

Experimental Study of Bio-fouling Control of *Limnoperna Fortunei* in Water Transfer Tunnels

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ABSTRACT: The golden mussel (*Limnoperna fortunei*) is a filter-collector macroinvertebrate species originating from southern China. It easily invades water transfer tunnels and attaches onto tunnel walls and structures with extremely high density, resulting in bio-fouling, pipe clogging, structure corrosion, a decrease in water transfer efficiency, and water pollution. It has become a prevalent problem and has caused concern all over the world. However, an effective and environment-friendly method of controlling golden mussel invasions has not been found yet. This study is aimed to find measures that would prevent golden mussel invasion and bio-fouling in the water transfer tunnels of the East River Water Source Project (ERWSP), which transports water from the East River to Shenzhen, southern China for 10 million people's production and daily life. Long-term samplings and observations of East River water were performed to study the golden mussel's invading pattern. Flume experiments were done to study the golden mussel's attachment onto 14 different materials and performance in turbulent flows. Measures of preventing golden mussel invasion in tunnels were proposed based on the experimental results. Furthermore, an integrated ecological prevention pool was designed and constructed for experimental study of golden mussel invasion prevention in scale model tunnels of the ERWSP. Results of long-term observations indicated that the golden mussel underwent the planktonic veliger stage in water. The planktonic veligers invaded into tunnels with water flow, and attached and developed into mussels on the tunnel walls. Attachment experiment results showed that after golden mussels attach to the tunnel wall, they successively experienced an unstable veliger-attachment stage and then a stable mussel-attachment stage with their byssuses. Golden mussels preferred geotextile cloth during the unstable attachment stage, but preferred bamboo material during the stable attachment stage. When the golden mussels attached stably on materials, the attachment force positively correlated to the mussel length. The average flow velocity that caused golden mussels to dislodge from materials showed a linear relationship with the product of the number and average diameter of the byssuses used for attaching. Flume experiment results indicated that the veligers were killed in high-frequency turbulence when the eddy scale was comparable with the veliger size. The killing rate of veligers positively correlated with the average flow velocity and action time of the turbulence. The integrated pool was designed to prevent golden mussel veligers from entering into tunnels by attracting veligers to attach onto geotextile cloth, attracting mussels to attach on bamboo, and killing veligers with high-frequency turbulence. An eight-month experiment of the pool showed that this method successfully controlled the golden mussel invasion and bio-fouling in the model tunnels. The mussel density on the attachment materials decreased sharply when the distance of the materials from the pool entrance increased; the turbulence was effective in killing veligers that escaped from the attachment materials. Absolutely no golden mussel attached on the model tunnels. Thus, the measure used in the integrated ecological prevention pool is recommended as a successful measure for controlling the golden mussel invasion and bio-fouling in water transfer tunnels.

KEY WORDS: Water transfer tunnels, Golden mussel invasion, Bio-fouling, Attachment attracting, Ecological prevention

1 INTRODUCTION

Inter-basin water transfer projects have been widely used to solve uneven distribution of water resources and water shortages in China. During the transferring of water resources, the golden mussel (*Limnoperna fortunei*), the filter-collector macro-invertebrate species originating from southern China has also been inadvertently transferred to new aquatic environments, resulting in a quick and uncontrolled spread of the species. For instance, the water transferred from East River to Hong Kong introduced golden mussels into the aquatic ecosystem of Hong Kong and has disrupted the native aquatic community (Morton, 1977). In the water transfer system of the Wuhan Iron and Steel Company in Hubei, central China, golden mussels clogged the supply pipes and caused great economic loss (GPS, 1973). In the past, the golden mussel was found only in southern China with the Yangtze River as its north boundary (Liu et al., 1979). However, the species was found in the Yellow River basin in the 1980s, and recently it has been found in the waters in Beijing (Ye et al., 2011). Golden mussels have also invaded the aquatic ecosystems and hydraulic structures in South America and other Asian countries (Darrigran, 2002; Boltovskoy et al., 2006). The clogging of cooling water pipes caused by dead golden mussels stopped raw water pumping to a water purification plant and caused the shutdown of a turbine dynamo-electric generator in a hydraulic power plant in Japan (Magara et al., 2001). Invasion and bio-fouling of golden mussels was also found in several most important nuclear power plants of the Plata Basin (Darrigran, 2002).

