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Evaluation of the Control Effect of Spartina alterniflora Based on eDNA: Biodiversity Responses to Plowing, Flooding and Mowing

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Abstract

The Yancheng National Nature Reserve for Rare Birds in Jiangsu Province is a vital coastal tidal wetland reserve. However, the invasive species *Spartina alterniflora* has caused significant ecological damage to its ecosystem. Therefore, controlling *S. alterniflora* and evaluating biodiversity restoration are of critical ecological importance. In this study, we implemented a combination of cutting, flooding, and plowing to suppress *S. alterniflora* in the reserve. Subsequently, eDNA technology was employed to sample and analyze fish and zooplankton communities. Community structure recovery was quantified using Shannon, Simpson, and Pielou indices. Biodiversity indices revealed distinct restoration patterns: plowed sites showed the poorest recovery of zooplankton and fish communities, while cut sites demonstrated optimal biodiversity restoration. Flooded sites exhibited intermediate but still significant recovery. The successful application of eDNA metabarcoding in this study underscores its value as a robust tool for assessing aquatic biodiversity restoration. While our results reveal treatment-specific recovery patterns, they also emphasize two critical research directions: first, the necessity for long-term monitoring of *S. alterniflora* management outcomes, and second, the importance of expanding taxonomic coverage to fully evaluate ecosystem recovery.

1. Introduction

Yancheng Wetland Rare Birds National Nature Reserve, located in Jiangsu Province, stands as the largest and most intact coastal wetland reserve along the Pacific Ocean's west coast and the Asian continent's edge. It serves as a critical hub on the East Asia-Australasia Flyway, playing a pivotal role in preserving regional biodiversity. In the 1980s, *Spartina alterniflora* (*S. alterniflora*), a highly adaptable plant native to the Atlantic coast, was introduced to Yancheng's coastal wetlands to protect the beaches(An, et al., 2007). However, *S. alterniflora* quickly became an "ecological invader." Its exceptional flood tolerance, hypoxia resistance, euryhalinity, and prolific reproductive ability enabled it to form large colonies by the 1990s, displacing native plant species and becoming a dominant invasive species(Li, et al., 2009; Strong, et al., 2013). This invasion has significantly altered habitat structures and biomass, damaged offshore habitats, led to a sharp decline in biodiversity, and threatened some local species with extinction(Shen, et al., 2009). This invasion has significantly altered habitat structures and biomass, damaged offshore habitats, led to a sharp decline in biodiversity, and threatened some local species with extinction. The negative impacts of *S. alterniflora*. alterniflora on the ecosystem's structure and function underscore the urgent need for effective control to safeguard the coastal environment, protect biodiversity, and reduce ecological damage(Li, et al., 2009).

Various control methods for *S. alterniflora* have been implemented(Zhang, et al., 2019), including biological control (introducing natural predators), chemical control (herbicides), and physical control (mowing, flooding, plowing, and mulching)(Xie et al., 2018). Mowing involves harvesting *S. alterniflora* before seed maturity to curb its growth and reproduction, but it often requires repeated treatments if not combined with other methods(Gao, et al., 2014).Studies, such as those by Yuan Yue and colleagues, they competitive relationship between Phragmites australis and *S. alterniflora* in the Yangtze River Estuary,

they also found that artificial mowing can effectively inhibit the growth of *S. alterniflora*(Tan, et al., 2010).Plowing refers to directly digging up *S. alterniflora* from the time when it germinates until its seeds mature, turning its roots upward and its stems and leaves downward and repeating this several times. The depth of plowing needs to be determined according to the actual situation to ensure that the roots of *S. alterniflora* can be damaged and a better control effect can be achieved. Flooding means that after mowing *S. alterniflora*, through the water transmission system and water diversion and drainage facilities, introducing fresh water from the land area or seawater from the sea area to flood the land for a certain period of time, so that the roots of *S. alterniflora* will rot and die due to lack of oxygen(Smith, et al., 2015; Yuan, et al., 2017). Field tests by Xie Baohua and others between 2016 and 2018 demonstrated the effectiveness of mowing and flooding in controlling *S. alterniflora* in the Yangtze River Delta(Yuan, et al., 2011).

Despite extensive efforts to control *S. alterniflora*, systematic studies assessing post-control biodiversity restoration remain limited. Biodiversity is vital for maintaining ecological balance, as species interdependence creates a complex, orderly ecosystem. More importantly, it is also a crucial means to measure the health status of the ecosystem. Monitoring biodiversity through traditional surveys or advanced techniques like environmental DNA (eDNA) provides critical insights into ecosystem health. eDNA technology, which involves extracting DNA from environmental samples (such as water, soil, air, etc.)(Ashish, et al., 2023; Buhle, et al., 2012), offers advantages in time efficiency, cost reduction, and minimal ecological impact. It employs metabarcoding and quantitative polymerase chain reaction (qPCR) to identify species and assess biodiversity. This method has proven more efficient and accurate than traditional approaches, with reduced risks of spreading invasive species or diseases(Deiner, et al., 2015). Compared with traditional biological monitoring methods, eDNA technology has significant advantages. In addition, the eDNA method shows higher detection ability and cost-effectiveness in species detection(Zhang, et al., 2022). Studies, such as those by Williford et al. in the Cedar Lake Estuary System in Texas have demonstrated eDNA's superior detection capabilities and broader species identification range(Williford, et al., 2023). Meanwhile, He, X and others conducted investigations at 54 strategic locations in the Northwest Atlantic, revealing that the eDNA method can reveal a wider range of species(He, et al., 2023).

