# Seasonal Dynamics of Zoobenthos Community in a Small Reservoir Cultured with Silver Carp and Bighead Carp (Postprint)

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## Abstract

Benthic animals are important natural food sources for fish, and assessing the standing stock of benthic animals in water bodies can guide the stocking density of fish in fishery production. To investigate the seasonal dynamics of benthic animal communities in freshwater ecological aquaculture reservoirs, the benthic animal community in Sanhe Reservoir was surveyed and analyzed in April, July, and October 2013, and January 2014. The study collected a total of 7 genera of benthic animals, belonging to the families Tubificidae, Chironomidae, and Ceratopogonidae, with no mollusks collected. The Relative Importance Index (IRI) calculation results indicated that *Clinotanypus* (IRI=7136), *Tubifex* (IRI=6734), and *Branchiura* (IRI=1384) were the dominant groups, accounting for 34.26%, 50.38%, and 10.96% of the total catch, respectively. The total density and biomass of benthic animals differed significantly among seasons ($P < 0.05$), both following the pattern of winter > spring > summer > autumn. Winter had the highest total density (4100 individuals/m²) and total biomass (10.14 g/m²), followed by spring (1446 individuals/m²; 1.07 g/m²), while summer (579 individuals/m²; 0.66 g/m²) was lower, and autumn (492 individuals/m²; 0.64 g/m²) was the lowest. Non-metric Multidimensional Scaling (MDS) and community similarity analysis indicated that the seasonal differences in benthic animal community structure were significant ($P = 0.001$), and the benthic animal communities in Sanhe Reservoir in 2013 could be clearly divided into three groups: spring community, summer-autumn community, and winter community. Pearson correlation analysis indicated that the total density of benthic animals was positively correlated with dissolved oxygen and nutrients, and significantly negatively correlated with other water physicochemical factors ($P < 0.05$). Redundancy analysis indicated that ammonia nitrogen, salinity, pH, and turbidity were significant influencing factors on the seasonal differences of benthic animal communities in Sanhe Reservoir ($P < 0.05$), while total nitrogen

had a marginally significant effect on the seasonal differences of benthic animal communities (P = 0.08).

## Full Text

## Preamble

### Seasonal Dynamics of Zoobenthos Communities in a Small Reservoir Stocked with Silver and Bighead Carp

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## Abstract

Zoobenthos is an important natural food resource for fishes. Assessing the standing crop of zoobenthos in water bodies can provide guidelines for fish stocking in fishery production. To explore the seasonal dynamics of zoobenthic communities in freshwater aquaculture reservoirs, investigations were conducted in April, July, and October 2013, and January 2014 to determine the composition of zoobenthic communities in the Sanhe Reservoir. The relationships between zoobenthic communities and environmental factors (water temperature, pH, turbidity, salinity, dissolved oxygen, chlorophyll a, NH -N, NO -N, total nitrogen, and total phosphorus) were analyzed.

In this study, seven genera of zoobenthos belonging to Tubificidae, Chironomidae, and Ceratopogouidae were detected. No mollusks were collected. The results of the index of relative importance (IRI) showed that *Clinotanypus* (IRI = 7136), *Tubifex* (IRI = 6734), and *Branchiura* (IRI = 1384) were dominant groups, accounting for 34.26%, 50.38%, and 10.96% of total abundance, respectively.

The total density and biomass of zoobenthos varied significantly among seasons ($P < 0.05$), with both highest in winter (4100 ind./$m^2$, 10.14 g/$m^2$), followed by spring (1446 ind./$m^2$, 1.07 g/$m^2$), and lowest in summer (579 ind./$m^2$, 0.66 g/$m^2$) and autumn (492 ind./$m^2$, 0.64 g/$m^2$). The zoobenthic communities in the Sanhe Reservoir mostly consisted of oligochaetes and chironomid larvae, exhibiting characteristics of higher density and lower biomass. *Tubifex* and *Branchiura* were dominant groups in summer and autumn, while Chironomidae was the most dominant group in spring. *Tubifex* were dominant groups in winter.