Invasion and bio-fouling by golden mussels in water transfer systems has drawn attention because it has resulted in a high resistance to water flow in tunnels, corrosion of pipe walls and even clogging of tunnels. Additionally, the invasion has caused water pollution and ecological imbalance in the regions that receive water infested with golden mussels (Darrigran, 2002). Therefore, quite a few researches have been done to study the golden mussels' biological and ecological features. It is found that golden mussels share several biological and ecological features with the North American invasive pest zebra mussels (*Dreissena polymorpha*), such as size, growth speed, and colonization on hard substrata by means of strong byssuses (Morton, 1979; Ricciardi, 1998). Both species are invasive by nature and endowed with a strong byssus for attaching onto their habitat, allowing them to easily invade natural and artificial aquatic systems, resulting in high-density attachment that causes serious bio-fouling. Invasion of golden mussels in a new habitat causes ecological imbalance that results in changes in the feeding habits of fish and, consequently, the composition of the macroinvertebrate community (Darrigran and Ezcurra, 2000; Penchaszadeh et al., 2000). Golden mussels colonize habitats with water temperature between 8-35°C, flow velocity between 0.1-2 m/s, water depth between 0.1-10 m, dissolved oxygen higher than 1.0 mg/L, pH-value higher than 6.4, and Calcium concentration above 3.0 mg/L (Morton, 1982; Márcia, 2006).

Finding strategies to reduce bio-fouling caused by golden mussel invasion has been studied for decades all over the world. Various chemical and physical measures of getting rid of golden mussels' attachment, such as coating pipe walls (Luo, 2006), poisoning with pesticide (Darrigran and Damborenea, 2006), spraying with hot water (Morton, 1982), trapping with filters (Darrigran, 2002), using ultraviolet irradiation, washing with high velocity flow, removing artificially with scrapers (Xu et al., 2009) have been employed. However, such measures can themselves contribute to water pollution, damage water transfer tunnels, or cost too much to be of practical use. Preventing golden mussels' invasion into water transfer tunnels is the most effective strategy to mitigate biofouling (Simberloff, 2001). Therefore, it is important to understand the invasion methods of golden mussels, and then possible to propose strategies to prevent their invasion. Ecological methods of preventing a golden mussel invasion in water transfer tunnels were attempted in the East River Water Source Project (ERWSP) in this study.

2 STUDY AREA AND METHODS

2.1 The East River Water Source Project

As shown in Fig. 1, the East River Water Source Project (ERWSP) transfers water from the East River and its tributary Xizhijiang River to Shenzhen, southern China for 10 million people. The ERWSP

consists of 17 km long double steel pipes (2.6 m in diameter), an 80 km long concrete tunnel (3.2 m wide and 4.0 m high), several sections of culverts, and numerous valve varve gates. The attachment density of golden mussels is 10,000 ind./m² on average and as high as 50,000 ind./m² on some sections. Thickness of golden mussel clusters exceeds 10 cm on pipe walls, valves, gates, and other structures, resulting in concrete wall corrosion and high resistance for water transferring (Fig. 2a). Additionally, fungi species growing on dead golden mussels results in water pollution (Fig. 2b). Artificial removal of golden mussels has been conducted year after year. However, golden mussel biofouling became more and more severe after each artificial removal.



Figure 1 Layout of the tunnels of East River Water Source Project: red line - water transfer tunnels, green line - water deliver tunnels



(a)

(b)

Figure 2 (a) Golden mussel biofouling on water transfer tunnels; (b) Fungi developed on dead golden mussels.

2.2 STUDY METHOD

Long-term samplings and observations of water samples taken from the East and Xizhijiang rivers were conducted to study the invasion way and performance in flows of golden mussels. Flume experiments were performed to study golden mussels' attachment. Furthermore, an integrated ecological prevention pool was designed and constructed based on the results of long-term observations and flume experiments to study golden mussels invasion and prevention methods in the scale model tunnels of the ERWSP.

2.2.1 Long-term samplings and observations

Some have thought that veligers of golden mussels develop inside the parental mussels and then enter into the water when the veligers have byssuses for attaching onto materials; therefore, their invasion

is limited to a short distance due to their poor crawl ability (GPS, 1973). However, according to an investigation of the 120 km long tunnels of the ERWSP, it was found that the whole tunnel system suffered from golden mussel biofouling, which means that golden mussels' invasion ability is far higher than their own crawl ability (Ye et al., 2011). One explanation is that golden mussels perhaps have plankton veligers in water, which can invade with water flow over long distances. Water samples were taken from the East and Xizhijiang rivers every week from January 2010 to December 2012 to detect golden mussels' plankton veligers. A water pumping system was designed and used to take water samples from different water depths. For each sample, 400 L of water was taken and filter through plankton net with mesh of 64 μm . The concentrated sample was observed under a microscope-camera system (SmartV Camera & MIVNT Image Analysis Software, Yongheng Shanghai). All of plankton veligers were photographed and documented with number, size, living status, and densities of veligers were calculated. Furthermore, living veligers were treated with high frequency turbulent flow (>30 Hz), which was generated by water flow passed through plates embedded with holes (diameter of holes: 10 mm, interval between holes: 2 mm, see details of the turbulent flow system in Xu (2012)). The living status of the veligers was observed after different action time of the turbulence.

