

Large scale northward expansion of warm water species *Skeletonema tropicum* (Bacillariophyceae) in China seas*

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Abstract *Skeletonema tropicum* is regarded as a species with an affinity to warm waters and it has never been reported in seas where temperatures drop below 11°C in winter. Previous studies in China reported that *S. tropicum* was restricted to subtropical and warm temperate seas (East and South China Seas), but the species was recently found during August cruises of 2009 and 2010 in Jiaozhou Bay, Yellow Sea, located several hundred kilometers to the north. Here, winter water temperatures often drop below 5°C. Identification of *S. tropicum* was confirmed under light and scanning electronic microscopes and maximum cell abundance in Jiaozhou Bay was estimated as 1.73×10^4 cell/L. This record of *S. tropicum* in Jiaozhou Bay represents a significant northward expansion in the geographic range of the species. Ship ballast water was identified as a possible carrier of *S. tropicum* from southern places along Chinese coastline, and in addition, thermal pollution from local power stations and seawater desalination plants may provide suitable conditions for species over-wintering.

Keyword: biogeography; ballast water; thermal pollution; *Skeletonema tropicum*; Yellow Sea

1 INTRODUCTION

Diatoms, as one of the most important primary producers in marine ecosystem, are sensitive to changes in the environment and regarded as important indicators of water masses with different physical and chemical characteristics (Sarno et al., 2005). One of the most widespread genera of coastal marine diatoms is *Skeletonema* Greville, which contains approximately 20 species (Zingone et al., 2005). One of these species, *Skeletonema tropicum* CLEVE was first described by Cleve (1900) in samples from the tropical waters of the Atlantic Ocean and Caribbean coast of South America, but was later found along the coast of Eastern USA, the Atlantic side of Panama, Guyana and Surinam, the East and South China Seas (Cheng and Liu, 1992; Cheng et al., 2008), coastal Japan (Ueno, 1993), Gulf of Mexico (Castillo et al., 1995), Italy (Sarno et al., 2005), the Pacific side of Panama (Kooistra et al., 2008), southern Brazil (Bergesch et

al., 2009) and India (Naik et al., 2010) (Fig.1).

On the basis of the geographic range of *S. tropicum*, it is regarded as a warm water species, restricted to tropical and subtropical areas. Hulburt and Guillard (1968) concluded that *S. tropicum* could not exist in regions where the seawater temperature drops below 13°C in winter; Ueno (1993) found that *S. tropicum* appeared in summer in the coastal waters of Japan but disappeared in early winter when the water temperature fell below 12°C; the occurrence of *S. tropicum* in the Mediterranean Sea and coastal waters of Uruguay showed similar temperature range, winter surface seawater temperatures were not below 11°C (Kooistra et al., 2008; González-Piana and

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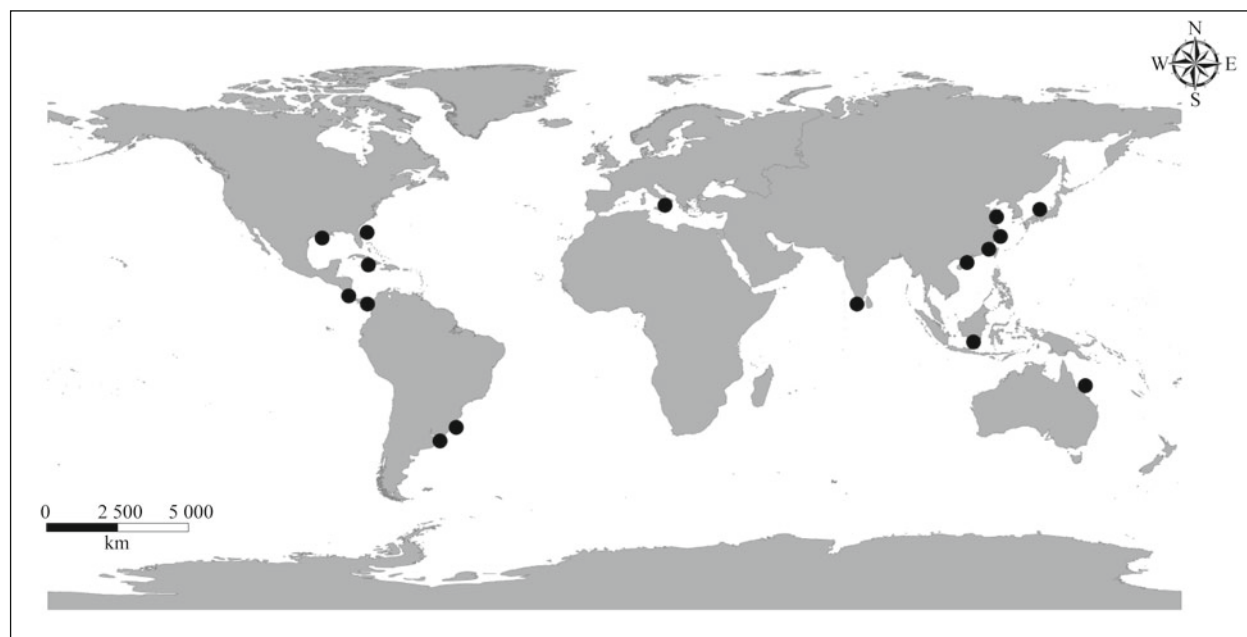


