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Optimization of fish to mussel stocking ratio: Development of a state-of-art pearl production mode through fish–mussel integration

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ABSTRACT

Integrated aquaculture has been widely used for pearl production in the freshwater pearl mussel *Hyriopsis cumingii* farming in China, but the production technology has not reached the state of the art. This study explored the optimal stocking ratio of fish to mussel (fish–mussel) through a 90-day experiment conducted in land-based enclosures. The integrated system included pearl mussel, grass carp, gibel carp, silver carp and bighead carp, with four fish–mussel stocking ratios by number: 1:1 (R1), 2:1 (R2), 3:1 (R3) and 4:1 (R4). The pearl yield was higher in the R2 enclosures than in the R1 and R4 enclosures, whereas the fish yield was higher in the R3 and R4 enclosures than in the R1 and R2 enclosures. The phosphorus (P) utilization efficiency was higher in the R2, R3 and R4 enclosures than in the R1 enclosures. The wastes of nitrogen (N) and P enhanced with the increase of fish–mussel ratio. Regression analyses indicated that the fish–mussel ratio was 2.3:1 for the maximal pearl yield, and 3.6:1 for the maximal fish yield, and 1.6–2.3:1 for the minimal N waste, and 1.9–2.9:1 for the minimal P waste. This study indicated that the suitable fish–mussel stocking ratio was 2:1 in the integrated culture of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp.

1. Introduction

Integrated culture has been widely used as a tool to improve production efficiency in freshwater and marine aquaculture (Troell et al., 2003; Neori et al., 2004). Species integration at multi-trophic levels offers an approach for improvement of ecological efficiency and reduction of environmental pollution of aquaculture systems (Neori et al., 2004; Shi et al., 2013), which is key to establish sustainability of aquaculture modes (Wang, 2004). The strategies in both stocking (such as species combination, density and stocking ratio) and husbandry management (such as feed and fertilizer supplement, aeration and water exchange) should be considered in optimization of aquaculture mode (Wang, 2004). In an integrated culture system, production efficiency and water quality can be affected by either species combination (Milstein et al., 2009; Wahab et al., 2011; Barcellos et al., 2012) or stocking ratio of farmed animals (Teichert-Coddington, 1996; Azim et al., 2002; Hossain and Islam, 2006; Uddin et al., 2006, 2007; Muangkeow et al., 2007, 2011). After determination of the species combination, stocking density becomes the primary factor in regulating growth, nutrient conversion ratio and economic return of integrated culture (Muangkeow et al., 2007). However, little research has focused on optimizing both species combination and stocking ratio in an integrated culture system due to a great time demand and labor costing.

Hyriopsis cumingii is a freshwater mussel commercially important for freshwater pearl production (Wang et al., 2009). In commercial farming, *H. cumingii* generally co-cultured with freshwater carps and the stocking ratio of fish to mussel (fish–mussel) is low. For instance, Yan et al. (2009) reported that the suitable fish–mussel stocking ratio is 1:10 in the integrated culture of *H. cumingii*, silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*. Recent studies revealed that pearl and fish yields and nutrient utilization efficiency were higher in the integrated system of *H. cumingii*, grass carp *Ctenopharyngodon idellus*, gibel carp *Carassius gibelio*, silver carp and bighead carp than in the integrated system of *H. cumingii*, silver carp and bighead carp (Tang et al., unpublished data). This result highlights the potential to enhance pearl and fish production using a novel integrated system of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp (grass carp and gibel carp are the major species in freshwater fish culture in China). It is necessary to test the suitable fish–mussel stocking

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ratio in the novel fish–mussel integrated system since the optimal stocking ratios in integrated culture depend on the species involved (Hossain and Islam, 2006; Uddin et al., 2006). The objective of the present study was to explore the suitable fish–mussel stocking ratio in integrated culture of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp, in an attempt to improve production efficiency.

2. Materials and methods

2.1. Experimental site, mussel, fish, pond and enclosures

A field experiment was conducted in Fengqiao farm (29°47′59.8″ N; 120°23′42.4″ E), Shaoxing, China, from July 23 to October 21, 2010. The integrated system comprised freshwater mussel *H. cumingii* as the principal species and four fish species (grass carp *C. idellus*, gibel carp *C. gibelio*, silver carp *H. molitrix* and bighead carp *A. nobilis*) as the co-cultured species. The mussel were purchased from a commercial mussel farm in Longyou, Quzhou, China, in September 2009, and the grass carp, gibel carp, silver carp and bighead carp from a freshwater fish farm in Deqing, Huzhou, China, in March 2010. Upon arrival, the mussel were put in net bags that hung in an earthen pond, and the fishes were reared in net pens suspended in the same pond and fed with a commercial formulated feed containing 28% crude protein (Kesheng Feed Stock Co., Ltd., Shaoxing, China). Prior to the experiment, the mussel with shell lengths >80 mm were selected and given grafted operation by which about 30 small pieces of the mantle epithelium collecting from the donor were planted into the mantle layer of the recipient mussel as pearl nuclear. The grafted mussels were hung in the earthen pond for one week to check survival.

