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Comparison of benthic macroinvertebrates in three polyculture models of ponds stocking mainly *Ctenopharyngodon idellus*

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ABSTRACT

We investigated the distributions of benthic macroinvertebrates in land-based fish ponds (three polyculture models), mainly stocking *Ctenopharyngodon idellus*, and studied which environmental variables would lead to variation in macroinvertebrate community structure. We sampled benthic macroinvertebrates in nine ponds, three times from June to October 2010. Meanwhile, many environmental variables were measured. Sixteen species of benthic macroinvertebrates were identified. Aquatic insects made up the majority component of the benthic fauna samples. *Glyptotendipes lobiferus* and *Limnodrilus hoffmeisteri* were the most dominant species. Insect and Oligochaeta abundance showed clear change, and their density variation tendency was different in each of the three models. Redundancy analyses (RDA), including the Monte Carlo permutation test and forward selection procedure, showed LOI, TN_S, TP_S, pH, T, Chl-*a*, NO_2^--N and TP to be the most important environmental variables acting on the benthic macroinvertebrate assemblages ($P < 0.05$). General linear modelling (GLM) was applied to determine the relationship between certain benthic macroinvertebrate species and selected individual environmental variables. The results indicated that *G. lobiferus* was significantly negatively correlated to LOI ($P = 0.042$) and *Endochironomus nigricans* was positively correlated to T ($P < 0.001$), TP_S ($P < 0.003$) and TN_S ($P = 0.018$). *Tanypus* sp. and *L. hoffmeisteri* were abundant in high TP_S, TN_S, LOI and Chl-*a*, and scarce at high pH. *Branchiura sowerbyi* abundance showed a significantly positive correlation to TP_S, TN_S, LOI and Chl-*a*. Only *Tanypus* sp. abundance showed a significant positive relationship to TP. The relationship between macroinvertebrates and environmental variables suggested that the physicochemical characteristics of sediment had a more significant influence on benthos fauna than the water in artificial and cultivated ponds. From the macroinvertebrate assemblages similarity analysis, there is evidence that *Polyodon spathula* can make valuable contributions to the biodiversity and ecosystem stability of fish ponds. Further studies need to be conducted to confirm the finding and clarify its mechanism.

Keywords: Macroinvertebrates; Community structure; Biodiversity; *Ctenopharyngodon idellus* ponds; Polyculture models; RDA

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1. Introduction

Farm ponds are widely found in agricultural landscapes. In China, they form an important part of the agricultural production system, being used, for example, in field irrigation and aquaculture. Aquaculture in farm ponds contributes over 70% of the freshwater aquaculture production (Fisheries statistics in 2005 in China). Furthermore, as the eutrophication and pollution of lakes and reservoirs becomes more and more serious, fish culture has been prohibited in many of them. Nevertheless aquaculture in farm ponds is indispensable for fish supply. Farm ponds are defined as bodies of water between 1 m^2 and 2 ha in area, including both artificial and natural bodies of water [1]. Notwithstanding their small size, they have been reported to be high in biodiversity [2,3]. Compared with lakes and reservoirs, ponds are reported to have lower productivity as a result of nutrient limitation [4,5]. However, different conclusions have also been drawn; Downing et al. demonstrated that ponds contribute significantly to the global carbon cycle, and may be more efficient than the oceans at assimilating carbon because of their abundance and high productivity [6].

Being an important biotic component of pond ecosystem, benthic macroinvertebrates play a relevant role in nutrient cycling and energy flow. As a critical energy link between the trophic base of a system and higher-level consumers [7], they can provide a trophic link between detritus, meiobenthos and fish [8], providing food for demersal fish and vertebrates [9,10]. Their burrowing and feeding activities can change the physical and chemical properties of sediment and ultimately accelerate the decomposition of organic elements and microorganism activity [11–13]. Furthermore, the macroinvertebrate community is an essential component for the assessment of aquatic environments and the understanding of biotic responses to regional environmental changes [14,15]. Benthic macroinvertebrate communities are ideal indicators of water and sediment quality because they contain a wide range of species from pollution sensitive to tolerant, and can be captured and identified easily. Chironomidae become an ideal and excellent tool for the assessment of freshwater pollution, and indicators of environmental changes [16]. *Glyptotendipes* and *Endochironomus* are a common and widespread species in eutrophic ponds and lakes [17]. *Tubifex* is the dominant species in waters with heavy organic contamination [18]. Ephemeroptera and Trichoptera are predominantly found in clean and oxygen-enriched water. Therefore, macroinvertebrate metrics are common components of biological water quality assessment studies [19].

Benthic macroinvertebrate assemblages in ponds are influenced by both abiotic and biotic factors. Abiotic factors include the physicochemical characteristics of sediment and water. It has been stated that the species and abundance of macroinvertebrate assemblages are closely related to the sediment type of the bottom, including the size and roughness of sediment [20,21]. Tubificidae, Ephemeroptera and Chironomidae mainly appear in sapropel bottoms; *Bellamya aeruginosa* in Donghu lake was mainly found in the sand substrate [22]. A large number of studies have shown that water area, hydroperiod, altitude, water chemistry, depth and temperature can dramatically affect benthic macroinvertebrate structure [2,23,24]. The hydroperiod and habitat characteristics of a pond can influence which macroinvertebrates occur in it [2]. As for biotic factors, predators (fish and amphibians) and aquatic vascular plants have been identified as important in determining the abundance of benthic macroinvertebrates, as well as the diversity index [25–27].

As important bodies of aquaculture water, farm ponds play a role both in the conservation of biological diversity and in the plentiful supply of goods for the aquatic product market [27–29]. However, few studies have focused on the fish pond ecosystem, and even fewer on the benthic macroinvertebrates within it. Many studies on macroinvertebrates are concerned with temporary and permanent ponds containing no fish. In the present study, we examined the distribution and structure of benthic macroinvertebrate assemblages in ponds mainly stocking *Ctenopharyngodon idellus*, and their relationship to some environmental variables, to verify the effects of these variables on the benthic fauna. Based on this comparative study, we also evaluated the stability of the pond ecosystem in order to provide a preliminary study on the healthy culture of polyculture ponds.

