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# Optimization of fish to mussel stocking [redacted]

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

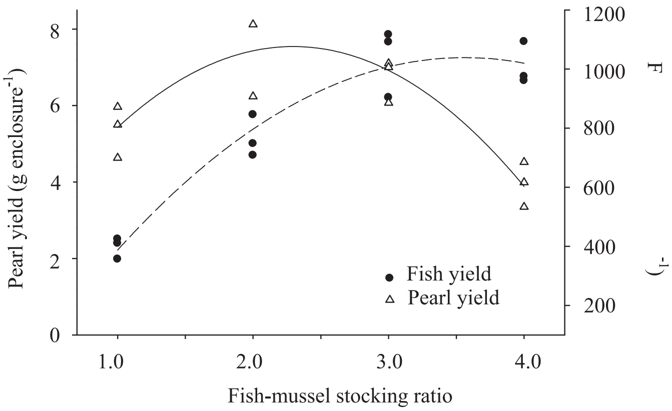

Pearl yield (g enclosure-1)
8
6
4
2
0
1200
1000
800
600
400
200
F
-1)
Fish yield
Pearl yield
1.0
2.0
3.0
4.0
Fish-mussel stocking ratio

**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 | $U_{N\text{-}N}$ (%) | $U_{N\text{-}P}$ (%) | $W_{N\text{-}N}$ (g $enclosure^{-1}$) | $W_{N\text{-}P}$ (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; $U_{N\text{-}N}$: nitrogen utilization efficiency; $U_{N\text{-}P}$: phosphorus utilization efficiency; $W_{N\text{-}N}$: nitrogen waste; $W_{N\text{-}P}$: 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, $U_{N\text{-}N}$, $W_{N\text{-}N}$ 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

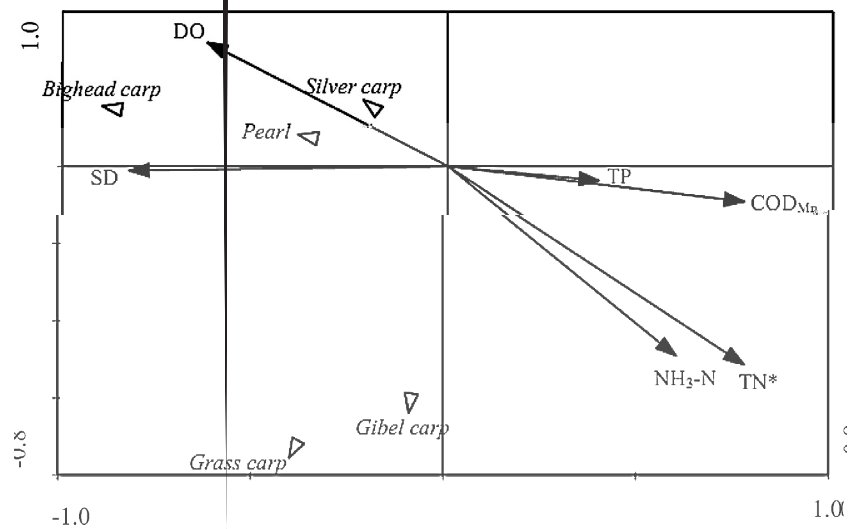
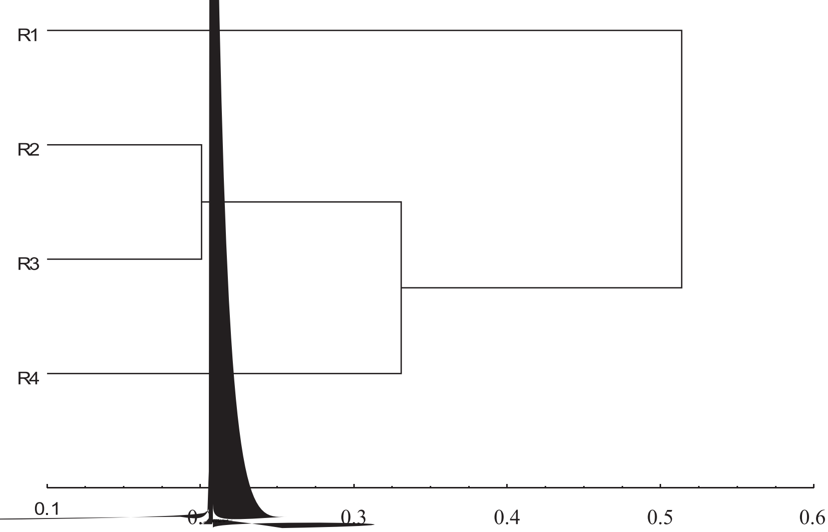



**Fig. 3.** Redundancy analysis (RDA) of pearl, grass carp, gibel carp, silver carp and bighead carp yields and water quality. SD: Secchi depth; DO: dissolved oxygen; $NH_3$-N: ammonia; TN: total nitrogen; TP: total phosphorus; $COD_{Mn}$: chemical oxygen demand. * represents $P < 0.05$.

*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



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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