*Original Research*

# Vertical-Flow Constructed Wetlands Applied in a Recirculating Aquaculture System for Channel Catfish Culture: Effects on Water Quality and Zooplankton

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**Abstract**

In recent decades, considerable attention has been paid to the serious water pollution caused by the fast-growing aquaculture industry. On the other side, water quality determines to a great extent the success or failure of an aquaculture operation. So highlighted is the need for sustainable development of aquaculture. In the present work, we established a recirculating aquaculture system (RAS) by vertical-flow constructed wetlands (CWs) for channel catfish (*Ictalurus punctatus*) culture, and assessed its effects on water quality improvement. The results indicated that the CWs applied in the RAS showed relatively higher removal efficiency for particulate matter (more than 55%) and lower, uneven removal efficiency for nutrients and organic matter (from -34.1% to 48.7%). Paired t-tests showed that only parameters of $NH_4^+$-N, TN, COD, $BOD_5$, TSS, and Chl-*a* were significantly ($p<0.05$) lowered after wetland treatment. Despite this, nutrients (but $NH_4^+$-N, $NO^{2-}$-N, and $NO^{3-}$-N), organic matter, and suspended solids (including plankton) in the recirculating ponds were significantly lowered compared to the control, indicating a decline in trophic status. Multivariate analyses revealed strong relationships between zooplankton community structure and the measured environment in the culture ponds. Cyanobacterial blooms that occurred heavily in the control were strongly restrained in the recirculating ponds. This led to water quality that was suitable for fish culture. Hereby, conclusions could be reached that the recirlulating treatment by the CWs achieved its aim of sustaining or extending water quality improvement in the RAS.

**Keywords:** water quality, recirculating aquaculture system, constructed wetland, zooplankton, relationship

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## Introduction

Aquaculture has been one of the fastest growing segments of the global food production sector in recent decades. By 2004, aquaculture had grown to be a U.S. $70 billion industry contributing almost one-half of fish products consumed by humans. China is by far the largest producer of aquacultural products, accounting for 67% of global production in terms of quantity and 49% of global value in 2006 [1]. However, its rapid growth and modernization also means that it may move toward intensive aquaculture with serious threats to aquatic systems that have been gradually publicized in China [2-4]. The quest for high profits could be accompanied by abuses, environmental impact, and more, which is why opposition is seen by some non-governmental organisations. It is important that well-balanced systems be developed that are sustainable.

Among the various threats, a pressing concern is the nutrient-rich wastewater, which is an important source of eutrophication in both inland and coastal zones [5]. Constructed wetlands (CWs) are ecologically beneficial and low-cost treatment alternatives, that have been proven capable of reducing suspended solids, 5-day biochemical oxygen demand ($BOD_5$), nitrogen, phosphorus, and heavy metals from wastewater of many sources [6]. They have rapidly increased in number, particularly since the late 1990s [7]. In aquaculture, CW systems have been used successfully for treating effluents from trout farms [8, 9], channel catfish [10], and shrimp ponds [6, 11]. Nevertheless, most of the previous studies focused on purifying performance and mechanisms; information on biotic factors, such as zooplankton, was lacking.

Zooplankton, as an essential component of all aquatic ecosystems, representing a key link in aquatic food webs. In aquaculture, zooplankton are not only a good indicator of trophic status of rearing water [12, 13], but also an important regulator for water quality through interactions among zooplankton, phytoplankton, bacterioplankton, benthos, and fish metabolism within food webs. Additionally, zooplankton are a good food source for reared fish, especially for fry, fingerlings, and juveniles. Therefore, zooplankton may closely relate to both quality and quantity of fish production in aquaculture industry.

For this paper, a recirculating aquaculture system (RAS) was established to culture juvenile channel catfish. Catfish farming in static ponds has gradually emerged as an important industry in China, but evidence suggests that catfish farming has apparently reached the limits of the production system as currently configured [14]. The RAS can be potentially used for intensive culture with limited pollutant discharge, thereby increasing fish production and reducing land and water usage as well as adverse environmental impacts. Through the analysis of interactions between zooplankton and the measured physicochemical parameters, and their indicating properties, a full assessment on the efficacy of the system can possibly be reached, which may provide a basis for the application of the technology from an ecological view of point.