Significant differences in zoobenthic communities among seasons were found by non-metric multi-dimensional scaling (MDS) and similarity analysis (P = 0.001). The zoobenthic communities in the Sanhe Reservoir showed three temporal communities: spring community, summer-autumn community, and winter community. Pearson correlation analysis indicated that total density of zoobenthos was positively correlated with dissolved oxygen and nutrients, but had significant negative correlations with water temperature, pH, salinity, turbidity, and chlorophyll-a content ($P < 0.05$). Redundancy analysis suggested that NH -N, salinity, pH, and turbidity were important factors with significant effects on seasonal variation of zoobenthic communities ($P < 0.05$). Total nitrogen concentration had marginally significant effects on seasonal differences of zoobenthic communities (P = 0.08).

**Keywords:** benthic fauna; density; biomass; seasonal dynamics; Sanhe Reservoir

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## Introduction

Zoobenthos is primarily composed of three major groups: oligochaetes, mollusks, and aquatic insects, and constitutes an important component of aquatic ecosystems. These organisms feed on benthic algae and organic detritus while serving as prey for carnivorous fish and birds, directly affecting the survival and reproduction of other aquatic animals [1-2]. They function as a critical hub for material cycling and energy flow in aquatic ecosystems. Since zoobenthos represents the main natural food source for benthivorous and some omnivorous fish species [3-4], studying zoobenthos community structure provides important guidance for fishery production. Surveys of zoobenthos standing crop can help determine fish stocking potential.

Environmental characteristics of lakes and watersheds change seasonally [5], and these variations in environmental factors directly affect the growth, reproduction, and population succession of macrozoobenthos [6-7], ultimately leading to shifts in zoobenthos communities [8]. Nutrient content in water bodies significantly influences zoobenthos distribution, with total phosphorus exerting particularly strong effects [9-10]. Fish predation also directly impacts zoobenthos communities [3].

This study selected the Sanhe Reservoir in Anlu City, Hubei Province as the research site to investigate zoobenthos composition, density, and biomass across seasons, while simultaneously measuring physicochemical parameters and nutrient content. The objectives were to understand the community structure and seasonal patterns of macrozoobenthos in small reservoirs used for ecological silver carp and bighead carp culture, and to further explore how water environmental factors influence seasonal distribution differences in macrozoobenthos communities. This research aims to enrich our understanding of zoobenthos community ecology and provide a scientific basis for ecological aquaculture in small and medium-sized reservoirs.

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## 1. Study Location

Anlu City is located in northeastern Hubei Province (113°10 -113°57 E, 31°04 - 31°29 N). The climate features short spring and autumn seasons, hot and rainy summers, with an average annual precipitation of 1100 mm and average annual temperature of 16.0°C. The average temperature is 2.8°C in January and 26.1°C in July. The city is an important agricultural and fishery hub in Hubei Province, with 15 reservoirs within its jurisdiction.

The Sanhe Reservoir is located 6.5 km northeast of Anlu City. It is a small reservoir primarily for flood control and irrigation, with secondary uses including fishery aquaculture. The reservoir has a surface area of 2.8 $km^2$ and an average depth of 6.5 m. The reservoir contractor annually stocks large numbers of silver and bighead carp fingerlings weighing 0.5–0.8 kg each. Fishing activities begin in late September and cease in early January of the following year.

[Figure 1: see original paper] Diagram of zoobenthos sampling points in Sanhe Reservoir

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## 2. Sampling Time and Methods

Sampling was conducted at five sites established in the Sanhe Reservoir. At each sampling point, a Peterson grab with a sampling area of 0.04 $m^2$ was used to collect sediment samples. Samples were filtered through a 0.5 mm stainless steel mesh sieve to collect zoobenthos specimens, which were then fixed and preserved in 50 mL plastic bottles with formalin solution. In the laboratory, specimens were identified to genus [11-12] and individual counts were recorded for each genus. After removing surface moisture with absorbent paper, specimens were weighed using an electronic balance (precision 0.0001 g).

Simultaneously with zoobenthos collection, water temperature (WT), pH, turbidity (Tur), salinity (Sal), and dissolved oxygen (DO) were measured at each sampling point using a YSI 6600V2 multi-parameter water quality analyzer. Water samples (1000 mL) were collected at each site and brought back to the laboratory for nutrient analysis, including ammonia nitrogen (NH -N), nitrate nitrogen (NO -N), total nitrogen (TN), total phosphorus (TP), and chlorophyll-a (Chl-a). Nutrient measurements were conducted according to national standards [13-14].

