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Assessment of Constructed Wetland in Nutrient Reduction, in the Commercial Scale Experiment Ponds of Freshwater Prawn *Macrobrachium rosenbergii*

Tariq Mahmood[1,2] · Jing Zhang[2] · Guosen Zhang[2]

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Abstract A free water surface constructed wetland (CW) was integrated into two commercial ponds of *Macrobrachium rosenbergii*, to evaluate the role of CW in reducing the excess nutrient concentration and other pollutants produced from the aquaculture waste. Hydraulic residence time was kept constant (24 h). There was a significant ($p < 0.05$) decrease in total suspended solids (TSS, 73.2 ± 15.4 %) and total nitrogen (TN, 39.6 ± 44.2 %) between wetland inflow and wetland outflow. The performance of the CW was highly impacted by the low concentration of dissolved nutrients at the inflow of CW. Results showed about 43.8 ± 24.6 % NO_3^-, 25.7 ± 23.0 % NH_4^+, 14.3 ± 1.0 % NO_2^-, 28.4 ± 18.8 % DIN and 13.1 ± 10.0 % PO_4^{3-} were removed. In agreement with previous published investigations, comparing values of pollutants before and after recirculation, this study concludes that a CW system can provide good water quality and minimize external water input.

Keywords *Macrobrachium rosenbergii* · Constructed wetland · Nutrient · Water re-circulation

Nutrients, particularly nitrate and phosphate, are of primary concern for aquaculture water quality. Uneaten feed and waste are the major sources of nitrogen in farmed ponds (Pasugdee et al. 2006). Feed input is one of the main external sources of nutrients in a culturing pond and if not managed properly may increase nutrients to lethal levels for cultured species. Effluents can also cause eutrophication into the source water (Boonyaratpalin 1983). Therefore water exchange is necessary to maintain good water quality in any kind of culture system. External input of freshwater for water exchange in cultured ponds can raise the production cost. At the same time freshwater shortage can create problems in maintaining good water quality in aquaculture ponds.

The use of constructed wetlands (CW) has been in practice over the last four decades in removing a variety of pollutants including sewage, industrial wastewater, agricultural, aquacultural, dairy farm effluent and storm runoff (APHA 1985; Tanner et al. 1995; Kadlec and Knight 1996; Reddy and D'Angelo 1997). Regarding aquaculture, previous studies demonstrated the performance of a CW in shrimp (*Litopenaeus vannamei*) aquaculture (Tilley et al. 2002; Lin et al. 2002, 2003, 2005; Shi et al. 2011; Zang et al. 2012) and in fish ponds (Sindilariu et al. 2009; Konnerup et al. 2011) towards removing pollutants including nitrogen, phosphorus, total suspended solids (TSS) and phytoplankton. Two types of CWs are commonly utilized classified on the basis of water flow—free water surface (FWS) and sub-surface flow (SSF). Both of these are considered to be very effective in removing nutrients, total nitrogen (TN), total phosphorus (TP) and TSS from the wastewater (Lee et al. 2009). As the removal of TSS, organic matter and nutrients are critical for recirculating aquaculture systems, a variety of biological, chemical and mechanical filters have been tried in recent years. Mechanical filters have the disadvantage of high manufacturing and operating costs and also produce sludge and require a high energy supply (Kristiansen and Cripps

✉ Tariq Mahmood
tariqnio@gmail.com

[1] School of Resources and Environmental Science, East China Normal University, 3663 Zhongshan Road North, Shanghai 200062, People's Republic of China

[2] State Key Laboratory of Estuarine and Coastal Research, East China Normal University, 3663 Zhongshan Road North, Shanghai 200062, People's Republic of China

1996; Lin et al. 2003). On the other hand a FWS-CW system is considered to mimic a natural wetland with low running costs and desirable results in which biological and chemical processes occur in a single unit.