Fig.1 Global biogeography of *Skeletonema tropicum*

Ferrari, 2009). Yamada et al. (2010) found *S. tropicum* in Dokai Bay, Japan, where seawater temperatures ranged from 13.2°C to 27.2°C. In China, *S. tropicum* has previously only been observed in the East and South China Seas, including Wenzhou, Xiamen, Daya Bay, Taiwan Straits and Haikou, which are all tropical or subtropical areas (Cheng and Liu, 1992; Cheng et al., 2008; Kooistra et al., 2008). The Yellow Sea is located in the northern region of the East China Sea, where the sea temperature can drop below 5°C in winter and increase to 27°C in summer. Despite numerous studies, *S. tropicum* was first found in the Yellow Sea in 2003, when Chen et al. (2007) isolated the cells of a *S. costatum*-like species from the Yellow Sea in October 2003 and grouped it as *S. tropicum* by the DNA sequence. However, it is uncertain whether the species had been transported into the Yellow Sea by currents or released with the ballast water of ships, or whether it had adapted to the environment of Yellow Sea, because Chen et al. (2007) only found it at one site and did not quantify the abundance.

During a scientific cruise in August 2009, a *S. tropicum*-like species was found in Jiaozhou Bay, a semi-enclosed basin in the western part of the southern Yellow Sea (Fig.2). Phytoplankton surveys of Jiaozhou Bay started in 1936, but *S. tropicum* had never previously been recorded in these surveys (Chin, 1936; Li and Huang, 1956; Qian and Wang, 1983; Guo, 1992; Liu, 2004; Yang et al., 2004; Li et

al., 2005). *Skeletonema costatum* was the only species from genus *Skeletonema* previously recorded in the phytoplankton assemblages of Jiaozhou Bay. To confirm the occurrence of *S. tropicum* in the bay and distinguish it from *S. costatum*, the two species were isolated from the mixed phytoplankton samples under the light microscope (Olympus IX 81) and cultured for the observation of scanning electronic microscope (SEM). The abundance of the two species in surface seawater was counted during nine cruises of 2009–2011 for detecting the seasonal variations. Environmental factors that could have led to the occurrence of *S. tropicum* in Jiaozhou Bay were investigated including seawater temperature increases over the last few decades, thermal pollution and increased releases of ballast water. The hypothesis that *S. tropicum* could extend its biogeographic range in Chinese seas due to the impact of global warming and human activity in coastal waters is discussed.

