Effect of Salinity Change Resulting From Ballast Water Discharge on the Recipient Freshwater Eco-System – Inputs to Scientific Ballast Water Management of Inland Ports

Min Yang
Shanghai Ocean University
Qiong Wang
Shanghai Ocean University
Wenjun Wu
Shanghai Ocean University
Xinyan Cheng
Yangshan Port Maritime Safety Administration
Huixian Wu (hxwu@shou.edu.cn)
Shanghai Ocean University

Research Article

Keywords: ballast water discharge, salinity change, freshwater ecosystem, plankton communities

Posted Date: March 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1444490/v1

Abstract

In nowadays, world trade and cargo transportation mainly rely on shipping, and inland ports provide valuable and essential services to the maritime transport. With the increase of trade volume, the discharge of ballast water from ships is also increasing, which brings in ecological challenges. The International Maritime Organization stipulates that the number of organisms in discharged ballast water should be limited to prevent biological invasion to recipient waters. However, ballast water still contains many substances of source water, and may affect the plankton community in the recipient waters. In this study, the effects of ballast water on nature freshwater plankton community were evaluated at laboratory scale. It was observed that the abundance and diversity of planktons decreased with increase of water salinity. Through 10 days of continuous monitoring, it was found that ≥ 50 μm organisms were more sensitive to rising salinity than 10–50 um organisms, when water salinity was not over 3.6‰, there was no significant difference on ≥ 50 μm organisms quantity and diversity compared with the freshwater control group after 10 days, and the biota maintained certain recovery ability, while 10–50 um organisms had recovery ability when water salinity was not over 6.5‰. However, when the water salinity was over 6.5‰, both species composition and organism density showed irreversible and rapid reduction. In addition, organism species *Platyias* sp., *Polyarthra* sp., *Tribonema* sp., *Navicula* sp. were found to be very sensitive to salinity change and could be considered as indicative organisms for monitoring and evaluating ecological effect of discharge of saline ballast water. This is of great significance for the scientific management of ballast water discharge in fresh waters in the future.

Highlights

- The change of salinity in inland port water is an important indicator to reflect the impact of international ships' discharge of ballast water on the port's ecological environment.
- ≥ 50 μm organisms in inland port waters are more sensitive to the changes in salinity from ships' ballast water discharge than 10–50 μm organisms.
- When the salinity change of inland port waters caused by ship ballast water discharge exceeds 6.5‰, the abundance and diversity of plankton communities will decrease irreversibly.
- *Platyias* sp., *Polyarthra* sp., *Tribonema* sp. and *Navicula* sp. in inland port water could be considered as indicative organisms of significant salinity variation.

1 Introduction

Shipping accounts for more than 90% of global cargo transportation in nowadays. Ballast water is utilized to maintain vessels’ hull balance, control the trim, stability and stresses when vessels sail without cargo (Rigby et al., 1999), it is estimated that approximately 10 billion tons of ballast water are transferred globally through oceangoing vessels each year (Romanelli et al., 2019). Ballast water is normally collected from the departure ports or the passing coastal waters (Sáenz Alcántara et al., 2018), and may contain many planktons, pathogens like Vibrio (Altug et al., 2012;Ng et al., 2018), heavy metal (RezaTolian et al.,2020), microplastic (Naik et al., 2019) as well as other substances, therefore, discharge of ballast water from ships can evoke multiple ecological risks to the receiving environment (Dock et al., 2019). The International Maritime Organization (IMO) is aiming at eliminating or reducing dangerous invasive organisms or species transmitted

through ballast water discharge. IMO requires treatment of ballast water by a ballast water management system before discharge to meet the D-2 standard (IMO, 2008a). Alternatively, the vessels can perform ballast water exchange (BWE) to exchange coastal ballast water in open sea of 200 nautical miles from the coast before the ballast water is allowed to be discharged in arriving ports (Bailey, 2015). The organisms carried in coastal ballast water will normally not be able to survive while being discharged into saline water. BWE reduces the risk of spreading non-indigenous species, particularly between freshwater regions (Gray et al., 2007; Santagata et al., 2008). However, the ballast water treatment technologies are not aiming at removing all harmful pollutants from or changing the water quality of discharged ballast water. The potential impacts to the local eco-system arisen from other characteristics other than non-indigenous organisms are to be investigated.

Nowadays, inland ports provide many economically valuable services and long-term benefits to the society. The inland ports are mostly freshwater ports, their eco-systems, such as Yangtze river eco-system, is one of the most valuable ecosystems on Earth (Hilt et al., 2017). In the recent years, freshwater ecosystems are severely altered or destroyed at a faster rate than ever in human history (WWF, 2020), they are exposed to a variety of chemical, biological and anthropogenic stressors (Jenny et al., 2020), such as the construction of an estuarine dam, discharge of industrial and urban sewage, aquaculture, discharge of ships' ballast water from all over the world, etc. Such changes are threatening biodiversity by increasing extinction rates and population declines (Barbosa et al., 2020). A recent study found that discharged ballast water greatly affected the salinity in recipient water area, in this study, the aged ballast water samples were translocated into water samples collected from the estuarine mouth, and the change in salinity was found to be detrimental to coastal species (Kuch et al., 2020). Previous studies also assessed that intruded seawater changed the nutrient concentrations and phytoplankton community in recipient water, and may further influence the growth of zooplankton through the trophical cascade (Yang and Tan, 2019). Abiotic factors may influence the survival and abundance of plankton and temporarily inhabit water bodies, salinity is a import one (Cruz et al., 2020;Cañedo-Argüelles et al., 2018). In many studies, most of the ballast water samples collected from various ports have a salinity exceeding 30‰, which is high-salt seawater(Oscar et al., 2021;Nor et al.,2021;Wang et al., 2020). This indicates a great impact on recipient water salinity by discharged ballast water, and therefore, the potential harm on freshwater ecosystem by discharged ballast water cannot be ignored.

As producers and consumers in the food web, planktons are important in the maintenance of the ecological balance and the service values in water ecosystems (Nogrady et al., 1993) They play a vital role in the ecological processes such as food chain, material transformation, energy flow, and information transmission (Park et al., 2007), its species distribution, community structure and biodiversity are associated with the water environment (Wu et al., 2008). Based on these connections, patterns like plankton richness and community composition can provide a sign on the vulnerability of specific inland ports or even the estuarine services and functions. These information provide guidance for decision-making to control the associated eco-system stress (Gomes and Bernardino, 2020). However, currently there are few studies on the impact of ballast water discharge on freshwater organisms. This study is to investigate the influence of saline ballast water on freshwater eco-system, and trace the consequent changes in composition and quantity of plankton communities. The study aims to enhance understanding about potential hazards from ballast water discharge hazards and serve for improved ballast water management strategy.

2 Materials And Methods

2.1 Experimental set-up

In this study, experiments on effect of saline water on nature freshwater plankton community were carried out, as outlined in Fig. 1. The saline water was prepared by adding sea salt to the tap water to reach a salinity of 30‰. This saline water was continuously monitored over 10 days to ensure that there were no plankton organisms in the saline water. This step was intended for simulating discharge of treated ballast water meeting the D-2 standard. The freshwater, as recipient water samples, was taken from the freshwater streams flowing into the DiShui Lake in Shanghai.

The prepared saline water was then translocated into the collected freshwater samples with different volume ratios, i.e. 0%, 5%, 10%, 20%, 40% and 80%, which represented different saline water salinities. In total six groups of samples were numbered as a0, a1, a2, a3, a4 and a5, with a0 as the blank control group. Each group was set three parallel samples, and the total volume of each group was 100 L. After translocation and well mixing of two water samples, daily measurements on phytoplankton and zooplankton in each group were carried out continuously for 10 days at the same time every day. The living plankton organisms were identified immediately after sampling.

2.2 Sample collection and analysis

Living organisms of size class ≥ 10–50 μm in minimum dimension (incl. phytoplankton and heterotrophic protists) were counted and analyzed using vital stains. A cell was recorded as living if it could be stained by FDA/CMFDA stains or exhibited movement. At the same time every day, the samples were stirred and mixed, then 10 ml water samples were randomly collected for ≥ 10–50 μm organisms analysis. After shaking and mixing again, each time randomly take 1ml for species identification and quantitative analysis.Repeated analysis for 6 times. Living organisms were stained with FDA/CMFDA stains in dark condition for 10 min (ETV,2010). Samples were analyzed counted for ≥ 10–50 μm organisms with green fluorescence under excitation of 465–495 nm. The heterotrophic protists were identified only at phylum level.

For living organisms of size class > 50 μm in minimum dimension (incl. zooplankton and protists), 5 L water samples were collected and then concentrated to 1L with plankton net for analysis, the diagonal mesh size of the plankton net was 50 μm, repeated for 3 times.Whole volume analysis was performed for > 50 μm organism counting, i.e. all organisms in the concentrated 1 L sample were identified and enumerated with a anatomical microscope. Samples were filled in a 5 m L bogorov serpentine counting chambers for counting, all direct counts were done using counting chambers placed under a stereoscopic microscope at the magnification ranging from 10x to 80x. A organism was considered dead if no visible movement was observed during an observation time of at least ten seconds, the unmoving organisms were then gently touched with the point of a dissecting needle or probe to elicit movement, before it was recorded as dead.

Abiotic parameters were measured in situ using a multi-parameter water quality meter (WTW). In situ measurements of abiotic parameters included temperature (T), dissolved oxygen (DO), pH, and salinity(S).

2.3 Statistical analyses

All data processing and plotting were conducted by using Excel, R and GraphPad Prism 5.0. Alpha diversity analysis was performed with the R vegan package. The principal component analysis (PCA) was performed

with R. Significant differences were established at a 95% confidence interval ($p < 0.05$). The inhibition, calculated every day, was defined as percentage of organism density in each group (a1 – a5) relative to the control group (a0) at each day. Environmental conditions, total density of plankton organisms, mortality and alpha diversity were compared among the different mixed water groups using a one-way ANOVA by Prism. (Dag et al., 2018). The significance level was set to 0.05.

3 Results

3.1 Environmental variables

After mixing of saline water into the freshwater samples, there were no significant changes on temperature (T), pH, salinity (S) and dissolved oxygen (DO) in all groups during 10 days ($P > 0.05$). Meanwhile, there were no significant differences of T, DO and pH among sample groups ($P > 0.05$), the temperature range was 4.1 ~ 6.7°C, pH was 7.5 ~ 7.9 and DO was 10.7 ~ 13.1 mg/l. However, the salinity was significantly different among sample groups ($P < 0.05$), salinity was higher in sample groups with higher volume ratio of saline water. After mixing the saline water into the freshwater samples, the salinity increased from initial salinity of 0.4‰ (a0) to about 2.1‰ (a1) and to highest about 24.8‰ (a5). It was considered that the influence on plankton community of freshwater ecosystems, which was analyzed in this study, may be caused by salinity change.

