Impact of Spatial and Environmental Variables on Aquatic Macroinvertebrates in Hilly Ponds

Dan Zhang
Chongqing University

Wang Kehong
Chongqing Three Gorges University

Zhang Guanxiong
Qufu Normal University

Liu Shuangshuang
Chongqing University

Wang Fang
Chongqing University

Pan Yuanzhen
Chongqing University

Yuan Xingzhong (✉️ 1072000659@qq.com)
Chongqing University

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Abstract

Ponds are “islands” of independent habitats and research hotspots of regional biodiversity. This paper got some basic information about habitat conditions and aquatic macroinvertebrates in hilly ponds. Then we tried to provide a theoretical basis for the regional biodiversity conservation and sustainable development of the pond networks among basins. In this paper, the environmental and spatial variables concerned about aquatic macroinvertebrates in the ponds with different small basins of Liangping District, which is located in the east of Sichuan Basin, China, were investigated. The results showed that drainage basin effect did not occur among basins. The aquatic macroinvertebrates in the hilly ponds were mainly affected by altitude, nutrients, pH and other environmental factors. Within the basin scale, pond isolation was also one of the important factors affecting community structure. Considering the spatial pattern of the macroinvertebrate assemblages in the hilly ponds, the protection and management of those ponds need to focus on pond network other than a single pond, improve the habitat quality of the ponds, and ensure the cost-effectiveness and ecological benefits.

1 Introduction

As the complex terrain, changeable climate and extremely uneven rainfall in hilly areas of China, agriculture was limited and often depended on the utilization of water infrastructures in these areas. Due to the limitation of terrain, it is difficult to build large-scale water infrastructures in hilly areas, while it is more suitable to build small and scattered water infrastructures. Thus a large number of ponds as water infrastructures are constructed as important facilities for agricultural production. In addition to irrigation, ponds are also used for aquaculture and recreation etc.. In recent years, more attention has been paid to the ecological value of ponds in China, and the related researches are increasing year by year (Fu, Xu, Wang, Yan, & Chaudhary, 2018; Li et al., 2013; Liu, Baoligao, Wu, Cu, & Mu, 2019; Sun et al., 2019). However, the understanding of ponds in hilly areas is obviously insufficient. It is still necessary to understand the characteristics of the assemblages and its impact factors.

Freshwater organisms are disappearing sharply around the world, and many aquatic ecosystems and its assemblages in the region are becoming more and more similar or homogeneous (Petsch, 2016). However, spatial and temporal variations on biodiversity of aquatic organisms, especially for aquatic macroinvertebrates, had been poorly studied within basin (McLean, Mushet, Sweetman, Anteau, & Wilttermuth, 2020). Ponds are scattered and relatively independent freshwater ecosystems that support many important species of macroinvertebrates. The important driving forces for the composition of these assemblages include environmental and spatial factors (Free et al., 2009). Environmental factors include area (Krasznai-K, Boda, Borics, Lukacs, & Varbiro, 2018), pH (Biggs et al., 2005), nutrients (Kadoya, Akasaka, Aoki, & Takamura, 2011), transparency (Robin et al., 2014), DO (Sun et al., 2018), altitude (Mabidi, Bird, & Perissinotto, 2017), etc.. While spatial factors include isolation (McAbendroth, Foggo, Rundle, & Bilton, 2005), connectivity (Iversen, Rannap, Briggs, & Sand-Jensen, 2017), etc..
A key challenge for biogeography and conservation biology is to understand spatial patterns and environmental drivers of freshwater biodiversity and community structure (Soininen, 2016). The ignorance about spatial process and environmental filters of aquatic macroinvertebrate assemblages in hilly ponds with lower altitude prevents regional ecological protection. A large number of natural ponds in hilly area have been declining due to over exploitation of human. Whether artificial ponds can offset the ecological losses caused by the disappearance of natural ponds need to be studied as soon as possible. Those studies determine the biodiversity and conservation values of these neglected and increasingly threatened habitats. To explore the composition and structure of aquatic macroinvertebrate assemblages in the ponds of hilly area and its relationship with environmental and spatial filters, it is hypothesized that: (1) Drainage basin effect of macroinvertebrates from the artificial ponds in hilly areas exists among different basins; (2) The aquatic macroinvertebrate assemblages in the artificial ponds are mainly driven by altitude, nutrients and pH; (3) Spatial processes can affect the diffusion and distribution of aquatic macroinvertebrate assemblages in hilly area. The results can help us understand the community characteristics and its impact factors of aquatic macroinvertebrates in the hilly ponds, provid basic data for regional ecological protection in hilly areas. Additionally, we can offer reasonable insights of basin management for pond managers.

2 Methods

2.1 Study area

Liangping district is located in the parallel ridge valley area in the east of Sichuan Basin. It belongs to subtropical monsoon climate with high humidity. The pH value of soil is 5.5–7.5. The main characteristics of rivers in Liangping are short length, small catchment and unstable runoff. Precipitation is the main resource of the surface water in Liangping.

2.2 Basin division and sampling sites

Basin division was based on DEM data with 1 m spatial resolution in Liangping district by ArcGIS 10.4.1 (Fig. 1 (d)). According to the basin division map, three different basins were selected, and then 40 ponds were randomly selected as sampling sites in each basin to get environmental and spatial information (Fig. 1). A total of 120 ponds were collected with both water samples and aquatic macroinvertebrate samples. The sampling time was June 24–29, 2020 (late spring). The sampling ponds were mainly constructed in the process of agricultural production and living, which were artificial or semi-artificial ponds, and usually with permanent inundation. The main functions of those ponds were aquaculture, irrigation, baitfish culture and landscape, etc..

