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Effects of water spinach *Ipomoea aquatica* cultivation on water quality and performance of Chinese soft-shelled turtle *Pelodiscus sinensis* pond culture

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ABSTRACT: The Chinese soft-shelled turtle *Pelodiscus sinensis* is a highly valued freshwater species cultured in China. A 122 d experiment was conducted to assess the effects of water spinach *Ipomoea aquatica* cultivation in floating beds on water quality, and growth performance and economic return of *P. sinensis* cultured in ponds. Two treatments, each in triplicate, with and without *I. aquatica* cultivation were designed. Results showed that the levels of total ammonia nitrogen (TAN), total nitrogen (TN), total phosphorus (TP), chlorophyll *a* (chl *a*) and turbidity in treatments with *I. aquatica* cultivation (IAC) were significantly ($p < 0.05$) lower than those in treatments without *I. aquatica* (control). Mean TN and TP concentrations in the IAC treatment were 27.9 and 42.5%, respectively, lower than in the control treatment at the end of the experiment. The presence of *I. aquatica* also has a positive effect on the performance of *P. sinensis*. Although no significant difference was found in specific growth rate (SGR) between the 2 treatments, mean survival rates, production and net income were significantly higher in the IAC treatment compared to the control ($p < 0.05$). These results suggest that *I. aquatica* cultivation in the pond system of turtles has a synergistic effect on overall economic return and is effective at improving turtle growth performance and water quality.

KEY WORDS: *Pelodiscus sinensis* · *Ipomoea aquatica* · Aquaculture effluent · Growth performance · Economic return

INTRODUCTION

The Chinese soft-shelled turtle *Pelodiscus sinensis* is the most common turtle species cultured in China (Li et al. 2013). Soft-shelled turtles have long been a part of traditional Chinese cuisine and are much sought after as a high-priced food item in restaurants. In the past, the great bulk of soft-shelled turtles consumed were wild-caught, leading to an over exploitation of wild turtles that resulted in a 'turtle crisis' in the country (Li et al. 2013). However, aquaculture developments over the past 4 decades have enabled the increasing demand for turtles to be met (Shi et al. 2008). The soft-shelled turtle is a highly valued aquaculture species in China with the 2014 yield reaching 341 000 t (Fisheries Administration of the People's Republic of China 2015). However, low survival rates and poor flesh quality of farmed turtles often compromise production and economic benefits (Ding 2000). Generally, the culture environment is an

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important factor influencing the survival, growth and quality of cultured species (Neal et al. 2010, Brito et al. 2014, Price et al. 2015). Also, the impacts of aquaculture effluents on water quality and diversity of adjacent natural water bodies have been addressed by many studies (Xie et al. 2004, De Silva 2012, Herbeck et al. 2014). Hence, improving culture environment is not only beneficial for better performance and flesh quality of the species, but also eases the negative impacts on the environment.

There are many approaches to improving a culture environment, and phytoremediation, especially using aquatic plants, is currently attracting much attention. It is considered promising because of its low cost, non-intrusiveness and safety (Li et al. 2009, Mook et al. 2012). Aquatic plants can effectively reduce total nitrogen, total phosphorus and chemical oxygen demand (Sooknah & Wilkie 2004). Water spinach *Ipomoea aquatica* Forsk. is an important crop in oriental cuisine and is also used in animal feeds. Water spinach grows well in moist soil or wetland systems and is commonly found creeping along muddy stream banks or floating in freshwater marshes and ponds (Li & Li 2009, Jampeetong et al. 2012). Floating bed technology makes it possible to plant this species on the water surface of fishponds, which not only improves the culture environment, but also provides additional economic benefits (Li & Li 2009).

Previous studies have demonstrated that planting *I. aquatica* in fishponds can efficiently remove nutrients and improve water quality (Li & Li 2009, Dai et al. 2012). Accordingly, we hypothesized that *I. aquatica* co-cultured in the pond systems of turtles could maintain water quality and provide better economic return. In this study, we compared selected water quality parameters, nutrient contents, and growth, survival and production of *P. sinensis* in ponds with and without *I. aquatica* cultivation. The aim of this study was to examine the effects of *I. aquatica* cultivation on water quality, growth performance and economic returns of *P. sinensis* cultured in earthen ponds. Results from this study will be useful to bring about efficient management and to maximize profitability of *P. sinensis* pond culture.