Table 2
Development of abiotic parameters of the six sample groups during 10 days period

	Temperature (°C)						Salinity (‰)					
	a0	a1	a2	a3	a4	a5	a0	a1	a2	a3	a4	a5
day 1	6.7	6.4	6.6	6.0	5.3	4.1	0.4	2.1	3.6	6.5	12.2	24.7
day 2	4.1	4.1	4.3	4.9	4.6	4.3	0.5	2.1	3.6	6.5	12.2	24.7
day 3	4.2	4.4	4.7	5.1	4.9	4.7	0.4	2.1	3.6	6.5	12.2	24.7
day 4	4.8	5.2	5.8	5.7	5.6	5.5	0.4	2.1	3.6	6.5	12.2	24.7
day 5	5.9	6.1	6.2	6.8	6.8	6.7	0.4	2.1	3.6	6.5	12.2	24.7
day 6	6.2	6.4	6.4	7.0	6.9	7.0	0.4	2.1	3.6	6.5	12.2	24.7
day 7	7.4	7.6	7.6	8.2	8.0	7.9	0.4	2.1	3.6	6.5	12.2	24.8
day 8	8.1	8.4	8.4	8.8	8.7	8.5	0.4	2.1	3.6	6.5	12.2	24.8
day 9	9.8	10.1	10.0	10.6	10.6	10.6	0.5	2.1	3.6	6.6	12.3	24.8
day 10	9.8	9.9	9.7	9.8	9.8	9.6	0.5	2.1	3.6	6.6	12.3	24.8
Average ± SD	6.7 ± 2.1	6.9 ± 2.1	7.0 ± 2.0	7.3 ± 2.0	7.1 ± 2.1	6.9 ± 2.3	0.4 ± 0.1	2.1 ± 0.0	3.6 ± 0.0	6.5 ± 0.1	12.2 ± 0.0	24.8 ± 0.1
	pH						Dissolved oxygen (mg/l)					
	a0	a1	a2	a3	a4	a5	a0	a1	a2	a3	a4	a5
day 1	7.9	7.5	7.6	7.6	7.7	7.7	10.7	11.0	10.9	11.5	11.7	13.1
day 2	7.7	7.9	7.8	8.0	8.0	7.9	12.0	12.1	12.1	11.8	11.9	12.9
day 3	8.1	8.0	8.1	8.0	8.0	8.0	12.0	12.5	11.4	12.1	12.1	12.7
day 4	8.0	8.0	8.1	8.0	8.0	8.0	11.7	11.5	11.8	11.9	12.1	12.6
day 5	8.3	8.3	8.2	8.2	8.2	8.2	11.9	11.4	11.2	11.8	12.1	12.5
day 6	8.1	8.1	8.1	8.0	8.0	8.1	11.5	11.3	11.3	11.9	11.9	12.1
day 7	8.0	8.0	8.0	8.2	8.2	8.2	11.5	11.2	11.0	11.4	11.6	11.8
day 8	7.9	8.1	8.0	8.1	8.1	8.1	11.0	11.1	10.9	11.2	11.4	11.7
day 9	8.3	8.3	8.3	8.1	8.1	8.3	10.5	10.8	10.6	10.9	11.0	11.1
day 10	8.3	8.3	8.3	8.4	8.4	8.4	10.5	11.2	11.0	10.9	11.0	11.0
Average ± SD	8.0 ± 0.2	8.0 ± 0.2	8.1 ± 0.2	8.1 ± 0.2	8.1 ± 0.2	8.1 ± 0.2	11.3 ± 0.6	11.4 ± 0.5	11.2 ± 0.5	11.5 ± 0.4	11.7 ± 0.4	12.2 ± 0.7

Community composition, organism density and inhibition rate, and biological diversity in the sample groups were analyzed and studied in the following chapters. The inhibition rate was defined as the ratio of reduced organism density in each group relative to the remaining biomass in the control group, inhibition rate was calculated each day for each group.

3.2 Effect of salinity change on organisms of size class ≧ 10–50 μm

3.2.1 Community composition

As shown in Fig. 1, during the 10 days of continuous monitoring, there were no significant changes recorded on the species composition of the control group a0. Changes on few certain species were observed, at phylum level, Cryptophyta decreased by 13.84% and Chlorophyta increased by 10.59%; at genus level, *Cryptomonas* sp. decreased by 16.04%, while the other phytoplankton species increased to varying degrees, among which *Chlamydomonas* sp. increased the most (5.72%). For group a1 ~ a4 (S = 2.1‰~12.2‰), the development of species composition during the 10 days was similar to group a0, that at the phylum level Cryptophyta decreased but Chlorophyta increased. For group a5 (S = 24.7‰), all 10–50 um organisms died out on day 8.

On day 1, the species composition and relative abundance in low salinity groups a1 and a2 (up to 3.6‰) had no significant change compared to the control group (Fig. 1). When salinity increased to 6.5‰ (group a3), the relative abundance of *Tribonema* sp. decreased significantly from 4.02–0.06%, though it recovered from day 7 to 4.77%. It was observed during the 10 days period that, when the salinity was 6.5‰, the biological communities in the receiving waters maintained their ability to recover from the impacts of salinity change. When salinity increased to 12.2‰ (a4) and 24.7‰ (a5), the *Tribonema* sp. and *Navicula* sp. all died and did not recover in the following days. In group a5 with salinity of 24.7‰, all organisms died on day 8, *Chlamydomonas* sp. in Chlorophyta had the longest survival time of 7 days under this high salinity condition.

The PCA grouped the different sample groups and explained 95.74% by the first two axes. The species composition of a3, a4 and a5 (S ≥ 6.5‰) were significantly different from that of a0 ($P < 0.05$), as shown in Fig. 2. As a proof, it could be seen from day 7, the species composition of groups a1 ~ a3 were similar to that of the control group a0. The most sensitive organisms to salinity change were found to be *Tribonema* sp., *Navicula* sp. and Protozoa, while *Chlamydomonas* sp. had the strongest tolerance to salinity change(Fig. 1).

3.2.2 Total organism density and inhibition rate

During the 10 days of continuous monitoring, the development of the 10–50 um organism density of a1, a2 and a3 were similar to that of the control group a0. The total organism density in these groups decreased first, then increased before it decreased eventually. The lowest density was observed on day 3 and the highest density on day 7.

Through one-way ANOVA analysis, no significant difference on the organism density was found between a1 and a0 during the 10 days period, however, for sample groups with salinity over 3.6‰ (a2 ~ a5), the organism density were significantly lower than that of a0. On day 1 after water mixing, the total organism density of 10–50 um organisms in group a1 ~ a5 decreased by 13.56%, 26.48%, 48.82%, 75.61% and 95.64% compared to

group a0, respectively(Fig. 3b). After 10 days, the total organism density in group a1, a2, a3,a4 and a5 decreased by 15.72%,53.85%,74.90%, 96.62% and 100.00%, respectively, compared to group a0.

For the sample groups with salinity change not over 6.5‰ (a1 ~ a3), their ecosystem seemed to possess certain recovery ability, the inhibition rate in a1 ~ a3 decreased from during day 3 to day 7. Although the inhibition rate gradually increased in the following days, it did not exceed 90% at the end. However, for groups with salinity change over 12.2‰ (a4 and a5), both species composition and organism density showed irreversible and rapid reduction. The organism density in group a5 decreased continuously during the study period and reached 100% mortality on day 8.

3.2.3 Biological diversity

The diversity index of each group were observed decreasing at first and then increasing during the 10 days period, except that the index for a5 showed no obvious change during the first 6 days and turned to be 0 on day 7 (Table 3). However, the evenness index and richness index showed no obvious changes in all groups during 10 days period (Fig. 4).

Table 3
Diversity index of ≥ 10–50 μm organisms of each group during 10 days period

	Shannon index						**Pielou index**					
Days	a0	a1	a2	a3	a4	a5	a0	a1	a2	a3	a4	a5
1	1.52	1.48	1.48	1.32	1.09	1.10	0.78	0.76	0.83	0.73	0.79	0.80
2	1.51	1.41	1.33	1.16	1.08	0.86	0.77	0.79	0.74	0.65	0.78	0.62
3	1.47	1.35	1.19	1.07	1.21	1.22	0.76	0.75	0.67	0.66	0.88	0.88
4	1.48	1.33	1.26	1.08	1.33	1.22	0.76	0.68	0.70	0.78	0.96	0.88
5	1.50	1.50	1.30	1.06	1.14	1.38	0.77	0.77	0.72	0.76	0.83	1.00
6	1.55	1.58	1.38	1.06	1.29	1.08	0.80	0.88	0.77	0.76	0.93	0.98
7	1.61	1.63	1.58	1.46	1.35	0.00	0.83	0.84	0.81	0.75	0.98	0.00
8	1.63	1.63	1.50	1.63	1.37	0.00	0.84	0.84	0.77	0.91	0.99	0.00
9	1.64	1.64	1.55	1.60	1.38	0.00	0.84	0.84	0.80	0.89	1.00	0.00
10	1.66	1.67	1.55	1.57	1.30	0.00	0.85	0.86	0.80	0.88	0.94	0.00
Average ± SD	1.56 ± 0.07	1.52 ± 0.13	1.41 ± 0.14	1.30 ± 0.24	1.26 ± 0.12	0.69 ± 0.61	0.80 ± 0.04	0.80 ± 0.06	0.76 ± 0.05	0.78 ± 0.09	0.91 ± 0.08	0.52 ± 0.46
	Simpson index						Margalef index					
Days	a0	a1	a2	a3	a4	a5	a0	a1	a2	a3	a4	a5
1	0.26	0.27	0.27	0.32	0.38	0.36	0.85	0.87	0.74	0.78	0.53	0.76
2	0.26	0.30	0.33	0.37	0.40	0.48	0.86	0.73	0.76	0.82	0.55	0.87
3	0.27	0.32	0.36	0.39	0.33	0.31	0.87	0.75	0.80	0.69	0.61	0.81
4	0.28	0.33	0.35	0.39	0.28	0.31	0.87	0.90	0.78	0.50	0.59	0.73
5	0.26	0.26	0.34	0.41	0.36	0.24	0.85	0.85	0.77	0.49	0.59	0.69
6	0.24	0.23	0.32	0.40	0.29	0.24	0.84	0.70	0.74	0.50	0.63	1.02
7	0.23	0.22	0.24	0.28	0.26	0.00	0.82	0.80	0.84	0.93	0.70	0.00
8	0.22	0.21	0.26	0.22	0.25	0.00	0.82	0.84	0.91	0.81	0.69	0.00
9	0.22	0.21	0.25	0.23	0.24	0.00	0.81	0.84	0.91	0.83	0.72	0.00
10	0.21	0.21	0.24	0.25	0.27	0.00	0.81	0.83	0.90	0.83	0.75	0.00
Average ± SD	0.25 ± 0.03	0.26 ± 0.05	0.30 ± 0.05	0.33 ± 0.08	0.30 ± 0.06	0.19 ± 0.18	0.84 ± 0.02	0.81 ± 0.06	0.82 ± 0.07	0.72 ± 0.16	0.64 ± 0.08	0.49 ± 0.43

It was observed from the Table 3 that, the average biological diversity became less with higher water salinity. For diversity index, the highest average value was 1.56 (a0) and the lowest was 0.69 (a5). For evenness index, the highest average value was 0.91 (a4) and the lowest was 0.52 (a5). For abundance, the highest average value was 0.84 (a0) and the lowest was 0.49 (a5). Through one-way ANOVA analysis, it was found that only a5 and a0 were significantly different on the diversity index, evenness index, and richness index ($P < 0.05$), as shown in Fig. 4.

