Impact of temperature and dissolved oxygen level on the population dynamics of naidids and their reproduction in biological activated carbon filters: a life table demographic study

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ABSTRACT

Aquatic macro-organisms, such as naidids, propagate excessively in biological activated carbon (BAC) filters. This has become a troublesome problem for drinking water plants. For successful control of naidid contamination risk, it is necessary to determine the population dynamics under different environmental conditions within drinking water plants, with special emphasis on BAC filters. In this study, field studies of naidid distribution in a drinking water plant were conducted, and the effects of temperature and dissolved oxygen (DO) on naidid population dynamics were investigated using the life table method. The results indicated that naidid pollution in the water plant occurred seasonally and was induced by the excessive propagation of naidids in the BAC filters. Increased temperature and DO increased the naidid intrinsic rate of natural increase and decreased the naidid population doubling time. The life table method was also used to acquire the reproductive parameters of naidids in BAC filters based on simulative experiments. These results indicated that naidids can reproduce asexually in BAC filters, and the population doubling time was 12.60 days.

Key words | biological activated carbon filters, life table, naidids, population doubling time, population dynamics, reproduction

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INTRODUCTION

Aquatic worms such as oligochaetes and nematodes are often the main benthic or staggered planktonic macro-organisms in source water (Luoto 2011). Once propagating excessively in source water and entering into drinking water works, these worms may penetrate through water treatment units and present in drinking water distribution systems (Van Lieverloo *et al.* 2012). Although there are no indications that these worms pose a threat to public health, their presence is not appreciated as most people associate the organisms with poor sanitation (Bichai *et al.* 2010).

Owing to increasing concerns regarding the contamination of source water, many drinking water plants in developing countries have recently installed ozone–biological activated carbon (O_3–BAC) filters to guarantee drinking water quality (Gibert *et al.* 2013). It is believed that this technology has increased the risk of contamination by aquatic macro-organisms, such as oligochaetes, nematodes, and the larvae of some insects (Schreiber *et al.* 1997; Smart & Harper 1999; Castaldelli *et al.* 2005).

Previous studies indicated that naidid reproduction in BAC filters was the main reason for increases of naidid populations in the effluent of BAC filters (Beaudet *et al.* 2000; Li *et al.* 2010; Wang *et al.* 2014). The level of naidid contamination was also found to depend on temperature

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and dissolved oxygen (DO) level in BAC filters (Beaudet *et al.* 2000; Li *et al.* 2010). However, it is still not clear how temperature and DO influence naidid reproduction in BAC filters.

To date, studies concerning naidid reproduction have mainly focused on population dynamics, yearly reproduction and biomass (Ibrahim 2007); however, few studies have focused on reproduction parameters such as generation time T, intrinsic rate of natural increase r_m and population doubling time t, despite their practical importance in the control of naidid contamination in drinking water plants. The life table method is one of the main approaches used to study population dynamics (Gabre *et al.* 2005; Golizadeh *et al.* 2009; Fernandez & Beltrán-Sánchez 2015). Life tables can be used for populations with generation overlap and stable age composition to the quantitative effects of environmental conditions on a host population (Mauri *et al.* 2003; Ren *et al.* 2016). Currently, this method has been widely used in reproduction studies of many macro-organisms, such as Diptera, Hymenoptera, and Cladocera (Mo & Liu 2006; Gama-Flores *et al.* 2007).

In the current study, we investigated: (1) the impact of temperature and DO on the population dynamics of naidids; (2) the reproduction of naidids in BAC filters. First, we conducted field studies of naidid distribution in a drinking water plant. We then investigated the effects of temperature and DO on population dynamics using the life table method. Finally, this method was also used to acquire the reproduction parameters of naidids in BAC filters based on experiments in which their growth was simulated.

MATERIALS AND METHODS

Field study of naidids

The field study was conducted at Bijiashan Water Plant in Shenzhen, Guangdong Province, China. Water samples included raw water and samples collected from the effluent of each purification unit. Wastewater samples included sludge from the sedimentation tank and backwashing water of the sand filters and BAC filters.