MATERIALS AND METHODS

Study location and experimental procedure

The experiment was conducted at a turtle breeding base in Honghu, Hubei Province, Central China, from June to October 2013. Juvenile *Pelodiscus sinensis* (mean [±SD] body weight 27.7 ± 2.1 g) were obtained from a turtle farm in Honghu and were acclimated for 6 wk in three 1 ha ponds that were filled to a depth of 100 cm prior to the commencement of the trials. During this period, turtles were maintained under an ambient day:night light cycle, in pond water temperature that ranged from 20.8 to 29.6°C and were fed daily with a commercial feed (46% crude protein).

Two experimental treatments, with and without *Ipomoea aquatica* cultivation, each in triplicate, were used in the study. The experiment was conducted in 6 randomly selected ponds each of 3000 m^2 (100 × 30 m) at the turtle breeding base. After acclimation for 6 wk, 3000 healthy turtles (mean body weight 55.6 ± 3.3 g) were selected (without considering the sex) and stocked randomly in each pond with a water depth of 1.2 m. There were no significant differences (*t*-test, $p > 0.05$) in body weight between the treatments. After stocking, 150 kg of silver carp *Hypophthalmichthys molitrix* (mean body weight 153.7 ± 9.9 g) and 100 kg of bighead carp *Aristichthys nobilis* (mean body weight 205.3 ± 15.2 g) were stocked into each pond to regulate water quality, as this is a common practice that is performed in Chinese turtle farming. The culture procedures complied with the animal welfare laws of the Government of China and the ethical rules of the Institutional Animal Care and Use Committee of the Institute of Hydrobiology (Approval ID: Keshuizhuan 08529).

Prior to stocking, water samples (500 ml) were collected from all experimental ponds. Turbidity, total ammonia nitrogen (TAN), total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD_{Mn}) were determined according to standard methods (APHA 1992). In the *I. aquatica* cultivation (IAC) treatment, each pond was planted with *I. aquatica* (cut stems 20.4 ± 3.1 cm) on floating beds of bamboo frames (80 × 1.25 m) with 20 cm plant spacing and 25 cm row pitch. A polyethylene net with 3 cm mesh was fixed on the frame. Three floating beds were set up in each pond, and the total area covered approximated 10% of each pond.

P. sinensis juveniles were reared for 4 mo and fed twice daily at 08:00 to 09:00 h and 16:00 to 17:00 h with a commercial dry pellet feed of 46% crude protein content (Hangzhou Haihuang Feed Development) and minced fillet of silver carp *H. molitrix* (approximately 15% crude protein content). The daily ration consisted of approximately 2% of the body weight of commercial feed and 6% body weight of minced fillet. This ration was chosen based on observations made during the acclimation period, and on the amount of feed that the juvenile turtles would consume in 1 h. The body weight of the turtles was measured every 2 wk, and uneaten food was estimated. The daily feed allowance was adjusted every 2 wk based on these observations.

During the experimental period, natural water from a nearby lake was supplemented when the water level of the ponds decreased below 10.0 cm. From July to October, *I. aquatica* was harvested every month, and weight and revenue recorded. At the end of the trial, the number and individual weights of turtles were recorded. Growth performance of the turtles was evaluated using survival, relative weight gain (WG), specific growth rate (SGR), absolute growth rate (AGR) and protein efficiency ratio (PER) (Nuwansi et al. 2016).

Water was sampled monthly at around 10:00 h for each pond and monitored for turbidity (HACH 2100Q) *in situ*. TAN, TN, TP and COD_{Mn} were analyzed following the standard methods for the examination of water and wastewater (APHA 1992). Chlorophyll *a* (chl *a*) concentration was determined using a fluorometer with methanol extraction of the filtrate (Holm-Hansen & Riemann 1978).

Economic analysis

The economic analysis followed that of Gomes et al. (2006). The total revenue (in US dollars; $1.00 US = $6.20 RMB) included that of harvested *P. sinensis*, silver and bighead carp and *I. aquatica*, sold at $14.52 kg^{-1}, $0.48 kg^{-1}, $1.13 kg^{-1} and $0.32 kg^{-1} in the local market, respectively. The total cost consisted of the cost of *P. sinensis* juveniles, fish juveniles, *I. aquatica* stems, commercial feed, minced fillet, floating bed, labor (4 mo), labor for picking *I. aquatica* and pond rent. *P. sinensis* juveniles, *I. aquatica* stems, commercial feed and minced fillet costs were $1.61 ind.$^{-1}$, $0.48 kg^{-1}, $1.29 kg^{-1} and $0.39 kg^{-1}, respectively. The costs of silver and bighead carp juveniles were $0.77 kg^{-1} and $1.29 kg^{-1}, respectively. Floating bed costs included the material, assembly and fixing in ponds. Labor costs included 4 farmers that handled routine work and their benefits in compliance with the laws of the country. Pond rental was $483.87 ha^{-1}.