2. Materials and methods

2.1. Study area

The study area is in Xinzhou aquaculture base, Hubei province, China (30°50′N, 114°58′E). The nine land-based ponds studied have the same area (110 m^2) and similar depth (1.2 ± 0.2 m, mean ± SD). These ponds have the same silt substrate, and were remoulded in March, 2010. Prior to this they were temporary ponds with no fish. The nine remoulded ponds were divided into three groups, with each group containing three replications in a randomized block design. Group one represents model 1 (including 75% *C. idellus*, 10% *Hypophthalmichthys molitrix*, 12% *Aristichthys nobilis*, 3% *Carassius auratus*), group two represents model 2 (75% *C. idellus*, 10% *H. molitrix*, 6% *A. nobilis*, 6% *P. spathula*, 3% *C. auratus*), and group three represents model 3 (75% *C. idellus*, 10% *H. molitrix*, 12% *P. spathula*, 3% *C. auratus*). Our study period was from June to October 2010. This five-mo period is the main aquaculture period in the center of China. All the ponds were sampled three times during the culture period, at the early stage (24 June), middle stage (23 August) and later stage (8 October).

Table 1
Results (mean ± SD) of physico-chemical analysis of water and sediment in three models (from June to October 2010)

Index	Model 1			Model 2			Model 3		
	24 June	23 August	8 October	24 June	23 August	8 October	24 June	23 August	8 October
TP, mg l^{-1}	0.045 ± 0.007	0.100 ± 0.005	0.130 ± 0.035	0.048 ± 0.018	0.090 ± 0.009	0.109 ± 0.038	0.072 ± 0.008	0.091 ± 0.005	0.106 ± 0.009
PO_3^--P, mg l^{-1}	0.066 ± 0.014	0.010 ± 0.014	0.001 ± 0.000	0.013 ± 0.004	0.010 ± 0.0016	0.002 ± 0.001	0.023 ± 0.017	0.014 ± 0.024	0.003 ± 0.000
NH_4^+-N, mg. l^{-1}	0.504 ± 0.079	0.212 ± 0.008	0.310 ± 0.257	0.484 ± 0.075	0.216 ± 0.006	0.075 ± 0.024	0.399 ± 0.160	0.149 ± 0.129	0.088 ± 0.019
NO_2^--N, mg. l^{-1}	0.005 ± 0.002	0.000	0.002 ± 0.000	0.027 ± 0.011	0.000	0.003 ± 0.001	0.006 ± 0.004	0.000	0.005 ± 0.001
NO_3^--N, mg. l^{-1}	0.131 ± 0.052	0.000	0.086 ± 0.032	0.178 ± 0.048	0.000	0.013 ± 0.005	0.101 ± 0.052	0.000	0.010 ± 0.011
TN, mg l^{-1}	0.855 ± 0.014	1.216 ± 0.012	1.396 ± 0.060	0.889 ± 0.092	0.947 ± 0.266	1.088 ± 0.074	1.029 ± 0.063	0.975 ± 0.027	1.043 ± 0.020
COD, mg l^{-1}	6.952 ± 0.467	6.344 ± 0.972	6.890 ± 1.212	6.578 ± 1.565	6.410 ± 0.986	5.980 ± 0.650	7.473 ± 0.724	5.929 ± 0.551	5.048 ± 0.755
DTP, mg g^{-1}	0.441 ± 0.016	0.483 ± 0.033	0.585 ± 0.004	0.367 ± 0.050	0.393 ± 0.030	0.490 ± 0.032	0.469 ± 0.052	0.613 ± 0.019	0.487 ± 0.028
DTN, mg g^{-1}	0.787 ± 0.021	1.015 ± 0.187	1.256 ± 0.098	0.503 ± 0.011	0.783 ± 0.029	0.906 ± 0.127	0.820 ± 0.054	1.457 ± 0.135	1.005 ± 0.138
DLOI, mg g^{-1}	3.268 ± 0.097	4.237 ± 0.247	4.896 ± 0.140	2.094 ± 0.374	4.361 ± 0.374	5.012 ± 0.065	3.539 ± 0.096	4.910 ± 0.220	4.858 ± 0.505
DO, mg l^{-1}	2.307 ± 0.506	2.187 ± 0.025	2.937 ± 0.494	4.263 ± 1.220	3.173 ± 0.990	4.497 ± 0.513	2.933 ± 0.300	3.467 ± 0.479	5.230 ± 0.862
Ph	7.977 ± 0.393	7.497 ± 0.129	7.227 ± 0.055	8.083 ± 0.395	7.580 ± 0.277	7.283 ± 0.109	7.820 ± 0.090	7.613 ± 0.198	7.667 ± 0.0681
T	28.967 ± 0.152	30.267 ± 0.321	20.800 ± 0.100	28.533 ± 0.378	30.500 ± 0.360	20.833 ± 0.321	29.233 ± 0.208	30.900 ± 0.264	21.267 ± 0.057
Chlorophyll *a*, μg l^{-1}	3.675 ± 1.556	5.434 ± 2.621	5.161 ± 0.947	4.052 ± 1.453	4.991 ± 2.395	5.018 ± 1.667	3.991 ± 0.996	5.146 ± 1.038	4.896 ± 2.351

2.2. Sampling

Macroinvertebrates (organisms > 1 mm) were obtained using a modified Petersen grab (with surface area of 0.0625 m^2); samples were taken from three random locations and then mingled. Three random locations in a 100 m^2 water ecosystem can be considered to give a representative sample of benthic macroinvertebrates [30]. Samples were passed through a sieve (450 µm mesh) and preserved using 8–10% buffered formalin until taxonomic identification was carried out. The species of most of the macroinvertebrates was identified using an optical microscope. Biomass was determined as the wet weight of each species after blotting on filter paper. For each pond, sediment samples were collected from three locations using self-regulating PVC pipes and pooled together. The samples were dried, pulverized and sieved with a 2 mm sieve. The rate of organics in the total sediment (LOI), total nitrogen content in the sediment (TN_S) and total phosphorus content in the sediment (TP_S) were determined according to the method of Asaduzzaman [31]. At each pond, nine water variables were measured, as shown in Table 1. Dissolved oxygen (DO), water temperature (T) and pH were measured with HQd Meters and IntelliCAL™ Probes (Hach Co., Loveland, CO, USA). Chemical oxygen demand (COD), Ammonia, as the ammonium ion (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), total nitrogen (TN), total phosphorus (TP), phosphate (PO_3^--P) and Chlorophyll-*a* (Chl-*a*) levels in the water were determined in the laboratory following standard methods [32,33].

2.3. Data analysis

The software packages CANOCO v4.5 [34] and STATISTICA 6.0 (Statsoft, Tulsa, Oklahoma, USA) were used for statistical analyses.

