

# Physicochemical water quality parameters in typical rice-crayfish integrated systems (RCIS) in China

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**Abstract:** To determine the variation of water quality in rice-crayfish (*Procambarus clarkii*) integrated systems (RCIS) in China, eleven water quality parameters were measured monthly in a typical RCIS located in Qianjiang City (Hubei Province) from July 2014 to June 2015, the parameters were analyzed with principal component analysis (PCA) and compared between the trenches and rice areas during the rice fallow period (Nov-May). The trench and rice area comprehensive results showed that pH (7.48-8.68),  $\text{NH}_4^+\text{-N}$  (0.2-1.09 mg/L),  $\text{NO}_2\text{-N}$  (<0.052 mg/L) and conductivity (435-951  $\mu\text{S/cm}$ ) were within the suitable ranges for *P. clarkii* and that turbidity (TU) was high during the crayfish harvesting and rice planting season. Annual averages of total nitrogen (TN), total phosphorus (TP), permanganate index ( $\text{COD}_{\text{Mn}}$ ), and chlorophyll *a* (Chl.*a*) content were <2 (except in Nov-Dec), 0.25, 10 mg/L, and 50 mg/m<sup>3</sup> (especially in Nov-May, <10 mg/m<sup>3</sup>), respectively. Dissolved oxygen (DO) was below 4 mg/L in Mar-Sep, with a minimum of ~ 1 mg/L, and much higher in Oct-Feb. The maximum and minimum monthly average water temperature (WT) were 31.4°C in July and 5.7°C in December, while the maximum and minimum instantaneous WT were 39.7°C and 2.5°C, respectively. PCA analysis showed that the first three axes, which were mainly correlated with DO, WT and nutrient level, described most information of the parameters, and parameters showed seasonal changes. Some differences were observed in water parameters between the trenches and rice areas, i.e., trenches generally had higher TU, WT and DO, and lower TN, TP and  $\text{COD}_{\text{Mn}}$ , although no significant differences were found in some months and some parameters. The study revealed relatively low water nutrient level, probable extreme WT and DO level in some seasons, and certain differences between the trenches and rice areas in typical RCIS in China. Accordingly, some measures should be taken to improve the negative parameters: 1) enhance the water fertility; 2) increase DO, especially in Mar-Sep; 3) increase the trench and water depth to avoid extreme WT. And water quality management should be addressed in both trenches and rice areas.

**Keywords:** rice-crayfish integrated system, co-culture, water quality parameters, trench, rice production area, PCA

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## 1 Introduction

The integration of rice and fish culturing has been successfully practiced for 2000 years and, in addition to food, provides social, economic and ecological benefits<sup>[1,2]</sup>. China has the longest history, the largest area and the highest fish yields of rice-fish coculture in the world<sup>[3]</sup>, with an area of about 1.5 million hm<sup>2</sup>

(24.49% of the total inland aquaculture areas), and a total yield of 1.56 million tons of fish in 2015<sup>[4]</sup>. Although integration of rice with crayfish (*Procambarus clarkii*) (RCIS) has a short history in China, and it has become popular and profitable, especially in the middle-lower reaches of Yangtze River<sup>[2]</sup>. Until 2009, RCIS accounted for about 60% of the total annual yield of *P. clarkii* in China, with a total area of 280 000 hm<sup>2</sup><sup>[5]</sup>, and the yield and culture area are increasing. A Chinese RCIS is typically operated as follows<sup>[2,6,7]</sup>: Peripheral trenches (2-4 m wide, ~1.5 m deep) and, if necessary, cross trenches (1-2 m wide, 0.8-1.2 m deep) are excavated, and a surrounding fence is set up using materials such as asbestos sheets or polypropylene mesh. Aquatic plants are set to cover 20%-50% of the trench area to provide shelter for crayfish. 300-750 kg/hm<sup>2</sup> of berried crayfish are stocked Oct-Nov, or 150 000-225 000 individual/hm<sup>2</sup> of juvenile crayfish are stocked Mar-May. Crayfish can be stocked annually, or only in the first year if a sufficient number of crayfish are left to reproduce. Supplementary feeding is necessary for high yield production. Trap harvesting of crayfish is carried out Apr-Jun. Middle-season rice is planted in June and harvested in October. The range of yields for crayfish and rice are 750-2250 kg/hm<sup>2</sup> and

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7 500-10 500 kg/hm<sup>2</sup>, respectively, depending on management, disease and climate.