At the outlet of each purification unit, we installed a bypass pipe with a water valve and then hung a nylon net with a pore diameter of 45 μm to collect naidids. The nylon nets were hung for 20 h each day, after which they were washed with tap water. All flushing water was collected and allowed to stand for 2 h. The supernatant was discarded, and the residual was transferred to a polyethylene bottle. Wastewater was collected in 10–50 L, then immediately filtered through a nylon net with a pore diameter of 45 μm, after which the same washing procedure was repeated. All naidid samples were fixed with 4% formaldehyde and naidids were counted using a stereoscopic microscope.

Materials and apparatus used for reproduction experiment

The population source was naidids collected from the backwashing water of BAC filters at Bijiashan Water Plant. Each reproduction experiment was conducted using naidids from the same batch, which were obtained as follows: larger individuals were selected and placed in cell culture plates with 96 wells, with each individual in one well and three plates in total; after 24 h, 30 newborn naidids were selected, and then divided into three replicates for each reproduction experiment.

Two respective series of experiments were performed to investigate the effects of temperature and DO on the reproduction of naidids. Both reproduction experiments were performed in cell culture plates with 96 wells with a well diameter of 0.5 cm and a well depth of 1 cm, which were placed in biochemical incubators under controlled light and temperature conditions. Before being added to the wells, distilled water was aerated, and then $Na_2S_2O_3$ was added to control the initial DO level in the distilled water. During the experiment, naidids were fed freshly blended spinach juice. Another series of experiments was conducted to investigate naidid reproduction in the BAC filters, which were also simulated in cell culture plates. In this case, the influent of the BAC filters at the Bijiashan Water Plant was added to the wells without implementing measures to control initial DO levels, and suspended particles collected from backwashing water of the BAC filters were served as feed.

Each experiment was repeated three times, and naidid observation and counting were conducted using stereoscopic microscope and light microscope.

Experimental procedure

For each experiment, 30 newborn naidids were placed individually into 30 wells that contained aerated distilled water with targeted initial DO levels or influent of the BAC filters at Bijiashan Water Plant.

For temperature and DO experiments, one drop of freshly blended spinach juice was added to each well, which was filled with 0.15 mL distilled water with the target initial DO level, and then all wells were sealed with plastic film in order to keep the fluctuation of DO level as small as possible. In simulation experiments, one drop of backwashing water of the BAC filters with some amount of suspended particles was added to each well to serve as feed, and each well filled with 0.15 mL distilled water was not sealed. The reproduction experiment was initiated by placing cell culture plates into biochemistry incubators with controlled temperature and light conditions. During the experiment, water and food were changed once per day. The survival and fissiparism results of the naidids were observed, which provided the survival rate l_x and reproduction rate m_x, in which x stands for the number of days since the beginning of the experiment; l_x was the survival rate on day x and m_x was the number of fissiparism of each naidid on day x. Every day, newborn naidids were selected, counted and thrown away. Each experiment lasted until all naidids were dead.

In the temperature experiments, temperatures were set to 16 °C, 23 °C, and 30 °C, with the same initial DO level of 8 mg/L. To investigate the effects of DO, initial DO levels were set to 3 mg/L, 5 mg/L, and 8 mg/L, and the temperature was 25 °C. The temperature for the simulated experiments was set to 25 °C. The light photoperiod was 12L:12D for all of these experiments.

Life table and reproduction parameter calculation

A life table for naidids in each experiment was constructed using the development time and survivorship rates of all the life stages and daily fecundity. The net reproductive rate (R_0) was calculated as follows (Pilkington & Hoddle 2007):

$$R_0 = \sum_{(x=0)}^{\infty} l_x m_x \tag{1}$$

The intrinsic rate of natural increase (r_m) was estimated by using the iterative bisection method and the Euler–Lotka equation with the age indexed from 0:

$$\sum_{(x=0)}^{\infty} e^{-r_m(x+1)} l_x m_x = 1 \tag{2}$$

The mean generation time (T) and population doubling time (t) were calculated as follows:

$$T = \frac{\sum_0^{\infty} x l_x m_x}{R_0} \tag{3}$$

$$t = \frac{\ln(2)}{r_m} \tag{4}$$

RESULTS

Naidid distribution pattern among water purification processes

In most water purification units, naidids were detected between late August and early October (Figure 1(a)), indicating the appearance was seasonal. Naidids were occasionally detected in raw water, settled water and effluent of the sand filter, while they were most frequently detected in effluent of the BAC filter with a maximum density of 68.9 ind./m^3. Among wastewater samples, naidids were only detected in the backwashing water of the BAC filter (Figure 1(b)), with the highest density being approximately 50 ind./L.

