Study on migration modes and correlative factors of Tubifex tubifex (Oligochaeta, Tubificidae) in source water

Huang Ting-lin, Nie Xiao-bao, Zhang Jin-song, Li Wei, Li Xiao-yu, Zhang Shuang and Wang Bao-shan

ABSTRACT

Excessive propagation of tubificid in eutrophic source water can result in its emergence in the drinking water plant, which has been a troublesome problem in recent years. The migration of tubificid, especially drift migration in the source water is the main cause of worm pollution in drinking water plant. Migration modes and correlative factors of Tubifex tubifex, dominating the tubificid frequently, were investigated to help drinking water plant to solve worm pollution. Migration modes were divided into superficial, deep and drift migrations, representing migrations occurring in surface sediment (0–2 cm), deep sediment (deeper than 2 cm) and from sediment to overlying water, respectively. The results showed that both temperature and dissolved oxygen (DO) affect the worm migration modes. The maximum drift efficiency occurred at 22°C while DO was controlled around 8 mg O2 l⁻¹. When the temperature was fixed at 20°C, maximum drift efficiency was observed at 2 mg O2 l⁻¹. Distinct increase of superficial migration and decrease of deep migration were observed at 28°C compared to 16 and 22°C. Low DO appeared to be the main reason for the increase of worm’s deep migration. The results confirmed that worm drift from sediment to overlying water, which was influenced by the temperature and DO content, was the main cause of worm pollution in the drinking water plant.

Key words | dissolved oxygen (DO), migration mode, temperature, Tubifex

INTRODUCTION

Tubificid, an aquatic benthic oligochaete known to be widely distributed, frequently dominates the macrobiotic community in freshwater habitats (Reynoldson 1987; Lucan-Bouche et al. 1999). Some tubifids like Tubifex tubifex, Branchitura sowerbyi and Limnodrilus hoffmeisteri are very resistant to pollution and often the last to disappear from a contaminated site (Reynoldson et al. 1999). Tubificids adopt a vertical position in the sediment with their anterior part in the mud to ingest large volumes of sediment for feeding and put their posterior end free in the water. Because of their special life-style, tubificids are particularly exposed to environmental pollutants, via the sediment, pore water and water column, through ingestion and/or epidermal contact (Leynen et al. 1999). They have been used as test organisms for sediment bioassays and to assess the acute toxicity of various metals and organic compounds (Vidal & Horne 2003; Mosleh et al. 2005).

Migration is a simple and effective way for most benthic invertebrates to enlarge population density and expand distribution range (Bilton et al. 2001; Romaniszyn et al. 2007). By migration, they can escape and hide from predators, avoid unfavorable environmental conditions and select suitable habitats (Merle et al. 1997; Tetsuya 2006). Tubificid worms, even their cocoons, will migrate or/and drift when habitat conditions change (Marian & Pandian 1984). For drinking water plants, most interests are concentrated on whether the
Migration from the sediment to the water will occur. One of the notorious results of such migration is that worms and their cocoons may enter plants along with the current of water lifted by pumps. The body sizes of tubificid worms such as *Tubifex tubifex* and *Limnodrilus hifmeisteri* are very small. For example, the body length and 8th segment width of *Tubifex tubifex* are about 5–25 mm and 0.11–1.65 mm respectively (Lazim & Learner 1986; Zhu et al. 2008). Once entering drinking water treatment system, they can easily penetrate sand filter and activated carbon filter and finally present in drinking water distribution systems for their small sizes and high stretching capability (Chen et al. 2005; Liu et al. 2009). Moreover, due to the microbiological processes in eliminating organic carbon in biological activated carbon (BAC) filters, providing plenty of food for the growth of tubificids, BAC filters are colonized by invertebrates including tubificids (Schreiber et al. 1997; Adam et al. 1998). The colonization of tubificid worms can lead to an output of organisms in high numbers. Although there are no indications that these organisms pose a threat to the public health, tubificid’s presence is not appreciated because most people associate the organisms with low hygiene (Lieverloo et al. 2004).

