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Tintinnid community throughout the Bohai Sea during the spring

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Abstract

As one of the important microzooplankton in marine pelagic habitats, tintinnids are classified as neritic genera, oceanic genera and cosmopolitan genera. Until now, we know little about the interaction between neritic and oceanic genera in continental shelves. Low species richness area was found in the mixing area between neritic and oceanic genera in the East China Sea. In this study, we investigated the spatial distribution of tintinnid community throughout the entire Bohai Sea in May (spring) of 2014 to find out: (1) whether the tintinnid assembalges differed in the three major bays bordering this shallow inland sea; (2) whether there was low species richness area in the Bohai Sea. Three genera and 11 species attributed to neritic and cosmopolitan taxa were reported from this region. Tintinnid abundance averaged (81±216) ind./L (0–1 234 ind./L). Tintinnid community differed within the three major bays, with each characterized by different tintinnid taxa. Referring to the average abundance of three bays, tintinnids were most abundant in the Laizhou Bay ((328±445) ind./L), and least abundant in the Liaodong Bay ((34±57) ind./L). A low species richness area occurred in the central Bohai Sea (20–40 m isobaths), in some stations of which no tintinnid was recorded. Our study demonstrates that tintinnid community varied greatly over small spatial scales, and low species richness area was found in the mixing area between neritic and oceanic genera within this inland sea.

Key words: tintinnid, community structure, spatial distribution, Bohai Sea, spring

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1 Introduction

Tintinnids are planktonic choreotrich ciliates with lorica, of which about 900 species have been recognized based on their diverse lorica morphologies (Zhang et al., 2012). They feed mainly on nanoplankton, and are grazed by mesozooplankton, so play important roles in the transfer of matter and energy among microbial and classic food webs (Calbet, 2008; Dolan et al., 2013). Investigations on the ecology of tintinnids contribute to understanding the functioning of planktonic food webs. According to their geographical distribution characters around the world, tintinnid genera are classified as neritic genera, oceanic genera (warm, boreal and austral types) and cosmopolitan genera (Pierce and Turner, 1993; Dolan et al., 2013). Neritic species expand from shallow coastal waters to deeper offshore waters while oceanic species from the open ocean intrude into the coastal water from opposite direction. Tintinnid species richness in the surface waters was low in their mixing area because both neritic and oceanic species richness decreased. Li et al. (2016) confirmed this hypothesis and found a low species richness area in the East China Sea. In this study, we meant to test this hypothesis in the Bohai Sea, which has one of the widest shelves in the world (Mouton, 1952).

The Bohai Sea is an inland sea with an average water depth of 18 m (Feng et al., 1999). Three surrounding bays (Bohai Bay, Laizhou Bay and Liaodong Bay) are shallower than the central sea (Fig. 1). During spring, water properties in the three bays differ from that in the central sea water (Hainbucher et al., 2004; Bian et al., 2016). Due to the flowing of cool, high salinity water from the Yellow Sea through the Bohai Strait at the easternmost end of the Bohai Sea (Lin et al., 2002; Wei et al., 2002; Zhou et al., 2009), water temperature at the end tips of these three bays was higher than in the central region (Huang et al., 1999), and salinity in each bay, particularly the Laizhou Bay due to runoff from the Huanghe River (Yellow River), is lower than the central sea. In addtion, water temperature in the Liaodong Bay is usually lower than the Bohai Bay and Laizhou Bay, as it is located further north in the Bohai Sea.

Reports on tintinnid community in the Bohai Sea are limited. Chen et al. (2018) mainly focused on two transects across the Bohai Sea, and found oceanic tintinnid species rarely occurred in the Bohai Sea. In addition, there were some tintinnid studies in small part of the Bohai Sea such as the Bohai Bay (Yu et al.,

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2016a, 2018) and Laizhou Bay (Zhang and Wang, 2000; Chen et al., 2014). There was no tintinnid data in the Liaodong Bay. In this paper, we study the tintinnid assemblage in surface waters throughout the Bohai Sea to analyze: (1) whether the tintinnid assembalges in the three bays are different; (2) whether there is low species richness area in the Bohai Sea as in the East China Sea (Li et al., 2016).