2.3 Environmental and spatial data

Environmental variables: altitude and area data were extracted in ArcGIS based on DEM data and remote sensing image map with resolution of 1m in Liangping district. The transparency of sampling sites was measured by Secchi disk. During the campaign, the coverage of aquatic macrophytes was estimated, and
the information of the main functions, aquaculture situation, water depth were investigated by the manager of each pond. A 1L water sampler was used to collect water samples at different sites of each pond for 5–8 times, and then stored in a 10L sampling container. A 500 ml sampling bottle of water was sampled in the 10 L sampling container and stored in a 4 °C incubator. Then dissolved oxygen (DO), water temperature (T), pH and conductivity were measured in the 10 L sampling container by HACH HQ30d, USA. Total nitrogen (TN), total phosphorus (TP), ammonium (NH$_4^+$), nitrate (NO$_3^-$) and nitrite (NO$_2^-$) were measured by the standard Chinese method after the water samples were brought back to the laboratory (Standard Method for the Examination of Water and Wastewater Editorial Board, 2002).

Spatial variables: Pond isolation is the number of other water bodies within 500m. Pond connectivity is the number of water bodies hydrologically connected to the sampling site by surface connection (Waterkeyn, Grillas, Vanschoenwinkel, & Brendonck, 2008). Pond isolation and connectivity were extracted from the remote sensing image with resolution of 1 m in Liangping district and field observation.

### 2.4 Macroinvertebrate field surveys

The sampling of aquatic macroinvertebrates was carried out by a pond net (diameter, 30cm; 0.25cm, mesh size). The samples were collected in different microhabitats of each pond for 3 min (Briers & Biggs, 2005). Then the samples were stored in 80% alcohol and brought back to the laboratory. The samples were identified under microscope, and identified to the lowest level by using relevant taxonomic keys (Keast, 1994), or consulting relevant experts.

### 2.5 Statistical methods

One-way ANOVA was used to identify the significance of environmental and spatial variables among different basins. One-way ANOVA was both performed by IBM SPSS Statistics 25. The community structure of aquatic macroinvertebrates was represented by taxon richness, the Shannon-Wiener diversity index and relative abundance. Principal component analysis (PCA) was used to compare the community structure similarity of aquatic macroinvertebrates in different ponds. Principal component analysis was performed in Past3. Canonical correlation analysis (CCA) was used to analyze the correlation between variables and aquatic macroinvertebrates. Before canonical correlation analysis, the data of aquatic macroinvertebrates were analyzed by detrended correspondence analysis (DCA) to confirm a unimodal rather than linear distribution. Then environmental variables that had significant correlation with macroinvertebrate assemblages were selected by a Monte Carlo permutation test. Canonical correlation analyses were carried out by the package CANOCO 5.

### 3 Results

#### 3.1 Environmental and spatial variables
The results of environmental and spatial variables of the ponds in Liangping district are shown in Table 1. Total phosphorus had no significant differences among the three basins, and the concentration of TP ranged from 0.09 to 0.55 mg/L. The concentration of TN in basin 2 was significantly lower than that of in basin 1 and basin 3, and the concentration of TN in three basins was 0.68–7.74 mg/L. Total nitrogen was mainly composed of ammonium and nitrate. The concentration of ammonium in basin 2 was also significantly lower than that of in basin 1 and basin 3. The concentration of ammonium in basin 2 ranged from 0.15 to 1.88 mg/L. There were significant differences in water temperature, DO and transparency among the three basins. The highest value of water temperature, DO and transparency were in basin 1, and the lowest values of those three variables were in basin 3. The water temperature of basin 1, 2 and 3 were 27-36.2 °C, 25.2–35.2 °C, 24.3–27.3 °C, respectively. The DO of basin 1, 2 and 3 were 5.42–20.33 mg/L, 4.42–17.04 mg/L, 2.94–10.98 mg/L, respectively. The transparency of basin 1, 2 and 3 were 10–57 cm, 12–45 cm and 8–48 cm, respectively. The average water depth of basin 3 was higher than that of in basin 1 and 2, and the average water depth of basin 3 was 1.37 m. The overall pH value of basin 3 was weakly acidic, while that of in basin 1 and 2 was weakly alkaline. The conductivity of basin 3 was significantly lower than that of in basin 1 and 2, the conductivity of basin 3 was ranged from 64.6–738 µs/cm. The area of the ponds in basin 2 was lower than that of in basin 1 and 3. The area of the ponds in basin 1 was 274–6581 m². The area of the ponds in basin 2 was 62-3409 m². The area of the ponds in basin 3 was 290–5302 m². There was no significant difference in altitude among the three basins, but the altitude gradient among sampling sites in basin 2 was relatively wider. The altitude of sampling sites in basin 1 was 439–519 m, and the altitude gradient was 80 m. The altitude of sampling sites in basin 2 was 254–755 m, and the altitude gradient is 501 m. The altitudes of sampling sites in basin 3 ranged from 411 to 553 m, with an altitude gradient of 142 m. The coverage of aquatic macrophytes in the ponds was low and the composition of macrophytes was simple. Macrophytes were mainly composed of Alternanthera philoxeroides. In several ponds, there had Typha orientalis, Najas minor, Potamogeton natans, Potamogeton crispus, Nymphoides peltata and other aquatic macrophytes. Pond isolation in basin 2 was significantly lower than that of in basin 1 and 3, indicating that there were fewer ponds and less adjacent aquatic ecosystems in basin 2. There had 0–27 other aquatic ecosystems within 500 m of the sampling ponds as shown in pond isolation. There were 0–15 other aquatic ecosystems hydrologically had surface connection to the sampling ponds as shown in pond connectivity.
Table 1
Environmental and spatial variables of the three basins.