Effects of temperature on naidid reproduction

The age-specific survival curve of naidids at 16 °C showed a typical concave shape (Figure 2(a)), indicating death primarily occurred among young individuals. The age-specific reproduction curve indicated that naidids only started to reproduce after they were 10 days old and that the reproduction rate was higher in the late stage (40–65 d) than in the early stage (10–30 d), while the average reproduction rate during the experiment was 0.14. The age-specific survival curve of naidids at 23 °C did not show a typical concave

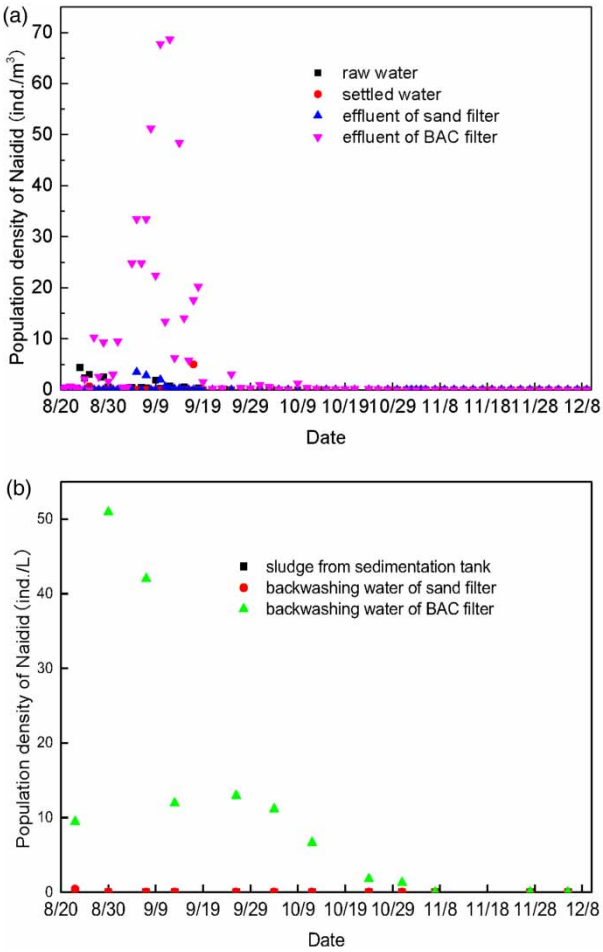

Figure 1 | Naidid distribution among the water purification processes of Bijiashan Water Plant: (a) raw water and samples collected from the effluent of each purification unit, (b) wastewater form sedimentation tank, sand filter and BAC filter. Field study was conducted from late August 2015 to early October 2015. Water samples were taken once every two days. Wastewater samples were taken once a week.

shape (Figure 2(b)); however, death still primarily occurred in the early stage. From the third day, naidids entered the reproduction phase, and no apparent regular pattern for the fluctuation of reproduction was observed. During the test period, the average reproduction rate was 0.33. When the temperature increased to 30 °C, the age-specific survival curve decreased and all individuals died within 39 days (Figure 2(c)). From the second day, naidids entered the reproduction phase and the average reproduction rate during the experiment was 0.55.

When temperature increased from 16 °C to 23 °C, both R_0 and r_m increased significantly ($p < 0.05$), with significant decrease of T and t ($p < 0.05$) (Table 1). When temperature was at 30 °C, no parameters changed significantly compared with those at 23 °C; however, all parameters significantly changed ($p < 0.05$) when compared with those at 16 °C.