In this study, we employed three control methods—mowing, enclosure and flooding, and plowing—to manage *S. alterniflora* in Yancheng Wetland Rare Birds National Nature Reserve. Post-treatment, we used eDNA barcoding to analyze fish and zooplankton community structures, assessing the restoration of biodiversity. The findings offer valuable guidance for future *S. alterniflora* control projects in coastal wetlands and provide recommendations for habitat construction and restoration in nature reserves(Deiner, et al., 2017).

2. Materials and methods

2.1 Research Area and Sampling

This study was carried out in the Yancheng Wetland Rare Birds National Nature Reserve in Jiangsu, China. After controlling *S. alterniflora* through three methods, namely mowing, enclosure and flooding, and ploughing, three stations were set up respectively, with a total of 9 sampling sites.

The investigation was conducted in November 2023, and field sampling was carried out in the Yancheng Wetland Rare Birds National Nature Reserve in Jiangsu. At each station, three one-liter surface water samples were collected using sterile bottles (Thermo Fisher Scientific™) and immediately transferred to a low-temperature incubator equipped with several ice packs (approximately 0–4°C) until filtration treatment was carried out(Li, et al., 2020). We set up six field replicates (or subsamples) at each station, and each subsample filtered a volume of 300–500 milliliters of water (approximately 3 liters of water for one station in total). Filtration was performed using Millipore 0.45μm hydrophilic nylon membranes (Merck Millipore). All the replicated eDNA membrane samples were placed in 5.0-milliliter centrifuge tubes respectively, and then immediately frozen and stored at -20°C until DNA extraction was carried out. At each station, autoclaved tap water (300 milliliters filtered) was used as a blank control to monitor possible contaminants(Li, et al., 2021).

2.2 DNA Extraction, PCR Amplification and Sequencing

All the filter membranes were extracted using the DNeasy PowerWater Kit (Qiagen). These six replicated samples of eDNA extraction and the blank controls were all subjected to subsequent processing. The primer set targeting the 18S rRNA gene

(F: 5'-GTACACACCGCCCGTC-3', R: 3'-TGATCCTTCTGCAGGTTCACCTAC-5') was selected for PCR amplification and analysis of zooplankton(Djurhuus, et al., 2018). The universal fish primers Mifish-U

(F: 5'-GTCGGTAAAACTCGTGCCAGC-3', R: 3'-GTTTGACCCTAATCTATGGGGTGATAC-5') developed by Miya et al. were selected for PCR amplification to analyze fish(Willemin, et al., 2025).

All PCRs were carried out in 30μl reaction mixtures according to the standard protocol. All PCR products were quantified and mixed in equimolar amounts for subsequent sequencing. Depending on the size of the PCR amplicons, the sequencing templates were sequenced on the Ion Proton sequencer (Life Technologies) and the Illumina MiSeq PE300 platform (Illumina) respectively.

2.3 Data analysis

In this study, three complementary diversity indices— the Shannon-Wiener index (H) ,the Simpson index (D)(Stoeckle, et al., 2021) and the Pielou index(J)—were employed to comprehensively assess the richness and diversity of zooplankton and fish communities across multiple sampling sites. The indices were calculated as follows:

H=- $\sum_{i=1}^{S} P_i \ln(P_i)$ (1)

D = 1- $\sum_{i=1}^{S} P_i^2$ (2)

Where Pi is the proportion of the total sequences contained in the i relative to the total sequences in the sample, and S represents the total number of the sample.

$J = \frac{H}{lnS}$ (3)

Where S is the total numbe in the sample, and *H* represent the Shannon .

3. Results

3.1 Analysis of Zooplankton Diversity

Through eDNA metabarcoding analysis and subsequent annotation against the NCBI database, our survey identified a diverse zooplankton community comprising 2 classes, 4 orders, 7 families, 8 genera, and 11 species. Among the 8 detected zooplankton taxa at the species and genus level, copepods dominated with 4 taxa, followed by rotifers with 3 taxa, and cladocerans represented by a single taxon. In terms of relative abundance based on DNA sequence counts, copepods constituted the largest proportion at 50.00%, followed by rotifers at 37.50%, while cladocerans accounted for the smallest proportion at 12.50% (Fig. 1).Among the sampling sites, the plowed site exhibited the lowest zooplankton diversity with only 5 species detected, while the mowed site showed the highest diversity with 9 species. The flooded site recorded an intermediate diversity of 6 zooplankton species (Fig. 2). Notably, *Acartia southwelli* (73.26%) and *Brachionus plicatilis* (14.64%) emerged as the dominant zooplankton species in this survey, with both species exceeding the dominance index threshold (Y ≥ 0.1) for classification as common species.

The Shannon index and Simpson index are used to represent the diversity of zooplankton. The larger the value of H, the higher the species diversity and the more even the distribution. And the closer the value of *D* is to 1, the higher the species diversity. The closer the value of the evenness index is to 1, the more evenly the species are distributed.