Therefore, to forecast and control worm pollution, it is necessary to investigate the migration modes and the influence factors of tubificids in source waters for drinking water plants. The migration and distribution of tubificid are reported in numerous literatures. Worms in sediment of different trophic levels show various migration modes (Lazim & Learner 1986; Collado & Schmelz 2001). Pollutants in sediment such as heavy metals have significant influence on worm migration (Singh et al. 2007). Meanwhile, the worm’s migration changes the physical and chemical properties, which promotes the degradation of organic matter and the export of nutrients in sediment (Ciatat et al. 2007).

Despite many studies investigating the worm’s migration in sediment, few works assessed transformation mechanism among different migration modes and the influence of environmental parameters on worm’s drift from sediment to overlying water. This study first aimed at investigating the degree of worm’s migration in laboratory. Second, the efficiencies of different migration modes and the influence of water temperature and dissolved oxygen (DO) were assessed based on the hypothesis that migration modes could be divided into surface, deep and drift migrations that represented migrations in superficial sediment (0–2 cm), deep sediment (deeper than 2 cm) and from sediment to overlying water respectively. Third, water flow was created to assess the influence of water flow on cocoon drift from sediment to overlying water.

### MATERIALS

**Origin of the worms, sediments and culture maintenance**

Worms were collected from Tangyu reservoir, one of the source water reservoirs of Xi’an city. They were transferred to the laboratory using plastic bags filled with site water and sediment. The samples were sieved through a 150 μm mesh. Worms were the mixture of *T. tubifex*, *L. hifmeisteri* and *B. sowerbyi*. Mature *T. tubifex* were selected and other worms were discarded. Mature worms were then cultured in a 50 cm diameter plastic vessel with a 5 cm layer of natural sediment of reservoir which had been frozen at −20 °C and gradually thawed and finally to ambient temperature to kill worms and cocoons that come from the reservoir. Culture water was dechlorinated tap water. Other culture conditions included water temperature (24±2 °C), illuminance (1000lux) and photoperiod (16L:8D).

**Migration experiment of worm and drift experiment of cocoon**

Migration experiments were carried out in a plexiglass tank (40 × 25 × 60 cm) with a 15 cm layer of sediment (Figure 1). The volume of the tank filled with sediment was divided into experimental and blank zones by a plexiglass column (15 cm high, 12 cm inside diameter). After filling the tank with 15 cm layer of sediment, worms were added into the experimental zone and dechlorinated tap water was pumped into the tank as overlying water until the water depth reached 40 cm. The plexiglass column was drawn out from sediment to allow worms migrate from the experimental zone to the blank zone. At the end of migration process, the plexiglass column was planted into sediment again for the separation of the two zones.
A plexiglass box (20 × 10 × 8 cm) was used to study the drift of cocoon at different water flow rates. The box was divided into the water distribution, drift and effluent zones by a perforation plate (8 cm height) and a baffle (6 cm height) (Figure 2). Drift zone was filled with a 5 cm layer of sediment and cocoons were deposited on the sediment surface. Dechlorinated tap water pumped by a peristaltic pump flowed through the box. The outlet of the effluent zone was wrapped with a 100 μm mesh. Drift cocoons were washed away by water flow and intercepted by the mesh.

METHODS

Sediment and worms addition

Sediment used for worms migration and cocoons drift was sampled from Tangyu reservoir too. Before addition, sediment was sieved through a 2 mm mesh to remove coarse fragment and macrofauna, and were frozen at −20°C for 1 week to kill most of the remaining worms. After thawing and homogenization, samples were added into experimental and blank zones. During sediment addition, the sediment surface should be kept as smooth as possible in order to avoid its influence on worm migration. 400 worms with body length around 3 cm were selected from culture vessel and gently added to experimental zone. Such number corresponded to a density of 48,000 worms m⁻², which is within the range normally found in Tangyu reservoir. The worms immediately burrowed into the sediment after being introduced. After the introduction of dechlorinated tap water as overlying water, homogeneous distribution of worms in the experimental zone was supposed to reach within 2 days.