2 Materials and methods

The investigation was conducted from May 17–26, 2014 in the Bohai Sea aboard R/V *Luchangyu 65678*. A total of 52 stations (Fig. 1) with depth 5–45 m were investigated in the Bohai Bay (7 stations), Laizhou Bay (7 stations), Liaodong Bay (20 stations), and the central sea (18 stations) according to Wang (2013).

At each station, surface temperature and salinity were recorded with a CTD sensor (Sea-Bird 19 plus). For chlorophyll *a* (Chl *a*) determination, 500 mL of water was filtered onto GF/F filters, which were frozen (–20°C). The filters were extracted with 90% acetone at –20°C in the dark for 24 h, and then Chl *a* concentrations were determined using a Turner Designs (Model II) fluorometer, calibrated with pure Chl *a* from Sigma (Strickland and Parsons, 1972).

For every tintinnid sample, 10 L of surface water was collected using Niskin bottles, and gently filtered through a small net of mesh pore-size 10 μm. About 150 mL samples were transferred into sample bottles and immediately fixed with Lugol's solution (1% final concentration). Samples were stored in a cool, dark environment until analysis. A subsample of 25 mL was settled in an Utermöhl counting chamber for at least 24 h, and then examined under inverted microscope (Olympus IX71) at 100× or 400× magnification. Tintinnids were counted, and the length and oral diameter of their lorica were measured. Species were identified based on lorica morphology (Hada, 1937; Yin, 1952; Zhang et al., 2012). Biogeographical types were determined following Pierce and Turner (1993) and Dolan et al. (2013).

Dominance index was calculated according to Xu and Chen (1989). Distributional data were presented as contour maps, established by Surfer 13 and bar charts by Grapher 5. PRIMER 6 was used for cluster analysis. Tintinnid abundance was fourth root transformed.

3 Results

3.1 *Hydrography*

Surface water temperature ranged from 10.15°C to 19.76°C (average (13.70±2.24)°C). Higher temperatures occurred nearer shore in each bay; lower temperatures occurred in the central sea (Fig. 2). The comparison of average temperature among the three bays showed that the highest average temperature occurred in the Laizhou Bay, and lowest in the Liaodong Bay (Fig. 3).

Surface water salinity ranged from 26.97 to 31.19 (averaging 29.69±1.05). Salinity was higher in the central sea and lower in each bay, and lowest average salinity occurred in the Laizhou Bay (Fig. 3). The distribution of temperature and salinity in surface waters showed that a weak flow of low temperature, high salinity water intruded into the central Bohai Sea (Fig. 2).

Chl *a* concentration ranged from 0.89 μg/L to 11.87 μg/L (average (3.8±2.62) μg/L), with higher values occurring mainly in coastal waters (Fig. 2). The average Chl *a* concentration was higher in the Bohai Bay and Laizhou Bay than in the Liaodong Bay (Fig. 3).

3.2 *Tintinnid species composition*

Three genera and 11 tintinnid species were identified in samples (Table 1, Fig. 4). Only two tintinnid geographical types were found in the Bohai Sea, i.e., neritic and cosmopolitan tintinnids. In terms of abundance in whole Bohai Sea, neritic tintinnids were more abundant than cosmopolitan ones, comprised 79.5% and 20.5% of total abundance, respectively.

