Potential risk of *Mesodinium rubrum* bloom in the aquaculture area of Dapeng'ao cove, China: diurnal changes in the ciliate community structure in the surface water*

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Abstract

Diurnal changes in the structure of the ciliate community in surface waters were studied in the aquaculture area of Dapeng'ao cove, China. Two periods of heavy rainfall occurred during the study period, intensifying water column stratification and influencing the water's properties. A total of 21 ciliate taxa from 15 genera were identified; the dominant species was *Mesodinium rubrum*. The maximum abundance of *M. rubrum* reached 3.92×10^4 indiv. dm^{-3}, contributing 95.1% (mean value) to the total ciliate abundance. Diurnal changes in *M. rubrum* abundance were highly variable, the driving force probably being irradiance and food availability. The results suggest that *M. rubrum* may form blooms in aquaculture areas when there is a suitable physical regime with enriched nutrients, which is potentially harmful to the fish-farming industry.

1. Introduction

Ciliates play an important role in transferring the production of pico- and nanoplankton to meso- and macrocarnivores (Stoecker & Michaels 1991, Pierce & Turner 1993). Ota & Taniguchi (2003) suggested that ciliate populations in the East China Sea may control primary producers through intensive grazing and also act as important nutrient regenerators. Because of their ubiquitous distribution, small size and rapid metabolic and growth rates, ciliates are considered a key part of the aquatic ecosystem (Dolan 1999). Some ciliates, such as the red-tide ciliate *Mesodinium rubrum*, belong to harmful algae bloom (HAB) species in the ocean. Blooms of *M. rubrum* are recurrent events in the world, sometimes extending over hundreds of square kilometres (Lindholm 1990). They have been found off Peru (Ryther 1967), in the Ria de Vigo (Villarino et al. 1995), and also in Southampton Water (Hayes et al. 1989), where such blooms occur every year from late May to August, peaking in abundance in July (Williams 1996).

Dapeng'ao cove has been subject to eutrophication due to elevated nutrient discharges from aquaculture and to the human population growth in this region since the 1990s (Wang et al. 2006). HABs have been recorded in Daya Bay since the 1980s, and have occurred in almost every one of the last ten years or so (Zhao et al. 2004, Liu & Wang 2004, Wang et al. 2006, Song et al. 2009), especially in spring and summer. Wang et al. (2008) stated that HAB species not previously recorded during 1991–2003 in the northern South China Sea included *Phaeocystis globosa*, *Scrippsiella trochoidea*, *Heterosigma akashiwo* and *M. rubrum*. Previous studies in Dapeng'ao cove focused on the phytoplankton community, so information on the ciliate community was rarely available. In the present study, we aimed to study the short-term dynamics of the ciliate community in the aquaculture area of Dapeng'ao cove, with special reference to the ecological dynamics of *M. rubrum*.

2. Material and methods

2.1. Study site

Dapeng'ao cove is located in the western part of Daya Bay, China (Figure 1). The experiment was carried out over a complete diurnal cycle (12–13 August 2009) at a fixed station located in the aquaculture cage area.