Table 1 lists the indices reflecting the richness and diversity of the zooplankton community. Among them, the Shannon index ranges from 0.56 to 1.12, the Simpson index ranges from 0.22 to 0.60, and the Pielou index ranges from 0.08 to 0.62. According to the estimation methods of different indices, the values of the plowed sampling points are the lowest. The data of the three indices in this sampling site all follow a similar distribution trend, indicating that the plowed area is the least diversified sampling point. The number of plankton species in this sampling point is the smallest, and the distribution of the number of individuals of each species is extremely uneven. It is very likely that a few dominant species account for a large number of individuals. The data of the mowed sampling points are slightly lower, which means that the evenness is poor, the dominant species are more active, and the species richness is not high. The flooded sampling site is the one with the best data, indicating that the plankton species in this sampling site are relatively rich, the distribution of the number of individuals of species is relatively even, the dominant species are not obvious, and the species diversity is relatively good.

3.2 Analysis of Fish Diversity

Through eDNA metabarcoding analysis and subsequent taxonomic annotation using the NCBI database, our survey identified a total of 30 fish species spanning 6genera, 11 families, and 26 orders. The fish community was predominantly composed of Cypriniformes and Perciformes, which collectively accounted for 40.00% of the total sequence reads. Cyprinodontiformes and Siluriformes each represented 6.60% of the sequences, while Beloniformes and Mugiliformes showed the lowest proportions at 3.30% each (Fig. 3).

As shown in Fig. 4, the mowed sampling site exhibited the highest fish diversity, with 24 species identified, while the plowed site showed the lowest diversity with only 8 species. The flooded site recorded an intermediate diversity of 17 fish species. Notably, *Oryzias latipes* emerged as the dominant fish species, accounting for 47.26% of the total sequences and exceeding the dominance index threshold ($Y \geq 0.1$) for classification as a common species.

Table 2 lists the indices reflecting the richness and diversity of the fish community. Among them, the Shannon index ranges from 1.40 to 2.25, the Simpson index ranges from 0.66 to 0.85, and the Pielou index ranges from 0.67 to 0.71. According to the estimation methods of different indices, again, the values of the plowed sampling points are the lowest, and the Shannon index and the Simpson index are also the lowest, indicating that the plowed area is the least diversified sampling point, with poor evenness and no prominent dominant species. The values of the three indices of the mowed sampling points are the highest, which means that the fish species are rich, and the number of individuals is evenly distributed among various species. The Simpson index is close to 1, indicating that there are almost no obvious dominant species, and the species diversity is extremely high. In the flooded sampling site, the fish species are relatively rich, the distribution of the number of individuals of species is relatively even, the dominant species are not obvious, and the species diversity is relatively good.

Table 1
Zooplankton Biodiversity Indices at Various Sampling Sites

	Simpson	Shannon	Pielou
Mowing	0.22	0.56	0.26
Plowing	0.04	0.14	0.08
Flooding	0.60	1.12	0.62

Table 2
Fish Biodiversity Indices at Various Sampling Sites

	Simpson	Shannon	Pielou
Mowing	0.85	2.25	0.71
Plowing	0.66	1.40	0.67
Flooding	0.76	1.95	0.69

4. Discussion

The zooplankton identified in this study encompassed 2 classes, 4 orders, 7 families, 8 genera, and 11 species. Among these taxa, copepods dominated in DNA sequence abundance, indicating their substantial prevalence. As keystone organisms in aquatic ecosystems, copepods play a vital role in diverse habitats—from rivers and estuaries to marine environments. Notably, they often account for more than 80% of the total zooplankton biomass in many aquatic systems, further emphasizing their ecological significance(Madhu, et al., 2007).

As common species, *Acartia lancicrus* and *Brachionus plicatilis* likely play a pivotal role in sustaining zooplankton community structure and ecological functions. Given that zooplankton dominate the abundance and biomass of multicellular animals in pelagic marine ecosystems, they were selected as a key focus of this study(Yan, et al., 2023). Hu et al. detected the biodiversity of zooplankton in Xixi Wetland, Zhejiang Province, China, in different seasons of 2022. The average Shannon index was between 1.00 and 1.50, the data of the Simpson index were around 0.50 to 0.60, and the Pielou index was around 0.70 to 0.75(Hu, et al., 2023). A comparison with undisturbed wetland data reveals varying degrees of ecological recovery across sampling sites. Notably, as shown in Fig. 5, the plowed site exhibits the lowest zooplankton recovery, characterized by a simplified community structure, indicating a need for targeted restoration efforts. The mowed and flooded sampling sites exhibit more pronounced biodiversity recovery, with zooplankton communities showing particularly even distribution at the mowed site. The community structure at this site has demonstrated significant recovery, indicating favorable ecological restoration outcomes.The investigation results of Hou et al. in Minghu National Wetland Park, Liupanshui, Guizhou Province, can also confirm this result(Hou, et al., 2025).