Tintinnopsis baltica, *C. mobilis* and *T. rapa* represent the

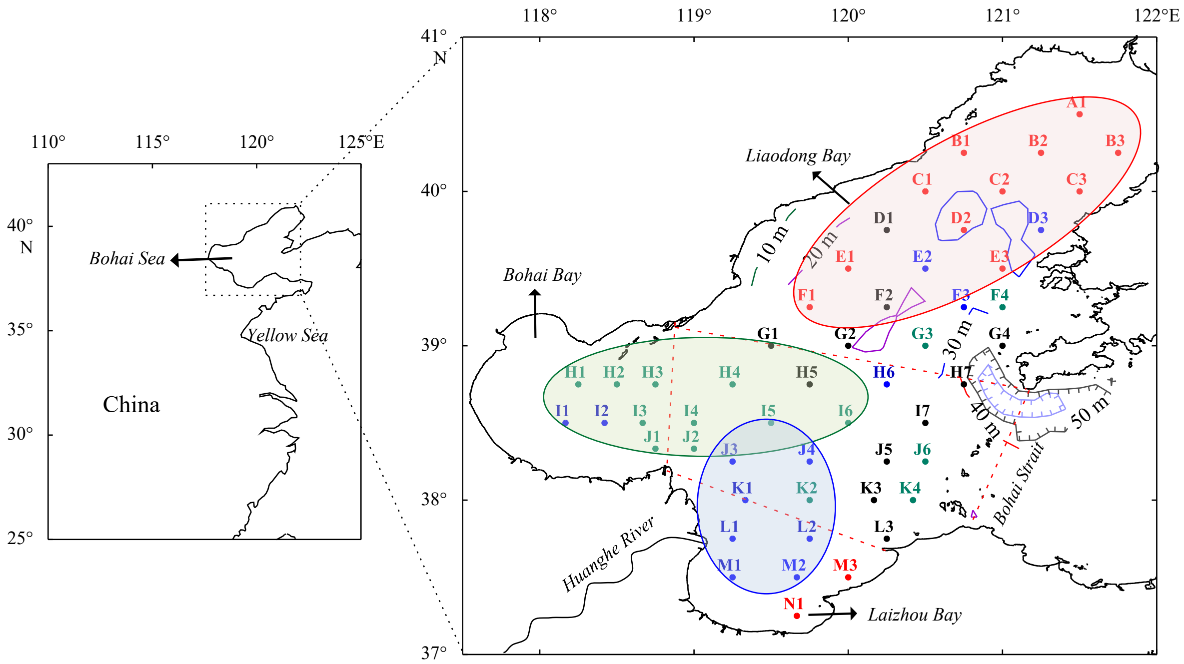

Fig. 1. Investigated stations in the Bohai Sea. The red dashed line depicts the division of the Bohai Sea (Wang, 2013) into bays and central sea. Contours indicate water depth. Different-colored dots (green, red, blue) indicate station groupings in cluster analysis. Black dots indicate stations with no tinitnnid recorded.

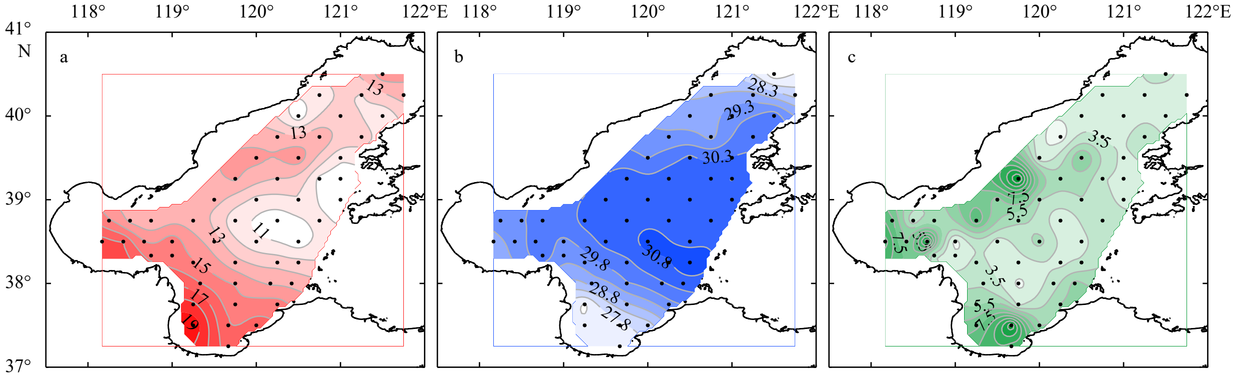

Fig. 2. Spatial distribution of temperature (°C, a), salinity (b) and Chl *a* concentration (μg/L, c) in surface waters of the Bohai Sea.

