

Noctiluca and copepods grazing on the phytoplankton community in a nutrient-enriched coastal environment along the Southwest Coast of India

¹Arunpandi, N., ^{1*}Jyothibabu, R., ¹Jagadeesan, L., ¹Gireeshkumar T.R., ¹Karnan, C., ²Naqvi, S.W.A

¹CSIR- National Institute of Oceanography, Regional Centre, Kochi, India

²CSIR- National Institute of Oceanography, Dona Paula, Goa, India

*Corresponding author: rjyothibabu@nio.org

Phone +91 (0) 484 2390814, Fax +91 (0) 484 2390618

Abstract

The relative grazing impact of *Noctiluca scintillans* (hereafter *Noctiluca*) and copepods (*Acrocalanus gracilis*, *Paracalanus parvus*, *Acartia danae*, and *Oithona similis*) on the phytoplankton community in an upwelling-mudbank environment along the southwest coast India is presented here. This study was carried out during the Pre-Southwest Monsoon (April - May) to the late Southwest Monsoon (August) period in 2014. During the sampling period, large hydrographical transformation was evident in the study area (off Alappuzha, Southwest coast of India); warmer Pre-Southwest Monsoon water column condition got transformed into cooler and nitrate rich hypoxic waters during the Southwest Monsoon (June - August) due to intense coastal upwelling. Copepods were present in the study area throughout the sampling period with a noticeable increase in their abundance during the Southwest Monsoon. On the other hand, the first appearance of *Noctiluca* in the sampling location was during the Early-Southwest Monsoon (mid - June) and thereafter their abundance increased towards the Peak-Southwest Monsoon. The grazing experiments carried out as per the food removal method showed noticeable differences in the feeding preferences of *Noctiluca* and copepods, especially on the different size fractions of phytoplankton. *Noctiluca* showed the highest positive electivity for the phytoplankton micro-fraction (av. 0.49 ± 0.04), followed by nano-fraction (av. 0.17 ± 0.04) and a negative electivity for the pico-fraction (av. -0.66 ± 0.06). In total ingestion of *Noctiluca*, micro-fraction contribution (83.7%) was significantly higher compared to the nano- (15.7%) and pico- (0.58%) fractions. On the other hand, copepods showed the highest positive electivity for the phytoplankton nano-fraction (av. 0.38 ± 0.04) followed by micro- (av. -0.17 ± 0.05) and pico- (av. -0.35 ± 0.05) fractions. Similarly, in total ingestion of copepods, nano-fraction (69.7%) was the highest followed by micro- (28.9%) and pico- (1.37%) fractions. The grazing pressure of *Noctiluca* on the total phytoplankton was found to be 27.7% of the standing stock and 45.6% of the production, whereas in the case of copepods, it was 9.95% of the standing stock and 16.6% of the production. The study showed that the grazing pressure of *Noctiluca* on the total phytoplankton as well as larger phytoplankton fraction was 2.8 and 8 folds higher than that of the copepods. This suggests the leading role of *Noctiluca* as an effective grazer of larger phytoplankton along the southwest west coast of India, especially during the Peak/Late Southwest Monsoon.

Key words: Chlorophyll *a*, size fraction, *Noctiluca*, grazing, copepods.

1. Introduction

Conventionally, copepods are considered the major grazers of phytoplankton in the secondary trophic level, although their feeding preferences and ingestion rates on different size classes of phytoplankton are not completely known (Calbet et al. 2000). Studies in the past have showed that size and composition of the prey in an environment significantly alter the grazing rates of copepods, and this, in turn, can even induce a shift in the dominance of copepod species (Stoecker and Capuzzo, 1990; Froneman, 2006). Seasonal shift in the composition and abundance of copepods is a characteristic of the Indian coastal waters in relation to the seasonal hydrographical transformations occurring semi-annually (Madhupratap et al. 1991; Smith and Madhupratap, 2005; Jagadeesan et al. 2013). It is also observed that the phytoplankton biomass built-up along the southwest coast of India is associated with the coastal upwelling during Southwest Monsoon period, which is believed to be the result of inefficient top-down control (grazing pressure) by zooplankton, especially copepods (Banse et al. 1996). Such ungrazed large phytoplankton stock in the upper euphotic layer eventually sinks down and contributes to the organic carbon pool in the water column along the western shelf of India during the late Southwest Monsoon (Naqvi et al. 2006). It is a fact that the actual quantification of copepods grazing on natural prey assemblages are less known not only from the Indian waters but, from the rest of the world ocean as well (Jagadeesan et al. 2013).

Noctiluca is a large (usually 150 to 700 μm in diameter) heterotrophic dinoflagellate capable of ingesting a variety of prey through food vacuoles (Elbrachter and Qi, 1998; Dela-Cruz et al. 2002; Frangopulos et al. 2011). When *Noctiluca* proliferates in the surface waters, they attribute either green/yellowish green or red colouration to the surface ocean; this has been commonly termed as 'red tide' and a list of such bloom records from Indian Seas has been presented (Table 1). *Noctiluca* by itself is a colourless organism with clear protoplasm and the green/yellowish green/red colour associated with it during massive proliferation is due to the coloured endo-symbionts (Sweeny, 1976; Hansen et al. 2004; Furuya et al. 2006; Harrison et al. 2011; Turkoglu, 2013; Gomes et al. 2014). The high abundance of red *Noctiluca* is a seasonal feature in the Arabian Sea during the Southwest Monsoon (Table 1), though the possible ecological role played by them in the lower level food web in the region is yet to be resolved.

During the Southwest Monsoon (June - September), the present study area (off Alappuzha) is influenced concurrently by two oceanographic events. One is the coastal upwelling that facilitates high nutrient availability in the surface waters, promoting luxuriant phytoplankton growth especially during the Peak and Late Southwest Monsoon (Ramasastriy, 1959; Silas, 1984; NIO Report 2008; Jagadeesan et al. 2016). The other one is the mud bank, defined as patches of calm littoral waters

with relatively high concentration of suspended sediments, which has special scientific and community interest due to the rich fishery associated with it (Bristow, 1938; Damodaran, 1973; Gopinathan and Qasim, 1974; Silas, 1984). The phytoplankton community in the study area is usually dominated by diatoms while sporadic incidences of *Noctiluca* red tide also occur during the Southwest Monsoon (Subrahmanyam et al. 1975; Nair et al. 1984). Recent studies elsewhere indicate the role of *Noctiluca*, an organism considered earlier as an autotroph, in controlling the phytoplankton community, especially in eutrophic conditions (Jocelyn et al. 2000; Turkoglu, 2013).

Based on the above background, we augmented a standard oceanographic sampling off Alappuzha, a mud bank-coastal upwelling region along the southwest coast of India, with a set of grazing experiments with the following objectives: (a) to understand the temporal hydrographical transformations and the associated fluctuations in the occurrence and abundance of copepods and *Noctiluca*, two potential grazers of phytoplankton in the study region (b) to estimate the feeding preferences (electivity index) of copepods and *Noctiluca* on the total and different size classes of phytoplankton (c) to measure the grazing rate of dominant copepods (>200µm in size) and *Noctiluca* on the total and the size fractions of phytoplankton, and (d) to infer the possible ecological role played by the dominant copepods and *Noctiluca* in the food web during the peak and late - upwelling periods.

2. Materials and methods

2.1. Sampling and methods

The present study was carried out as a part of the ‘Alappuzha Mudbank Process Studies (AMPS),’ an initiative of CSIR-National Institute of Oceanography, India to investigate the Alappuzha Mudbank. Under AMPS, 18 time series field sampling sessions were carried out in the coastal waters of Alappuzha, where mudbank occur during the Southwest Monsoon period, on weekly/biweekly intervals from April to September, 2014 (Fig. 1). During the field observations, the vertical profiles of temperature and salinity were measured using a calibrated Seabird CTD conductivity, temperature and depth (SEACAT SBE 19 plus) profiler. In order to measure the temporal change in the concentration of nutrients, dissolved oxygen, surface water samples were collected using a Niskin sampler. Dissolved oxygen was measured following Winkler’s method and nitrate using standard procedures of Grasshoff et al. (1983). The prevalence of coastal upwelling and mudbank was observed concurrently in the study area during the Southwest Monsoon (June - September) (Fig. 2).

2.2. Abundance of *Noctiluca* and copepods

In order to track the occurrence of *Noctiluca* in the study area during the time series observations, 2 litres of surface water samples were collected and preserved in Lugol's iodine. In the laboratory, the preserved samples were concentrated by siphoning using a 20 μ m mesh. The sample volume was concentrated up to 10 ml and inspected under the microscope for the presence of *Noctiluca*; if present, their counts were taken using a Sedgwick rafter counting chamber. For measuring the copepod density and their species composition, zooplankton samples were collected using horizontal tows of WP-2 net (200 μ m) fitted with a Hydrobios flow meter (Postel et al. 2000). In the laboratory, the zooplankton groups were sorted out and the abundance of copepods were estimated. Sorted copepods were further analysed for species-level identification using standard literature (Sewell, 1929; Kasturirangan, 1963).

2.3. Isolation of *Noctiluca* and copepods

When *Noctiluca* cells were found abundant in the routine zooplankton net collections in July, a separate net tow was carried out in slow speed to collect *Noctiluca* and copepods for conducting the grazing experiments. The cod end of the net was carefully transferred into 20 litre-volume dark containers filled with seawater from the same environment and transported to the laboratory. Additionally, surface seawater (as natural food suspension) was also collected using Niskin samplers, filled in 20 litre carboys and transported to the laboratory. The live plankton brought to the laboratory was acclimated in large tanks for 3 hours, during which *Noctiluca* cells concentrated on the sides of the tank at the surface. They were separated initially to another acclimation tank and then placed in conical flasks with 0.2 μ m filtered natural sea water. Dominant copepods were separated from the acclimatized tanks by large pipettes and placed separately in 250 ml beakers with 0.2 μ m (Whatman GF/F) filtered natural sea water. Maximum care was taken while handling the live specimens for grazing experiments and only undamaged *Noctiluca* and copepod specimens were used for experiments.

2.4. Grazing experiment

2.4.1. Experiment setup

Three standard experimental methods available for the quantification of zooplankton grazing include gut fluorescence, food removal, and mandibles in the stomach (Bamsteadt et al. 2000). Each of these methods has merits and demerits and in the present case, food removal method was used to quantify the grazing of *Noctiluca* and copepods. In this method, chlorophyll *a* was used as an index of phytoplankton biomass and incubation experiments were conducted using 1litre borosil screw cap glass bottles. Prior to conducting the experiments, all experimental bottles were acid-washed (10%

HCL) and cleaned with mild soap (Labolene), after which they were rinsed thrice with distilled water. The experimental bottles were filled with the seawater collected from the study region with natural food assemblages. The large and visible grazers of copepods and *Noctiluca* were removed from the natural food assemblages introduced into the experimental bottles. The number of *Noctiluca* and copepods included in the experimental bottles were maintained similar to their abundance in the study area, based on which 150 individuals of *Noctiluca* and 20 individuals of the dominant copepods were introduced into the experimental bottles.

Experiments were designed with initial, control and experimental bottles with a separate set of experimental bottles for *Noctiluca* and copepods (Bamsteadt et al. 2000). In both cases, initial and control bottles were filled with natural seawater containing food assemblages, but in the experimental bottles both food assemblages and *Noctiluca*/ copepods were included. Incubation experiments were conducted in the laboratory and the temperature and light conditions were maintained almost near to the field conditions. Triplicates were maintained for all incubation bottles in the experiments (i.e., 3 control and 3 treatments). The natural food assemblages were filled into all bottles without air bubbles. After including live specimens, all bottles were closed with cap (Bamsteadt et al. 2000). The control and experimental bottles were incubated for 24 hrs in the laboratory at near-ambient temperature of the natural surface seawater (~29°C) and the bottles were gently mixed every 4 hours to avoid the settling of the food assemblages.

Total and size-fractionated chlorophyll *a* in all the incubation bottles was measured at the beginning and at the termination of the experiments. Total chlorophyll *a* was measured by concentrating 500 ml of water samples on Millipore filters (0.2µm) and extracting the pigment using 10 ml 90% acetone. The extracted chlorophyll *a* was measured using a Trilogy fluorometer (TURNER Designs, USA) following the standard procedure (UNESCO, 1994). The size-fractionated chlorophyll *a* was measured by adopting serial filtration of water samples through different filters adopting the procedure followed in Jyothibabu et al. (2013). The size-based phytoplankton thus separated on filters was estimated for chlorophyll *a* to quantify the biomass retained in different size fractions of the phytoplankton community.

