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The effects of two free-floating plants (*Eichhornia crassipes* and *Pistia stratiotes*) on the burrow morphology and water quality characteristics of pond loach (*Misgurnus anguillicaudatus*) habitat

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ABSTRACT

Loach exhibit conspicuous drilling behaviors in the mud of shallow waters, yet their burrow morphology and the factors affecting this behavior have received little attention. We characterized the burrow morphology and water quality of the pond loach *Misgurnus anguillicaudatus* in three scenarios: in tanks without plants, tanks with the free-floating plant water hyacinth *Eichhornia crassipes*, and tanks with water lettuce *Pistia stratiotes*. Water hyacinth effectively removed water TN, COD, NO_3-N and NH_4-N, and water lettuce removed water TP and NH_4-N. Water hyacinth and water lettuce markedly reduced water turbidity and DO, increased TOC and EC. Water hyacinth purified water more effectively than water lettuce, providing a suitable habitat for loach feeding, living and burrowing. The burrow structures were V-shaped, Y-shaped, inverted L-shaped, or complicated dendritic networks composed of multiple V shapes. The hyacinth treatment was characterized by the greatest burrow volume, length, depth, and structural complexity, but the opening size was reduced by dense root mat coverage. Burrows in the water lettuce treatment were characterized by intermediate volume, length, branches and sinuosity, but they had the largest opening and pit size. The control treatment had a flat bottom with the smallest, shortest burrows. This study indicates that free-floating plants improve habitat suitability and change burrow morphology and may be used to improve loach breeding methods.

1. Introduction

The pond loach *Misgurnus anguillicaudatus*, well known for its nutritional value, tender meat, and palatability, is considered “ginseng of the water” in China (Lei & Wang, 1990; Wang et al., 2010). There has been growing interest in culturing and breeding loach in China because it is an excellent freshwater aquaculture species. In recent years, the market demand and price of loach have increased steadily, and as has the need to improve culturing techniques (Hu, Chu, & Wang, 2012; Wang, Hu, Wang, & Cao, 2009).

Many loach species possess intensive mud-drilling habits and burrowing activities which provide protection (Natuhara, 2013). Loach mainly inhabit shallow water with a depth less than 1 m. These species are benthic fishes in ponds and exhibit high tolerance to harsh environmental conditions, such as elevated ammonia and nitrate, hypoxia, and dehydration and a broad range of temperatures, from 0 to 38 °C (Keller & Lake, 2007; Koetsier & Urquhart, 2012; Silva, Coimbra, Steffensen, & Wilson, 2008). Their adaption to such conditions is attributed to the buffering function of their burrows in the mud.

Burrows provide ideal locations for fish mating, spawning, egg incubation, and escape from predators (Atkinson & Taylor, 1991). Resin casting is currently the most common method used to study burrow morphology (Dinh, Qin, Dittmann, & Tran, 2014; Frey, Basan, & Scott, 1973; Wang, Bertness, Li, Chen, & Lü, 2015) and was used in the present study. Burrow casting can reveal fish behavior patterns, adaptation characteristics and properties of the bottom sediment (Lee & Koh, 1994). Burrow casting is also an

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important approach to understanding the physiological demands experienced by loach (Seike & Nara, 2007; Wang et al., 2015). Studies on loach burrow morphology are very important to clarify their behavioral ecology and to increase our understanding of loach growth, feeding, and reproduction. Burrow morphology also reflects the utilization and preference of loach with respect to bottom sediment. Several studies have examined burrows of goby and mudskipper in mudflats (Dinh et al., 2014), but little is known about loach burrows and the factors influencing their morphology.

Free-floating plants are important primary producers and structural elements in freshwater that greatly increase habitat complexity and heterogeneity, elevate fish biodiversity, and improve habitat conditions that maintain ecosystem health (Bond & Lake, 2003; Kadye, Magadza, Moyo, & Kativu, 2008; Marchetti & Moyle, 2001). Free-floating plants are frequently cultivated on the surface of culture water to purify the water, serve as livestock feed, and enhance fish survival and growth rates, thereby enhancing multiple aspects of water utilization efficiency.

