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Spatial pattern of the planktonic ciliate community and its relationship with the environment in spring in the northern Beibu Gulf, South China Sea

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Key words: Tintinnid, aloricate ciliate, community, nitrogen, phosphorus

Abstract

Surface water samples were collected from 19 sites in the northern Beibu Gulf in April 2011. Species composition and abundance of planktonic ciliates were investigated. They were combined with environmental data to obtain the spatial pattern of the ciliate community and its relationship with the environment. A total of 36 species belonging to 13 genera and 3 orders (Oligotrichida, Haptorida and Tintinnida) were identified, including 10 dominant species. All 19 samples were divided into three groups using cluster analysis and multidimensional scaling (MDS) on the basis of the ciliate species composition and abundance. Group 1 was the least abundant. The species of the order Oligotrichida dominated in Group 2. In contrast, *Mesodinium* species were of increased importance in Group 3. Redundancy analysis (RDA) revealed that ciliate communities were more closely related to the level of nitrogen (total nitrogen, nitrate nitrogen, nitrite nitrogen) and phosphorus (total phosphorus, active phosphorus) rather than other variables. As the levels of nitrogen and phosphorus increased, communities dominated by species from the order Oligotrichida were replaced by those dominated by *Mesodinium* species.

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INTRODUCTION

Planktonic ciliates are microplankton ranging from 20 to 200 micrometers in size. They are not only a significant component of microplankton communities in the ocean, but they also play a crucial role in microbial food webs (Azam et al. 1983). As important consumers of pico- and nano- plankton, they are able to exploit nutriments unavailable to the larger organisms (Montagnes et al. 1988). Thus, planktonic ciliates may act as important trophic intermediates from the microbial loop to higher trophic levels in the sea. By virtue of their rapid response to environmental changes, planktonic ciliates may play a part in indicating the water quality in aquatic ecosystems (Xu et al. 2008, Jiang et al. 2012, Kim et al. 2012).

The Beibu Gulf is a semi-enclosed gulf in the South China Sea, surrounded by the land territories of China and Vietnam. Rich nutrients and low salinity conditions exist in the littoral zone while oligotrophic and hyperhaline conditions exist at the continental slope and in the open sea (Liu et al. 2010). The Beibu Gulf in the South China Sea has a typical subtropical climate influenced by the East Asian Monsoon; southwesterly winds prevail in summer and northeasterly winds in winter (Sun 2005, Chen et al. 2011, Ling et al. 2011). The whole Beibu Gulf is influenced by several water masses and currents (Sun 2005). The existing upwelling and downwelling are affected by the monsoon and topography. Not only is the thermocline caused by the seasonal stratification of the seawater, but also

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other conditions, including light intensity, temperature, salinity and nutrients are significantly different in the whole Beibu Gulf (Liu et al. 2010). Moreover, with the rapid development of economy along the coast of Guangxi, the influence of human on the ecology of Guangxi offshore waters has become more obvious over the recent years. The increasing amounts of pollutants entering the coastal waters have led to the deterioration of the water quality in the Beibu Gulf (Xin et al. 2010). Because of the complex ecological characteristics and situation in the Beibu Gulf, it has become necessary to conduct research on the biological community and its response to environmental changes.

So far there have been several studies on the spatial patterns of marine planktonic ciliates (Sanders 1987, Montagnes et al. 1988, Zhang and Wang 2000, Kchaou et al. 2009, Jiang et al. 2011, Kim et al. 2012). There is, however, limited information about marine ciliate communities in China, and relevant research in China has mostly been conducted in the Bohai and Yellow Seas (Song and Bradbury 1998, Zhang and Wang 2001, Zhang et al. 2002, Zhang et al. 2008, Jiang et al. 2011, Yu et al. 2011) and the South China Sea (Gómez 2007, Su et al. 2007, Zhang et al. 2010, Feng et al. 2010, Liu et al. 2010). Despite a number of investigations into other plankton community structures in the Beibu Gulf, there are no data available on ciliate community structures. The objectives of this research are as follows: (1) to record the species composition and abundance of ciliates occurring in spring; (2) to determine the spatial pattern of ciliate communities; (3) to investigate the relationship between ciliate communities and environmental factors in the northern Beibu Gulf. The results of this study will provide valuable data for further studies on ciliate community ecology.

