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# Uptake of farming wastes by silver carp *Hypophthalmichthys molitrix* in polyculture ponds of grass carp *Ctenopharyngodon idella*: Evidence from C and N stable isotopic analysis

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ABSTRACT

Understanding and reducing the environmental impacts of organic waste derived from fish farming activities are essential for improving the sustainable development of aquaculture and optimizing intensive farming techniques. To evaluate the feasibility and capability of using filter-feeding silver carp *Hypophthalmichthys molitrix* as biofilters to remove the farming wastes, including feed residues and fish feces, derived from the aquaculture of grass carp *Ctenopharyngodon idella*, five mesocosmic enclosures (A, B, C, D, E) combining grass carp (GC) and silver carp (SC) with different proportions were developed as experimental enclosures. For controls, silver carp from the same population were simultaneously transplanted to reference enclosures (F) where the co-culture of grass carp was absent. After 5 month acclimation, samples of SC tissue, particulate organic matter (POM), fish feed and fish feces (GC and SC feces) were collected for measurements of carbon and nitrogen isotopic ratios. Enrichment of $^{13}C$ and $^{15}N$ in the tissues of SC from the experimental enclosures compared to those from the controls indicated the uptake and assimilation of isotopically heavier farming wastes, i.e., fish feed and fish feces. Based on the isotope mixing model, the proportions of SC biomass assimilated from POM, fish feed, GC feces and SC feces to SC dietary consumption were 62.0, 24.4, 10.7, and 2.9% in A, 61.5, 26.9, 9.0, and 2.6% in B, 65.3, 24.3, 6.2, and 4.2% in C, 70.9, 20.6, 4.8, and 3.7% in D, and 72.1, 20.6, 3.5, and 3.9% in E, respectively.



## 1. Introduction

Grass carp (GC) *Ctenopharyngodon idella* is the largest freshwater fish species for commercial aquaculture in China in terms of production and the farming scale has been increasingly expanded in the last decades. At present, the production of this species has reached 4,222,000 tons or 18.0% of the total aquaculture production of all freshwater fish species in China (MOAC, 2011). For maximizing the commercial profit, the stocking density has been continuously grown as the result of the increasing use of artificial formulated feed (Lin and Song, 2010). Along with the development of farming intensity in terms of scale, production and density, worldwide concerns are evoked about the negative impacts that farming activities imposed on the environment and the subsequent threat to sustainable development of aquaculture in both fresh- and sea waters (Fan et al., 2011; Wu et al., 1994).

Although modern fish feed becomes more closely aligned with the dietary requirements of fish culture, fish farming activities have resulted in marked pollution to the farming and adjacent waters owing to the substantial and inevitable release of organic and inorganic wastes from uneaten feed, feces and dissolved excretory products. Zhou and Wen (2004), for example, estimated that approximately 20–27% N and 8–24% P in feed were retained by fish, while 54–77% N and 72–89% P in feed were released into the water column which could become solid organic wastes in the form of uneaten pellets and feces. The build-up of solid wastes within the aquaculture system should be prevented as it can cause oxygen depletion and ammonia toxicity when the wastes decompose with a series of negative ecological impacts. Wastes without treatment, if continuously discharged into the aquatic environment, could also result in a significant elevation of the total organic matter contents and cause considerable economic loss (Cao et al., 2007).

Sliver carp (SC) *Hypophthalmichthys molitrix* have specialized fused gill rakers with an average pore size of 20–25 μm (Opuszynski, 1981), and are capable of consuming feed particles as small as 8 μm. Food consumption by silver carp is strongly dependent on food availability in the environment (Chen, 1990; Fukushima et al., 1999; Spataru and Gophen, 1985). Hence, filter-feeding silver carp take up particulate matter with considerable efficiency owing to such nature of their high filtration rate in aquaculture waters (Burke et al., 1986; Cremer and Smitherman, 1980; Dong and Li, 1994). Leventer and Teltsch (1990), for example, reported that significant reduction in suspended organic

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matter was observed when silver carp were introduced into the Netofa reservoirs in Israel at an initial density of 300–4500 fish individuals per hectare. Liu and Xie (2002) revealed that silver carp can play a decisive role in controlling the occurrence of algal blooms caused by eutrophication, as long as the stocking biomass of silver carp remains at or exceeds 50 g per cubic meter of lake water. Thus, integrated polyculture of silver carp with other species such as grass carp can potentially remove the farming wastes in the form of feed residues and fecal pellets, and function as a self-regulator for cycling of particulate matter.

Utilization efficiency of excessive nutrients in the suspended farming wastes by silver carp in the polyculture systems, however, has not been documented so far. Ingestion and absorption of various food sources by aquatic organisms have traditionally been examined by means of direct gut content analysis (Xie, 1999). However, trophic models based on dietary observations can only represent an instant snapshot of food ingested by the consumers. The application of stable isotopes may overcome such limitations. Carbon and nitrogen stable isotopes change in a predictable way when transferred along trophic pathways (Peterson and Fry, 1987). Moreover, the stable isotope approach offers distinct advantages over conventional dietary techniques relying on the morphological identification because (1) evaluation of food sources is based on assimilated nutrient instead of ingested food, and (2) assimilated matter represents time-integrated utilization of food (Hobson and Welch, 1992). Stable isotope analysis may be particularly useful in revealing the food sources of aquatic animals because the recognition of gut content is difficult, even impossible sometimes, due to the small size of, and digestive damage to, the food particles. There has been increasing application of stable isotopes as tracers to follow the flux of organic matter or pollutants along food chains or food webs in both terrestrial and aquatic environments (e.g., Gao et al., 2006, 2011; Sarà et al., 2007; Sun et al., 2012; Vizzini et al., 2002).