2 MATERIAL AND METHOD

2.1 Study area and sampling method

Jiaozhou Bay (35°55'–36°18'N, 120°04'–120°23'E) (Fig.2) is a semi-enclosed coastal bay, connected to the southern Yellow Sea, China through a narrow channel (~2.5 km). It has a surface area of 390 km² and an average water depth of 6–7 m. Average seawater temperature is about 5.6°C in winter and

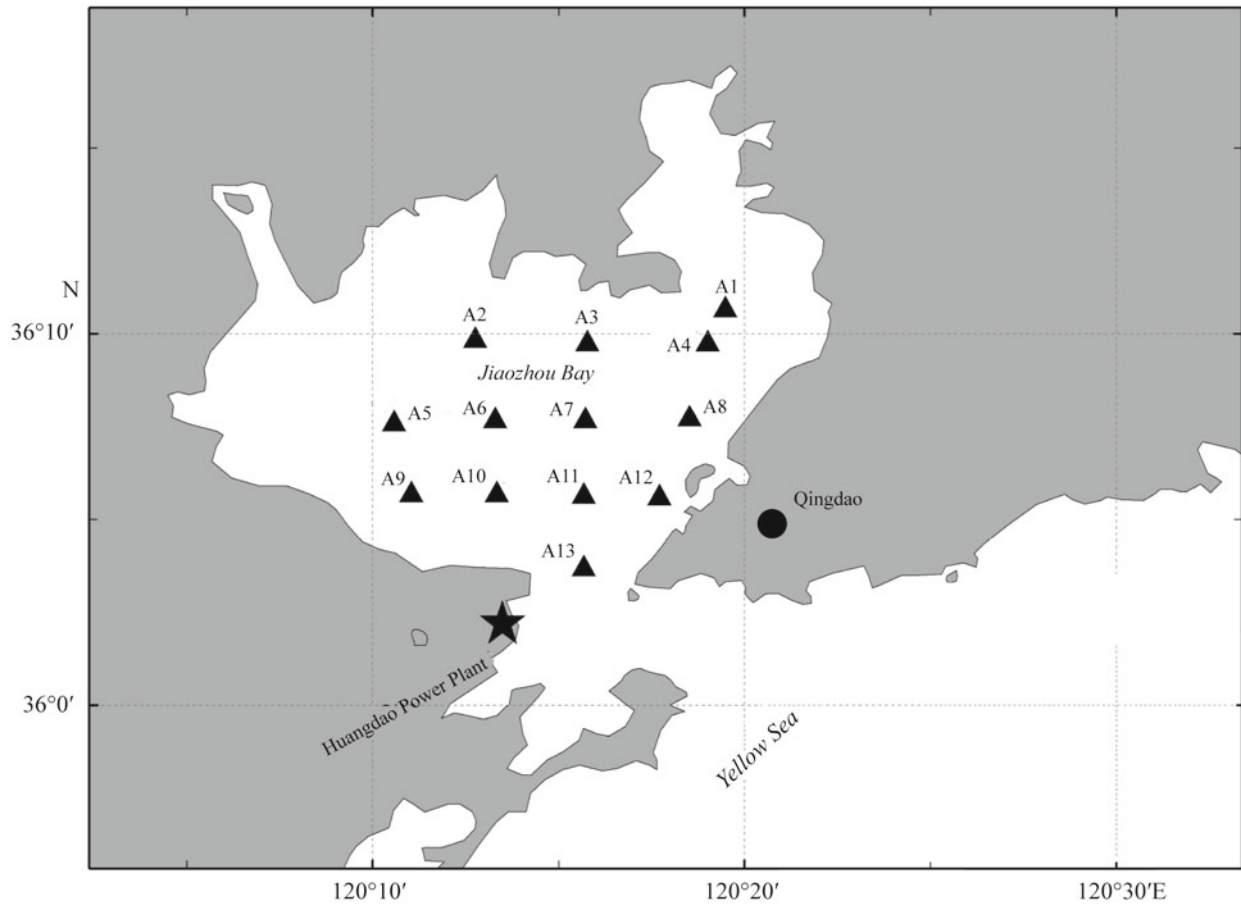


Fig.2 Sampling stations in the Jiaozhou Bay, Yellow Sea, China

27.8°C in summer; the dramatic seasonal changes resulted from cold Siberian winds in winter and warm summer monsoons (Liu, 2006).

Phytoplankton samples were collected at 13 stations during nine cruises in May and August 2009, February, April, June, August, October 2010 and January and March 2011 (Fig.2). Seawater was collected at each station using a 5-L Niskin bottle, and a 500-mL sample was fixed with formalin (final concentration 5%) and stored in polyethylene bottles for species identification and cell enumeration. The temperature and salinity in surface seawater were measured in-situ using a YSI 6920 multi-parameter water quality monitor.

