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Effects of Terrestrial Inputs on Mesozooplankton Community Structure in Bohai Bay, China

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Abstract: Zooplankton play a pivotal role in connecting primary producers and high trophic levels, and changes in their temporal and spatial distribution may affect the entire marine ecosystem. The spatial and seasonal taxonomic composition patterns of mesozooplankton in Bohai Bay were investigated in relation to a number of water parameters. Bohai Bay is a eutrophic semi-enclosed bay with dynamic physico-chemical conditions influenced by terrestrial inputs and seawater intrusion. The results showed that under the condition of terrigenous input, the diversity of mesozooplankton species near the eutrophic Haihe River Estuary and Jiyun River Estuary was lower than that in the central Bohai Bay, with gelatinous *Oikopleura dioica* as the dominant species. The mesozooplankton diversity was highest in the bay mouth affected by seawater intrusion, and the dominant oceanic species, mainly copepods *Corycaeus affinis*, *Calanus sinicus*, and *Oithona similis*, entered the inner bay from the bay mouth. Meanwhile, the abundance of mesozooplankton in summer was significantly higher than that in autumn. Compared with historical data, the dominant species in Bohai Bay has evolved from arrow worm *Sagitta crassa* to copepod *Paracalanus parvus*, probably due to global warming, indicating the effects of human activities on the succession of mesozooplankton community.

Keywords: mesozooplankton; spatiotemporal distribution; seasonal variation; eutrophic estuary; diversity

1. Introduction

Zooplankton plays a pivotal role in connecting primary producers and high trophic levels [1]. At the same time, as an important part of the marine food chain, the change in their temporal and spatial distribution may affect the whole marine ecosystem, thus making zooplankton an ideal component to detect the dynamics of the marine ecosystem [2]. Zooplankton communities are closely related to environmental factors and are highly sensitive to climate change and human activities [3]. Therefore, it is very important to understand the zooplankton community under the influences of both climate change and human activities. Among diverse marine habitats, coastal waters are most closely related to human society, supporting rich fishing grounds and economic benefits with high productivity, which are largely connected to the zooplankton community [4].

In estuarine and coastal waters, terrestrial inputs bring a high amount of nutrients, leading to changes in nutrient levels in nearshore waters [5]. Inorganic nutrients such as nitrogen, phosphorus and silicon synthesize organic matter through photosynthesis of phytoplankton and transfer matter and energy to zooplankton through the food web [6]. However, the excessive inputs of nutrients can stimulate massive algal growth, decrease the abundance and diversity of zooplankton and caused a shift in the dominant species [7]. For

example, in the estuary area of Laizhou Bay, the abundance and diversity of mesozooplankton decreased due to the terrestrial inputs that induced eutrophication [8]. In Daya Bay, the mesozooplankton diversity near the eutrophic Dan'ao estuary is lower than that in the middle of the Bay, in which the small herbivore copepods of *Acartia* spp. and *Paracalanus* spp. are dominant [9]. The abundance and diversity index of copepods in Manar Bay was also found to be higher in the outer than in the inner Bay [10]. Eutrophication can also create low-oxygen zones in the enclosed marine lake in the Croatian island of Mljet, resulting in limitation of the growth of mesozooplankton to such an extent that their abundance and diversity are reduced [11].

Changes in hydrology can also affect mesozooplankton communities. The invading seawater can change the nutrient concentrations and mesozooplankton community [12–16]. For example, the highest diversity of mesozooplankton in the bay mouth of Daya Bay is a result of seawater intrusion [10]. In another case in Xiangshan Bay, the strong influence of seawater exchange resulted in mesozooplankton adapting to high salinity, such as copepod *Paracalanus aculeatus* and arrow worm *Sagitta bedoti*, to be brought to Xianshan Bay from the waters nearby [17].

Bohai Bay is a typical semi-closed bay located in the west of the Bohai Sea. It is connected with the central part of the Bohai Sea to the East, and the other three sides are surrounded by five coastal cities, namely Tangshan, Tianjin, Cangzhou, Binzhou and Dongying. A number of aquaculture ponds and factories and farmlands are located near Tianjin City. Large amounts of nitrogen and phosphorus pollutants, generated by anthropogenic activities, enter the coastal waters through surface runoff (e.g., Haihe River), leading to eutrophication and thus influence the marine ecosystem of Bohai Bay [18,19]. In the Haihe River estuary, an anoxic zone (dissolved oxygen, DO < 2 mg/L) has been formed due to increased terrigenous inputs during summer rainfall periods [20,21]. The results of a hydrodynamic model simulation show that there are two vortices in Bohai Bay, with the clockwise vortex in the northwest of the bay and the counterclockwise vortex in the south of the estuary [22]. At the same time, there is a relatively stable counterclockwise weak circulation in Bohai Bay [4]. The seawater of the Yellow Sea driven by currents enters the central part of the Bohai Sea from the north of the Bohai Strait, then enters the north side of the mouth of the Bohai Bay through the Liaodong Bay, while the seawater flows out through the south side of the mouth of Bohai Bay [22]. As the connection between land and the central Bohai Sea, Bohai Bay is affected by human activities and the circulation of the Bohai Sea [4]. Therefore, it becomes increasingly important to understand the changes of zooplankton communities in Bohai Bay under the influences of multiple physico-chemical factors, such as terrestrial inputs and seawater intrusion.

However, research on the temporal and spatial changes in the mesozooplankton community in response to terrigenous inputs and seawater intrusion in Bohai Bay is still limited. Thus, the purpose of this study is to (1) evaluate the temporal and spatial community structure and dominant species composition of mesozooplankton in Bohai Bay, and (2) analyze the main environmental factors affecting the composition and distribution of the dominant species of mesozooplankton in Bohai Bay. Furthermore, this study updated the observation data of mesozooplankton in Bohai Bay, so as to further understand the impacts of terrestrial inputs and seawater intrusion on the changes of mesozooplankton community structure.

2. Materials and Methods

2.1. Study Area and Sampling Stations

Bohai Bay (117°72′–119°14′ E and 37°98′–139°98′ N) covers an area of 15,900 km^2 and its water depth ranges from 3.5 to 33 m (average 15.73 m). Over the past three decades, some important economic and technological development areas have been established around the bay. About one billion tons of waste water which are not fully treated is annually discharged into the Bohai Bay from Tianjin City, posing a potential risk to the marine ecosystem [18]. There are two major inflow rivers in the study area [23]. The Haihe River is the largest