Macrobrachium rosenbergii, De Man 1879, commonly called the giant freshwater prawn, is one of the most commercially important aquaculture species in the world, widely cultured in many countries. Global production of giant prawn was reviewed in detail by New and Nair (2012). China is the largest producer of giant freshwater prawn and this significantly contributes to the Chinese economy (New 2005). Chinese production of *M. rosenbergii* constituted 1.23×10^5 tons out of a total freshwater aquaculture production of 2.48×10^7 tons during 2011 (FAO 2013). Intensive culture is a common practice in China for *M. rosenbergii* where the post-larvae stocking density ranges between 0.9×10^6 and 1.5×10^6 ind. ha^{-1} (average 1.2×10^6 ind. ha^{-1}) with a prawn harvest size of 10–17 g (Hongtuo et al. 2012). Adequate water quality is the key element in any kind of aquatic farming system. Impaired water quality can cause stress and diseases to cultured species, which ultimately impact their growth and production, can result in eutrophication of receiving water (Konnerup et al. 2011).

In this study a CW was integrated into commercial ponds of freshwater prawn (*Macrobrachium rosenbergii*) in order to determine the performance of FWS-CW without external water exchange. The objectives of the study were to investigate the function of a CW in reducing the dissolved nutrient concentrations in aquaculture systems and impacts of this water re-circulation through a CW on the water quality of grow-out ponds.

Materials and Methods

The study area is located on the South West Jinshan District of Shanghai, China (between 30.78°N and 121.16°E). The CW was built in the Shanghai Jinshan Shrimp Farms (Fig. 1). The construction of the CW was started 2 months before the stocking of the ponds with fresh water prawn post-larvae. The CW was composed of a slope unit, macrophyte unit and reservoir (Fig. 1). The experimental re-circulating aquaculture system consisted of two grow-out ponds (68.4 m long × 26.6 m wide × 2.5 m high) and the FSW-CW (Fig. 1). A submersible pump was used to move the effluent of the two ponds (ponds 1, 2 on Fig. 1) through the wetland. The water flow was controlled by a level controller and maintained a constant flow rate of 25 m^3 h^{-1} throughout the culture period. The CW was planted with two species *Typha angustifolia* and *Canna indica*, at a density of approximately 40 plants m^{-2} over the whole region during this study.

Post-larvae (PL) of prawn (*Macrobrachium rosenbergii*) were introduced into culture ponds at a stocking density of 60 PL m^{-2} on May 31, 2011. The stocking weight was 2–3 mg per larvae in each pond. Two pairs of paddle wheel aerators were installed into experimental and control ponds. These aerators were operated during low dissolved oxygen concentration periods. The prawns were fed by hand with commercial feed three times a day with a feeding rate of 10 %–20 % of wet weight body mass during the initial stage and at about 3 % for later stages. The commercial feed (Ming Hui, China) was composed of 41 % protein, 6.5 % fat, 16 % ash and 12 % water. No external water discharge or displacement occurred in ponds; except to control evaporation losses which were not large and mostly counteracted by rainfall events because culture was carried out during the summer period. The water re-circulation (25 m^3 h^{-1}) through the CW for two ponds was started after 1 month of PL stocking. Prawns were harvested in the month of September after 118 days of culture.

Water samples were collected from the CW weekly (10 weeks, 10 paired of samples) at the inflow and outflow of the FWS-CW at the starting and closing of water circulation. The hydraulic retention time (HRT) was maintained at 24 h during the whole experimental recirculation period. The samples from the CW were analyzed for dissolve nutrients (NH_4^+, NO_3^-, NO_2^- and PO_4^{3-}) and TSS (TSS, dry weight in mg L^{-1}). Dissolved nutrients water samples were filtered through Whatman GF/F (0.7 µm pore size), pre-combusted (450°C, 4 h) and pre-weighed filter papers and stored in polypropylene bottles. The filtrates were immediately dosed with saturated $HgCl_2$ (ca. 1.5×10^{-3} v/v) and stored in the dark for one to 2 months, in order to stop biological activities before analysis (Liu et al. 2011). Samples were analyzed in triplicate for NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} using the Skalar SAN^{plus} Continuous Flow Autoanalyzer. The precisions for different nutrients were estimated as NH_4^+ (0.21 %), NO_3^- (0.98 %), NO_2^- (0.66 %), and PO_4^{3-} (0.87 %).

Dissolved oxygen, temperature and pH were measured with the help of a probe unit (WTW Multi 197-I USA). Concentrations of total suspended solids (TSS) were determined by weighing the dried filter, subtracting the original weight of the empty filter and dividing it by the respective volume of water filtered. Particulate matter on GF/F was analysed for total nitrogen (TN) by VARIO EL III, CHNOS Elemental Analyzer with standard acetanilide (acet = N 71.09 %).