2.2 Species identification and cell numeration

The morphology of *S. tropicum*-like species and *S. costatum* were observed and photographed under light microscopes (Olympus IX 81 and Olympus E-510 camera, respectively) at magnification of 400× and 1 000×. The ranges of cell sizes of *S. tropicum*-

like species and *S. costatum* were estimated by measuring 100 cells of each sample, using digital camera software (IX2-BSW1.6). The two species were isolated from the mixed phytoplankton samples under the light microscope (Olympus IX 81) and cultured for the observation by scanning electronic microscope (SEM). The filtered samples were washed in distilled water, dehydrated in a series of 30%, 50%, 70%, 90%, and 2×100% ethanol, critical-point dried, and then sputter coated with gold-palladium. The chemical treated samples were observed under an SEM (JSM-5610LV) and images were recorded. Microstructures of the two species were compared to previous descriptions of *S. tropicum* and *S. costatum* (Ueno, 1993; Castillo et al., 1995; Sarno et al., 2005; Naik et al., 2010). Seawater (25 mL) of the mixed phytoplankton samples were taken and placed in a 25-mL glass chamber for cell numeration. After 24-h settlement, cell abundances of the two species were counted under light microscope (Utermöhl, 1958).

3 RESULT

3.1 The morphological description of *S. tropicum*-like species and *S. costatum*

Previous studies on *Skeletonema* genus show that the valve diameter, chloroplast number and process density of valves are key parameters to distinguish *S. tropicum* and *S. costatum* (Ueno, 1993; Castillo et al., 1995; Sarno et al., 2007; Yamada et al., 2010). *S. costatum* is characterized by cylindrical cells-forming chains in long tubular processes associated with a peripheral ring of fultoportulae, valve diameter 5–16 μm and each cell contains one to two chloroplasts (Table 1). In contrast, the valve diameter of *S. tropicum* has a wider range than *S. costatum*, and is commonly over 20 μm , which is rare for

S. costatum (Table 1). Our results show that the valve diameter of the *S. tropicum*-like species from the Jiaozhou Bay ranged 6.8–25.7 μm , but that *S. costatum* only varied 4.3–15.1 μm (Table 1), suggesting that the two species found in the Jiaozhou Bay have different ranges of cell sizes. The number of chloroplasts is regarded as the most important characteristics to distinguish *S. tropicum* from *S. costatum*. *S. costatum* contains 1 or 2 chloroplasts in each cell, while *S. tropicum* usually has more than two chloroplasts and up to 15 chloroplasts in each cell (Table 1). In this study, 3–8 chloroplasts were found in each cell of the *S. tropicum*-like species (Fig.3a), and only 1–2 chloroplasts in each cell of *S. costatum* (Fig.3d). Again, this demonstrates a major difference between the two species from Jiaozhou Bay.

Table 1 Morphological comparison between *Skeletonema tropicum* and *Skeletonema costatum*

Parameters	Species		Reference
	<i>S. tropicum</i>	<i>S. costatum</i>	
Cell diameter (μm)	4.5–21	5–16	Cheng and Liu, 1992
	3.9–27.0	2.0–26.7	Ueno, 1993
	13–20	4–15	Castillo et al., 1995
	23	6	Guo and Qian, 2003
	-	5–16	Zingone et al., 2005
	5.3–10	-	Sarno et al., 2005
	-	8–12	Sarno et al., 2007
	5.8–35	5.4–14	Bergesch et al., 2009
	5.1–15.6	-	González-Piana and Ferrari, 2009
	9–15	4–12	Naik et al., 2010
6.8–25.7	4.3–15.1	Our study in Jiaozhou bay	
Chloroplast number (ind./cell)	5–15	1–2	Hulburt and Guillard, 1968
	>2	-	Ueno, 1993
	4–9	1–2	Castillo et al., 1995
	5–10	-	Sarno et al., 2005
	1–7	1–2	Sarno et al., 2007
	2–4	-	Cheng et al., 2008
	-	2	Bergesch et al., 2009
	1–7	1–2	Naik et al., 2010
	many	1–2	Yamada et al., 2010
3–8	1–2	Our study in Jiaozhou bay	
Process density(ind./valve)	8–36	10–38	Ueno, 1993
	16–28	12–24	Castillo et al., 1995
	15–24	10–20	Our study in Jiaozhou bay