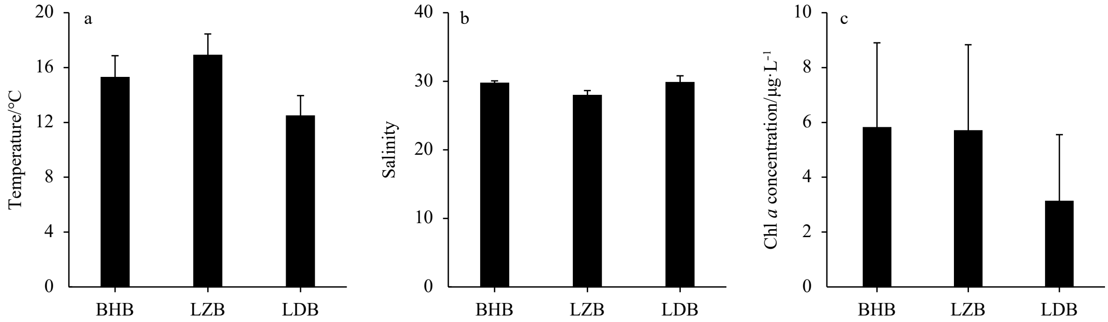

Fig. 3. Average temperature (a), salinity (b) and Chl *a* concentration (c) in the Bohai Bay (BHB), Laizhou Bay (LZB) and Liaodong Bay (LDB).

Table 1. List of tintinnid species in the Bohai Sea

	Maximum abundance/ind.·L^{-1}	Occurrence frequency	Dominance index
Cosmopolitan			
Codonellopsis lusitanica	168	0.08	0.00
C. mobilis	118	0.40	0.03
Neritic			
Tintinnidium mucicola	41	0.14	0.01
Tintinnopsis baltica	1 219	0.27	0.19
T. beroidea	8	0.31	0.00
T. brasiliensis	6	0.06	0.00
T. directa	8	0.02	0.00
T. kofoidi	3	0.02	0.00
T. lohmanni	8	0.06	0.00
T. radix	3	0.02	0.00
T. rapa	217	0.33	0.03

three most dominant species according to dominance index. *Tintinnopsis baltica* was more abundant in waters with temperature of 15–20°C (Fig. 5), and the maximum abundance was recorded in the Laizhou Bay (Fig. 6). *Codonellopsis mobilis* was more abundant in waters with temperature of 10–17°C (Fig. 5) with maximum abundance in the Liaodong Bay (Fig. 6). *Tintinnopsis rapa* was more abundant in waters of 10–17°C (Fig. 5), and the maximum abundance was recorded in the Liaodong Bay (Fig. 6).

3.3 *Tintinnid community composition in the three bays*

Tintinnid assemblages and some dominant taxa differed among the three bays. In the Bohai Bay, *Tintinnopsis baltica* was dominant, contributing 79.5% to total tintinnid abundance. In the Laizhou Bay, *Tintinnopsis baltica* was also dominant, with a contribution of 85.8% to total tintinnid abundance. In the Liaodong Bay, *Tintinnopsis rapa* and *Codonellopsis mobilis* contributed 53.4% and 37.1% to total tintinnid abundance, respectively (Fig. 7). Average abundance of tintinnids was highest in the Laizhou Bay ((328±445) ind./L), where the value was ~1.3 and 8.8 times higher than that in the Bohai Bay ((143±297) ind./L) and Liaodong Bay ((34±57) ind./L), respectively.

Using Bray-Curtis similarity index and cluster analysis, stations (except for stations with no tintinnid recorded) were clustered into three groups. Group I mainly included most stations in the Bohai Bay (except for Stations I1 and I2) and some stations in the central sea adjacent to the Bohai Bay. Group II mainly included most stations in the Liaodong Bay (except for Stations D3, E2, F3, F4, and G3). Group III mainly included most stations in the Laizhou Bay (except for Stations M3 and N1) and some stations in the central sea adjacent to the Laizhou Bay (Figs 1 and 8). Each group represented tintinnid community from single bay and its adjacent area, and three groups tended to converge in the central sea.

