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# Improvement of fish and pearl yields and nutrient utilization efficiency through fish–mussel integration and feed supplementation

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ABSTRACT

A 153-day experiment was conducted in land-based enclosures to explore the efficacy of fish–mussel integration in pearl yield, fish yield and nutrient utilization. The freshwater mussel *Hyriopsis cumingii* were integrated with either a four-fish species combination (grass carp, gibel carp, silver carp and bighead carp) or a two-fish species combination (silver carp and bighead carp). Fish in each combination received either formulated feed supplementation or no formulated feed. Fish yield, nitrogen utilization efficiency and wastes of nitrogen and phosphorus were higher in the enclosures received formulated feed supplementation than in those received no formulated feed. Production performance (evaluated with pearl weight and soft tissue weight of each mussel, pearl and fish yields, nitrogen utilization efficiency and nitrogen wastes) was better in the enclosures of mussel integrated with four fish species and fed with formulated feed than in those of mussel integrated with two fish species without feeding formulated feed. The total nitrogen, total phosphorus, chemical oxygen demand and calcium in the water column were higher, while the Secchi depth and dissolved oxygen were lower, in the enclosures stocked with four fish species and fed formulated feed than in those stocked with two fish species and without feeding formulated feed. This study indicates that formulated feed supplementation to a fish–mussel integrated system can enhance fish and pearl yields and nitrogen utilization efficiency. The combination of four fish species with complementary feeding habits is more productive than that of two fish species in a fish–mussel integrated system.

*Statement of relevance:* We declare that:

1. This study is original and has not been published elsewhere.
2. All data involved in the manuscript are checked and reliable.
3. All the authors have read and accepted the manuscript as it is submitted.
4. All the authors approve the version to be published.

We declare that nobody who qualifies for authorship has been excluded from the list of authors.
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## 1. Introduction

The rapid expansion of aquaculture industry has caused environmental pollution and disturbance to aquatic ecosystems. Strategies of aquaculture development, including the plan of the site and scale for aquaculture and technologies for aquaculture operation, are essential to sustain aquaculture industry development. The use of a proper aquaculture mode can develop a farming system leading to highly productive, profitable and environment-friendly aquaculture operation (Wang, 2004). The traits contributing to an aquaculture operation mode include economic income (market price of the major species), stocking structure (number of species combined, species ratio and stocking density) and husbandry management (regimes of feeding and fertilization, water exchange, waste management and disease control). It is important to optimize stocking structure and husbandry management for establishing a sustainable aquaculture mode at a commercial scale.

Polyculture and/or integrated culture have been widely used in freshwater and marine aquaculture to improve yield and nutrient utilization efficiency (Milstein, 1992; Neori et al., 2004). In practice, polyculture is farming multiple species in a system, while integrated culture refers to a combination of various types of aquaculture operation (Neori et al., 2004; Troell et al., 2003; Zhong and Power, 1997). Integrated culture is a useful approach to optimize aquaculture modes for sustainable production. For example, integrated multi-trophic

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aquaculture (IMTA) has been widely used to improve nutrient utilization efficiency by recycling nutrients between different trophic levels in aquaculture farming (Neori et al., 2004; Troell et al., 2003). In the past decade, various taxa of aquatic species have been integrated in aquaculture, such as fish and prawn (Asaduzzaman et al., 2009; Uddin et al., 2006), fish and bivalve (MacDonald et al., 2011; Sarà et al., 2009) and shrimp and bivalve (Tendencia, 2007; Yokoyama et al., 2002). However, the relationship between species combination and nutrient supplementation in integrated culture systems has been rarely evaluated.

*Hyriopsis cumingii* is a commercially important freshwater pearl mussel contributing to over 95% pearl production in the world (Wang et al., 2009). In commercial farming, *H. cumingii* is usually co-cultured with planktivorous fishes in earthen ponds, and the ponds are fertilized with poultry manure to develop natural food for mussel and fish (Yan et al., 2009). Water in the pond is exchanged frequently to maintain water quality to satisfy the growth of mussel and fish. The traditional mode for *H. cumingii* farming results in high nutrient loading in ponds and serious eutrophication in the water bodies surrounding the mussel farms (Wang et al., 2006), and should be improved to enhance the sustainability of freshwater pearl industry. Wang et al. (2009) reported that the pearl yield and the growth of *H. cumingii* were enhanced by stocking gibel carp and bighead carp with formulated feed supplementation. We thus hypothesize that the increase of the number of fish species and feed supplementation in a fish–mussel integrated system can further improve pearl yield and growth of *H. cumingii* because fish activities are possibly beneficial to mussel growth through trophic complementation. In addition, the co-cultured fish may be benefited from filtration of the mussel on particle organic matters in water column. In the present study, we examined the effects of fish species combination and formulated feed supplementation on pearl and fish yields and nutrient utilization efficiency in a fish–mussel integrated system. This study aimed to test if the improvement of species combination in the fish–mussel integrated system and feeding the co-cultured fish formulated feed can enhance pearl yield and nutrient utilization efficiency but reduce waste accumulation in the integrated system.