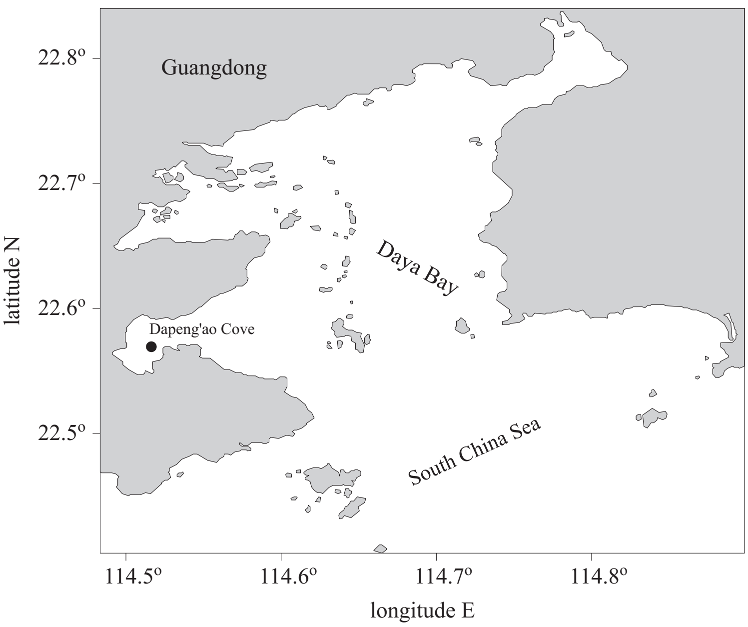

Figure 1. Study site (solid dot) in the aquaculture area of Daya Bay

2.2. Sample collection and analysis

Photosynthetically Active Radiation (PAR) was monitored continuously with a Quantum Sensor (LI-COR LI-190SZ) installed on the roof of the Marine Biological Station (22.55°N, 114.53°E) at Daya Bay. This instrument makes a measurement every second in the 400–700 nm wave bands. Water samples were collected at 3 hr intervals, from 12:00 hrs on 12 August to 12:00 hrs on 13 August. Water samples were collected from the surface layer (about 0.5 m depth) using a 5.0 L Niskin bottle. Temperature and salinity were measured in the surface water continuously over the investigation period using an YSI 6600 environmental monitoring system (Yellow Springs Instrument Co., USA). Inorganic nutrient concentrations were analysed using an auto-analyser (Quickchem 8500, USA).

Chlorophyll *a* (Chl *a*) was divided into micro- ($\leq$ 20 μm), nano- (2–20 μm) and pico- ($\leq$ 2 μm) size fractions by filtering the water samples sequentially through 20 μm polycarbonate filters, 2 μm polycarbonate filters and GF/F filters (Whatman). Filters containing pigments were stored at -20°C and analysed according to Parsons (1984). Water samples for ciliates were preserved with 1% Lugol's iodine solution. 10 ml of the subsamples were introduced into a sedimentation chamber and allowed to settle for at least 24 h. The bottom area of the whole chamber was examined under an inverted microscope to identify and count species. Protargol stain was used as necessary to aid species identification (Berger 1999). Taxonomic classification of ciliates was based on Kahl (1930–1935), Carey (1992), Foissner (1993) and Berger (1999). Pearson correlation analysis was conducted using SPSS 13 between abiotic and biotic parameters.

3. Results

3.1. Environmental variables

Two rainfall events occurred between 02:30 and 06:00 hrs and between 09:20 and 11:50 hrs on 13 August. The detailed environmental changes as well as biological factors were described in our previous publication (Liu et al. 2011). Owing to the heavily overcast conditions associated with the precipitation, the incident solar irradiance was extremely variable (Figure 2). Sea surface temperature (SST) ranged from 28.87°C to 29.91°C, sea surface salinity from 26.52 to 30.91 (Liu et al. 2011). Water stratification was enhanced after the rainfall, with picophytoplankton

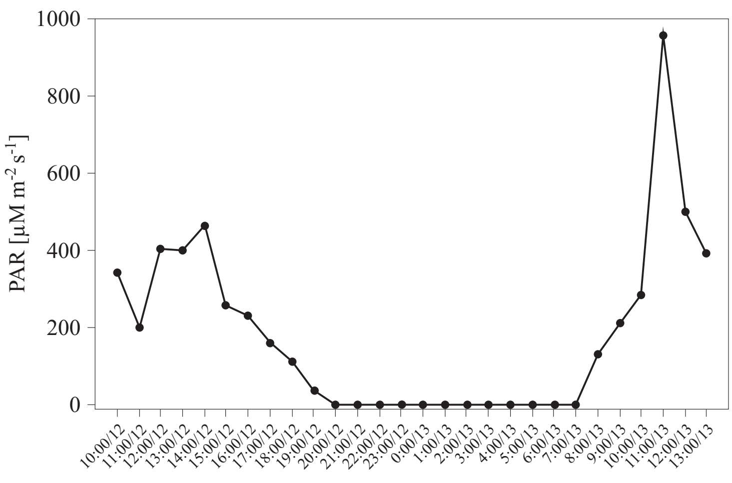

Figure 2. Diurnal changes in PAR at the Marine Biology Research Station, Daya Bay

(< 3 μm) dominating the phytoplankton biomass before the rainfall and nanophytoplankton (3–20 μm) dominant thereafter (Figure 3).