3.4 *Spatial distribution of tintinnid species and abundance*

Average species richness throughout the Bohai Sea was 2. Species richness was greater in bays than in deeper central-sea waters. No tintinnid occurred in some stations of central sea (Fig. 9a). Average abundance throughout the sea was (81±216) ind./L (0–1 234 ind./L). Higher abundance of total tintinnids was distributed in bays, especially near the Huanghe River Estuary. Tintinnid abundance was low in the central Bohai Sea, with average abundance of (14±37) ind./L (Fig. 9b).

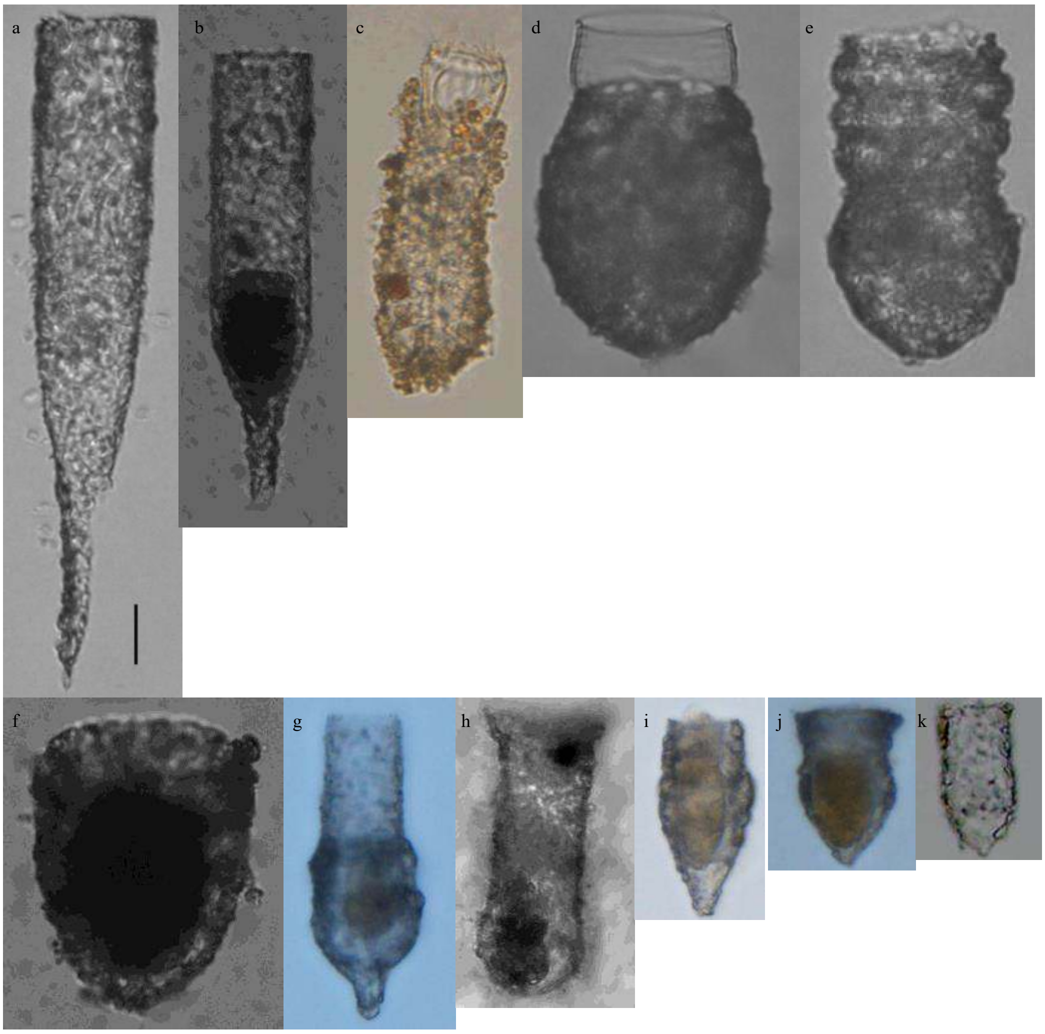

Fig. 4. Photos of tintinnid species in the Bohai Sea. a. *Tintinnopsis radix*, b. *Tintinnopsis kofoidi*, c. *Tintinnidium mucicola*, d. *Codonellopsis mobilis*, e. *Tintinnopsis lohmanni*, f. *Tintinnopsis brasiliensis*, g. *Codonellopsis lusitanica*, h. *Tintinnopsis directa*, i. *Tintinnopsis rapa*, j. *Tintinnopsis baltica*, and k. *Tintinnopsis beroidea*. Scale bar: 20 μm.