Effects of initial DO on naidid reproduction

When the initial DO was 3 mg/L, the age-specific survival rate curve decreased almost linearly in the first 25 days (Figure 2(d)), then remained stable at 3.3% until day 33, after which all naidids died. The age-specific reproduction rate curve indicated that naidid reproduction mainly occurred in the middle of the experiment. When the initial DO level was 5 mg/L, the age-specific survival rate curve had a concave shape (Figure 2(e)) and the mortality in the first 5 days was 37%. Naidids entered the reproduction phase after day 5, at which time reproduction mainly occurred in the mid-term, while it stopped after day 24. During the experiment, the average reproduction rate was 0.14. At initial DO levels of 8 mg/L, the age-specific survival rate decreased linearly with a slight concave shape (Figure 2(f)), and the mortality was 70% in the first 18 days, which was much higher than that at 3 mg/L and 5 mg/L. From the first day, naidids entered the reproduction phase, while the reproduction rate increased dramatically and reached 0.95. at day 5. Throughout the experiment, the average reproduction rate was 0.62.

When the initial DO increased from 3 mg/L to 5 mg/L, no parameters significantly changed (Table 1). When the initial DO was 8 mg/L, all parameters significantly changed ($p < 0.05$) compared with those of 5 mg/L.

Naidid reproduction simulation experiment in BAC filters

During the simulation experiment, the age-specific survival rate curve decreased linearly (Figure 2(g)), and all naidids were dead on day 76. The age-specific reproduction rate curve indicated that naidids entered the reproduction phase quickly, with the reproduction rate on day 1 being 0.16. The average reproduction rate was 0.1 during the simulation experiment, and r_m and t were 0.06 d^{-1} and 12.6 d, respectively (Table 1).