Materials and Methods

Study sites and sampling

Nineteen sites were located in the northern Beibu Gulf (108.25°~109.88° E, 20.05°~21.46° N), which is surrounded by Vietnam, Guangxi and Leizhou Peninsula, China (Fig. 1). The cruise was carried out on the vessel "Tianying" from April 20 to 26, 2011.

Temperature and salinity of seawater were measured by using conductivity-temperature-depth (CTD) oceanic profilers (SBE-917) via probes. Seawater samples were collected from the surface

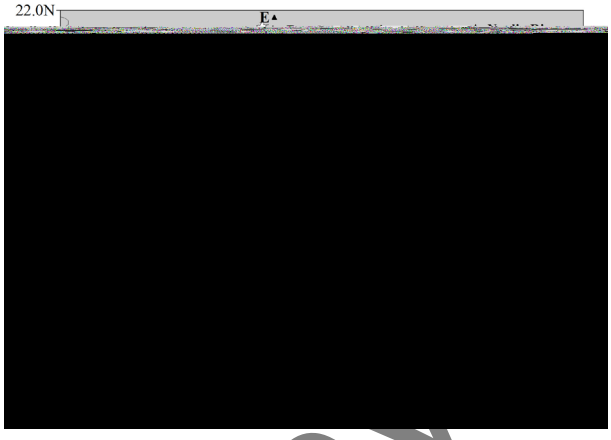

Fig. 1. Sampling sites of planktonic ciliates in the northern Beibu Gulf, April 2011

layers using Niskin bottles. A total of 19 water samples were obtained and fixed with acid Lugol's iodine solution (final concentration 2%) in plastic bottles. Chemical parameters, including pH, dissolved oxygen (DO), total organic carbon (TOC), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonium nitrogen (NH_4-N), total nitrogen (TN), active phosphorus (AP), total phosphorus (TP) and active silicon (ASi) were measured according to the standard methods defined in the Offshore Marine Chemical Survey Technical Regulations (908 Office of the State Oceanic Administration 2006). Chlorophyll-*a* (Chl *a*) concentration was measured using a Turner Fluorometer (10-AU-005).

Identification and enumeration

1 l Lugol's fixed seawater was left to settle for more than 48 hours resulting in 15 ml of a concentrated sample (Utermöhl 1958). Each time, 0.1 ml of a well-mixed concentrated sample was placed on a microscope slide and the ciliates were counted under a light microscope at a magnification of 400×. Ten slide replicates were examined for each concentrated sample. Tintinnids were identified on the basis of lorica morphology and species description provided by Kofoid and Campbell (1929, 1939), Hada (1932, 1937, 1938), Nie (1934) and Lynn (2008). Aloricate ciliates were identified following Leegaard (1915), Song et al. (2009) and Lynn (2008). The classification system mainly referred to Lynn (2008). All ciliates were finally identified to the lowest possible taxa.

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Data analysis

Dominance (Y) of species was calculated by the following equation:

$$= (—) \times$$

where n_i = abundance of the i^{th} species; N = total number of individuals; and f_i = occurrence of the i^{th} species.

Species number and abundance were calculated for each sample. The horizontal distribution pattern of planktonic ciliates in the northern Beibu Gulf was displayed using surfer 8 software. Cluster analysis and multidimensional scaling (MDS) of ciliate communities were analyzed using the PRIMER v5.0 package, in which the Bray-Curtis similarity matrix was computed on square root-transformed biotic data and the Euclidean distance matrix on log-transformed abiotic data (Clarke and Gorley 2001). The variance between groups of samples was tested using the submodule ANOSIM in PRIMER v5.0, and the submodule SIMPER (Similarity Percentage Analysis) was applied to find the species with a cumulative contribution of 90% in each group. Redundancy analysis (RDA) was applied to obtain further information on the relationship between ciliate communities and environmental parameters by the Canoco for Windows 4.5 package (ter Braak and Smilauer 2002).

RESULTS

Environmental parameters

Surface temperature and salinity were within the range of 20.7-22.6°C and 32.099-32.886, respectively. Generally, the surface water had a higher temperature at the head of the Beibu Gulf and higher salinity in the southwest of the study area. DO and pH were within the range of 7.65-8.79 mg l^{-1} and 8.24-8.38, respectively. The chlorophyll-*a* concentrations varied from 0.039 to 3.87 μg l^{-1}, sharply decreasing in a westerly direction from the west of the Leizhou Peninsula.