Statistical analysis

All data are expressed as mean ± SD. A *t*-test was used to determine differences between treatments. Differences were considered significant at $p < 0.05$. All analyses were performed using SPS 16.0 statistical package (SPSS).

RESULTS

Water quality

Trends in TAN, TN, TP, COD_{Mn}, chl *a* concentrations and turbidity between the 2 treatments did not differ significantly ($p < 0.05$) at the commencement of the experiment. A high degree of fluctuation in TAN and TN levels was observed during the culture period. TAN concentrations varied significantly between treatments and sampling dates, with lower mean values in IAC treatments compared to the control treatments in October ($p < 0.05$; Fig. 1A). TN concentrations in the IAC treatment were significantly lower than those in the control treatment in the last 4 mo ($p < 0.05$; Fig. 1B). The mean TN concentration in the IAC treatment was 27.9% lower compared to the control at the end of the experiment. TP concentrations were significantly affected by the presence of *Ipomoea aquatica* ($p < 0.05$; Fig. 1C); the mean TP concentration in the IAC treatment was 42.5% lower compared to the control treatment at the end of the experiment. The COD_{Mn} levels did not significantly differ between the 2 treatments during the whole experimental period ($p > 0.05$; Fig. 1D), however chl *a* concentration and turbidity were significantly influenced by *I. aquatica*, with lower mean values in IAC ponds compared to the controls from August to October, respectively ($p < 0.05$; Fig. 1E,F).

Growth performance

For *Pelodiscus sinensis*, mean survival rate in the IAC treatment was significantly higher than that in the control treatment ($p < 0.05$). No significant differences between the 2 treatments were observed in initial weight, final weight, WG, SGR or AGR, indicating that growth of *P. sinensis* was not significantly influenced by *I. aquatica* cultivation. Significant differences were observed in mean production in the IAC treatment, which was significantly higher (2496.7 ± 58.4 kg ha^{-1}) than that in the control (2180.0 ± 49.7 kg ha^{-1}) treatment ($p < 0.05$). There was no significant difference in PER between the 2 treatments ($p > 0.05$) (Table 1).

The survival rate of silver carp was not significantly different between treatments ($p > 0.05$), but final weight, WG, SGR and AGR in the IAC treatment was