## 2. Materials and methods

### 2.1. Experimental pond, enclosures and animals

A field experiment was conducted at Fengqiao farm (29°47′59.8″N and 120°23′42.4″E) located in Zhuji City (Shaoxing, China) from May 20 to October 20, 2010. The *H. cumingii* were purchased from a pearl mussel farm in Lanxi City (Jinhua, China) in September 2009, and the grass carp *Ctenopharyngodon idellus*, gibel carp *Carassius gibelio*, silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis* were purchased from a fish farm in Deqing County (Huzhou, China) in March 2010. Upon arrival, the mussel were placed in cages (35 cm × 35 cm × 10 cm) that were suspended at 20 cm under water surface in an earthen pond (1.33 ha), and the fish were stocked in net pens (2 m × 3 m × 1.5 m) that were suspended in the same pond. Prior to the experiment, the fish were fed with a formulated feed containing 28% crude protein (Kesheng Feed Co. Ltd., Shaoxing, China). The recipient mussel (shell length >80 mm) were grafted with pieces of the mantle epithelium tissue received from the donor mussel (about 30 mantle pieces were planted into the mantle of each recipient mussel). After the grafted operation, the mussels were resuspended in the pond.

The experiment was conducted in land-based enclosures (1.7 m high, 6.4 m diameter, 31.9 $m^2$ area) that were constructed in the center of the earthen pond. Each enclosure comprised a tube made from a polyethylene (PE) sheet that were placed on the bottom of the pond and buried 20 cm deep. Twenty timber piles were buried into the substrate at 50 cm deep along with the wall (inside and outside) of the PE tube, and two bamboo rings formed a frame inside the wall to hold the PE tube in a cylindrical shape. Each enclosure contained about 32,000 L pond water (1.0 m deep). A polyvinyl chloride (PVC) tube (20 cm diameter) was buried under each enclosure to allow water exchange between the enclosure and the pond. The enclosures used in the experiment are shown in Fig. 1.

### 2.2. Experimental design and procedure

A 2 × 2 factorial layout comprised two combinations of fish species (four fish species versus two fish species) and two regimes of formulated feed supplementation (feeding or no-feeding formulated feed). Four treatments included (enclosures): (1) grass carp, gibel carp, silver carp and bighead carp fed with formulated feed (GISB-F), (2) grass carp, gibel carp, silver carp and bighead carp without feeding formulated feed (GISB-NF), (3) silver carp and bighead carp fed with formulated feed (SB-F), and (4) silver carp and bighead carp without feeding formulated feed (SB-NF). Twelve enclosures were totally used with three replicates for each treatment.

The pond was drained during enclosure construction, and was refilled with river water prior to the field experiment. The PVC tubes under the enclosures were kept open on the bottom to allow slow water exchange between the pond and enclosures. The filling process ceased until the water depth in the enclosures reached 110 cm, and then the PVC tubes were closed to stop water exchange between the pond and enclosures.

At the start of the experiment, *H. cumingii* (63.2 ± 10.9 g), grass carp (26.0 ± 3.7 g), gibel carp (32.7 ± 4.0 g), silver carp (31.8 ± 7.2 g) and bighead carp (46.2 ± 13.2 g) were randomly stocked into the enclosures. The stocking densities of mussel and fishes are shown in Table 1. The mussels were stocked at half of the density (1.2–1.5 mussel $m^{-2}$) used in commercial farming due to lack of water exchange between the pond and the enclosures. The mussels were put in net bags (2 cm mesh) at 2 mussel $bag^{-1}$, and five net bags were hung at 40 cm deep in each enclosure. The grass carp and gibel carp were stocked in net pens (1 m × 1 m × 1.5 m) suspended in the enclosures to ensure that the dropped pellets could be ingested by these

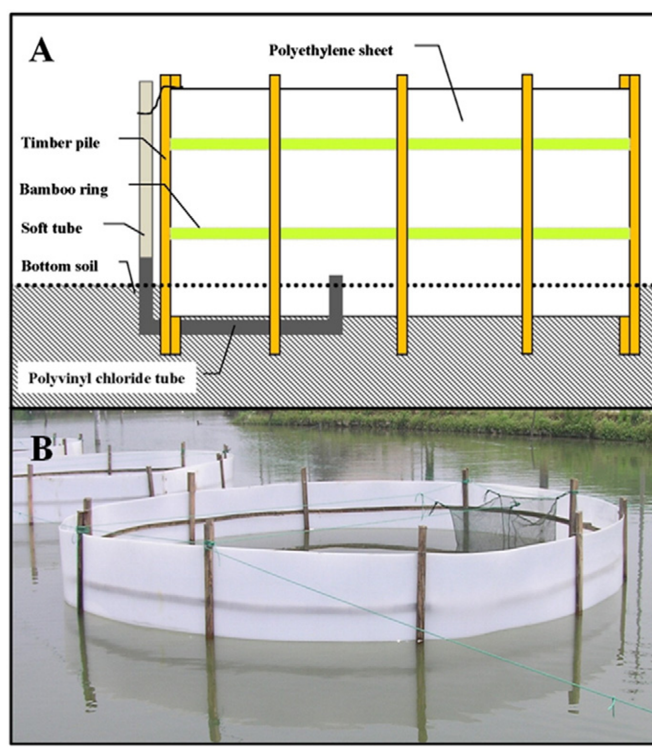


**Fig. 1.** The land-based enclosures used in the experiment. (A) The structure of the enclosure; (B) the enclosures used in the experiment.