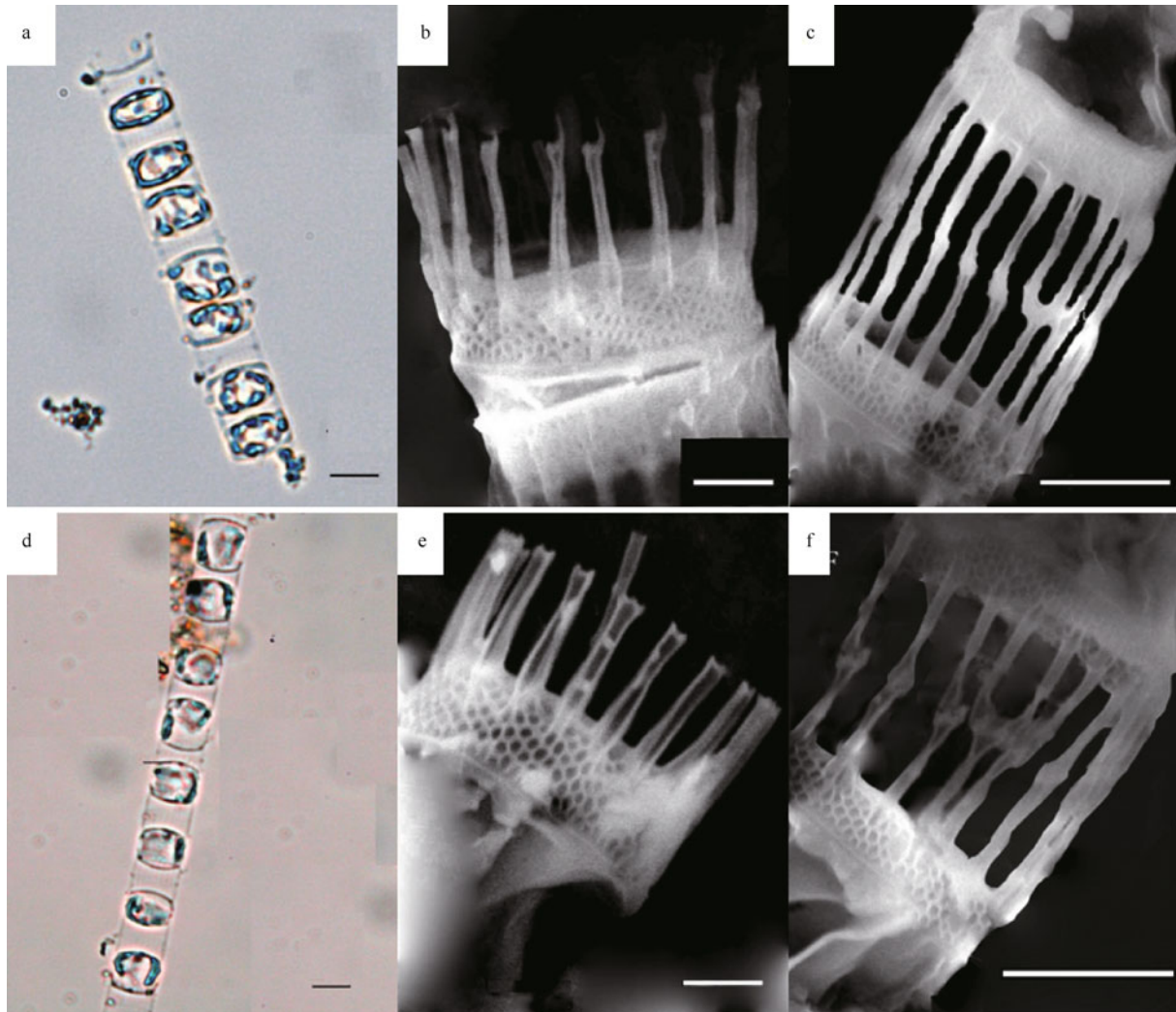


Fig.3 Observation of *Skeletonema tropicum* and *Skeletonema costatum* under LM and SEM

a. LM of *S. tropicum* (scale bar: 10 μm); b. SEM of *S. tropicum* showing terminal valve (scale bar: 2 μm); c. SEM of *S. tropicum* showing intercalary valve (scale bar: 5 μm); d. LM of *S. costatum* (scale bar: 10 μm); e. SEM of *S. costatum* showing terminal valve (scale bar: 2 μm); f. SEM of *S. costatum* showing intercalary valve (scale bar: 5 μm).