2.2.2 Experiments of golden mussels' attachment

Preliminary experiments were conducted to observe attachment of golden mussels onto 14 different materials: bamboo, plastic plane with lumpy surfaces, smooth PP plane, rough PP plane, PP plane with cement mortar, geotextile cloth (four different types), woven net, smooth glass, hemp rope, ceramic tile, concrete slab. It was found that attachment densities of golden mussels were similar on the types of geotextile cloth, but very different on the other nine materials. According to the preliminary experimental result, representative materials: bamboo, plastic plane with lumpy surfaces, PP plane (one rough surface and one cement mortar surface), geotextile cloth, and woven net were chosen and set in a flume for a long-term attachment experiment. Figure 3a shows the layout of the experimental flume, which consisted of a fore bay (I) and four sections (II-V) of open channels with different widths and different bed slopes to obtain different flow velocities. Water was pumped from the Xizhijiang River to the flume, flowing from section I to section V then drained out from the flume. Four frames fixed with the representative materials, as shown in Fig. 3b, were set in the sections II-V. Flow velocities of these sections were kept between 0.3-0.6 m/s, which is a suitable flow velocity for golden mussels' attachment (Xu, 2012). The attachment densities of golden mussels were measured for different attachment times: one day, one week, two weeks, and four weeks after the start of the experiment, respectively. The attachment force of the stably attached mussels on different materials was measured using a Digital Push & Pull Tester (0.01-50 N, SH-50, Wenzhou). High-velocity flows were used to flush the attached mussels to test the resistance of golden mussels' attachment to flushing flow.

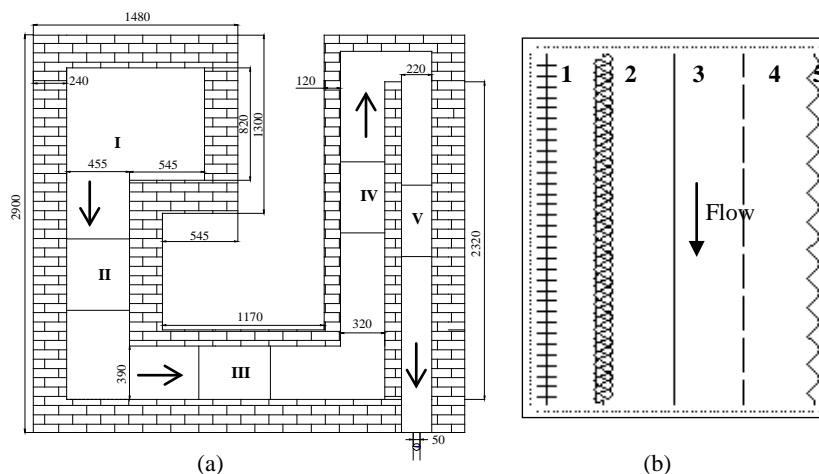


Figure 3 (a) Layout of experimental flume, in which flow depth was 0.5 m; (b) Layout of the materials in the frame: 1- bamboo, 2- plastic plane with lumpy surfaces, 3- PP plane (one rough surface and one cement mortar surface), 4- geotextile cloth, 5- woven net.

