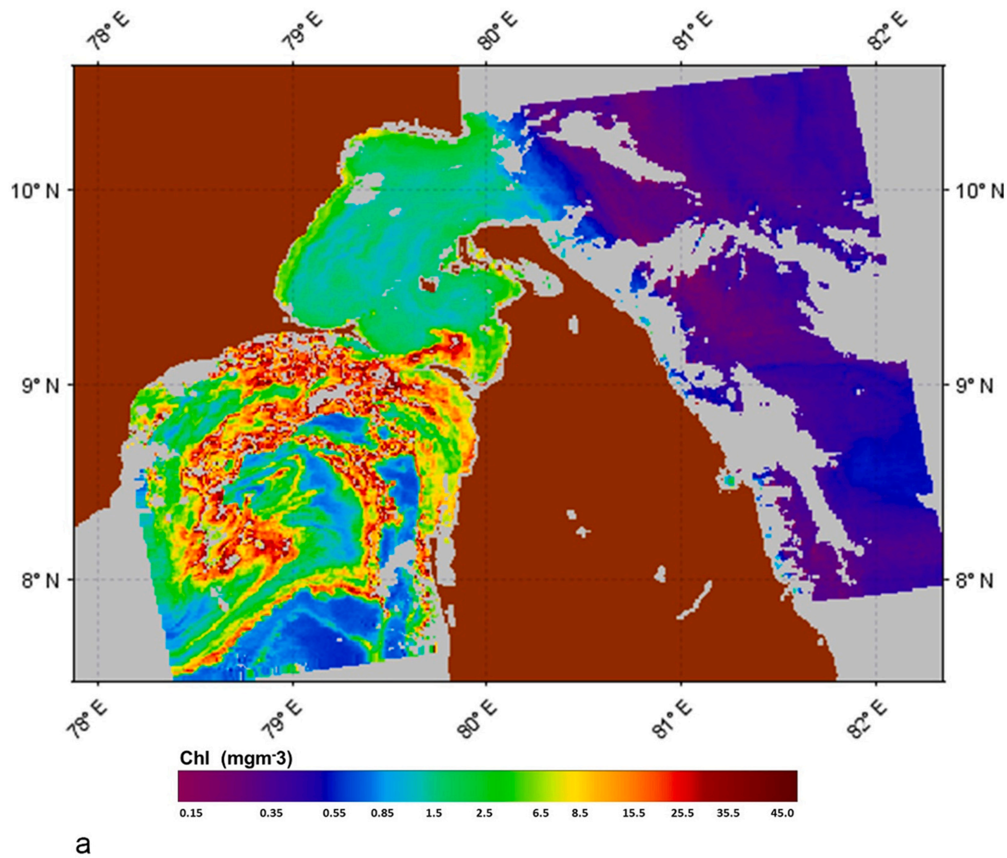
the clear open oceans and shelf regions (Sarangi et al., 2001). In this study, anomalously high chlorophyll concentrations occurring in patches and filaments, as seen from ocean colour images, are associated with the appearance of Noctiluca bloom both in central-eastern GoM and in the coastal regions. According to the MODIS-Aqua-derived OSABI (ocean surface algal bloom index), *N. scintillans* bloom seems to reach the maximum in winter (November–February) and minimum in summer (June–September), especially in the northern Arabian Sea (Tholkapiyan et al., 2014).

Kitatsuji et al., 2019 observed rapid growth of *N. scintillans* population just after increasing chlorophyll concentration and indicated the active feeding of *N. scintillans* on diatoms ending the diatom bloom in coastal waters. In our present observation the in-situ measured chlorophyll-a concentration during the non-bloom period was 0.0854 Chl a/m^3 and the chlorophyll-a concentration during the bloom period was highest at 8.8112 Chl a/m^3during 2021, followed by 2020 (8.144 Chl a/m^3) and 2019 (7.784 Chl a/m^3). The high chlorophyll concentration during the bloom period was also correlated with the satellite image analysis. The abundance of *N. scintillans* correlates with increased chlorophyll concentration. Our findings are also similar to that reported by Fonda-Umani et al. (1983).