The experiment was conducted in land-based enclosures (3.18 m diameter, 7.94 m^2 area) that were constructed in an earthen pond (1.33 ha). Each enclosure comprised a polyethylene (PE) tube that were buried into the sediment soil to a depth of 20 cm, 12 timber piles that were inserted into the soil bottom around both inside and outside of the PE tube to maintain the tube standing vertically on the bottom of the pond, and two bamboo rings that were circled around inside of the PE tube to support the tube. Each PE tube was made of a PE sheet (10 m length × 1.7 m width). A polyvinyl chloride tube (20 cm diameter) was buried under each enclosure to allow water exchange.

2.2. Fish–mussel stocking ratio and procedure of the field experiment

Four stocking ratios of fish to mussel by number were 1:1 (R1), 2:1 (R2), 3:1 (R3) and 4:1 (R4). The mussel density in all the treatments was 1.2 ind. m^{-2}, and the fish density in the R1, R2, R3 and R4 treatments was 1.2, 2.4, 3.6 and 4.8 ind. m^{-2}, respectively. The stocking ratio between grass carp, gibel carp, silver carp and bighead carp in each treatment was set at 6:2:1:1, which is widely used in freshwater fish pond in China.

At the beginning of the experiment, the pond was filled with river water (water depth in the enclosures was 1.1–1.2 m). Each enclosure was fertilized with 1 kg of duck manure. The mussels were put in net bags (2 ind. bag^{-1}) and then 5 bags were suspended in each enclosure at 30 cm deep. The grass carp, gibel carp, silver carp and bighead carp were distributed into the enclosures. The number of mussel, grass carp, gibel carp, silver carp and bighead carp in enclosures R1, R2, R3 and R4 was 10, 6, 2, 1 and 1; 10, 12, 4, 2 and 2; 10, 18, 6, 3 and 3; and 10, 24, 8, 4 and 4, respectively. Each fish–mussel stocking ratio was in three replicator, therefore, totally 12 enclosures were used. The shell length and whole weight of the mussel and body weight of different fishes were measured as described in Wang et al. (2009). Three groups, each including 10 mussel, 5 grass carp, 5 gibel carp, 5 silver carp and 5 bighead carp, were randomly sampled and stored at −20 °C for the analysis of nitrogen (N) and phosphorus (P) contents.

During the experiment, all fish species were fed with the commercial formulated feed at 0800 and 1700 h daily. Totally, 564 ± 20, 1144 ± 11, 1715 ± 46 and 2306 ± 114 g feed (mean ± S.D., $n = 3$) were supplied to enclosures R1, R2, R3 and R4, respectively. At the end of the experiment, the mussel, grass carp, gibel carp, silver carp and bighead carp were captured from the enclosures. The shell length, whole weight, pearl number and the pearl weight of the mussel, and the body weight of each fish species were measured. Samples including 5 mussel, 5 grass carp, 5 gibel carp, 3 silver carp and 3 bighead carp were randomly collected from each treatment for the analysis of N and P contents. The contents of N and P in the formulated feed, duck manure, mussel and fishes were analyzed with the method described in AOAC (2005).

2.3. Water quality monitoring

Water temperature and dissolved oxygen (DO) in the enclosures were measured with a YSI 550A DO meter (YSI scientific Inc., Yellow Springs, OH, USA), and the Secchi depth (SD) was measured with a Secchi disk at 0600–0800 h daily. Water samples were collected with a 5-L sampling vessel in the morning (0800–1000 h) fortnightly, and the contents of ammonia, total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD_{Mn}) were measured with the method described in APHA (2005).

2.4. Calculation and statistical analysis

The growth rates in shell length and whole weight of mussel, pearl yield and mussel yield were calculated as described in Wang et al. (2009). The fish yield of each species was estimated as weight of the captured fish/mean recaptured rate of the fishes (the mean recaptured rate was calculated as 100 × number of the fish captured/number of the fish stocked). In the present study, the mean recaptured rate of the fishes was 58.1% (36.7–93.3%). Feed conversion ratio (R_{FCR}), nutrient utilization efficiency (U_N; %) and nutrient waste (W_N; g $enclosure^{-1}$) were calculated as:

$$R_{FCR} = \frac{I_p}{(W_t - W_0)}$$

$$U_N = 100 \times \frac{(W_{mt} \times C_{Nmt} + W_{grt} \times C_{Ngrt} + W_{gt} \times C_{Ngt} + W_{st} \times C_{Nst} + W_{bt} \times C_{Nbt} - W_{m0} \times C_{Nm0} - W_{gr0} \times C_{Ngr0} - W_{g0} \times C_{Ng0} - W_{s0} \times C_{Ns0} - W_{b0} \times C_{Nb0})}{(I_p \times C_{Np} + I_d \times C_{Nd})}$$

$$W_N = (I_p \times C_{Np} + I_d \times C_{Nd}) \times \left(1 - \frac{U_N}{100}\right)$$

where W_0 (g) is total initial body weight of grass carp, gibel carp, silver carp and bighead carp, and W_t (g) is total final body weight; W_{mt} (g), W_{grt} (g), W_{gt} (g), W_{st} (g) and W_{bt} (g) are final weights of the mussel, grass carp, gibel carp, silver carp and bighead carp, and W_{m0} (g), W_{gr0} (g), W_{g0} (g), W_{s0} (g) and W_{b0} (g) are initial weights; C_{Nmt} (%), C_{Ngrt} (%), C_{Ngt} (%), C_{Nst} (%) and C_{Nbt} (%) are contents of N or P of the mussel, grass carp, gibel carp, silver carp and bighead carp at the end of the experiment, and C_{Nm0} (%), C_{Ngr0} (%), C_{Ng0} (%), C_{Ns0} (%) and C_{Nb0} (%) at the start; C_{Np} (%) and C_{Nd} (%) are contents of N or P in the formulated feed and duck manure; I_p (g) and I_d (g) are