Cleve (1900) suggested that *S. tropicum* has more connecting processes when compared to *S. costatum*. However, more recent studies have found that the difference in the numbers of connecting processes was not always different between the two species and that this distinguishing characteristic should be used with care (Ueno, 1993). The numbers of connecting processes can vary with valve size, as larger valves usually have more processes. Thus, it is only the maximum number of processes/valve that is slightly higher for *S. tropicum* when compared to *S. costatum* (Table 1). In this study, 16–28 processes/valve were observed for *S. tropicum* (Fig.3b, c) and 12–24 processes/valve for *S. costatum* (Fig.3e, f). This

indicates that there are only slight differences in these characteristics between the two species. However, the microstructure of the connecting processes of the two species is quite different. In SEM images, Naik et al. (2010) found that *S. costatum* has rimoportula processes and the end of the process was more like a protrusion; *S. tropicum* has fultoportula processes and the tips of process often showed a claw-like projection. In our study, using SEM, the *S. tropicum*-like species from the Jiaozhou Bay had fultoportula processes (Fig.3c) and the end of the processes had claw-like projections (Fig.3b). In contrast, *S. costatum* had rimoportula processes and the end of the processes was more like a protrusion (Fig.3e, f). Based on these

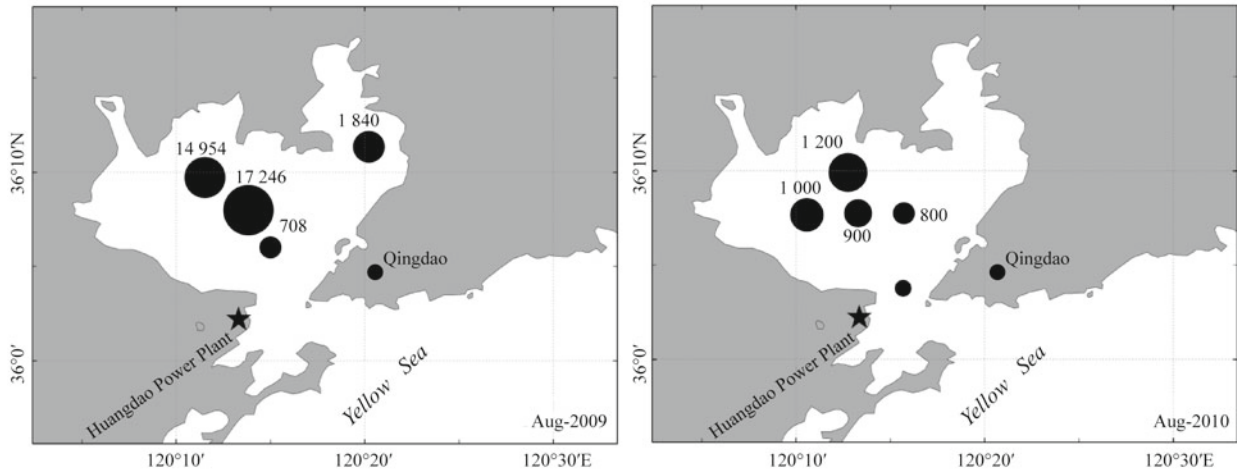


Fig.4 The occurrence and abundance of *Skeletonema tropicum* in the Jiaozhou Bay during the cruises of August 2009 and 2010

distinct taxonomic characteristics, the *S. tropicum*-like species in the Jiaozhou Bay was confirmed as *S. tropicum*.