|       | NH$_4^+$ (mg/L) | NO$_2^-$ (mg/L) | NO$_3^-$ (mg/L) | TN (mg/L) |
|-------|-----------------|-----------------|-----------------|-----------|
| Basin 1 | 0.82 ± 0.39a    | 0.0020 ± 0.0009a| 1.05 ± 0.43a    | 2.28 ± 0.90a |
| Basin 2 | 0.69 ± 0.45b    | 0.0019 ± 0.0008a| 0.89 ± 0.58b    | 1.89 ± 1.01b |
| Basin 3 | 0.93 ± 0.62a    | 0.0019 ± 0.0005a| 1.06 ± 0.64ab   | 2.37 ± 1.33a |

|       | TP (mg/L) | T (°C) | Average water depth (m) | pH      |
|-------|-----------|--------|-------------------------|---------|
| Basin 1 | 0.28 ± 0.10a | 32.4 ± 2.1a | 1.5 ± 0.6a                   | 8.96 ± 0.62a |
| Basin 2 | 0.27 ± 0.12a | 29.9 ± 3.0b | 1.6 ± 0.7a                   | 8.77 ± 0.46a |
| Basin 3 | 0.29 ± 0.11a | 26.3 ± 0.7c | 1.9 ± 0.5b                   | 6.46 ± 0.73b |

|       | DO (mg/L) | Conductivity (µs/cm) | Transparency (cm) | Area (m$^2$) |
|-------|-----------|----------------------|------------------|-------------|
| Basin 1 | 10.83 ± 3.67a | 324.1 ± 101.2a       | 34 ± 8a           | 1813 ± 1593a |
| Basin 2 | 8.94 ± 2.78b | 340.9 ± 116.0a       | 28 ± 9b           | 663 ± 656b   |
| Basin 3 | 6.12 ± 1.81c | 236.3 ± 109.8b       | 19 ± 8c           | 1446 ± 1144a |

|       | Altitude (m) | Coverage of aquatic macrophyte (%) | Pond isolation | Pond connectivity |
|-------|--------------|------------------------------------|----------------|------------------|
| Basin 1 | 460 ± 18a   | 4 ± 10a                             | 6 ± 4a         | 2 ± 2a           |
| Basin 2 | 468 ± 157a  | 9 ± 20a                             | 4 ± 2b         | 1 ± 1b           |
| Basin 3 | 454 ± 34a   | 4 ± 10a                             | 7 ± 6a         | 1 ± 2ab          |

*Data in the Table are Mean ± SE. Different letters represent the significant difference at $p < 0.05$.

3.2 Spatial pattern of macroinvertebrates

A total of 46 aquatic macroinvertebrates were collected, that belonged to 25 families and 41 genera and 46 species (Table 2). Forty-one species were collected in basin 2, while 32 and 31 species were collected in basin 1 and basin 3, respectively. Nine taxa of Gastropoda, one taxon of Crustacea and thirty-six taxa
of Insecta were collected in this experiment. Thirteen taxa of Insecta belonged to Chironomidae. Those taxa of *Parafossarulus* sp., *Dytiscus marginalis*, Gyrinidae sp., *Tanytarsus* sp., *Chironomus kiiensis*, *Micropsecra* sp. and *Eukiefferiella brehmi* were only collected in basin 2.
Table 2
Spatial pattern of aquatic macroinvertebrate assembles in different basins.