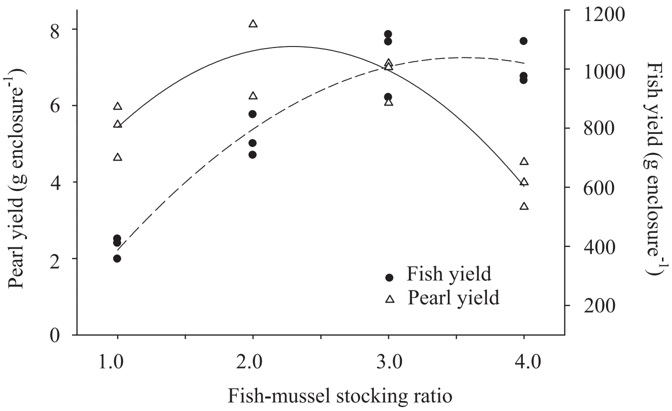

Fig. 1. Relationships between the fish–mussel stocking ratio and pearl yield or between the fish–mussel stocking ratio and fish yield.

amounts of the formulated feed and duck manure supplied to the enclosures.

The differences in the growth rates in shell length or whole weight of mussel, pearl yield, mussel yield, fish final body weight, fish yield (total yield of four fish species), R_{FCR}, U_N, W_N and water quality parameters (SD, DO, ammonia, TN, TP and COD_{Mn}) were examined with one-way ANOVA. The differences in the above variables between the treatments were examined with Tukey's honest significant difference (HSD) test. The relationships between fish–mussel stocking ratio and pearl yield, fish yield or W_N were examined with regression analysis. The relationships between pearl yield, fish yield of each species (grass carp, gibel carp, silver carp or bighead carp) and water quality (SD, DO, ammonia, TN, TP and COD_{Mn}) were examined with redundancy analysis (RDA). Cluster analysis was performed to evaluate production efficiency with the variables including pearl yield, fish yield, utilization efficiency and waste of N. The significant level was set at $P < 0.05$. ANOVA and cluster analysis were performed with SPSS (version 19.0; IBM Corp., Armonk, USA), and RDA was performed with Canoco for Windows (version 4.5; Microcomputer Power, Ithaca, USA).

3. Results

3.1. Survival, pearl yield and growth of the mussel

The survival of the mussel was 97–100%, and no significant difference was found in survival between the treatments ($P > 0.05$). The growth rate in whole body weight of mussel and mussel yield were higher in R2 enclosures than in R4 enclosures ($P < 0.05$), while R1 and R3 enclosures were intermediate and not significantly different from either ($P > 0.05$, Table 1). No significant differences were found in the growth rate in shell length of mussel between the treatments ($P > 0.05$, Table 1). The pearl yield was higher in R2 enclosures than in R1 and R4 enclosures ($P < 0.05$), while no significant difference was found in the pearl yield between R2 and R3 enclosures ($P > 0.05$, Table 1). The regression equation between pearl yield (Y_P; g enclosure^{-1}) and the fish–mussel stocking ratio (R) was $Y_P = 0.906 + 5.782 \times R - 1.259 \times R^2$ ($n = 12$, $r^2 = 0.807$, $P = 0.0006$, Fig. 1).

3.2. Fish yield and feed conversion ratio

The final body weight of bighead carp decreased with the increase of fish–mussel stocking ratio. The fish yield (total yield of four species) was higher in R3 and R4 enclosures than in R1 and R2 enclosures ($P < 0.05$, Table 2). The R_{FCR} was lower in R1, R2 and R3 enclosures than in R4 enclosures ($P < 0.05$, Table 2). The regression equation between fish yield (Y_F; g enclosure^{-1}) and fish–mussel stocking ratio (R) was $Y_F = -221.44 + 707.98 \times R - 99.44 \times R^2$ ($n = 12$, $r^2 = 0.933$, $P < 0.0001$, Fig. 1).