Density and biomass were reported on a per m^2 basis, and the data was $\ln(X + 1)$ – transformed [23] in order to modify the range of data. The temporal and spatial variation of macroinvertebrate density and biomass in different culture models was analyzed using STATISTICA 6.0. The ecological status of the macroinvertebrate community was assessed using Shannon's diversity index [35], Margalef's index [36] and Simpson's diversity index [37].

Canonical correspondence analysis (CANOCO) was used to perform a direct gradient analysis and identify environmental factors potentially influencing biotic assemblages [38]. Direct ordination analyses were used to look for significant relationships between environmental variables and macroinvertebrate assemblages. Species assemblage matrices were taken as response variables, and environmental variables as explanatory ones. Analyses were performed based on abundance and biomass data using CANOCO v4.5. A detrended correspondence analysis (DCA) was performed on the species data to determine whether linear or unimodal ordination techniques were appropriate. The results of DCA axis gradient lengths were below two standard deviation units. Therefore, we used linear-based redundancy analyses (RDA) [39]. Subsequent analyses were based on linear species-response models. The significance of the environmental variables was assessed using manual forward selection, and a Monte Carlo permutation test ($P < 0.05$, 499 permutations) was used to determine the statistical significance of the species-environment relationships. Additionally, a series of partial RDAs were used to estimate the importance of variables on the species assemblage structure following the methods of DeSellas et al. [40].

In order to determine the relationship of certain benthic macroinvertebrate species to individual environmental variables, species response curves, using a general linear regression model with Poisson distributions, were generated in CANOCO 4.5 for the four most common species (*G. lobiferus*, *Tanypus* sp., *L. hoffmeisteri*, *B. sowerbyi*) and the Shannon–Wiener index (*H'*). We generated species response curves for the main water and sediment nitrogen and phosphorus indices (TN, TP, NH_4^+-N, NO_3^--N, NO_2^--N, PO_3^--P, LOI, TN_S, TP_S), T, DO, pH, and Chl-*a*; values with $P < 0.05$ were considered significant and curves are shown in Fig. 5.

By taking biological and environment variables, redundancy analysis (RDA) was used to assess similarity among the 27 samples (three samples from each of the nine ponds). The RDA plot (Fig. 3) was focus on inter-sample distance, in order to interpret the Euclidean distance between different samples.

3. Results

3.1. Environmental variables

Environmental factors showed spatial and temporal differences between sampling ponds during the cultivation period (Table 1).

3.2. Benthic macroinvertebrate assemblages

In total, 16 taxa were identified in the nine sampling ponds (Table 2). Three taxa were Oligochaeta, ten were aquatic insects and three were Mollusca. Insect larvae were dominant in benthic macroinvertebrate samples. *G. lobiferus* was the most common species, followed by *Tanypus* sp., and the third was *E. nigricans*. Their occurrence rate was 92.3%, 66.7%, and 40.7% respectively in 27 samples (Table 2). The occurrence rates of other insects were relatively low. For Oligochaeta, *L. hoffmeisteri* was the most common species (92.3%). Gastropoda were rarely found in benthic samples; *Bellamya purificata* and *Cipangopaludina chinensis* were scarce in every pond and *Unio douglasiae* was found in just two ponds.

3.2.1. Density and biomass in three model ponds

From Table 3, we know that Oligochaeta dominated the benthic macroinvertebrates collected in model 1; average density was 344.89 ind m^{-2}. Insects were the dominant group found in models 2 and 3, with average densities of 272.39 and 300.84 ind m^{-2}. Despite their low density (10–14 ind m^{-2}), the Mollusca in ponds composed the largest part of the total biomass (94–97%) due to the large size and weight of individuals; average biomass was 46.9, 77.4 and 51.1 mg m^{-2} respectively in models 1, 2 and 3.

Table 2
List of species of benthic macrozoobenthos in ponds

Taxon		Model 1			Model 2			Model 3		
		1#	2#	3#	4#	5#	6#	7#	8#	9#
Insects	*Glyptotendipes lobiferus*	+++	+++	+++	+++	++	+++	+++	+++	++
	Endochironomus nigricans	++	+	++		+	+	+ +	+	+
	Tanypus sp.	+++	+++	+	++	+	++	++	++	++
	Dicrotendipes			+	+	+				
	Rheotanytarsu exiguous	+			+++	++		+		+
	Procladius choreus			+	++	+	+			
	Tendipus plumosus		+				+			
	Polypedilum scalaenum	+			+	+++	+		+	+
	Chaoborus sp.	+		+++	+	+				+
	Limmnophilus			+	+	+			+	
Oligochaeta	*Limnodrilus hoffmeisteri*	+++	+++	+++	++	+++	+++	+++	+++	++
	Tubifex sinicus		++	++	+	+	+		+	
	Branchiura sowerbyi	+	+++	++	+++	+++	+	+++	+++	+++
Mollusca	*Bellamya purificata*	++	++		++	+	++	+++	+	+
	Cipangopaludina chinensis	+	+++	+				+	+	
	Unio douglasiae				+++	++				

Note: "+" means that this species was found in one of the three samples of the given pond, "++" means that it was found in two, "+++" means that it was found in all three samples. "1#, 2#, 3#, 4#, 5#, 6#, 7#, 8#, 9#" represent the nine ponds.

Table 3
Average density (ind m^{-2}) and biomass (g m^{-2}) of benthic macroinvertebrate species in three models (from June to October 2010)

Pond	Time	Insects		Oligochaeta		Mollusca	
		Density	Biomass	Density	Biomass	Density	Biomass
Model 1	24 June	195.57	0.44	64.00	0.20	10.67	22.65
	23 August	135.13	1.03	317.64	2.18	13.35	58.43
	08 October	376.90	1.62	653.02	2.72	16.00	71.96
	Average	235.87	1.03	344.89	1.70	13.34	51.01
Model 2	24 June	400.00	0.62	78.20	0.23	8.90	11.83
	23 August	103.10	0.60	253.64	1.10	13.35	92.25
	08 October	314.08	1.52	171.24	0.91	8.90	83.19
	Average	272.39	0.91	167.69	0.75	10.38	79.09
Model 3	24 June	184.90	0.29	182.53	0.43	8.00	16.08
	23 August	509.63	2.78	451.11	3.98	13.35	57.20
	08 October	208.00	0.60	171.83	1.18	13.30	80.06
	Average	300.84	1.22	268.49	1.86	11.55	51.11

3.2.2. Variation of density and biomass

Fig. 1 shows the variation of density (Fig. 1a) and biomass (Fig. 1b) of macroinvertebrates in three models during cultivation. The abundance of insects and Oligochaeta showed clear change, but the Mollusca were relatively stable. In model 1, insect density first decreased and then increased; Oligochaeta increased continuously, and their biomass continued to rise during cultivation. In model 2, the trend of the insects was the same as in model 1, but the Oligochaeta showed an inverse trend. The biomass of insects and Oligochaeta continued to rise with different growth rates. In model 3, density and biomass showed a consistent variation trend, first increasing dramatically, and finally decreasing significantly.