Research on domestic RCIS has focused on farming techniques<sup>[6,7]</sup>, and few reports have focused on water quality. Water, in deed, is the most important single factor for all aquacultural activities. The growth and survival of crayfish inevitably depend on water quality<sup>[8]</sup>, and crayfish culture strongly effects water quality<sup>[9,10]</sup>. The inundated rice fields used for crayfish culture comprise a special type of wetlands: 1) Rice fields are shallow closed water systems. They are strongly affected by weather conditions, and display large spatial and temporal variations in turbidity, temperature, pH, dissolved oxygen (DO) and nutrient levels<sup>[11]</sup>. 2) Rice fields used for RCIS are mainly maintained and influenced by agricultural activities<sup>[11]</sup>. Water quality changes with cultivation, such as plowing, rice transplanting, fertilization, feeding and crayfish harvesting. 3) Crayfish bioperturbation on the rice field system transforms nutrients from sediments (containing detritus, crayfish feces and residual feed) to water<sup>[12]</sup>. 4) Nutrients of decomposing rice stubble are continuously released to water in rice fallow period. 5) The limited water volume in rice fields limits buffering and self-purification capacity<sup>[13]</sup>. 6) The trenches and rice areas in RCIS share different engineering structure and maintenance, probably presenting different water conditions. In short, changes and trends in water quality are partly caused by the special structure and management of RCIS, and they can indicate strengths and weaknesses of the current operation. For instance, Zhang et al.<sup>[13]</sup> observed significant differences of water DO, NH<sub>3</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and PO<sub>4</sub><sup>3-</sup>-P between trenches and rice areas in some stages in rice-crab (*Eriocheir sinensis*) coculture system. Another example, with a water depth similar to trenches in RCIS, Ni et al.<sup>[14]</sup> reported frequent water temperature (WT) exceeding 32.2°C, which is detrimental or even lethal to *P. clarkii*<sup>[15]</sup> in crayfish ponds during summer. Although Mao et al.<sup>[16]</sup> reported on the stability of water quality parameters in a Chinese RCIS, but only nutrients (ammonia, nitrate, nitrite and available phosphorus) were measured, and temporal variations were not reported. Thus, variations of water quality parameters of RCIS in China is still to be understood.

The purposes of this study were to: 1) understand the variations of water quality parameters in typical RCIS in China; 2) determine the differences of water quality between the trench and rice area. Eleven physicochemical parameters of water quality, in both trenches and rice areas of a typical RCIS in Qianjiang City, Hubei Province, China, were measured monthly from July, 2014 to June, 2015. Water parameters were then analyzed with PCA, and trench and rice area water were compared during the rice fallow period. Qianjiang City was selected because it is the birthplace of RCIS and “The Land of Crayfish” in China<sup>[5,7]</sup>. The results can reveal the changing patterns of water quality parameters of RCIS, and help RCIS farmers to improve aquaculture practices, such as how to prevent extreme water quality factors, how to improve the field engineering and so on.

## 2 Materials and methods

### 2.1 Study site

The study was conducted at Zhangjiayao Farm, Houhu Management District, Qianjiang City, Hubei Province, China (Figure 1a). Qianjiang City (112°29'-113°01'E, 30°04'-30°39'N) is located in the hinterland of Jiangnan Plain, where rich water resources and wetlands provide excellent crayfish habitats.

### 2.2 Agronomic and aquacultural management

There were 16 rice plots in the study aquafarm (Figure 1b and 1c). Each plot was 2.33 ha: 0.33 ha for trenches (four peripheral trenches and a cross one) and 2 ha for rice area. Peripheral trenches of each plot were 1.5 m deep by 4.0 m wide and the cross trench was 1.0 m deep by 1.8 m wide. Plot inlets and outlets were screened. *Elodea nuttallii* and *Alternanthera philoxeroides* were planted in the trenches on March 5, 2014, and maintained at about 20% coverage and *E. nuttallii* was also planted in the rice areas during the rice fallow period. Juvenile crayfish (weight of 3-5 g, 15 000 individual/hm<sup>2</sup>) were released on March 20. Crayfish were fed daily with commercial pellets (28% crude protein, 4% crude lipid, 8% crude fiber, Hubei Kewang Graziery Limited Liability Company) at a rate of 3%-8% of elevated total crayfish weight. The daily amount varied, but totaled ~750 kg/hm<sup>2</sup>. Crayfish harvest lasted from April 25 to June 10, and sufficient crayfish were left as breeding stock. The annual crayfish yield was 1560 kg/hm<sup>2</sup>.