**Table 1**
Stocking density and feed supplement in the fish–mussel integrated system.

| Treatment | Stocking density (mussel or fish enclosure$^{-1}$) | | | | | Feed supplement (kg enclosure$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|
| | Mussel | Grass carp | Gibel carp | Silver carp | Bighead carp | Pellet feed | Powder feed | Forage grass |
| GISB-F | 20 | 15 | 5 | 5 | 5 | 6.06 | 8.00 | 0.00 |
| GISB-NF | 20 | 15 | 5 | 5 | 5 | 0.00 | 0.00 | 14.00 |
| SB-F | 20 | 0 | 0 | 5 | 5 | 0.00 | 8.00 | 0.00 |
| SB-NF | 20 | 0 | 0 | 5 | 5 | 0.00 | 0.00 | 0.00 |

GISB-F: grass carp, gibel carp, silver carp and bighead carp fed with formulated feed; GISB-NF: grass carp, gibel carp, silver carp and bighead carp without feeding on formulated feed; SB-F: silver carp and bighead carp fed with formulated feed; SB-NF: silver carp and bighead carp without feeding on formulated feed.

fishes. The silver carp and bighead carp were released into the enclosures. Mussel shell length and body weight, and fish weight were separately measured as described in Wang et al. (2009). Three groups of samples each comprising 10 mussels, 4 grass carp, 4 gibel carp, 4 silver carp and 4 bighead carp were randomly collected, and stored at −20 °C until analysis of nitrogen and phosphorus contents.

The experiment lasted 153 days. Grass carp and gibel carp in the GISB-F enclosures were fed with a commercial pellet feed containing 4.9% nitrogen and 2.9% phosphorus (Kesheng Feed Co. Ltd., Shaoxing, China) at 08:00 and 17:00 h daily, while the silver carp and bighead carp in the GISB-F and SB-F enclosures were fed with a commercial powder feed containing 5.0% nitrogen and 3.1% phosphorus (Kesheng Feed Co. Ltd., Shaoxing, China). No formulated feed was supplied to the GISB-NF and SB-NF enclosures. However, the grass carp in the GISB-NF enclosures were fed with forage grass (contents of nitrogen and phosphorus were 2.3% and 0.2%, respectively) every morning. The feeding rate for grass carp and gibel carp throughout the experiment was 7–8% of initial body weight per day, but was adjusted daily according to the amount of unfed feed on the feeding trays in each net pen. The formulated feed supplements in the fish–mussel integrated system are shown in Table 1. During the experiment, each of the enclosure was fertilized with 3.00 kg duck manure (contents of nitrogen and phosphorus were 2.0% and 4.9%, respectively), 0.63 kg urea (nitrogen content was 46.7%) and 0.16 kg potassium dihydrogen phosphate ($KH_2PO_4$, phosphorus content was 22.8%) to boost the growth of plankton. Complete water exchange did not occur but the pond was occasionally filled or drained to adjust the change of water level due to evaporation or precipitation.

At the end of the experiment, the shell length, whole body weight, pearl number and pearl weight of each mussel were measured as described in Wang et al. (2009). The grass carp and gibel carp were captured from the net pens and weighed in bulk. The silver carp, bighead carp and wild fishes were captured with electrofishing (HlenSig™-FS08-DC12V-8000AV, Haomenshijia Electric Factory, Zhongshan, China), and weighed in bulk. Five mussels, 2 grass carp, 2 gibel carp, 2 silver carp, 2 bighead carp and 2 wild fish were sampled from each enclosure, and stored at −20 °C for analysis of nitrogen and phosphorus contents.

### *2.3. Water quality measurements and chemical analyses*

During the experiment, water temperature and dissolved oxygen in the enclosures were daily measured with a 550A DO meter (YSI Inc., Yellow Springs, Ohio, USA) in the morning and evening, and Secchi depth was measured with a Secchi disk in the morning. Water samples were collected from the enclosures at an interval of two weeks, and concentrations of calcium ($Ca^{2+}$), ammonia, total nitrogen, total phosphorus and chemical oxygen demand ($COD_{Mn}$) were measured with the methods described in APHA (2005). Contents of nitrogen and phosphorus in mussel, fishes, formulated feeds (pellet feed and powder feed), forage grass, duck manure and chemical fertilizers were analyzed with the methods described in AOAC (2005).

### *2.4. Calculation and statistical analysis*

Pearl yield ($Y_P$, g enclosure$^{-1}$), mussel yield ($Y_M$, g enclosure$^{-1}$) and growth rate in shell length ($G_{SL}$, % d$^{-1}$) and whole weight ($G_W$, % d$^{-1}$) of the mussel were calculated as described in Wang et al. (2009). Fish yield ($Y_F$, g enclosure$^{-1}$) was calculated as the yield of each fish species or total yield. Yields of silver carp, bighead carp and wild fishes were estimated as: weight of fish captured from each enclosure / capture rate, where capture rate (%) was estimated as: 100 × number of fish (both silver carp and bighead carp) captured from each enclosure / number of fish stocked in the enclosure. In this study, capture rates of silver carp and bighead carp varied from 36.7 to 93.3% among the enclosures,