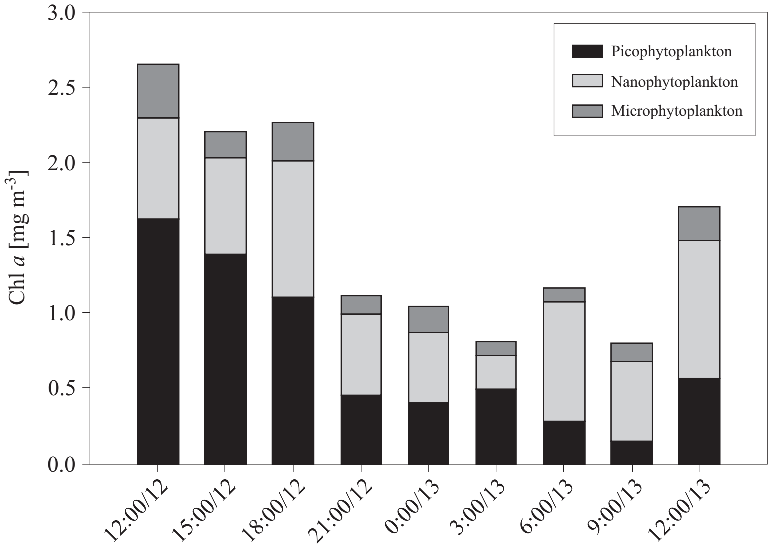

Figure 3. Diurnal variation in size-fractionated Chl *a*

3.2. Ciliate composition and abundance

A total of 21 ciliate taxa from 15 genera were identified, most of them to species level (Table 1). *Mesodinium rubrum*, *Paudella longa*, *Tintinnopsis tocantinencis* and *Strombidium conicum* were detected during the whole investigation period.

Table 1. Diurnal variation in ciliate species and abundance

Species	12 August		13 August						
	12:00	15:00	18:00	21:00	00:00	03:00	06:00	09:00	12:00
Didinium nasutum	12	7	7						
Eutintinnus fraknoii			7		3	6			
Eutintinnus stramentus	2	4		4					
Euplotes sp.	2						3		
Favella ehrenbergi	2	7				3			
Helicostomella longa	6						6	6	3
Laboea strobila	12	13	11						
Lacrymaiay sp.	2								
Mesodinium rubrum	17290	39168	21910	3600	1840	768	520	2392	10962
Metacylis oviformis		4							
Paudella longa	82	84	193	29	34	65	29	6	18

Table 1. (*continued*)

Species	12 August		13 August						
	12:00	15:00	18:00	21:00	00:00	03:00	06:00	09:00	12:00
Strombidium major	82	176	63	8				16	15
Strombidium conicum	25	144	98	27	22	27	11	39	27
Strombidium tintinnodes	40		28		28				18
Strombidium styliferum					2				
Tintinnopsis tocantinencis	4	13	7	24	24	5	3	6	21
Tintinnopsis					2	5			
Tintinnopsis sp.	8	13	35	65	32		6	6	12
Vorticella sp.		4	4						
Leprotintinnus nordquisti				3					9
Zoothamnium sp.									3
Total	17569	39637	22363	3756	1988	876	584	2471	11088

Numbers of ciliate species ranged from 7 to 14, and their abundance from 0.06 to 3.96×10^4 indiv. dm^{-3}. Numbers of species and abundance were both low during the hours of darkness. The abundance of *M. rubrum* ranged from 0.05 to 3.92×10^4 indiv. dm^{-3}, making up over 90% of the ciliate abundance (Figure 4), followed by *P. longa* and *Strombidium major*. Temperature showed a positive relationship with the abundance of *M. rubrum* ($p < 0.05$) and picophytoplankton biomass displayed a positive relationship with ciliate abundance ($p < 0.01$).

