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Relationship between environmental factors and plankton in the Bayuquan Port, Liaodong Bay, China: a five-year study*

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Abstract To understand the relationship between the plankton community and environmental factors and water quality in the Bayuquan Port of Liaodong Bay, China, and investigations were carried out during six different periods (April 2009, April 2010, October 2011, April 2012, October 2012, and April 2013). This area was characterized by high levels of nutrient and suspended solids (SPS) during survey periods, and eutrophication led to the occurrence of red tides in April and October 2012 and April 2013. Our analyses revealed that the plankton communities of Bayuquan Port lacked stability and were affected seriously by external disturbance (e.g., oceanographic engineering and river runoff). Our data indicate that oil, dissolved inorganic nitrogen (DIN), SPS, and chlorophyll *a* (Chl-*a*) were key factors that regulated the phytoplankton and zooplankton communities. The partial redundancy analysis (partial RDA) suggested that oil and SPS were the most important environmental variables affecting the phytoplankton community in April 2010 and 2012, whereas DIN concentration played a governing role in zooplankton dynamics. Oil and Chl-*a* concentrations affected significantly the zooplankton community in October 2012. Therefore, the plankton communities could reflect both dynamic changes in coastal environmental factors and the ongoing eutrophication process caused by anthropogenic activities in this area.

Keyword: phytoplankton; zooplankton; ABC curve; environmental variables; ecological stability; Bayuquan Port

1 INTRODUCTION

Port and harbor areas play critical roles in regional economic development through the storage and transport of goods from inland areas and other ports (Buruaem et al., 2012). Marine and coastal environments are subjected to port operating activities (e.g., harbor construction, basin dredging, and wastewaters) (Ruggieri et al., 2011; Buruaem et al., 2012), which negatively influence water quality and functioning of aquatic ecosystems either directly or indirectly (Rossi and Jamet, 2008; Wu et al., 2010). Anthropogenic activities in ports are closely associated with water pollution and the spreading of contaminants among different environmental compartments (e.g., water, biota, and sediment) (Riba et al., 2003; Pereira et al., 2007), resulting in decreased water quality and biodiversity, loss of critical habitats, and an overall decrease in the quality of life of local inhabitants (Herrera-Silveira and Morales-Ojeda, 2009). Additionally, freshwater runoff and neighboring industrial installations around ports pose adverse affects on the coastal ecosystem (Darbra et al., 2004; Jiang et al., 2013). Therefore, coastal ecosystems are becoming increasingly degraded because of intensive large-scale human activities (Ferraro et al., 1991; McGlathery et al., 2007).

Regular monitoring programs that help us to understand how anthropogenic activities affect the

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coastal water environment are essential for collecting huge datasets, preventing and controlling deterioration of the environment, and effective management of coastal ecosystems (Simeonov et al., 2003; Singh et al., 2004; Wu et al., 2010; Praveena and Aris, 2013). The coastal water quality around a port is evaluated based on physical, chemical, and biological parameters, and these factors affect the plankton community, including phytoplankton and zooplankton (Casé et al., 2008; Karthik et al., 2012; Zheng et al., 2014). Phytoplankton lives at the base of the marine food chain fuel, the primary consumers in aquatic ecosystems (Ananthan et al., 2005; Tas and Gonulol, 2007; Tian et al., 2014); understanding relationships between plankton communities and environmental factors is crucial to understanding the dynamics of coastal ecosystems. The relationship has been studied previously intensively (Fietz et al., 2005; Hassan et al., 2008; Nowrouzi and Valavi, 2011). Environmental factors, such as water temperature and nutrient concentrations, influence plankton biomass, composition, and succession in aquatic systems (Shapiro, 1997; Salmaso and Braioni, 2008). Additionally, plankton is the first group affected by contamination; as such, plankton can provide important information that can be used to predict the environmental impact of pollution (Debelius et al., 2009).

Bayuquan Port is the new part of Yingkou Harbor, which is located at the mouth of the Liaohe River in Liaodong Bay in the Bohai Sea in the western area of Liaodong Peninsula (Gao et al., 1994; Wang and Yu, 1997). Bayuquan Port is an important location in Liaoning Province, China, as it is the bridgehead between inland areas and the ocean and thus plays an important role in regional economic development. However, in the last three decades, continuous port construction, including large-scale tidal flat reclamation, breakwater engineering, basin dredging, and waste discharge, has significantly affected the environment of coastal areas and degraded the marine ecosystem (Wang et al., 1995; Tang, 2004; Liu et al., 2009).

To date, few studies have examined the relationship between plankton communities and environmental variables in Bayuquan Port. However, understanding the plankton community structure and its relationship with environmental variables is crucial to identifying the key factors that affect plankton population dynamics and the ecological stability of the coastal environment. The results of this study may provide a good reference for scientific management and planning near the port region in Liaodong Bay.

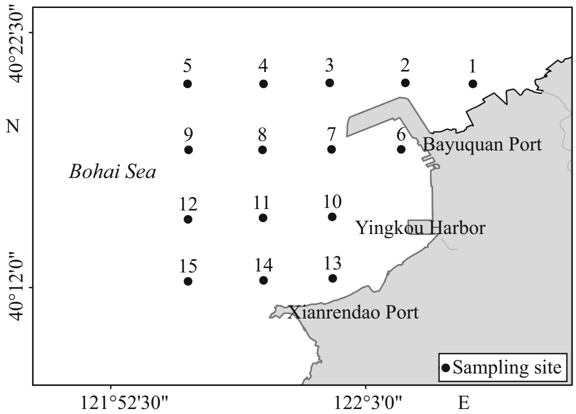

Fig.1 Locations of sampling stations in the Bayuquan Port of the Liaodong Bay during the period 2009–2013

2 MATERIAL AND METHOD

2.1 Study site

Yingkou Harbor (Fig.1), which includes the Xianrendao Port (122°00′E, 40°11′N) and the Bayuquan Port (122°06′E, 40°16′N), was the first harbor in Liaoning Province, China; it began operation in 1864 (Wang and Yu, 1997). Bayuquan Port is located along the east coast of Liaodong Bay at the foot of Taizi Mountain in the Bohai Sea; it is about 30 nautical miles by sea from the Xianrendao Port, and the Yingkou Harbor is one of the major harbors in Northeast China, with a wide range of different activities. Today, the Bayuquan Port plays an important role in economic development, as it is used for the transfer of goods from Dalian Port (e.g., ore, iron and steel, oil, and chemicals) and from inland cities (e.g., Shenyang, Yingkou, Anshan, Fushun, Benxi, Liaoyang, and Panjing). With the rapid development of the economy and continuous construction of Bayuquan Port in the last three decades, the coastal environment has experienced significant stress.

2.2 Field sampling and laboratory analyses

Environmental variable data and plankton samples (phytoplankton and zooplankton) were collected from the coastal area of Bayuquan Port from April 2009 to April 2013. Data were collected at 15 stations from the inner harbor (off-shore of Bayuquan Port) to the open sea (Liaodong Bay in the Bohai Sea) (Fig.1). In total, six complete surveys were completed (April 2009, April 2010, October 2011, April 2012, October 2012, and April 2013), during which more than 200 samples of water and plankton were collected.

At each station, physical factors (temperature (T), pH, salinity (S), and dissolved oxygen (DO) concentration) were measured using an YSI water quality meter (YSI Model 85 Conductivity System, Yellow Springs, Ohio, USA) at 0.5–1 m depth: values ranged from 1.4 to 19.4°C, salinity of 7.80 to 8.40, 28.94 to 31.55, and DO 7.26 to 8.54 mg/L, respectively. The meter was calibrated prior to each sampling campaign. Seawater samples for analysis of nutrient, suspended solids (SPS), chemical oxygen demand (COD), and chlorophyll *a* (Chl-*a*) concentrations were collected in acid cleaned polyethylene bottles at the sea surface (0.5–1 m depth) using a Niskin water sampler. Seawater samples (approximately 1 L) for analysis of petroleum hydrocarbon content (i.e., oil concentration in the seawater) were collected in pre-cleaned amber glass bottles at 10 cm below the surface layer using a custom-made stainless steel and Teflon sampling device attached to a weighed metal frame (Kim et al., 2013).