3.2.3. Variation of density of dominant species

The dominant species in the three models were *L. hoffmeisteri*, *B. sowerbyi*, *G. lobiferus* and *Tanypus* sp. Fig. 2 illustrates that the density of the dominant taxa changed markedly during the culture period, and that their

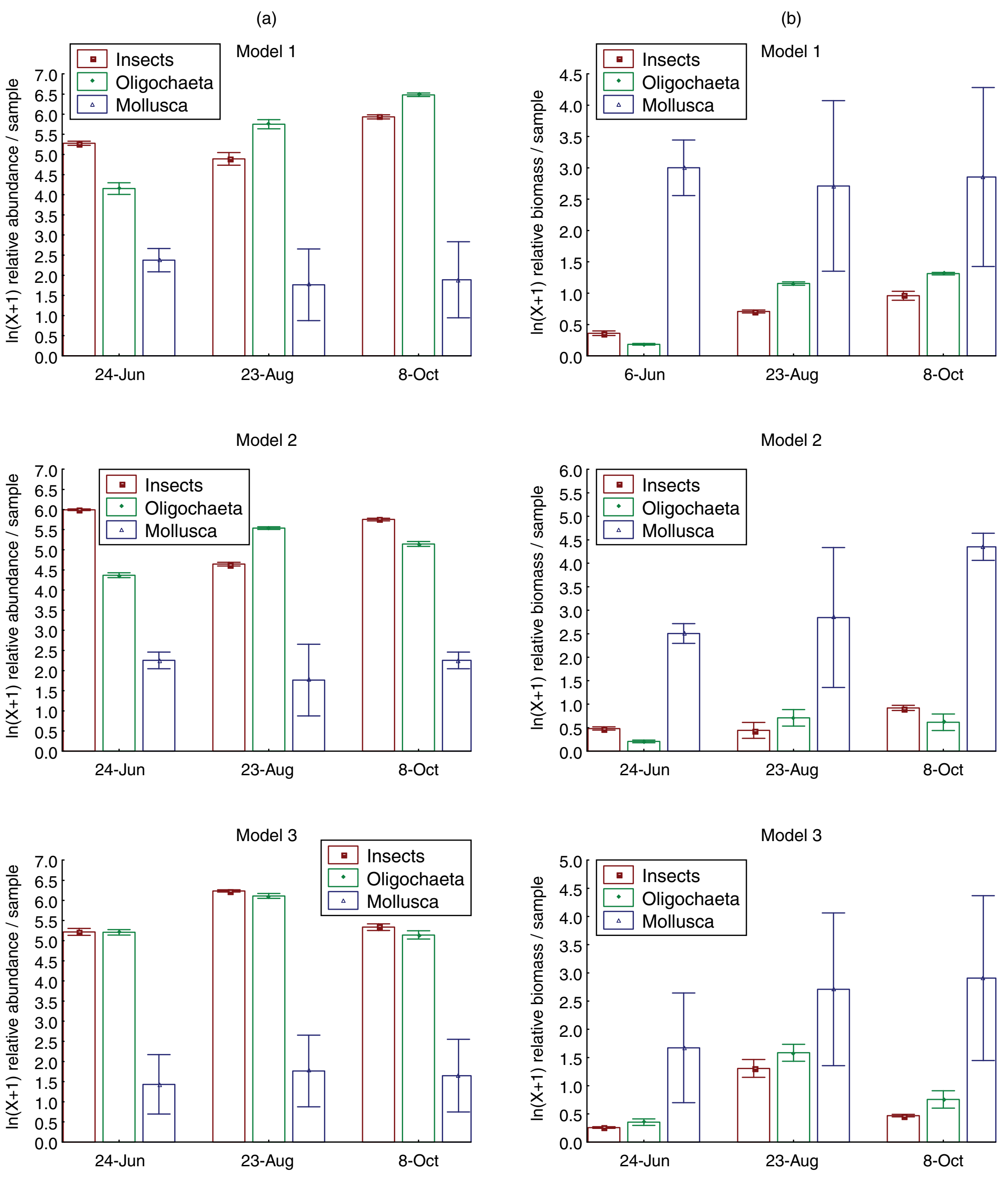


Fig. 1. Relative abundance (a) and biomass (b) of Oligochaeta, aquatic insects and Mollusca in three models. Data is expressed as the mean ± SE (n = 9).

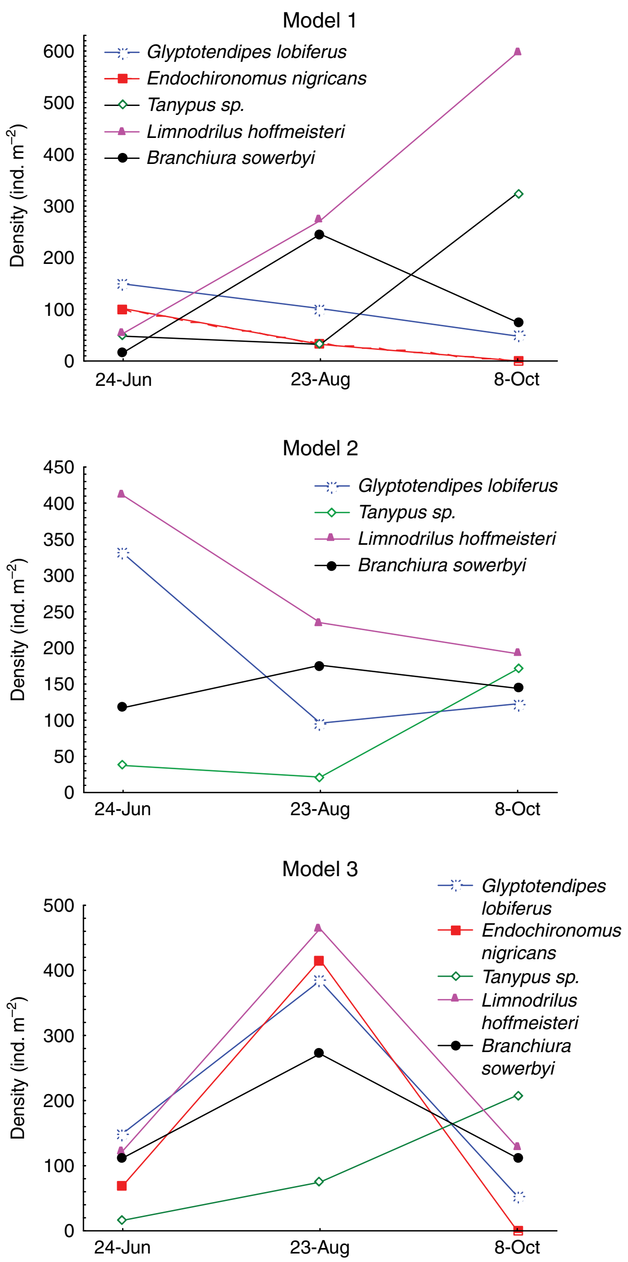


Fig. 2. Density variation of dominant species in the three models.