**Table 2**
Shell length, whole weight, growth rate, pearl and mussel yield of *Hyriopsis cumingii* in the fish–mussel integrated system (mean ± S.D., $n = 3$).

| Treatment | Shell length (mm) | | Whole weight (g) | | Growth rate (% d$^{-1}$) | | Pearl yield | Mussel yield |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Shell length | Whole weight | (g enclosure$^{-1}$) | (g enclosure$^{-1}$) |
| GISB-F | 87.3 ± 0.4 | 101.2 ± 1.7 | 65.0 ± 6.2 | 102.8 ± 5.8 | 0.106 ± 0.017 | 0.391 ± 0.041 | 15.8 ± 0.9 | 757 ± 19 |
| GISB-NF | 86.3 ± 1.5 | 98.8 ± 3.4 | 60.1 ± 3.9 | 93.9 ± 8.0 | 0.097 ± 0.016 | 0.382 ± 0.152 | 13.9 ± 3.1 | 677 ± 238 |
| SB-F | 87.6 ± 2.0 | 99.9 ± 3.5 | 61.2 ± 5.1 | 103.1 ± 11.2 | 0.093 ± 0.011 | 0.455 ± 0.029 | 14.6 ± 2.5 | 838 ± 122 |
| SB-NF | 87.7 ± 1.8 | 97.2 ± 1.9 | 62.6 ± 6.7 | 90.8 ± 10.9 | 0.072 ± 0.009 | 0.302 ± 0.077 | 10.2 ± 3.4 | 564 ± 152 |

GISB-F: grass carp, gibel carp, silver carp and bighead carp fed with formulated feed; GISB-NF: grass carp, gibel carp, silver carp and bighead carp without feeding on formulated feed; SB-F: silver carp and bighead carp fed with formulated feed; SB-NF: silver carp and bighead carp without feeding on formulated feed.

**Table 3**
Pearl weight, shell weight, soft tissue weight and the weight ratios of *Hyriopsis cumingii* in the fish–mussel integrated system (mean ± S.D., $n = 3$).

| Treatment | Pearl weight (g pearl$^{-1}$) | $W_{sh}$ (g mussel$^{-1}$) | $W_{st}$ (g mussel$^{-1}$) | $W_p/W_{st}$ (%) | $W_p/W_{sh}$ (%) | $W_p/W_m$ (%) | $W_{st}/W_m$ (%) | $W_{sh}/W_m$ (%) |
|---|---|---|---|---|---|---|---|---|
| GISB-F | $0.026 \pm 0.001^{a}$ | 46.3 ± 7.9 | $26.6 \pm 5.5^{a}$ | 3.0 ± 1.3 | 1.7 ± 0.7 | 0.8 ± 0.1 | 26.5 ± 3.9 | 45.8 ± 1.4 |
| GISB-NF | $0.023 \pm 0.005^{ab}$ | 42.1 ± 9.8 | $22.6 \pm 6.2^{bc}$ | 3.1 ± 0.9 | 1.6 ± 0.5 | 0.7 ± 0.1 | 24.0 ± 1.4 | 44.8 ± 0.9 |
| SB-F | $0.023 \pm 0.005^{ab}$ | 45.1 ± 8.8 | $25.4 \pm 5.8^{ab}$ | 2.9 ± 0.9 | 1.6 ± 0.5 | 0.7 ± 0.1 | 24.6 ± 1.4 | 43.7 ± 1.9 |
| SB-NF | $0.015 \pm 0.005^{b}$ | 40.8 ± 9.23 | $20.7 \pm 4.5^{c}$ | 2.4 ± 0.8 | 1.2 ± 0.4 | 0.6 ± 0.1 | 22.8 ± 0.9 | 44.9 ± 0.8 |

GISB-F: grass carp, gibel carp, silver carp and bighead carp fed with formulated feed; GISB-NF: grass carp, gibel carp, silver carp and bighead carp without feeding on formulated feed; SB-F: silver carp and bighead carp fed with formulated feed; SB-NF: silver carp and bighead carp without feeding on formulated feed.
$W_{sh}$: shell weight; $W_{st}$: soft tissue weight; $W_p$: pearl weight; $W_m$: whole weight. The superscripts present the results of Tukey's HSD test, and the values with different superscripts in the same column are significantly different ($P < 0.05$).

**Table 4**
Fish yield (g enclosure$^{-1}$) in the fish–mussel integrated system (mean ± S.D., $n = 3$).

| Treatment | Grass carp | Gibel carp | Silver carp | Bighead carp | Wild fishes | Total fish yield |
|---|---|---|---|---|---|---|
| GISB-F | $1990 \pm 1022^a$ | $225 \pm 95^a$ | $2544 \pm 435^a$ | $1232 \pm 190^a$ | $2691 \pm 952^a$ | $8682 \pm 76^a$ |
| GISB-NF | $371 \pm 161^b$ | $-19 \pm 9^b$ | $1446 \pm 107^b$ | $219 \pm 84^b$ | $573 \pm 255^b$ | $2591 \pm 260^c$ |
| SB-F | | | $2928 \pm 69^a$ | $1055 \pm 367^a$ | $1059 \pm 439^b$ | $5041 \pm 108^b$ |
| SB-NF | | | $1715 \pm 488^b$ | $333 \pm 70^b$ | $463 \pm 195^b$ | $2511 \pm 506^c$ |