river flowing into the bay with a freshwater discharge of approximately 228×10^8 m^3 annually, while the Jiyun River with a freshwater discharge of approximately 1.6×10^8 m^3 annually. Sampling was conducted at 17 stations in summer (July) and 19 stations in autumn (October) in 2020 (Figure 1). In order to study the effects of these two rivers on the zooplankton community, we set up sampling stations at the estuary of Haihe River (stations H2 and H4 in summer and stations H1 and H5 in autumn) and the estuary of Jiyun River (station J1). Other sampling stations were distributed along the coastal waters and in the open waters in Bohai Bay, among which Station B1 is located near a shellfish farming area.
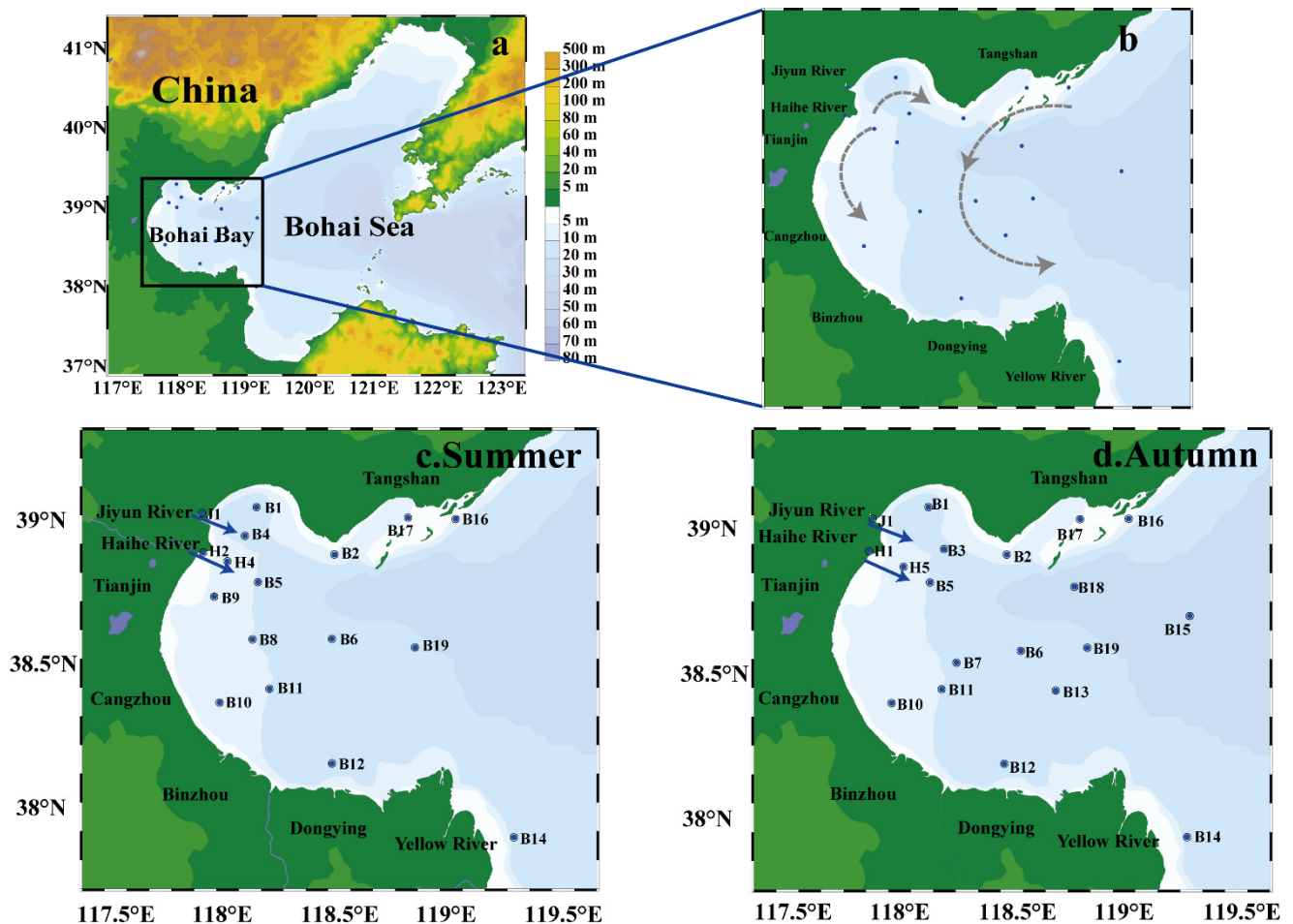

Figure 1. The locations of sampling stations in Bohai Bay where (**a**) is the geographical location of Bohai Bay, (**b**) is Bohai Bay current drawn according to the research results of Wang et al. [24], (**c**) and (**d**) are sampling stations in summer and autumn, respectively. Arrows show estuaries.

2.2. Sample Collection and Analysis

Mesozooplankton samples were collected using vertical hauls from about 1 m above the seafloor to the sea surface using a plankton net (50 cm mouth diameter, 200 µm mesh size). The net was equipped with a calibrated flowmeter (Hydrobios) to measure the volume of filtered seawater. Seawater samples were collected and measurements of temperature and salinity were performed using a SeaBird conductivity/temperature/depth (CTD) meter (SBE 19 Plus V2) equipped with Niskin bottles. Seawater samples were collected from surface depth (2 m) for the determination of nutrient concentration, chlorophyll *a* (Chl *a*) concentration, DO concentration and pH.

The net samples collected were stored in 500 mL polyethylene (PE) bottles and fixed with 5% formaldehyde solution (final concentration). Prior to microscopic analysis, large

zooplankton, such as hydromedusae, were picked out and counted. For microscopic analysis, at least 1% of the total volume of the remaining sample (depending on the sample density) was placed in the plankton counting frame (DSJ). Mesozooplankton abundances (ind. m^{-3}) for each station were calculated using the total volume of filtered water. A total of 36 subsamples were examined under the microscope (Motic Panthrea L and Motic SMZ-168 SERIES) at 40–100× magnification and mesozooplankton taxa were identified to the lowest possible taxonomic level.

The concentration of Chl *a* was determined using the extraction fluorescence method. Samples were collected by filtration of 1 L seawater through Whatman GF/F filters (0.7 μm porosity) using a diaphragm vacuum pump under a vacuum of less than 100 mm Hg. The filter was placed into a 10 mL brown glass tube, then 5 mL of acetone with a volume fraction of 90% was added and the glass tube was stored in the dark at 4 °C for 24 h. The Chl *a* biomass was measured using the Turner Fluorometer (model 10-AU). The Chl *a* fluorescence was measured in non-acidified mode and calculated according to Parsons' formula [25].

Nutrient samples (300 mL) were filtered through a 0.45 μm cellulose acetate membrane filter to remove large particles and then quickly frozen at −20 °C and analyzed as soon as possible. Nutrients, mainly including nitrate (NO_3-N), nitrite (NO_2-N), and ammonia (NH_4-N), phosphate (PO_4-P), and silicate (SiO_3-Si), were determined using the Technicon AA3 automatic analyzer (Bran + Luebbe) following the method reported by Brzezinski and Karl [26,27]. In addition, we set a minimum nutrient concentration of 0.01 μmol/L to avoid detection limit issues. The concentration of dissolved inorganic nitrogen (DIN) was calculated as the sum of the concentrations of NO_3-N, NO_2-N, and NH_4-N. Dissolved inorganic phosphorus (DIP) was generalized as PO_4-P and dissolved inorganic silicate (DSi) was generalized as SiO_3-Si.

The pH of the water sample was determined using a pH meter. DO was determined immediately by the Winkler method after the CTD arrived on deck.

2.3. Statistical Analysis

As a measure of the diversity of the mesozooplankton community, we calculated the dominance index (*Y*), the Shannon–Wiener diversity index (*H′*), and Pielou's evenness index (*J*) as follows:

$$Y = \frac{n_i}{N} f_i$$

$$H' = - \sum_{i=1}^{S} P_i log_2 P_i$$

$$J = \frac{H'}{log_2 S}$$

where *S* is the mesozooplankton species in each sample, also known as species richness; *N* is the total number of individuals in all samples, n_i is the number of individuals of the *i*th species, P_i is the abundance of the *i*th species, $P_i = n_i/N_i$, and f_i is the frequency of occurrence of the *i*th species in a single sample. The dominant species was identified using the ranking list of the dominance index.