All chemical factors were analyzed within 24 h of sampling. Ammonium (NH_4-N), nitrate nitrogen (NO_3-N), nitrite (NO_2-N), soluble reactive phosphorus (SRP), Chl-*a*, and COD were analyzed according to standard methods (GB 17378.4-2007; Wu et al., 2010). The three nitrogen sources (ammonium (NH_4-N), nitrate nitrogen (NO_3-N), and nitrite (NO_2-N)) were integrated in this work into a single value of dissolved inorganic nitrogen (DIN). SPS level was determined by filtering 1 L of seawater through pre-dried and pre-weighed 1.2 μm Millipore GF/C filter paper (Sahu et al., 2013). Samples for Chl-*a* determination were filtered using Whatman GF/F filters; the Chl-*a* collected on the GF/F filters was extracted with 90% acetone and then measured using a UV-visible spectrophotometer (TU-1810, Purkinje General Instrument Co. Ltd., Beijing, China). The oil concentrations were determined using the TU-1810 UV-visible spectrophotometer following China's Marine Monitoring Standards (GB 17378.6-2007).

The geographic coordinates for each phytoplankton and zooplankton sample were recorded using GPS. Vertical I (mesh size: 500 μm; inner diameter of net mouth: 50 cm; length: 145 cm), II (mesh size: 150 μm; inner diameter of net mouth: 31.6 cm; length: 110 cm), and III (mesh size: 76 μm; inner diameter of net mouth: 37 cm; length: 140 cm) type plankton net tows were conducted from the bottom to the sea surface to collect large zooplankton, medium-sized zooplankton, and phytoplankton, respectively. Samples were fixed with neutral Lugol's solution. Phytoplankton samples were quantitatively analyzed and identified to species or genus level in a Fuchs-Rosenthal slide using an Olympus CX21 microscope (Olympus Corporation, Tokyo, Japan) at 400× magnification. Zooplankton was identified to species or genus level and counted at 100× magnification, and then the entire sample was scanned at 50× for rare species. During the summer investigation, we found a certain number of jellyfish in the sea surface, but we did not make a statistical investigation on them. Population densities were estimated from the counts as numbers per milliliter based on the volume of water sampled by the net and assuming 100% sampling efficiency.

2.3 Statistical analyses

The dominant species were identified using the index of relative importance (IRI) (Pinkas, 1971). The IRI value for plankton was calculated as follows:

$$IRI=(W+N)\times F,$$

where W is the percent composition by biomass, N is the percent composition by number, and F is the percent frequency of occurrence.

Using equations from the literature, phytoplankton cell volume and biomass were calculated (Strathmann, 1967; Strickland, 1970; Sun et al., 1999). Zooplankton specimens were weighed and biomass were calculated using equations from related studies (Iguchi and Ikeda, 1998; Ikeda and Shiga, 1999; Satapoomin, 1999; Zuo et al., 2008).

The ABC curve method can be used to analyze the community response to environmental change and human activities at different times and under different stress situations (Yemane et al., 2005). The difference between the biomass curve and the abundance curve is given by the W statistic, which represents the area between them. A negative sign for the W statistic indicates that the biomass curve lies below the abundance curve and suggests a disturbed community, whereas a positive sign indicates that the biomass curve lies above the abundance curve and is indicative of an undisturbed community (Warwick, 1986; Yemane et al., 2005). In moderately disturbed communities, both curves roughly coincide and the W statistic value is close to zero. Herein, values of the W statistic were used to identify community disturbance in Bayuquan Port, and the ABC curves were constructed to examine the ecological characteristics of phytoplankton and zooplankton assemblages. For each survey, the W value for each ABC plot derived from the 15 sampling stations is given.

The effect of environmental factors on the phytoplankton and zooplankton communities was analyzed using CANOCO version 4.5 (Microcomputer Power, Ithaca, NY, USA). The phytoplankton and zooplankton species data were analyzed using detrended correspondence analysis (DCA) to identify the ordination methods (Hill and Gauch, 1980). If the maximum gradient length of the four axes is lower than 3, redundancy analysis (RDA) is appropriate (Peng et al., 2012). Principal component analysis (PCA) can explain most of the variance of the original multivariate data (Jolliffe, 1986), so that PCA was used to select the variables that made remarkable and independent contributions to the phytoplankton and zooplankton communities (Tian et al., 2014). Only those species that represented >5% of the total in each sample were included in the analysis (Leira and Sabater, 2005). All data were $\log_{10}(x+1)$ transformed before multivariate ordination analysis was conducted. Monte Carlo simulation was used to test the significance ($P<0.05$) between the environmental variables and the plankton data in the RDA analysis.

3 RESULT

3.1 Environmental characteristics

The box plot allowed us to visually assess the annual and seasonal variability of SPS, COD, PO_4-P, DIN, oil, and Chl-*a* data (Fig.2) in Bayuquan Port during the period from 2009 to 2013. The average concentration of SPS over the sampling time was 40.6 mg/L (range, 11.2–94.7 mg/L). SPS levels were kept at a high level, especially in April 2013, the average concentration of SPS was significantly higher compared to these during the survey in April 2009, 2010, and 2012 and October 2011 and 2012 ($P<0.01$; Fig.2). Average concentration of COD was 1.91 mg/L (range, 1.43–3.56 mg/L). The average COD concentration in April 2013 was 3.56 mg/L, which was significantly higher than those of the other five surveys (Fig.2). The average concentration of PO_4-P was 0.016 mg/L throughout the study period (range, 0.003 mg/L (April 2012) to 0. 031 mg/L (April 2009)) (Fig.2). The average DIN concentration over the study period was 0.763 mg/L (rang, 0.131–1.058 mg/L). The highest average DIN values were found in April 2009, and across the entire survey the DIN concentration has been at a higher concentration level compared to these in April of 2013 ($P<0.01$; Fig.2). The average concentration of oil was 0.032 mg/L through the study period, with minimum averages in October 2012 (0.027 mg/L) and maximum averages in April 2013 (0.048 mg/L) (Fig.2). The average Chl-*a* concentration over the study period was 3.78 μg/L (range, 0.67–8.12 μg/L. Higher Chl-*a* concentrations were recorded in April 2013 compared with the other survey periods (Fig.2).

3.2 Plankton composition and distribution

During the entire survey, 69 taxa of phytoplankton and 23 taxa of zooplankton were identified. Over 80% and 60% of the taxa were diatoms and copepods, respectively; these groups were the most dominant taxa in the total population at all stations. Meanwhile, our results also reveal that diatoms have numerous abundance advantages over dinoflagellates throughout the five year investigation period.

The dominant taxa based on IRI values which is above 1 000 show that the diatom genus *Coscinodiscus* were the most frequent types of phytoplankton in the study area, and they included *Coscinodiscus subtilis*, *C. gigas*, *C. asteromphalus*, *C. radiatus*, *C. granii*, *C. oculusiridis*, and *C. subtilis*. The sum of the dominant taxa occurred in very high abundances and accounted for >55% to the total phytoplankton population in each survey period. Massive blooms of *Chaetoceros lorenzianus*, *Melosira sulcata*, *Skeletonema costatum*, *Eucampia cornuta*, and *Pseudo-nitzschia delicatissima* occurred during April and October 2012 and April 2013, and these bloom species were the most abundant species at each station in the study area during the red tide (Fig.3). The harmful algal blooms (HAB) species *M. sulcata*, *S. costatum*, and *C. lorenzianus* accounted for 65% of the total population in April 2012, whereas *C. lorenzianus*, *P. delicatissima*, and *E. cornuta* accounted for 56% of the total population in October 2012 (Fig.3). *S. costatum* also caused a HAB in April 2013, accounting for 86% to the total abundances (Fig.3).

The average total abundance values of phytoplankton during April and October 2012 and April 2013 were $7\,157.20\times10^4$, $3\,919.66\times10^4$, and $3\,311.66\times10^4$ cells/m^3, respectively; these values were significantly higher than those in April 2009 and 2010 and October 2011($P<0.01$; Fig.4). HAB species accounted for more than 56% of total phytoplankton abundance in April and October 2012 and April 2013.

Among the zooplankton taxa, *Paracalanus parvus*, *Acartia clausi*, *Oithona similes*, *Paracalanus crassirostris*, *Acartia hongi*, nauplius larvae, copepodite, *Oikopleura dioica*, and *Sagitta crassa* were the most abundant during the entire study period.