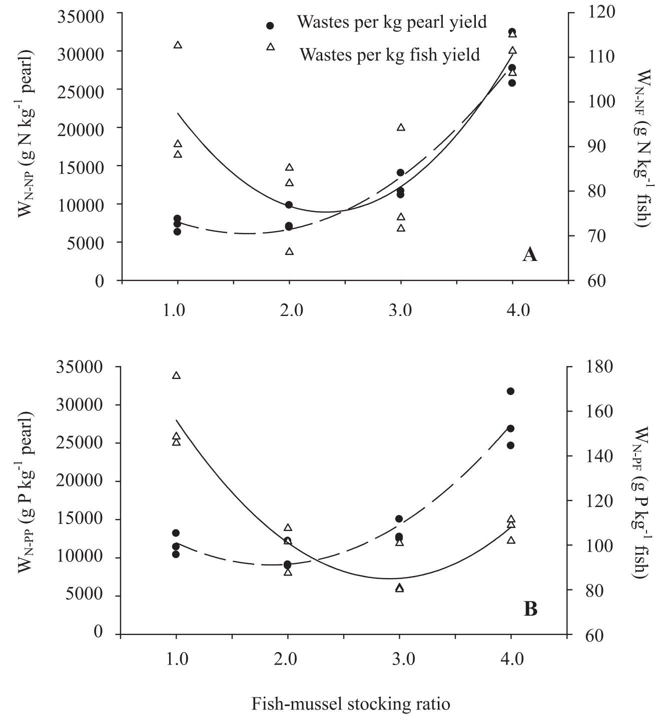

Fig. 2. Relationships between the fish–mussel stocking ratio and nitrogen waste (A) or between the fish–mussel stocking ratio and phosphorus waste (B). $W_{N\text{-}NP}$: nitrogen waste of per unit pearl yield; $W_{N\text{-}NF}$: nitrogen waste of per unit fish yield; $W_{N\text{-}PP}$: phosphorus waste of per unit pearl yield; $W_{N\text{-}PF}$: phosphorus waste of per unit fish yield.

3.3. Nutrient utilization efficiency and wastes production

The N utilization efficiency ($U_{N\text{-}N}$) and P utilization efficiency ($U_{N\text{-}P}$) tended to increase with increasing the fish–mussel stocking ratio from 1:1 to 2:1, and then tended to decrease with increasing the fish–mussel stocking ratio from 2:1 to 4:1. $U_{N\text{-}P}$ was lower in R1 enclosures than in R2, R3 and R4 enclosures ($P < 0.05$, Table 3). The wastes of N ($W_{N\text{-}N}$) and P ($W_{N\text{-}P}$) increased with the increases of fish–mussel stocking ratio. The $W_{N\text{-}N}$ and $W_{N\text{-}P}$ were highest in R4 enclosures but lowest in R1 enclosures ($P < 0.05$, Table 3). The wastes of N and P per kg pearl yield ($W_{N\text{-}NP}$ and $W_{N\text{-}PP}$) or per kg fish yield ($W_{N\text{-}NF}$ and $W_{N\text{-}PF}$) were significantly correlated with the fish–mussel stocking ratio (Fig. 2). The regression equations were $W_{N\text{-}NP} = 16{,}374.013 - 12{,}665.957 \times R + 3906.117 \times R^2$ ($n = 12$, $r^2 = 0.951$, $P < 0.0001$); $W_{N\text{-}PP} = 22{,}673.779 - 14{,}699.93 \times R + 3971.297 \times R^2$ ($n = 12$, $r^2 = 0.927$, $P < 0.0001$); $W_{N\text{-}NF} = 143.286 - 58.549 \times R + 12.588 \times R^2$ ($n = 12$, $r^2 = 0.704$, $P = 0.0042$); and $W_{N\text{-}PF} = 249.81 - 113.347 \times R + 19.479 \times R^2$ ($n = 12$, $r^2 = 0.882$, $P < 0.0001$).

3.4. Water quality and relationship between production performance and water quality

During the experiment, water temperature in the enclosures fluctuated from 20 to 34 °C (mean value 28 °C). The concentrations of ammonia, TN and COD_{Mn} increased, while the DO decreased, with the increase of fish–mussel stocking ratio (Table 4). The ammonia, TN and COD_{Mn} were higher, and the DO was lower, in R4 enclosures than in R1 enclosures ($P < 0.05$).

Table 1
Growth rate, pearl yield and mussel yield of *Hyriopsis cumingii* in the experimental enclosures.

Treatment	Growth rate (% d^{-1})		Pearl number	Pearl weight	Pearl yield	Mussel yield
	Shell length	Whole weight	(pearl $mussel^{-1}$)	(g $mussel^{-1}$)	(g $enclosure^{-1}$)	(g $enclosure^{-1}$)
R1	0.066 ± 0.031	0.169 ± 0.116^{ab}	32 ± 1	0.54 ± 0.07^{bc}	5.4 ± 0.7^{bc}	138 ± 92^{ab}
R2	0.108 ± 0.037	0.270 ± 0.078^{a}	32 ± 2	0.76 ± 0.12^{a}	7.6 ± 1.2^{a}	219 ± 71^{a}
R3	0.057 ± 0.030	0.126 ± 0.055^{ab}	31 ± 1	0.67 ± 0.06^{ab}	6.7 ± 0.7^{ab}	109 ± 49^{ab}
R4	0.043 ± 0.013	0.045 ± 0.015^{b}	30 ± 2	0.40 ± 0.06^{c}	4.0 ± 0.7^{c}	37 ± 12^{b}

R1: fish–mussel stocking ratio was 1:1; R2: fish–mussel stocking ratio was 2:1; R3: fish–mussel stocking ratio was 3:1; R4: fish–mussel stocking ratio was 4:1.
Data are expressed as mean ± S.D. ($n = 3$). The superscripts present results of Tukey's HSD test, and the data with different superscripts within the same column are significantly different ($P < 0.05$).

Table 2
Final body weight (g $ind.^{-1}$), yield (g $enclosure^{-1}$) and feed conversion ratio of fish in the experimental enclosures.