3.3 Effect of salinity change on organisms of size class ≥ 50 μm

3.3.1 Community composition

As shown in Fig. 5, during the 10 days of continuous monitoring, there were significant changes recorded on the species composition of the control group a0. At class level, Crustacea Branchiopoda was observed only on day 1 (0.50%), and Sarcodina was observed on day 2 (5.65%). After 10 days, Rotifera and Sarcodina increased while other species decreased; The development of species composition in a1 and a2 were similar to that of a0. The existence of Crustacea Branchiopoda was observed only on day 1, and the dominant specie was always Rotifera. However, the development in a3, a4 and a5 were different from that of a0. After 10 days, the dominant species in a3 were Ciliata and Sarcodina, while no organisms in a4 and a5 survived.

At class level, when salinity increased over 6.5‰ (a3 ~ a5), the relative abundance of Crustacea Copepoda and Crustacea Branchiopoda were significantly different from a0 (Fig. 5a). On day 1 the relative abundance of Crustacea Copepoda in a3 was 11.41%, which was much higher than that of a0 (2.31%); the relative abundance of Crustacea Branchiopoda in a3 and a4 were 3.36% and 4.35%, respectively, both higher than that of a0 (0.50%); all organisms in a5 died on day 1. After 10 days, Ciliata and Sarcodina were the species found in a3 and no living organisms were found in a4 and a5; mainly Rotifera was found in a0 (97.92%).

At genus level, when salinity increased to 3.6‰ (a2 ~ a4), the relative abundance of *Polyarthra* sp. decreased significantly on day 1, only 4.69% in a3 and no detection in a4 and a5. *Platyias* sp. was only found in a0 and a1 with relative abundance of 3.13% and 1.02%, respectively. When salinity increased to 24.7‰ (a5), all organisms died on day 1 and did not recover in the following days.

PCA grouped different sample groups and explained 71.46% with the first two axes. The species composition of a3, a4 and a5 ($S \geq 6.5‰$) were significantly different from that of a0 ($P < 0.05$), as shown in Fig. 6. The species composition of groups a1 and a2 were similar to that of the control group a0(Fig. 5b). The most sensitive organisms to salinity change were found to be *Platyias* sp., while *Synchaeta* sp., *Tintinnidiumn* sp. and *Arcella* sp. were more tolerant to salinity change. After 10 days, no organisms were found in a4, only *Tintinnidiumn* sp. and *Arcella* sp. were found in a3, while the dominant specie in a0 was *Synchaeta* sp., accounting for 61.63%.

3.3.2 Total organism density and inhibition rate

During the 10 days of continuous monitoring, the development of ≥ 50 μm organism density of a1 and a2 were similar to that of the control group a0. The total organism density in these groups decreased first, then increased to their peak values on day 5–6 before they dropped again. Through one-way ANOVA analysis, no

significant difference on the organism density was found between a1 and a0 during the 10 days period, however, for sample groups with salinity over 3.6‰ (a2 ~ a5), the organism density were significantly lower than that of a0 which was similar to the results for 10–50 um organism density. Different from 10–50 um organisms, when the salinity exceeded 6.5‰, i.e. group a3 ~ a5, no significant changes with the salinity increase were observed in these groups.

On day 1 after water mixing, the total organism density of ≥ 50 um organisms in group a1 ~ a5 decreased by 18.98%, 36.77%, 75.39%, 96.20% and 100.00% compared to group a0, respectively, the density reduction in each group was more than the density reduction of 10–50 um organisms. After 10 days, the total organism density decreased by 72.26% in group a1 and over 99.83% in group a3 ~ a5, compared to group a0.

For the sample groups with salinity change not over 3.6‰ (a1,a2), their ecosystem seemed to possess certain recovery ability, the inhibition rate in a1and a2 showed a decreasing trend after the first day/few days of inhibition increase. This was similar to 10–50 um organisms but lower recovery ability was observed for ≥ 50 um organisms. The inhibition rate kept increasing after day 8, reaching 98.92% and 99.83% for a2 and a3 on day 10, respectively. For groups with salinity change over 12.2‰ (a4 and a5), both species composition and organism density showed irreversible and rapid reduction. The organism density in group a4 decreased continuously during the study period and reached 100% mortality on day 6, while in group a5 the inhibition rate/mortality reached 100% on day 1.

3.3.3 Biological diversity

The diversity index of each group were monitored during 10 days period and summarized in Table 4. The index for a4 turned to be 0 on day 6, and the index for a5 reached 0 on day 1. For the other groups, no obvious changes were observed on the evenness index and richness index, as shown in Fig. 8.

Table 4
Diversity index of ≥ 50 µm organisms of each group during 10 days period

	Shannon index						**Pielou index**					
Days	a0	a1	a2	a3	a4	a5	a0	a1	a2	a3	a4	a5
1	1.56	1.47	1.29	1.12	1.24	0.00	0.63	0.61	0.62	0.51	0.69	0.00
2	1.27	1.00	1.40	0.68	0.33	0.00	0.61	0.46	0.67	0.35	0.47	0.00
3	1.33	1.37	0.98	0.65	1.01	0.00	0.58	0.59	0.47	0.40	0.92	0.00
4	1.29	1.36	1.31	0.38	0.78	0.00	0.56	0.59	0.60	0.28	0.48	0.00
5	1.26	1.03	1.06	0.69	0.74	0.00	0.55	0.45	0.54	0.36	0.67	0.00
6	1.16	1.27	0.86	0.56	0.00	0.00	0.53	0.61	0.48	0.81	0.00	0.00
7	1.27	1.04	0.94	0.65	0.00	0.00	0.58	0.65	0.52	0.59	0.00	0.00
8	1.40	1.20	0.86	1.08	0.00	0.00	0.64	0.58	0.41	0.67	0.00	0.00
9	1.36	0.91	1.01	0.46	0.00	0.00	0.62	0.47	0.56	0.42	0.00	0.00
10	1.07	1.25	0.79	0.69	0.00	0.00	0.49	0.60	0.57	1.00	0.00	0.00
Average ± SD	1.30 ± 0.13	1.19 ± 0.18	1.05 ± 0.21	0.70 ± 0.24	0.41 ± 0.49	0.00 ± 0.00	0.58 ± 0.05	0.56 ± 0.07	0.55 ± 0.08	0.54 ± 0.23	0.32 ± 0.36	0.00 ± 0.00
	Simpson index						Margalef index					
Days	a0	a1	a2	a3	a4	a5	a0	a1	a2	a3	a4	a5
1	0.26	0.30	0.36	0.54	0.41	0.00	0.90	0.83	0.60	0.74	0.56	0.00
2	0.39	0.51	0.34	0.70	0.82	0.00	0.59	0.69	0.62	0.55	0.12	0.00
3	0.33	0.34	0.51	0.71	0.39	0.00	0.72	0.74	0.62	0.36	0.26	0.00
4	0.38	0.31	0.34	0.84	0.65	0.00	0.71	0.72	0.67	0.30	0.45	0.00
5	0.35	0.45	0.44	0.72	0.59	0.00	0.71	0.70	0.49	0.58	0.25	0.00
6	0.41	0.35	0.58	0.62	0.00	0.00	0.61	0.56	0.43	0.14	0.00	0.00
7	0.37	0.42	0.54	0.63	0.00	0.00	0.64	0.38	0.45	0.22	0.00	0.00
8	0.31	0.43	0.55	0.48	0.00	0.00	0.63	0.57	0.64	0.46	0.00	0.00
9	0.32	0.57	0.51	0.77	0.00	0.00	0.62	0.50	0.46	0.23	0.00	0.00
10	0.45	0.44	0.61	0.50	0.00	0.00	0.62	0.60	0.36	0.15	0.00	0.00
Average ± SD	0.36	0.41	0.48	0.65	0.29	0.00	0.67	0.63	0.53	0.37	0.17	0.00

It was observed from the Table 4 that, the average biological diversity became less with higher water salinity. For diversity index, the highest average value was for 1.3 (a0) and the lowest was 0 (a5). For evenness index,

the highest average value was 1.0 (a1) and the lowest was 0 (a5). For abundance, the highest average value was 0.67 (a0) and the lowest was 0 (a5). Through one-way ANOVA analysis, it was found that a4 and a5 were significantly different from a0 on the diversity index and evenness index, while a3-a5 were significantly different from a0 on the richness index ($P < 0.05$), as shown in Fig. 8.

4 Discussion

It was demonstrated in this study that, salinity increase in the recipient freshwater may lead to significant changes in the composition of plankton community, organism density, and biological diversity. In the sample group mixed with high salinity water (24.8‰), an immediate reduction of 96.16% for ≥ 10–50 μm organisms and 100% for ≥ 50 μm organisms were identified on day 1 after water mixing. After 10 days, only when the salinity was 2.1‰, the total organism density decreased by 15.72% for ≥ 10–50 μm organisms and by 72.26% for ≥ 50 μm organisms, while the total organism density decreased by 96.62% for ≥ 10–50 μm organisms and by 100% for ≥ 50 μm organisms in sample group with salinity of 12.2‰, all organisms died in sample group with salinity of 24.8‰. These results aligned with the findings in previous study by Lin et al. (2017) on decline of zooplankton biomass and Lind L. (2018) decline of cladocerans due to salinity increase. Greco et al (2021) and Moffett (2020) also found in their studies that the diversity and richness of planktons decreased with increasing salinity after 10 days. The salinization phenomenon in the recent years has severely affected the planktons. The species composition of the zooplanktons has become much poorer, and the taxonomic diversity decreased 1.4 ~ 1.5 times than before (Afanasyev, 2019). Mo et al. (2021) demonstrated that at low salinities, even small increases in salinity are sufficient to exert selective pressure to reduce the plankton diversity and alter assembly mechanism and network stability of the organism community. In this study, biodiversity in sample groups has surprisingly shown a slight upward development after salinity increase. When the salinity change was less than 6.5‰, after 7 days, the freshwater planktons have demonstrated certain recovery ability, the plankton biomass had positive response to the salinity change after some time. It may be explain as the ecosystem's adaptability to the ecological disasters with time, and the species diversity, average size and total number of species in the biological community may increase as a result (Pennesi, C. 2017;Nickolai, 2021). It was thus reasonable to assume that, in real ballast water operations, the biological indicators such as species composition and quantity development may tend to be stable shortly after ballast water de-ballasting, given a relatively mild salinity level of the discharged water.