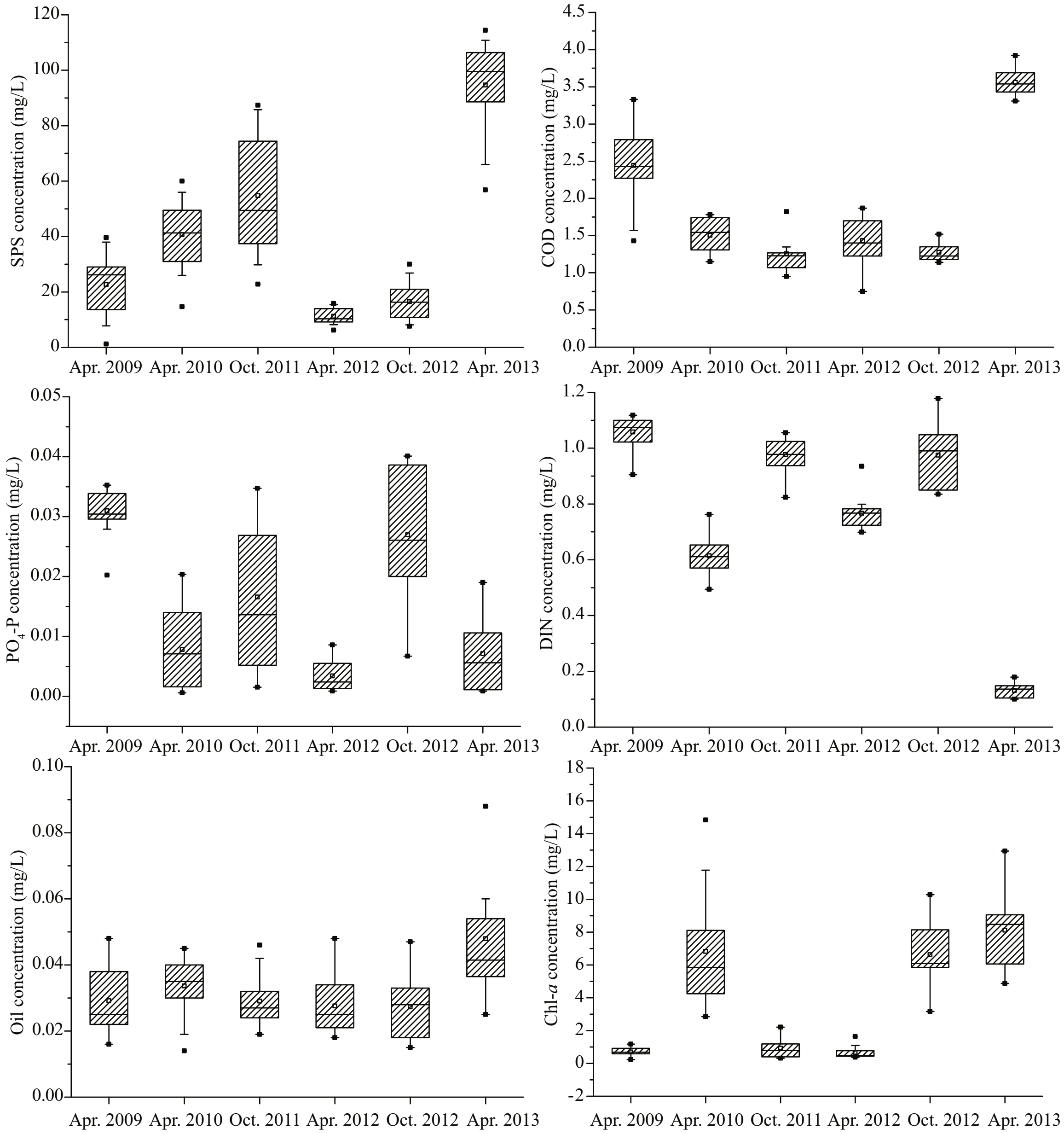

Fig.2 Variation of SPS, COD, PO_4-P, DIN, Oil and Chl-*a* in Bayuquan Port during the period 2009–2013

The white square indicates the mean and the black line indicates the median.

The copepods clearly dominated the zooplankton community, with the various life cycle stages (adults, nauplius larvae, and copepodite) in the samples accounting for more than 85% of the total number of zooplankton in each survey. After copepods, the other dominant groups were *O. dioica* and *S. crassa*, in which *S. crassa* was dominant in terms of biomass.

Zooplankton abundance ranged from 179.36 (April 2013) to 11 978.75 ind/m^3 (April 2012) during the survey period (Fig.4). Average total abundance of zooplankton in April 2012 was significantly higher than that of the other five sampling periods (P<0.01; Figs.1–4). The abundance of zooplankton in April 2013 was significantly lower than that of the other five sampling periods (P<0.01).

3.3 Characteristics of the ABC curve

For the phytoplankton community in April 2009, the abundance curve was above the biomass curve in some places, and the W statistic was negative. In April 2013, the W statistic also was negative (Fig.5). In the other four surveys (April 2010; October 2011; April 2012; October 2012), the biomass curves were above the abundance curves and the value of W was positive. For zooplankton, with the exception of October 2010 and April 2012, the value of W was positive (Fig.6).

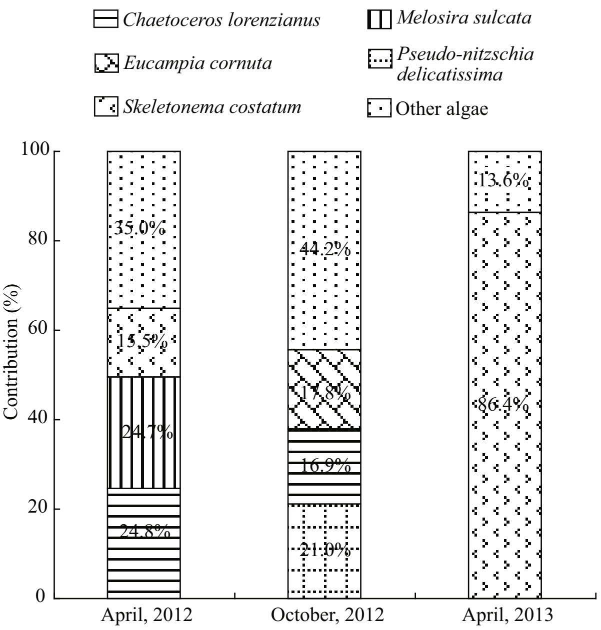

Fig.3 Contributions (%) of five dominant species which caused red tides to the total abundance of phytoplankton community in different periods

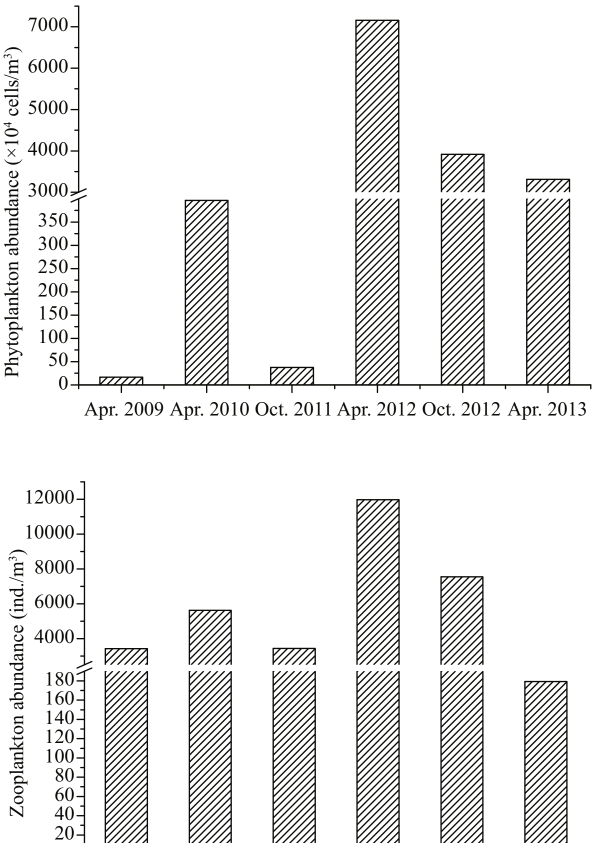

Fig.4 Distribution of the phytoplankton and zooplankton abundance in different periods during 2009–2013 in the study area

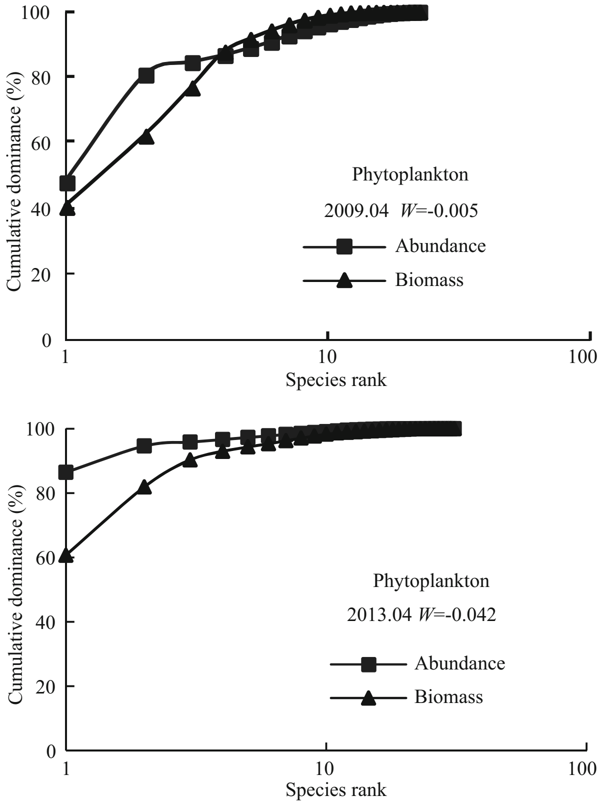

Fig.5 The ABC curves and *W* statistic of phytoplankton community in April of 2009 and 2013