## Materials and Methods

### System Configuration

The RAS consisted of five parallel culture ponds and a group of CWs. Each pond had an area of 200 $m^2$, with concrete wall and mud bottom. The total wetland area was 320 $m^2$. Pond P1 was set as the control without water recirculating, and hence corresponded to stagnant water conditions; P2-P4 were set to water recirculating; and P5 for water recruitment (for P1-P4) due to evaporation (Fig. 1a). Pumped water was purified by the CWs before flowing back to the recirculating ponds. The CWs comprised four equally sized consecutive chambers: down-flow with *Canna indica* L. followed by up-flow with *Typha latifolia* L., *Acorus calamus* L., and sisal (Fig. 1b). Substrate was washed gravel, with a particle size ranging from 0.8 to 6.4 cm in diameter. The wetlands and culture ponds were connected by PVC pipes and a concrete ditch. Tube diffusers, connected to an air compressor, were installed beside an aeration tank to supply oxygen. The system was established in October 2003, and had been used for fish culture for more than two years before this study.

### Operation and Management of the System

Before fish stocking, all ponds were drained, silt removed, and they were disinfected with lime. Water pumped from a nearby lake was purified by the CWs, and then flowed into the culture ponds to a depth of 1.2 m. In April 2006, the juvenile channel catfish (*Ictalurus punctatus*), with 19.8±2.9 cm (mean±SE) in full length, were introduced into the culture ponds. To differentiate water quality among the culture ponds, stocking densities were 10,000, 10,000, 20,000, and 30,000 fish/ha for ponds P1-P4, respectively. Density setup was determined with the reference of the yield-maximizing stocking density (30,000 fish/ha) [15]. As a rule, trophic status increased with

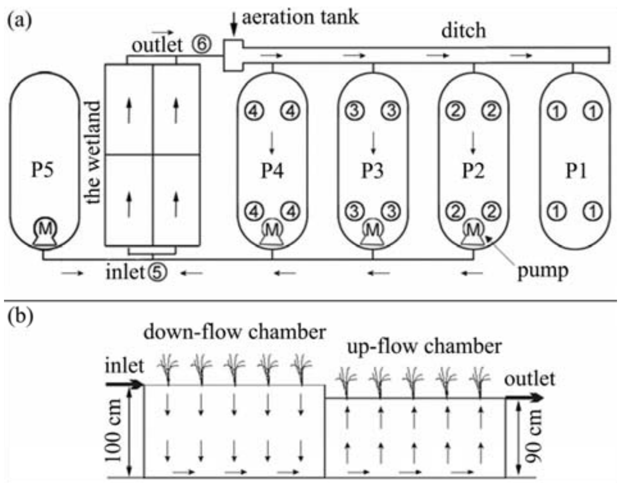


Fig. 1. Schematic diagram of the RAS (a) and the CWs (b); P1-P5 represent five culture ponds and numbers of 1-6 in circles represent sampling sites; arrows show water flow direction.

increasing stocking densities in the culture ponds in that daily feeding ration (3% of fish biomass) was based upon the standing crop (measured monthly). Feed (granular) was offered by Gaolong Feedstuff Co., Ltd. (in Donghu Development Zone, Wuhan, China) and contained more than 32% crude protein. By comparison, we explored whether trophic status of water body of culture pond even at a higher stocking density could still maintain a lower level compared to the control. Water exchange rate was 10% daily before October 2006, and thereafter increased to 15%. Correspondingly, the hydraulic loading rate increased from 225 to 337.5 mm/d and the theoretical hydraulic residence time (HRT) in the CWs decreased from 1.3 to 0.87 days.

## Methods for Sampling and Treatment

Sampling was conducted fortnightly from mid-May to mid-December in 2006. Water samples were obtained by mixed water taken from each corner of the pond at different depths. The inflow was sampled by intercepting the pumping flow, while the outflow was sampled at the outlet of the CWs (Fig. 1a). The parameters for water quality were determined by the standard methods [16], except dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC), temperature (Temp), and pH that were determined *in situ* with an Orion 5-Star Portable pH/ORP/DO/Conductivity Multimeter (Thermo Fisher Scientific Company USA).