Middle-season rice (Huanghuazhan) was transplanted on June 20th. Before rice seedling transplantation, the soil and remaining rice straw were ploughed and compound fertilizer was broadcast (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O: 17-17-17, 600 kg/hm<sup>2</sup>). Ten days after transplanting, tillering fertilizer and urea (150 kg/hm<sup>2</sup>) were broadcast. Rice was harvested in October 16, with rice straw (height of ~35 cm, with roots) left. The annual rice yield was 8900 kg/hm<sup>2</sup>. Water addition was performed on October 25 to half-submerge rice straw, and the water level was maintained during the rice fallow period Nov-May.

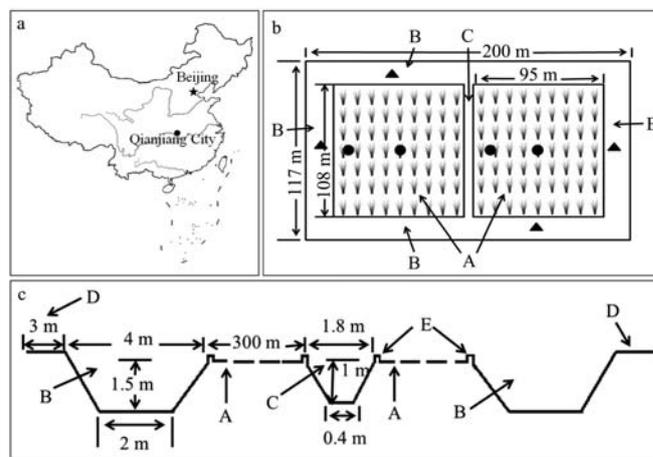


Figure 1 (a) location of study site; (b) plane figure of rice-crayfish plot (dimensions, trench sampling points ▲ and rice area sampling points ●); (c) cross-section of RCIS plot (A, rice area; B, peripheral trench; C, cross trench; D, plot dike; E, trench dike)

Figure 1 Study site and design of RCIS plots

### 2.3 Water quality parameters monitoring

Four rice plots were selected as replicates and water samples were collected at 8:00-10:00 on the 22<sup>nd</sup> of each month from July 2014 to June 2015. Four 500 mL samples were collected in both the peripheral trenches (triangles in Figure 1b) and rice areas (circles in Figure 1b) of each plot. The four samples from each area were combined to give two 2000 mL composite samples for each plot. TU, WT, DO, conductivity (Cond) and pH were tested in situ using a HACH 2100Q turbidimeter and a YSI Pro Plus Multi-Parameter water quality meter. TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N, TP, COD<sub>Mn</sub> and Chl.*a* were measured according to APHA methods<sup>[17]</sup> in the laboratory. Water during the rice growing season (Jun-Oct) could not be sampled because there was little or no water in the rice areas.

WT changes quickly with air temperature and monthly monitoring is not sufficient to characterize the annual variations. To obtain highly resolved temporal data, a HOBO Pendant Temperature/Light data logger (Onset Computer Corporation) was placed half a meter below the water surface to record values every two hours during the entire study period.

#### 2.4 Statistical analyses

All calculations and statistical analyses were performed using Excel 2003 and R 3.2.4, respectively. The T tests ( $p < 0.05$ ) was used to compare differences between the trench and rice area water parameters. The PCA was performed on a correlation matrix of standardized and transformed data to reduce the dimensionality, with “ade4”, “psych” and “factoextra” packages.

### 3 Results

#### 3.1 Trench water parameters

Water pH and Cond varied at 7.48-8.68 (Figure 2a), and 435-951  $\mu\text{S}/\text{cm}$  (Figure 2d), respectively. Maximum and minimum values of average WT were respectively 31.4°C in July and 6.5°C in December (Figure 2b), and the maximum and minimum instantaneous WT were 39.7°C in July and 2.5°C in January (Figure 3). DO remained above 4 mg/L in Oct-Mar and  $\leq 3$  mg/L in Apr-Jun, and the lowest value of 1.36 mg/L was recorded in June (Figure 2c). TU remained relatively low ( $< 10$  NTU) in Oct-Mar, and rose sharply in the fishing and planting season (Figure 2e).