Treatment	Grass carp		Gibel carp		Silver carp		Bighead carp		Total yield	R_{FCR}
	Wt	Yield	Wt	Yield	Wt	Yield	Wt	Yield		
R1	26.3 ± 5.6^{ab}	124 ± 33^{b}	22.1 ± 0.1	24 ± 0^{b}	185.2 ± 15.7^{a}	170 ± 15^{c}	66.8 ± 8.1^{a}	57 ± 8^{b}	397 ± 36^{c}	1.51 ± 0.15^{b}
R2	34.0 ± 5.1^{a}	342 ± 62^{a}	20.7 ± 3.1	40 ± 14^{ab}	137.9 ± 7.3^{b}	252 ± 13^{b}	61.0 ± 6.2^{a}	102 ± 14^{ab}	767 ± 71^{b}	1.56 ± 0.16^{b}
R3	30.1 ± 3.8^{ab}	439 ± 67^{a}	20.6 ± 4.0	62 ± 24^{ab}	147.2 ± 10.6^{b}	407 ± 31^{a}	44.9 ± 10.6^{b}	103 ± 30^{a}	1037 ± 116^{a}	1.71 ± 0.19^{b}
R4	23.6 ± 3.6^{b}	432 ± 82^{a}	20.6 ± 1.7	79 ± 12^{a}	114.6 ± 9.8^{c}	414 ± 37^{a}	26.1 ± 2.4^{c}	62 ± 9^{ab}	1010 ± 73^{a}	2.34 ± 0.06^{a}

R1: fish–mussel stocking ratio was 1:1; R2: fish–mussel stocking ratio was 2:1; R3: fish–mussel stocking ratio was 3:1; R4: fish–mussel stocking ratio was 4:1; Wt: final body weight; R_{FCR}: feed conversion ratio.
Data are expressed as mean ± S.D. ($n = 3$). The superscripts present results of Tukey's HSD test, and the data with different superscripts within the same column are significantly different ($P < 0.05$).

Table 3
Utilization efficiency and waste of nitrogen and phosphorus in the experimental enclosures.

Treatment	U_{N-N} (%)	U_{N-P} (%)	W_{N-N} (g $enclosure^{-1}$)	W_{N-P} (g $enclosure^{-1}$)
R1	20.2 ± 3.0	7.1 ± 0.7^{b}	38.2 ± 1.7^{d}	61.8 ± 0.8^{d}
R2	23.0 ± 3.0	10.6 ± 1.1^{a}	59.2 ± 2.7^{c}	75.3 ± 1.2^{c}
R3	22.3 ± 2.9	12.0 ± 1.5^{a}	82.0 ± 3.5^{b}	89.6 ± 1.9^{b}
R4	17.1 ± 0.7	9.8 ± 0.4^{a}	111.8 ± 4.0^{a}	108.2 ± 2.8^{a}

R1: fish–mussel stocking ratio was 1:1; R2: fish–mussel stocking ratio was 2:1; R3: fish–mussel stocking ratio was 3:1; R4: fish–mussel stocking ratio was 4:1; U_{N-N}: nitrogen utilization efficiency; U_{N-P}: phosphorus utilization efficiency; W_{N-N}: nitrogen waste; W_{N-P}: phosphorus waste.
Data are expressed as mean ± S.D. ($n = 3$). The superscripts present results of Tukey's HSD test, and the data with different superscripts within the same column are significantly different ($P < 0.05$).

Table 4
Water quality in the experimental enclosures.

Treatment	Secchi depth (cm)	Dissolved oxygen (mg L^{-1})	Ammonia (mg L^{-1})	Total nitrogen (mg L^{-1})	Total phosphorus (mg L^{-1})	COD_{Mn} (mg L^{-1})
R1	25 ± 2	3.68 ± 0.34^{a}	0.21 ± 0.03^{b}	0.63 ± 0.01^{d}	0.14 ± 0.06	10.1 ± 0.8^{b}
R2	23 ± 5	3.23 ± 0.47^{ab}	0.26 ± 0.02^{ab}	0.69 ± 0.03^{c}	0.16 ± 0.07	10.8 ± 0.8^{b}
R3	19 ± 3	2.62 ± 0.29^{b}	0.30 ± 0.01^{ab}	0.84 ± 0.02^{b}	0.19 ± 0.08	11.8 ± 0.8^{ab}
R4	17 ± 3	2.39 ± 0.16^{b}	0.36 ± 0.07^{a}	0.90 ± 0.01^{a}	0.25 ± 0.09	12.8 ± 0.7^{a}

R1: fish–mussel stocking ratio was 1:1; R2: fish–mussel stocking ratio was 2:1; R3: fish–mussel stocking ratio was 3:1; R4: fish–mussel stocking ratio was 4:1; COD_{Mn}: chemical oxygen demand.
Data are expressed as mean ± S.D. ($n = 3$). The superscripts present results of Tukey's HSD test, and the data with different superscripts within the same column are significantly different ($P < 0.05$).

The SD, DO, ammonia, TN, TP and COD_{Mn} explained 63.6% of the variability in pearl yield and fish (grass carp, gibel carp, silver carp and bighead carp) yield. The first and second ordination axis had the highest eigenvalue (0.37 and 0.23), respectively. The forward selection procedure indicated that TN was the dominant factor affecting pearl and fish yields ($F = 4.02$, $P = 0.014$, Fig. 3). The production efficiency (evaluated with pearl yield, fish yield, U_{N-N}, W_{N-N} and COD_{Mn}) in R2 enclosures was close to that in R3 enclosures, while the production efficiency in R1 enclosures was apart from that in R2, R3 and R4 enclosures (Fig. 4).