Crustacean samples were collected by sieving 10 l of the mixed water through a 64-μm plankton net and preserved in 4% formalin solution. Another liter of the mixed water was fixed in Lugol's solution for rotifer quantification. In the laboratory, crustacean samples were counted completely and measured using a dissecting microscope at 40-× magnification. Rotifer samples were concentrated to 30 ml and subsamples were placed in a count-frame, counted using an inverted microscope at 160-× magnification. Zooplankton were identified to genus/species level with reference to Chiang and Du [17], Shen and Tai [18], Wang [19], Zhang and Huang [20], and Jersabek et al. [21]. Biomass was estimated using formulae given by Huang [22].

## Data Analysis

Prior to analysis, data were inspected for normality with Shapiro-Wilks tests. Paired t-test was used to compare the differences between the simultaneously measured in- and outflow values to test the treatment efficiency of the CWs. Nevertheless, independent t-test or Mann-Whitney u-test was selected to compare the differences among the culture ponds by normality of the data. These analyses were performed in the program SPSS 13.0 for Windows. Further, ordination techniques were used to describe relationships between zooplankton community structure and the underlying environmental gradients in the culture ponds. As a result, a linear-based redundancy analysis (RDA) for density and a unimodal canonical correspondence analysis (CCA) for biomass were utilized, since preliminary detrended correspondence analyses (DCA) showed a short gradient length on density data (SD<2), while a long one on biomass data (SD>2) [23]. To abstain from multicollinearity, principal component analysis (PCA) was used to reduce the number of environmental variables. In PCA, we chose "Varimax," "Save as variables," and "Sorted by size," with other default options, to extract principal components that were treated as new environmental variables in later ordinations [24]. The biotic data in RDA and CCA were log(x+1)-transformed. The statistical significance of the first and all the ordination axes was tested by a Monte Carlo permutation test (499 unrestricted permutations). DCA, RDA, and CCA were performed by Canoco 4.5 for Windows, while PCA was completed using SPSS 13.0 for Windows.

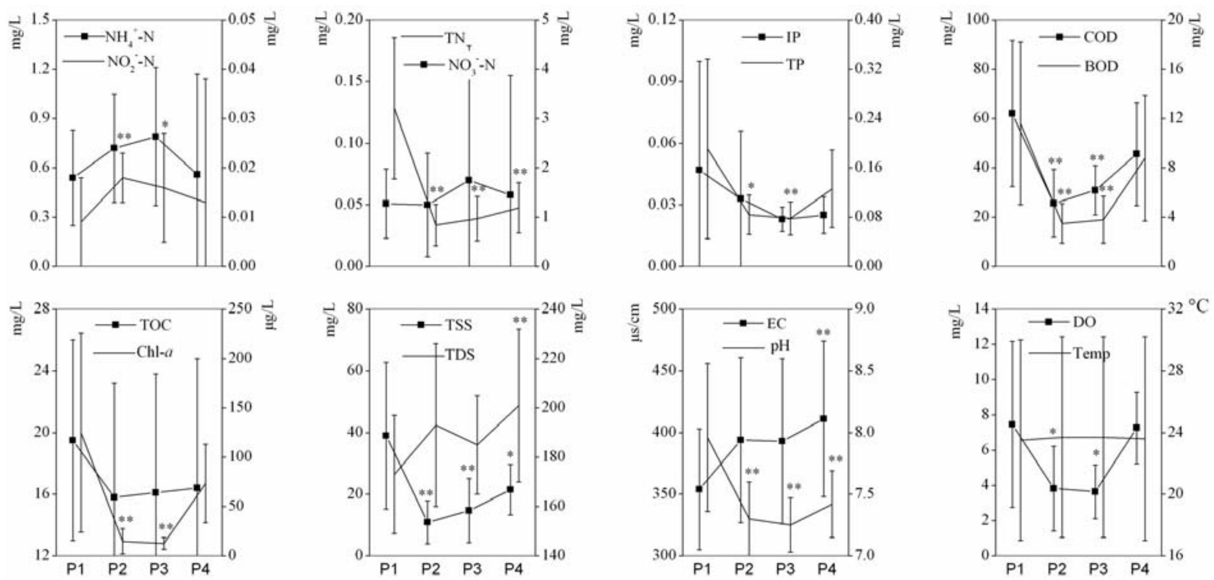


Fig. 2. Mean values (±SD) for all water-quality parameters in the culture ponds.
Significant differences between the control and the recirculating ponds are marked with asterisks: *p<0.05, ** p<0.01.