Environmental factor distribution was generated using Ocean Data View 4.7.6 (Alfred-Wegener-Institut (AWI), Bremerhaven, Germany). Pie charts and histograms were generated by ArcGis 10.7 (Environmental Systems Research Institute (Esri)) in order to observe the abundance, species numbers and composition at different stations. Hierarchical cluster analysis and heatmaps were performed and created using the "pheatmap" package in R statistical software (version 4.0.3, R Core Team, Vienna, Austria, 2020). The stations were clustered according to the environmental factors and diversity in each sampling station. Environmental data (temperature, salinity, Chl *a*, DIN, DIP, DSi, DO, pH) and zooplankton abundance and diversity (Shannon–Wiener diversity index and species richness) were used in the cluster analysis. Principal coordinate analysis (PCoA) was performed using the R package "vegan" (version 2.5–7), and all species at different stations were subjected to dimensionality reduction analysis to study the similarity of community composition at

different stations. Redundancy analysis (RDA) was performed using CANOCO 5.0 for Windows (Microcomputer Power, Ithaca, NY, USA) to identify the structuring effects of environmental conditions on the distribution of the dominant mesozooplankton species. The boxplot was generated using Origin 2021 (Origin Lab, Northampton, MA, USA). In all tests, statistical significance was accepted at *p* < 0.05.

3. Results

3.1. Environmental Variables

In summer and autumn, the distribution of environmental factors in surface water was different (Figure 2). In summer, the sea surface temperature (SST) ranged from 24.79 to 28.56 °C (Table S1), and the seawater salinity was in the range of 23.44–32.15 psu. The lowest SST and highest salinity were found in stations B14 (24.79 °C) and B1 (32.15 psu), respectively, while the lowest salinity occurred near the Haihe River estuary (H2, 23.44 psu). The SST in autumn was lower than that in summer, with a range between 13.39 and 17.22 °C. Salinity ranged from 6.63 to 31.82 psu. The salinity of the estuarine area was low, with the value of 6.63 in Haihe River estuary (H1) and 19.64 in Jiyun River estuary (J1).
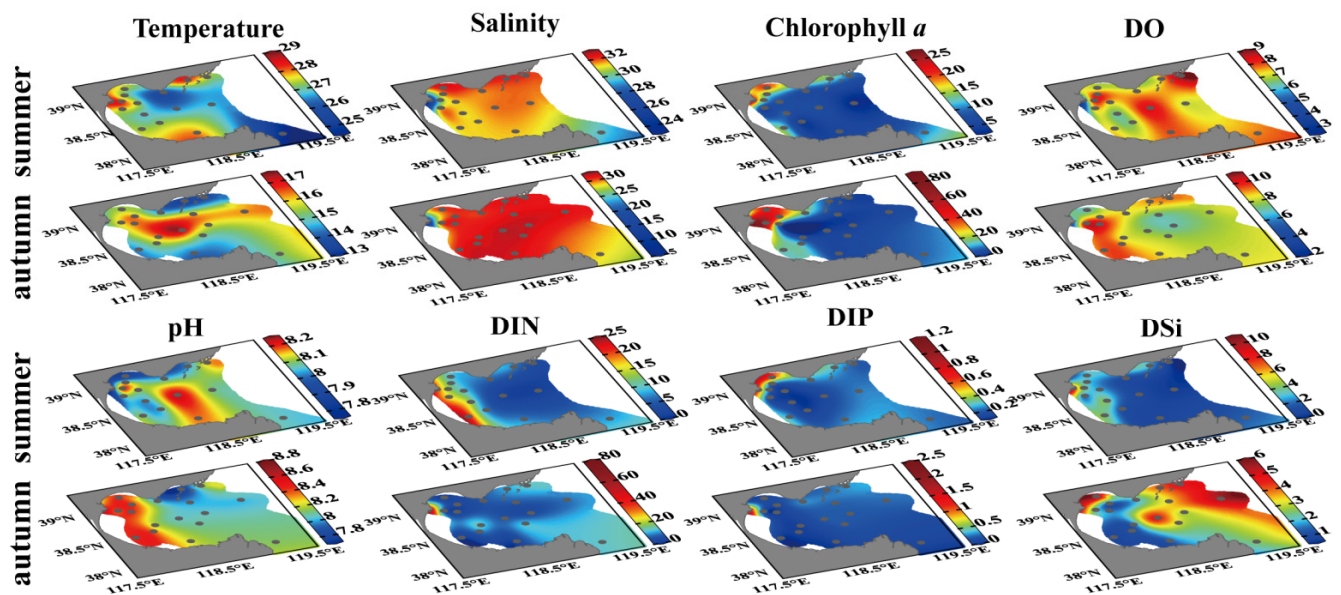

Figure 2. Distribution of the (2 m) temperature (°C), salinity, chlorophyll *a* (μg/L), dissolved oxygen (DO mg/L), pH, dissolved inorganic nitrogen (DIN, μmol/L), dissolved inorganic phosphorus (DIP, μmol/L), and dissolved inorganic silicate (DSi, μmol/L) in surface waters in summer and autumn in Bohai Bay.

The distribution of Chl *a* was high in the inner bay and gradually declined toward the outer bay in both seasons, with the highest values near the Haihe River estuary (H2, 25.16 μg/L in summer and H1, 88.89 μg/L in autumn). The concentrations of DO ranged from 2.87 to 8.95 mg/L in summer and 6.62 to 10.13 mg/L in autumn. The lowest DO occurred near estuaries (H2, 2.87 mg/L in summer and J1, 6.62 mg/L in autumn). The pH ranged from 7.77 to 8.24 in summer and from 7.70 to 8.71 in autumn. The distribution pattern of DIN and DIP concentrations was similar as that of Chl *a*. In summer, the highest DIN (24.00 μmol/L) and DIP (1.10 μmol/L) were observed near the Jiyun River estuary. While in autumn, the highest DIN (78.92 μmol/L) and DIP (2.25 μmol/L) were observed near the Haihe River estuary. The concentration of DSi in summer and autumn ranged from 0.30 to 10.04 μmol/L and from 0.80 to 5.85 μmol/L, respectively.

3.2. Mesozooplankton Composition and Abundance

A total of 105 mesozooplankton taxa were identified during summer and autumn in Bohai Bay (Table S2). Among them, 87 species belonging to 12 groups were identified in summer, while 67 species belonging to 12 groups were identified in autumn (i.e., copepods, hydromedusae, chaetognaths, tunicates, amphipods, cladocerans, decapods, euphausiids, mysids, polychates, ostracods and planktonic larvae, Tables S3 and S4). Mesozooplankton were composed mainly of copepods and pelagic larvae in both summer and autumn. Copepods accounted for the largest proportion (32 species, 30.48%).