In estuaries and coastal ecosystems which were normally subject to constant changes, salinity was an important factor that may influence plankton community structure (Cruz,2020;Greenwald,1993). Salinity can expose sensitive organisms to osmotic stress and promote the replacement of salinity-sensitive species by salinity-tolerant taxa (Stefanidou, 2018). The responses of phytoplankton and protozoa to chloride increases were group-specific and depend on nutrient levels. The previous survey and identification results showed that *Ankistrodesmus falcatus* or *Scenedesmus* in Chlorophyta survived in the high-salinity ballast water, even though they were freshwater species (Wu, 2019). In this study, it was observed that *Chlamydomonas* sp. in Chlorophyta had a strong tolerance to salinity increase. Song et al. (2020) inferred that changes of water-mass conditions, especially a sharp reduction in salinity to possibly low-brackish conditions (< 10 psu), were the primary causes of concurrent changes in the micro-algal community, reflecting tolerance of low-salinity conditions by green algae. Besides, this study also found that the *Navicula* sp. in Bacillariophyta and *Tribonema* sp. in Xanthophyta were very sensitive to salinity, and can not survive beyond 6.5‰. One of the

main mechanisms that allow algae to exist in high saline environment was that with the production of exopolysaccharide (EPS), a large number of EPS was released into the environment, and cells survive in it (Abdullahi,,2006 Steele, 2014).

Surprisingly, zooplankton was more sensitive to rising salinity than phytoplankton, in terms of species composition or density, which may be attributed to different physiological characteristics of the two organism groups, such as size and volume. Through 10 days of continuous monitoring, it was found that when water salinity was not over 3.6‰, there was no significant difference on ≥ 50 μm organisms quantity and diversity compared with the freshwater control group, and the biota maintained certain recovery ability, while 10–50 um organisms had recovery ability when water salinity was not over 6.5‰.Phytoplanktons responded to salinity change mainly through the indirect top-down effects of zooplankton grazing (Emma, 2020).In the previous studies, large herbivore species (e.g. Daphnia) have disappeared, replaced by more salt-tolerant and smaller species (e.g.rotifers), and increased salinity may weaken top-down control (Thompson and Shurin,2012; coldsnow and Relyea,2018). Besides, species differences may drive the change of chloride sensitivity. In our experiment, zooplankton groups differed in the relative severity of their chloride response, copepods experienced the greatest declines, followed by cladocerans and then rotifers. In previous studies, cladocerans tended to be the most sensitive then rotifers(Van Meter et al. 2011; Stoler et al. 2017b). Rotifers were generally the most tolerant, only showing declines in one study (Stoler et al. 2017b). While copepods were more variable in their response to chloride, sometimes declining (Petranka and Francis 2013; Lind et al. 2018) or not responding to chloride (Stoler et al. 2017a, Sinclair and Arnott 2018).The driving factors of chloride sensitivity change in the study were not clear. The reason may be include hydrochemical differences and food quantity and quality (Elphick et al. 2011).Variation in responses to salinity by plankton have been linked to predation, disturbance, adaptation, dispersal, and water chemistry (Coldsnow et al. 2017; Hintz and Relyea 2017; Hintz et al. 2019). Therefore, regional context and water chemistry must be considered in the study of salt impact assessment of ballast water discharge.

Changes on the biological composition may result in loss of certain essential functions of the local ecosystem. As the relative abundance shifts from large crustaceans to small rotifers, the total biomass will decrease (Fig. 3 and Fig. 7). Some species of both zooplankton and phytoplankton can be used as indicator organisms on water quality change as they are very sensitive to salinity change. In this study, *Platyias* sp., *Polyarthra* sp., *Tribonema* sp.and *Navicula* sp. were found to be sensitive to salinity change, and these species were also found to be existing in freshwater ecosystem (like the Yangtze River Basin in China) with large quantities, they are thus considered as suitable indicator organisms for monitoring water quality changes in freshwater ecosystem of inland ports. The inland ports like Yangtze River Basin are important shipping transportation routes with heavy ship traffic, it is very important and essential to monitor its water quality continuously. In the future, scientific management of ballast water discharge in Yangtze River Basin can be designed by measuring biomass of selected indicator organisms or by continuous monitoring salinity change in this water area with salinity meter. These results are important inputs on evaluating effects of ballast water discharge on bio-diversity and ecosystem service losses in this freshwater environment.

5 Conclusion

Increased salinity in freshwater systems may leads to concerns on ecosystem health, quality of drinking water and possible damage to fisheries productivity. However, compared to other contemporary sources of pressure to the eco-systems (e.g. pollution emissions, biological invasions, etc.), the impacts of salinity change on receiving water eco-systems resulting from ballast water discharge were poorly understood. This study explored how discharge of saline ballast water may affect natural plankton communities in freshwater recipients. The study demonstrated that the quantity and diversity of planktons decreased with the increased salinity. However, through the continuous monitoring of 10 days, it was found that in water samples with salinity not over 3.6‰, the ≥ 50 μm organisms demonstrated certain recovery ability, and there was no significant difference on organism quantity and diversity compared with the freshwater control group after 10 days. When the water salinity was over 6.5‰, both species composition and organism density showed irreversible and rapid reduction. In addition, it was identified from this study that organisms of size class ≥ 50 μm were more sensitive to salinity change than 10–50 um organisms, which may be attributed to different physiological characteristics of the two organism groups, such as size and volume. *Platyias* sp., *Polyarthra* sp., *Tribonema* sp. and *Navicula* sp.were found to be very sensitive to salinity change and could be considered as indicative organisms for monitoring and evaluating ecological effect of discharge of saline ballast water. This is of great significance for the scientific management of ballast water discharge in fresh waters.

Declarations

CRediT authorship contribution statement

Min Yang: Conceptualization, Data curation, Formal analysis, Investigation, Writing-original draft, Writing-review & editing, Visualization, Project administration. **Qiong Wang**: Conceptualization, Data curation, Formal analysis, Writing-original draft, Writing-review&editing, Visualization. **Wenjun Wu**: Review & editing. **Xinyan Cheng**: Investigation. **Huixian Wu**: Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study is a key project funded by the National Key Research and Development Program of China (No. 2019YFC0810904), The Ship's High Technology Research Program of Ministry of Industry and Information Technology (No. [2019] 360) and Shanghai Science and Technology Commission Scientific Research Project (No.19DZ2292500). We are very grateful to the officers and crews of all the vessels that were boarded and sampled. The comments and suggestions of the reviewers are greatly appreciated.

References

1. Rigby G, Hallegraeff G, Sutton C. Novel ballast water heating technique offers cost-effective treatment to reduce the risk of global transport of harmful marine organisms. Marine Ecology Progress, 1999, 191:289-293. DOI:10.3354/meps191289
2. Romanelli G, Berto D, Cala Ce N, et al. Ballast water management system: Assessment of chemical quality status of several ports in Adriatic Sea. Marine Pollution Bulletin, 2018, 147:86-97. DOI:10.1016/j.marpolbul.2017.12.030
3. E. Sáenz Alcántara .Comparative Study of Approved Imo Technologies for Treatment of Ballast Waters Universitat Politècnica de Catalunya (2018) Master's thesis.
4. Altug G, Gurun S, Cardak M, et al. The occurrence of pathogenic bacteria in some ships' ballast water incom ing from various marine regions to the Sea of Marmara, Turkey. Marine Environmental Research, 2012, 81:35-42. DOI:10.1016/j.marenvres.2012.08.005
5. Ng C, Goh S G, Saeidi N, et al. Occurrence of Vibrio species, beta-lactam resistant Vibrio species, and indicator bacteria in ballast and port waters of a tropical harbor. Science of the Total Environment, 2017, 610-611:651-656. DOI:10.1016/j.scitotenv.2017.08.099
6. Tolian R, Makhsoosi A H, Bushehri P K. Investigation of heavy metals in the ballast water of ship tanks after and before the implementation of the ballast water convention: Bushehr Port, Persian Gulf. Marine Pollution Bulletin, 2020, 157(1):111378. DOI:10.1016/j.marpolbul.2020.111378
7. Naik R K, MM Naik, D'Costa P M, et al. Microplastics in ballast water as an emerging source and vector for harmful chemicals, antibiotics, metals, bacterial pathogens and HAB species: A potential risk to the marine environment and human health. Marine Pollution Bulletin, 2019, 149:110525. DOI 10.1016/j.marpolbul.2019.110525
8. Dock A, Linders J, David M, et al. Is human health sufficiently protected from chemicals discharged with treated ballast water from vessels worldwide?–A decadal perspective and risk assessment. Chemosphere, 2019, 235: 194-204. DOI:10.1016/j.chemosphere.2019.06.101
9. International Maritime Organization(IMO). Guidelines for approval of ballast water management systems (G8). 2008a, Annex 4 Resolution MEPC 174 (58), 3-5.
10. Bailey S A . An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. Aquatic Ecosystem Health & Management, 2015, 18(3):261-268. DOI:10.1080/14634988.2015.1027129
11. Gray D K, Johengen T H, Reid D F, et al. Efficacy of open-ocean ballast water exchange as a means of preventing invertebrate invasions between freshwater ports. Limnology and Oceanography, 2007, 52(6): 2386-2397. DOI:10.2307/4502388
12. Santagata S, Zita R Gasiūnaite, Verling E, et al. Effect of osmotic shock as a management strategy to reduce transfers of nonindigenous species among low-salinity ports by ships. Aquatic Invasions, 2008, 3(1):61-76. DOI:10.3391/ai.2008.3.1.10
13. Hilt S, Brothers S, Jeppesen E, et al. Translating regime shifts in shallow lakes into changes in ecosystem functions and services. BioScience, 2017, 67(10): 928-936. DOI:10.1093/biosci/bix106
14. Almond R E A, Grooten M, Peterson T. Living Planet Report 2020-Bending the curve of biodiversity loss. World Wildlife Fund, 2020.