Experimental design

After homogeneous distribution of worms reached, the plexiglass column was drawn out from sediment and worms began to migrate. To assess the effect of the DO content and temperature on T. tubifex migration, the DO contents of overlying water were controlled to be around 0.5 mg O₂ l⁻¹, 2 mg O₂ l⁻¹ and 8 mg O₂ l⁻¹ respectively by aeration of N₂/air and the temperatures were controlled to be 16°C, 22°C and 28°C respectively by heat bar. The temperature was fixed at 20°C while investigating the effect of DO and DO was controlled at around 8 mg O₂ l⁻¹ while assessing the influence of temperature. During migration, overlying water was changed twice each day to avoid obvious DO fluctuations and frequent switch between aeration of N₂ and air. Migrations under each combined condition lasted for 10 d to avoid the first emergence of young worms, which normally occurs after 12 d at 30°C (Paoletti 1989). At the end of migration process, overlying water was drawn out from the tank by a siphon pipe, and sediments of experimental and blank zones were taken out. The worms were gently sieved out of the sediments by a 150 μm mesh to

Figure 1 | Diagram of worm migration experimental system.

Figure 2 | Diagram of cocoon drift experimental system.

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assess total migration efficiency and worm density of both zones.

Another set of experiments were conducted to investigate the worm’s migration modes. Experimental procedures of migration were the same as mentioned previously. Before overlying water being drawn out, the worms drifted from sediments to overlying water were collected by net (150 μm mesh). Superficial sediments (0–2 cm) and deep sediments of both zones were taken out one by one and worms were sieved and counted.

For cocoon drift study, 200 cocoons were deposited on the sediment surface, which is the usual position for the worm to lay its cocoon in nature. Cocoon drift was conducted at flow rates of 100, 500, 1500, 2000 and 2500 ml min⁻¹ without DO and temperature control.

Estimation of migration efficiency of different modes

The worms in overlying water procured by net at the end of migration were defined as drift worms, of which the number was designated as Adr. The worms in 2 cm layer of sediment in the blank zone below the water/sediment interface were considered as superficial migration worms, of which the number was designated as As. Worms in the experimental zone were viewed as failing to migrate with number of Af. Therefore, the number of worms adopting deep migration, designated as Adr, could be calculated by the following calculation: Adr = 400 – Adr – As – Af. In this way, laborious task of sieving plenty of deep sediments in blank zone was avoided. Cocoons drift efficiency could be easily obtained from the number intercepted by mesh.

RESULTS

Migration of worm

Obvious migration occurred during 10 days. There were many worms staying on the water/sediment surfaces in both experimental and blank zones except for DO 0.5 mg O₂ l⁻¹, which meant superficial migration happened. Some worms were collected during overlying water change, showing drift migration occurred too. At the same time deep migration was observed in deep sediment. Total migration efficiencies were above 80% under all tested conditions (Table 1 and 2). Both water temperature and DO influenced total migration efficiency and final worm density distribution. For temperature, the highest total migration efficiency 99.6% was observed at 28°C. The lowest population density of 200 worms m⁻² in the experimental zone and the highest of 5300 in the blank zone occurred at 28°C too, which meant worm exhibited stronger migration trend at 28°C than at 16°C and 22°C. When temperature was fixed at 16°C, the lowest total migration efficiency 87.9% occurred at 8 mg O₂ l⁻¹ and population density in experimental zone was higher than that in blank zone at the same time. On the contrary, at 0.5 and 2 mg O₂ l⁻¹, population densities in the experimental zone were lower than those in the blank zone. The highest total migration efficiency was observed at 2 mg O₂ l⁻¹. Below 2 mg O₂ l⁻¹, decrease of DO could not induce further migration of worm, instead, decreased total migration efficiency.

