Dynamics of a Heterotrophic Dinoflagellate, *Protoperidinium divergens*, in the South-Eastern Coastal Waters of the Bay of Bengal

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**Authors’ contributions**

The work was carried out in collaboration among all authors. All authors read, reviewed and approved the final manuscript.

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**ABSTRACT**

This research is the first attempt to present temporal distribution of heterotrophic dinoflagellate *Protoperidinium divergens* and their relationship with diatom and environmental factors in the Maheshkhali channel, south-east coast of the Bay of Bengal, Bangladesh. The initiation of higher abundances of *P. divergens* were observed in October and reached its peak in November when diatoms were the most abundant phytoplankton. *Protoperidinium divergens* preferred comparatively low temperature from 20-22°C and high concentration of phosphate-phosphorus from 31-37 µM. It had insignificant relationship with nitrate-nitrogen. Lower abundances of *P. divergens* were

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observed during pre-monsoon and monsoon due to lower abundances of diatom and higher water temperature (>23°C). *Protoperidinium divergens*, therefore, were mainly controlled by the availability of diatom abundance at comparatively lower temperature and higher concentration of phosphate-phosphorus. This research is important to estimate the potential trophic impact of *P. divergens* in the Maheshkhali channel.

Keywords: *Protoperidinium divergens*; temperature; phosphate-phosphorus; diatom; Maheshkhali channel.

1. INTRODUCTION

Heterotrophic dinoflagellates are prevalent protists in marine environments, which play a vital role as an intermediate link between the microbial loop and higher trophic levels [1,2] and they also have an important role in the carbon cycling and energy flow in the marine planktonic community [3].

Heterotrophic dinoflagellates have been reported to feed on prey such as bacteria, flagellates, diatoms, other dinoflagellates, ciliates and metazoans [4,5,6] that varies from species to species. For instance, *P. pallidum* prefers diatom, while *P. steinii* feed better on both diatoms and dinoflagellates [7]. *Protoperidinium* cf. *divergens* can feed on adult copepods, copepod eggs and nauplii [8]. *Protoperidinium* spp. feed on prey cells by using a pseudopod “veil”, called the pallium, to envelop the prey, with subsequent external digestion [4,9]. This feeding adaptation enables *Protoperidinium* to consume prey as large as or larger than themselves (including chain forming diatoms) [7], and thus they can compete with mesozooplankton for food resources (within the trophic levels). Although there are many studies on feeding habit of *Protoperidinium*, little is known about the ecologies of species in this genus.

An extensive bloom of *Protoperidinium* Bergh was observed, for the first time, in the west coast of the Bay of Bengal in 2008 [10]. Heterotrophic dinoflagellates were comprised as high as 80% (average 59%) of total microzooplankton biomass in the Bay of Bengal and the Andaman Sea [11]. *Protoperidinium* abundance in the region is mainly controlled by food availability and nutrient. For instance, coastal waters especially at the Ganges-Brahmaputra riverine systems support a comparative abundance of diatoms and in turn *Protoperidinium* population throughout the year [12]. *Protoperidinium* abundance might have been occurred due to nutrient enrichment during the south-west monsoon and also influenced by moderate wind driven advection during the northeast monsoon [12]. Although, consistent with the Bay of Bengal, heterotrophic and mixotrophic dinoflagellates (i.e. *Ceratium furca*, *C. tripos*, *C. fusus*, *Dinophysis caudata*, *Prorocentrum micans* and *Gonyaulax polygramma*) were also dominant in the Maheshkhali channel [13], there is very few study on a particular dinoflagellate species in the Maheshkhali channel.

This research was undertaken to present the temporal distribution of heterotrophic *Protoperidinium divergens* and their relationship with diatom and environmental factors in the Maheshkhali channel, south-east coast of the Bay of Bengal. This finding can be used to estimate the potential trophic impact of *P. divergens* in the Maheshkhali channel.

2. MATERIALS AND METHODS

2.1 Study Area

The present study was carried out in the Maheshkhali channel, situated at the south-eastern coast of the Bay of Bengal, Bangladesh (Fig. 1). Three sampling sites were selected at the mouth of this channel. The channel includes Bakkhali River in the north and the Bay of Bengal in the south. The Maheshkhali channel is very important as a large fishing ground and a centre for recreation. Different traditional capture fisheries and commercial shrimp farms have developed around the estuary. This channel considered as highly productive because of excessive nutrients invaded from industrial wastes, agricultural lands, rural and urban sewages and from the adjacent shrimp and bivalve farms, which sometimes induces the growth of many harmful algae [14].