4. Discussion

Stocking strategies in integrated culture or polyculture include variation of species combination and stocking ratios between farmed organisms (Azim et al., 2001). The stocking ratio can affect the quantity and quality of products in integrated culture or polyculture, as well as nutrient utilization efficiency (Teichert-Coddington, 1996; Ridha, 2006; Milstein et al., 2009). In the present study, the pearl yield was higher at the fish–mussel stocking ratio 2:1 than at the fish–mussel stocking ratios 1:1 and 4:1. The regression equation indicates that the fish–mussel stocking ratio for maximal pearl yield is 2.3:1. These results indicated that the use of fish–mussel stocking ratio 2:1 in the integrated culture of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp can enhance pearl yield. Yan et al. (2009) reported the suitable stocking ratio was 1:10 in the integrated culture of *H. cumingii*, silver carp and bighead carp. Therefore, the suitable fish–mussel stocking ratio in the integrated culture of *H. cumingii* and different fish species is dependent on the species combination used, and the fish–mussel stocking ratio must be evaluated when the species combination is changed. In the integrated culture of shrimp *Macrobrachium*

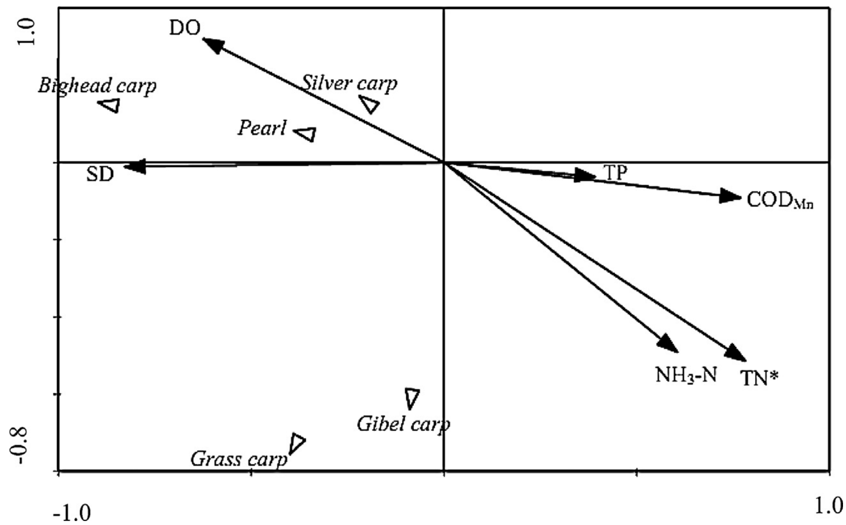

Fig. 3. Redundancy analysis (RDA) of pearl, grass carp, gibel carp, silver carp and bighead carp yields and water quality. SD: Secchi depth; DO: dissolved oxygen; NH_3-N: ammonia; TN: total nitrogen; TP: total phosphorus; COD_{Mn}: chemical oxygen demand. * represents $P < 0.05$.

rosenbergii and fishes, the suitable fish-shrimp stocking ratio was 3:1 in the integrated culture of shrimp and tilapia (Uddin et al., 2006), but was 1:1 in the integrated culture of shrimp, catla *Catla catla*, rohu *Labeo rohita* and silver carp (Hossain and Islam, 2006).

Azim et al. (2002) reported that fish yield was higher in the polyculture system of rohu, catla and kalbaush *Labeo calbasu* (rohu: catla: kalbaush was 12:8:3) than in the polyculture system of rohu and catla (rohu: catla was 12:8). A previous study indicated that adding grass carp and gibel carp to the integrated system of *H. cumingii*, silver carp and bighead carp and feeding these fishes with formulated feed could enhance fish yield (Tang et al., unpublished data). In the present study, fish yield was higher at the fish–mussel stocking ratio 3–4:1 than at the fish–mussel stocking ratio 1–2:1. The fish–mussel stocking ratio (3.6:1) for maximal fish yield was higher than that (2.3:1) for maximal pearl yield, suggesting the benefits to enhance pearl yield or fish yield in the integrated culture of *H. cumingii* and fishes can be regulated through adjusting fish density. We recommend that the suitable fish–mussel stocking ratio is 2:1 in the integrated culture of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp since at such a fish–mussel stocking ratio, pearl yield, size of a single pearl and fish size at harvest were greater than those at the fish–mussel stocking ratio 3:1. The production of larger pearls and fishes is more profitable than the production of smaller pearls and fishes due to the higher market price of larger pearls and fishes. Teichert-Coddington (1996) indicated that the economic profit from polyculture of tilapia and tambaqui depended on the market price and body size of the fishes at harvest, rather than the total fish yield.