The abundance of mesozooplankton was higher in summer. Mesozooplankton abundance ranged between 342.22 and 23,704.17 ind. m^{-3} in summer and between 685.11 and 10,807.00 ind. m^{-3} in autumn. The highest abundance was found in station B6 in summer which is located among the stations far from the estuaries, while the lowest abundance found in station B1 in summer near a shellfish farming area. At the same time, the distribution of species richness was similar to the distribution of abundance (Figure 3).
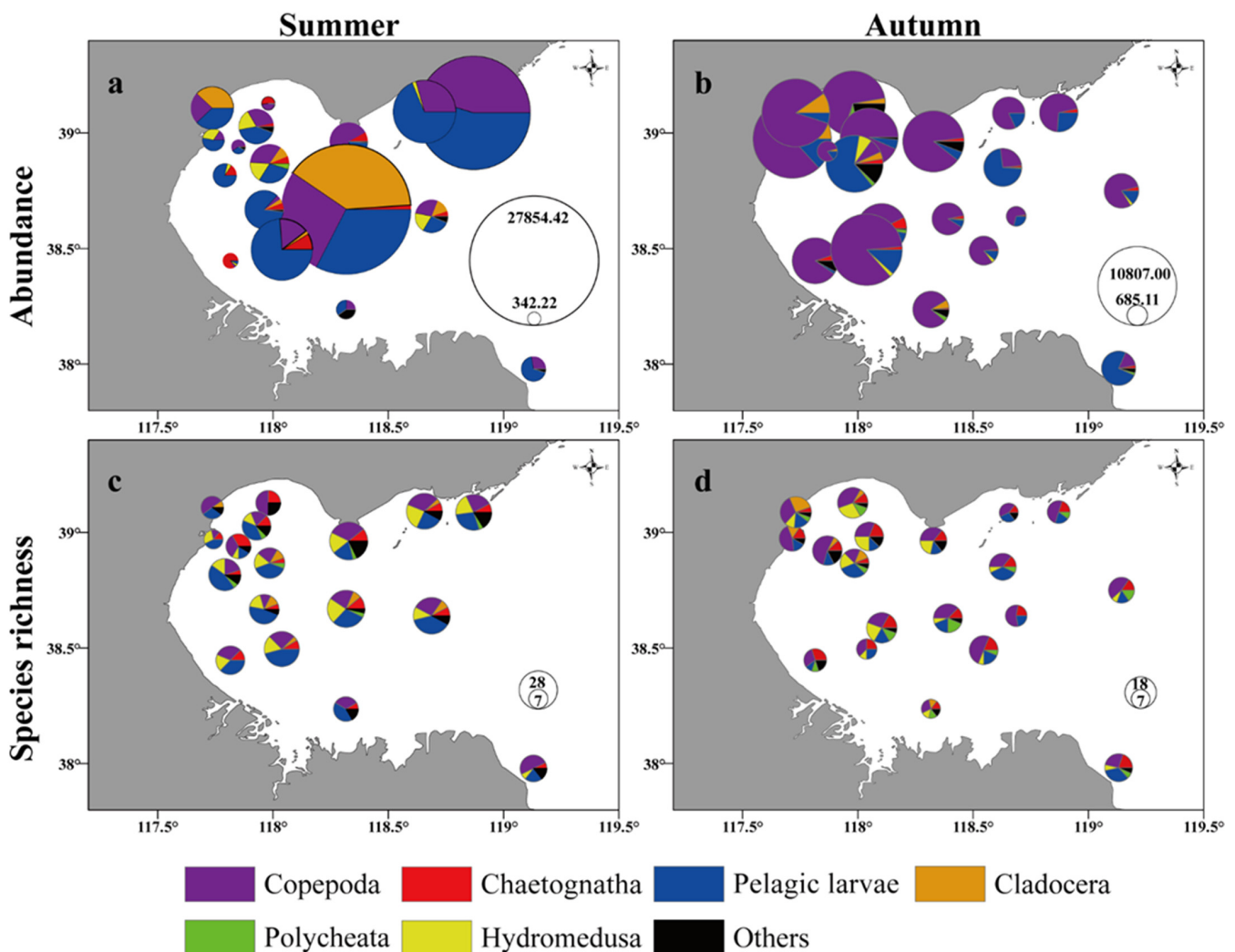

Figure 3. Abundance (ind. m^{-3}), (**a**,**b**) and species richness (**c**,**d**) of mesozooplankton in summer (**a**,**c**) and autumn (**b**,**d**).

There were six dominant species in both summer and autumn in Bohai Bay (Figure 4, Tables S5 and S6). The dominant species of mesozooplankton and their frequency, abundance and dominance are shown in Table 1. Three species were dominant in both seasons, they were copepod *Paracalanus parvus*, tunicata *Oikopleura dioica*, and arrow worm *Sagitta*

crassa. In addition, the dominant species in summer were two cladocera species *Pseude-vadne tergestina*, *Penilia avirostris* and jellyfish *Pleurobrachia globosa*. The dominant species in autumn were three copepods *Corycaeus affinis*, *Calanus sinicus*, and *Oithona similis*. Among them, the distribution of *Paracalanus parvus* shifted from the outer bay in summer to the inner bay in autumn. The abundance of *Sagitta crassa* was not high but it was widely distributed in the Bohai Bay. *Oikopleura dioica* was mainly distributed in the eastern part of Bohai Bay. Cladocerans *Pseudevadne tergestina* and *Penilia avirostris* appeared in the inner bay in summer, while *Oithona similis* was distributed near the estuarine area of the inner bay in autumn.
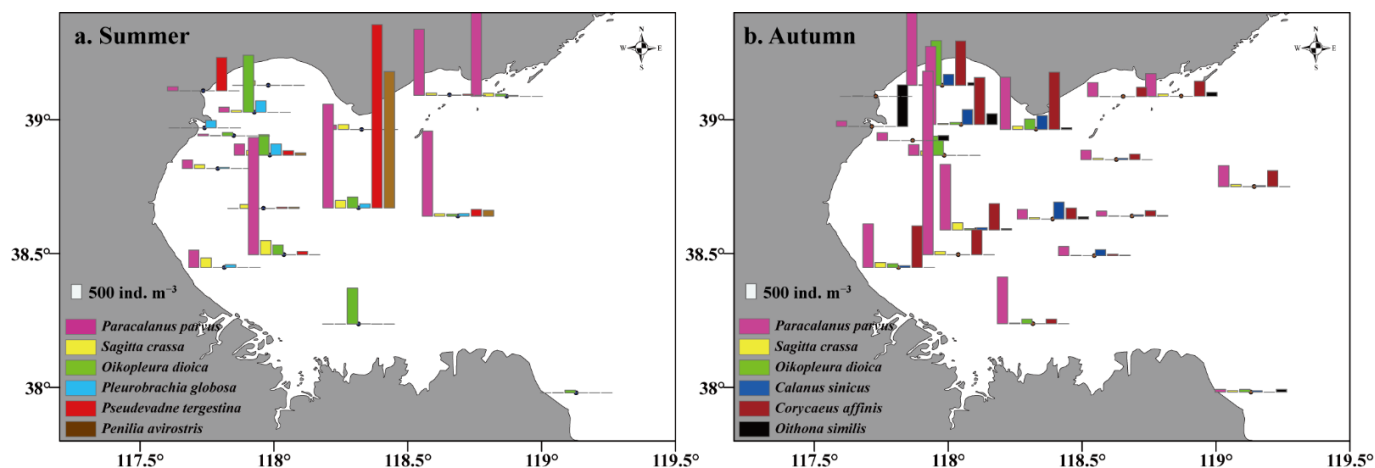

Figure 4. Distribution and seasonal changes in the abundance (ind. m^{-3}) of predominant mesozoo-plankton species (*Y* > 0.02). Note the different species in figures (**a**,**b**).

Table 1. Dominant mesozooplankton species of Bohai Bay in summer and autumn (F, frequency, *Y*, dominant index, x, average abundance, ind. m^{-3}).