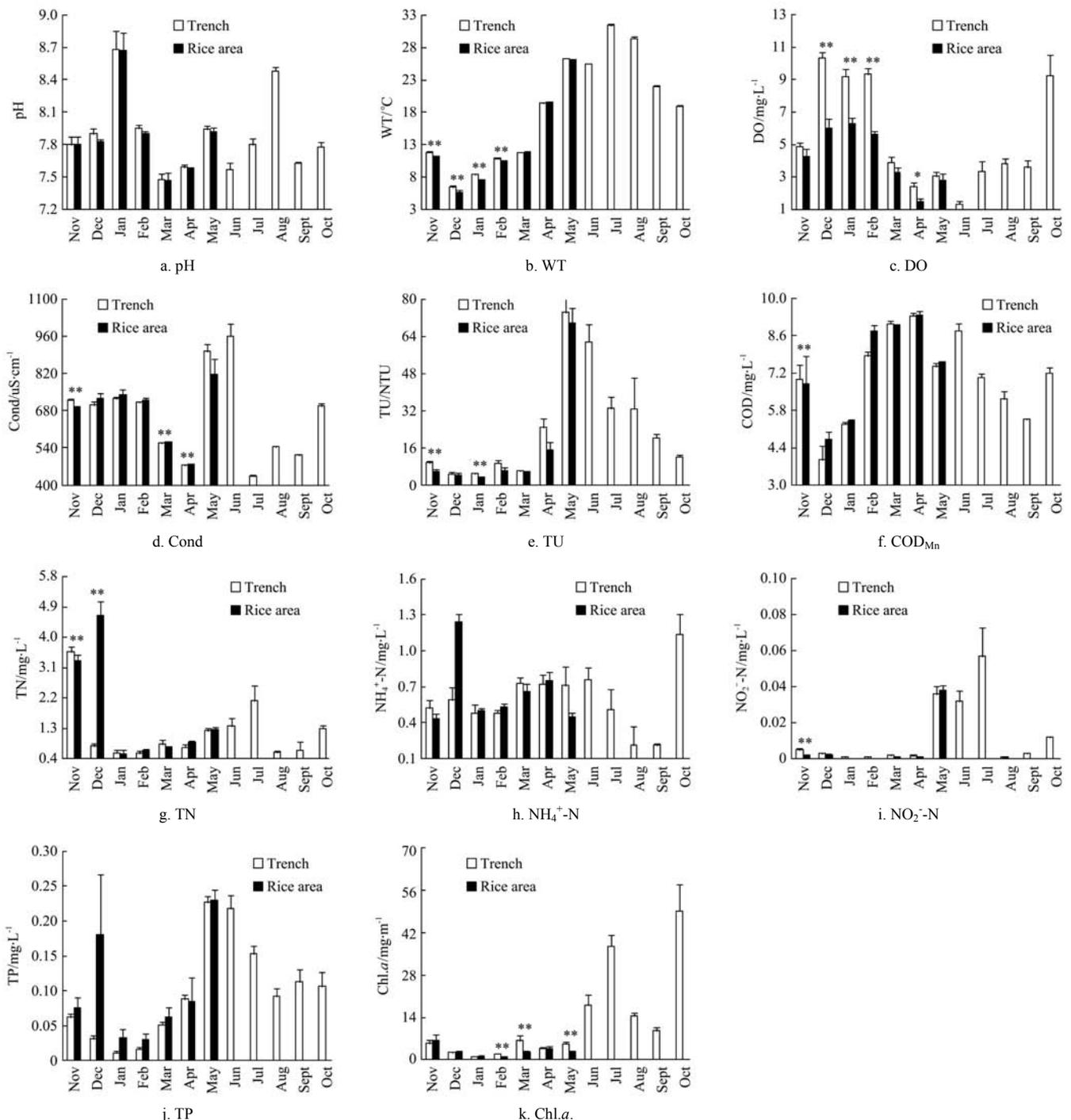


Figure 2 Water quality parameters in trenches and rice areas of RCIS (mean  $\pm$  SE). Asterisks represent significant differences between trench and rice area water (\*  $p < 0.05$ , \*\*  $p < 0.01$ )

COD<sub>Mn</sub> varied at 3.74-9.36 mg/L, with a minimum and maximum in December and April, respectively (Figure 2f). TN generally increased Jan-Jul, decreased sharply to the minimum of 0.59 mg/L in August, increased rapidly through November to 3.57 mg/L and decreased until January (Figure 2g). NH<sub>4</sub><sup>+</sup>-N showed two relatively stable periods Nov-Feb and Mar-Jul, decreased Aug-Sep to the minimum of 0.21 mg/L, and then rose sharply to 1.14 mg/L in October (Figure 2h). NO<sub>2</sub><sup>-</sup>-N was nearly always <0.01 mg/L except May-Jul, when it peaked at 0.035-0.052 mg/L (Figure 2i). TP increased from January, peaked in May-Jun at 0.22 mg/L and then declined until the next January, excepting a slight rise in September (Figure 2j). Chl.*a* was below 10 mg/m<sup>3</sup> in Nov-May, and relatively high in Jul-Oct (Figure 2k).

