Aquacultural Engineering 66 (2015) 11–16

Contents lists available at ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

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

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ARTICLE INFO

*Article history:*
Received 11 August 2014
Accepted 22 January 2015
Available online 31 January 2015

*Keywords:*
*Hyriopsis cumingii*
Fish–mussel integration
Stocking ratio
Pearl yield
Fish yield
Nutrient utilization efficiency

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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http://dx.doi.org/10.1016/j.aquaeng.2015.01.002
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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