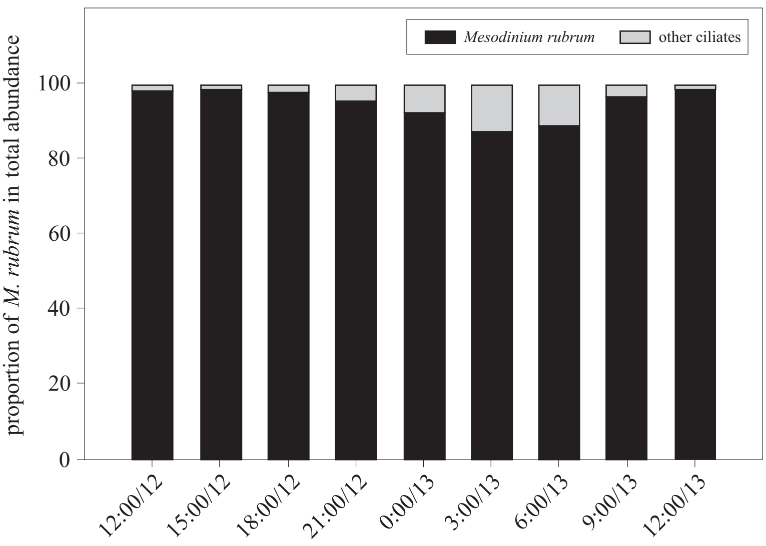

Figure 4. Diurnal variation in *Mesodinium rubrum* with respect to total ciliate abundance

4. Discussion

The bloom dynamics of *Mesodinium rubrum* has been well studied and it is known that populations of this species may undergo diel vertical migrations to exploit nutrient-rich water masses and optimal light levels (Lindholm et al. 1990, Passow 1991). Irradiance-driven nitrate uptake and the capacity for the dark uptake of ammonium and dissolved organic nitrogen combined with potential internal recycling, gives *M. rubrum* obvious advantages for producing blooms (Frances et al. 1990). In the present study, the low abundance of *M. rubrum* during the night, an observation consistent with previous studies, indicated that irradiance intensity may play an important role in modulating the vertical migration of *M. rubrum*.

The heavy rainfall could have been another important reason inhibiting ciliate abundance during the night. The maximum precipitation was recorded at night and increased the turbidity of the surface water. In addition, the phytoplankton biomass was obviously reduced in the upper layer (Liu et al. 2011). Therefore, all the environmental and biological disadvantages mentioned above resulted in a dramatic decrease in ciliate abundance during the night. Since SST decreased when irradiation was low, and this could also have been partly due to the night-time precipitation, it is reasonable to find a significant positive correlation between SST and the ciliate abundance, as suggested by Table 2. The picophytoplankton biomass was also positively correlated with ciliate abundance, which can be attributed to the change in the phytoplankton community structure caused

Table 2. Correlation between *Mesodinium rubrum* abundance and environmental variables

Parameters	Abundance	Temperature	Salinity	NH_4^+	NO_3^-	PO_4^{3-}	SiO_3^{2-}	Pico-	Nano-
Temperature	0.73*								
Salinity	0.30	0.73*							
NH_4^+	–0.05	0.12	–0.28						
NO_3^-	–0.03	0.20	–0.20	0.82**					
PO_4^{3-}	–0.25	0.04	–0.20	0.79**	0.96**				
SiO_3^{2-}	0.02	0.06	–0.38	0.86**	0.91**	0.89**			
Pico-	0.83**	0.66*	0.32	–0.20	–0.12	–0.37	–0.21		
Nano-	0.39	0.26	–0.15	0.27	0.34	0.26	0.52	0.28	
Micro-	0.48	0.58	0.23	0.08	0.28	0.06	0.11	0.79**	0.46

Significant level: $^{*}p < 0.05$; $^{**}p < 0.01$.

by the precipitation. This indicates that physical driving factors may also be playing important short-term roles in the microbial food web.

M. rubrum was the dominant ciliate species and the maximum abundance reached 3.92×10^4 indiv. dm^{-3}, with percentages of always $> 85\%$ of the total ciliate abundance, even during the period of heavy rainfall. This suggests that *M. rubrum* is a very competitive bloom species in Dapeng'ao cove, and when there is a suitable physical regime with enriched nutrients, the risk of a *M. rubrum* bloom will increase and harm the aquaculture industry in Daya Bay.

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