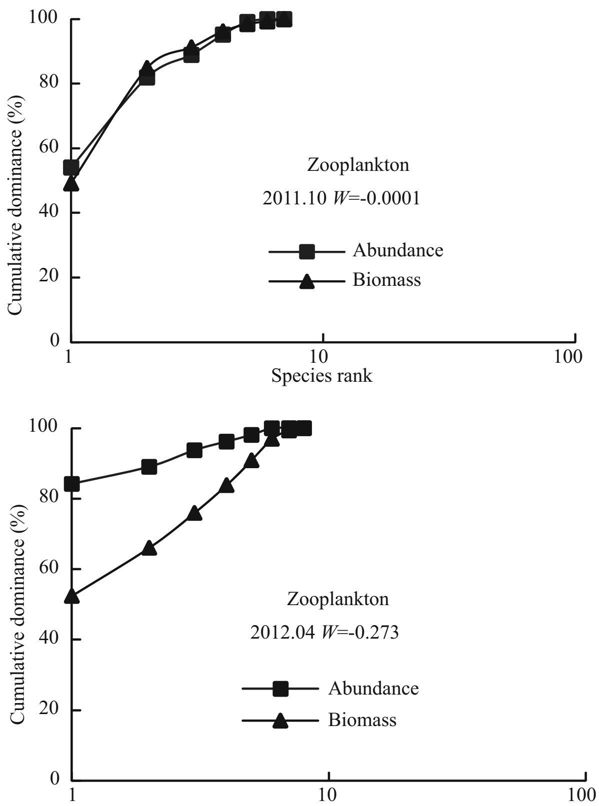

Fig.6 The ABC curves and *W* statistic of zooplankton community in October, 2011 and April, 2012

Table 1 Correlation coefficients between the environmental variables and axes for plankton community in Bayuquan Port around Liaodong bay during 2009–2013

Sampling time	Environ variables	Axis 1	Axis 2	Axis 1	Axis 2
		Phytoplankton		Zooplankton	
April, 2009	SPS	0.047 7	0.603	-0.058 4	0.072 5
	COD	-0.299 7	0.103 6	0.094 2	0.108 1
	PO_4-P	-0.123 1	0.435 4	0.136	-0.121 4
	DIN	-0.138 2	0.393 5	-0.204 5	-0.063 8
	Oil	0.306 5	-0.334 4	0.185 2	-0.441
	Chl-*a*	-0.222 1	-0.445 7	0.458 7	-0.093 4
April, 2010	SPS	-0.191 7	0.164 6	-0.346 9	0.337
	COD	-0.032 3	-0.374 8	-0.138 8	0.064 2
	PO_4-P	-0.068 1	0.572 8	0.232 2	-0.152 2
	DIN	0.379 9	0.151 4	0.594 9	-0.161 7
	Oil	-0.611 7	0.311 4	-0.221 7	-0.449 7
	Chl-*a*	-0.206	-0.022	-0.140 1	-0.156 6
October, 2011	SPS	-0.404 5	-0.088 2	0.292 8	0.109
	COD	-0.101 7	-0.236 7	0.341 4	-0.120 3
	PO_4-P	0.081 3	-0.069 5	-0.041 2	0.053 7
	DIN	-0.276	-0.205 2	0.199 5	-0.212 6
	Oil	-0.196 8	-0.377 1	0.431 9	0.152 5
	Chl-*a*	0.222	-0.205 4	0.117 8	-0.351 7
April, 2012	SPS	0.460 4	-0.563 5	-0.332 2	0.445 1
	COD	0.054 4	-0.206 5	0.042	0.155 3
	PO_4-P	-0.125 3	-0.368 2	0.033 5	-0.359 8
	DIN	0.610 6	0.079 7	0.483 2	0.571 1
	Oil	0.665 5	-0.320 8	-0.181 9	0.66
	Chl-*a*	0.081 7	-0.143 7	0.464 3	0.040 1
October, 2012	SPS	0.457	-0.265 2	-0.010 5	0.180 3
	COD	0.015 8	-0.051 5	0.269 9	-0.607 3
	PO_4-P	0.150 3	-0.427 9	0.081 5	0.332 5
	DIN	0.086 3	-0.243 4	0.211 7	-0.291 6
	Oil	-0.290 2	-0.495 7	-0.661 7	0.022 7
	Chl-*a*	-0.462 6	0.459 4	0.233 7	-0.728 3
April, 2013	SPS	-0.195 7	-0.071 4	-0.097 3	-0.718 3
	COD	-0.048 2	-0.157 8	-0.223 3	0.068 1
	PO_4-P	0.247 7	0.327 5	-0.152 9	-0.189 2
	DIN	-0.258	-0.437 5	0.182 5	-0.143 4
	Oil	-0.277 8	0.053 8	0.110 5	0.498 4
	Chl-*a*	-0.067	-0.440 6	-0.152 7	0.035

The curves roughly coincided for zooplankton community in the October 2010 and April 2013 surveys. In April 2012, the abundance curve overhung the biomass curve. For the other three surveys, the biomass curves were above the abundance curves and had small positive *W* values.

3.4 Principal component analysis

The PCA results for the phytoplankton community are given in Table 1. In April 2009, the first two axes together accounted for 60.2% of the total variance of the data set. Based on these results, we used the first two axes as the principal component axes. Axis 1 was correlated positively with oil and negatively with COD, and axis 2 positively with SPS, PO_4-P, and DIN and negatively with Chl-*a* (Table 1). In April 2010, we used the first two axes as the principal component axes, as they accounted for 64.1% of the total variation. Axis 1 was correlated positively with DIN and negatively with Chl-*a* and oil (oil had the strongest correlation); axis 2 showed positive correlation with SPS, PO_4-P, DIN, and oil (PO_4-P had the strongest correlation) and negative with COD and Chl-*a* (Table 1). In October 2011, the first two axes together accounted for 46.0% of the total variance of the data set. Axis 1 was y correlated positivelwith Chl-*a* and negatively with SPS and DIN; axis 2 showed negative correlations with all environmental variables, with the strongest correlation for oil (Table 1). In April 2012, the first two axes together accounted for 60.5% of the total variance of the data set. Axis 1 was correlated positively with SPS, DIN, and oil, and axis 2 negatively with all environmental variables (SPS had the strongest correlation) except for DIN (positive; Table 1). In October 2012, the first two axes together accounted for 88.4% of the total variance of the data set. Axis 1 had a large positive coefficient for SPS and a large negative coefficient for Chl-*a*; axis 2 was correlated negatively with all environmental variables (oil had the strongest correlation) except for Chl-*a* (positive; Table 1). In April 2013, the first two axes together accounted for 77.7% of the total variance of the data set. Axis 1 was correlated negatively with all environmental variables (oil had the strongest correlation) except for PO_4-P (positive); axis 2 had large negative coefficients for DIN and Chl-*a* and a large positive coefficient for PO_4-P (Table 1).

The PCA results for the zooplankton community are also given in Table 1. In April 2009, the first two axes explained 68.7% of the variance. Therefore, we considered only the first two axes. Axis 1 had a large positive coefficient for Chl-*a*, and axis 2 was strongly negatively correlated with oil (Table 1). In April 2010, the first two axes explained 70.8% of the variance. Axis 1 had a strong positive coefficient for DIN, and axis 2 was strongly correlated negatively with oil (Table 1). In October 2011, more than half of the total system variability (98.2%) was due to the first axis (56.7%). Axis 1 had strong positive coefficients for COD and oil, and axis 2 was strongly correlated negatively with Chl-*a* (Table 1). In April 2012, the first two axes explained 74.4% of the variance. Axis 1 had large positive coefficients for DIN and Chl-*a* (DIN had the strongest correlation), and axis 2 was strongly and positively correlated with oil, DIN, and SPS (oil had the strongest correlation) (Table 1). In October 2012, the first two axes explained 86% of the variance. Axis 1 had a strong negative coefficient for oil, and axis 2 was strongly and negatively correlated with Chl-*a* and COD (Chl-*a* had the strongest correlation) (Table 1). In April 2013, more than half of the total system variability (96.8%) was due to the first axis (54.8%) Axis 1 had a large negative coefficient for COD and a large positive coefficient for DIN, and axis 2 had a strong negative coefficient for SPS and a large positive coefficient for oil (Table 1).