The stocking ratios in integrated culture or polyculture can affect feed utilization efficiency (Teichert-Coddington, 1996; Milstein et al., 2008) and water quality (Hossain and Islam, 2006; Asaduzzaman et al., 2009; Yuan et al., 2010). In the present study, the utilization efficiency of N and P tended to increase with increasing fish–mussel stocking ratio from 1:1 to 2:1. However, the wastes of N and P increased with increasing fish–mussel stocking ratio from 1:1 to 4:1. These results suggest that accumulation of N and P wastes in the integrated system cannot be reduced by improvement of fish–mussel stocking ratio because feed supplementation increased with the increase of fish density. The fish–mussel stocking ratio for the minimal N wastes per kg pearl yield or per kg fish

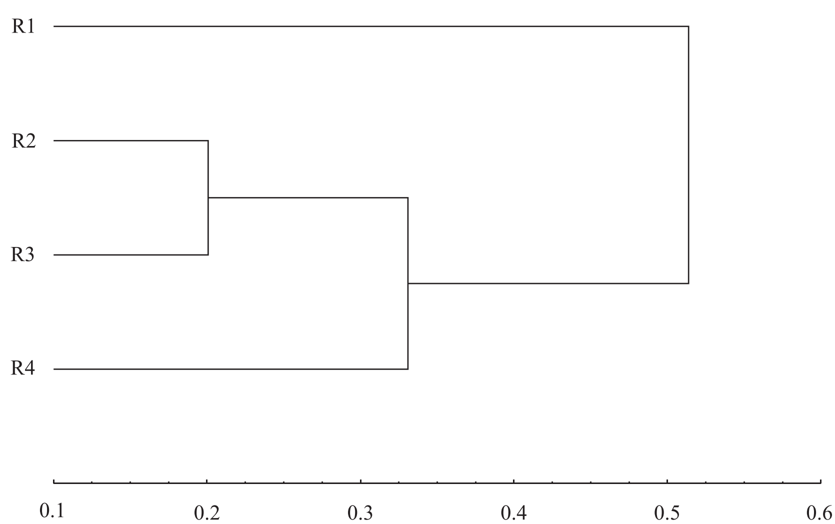

Fig. 4. Dendrogram of cluster analysis on production efficiency of fish–mussel integration during the experiment. Production efficiency was evaluated with pearl yield, fish yield, nitrogen utilization efficiency, nitrogen waste and chemical oxygen demand. R1: fish–mussel stocking ratio was 1:1; R2: fish–mussel stocking ratio was 2:1; R3: fish–mussel stocking ratio was 3:1; R4: fish–mussel stocking ratio was 4:1.

yield were 1.6:1 or 2.3:1, and the fish–mussel stocking ratio for the minimal P wastes per kg pearl yield or per kg fish yield were 1.9:1 or 2.9:1. These results suggest that the suitable fish–mussel stocking ratio for minimal wastes of N and P in the integrated system of *H. cumingii* and fishes should not exceed 2:1. The concentrations of ammonia, TN and COD_{Mn} increased, while the DO decreased with the increase of fish–mussel stocking ratio from 1:1 to 2:1. In the enclosures with fish–mussel stocking ratio at 3:1 or 4:1, low DO ($<3\,mg\,L^{-1}$) was frequently observed in the morning. These results suggest that a high fish–mussel stocking ratio may result in more N, P and organic matter accumulation and lower DO in the integrated system. Therefore, aeration must be performed in the integrated culture of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp when the fish–mussel stocking ratio exceeds 1:1.

In a system of integrated culture and/or polyculture, only the organisms with positively synergetic interaction in nutrient utilization and social behavior are used as the principal or co-cultured species. Milstein et al. (2009) reported that the yield of a fish polyculture system was affected by the stocking ratio between fish species living in water column or near bottom. In commercial farming ponds, grass carp and gibel carp are generally fed with formulated feed (termed as feed consumer), and *H. cumingii* and silver carp feed on phytoplankton and detritus (termed as phytoplankton consumer). Bighead carp feed on zooplankton in a natural environment (termed as zooplankton consumer) but also eat formulated feed in commercial farming ponds. Moreover, *H. cumingii* and silver carp distribute near water surface (termed as top feeder), and grass carp and bighead carp distribute in the middle layer of the water column (termed as middle feeder), and gibel carp distribute near bottom (termed as bottom feeder). In the integrated system of mussel, grass carp, gibel carp, silver carp and bighead carp with the fish–mussel stocking ratio set at 2:1, the stocking ratio of feed consumer:phytoplankton consumer:zooplankton consumer was 8:6:1, while the stocking ratio of top feeder:middle feeder:bottom feeder was 6:7:2. It is reasonable to believe that the increase of stocking proportion of gibel carp (an omnivorous bottom feeding fish) can improve nutrient flux between water column and bottom soil in the fish–mussel integrated system. For instance, the stocking ratio of top:middle:bottom fish feeders can be adjusted to 7:7:4. If grass carp, gibel carp, silver carp and bighead carp are used to integrate with mussel, the stocking ratio of these four fishes is suggested at 6:4:1:1 and the overall fish to mussel ratio is suggested at 2:1. These stocking ratios remain to be tested to confirm the efficacy of pearl and fish production at field situation.

In conclusion, the fish–mussel stocking ratio can affect pearl and fish yields, utilization efficiency and wastes of nitrogen and phosphorus in the integrated system of *H. cumingii*, grass carp, gibel carp, silver carp and bighead carp. The fish–mussel stocking ratio for maximum pearl and fish yields and nutrient utilization efficiency is 2:1 when the mussel density is set at $1.2\,ind.\,m^{-2}$ and the stocking ratio of grass carp:gibel carp:silver carp: bighead carp is set at 6:2:1:1.

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References

AOAC, 2005. Official Methods of Analysis, 18th edition (online). International Association of Analytical Communities, Gaithersburg, MD, USA.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association, Washington, DC, USA.