| Taxa                  | Basin 1 | Basin 2 | Basin 3 | Taxa                  | Basin 1 | Basin 2 | Basin 3 |
|-----------------------|---------|---------|---------|-----------------------|---------|---------|---------|
| *Bellamya* sp.        | +       | +       |         | *Scirtidae* sp.       |         |         | +       |
| *Viviparus* sp.       | +       | +       |         | *Stratiomys* sp.      | +       | +       | +       |
| *Cipangopaludina* sp. | +       | +       | +       | *Ranatra* sp.         | +       | +       | +       |
| *Gyraulus* sp.        | +       | +       |         | *Laccotrephes japonensis* | +       |         |         |
| *Polypylis* sp.       | +       | +       | +       | *Diplonychus esakii*  | +       | +       | +       |
| *Physa* sp.           | +       | +       |         | *Sigara* sp.1         | +       | +       | +       |
| *Radix* sp.           | +       | +       | +       | *Sigara* sp.2         | +       | +       | +       |
| *Galba pervia*        | +       |         |         | *Gerris* sp.          | +       | +       | +       |
| *Parafossarulus* sp.  | +       |         |         | *Hydroptila* sp.      | +       | +       |         |
| *Caridina* sp.        | +       | +       | +       | *Culicidae* sp.       | +       | +       | +       |
| *Baetis* sp.          | +       | +       |         | *Chironomus* sp.      | +       | +       |         |
| *Megalestes* sp.      | +       | +       | +       | *Dicrotendipes* sp.   | +       | +       | +       |
| *Pseudothemis* sp.    | +       | +       | +       | *Dicrotendipes* tritomus | +       | +       | +       |
| *Macromidia* sp.      | +       | +       |         | *Polypedilum* sp.     | +       | +       |         |
| *Anax* sp.            | +       | +       | +       | *Parachironomus* sp.  | +       | +       | +       |
| *Anotogaster* sp.     |         |         | +       | *Chironomus anthracinus* | +       | +       |         |
| *Cybister* sp.1       | +       | +       | +       | *Tanytarsus* sp.      | +       |         |         |
| *Cybister* sp.2       |         | +       |         | *Chironomus kiiensis* | +       |         |         |
| *Dytiscus marginalis* | +       |         |         | *Micropsectra* sp.    | +       |         |         |
| *Haliplus* sp.        | +       | +       |         | *Cricotopus* sp.      | +       | +       | +       |
| *Typhaea* sp.         | +       | +       |         | *Polypedilum laetum*  | +       | +       | +       |
| *Spercheus emarginatus* | +     | +       | +       | *Krenosmittia* sp.    | +       |         |         |
| *Gyrinidae* sp.       |         | +       |         | *Eukiefferiella brehmi* | +       |         |         |
According to Table 3, the mean taxon richness and Shannon-Wiener index of basin 3 in Liangping district were significantly lower than those in basin 1 and basin 2. The taxon richness of aquatic macroinvertebrates in ponds of basin 1 ranged from 3 to 15. The taxon richness of aquatic macroinvertebrates in ponds of basin 2 ranged from 3 to 19. The taxon richness of aquatic macroinvertebrates in ponds of basin 3 ranged from 3 to 14.

Table 3

|                  | Basin 1 | Basin 2 | Basin 3 | total |
|------------------|---------|---------|---------|-------|
| total taxon richness | 32      | 41      | 31      | 46    |
| mean taxon richness    | 7 ± 3a  | 8 ± 4a  | 6 ± 3b  | 7 ± 4 |
| Shannon-Weiner index  | 1.45 ± 0.38a | 1.55 ± 0.47a | 1.24 ± 0.35b | 1.41 ± 0.42 |
| % of total taxon richness supported | 70%  | 89%      | 67%      | 100%  |

*Data in the Table are Mean ± SE. Different letters represent the significant difference at \( p < 0.05 \).

As shown in Fig. 2, the aquatic macroinvertebrate assemblages in all basins were mainly composed of Mollusca and Insecta, and the dominant species of Insecta including Odonata, Hemiptera, Coleoptera and Diptera. Diptera accounted for 31% and 29% of the macroinvertebrate assemblages in basin 1 and 2, respectively. In basin 3, Hemiptera and Diptera both accounted for 23% of the macroinvertebrate assembles.

3.3 Principal component analysis based on aquatic macroinvertebrate profiles

The first two PCs explained 52.78% of the total variance (Fig. 3). There was little significant difference in macroinvertebrate assemblages among basins. Meanwhile, macroinvertebrate assemblages in several ponds were significantly different from that of other ponds. The loading values of different species for the first two PCs were shown in Fig. 4. As shown in Figs. 3 and 4, the ponds 1–6, 1–7 and 1–40 in basin 1 were mainly affected by *Baetis* sp. and *Sigara* sp.2 along PC2, which resulted in significantly different from other ponds. In basin 2, ponds 2–12 and 2–14 were mainly affected by *Sigara* sp.1 and *Sigara* sp.2 along PC1, which resulted in significantly different from other ponds. In basin 3, ponds 3–14, 3–17, 3–20 and 3–29 were mainly affected by *Sigara* sp.1 and *Sigara* sp.2 along PC1, which resulted in significantly different from other ponds.

3.4 Relationship between variables and aquatic macroinvertebrates
The longest length of the DCA axis for aquatic macroinvertebrate data in basin 1, 2 and 3 were 3.54, 3.53 and 3.73, respectively. The results indicated that CCA can be used to characterize the relationship between macroinvertebrate assemblages and variables with significant relationship. The first axis of DCA explained 16.61%, 11.61% and 17.17% of aquatic macroinvertebrate profiles in basin 1, 2 and 3, respectively. The other three axes of DCA explained 20.18%, 21.09% and 22.63% of the variables in basin 1, 2 and 3, respectively. There were 6, 3 and 2 variables in basin 1, 2 and 3 respectively, which had significant correlation with aquatic macroinvertebrate assemblages ($p \leq 0.05$). According to CCA plot of basin 1, the eigenvalues of the first axis and the second axis were 0.1628 and 0.1257, respectively. Environmental and spatial variables accounted for 23.7% of the variation information of species composition. For CCA of basin 2, the eigenvalues of the first axis and the second axis were 0.1679 and 0.1256, respectively. Environmental and spatial variables accounted for 13.7% of the variation information of species composition. According to CCA of basin 3, the eigenvalues of the first axis and the second axis were 0.1689 and 0.0405 respectively. Environmental and spatial variables accounted for 8.9% of the variation information of species composition.