2.4.2. Grazing calculations

Ingestion rate (I) was calculated using Frost (1972)

$$I = F \times C$$

Clearance rate (F) - is the volume of food suspension from which *Noctiluca* /copepods would have to remove all cells in the unit of time to provide its measured ingestion; C - mean concentrations of the prey

Clearance rate (F) was calculated by the equation:

$$F = [\ln(C_0 - C_e) - \ln(C_0/C_e)] \times V / (t \times N)$$

C₀ - initial concentrations of the prey; C_e - prey concentrations in control and experimental bottles; V - Volume of the incubation bottle in litre; N - number of organisms added to the incubation bottle and t - incubation time.

$$C = C_0 [e^{Kt} - 1] / K$$

Growth rate of phytoplankton (K) excluding the micro zooplankton grazing:

$$K = \ln (C_0/C_e)$$

Daily ration (DR) was calculated using the formula

$$DR = (I/B)$$

Where I is the ingested carbon (phytoplankton) by *Noctiluca* or copepods per day, B is the body mass (Carbon) of *Noctiluca* or copepods.

2.4.2. Electivity index and grazing pressure

In addition to the ingestion rates of copepods and *Noctiluca*, their preference in different size categories of phytoplankton was quantified through estimating the electivity index E* (Vanderploeg & Scavia, 1979). E* index compares the proportion of a particular prey type in the natural assemblages with respect to the predators' diet. The steps involved in the calculation of E* are presented below.

The concentration of the chlorophyll *a* in each prey type (Micro, Nano and Pico fractions) consumed by copepods (R_i) in each experimental vessel was determined as follows:

$$R_i = \frac{(N_{ic} + N_{tc})}{2} - N_{ft}$$

Where i was the prey item, N_{ic} was the mean chlorophyll *a* present in the initial bottles, N_{tc} was the mean chlorophyll *a* present in the control bottles at the end of the incubation and the N_{ft} was the mean number of individuals present in each treatment bottle at the end of the incubation. The proportion of each prey type (Micro, Nano and Pico fractions) in the diet (*r_i*) of copepods/*Noctiluca* and in the available medium (*ni*) were calculated as:

$$r_i = \frac{R_i}{\sum_{j=1}^m R_j}$$

$$n_i = \frac{N_{ic}}{\sum_{j=1}^m N_{jc}}$$

Where m is the number of prey types (Micro, Nano and Pico) and R_i and N_{ic} were described above; E^* for each prey type was calculated based on the formula

$$E_i^* = \frac{W_i - \frac{1}{m}}{W_i + \frac{1}{m}}$$

Where W_i was defined by the following equation

$$W_i = \frac{\frac{r_i}{n_i}}{\sum_{j=1}^m \frac{r_j}{n_j}}$$

E^* values range from -1 to 1. A value of 0 indicates neutral selection, positive index represents positive selectivity or preference and the negative index represents negative selectivity or avoidance.

For expressing the results in terms of carbon units, all biological data (chlorophyll *a*, the abundance of *Noctiluca* and copepods) were converted into organic carbon units. Chlorophyll *a* was converted to organic carbon considering a factor 50. Copepod biomass carbon was calculated by measuring their Equivalent Spherical Diameter (ESD) using a FlowCAM. The biovolume of copepods was calculated using the formula

$$BV (mm^3) = 4/3 \pi (ESD/2)^3$$

Biovolume was converted into wet weights assuming the specific gravity of copepods to be 1 and, therefore, $1mm^3 = 1 \text{ mg wet mass}$ (Winberg, 1971; Omari and Ikeda, 1984). Wet mass units were converted into dry matter using standard formulae (Madhupratap et al. 1991) and subsequently, dry weight was converted into carbon units considering 34.2% of the dry mass as carbon content (Madhupratap et al. 1991). For *Noctiluca* cells, $91.2 \text{ ngC Cell}^{-1}$ was used for converting their cell abundance into carbon biomass (Tada et al. 2004). The grazing pressure (%) of the dominant copepods and *Noctiluca* on phytoplankton biomass and production was estimated by dividing the total ingestion of the grazer by the standing stock and production of the phytoplankton.

2.5. Statistical analysis

The ecological interrelationship between copepods, *Noctiluca* and phytoplankton stock were analysed using RDA (CANOCO 4.5). The data were tested initially using Detrended Correspondence Analysis for finding the appropriate ordination techniques. The Detrended Correspondence Analysis results showed axis gradient length < 2 , suggesting the use of linear multivariate RDA as the most appropriate (Leps and Smilauer, 2003). The biological variables were log transformed prior to the analysis. The ordination significance was tested with Monte Carlo permutation tests (499 unrestricted permutations) ($p < 0.05$). The result of the RDA is presented in the form of Triplots in which samples are displayed by points, and biological (dashed blue) and environmental variables (red lines) are displayed by arrows.

3. Results

3.1. Temporal changes in environmental features

The vertical distribution of temperature and dissolved oxygen representing 18 field sampling sessions clearly display the major seasonal hydrographical transformation in the study area (Fig. 2). The study period consisted of Pre -Southwest Monsoon (April - May), Early-Southwest Monsoon (June), Peak-Southwest Monsoon (July-August) and Late-Southwest Monsoon (September) periods. The most striking hydrographic feature during the Pre-Southwest Monsoon was the warm water column while during the southwest Monsoon it was the surfacing of the cool and hypoxic upwelled water. Associated with the onset of the coastal upwelling by the Early Southwest Monsoon, a noticeable increase in nitrate was evident in the water column (Fig. 3a), which caused increased phytoplankton biomass during the period (Fig. 3b). Although there were fluctuations in the concentrations of nutrients and chlorophyll a within the Southwest Monsoon period itself, their overall increase from the Pre-Southwest Monsoon conditions was evident during the Southwest Monsoon, especially during the Peak and Late - Southwest Monsoon. The turbidity in the study area showed a noticeable increase during early Southwest Monsoon, as it was associated with mudbank formation in the region; this signature continued till August (Fig. 3b).

3.2. Abundance of copepods and *Noctiluca*

The occurrence and temporal abundance of copepods and *Noctiluca* in the study area was monitored during the entire sampling period. As evident in Fig. 3c, copepods were present in the study area throughout the sampling period with a noticeable increase in their abundance during the Southwest Monsoon. During the experimental sample collection in July, the copepod abundance was 14187 No.m^{-3} , wherein cyclopoid and calanoid copepods together contributed 83.79% of the total

abundance (Table 2). In general, smaller copepods *Oithona similis*, *Acartia danae*, *A. erythraea*, *Acrocalanus gracilis*, and *Paracalanus parvus* dominated the study area during the Southwest Monsoon (Jagadeesan et al., 2016). The first appearance of *Noctiluca* in the study region was recorded during the Early-Southwest Monsoon (mid - June) and thereafter their abundance increased towards the Peak-Southwest Monsoon. Also, it was noticed that the abundance peak of *Noctiluca* was during mid-August, whereas the peak copepod abundance was mid-July (Fig. 3c).

3.3. Phytoplankton size fractions and growth rate

The colour of the water samples collected from the study area for carrying out the grazing experiment is presented in Fig. 4a. It appeared straw/brown- coloured due to the presence of a mixed diatom bloom (*Fragilaria* and *Coscinodiscus*). The photomicrographs of the fractionated phytoplankton stock based on size are presented in Fig. 4 (a -g). Total chlorophyll *a* in the study area during the experimental sample collection was exceptionally high (10.17 mg m^{-3}), in which 53% was contributed by micro-plankton, 46% by nano-plankton and 1% by pico-plankton (Fig. 5a). *Fragilaria* and *Coscinodiscus* were the most dominant forms in the micro-plankton fraction and they together contributed 85% of the total abundance. *Skeletonema coastatum* and *Chaetoceros* sp. formed the most dominant nano-size fraction. *Synechococcus* and pico-eukaryotes mainly contributed to the pico- fraction of the phototrophs. The growth rate of the total and size-fractionated phytoplankton community in the experimental samples was estimated during the grazing experiments. The growth rate of the total phytoplankton community in the experimental water was 0.47 ± 0.06 . The growth rate of micro-plankton (0.46 ± 0.08) was comparable with nano-phytoplankton (0.49 ± 0.11), but noticeably higher than pico-plankton (0.22 ± 0.03) (Fig. 5b).

3.4. Feeding response of copepods and *Noctiluca* on phytoplankton

Before the actual start of the grazing experiments, utilizing the light and fluorescent microscopy, we verified qualitatively the primary feeding response of copepods and *Noctiluca* on phytoplankton. For this purpose, a few individuals (5-10) of dominant copepods and *Noctiluca* acclimated in laboratory conditions and fed with natural food suspension were inspected under an Olympus epifluorescence microscope. Few of the photomicrography evidences are presented in Fig. 6a-d. Representative specimens of dominant copepods and *Noctiluca* were also imaged under the epifluorescence microscope after grazing incubation experiments with different size fractions of the phytoplankton community. All four dominant copepods tested for feeding habit incubations showed that they preferred to feed on nano-phytoplankton as their guts were completely filled with red chlorophyll auto-fluorescence after the incubation. However, when the copepods were incubated

with larger phytoplankton such as *Coscinodiscus* and *Fragilaria*, they underperformed and showed incomplete feeding, traditionally referred to as ‘sloppy feeding.’ The light and fluorescence microscopic evidences of sloppy feeding of copepods on larger phytoplankton cells are presented in Fig. 6(e-h). A video has also been captured to present the sequence of events involved in sloppy feeding, wherein copepods orient closer to the large *Coscinodiscus* cells and after making a protrusion on the cell wall, suck in the protoplasm of the prey fully/ partially and ignore the cell wall and remnants (Supplementary material 1). Alternatively, the *Noctiluca* cells after incubation with micro-size phytoplankton assemblage have been presented in panels Fig. 7(a-e); these provide clear evidences of their feeding response to long *Fragilaria* chains and large *Coscinodiscus*. More importantly, *Noctiluca* were found to be efficient in engulfing long *Fragilaria* chains, with some of the ingested chains being many folds larger than the grazer cell diameter itself (Fig. 7d & e). After the incubation with micro-phytoplankton assemblages, *Noctiluca* cells showed large *Coscinodiscus* cells in their gullet region (Fig. 7a-c).

3.5. Daily ration (DR), electivity index and grazing

The contribution of different size fractions of phytoplankton to the ingestion of copepods and *Noctiluca* presented in Fig. 8a. The ingestion of total phytoplankton and DR of copepods were found to be lower ($5.17 \pm 0.23 \mu\text{g.C.day}^{-1}$ and 0.69 ± 0.11 , respectively) compared to *Noctiluca* ($0.74 \pm 0.08 \mu\text{g.C.day}^{-1}$ and 8.04 ± 0.16 , respectively). Detailed size-fractionated analysis of chlorophyll *a* showed that *Noctiluca* and copepods could ingest all size categories of phytoplankton but with varying preferences (electivity) (Fig. 8b). *Noctiluca* showed high positive electivity (E^*) for micro-plankton ($E^* = 0.49 \pm 0.04$) followed by nano-plankton ($E^* = 0.17 \pm 0.04$) and negative electivity for pico-plankton ($E^* = -0.66 \pm 0.06$). On the other hand, copepods showed the highest positive electivity index for phytoplankton nano-fraction (av. 0.38 ± 0.04) followed by micro-fraction (av. -0.17 ± 0.05) and least for the pico-fraction (av. -0.35 ± 0.05). Copepod community ingestion on total phytoplankton was 50.6 mgC m^{-3} wherein 14.16 mgC m^{-3} was ingested from micro-fraction, 36.24 mgC m^{-3} was from nano-fraction and 0.197 mgC m^{-3} from pico-fractions. The grazing pressure exerted by copepod community ingestion on phytoplankton was 9.95 % of the total standing stock and 16.59% of the production. In the micro-plankton fraction, grazing pressure of the copepod community was 5.27% of the standing stock and 8.96% of the production. In the case of nano-fraction, grazing pressure of copepods was 15.62% of the standing stock and 24.98% of the production, whereas in the pico-plankton fraction, grazing pressure of copepods community was 2.47% of the standing stock and 10.11% of the production (Fig. 9).