15. Jenny J P, Anneville O, Arnaud F, et al. Scientists' Warning to Humanity: Rapid degradation of the world's large lakes. Journal of Great Lakes Research, 2020. DOI:10.1016/j.jglr.2020.05.006
16. Barbosa L, Amorim C A, Parra G, et al. Advances in limnological research in Earth's drylands[J]. Inland Waters, 2020. DOI:10.1080/20442041.2020.1728179
17. Kuchi N, Khandeparker L. Influence of salinity stress on bacterial diversity from a marine bioinvasion perspective: evaluation through microcosm experiments. Current Science, 2020, 119(3):507-525. https://doi.org/10.18520/cs%2Fv119%2Fi3%2F507-525
18. Yang X, Tan Y. Effects of shelf seawater intrusion on phytoplankton community structure in Daya Bay in the summer. Mar. Sci., 2019, 43: 96-105.
19. M Cañedo-Argüelles, Kefford B , Schäfer, Ralf. Salt in freshwaters: Causes, effects and prospects - Introduction to the theme issue. Philosophical Transactions of The Royal Society B Biological Sciences, 2018, 374(1764). DOI:10.1098/rstb.2018.0002
20. Casas-Monroy O , Bailey S A . Do Ballast Water Management Systems Reduce Phytoplankton Introductions to Canadian Waters?. Frontiers in Marine Science, 2021. DOI:10.3389/fmars.2021.691723
21. Salleh N A, Rosli F N, Akbar M A, et al. Pathogenic hitchhiker diversity on international ships' ballast water at West Malaysia port. Marine pollution bulletin, 2021, 172: 112850. https://doi.org/10.1016/j.marpolbul.2021.112850
22. Wang L, Wang Q, Xue J, et al. Effects of holding time on the diversity and composition of potential pathogenic bacteria in ship ballast water. Marine Environmental Research, 2020, 160(9):104979. DOI:10.1016/j.marenvres.2020.104979
23. Nogrady T, Wallace RL, Snell TW. 1993. “Morphology and internal organization”. In Biology, ecology and systematics, Edited by: Rotifera. Vol. 1, 142The Hague: SPB Academic Publishing.
24. Park K S, Shin H W. Studies on phyto-and-zooplankton composition and its relation to fish productivity in a west coast fish pond ecosystem. Journal of Environmental Biology, 2007, 28(2S):415. DOI:10.1016/j.jconhyd.2006.08.012
25. WU Zhenbin, A Liu, S Zhang,et al. Short-term effects of drawing water for connectivity of rivers and lakes on zooplankton community structure. Journal of Environmental Sciences, 2008, 20(4):419-423. DOI:10.1016/S1001-0742(08)62073-2
26. Gomes L, Bernardino A F. Drought effects on tropical estuarine benthic assemblages in Eastern Brazil. Science of The Total Environment, 2020, in press:135490. DOI:10.1016/j.scitotenv.2019.135490
27. ETV. Environment Technology Verification Program document Generic Protocol for the Verification of Ballast Water Treatment Technologies, 2010.
28. Lin Q, Xu L, Hou J, et al. Responses of trophic structure and zooplankton community to salinity and temperature in Tibetan lakes: Implication for the effect of climate warming. Water Research, 2017, 124(nov.1):618–629. DOI:10.1016/j.watres.2017.07.078.
29. Lind L, Schuler M S, Hintz W D, et al. Salty fertile lakes: how salinization and eutrophication alter the structure of freshwater communities. Ecosphere, 2018, 9. DOI:10.1002/ecs2.2383
30. Greco D A, Arnott S E, Fournier I B, et al. Effects of chloride and nutrients on freshwater plankton communities[J]. Limnology and Oceanography Letters, 2021. https://doi.org/10.1002/lol2.10202

31. Emma R. Moffett, Henry K. Baker, Christine C. Bonadonna, et al.Cascading effects of freshwater salinization on plankton communities in the Sierra Nevada. Limnology and Oceanography Letters, 2020. https://doi.org/10.1002/lol2.10177
32. Afanasyev D F, Mirzoyan Z A, Martynyuk M L, et al. Early Summer Zooplankton in the Sea of Azov during the Period of Its Salinization. Inland Water Biology, 2019. DOI:10.1134/S1995082919050031
33. Mo Y, Peng F, Gao X, et al. Low shifts in salinity determined assembly processes and network stability of microeukaryotic plankton communities in a subtropical urban reservoir. Microbiome, 2021, 9(1). DOI:10.1186/s40168-021-01079-w
34. Pennesi C, Danovaro R. Assessing marine environmental status through microphytobenthos assemblages colonizing the Autonomous Reef Monitoring Structures (ARMS) and their potential in coastal marine restoration.. Marine Pollution Bulletin, 2017,125, 56–65. DOI:10.1016/j.marpolbul.2017.08.001
35. Anufriieva E . Microphytobenthos in the Hypersaline Water Bodies, the Case of Bay Sivash (Crimea): Is Salinity the Main Determinant of Species Composition? Water,2021,13. DOI:10.3390/w13111542
36. Cruz J, Teodósio M A, Garrido S, et al. Plankton community and copepod production in a temperate coastal lagoon: What is changing in a short temporal scale?. Journal of Sea Research, 2020, 157: 101858. DOI:10.1016/j.seares.2020.101858
37. Greenwald G M, Hurlbert S H. Microcosm analysis of salinity effects on coastal lagoon plankton assemblages. Hydrobiologia, 1993, 267(1):307-335. DOI:10.1007/BF00018810
38. N Stefanidou, Genitsaris S, Lopez-Bautista J, et al. Unicellular Eukaryotic Community Response to Temperature and Salinity Variation in Mesocosm Experiments. Frontiers in Microbiology, 2018, 9. DOI:10.3389/fmicb.2018.02444
39. WU, Huixian, SHEN, et al. Survivorship characteristics and adaptive mechanisms of phytoplankton assemblages in ballast water. Journal of Oceanology & Limnology, 2019. DOI:CNKI:SUN:HYFW.0.2019-02-017
40. Song Y, Gilleaudeau G J, Algeo T J, et al. Biomarker evidence of algal-microbial community changes linked to redox and salinity variation, Upper Devonian Chattanooga Shale (Tennessee, USA). Geological Society of America Bulletin, 2020, 133(1-2). DOI:10.1130/B35543.1
41. Abdullahi A S, Underwood G J C, Gretz M R. Extracellular matrix assembly in diatoms (Bacillariophyceae). V. Environmental effects on polysaccharide synthesis in the model diatom, Phaeodactylum tricornutum. Journal of Phycology, 2010, 42(2):363-378. DOI:10.1111/j.1529-8817.2006.00193.x
42. Steele D J, Franklin D J, Underwood G. Protection of cells from salinity stress by extracellular polymeric substances in diatom biofilms. Biofouling, 2014, 30(8):987-998. DOI:10.1080/08927014.2014.960859
43. Thompson P L, Shurin J B. Regional zooplankton biodiversity provides limited buffering of pond ecosystems against climate change. Journal of Animal Ecology, 2012, 81(1). DOI:10.1111/j.1365-2656.2011.01908.x.
44. Coldsnow, Kayla, D, et al. Toxicity of Various Road-Deicing Salts to Asian Clams (Corbicula fluminea). Environmental toxicology and chemistry, 2018,37: 1839– 1845. DOI:10.1002/etc.4126.
45. Canadian Council of Ministers of the Environment. 2011. Canadian water quality guidelines for the protection of aquatic life: Chloride, p. 1-16. Canadian environmental quality guidelines 1999. Winnipeg: Canadian Council of Ministers of the Environment.

46. Meter R J V, Swan C M, Leips J, et al. Road Salt Stress Induces Novel Food Web Structure and Interactions. Wetlands, 2011, 31(5):843-851. DOI:10.1007/s13157-011-0199-y.
47. Stoler A B, Walker B M, Hintz W D, et al. Combined effects of road salt and an insecticide on wetland communities. Environmental Toxicology & Chemistry, 2017, 36(3):771– 779. DOI:10.1002/etc.3639.
48. James, W, Petranka, et al. Effects of Road Salts on Seasonal Wetlands: Poor Prey Performance May Compromise Growth of Predatory Salamanders. Wetlands: The Journal of the Society of Wetland Scientists, 2013, 33(4):707-715. DOI:10.1007/s13157-013-0428-7.
49. Lind L, Schuler M S, Hintz W D, et al. Salty fertile lakes: how salinization and eutrophication alter the structure of freshwater communities. Ecosphere, 2018, 9. DOI:10.1002/ecs2.2383.
50. Sinclair J S, Arnott S E. Local context and connectivity determine the response of zooplankton communities to salt contamination. Freshwater Biology, 2018, 63: 1273–1286. DOI:10.1111/fwb.13132.
51. Stoler A B, Hintz W D, Jones D K, et al. Leaf litter mediates the negative effect of road salt on forested wetland communities. Freshwater science, 2017:415-426. DOI:10.1086/692139.
52. Elphick J R F, Bergh K D, Bailey H C. Chronic toxicity of chloride to freshwater species: Effects of hardness and implications for water quality guidelines. Environmental Toxicology & Chemistry, 2011, 30(1):239-246. DOI:10.1002/etc.365.
53. Coldsnow K D, Mattes B M, Hintz W D, et al. Rapid evolution of tolerance to road salt in zooplankton. Environmental Pollution, 2017, 222(mar.):367-373. DOI:10.1016/j.envpol.2016.12.024.
54. Hintz W D, Relyea R A. A salty landscape of fear: responses of fish and zooplankton to freshwater salinization and predatory stress. Oecologia, 2017,185: 147–156. DOI:10.1007/s00442-017-3925-1.
55. Hintz W D, Jones D K, Relyea R A. Evolved tolerance to freshwater salinization in zooplankton: life-history trade-offs, cross-tolerance and reducing cascading effects. Philosophical Transactions Biological Sciences, 2019, 374(1764). DOI:10.1098/rstb.2018.0012.