Dominant Species	Group	Summer			Autumn		
		F	x	Y	F	x	Y
Paracalanus parvus	Copepoda	0.76	504.97	0.15	0.95	1403.17	0.34
Pseudevadne tergestina	Cladoceera	0.41	469.52	0.08	0.32	70.64	0.01
Oikopleura dioica	Tunicate	0.53	220.27	0.05	0.53	188.89	0.03
Sagitta crassa	Chaetognatha	0.94	118.10	0.04	1.00	77.15	0.02
Penilia avirostris	Cladoceera	0.24	283.02	0.03	0.16	41.86	0.01
Pleurobrachia globosa	Hydromedusa	0.71	79.85	0.02	0.58	40.53	0.01
Corycaeus affinis	Copepoda	0.41	49.87	0.01	0.79	657.61	0.13
Calanus sinicus	Copepoda	0.47	13.96	0.003	0.53	123.85	0.02
Oithona similis	Copepoda	0.17	4.07	0.0008	0.58	154.77	0.02

3.3. Diversity Indices of the Mesozooplankton Community

Different biodiversity indices express the diversity of mesozooplankton communities or assemblage in different ways, but the best way to assess community diversity is to use these indices in combination. Overall, the values of diversity index (*H'*) and Pielou evenness index (*J*) showed similar distributions (Figure 5). The values of *H'* ranged from 0.28 to 2.53 in summer and 1.25 to 2.16 in autumn. The *H'* value was highest at station B6 located in central bay both in summer (2.53) and in autumn (2.16). *J* ranged from 0.08 to 0.54 in summer, with the highest value at station B5 (0.54), while in autumn ranged from 0.38 to 0.59, with the highest value at station B19 (0.59).
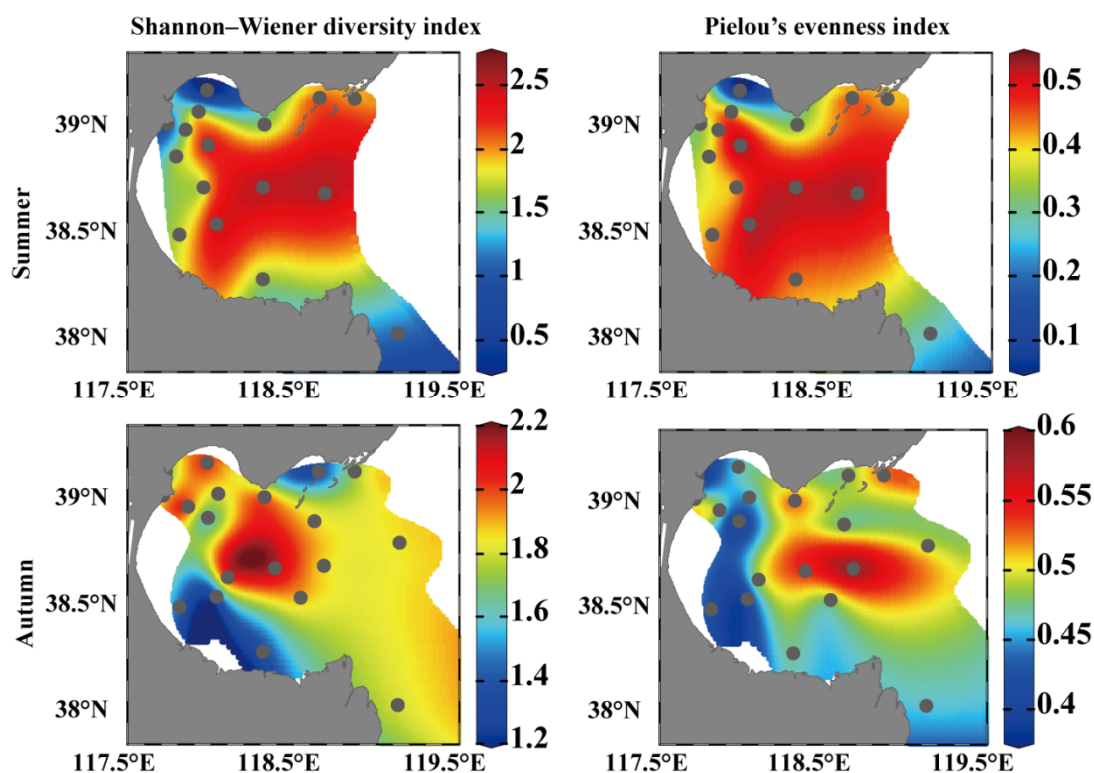
Figure 5. The horizontal distribution of mesozooplankton species diversity indices in summer and autumn. Shannon–Wiener diversity index (H'), Pielou's evenness index (J).

3.4. Relationship between Mesozooplankton Community and Environmental Factors

Hierarchical cluster analysis related to environmental variables clustered the stations in three groups (Figure 6), which is similar to the results of PCoA analysis showing site ranking based on the similarity or dissimilarity of community composition (Figure 7). Combined with Figure 8, the abundance and diversity indices of group 1 near the bay mouth were higher than those of group 3 near the estuary in summer. In autumn, group 2 near the estuary had the highest diversity.