N. scintillans bloom has been recorded for three consecutive years, 2019, 2020 and 2021 during the end of south-west monsoon and extending to post-monsoon (August–October) in GoM. Further, Bay of Bengal waters have a predominance of green *N. scintillans* (Harrison et al., 2011). Numerous studies have shown that the south-west monsoons are distinguished by the upwelling of nutrients and the growth of diatom-dominated phytoplankton along the Arabian Sea's south-east coast. The diatom population is thought to have been transported to the eastern part of GoM by the prevailing water current movements and wind direction. The few *Noctiluca* cells that are currently in GoM would have had the chance to feed on the diatom population and expand rapidly, albeit being few in number per litre.

5. Conclusion

Earlier researchers had mentioned *N. scintillans* blooms in GOM, but they were intermittent and of lower intensity. These blooms, which are harmful to both free-ranging and caged fish as well as other creatures, have become more frequent and intense in recent years. By using effective cage management techniques in 2020, the rate of fish mortality in sea cage culture caused by acute hypoxia could be reduced. However, in 2021, complete mortality of cage reared fishes happened due to the bloom. Histologically, all fish gills displayed hypertrophy of the primary lamellae and medullary cavity together with erosion and necrosis of secondary lamellae lesions. Acute respiratory lesions were found in this acute hypertrophy. Hence it is inferred that extremely low dissolved oxygen levels and elevated ammonium concentrations during bloom caused the mass fish mortality. Recent developments in ocean colour remote sensing have made it possible to map abnormally high chlorophyll concentrations, revealing the spatial and temporal dispersion of the GOM bloom as demonstrated in this work. It will be possible to



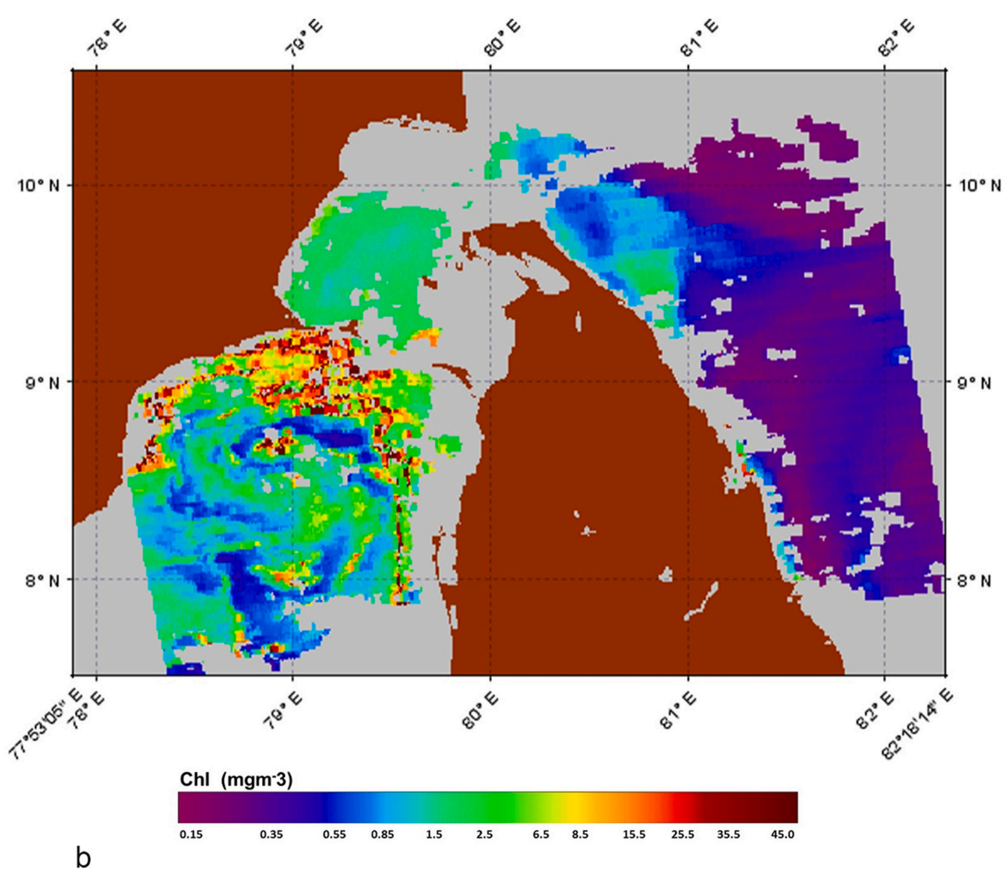


Fig. 14. The Similar pattern of bloom–like chlorophyll distribution is observed during 9th September 2021, 13, and 18 October 2021 (Fig. 14 a, b, c).