The difference between inflow and outflow of each parameter concentration of the CW was calculated for collected samples (Chang et al. 2007; Shi et al. 2011). The removal percentage (RP) of pollutants by the CW was calculated as follows:

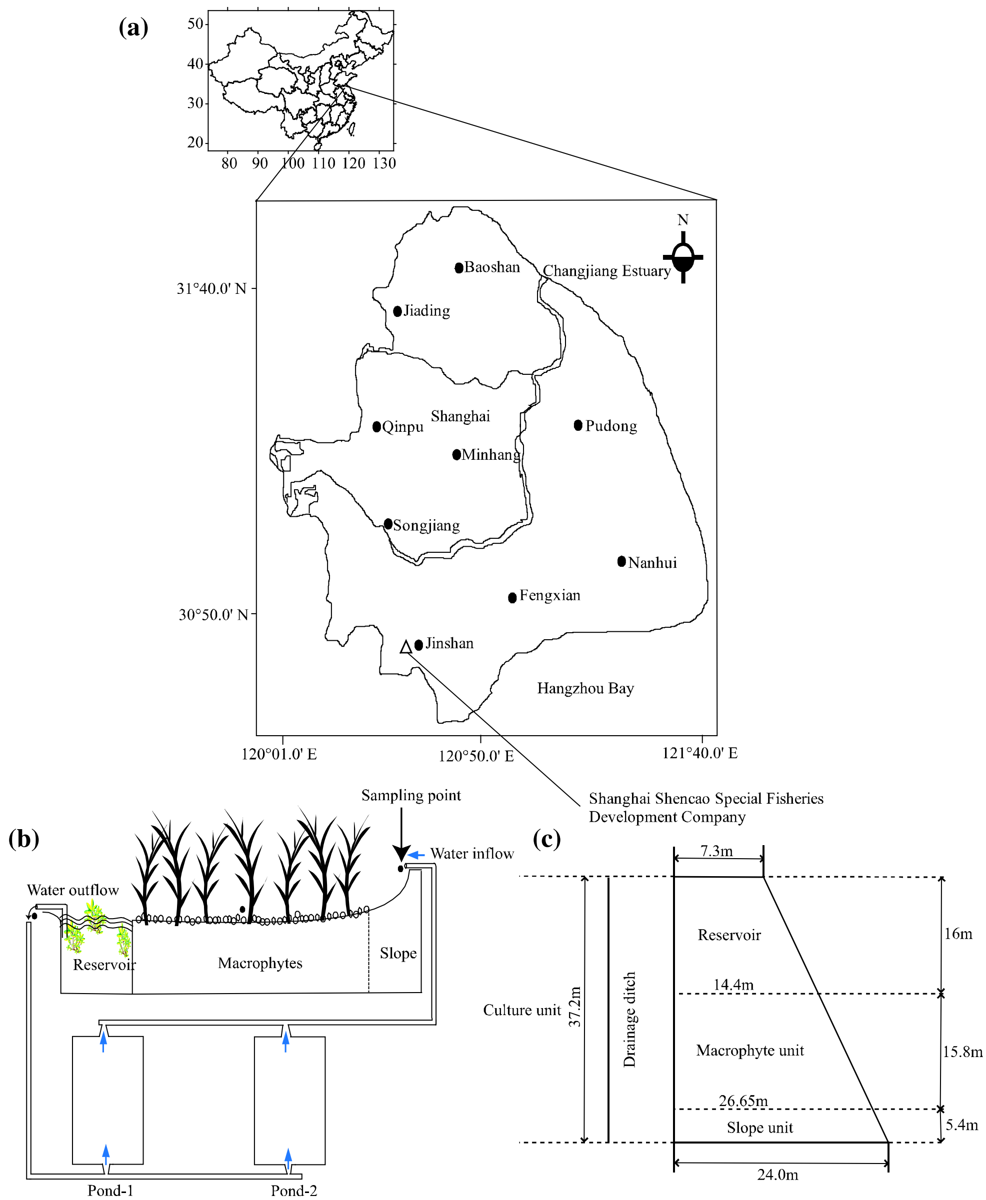

Fig. 1 Schematic diagram of constructed wetland. **a** Study area, **b** complete culture unit in water from pond 1 and 2 *outlet* enters through slope unit into wetland and passes through central macrophytes unit composed of vegetation, gravel and clay, than water is taken out from reservoir throughout flow into pond 1 and 2 *inlet*. **c** Size dimensions of different areas in constructed wetland