The grazing of *Noctiluca* on total phytoplankton was 140.6 mgC m^{-3} in which 117.3 mgC m^{-3} was from the micro-fraction, 23.13 mgC m^{-3} from nano-plankton, and 0.115 mgC m^{-3} from the pico-fraction. The grazing pressure exerted by *Noctiluca* on the total phytoplankton standing stock was 27.65% and production was 45.56%. In the case of micro- fractions, the grazing pressure of the *Noctiluca* was 43.71% of the standing stock and 74.30% of the production (Fig. 9b). Similarly, the grazing pressure exerted by *Noctiluca* on nano-fractions was 9.97% of the standing stock and 15.94% of the production, whereas, in the case of pico-fractions, it was 1.43% of the standing stock and 5.87% of the production. The overall results of the grazing experiments showed that the ingestion of *Noctiluca* on total phytoplankton stock was 2.8 times higher than the copepods (Fig. 9a). More importantly, it was found that *Noctiluca* ingestion on micro- fraction was 8.3 folds higher than that of copepods (Fig. 9b). Similarly, copepods ingestion was 1.6 folds high compared to *Noctiluca* in the case of nano-fraction (Fig. 9c). The ingestion of both *Noctiluca* and copepods were low and comparable in the case of pico-fractions (Fig. 9d).

3.6. Ecological inter-relationships

In the RDA Triplot, samples are displayed by green circles, and environmental and biological variables by solid red and dashed blue arrows, respectively (Fig. 10). The direction of a parameter arrow indicates their increasing gradient and the angle between two parameter arrows represents how closely they are correlated. The seasonal shift in the hydrography and overall ecological inter-relationships between biological components and also with the environment was well-reflected in the RDA Triplot (Fig. 10). The results of the complete RDA showed that environmental variables together explained 82.8 % of variations in biological parameters. The Monte Carlo test indicated that the ordination of all RDA axes were significant ($P < 0.05$). The increasing gradients of dissolved oxygen, temperature and decreasing gradients of turbidity and nutrients were oriented on the left side of the RDA plot, which represented the Pre- Southwest Monsoon samples. On the other hand, the decreasing gradients of temperature and dissolved oxygen and increasing gradients of turbidity and nutrients were oriented on the right side of the plot associated with the Peak- and Late-Southwest Monsoon samples. The increasing gradients of copepods and *Noctiluca* and chlorophyll *a* were oriented towards the right hand side of the Triplot, indicating their high values during the Southwest Monsoon samples. The chlorophyll *a* axis was oriented in the opposite direction of the dissolved oxygen and temperature, which was in close association with the increasing gradients of nutrients and turbidity; this represented the samples during the upwelling and mudbank period. The *Noctiluca* abundance axis showed the same orientation of the chlorophyll *a* axis, representing their positive inter-relationship.

4. Discussion

World over in marine environments, copepods contribute on an average 70% of the total grazer abundance at the secondary trophic level (Madhupratap et al. 2001; Jagadeesan et al. 2013) and an exception to this situation occurs during eutrophic/diatom blooming conditions where *Noctiluca* proliferate and outnumber copepods (Nakamura, 1998; Frangopulos et al. 2011; Elbrachter and Qi, 1998; Dela-Cruz et al. 2002; Naqvi et al. 1998; Sahayak et al. 2005). The proliferation of *Noctiluca* in marine environments is conventionally termed as 'bloom' considering them as a part of the phytoplankton community. However, advanced research in the recent decades has provided enough evidences to revise the conventional view and position *Noctiluca* as obligate heterotrophs as at times they function as the dominant grazer/ carnivore in the secondary trophic level (Elbrachter and Qi, 1998; Busky, 1995; Nakamura, 1998). In this context, it is relevant to note that several earlier 'swarm' (a better terminology) incidences of *Noctiluca* in different parts of the world were found associated with diatom blooms caused by natural or anthropogenic nutrient enrichment of marine environments (Nakamura, 1998; Frangopulos et al. 2011; Elbrachter and Qi, 1998; Dela-Cruz et al. 2002; Naqvi et al. 1998; Sahayak et al., 2005). Earlier studies along the west coast of India also provide the same message that *Noctiluca* swarms occur in this region with close association with high diatom abundance, but was conveniently considered as a plankton successional feature during the Southwest Monsoon (Nair et al. 1984; Sawant and Madhupratap, 1996).

Two fundamental processes that make the coastal waters of the west coast of India biologically productive during the Southwest Monsoon period are the coastal upwelling and the land runoff /river influx (Madhupratap et al. 1992; Jyothibabu et al. 2010). The vertical distribution of the temperature and dissolved oxygen in the study area indicate the start of the coastal upwelling by the first week of June, intensification during July - August and withdrawal by September. This temporal pattern of coastal upwelling is quite typical of the Southwest coast of India during the southwest Monsoon (Nair et al. 1984; Madhupratap et al. 1990; Smith and Madhupratap et al. 2005; Jyothibabu et al. 2006; 2008; 2010). On the other hand, the presence of high turbidity observed in the study area by mid - June represents the formation of the mudbank in the region more or less simultaneously, every year (Silas, 1984). As evident in the Triplot, the coastal upwelling process increased the availability of nutrients in the water column during the Southwest Monsoon, which eventually supported the high phytoplankton biomass (Fig. 10). During the present experimental sampling in mid-July, the phytoplankton stock was exceptionally high (chlorophyll *a* 10.17 mg m⁻³), which can be attributed to the elevated concentrations of the nutrients associated with the coastal upwelling. Size-fractionated phytoplankton stock measured during the Peak -Southwest Monsoon period in the present study

showed that micro-plankton contributes >50% to the total phytoplankton due to high abundance of *Fragilaria* and *Coscinodiscus*. Many earlier studies support the present observations of dominant large phytoplankton along the Southwest coast of India during the Peak Southwest Monsoon mainly due to the proliferation of *Fragilaria* and *Coscinodiscus* (Subrahmanyam, 1959; Nair et al. 1984; Banse et al. 1996).

The temporal change in copepod abundance showed a clear increase during the Southwest Monsoon period, which is a typical feature of the Southwest coast of India (Jyothibabu et al. 2010; Jagadeesan et al. 2013). Copepod abundance during the experimental sampling was high in the study area (14187 No.m⁻³), predominantly contributed by the cyclopoid *Oithona similis* (~65%) and their predominance was associated with the upwelling process, especially the presence of hypoxic waters (Jagadeesan et al. 2016). During the Pre -Southwest Monsoon, *Noctiluca* was found absent in the study area and their first occurrence was noticed in late – June; their abundance increased by July and peaked in August. Similar temporal pattern of *Noctiluca* was noticed earlier in the Alappuzha mudbank (Nair et al. 1984) as well as some other parts along the southwest coast of India (Sawant and Madhupratap, 1996; Madhupratap et al. 2001; Jyothibabu et al. 2006; 2008). A few earlier observations along the Southwest coast of India reported the abundance of *Noctiluca* with a density as high as >10⁵ cells L⁻¹ forming extensive red tides (Nair et al., 1984; Sahayak et al. 2005), but in the present case such extensive red tides were not discernible.

The first appearance of *Noctiluca* and their subsequent proliferation occurred in the study area when the chlorophyll *a* concentration was significantly high (>10 mg m⁻³). Similar observations on the occurrence of *Noctiluca* were recorded elsewhere as well (Nakamura, 1998). The grazing experiment conducted in the present study showed that both *Noctiluca* and copepods could ingest all size fractions of phytoplankton in natural environment, but their grazing preferences and efficiency significantly varied with different size fractions of the prey. Copepods preferred nano-size fractions of phytoplankton and ingested 80% of their daily ration from nano-fractions, as reported in some of the earlier observations (Calbet et al. 2000 and Kleppel, 1993). It was noticed earlier that copepods consume more phytoplankton in the size range 5 - 20 μM (Paffenhofen et al. 1982; Sautour et al. 2000), though they also attempt to graze upon larger phytoplankton with poor success rates. This is due to their small mouth size, which leads to inefficient or sloppy feeding (Banse, 1986). It was clearly recorded in the present study how copepods try to feed on larger micro-plankton such as *Coscinodiscus* and *Fragilaria*. Complete ingestion of the phytoplankton cell by filter feeding mechanism is not possible for copepods living in nutrient-enriched waters due to their small size and also the dominance of larger sized phytoplankton fractions in such environments. Instead, copepods

in such conditions adopt specialised ways of grazing the larger phytoplankton by breaking the cell wall and ingesting the protoplasm. A video of such a feeding event has been recorded (supplementary material 1), wherein, copepods rupture the prey cell wall and suck in the prey protoplasm into their alimentary track; however, while doing so, a part of the prey biomass is discarded as remains, popularly known as sloppy feeding.

Noctiluca are enabled with at least two distinctive feeding mechanisms - one for feeding of individual particles and the other for mucoid feeding of several cells connected by mucus threads (Uhlir and Sahling, 1990). The mucoid feeding is very advantageous for *Noctiluca* during phytoplankton bloom conditions, where scavenging and aggregating particles in mucoid threads become very efficient (Uhlir and Sahling, 1990; Kiorboe et al. 1998; Shanks & Walters, 1996 ; Tiselius and Kiorboe, 1998). Usually, *Noctiluca* feeds on phytoplankton aggregates with particle sizes ranging from a few millimetre to centimetre and is capable of forming feeding web surrounding even large aggregates of particles (Omori and Hamner, 1982; Tiselius and Kiorboe, 1998). This was very clear in the present study as well wherein *Noctiluca* cells under microscope showed ingested *Fragilaria* and *Coscinodiscus* inside their body; this also indicated their feeding preference for larger phytoplankton compared to nano- and pico-fractions (Fig. 7).

The ingestion of *Noctiluca* on phytoplankton during the present study was found to be high ($0.74 \pm 0.08 \mu\text{g C.day}^{-1}$ with a DR of 8.04 ± 0.16), which could be the result of their high carbon demand for growth (Busky, 1995; Nakamura, 1998; Tiselius and Kiorboe, 1998). This high carbon requirement of *Noctiluca* could be the main reason for their high positive preference for microplankton resources available in the environment (Busky, 1995; Nakamura, 1998; Tiselius and Kiorboe, 1998). This is further supported by the observation of Tiselius and Kiorboe (1998), who showed significantly high growth rate of *Noctiluca* during blooms of larger diatoms (0.5 d^{-1}) compared to the environment dominated by smaller flagellate ($0 - 0.25 \text{ day}$) (Lee and Hirayama, 1992). In the present study, the high abundance of *Noctiluca* was found in July (190 cells.L^{-1}) and August ($1600 \text{ cells.L}^{-1}$), which coincided with the blooms of larger diatoms.

In the present study, the grazing pressure of copepods on total phytoplankton was 9.95% of the standing stock and 16.59% of the production; similar ranges have been reported from some other parts of the world (Landry et al. 1994; Bollens and Landry, 2000; Bautista and Harris, 1992). On the other hand, the grazing pressure of *Noctiluca* on total phytoplankton was 27.65% of the standing stock and 45.56% of the production, being 2.8 folds higher than that of the copepods. Detailed size-fractionated grazing analysis showed that the grazing pressure of copepods was mainly on nano-fractions, whereas the grazing pressure of *Noctiluca* was on micro-plankton. These observations

provide a valuable insight into the co-occurrence of the seasonal phytoplankton blooms and swarming of *Noctiluca* along the west coast of India during the Peak/Late Southwest Monsoon periods. We appreciate the view of Banse et al., (1996) that the inefficient grazing of dominant copepods fail to control the micro-plankton blooms during the Southwest Monsoon period and propose that in such cases *Noctiluca* could perform better and function as the major grazer of the phytoplankton stock. In our future studies, we would like to address these aspects in a more detailed manner so as to improve our understanding on the seasonal phytoplankton blooms along the Southwest coast of India during Southwest Monsoon and their top-down control by various potential grazers.

5. Summary and conclusion

Based on 18 weekly/biweekly filed observations off Alappuzha, southwest coast of India, supported by grazing experiments, we presented here the relative grazing impact of dominant copepods and *Noctiluca* on the larger-sized phytoplankton community supported by nutrient enrichment associated with coastal upwelling and mudbank formation. In the experiment, copepod and *Noctiluca* consumed all the size ranges of the phytoplankton but their prey size preferences varied significantly. *Noctiluca* showed high positive electivity for micro-plankton ($E^* = 0.49 \pm 0.04$) followed by nano-plankton ($E^* = 0.17 \pm 0.04$) and negative electivity for pico-plankton ($E^* = -0.66 \pm 0.06$), whereas copepods showed their highest positive electivity for nano-fraction (av. 0.38 ± 0.04) followed by the micro-fraction (av. -0.17 ± 0.05) and the least for pico-fraction (av. -0.35 ± 0.05). The grazing pressure of copepods on the total phytoplankton biomass was found to be 9.95% of the standing stock and 16.59% of the production, whereas in the case of *Noctiluca*, it was 27.65% of the biomass and 45.56% of the production. Overall, the ingestion of *Noctiluca* on the total and micro-fraction phytoplankton was 2.8 and 8 folds higher than that of the dominant copepods. This study unravels the dominant role of *Noctiluca* as a grazer of larger phytoplankton along the southwest west coast of India during the Peak/Late Southwest Monsoon. The inefficient and sloppy feeding of copepods on larger diatoms make them less competent to control large diatom blooms in the region, and hence, such blooms are usual along the west coast of India during the Peak - and Late - Southwest Monsoon.