Worm migration modes at different temperature

The relationship between temperature and worm migration modes was shown in Figure 3. The threshold temperature for worm drift was about 16°C. Drift efficiency was remarkably lower compared to those of superficial and deep migrations. The highest drift, occurring at 22°C, was only 1.01%. Both superficial and deep migration efficiencies had inappreciable

Table 1 | Worm migration efficiencies and population densities at different temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Population density of blank zone (x 10⁴ worms m⁻²)</th>
<th>Population density of experimental zone (x 10⁴ worms m⁻²)</th>
<th>Total migration efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.47</td>
<td>0.58</td>
<td>87.9</td>
</tr>
<tr>
<td>22</td>
<td>0.44</td>
<td>0.86</td>
<td>82.1</td>
</tr>
<tr>
<td>28</td>
<td>0.53</td>
<td>0.02</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Table 2 | Worm migration efficiencies and population densities at different DO contents

<table>
<thead>
<tr>
<th>DO (mg O₂ l⁻¹)</th>
<th>Population density of blank zone (x 10⁴ worms m⁻²)</th>
<th>Population density of experimental zone (x 10⁴ worms m⁻²)</th>
<th>Total migration efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.50</td>
<td>0.28</td>
<td>94.2</td>
</tr>
<tr>
<td>2</td>
<td>0.53</td>
<td>0.07</td>
<td>98.5</td>
</tr>
<tr>
<td>8</td>
<td>0.49</td>
<td>0.52</td>
<td>87.9</td>
</tr>
</tbody>
</table>
changes when temperature increased from 16 °C to 22 °C and reached a stable level about 44% and 56% respectively. Distinct increase of superficial migration and decrease of deep migration were observed at 28 °C compared to 16 and 22 °C. At 28 °C, surface migration efficiency reached 71.55%, much higher than 28.03% of deep migration.

**Worm migration modes at different DO contents**

The worm migration modes and their efficiencies under different DO contents were presented in Figure 4. Under high DO (8 mg O₂ l⁻¹), superficial migration efficiency was very close to deep migration while worm drift was not observed. Obvious decrease of surface migration and increase of deep migration were observed with the reduction of DO, except that there was little increase of superficial migration at 0.5 mg O₂ l⁻¹. Under low DO (2, 0.5 mg O₂ l⁻¹), deep migration were nearly 92%, possessing the absolute superiority in all migration modes. The highest drift efficiency of 4.02% occurred at 2 mg O₂ l⁻¹, and then decreased to 0.66% at 0.5 mg O₂ l⁻¹. Movements of worms under different DO contents were also observed. At 8 mg O₂ l⁻¹, many worms were seen extruding partly at the sediment surface and waving their posterior body parts. The length of worm body part stayed outside in overlying water was short and only slow waving was observed. At 2 mg O₂ l⁻¹, some worms still stayed on water/sediment interface, waving most of their posterior body parts to get more DO. There were nearly no worms staying on water/sediment interface when DO dropped to 0.5 mg O₂ l⁻¹.

**Drift of cocoon**

The cocoon drift efficiencies at different water flow rates were shown in Figure 5. It is obvious that cocoon can not move by itself and water flow may be the only reason for its drift. The threshold water flow rate for cocoon drift was about 1000 ml min⁻¹. After 1000 ml min⁻¹, the drift efficiency increased with the water flow rate. Under the tested conditions, the highest drift efficiency of 4.55% occurred at 2500 ml min⁻¹. During the test, Cocoons filled with different numbers of egg (between 1 and 7) were founded in effluent, indicating the number of cocoon egg had little effect on the drift.