Fish biodiversity in the study area comprised 30 species across 27 genera, 11 families, and 7 orders. Cypriniformes emerged as the dominant group in the fish community, accounting for the highest proportion of sequences. As a widely distributed species, the medaka (*Oryzias latipes*) exhibits a broad ecological niche and strong adaptability, playing a crucial role in maintaining fish community stability and ecosystem balance(Wittbrodt, et al., 2002). As a model organism, the medaka exhibits strong reproductive capacity and environmental adaptability. Its high abundance suggests that local food resources, water quality, and habitat conditions have been restored to a certain degree. Logan M. Cutler et al. compared seasonal variations in prey fish diversity across wetland and lake ecosystems. Their results showed that all sampled sites exhibited lower diversity indices in autumn compared to the values

observed in our study, suggesting that our study area has achieved a certain level of ecological restoration(Cutler, et al., 2024). Rajesh Debnath et al. (Year) assessed fish community diversity in the Brahmaputra River Basin's open wetlands (India). Their study reported the following biodiversity indices: Shannon (2.10–2.90), Simpson (0.85–0.93), and Pielou (0.82–0.93)(Debnath, et al., 2022). As shown in Fig. 6, omparative analysis revealed that the mowed sample plot exhibited the highest fish species richness among all study sites, with relatively even species distribution. These findings suggest successful ecological restoration in this habitat. While the flooded sample plot showed slightly lower species richness compared to the mowed plot, it still demonstrated significant restoration progress. The community structure exhibited clear stabilization trends. The plowed sample plot exhibited the lowest fish species richness among all study sites, characterized by the absence of dominant species and uneven community distribution. These findings indicate significantly impaired restoration of fish biodiversity in this habitat.

Biodiversity assessment constitutes a fundamental component of environmental protection and monitoring programs. However, traditional zooplankton community characterization methods are often invasive, labor-intensive, and potentially environmentally damaging. These limitations highlight the need for alternative assessment approaches(Wheeler, et al., 2004). Unlike traditional methods, eDNA analysis —using DNA shed by organisms into the environment—delivers higher sensitivity, greater cost-efficiency, faster processing, and reduced ecological impact in biodiversity studies(Flynn, et al., 2015; Gauvin, et al., 2024). eDNA technology has been successfully utilized in assessing zooplankton community diversity, demonstrating its efficacy in biodiversity monitoring. Currently in China, eDNA-based biodiversity research has primarily focused on lacustrine and riverine fish communities, while its application to wetland aquatic biodiversity remains largely unexplored. These findings demonstrate the considerable potential of eDNA metabarcoding for characterizing dominant zooplankton taxa in nearshore environments(Gallego, et al., 2020). As a non-invasive monitoring approach, eDNA technology has revolutionized fish biodiversity assessments, offering distinct advantages over traditional capture methods by overcoming their inherent spatial and temporal limitations(Zhang, et al., 2020). This approach demonstrates particular efficacy in detecting cryptobenthic and rare fish species that often evade traditional survey methods, while its minimally invasive nature significantly reduces sampling-induced ecosystem disturbance compared to conventional capture-based techniques(Li, et al., 2022). While eDNA-derived fish assemblages generally reflect authentic species distributions within the sampling area, meteorological data indicate sustained gale-force winds during critical November 2023 sampling windows, potentially compromising detection sensitivity through water column mixing and eDNA dispersion(Long, et al., 2022). Additionally, the detection sensitivity was likely compromised by the non-reproductive status of certain fish species during our sampling campaign. This physiological factor may have contributed to underestimation of species presence, particularly for those with seasonal spawning behaviors. Consequently, more comprehensive investigations with extended monitoring durations are required to better understand fish diversity recovery patterns.

Despite distinct life history strategies between zooplankton and fish leading to differential recovery patterns, our findings consistently demonstrate that mechanical plowing exerts negative impacts on

biodiversity restoration for both taxa in *S. alterniflora* -invaded ecosystems. In contrast, mowing emerges as the most sustainable control method, showing optimal outcomes for coastal biodiversity recovery. The study further confirms the transformative potential of eDNA metabarcoding as a monitoring tool. Its non-invasive nature, high taxonomic resolution, and capacity for longitudinal assessment position eDNA technology as an indispensable component of future coastal biodiversity assessment frameworks, particularly in quantifying ecosystem responses to restoration interventions.

Declarations

Competing interest

The authors have no relevant financial or non-financial interests to disclose.

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Author contributions

Conceptualization: Yanming Sui; Methodology: Wanjun Feng, Dagui Liao; Formal analysis and investigation: Yanan Wei, Cong Yan; Writing-original draft preparation: Yanan Wei; Writing - review and editing: Yanan Wei, Jiyi Chen; Funding acquisition: Linlan Lv; Resources: Yanming Sui; Supervision: Linlan Lv.

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Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Figures