trends were different from each other. *L. hoffmeisteri* was the most abundant in each model. In model 1, *L. hoffmeisteri* reached up to 597.3 ind m^{-2} in the late period; in model 2, up to 410.6 ind m^{-2} in the early period; in model 3, up to 464 ind m^{-2} in the mid period. *Tanypus* sp. showed a similar trend in model 1 and model 2, firstly decreasing and then increasing. In model 3, however, it increased continuously. The highest density of *Tanypus* sp. in the three models was 325.3, 170.7 and 208 ind m^{-2} respectively. Although Mollusca density was very low in all three models (5.3–21.3 ind m^{-2}), this taxa made up the largest percentage of the total biomass due to the large size of individual Mollusca and the weight of their shells.

3.2.4. Biodiversity indices

On the basis of 27 samples, a comprehensive biological assessment of the water quality of the three models was implemented using Shannon–Wiener (H'), Margalef (D) and Simpson indices (S) (Table 4). The results indicated that biodiversity gradually reduced during the culture period in model 1, and firstly decreased then increased in model two judging by the three indices. However, H' and D showed the same trend in model 3, which was different from S.

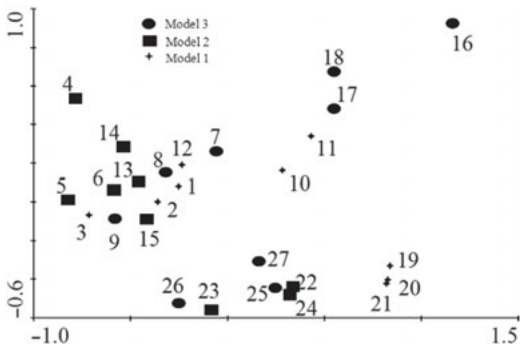
3.2.5. Similitude analyses

By taking biological and environment factors, the Euclidean distance between different samples was interpreted by RDA. From Fig. 3 we know that sample 1–15 had higher similarity than other samples. Sample 19–21 had the high similarity because of the very short Euclidean distance. Sample 22–27 had relative high similarity than other samples. Sample 10, 11, 17, 18 had relative high similarity. The sample 16 had high distance with all other samples. Thus, the 27 samples can be divided into 4 major groups. Group I included sample 1–15; group II included 19–21; group III included sample 10, 11, 17, 18; group IV included sample 22–27. Thus, the early culture period in three models (sample 1–9) and mid culture period in model 2 (sample 13–15) were put into group I,

Table 4
Biodiversity of macrobenthos in three models during culture period (from June to October 2010)

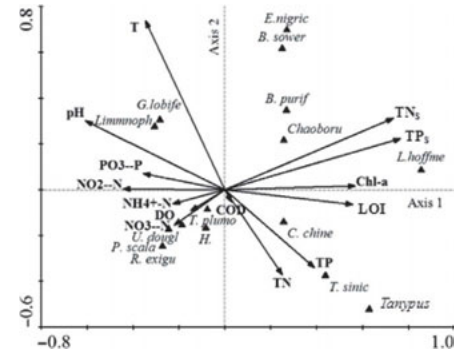
Time	Model 1			Model 2			Model 3		
	H′	*D*	*S*	*H′*	*D*	*S*	*H′*	*D*	*S*
24 June	2.42 ± 0.29	1.13 ± 0.25	4.44 ± 1.18	1.78 ± 0.62	1.13 ± 0.28	2.81 ± 0.74	2.03 ± 0.29	0.85 ± 0.16	3.59 ± 0.85
23 August	1.91 ± 0.22	0.69 ± 0.11	3.14 ± 0.61	1.69 ± 0.51	0.79 ± 0.42	2.56 ± 0.70	1.76 ± 0.20	0.57 ± 0.14	3.64 ± 0.16
08 October	1.81 ± 0.39	0.66 ± 0.16	3.05 ± 0.94	2.32 ± 0.36	0.97 ± 0.28	4.33 ± 0.87	1.96 ± 0.36	0.73 ± 0.27	3.39 ± 0.77

Note: H': Shannon–Wiener index, D: Margalef index, S: Simpson index; data is expressed as the mean ± SD (n = 9).



1-9 correspond, respectively, to the samples of 9 ponds taken on 24-June 2010; 10-18 account for the 9 pond samples taken on 23-August 2010; and 19-27 account for the 9 pond samples taken on 08-Oct 2010.

Fig. 3. Redundancy analysis (RDA) plot of each sample.



Species abbreviations: B. purify – B. purificata; B. sower - B. sowerbyi; Chaoboru -Chaoborus sp.; C.chine – C. chinensis; E. nigric – E. nigricans; G. lobife – G. lobiferus; L. hoffme - L. hoffmeisteri; Limmnoph – Limmnophilus; Tanypus –Tanypus sp.; P. scala – P. scalaenum; R.exigu – R. exiguous; T. plumo – T. plumosus; T. sinic – T. sinicus; U. dougl – U. douglasiae

Fig. 4. Redundancy analysis (RDA) biplot of species and environmental variables.

the late period in model 1 (sample 19–21) were put into group II, the late period in model 2 (sample 22–24) and model 3 (sample 25–27) were put into group IV.