**DISCUSSION**

**Influence of temperature on worm migration efficiency and modes**

*T. tubifex* has been reported to be an adaptable species to temperature and DO: normal growth and development and
reproduction of the worm are observed from 0°C to 34°C (Chapman & Brinkhurst 1987) and the worm can survive in both aerobic and anaerobic environment by adopting different metabolic pathways (Hoffmann et al. 1987). Despite of its strong adaptability to temperature and DO, the changes of both temperature and DO could result in changes of the worm’s migration degree and modes based on results of the present experiment. Several studies (Chapman et al. 1982; Paoletti 1989; Li 2003) demonstrated that temperature around 22°C is much suitable for the colonization and survival of T. tubifex. Therefore, the worm was intended to stay in such suitable environment rather than migrating to find new habitat. It could explain why the lowest total migration efficiency occurred at 22°C. Tetsuya (2006) found that T. tubifex moved deeper in sediment in summer to elude high temperature. However, worm in this test exhibited higher superficial migration efficiency than that of deep migration at 28°C, indicating that it seemed to prefer to superficial sediment at high temperature. Tetsuya’s study was conducted at a littoral site of the south basin of Lake Biwa and the depth of sediment ranged from 76 to 125 cm. Sediment temperatures in the deep layers seemed to converge at around 20–21°C in the summer months. In our case, the thickness of sediment was only 15 cm, which might result in homogeneous distribution of temperature in the whole sediment at high temperature. As noted previously, 22°C seemed to be more suitable for worm colonization. High temperature (28°C) in our test eventually resulted in worm migration from deep sediment to superficial for high DO content in overlying water. It may therefore be hypothesized that the depth of sediment may play a crucial role in determining the worm migration at high temperatures.

Influence of DO on worm migration efficiency and modes

T. tubifex has several mechanisms of metabolic modes (Hoffmann et al. 1987), which enable the worm to occupy habitats of extremely variable oxygen content. Under normoxic conditions the metabolism of the worm is mainly aerobic. Under hypoxic conditions, metabolism of energy sources via aerobic and anaerobic pathways is observed (Famme & Knudsen 1985). The experimental results of different DO contents confirmed T. tubifex is not only aerobic but also anaerobic worm. The worm preferred high (8 mg O₂ l⁻¹) or low (0.5 mg O₂ l⁻¹) DO contents to moderate (2 mg O₂ l⁻¹) DO content, which might be induced by the particular metabolic mechanism. Therefore, the highest total migration efficiency was observed at the moderate DO (2 mg O₂ l⁻¹). Similar observation was made by Merle et al. (1997). Merle et al. suspected that the migration in sediment is reduced under anoxic conditions in order to conserve energy. Mosleh et al. (2005) found that T. Tubifex requires high energy to overcome the stress situation such as drouth and extreme anoxic conditions. Under strong environmental constraints, T. tubifex has two alternatives to survive: either to follow the downward water movement as an active survival strategy or to remain within the sediment till the environmental constraints disappear (a passive life strategy) (Robertson et al. 1995). The former strategy results in energy consumption while the later guarantees necessary energy for worm’s survival. Based on the findings of the present investigation and available literature, it may be hypothesized that not only metabolic mechanisms but also survival strategies play crucial roles in determining the worm migration efficiency and modes. At high DO content (8 mg O₂ l⁻¹), the worms...
migration would not be restricted by DO or energy and almost average distribution of superficial and deep migration occurred (Figure 4). Along with the decrease of DO, more and more worms began to move to deep sediments, adopting passive life strategy and switching their metabolic mechanism from aerobic to anaerobic. Therefore, deep migration efficiency was much higher than that of superficial migration at 2 mg O$_2$ l$^{-1}$. However, there were still a few worms that attempted to get more DO by lengthening their body part in overlying water and increasing waving frequency, which resulted in the increase of drift migration efficiency. Since quick wave of worm’s tail caused large energy consumption, almost all the worms would give up initiating anaerobic metabolic mechanism if DO decreased further. This could be explained by the phenomenon that there were nearly no worms staying on water/sediment interface when DO dropped to 0.5 mg O$_2$ l$^{-1}$.