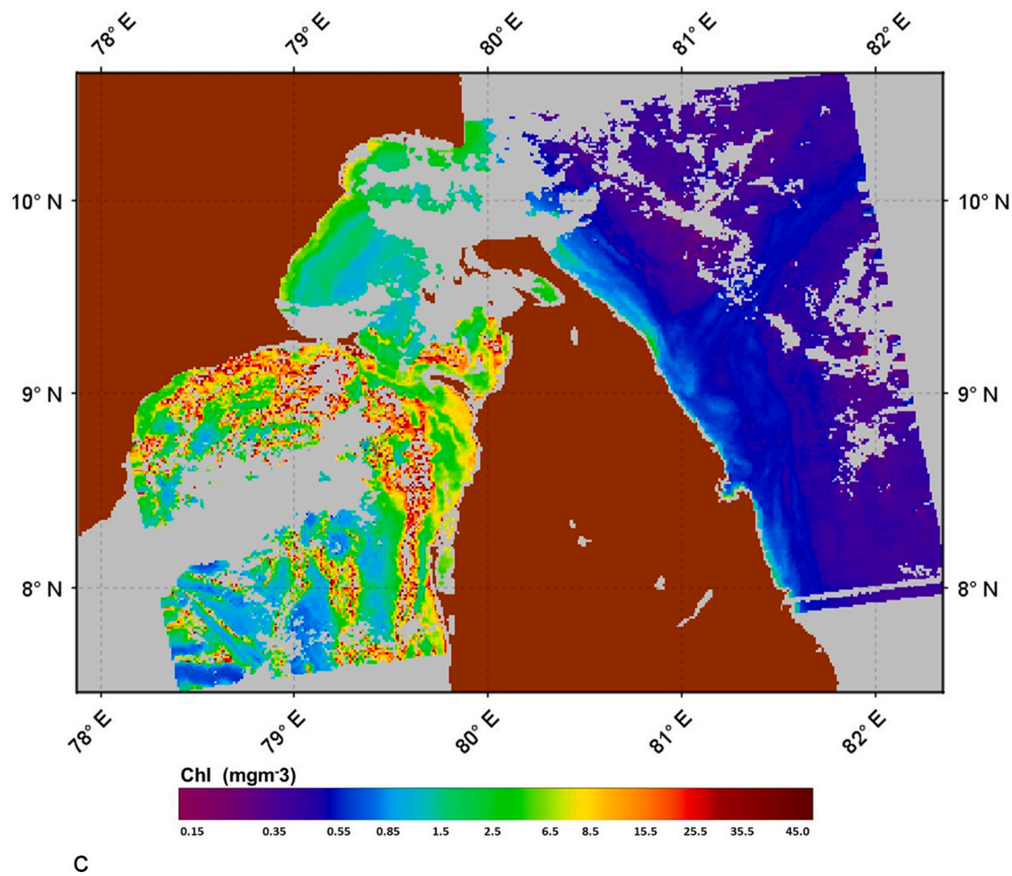


Fig. 14. (*continued*).

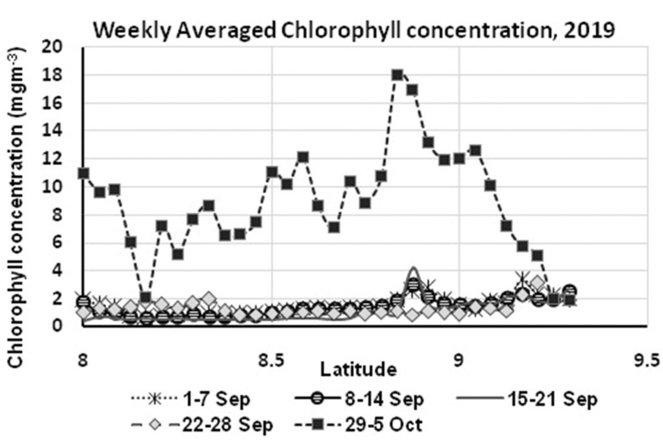


Fig. 15. Graph. The minimum chlorophyll concentration during the 1–4 weeks in the central eastern waters of GoM (8°-8.7°) ranged between 0.43 and 0.79 mgm^{-3} during September 2019.