RDA was conducted to further demonstrate the preferred environment for dominant species. The results of the RDA analysis showed that the distribution and hierarchical clustering of the stations and the results of PCoA were similar. In summer, *Paracalanus parvus* showed a negative correlation with temperature and a positive correlation with salinity (Figure 9a). According to the distribution of the three groups of stations (Figure 9a), group 1 near the mouth of the bay was mainly negatively correlated with temperature and nutrients. Group 2 was positively correlated with temperature and salinity. The areas from the bay mouth to the outer bay had higher salinity. Coastal and oceanic species, such as *Oikopleura dioica* and *Paracalanus parvus*, which were positively correlated with salinity and negatively correlated with temperature, appeared near B5. In autumn, most dominant species showed a positive correlation with temperature (Figure 9b). Group 3 was close to the estuary with high temperature, low salinity and high nutrient concentrations, and there were almost no dominant species. In autumn, most dominant species were positively correlated with temperature (Figure 9b). Group 1 in autumn was mainly negatively correlated with DSi, stations of which were influenced by seawater intrusion and intra-bay circulation in autumn. Group 2, includes stations in the estuary area, were characterized by high nutrients levels. Group 3 was regulated by multiple factors and the DSi concentration was higher, indicating that DSi was brought to the sea surface by seawater intrusion.
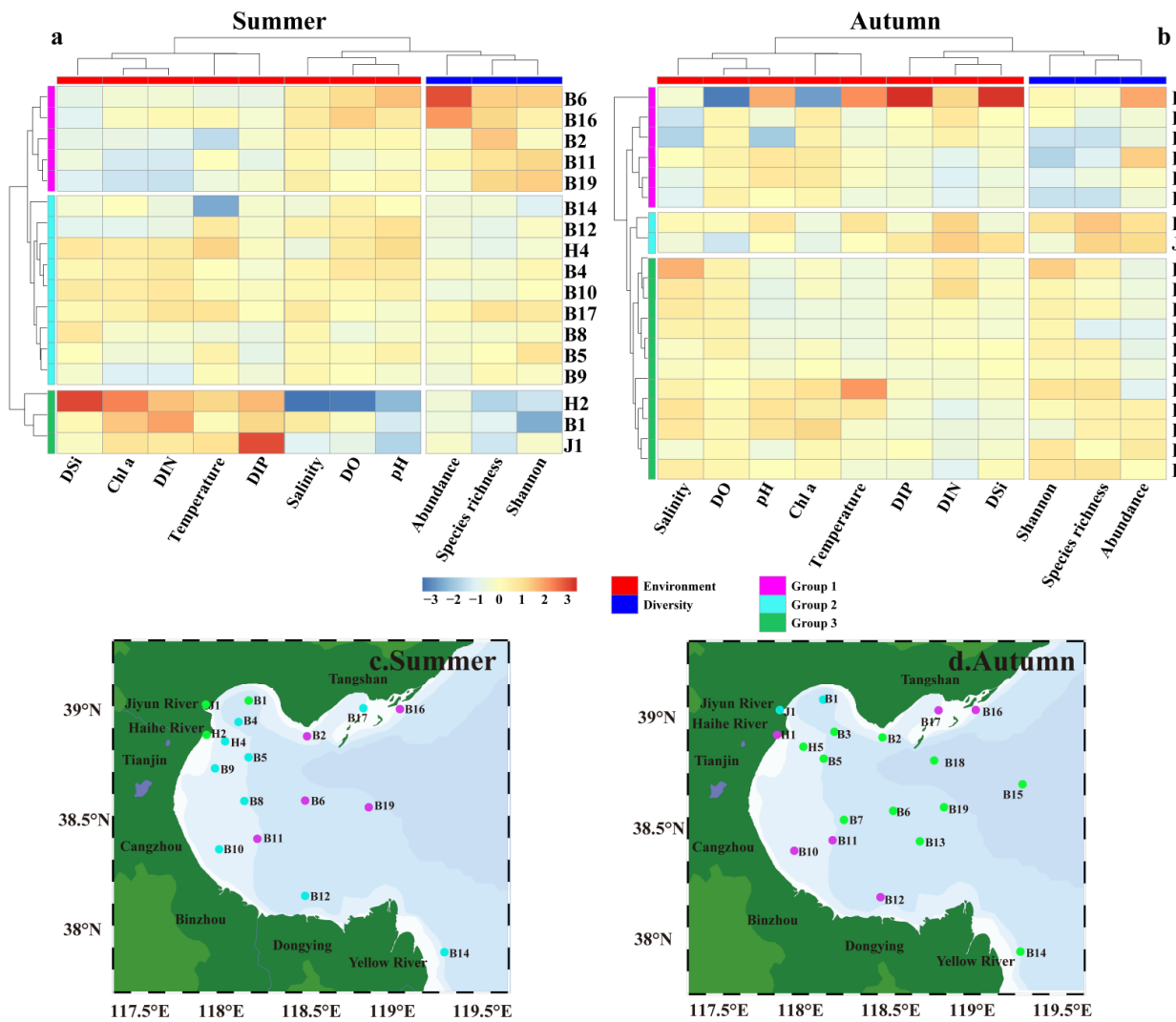
Figure 6. Heat map presenting the (**a**,**b**) hierarchical cluster of environmental variation and meso-zooplankton abundance and diversity. (**c**,**d**) were station maps drawn according to the results of hierarchical clustering. (Shannon, Shannon–Wiener diversity index).
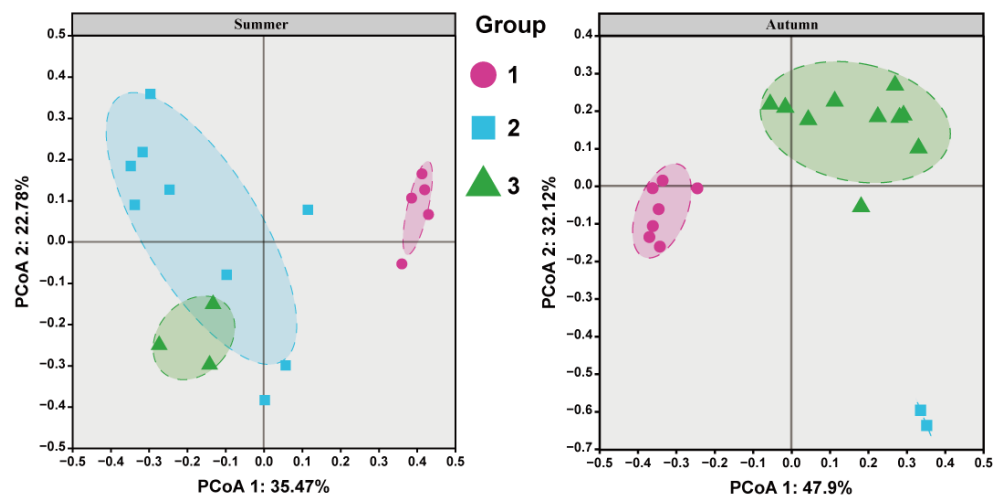

Figure 7. Principal coordinates analysis the groups. *p* < 0.05. Different colors and shapes represent different groups in summer and autumn. Percentages of total variance are explained by *axes* 1 and 2.
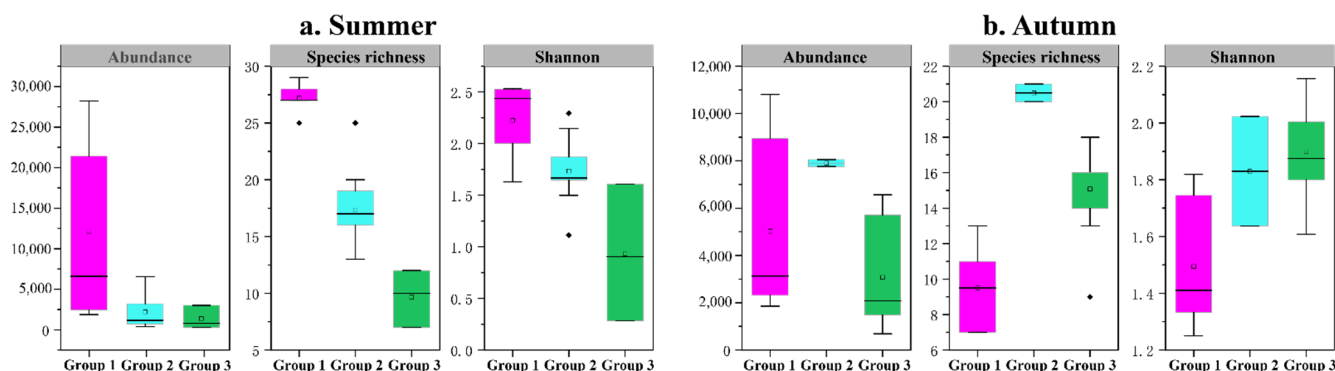
Figure 8. Abundance and diversity of mesozooplankton from the 3 groups in summer (**a**) and autumn (**b**). (Shannon, Shannon–Wiener diversity index).
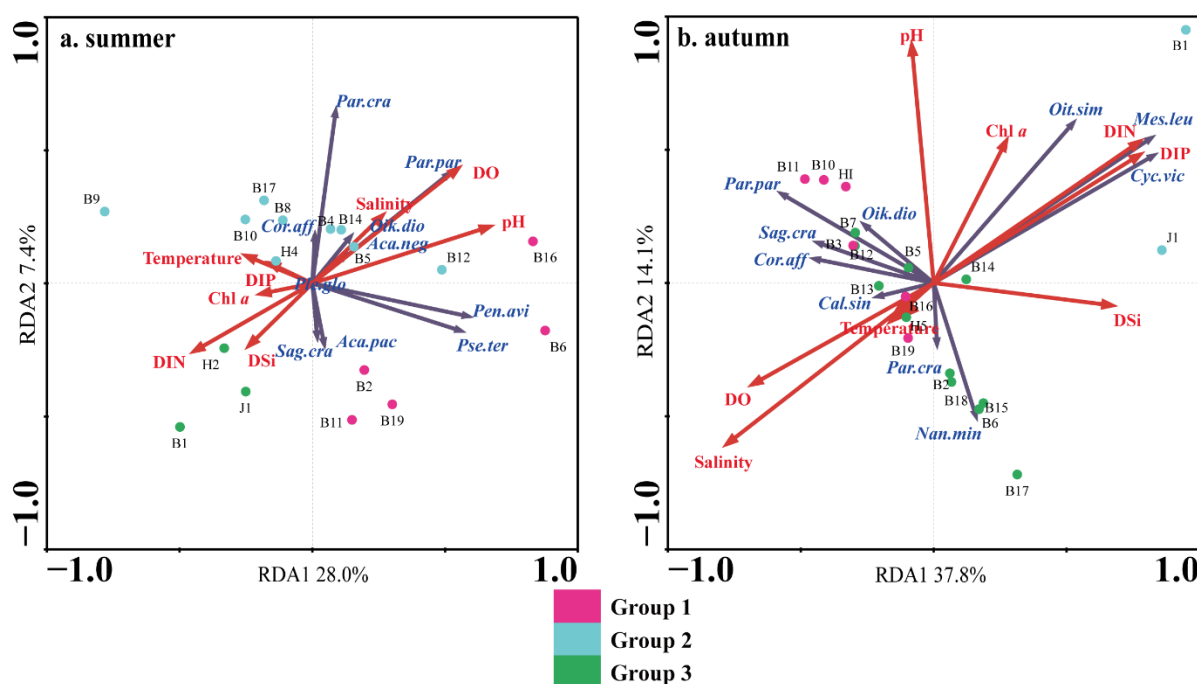