Table 1. Mean concentrations (±SD) of pollutants in in- and outflow and their removal by the CWs.

| Item | Inflow | Outflow | Removal rate (%) | p-value |
|---|---|---|---|---|
| Ammonium nitrogen ($NH_4^+$-N, mg/L) | 0.62±0.36 | 0.41±0.20 | 34.2 | 0.006* |
| Nitrite nitrogen ($NO^{2-}$-N, mg/L) | 0.041±0.065 | 0.021±0.028 | 48.7 | 0.231 |
| Nitrate nitrogen ($NO^{3-}$-N, mg/L) | 0.058±0.077 | 0.056±0.067 | 3.1 | 0.834 |
| Total nitrogen (TN, mg/L) | 1.21±0.41 | 0.63±0.26 | 48.2 | 0.001* |
| Total phosphorus (TP, mg/L) | 0.09±0.03 | 0.08±0.05 | 16.7 | 0.442 |
| Inorganic phosphorus (IP, mg/L) | 0.017±0.015 | 0.023±0.014 | -34.1 | 0.653 |
| Chemical oxygen demand (COD, mg/L) | 36.5±18.3 | 27.2±13.8 | 25.6 | 0.009* |
| 5-day biochemical oxygen demand ($BOD_5$, mg/L) | 4.3±1.9 | 1.9±1.2 | 55.6 | 0.000* |
| Total organic carbon (TOC, mg/L) | 15.8±7.2 | 12.7±6.1 | 19.5 | 0.135 |
| Total suspended solids (TSS, mg/L) | 12.4±7.7 | 5.2±2.2 | 57.6 | 0.001* |
| Chlorophyll *a* (Chl-*a*, μg/L) | 25.5±13.5 | 4.7±2.6 | 81.6 | 0.000* |

Significant differences between the in- and outflow values are marked with asterisks.

Table 2 Mean density and biomass (±SD) of zooplankton in in- and outflow and their removal by the CWs.

| Taxon | Density (ind./L) | | | Biomass (mg/L) | | |
|---|---|---|---|---|---|---|
| | Inflow | Outflow | Removal rate (%) | Inflow | Outflow | Removal rate (%) |
| Rotifera | 1330±1922.1 | 175±357.2 | 86.8 | 0.46±0.81 | 0.03±0.05 | 93.5 |
| Cladocera | 8.3±14.9 | 1.6±5.0 | 80.7 | 0.15±0.23 | 0.03±0.09 | 81.0 |
| Copepoda | 51.7±47.5 | 3.1±5.6 | 94.1 | 1.03±0.91 | 0.06±0.12 | 93.8 |

## Results

### Pollutant and Plankton Removal by the CWs

Naturally, the CWs showed a high removal efficiency for particulate matter, which was indicated by the relatively higher removal rates (more than 55%) of $BOD_5$, TSS, Chl-*a*, zooplankton density, and biomass. The average removal rates of various forms of nitrogen reached more than 30%, with the exception of $NO^{3-}$-N (3.1%), while the removal efficiencies for TP, COD, and TOC were rather low, and even a minus one for IP. Paired t-tests showed that only parameters of $NH_4^+$-N, TN, COD, $BOD_5$, TSS, and Chl-*a* for the inflow were significantly higher compared to the outflow (Tables 1 and 2).

### Water Quality Parameters in the Culture Ponds

After the recirculating treatment, the concentrations of TN and TSS as well as pH value were significantly ($p<0.05$, n=28) lowered in the recirculating ponds (P2-P4) compared to the control (P1). Similarly, the concentrations of TP, COD, $BOD_5$, Chl-*a,* and DO were significantly ($p<0.05$, n=28) lower in the recirculating ponds (only P2 and P3) than in the control, while $NH_4^+$-N, $NO^{2-}$-N, and $NO^{3-}$-N seem to display converse trends. Only TN, COD, and TSS in the recirculating ponds increased obviously with the increasing stocking densities (Fig. 2).

### Zooplankton Standing Crop in the Culture Ponds

Zooplankton assemblages, as parts of suspended solids, could be easily removed by the CWs. Like nutrients (TN, TP), zooplankton standing crop was also lowered apparently by the recirculating treatment. Furthermore, statistical differences in density or biomass of rotifers and copepods were found ($p<0.05$, n=28), while no significant difference in cladoceran's, although the later showed an obvious decreasing trend. Additionally, zooplankton standing crop showed no obvious trends with the increasing stocking densities (Fig. 3).