3.3. Relationship between benthic macroinvertebrates and environmental variables

CANOCO software has been widely used in analyzing the relationship between biological community diversity and its environmental factors. The construction of ordination diagrams can effectively reflect vast quantities of information in data. A RDA was carried out to describe the relationship between the 14 selected environmental variables (T, DO, TN, TP, NH_4^+-N, NO_3^--N, NO_2^--N, PO_3^--P, LOI, TN_S, TP_S, COD, pH and Chl-*a*) and the macroinvertebrate assemblages (Fig. 4). The RDA-biplot based on the first four axes explained 66% of the variance in species data, and 93% of the fitted species correlated with the environmental variables (Table 5). The Monte Carlo permutation test was significant on the first axis (F = 7.459, P = 0.024) and on all axes (F = 2.051, P = 0.012) (Table 5). Table 6 shows the inter-set correlation values and illustrates the relationship of the environmental variables to the canonical axes. TP_S, TN_S, LOI and Chl-*a* showed a highly positive correlation (r = 0.70, 0.67, 0.51, 0.52 respectively), and pH, NO_2^--N and T were negatively correlated (r = −0.56, −0.41, −0.32) to species axis one. Other environmental variables showed a short correlation (r < 0.5). T was positively correlated (r = 0.67) to axis 2. The forward selection procedure showed TP_S, TN_S, pH, T, LOI, Chl-*a*, NO_2^--N and TP as the most important environmental variables acting on the benthic macroinvertebrate assemblages (P < 0.05).

Fig. 4 depicts the individual species distributions in relation to the environmental variables. The arrows of NH_4^+-N, DO, NO_3^--N, PO_3^--P and COD were significantly shorter than other environmental arrows.

Table 5
Summary statistics (eigenvalues, cumulative percentage of variance explained by axes 1–4 and significance of the first and of all canonical axes) of the redundancy analysis (RDA)

Axes	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalue	0.383	0.17	0.057	0.046
Species-environment correlation	0.914	0.908	0.711	0.690
Cumulative percentage variance				
Of species data	38.3	55.4	61.0	65.6
Of species–environment relationship	54.3	78.5	86.5	93.1
Monte Carlo test of significance				
Significance of first canonical axis		F ratio = 7.459		P = 0.024
Significance of all canonical axes		F ratio = 2.051		P = 0.012

Table 6
Redundancy analysis inter-set correlations of environmental variables against the canonical axes for the benthic macroinvertebrate data

Environmental variable	Axis 1	Axis 2	Axis 3	Axis 4
TP	0.3568	−0.3096	0.1191	0.3527
PO_3^--P	−0.3255	0.0636	−0.0360	−0.1617
NH_4^+-N	−0.2080	−0.0563	0.1728	−0.3940
NO_2^--N	−0.4053	0.0029	0.3722	−0.2321
NO_3^--N	−0.2021	−0.1405	0.2444	−0.4124
TN	0.2285	−0.3384	0.1790	0.2716
COD	0.0267	−0.0492	−0.0208	−0.3130
DTP	0.7003	0.2027	0.0017	0.3153
DTN	0.6710	0.2837	−0.0458	0.2809
DLOI	0.5096	−0.0592	−0.2175	0.3412
DO	−0.1363	−0.0840	0.0395	0.2684
pH	−0.5558	0.2729	−0.0103	−0.1464
T	−0.3167	0.6703	−0.0776	−0.2504
Chl-*a*	0.5193	0.0149	−0.0411	0.2610

Note: Variables in bold were found to be significant in the forward selection procedure $P < 0.05$.

These environmental variables had a low influence on species abundance. Most of the macroinvertebrate species, especially the main dominant species (*L. hoffmeisteri*, *B. sowerbyi*, *Tanypus* sp.) showed a significantly positive correlation to TN_S, TP_S, LOI and Chl-*a*. *Tanypus* sp. and *Tubifex sinicus* abundance showed a significantly positive correlation to TP and TN, and a negative correlation to T and pH. But *G. lobiferus* and *Limmnophilus* showed a positive relationship to T and pH. *L. hoffmeisteri* had the closest relationship to Chl-*a* concentration in water and showed a significantly positive correlation to TN_S, TP_S and LOI, but a negative correlation to T and pH. The rare species (*U. douglasiae*, *Polypedilum scalaenum*, *Rheotanytarsu exiguous*, *Tendipus plumosus,* occurrence rate <0.2) and the Shannon–Wiener index (H') seemed to have a closer relationship to DO, NO_3^--N and NH_4^+-N. However, there was so little data that it may not reflect the real situation.

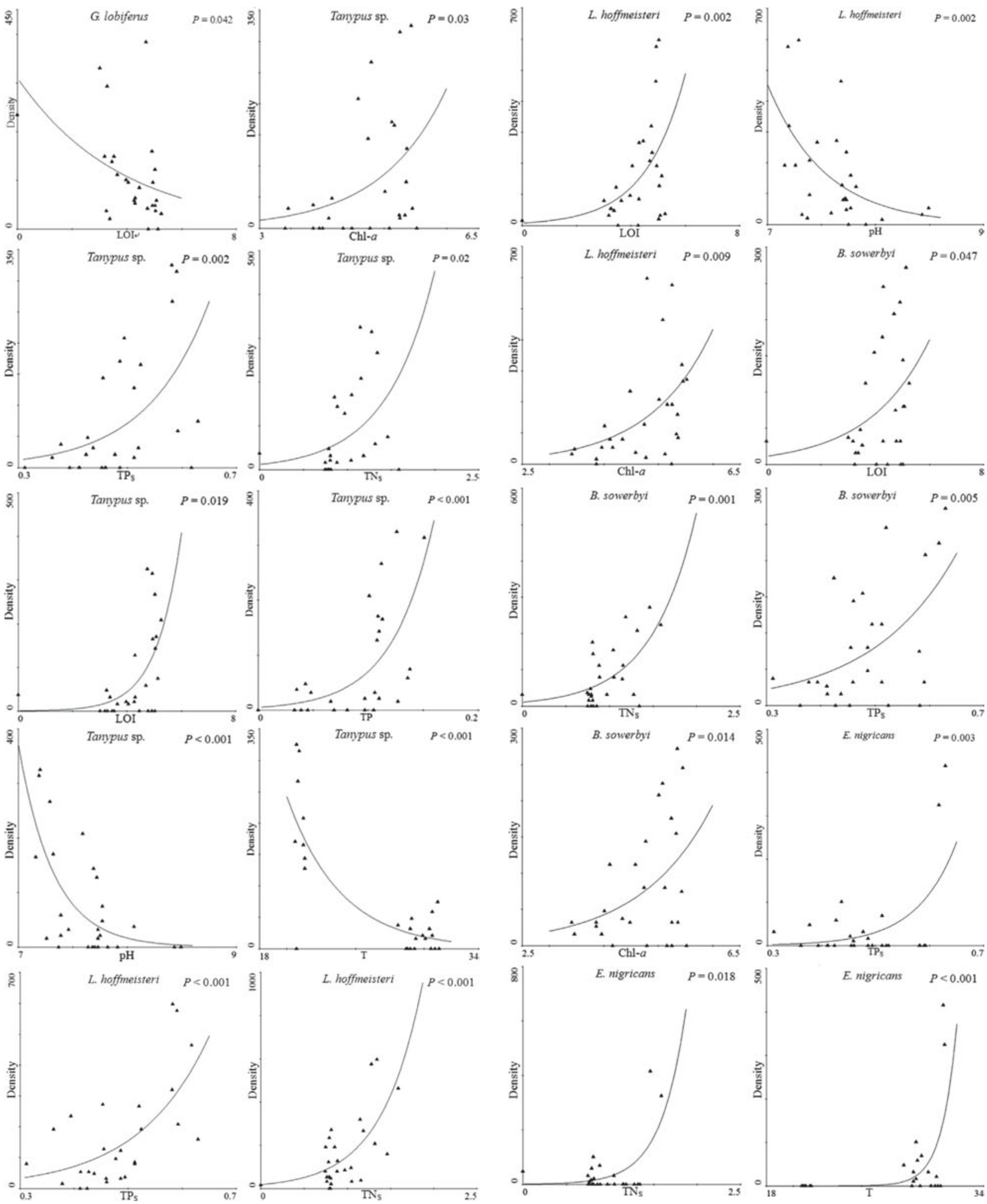
In order to determine whether each of the environmental variables (the significant variables in the forward selection procedure, based on Monte Carlo permutations ($P < 0.05$, 499 permutations), in Table 6) independently influenced macroinvertebrate assemblages, a series of partial RDAs were run with one variable as the only explanatory variable, and others as a covariable. The results showed that Chl-*a*, LOI, pH, T and TP remained significant (Significance of all canonical axes $P < 0.05$).