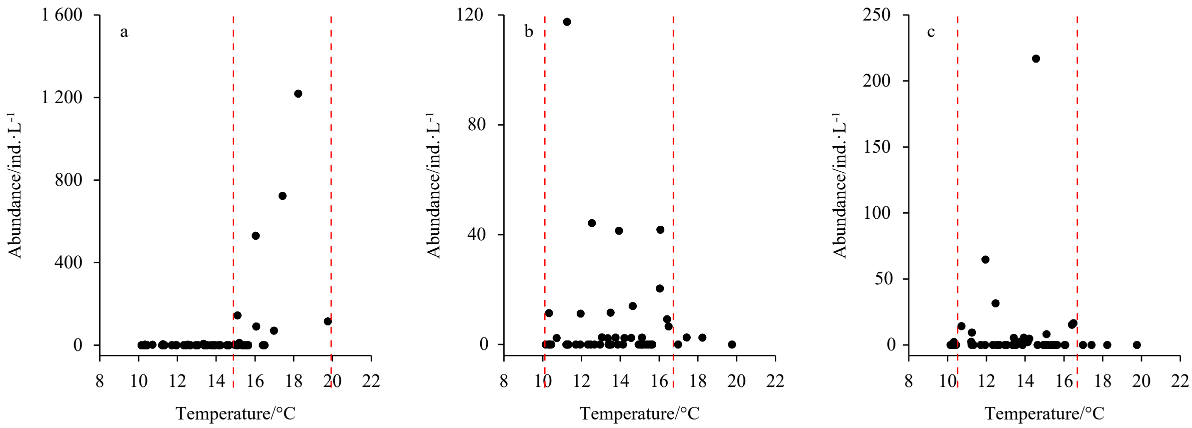

Fig. 5. Relationship between tintinnid abundance and temperature. a. *Tintinnopsis baltica*, b. *Codonellopsis mobilis*, and c. *Tintinnopsis rapa*.

4 Discussion

4.1 *Differences of tintinnid community in the three bays*

Studies on tintinnids of the Bohai Sea are scarce compared to the neighboring Yellow Sea and East China Sea. We found obvious difference in tintinnid composition among the three bays. *Tintinnopsis baltica* occurred in waters of higher temperature, mainly within the Bohai Bay and Laizhou Bay, while the hypo-

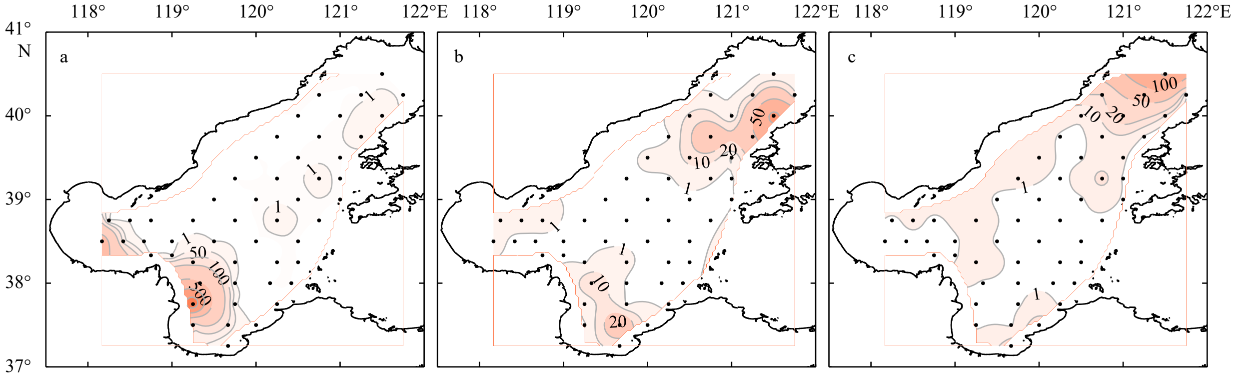

Fig. 6. Abundance (ind./L) distributions of *Tintinnopsis baltica* (a), *Codonellopsis mobilis* (b) and *Tintinnopsis rapa* (c).