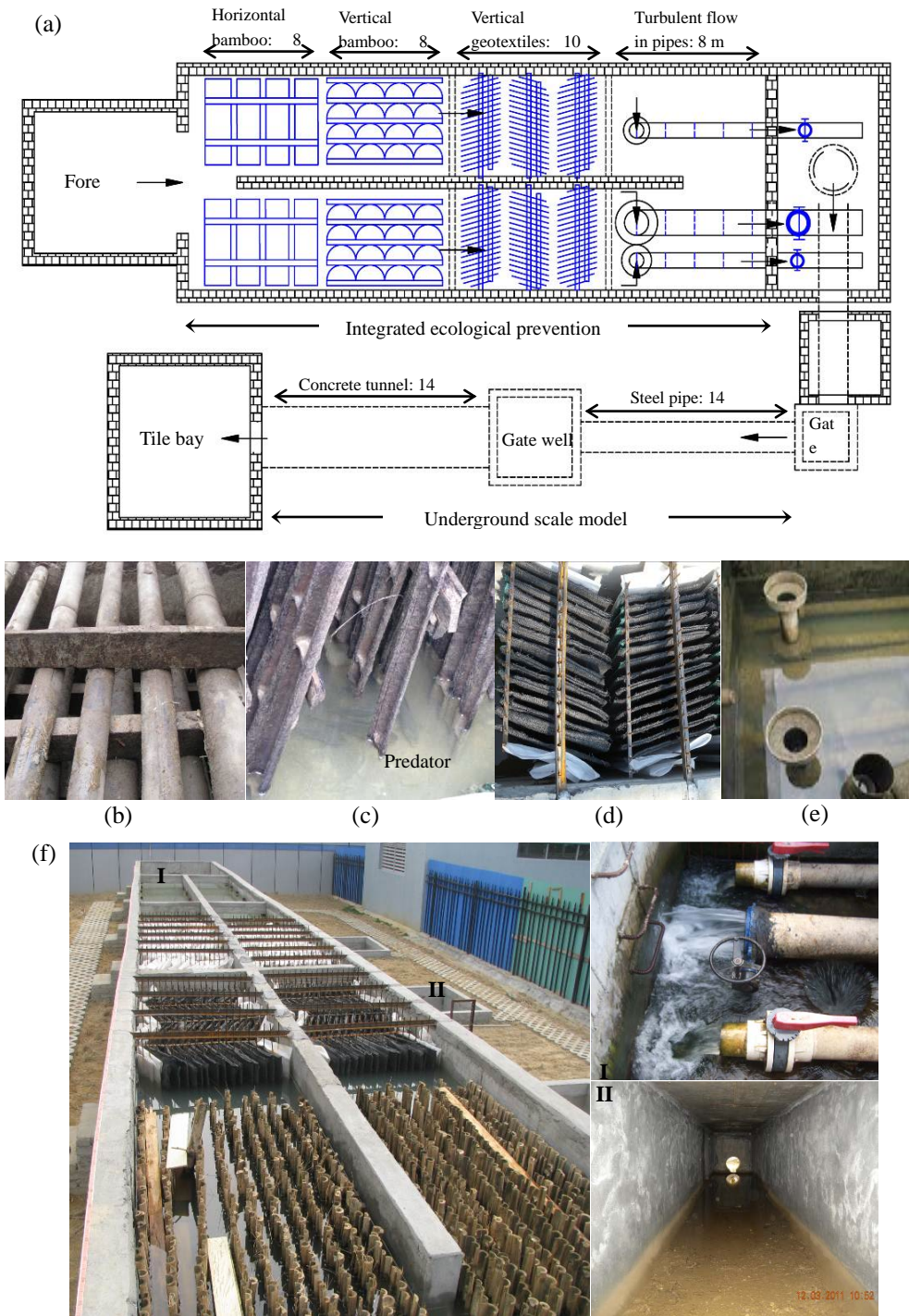


Figure 4 The layout of the experimental system: (a) Demonstration of the integrated ecological prevention pool; (b) Horizontal bamboo rafts; (c) Vertical bamboo rafts and predator fishes of golden mussels; (d) Vertical geotextile cloth frames; (e) Inlet of turbulent flow pipes; (f) I-outlet of turbulent flow pipes, II-concrete scale model tunnel

2.2.3 Long-term experiment of integrated ecological prevention

Based on the results of the long-term observations and attachment experiments, an ecological prevention strategy was proposed that combined two techniques: attracting golden mussels to attach onto their favorite materials and killing golden mussel veligers with high frequency turbulent flow. An

integrated ecological prevention pool was designed and constructed for the experimental study of golden mussel invasion prevention in scale model tunnels of the ERWSP. Figure 4 shows the layout and materials of the experimental system, which included the prevention pool and the scale model tunnels.

The experiment was performed from March 2011 to October 2011, covering the reproduction peaks of golden mussels. During the experiment, water was pumped from the Xizhijiang River to the fore bay (Pump discharge: 240 m³/h), then flowed through to the prevention pool (total length: 34 m, width: 2.2 m, height: 1.6 m), which consisted of three attachment sections: horizontal bamboo rafts (8 m long), vertical bamboo rafts (8 m long), vertical geotextile cloth frames (10 m long), and a section of high frequency turbulent flows created in the three pipes embedded with hole-plates (as described in 2.2.1)(as shown in Fig. 4a). In the sections of bamboo rafts (Fig. 4b and c), the average velocity was kept between 0.4-0.6 m/s and the average water depth was about 1.5 m. The predators of golden mussels, *Carassius auratus* and *Channa argus*, were cultured to prey on the attached golden mussels in these sections. In the section of geotextile cloth frames (Fig. 4d), the average velocity was between 0.2-0.3 m/s and the average water depth was about 1.5 m. In the section of high frequency turbulent flows, the inlets of the pipes were close to the water surface of the pool (Fig. 4e) and the outlets of the pipes were at the pool bottom and discharged water freely out from the pool (Fig. 4f). Therefore, the 1.5 m waterhead drove the water flows through the pipes embedded with hole-plates and created the high frequency turbulent flows. Afterwards, the water discharged from the pipes flowed down to the underground scale model tunnels (Fig. 4 a: 14 m long steel pipe and 14 m long concrete tunnel).