GISB-F: grass carp, gibel carp, silver carp and bighead carp fed with formulated feed; GISB-NF: grass carp, gibel carp, silver carp and bighead carp without feeding on formulated feed; SB-F: silver carp and bighead carp fed with formulated feed; SB-NF: silver carp and bighead carp without feeding on formulated feed.
The superscripts present the results of Student's *t*-test or Tukey's HSD test, and the values with different superscripts in the same column are significantly different ($P < 0.05$).

and mean value (58.1%) of capture rates was used to estimate yield of silver carp, bighead carp and wild fishes. Feed conversion ratio ($R_{FCR}$), nutrient utilization efficiency ($U_N$, %), and nutrient wastes ($W_N$, g enclosure$^{-1}$) were calculated as below:

$$R_{FCR} = (I_{pl} + I_{pd})/(W_t - W_0)$$
$$U_N = 100 \times (N_{mt} + N_{ft} - N_{m0} - N_{f0})/(I_{pl} \times C_{Npl} + I_{pd} \times C_{Npd} + I_g \times C_{Ng} + I_d \times C_{Nd} + I_c \times C_{Nc})$$
$$W_N = (I_{pl} \times C_{Npl} + I_{pd} \times C_{Npd} + I_g \times C_{Ng} + I_d \times C_{Nd} + I_c \times C_{Nc}) \times (1 - U_N/100)$$

where, $I_{pl}$ (g) is the amount of pellet feed supplied to each enclosure; $I_{pd}$ (g) is the amount of powder feed supplied to each enclosure; $W_t$ (g) is the total weight of fish harvested; $W_0$ (g) is the total weight of fish stocked; $N_{mt}$ (g) and $N_{ft}$ (g) are the total nutrient contents of the mussel and fishes harvested (total nutrient content was calculated as total weight of the animal harvested × contents of nitrogen or phosphorus of the animal); $N_{m0}$ (g) and $N_{f0}$ (g) are the total nutrient contents of the mussel and fishes stocked; $C_{Npl}$ (%), $C_{Npd}$ (%), $C_{Ng}$ (%), $C_{Nd}$ (%) and $C_{Nc}$ (%) are the contents of nitrogen or phosphorus in the pellet feed, powder feed, forage grass, duck manure and chemical fertilizer; and $I_g$ (g), $I_d$ (g) were and $I_c$ (g) are the amounts of grass, duck manure and chemical fertilizer supplied to the enclosures.

The differences in shell length, body weight and pearl weight of the mussel, $G_{SL}$, $G_W$, $Y_P$, $Y_M$, $Y_F$, $R_{FCR}$, $U_N$, $W_N$ and water quality parameters (Secchi depth, dissolved oxygen, $Ca^{2+}$, ammonia, total nitrogen, total phosphorus and $COD_{Mn}$) between the treatments were tested with two-way ANOVA. Further comparisons between the treatments were performed with Tukey's honestly significant difference (HSD) test. Student's *t*-test was performed to examine the difference in yield of grass carp and gibel carp between the treatments GISB-F and GISB-NF and the difference in $R_{FCR}$ between the treatments GISB-F and SB-F. The significant level was set at $P < 0.05$. Statistical analysis was performed with SPSS 19.0 (IBM® SPSS® Statistics).

## 3. Results

### 3.1. Survival, pearl yield and growth of mussel

The survival of mussel was 100% in all the enclosures. The pearl number of each mussel was 31 ± 1, 32 ± 1, 31 ± 2 and 33 ± 1 (mean ± S.D., $n = 3$) in the GISB-F, GISB-NF, SB-F and SB-NF treatments, respectively. No significant differences were found in the pearl yield, final shell length, final whole weight, and growth rates of shell length and whole weight of mussel between the treatments (ANOVA, $P > 0.05$, Table 2). The pearl yield and growth rates in shell length and whole weight of mussel were slightly high in the GISB-F treatment compared with those in the GISB-NF treatment, while the pearl yield and growth rates in shell length and whole weight were slightly high in the SB-F treatment compared with those in the SB-NF treatment. At the end of the experiment, weight of pearl and soft tissue of mussel were higher in the GISB-F treatment than in the SB-NF treatment (ANOVA, $P < 0.05$, Table 3), while no significant differences were found in the shell weight and weight ratios of various parts of mussel between the treatments (ANOVA, $P > 0.05$).

### 3.2. Survival and yield of fishes

No dead fish was found during the experiment. The yields of grass carp and gibel carp were higher in the GISB-F treatment than in the GISB-NF treatment (Student's *t*-test, $P < 0.05$, Table 4), while the yields of silver carp and bighead carp were higher in the GISB-F and SB-F treatments than in the GISB-NF and SB-NF treatments (HSD test, $P < 0.05$). Total fish yield was higher in the GISB-F and SB-F treatments than in the GISB-NF and SB-NF treatments (HSD test, $P < 0.05$).