Figures

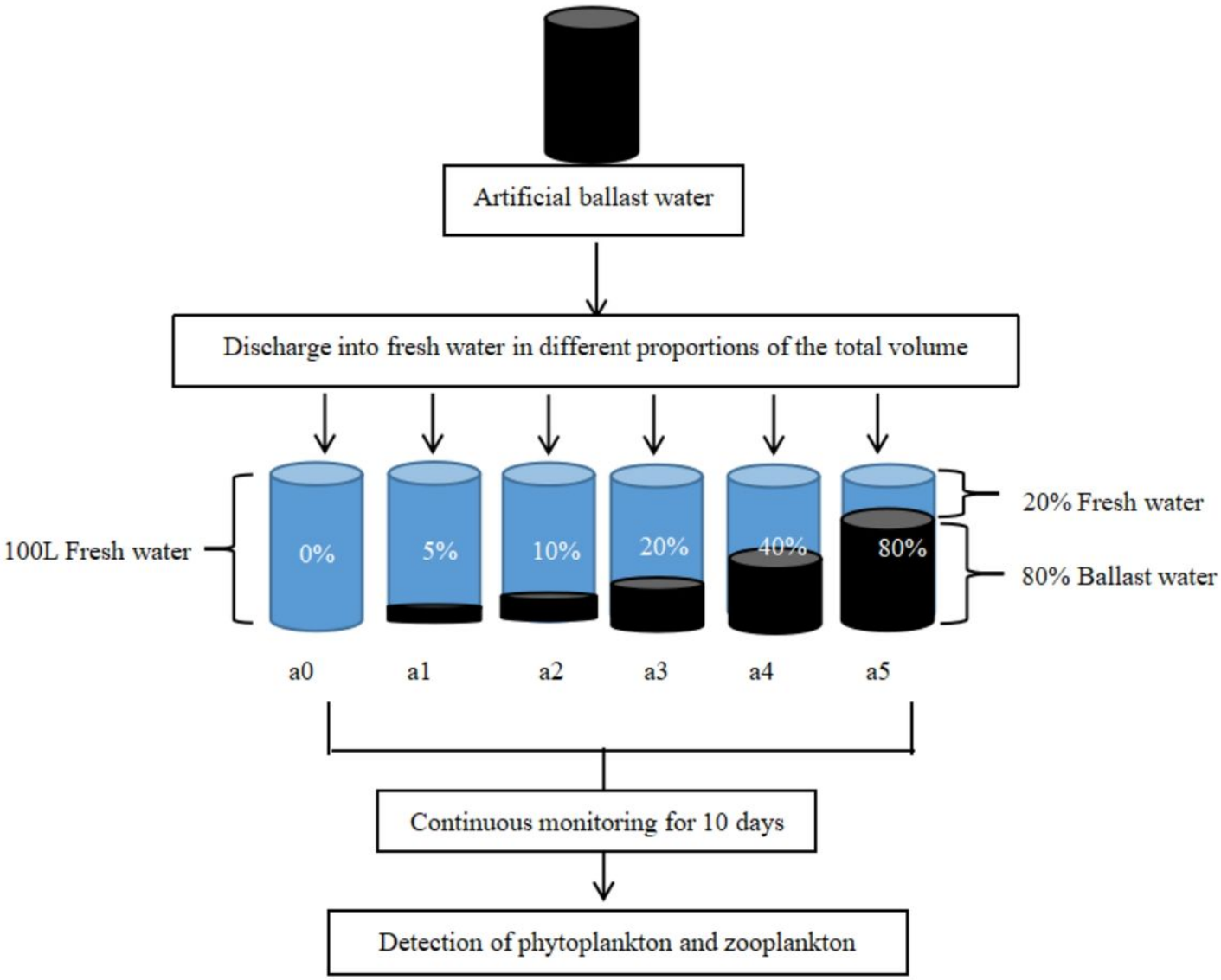

Figure 1

The experimental design (Blue represents fresh water and black represents saline water (as ballast water))

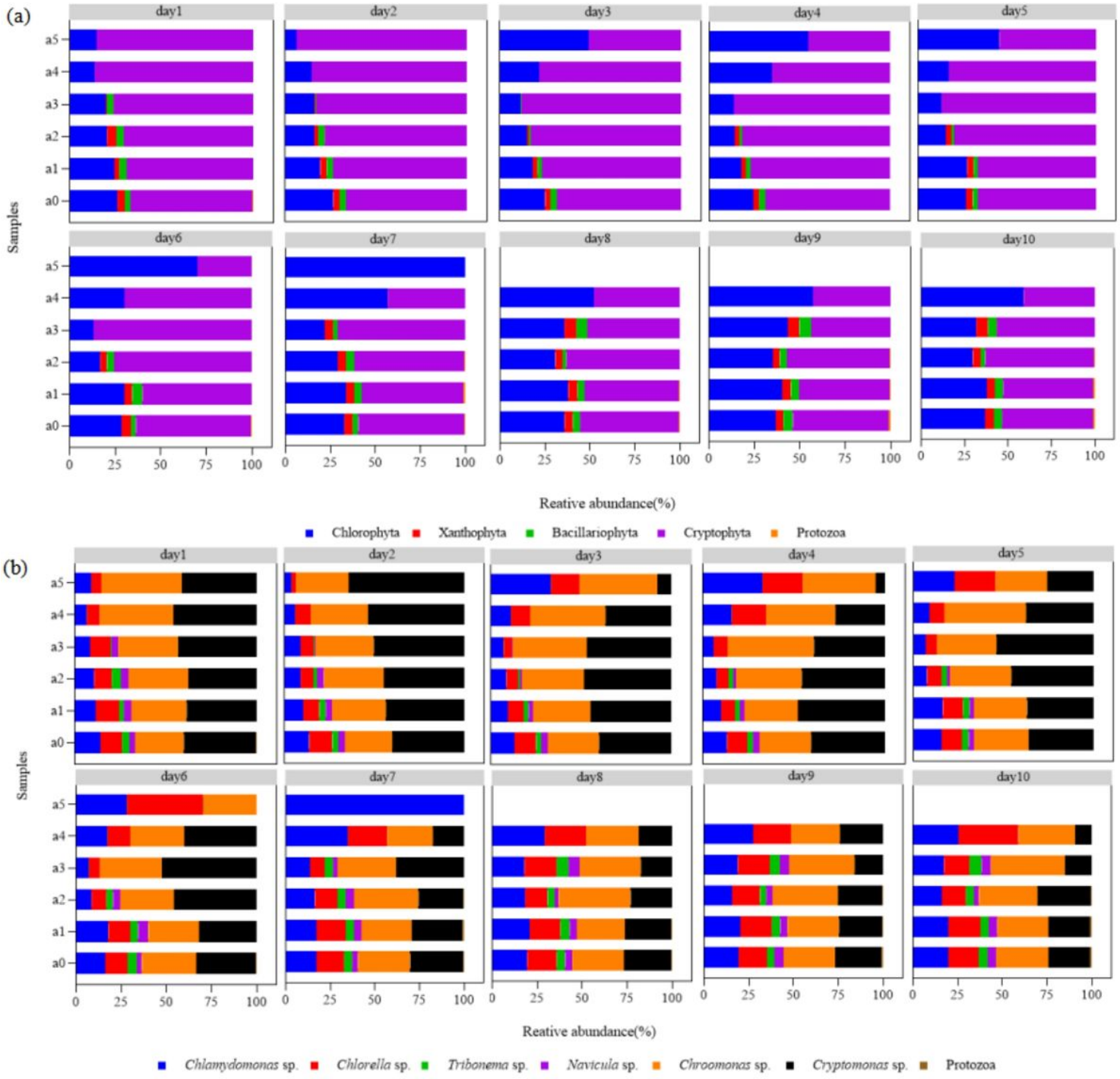

Figure 2

Effects of salinity changes on the composition of ≥10-50 μm organisms during 10 days period. (a) phylum level, (b) genus level.

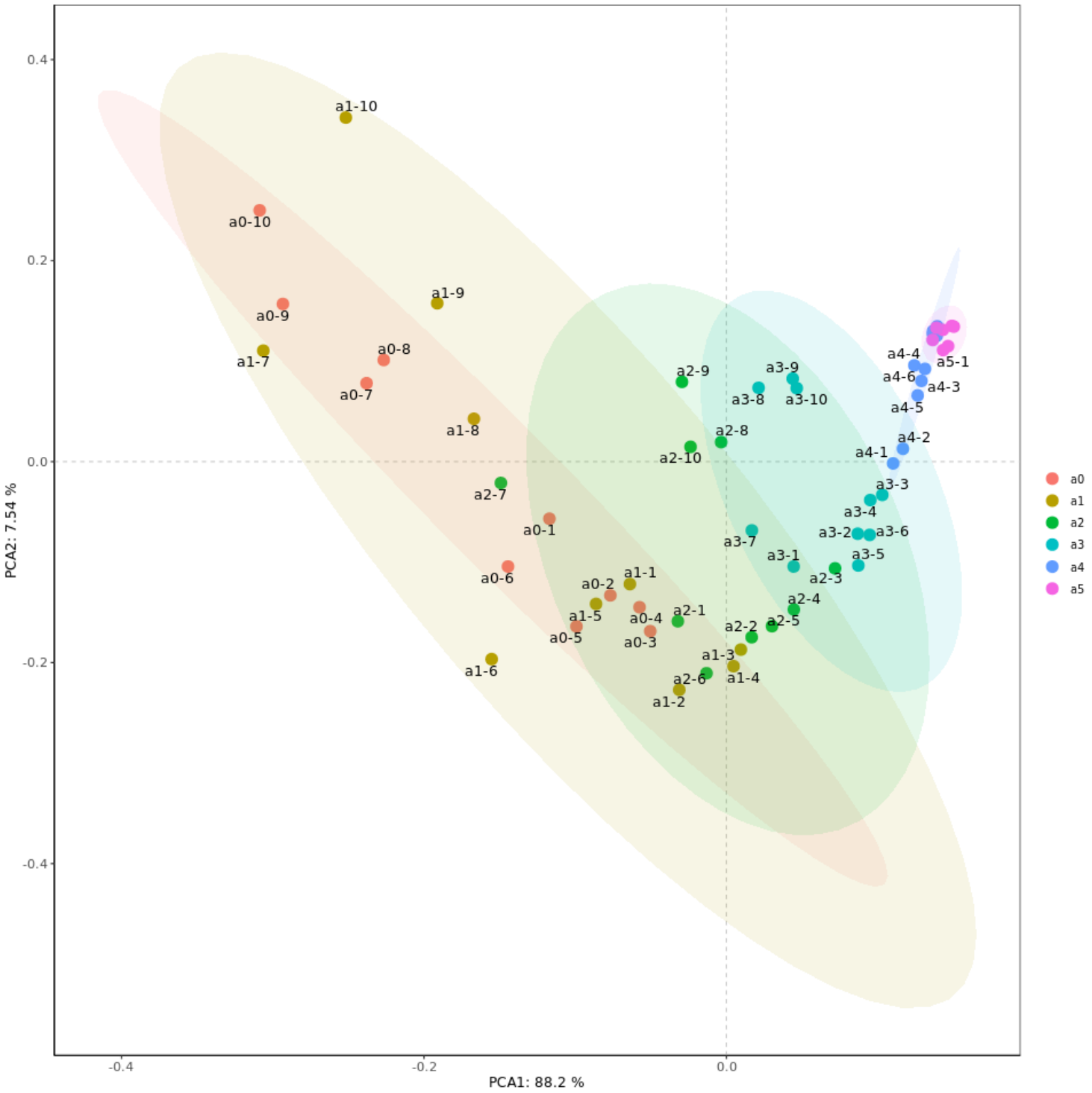

Figure 3

PCA analysis of community composition of ≥10-50 μm organisms in sample groups with different volume ratio of saline water