3.5 Relationships between plankton and environmental factors

RDA is a way to visualize correlations between the phytoplankton community and environmental variables and years (Fig.7). In April 2009, 72.3% of the cumulative variance of the species-environment relationship was represented by the first two axes. All canonical axes accounted for 49.8% of the variation in the phytoplankton data. DIN concentration was related positively to the abundance of *S. costatum*. In April 2010, 75.2% of the cumulative variance of the species-environment relationship was represented by the first two axes. All canonical axes accounted for 56.2% of the variation in the phytoplankton data. Oil concentration was positively related to abundance of *Chaetoceros debilis* and negatively related to the abundance of both *Biddulphia biddulphiana* and *Chaetoceros castracanei*. In October 2011, 56.2% of the cumulative variance of the species-environment relationship was represented by the first two axes. All canonical axes accounted for 44.2% of the variation in the phytoplankton data. Oil and SPS concentrations were positively correlated with *Eucampia zodiacus* abundance. DIN concentration was positively and negatively correlated with *Coscinodiscus* sp. and *E. zodiacus*, respectively. In April 2012, 75.2% of the cumulative variance of the species-environment relationship was represented by the first two axes. All canonical axes accounted for 62.4% of the variation in the phytoplankton data. DIN concentration was positively related to the abundance of *Guinardia delicatula*. In October 2012, 86.2% of the cumulative variance of the species-environment relationship was represented by the first two axes. All canonical axes accounted for 63.8% of the variation in the phytoplankton data. Chl-*a* concentration was related positively to the abundance of *Chaetoceros curvisetus*. In April 2013, 76.4% of the cumulative variance of the species-environment relationship was represented by the first two axes. All canonical axes accounted for 49.7% of the variation in the phytoplankton data. Chl-*a* concentration was positively related to abundances of both *P. delicatissima* and *Thalassiosira* sp.

Figure 8 shows the RDA ordination results for zooplankton functional groups and environmental factors. In April 2009, the eigenvalues for RDA axis 1 (0.210) and RDA axis 2 (0.079) explained 72.8% of the variance of the species-environment relationship. All canonical axes accounted for 39.7% of the variation in the zooplankton data. Chl-*a* concentration was related positively to the abundance of copepodite. In April 2010, the eigenvalues for RDA axis 1 (0.239) and RDA axis 2 (0.130) explained 81.0% of the variance of the species-environment relationship. All canonical axes accounted for 45.6% of the variation in the zooplankton data. Oil concentration was correlated positively with *Acartia clausi* abundance. In October 2011, the eigenvalues for RDA axis 1 (0.316) and RDA axis 2 (0.074) explained 81.8% of the variance of the species-environment relationship. All canonical axes accounted for 47.6% of the variation in the zooplankton data. Oil and SPS concentration were positively correlated with *Acartia bifilosa* abundance and negatively to *P. parvus* abundance, respectively. In April 2012, the eigenvalues for RDA axis 1 (0.241) and RDA axis 2 (0.176) explained 84.1% of the variance of the species-environment relationship. All canonical axes accounted for 49.6% of the variation in the zooplankton data. In October 2012, the eigenvalues for RDA axis 1 (0.402) and RDA axis 2 (0.296) explained 85.6% of the variance of the species-environment relationship. All canonical axes accounted for 81.6% of the variation in the

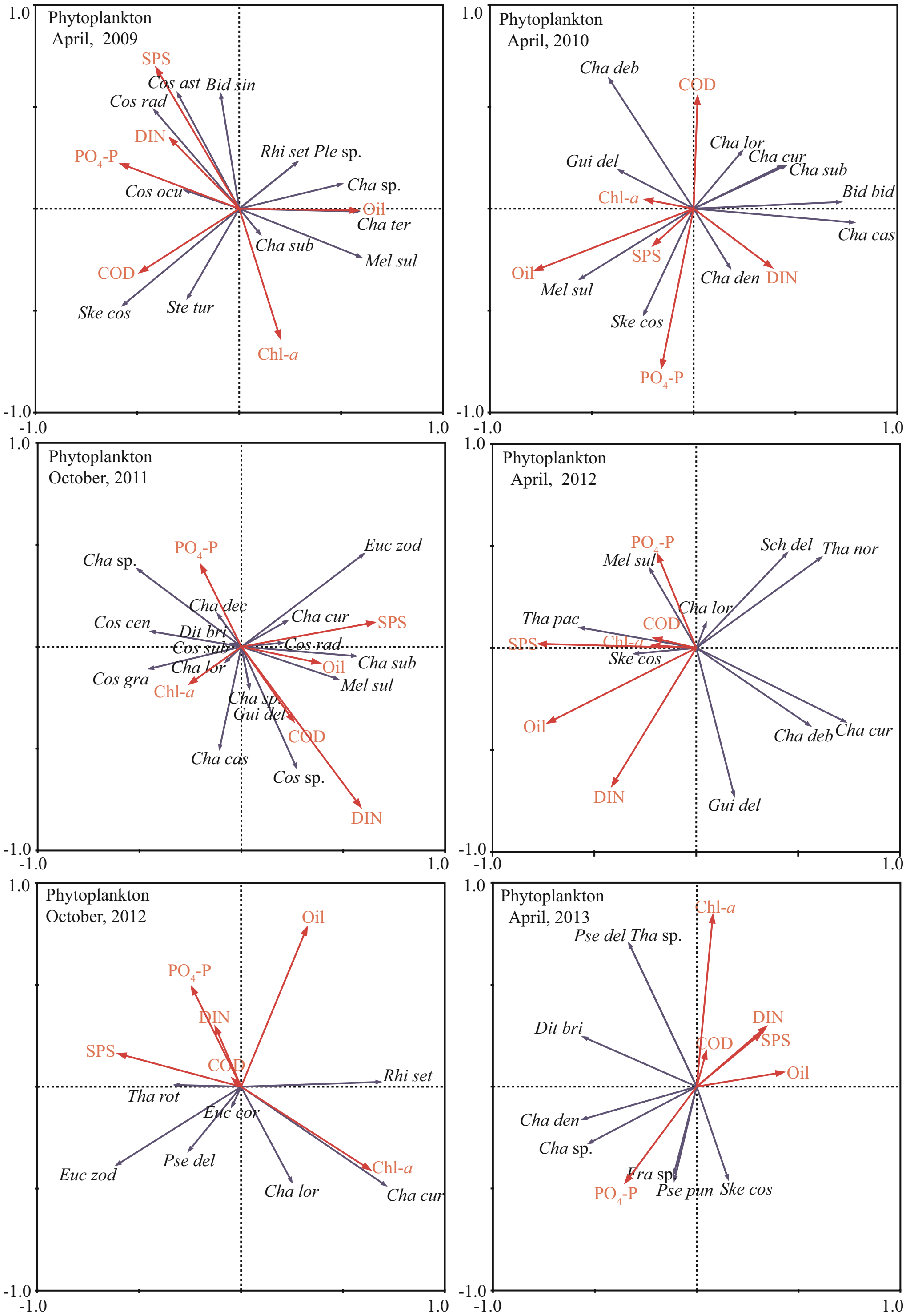

Fig.7 Ordination biplot of environmental variables and phytoplankton species assemblages obtained by RDA during the survey time

Abbreviations: *Bid bid*: *Biddulphia biddulphiana*; *Bid sin*: *Biddulphia sinensis* Greville; *Cha* sp.: *Chaetoceros* sp.; *Cha ter*: *Chaeloceras teres*; *Cha cas*: *Chaetoceros castracanei*; *Cha cur*: *Chaetoceros curvisetus*; *Cha deb*: *Chaetoceros debilis*; *Cha dec*: *Chaetoceros decipiens*; *Cha den*: *Chaetoceros densus*; *Cha lor*: *Chaetoceros lorenzianus*; *Cha sub*: *Chaetoceros subsecundus*; *Cos* sp.: *Coscinodiscus* sp.; *Cos ast*: *Coscinodiscus asteromphalus*; *Cos cen*: *Coscinodiscus centralis*; *Cos gra*: *Coscinodiscus granii*; *Cos ocu*: *Coscinodiscus oculus-iridis*; *Cos rad*: *Coscinodiscus radiatus*; *Cos sub*: *Coscinodiscus subtilis*; *Dit bri*: *Ditylum brightwelli*; *Euc cor*: *Eucampia cornuta*; *Euc zod*: *Eucampia zodiacus*; *Fra* sp. : *Fragilaria* sp.; *Gui del*: *Guinardia delicatula*; *Mel sul*: *Melosira sulcata*; *Ple* sp. : *Pleurosigma* sp.; *Pse del*: *Pseudo-nitzschia delicatissima*; *Rhi set*: *Rhizosolenia setigera*; *Sch del*: *Schroderella delicatula*; *Ske cos*: *Skeletonema costatum*; *Ste tur*: *Stephanopyxis turris*; *Tha* sp: *Thalassiosira* sp.; *Tha nor*: *Thalassiosira nordenskioldii*; *Tha pac*: *Thalassiosira pacifica*; *Tha rot*: *Thalassiosira rotula*.