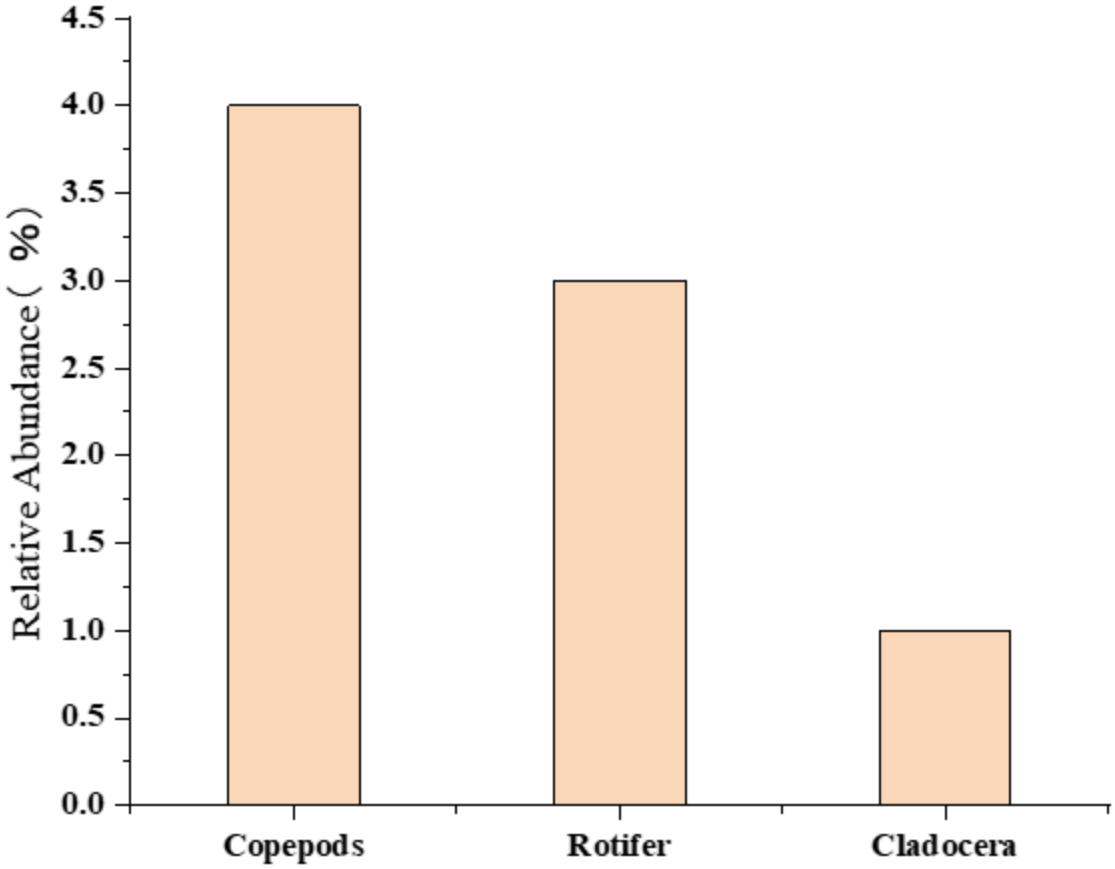


Figure 1

Proportion of Zooplankton Sequence Numbers

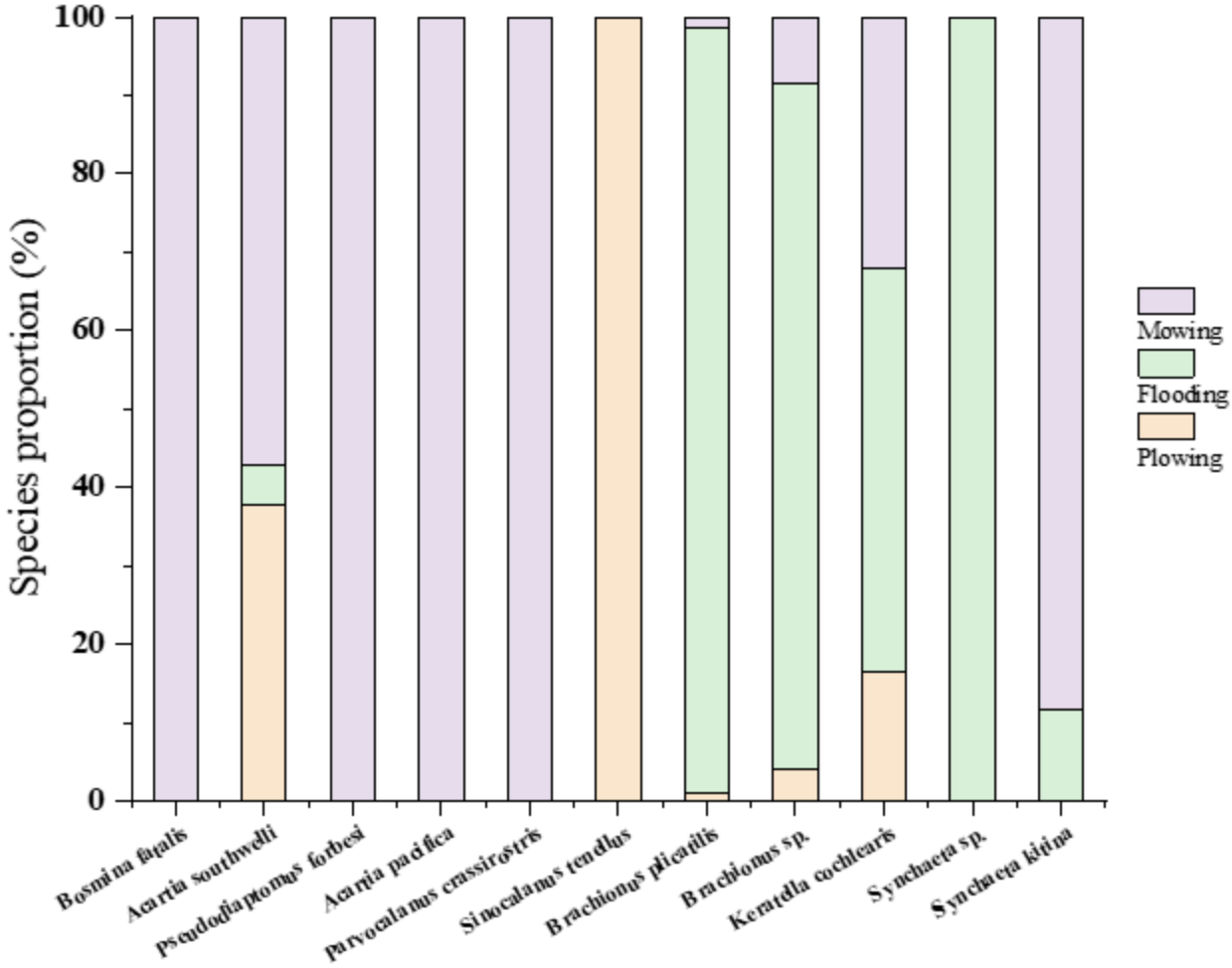


Figure 2