Acknowledgments

The authors thank the Director, CSIR- National Institute of Oceanography (CSIR-NIO), India for facilities and support. We thankfully acknowledge all of our colleges in National Institute of Oceanography, India who helped in carrying out the field work. The first author acknowledges

Department of Science and Technology (DST) for providing him DST-INSPIRE Fellowship. This is NIO contribution XXXX.

References

- Bamstedt, U., Gifford, D.J., Irigoien, X., Atkinson, A. & Roman, M. (2000). Feeding, in ICES Zooplankton Methodology Manual, San Diego, CA, USA: Academic Press.
- Banse, K. & McClain, C. R. (1986). Winter blooms of phytoplankton in the Arabian Sea as observed by the Coastal Zone Color Scanner. *Marine Ecology Progress Series*, 34, 201-211.
- Banse, K., Vijayaraghavan, S. & Madhuratap, M. (1996). On the possible causes of the seasonal phytoplankton blooms along the southwest coast of India. *Indian Journal of Marine Science*, 25, 283-289.
- Bautista, B. & Harris, R. P. (1992). Copepod gut contents, ingestion rates and grazing impact on phytoplankton in relation to size structure of zooplankton and phytoplankton during a spring bloom. *Marine Ecology Progress Series*, 82, 41-50.
- Bindu, S., Dineshababu, A. P., Saravanan, R., Bhat, G. S. & Lavanya, S. (2014). Occurrence of *Noctiluca scintillans* bloom off Mangalore in the Arabian Sea. *Indian Journal of Fisheries*, 61, 42-48.
- Bollens, G. C. R. & Landry, M. R. (2000). Biological response to iron fertilization in the eastern equatorial Pacific (IronEx II). II. Mesozooplankton abundance, biomass, depth distribution and grazing. *Marine Ecology Progress Series*, 201, 43-56.
- Bristow, R. C. (1938). History of Malabar mud balls, " Vol. I, Cochin Port Authorities (not for circulation).
- Buskey, E. J. (1995). Growth and bioluminescence of *Noctiluca scintillans* on varying algal diets. *Journal of Plankton Research*, 17, 29-40.
- Calbet, A., Landry, M. R. & Scheinberg, R. D. (2000). Copepod grazing in a subtropical bay: species-specific responses to a midsummer increase in nanoplankton standing stock. *Marine Ecology Progress Series*, 193, 75-84.
- Damodaran, R. & Kurian, C. V. (1973). *Studies on the benthos of the mud banks of the Kerala coast*. Bull. Dept. Mar. Sci. Univ. Cochin VI, 1-112.
- Dela-Cruz, J., Ajani, P., Lee, R., Pritchard, T. & Suthers, I. (2002). Temporal abundance patterns of the red tide dinoflagellate *Noctiluca scintillans* along the southeast coast of Australia. *Marine Ecology Progress Series*, 236, 75-88.
- Devassy, V. P. (1989). Red tide discolouration and its impact on fisheries. In: Okaichi T, Anderson, D, M. Nemoto T eds. *Red Tides: Biology, Environmental Science, and Toxicology*. Elsevier Science Amsterdam, Netherlands. p.57-60.
- Devassy, V. P. & Nair, S. R. S. (1987). Discolouration of water and its effect on fisheries along the Goa coast. *Mahasagar*, 20, 121-128.

- Dwivedi, R. M., Chauhan, R., Solanki, H. U., Raman, M., Matondkar, S. G. P., Madhu, V. R. & Meenakumari, B. (2012). Study of ecological consequence of the bloom (*Noctiluca miliaris*) in off shore waters of the Northern Arabian Sea. *Indian Journal of Geo-Marine Sciences.*, 14, 304-305.
- Elbrachter M, Qi, Z (1998). Aspects of *Noctiluca* (Dinophyceae) population dynamics. Physiological ecology of harmful algal blooms (DM Anderson, AD Cembella, GM Hallegraeff, eds) *Springer, Berlin, Heidelberg*, 315-335.
- Frangopulos, M. X., Spyarakos, E. & Guisande, C. S. (2011). Ingestion and clearance rates of the red *Noctiluca scintillans* fed on the toxic dinoflagellate *Alexandrium minutum* (Halim). *Harmful Algae*, 10, 304-309.
- Froneman, P. W. (2006). The importance of phytoplankton size in mediating trophic interactions within the plankton of a southern African estuary. *Estuarine, Coastal and Shelf Science*, 70, 693-700.
- Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behavior Of the marine planktonic copepod *Calanus pycnificus*. *Limnology and Oceanography*, 17, 805-815.
- Furuya, K., Saito, H., Sriwoon, R., Omura, T., Furio, E. E., Borja, V. M. & Lirdwitayaprasit, T. (2006). Vegetative growth of *Noctiluca scintillans* containing the endosymbiont *Pedinomonas noctilucae*. *African Journal of Marine Science*, 28, 305-308.
- Gomes, H. D. R., Goes, J. I., Matondkar, S. G. P., Buskey, E. J., Basu, S., Parab, S. & Thoppil, P. (2014). Massive outbreaks of *Noctiluca scintillans* blooms in the Arabian Sea due to spread of hypoxia. *Nature communications*, 5.
- Gopinathan, C. K. & Qasim, S. Z. (1974). Mud banks of Kerala-their formation and characteristics. *Indian Journal of Marine Sciences*, 3, 105-114.
- Grasshoff, K. E. & Kremling, M. K. (1983). Methods of seawater analysis. Verlag Chemie. *Weinheim, Germany*, 4, 9.
- Hansen, P. J., Miranda, L. & Azanza, R. (2004). Green *Noctiluca scintillans*: a dinoflagellate with its own greenhouse. *Marine Ecology Progress Series*, 275, 79-87.
- Harrison, P. J., Furuya, K., Glibert, P. M., Xu, J., Liu, H. B., Yin, K., Lee, J. H. W., Anderson, D. M., Gowen, R. & Al-Azri, A. R. (2011). Geographical distribution of red and green *Noctiluca scintillans*. *Chinese Journal of Oceanology and Limnology*, 29, 807-831.
- Jagadeesan, L., Jyothibabu, R., Anjusha, A., Mohan, A. P., Madhu, N. V., Muraleedharan, K. R. & Sudheesh, K. (2013). Ocean currents structuring the mesozooplankton in the Gulf of Mannar and the Palk Bay, southeast coast of India. *Progress in Oceanography*, 110, 27-48.
- Jagadeesan, L., Jyothibabu, R., Arunpandi, N., Karnan, C. & Balachandran, K. K. (2016). Influence of Coastal Upwelling and Mud Bank in Shaping the Meso-zooplankton and Dominance Pattern of Copepods along the South West Coast of India during the Southwest Monsoon. (Under Review in *Progress in Oceanography*)

- Jocelyn, D. C., Penelope, A., Randall, L. & Suthers, I. (2000). *Noctiluca scintillans* "An indicator of coastal eutrophication. In: *HAB ninth conference Tasmania*, www.utas.edu.au/docs/ plant-science/HAB2000/abstracts/docs.
- Jyothibabu, R., Devi, C. R. A., Madhu, N. V., Sabu, P., Jayalakshmy, K. V., Jacob, J., Habeebrehman, H., Prabhakaran, M. P., Balasubramanian, T. & Nair, K. K. C. (2008). The response of microzooplankton (20-200 μm) to coastal upwelling and summer stratification in the south-eastern Arabian Sea. *Continental Shelf Research*, 28, 653-671.
- Jyothibabu, R., Madhu, N. V., Habeebrehman, H., Jayalakshmy, K. V., Nair, K. K. C. & Achuthankutty, C. T. (2010). Re-evaluation of 'paradox of mesozooplankton' in the eastern Arabian Sea based on ship and satellite observations. *Journal of Marine Systems*, 81, 235-251.
- Jyothibabu, R., Madhu, N. V., Jayalakshmi, K. V., Balachandran, K. K., Shiyas, C. A., Martin, G. D. & Nair, K. K. C. (2006). Impact of freshwater influx on microzooplankton mediated food web in a tropical estuary (Cochin backwaters- India). *Estuarine, Coastal and Shelf Science*, 69, 505-518.
- Jyothibabu, R., Mohan, A. P., Jagadeesan, L., Anjusha, A., Muraleedharan, K. R., Lallu, K. R., Kiran, K. & Ullas, N. (2013). Ecology and trophic preference of picoplankton and nanoplankton in the Gulf of Mannar and the Palk Bay, southeast coast of India. *Journal of Marine Systems*, 111, 29-44.
- Kasturirangan, L. R. (1963). a key for the identification of the more common planktonic copepoda of indian coastal waters. C. S. I. R., New Delhi, *Ind. Nat. Comm. on Oceanic Res.*, 2nd publ., 87pp.
- Katti, R. J. & Gupta, C. (1988). On the occurrence of "green tide" in the Arabian Sea off Mangalore. *Current Science, Bangalore*, 57, 380-381.
- Kiarboe, T. & Titelman, J. (1998). Feeding, prey selection and prey encounter mechanisms in the heterotrophic dinoflagellate *Noctiluca scintillans*. *Journal of Plankton Research*, 20, 1615-1636.
- Kleppel, G. S. (1993). On the diets of calanoid copepods. *Marine ecology progress series*, 99, 183-183.
- Landry, M. R., Lorenzen, C. J. and Peterson, W. K. (1994) Mesozooplankton grazing in the southern California Bight. 2. Grazing impact and particulate flux. *Marine ecology progress series*, 115, 73-85.
- Lee, J. K., & Hirayama, K. (1992). Effects of salinity, food level and temperature on the population growth on *Noctiluca scintillans* (Macarthy). *Bulletin of the Faculty of Fisheries, Nagasaki University*. 71, 163-168.
- Leps, J. & Smilauer, P. (2003). *Multivariate analysis of ecological data using CANOCO*. Vol., Cambridge university press, Cambridge, 282 pp.
- Madhu, N. V., Jyothibabu, R., Maheswaran, P. A., Jayaraj, K. A. & Achuthankutty, C. T. (2012). Enhanced chlorophyll *a* and primary production in the northern Arabian Sea during the

spring intermonsoon due to green *Noctiluca scintillans* bloom. *Marine Biology Research*, 8, 182-188.

Madhupratap, M., Achuthankutty, C. T. & Nair, S. R. S. (1991). Zooplankton of the lagoons of the Laccadives: diel patterns and emergence. *Journal of plankton research*, 13, 947-958.

Madhupratap, M., Gopalakrishnan, T. C., Haridas, P. & Nair, K. K. C. (2001). Mesozooplankton biomass, composition and distribution in the Arabian Sea during the fall intermonsoon: implications of oxygen gradients. *Deep Sea Research Part II: Topical Studies in Oceanography*, 48, 1345-1368.

Madhupratap, M., Haridas, P., Ramaiah, N. & Achuthankutty, C. T. (1992). Zooplankton of the southwest coast of India: abundance, composition, temporal and spatial variability in 1987. In: *Oceanography of the Indian Ocean* (ed) B N Desai (New Delhi: Oxford & IBH Publishing Co) pp. 99–112.

Madhupratap, M., Nair, S. R. S., Haridas, P. & Padmavati, G. (1990). Response of zooplankton to physical changes in the environment: coastal upwelling along the central west coast of India. *Journal of Coastal Research*, 6, 413–426.

Matondkar, S. G. P., Basu, S., Parab, S. G., Pednekar, S., Dwivedi, R. M., Raman, M., Goes, J. I. & Gomes, H. (2012). The bloom of the dinoflagellate (*Noctiluca miliaris*) in the North Eastern Arabian Sea: Ship and Satellite study. In: Proceedings of the 11th Biennial Conference of Pan Ocean Remote Sensing Conference (PORSEC). Kochi, Kerala, India.