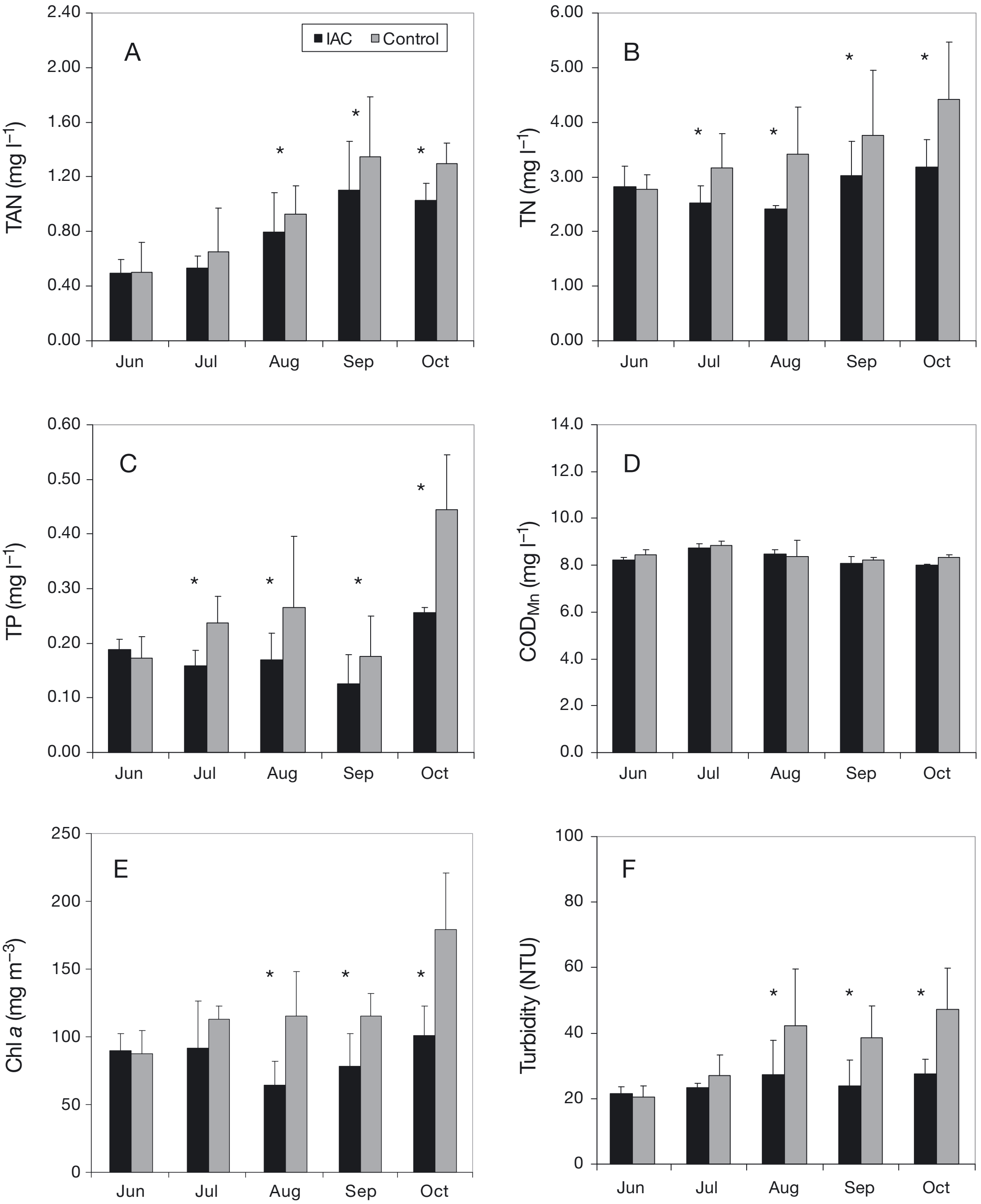

Fig. 1. Fluctuations in (A) total ammonia nitrogen (TAN), (B) total nitrogen (TN), (C) total phosphorus (TP), (D) chemical oxygen demand (COD$_{Mn}$), (E) chlorophyll *a* (chl *a*) concentrations and (F) turbidity in the Chinese soft-shelled turtle *Pelodiscus sinensis* ponds during the 4 mo experimental period. Values are means (±SD) of 3 replicate ponds per sampling time in each treatment. IAC: *Ipomea aquatica* treatment. (*) indicates significant difference between treatments ($p < 0.05$)

significantly lower than those in the control treatment ($p < 0.05$). For bighead carp, mean survival rate in the IAC treatment was higher than that in the control treatment, while final weight, WG, SGR and AGR in the IAC treatment was lower than those in the control treatment, but these differences were not significant ($p > 0.05$) (Table 1).

Economic analysis

The cost–benefit analysis of *P. sinensis* juveniles cultured in ponds for 4 mo under 2 treatments is shown in Table 2. Total revenue and total costs were both higher in the IAC treatment than in the control and net income was higher in the IAC treatment. The

Table 1. Growth performance of Chinese soft-shelled turtle *Pelodiscus sinensis* and 2 carp species (silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*) cultured for 4 mo under 2 different treatments in aquaculture ponds. Data are means ± SD of 3 replicate ponds. For each row, means with different superscript letters are significantly different from each other ($p < 0.05$). IAC: *Ipomoea aquatica* treatment; WG: weight gain; SGR: specific growth rate; AGR: absolute growth rate; PER: protein efficiency ratio