Comparison of Zooplankton Distribution

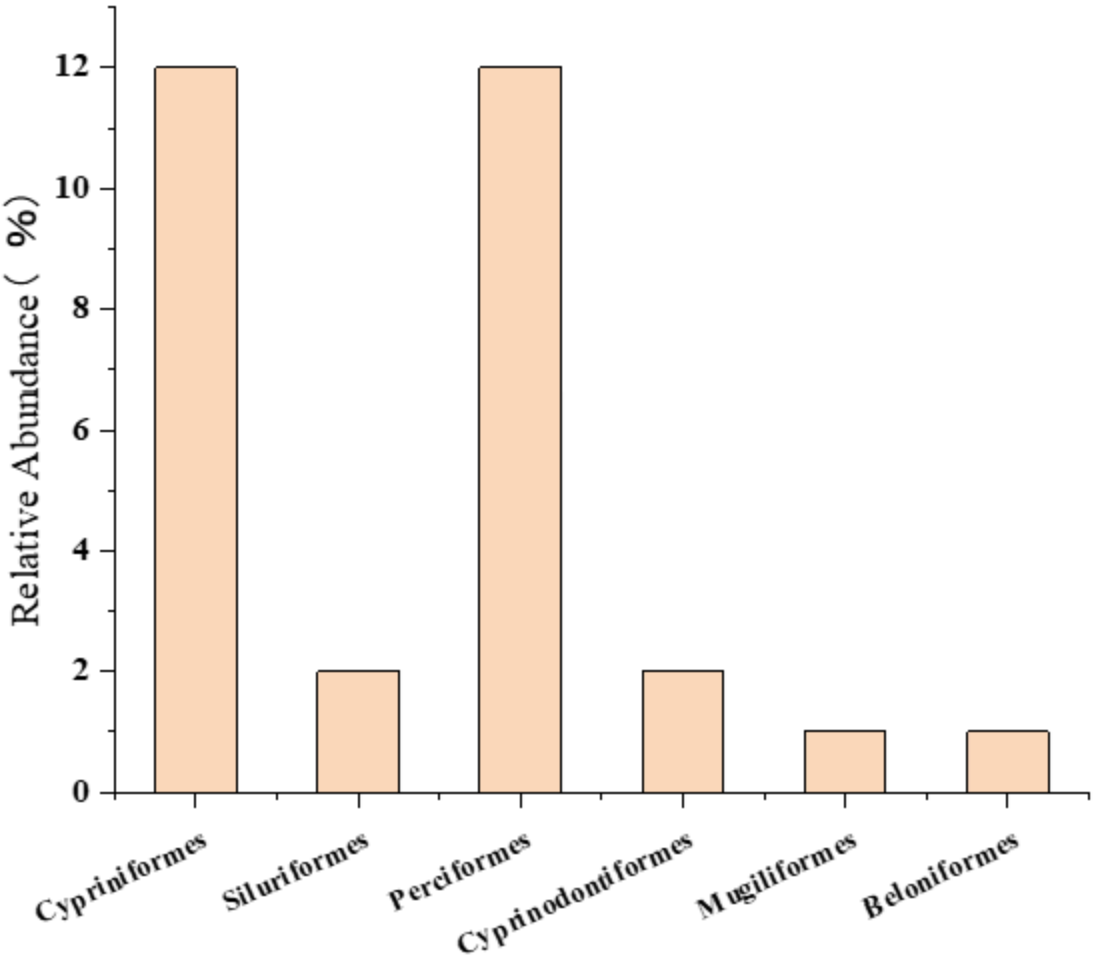


Figure 3

Proportion of Fish Sequence Numbers

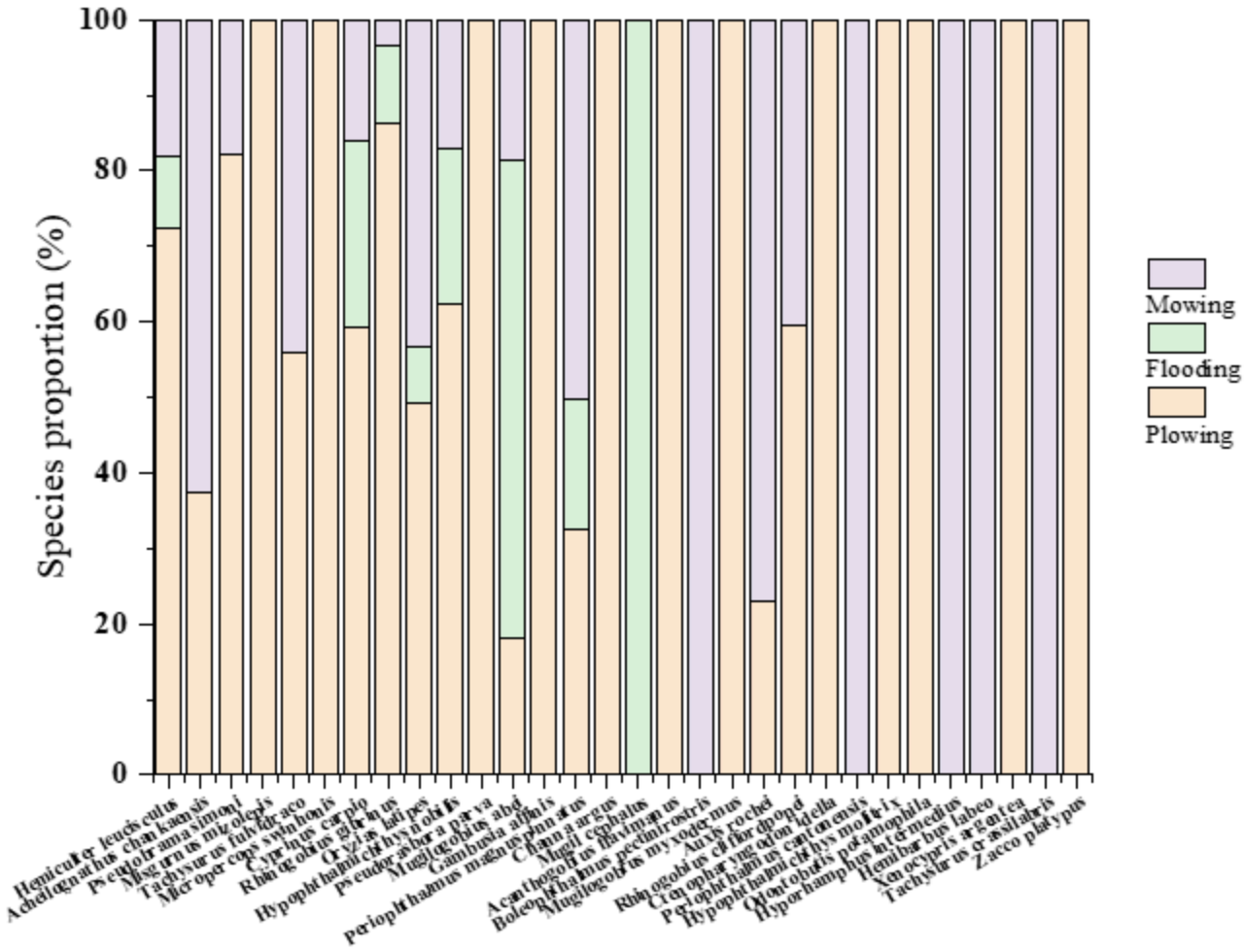