**Influence of water flow on cocoon drift**

Since cocoon of *T. tubifex* can not move by itself, water flow may be the only reason for its drift in the present experiment. Water flow causes the shear stress on sediment and if shear stress exceeds the sediment entrainment threshold, the sediment becomes unstable and particles are partially or fully set in motion (Gibbins et al. 2009). Considering the density and size of cocoon have the same order as that of particles in sediment, cocoons would passively drift from sediment to overlying water provided water flow is high enough. The drift efficiency increased with the water flow rate, confirming the opinion that water flow plays a crucial role in cocoon drift for the fact that the higher water flow rate, the stronger shear stress.

**Worm drift and its pollution in drinking water plant**

For drinking water plants, much attention should be paid to the drift of worms and their cocoons, which are the key reasons for worm pollution in drinking water plants. Compared to other aquatic animals such as fish, diptera and mayfly, tubificid worms exhibit poor migration ability and small movement range. For example, tubificid migration just accounts for 0.62% of the total invertebrates’ migration in Upper Rhone River (France) (Cellot & Juget 1998). At epibentho, the worm undulates the posterior part in the overlying water without net building behavior in sediment. Therefore, it exhibits stronger migration, especially drift ability compared to endobenthos. For example, in all the oligochaetes in Scandinavia, tubificid worms show highest drift capacity (Milbrink 1999). Recently some drinking water plants in China have been reported on tubificid pollution (Chen et al. 2005), confirming the occurrence of worm drift from sediment to overlying water. In the present test, worm drift could be induced by changes of temperature and DO. At 20°C, the highest drift of 4.02% occurred at moderate DO content (2 mg O$_2$ l$^{-1}$). If DO content was fixed (8 mg O$_2$ l$^{-1}$), the highest drift of 1.02% occurred at 22°C. Based on the present results, the worm drift seemed to be more sensitive to DO than to temperature since the increment induced by DO change was up to 4.02%, compared to 1.02% induced by temperature. It may therefore be hypothesized that the decrease of DO plays a crucial role in the breaking out of worm pollution in drinking water plants. Though the absolute value of drift efficiency is very small, severe worm pollution in the drinking water plant will be induced in the source water. For example, for a drinking water plant using reservoir as its water supply source with scale of 6 x 10$^5$ m$^3$ d$^{-1}$, if the drift efficiency is 1%, there will be about 2,400,000 worms entering into the plant each day, with the hypothesis that homogeneous distribution of worm occurs in the reservoir, of which worm population density in sediment is 4 x 10$^3$ worms m$^{-2}$ and mean water depth is 10 m. Moreover, the influence of cocoon drift has not been taken into account. It is obviously that cocoons entering into plants will aggravate the worm pollution. As showed in our experiments, cocoon drift occurred while the rate of water flow was higher than 1000 ml min$^{-1}$.

**CONCLUSIONS**

This study clearly demonstrated that *T. tubifex* could carry out superficial, deep and drift migration and that the worm’s cocoon could drift from sediment to overlying water provided the water flow exceeded the threshold. Our results showed that *T. tubifex* migration modes and efficiencies could be influenced by both temperature and DO. Temperature
performs the effect on worm migration by the worm preference to temperature around 22°C. Not only metabolic mechanisms but also survival strategies play crucial roles in determining the worm migration efficiency and modes. Worm drift, the main cause of worm pollution in the drinking water plant, is more sensitive to DO than to temperature. Although the absolute value of worm drift efficiency is very small, severe worm pollution in the drinking water plant may be caused by the worm's drift. Moreover, cocoon drift, caused by water flow rate higher than 1000 ml min⁻¹, can aggravate the worm pollution. The present study clearly suggests that the investigations of the worm's migration modes are helpful to the forecast and control of worm pollution in water plant. As the experimental conditions were different to nature conditions, more investigations such as the effect of nutrients in sediment, the effect of other zoobenthos and the cross effect of DO and temperature, are still required to better estimate the worm's migration.

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