Mohamed, K. S., Kripa, V., Jugnu, R., Radhakrishnan, P., Alloyicious, P. S., Jenni, B., Joseph, M. & Velayudhan, T. S. (2007). Mortality of farmed pearl oyster *Pinctada fucata* (Gould, 1850) due to the blooming of *Noctiluca scintillans* and *Cochlodinium sp.* at Kollam Bay, Kerala. *Journal of the Marine Biological Association of India*, 49, 213-218.

Nair, P. N., Gopinathan, C. P., Balachandran, V. K., Mathew, K. J., Regunathan, A., Rao, D. S. & Murty, A. V. S. (1984). Ecology of mudbanks-Phytoplankton productivity in Alleppey mudbank. *CMFRI Bulletin*, 31, 28-35.

Nakamura, Y. (1998). Biomass, feeding and production of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *Journal of plankton research*, 20, 2213-2222.

Naqvi, S. W. A., George, M. D., Narvekar, P. V., Jayakumar, D. A., Shailaja, M. S., Sardessai, S., Sarma, V., Shenoy, D. M., Naik, H. & Maheswaran, P. A. (1998). Severe fish mortality associated with 'red tide' observed in the sea off Cochin. *Current Science*, 75, 543.

Naqvi, S. W. A., Naik, H., Pratihary, A., D'souza, W., Narvekar, P. V., Jayakumar, D. A., Devol, A. H., Yoshinari, T. & Saino, T. (2006). Coastal versus open-ocean denitrification in the Arabian Sea. *Biogeosciences*, 3, 621-633.

Nayar, S., Gupta, T. R. C. & Prabhu, H. V. (2001). Bloom of *Noctiluca scintillans* MaCartney in the Arabian Sea Off Mangalore, Southwest India. *Asian Fisheries Science*, 14, 77-82.

NIO Report, (2008). Near – shore dynamics of the southwest coast of India with special references to upwelling and mud bank. Centre for Marine Living Resources and Ecology (MoES), Kochi, pp. 257.

- Omori, M. & Ikeda, T. (1984). *Methods in marine zooplankton ecology*. John Wiley, Inc. New York, First Edition, 332 pp.
- Omori, M. & Hamner, W. M. (1982). Patchy distribution of zooplankton: behavior, population assessment and sampling problems. *Marine biology*, 72, 193-200.
- Padmakumar, K. B., Sanilkumar, M. G., Saramma, A. V., Sanjeevan, V. N. & Menon, N. R. (2008). "Green tide" of *Noctiluca miliaris* in the Northern Arabian Sea. *Harmful Algae News*, 36, 12.
- Padmakumar, K. B., Sreeranjima, G., Fanimol, C. L., Menon, N. R. & Sanjeevan, V. N. (2010). Preponderance of heterotrophic *Noctiluca scintillans* during a multi-species diatom bloom along the southwest coast of India. *International Journal of Oceans and Oceanography*, 4, 45-53.
- Paffenhofer, G. A., Strickler, J. R. & Alcaraz, M. (1982). Suspension-feeding by herbivorous calanoid copepods: a cinematographic study. *Marine biology*, 67, 193-199.
- Postel, L., Fock, H. & Hagen, W. (2000). 4 Biomass and abundance, in ICES Zooplankton Methodology Manual, edited by R. Harris *et al.*, Elsevier, New York. pp. 83–192,
- Ramasastri, A. A. & Myrland, P. (1959). Distribution of temperature, salinity and density in the Arabian Sea along the South Malabar Coast (South India) during the post-monsoon season. *Indian Journal of Fisheries*, 6, 223-255.
- Rao, D. S., Mathew, K. J., Gopinathan, C. P., Regunathan, A. & Murty, A. V. S. (1980). Mud banks and coastal erosion in relation to fisheries. *Marine Fisheries Information Service, Technical and Extension Series*, 19, 1-10.
- Sahayak, S., Jyothibabu, R., Jayalakshmi, K. J., Habeebrehman, H., Sabu, P., Prabhakaran, M. P., Jasmine, P., Shaiju, P., Rejomon, G. & Thresamma, J. (2005). Red tide of *Noctiluca miliaris* off south of Thiruvananthapuram subsequent to the 'stench event' at the southern Kerala coast. *Current Science*, 89, 1 472–1 473.
- Sautour, B. T., Artigas, L. F., Delmas, D., Herbland, A. & Laborde, P. (2000). Grazing impact of micro-and mesozooplankton during a spring situation in coastal waters off the Gironde estuary. *Journal of plankton research*, 22, 531-552.
- Sawant, S. S. & Madhupratap, M. (1996). Seasonality and composition of phytoplankton in the Arabian Sea. *Current Science*, 47, 869–873.
- Sewell, R. B. S. (1929). *The copepoda of Indian seas*. *Mem. Indian Musueum*, 10, 1–221.
- Shanks, A. & Walters, K. (1996). Feeding by a heterotrophic dinoflagellate (*Noctiluca scintillans*) in marine snow. *Limnology and oceanography*, 41, 177-181.
- Silas, E. G. (1984). Mudbanks of Kerala-Karnataka; Need for integrated study. *CMFRI Bulletin*, 31, 2-7.
- Smith, S. L. & Madhupratap, M. (2005). Mesozooplankton of the Arabian Sea: patterns influenced by seasons, upwelling, and oxygen concentrations. *Progress in Oceanography*, 65, 214-239.

- Stoecker, D. K. & Capuzzo, J. M. (1990). Predation on protozoa: its importance to zooplankton. *Journal of Plankton Research*, 12, 891-908.
- Subrahmanyam, R. (1959). Studies on the phytoplankton of the west coast of India *Proceedings of the Indian Academy of Sciences-Section B*. Vol. 50. Springer, pp. 189-252.
- Subrahmanyam, R., Gopinathan, C. P. & Pillai, C. T. (1975) Phytoplankton of the Indian Ocean: some ecological problems. *Journal of the Marine Biological Association of India*, 17, 608-612.
- Sweeney, B. M. (1976). *Pedinomonas Noctilucae* (Prasinophyceae), The flagellate symbiotic in *Noctiluca* (Dinophyceae) in Southeast Asia. *Journal of Phycology*, 12, 460-464.
- Tada, K., Pithakpol, S. & Montani, S. (2004). Seasonal variation in the abundance of *Noctiluca scintillans* in the Seto Inland Sea, Japan. *Plankton Biology and Ecology*, 51, 7-14.
- Thibodeau, P. S., Roesler, C. S., Drapeau, S. L., Prabhu Matondkar, S. G., Goes, J. I. & Werdell, P. J. (2014). Locating *Noctiluca miliaris* in the Arabian Sea: An optical proxy approach. *Limnology and oceanography*, 59, 2042-2056.
- Thrisiamma, J., Shaiju, P., Laluraj, C. M., Balachandran, K. K., Nair, M., George, R., Nair, K. K. C., Sahayak, S. & Prabhakaran, M. P. (2008). Nutrient environment of red tide-infested waters off south-west coast of India. *Environmental monitoring and assessment*, 143, 355-361.
- Tiselius, P. & Kiarboe, T. (1998). Colonization of diatom aggregates by the dinoflagellate *Noctiluca scintillans*. *Limnology and oceanography*, 43, 154-159.
- Turkoglu, M. (2013). Red tides of the dinoflagellate *Noctiluca scintillans* associated with eutrophication in the Sea of Marmara (the Dardanelles, Turkey). *Oceanologia*, 55, 709-732.
- Uhlig, G. & Sahling, G. (1990). Long-term studies on *Noctiluca scintillans* in the German Bight population dynamics and red tide phenomena 1968-1988. *Netherlands Journal of Sea Research*, 25, 101-112.
- UNESCO, (1994). The Salamanca Statement and Framework for action on special needs education: adopted by the World Conference on Special Needs Education; Access and Quality. Salamanca, Spain, 7-10 June 1994. UNESCO.
- Vanderploeg, H. A. & Scavia, D. (1979). Calculation and use of selectivity coefficients of feeding: zooplankton grazing. *Ecological modelling*, 7, 135-149.
- Venugopal, P., Haridas, P., Madhupratap, M. & Rao, T. S. S. (1979). Incidence of red water along South Kerala coast. *Indian Journal of Marine Science*, 8, 94-97.
- Winberg, G. G., Patalas, K., Wright, J. C., Hillbricht-Ilkowska, A., Cooper, W. E. & Mann, K. H. (1971). Methods for calculating productivity. *A manual on methods for the assessment of secondary productivity in fresh waters*. IBP Handbook, 17, 296-316.

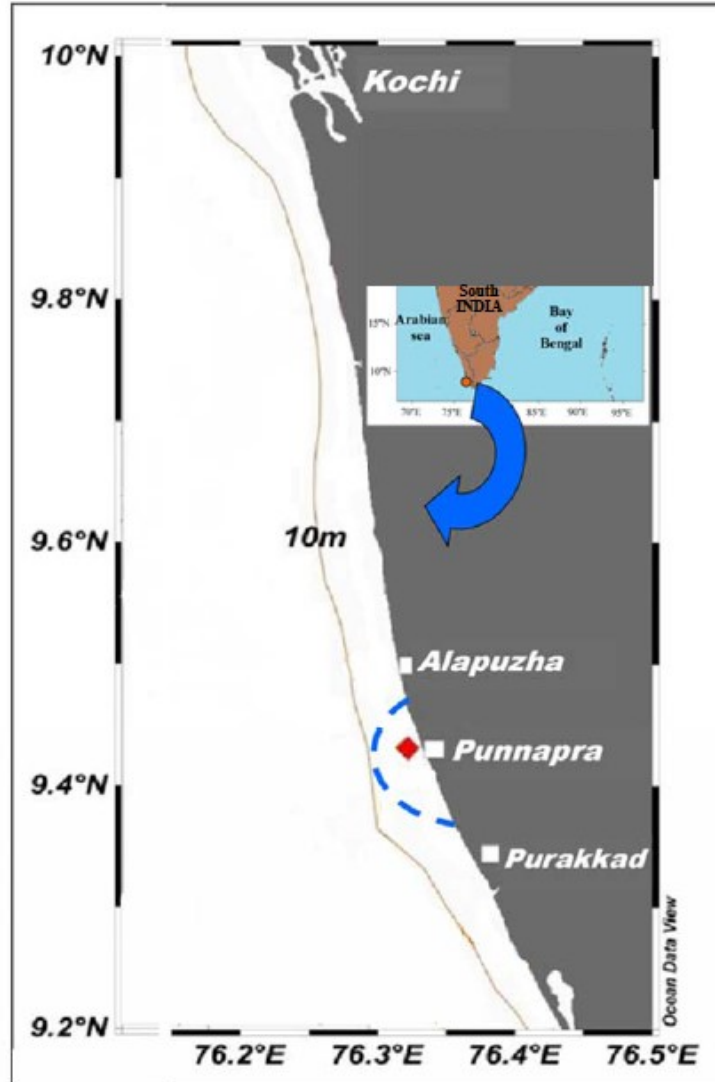


Fig. 1 - Sampling location off Alappuzha, southwest coast of India indicated as a red dot. Blue discontinuous semicircle indicate the area where mud bank existed during the Southwest Monsoon period. 18 weekly/biweekly time series sampling were carried out from April to September 2014 for hydrographical and biological parameters.