### Relationship between Zooplankton and Their Environment in the Culture Ponds

The 16 measured environmental variables were employed to explain spatio-temporal variation in zooplankton community structure in the culture ponds. Before ordinations, variable reduction via PCA was performed. As a result, only five components were extracted from the environmental data set with 75.5% of cumulative variance

Table 3. Summary of RDA and CCA analysis.

| Item | In density-RDA | | In biomass-CCA | |
|---|---|---|---|---|
| Axis | 1 | 2 | 1 | 2 |
| Eigenvalues | 0.329 | 0.114 | 0.330 | 0.083 |
| Species-environment correlations | 0.718 | 0.657 | 0.816 | 0.586 |
| Cumulative percentage variance | | | | |
| of species data | 32.9 | 44.3 | 44.8 | 56.0 |
| of species-environment relation | 70.8 | 95.3 | 79.9 | 100.0 |
| Total variance explained | 46.5% | | 56.0% | |
| Monte Carlo permutation test | F-ratio | P-value | F-ratio | P-value |
| on the first axis | 24.549 | 0.002 | 40.504 | 0.002 |
| on all axes | 8.692 | 0.002 | 12.725 | 0.002 |

explained. Factor scores were simultaneously calculated by the method of regression and saved as new variables used in later RDA and CCA. According to factor loadings,

component 1 was mainly determined by TN (0.85), $BOD_5$ (0.76), Chl-*a* (0.67), and TSS (0.60);

component 2 by TDS (0.88), EC (0.88), temperature (-0.87), and TOC (0.69);

component 3 by pH (0.80), DO (0.79), COD (0.69), and $NH_4^+$-N (-0.57);

component 4 by $NO^{3-}$-N (0.94) and $NO^{2-}$-N (0.87); and

component 5 by IP (0.78) and TP (0.67).

With the submission of the five synthetic variables to ordinations, the first two axes accounted for 44.3% of cumulative variance in density and 56.0% in biomass. In addition, variance in zooplankton-environment relationship explained by the first two axes was 95.3% in density and 100% in biomass, which showed a strong relationship between zooplankton and the measured environment. The total variance explained was 46.5% in density and 56.0% in biomass. The Monte Carlo permutation test was significant ($p<0.05$) on the first axis and on all axes in both RDA and CCA (Table 3).

In the RDA ordination plot, Factor_1 and Factor_2 had long projections on the corresponding axes; while in CCA plot, it was Factor_1, Factor_2, Factor_3, and Factor_4 that had long projections on the corresponding axes. At the same time, density and biomass of rotifers were positively correlated with Factor_1, while those of crustaceans were negatively correlated with Factor_2 (Figs. 4a and b).