### 3.3. Nutrient utilization efficiency and nutrient waste

No significant difference was found in the $R_{FCR}$ between the treatments GISB-F and SB-F (Student's *t*-test, $P > 0.05$, Table 5). The nitrogen utilization efficiency ($U_{N-N}$) was higher in the GISB-F treatment than in the GISB-NF and SB-F treatments (HSD test, $P < 0.05$), while no significant difference was found in phosphorus utilization efficiency ($U_{N-P}$) between the treatments (ANOVA, $P > 0.05$). The nitrogen waste ($W_{N-N}$) and phosphorus waste ($W_{N-P}$) were higher in the GISB-F and SB-F treatments than in the GISB-NF and SB-NF treatments (HSD test, $P < 0.05$).

### 3.4. Water quality

During the experiment, water temperature fluctuated from 20 to 34 °C (mean = 28 °C). In August and September, water temperatures exceeded 30 °C. The Secchi depth and dissolved oxygen were lower, but the $COD_{Mn}$ were higher in the GISB-F treatment than in the GISB-NF and SB-NF treatments (HSD test, $P < 0.05$, Table 6). The concentrations of total nitrogen were higher in the GISB-F treatment than in the GISB-NF treatment

**Table 5**
Feed conversion ratio, nutrient utilization efficiency, nitrogen waste and phosphorus waste in the fish–mussel integrated system (mean ± S.D., $n = 3$).

| Treatment | $R_{FCR}$ | Utilization efficiency (%) | | Waste (g enclosure$^{-1}$) | |
|---|---|---|---|---|---|
| | | Nitrogen | Phosphorus | Nitrogen | Phosphorus |
| GISB-F | $2.39 \pm 0.42$ | $19.1 \pm 0.6^a$ | $19.7 \pm 0.1$ | $844.2 \pm 6.2^a$ | $484.8 \pm 0.3^a$ |
| GISB-NF | | $9.4 \pm 0.8^c$ | $17.3 \pm 2.1$ | $605.9 \pm 5.0^c$ | $178.9 \pm 4.6^c$ |
| SB-F | $2.02 \pm 0.16$ | $14.8 \pm 0.5^b$ | $15.7 \pm 0.5$ | $638.7 \pm 3.9^b$ | $361.7 \pm 2.2^b$ |
| SB-NF | | $16.7 \pm 3.1^{ab}$ | $18.2 \pm 3.6$ | $291.3 \pm 10.7^d$ | $150.3 \pm 6.7^d$ |

GISB-F: grass carp, gibel carp, silver carp and bighead carp fed with formulated feed; GISB-NF: grass carp, gibel carp, silver carp and bighead carp without feeding on formulated feed; SB-F: silver carp and bighead carp fed with formulated feed; SB-NF: silver carp and bighead carp without feeding on formulated feed.
$R_{FCR}$: feed conversion ratio.
The superscripts present the results of Tukey's HSD test, and the values with different superscripts in the same column are significantly different ($P < 0.05$).

**Table 6**
Water quality in the fish–mussel integrated system (mean ± S.D., $n = 3$).

| Treatment | Secchi depth (cm) | Dissolved oxygen (mg $L^{-1}$) | $Ca^{2+}$ (mg $L^{-1}$) | Ammonia (mg $L^{-1}$) | Total nitrogen (mg $L^{-1}$) | Total phosphorus (mg $L^{-1}$) | $COD_{Mn}$ (mg $L^{-1}$) |
|---|---|---|---|---|---|---|---|
| GISB-F | $42 \pm 3^{b}$ | $6.12 \pm 0.33^{b}$ | $23.7 \pm 0.7^{a}$ | $0.36 \pm 0.13$ | $2.49 \pm 0.23^{a}$ | $0.42 \pm 0.13^{a}$ | $14.0 \pm 0.6^{a}$ |
| GISB-NF | $55 \pm 6^{ab}$ | $8.57 \pm 0.55^{a}$ | $22.8 \pm 1.9^{ab}$ | $0.18 \pm 0.11$ | $1.81 \pm 0.32^{b}$ | $0.20 \pm 0.02^{b}$ | $9.2 \pm 0.7^{c}$ |
| SB-F | $49 \pm 4^{ab}$ | $7.33 \pm 0.46^{b}$ | $23.0 \pm 1.1^{ab}$ | $0.24 \pm 0.07$ | $2.39 \pm 0.18^{ab}$ | $0.26 \pm 0.02^{ab}$ | $11.2 \pm 1.0^{b}$ |
| SB-NF | $60 \pm 7^{a}$ | $8.87 \pm 0.53^{a}$ | $19.9 \pm 0.8^{b}$ | $0.15 \pm 0.01$ | $2.14 \pm 0.17^{ab}$ | $0.19 \pm 0.03^{b}$ | $9.1 \pm 0.6^{c}$ |

GISB-F: grass carp, gibel carp, silver carp and bighead carp fed with formulated feed; GISB-NF: grass carp, gibel carp, silver carp and bighead carp without feeding on formulated feed; SB-F: silver carp and bighead carp fed with formulated feed; SB-NF: silver carp and bighead carp without feeding on formulated feed.
$Ca^{2+}$: calcium; $COD_{Mn}$: chemical oxygen demand.
The superscripts present the results of Tukey's HSD test, and the values with different superscripts in the same column are significantly different ($P < 0.05$).