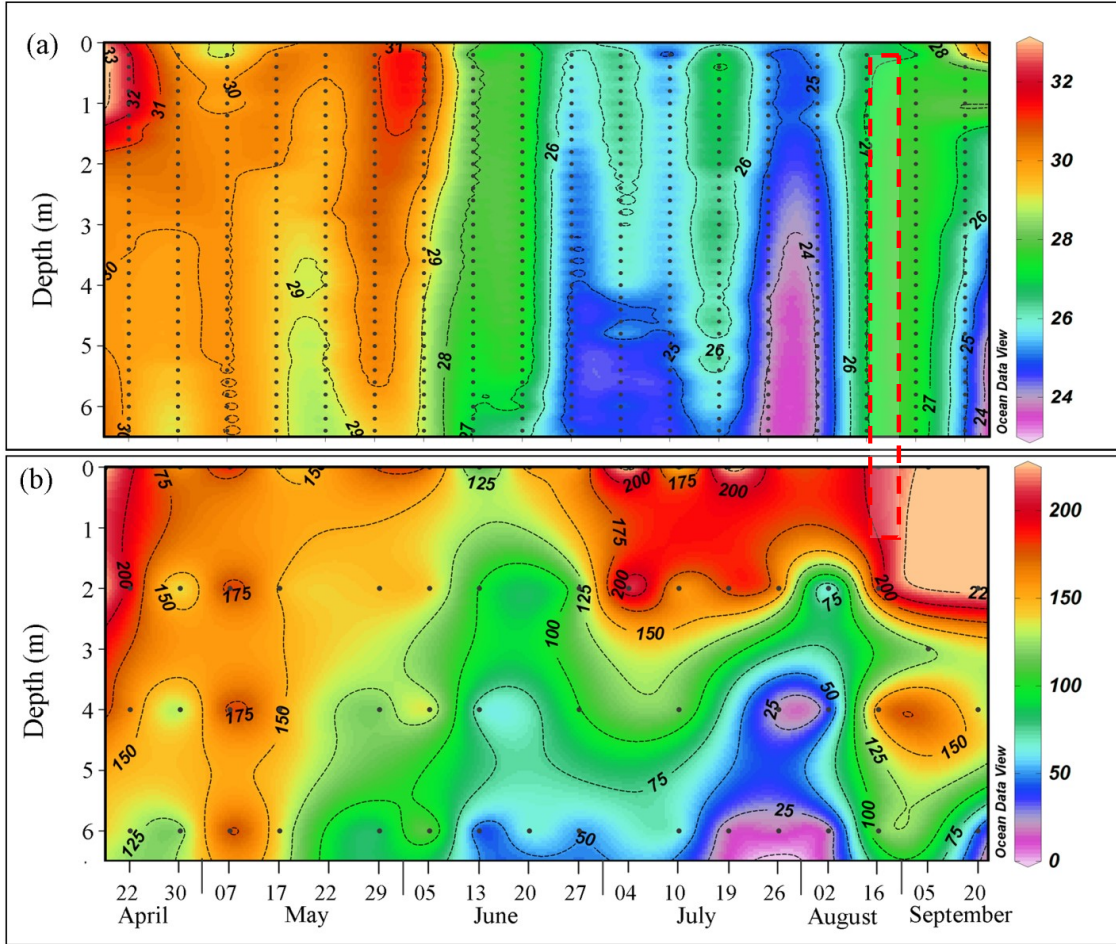


Fig. 2 - Temporal change in the vertical distribution of (a) temperature and (b) dissolved oxygen during the study period between April and September 2014. The presence of *Noctiluca* were observed in the study area during the Southwest Monsoon (June – September). The red discontinuous rectangle in panel 1 shows the time when water samples were collected for conducting the grazing experiments.

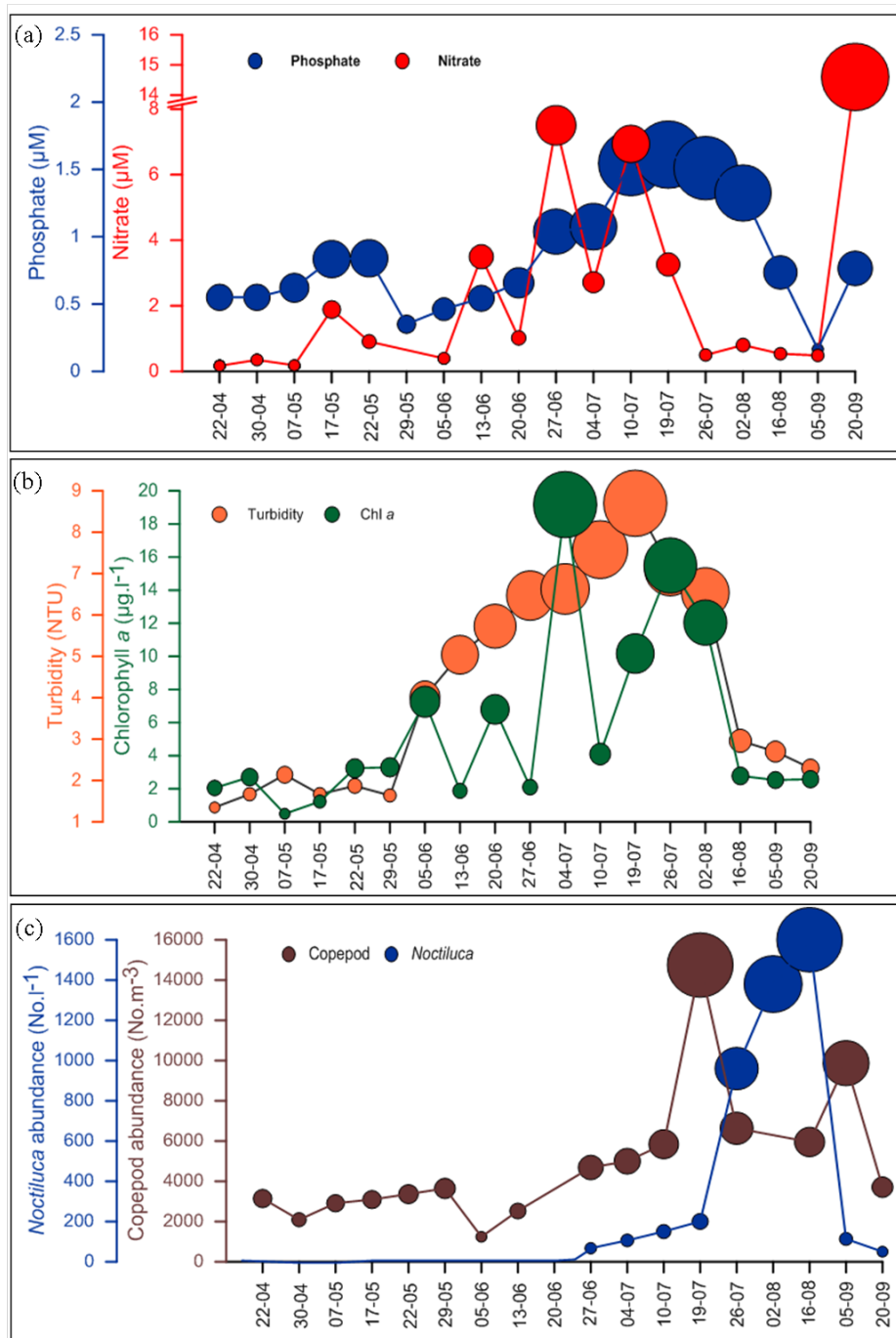


Fig. 3 - Temporal variations in the (a) nutrients (nitrate and phosphate), (b) chlorophyll *a* and turbidity and (c) abundance of copepods and *Noctiluca* during the study period. A general increase of nutrients was evident during the upwelling period in panel (a) and a similar increasing trend in chlorophyll *a* in panel (b). The presence of *Noctiluca* were observed in the study area from late-June to late September indicated by blue bubbles in panel (c). Copepods were found throughout the observations but, their abundance significantly increased during the Southwest Monsoon especially during mid-July.

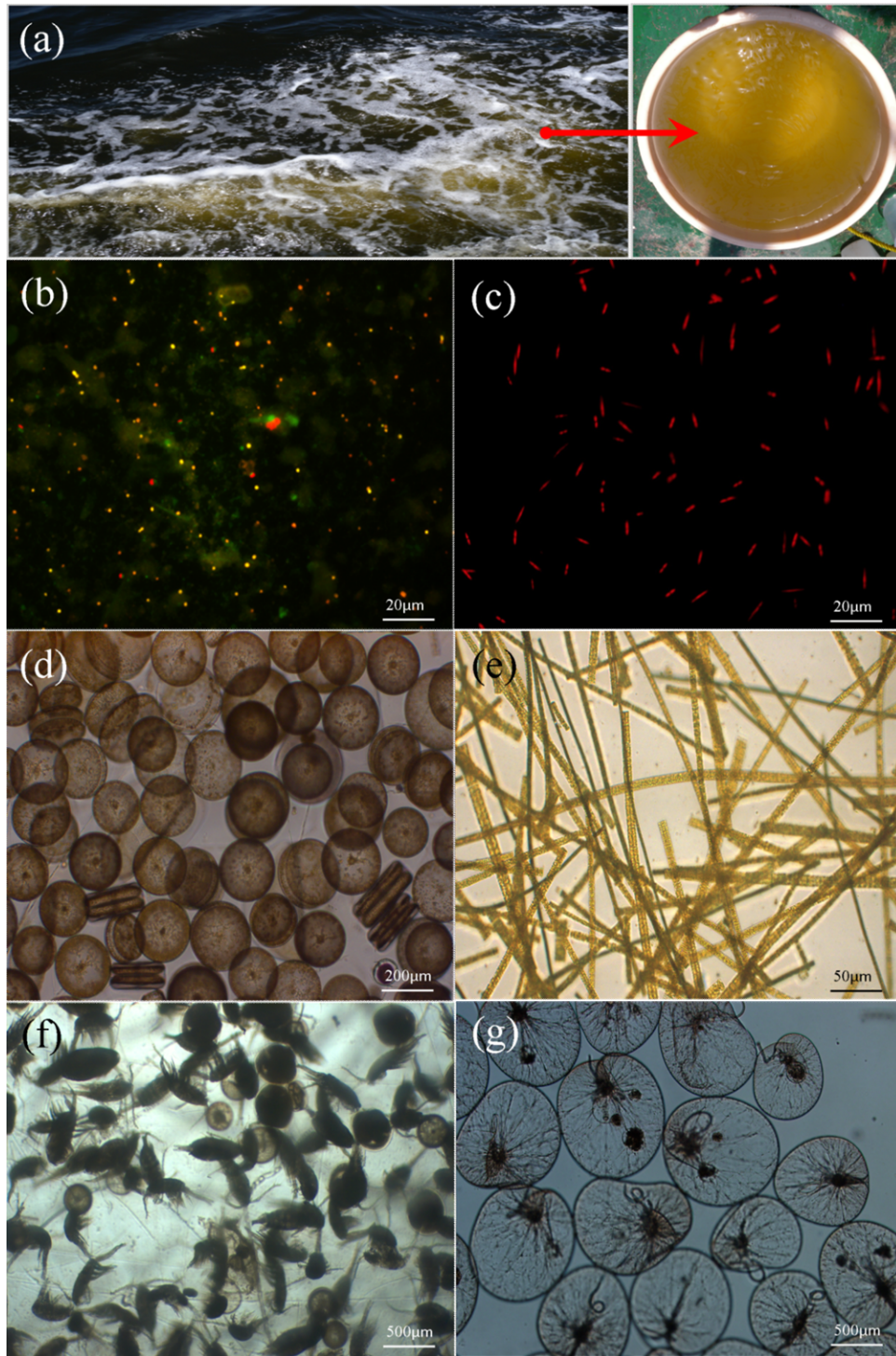


Fig. 4 - Panel (a) shows the discoloration of the water column in the study area due to the proliferation of macro-phytoplankton *Fragilaria* and *Coscinodiscus*. *Noctiluca* cells were also found in high abundance along with the diatom bloom (Figure 3c for further details). Panels (b –g) represent the relative size of different plankton food web components experimented in the present study. Fluorescence microscope images of phytoplankton (b) pico-fraction and (c) nano-fraction. Subsequent panels indicate light microscope images of (d) *Coscinodiscus*, (e) *Fragilaria*, (f) Copepods and (g) *Noctiluca*.