2.2 Methods

Water samples were collected from January to December 2002, covering three seasons: monsoon (June-September), post-monsoon (October-January), and pre-monsoon (February-May). Monthly plankton samples were collected
using a 25 µm mesh size net [15]. The samplings were made in during daytime at high tide. For qualitative plankton study a plankton net was towed just under the water surface for one minute at a speed of approximately 1 m/s. From the net the collected samples were drained in a polyethylene bottle and was preserved with 5% buffered formalin in sea water. For quantitative study, a known volume (100 liters) of sub-surface water was passed through a plankton net (mesh 25 µm) and the concentrate was collected from the bucket and preserved in 5% buffered formalin in sea water. The quantitative estimation of phytoplankton was done by Sedgewick-Rafter counting chamber (S-R cell) method using an Olympus binocular microscope. Phytoplankton were identified up to species level as possible and enumerated. Taxonomic study was based on Newell and Newell [16], Taylor et al. [17] and Steidinger and Tangen [18].

During sampling, surface water temperature and salinity were determined using a Celsius thermometer and a Handheld Refractometer, respectively. Nitrate-nitrogen (NO₂-N) and phosphate-phosphorus (PO₄-P) concentration were measured in the laboratory by HACH kit (DR 2010). Difference between different groups were analyzed by one-way ANOVA with LSD (Least Significant Difference) at significance level p=.05 using SPSS. Principal component analysis (PCA) was done to elucidate relationships between phytoplankton (i.e. Protoperidinium divergens and diatom) and environmental parameters (i.e. temperature, salinity, nitrate-nitrogen and phosphate-phosphorus) by R-language using “vegan” and “ggplot2” packages. Stepwise multiple linear regression was performed by IBM-SPSS Statistics 21.

3. RESULTS AND DISCUSSION

3.1 Results

Comparatively higher abundance of Protoperidinium divergens were observed during post-monsoon (Fig. 2). The cell density started to increase in October (28×10³ ± 8.46×10³ cells l⁻¹) showed the peak (66.14×10³ ± 26.65×10³ cells l⁻¹) in November and again decreased (14.92×10³ ± 2.47×10³ cell l⁻¹) sharply in the next month December (Fig. 2). Comparatively low abundances of P. divergens were observed during pre-monsoon (mean 2.66×10³ ± 2.23×10³ cells l⁻¹) and monsoon (mean 5.22×10³ ± 5.61×10³ cells l⁻¹). Remarkably, P. divergens was absent in June. On the other hand, the initiation of higher abundance of diatom were observed
from May that was gradually increased on consecutive months and showed its peak (2002.67×10^3 ± 128×10^3 cells l^-1) in November (Fig. 2).

*Protoperidinium divergens* showed inverse relation with temperature. For instance, comparatively lower temperature was recorded in October (21.17 ± 0.76°C) and November (20.83 ± 0.29°C) during higher abundance period of *P. divergens* (Fig. 3A). In contrast, comparatively high temperature were recorded in August (31.67 ± 0.24°C), September (29.67 ± 0.24°C) and April (29.83 ± 0.24°C) during low abundance period of *P. divergens*. Higher abundance of *P. divergens* was observed at salinity 13.67 ± 1.5 in October and the peak abundance in November at salinity 25.67 ± 0.24 (Fig. 3B). It can be noted here that, *P. divergens* was absent at the intermediate level of salinity (26.6 ± 0.41) in June and the lowest...
abundance was observed at the highest salinity in April. Although comparatively higher concentration of nitrate-nitrogen (9.89 ± 0.15 µM) was observed during high abundance period of *P. divergens* in November, the highest concentration of nitrate-nitrogen (15.7 ± 1.47 µM) was recorded during lower abundance phase of *P. divergens* in August (Fig. 3C). The highest peak of phosphate-phosphorus value was found in November (34.43 ± 2.98 µM) during high abundance period of *P. divergens*, the second peak of phosphate-phosphorus (32 ± 4.2 µM in August) was unable to enhance *P. divergens* abundance (Fig. 3D). The lowest concentration of phosphate-phosphorus was recorded in June (6.11 ± 2.44 µM) during early monsoon. Higher abundance of *P. divergens* (30.85×10^3 ± 25.36×10^3 cells l^-1) was shown at comparatively low water temperature (20-22°C) that was significantly different than the abundance at temperature range of 23-26°C (1.74×10^3 ± 1.9×10^3 cells l^-1, *p=0.003*) (Fig. 4A). There was no significant difference between the *P. divergens* abundance at higher temperature range of 27-29°C (5.5×10^3 ± 5.1×10^3 cells l^-1) and 30-32°C (2.67×10^3 ± 2.9×10^3 cells l^-1, *p=0.755*). During the study period, *P. divergens* shown wide range of salinity tolerance. There was no significant difference among different ranges of salinity (Fig. 4B). Comparativel

In principal component analysis, the first and second axis explained 53.8% and 28.1% of total variance in environmental parameters, diatom and *P. divergens*, respectively (Fig. 5). *Protoperidinium divergens*, diatom and phosphate-phosphorus were negatively related with the first axis, while salinity was positively related with the first axis (Fig. 5). Temperature and nitrate-nitrogen were negatively related with the second axis. The stepwise multiple regression demonstrated that *P. divergens* were significantly negatively and positively related with temperature and phosphate-phosphorus, respectively (Table 1).