$$RP\ (\%) = [(C_i - C_e)/C_i] \times 100 \quad (1)$$

where C_i and C_e are the concentrations (mg L^{-1}) of water parameters at inflow and outflow of the CW respectively.Hydraulic Loading Rate (HLR) was calculated as

$$HLR\ \left(m\ day^{-1}\right) = Q/A_w \quad (2)$$

where Q is the flow of wastewater through the CW and A_w is the total surface area (m) of the CW.Mass loading rate (MLR) and R_{mass} of pollutants were calculated as

$$MLR\ \left(g\ m^{-2}day^{-1}\right) = (C_i \times HLR)/1000 \quad (3)$$

$$R_{mass}\left(g\ m^{-2}day^{-1}\right) = [(C_i - C_e) \times HLR]/1000 \quad (4)$$

Statistical calculations were performed with SPSS 17 and Grapher 10 (Golden Software USA). The paired *t* test was applied to calculate the difference between inflow and outflow of the CW.

Results and Discussion

The HRT was maintained constant at 24 h throughout the experiment and HLR was calculated 0.8 m day^{-1}. The average concentrations of NH_4^+, NO_3^-, NO_2^- and PO_4^{3-}, DIN (NH_4^++NO_2^-+NO_3^-), TN, TSS, removal percentages and rate at inflow and out flow of CW are listed in Table 1.

Table 1 Average concentration of pollutants at the inflow and outflow of the experimental CW

Water parameters	CW (mg L^{-1})		Removal percentage (%)	Removal rate (gm^{-2} day^{-1})	*t* values	*p* values
	Inflow (n = 10)	Outflow (n = 10)				
NH_4^+	0.1 ± 0.04	0.07 ± 0.01	25.7 ± 23.0	0.04 ± 0.02	−0.54	0.602
NO_3^-	0.03 ± 0.01	0.014 ± 0.07	43.8 ± 24.6	0.02 ± 0.01	−1.73	0.118
NO_2^-	0.01 ± 0.01	0.008 ± 0.01	14.3 ± 1.0	−0.002 ± 0.002	−3.0	0.015
DIN	0.17 ± 0.02	0.09 ± 0.06	28.4 ± 18.8	0.028 ± 0.026	−0.97	0.355
PO_4^{3-}	0.06 ± 0.02	0.05 ± 0.02	13.1 ± 10.0	0.006 ± 0.005	−1.49	0.169
TN	110.3 ± 43.3	55.3 ± 26.2	39.6 ± 44.2	44.5 ± 31.0	4.31	0.003
TSS	128.1 ± 74.5	27.5 ± 16.8	73.2 ± 15.4	81.5 ± 63.5	3.85	0.005

Removal efficiency and mass removal rate of various water parameters. Showing highest removal percentage of suspended solids (α-value = 0.05)