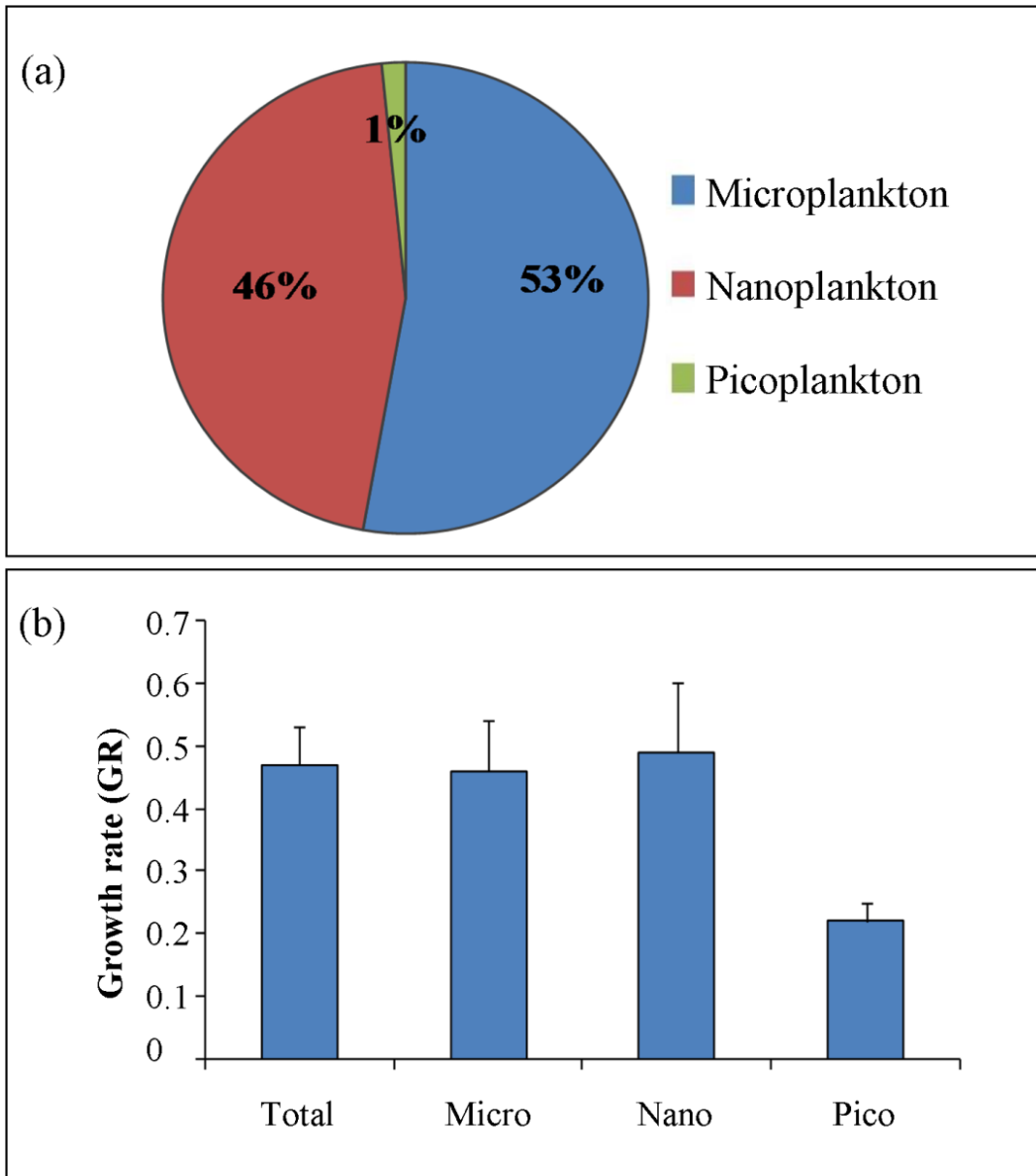


Fig. 5 - Panel (a) contribution of different phytoplankton size fractions to the total community biomass, and panel (b) growth rate (mean \pm SD) of total and different size fractions of phytoplankton in the experimental water sample.

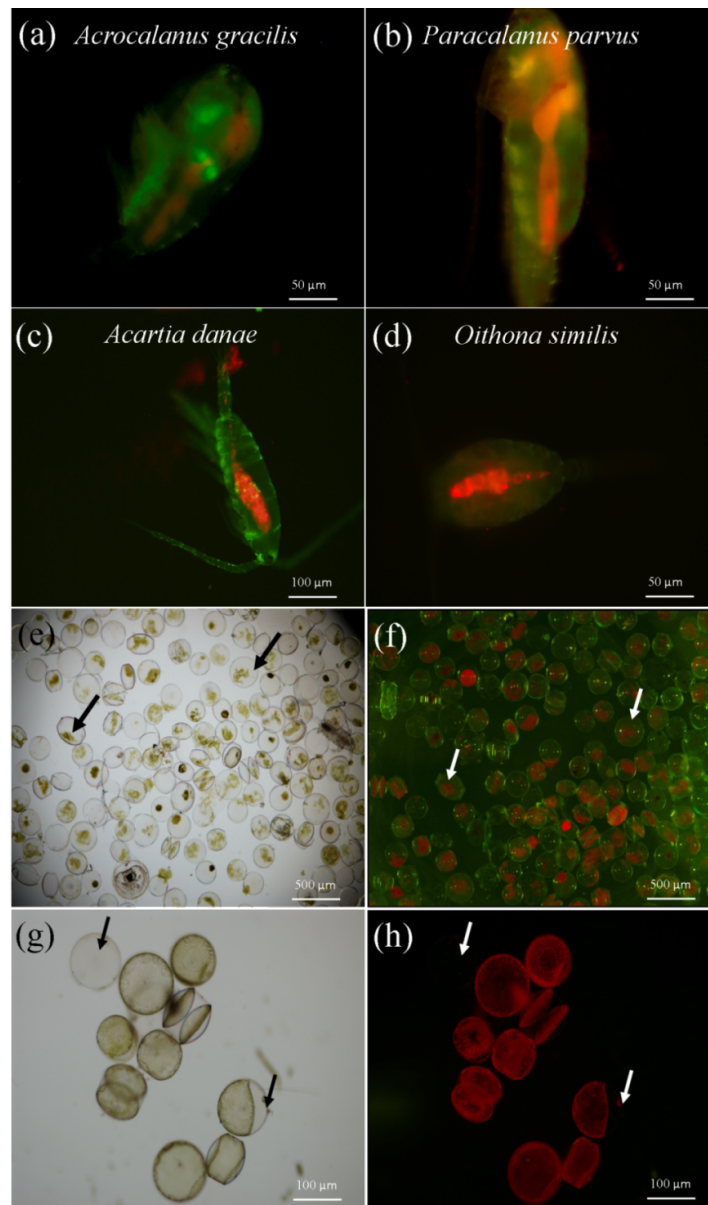


Fig. 6 - Panels (a – d) show the observational evidences for the feeding of the dominant copepods on phytoplankton. Fluorescence microscope images showing the chlorophyll *a* red gut fluorescence in *Acrocalanus gracilis*, *Paracalanus parvus*, *Acartia danae* and *Oithona similis*. Subsequent panels (e – h) show light and fluorescence microscopic evidences for the sloppy feeding of dominant copepods on larger phytoplankton cells. The black and white arrows represent the partially consumed cells by the copepods. A video has been provided to visualize the sequence of events involved in the sloppy feeding, wherein, copepods make protrusions on the cell wall of *Coscinodiscus* sucks in the prey protoplasm fully/ partially and ignore the remnants (Supplementary material 1).

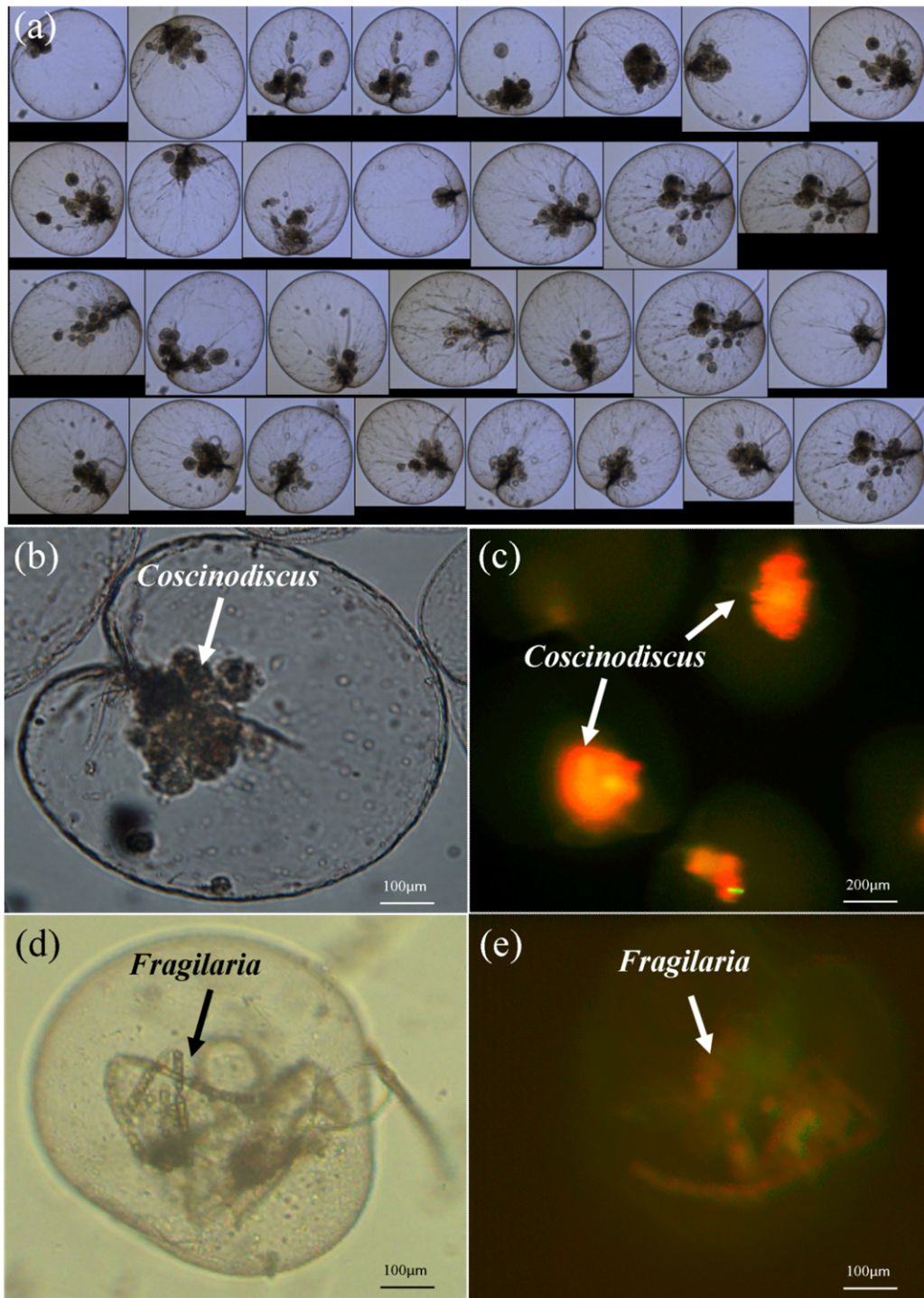


Fig. 7 - The qualitative analysis of the separated individuals from the experimental bottles clearly visualized the presences of the larger phytoplankton cells inside of the food vacuoles of *Noctiluca*. The black and white arrows in the images pointed out the presence of the larger diatoms inside of the food vacuole.