Parameter	Turtle		Silver carp		Bighead carp	
	IAC	Control	IAC	Control	IAC	Control
Survival (%)	81.3 ± 2.7^{a}	72.4 ± 1.6^{b}	93.3 ± 3.3^{a}	90.1 ± 2.8^{a}	91.7 ± 2.9^{a}	89.3 ± 2.2^{a}
Initial weight (g)	55.3 ± 2.1^{a}	57.1 ± 2.6^{a}	153.7 ± 9.9^{a}	153.7 ± 9.9^{a}	205.3 ± 15.2^{a}	205.3 ± 15.2^{a}
Final weight (g)	307.2 ± 5.5^{a}	301.1 ± 3.7^{a}	680.6 ± 23.6^{a}	777.0 ± 20.9^{b}	785.9 ± 25.3^{a}	869.0 ± 30.7^{a}
WG (g)	251.9 ± 5.1^{a}	244.0 ± 3.3^{a}	526.9 ± 15.4^{a}	623.3 ± 14.9^{b}	580.6 ± 19.8^{a}	663.7 ± 23.9^{a}
SGR (% d^{-1})	1.40 ± 0.03^{a}	1.36 ± 0.02^{a}	1.22 ± 0.04^{a}	1.33 ± 0.02^{b}	1.10 ± 0.03^{a}	1.18 ± 0.03^{a}
AGR (g d^{-1})	2.06 ± 0.11^{a}	2.0 ± 0.09^{a}	4.32 ± 0.16^{a}	5.11 ± 0.21^{b}	4.76 ± 0.19^{a}	5.44 ± 0.23^{a}
Production (kg ha^{-1})	2496.7 ± 58.4^{a}	2180.0 ± 49.7^{b}	2066.7 ± 63.7^{a}	2276.7 ± 77.4^{b}	1168.3 ± 82.1^{a}	1260.0 ± 79.2^{a}
PER	0.81 ± 0.04^{a}	0.74 ± 0.03^{a}				

Table 2. Cost and return analysis of Chinese soft-shelled turtle *Pelodiscus sinensis* cultured for 4 mo under 2 different treatments in ponds. IAC: *Ipomoea aquatica* treatment. Values are in US dollars ($1.00 US = $6.20 RMB)

Parameter	Treatment	
	IAC	Control
Total revenue	**12 871.38**	**10 251.06**
P. sinensis harvest (kg)	749.00	654.00
Revenue kg^{-1} of *P. sinensis*	14.52	14.52
Revenue of *P. sinensis*	10875.48	9496.08
Fish (bighead carp and silver carp) harvest (kg)	620.00 + 350.50	683.00 + 378.00
Revenue kg^{-1} of fish	0.48/1.13	0.48/1.13
Revenue of fish	693.66	754.98
I. aquatica harvest (kg)	4069.50	0
Revenue kg^{-1} of *I. aquatica*	0.32	0.32
Revenue of *I. aquatica*	1302.24	0
Total cost	**9067.14**	**8140.77**
P. sinensis juveniles	4838.71	4838.71
Fish juveniles	232.25	232.25
I. aquatica stems	217.74	0
Commercial feed	1347.26	1229.59
Minced fillet	1212.47	1106.67
Floating bed investment	129.03	0
Labor (4 mo)	387.10	387.10
Cost for picking *I. aquatica*	387.10	0
Pond rent	145.16	145.16
Other costs	170.32	201.29
Net income	**3804.24**	**2110.29**
Net income ha^{-1}	**12 680.80**	**7034.30**

revenue obtained from *P. sinensis* was the dominant component of the total revenue in both treatments. In addition, the revenue from *I. aquatica* in the IAC treatment was $1302.24, accounting for 10.12% of total revenue. The major components of total cost were *P. sinensis* juveniles and feeds, with turtles representing 53.37 and 59.44% of the total cost for the IAC and control treatments, respectively, and feeds representing 28.23 and 28.70%.

DISCUSSION

Water quality management is an important component of the production and quality of aquatic animals in aquaculture, and aquaculture effluent is recognized as a serious global problem because of its influence on surrounding watersheds (Othman et al. 2013, Zhang et al. 2014). Previous studies have reported that floating bed technology using aquatic vegetables in culture ponds is an effective method of removing nutrients and improving water quality (Li et al. 2007, Li & Li 2009, Song et al. 2009). In the present study, the concentrations of TAN, TN, TP, chl *a* and turbidity were significantly affected (lowered) by *Ipomoea aquatica* cultivation. The mean TN and TP concentrations in the IAC treatment were 27.9 and 42.5% lower compared to the control, respectively, suggesting that this aquatic vegetable can efficiently remove nitrogen and phosphorus nutrients from aquaculture wastewater in turtle culture ponds. Firstly, *I. aquatica* can accumulate nutrients in its leaves, stems and other tissues. In addition, periphyton that grow on the flourishing roots of *I. aquatica* may also contribute to absorbing nitrogen and phosphorus (Wu et al. 2014, Basílico et al. 2016). The low concentrations of chl *a* and turbidity in the IAC treatment were attributed to inhibition of algal growth due to the low concentration of TN and TP in the water and the reduced illumination induced by *I. aquatica* cultivation. Therefore, we suggest that using floating beds of aquatic vegetables in turtle culture ponds has the potential to improve the aquaculture environment and decrease wastewater discharge.