Species composition

A total of 36 species belonging to 13 genera and 3 orders (Oligotrichida, Haptorida and Tintinnida) were identified (Table 1). Oligotrichida was the most abundant order in terms of both species number and abundance. Oligotrichida, Haptorida and Tintinnida accounted for 74.48%, 24.09% and 1.43% of the

Table 1

List of ciliate species recorded in the northern Beibu Gulf, April 2011

Species	Abundance (ind l^{-1})		Occurrence
	Range of variation	Mean	
Oligotrichida			
Apostrombidium pseudokielum	0~60	5	15.79%
Omegastrombidium elegans	0~30	5	26.32%
Omegastrombidium jankowskii	0~105	17	47.37%
Parallelostrombidium paralatum	0~75	13	36.84%
Parastrombidium faurei	0~60	5	15.79%
Rimostrombidium caudatum	0~555	57	52.63%
Rimostrombidium kahli	0~30	4	21.05%
Rimostrombidium orientale	0~150	21	42.11%
Spirostrombidium cinctum	0~180	40	57.89%
Spirostrombidium platum	0~45	5	15.79%
Spirostrombidium schizostomum	0~15	2	10.53%
Spirotontonia turbinata[3]	0~630	216	89.47%
Strombidinopsis cheshiri	0~75	13	42.11%
Strombidinopsis elegans[6]	0~1605	169	52.63%
Strombidinopsis elongata	0~90	13	31.58%
Strombidinopsis minima	0~90	9	26.32%
Strombidium apolatum	0~330	61	57.89%
Strombidium capitatum	0~420	81	52.63%
Strombidium conicum[5]	0~930	186	73.68%
Strombidium globosaneum[10]	0~345	93	73.68%
Strombidium inclinatum[7]	0~945	118	68.42%
Strombidium montagnesi	0~375	77	57.89%
Strombidium paracalkinsi	0~795	92	57.89%
Strombidium parastyliferum	0~345	65	63.16%
Strombidium sp.	0~300	72	68.42%
Strombidium styliferum[9]	0~480	94	73.68%
Strombidium sulcatum	0~420	76	57.89%
Strombidium tintinnodes[2]	0~780	310	94.74%
Strombidium wilberti [8]	0~825	137	57.89%
Haptorida			
Mesodinium rubrum[1]	15~2220	474	100.00%
Mesodinium velox[4]	0~975	216	78.95%
Tintinnida			
Codonella rapa	0~75	5	10.53%
Stenosemella pacifica	0~165	21	42.11%
Tintinnopsis schotti	0~15	2	15.79%
Tintinnopsis tubulosoides	0~45	4	10.53%
Tintinnopsis urnula	0~120	9	15.79%

numbers in [ ]-ranks of 10 dominant ciliates

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total abundance, respectively. The average abundance of Tintinnids and aloricate species was 41.05±16.54 ind. l^{-1} and 2743.42±344.91 ind. l^{-1}, respectively.

There were 10 dominant species (dominance $Y \geq 0.02$), the cumulative abundance of which exceeded 72% of the total abundance. They were: *Mesodinium rubrum* (Y=0.1704), *Strombidium tintinnodes* (Y=0.1056), *Spirotontonia turbinata* (Y=0.0693), *Mesodinium velox* (Y=0.0611), *Strombidium conicum* (Y=0.0491), *Strombidinopsis elegans* (Y=0.0319), *Strombidium inclinatum* (Y=0.0289), *Strombidium wilberti* (Y=0.0284), *Strombidium styliferum* (Y=0.0248) and *Strombidium globosaneum* (Y=0.0247). Among the dominant species, *Mesodinium rubrum* was the only species recorded in all samples, and occurrence of each dominant species was more than 50%.

