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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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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 *H riopsis 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 c5.8(i)t0We declare that no fied for authorship has been excluded from the list of aut

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 estab

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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.

H riopsis 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 *Ctenophar ngodon idellus*, gibel carp *Carassius gibelio*, silver carp *H pophthalmichth s molitrix* and bighead carp *Aristichth s 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

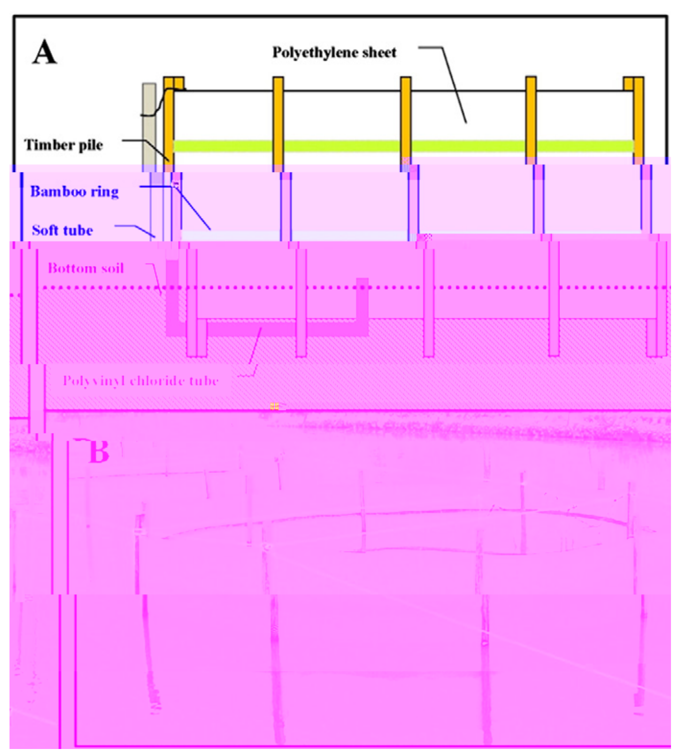

Fig. 1. The land-based enclosures used in the experiment. (A) The structure of the enclosure; (B) the enclosures used in the experiment.

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 ± 0.001^{a}	46.3 ± 7.9	26.6 ± 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 ± 0.005^{ab}	42.1 ± 9.8	22.6 ± 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 ± 0.005^{ab}	45.1 ± 8.8	25.4 ± 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 ± 0.005^{b}	40.8 ± 9.23	20.7 ± 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 ($P < 0.05$).

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:

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

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 ± 3^{b}	6.12 ± 0.33^{b}	23.7 ± 0.7^{a}	0.36 ± 0.13	2.49 ± 0.23^{a}	0.42 ± 0.13^{a}	14.0 ± 0.6^{a}
GISB-NF	55 ± 6^{ab}	8.57 ± 0.55^{a}	22.8 ± 1.9^{ab}	0.18 ± 0.11	1.81 ± 0.32^{b}	0.20 ± 0.02^{b}	9.2 ± 0.7^{c}
SB-F	49 ± 4^{ab}	7.33 ± 0.46^{b}	23.0 ± 1.1^{ab}	0.24 ± 0.07	2.39 ± 0.18^{ab}	0.26 ± 0.02^{ab}	11.2 ± 1.0^{b}
SB-NF	60 ± 7^{a}	8.87 ± 0.53^{a}	19.9 ± 0.8^{b}	0.15 ± 0.01	2.14 ± 0.17^{ab}	0.19 ± 0.03^{b}	9.1 ± 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 *M. tilus 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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