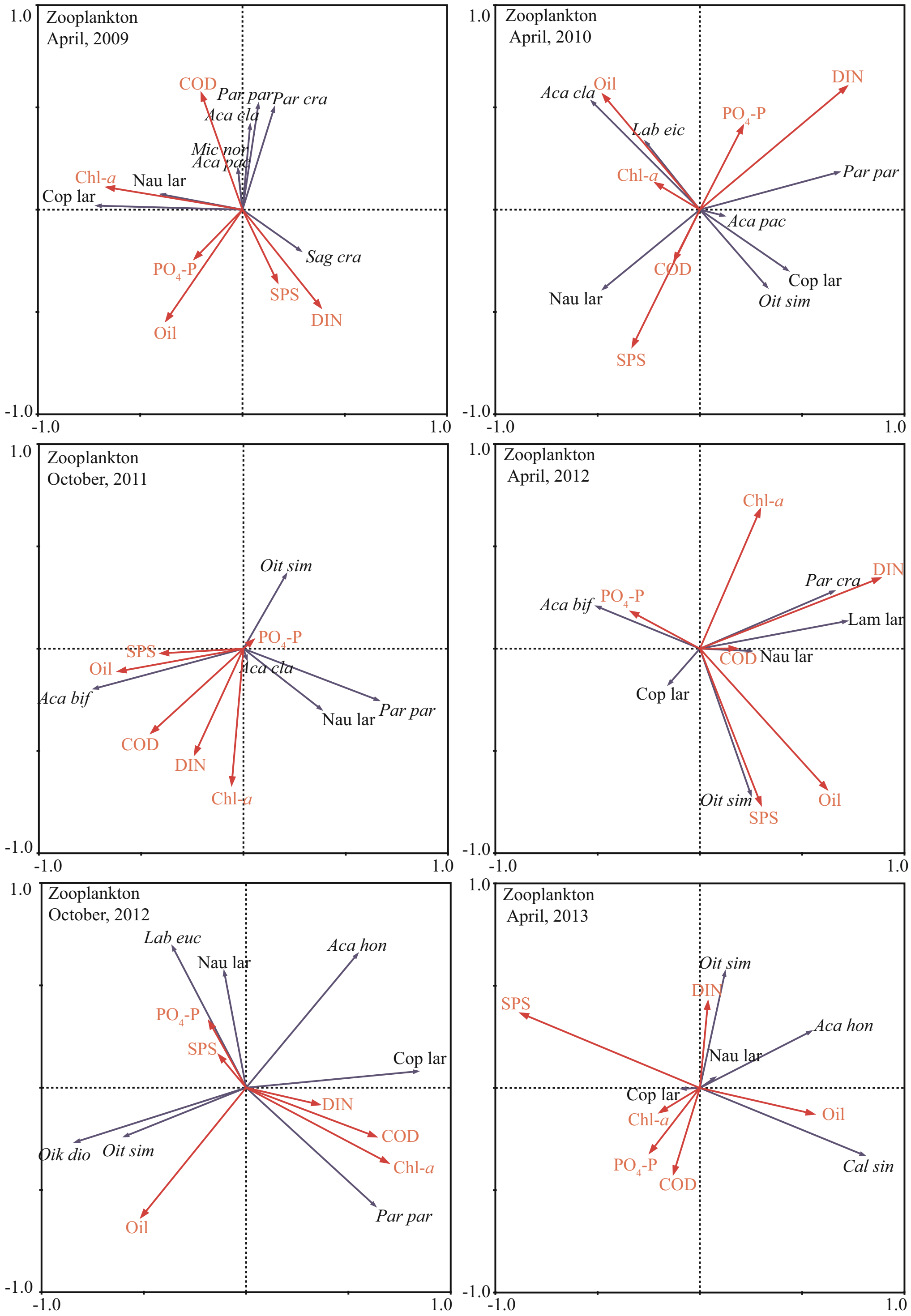

Fig.8 Ordination biplot of environmental variables and zooplankton species assemblages obtained by RDA during 2009–2013

Abbreviations: *Aca bif*: *Acartia bifilosa*; *Aca cla*: *Acartia clausi*; *Aca hon*: *Acartia hongi*; *Aca pac*: *Acartia pacifica*; *Cal sin*: *Calanus sinicus*; Cop lar: Copepodite; *Lab eic*: *Labidocera eichaeta*; Lam lar: Lamellibranchia larvae; *Mic nor*: *Microsetella norvegica*; Nau lar: Nauplius larva; *Oik dio*: *Oikopleura dioica*; *Oit sim*: *Oithona similis*; *Par cra*: *Paracalanus crassirostris*; *Par par*: *Paracalanus parvus*; *Sag cra*: *Sagitta crassa*.

zooplankton data. In April 2013, the eigenvalues for RDA axis 1 (0.192) and RDA axis 2 (0.132) explained 40.3% of the variance of the species-environment relationship.

Based on the RDA results, different environmental variables explained the variability in the phytoplankton and zooplankton composition, with partial RDA in April 2010 and 2012 and October 2012, respectively

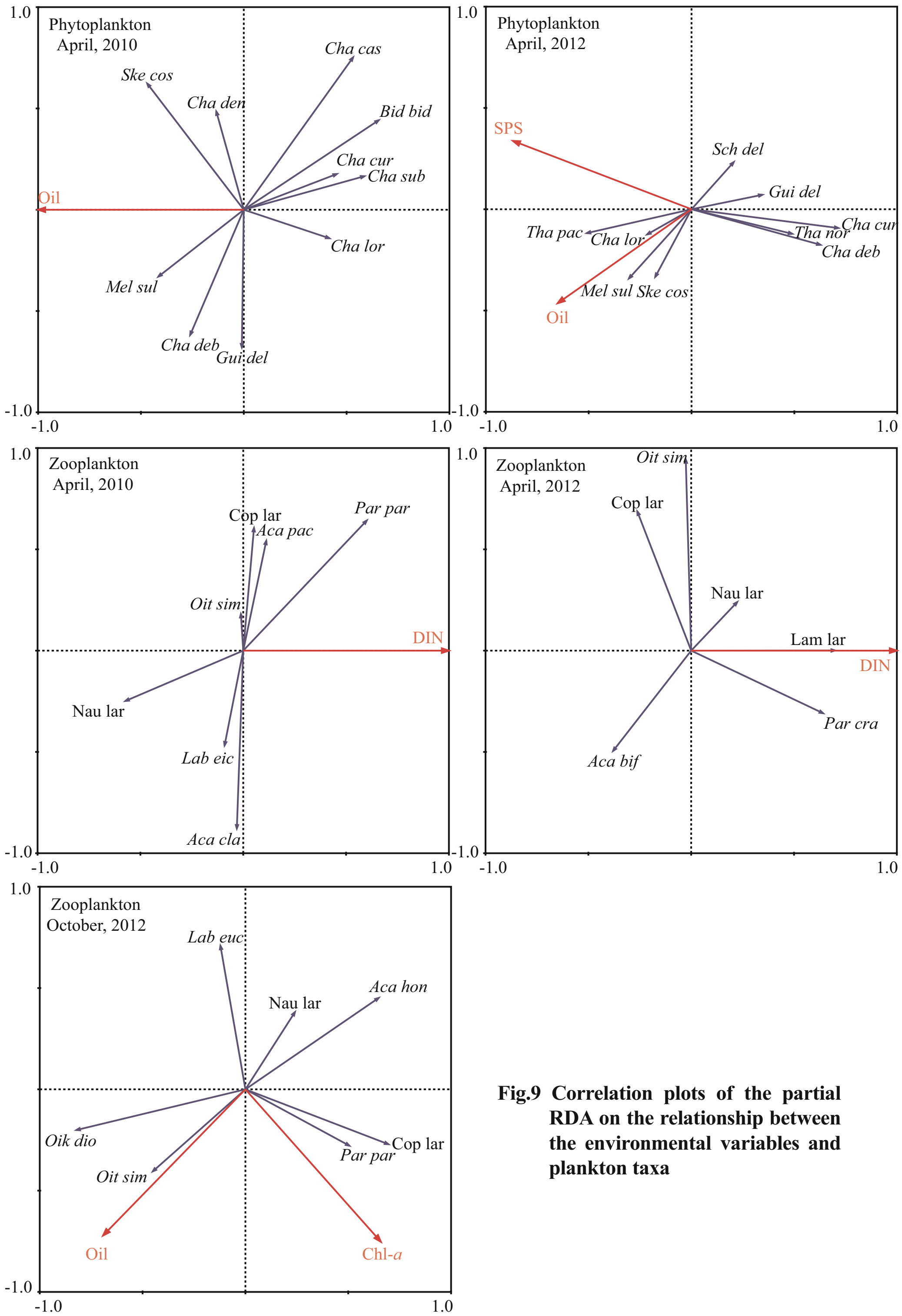

Fig.9 Correlation plots of the partial RDA on the relationship between the environmental variables and plankton taxa