follow the development of the bloom and send out notifications to cage-culture farmers to harvest the fish before the blooms spread to coastal areas using images of chlorophyll concentration determined from ocean colour and current patterns derived from models in close to real time. The brooders that are kept in sea cages in open waters can either be onshore facilities or temporarily moved to a deeper area in Palk Bay (4-5 ft. depth). Research is currently being done on the identification of monospecific blooms like *N. scintillans*, and it will soon be feasible to identify and monitor this bloom using cutting-edge hyperspectral sensors by investigating the bio-optical characteristics and pigment composition. By offering low-cost, independent data with excellent resolution, the PhytoMOPS (Phytoplankton Morphology and Optical Properties Sensor) gadget will overcome flaws in current monitoring methods. The device will serve as a "early warning" system, identifying HABs as they emerge and allowing governing and statutory bodies to make timely, informed decisions.

CRediT authorship contribution statement

Palsamy Rameshkumar*: Conceptualisation, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. S.Thirumalaiselvan*: Conceptualisation, Methodology, Water sample analysis, Formal analysis, Investigation. Mini Raman: Writing – review & editing, Visualisation, Satellite data validation, Data curation. L.Remya: Writing – review & editing, Visualisation Supervision. R. Jayakumar: Project administration, Funding acquisition, review & editing. M.Sakthivel: Writing – review & editing, Validation, Resources. G. Tamilmani: Review & editing, Visualisation. M.Sankar: Methodology, Formal analysis, Investigation. K.K.Anikuttan-Writing – review & editing, Visualisation. Nandini N. Menon: Writing – review & editing, Visualisation. Raju Saravanan: Supervision, Writing – review & editing. T. T.Ravikumar: Histology, Water sample analysis. IyyapparajaNarasimapallavan: Software, Data curation. N.Krishnaveni: Software, Datacuration. V. Muniasamy: Sample collection, Water sample analysis. S.M. Batcha: Sample collection, Water sample analysis. A. Gopalakrishnan: Project administration, Funding acquisition, Supervision. * Both authors are equally contributed.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

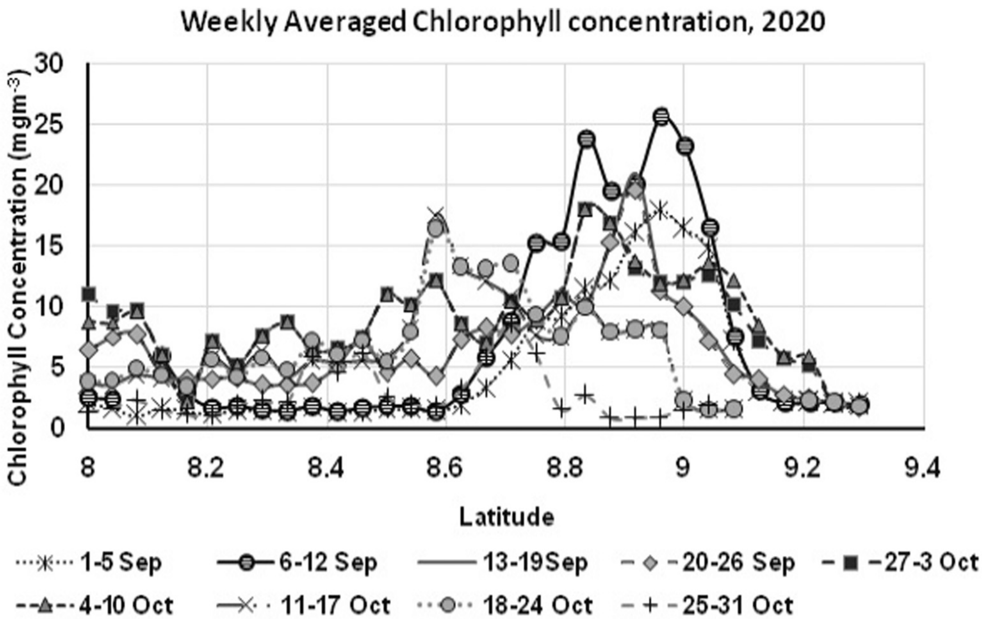


Fig. 16. Graph. Maximum value of 25.6 and 20.9 mgm^{-3} chlorophyll was observed during the 2nd and 3rd week of September along 8.95°-8.91° N latitude close to the coastal waters near the cage site.

the work reported in this paper. The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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