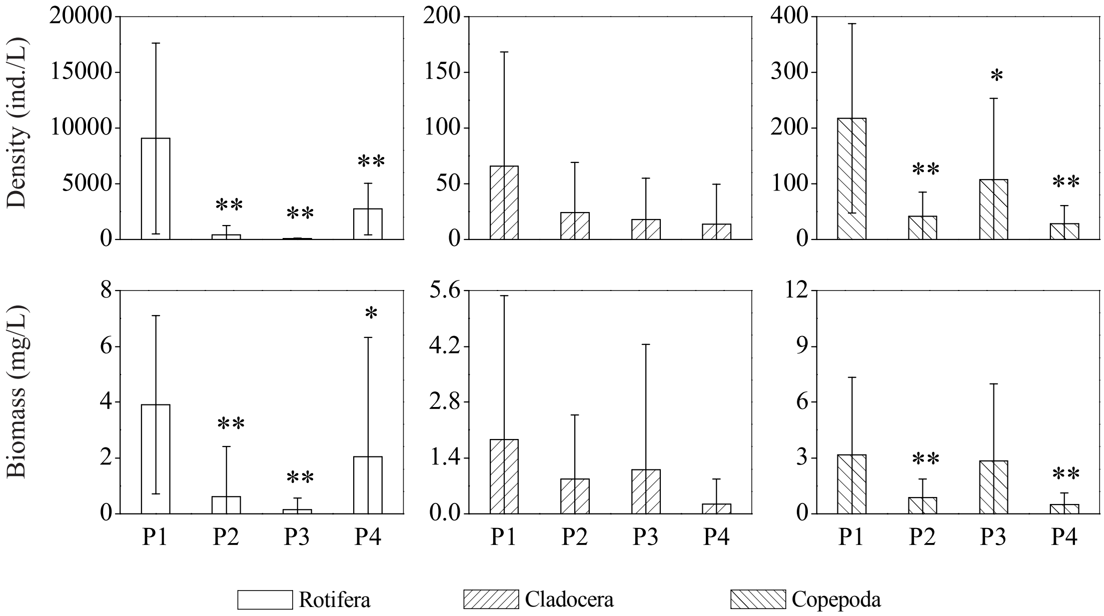


Fig. 3. Mean density and biomass (±SD) of zooplankton in the culture ponds.
Significant differences between the control and the recirculating ponds are marked with asterisks: *$p<0.05$, **$p<0.01$.

## Discussion

### Pollutant Removal by CWs

The processes involved in nutrient removal in CWs include decomposition of organic matter, uptake of nutrients by plants and bacteria, nitrification-denitrification, and absorption of ions by plants and soil [25-27], whereas suspended solids are primarily removed by sedimentation and filtration [28]. In the present work, suspended solids (including plankton) were well removed by the CWs, while removal rates for nutrients and organic matter were rather low. Furthermore, the CWs displayed lower efficiency in removing pollutants compared to other systems [6, 11, 27]. This was possibly due to the following reasons. First, pollutant loading rate or HRT for the CWs was lower compared to other wetland systems [6, 11, 27]. For example, in the studies by Tilley et al. [6], the mass loading rates for TP and $BOD_5$ were 0.55 and 18.7 kg/ha·d, respectively, with an HTR of 24 h in the wetland. Similarly, in the studies by Yang et al. [27], the mass loading rate for $BOD_5$ reached 19.0 kg/ha·d, corresponding to an HRT of 5 days. In the present study, the mass loading rates for TN, TP, and $BOD_5$ were only 2.72, 0.20, and 9.7 kg/ha·d, respectively, with an HRT of 1.3 days under the condition of 10% water exchange. Secondly, the system had been used to treat aquacultural wastewater for more than two years before this study, while phosphate removal typically depends on the nature of filter media and maturity of CWs [29]. The more CWs mature, the lower removal efficiency CWs display because sorption sites become more saturated.

### Response of Water Quality Parameters to Recirculating Treatment

After the recirculating treatment, the concentrations of nutrients, organic matter, and suspended solids in the recirculating ponds maintained relatively lower levels compared to other culture systems [6, 28, 30]. This was partly due to the purifying effect of the CWs, which was further demonstrated by the decreasing trends for nutrients (but $NH_4^+$-N, $NO^{2-}$-N, and $NO^{3-}$-N), organic matter and suspended solids in the recirculating ponds compared to the control. Lin et al. [28] studied an outdoor RAS and reported that the concentrations of suspended solids, Chl-*a*, turbidity and $NO^{3-}$-N in the culture tank were significantly ($p \leq 0.05$) lower than those in the control, which simulated static pond culture without wetland treatment. In this study, the concentrations of $NH_4^+$-N, $NO^{2-}$-N, and $NO^{3-}$-N were unexpectedly higher in the recirculating ponds than in the control. The main reason was that cyanobacterial blooms occurred heavily in the control pond during the investigation. Dissolved nutrients, such as $NH_4^+$-N, $NO^{2-}$-N, and $NO^{3-}$-N, had been incorporated into algae mostly.

### Response of Zooplankton to the Recirculating Treatment

As in water quality parameters, zooplankton standing crop in the recirculating ponds also lowered significantly compared to the control (Fig. 3). This could possibly be explained by the following. First, as stated previously, zooplankton were effectively removed by the CWs. Second, zooplankton community structure was strongly associated with the measured environment, as unraveled by the multivariate analyses. Since the recirculating treatment changed the environmental conditions in the culture ponds significantly, it would also lead to changes in community structure. Nevertheless, unlike water quality parameters, zooplankton standing crop in the recirculating ponds showed no obvious trends with the increasing stocking densities (Fig. 3).