Asaduzzaman, M., Wahab, M.A., Verdegem, M.C.J., Mondal, M.N., Azim, M.E., 2009. Effects of stocking density of freshwater prawn *Macrobrachium rosenbergii* and addition of different levels of tilapia *Oreochromis niloticus* on production in C/N controlled periphyton based system. Aquaculture 286, 72–79.

Azim, M.E., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., Huisman, E.A., Verdegem, M.C.J., 2001. Optimization of stocking ratios of two Indian major carps, rohu (*Labeo rohita* Ham.) and catla (*Catla catla* Ham.) in a periphyton-based aquaculture system. Aquaculture 203, 33–49.

Azim, M.E., Verdegem, M.C.J., Rahman, M.M., Wahab, M.A., van Dam, A.A., Beveridge, M.C.M., 2002. Evaluation of polyculture of Indian major carps in periphyton-based ponds. Aquaculture 213, 131–149.

Barcellos, L.J.G., Quevedo, R.M., Kreutz, L.C., Ritter, F., Pandolfo, A., Hemkemeier, M., Colla, L., Silva, L.B., Koakoski, G., da Rosa, J.G.S., 2012. Comparative analysis of different fish polyculture systems. J. World Aquac. Soc. 43, 778–789.

Hossain, M.A., Islam, M.S., 2006. Optimization of stocking density of freshwater prawn *Macrobrachium rosenbergii* (de Man) in carp polyculture in Bangladesh. Aquac. Res. 37, 994–1000.

Milstein, A., Kadir, A., Wahab, M.A., 2008. The effects of partially substituting Indian carps or adding silver carp on polycultures including small indigenous fish species (SIS). Aquaculture 279, 92–98.

Milstein, A., Wahab, M.A., Kadir, A., Sagor, M.F.H., Islam, M.A., 2009. Effects of intervention in the water column and/or pond bottom through species composition on polycultures of large carps and small indigenous species. Aquaculture 286, 246–253.

Muangkeow, B., Ikejima, K., Powtongsook, S., Yi, Y., 2007. Effects of white shrimp, *Litopenaeus vannamei* (Boone), and Nile tilapia, *Oreochromis niloticus* L., stocking density on growth, nutrient conversion rate and economic return in integrated closed recirculation system. Aquaculture 269, 363–376.

Muangkeow, B., Ikejima, K., Powtongsook, S., Gallardo, W., 2011. Growth and nutrient conversion of white shrimp *Litopenaeus vannamei* (Boone) and Nile tilapia *Oreochromis niloticus* L. in an integrated closed recirculating system. Aquac. Res. 42, 1246–1260.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391.

Ridha, M.T., 2006. Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. Aquac. Res. 37, 172–179.

Shi, H.H., Zheng, W., Zhang, X.L., Zhu, M.Y., Ding, D.W., 2013. Ecological-economic assessment of monoculture and integrated multi-trophic aquaculture in Sanggou Bay of China. Aquaculture 410–411, 172–178.

Teichert-Coddington, D.R., 1996. Effect of stocking ratio on semi-intensive polyculture of *Colossoma macropomum* and *Oreochromis niloticus* in Honduras, Central America. Aquaculture 143, 291–302.

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A.H., Kautsky, N., Yarish, C., 2003. Integrated mariculture: asking the right questions. Aquaculture 226, 69–90.

Uddin, S., Ekram-Ul-Azim, M., Wahab, A., Verdegem, M.C.J., 2006. The potential of mixed culture of genetically improved farmed tilapia (*Oreochromis niloticus*) and freshwater giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. Aquac. Res. 37, 241–247.

Uddin, M.S., Rahman, S.M.S., Azim, M.E., Wahab, M.A., Verdegem, M.C.J., Verreth, J.A.J., 2007. Effects of stocking density on production and economics of Nile tilapia (*Oreochromis niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) polyculture in periphyton-based systems. Aquac. Res. 38, 1759–1769.

Wang, Y., 2004. Optimization of culture model in seawater pond: concepts, principles and methods. J. Fish. China 28, 568–572 (in Chinese with English abstract).

Wang, Y., Wang, W.L., Qin, J.G., Wang, X.D., Zhu, S.B., 2009. Effects of integrated combination and quicklime supplementation on growth and pearl yield of freshwater pearl mussel, *Hyriopsis cumingii* (Lea, 1852). Aquac. Res. 40, 1634–1641.

Wahab, M.A., Kadir, A., Milstein, A., Kunda, M., 2011. Manipulation of species combination for enhancing fish production in polyculture systems involving major carps and small indigenous fish species. Aquaculture 321, 289–297.

Yan, L.L., Zhang, G.F., Liu, Q.G., Li, J.L., 2009. Optimization of culturing the freshwater pearl mussels, *Hyriopsis cumingii* with filter feeding Chinese carps (bighead carp and silver carp) by orthogonal array design. Aquaculture 292, 60–66.

Yuan, D.R., Yi, Y., Yakupitiyage, A., Fitzimmons, K., Diana, J.S., 2010. Effects of addition of red tilapia (*Oreochromis* spp.) at different densities and sizes on production, water quality and nutrient recovery of intensive culture of white shrimp (*Litopenaeus vannamei*) in cement tanks. Aquaculture 298, 226–238.