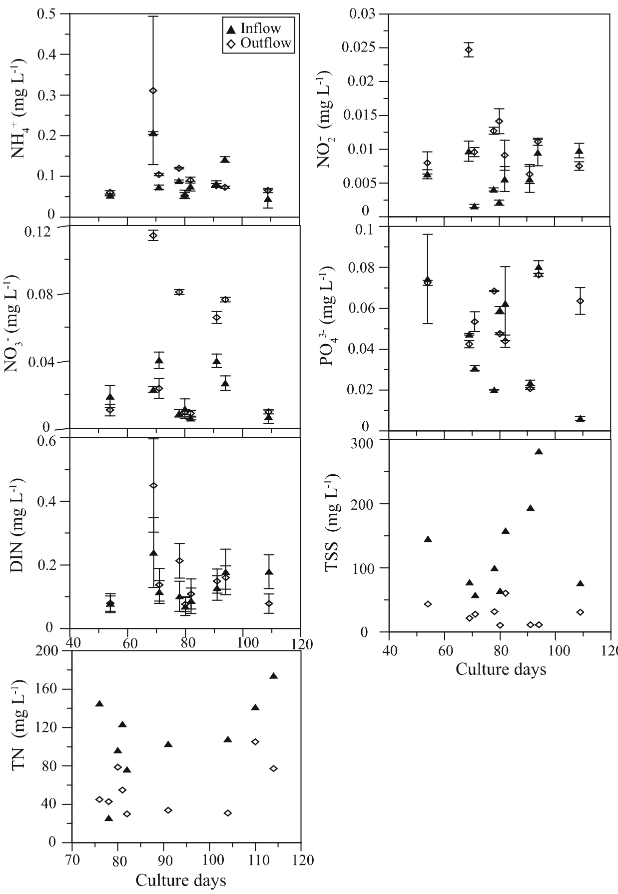

Fig. 2 Concentration of nutrient and total suspended material (TSS) in the inflow and outflow of constructed wetland, showing the values with respect to cultured days and circulation in experimental pond 1 and 2 during 118 days growing periods of giant prawn *M. rosenbergii*. (±) are the standard deviation calculated for each sample

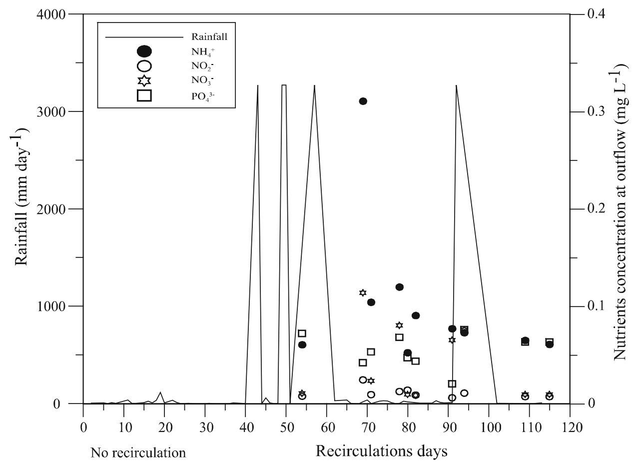

Fig. 3 Concentration of nutrient (mg L^{-1}) at outflow of constructed wetland and rainfall (mm day^{-1}), showing the values with respect to water recirculation through wetland during the growing periods of giant prawn *M. rosenbergii*

Nutrient concentrations in the rainwater were measured as NH_4^+ (0.27 ± 0.0004 mg L^{-1}), NO_2^- (0.006 ± 0.00002 mg L^{-1}), NO_3^- (0.23 ± 0.0002 mg L^{-1}), PO_4^{3-} (0.00002 ± 0.0003 mg L^{-1}) and DIN (0.51 ± 0.0002 mg L^{-1}). The concentrations of dissolved nutrients and TSS during water re-circulation at the inflow and outflow of the CW are shown in Fig. 2. The nutrient concentrations at the CW outflow and rainfall is given in Fig. 3. The rainfall data were collected from the China Meteorological Data Sharing Service System *(cdc.cma.gov.cn)*. The removal efficiency and concentration of nutrients were influenced by the higher than average rainfall during the experiment. Overall no significant difference was found in the concentrations of NH_4^+, NO_3^- and PO_4^{3-} between inflow and outflow of CW. Nitrite showed significant difference ($p < 0.05$) with slightly higher values at outflow of CW. The minimum concentration of NH_4^+, NO_2^-, NO_3^- and PO_4^{3-} at the CW inflow was found as 0.04 ± 0.05, 0.002 ± 0.003, 0.01 ± 0.01 and 0.01 ± 0.03 mg L^{-1} and maximum 0.21 ± 0.05, 0.01 ± 0.003, 0.04 ± 0.01, 0.08 ± 0.03 mg L^{-1}, respectively. While at the outflow of the CW the minimum concentration of the corresponding nutrients were 0.05 ± 0.08, 0.006 ± 0.005, 0.01 ± 0.04 and 0.02 ± 0.02 mg L^{-1} and maximum 0.31 ± 0.08, 0.02 ± 0.01, 0.11 ± 0.04, and 0.08 ± 0.03 mg L^{-1}, respectively. Significant difference ($p < 0.05$, Paired t test) was found between inflow and outflow concentrations of TSS ($t = 3.85$, $p = 3.85$) and TN ($t = 4.31$, $p = 0.003$) and were effectively reduced by the CW treatment.