Silver carp and bighead carp, 2 planktivorous filter-feeding fishes, are usually co-cultured in turtle rearing ponds, and this is a common practice in Chinese turtle farming. These fish do not compete with the turtles for food, and also play a role in improving the water quality by feeding on phyto- and zooplankton. Accordingly, in our study, silver carp and bighead carp of the same biomass were stocked in both treatments. The study did not permit us to ascertain the interrelationship (if any) that existed between *I. aquatica* cultivation and growth of the 2 carp species. However, the efficiency of phosphorus and nitrogen absorption by *I. aquatica* could be explained further through comparing the biomass of the carps. The weight gain of 2 carp species was higher in the control ponds, suggesting that more nitrogen and phosphorus was accumulated by the carps in that treatment. In addition, concentrations of nitrogen and phosphorus in the control treatment were higher than those in the IAC treatment at the end of the experiment, indicating a reduction in the rate of nitrogen and phosphorus absorption by *I. aquatica* actually higher than 27.9 and 42.5 %, respectively.

In the present study, there were no significant differences in WG, SGR, AGR and PER between the 2 treatments, but there was a difference in survival, suggesting that *I. aquatica* cultivation significantly affected survival rather than the growth of *Pelodiscus sinensis* in ponds. Although this is the first study to report the effects of *I. aquatica* cultivation on *P. sinensis* growth and survival in pond culture, similar observations on cultured fish species, such as crucian carp *Carassius auratus* (Chen et al. 2010), have been made. However, different results were found in fish–seaweed cohabiting systems (Lombardi et al. 2006, Portillo-Clark et al. 2013). Portillo-Clark et al. (2013) reported that cohabitation with green feather alga *Caulerpa sertularioides* had a significant positive effect on biomass and growth rather than survival rates of juvenile yellow leg shrimp *Farfantepenaeus californiensis*. The inconsistency was probably dependent on the difference of the interaction between co-cultured plants and animals. Similarly, shrimp and turtle cultivation generates residues that are very rich in nutrients, which provides favorable conditions for the co-cultured plants and the associated microbial community (Duan et al. 1995, Portillo-Clark et al. 2013). Green feather algae offer an abundant living surface for microbial communities because of their highly 'feathered' morphology, which may promote the development of microbial biofilms that can enrich the diet of shrimp and lead to increased growth (Portillo-Clark et al. 2013). In our study, *I. aquatica* was cultivated on floating beds, which can strip nutrients from water through the roots rather than provide food for turtles. This is likely the main reason why significant differences did not exist in the growth of turtles in our study. However, the floating beds were able to provide a platform for turtles to bask in the light, which is advantageous for killing bacteria and reducing the occurrence of disease (Huang & Ben 2001). This, in addition to a better aquaculture environment because the N and P nutrients were absorbed by *I. aquatica*, could explain the higher survival in the IAC treatment.

Several factors affect the economic return of aquaculture systems such as yield, sale prices, feed costs, fingerlings or juveniles costs, system investments and operating costs (Muangkeow et al. 2007, de Oliveira et al. 2012). In the present study, total revenues and total costs were highest in the IAC treatment, as was the net income. These findings suggest that *I. aquatica* cultivation in the turtle ponds was effective at improving profitability. Economic analysis in this study mainly emphasized yield, turtle juveniles and feed costs. The feed costs accounted for 28.23 and 28.70 % of the total costs in the IAC and control teatments, respectively, which is lower than that for cage production of various fish species, which range from 30 to 60 % of total costs (Huguenin 1997, Silva et al. 2007). Compared to the control treatment, revenue of *I. aquatica* in the IAC treatment is one of the most important components (10.12 %) of the total revenue. Thus, *I. aquatica,* as a co-product in turtle culture ponds, can generate extra economic benefits. The present findings suggest that farmers can use floating beds to cultivate *I. aquatica* in turtle culture ponds for maximizing profitability.

In summary, this study has demonstrated that coculture of *I. aquatica* in *P. sinensis* culture ponds has positive effects on nutrient stripping and economic returns, which allows for optimal utilization of the available pond space, contributing substantially to improving the economic output and water quality. Further studies incorporating higher covered areas and in combination with stocking densities might maximize economic outputs and nutrient removal further.

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