In the present study, we set up polyculture models combining 2 freshwater fish species with different feeding habits, i.e., the predating grass carp (GC) and filter-feeding silver carp (SC). The samples of silver carp tissue and its potential food sources, including particulate organic matter (POM), fish feed and feces egested by GC and SC, were collected. The carbon and nitrogen stable isotopic ratios of the samples were measured and the contributions of the various food sources to the silver carp were evaluated using the stable isotope mixing model. The objectives of this study were to identify the potential food sources of silver carp reared in the polyculture system of grass carp, with the emphasis on quantifying the removal efficiency of farming wastes by the silver carp. Based on this quantification, the feasibility and capability of silver carp as biofilters to ameliorate the environmental impacts derived from grass carp farming were evaluated.

## 2. Materials and methods

### 2.1. Experiment and sampling scheme

The experimental pond for fish culture was located in Jinan City, Shandong Province, China (36°69′N, 116°86′E). The freshwater used in the pond was pumped from a nearby well. The area of the pond was approximately 5 ha with water depth ranging from 1.6 to 1.7 m. Enclosures of same size (L × W × D = 8 × 8 × 2.5 m), which divided the culture pond into different parts, were constructed within the pond. Waterproof enclosures were made of polyvinyl plastics and supported with timber piles around the boundaries of the enclosures. The separation of the pond area by enclosures avoided water exchange among different experimental treatments. In addition, the top of the enclosures was covered with 1 cm mesh net to prevent fish from jumping out of the enclosures.

A total of 18 replicates, including 15 experimental and 3 control enclosures, were used in the present study. Juvenile grass and silver carp with body weight of ~100 g were released to the enclosures in April 2009. In the experimental enclosures, grass carp and silver carp were cultured with a series of inter-species ratios in terms of fish individuals, i.e., 8:2, 7:3, 6:4, 5:5 and 4:6 (referred as enclosures A, B, C, D and E, respectively). In the 3-replicate control enclosures (enclosure F), only silver carp were cultured. The fish stocking densities in all enclosures were 1 individual per square meter. The total biomass of the released fish did not differ significantly among the enclosures (ANOVA, $p > 0.05$). Artificial feed were supplied to the grass carp cultured in the experimental enclosures with the ration of 5% of total grass carp biomass every day. The ingredients of the feed consisted of 28% protein, 6% crude fat, 33% carbohydrate and other minor constituents such as ash, minerals and vitamins. No feed was supplied to the control enclosures. In September 2009, the fish were harvested and the difference between the final and initial average body weight for each enclosure was calculated to evaluate the fish growth. Samples of silver carp and their potential food sources including POM, fish feed and feces egested by GC and SC were collected for stable isotope analysis (SIA). The 5-month culture period from April to September was assumed to be long enough for silver carp to acclimatize to the food conditions and complete turnover of their muscle reserve so as to meet the requirement of isotopic equilibrium for SIA (Bosley et al., 2002; Buchheister and Latour, 2010; Xia et al., 2013).

After fish collection, the muscle of SC was dissected and dried at 60 °C for over 72 h to constant dry weight. Muscle tissue of 3 SC individuals from the same enclosure was pooled, homogenized and sieved through a 0.5 mm mesh size screen, and was used as one replicate. Silver carp collected from the enclosures with same inter-species ratio were used as 3 replicates. The tissue powder was tightly sealed in glass Petri dish and stored in an ultralow temperature freezer (−80 °C) for future analyses.

Suspended particulate matter (SPM) was collected via filtration of water from each enclosure using glass fiber filters (Whatman GF/F) which were pre-combusted at 450 °C for 6 h to remove any possible contamination of organic matter (Gao et al., 2006). The vacuum filtration was conducted under the suction of lower than one-third atmospheric pressure to avoid the damage of phytoplankton cells. Prior to SPM collection, feed supply to the enclosures was intentionally ceased for 3 days allowing for the complete settlement of the uneaten artificial feed fragments so as to minimize or avoid the contamination of fish feed to the natural SPM. A direct check under microscopic observation for SPM immediately after collection did not find obvious feed fragments. Major constituents in the particulate matter included phytoplankton and detrital particles. Particulate organic matter (POM) was obtained by means of HCl-treatment of SPM following Yokoyama et al. (2002). The SPM samples were treated with 1.2 N isotonic HCl to remove carbonates until no more $CO_2$ bubbles were produced and the decarbonated POM was dried and stored at −80 °C freezer for future SIA.

To collect fish feces, the grass carp and silver carp were individually contained in a tank and cultured with flowing water pumped from the enclosures where the fish were collected. The egested feces which settled on the bottom of the culture tank were collected carefully with a pipette by means of siphon. After collection, the fish feces were HCl-treated for decarbonation and dried following the method used for SPM. Since the isotopic signature of the fish residue produced in the culture ponds was same as that of the original fish feed, fish feed was directly used as feed residue for stable isotope analysis.

### 2.2. Measurements of environmental conditions

At the end of the experiment, 100 ml freshwater samples were collected from the enclosures. The collected water samples were filtered through pre-combusted and weighed 47 mm filters (Whatman GF/F) and rinsed with distilled water. The filters were then dried in an oven at 80 °C for 24 h, weighed to the nearest 0.1 mg, ashed in a muffle furnace at 450 °C for 6 h to combust the organic matter and reweighed to determine the total particulate matter (TPM: mg $l^{-1}$) and particulate