Water samples were taken from the fore bay (Fig. 4a) and the end of prevention pool to assess the reduction of densities of living veligers during the experiment. At the end of the experiment, the pumps were switched off and water was drained out from the whole experiment system. The attachment densities of golden mussels on the walls and the attachment materials in the prevention pool were measured. The densities of golden mussels on the pool walls and the underground scale model tunnels were also measured. The efficiency of the integrated ecological prevention pool was assessed by comparing the attachment densities of golden mussels in the pool and in the model tunnels.

3 RESULTS AND DISCUSSION

3.1 Golden mussel veligers

After our long-term frequent water samplings and observations, the golden mussel veligers were finally found in the water samples of both the East and Xizhijiang rivers. By comparing these samples with the features of golden mussel veligers reported in South America (Dos Santos, 2005; Cataldo, 2005), it was confirmed that golden mussel veligers were released into the water and experienced about a one month period of varying plankton stages in water. Figure 5 shows the three different veliger stages: D-shape veligers, pediveligers, and plantigrade veligers. This is the first time that golden mussel veligers were found living in water in China, which is essential for the further study of preventing golden mussels' invasion.



Figure 5 Different plankton veliger stages of golden mussel in water: (a) D-shape veliger, length=140 μm; (b) Pediveliger, length=190 μm; (c) Plantigrade veliger, length=320 μm.

Our observations showed that plankton veligers invade with water flow to every section of the water

transfer tunnels, resulting in serious biofouling. The densities of the veligers varied from several hundred up to more than 10,000 ind./m³ in different months, and peaked from May to August, which was the period of the most intensive invasion of golden mussels. Therefore, preventing the plankton veligers from invading in tunnels during this period is considered to be the most effective control strategy.

Figure 6a and b show the statuses of the veligers after they were treated with high frequency turbulence. Either the tissues of the veligers were broken and released out from the shells or the shells were damaged due to the high frequency turbulence. As shown in Fig. 6c, the rate of the veligers killed by the turbulence increased with the action time of the turbulence. More than 80% of the veligers were killed when the action time was over 5 min, and all of the veligers were killed when the action time was over 10 min. The veligers of zebra mussels were also observed being killed in the turbulent flow created by an aerating pump when the eddy scale was comparable with the veliger sizes (Rehmann et al., 2003). Therefore, such high frequency turbulence (>30 Hz) can be used to control the invasion of golden mussels and zebra mussels. However, the energy consumption requirements of creating this turbulence may restrict its application in practice.

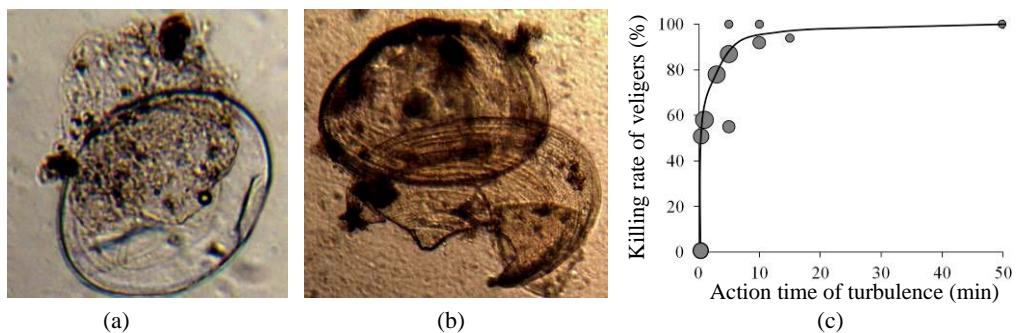


Figure 6 Status of veligers after treated with high frequency turbulence: (a) The tissues of the veligers were broken and released out from the shells; (b) The shells of the veligers were damaged; (c) the relation between the killing rate of veligers and action time of the turbulence, the different plot sizes indicate the different densities of the veligers.

3.2 Attachment characters of golden mussels

Observation of the attachment activities of the veligers indicated that when they developed into plantigrade veligers, they began to attach onto various materials. They experienced an unstable veliger-attachment stage and then a stable mussel-attachment stage with their byssuses, successively. The attachment of veligers with lengths of 200-400 μm was unstable. These veligers usually moved from their original attachment places and back to water flow to find new places. When the veligers grew longer than 450 μm , they produced byssuses and used them to attach onto materials firmly. Once attached, these veligers hardly moved and developed into mussels in their current location. Figure 7 shows the characters of the stable mussel-attachment of golden mussels.