Figure 4

Comparison of Fish Distribution

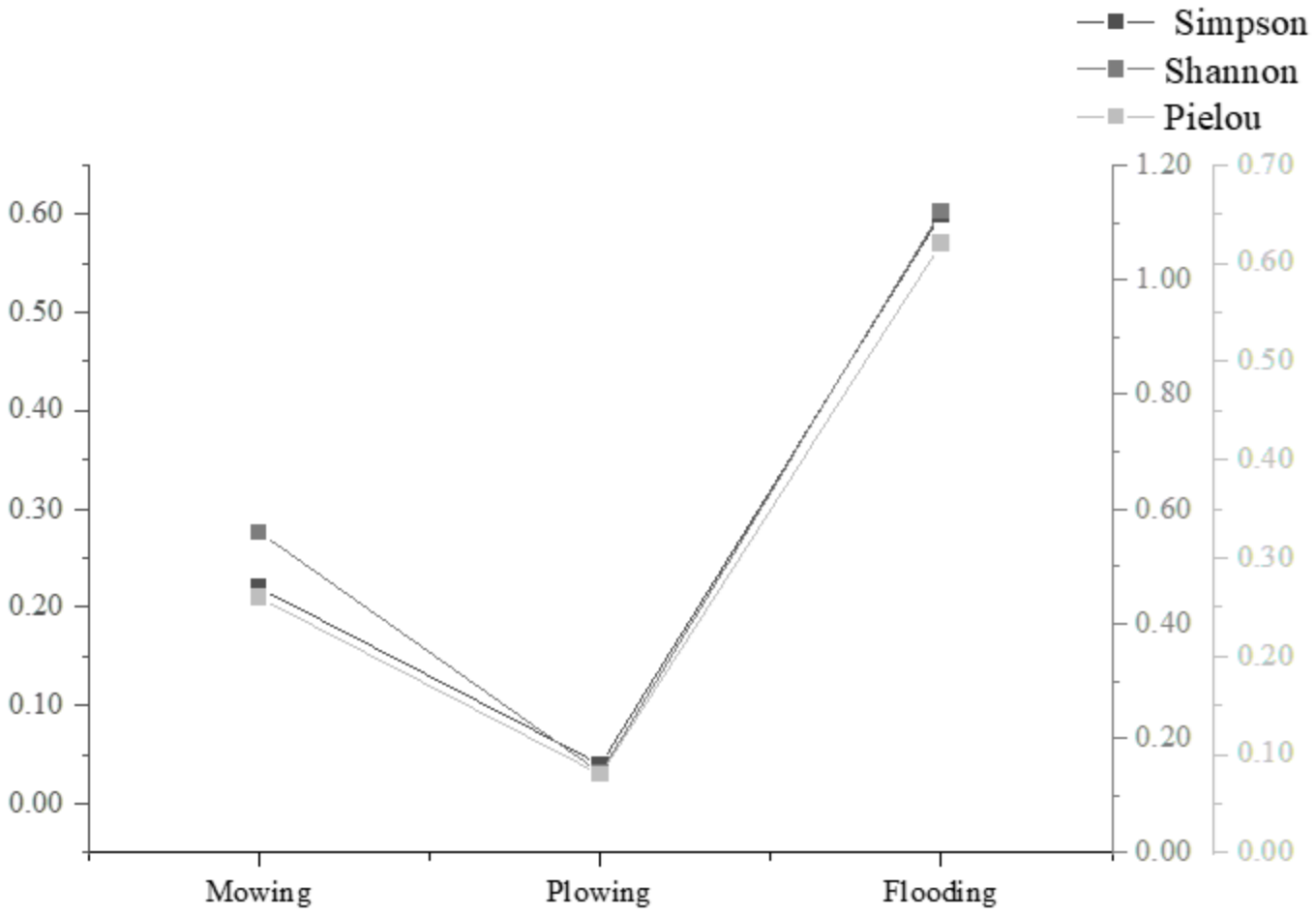


Figure 5

The Simpson index, Shannon index, and Pielou index of Zooplankton in