3.2 The cell abundance and distribution of *S. tropicum* in the Jiaozhou Bay

The seawater samples collected from the Jiaozhou Bay in 2009, 2010, and 2011 were checked for the presence of *S. tropicum* and cell abundances of the species were recorded (Fig.4). *S. tropicum* was found in the cruises of August 2009 and August 2010 only, when sea temperature was about 25°C (Fig.5). *S. tropicum* was distributed mainly in the centre of the bay, and the cell abundance in August 2009 displayed higher values than in August 2010, with a maximum of 1.73×10^4 cell/L (Fig.4).

Sea temperature and salinity in the Jiaozhou bay determined the occurrence and abundance of *S. tropicum*. *S. tropicum* is known to grow well in warm waters. Ueno (1993) found that the cell abundance of *S. tropicum* dropped quickly in temperature below 25°C, and disappeared when seawater temperature drop below 12°C. During the sampling, the highest sea temperature in the Jiaozhou Bay was in August with a range of 24–26°C, which met the temperature condition as *S. tropicum* required. However, the sea temperature was generally below 20°C in summer and autumn months (June and October) as observed in previous studies (Lin et al., 2005) and our cruise (Fig.5a). Thus, the growth of *S. tropicum* in Jiaozhou Bay decreased considerably after August, and result in low abundances, making the detection difficult. Castillo et al. (1995) found that *S. tropicum* grows optimally at salinity above 29°C.

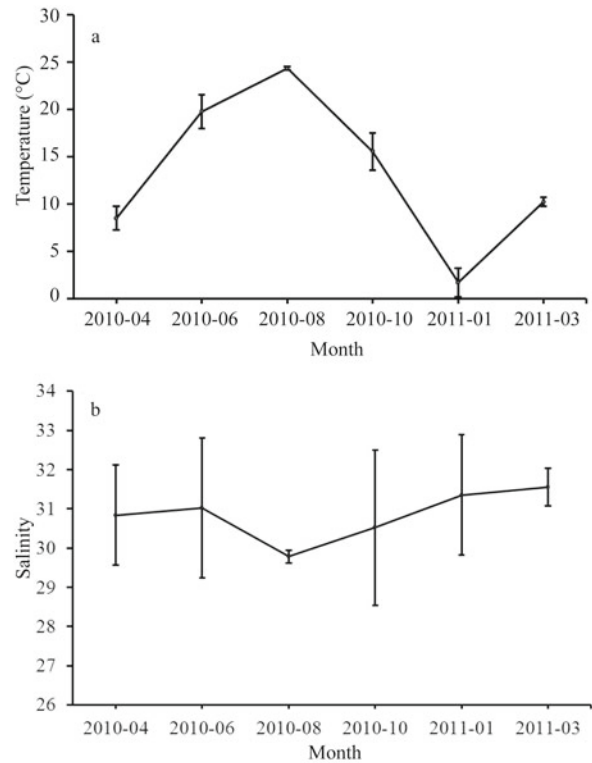


Fig.5 The annual variation of sea temperature and salinity in the Jiaozhou Bay during sampling period

The salinity in Jiaozhou Bay was above 29°C for most of the survey period (Fig.5b), which is good for the growth of *S. tropicum*.

4 DISCUSSION

In this study, the occurrence of *S. tropicum* in the Jiaozhou Bay was confirmed by investigating the

morphology of *S. tropicum*-like species, by comparing this species to the co-occurring *S. costatum* and quantifying its abundance in August 2009 and 2010. Our findings show that the geographic distribution of *S. tropicum* along the China coastline had been expanded for many hundreds of kilometers. *S. tropicum* is regarded as a warm water species that could not survive water temperature below 13°C (Kooistra et al., 2008; González-Piana and Ferrari, 2009); it distributed only in the South China Sea and the East China Sea, and the first record of *S. tropicum* in China seas was in the Qiongzhou Straits in 1973, and then it was also found in Xiamen Harbor and Taiwan Straits (Cheng and Liu, 1992; Guo and Qian, 2003; Kooistra et al., 2008). Historical evidence suggests that *S. tropicum* is not a species that naturally occurred in the Jiaozhou Bay, located in the southern Yellow Sea of China where seawater temperature can increase to 26°C in summer and drop below 5°C in winter. The earliest phytoplankton investigation in the Jiaozhou Bay was carried out in 1936 (Chin, 1936); despite regular surveys, no *S. tropicum*-like species were recorded until Chen et al. (2007) isolated it from the Yellow Sea (outside of Jiaozhou Bay). Due to lack of detailed study, it is difficult to know whether this species was transported into the Yellow Sea by occasional currents or has been introduced through the release of ship ballast waters. Thus, it is important to consider how *S. tropicum*, a warm water species, has moved to the Jiaozhou Bay and adapted to local colder seawater temperatures.