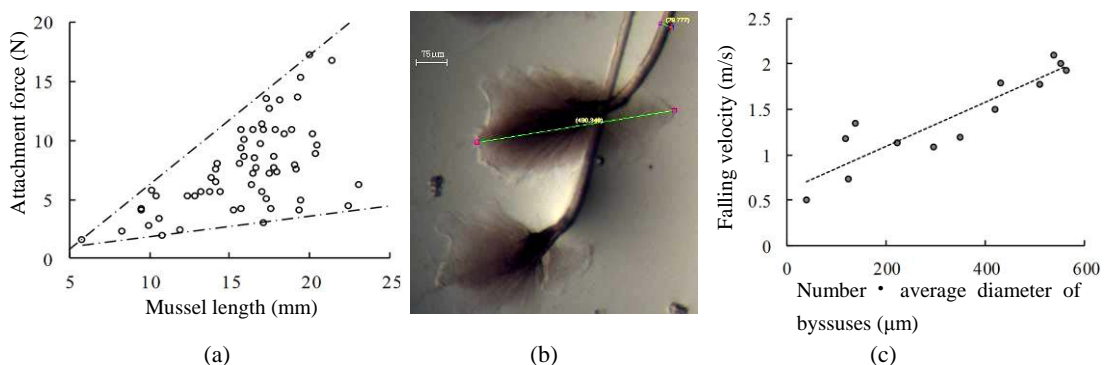
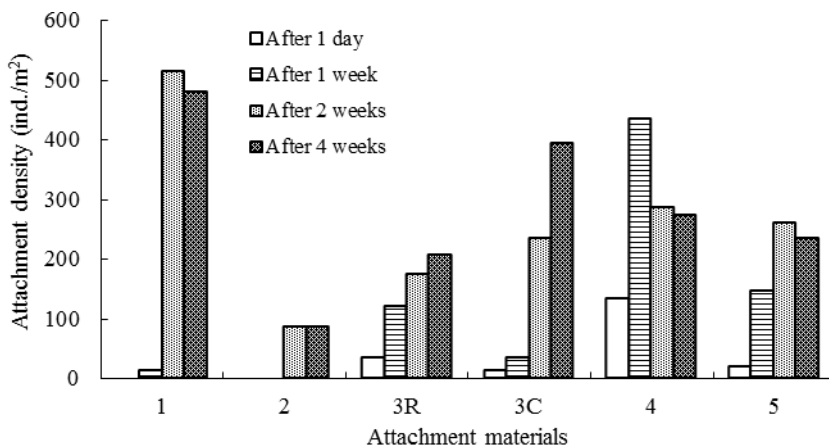


Figure 7 (a) The relationship between attachment force and length of golden mussels; (b) The byssus structure: diameter of byssus is among 10-70 μm , diameter of sucker is >1,000 μm ; (c) The relationship between falling velocity and the product of the number and average diameter of the byssuses.

As shown in Fig. 7a, the attachment force is positively correlated to the mussel length. Normally, longer mussels produce larger and stronger byssuses (Xu et al. 2012). The inflated suckers of the byssuses attached firmly on materials and were difficult to remove (Fig. 7b). As shown in Fig. 7c, the average flow velocity that caused the golden mussels to dislodge from a material (called “falling velocity”) shows a linear relationship with the product of the number and average diameter of the byssuses that were used for attaching. All of the attached golden mussels fell off when the flow velocity reached 2.2 m/s. In the water transfer tunnels of ERWSP, it was also found that the attachment density of golden mussels was very low at the sections with flow velocity higher than 2.0 m/s (Ye et al., 2011).

Figure 8 shows the attachment densities of golden mussels on different materials after different attachment times. After one day, a few golden mussels attached onto geotextile cloth, PP plane, and woven net. After one week, the attachment densities on all materials increased. To be specific, the density on the geotextile cloth reached its maximum. After two weeks, the attachment density on most materials increased. Especially on the bamboo the density increased dramatically and became much higher than the other materials. However, the density on geotextile decreased significantly compared to its density at one week. After four weeks, except for PP plane cement mortar surface, the densities on the materials seldom changed compared to their density at two weeks. Therefore, a time of two weeks is considered to be the threshold for golden mussels’ stable attachment. Golden mussels’ attachment shows preference to specific materials. Bamboo was the most favoured habitat for golden mussels’ stable attachment, while geotextile cloth was the most popular material for mussels during the unstable attachment stage. Therefore, using geotextile cloth is recommended to absorb the golden mussels during the unstable attachment stage. The geotextile cloth should be replaced every one week to make sure more golden mussels are taken out from the water flow with the material. Bamboo can be used as a suitable habitat material to attract golden mussels stably attached onto the current habitat. With addition of these two materials, less golden mussels invade into water transfer tunnels. It is important that the bamboo habitat should be replaced before the attached mussels reach sexual maturity. It is found that the golden mussels become sexual mature when their shell lengths are over 8 cm (GPS, 1973). The plastic plane with lumpy surfaces, which releases toxicity, is the least welcomed attachment material and supposed to be used to avoid golden mussels’ attachment in certain circumstance.