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## 3. Physicochemical Indicators and Nutrient Measurement

Nutrient content measurements, including ammonia nitrogen, were performed following standard protocols [13-14].

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## 4. Data Statistics

The index of relative importance (IRI) was used to determine dominant zoobenthos groups, calculated as: $IRI = (F + N) \times W$ [15], where W is the percentage of biomass of a particular group relative to total macrozoobenthos biomass, N is the percentage of density of that group relative to total density, and F is the frequency of occurrence of that group.

SPSS 13.0 and Statistica 7.0 were used for one-way ANOVA of water environmental factors, zoobenthos density, and biomass. PRIMER 5.0 was used for non-metric multi-dimensional scaling (MDS) ordination and similarity analysis (ANOSISIM) of zoobenthos communities across four seasons. Pearson correlation coefficients were calculated to analyze relationships between zoobenthos abundance and water environmental factors. CANOCO for Windows 4.5 was used for correspondence analysis between zoobenthos and water environmental factors, with Monte Carlo tests to identify environmental factors with significant effects on zoobenthos communities. All data were log (x+1) transformed before statistical analysis. $P < 0.05$ indicated significant differences or effects.

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## 5. Results

### 5.1 Seasonal Characteristics of Water Environmental Factors

Water environmental factors in the Sanhe Reservoir showed significant seasonal variation (Figure 2). Water temperature was highest in summer (average 28.82°C) and lowest in winter (7.43°C). Dissolved oxygen content showed the opposite pattern, being highest in winter (12.08 mg/L) and lowest in summer (5.85 mg/L). Chlorophyll-a content was highest in autumn (26.2 g/L) and lowest in spring (11.02 g/L).

The concentrations of ammonia nitrogen and nitrate nitrogen showed opposite trends from spring to winter: ammonia nitrogen increased progressively, while nitrate nitrogen decreased. Salinity and turbidity showed consistent seasonal patterns, both being significantly lower in winter than in other seasons. Total nitrogen and total phosphorus concentrations followed the same seasonal trend as ammonia nitrogen, with lowest values in spring (0.497 mg/L and 0.011 mg/L, respectively). Total nitrogen concentration was highest in summer (1.203 mg/L), while total phosphorus concentration was highest in winter (0.095 mg/L).

[Figure 2: see original paper] Seasonal differences of environmental factors in Sanhe Reservoir

### 5.2 Zoobenthos Species Composition

This survey identified oligochaetes and aquatic insects belonging to Tubificidae, Chironomidae, and Ceratopogouidae. No mollusks were collected. The genus *Tubifex* had high frequency across all seasons and was a common group. Based on the index of relative importance, *Clinotanypus* (IRI = 7136), *Tubifex* (IRI = 6734), and *Branchiura* (IRI = 1384) were identified as dominant groups, accounting for 34.26%, 50.38%, and 10.96% of total captured individuals, respectively. Chironomid larvae of the genus *Pelopia* were present in all seasons.

Group composition, capture number, IRI, and abbreviation of zoobenthos in Sanhe Reservoir

### 5.3 Seasonal Changes in Zoobenthos Density and Biomass

Total zoobenthos density differed significantly among seasons ($P < 0.05$). Winter density (4100 ind./$m^2$) was significantly higher than other seasons, while autumn density (492 ind./$m^2$) was the lowest. Statistical analysis of total biomass showed winter biomass (10.14 g/$m^2$) was extremely significantly higher than other seasons ($P < 0.001$). Autumn had the lowest total density and biomass.

The density and biomass of the dominant genus *Tubifex* were similar in winter and spring, and significantly higher than in summer and autumn ($P < 0.05$). The densities of *Clinotanypus* and *Branchiura* were highest in winter, followed by summer and autumn. The biomass of *Clinotanypus* was highest in winter and spring, while *Branchiura* biomass showed no significant differences among seasons.