(Fig.9; Table 2). In April 2010, oil had a significant (P<0.05, Monte Carlo permutation test) relationship with the phytoplankton community, whereas in April 2012 the significant environmental variables were oil and SPS. In April 2010 and 2012, Monte Carlo permutation testing revealed a significant correlation between the DIN concentration and the zooplankton community (P<0.05). In October 2012, RDA with forward selection showed that oil (F=2.662, P=0.046) and Chl-*a* (F=2.557, P=0.048) explained the variability in the zooplankton community composition.

Table 2 The variation partitioning analysis of the significant environmental factors on the phytoplankton and zooplankton communities

Environmental factor	Eigenvalues	Percentage of variation explained solely	F-ratio	P-value
April, 2010	Phytoplankton			
Oil	0.207	20.7	3.388	0.002
April, 2012	Phytoplankton			
Oil	0.174	17.4	2.107	0.046
SPS	0.173	17.3	2.085	0.048
All the above together	0.268	26.8	1.647	0.084
April, 2012	Zooplankton			
DIN	0.208	20.8	2.631	0.022
October, 2012	Zooplankton			
Oil	0.250	25.0	2.662	0.046
Chl-*a*	0.242	24.2	2.557	0.048
All the above together	0.506	50.6	3.586	0.002

Variation partitioning analysis of the partial RDA was performed to estimate the influence of each significant variable (Zhang et al., 2011). The results showed that in April 2010 oil explained 20.7% of the phytoplankton community variation (Fig.9; Table 2). In April 2012, oil and SPS individually explained 17.4% and 17.3% of the phytoplankton variation, respectively (Fig.9; Table 2). DIN concentration explained 17.2% and 20.8% of the zooplankton community variation without the other environmental variables in April 2010 and 2012, respectively (Table 2). In October 2012, oil alone explained 25.0% of the phytoplankton variation, whereas Chl-*a* explained 24.2% of the zooplankton variation (Fig.9; Table 2).

The variation in plankton composition could be summarized on the first two axes of the partial RDA (Fig.9). In April 2010, the dominant species of phytoplankton (*C. curvisetus*, *Chaetoceros subsecundus*, and *C. lorenzianus*) were located on the right-hand side of the biplot, and they were negatively correlated significantly with oil. In April 2012, the dominant species of phytoplankton were *M. sulcata*, *S. costatum*, and *C. lorenzianus*, which were correlated positively with SPS and oil; *Schroderella delicatula* was correlated strongly with axis 2 and negatively correlated with oil. In April 2010, the dominant species of zooplankton were *P. parvus*, copepodite, and *A. clausi*. *P. parvus* was correlated positively with DIN and copepodite, and *A. clausi* was significantly correlated with axis 2. In April 2012, the dominant species of zooplankton were *Oithona similis*, nauplius larvae, and *P. crassirostris*. *O. similis* was correlated significantly with axis 2. Nauplius larvae and *P. crassirostris* were correlated positively with DIN. In October 2012, the dominant species of zooplankton were *O. dioica*, nauplius larvae, and *P. parvus*. Oil was correlated positively with *O. dioica* and negatively with nauplius larvae and *P. parvus*. Chl-*a* was correlated positively with *P. parvus* and negatively with *O. dioica* and nauplius larvae.

4 DISCUSSION

4.1 Environmental characteristics of Bayuquan Port

Liaodong Bay, especially the Bayuquan Port area, has been affected strongly by various anthropogenic pressures. In the last three decades, the water quality of Yingkou Bay has deteriorated dramatically because of urbanization and industrial and port development. The Bohai Sea received 0.8 billion tons of industrial sewage water in 1998 (State Ocean Administration, 2000), and today Bohai Bay receives about 1 billion tons of wastewater each year (Duan et al., 2010). The main rivers that empty directly into Liaodong Bay include the Liaohe, Daliaohe, Dalinghe, Xiaolinghe, and Shuangtaizihe, which carry anthropogenic materials as well as nutrients and suspended solids, thereby increasing human impacts on water quality in the bay. This riverine input is considered the main source of nutrients (Zhang et al., 2004; Wang et al., 2009; Duan et al., 2010) in the bay, and Wang et al. (2009) reported that Liaohe River was mainly responsible for the eutrophication of Liaodong Bay. In our study, the average DIN concentration was 0.763 mg/L, which exceeds the guideline (TN>0.5 mg/L) for eutrophic status (Twist et al., 1998); the concentration was only lower than the guideline value in April 2013 (0.131 mg/L), possibly due to decreased river runoff or regulated discharge of industrial sewage water. The mean PO_4-P concentrations during April 2009 and October 2011 and 2012 were 0.031, 0.017, and 0.027 mg/L, respectively, which are close to or beyond the benchmark concentration (TP>0.02 mg/L) for eutrophic status (Twist et al., 1998). These data suggest that this area was a potential eutrophication risk for Bayuquan Port. Meanwhile, red tides, which are linked closely to eutrophication, have been occurring more frequently and spreading ubiquitously in recent years (Sündermann and Feng, 2004). In addition, SPS levels were high during the survey period in the Bayuquan

Port area. Reasons for the high concentration of SPS include sediment resuspension due to wind-induced waves, port dredging, and ocean engineering projects (e.g., reclamation projects and breakwater construction). High SPS concentrations can have direct or indirect impacts on the plankton community by reducing water transparency, nutrient release rate, or adsorption efficiency and affecting phytoplankton photosynthesis, which in turn directly affects primary consumers (Arruda et al., 1983; Bilotta and Brazier, 2008). Additionally, hydrological conditions changing, such as sea surface temperature and wind speed, would significantly affect the abundance and distribution of diatoms and dinoflagellates and also possibly increase the occurrence of HAB species (Reid et al., 1998; Moore et al., 2009; Hallegraeff, 2010; Hinder et al., 2012). Continuous port construction, long-term sea ice trends along the Bayuquan Port, sea temperature and summer windiness conditions have created favorable conditions for the growth of diatoms and harmful algal bloom (Cao et al., 2005; Hinder et al., 2012). Moreover, the unstable ecosystems lead to a decrease in copepod abundance and biomass compared to the other parts of the Liaodong Bay (Song et al., 2010; Lynam et al., 2011), and its shows a synergistic positive effect the climate conditions and anthropogenic stresses may benefit outbreaks of jellyfish (Lynam et al., 2011; Condon et al., 2013).

4.2 Annual and seasonal variation in phytoplankton and zooplankton communities

According to the particle size, phytoplankton can be divided into picophytoplankton (0.2–3 μm), nano-phytoplankton (3–20 μm) and microphytoplankton (20–200 μm) (Hong et al., 1999). "The specifications for marine biological survey of China" (GB/T 12763.6-2007) provides two methods to collect phytoplankton (i.e., water sampling and trawl with vertical III plankton net (mesh size: 76 μm)), however, mainly relied on microscopic identification methods and the scope of the identification was confined to microphytoplankton, but for picophytoplankton and nanophytoplankton species identification method has not yet been specified. Due to fewer phytoplankton species in the northern sea area of China, plankton net trawl can be have higher efficiency compared with water sampling for phytoplankton collection, while it is difficult to trawl to collect picophytoplankton, nanophytoplankton and microphytoplankton with an aperture to achieve. For picophytoplankton and nanophytoplankton investigation only through water sampling and molecular biological identification, but cannot be to determine their number and particle size. This research is mainly aimed at microphytoplankton which were collected by vertical III plankton net, including some chain nanophytoplankton, and zooplankton investigation also only use plankton net (Vertical I and II), in order to link the consistency of phytoplankton and zooplankton investigation and to make full use of the history sample data, and therefore the phytoplankton investigation in this paper can only refer to the plankton nets provided by "The specifications for marine biological survey of China" (GB/T 12763.6-2007).