The results of PCA analysis described 41.97%, 18.02% and 13.26% of the variance in the water parameters in the first three axes, with the eigenvalues of 3.77, 1.62 and 1.19, respectively. DO (0.702) showed the highest positive correlation with the first PCA axis, whereas TP (-0.822), NO<sub>2</sub><sup>-</sup>-N (-0.802), WT (-0.789), TN (-0.74), Chl.*a* (-0.543) and COD<sub>Mn</sub> (-0.529) exhibited a negative correlation with this axis. The first PCA axis was related to the eutrophic conditions, temperature and DO in trench water. COD<sub>Mn</sub> (0.628) was positive correlated with the second PCA axis, while DO (-0.56) and Chl.*a* (-0.546) were negative correlated with this axis (Figure 4A).

The location of winter (Wi) was separated from the others in the ordination diagram (Figure 4A). Water in winter showed the highest DO, while summer (Su) exhibited highest eutrophic level and WT, and spring (Sp) and autumn (Au) located in the middle.

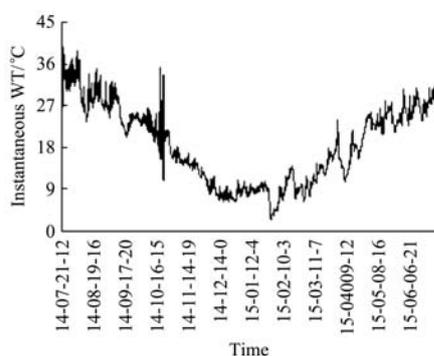


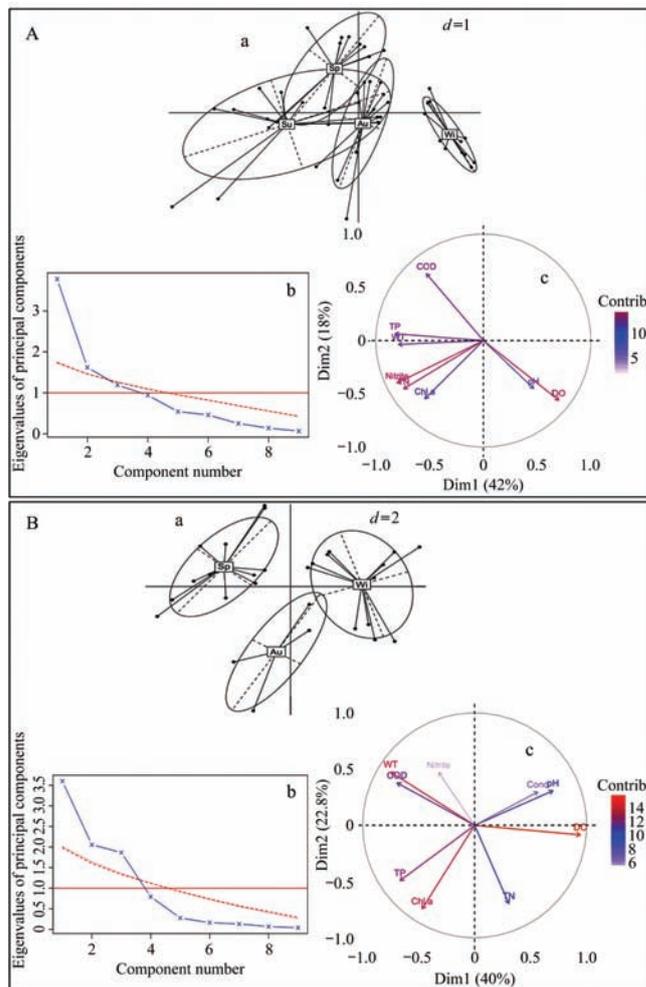
Figure 3 Instantaneous temperature of trench water from July, 2014 to June, 2015

### 3.2 Rice area water parameters during the rice fallow period

During the rice fallow period, the dynamic interaction of rice area water with trench water was reflected in all parameter values, excepting a few occasions with TN, NH<sub>4</sub><sup>+</sup>-N and TP (Figure 2). The range for each parameter was as follows: pH, 7.47-8.67; WT, (5.7-26.1)°C; DO, 1.48-6.28 mg/L; Cond, 480-819 μS/cm; TU, 4.28-69.83 NTU; COD<sub>Mn</sub>, 4.72-9.36 mg/L; NO<sub>2</sub><sup>-</sup>-N, 0-0.038 mg/L and Chl.*a*, 0.90-6.50 mg/m<sup>3</sup>. TN peaked in Nov-Dec, declined drastically in January, and then showed a slow increase (Figure 2g). NH<sub>4</sub><sup>+</sup>-N and TP displayed seasonal variations similar to TN, excepting in May (Figure 2h and 2j).