The performance of the CW is dependent upon many factors such as precipitation, evaporation, hydraulic loading rate, influent concentration and water depth which can affect the removal of organics, nutrients and trace metals (EPA 1988). Hence external factors can influence the removal efficiency of a CW. One example is high rainfall events which, by dilution, create low nutrient concentrations affecting the removal efficiency of a CW as well as the nutrient concentration in grow-out ponds. Overall, the CW displayed lower efficiency in removing the nutrients in this study. For this reason only the reduced nutrient concentrations at the CW outflow were taken into account to determine the reduction efficiency of the CW. In this study the CW showed 43.8 % removal of NO_3^- compared to other nutrients implying a comparatively high denitrification rate in the CW which demonstrates the occurrence of anaerobic zones in the CW (Van Rijn et al. 2006). The concentration of NO_2^- at the outflow of the CW during most of the experimental recirculation period was reduced by about 14 %. One explanation could be the lower concentration of NO_2^- in the culture ponds, second could be the lack of suitable environment which can boost the nitrification process within the CW unit as reduction of NH_4^+ and NO_2^- is mainly attributed to nitrification (Lin et al. 2005). This assumption is further supported by the low treatment of NH_4^+ in wetland and high pH values (>8.6) in the experimental ponds cause a lower nitrification rate, because nitrification produces hydrogen ions that neutralise alkalinity resulting in a lower pH (Lin et al. 2005). Like other nutrients NH_4^+ was impacted by external factors as indicated by comparing the overall CW performance and reduced values responsible for 25.7 % removal and decreasing trend of NH_4^+ with time indicated the occurrence of mineralization (ammonification) throughout the recirculation period (Lin et al. 2005). Phosphate is not a critical nutrient for aquaculture species generally but high concentrations can cause algal blooms in culture units. It is hypothesized that the removal of PO_4^{3-} at 13.1 % and an irregular distribution pattern in concentrations between inflow and outflow are the result of lower uptake rate by the CW plants, absorption by the TSS and high precipitation (Reddy and Debusk 1985). The removal percentage of TSS in this study was highest at about 73 % ($p < 0.005$) compared with other pollutants, consistent with removal levels of 67 %–72 % reported in the literature for other CWs (Schulz et al. 2004). The nitrification and denitrification rate in a circulation system relies on high loads of nutrient and organic matters (Van Rijn et al. 2006), but in our study the precipitation rate and continuous pond water recirculation through the CW diluted the nutrient and organic loads with the passage of time. The outcome is a reduction in nutrient concentration and an apparent reduction in efficiency of the CW, leading to generally insignificant differences among the nutrients between inflow and outflow.

In a FWS-CW, hydraulic retention time (HRT) and hydraulic loading rate (HLR) or mass/pollutant loading rate (MLR) have a great impact on the removal efficiency (Chang et al. 2007). In order to achieve the successful treatment performance of a CW, the HRT (24 h.) was kept constant throughout the investigated period. Generally, it is considered that a low HLR resulted in a high removal efficiency of nutrient in wetland (Lin et al. 2003; Chang et al. 2007). In this study the HRT is lower than the typical range of 4–5 days but HLR (0.8 m day^{-1}) is higher than typical ranges of 0.014–0.047 m day^{-1} suggested by Metcalf and Eddy Inc (1991) for the waste water treatment through a CW. The existence of various biological and chemical processes within the CW depends on the HLR of pollutants (Lin et al. 2003). In the present study most of the total suspended solids (TSS) removed (73 %) from the CW were at a removal rate (R_{mass}) of 81 g m^{-2} day^{-1} at an MLR of 102 g m^{-2} day^{-1}. Both these loading and removal rates are higher than those found in previous studies for other FSW-CW systems. Muller (2000) observed a maximum R_{mass} of 15 g m^{-2} day^{-1} at an MLR of 36.8 g m^{-2} day^{-1} with a HRT of 3.5–5.5 h in the waste water treatment through their CW. Therefore, at that level of HRT there was no re-suspension of settled out solids. Lin et al. (2003) reported the mean R_{mass} of TSS to be 7.8 g m^{-2} day^{-1} and an MLR of 10.8 g m^{-2} day^{-1} at a HLR of 0.3 m day^{-1} indicated the high sedimentation rate in the CW. For an intensive shrimp culture pond, Lin et al. (2005), reported an R_{mass} for the TSS at 26.7 g m^{-2} day^{-1} with a HLR of 1.54–1.95 m day^{-1} producing a 55 % nutrient reduction in a FWS-SF CW. Varying HRTs and HLR did not affect the TSS removal in the CW (Schulz et al. 2004); therefore a comparatively higher sedimentation rate was observed in this CW in the present study than in previous investigations. Higher TSS loadings (8.6–43.2 g m^{-2} day^{-1}) are capable of causing physical clogging of the CW soil matrix affecting flow-through and decreasing the removal performance of CWs (Kadlec and Knight 1996). This could be a reason why the performance efficiency of the CW in our study was impacted. It indicates that in the present study, the HLR contained a huge quantity of TSS which could be removed by sedimentation and recirculation in CW (Lin et al. 2003). Another reason for the overall lower efficiency of this CW regarding the nutrient reduction can be a lower R_{mass} of DIN and PO_4^{3-}