In CCA plot of basin 1 (Fig. 5 (a)), transparency, DO, NH$_4^+$, NO$_2^-$, TN and pond isolation were significantly correlated with species composition. Several species of Chironomidae, such as *Chironomus* sp., *Cricopopus* sp., *Polyphemus laetum*, and *Gyraulus* sp., showed strong tolerance of nutrient pollution, and were positively correlated with NH$_4^+$, NO$_2^-$, TN and transparency. The species which were sensitive to nutrients and transparency included Culicidae sp., *Spercheus emarginatus* and *Diplonychus esakii*, etc.. The lower value of pond isolation, the less number of other aquatic ecosystems within 500 m. The negative correlation between species and pond isolation might be explained by the strong migration ability of the species. *Cybister* sp.1 and *Sigara* sp.1 both showed negative correlation with pond isolation. In basin 1, the species composition was also affected by DO, and the species had positive correlation with DO included *Stratiomys* sp., *Dicrotendipes* sp., etc.. In the CCA plot of basin 2 (Fig. 5 (b)), altitude, area and pond isolation had significant correlation with species composition. The gradient of altitude among sampling sites in basin 2 was larger than that of in basin 1 and 3. The results showed that there was a positive correlation between altitude and *Dicrotendipes tritomus*, Culicidae sp., *Baetis* sp., *Pseudothemis* sp.. Meanwhile those species had negative relationship with pond isolation. *Chironomus* sp., *Physa* sp., *Galba pervia* showed positively correlation with area. In CCA plot of basin 3 (Fig. 5 (c)), pH and T were the main variables affecting species composition. Species with a positive correlation with pH were more adaptable to alkaline environment, such as *Spercheus emarginatus* and *Physa* sp.. Species with negative relationship with pH were more suitable for acidic environment, such as *Diplonychus esakii*, *Haliplus* sp. and *Dicrotendipes* sp.. *Bellamya* sp. and *Pseudothemis* sp. were positively correlated with T, showed a preference for warmer environment. *Megalestes* sp. and *Stratiomys* sp. were negatively correlated with T, and preferred cold environment.

### 4 Discussion

#### 4.1 Macroinvertebrate assembles
Forty-six taxa of aquatic macroinvertebrates belonging to 41 genera and 25 families were collected in hilly ponds of Liangping District in late spring. They were dominated by Diptera, but less Crustacea, Ephemeroptera and Trichoptera. Compared with other ponds, the taxa of macroinvertebrates were less, and the composition of the assemblages was different. For example, a survey of macroinvertebrate communities in 51 alpine lakes and ponds in Spain identified 84 taxa with an average richness of 18.8 (Garcia-Criado, Martinez-Sanz, Valladares, & Fernandez-Alaez, 2017). A total of 228 taxa of macroinvertebrates belonging to 68 families and 21 orders were found in 95 urban and rural ponds in the UK, among which Coleoptera was dominant (Hill, Heino, White, Ryves, & Wood, 2019). Based on the investigation of 30 urban and rural ponds in the UK, 192 taxa of 14 orders were collected, among which Coleoptera was dominant (Thornhill, Batty, Death, Friberg, & Ledger, 2017). The taxa collected in this experiment were less, which might be due to the high concentration of nutrients in the ponds. The diversity of aquatic macroinvertebrates was usually negatively correlated with the concentration of nutrients. We found that the concentration of TN was higher than 2 mg/L, and the concentration of TP was higher than 0.2 mg/L in most ponds, which meant on the state of eutrophication. Figure 5(a) also showed that a large number of invertebrates were inhibited by nitrogen. In this experiment, the major function of most ponds was aquaculture. The management behavior of managers, such as feeding and disinfection, would also have a strong interference on the aquatic food chain, thus reducing its biodiversity. Therefore, the results of this experiment identified a subset of aquatic macroinvertebrate assemblages in hilly region, and also showed that the value of the regional biodiversity was damaged. It had great potential to improve the habitat quality and ecosystem services of the ponds.

4.2 Spatial pattern

With the increase of physical distance, the limitation of taxa diffusion would increase, which might form spatial correlates (Legendre and Legendre, 1998). Therefore, we usually assumed that habitats with closer distance had more similar communities than habitats that were far apart, and the intensity of their impact depended on the characteristics of individual diffusion ability (Razeng et al., 2016). For the study of freshwater organisms, the study area might be a single basin or basins. It was generally believed that biological diffusion was more likely to occur within a basin than among basins (Jani Heino et al., 2015). Therefore, the spatial correlates of species are more likely to occur among basins. Aquatic assemblages can be affected by both spatial and environmental variables. However, it is not clear at what spatial scale, spatial correlates is more likely to occur. It is generally believed that larger spatial scales are more prone to spatial correlates. Based on the investigation of aquatic organisms in three basins in Finland with a total area of 63609 km², it found that the aquatic communities in different basins were mainly determined by the environmental variables and basin effects (Jani Heino, Soininen, Alahuhta, Lappalainen, & Virtanen, 2017). Based on the investigation of aquatic insects in the ponds of Stockholm, Sweden, which covers an area of 2700 km², little significant correlation was found between spatial distribution and diversity of aquatic insect (J. Heino et al., 2017). However, some studies had shown that the scale of study area did not necessarily correlate with spatial correlates. For example, the spatial correlates of taxa in the ponds of Oxford County was reported which study area was 2608 km² (Briers & Biggs, 2005). However, in the survey of 51 alpine lakes and ponds in Castilla y León of Spain with an
administrative area of 94223 km$^2$, no spatial correlates of taxa was reported in most areas (Garcia-Criado et al., 2017). This supports the hypothesis that a common typology can be applied to a group of aquatic ecosystem with irregular distribution in a large-scale region. The investigation area of this experiment was 1892.13 km$^2$, and the spatial correlates of taxa were not obvious. There are two possible reasons for this phenomenon. One is that compared with other spatial correlates studies, the scale of the study area is smaller, the geographical barrier of species among basins is smaller, and it is easy for macroinvertebrates to diffusion. Although geographical factors were the main factors controlling the composition structure of macroinvertebrates in wetlands, but the differences among basins in small-scale region were negligible (Batzer & Ruhi, 2013). Second, the altitude of the study area is low. Biogeographical effects (among basins), such as historical effects and climate stress, have little impact on aquatic communities in lowland areas. In lowland areas, there might be similar communities in different basins (Hoeinghaus, Winemiller, & Bimbaum, 2007).