(HSD test, $P < 0.05$), while the concentrations of total phosphorus and $Ca^{2+}$ were higher in the GISB-F treatment than in the SB-NF treatment (HSD test, $P < 0.05$). No significant difference was found in the ammonia concentration between the treatments (ANOVA, $P > 0.05$).

## 4. Discussion

Previous studies have showed that integration between fish and bivalve species in aquaculture can benefit the growth of bivalves (MacDonald et al., 2011; Peharda et al., 2007; Reid et al., 2010; Sarà et al., 2009). Wang et al. (2009) found that adding gibel carp in the fish–mussel integrated system with only one fish species (bighead carp) and supplying formulated feed could enhance pearl yield and growth of *H. cumingii*. In the present study, fish yield was the highest, while pearl yield was slightly high in the enclosures stocked with four fish species (grass carp, gibel carp, silver carp and bighead carp) and supplied with formulated feed. This result supports the hypothesis that integration with more fish species and feed supplementation can enhance both pearl and fish yields in a fish–mussel integrated system. Production performance (pearl and fish yields, nitrogen utilization efficiency and nitrogen wastes) was better in the enclosures received formulated feed supplementation than those without feeding formulated feed regardless fish composition. This result indicates that natural food production is not sufficient to achieve the fish and mussel growth potential and there is a need to supply formulated feed to enhance production efficiency in the fish–mussel integrated system. The conclusion is supported by the fact that the yield of wild fishes was higher in the enclosures fed with formulated feed than in the enclosures without feeding formulated feed, and gibel carp exhibited negative yield in the enclosures stocked with four fish species without feeding formulated feed. Therefore, both the number of fish species and nutrient supplementation should be considered to optimize the production efficiency in a fish–mussel integrated system.

Most bivalves are filter feeders and play an important role as environmental cleaners (Jones et al., 2001; Stadmark and Conley, 2011) or disease controllers (Molloy et al., 2011; Tendencia, 2007) in fish or shrimp farming. Bivalves can feed on organic particles of various sizes such as phytoplankton, bacteria and detritus (Borrero and Hilbish, 1988; Miranda et al., 2010; Wang et al., 2009). Nitrogen wastes including uneaten feed and feces in fish farming can be utilized by bivalves as food (Gao et al., 2006; MacDonald et al., 2011; Reid et al., 2010; Sarà et al., 2009). In fish polyculture ponds, herbivorous grass carp and omnivorous gibel carp are usually fed with formulated feed. When these two species are co-cultured with mussel in the same system and fed with formulated feed, the wastes derived from formulated feed can be partially consumed by mussel. In the present study, therefore, the enhanced pearl yield in the enclosures supplied with formulated feed is attributable to the waste produced from fish farming.

Mussels need calcium from the environment for bio-mineralization in shell or pearl. In commercial farming ponds, calcium concentration is limited when the stocking density of mussel is high, and the frequent addition of calcium rich water or lime (calcium oxide) is necessary to supply adequate calcium as pearl formation and mussel growth are limited by calcium deficiency (Wang et al., 2009). In the present study, calcium concentrations were slightly high in the enclosures received formulated feed than in those without feed supplementation. This result suggests that feed supplementation to a fish–mussel integrated system can also provide calcium for mussel growth due to formulated fish feed generally contains 1.5% calcium dihydrogen phosphate or calcium hydrogen phosphate (Liu et al., 2011). Therefore, the frequency of lime or water exchange can be reduced in the fish–mussel integrated system when formulated feed is supplied.

To our best knowledge, the present study is perhaps the first of such study to evaluate nutrient utilization efficiency and waste production in pearl mussel farming. The nutrient utilization efficiency was 9.4–19.1% for nitrogen and 15.7–19.7% for phosphorus regardless of fish species combinations and feed supplementation, while nitrogen wastes were 43,535 ± 11,250 g N (kg pearl gain)$^{-1}$ or 145 ± 58 g N (kg fish gain)$^{-1}$ and phosphorus wastes were 21,288 ± 8022 g P (kg pearl gain)$^{-1}$ or 65 ± 10 g P (kg of fish gain)$^{-1}$. This result reveals that nitrogen wastes for 1 kg pearl gain are nearly 300 times higher than that [52–88 g N (kg of fish gain)$^{-1}$] for 1 kg fish gain in net pen culture of marine fishes (Wang et al., 2007, 2008). Nitrogen utilization efficiency was higher in the enclosures stocked with four fish species than in those with two fish species when formulated feed was supplemented. Meanwhile, nitrogen utilization efficiency was higher in the enclosures stocked with four species of fish and fed with formulated feed than in those stocked with the same number of fish species but fed with forage grass. These results indicate that stocking with more fish species with complementary feeding habits and provision with feed supplementation can enhance nitrogen utilization efficiency in the fish–mussel integrated system. The amount of nitrogen and phosphorus wastes was greater in the enclosures fed with formulated feed than in those without feeding, suggesting that mussel cannot absorb all the wastes from formulated feed. Therefore, there is still a room to improve nutrient balance between mussel uptake and waste production through fish feeding by further optimization of the ratio of fish to mussel in the fish–mussel integrated system.