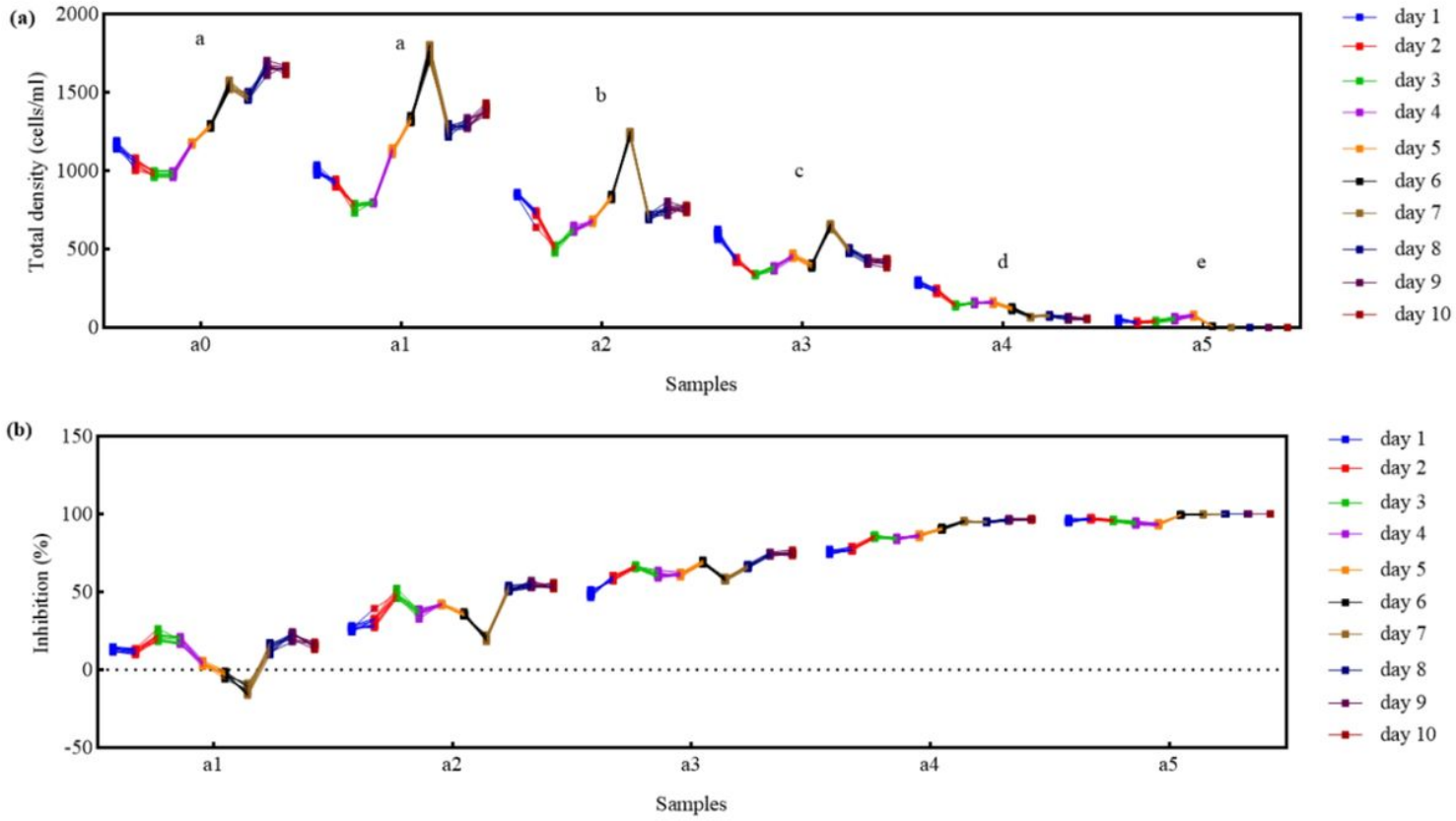

Figure 4

Total organism density (a) and daily inhibition (b) for 10-50 um organisms in different sample groups were studied. Different lowercase letters on the chart represent significant differences at the P=0.05 level, the different letters between the sample groups mean P <0.05, conversely the presence of any same letter means P> 0.05. Results of parallel samples were presented for each group.

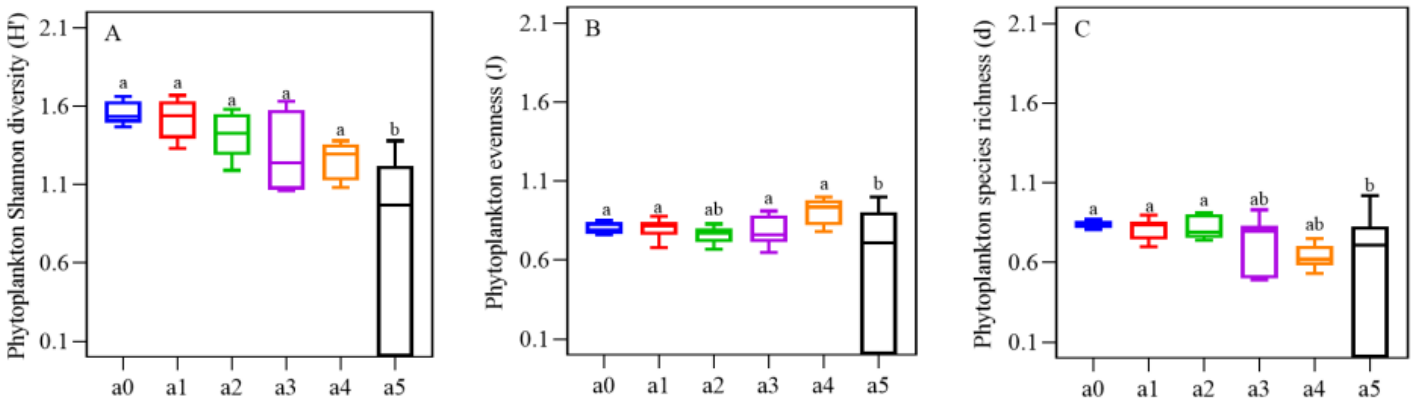

Figure 5

Margalef's species richness (A), Shannon-Wiener diversity (B) and Pielou evenness (C) for ≥10-50 μm organisms in different sample groups were studied. Different lowercase letters of the same index on the chart represent significant differences at the P=0.05 level, the different letters between the sample groups mean P <0.05, conversely the presence of any same letter means P> 0.05.

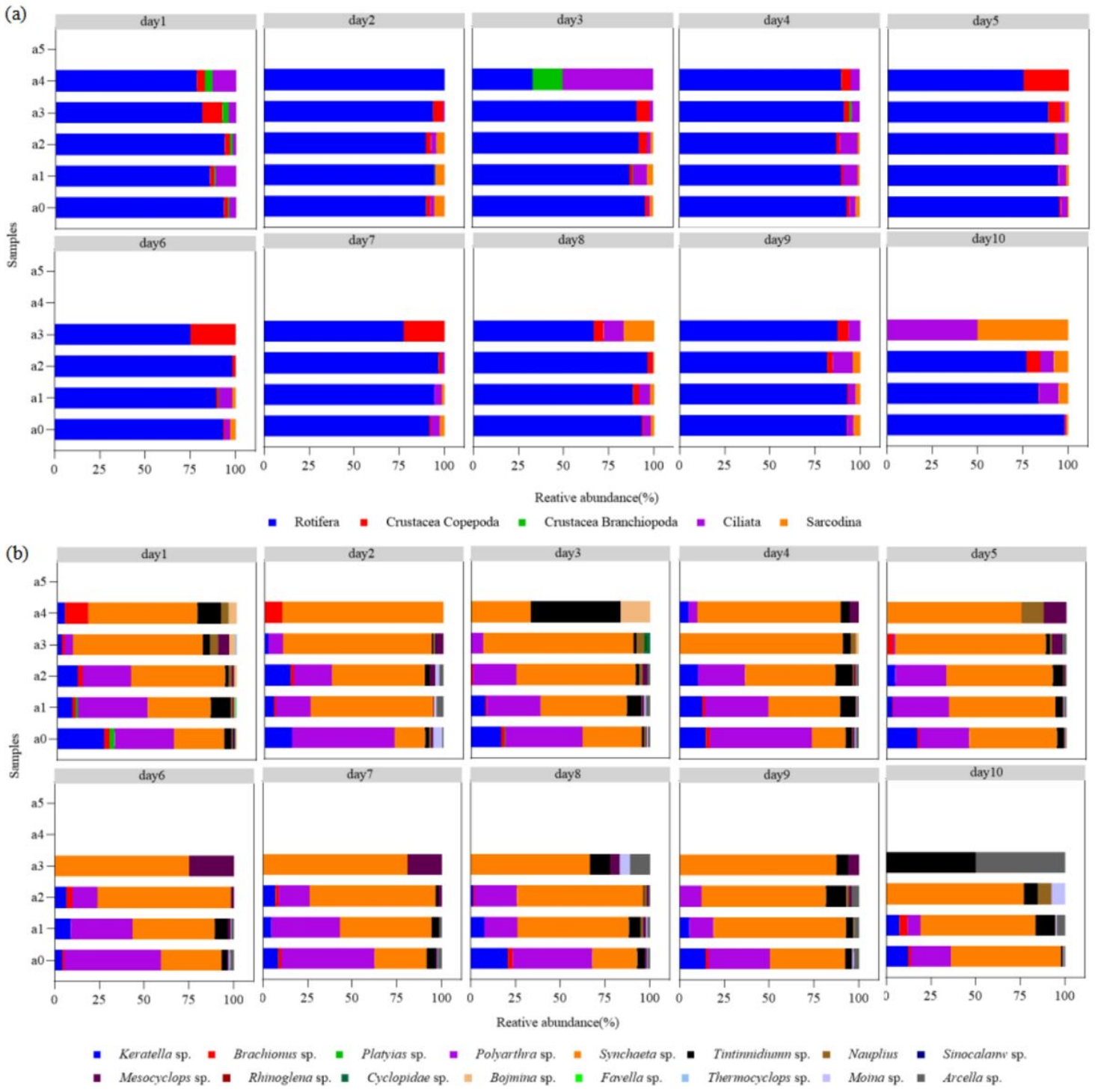

Figure 6

Effects of salinity changes on the composition of ≥50 μm organisms during 10 days period. (a) class level, (b) genus level