The generalized linear model with Poisson distributions was applied to generate dominant species response curves for Chl-*a*, LOI, TP_S, TN_S, NO_2^--N, pH, T and TP in CANOCO 4.5. The results are shown in Fig. 5. *G. lobiferus* only showed a significant relationship to LOI ($P = 0.042$), and was relatively more abundant in low LOI. *E. nigricans* was positively correlated to T ($P < 0.001$), TP_S ($P < 0.003$) and TN_S ($P = 0.018$). *Tanypus* sp. and *L. hoffmeisteri* were closely related to TP_S, TN_S, LOI, pH and Chl-*a*. They were abundant in high TP_S, TN_S, LOI and Chl-*a*, but infrequent at high pH (7.0–9.0). *Tanypus* sp. density decreased as the water temperature increased. *B. sowerbyi* abundance showed a significantly positive correlation to TP_S, TN_S, LOI and Chl-*a*. The only species which showed a significant correlation to TP was *Tanypus* sp.

4. Discussion

4.1. Macrobenthos assemblage structure and composition in three models of culture ponds

As an important habitat for aquatic macroinvertebrates, the pond ecosystem has been paid much attention to its different components, the macroinvertebrate assemblages were often a matter of atenton [1,41,42]. The number of species (10 aquatic insects, 3 Oligochaeta and 3 Mollusca) was quite low in the studied fish ponds compared with other similar ones. Florencio et al. reported 123 different taxa in Doñana ponds [43]. Bilton et al. recorded 165 macroinvertebrate species, including 38 genera of Chironomidae, 23 Trichoptera, 19 Hemiptera, 10 Gastropoda, 8 Odonata, 2 Macrocrustacea and 1 Ephemeroptera in Lizard Peninsula and New Forest



Note: A line-of-fit was estimated by the generalized linear model for taxa that were significantly related to an environmental variable.

Fig. 5. Results of the generalized linear models with Poisson distributions for the five most abundant benthic macroinvertebrate taxa found in the studied ponds.

ponds in southern England [29]. However, a similarly low species richness value of macroinvertebrates was also found in other artificial ponds [44,45], and was mainly attributed to aquaculture management and continuously fluctuating water levels. Furthermore, the small size of artificial ponds would make macroinvertebrate assemblages highly vulnerable to threats related to the intensification of anthropogenic intervention and environmental changes. Moreover, demersal fish and amphibians in ponds have been identified as important in determining the abundance of benthic macroinvertebrates [25,26]. *C. auratus* would have the effect of restricting the abundance of macroinvertebrates in the studied ponds. A low number of species found in the nine ponds might also be due to the short sampling duration and small sampling area. 52 taxa of macroinvertebrates were found in 25 Macun cirque ponds between 2002 and 2004 by Oertli et al. [46].

In the studied ponds, the richest group was aquatic insects, which occupied 62.5% of the total. Although just three species of Oligochaeta were found in the studied ponds, *L. hoffmeisteri* was the major dominant macroinvertebrate species (Fig. 2), with wide ecological tolerances and extensive geographical ranges. Mollusca had the lowest density (Fig. 1a) but the largest biomass (Fig. 1b), which was closely related to their body size and heavy shell. Wabab and Stirling found that Oligochaeta and aquatic insects were the predominant species in earthen trout ponds in central Scotland [47]. It has also been shown that the dominant macroinvertebrate species in freshwater ponds in Ranchi and in carp ponds in Croatia were Oligochaeta and aquatic insects [48]. The taxon composition and ecological characteristics of benthic macroinvertebrates are closely related to the benthic environment. The community distribution of macrozoobenthos can be influenced by physicochemical properties and the organic matter content in sediment. The values for the weight and mean size of macroinvertebrates were highest in organically rich silts and clays, and lowest in sands poor in organic matter [49]. For macroinvertebrates, especially Oligochaeta and aquatic insects, biomass relates to sediment type and generally shows the following order: sapropel > ooze > clay > sand [22]. In the studied ponds, the sediments were mainly composed of terrestrial clay and silt, and were rich in organic detritus; this was a suitable habitat for Oligochaeta and Chironomidae, which were the dominant species in the fish ponds.

4.2. Macroinvertebrate assemblage variation in three pond models

The overall variation of different species abundance in the three models appeared chaotic and followed no simple rule. Insect and Oligochaeta abundance in model 1 and model 2 showed similar trends in the early and mid culture periods, but different trends in the later period. However, they were all significantly different from model 3 (Fig 1a). In all nine ponds, Mollusca abundance was relatively stable.

Different taxa of macroinvertebrates show wide differences in their life strategies, such as in propagating, feeding, development or dispersal, and other particularities of their life cycle [50,51]. The emergence of macroinvertebrate larvae would lead to temporal variation in their relative abundance, which may be linked to phenology and the life-history patterns of individuals such as emergence, wintering, recruitment and dispersal [52,53]. The increasing density of aquatic insects can be caused by the hatching of eggs from their adults, while decreasing density may be directly related to adult emergence. This would explain why aquatic insect abundance recorded in August 2010 was lower than that in June 2010 and October 2010 in models 1 and 2.