4.3 Impact of environmental and spatial variables on the assembles

In large-scale research, spatial correlation is more likely to occur. However, in small-scale and scattered wetlands, the spatial variation of aquatic macroinvertebrate community was mostly explained by local environmental variables (Tornwall, Pitt, Brown, Hawley-Howard, & Baldwin, 2020). This experiment involved area of 1892.13 km$^2$. The differences of altitude and surrounding habitat led to the differences of habitat conditions among sampling sites. Meanwhile, there were different environmental factors affecting the aquatic macroinvertebrate community in different basins. Nutrients had a certain influence on the distribution of macroinvertebrates in ponds (Smith, Vaala, & Dingfelder, 2003). It was widely recognized that high concentrations of nutrients could reduce the richness and diversity of aquatic macroinvertebrates (Thornhill et al., 2017). In particular, species that were sensitive to high concentration of nutrients, such as Culicidae sp., Spercheus emarginatus and Diplonychus esakii, decreased with the increasing concentration of nutrients. Dissolved oxygen is another factor affecting the distribution of aquatic macroinvertebrates. For different species, the influence of DO is different. Studies had shown that DO was an important factor of snail distribution, with positive correlation (Vansomeren, 1946) and negative correlation (Hurley, Hearnden, & Kay, 1995). Dissolved oxygen may have a great impact on dragonflies. Larvae of dragonfly mainly relied on DO to breathe (Griffiths C, Day J & Picker M, 2015). In addition, higher diversity of water beetles in ponds was associated with lower dissolved oxygen, which might indicate the presence of carnivorous vertebrates (Deacon, Samways, & Pryke, 2018). In this paper, the difference with these studies was that DO was only significantly correlated with macroinvertebrate assemblages in basin 1. Gastropods, Odonata and water beetles had little correlation with DO. Area has always been one of the important factors to aquatic organisms in ponds. The area of ponds can affect the microhabitat structure and number. Based on the traditional island biogeography (MacArthur and Wilson 1967), it could be concluded that high quality and large ponds had higher biodiversity, including biodiversity of invertebrates. According to the habitat diversity hypothesis, the impact of area on species richness is mainly realized through biodiversity. The larger the area, the greater the species diversity
(Ricklefs & Lovette, 1999). The habitats with large area, permanent inundation and alkalinity tended to have high biodiversity (Hoverman et al., 2011). However, some studies had shown that a group of small ponds was a key contributor to the biodiversity of regional invertebrates (Boix et al., 2012; Scheffer et al., 2006; Wood, Greenwood, Barker, & Gunn, 2001). The decrease of invertebrate biodiversity in the larger ponds might be due to the increase of fish and waterfowl in the ecosystem, which led to the exclusion of some invertebrates and the decrease of plant complexity (Schilling, Loftin, & Huryn, 2009). Or several ponds could increase the number of niche (P. Williams et al., 2004). Study showed that when the area gradient was narrow, the correlation between area and macroinvertebrate community might not be significant (Moraes, Stenert, Rolon, & Maltchik, 2014). However, it was interesting that the gradient of pond area in basin 2 was the narrowest, but the significant correlation between area and species only occurred in basin 2 in this paper. The gradient of altitude among ponds in basin 2 was 501 m, which was larger than the other two basins. There was a significant correlation between species and altitude in basin 2. This proves that there is a significant correlation between altitude and assemblages in low altitude areas. In addition to the above factors, temperature also has a significant impact on the spatial distribution of invertebrates. Among all organisms of wetland, invertebrates might be one of the animals most affected by temperature, because as ectotherms, their life cycle and growth rate were controlled by temperature (Kingsolver et al., 2011).

(1) pH

Another environmental factors as pH usually had a significant impact on the invertebrate assemblages in ponds (da Rocha et al., 2016; Epele & Miserendino, 2016; Mabidi et al., 2017), but the relationship between them was less investigated in field (Spyra, 2017). Variation of pH could cause changes in food supply and affect the growth, development and survival of species indirectly (Friday, 1987). Different aquatic macroinvertebrates have different sensitive degree to pH. Larvae of dragonfly were sensitive to different pH gradients, as *Anax* had a wide range of pH (Jooste, Samways, & Deacon, 2020). One of the environmental problems faced by invertebrates in ponds is acidification. In the past decades, the negative effects of acidification on function and diversity of ecosystem had become more and more obvious (Guerold et al., 2000). Acidification induced by human had a greater impact on snails than natural acidification (Petrin, Laudon, & Malmqvist, 2007). Mollusca were the most sensitive species to acidification, so they were greatly affected by pH (Skowronska-Ochmann, Cuber, & Lewin, 2012; Sowa, Krodkiewska, Halabowski, & Lewin, 2019). In the study of forest ponds, 14 gastropods were collected in alkaline ponds (pH > 7.2), 18 gastropods were collected in neutral ponds (pH 6.8–7.2), 18 gastropods were collected in acidic ponds (pH 6 ≤ pH < 6.6), and 8 gastropods were collected in acidic pond system (pH 4.4-6) (Spyra, 2017). When the pH value of water was lower than 5.2, gastropods might not survive (Singh & Agrawal, 2008). There was no reference value for the pH tolerance range of gastropods, and it was considered that 5.5–9.5 was the adaptive range of gastropods (Spyra, 2017).