The first three axes described 40.02%, 22.84% and 20.72% of the variance in the water parameters in the PCA analysis, with the eigenvalues of 3.60, 2.06 and 1.86, respectively. DO (0.945), pH (0.67) and Cond (0.564) showed the highest positive correlation with the first PCA axis, whereas WT (-0.749), COD<sub>Mn</sub> (-0.693) and TP (-0.666) exhibited a negative correlation with this axis. Chl.*a* (-0.743) and TN (-0.67) were negative correlated with the second axis (Figure 4B).

The location of winter (Wi) in the ordination diagram indicated its strong correlation with Cond, pH and DO, while spring (Sp) with WT, COD<sub>Mn</sub> and TP, and autumn (Au) with Chl.*a* and TN (Figure 4B).



Note: Sp: spring; Su: summer; Au: autumn; Wi: winter. a: ellipsoid represents samples for each season ordinated in the PCA plane; b: Parallel analysis scree plots of the eigenvalues of each PCA axis (principal components were integrally determined by the eigenvalues >1, the drastic spindal in breakline and the intersection of the breakline and dotted line); c: the correlation circle of environmental variables (COD means COD<sub>Mn</sub>, and the darker color means higher contribution). TU and NH<sub>4</sub><sup>+</sup>-N were removed to satisfy the requirements in PCA analysis as they were significantly related to WT, TN ( $p < 0.05$ ), respectively.

Figure 4 PCA analysis of trench (A) and rice area (B) water parameters

### 3.3 Differences in parameter values between trenches and rice areas

Mean values measured during the rice fallow period of all parameters were compared between the trench and rice area water. No significant differences were detected for pH, NH<sub>4</sub><sup>+</sup>-N or TP (Figure 2a, 2h, and 2j). The mean WT of trench water was significantly higher than rice area water in Nov-Feb ( $p < 0.01$ ) (Figure 2b). A significant higher level of DO of trenches was observed in December, January, February and April ( $p < 0.05$ ) (Figure 2c). Except in November and May, the trench Cond was lower than the rice area, and significant differences were detected in November, March and April ( $p < 0.01$ ) (Figure 2d). TU in trenches was higher than in rice areas, but significant differences were observed only in November and January ( $p < 0.01$ ) (Figure 2e). The differences in COD<sub>Mn</sub> and NO<sub>2</sub><sup>-</sup>-N between the trench and rice

area water were only significant in November ( $p < 0.05$ ) (Figure 2f and 2i). TN of the trench water was significantly higher in November, and significantly lower in December ( $p < 0.01$ ) than that of rice area water (Figure 2g). Chl.*a* of the trench was higher than the rice area, excepting in November, and significant differences were observed in February, March and May ( $p < 0.01$ ) (Figure 2k).

## 4 Discussion

Water is the most important single factor for all aquacultural activities. Survival, growth and immunity of *P. clarkii* are dependent on the presence and the quality of water<sup>[8]</sup>, which have been summarized by Ren et al.<sup>[18]</sup>. Unsuitable water pH, temperature, DO,  $\text{NH}_4^+\text{-N}$   $\text{NO}_2^-\text{-N}$ , salinity and heavy metal are detrimental and even lethal to *P. clarkii*. For instance, a WT beyond 32.2°C, and a DO under 3 mg/L, have been considered to be hypoxic and growth inhibitory to *P. clarkii*, respectively<sup>[15,19]</sup>. Rice growth is also affected by water parameters. It is known that rice seed germination increases with increasing water transparency<sup>[20]</sup>, and increased turbidity may decrease the efficiency of some chemicals commonly added to rice fields<sup>[21]</sup>.

### 4.1 Water parameter dynamics in trenches and rice areas

Yue et al.<sup>[22]</sup> reported that the maximum survival and weight gain, and highest moult frequency of juvenile *P. clarkii* occurred at pH 7.8 compared with pH of 6.8 and 8.8, and suggested the pH range 7.8-8.5 for juvenile *P. clarkii*. Water pH in this study ranged from 7.47 to 8.67, which is close to the range suggested by Yue.