Figure 9. Redundancy analysis (RDA) result of the dominant mesozooplankton species and environmental variables of the 3 groups in Bohai Bay in summer (**a**) and in autumn (**b**). (*Par.par, Paracalanus parvus; Oik. dio, Oikopleura dioica; Pse. ter, Pseudevadne tergestina; Sag. cra, Sagitta crassa; Pen. avi, Penilia avirostris; Ple. glo, Pleurobrachia globosa; Cor. aff, Corycaeus affinis; Cal. sin, Calanus sinicus; Oit. sim, Oithona similis; Par.cra, Paracalanus crassirostris; Aca.neg, Acartia negligens; Aca.pac, Acartia pacifica; Cyc.vic, Cyclops vicinus; Mes.leu, Mesocyclops leuckarti; Nan.min, Nannocalanus minor*).

4. Discussion

4.1. Comparison with Historical Data

An analysis of long-term changes in plankton based on long-term observational data is one of the key scientific issues in marine ecological research [28]. The results of the present study were different from the historical records (Table 2) since cladocerans *Penilia avirostris* and *Pseudevadne tergestina* became the dominant species in Bohai Bay in summer. Cladocerans are generally typical nearshore saltwater animals and serve as an important link between microalgae and fish [29]. *Pseudevadne tergestina* is a warm-water species of cladocera with a distinct seasonal distribution, usually beginning to appear in spring, peaking in summer, declining in autumn, and disappearing completely in winter [13], which was also the case in the present study. Meanwhile, *Penilia avirostris* mainly feeds on dinoflagellates [30]. During the present survey, a dinoflagellate bloom dominated

by *Akashiwo sanguinea* occurred, as a result of the warming and eutrophication of the waters of Bohai Bay, while these blooms probably favored the proliferation of *Penilia avirostris*. Moreover, the phytoplankton community in Bohai Bay experienced a shift from an absolute dominance of diatoms to a co-dominance of diatoms and dinoflagellates in recent years [31–34], resulting in a shift of zooplankton community with copepod *Calanus sinicus* replaced by cladocerans *Penilia avirostris* and *Pseudevadne tergestina* dominating in Bohai Bay in summer.

Table 2. Historical changes in the dominant mesozooplankton species in Bohai Bay.

Time	2020 This Study	2013 Wu et al. [35]	2005–2009 Yang et al. [36]	2003–2004 Fan et al. [37]	1959 Bi et al. [29]
Summer	*Paracalanus parvus* *Pseudevadne tergestina* *Oikopleura dioica* *Sagitta crassa* *Penilia avirostris* *Pleurobrachia globosa*	*Paracalanus parvus* *Acartia bifilosa* *Acartia pacifica* *Oithona similis* *Calanus sinicus* *Sagitta crassa* *Paracalanus crassirostris*	*Sagitta crassa* *Calanus sinicus* *Acartia bifilosa* *Paracalanus parvus*	*Sagitta crassa* *Labidocera euchaeta* *Paracalanus parvus* *Paracalanus crassirostris* *Oithona similis*	*Paracalanus parvus* *Paracalanus crassirostris* *Oithona similis* *Labidocera euchaeta*
Autumn	*Paracalanus parvus* *Corycaeus affinis* *Oikopleura dioica* *Oithona similis* *Sagitta crassa* *Calanus sinicus*	*Sagitta crassa* *Calanus sinicus* *Labidocera euchaeta* *Acartia pacifica* *Acartia bifilosa* *Pleurobrachia globosa*		*Paracalanus parvus* *Paracalanus crassirostris* *Oithona similis*	

In the present study, the most dominant species of mesozooplankton was the copepod *Paracalanus parvus*. Historical records show that from 2003 to 2009, the most dominant species is arrow worm *Sagitta crassa* [29]. However, since the outbreak of small copepods represented by *Paracalanus parvus* in 2006, *Sagitta crassa* was gradually replaced. Moreover, it was found that *Calanus sinicus* has changed from high abundance to low abundance since 2009 [36]. The shift to smaller mesozooplankton species could be attributed to the change of phytoplankton size due to global warming favoring pico- and nano-phytoplankton [38], affecting the structure of the zooplankton community. By comparing with historical data, the mesozooplankton community appears to be responding to the long-term changes in phytoplankton community in Bohai Bay [39–42].

4.2. Comparison with Other Bay Ecosystems

Based on our results, the abundance and diversity of the zooplankton community were higher in the outer bay than those in the inner bay in summer. This is consistent with the results from other bays, namely Hailing Bay, Laizhou Bay, Daya Bay, Gulf of Cadiz, and Masan Bay, which are all influenced by terrestrial inputs (Table 3). Copepods are the most dominant group in these bays, with *Paracalanus parvus* being the dominant species. The rivers in the Masan Bay bring a huge amount of nutrients in the bay stimulating excessive growth of algae, depletion of oxygen in water column, and finally leading to a reduction of zooplankton community [43]. As a result, zooplankton abundance is negatively correlated with nutrients levels in most bays mentioned above. Nutrient concentration in Laizhou Bay in 2009 was much lower than those in other bays. An appropriate amount of nutrients could enhance the growth of phytoplankton and thus provide a potential food source for zooplankton. Similar to our results, a large number of cladocerans, such as *Penilia avirostris*, was also found in Daya Bay, Masan Bay and Gulf of Cadiz with low oxygen conditions in summer. The cladoceran *Penilia avirostris* generally appeared in the inner bay probably due to the higher sea surface temperature in estuarine waters caused by the inputs of warmer freshwater. Moreover, their abundance was negatively correlated with low DO, indicating that they are tolerant to low oxygen conditions [44].