Note: 1-bamboo, 2-plastic plane with lumpy surfaces, 3R-PP plane with rough surface, 3C-PP plane cement mortar surface, 4- geotextile cloth, 5- woven net

Figure 8 The attachment densities of golden mussels on different materials after different attachment times.

3.3 Integrated ecological prevention

As recommended in 3.2, bamboo rafts were used as habitat materials for golden mussels’ stable attachment and geotextile flames were used to absorb the golden mussels that were in the unstable-stage and escaped from the bamboo rafts in the integrated ecological prevention pool. During the eight-month experiment, the predators of golden mussels, *Carassius auratus* and *Channa argus*, played important roles in restraining both attachment densities and shell lengths of the golden mussels. Most of the golden

mussels on the bamboo rafts were short than 8 cm and were not sexual mature. The pipes created high frequency turbulent flows in the end of the integrated ecological prevention pool and worked as the final guarantee to reduce the living veligers.

The attachment densities of golden mussels on the bamboo rafts and geotextile frames at different water depths and distances from the fore bay were compared in Fig. 9. Firstly, the orientation of the attachment materials affected the attachment densities of golden mussels. The attachment densities of the horizontal bamboo were much lower than the attachment densities of the vertical bamboo rafts and the vertical geotextile frames, because the horizontal bamboo rafts were more prone to precipitation of silt and clay. Clay and silt precipitates interfere with filter-feeding, breathing, and attachment of golden mussels (Morton, 1977). In the water transfer tunnels, it is also found that the walls with less clay and silt precipitates have higher attachment densities of golden mussels than those with more clay and silt precipitates (Ye et al., 2011). Furthermore, for the sections with vertical bamboo rafts and geotextile frames, the attachment densities decreased as the distance from the fore bay increased, and it reduced to nearly zero ind./m² at the end of the sections, which indicated that nearly all of the golden mussels were prevented in the attachment sections of the prevention pool. Nevertheless, the attachment densities at different water depths were also different. Mostly the densities were the highest at the bottom (H=1-1.5 m), followed by those at the middle (H=0.5-1 m), and the lowest at the surface (H=0-0.5 m). This may be due to the photopathy and benthic characteristics of golden mussels (Xu et al., 2009). The settling characteristic of the veligers also results in more veligers at deeper water depths (Xu et al., 2013).

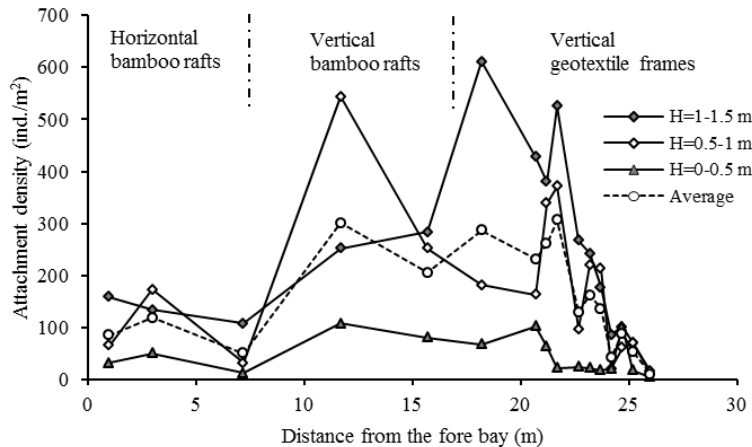


Figure 9 Attachment densities of golden mussels on attachment materials at different water depths and distances from the fore bay.

Figure 10a shows the monthly average density and reduction rate of living veligers at the fore bay and the end of the prevention pool during the experiment period. The density of living veligers decreased significantly after the water passed through the pool. The reduction rate of living veligers was over 80%. Very few living veligers, most of which were in their D-shape and preveliger stages and not ready for stable attachment, entered into the scale model tunnels, but did not cause biofouling (as shown in Fig. 10b and c).