4.3. Relationship between environmental variables and biological metrics

Variation of macroinvertebrate abundance is closely related to the environmental variables of a pond. The distribution, species composition and development of benthic macroinvertebrates depend on many factors such as water temperature, the chemical and physical properties of the water and sediments. Habitat structure, pond area, macrophyte species richness, water chemistry and sediment characteristics have been identified as important factors in determining the structure of invertebrate communities [39,54]. In temporal ponds, hydroperiod has been one of most studied factors [38,43,50]. However, hydroperiod has not been considered in this study, because the level of the water in the studied ponds was relatively stable during the culture period.

The relationship between species and environmental conditions has traditionally been studied using multivariate analyses. Among the variety of multivariate methods, RDA has often been successfully applied to the study of the effects of environmental conditions on species assemblages [55,56]. Previous studies have come to comprehensive conclusions about the correlation between the density of benthic macroinvertebrates and water temperature, nutrients in the water and sediment composition [57,58]. Stewart and Downing found that organic matter in sediment had a stronger impact on variation of the macroinvertebrate community than nutrient concentrations in the water (nitrogen and phosphorus) [59]. Results from RDA suggested that the most dominant species abundance was more closely related to nitrogen and phosphorus contents in sediment than those in the water, which may support the theory that nutrients in sediment have a stronger impact on macroinvertebrate abundance than those in the water. It is possible that high external nutrient loads, particularly from the feeding of fish in ponds, have more effect on sediment nutrients than water nutrients. In the studied fish ponds, we mainly supplied artificial feed for *C. idellus*. It has been confirmed that parameters such as nutrients and organic matter concentration of the sediments strongly influence the benthic assemblage structure, especially for Oligochaeta and Chironomidae [60,61].

Although benthic macroinvertebrates are widely distributed in many freshwater ecosystems, different species show various ecological adaptations, such as different trophic levels, temperature, pH, organic matter content in sediment and dissolved oxygen levels [62]. Oligochaeta are the most abundant group in many freshwater ecosystems. *L. hoffmeisteri* was frequently considered living in extreme environmental situations, such as high trophic levels, high organic matter content, and low oxygen levels at the water-sediment interface [63]. It is a known eutrophic taxon in lentic systems. It was also the dominant benthic macroinvertebrate species in the ponds studied here.

In order to determine which chemical and physical variables were the most influential in determining the characteristics of benthic macroinvertebrate assemblages, further analysis using generalized linear models (GLMs) was carried out, and *Tanypus* sp. (P = 0.019), *L. hoffmeisteri* (P = 0.002) and *B. sowerbyi* (P = 0.047) were positively related to LOI. Many species, such as Chironomidae larvae and Oligochaeta, which mainly feed on organic matter in sediment, have a significantly positive relationship with LOI. But *G. lobiferus* abundance showed a negative correlation to LOI. This seemed inconsistent with previous conclusions, which showed that Chironomidae were frequently tube dwellers and fond of organically rich sediments [64]. *G. lobiferus* was verified to be a filter-feeding species which mainly consumes particulate matter in the water column [65]. They often occur in eutrophic and lentic water. The species *L. hoffmeisteri* has usually been found in sediment composed of fine particles, with high organic matter content [66]. *B. sowerbyi* is abundant in tropical limnic environments with sediment rich in organic matter [67]. Ducrot et al. also showed that particles of low size and high organic matter content favored the growth and reproduction of *B. sowerbyi* [68]. In previous studies, *B. sowerbyi* and *L. hoffmeisteri* have been recognized as benthic groups very tolerant to high concentrations of organic pollution [66,69].

The use of artificial feed in the studied ponds is believed to dramatically increase nitrogen and phosphorus levels in sediment. High nutrient enrichments are known to favor Oligochaeta and Chironomidae larvae [62]. The GLM results showed that TP_S and TN_S had significantly positive relationships to *Tanypus* sp., *L. hoffmeisteri*, *B. sowerbyi* and *E. nigricans*. Only *Tanypus* sp. showed a close relationship with TP. Nutrients such as nitrogen and phosphorus are important for life in aquatic ecosystems as they are essential for the survival and growth of aquatic organisms. However, because of anthropogenic interventions such as aquaculture, sanitary wastewater and field irrigation, excessive nitrogen and phosphorus loading may lead to water eutrophication. In our studied ponds, the increasing of nitrogen and phosphorus content in sediment was mainly due to artificial feed. Consequently, the eutrophic species such as *Tanypus* sp., *L. hoffmeisteri*, *B. sowerbyi* and *E. nigricans* become the dominant species.

The Chl-*a* content in water reflects the primary productivity in water, and directly influences secondary production in water. Lewis and McCutchan observed an increase in the abundance of herbivorous taxa of benthic macroinvertebrates with increasing Chl-*a*, and found that the highest Chl-*a* concentrations were associated with the highest macroinvertebrate abundance [70]. The RDA ordination (Fig. 4) demonstrated a significant relationship between Chl-*a* and Axis 1, indicating the importance of primary productivity in determining macroinvertebrate community abundance and distribution. The results of the GLMs also showed that Chl-*a* had a significantly positive relationship to *Tanypus* sp., *L. hoffmeisteri* and *B. sowerbyi*. It has been verified that Chl-*a* was one of the key factors affecting benthic macroinvertebrate abundance in lakes [71,72].

5. Conclusions

Our results indicated that Oligochaeta and Chironomidae were the dominant taxa in fish ponds and that the species richness of benthic macroinvertebrates was significantly lower than that found in reservoirs and lakes, mainly due to the small sampling area.

The most dominant species abundance was more closely related to nitrogen and phosphorus contents in sediment than those in water, which may support the theory that nutrients in sediment have a stronger impact on macroinvertebrate abundance than those in water.

According to similitude analyses of the samples, the macroinvertebrate community and environment variables of model 2 and 3 ponds were found to be very similar to each other, while those of model 1 ponds were found to be greatly different from model 2 and 3 ponds. These difference mainly due to the presence of *P. spathula* in addition to that of the demersal fish (*C. auratus*). The results seemed to illustrate that different polyculture models of artificial ponds had significant influence on the benthic macroinvertebrate community. Similar studies should certainly be carried out to further investigate the influence of different culture models on benthic macroinvertebrate assemblages in artificial ponds. It has a practical instructive purpose for aquaculture, especially in China.

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