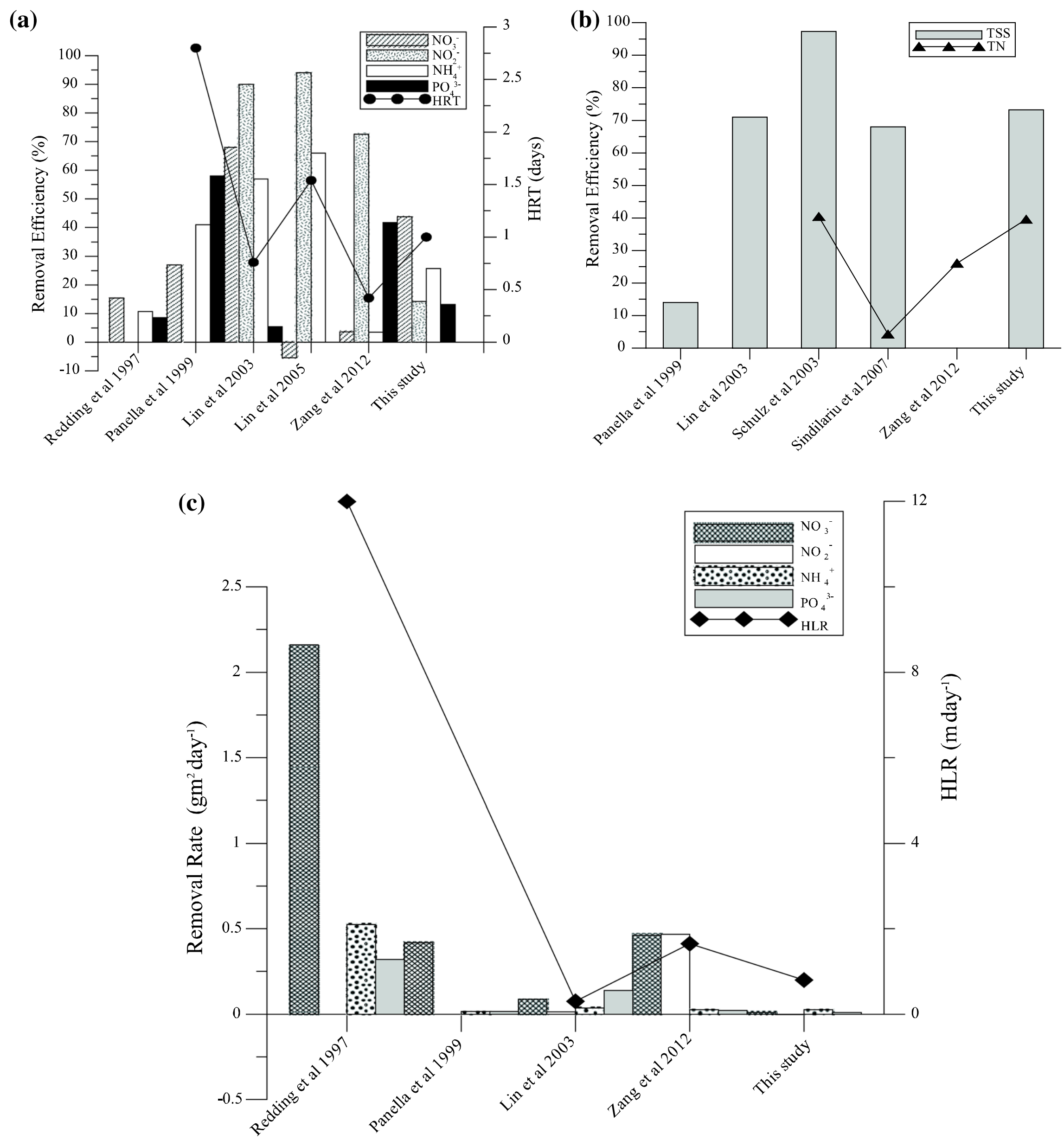

Fig. 4 The comparison of FWS-SF CW from literature to this study. **a**, **b** The comparisons of removal efficiency of dissolved nutrient, total suspended solids (TSS), total nitrogen (TN) and hydraulic retention time (HRT). **c** The comparison of removal rate and hydraulic loading rate (HLR)

than in other studies. Similar results of low efficiency due to less pollutant R_{mass} were found by Zhang et al. (2010). In the present study the R_{mass} of NH_4^+, NO_3^-. NO_2^-, DIN and PO_4^{3-} are 0.07, 0.02, 0.004, 0.11 and 0.03 g m^{-2} day^{-1}, respectively. These R_{mass} are lower than the previously reported for DIN = 0.21 g m^{-2} day^{-1}, PO_4^{3-} = 2.56 g m^{-2} day^{-1} (Lin et al. 2003), NH_4^+ = 0.18–1.10 g m^{-2} day^{-1} (Chang et al. 2007). Shi et al. (2011) demonstrated that the mass removal rate of NH_4^+ increased from 0.05 to 3.25 g m^{-2} day^{-1} and NO_2^- from 0.008 to 0.320 g m^{-2} day^{-1}, with increased MLR. The performance of the CW became stable with the passage of time as indicated by slight increase in the removal rate of NH_4^+ and NO_3^- after 1 month of recirculation. Similar results were reported by Lin et al. (2002). It also shows that there is an increased uptake rate of macrophytes with the growth and enhanced reduction capacity of the CW with time. The slightly lower and irregular nutrient pattern at the outflow of the CW indicates its reduction efficiency. However, the present study did not show the maximum nutrient removal observed in other studies. Comparison of water quality before and after recirculation clarifies the role of the CW in reducing pollutants. The removal efficiency of nutrients, as calculated by the reducing values (differences between the CW inflow and outflow), are still within the limits of CW treatment capacity values reported from other studies (Redding et al. 1997; Panella et al. 1999; Lin et al. 2003; Schulz et al. 2003; Lin