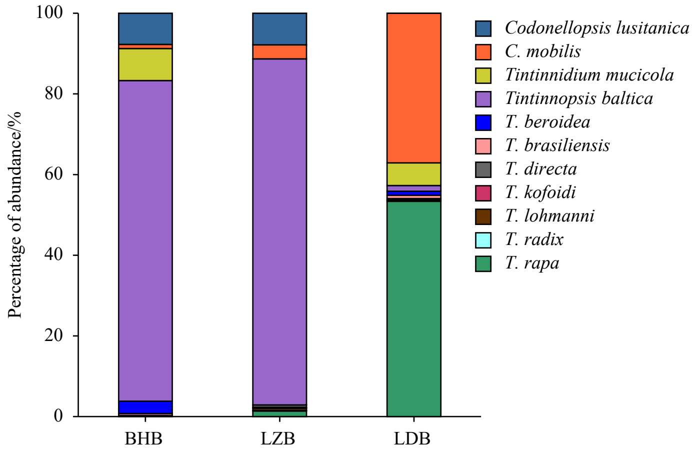

Fig. 7. Percentage of each species among total tintinnid abundance in the Bohai Bay (BHB), Laizhou Bay (LZB) and Liaodong Bay (LDB).

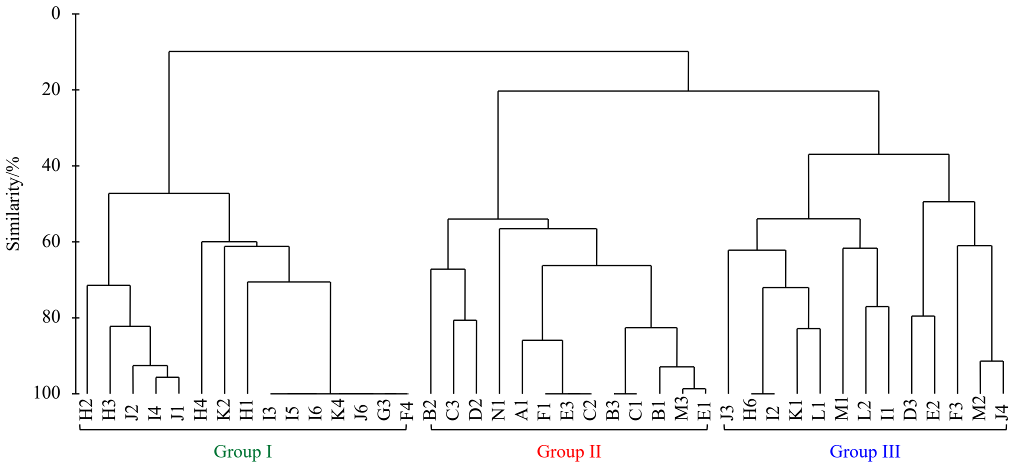

Fig. 8. Hierarchical cluster analysis of tintinnid community structures in the Bohai Sea.

thermophilous *C. mobilis* and *T. rapa* occur mainly within the cooler waters of the Liaodong Bay. Dominant tintinnid species can change in response to seasonal variation in temperature (Feng et al., 2018). Our study confirmed these findings that the difference of species composition among the three bays might be concerned with regional temperature variation in the Bohai Sea.