Horizontal distribution

Distribution of ciliate abundance

In April, the abundance of total ciliates ranged from 555 ind, l^{-1} frdan(d)-1(a)(d)-1(/TT1 1 Tf-16.9P(483.96 TJ0.1[7(e)3(dC816.96 120.96 449.4 Tmp)3(01 Tc 0.218 Tw 1.2835(ar)-5(o)-2(m)9(5 11.04 529(fmi) TditTd( )6oyiy1.2835(ar)t..e)3(th)-5

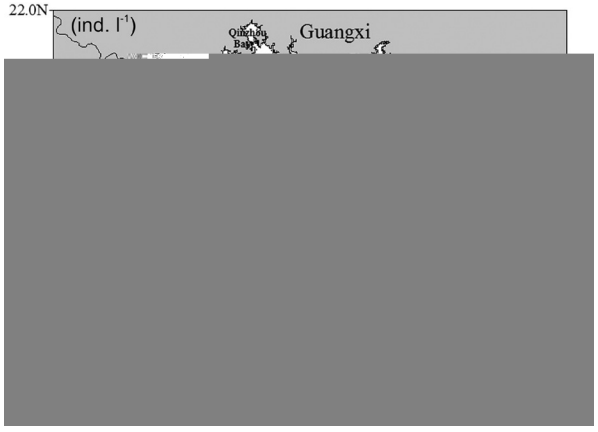

Fig. 3. Distribution pattern of aloricate ciliate abundance in the surface waters of the northern Beibu Gulf

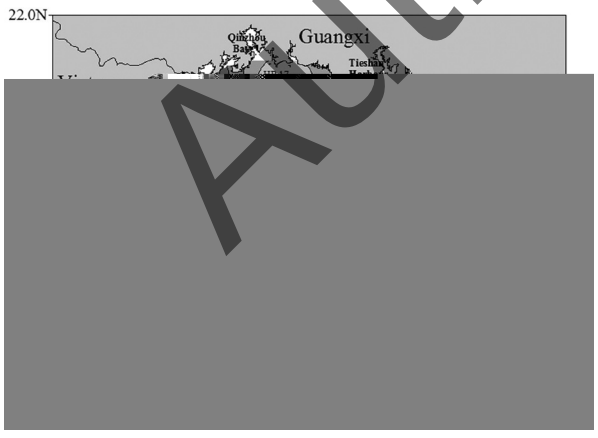

Fig. 4. Distribution pattern of Tintinnid abundance in the surface waters of the northern Beibu Gulf

Fig. 5. Distribution pattern of the number of total ciliate species in the surface waters of the northern Beibu Gulf

Fig. 6. Distribution pattern of the number of aloricate ciliate species in the surface waters of the northern Beibu Gulf

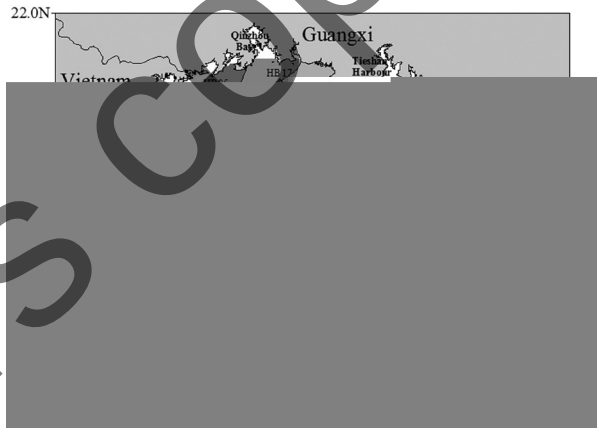

Fig. 7. Distribution pattern of the number of Tintinnid species in the surface waters of the northern Beibu Gulf

groups at a similarity level of 40% (Fig. 8): Group 1 comprised the sample from site HB16, which was situated in the coast of Guangxi. This group was different mainly due to the lowest abundance. Group 2 included samples from sites HB17, HB19, HB28, HB29. Species *Strombidium tintinnodes* and *Spirotontonia turbinate* were the main contributors to the specificity of Group 2. Group 3 consisted of the samples from the other sites (HB01, HB03, HB05, HB12, HB14, HB18, HB21, HB23, HB24, HB26, HB30, HB32, HB35 and HB40), the common feature of which was a large contribution of *Mesodinium rubrum*, *Strombidium tintinnodes* and *Mesodinium velox*. Group 3 was further divided into four subgroups. The first subgroup (HB05, HB35, HB23, HB24, HB40) dominated by *Mesodinium velox* and *Mesodinium rubrum* displayed the