The temperature range suitable for *P. clarkii* is (21-30)°C, with the optimum ~ 23°C<sup>[23,24]</sup>, and temperature below 12.8°C or above 32.2°C has been determined to be detrimental to this crayfish. Research shows that *P. clarkii* has normal feeding and moulting activities at temperature above 12.8°C and inhibited growth at temperature above 32.2°C<sup>[15]</sup>. Li et al.<sup>[25]</sup> reported that juvenile *P. clarkii* stopped feeding at a temperature of 5°C-6°C and died at 1°C. The maximum and the minimum instantaneous WT were respectively 39.7°C and 2.5°C in this study, and there were 22 and 8 days with instantaneously beyond 32.2°C and below 5°C, respectively. Lethal WT could thus occur in Jul-Aug and Dec-Feb. Deepen the trench depth to  $\geq 2$  m and increase the trench dike height (to prevent water to enter rice area in the vegetative period of rice) to increase water depths can decrease the stress imposed by the existing extremes in summer and winter.

Hypoxic conditions (DO < 3 mg/L) is a major limiting factor in crayfish culture<sup>[19]</sup>. Research indicates that a DO level of 2 mg/L stresses *P. clarkii* and 1 mg/L is lethal to juveniles<sup>[19,26]</sup>. Typically, nocturnal oxygen consumption due to respiration of crayfish, plants and aerobe and chemical oxidation processes, leads to minimum DO at dawn. In this investigation, DO did not exceed 3 mg/L in Apr-Jul and the minimum was < 1.5 mg/L. DO at dawn is thus speculated to be lower than the measured values because photosynthesis had begun when water samples were collected (8:00-10:00 AM). DO at dawn in Mar-Sep is probably at hypoxic levels and stress *P. clarkii* in Apr-Jul. High stocking densities further aggravate hypoxic conditions and measures, such as mechanical aeration and water exchange, should be conducted during the warm season to increase DO levels.

Large variations in TU are common in rice paddies<sup>[11]</sup>. Crayfish bioperturbation of sediments, and foraging and fragmentation on macrophytes both further increase turbidity<sup>[9,10]</sup>, as does crayfish harvesting, plowing and rice transplanting. These factors all lead to higher TU in Apr-Sep. Low TU in Oct-Mar was

attributed to the suspension of farming and inactivity of crayfish during the low temperature period. High TU suppresses photosynthesis of hydrophytes and phytoplankton<sup>[27]</sup>, and affects visually dependent predator-prey interactions<sup>[28]</sup>, some measures, such as minimizing bottom disturbance, should be adopted to reduce TU in Apr-Sep.

The main sources of nitrogen and phosphorus in RCIS water is nutrient content of inflow, crayfish feed and feces, fertilizer, aquatic plants, sediment and decomposition of rice straw in rice fallow period. In this study, TN and TP increased slowly Jan-Mar, primarily from slow sediment release of nutrients after feeding suspension and crayfish become inactive. As WT increases in the spring, crayfish become more active, decomposition increases and the release of nutrients from sediment accelerates<sup>[12]</sup>. The addition of feed pellets, sediment disturbance caused by crayfish harvesting and intermittent fertilization during rice transplantation all contributed to the increased TN and TP in Apr-Jul. TN and TP both declined markedly in Aug-Sep, due to decreased feeding, lower crayfish biomass and more frequent water exchange during the hot season. After that, refeeding increased TN and TP instantly. An obvious increase in TN and TP in rice area water Nov-Dec was observed and is attributed to the rapid decomposition of rice straw and roots<sup>[29]</sup>. As large quantities of commercial feed and fertilizer were added to the coculture system during the study, the relatively low levels of water TN and TP would be owing to crayfish and aquatic plant consumption<sup>[30]</sup>, water exchange, and sedimentation<sup>[31]</sup>. Macrophytes in RCIS can absorb large amount of N and P<sup>[30]</sup>, and reduce turbidity<sup>[32]</sup>. Further study is needed to evaluate nitrogen and phosphorus sedimentation and loss in water exchange in RCIS. And a good sediment management and maintaining a healthy hydrophyte population should be emphasized.

The factors driving the variation of TN and TP seemed to also drive the variations in  $\text{COD}_{\text{Mn}}$  and Chl.*a*. Typically, the relationships of Chl.*a* and WT, TN, TP and TU are positive<sup>[33]</sup>. In the study, Chl.*a* did generally increase with TN and TP, but no obvious relationship between Chl.*a* and TU was observed.