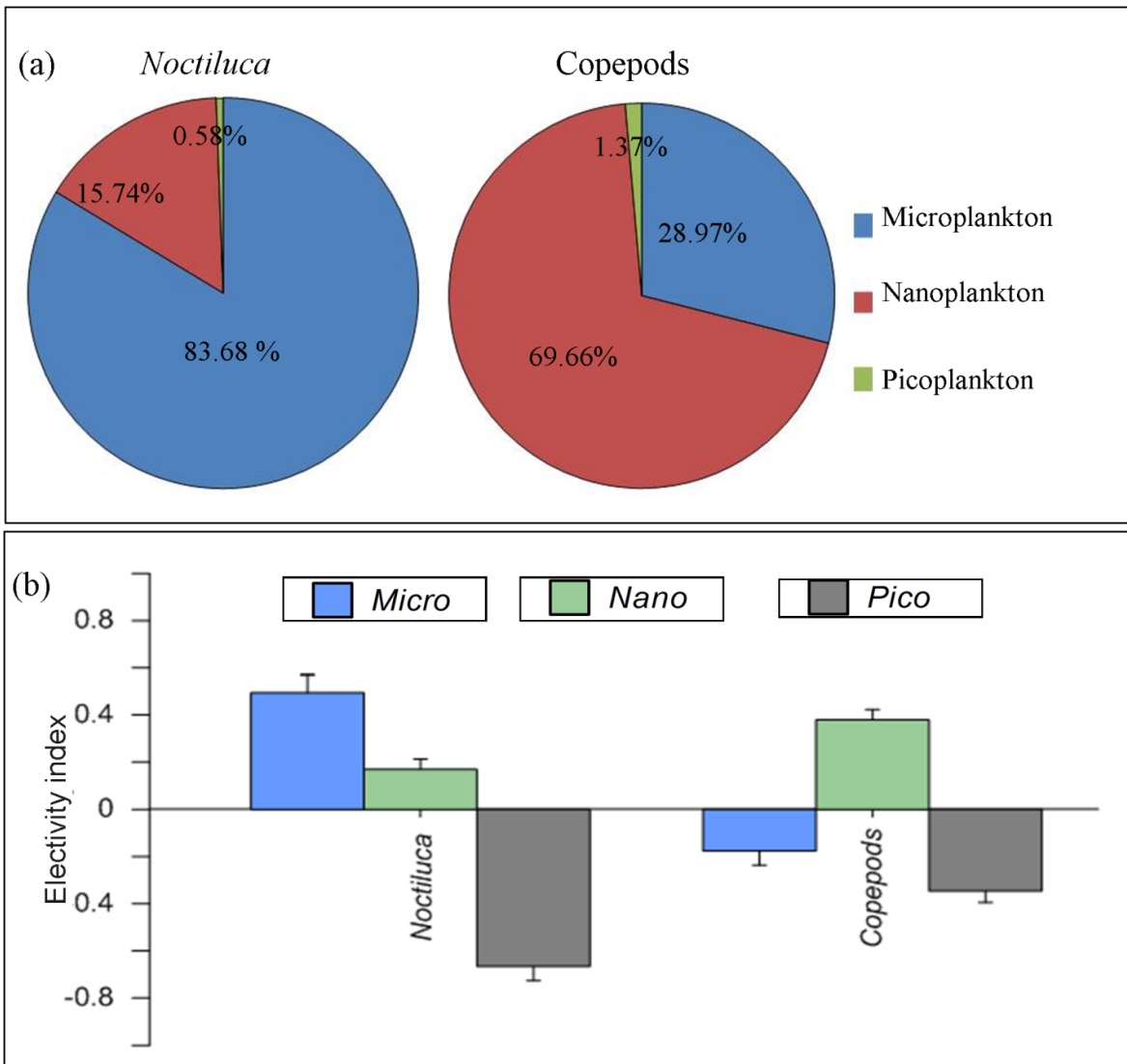


Fig. 8 - Panel (a) represents the contribution of different size fractions of phytoplankton to the ingestion of copepods and *Noctiluca*. Panel (b) shows the food electivity index of *Noctiluca* and copepods. E* values ranged from -1 to 1. The bars towards positive value indicate the preference and negative value indicates non- preference.

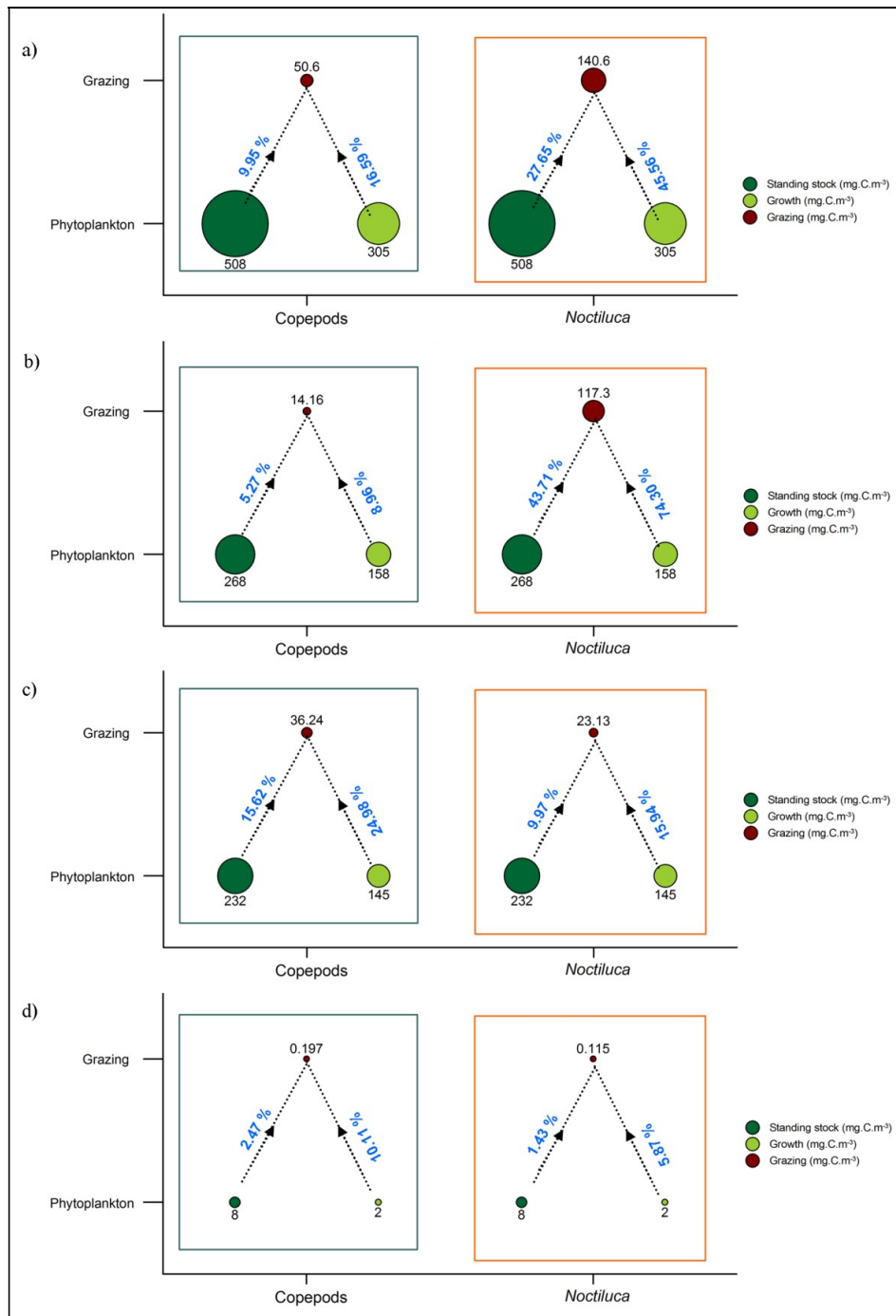


Fig. 9 - Grazing pressure (in percentage) exerted by dominant copepods (brown bubbles; left side panels) and *Noctiluca* (brown bubbles; right side panels) on the phytoplankton standing stock (dark green bubbles) and production (light green bubbles). Notations (a) total phytoplankton biomass, (b) micro - phytoplankton biomass, (c) nano-phytoplankton biomass, (d) pico- phytoplankton biomass.

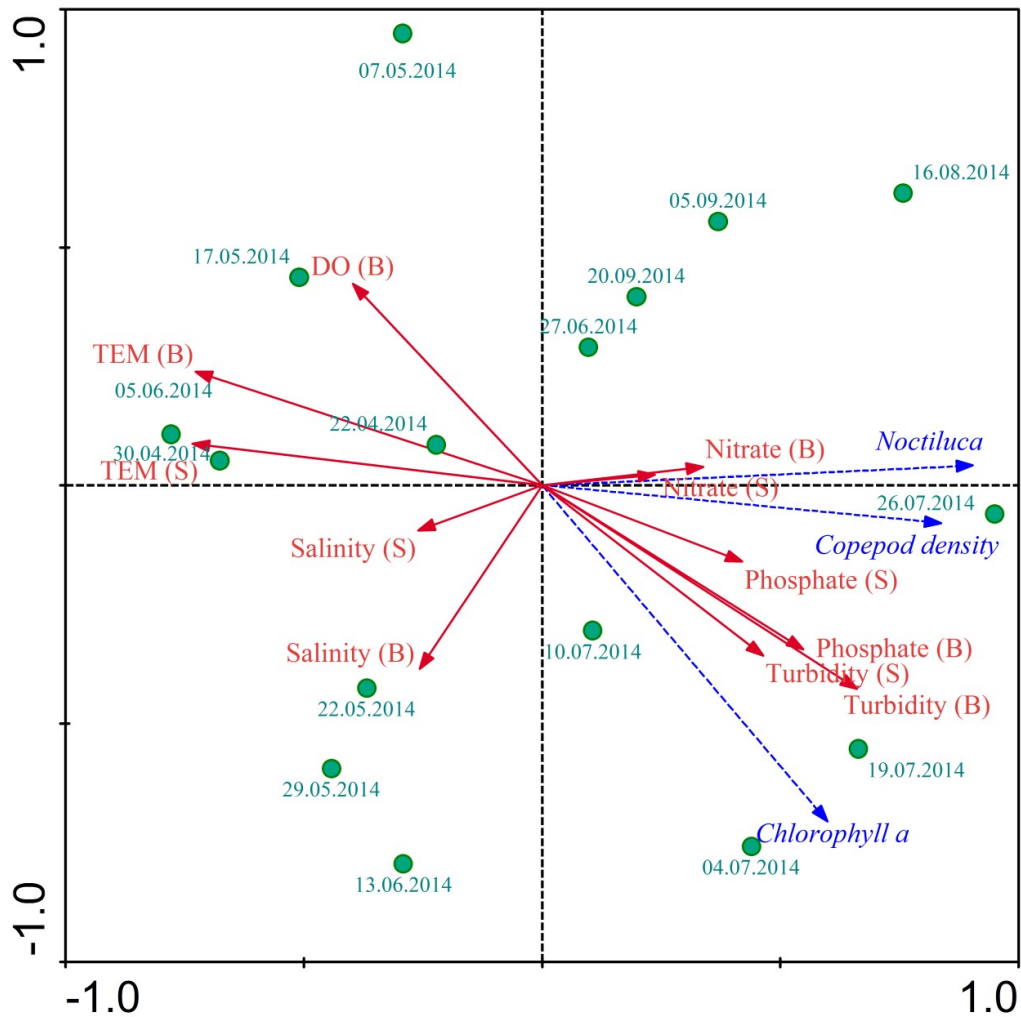


Fig. 10 - RDA Triplot showing the overall ecological inter-relationship of copepods and *Noctiluca* with other environmental variables during the study period. It shows high concentration of all biological parameters towards low temperature, high nutrients and turbidity indicating Southwest Monsoon period. Here (S) indicates as surface and (B) indicates as bottom waters.

No.	Source	Place	Period
Red <i>Noctiluca</i>			
1	Venugopal et al., 1979	Off Cochin	August, 1976
2	Rao, 1980	Alappuzha mud bank	September-November 1983
3	Devassy, 1989	Central west coast of India	September 1973
4	Naqvi et al., 1998	Off Kerala	August 1998
5	Nayar et al., 2001	Off Mangalore	November 1998
6	Sahayak et al., 2005	Kerala coast	September 2004
7	Mohamed et al., 2007	Kollam bay	September 2003
8	Thresiamma et al., 2008	South west coast of India	September 2004,
9	Padmakumar, 2010	Off Kerala	August,2008
10	Bindu et al., 2014	Mangalore Coast	May-June 2011
11	Jyothibabu (unpublished)	Off Kochi	September 2013
Green <i>Noctiluca</i>			
1	Devassy and Nair, 1987	Off Goa	February to April 1987
2	Katti et al., 1988	Mangalore	January 1987
3	Padmakumar et al., 2008	Off Gujarat	March 2007
4	Matondkar et al., 2012	Arabian Sea	January-April, 2003-2011
5	Dwivedi et al., 2012	North-eastern Arabian Sea	February-early March
6	Madhu et al., 2012	north-eastern Arabian Sea	March 2000
7	Gomes et al., 2014	Northern Arabian Sea	February 2009
8	Thibodeau et al., 2014	North-Eastern Arabian Sea	Nov – Dec 2009

Table 1 - Earlier records of *Noctiluca* red tide along the west coast India. It is evident here that red *Noctiluca* occur mostly during the Southwest Monsoon period (June – September), whereas, green *Noctiluca* occur during the Northeast Monsoon (October to February). Red *Noctiluca* were reported mainly from southeastern Arabian Sea, whereas the green *Noctiluca* from the central and northern Arabian Sea.

Sl.No.	Parameters	Surface	Bottom
Physico-chemical			
1	Salinity	32.66	34.04
2	Temperature (°C)	27.35	24.82
3	Turbidity (NTU)	8.7	13.47
4	Nitrate (μM)	3.26	10.06
5	Phosphate (μM)	1.61	2.02
6	Silicate (μM)	4.6	20.9
Biological			
7	Chlorophyll <i>a</i> (mg m^{-3})	10.17	-
8	Micro-fraction (mg m^{-3})	5.37	-
9	Nano-fraction (mg m^{-3})	4.64	-
10	Pico-fraction (mg m^{-3})	0.16	-
11	Zooplankton biomass (ml m^{-3})	11.46	-
12	Zooplankton density (No. m^{-3})	14745	-
13	Copepods density (No. m^{-3})	14187	-
14	<i>Noctiluca</i> density (cells L^{-1})	Av.190	-

Table 2 – Physico-chemical and biological characteristics of the sampling area during the experimental water sample collection in July 2014.