The growth rates of shell size and soft tissue can be allometric in bivalves. For instance, the shell growth of mussel *Mytilus edulis* can exceed the growth of soft tissue (Borrero and Hilbish, 1988; Hilbish, 1986). Stirling and Okumus (1994) reported that the shell morphology of mussel *M. edulis* changes under different environmental conditions. In the present study, pearl weight, soft tissue weight and the ratio of pearl weight to shell weight were slightly high in the mussel hung in the enclosures stocked with grass carp, gibel carp, silver carp and bighead carp and fed with formulated feed. This result indicates that fish species combination and nutrient supplementation can affect the shell morphology (the ratio of soft tissue weight to shell weight) of mussel.

In conclusion, pearl and fish yields and nutrient utilization efficiency in the fish–mussel integrated system depend on both the number of fish species and feed supplementation. Production performance (yields, nitrogen utilization efficiency and nitrogen wastes) is benefited by integrating with four fish species (grass carp, gibel carp, silver carp and bighead carp) with complementary feeding habits and provision of formulated feed.

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## References

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

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. 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.

Borrero, F.J., Hilbish, T.J., 1988. Temporal variation in shell and soft tissue growth of the mussel *Geukensia demissa*. Mar. Ecol. Prog. Ser. 42, 9–15.

Gao, Q.F., Shin, P.K.S., Lin, G.H., Chen, S.P., Cheung, S.G., 2006. Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. Mar. Ecol. Prog. Ser. 317, 273–283.

Hilbish, T.J., 1986. Growth trajectories of shell and soft tissue in bivalves: seasonal variation in *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 96, 103–113.

Jones, A.B., Dennison, W.C., Preston, N.P., 2001. Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study. Aquaculture 193, 155–178.

Liu, X.Y., Wang, Y., Ji, W.X., 2011. Growth, feed utilization and body composition of the Asian catfish (*Pangasius hypophthalmus*) fed at different dietary protein and lipid levels. Aquacult. Nutr. 17, 578–584.

MacDonald, B.A., Robinson, S.M.C., Barrington, K.A., 2011. Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multi-trophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. Aquaculture 314, 244–251.

Milstein, A., 1992. Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231, 177–186.

Miranda, A., Lizarraga-Armenta, J., Rivas-Vega, M., Lopez-Elias, J.A., Nieves-Soto, M., 2010. Pacific oyster, *Crassostrea gigas*, cultured with tilapia, *Oreochromis mossambicus* × *Oreochromis niloticus* in a recirculation system. J. World Aquacult. Soc. 41, 764–772.

Molloy, S.D., Pietrak, M.R., Bouchard, D.A., Bricknell, I., 2011. Ingestion of *Lepeophtheirus salmonis* by the blue mussel *Mytilus edulis*. Aquaculture 311, 61–64.

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.

Peharda, M., Župan, I., Bavčević, L., Frankić, A., Klanjšček, T., 2007. Growth and condition index of mussel *Mytilus galloprovincialis* in experimental integrated aquaculture. Aquac. Res. 38, 1714–1720.

Reid, G.K., Liutkus, M., Bennett, A., Robinson, S.M.C., MacDonald, B., Page, F., 2010. Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon (*Salmo salar*) feed and fecal particulates: implications for integrated multi-trophic aquaculture. Aquaculture 299, 165–169.

Sarà, G., Zenone, A., Tomasello, A., 2009. Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of integrated multi-trophic aquaculture within the Tyrrhenian Sea. Hydrobiologia 636, 129–136.

Stadmark, J., Conley, D.J., 2011. Mussel farming as a nutrient reduction measure in the Baltic Sea: consideration of nutrient biogeochemical cycles. Mar. Pollut. Bull. 62, 1385–1388.

Stirling, H.P., Okumus, I., 1994. Growth, mortality and shell morphology of cultivated mussel (*Mytilus edulis*) stocks cross-planted between two Scottish sea lochs. Mar. Biol. 119, 115–123.

Tendencia, E.A., 2007. Polyculture of green mussels, brown mussels and oysters with shrimp control luminous bacterial disease in a simulated culture system. Aquaculture 272, 188–191.

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.

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

Wang, X.D., Wang, W.L., Dong, X.Q., Zhu, S.B., Wang, Y., 2006. Effect of different models of stocking and management on growth and yield of freshwater pearl mussel *Hyriopsis cumingii*. J. Shanghai Fish. Univ. 15, 315–320 (in Chinese with English abstract).

Wang, Y., Kong, L.J., Li, K., Bureau, D.P., 2007. Effects of feeding frequency and ration level on growth, feed utilization and nitrogen waste output of cuneate drum (*Nibea miichthioides*) reared in net pens. Aquaculture 271, 350–356.

Wang, Y., Li, K., Han, H., Zheng, Z.X., Bureau, D.P., 2008. Potential of using a blend of rendered animal protein ingredients to replace fish meal in practical diets for malabar grouper (*Epinephelus malabricus*). Aquaculture 281, 113–117.

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.

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.

Yokoyama, H., Higano, J., Adachi, K., Ishihi, Y., Yamada, Y., Pichitkul, P., 2002. Evaluation of shrimp polyculture system in Thailand based on stable carbon and nitrogen isotope ratios. Fish. Sci. 68, 745–750.

Zhong, Y., Power, G., 1997. Fisheries in China: progress, problems, and prospects. Can. J. Fish. Aquat. Sci. 54, 224–238.