Table 3. The comparison of mesozooplankton with the multiple bay data. (All data were from references. "−" indicates that it does not appear in the reference.

Time	Research Area		References Method and Mesh Size	Number of Species and Larvae	Abundance (ind. m^{-3})	Reference
July–October 2020	Bohai Bay	117°72′−119°14′ E 37°98′−139°98′ N	Microscope 200 μm	105	342.22−23,704.17	This study
July–December 2015	Daya Bay	114°30′−114°50′ E 22°30′−22°50′ N	Microscope 505 μm	131	56.3−1129.2	Xiang et al., 2021 [9]
April 2004−February 2006	Masan Bay	128°34′−128°46′ E 35°00′−35°12′ N	Microscope 200 μm	−	94−2300	Jang et al., 2015 [43]
2001–2015	Gulf of Cadiz	6°00′−8°00′ W 36°00′−37°00′ N	Microscope 200 μm	120	280.0−30,000.0	Llope, et al., 2020 [44]
February–November 2015 April 2016	Hailing Bay	111°43′−111°57′ E 21°28′−21°38′ N	Microscope 200 μm	132	12.88−5652.85	Gong et al., 2019 [45]
August 2009	Laizhou Bay	119°10′−120°30′ E 37°10′−37°80′ N	Microscope 200 μm	38	101.3−3620.0	Liu et al., 2012 [46]

4.3. Effects of Terrestrial Inputs on Mesozooplankton Communities

The water environment of Bohai Bay is affected by various nearshore anthropogenic impacts, such as industrial and domestic sewage and aquaculture [47]. In summer and autumn, we observed the environmental characteristics of low salinity and high Chl *a* in the estuary area of the inner bay, especially in the estuary of the Haihe river. High nutrient concentrations together with low salinity values suggests that high Chl *a* was caused by eutrophic terrestrial inputs. Terrestrial inputs are a source of dissolved nutrients and promote Chl *a* concentration [9]. In the present study, the stations with high Chl *a* concentration had low mesozooplankton diversity, especially at near-shore stations which suffered higher nutrient inputs. The increase in nutrients can stimulate primary production in the water column, leading to reduction of sensitive species through depletion of oxygen due to microbial decomposition of organic matter [43]. Usually only a few tolerant species can survive in hypoxic waters induced by eutrophication, which in turn results in low species diversity [48]. Moreover, the fact that most mesozooplankton species were negatively correlated with nutrients indicates that eutrophication had a certain inhibitory effect on the development of mesozooplankton communities [36].

In summer, the tunicate *Oikopleura dioica* was dominant near the estuary with low mesozooplankton diversity. It is an opportunistic species which mainly feeds on microscopic organic matter < 20 μm [49]. Moreover, it is usually found to have a fast response to the phytoplankton bloom [50]. In our present study, the high abundance of *Oikopleura dioica* in the field all occurred during the blooms period. Acuña found that the highest abundance of *Oikopleura dioica* occurs during the summer blooms in the offshore waters of Plymouth, UK [51]. Similar results were obtained by Nasquez-Yeomans in the western Caribbean where the *Oikopleura dioica* was the only apendicularian species presented during the blooms [52]. *Oikopleura dioica* was also found in large numbers in the waters near the estuary in the Jiaozhou bay, where phytoplankton blooms often occurred [53]. Compared with the open sea area, *Oikopleura dioica* was more likely to proliferate in nearshore eutrophic waters with a rapid response to nutrient enrichment. Apart from eutrophication, the variations of salinity in estuary areas caused by freshwater inputs can also influence the zooplankton composition. The appearance of some cladocera species in the estuary area such as *Pseudevadne tergestina* and *Penilia avirostris,* which prefer to live in environments with low salinity, proves that the inputs of freshwater have changed the species composition of mesozooplankton. These results show that the terrestrial inputs can play an important role in shaping the structure of mesozooplankton community through the bottom-up mechanism as well as the changes in environmental conditions.

In addition, aquaculture may also alter mesozooplankton abundance and species composition. Selective filter feeding of shellfish could exert directional selective pressure

on plankton community species, leading to a shift in phytoplankton communities towards pico-plankton species [54]. The changes in the phytoplankton community could in turn affect the mesozooplankton community. In this study, the abundance and species richness of mesozooplankton in the shellfish aquaculture area was lower than those in surrounding waters, which may be due to the changes in phytoplankton community structure.

4.4. Effects of Seawater Intrusion on Mesozooplankton Communities

The highest mesozooplankton abundance and species richness were observed at the mouth of the outer bay in summer (Figure 3), which can be related to the low temperature seawater brought by the counter-clockwise residual current in Bohai Bay. In the present study, temperature was the main environmental factor affecting the spatial distribution of the mesozooplankton communities in summer. Copepods with higher dominance such as *Paracalanus parvus* were mainly distributed in far-shore waters, showing a negative correlation with temperature. The growth rate of the copepod *Paracalanus parvus* would be inhibited if the water temperature went above 20 °C [55]. When the nearshore water temperature was higher than 26 °C, the high value area of *Paracalanus parvus* had a tendency to move outward [56]. In Bohai bay, the counterclockwise residual current brings cooler seawater to the mouth of the bay, favoring the *Paracalanus parvus* to gather at the mouth of the bay.

In autumn, the seawater intrusion was stronger, and after the mixing of marine and fresh water there was no obvious gradient change in water temperature and salinity from the mouth of the bay to the inner bay (Figure 2). The distribution of oceanic species showed that the seawater intrusion had a great influence on the mesozooplankton community structure in the bay. The copepods *Corycaeus affinis*, *Calanus sinicus*, and *Oithona similis* were dominant only in autumn. These three copepod species are widely distributed in the Bohai Sea and the northern part of the Yellow Sea, which are transported to the nearshore areas of the Bohai Bay by coastal currents in autumn [57]. In the present study, the oceanic species were only concentrated near the mouth of the bay in summer, while in autumn they were widely distributed in the inner bay. Similar results were also found in the study of Daya Bay, in which invasive water resulted in higher abundance and species richness of copepods in the bay [9,58].

In autumn, the monsoon-induced vertical mixing can breakdown the stratification in the water column, forming a mass of low temperature and high salinity water, which inhibits the growth and reproduction of mesozooplankton [57,59]. While in summer, the appropriate water temperature at the mouth of Bohai Bay favors the proliferation of mesozooplankton resulting in a higher abundance than that in autumn. Overall, the changes in the mesozooplankton community were associated with seawater intrusion, coastal currents, and monsoon strength. However, long-term-scale analyses are also needed to monitor the dynamics of planktonic community responses to environmental changes.

5. Conclusions

The spatial and seasonal taxonomic composition patterns of mesozooplankton in Bohai Bay were studied in relation to a number of water parameters. In summer, the mesozooplankton diversity in the eutrophic estuarine area was lower than that in the central Bohai Bay, while more oceanic species entered the bay in autumn, probably due to the strong seawater intrusion. In addition, a shift of dominant species from arrow worm *Sagitta crassa* to copepod *Paracalanus parvus* was noted compared with historical data, probably due to the influences of global warming and eutrophication caused by human activities. Eutrophication caused by terrigenous inputs has influenced the zooplankton community structure in multiple regions when compared with studies from other bays. In summary, the changes in the mesozooplankton community were related to terrestrial inputs and seawater intrusion. Additionally, long-term monitoring and analysis of the mesozooplankton community in Bohai Bay is needed to further understand the impact of human activities on zooplankton and their role in this ecosystem.

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