different sample plots

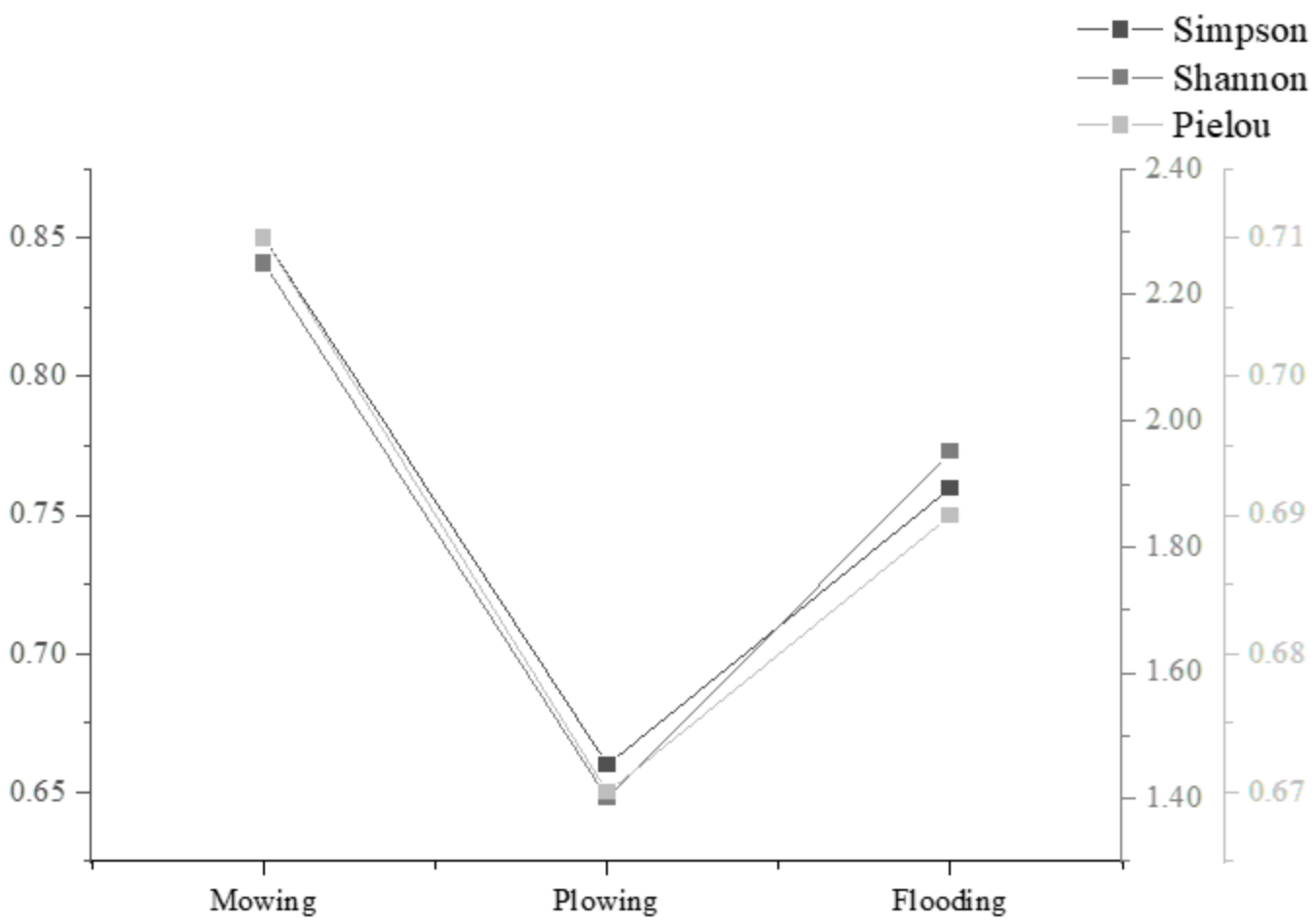


Figure 6

The Simpson index, Shannon index, and Pielou index of Fish in different sample plots