Density (ind./$m^2$) of zoobenthos in Sanhe Reservoir
Biomass (g/$m^2$) of zoobenthos in Sanhe Reservoir

### 5.4 Seasonal Patterns of Zoobenthos Communities

Community similarity analysis revealed significant differences in zoobenthos communities among the four seasons in Sanhe Reservoir (ANOSIM, $P = 0.001$). Based on non-metric multi-dimensional scaling (MDS) of zoobenthos individuals, the 2013 Sanhe Reservoir zoobenthos communities could be clearly divided into three groups: spring community, summer-autumn community, and winter community.

[Figure 3: see original paper] Non-metric multi-dimensional scaling (MDS) ordination of zoobenthos communities in Sanhe Reservoir

### 5.5 Correlation Analysis Between Zoobenthos and Water Environmental Factors

Pearson correlation analysis showed that total zoobenthos density was significantly negatively correlated with water temperature, turbidity, and chlorophyll-a content ($P < 0.05$), and significantly positively correlated with dissolved oxygen and total phosphorus ($P < 0.05$). The dominant genus *Tubifex* was significantly negatively correlated with water temperature and chlorophyll-a ($P < 0.05$), and significantly positively correlated with nitrate nitrogen ($P < 0.05$). *Clinotanypus* was significantly positively correlated with dissolved oxygen, ammonia nitrogen, and total phosphorus ($P < 0.05$). *Branchiura* was significantly negatively correlated with salinity ($P < 0.05$). Other zoobenthos groups showed no significant correlations with water environmental factors.

Pearson correlation coefficients for the relationship between zoobenthos density and environmental factors

### 5.6 Redundancy Analysis of Zoobenthos and Water Environmental Factors

Detrended correspondence analysis (DCA) of zoobenthos individuals showed a maximum gradient length of 2.027 for four axes, indicating that redundancy analysis (RDA) was appropriate for analyzing zoobenthos data and environmental factors. The RDA ordination diagram showed that the genus *Tubifex*, which had high abundance in all four seasons, was influenced by physicochemical factors and nutrient concentrations. The other two dominant groups, *Branchiura* and chironomid larvae, were distributed in the fourth quadrant. Total nitrogen and dissolved oxygen showed positive correlations. Monte Carlo tests identified ammonia nitrogen, salinity, pH, and turbidity as factors with significant effects on zoobenthos distribution ($P < 0.05$). Total nitrogen was a marginally significant factor ($P = 0.08$). Nitrate nitrogen and other physicochemical factors showed no significant effects on zoobenthos distribution.

[Figure 4: see original paper] Ordination diagrams of redundancy analysis between zoobenthos assemblage and water environmental factors

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## 6. Discussion

### 6.1 Zoobenthos Community Composition

Previous studies have reported that lake substrate and submerged vegetation distribution significantly affect mollusk distribution [16]. For example, the mollusk *Corbicula* prefers sandy substrates and is least abundant in clay substrates [17]. The mutualistic theory suggests that snails and epiphytic algae on aquatic plants can form mutualistic systems, where epiphytic algae provide food for snails [18]. Research has also found that mollusk scarcity is a common phenomenon in reservoirs [19-20]. Studies on macrozoobenthos have only collected mollusks in shallow littoral zones [19], with mollusk distribution being more concentrated in littoral zones than in pelagic zones [21].

Studies by Ma Xufa et al. in Daoguanhe Reservoir, Hubei, indicated that organic sedimentation from aquaculture can alter zoobenthos structure and function [22]. In high-density aquaculture water bodies, macrozoobenthos composition is simple, dominated by oligochaetes and chironomid larvae [23]. This study only collected oligochaetes and aquatic insects in Sanhe Reservoir, with only a few *Anodonta* and *Bellamya* found in the reservoir' s littoral shallow areas and dam rubble zones. The main reason is that Sanhe Reservoir is a small reservoir primarily stocked with silver and bighead carp, with muddy bottom substrate and no submerged vegetation distribution at sampling sites located in pelagic zones. Based on these results, we can infer that in the pelagic zones of reservoirs with high-density fish stocking, organic sedimentation and lack of submerged vegetation create unsuitable habitats for mollusks such as shellfish and snails. The habitat suitable for mollusks is relatively narrow, resulting in zoobenthos communities dominated by oligochaetes and insect larvae.