Tintinnid abundance in the Liaodong Bay was lower than in the Bohai Bay and Laizhou Bay, possibly because of lower temperature and Chl *a* concentration in this region. The Liaodong Bay, in the northeast Bohai Sea, had average temperatures of 3–4°C lower than those in the Bohai Bay and Laizhou Bay. Tintinnids might have lower growth rates in cooler waters (Montagnes, 1996). Lower Chl *a* concentration might also contribute to low tintinnid abundance, because of the trophic coupling between

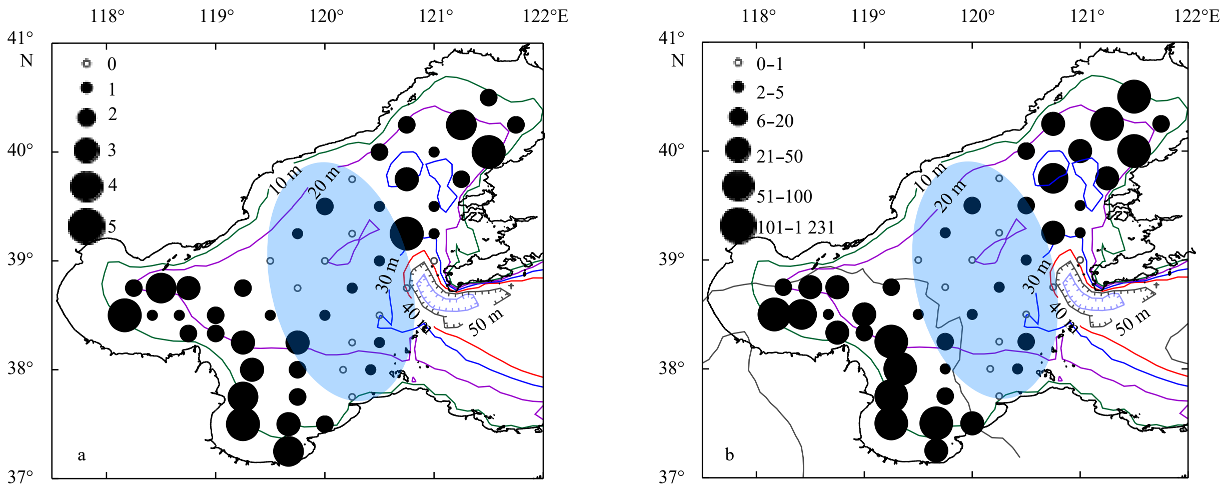

Fig. 9. Spatial distribution of species richness (a) and abundance (ind./L, b) of tintinnids in the Bohai Sea. Blue shaded area highlights the low species richness and abundance area.

phytoplankton and tintinnid abundance (Dolan et al., 2006).

4.2 *Low species richness in the central Bohai Sea*

The shelf of Bohai Sea and Yellow Sea was one of the widest shelves in the world (Mouton, 1952). The Bohai Sea was located in the innermost part of the long shelf, and thereby was influenced by the oceanic species from the Yellow Sea. The low species richness area should be more prominent than that in the East China Sea, which was supported in our study. First, no tintinnid was recorded in half stations of the low species richness area in the Bohai Sea. Second, the low species richness area was located in shallower water than that in the East China Sea (Li et al., 2016).

As proposed by Li et al. (2016), the mechanism shaping the low species richness in the central Bohai Sea may be attributable to interactions between neritic and oceanic communities. From coastal to oceanic seas, neritic species richness decreased while oceanic species richness increased along with the increase in salinity. As a result, species richness of both neritic and oceanic communities decreased in their mixing area. Oceanic tintinnids seldom occurred in the Bohai Sea (Chen et al., 2018), an area in which we report only neritic and cosmopolitan species. Additionally, while Chen et al. (2018) reported one uncommon (<24 ind./L) warm-water species (*Ascampbelliella armilla*) in summer, we report no warm-water species during spring. Warm water species intrusion into the Bohai Sea might occur rarely.

In our study, most stations with low species richness were located in the 20–40 m isobaths, which was shallower than the 50–80 m isobaths reported by Li et al. (2016) for the East China Sea. This may be due to weaker water turbulence in the Bohai Sea than in the East China Sea (Feng et al., 1999). Tintinnids, especially neritic tintinnid with agglutinated lorica, need turbulence to suspend in the water column (Yu et al., 2016b). This might be the reason that low species richness area appeared in the shallower waters in the Bohai Sea than in the East China Sea.

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