Figure 10b shows the attachment density of golden mussels on the walls from the fore bay to the tile bay after eight months' experiment. The density of golden mussels decreased dramatically from the wall of the fore bay to the wall of the section of the prevention pool with bamboo rafts. Then it reduced to zero ind./m² on the wall of the section of geotextile frames of the pool. No mussels were found on the wall of the scale model tunnels. It is thought that when water flowed into the section of bamboo rafts, the veligers that were ready for stable attachment were attracted to attach on the bamboo. Then water flowed into the section with geotextile frames; the veligers that were in an unstable stage of their lifecycle were absorbed on the geotextile frames. Therefore, most of the veligers were stopped in the prevention pool, and the invasion in the tunnels was controlled.

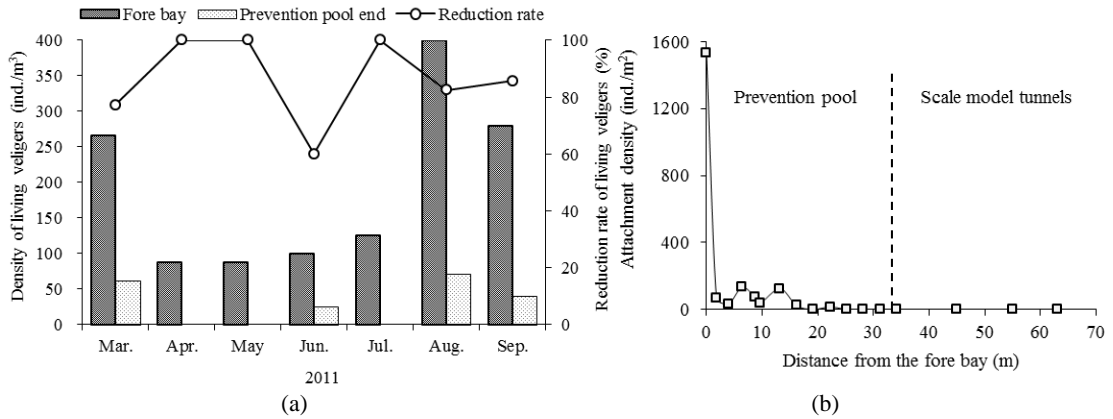


Figure 10 (a) Density and reduction rate of living veligers at the fore bay and the prevention pool end during the experiment period; (b) Attachment density of golden mussels at walls from the fore bay to the tile bay.

Figure 11 compared the attachment of golden mussels on the gate wells in the tunnels of ERWSP and the scale model tunnels. Without prevention measures of golden mussels' invasion, the gate well in the tunnel of ERWSP suffered from serious golden mussel biofouling (Fig. 11a). Conversely, in the scale model tunnels there were absolutely no golden mussels attached (Fig. 11b) because the integrated ecological prevention pool successfully controlled the golden mussel invasion.



Figure 11 Attachment of golden mussels in 2011: (a) The wall of gate well in the tunnels of ERWSP; (b) The wall of gate well in the scale model tunnels.

4 CONCLUSIONS

Golden mussels underwent planktonic veliger stages in water. The movement ability of both the veligers and mussels was poor. Their invasion in water transfer tunnels was achieved because planktonic veligers were transported by water flow. Planktonic veligers were killed in high frequency turbulence when the eddy scale was comparable with the veliger size. The killing rate of veligers was positively correlated with the action time of the turbulence. After the veligers entered a tunnel, they begin to attach and grew into mussels on the tunnel walls. After attachment, they developed from the unstable veliger-attachment stage to the stable mussel-attachment stage with their byssuses. They preferred geotextile cloth during the unstable attachment stage and bamboo material during the stable attachment stage. When the golden mussels attached stably and firmly on materials, they could withstand the flushing of water flow. Their attachment force was positive to their mussel length. The average flow velocity that caused golden mussels to dislodge from materials showed a linear relation with the product of the number and average diameter of the byssuses used for attaching.

The integrated prevention pool was designed and constructed to prevent golden mussel veligers from entering into tunnels by attracting mussels to attach stably onto bamboo and absorbing veligers to attach on

geotextile cloth. High frequency turbulence was also used as a final guarantee at the end of the prevention pool to reduce the amount of living veligers that may enter into tunnels. An eight month experiment using this prevention pool indicates that these methods successfully controlled the golden mussel invasion and bio-fouling in the model tunnels. The sections of attachment materials were very effective in preventing the veligers from invading in the scale model tunnels. The mussel density on the attachment materials decreased sharply as the distance from the pool entrance increased and reduced to nearly zero at the end of pool. The predator fishes restrained the densities and shell lengths of the stably attached golden mussels. The high frequency turbulence was effective in killing veligers that escaped from the attachment sections. Absolutely no golden mussel attached in the scale model tunnels. Therefore, the integrated prevention pool is recommended as a successful measure of controlling golden mussels' invasion and bio-fouling in the water transfer tunnels.

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