Three aspects show the effect of pH on invertebrates in ponds as follows. First, a large number of studies had shown that the higher pH value was, the greater taxa richness of invertebrates in ponds was (J. Heino, 2000; Kochjarova et al., 2017; McDevitt-Galles, Calhoun, & Johnson, 2018; Nicolet et al., 2004). For
example, the abundance of Aeshnidae, Coenagrionidae, Corixidae and Gerridae was higher in the sampling sites with higher conductivity and pH value (Dalu & Chauke, 2020). This might be due to the fact that acidity could increase the toxicity level of some metals (such as Al, Cd, Pb, Zn), thus directly or indirectly affecting macroinvertebrates (Herrmann et al., 1993). Moreover, under alkaline conditions, generalists, such as Hemiptera, were more competitive than obligate species (Jooste et al., 2020). Secondly, some studies suggested that the functional diversity of invertebrates was smaller under high pH conditions (Interagency Freshwater Group, 2015). Related studies were mainly focused on water beetles (Arnott, Jackson, & Alarie, 2006; Roth, Zoder, Zaman, Thorn, & Schmidl, 2020) and Oligochaeta (Krodkiewska, Strzelec, & Spyra, 2016). It might be for the reason that under natural conditions, lower pH values were associated with higher residues and humus of vegetation (Vuorenmaa, Forsius, & Mannio, 2006). The accumulation of detritus also increased the diversity of habitat structure, thus increasing species richness (Schmidl, J, 2003). Thirdly, some studies found that pH value had no significant effect on density of invertebrates (Simpson, Bode, & Colquhoun, 1985; Winterbourn & Collier, 1987). Therefore, under certain conditions, pH will have an impact on aquatic invertebrates, and the impact on different species is different. Some invertebrates such as Odonata and aquatic beetles may prefer alkaline water, while some invertebrates such as aquatic beetles and Oligochaeta may prefer acidic water. In this paper, there was a significant positive correlation between pH and *Spercheus emarginatus* which belonged to aquatic beetles, and a significant negative correlation between pH and *Diplonychus esakii* which belonged to aquatic beetles.

(2) Isolation

According to the theory of island biogeography, biological assemblages of ponds should affected by the size and isolation degree (Holland & Jain, 1981; Ripley & Simovich, 2009). Island size could affect the survival rate and available niche, while isolation affected the migration rate (MacArthur RH and Wilson EO, 1967). The effect of isolation on biological communities was related to the migration ability of organisms among habitats (Hanski, 1999). Species with strong migration ability are less likely to species extinction. Aquatic macroinvertebrates can migrate from one pond to another by active or passive diffusion, thus increasing species exchange among ponds. Meanwhile, not all species responded to habitat size and area (Scheffer et al., 2006). In recent years, little attention had been paid to the index of isolation for ponds (Brooks & Colburn, 2012). Some studies had also shown that the richness of aquatic or terrestrial invertebrates was not related to isolation of habitat (Brooks & Colburn, 2012; Jonsson, Yeates, & Wardle, 2009; Moraes et al., 2014; Scheffer et al., 2006). However, other studies had shown that the increase of isolation among ecosystems could reduce the migration rate and increase the risk of species extinction (MacArthur RH and Wilson EO, 1967). Isolation was an important factor affecting the distribution of gastropods (Bronmark, 1985) and the migration pattern of predatory aquatic insects (Wilcox, 2001). Briers and Biggs believed that the isolation degree of ponds had a significant impact on invertebrate community, and the invertebrate community structure among adjacent sampling sites was more similar than that among distant ponds (Briers & Biggs, 2005). Significant correlates between isolation degree and species assemblages were found in both basin 1 and basin 2 which was in line with previous studies.
4.4 Implication for biodiversity conservation of ponds

Different aquatic ecosystems support different community structures of aquatic macroinvertebrates. The physical and chemical characteristics of water, such as pH, DO and transparency, are important factors affecting the community structure of aquatic macroinvertebrates. At the same time, due to the regularity of regional water quality, regional aquatic macroinvertebrates usually show relevant regularity. So we can understand the characteristics of regional biodiversity, the scale and species to be protected. To a certain extent, artificial ponds of hilly area in China maintain the scale of local aquatic species, expands the habitat area, and improves the functional connectivity of most ponds. As a “stepping stone” of habitat, it provides convenience for the migration and diffusion of aquatic organisms. However, hilly ponds are also faced with over utilization and serious agricultural non-point source pollution. In addition to the establishment of statutory reserves, we need to shift the unreasonable utilization of ponds to the sustainable development of biodiversity resources desperately. The cost-effectiveness of protection management must be considered, and protection decisions also be constrained by multiple conflicts of interest. Therefore, we need to conserve the habitat and biodiversity of ponds from the aspects of landscape scale, habitat conditions and industrial structure etc.. In terms of landscape scale, we should focus on the landscape level of ponds rather than single pond (Briggs, Pryke, Samways, & Conlong, 2019; Hill et al., 2018; Thornhill et al., 2017). In terms of habitat conditions of ponds, the restoration of ponds habitat should pay attention to nutrients (such as TN, NH$_4^+$), distribution pattern (such as isolation), temperature and climate (such as altitude), habitat structure (such as area). At the same time, we should be alert to the loss of biodiversity caused by acidification of human activities. In China, a large number of artificial ponds are permanent, and they may become temporary wetlands for no artificial management. As a part of the protection and management strategy, it was encouraged to maintain the pond network with different length of hydrological cycle and environmental characteristics (Hill et al., 2017). In terms of industrial structure, we can introduce more sustainable utilization modes into the production and utilization of ponds, increase the economic output of ponds, and realize biodiversity conservation with low-cost.