Generally, the length of an environmental arrow in a constrained ordination plot indicates the importance of the variable. More formally, the length is equal to the multiple correlation of the variable with the displayed ordination

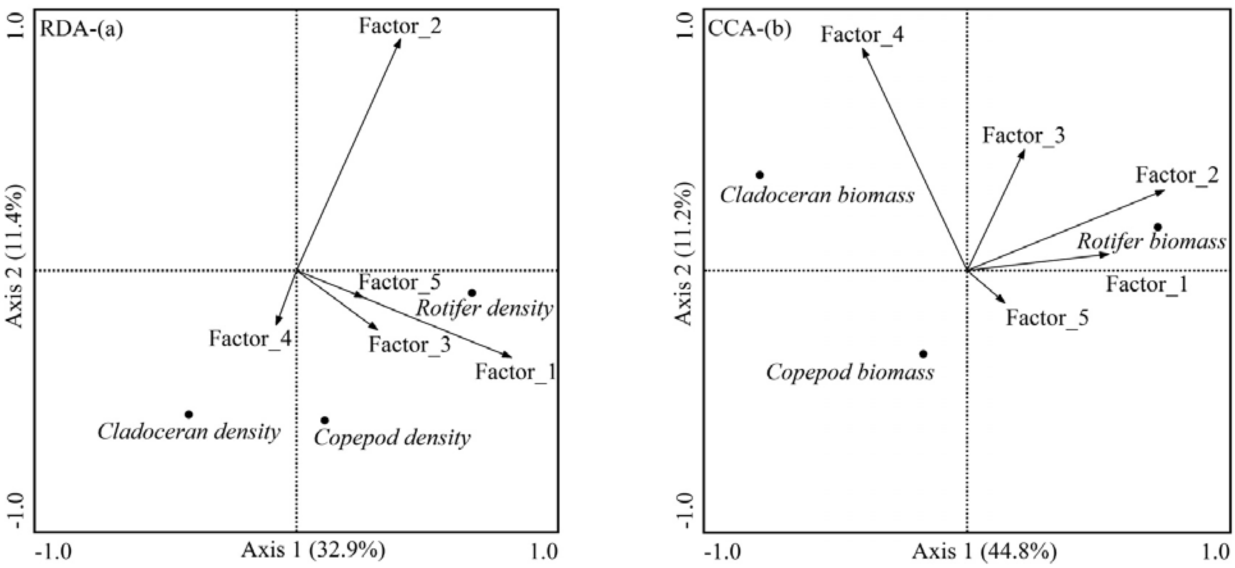


Fig. 4. Ordination plots based upon zooplankton community data and new synthetic environmental variables.

axes, to the maximum rate of change of the variable, and to the size of the effect that the corresponding variable has on the ordination scores while neglecting other variables [31]. In the present RDA and CCA ordinations, both Factor_1 and Factor_2 (the first two synthetic variables) had long projections on the corresponding axes (Axis 1 or 2) implying their importance in shaping zooplankton community structure. While in PCA, the first component was mainly determined by TN, $BOD_5$, Chl-*a*, and TSS, and the second one by TDS, EC, and temperature. Hence, it was conclusive that these factors separated by the two components from the measured environment should be the major determinants of zooplankton community structure in the culture ponds.

In fact, the first synthetic environmental variable in the ordinations emphasized the importance of food sources (i.e. phytoplankton) for zooplankton, while the second one reflected the physical conditions of the rearing water. Nevertheless, phosphates, commonly considered the limiting factor for phytoplankton growth, showed a weak link to zooplankton community structure in the culture ponds. In the ordinations, the density and biomass of rotifers were positively correlated with Factor_1, while those of crustaceans were negatively correlated with Factor_2. Hence, it could be determined that phytoplankton in the culture ponds played an important role in shaping the rotifer community, while the crustacean zooplankton community was mainly associated with the physical conditions among the measured environment.

## Conclusions

The vertical-flow CWs applied in the RAS showed relatively higher removal efficiency for particulate matter (more than 55%), while lower, uneven removal efficiency for nutrients and organic matter (from -34.1% to 48.7%). After the recirculating treatment, nutrients (but $NH_4^+$-N, $NO^{2-}$-N, and $NO^{3-}$-N), organic matter and suspended solids including plankton in the recirculating ponds decreased apparently to low levels compared to the control. Cyanobacterial blooms occurring heavily in the control were strongly restrained in the recirculating ponds. Hence, conclusions could be reached that the recirlulating treatment by the CWs achieved its aim of sustaining or extending water quality improvement in the RAS.

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