Ammonia nitrogen is a ubiquitous concern and one of the major limiting factors in crustacean aquaculture, affecting growth, survival and physiology<sup>[34]</sup>. A "safe" concentration of  $\text{NH}_4^+\text{-N}$  to juvenile *P. clarkii* was reported as 7.94 mg/L<sup>[35]</sup>, which is much higher than the measured concentration, indicating a low acute ammonia toxic risk for crayfish. The proportion of  $\text{NH}_4^+\text{-N}$  in TN was relatively high in this study, sometimes more than 60%, and this is consistent with the results of several other studies<sup>[12,16,36]</sup>. Ammonia is produced partially by fertilization, ammonification in sediment and water, organisms corpse decay, and crayfish excretion (60%-90% of TN is ammonia in freshwater crustacea)<sup>[37]</sup>. These processes lead to a high proportion of  $\text{NH}_4^+\text{-N}$  in TN.

Nitrite is formed from the aerobic nitrification of  $\text{NH}_4^+\text{-N}$  and continued oxidation converts  $\text{NO}_2^-\text{-N}$  to  $\text{NO}_3^-\text{-N}$ . However, nitrification is often limited in rice-crayfish systems for the anoxic condition in sediments<sup>[36]</sup>, leading to low  $\text{NO}_2^-\text{-N}$  levels in this study (0-0.057 mg/L), and far below the "safe" concentration of nitrite to *P. clarkii* (2.8 mg/L)<sup>[38]</sup>. Thus, no nitrite toxicity to crayfish was suspected in the investigated water of this study.

PCA analysis showed that water quality parameters in both trenches and rice areas were most correlated with DO, and then with WT and nutrient level, indicating the especial importance of DO, WT and nutrient level monitoring and management. It also indicated the differences of water quality parameters among different seasons.

## 4.2 Comparison of water quality between trenches and rice areas

WT was higher in the trenches, which serve as a warmer environment for crayfish in winter. During the fallow season, oxygen consumption by rice straw decomposition and zooplankton nourished by the decomposition resulted in lower DO in the rice area water. Water TU in rice areas was lower than in trenches because the sediment in rice areas was more compact and less easily disturbed than in trenches, and some suspended solids adhered to rice straw. The decomposing rice straw produced a massive zooplankton bloom (personal observation) that then consumed phytoplankton, resulting in lower Chl.*a* content in rice area water than in trenches. Cond, COD<sub>Mn</sub>, TN, TP and NH<sub>4</sub><sup>+</sup>-N in rice area water were generally higher than in trenches, mainly due to the decomposing rice straw, and generally consistent with that in rice-crab coculture systems<sup>[13]</sup>. The differences in water quality between the trenches and rice areas indicate that the two areas constitute different habitats in the rice-crayfish coculture system.

Generally, this work is a primary study of variations of water quality parameters of the most typical RCIS in China, and some underlying driving forces of bio-, physical-, chemical progress are quite complicated, and how these parameters will change with the variations of maintenance in RCIS (e.g. different crayfish stocking densities, field engineering, feeding strategies, and water managements, etc) are still to be understood. Thus, underlying mechanism of these variations and how to maintain a better water environment in Chinese RCIS should be further explored. Furthermore, as severe extreme water parameters (especially for WT and DO) are lethal for crayfish, real-time water quality monitoring systems<sup>[39]</sup> should be used for judgement of extreme water factors.

## 5 Conclusions

A monthly measurement of 11 water quality parameters of CRIS in China was conducted, and these parameters were compared between the trenches and rice areas in the rice fallow period. The following conclusions were obtained:

1) Water pH, NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and Cond were within the suitable ranges for *P. clarkii*, while TU was high during the crayfish harvesting and rice planting season.

2) Annual average TN, TP, COD<sub>Mn</sub> and Chl.*a* were <2 (except in Nov-Dec), 0.25 and 10 mg/L, and <50 mg/m<sup>3</sup>, respectively, indicating a relatively low water nutrient level.

3) Extreme WT (>32.2°C and <5°C) may appear in Jul-Aug and Dec-Feb, respectively, and hypoxic conditions (DO<3 mg/L) may appear in Mar-Sep.

4) Water quality parameters in both trenches and rice areas were most correlated with DO, and then with WT and nutrient level, and showed certain seasonal differences.

5) There were certain differences in water parameters between the trench and rice area, i.e., trenches generally had higher TU, WT and DO, and lower TN, TP and COD<sub>Mn</sub>. Although no significant differences were found in some months and some parameters, water quality management should be addressed in both trenches and rice areas.

6) Some measures should be conducted to enhance the water fertility, increase DO (especially Mar-Sep), increase the trench and water depth to avoid extreme WT in summer and winter, and improve water quality monitor and anticipation.

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