![Fig. 4](image-url)
Discussion

3.2.1 Predator-prey relationship

Generally, diverse heterotrophic feeding mechanisms support *Protoperdinium* species to maintain the static population in diverse environmental and regional conditions [19]. Consistent with previous studies [4,20], diatom was the most important prey for *Protoperdinium divergens* in the Maheshkhali channel where higher abundance of *P. divergens* was observed concurrently with higher abundance of diatoms (Fig. 2). In principal component analysis (PCA), *P. divergens* was strongly significantly related with abundances of diatom (Fig. 5). Similarly, in the northern part of the Bay of Bengal, *Protoperdinium* abundance mostly depends on diatom population [12]. *Protoperdinium* spp. mostly prey on diatom species such as *Skeletonema costatum*, *Leptocylindrus danicus*, *Thalassiosira* sp., *Ditylum brightwellii* [21]. It is noted here that, higher abundance of *Skeletonema costatum*, *Leptocylindrus danicus*, *Thalassiosira* sp. were found in the Maheshkhali channel during post-monsoon [13]. In contrast, *P. divergens* sometimes ingest dinoflagellates *Gymnodinium sanguineum* and *Gonyaulax polyedra* [22]. This contrast features might be due to inter-species and inter-regional differences.

3.2.2 Environmental factors and abundance of *Protoperdinium divergens*

In this study, *Protoperdinium divergens* preferred comparatively lower temperature (20-22°C) (Fig. 4A, Table 1). In the previous studies, *Protoperdinium* blooms were observed at 29°C in the west coast of the Bay of Bengal [10] and from 21.36-29.0°C in Scandinavian countries [23]. Heterotrophic dinoflagellates, generally, can be indirectly stimulated by water temperature, mediated through an increase in the abundance of algal prey that may be directly stimulated by specific environmental parameters. In this study,
the prey diatom species generally thrive in cold, turbulent and nutrient-rich environment [24,25,26]. It is attributed that comparatively lower abundances of *P. divergens* observed when temperature exceeded >23°C (Fig. 4A, Table 1). Consistently, heterotrophic dinoflagellates (i.e. *Noctiluca scintillans*) sometimes cannot tolerate higher temperature (>27°C), where diatom may be the most important prey items [27].

In this study, *Protoperidinium divergens* had no significant relationship with salinity and showed slightly higher abundance at salinity range from 25-29 (Fig. 4B). Consistently, higher abundance of mixotrophic and heterotrophic dinoflagellates were observed at salinity range from 25-27 in the Maheshkhali channel [13]. However, *Protoperidinium divergens* preferred comparatively higher salinity such as 31-32.6 in Andaman Sea [11] and 29 in the west coast of the Bay of Bengal [10]. Alkawri and Ramaiah [28] observed heterotrophic and mixotrophic dinoflagellates in the coast of Goa (west coast of the Bay of Bengal) over wide ranges of water temperatures, salinities and nutrient concentrations. This could be attributed to the fact that their active swimming-cell stages can adapt to the ecological variations [18,29].

*Protoperidinium divergens* cell abundances were significantly positively related with phosphate-phosphorus and had no significant relationship with nitrate-nitrogen (Fig. 4C, D; Table 1). Consistently, heterotrophic and mixotrophic dinoflagellates in the south-western coastal waters (Zurai estuary) of the Bay of Bengal were found during monsoon period when estuaries were rich in inorganic phosphate [30]. The sources of phosphate in the Maheshkhali channel are mostly from the large number of tributaries, industrial effluents, and shrimp and bivalve farm wastes. In contrast, *Protoperidinium* blooms off the west coast of the Bay of Bengal had been reported when nitrate was below the detectable range while the concentrations of phosphate and silicate were also low (0.245 and 1.68 µm L⁻¹, respectively) [10]. Remarkably, comparatively lower abundances of *P. divergens* were recorded during the highest concentration of nitrate and phosphate in August (Fig. 3C, D) and it might be due to (1) comparatively lower abundance of the prey diatom species and (2) non-suitable environmental parameter such as higher water temperature (>23°C). In general, nutrient can affect indirectly heterotrophic dinoflagellates (i.e. *Pfiesteria* spp., *Karlodinium veneficum*, etc.), mediated through an increase in the abundance of algal prey that may be directly stimulated by specific nutrient forms [31,32,33].

4. CONCLUSION

For the first time, this study showed the detail documents on the bloom of *Protoperidinium divergens* in the Maheshkali channel. It is attributed that the abundance of *P. divergens* are mainly controlled by the availability of the prey items in the context of tolerance of environmental parameters. For instance, along with the prey diatoms, higher abundances of *P. divergens* were favoured by comparatively lower water temperature and higher concentration of phosphate-phosphorus. In contrast, the decreased abundance of *P. divergens* was due to lower abundance of the prey diatoms and higher water temperature (>23°C). Further research is needed to know the heterotrophic dinoflagellates community composition and their effects on food web structure in the Maheshkali channel, Bay of Bengal, Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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