In April 2009 and 2010 and October 2011, phytoplankton abundance around Bayuquan Port was similar to values reported from previous surveys, such as 132.3×10^4 and 25.1×10^4 cell/m^3 along the shore of the Bohai Sea in May and October 1998, respectively (Wang, 2003); 25.1×10^4 cell/m^3 in the area southeast of Liaodong Bay along Yingkou Port from July to September 2005 (Song et al., 2007); and 35.66×10^4 cell/m^3 in the area north of Liaodong Bay in May 2009 (Luan et al., 2009). However, when red tides occurred in the Bohai Sea from 2009 to 2012, the phytoplankton abundance increased sharply (Yin et al., 2014). From 1998 to May 2003, 49 red tides were observed in the north sea of China; 20 of them occurred in Liaodong Bay, accounting for 41% of the total, and most of them occurred near the shore of Bayuquan Port (Cao et al., 2005). From May to October 2012, two red tide events were observed in Bahai Bay, located in the western part of Bohai Sea, and they were caused by *S. costatum*, *P. delicatissima*, *Chaetoceros* sp., and *E. zoodiacus* (Yin et al., 2014). We found similar results in our study. All of these data indicate that red tides have increased in frequency and scale in recent years. Several studies have suggested that the occurrence of red tides is strongly associated with human activities in coastal zones, especially the anthropogenic loadings that lead to eutrophication (Tang et al., 1998; Sellner et al., 2003). Eutrophication appears to be the key factor for development of red tides in coastal and bay waters (Paerl, 1997; Lim et al., 2005; Wang et al., 2008). In Liaodong Bay, the mass introduction of nutrients from river runoff, sewage and industrial wastewater, semi-closed water, upwelling relaxation (sediment resuspension), and other anthropogenic loadings can invoke eutrophication and lead to red tides.

Bi et al. (2001) reported that the average zooplankton abundance in the Bohai Sea was

3 841 ind/m^3 in the 1950–1960s, which is consistent with our results for April 2009 and 2010 and October 2011 and 2012. Song et al. (2010) reported zooplankton abundance of 17 978 ind/m^3 in Yingkou Port from July to September 2005, which is similar to our results for April 2012. Grazing by copepods is thought to have a significant impact on diatom blooms, sometimes suppressing their deterioration (Tiselius, 1988; Bathmann et al., 1990; Liu et al., 2009), which in turn can lead to increased zooplankton abundance such as that seen in April and October 2012 in this study. However, zooplankton abundance in April 2013 was significantly lower than that of the other sampling periods. This may have been due to the increased abundance of red tide species and dramatic rise in SPS concentration, which can alter the food web dynamics and reduce phytoplankton diversity. The resulting changes in the food chain and feeding environment can lead to fluctuations in the food available for zooplankton.

Nutrient levels play an important role in marine biodiversity and influence competition and community structure in the marine environment (Raghukumar and Anil, 2003; Worm et al., 2006; Gaonkar et al., 2010). In addition, several studies speculated that human activities and environmental factors influence the spatial and temporal distributions of the abundance and biomass of aquatic organisms (DelValls et al., 1998; Magni et al., 2005, 2006). In the present study, the ABC curves and their corresponding W values for the phytoplankton and zooplankton communities indicate the presence of a moderate to high range of pollution in the study area. The plankton communities were affected significantly by external disturbances, suggesting that the environmental variables give increasingly adverse pressure to the plankton community around coastal in the Bayuquan Port. The increased levels of eutrophication and anthropogenic stresses in Liaodong Bay have already affected the abundance of phytoplankton and zooplankton and keeping them in a sharp fluctuation level in the Bayuquan Port.

4.3 Environmental factors regulating the plankton assemblages

Phytoplankton community structure and abundance could make a rapidly response to vary in the environmental factor, and some of them may play a pivotal role in phytoplankton diversification (Peng et al., 2013). Our results suggest that the phytoplankton composition, abundance, and succession in Bayuquan Port were strongly influenced by oil, DIN, SPS, PO_4-P, and Chl-*a*, which contributed greatly to axis 1 and axis 2 throughout the study period. The annual and seasonal dynamics of phytoplankton composition and abundance and dominant species were most likely controlled by the environmental conditions of the habitat (Peng et al., 2013).

According to the RDA, *C. debilis*, *E. zodiacus*, *B. biddulphiana*, and *C. castracanei* were correlated strongly with oil concentration. The partial RDA results suggest that the phytoplankton community was affected significantly by the oil and SPS levels in April 2010 and 2012. Furthermore, the abundances of *Coscinodiscus* sp., *G. delicatula*, and *Stephanopyxis turris* were closely associated with high DIN concentration and *E. zodiacus* was the dominant species in October 2011 and closely associated to the low DIN. *C. curvisetus*, *P. delicatissima*, and *Thalassiosira* sp. abundances also were significantly correlated with high Chl-*a* concentration. The above results suggest that phytoplankton species use different strategies to adapt to the environmental conditions and changes.

It is recognized generally that estuarine/coastal marine red tides are closely linked with the nutrient concentrations in the water column (Anderson et al., 2002; Glibert and Burkholder, 2006). In our study, the red tide forming diatoms were *C. lorenzianus*, *M. sulcata*, *S. costatum*, *E. cornuta*, and *P. delicatissima*, and blooms were caused by high DIN loading from river runoff and sediment resuspension at Bayuquan Port during April and October 2012 and April 2013. *S. costatum* is known to be associated with high nutrients concentrations (Patil and Anil, 2011; Peng et al., 2013). Furthermore, the environmental factors (oil, SPS, and Chl-*a*) also contributed to the phytoplankton blooms.

In the present study, the PCA results suggest that the zooplankton community in Bayuquan Port was strongly influenced by oil, DIN, SPS, and Chl-*a* during the survey period. The results indicate that changes in oil, SPS, and Chl-*a* levels could have a great impact on the zooplankton species (e.g., *A. clausi*, *A. bifilosa*, *P. parvus*, and copepodite) present in Bayuquan Port. The partial RDA results suggest that annual changes in DIN concentration were more pronounced and may have had a greater impact on the zooplankton community in April 2010 and 2012 than during the other sampling periods. The oil and Chl-*a* were significantly associated with zooplankton composition and abundance. Previous studies have shown that zooplankton play an important role in the phytoplankton community via grazing pressure, thereby affecting its abundance and biomass (Morales et al., 1991; Dam and Peterson, 1993; Zhang et al., 1995). Thus, it is most likely that zooplankton abundance increased in conjunction with algae blooms during the surveys in the Bayuquan Port. However, the RDA results indicate that an increased SPS concentration can have a negative effect on zooplankton abundance, which may explain the dramatic reduction in zooplankton abundance in April 2013. Based on these findings, we infer that the zooplankton community in Bayuquan Port was affected strongly by both environmental factors and the phytoplankton community.

5 CONCLUSION

Red tides occur frequently in Bayuquan Port from 2012 to 2013. Eutrophication and high concentrations of suspended solids showed negative effects on phytoplankton and zooplankton community. Zooplankton abundance was closely associated with the phytoplankton community when the red tide occurred. Significant relation was found between plankton communities and some environmental factors e.g., oil, dissolved inorganic nitrogen (DIN), suspended solids (SPS), and chlorophyll *a* (Chl-*a*). Generally, eutrophication processes were driven by river runoff and increased the SPS levels, which were caused by intensive marine engineering and rapid industrialization and urbanization over the past three decades. These factors have heavily stressed the marine environment and greatly degraded plankton ecosystems in Bayuquan Port in Liaodong Bay.

Now, port construction has become a key issue for changing coastal port cities in economic and social, but environmental terms serves as a new challenge for them. How can the potential and foreseeable coastal contamination problems in the process of port reconstruction in waterfront be resolved? On the basis of the scientific assessment and protection of the marine environment along the Bayuquan Port of Liaodong Bay, Bohai Sea, the following goals need to be addressed:

First, we should be learning the experienced of port construction in the developed countries. Second, to further strengthen environmental supervision in the port waters, promote environmental remediation and ecological restoration. Third, in addition to the efforts of planners, designers and local government, the process needs multidisciplinary efforts from project feasibility studies in academia at home and abroad to focus on the marine environmental impacts of the port redevelopment, in line with sustainable development, to seek multiple appropriate redevelopment models. Furthermore, we would pay more attention to the particles size structure of the plankton community under different marine environmental conditions in the harbor water area in the future, as well as distribution and succession of alien invasive micro-organisms, and the interaction between environmental factors and outbreaks of jellyfish deserving of further study in experimental and in situ conditions.

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