### 6.2 Seasonal Dynamics of Zoobenthos Communities

Zoobenthos communities in Sanhe Reservoir showed significant seasonal differences, with density and biomass in summer and autumn being significantly lower than in spring and winter. These results are similar to findings by Jiang Wanxiang et al. [10] and Chen Libin et al. [24], but differ from reports by Shu Fengyue et al. [25]. The former two studies reported that zoobenthos was mainly composed of oligochaetes and aquatic insect larvae, with mollusks contributing little to density and biomass. Their research also found lower zoobenthos density in summer and autumn and higher density in spring and winter. However, due to the presence of mollusks, zoobenthos biomass peaked in autumn [25].

Reports indicate that oligochaete reproduction occurs mainly in winter and spring [26]. Some chironomid larvae, such as *Tokunagayusurika sexpapilosa* and *Propsilocerus akamusi*, emerge in summer and autumn [27], leading to decreased zoobenthos density [25]. Certain chironomid larvae exhibit vertical migration habits, diving deeper in warm seasons and moving upward in cold seasons [24,28], which contributes to lower zoobenthos density in summer and autumn. This pattern relates both to life history differences between oligochaetes and chironomid larvae [26] and to their behavioral habits, such as vertical migration [29]. Although chironomid larvae can reproduce from spring to autumn, their density was highest in winter in Sanhe Reservoir.

These results differ from other reservoir studies [30-31] and are necessarily linked to fishery aquaculture practices. Fish fry are stocked in spring, and their predation on zoobenthos reduces density in summer and autumn. After fishing in late autumn, fish standing crop in the reservoir decreases substantially, reducing predation pressure on zoobenthos in winter and resulting in high densities of oligochaetes and aquatic insect larvae.

### 6.3 Effects of Environmental Factors on Zoobenthos Communities

Water physicochemical properties directly affect zoobenthos communities. Studies have reported that oligochaetes, mollusks, and chironomid larvae prefer alkaline water bodies [8], while extremely high or low pH reduces zoobenthos reproductive capacity, density, and biomass [32]. This study found that pH had significant effects on seasonal distribution of zoobenthos in Sanhe Reservoir. Other reports have identified dissolved oxygen levels in lake hypolimnion as important factors affecting zoobenthos distribution [33-34]. This study found a significant positive correlation between zoobenthos density and dissolved oxygen content in Sanhe Reservoir.

Jiang Wanxiang et al. found that significant influencing factors for macrozoobenthos communities differed among seasons, with water conductivity being the main factor in summer and autumn [10]. Pearson correlation analysis in this study showed that salinity was significantly negatively correlated with zoobenthos density ($P < 0.05$), consistent with previous research.

Water nutrient content also importantly affects zoobenthos density and distribution. Studies have shown positive correlations between zoobenthos density and phosphorus content in lakes [9]. This study found that nutrient content in Sanhe Reservoir increased significantly from autumn to winter, with ammonia nitrogen and total phosphorus peaking in winter. This increase may result from: (1) vertical water exchange in winter releasing nitrogen and phosphorus from sediment into the upper water column [35]; and (2) reduced algal density and nutrient utilization by primary producers due to winter temperature and light conditions [36]. The increased nutrient content in winter is an important factor contributing to the highest zoobenthos density and biomass in this season. Pearson correlation analysis showed positive correlations between zoobenthos density and total nitrogen and nitrate nitrogen, with only total phosphorus having a significant effect, consistent with reports by Lü Guangjun et al. [9].

In addition to water physicochemical factors and nutrients, fish predation directly affects zoobenthos communities. According to fishing records, the reservoir contractor stocks large numbers of silver and bighead carp fingerlings in March [37]. Fish metabolism is vigorous in autumn, creating strong predation pressure on zoobenthos. Additionally, the reservoir contains some benthivorous fish such as *Pelteobagrus fulvidraco*. Therefore, fish feeding activity is a direct cause of significantly lower zoobenthos density in summer and autumn compared to winter and spring.

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*Note: Figure translations are in progress. See original paper for figures.*