Coastal currents were assumed to be one of possible transportation for the *S. tropicum* northward expansion. The Jiaozhou Bay is a semi-enclosed basin, connected with the southern Yellow Sea, China, through a narrow channel (~2.5 km long). Water exchange between bay and ocean is controlled by the residual tidal currents of the Yellow Sea, which run in a clockwise circulation, moving up along the western coast and returning down in the eastern coast (Fig.2). The average residence time is about 52 days, ranging from less than 20 days near the bay channel to over 100 days in the northwest of the bay due to the limited tidal and near shore currents (Liu et al., 2004).

The increased ballast water brought by coastal ships was another possible carrier for the migration of *S. tropicum* from tropical to temperate seas. Qingdao Port is the largest port in Shandong Peninsula, and located in the east of the Jiaozhou Bay, facing into the Yellow Sea (Fig.2). Over the last few decades, the port has been developed quickly and currently deals

with over 100 million tons of goods, and trades with 450 shipping ports in over 130 countries. Although no studies have been carried out in Qingdao Port, the introduction of alien species from ship ballast water has been suggested in Yantai Port (northern Yellow Sea) and Rizhao Port (southern Yellow Sea) in Shandong Peninsula (Li et al., 2006a, b). Thus, release of ballast water from cargo ships represents a possible pathway for the introduction of alien species (e.g., *S. tropicum*) to Jiaozhou Bay.

Climate is an important determinant for the geographic range of species (McCarty, 2001). Once introduced into the Jiaozhou Bay, *S. tropicum* needs a suitable sea temperature for growth and reproduction. A previous study in the Jiaozhou Bay region has suggested that average air temperatures have increased 0.56°C in summer and 2.62°C in winter since 1900 (Jiao, 2001); Liu (2006) also noted that the sea surface temperature in Jiaozhou Bay displays a slight increase since the 1960s, but not more than 2°C. The survey data from our study and Jiang (2011) in the Jiaozhou Bay (Fig.5a) show that seawater temperatures can still drop below 5°C in winter. These data suggest that the sea temperature is not warm enough for *S. tropicum* to overwinter in the bay.

Compared with climate change, thermal pollution can significantly improve the sea temperature. In the late 1980s, Huangdao Power Plant was built up in the Jiaozhou Bay. Temperature difference between cooling water from Huangdao Power Plant and natural seawater ranged 5.5–11.5°C, and the seawater temperatures in the outlet channel ranged 9.5–38°C, which significantly affected the abundance of benthic macroalgae (Qian et al., 1993). The extent of the thermal pollution is approximately 0.25 km³ around the outlet, and the average increase of seawater temperature in winter in this zone was 4.82°C (Wang et al., 1998). During 2006–2010, the seawater desalination plants were built and linked to Huangdao Power Plant to increase energy efficiency. As a result, the thermal pollution-affected area increased significantly and transferred by tide current (Wu et al., 2008; Wang, 2009). Thus, it may be feasible that thermal pollution from Huangdao Power Plant and seawater desalination plants in the Jiaozhou Bay provide local conditions that allow populations of *S. tropicum* to survive over the winter.

In conclusion, the positive identification of *S. tropicum* in the Yellow Sea has extended the geographic range of this warm water species by several hundreds of kilometers, into temperate

waters. It was assumed that the range extension of this species is through an accidental introduction of an alien species from the ballast water released by cargo ships travelling from further south. Thermal pollution from power stations and a desalination plant may then provide localized conditions that allow *S. tropicum* to overwinter in the Jiaozhou Bay. Additional research is required to investigate the possible links between this large extension in the range of *S. tropicum* along Chinese coastline, and the environmental impacts of ballast water releases and thermal pollution.

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