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Table 3

Comparison of ciliate data in different parts of the China Sea

No.	Area	Season of sampling	Total abundance (10^3 ind. l^{-1})	Abundance of aloricate ciliates (10^3 ind. l^{-1})	Abundance of Tintinnids (10^3 ind. l^{-1})	Reference
1	Bohai Sea	Fall	0.02-0.77			Zhang and Wang 2002
2	Laizhou Bay, Bohai Sea	Summer	0-2.38			Zhang and Wang 2000
3	Laizhou Bay, Bohai Sea	Summer	0-0.27			Zhang et al. 2002
4	Jiaozhou Bay, Yellow Sea	Summer		<21.30		Zhang and Wang 2001
		Spring			<1.72	
5	Yellow Sea	Spring	0.04-3.42			Zhang et al. 2002
6	Southern Yellow Sea	Summer			0.158	Zhang et al. 2008
7	Eastern China Sea			0-0.27	0-0.15	Zhang et al. 2001
8	South China Sea	Winter	<20			Gómez 2007
9	Northern South China Sea	Fall	0.009-0.102			Su et al. 2007
10	Northern South China Sea	Fall	0-5.757			Zhang et al. 2010
11	Northern South China Sea	Summer	0.05-2.425		0-2.175	Liu et al. 2010
12	Northern South China Sea (>76 ..m)	Fall			0-0.0418	Feng et al. 2010
13	Shantou coastal water, South China Sea	Spring			0.120-2.265	Liu et al. 2011
		Fall			0.516-1.520	
14	Northern Beibu Gulf, South China Sea	Spring	0.555-5.985	0.555-5.970	0-0.285	This study

by environmental factors were well reflected in the two-dimensional plot. Furthermore, it was demonstrated that ciliate communities were sensitive to environmental changes and may respond strongly to changes in the aquatic environment.

The result of spearman correlation analysis in this study coincided with Yu et al. (2011), i.e. the abundance of total ciliates, Tintinnids and aloricate ciliates in surface waters was not significantly correlated with temperature or salinity. However, our result indicated that they were significantly affected by the level of total phosphorus in the surface water in the northern Beibu Gulf. Both clustering and RDA analysis demonstrated that the primary factor in classification of ciliate communities in the study area was the levels of nitrogen and phosphorus nutrients, rather than other physicochemical or biological factors, i.e. temperature, salinity, dissolved oxygen, pH, chlorophyll-*a*, etc. The research in the Hangzhou section of the Grand Canal, northern China indicated that specific protozoan species (e.g. *Carchesium polypinum* and *Vorticella putrina*) were significantly related to COD, either alone or in combination with TP and/or TN (Shi et al. 2012). The spatial variations of protist community structures in Jiaozhou Bay, northern China were correlated with nitrate nitrogen (NO_3-N) and soluble reactive phosphates (SRP). It seems to be a consensus that ciliate communities are significantly influenced by the level of nitrogen and phosphorus in the water environment. Nevertheless, the chemical forms of nitrogen and phosphorus, or the extent of impact exerted by other factors on the communities, will depend on local conditions.

According to Sanders (1995) and Yu et al. (2011), the abundance of ciliates is affected by multiple environmental factors, such as light intensity, turbulence, temperature, nutrient level, and predator-prey interactions. Both top-down and bottom-up interactions may control the population of ciliates. Oligotrichs, Tintinnids and *Mesodinium* spp. were dominant in the study area. Oligotrichs and *Mesodinium* spp. were autotrophic or heterotrophic species, while all the Tintinnids were heterotrophic (Tan et al. 2010). Heterotrophs feed on phytoplankton and bacteria, whereas autotrophs can absorb nutrients directly, and they both promptly respond to prey concentration. Nutrient levels in the seawater, therefore, affect the growth of phytoplankton and autotrophic ciliates, and have indirect influence on the total ciliate abundance (Sanders 1995). The atomic Si:N:P ratio of 16:16:1 (Redfield 1958) could be used as a criterion to determine which is the limiting element in the phytoplankton growth in certain waters (Lane et al. 2004). According to Xin et al. (2010), our study area appeared phosphorus deficient throughout the year, except summer, and was oligotrophic in spring as a consequence of lower concentration of nitrogen and phosphorus. This conclusion was consistent with our findings. Hence the level of nitrogen and phosphorus in this survey played an essential part in ciliate communities.

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