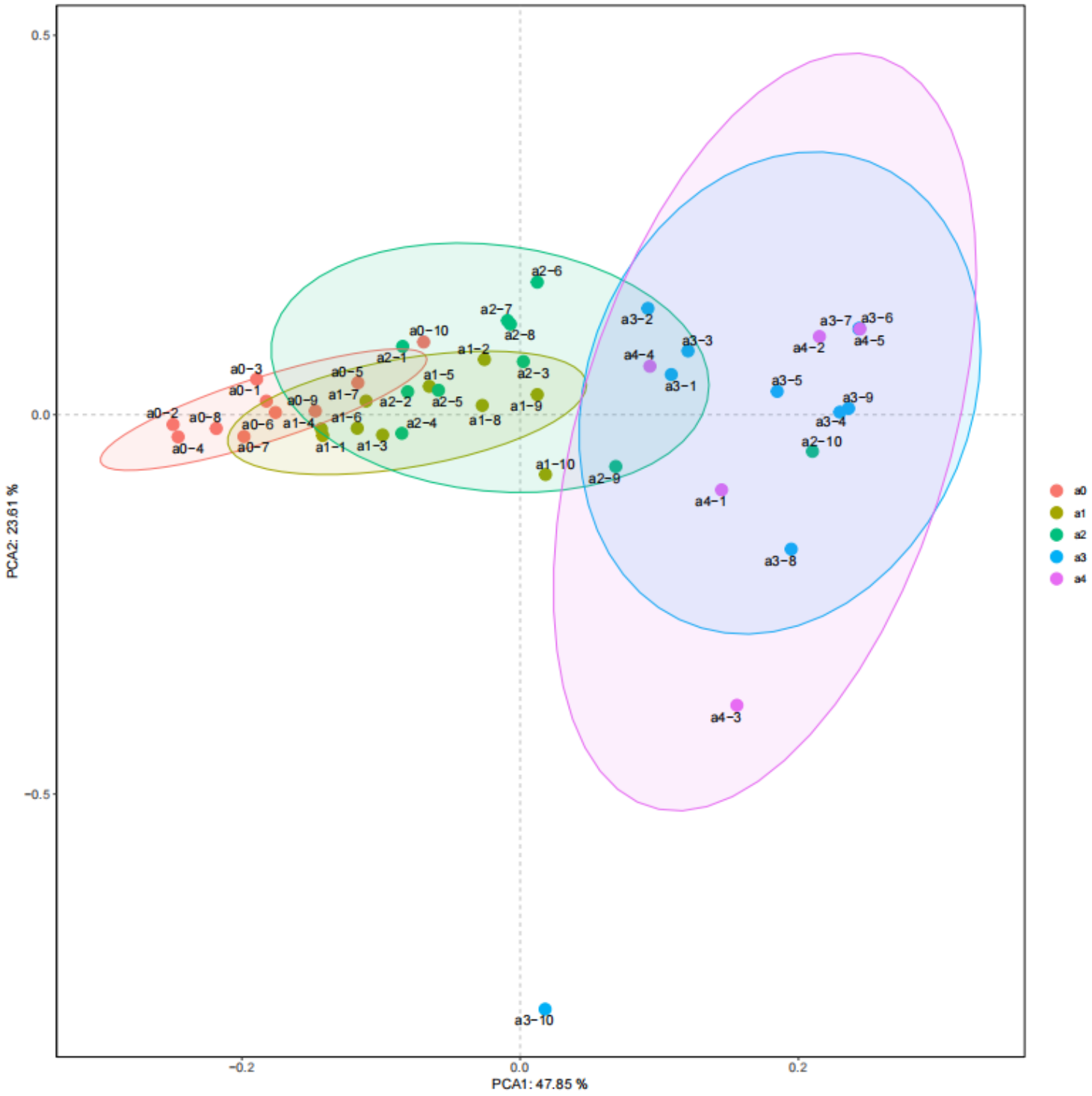

Figure 7

PCA analysis of community composition of ≥50 μm organisms in sample groups with different volume ratio of saline water

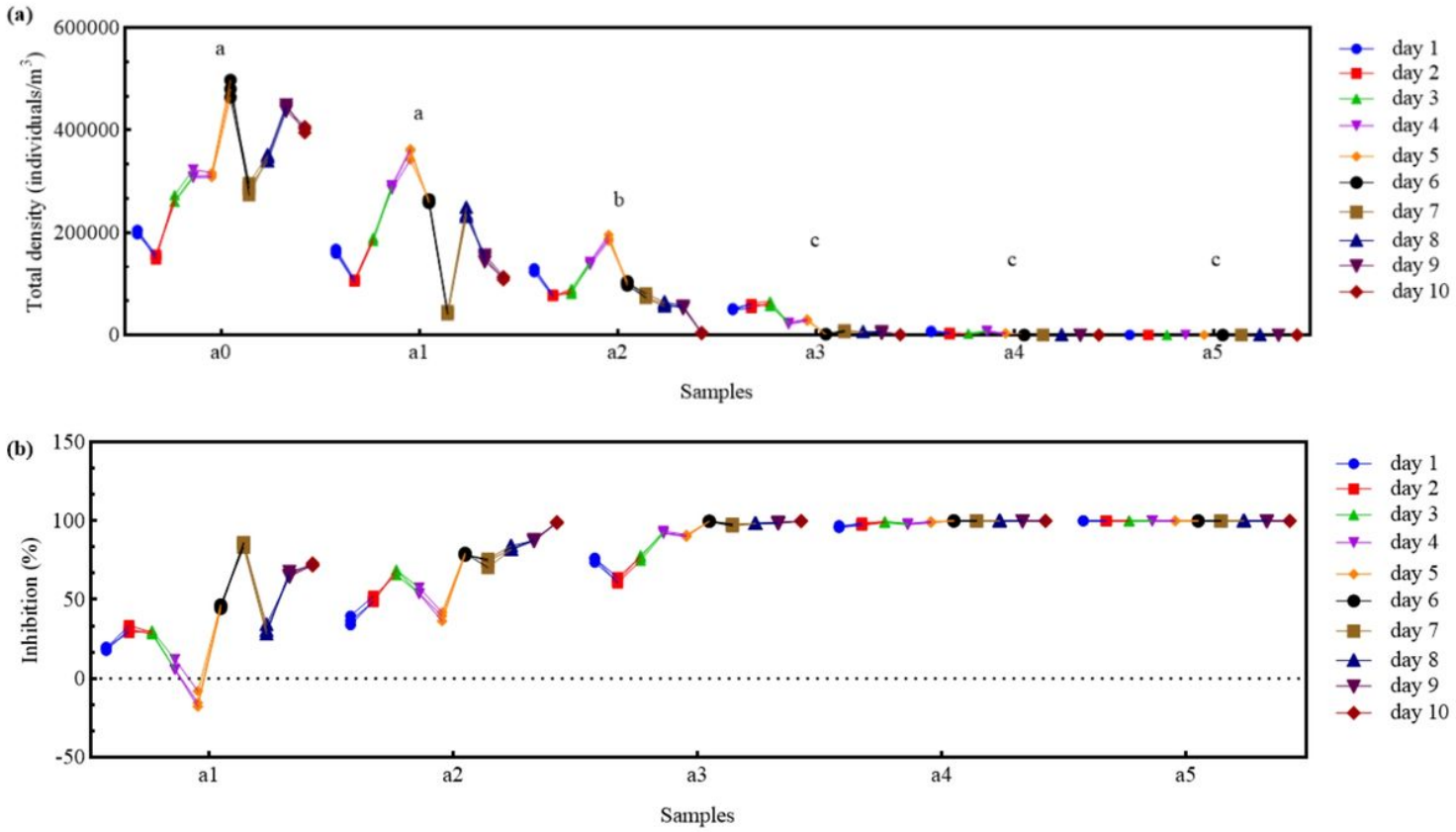

Figure 8

Total organism density (a) and daily inhibition (b) for ≥50 um organisms in different sample groups were studied. Different lowercase letters on the chart represent significant differences at the P=0.05 level, the different letters between the sample groups mean P <0.05, conversely the presence of any same letter means P> 0.05.Results of parallel samples were presented for each group.

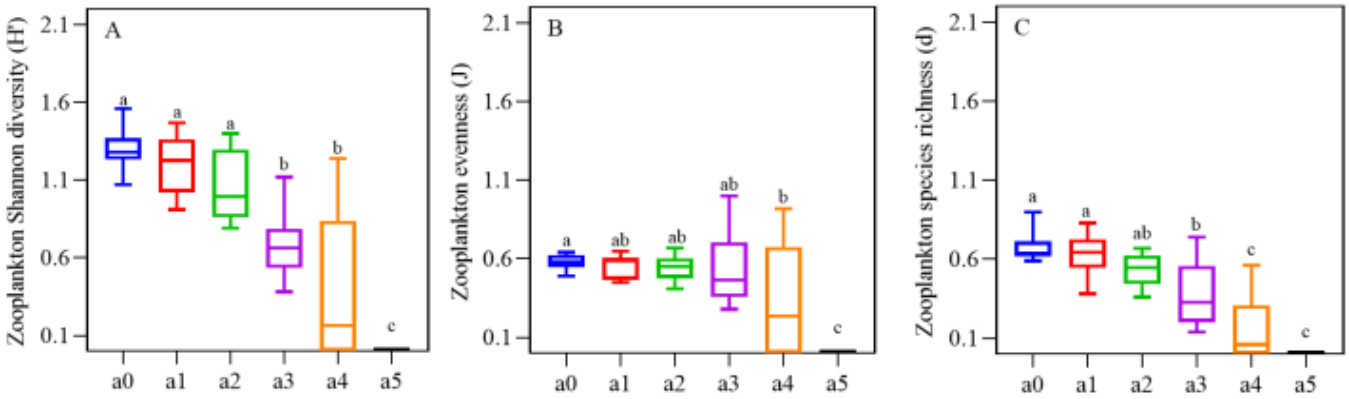

Figure 9

Margalef's species richness (A), Shannon-Wiener diversity (B) and Pielou evenness (C) for ≥50 μm organisms in different sample groups were studied. Different lowercase letters of the same index on the chart represent significant differences at the P=0.05 level, the different letters between the sample groups mean P <0.05, conversely the presence of any same letter means P> 0.05.