5 Conclusion

Hilly ponds support special biological assemblages and contribute to regional biodiversity. However, in this paper, the habitat quality of the ponds was damaged, resulting in the low taxa richness of the aquatic macroinvertebrate assembles. It found that there was little difference of the macroinvertebrate assemblages among basins. These hilly ponds were mainly affected by local environmental factors and spatial processes, including altitude gradient, nutrients, area, temperature, pH and pond isolation etc.. On the premise of understanding the local ponds habitat conditions and aquatic assemblages status, we put forward the strategy of biodiversity conservation of regional ponds. We should focus on the pond network, control the cost-effectiveness and increase the ecological benefits by developing sustainable wetland economy.
Declarations

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Author contributions

Zhang Dan: Conceptualization, data curation, formal analysis, writing-original draft, writing-review & editing. Wang Kehong: Conceptualization. Zhang Guanxiong: Data curation. Liu Shuangshuang: Formal analysis. Wang Fang: Formal analysis. Pan Yuanzhen: Data curation. Yuan Xingzhong: Conceptualization, funding acquisition, project administration, supervision.

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Competing interests

None declared.

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Figure 1

Basin division of Liangping district, Chongqing, China (d). The sampling sites of basin 1 are from 1-1 to 1-40 (a). The sampling sites of basin 2 are from 2-1 to 2-40 (b). The sampling sites of basin 3 are from 3-1 to 3-40 (c). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

The faunal composition of aquatic macroinvertebrates in each basin.

Figure 3
PCA analysis of aquatic macroinvertebrate assembles in ponds of different basins based on PC1 by PC2 axes.

**Figure 4**

Loading values of each species for the first two PCs based on the PCA analysis of aquatic macroinvertebrates profiles in the sampling sites from the three basins. Abbreviation: Be (Bellamya sp.), Vi (Viviparus sp.), Ci (Cipangopaludina sp.), Gy (Gyraulus sp.), Po (Polypylis sp.), Ph (Physa sp.), Ra1 (Radix sp.), Ga (Galba pervia), Pa (Parafossarulus sp.), Ca (Caridina sp.), Ba (Baetis sp.), Me (Megalestes sp.), Ps (Pseudothemis sp.), Ma (Macromidia sp.), An1 (Anax sp.), An2 (Anotogaster sp.), Cy1 (Cybister sp.1), Cy2 (Cybister sp.2), Dm (Dytiscus marginalis), Ha (Haliplus sp.), Ty (Typhaea sp.), Se (Spercheus emarginatus), Gy (Gyrinidae sp.), Sc (Scirtidae sp.), St (Stratiomys sp.), Ra2 (Ranatra sp.), Lj (Laccotrephes japonensis), De (Diplonychus esakii), Si1 (Sigara sp.1), Si2 (Sigara sp.2), Ge (Gerris sp.), Hy (Hydroptilida sp.), Cu (Culicidae sp.), Ch (Chironomus sp.), Di (Dicrotendipes sp.), Dt (Dicrotendipes
tritomus), Po (Polypedilum sp.), Pa (Parachironomus sp.), Ca (Chironomus anthracinus), Ta (Tanytarsus sp.), Ck (Chironomus kiiensis), Mi (Micropsectra sp.), Cr (Cricotopus sp.), Po (Polypedilum laetum), Kr (Krenosmittia sp.), Eb (Eukiefferiella brehmi).

Figure 5

CCA plot showing the relationship of aquatic macroinvertebrate assembles with the significant environmental and spatial variables in three basins. The first axis is horizontal and second axis vertical. Only those species contributing more than 1% of the total macroinvertebrate abundance are shown in the graph for clarity. Only significant environmental and spatial variables ($p \leq 0.05$) are shown. Abbreviation: Be (Bellamya sp.), Vi (Viviparus sp.), Gy (Gyraulus sp.), Ph (Physa sp.), Ra (Radix sp.), Ga (Galba pervia), Ca (Caridina sp.), Ba (Baetis sp.), Me (Megalestes sp.), Ps (Pseudothemis sp.), Cy1 (Cybister sp.1), Cy2 (Cybister sp.2), Ha (Haliplus sp.), Ty (Typhaea sp.), Se (Spercheus emarginatus), St (Stratiomys sp.), De (Diplonychus esakii), Si1 (Sigara sp.1), Si2 (Sigara sp.2), Cu (Culicidae sp.), Ch (Chironomus sp.), Di (Dicrotendipes sp.), Dt (Dicrotendipes tritomus), Cr (Cricotopus sp.), Po (Polypedilum laetum).