The water hyacinth *Eichhornia crassipes* and the water lettuce *Pistia stratiotes* are widely distributed species in tropical regions and are used extensively in the shallow water of loach culture ponds (Akinbile & Yusoff, 2012; Mbati & Neuenschwander, 2005). These two floating plant species attract fish into plant clusters on the water surface and thus facilitate the harvest of marketable loach (Huang & Huang, 2011). They also have well-developed roots that directly affect pond sediment (Padial, Thomaz, & Agostinho, 2009; deNeiff & Carignan, 1997). The flourishing above-ground structure of floating macrophytes, e.g., stem and leaves, forms a microhabitat of crown closure on the water surface. The well-developed roots directly contact pond sediment, with potentially strong effects on the sediment properties. These impacts likely change the burrowing behavior and inhabiting characteristics of loach, changes that may affect other aspects of loach culture. However, the interactive effects of floating plants and loach burrowing remain largely unexplored, in part due to the difficulty of casting loach burrows in the soft mud of shallow ponds. By analyzing the effects of the two floating plants on loach burrows, we explore the role of plants in loach reproduction and growth and further optimize the technologies for high-efficiency integrated aquaculture with approaches that combine planting and breeding.

Our study aimed to determine the primary morphological characteristics of loach burrows and analyze the differences in root distribution, stem and leaf status, and burrow morphology among the three treatments. These analyses illustrate the effects of planting the floating macrophytes *E. crassipes* and *P. stratiotes* on the morphology of loach burrows. This study may provide useful data and new insights for the high-efficiency culture of loaches and application of aquatic plants.

2. Materials and methods

2.1. Study methods

This study was conducted in a greenhouse in the Zhuanghang Experimental Station of Shanghai Academy of Agricultural Sciences, Zhuanghang Town, Fengxian District, Shanghai, China (30°53′25″N, 121°23′20″E), from July to August 2013. Plastic rectangular tanks (size: 57 cm L × 37 cm W × 35 cm H) were used as experimental units. Dried and crushed soil was placed into tanks at 20 cm depth. This soil was soaked for one week to give the soil homogeneous texture and moderate viscosity for facilitating loach burrowing and casting, after which the culture water was added to a 20 cm depth for stocking loach.

Floating plants were introduced into tanks to establish three treatments: *E. crassipes*, *P. stratiotes*, and a control. Each treatment was replicated four times. Ten *E. crassipes* individuals, with a total weight of 372.9 g, and seven *P. stratiotes* individuals, with a total weight of 359.5 g, were placed in each tank at approximately natural densities. Fresh plants were weighed and the root length, height and density were recorded. Water hyacinth (family Pontederiaceae, genus *Eichhornia*) has smooth and oval-shaped leaf blades with a 4–12 cm width. The perennial herb water lettuce (family Araceae, genus *Pistia*) has longer roots and greater plant weight and leaf number, but lower plant height above the water than water hyacinth (Table 1, Fig. 1). Water hyacinth has more interstitial space and therefore allows greater light infiltration than water lettuce.

The loach used in this experiment was obtained from a local aquaculture market. The fish were sterilized with 5% NaCl solution for 10 min and used to stock the pool. The weights and lengths of loach were recorded. Three males and three females were placed into each tank. The mean wet weights for the male and female fish were 11.8 ± 0.9 g and 19.5 ± 1.5 g, respectively. Their mean body lengths were 128.5 ± 3.0 mm and 155.5 ± 5.38 mm, respectively. The weights and body lengths have no significant difference among the three treatments ($P > 0.05$). During the experimental period (July to August), the water temperature was relatively high in all treatment in the greenhouse (31.9–33.2 °C). The atmospheric pressure was lower in the experimental environment (984.1–996.5 kpa). Loach exhibited active burrowing behavior in tanks.

Water was sampled from the tanks at 09:00 at 10 day intervals and monitored *in situ* for pH, temperature, electrical conductivity (EC), dissolved oxygen (DO), and turbidity using a portable multi-parameter water analyzer (HI9828, Hanna, Italy). Water total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_4-N), and nitrate nitrogen (NO_3-N) were measured using the alkaline potassium persiflage digestion-UV spectrophotometric method (GB 11894-89), the alkali fusion Mo-Sb anti-spectrophotometric method, Nessler's reagent colorimetric method, and the phenol disulfonic acid spectrophotometric method, respectively. COD_{Mn} was determined according to permanganate index methods. Total

Table 1
Plant characteristics of water hyacinth and water lettuce.

Plant Species	N	Weight (g)	Stem height (cm)	Leaf number	Root length (cm)
E. crassipe	10	37.3 ± 4.5	11.6 ± 0.3	8.5 ± 0.4	18.9 ± 1.2
P. stratiotes	7	51.4 ± 4.6	9.7 ± 0.8	12.1 ± 0.7	22.2 ± 0.9
T test:*t*(p)		−2.13(<0.05)	2.51(<0.05)	−4.81(<0.001)	−2.14(<0.05)

Fig. 1. The appearance of water hyacinth *Eichhornia crassipes* (A), water lettuce *Pistia stratiotes* (B).

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