et al. 2005; Zang et al. 2012). In comparison with previously reported data for FWS-CWs (Fig. 4a, b), this study revealed a higher removal rate of TSS and low rate of NO_2^- and PO_4^{3-} removal. The difference of nutrient removal from previously reported investigations may be due to differences in operating conditions such as the HLR, HRT, pollutants loading rate, inflow concentrations, precipitation and CW size. In our study the high accumulation rate of suspended solids in the CW lead to slightly higher reduction in TN which indicate that stabilization and mineralization of solids also take place in the CW (Lin et al. 2005). Considered overall, the performance of a CW is not satisfactory in respect of removal of dissolved nutrients but does show great ability to remove TSS and TN.

This study demonstrated the performance of a CW integrated into the intensive grow-out ponds of giant fresh water prawn. Water from the two ponds was re-circulated through the CW after 1 month of stocking. The wetland effectively removed 73.2 ± 15.4 % and 39.6 ± 44.2 % of TSS and TN respectively from the aquaculture waste but performed less desirably with dissolved nutrients (Table 1). This lack of performance from the CW is hypothesized to be a result of dilution of inflow concentrations with high rainfall. This can influence the rates of chemical and biological processes occurring within the CW. However reductions in nutrient concentrations (reduced values at the CW outflow) were found by comparing with previous literatures. These results illustrate that a CW can improve and maintain a good water quality and environment in freshwater prawn ponds at intensive stocking densities. As the focus of this study was to determine the dissolved nutrient species and role of CW therefore further study is required to determine the economical feasibility of CW and growth rate of prawns through CW circulation.

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