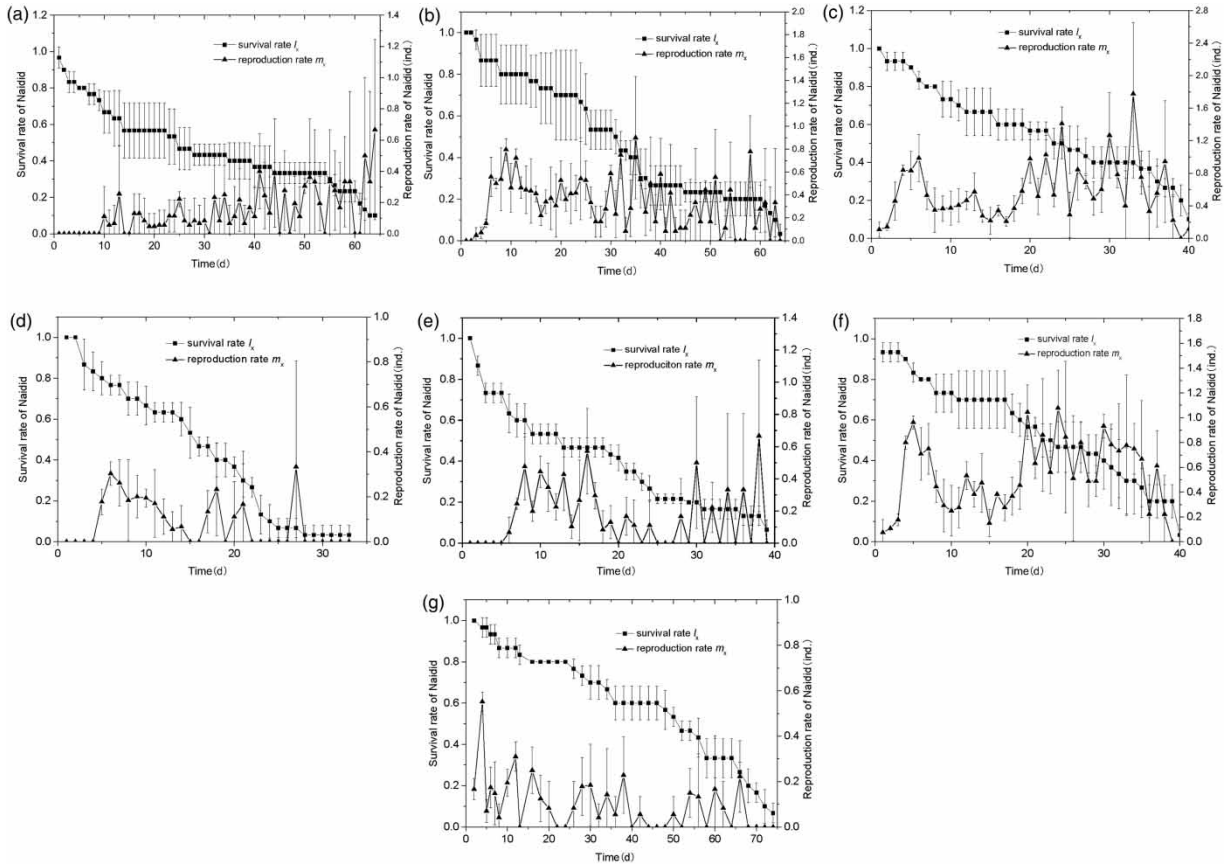

Figure 2 | Naidid age-specific survival rate and age-specific reproduction rate with different temperatures and DO: (a) 16 °C, 8 mg/L, (b) 23 °C, 8 mg/L, (c) 30 °C, 8 mg/L, (d) 25 °C, 3 mg/L, (e) 25 °C, 5 mg/L, (f) 25 °C, 8 mg/L, and (g) in reproduction simulation experiments. Reproduction experiments were performed on a 96-well cell culture plate with initial DO level of 3–8 mg/L and temperature of 16–30 °C, and blended spinach juice (a–f) and suspended particles collected from backwashing water of the BAC filters (g) were used as feed. The error line represents the standard deviation of repeated samples ($N = 3$).

DISCUSSION

Field study results indicated that naidid contamination was seasonal. Moreover, we confirm that naidid contamination mainly resulted from the excessive reproduction of naidids in the BAC filter as worm density in its backwashing water was almost 1,000 times higher than in the influent (Figure 1), which was similar to the results reported by Beaudet *et al.*

Table 1 | Naidid reproduction parameters in temperature and DO experiments and simulated experiments

Temperature (°C)	DO (mg/L)	Net reproductive rate R_0	Mean generation time T (d)	Intrinsic rate of natural increase r_m (d^{-1})	Population doubling time t (d)
16	8 mg/L	3.17 ± 0.55^{b}	37.20 ± 4.30^{a}	0.03 ± 0.01^{a}	22.96 ± 4.76^{c}
23	8 mg/L	10.40 ± 2.55^{a}	21.70 ± 2.04^{b}	0.11 ± 0.02^{c}	6.56 ± 1.12^{a}
30	8 mg/L	11.20 ± 2.39^{a}	18.06 ± 1.35^{bc}	0.13 ± 0.02^{cd}	5.22 ± 0.10^{a}
25	3 mg/L	1.93 ± 0.21^{b}	10.26 ± 1.21^{d}	0.06 ± 0.01^{b}	11.20 ± 3.23^{b}
25	5 mg/L	2.50 ± 0.17^{b}	12.99 ± 1.52^{d}	0.07 ± 0.01^{b}	9.82 ± 0.50^{b}
25	8 mg/L	12.20 ± 1.25^{a}	17.89 ± 0.59^{c}	0.14 ± 0.01^{d}	4.98 ± 0.31^{a}
25	–	2.87 ± 0.40	19.02 ± 2.84	0.06 ± 0.002	12.60 ± 0.42

Numbers with different superscripts are significantly different.

(2000) and Wang *et al.* (2014). At the end of September, naidids were not detected in the effluent of the BAC filters (Figure 1), indicating the reproduction of naidids was completed. However, naidids were detected in the backwashing water from the BAC filters until the end of October, indicating that backwashing had limited removal effects on the naidids in the BAC filters. Previous studies (Weeks *et al.* 2007; Wang *et al.* 2014) have reported that the adhesion of some aquatic worms to BAC filter media seemed to be sufficient to completely prevent themselves from being removed if the BAC filter was backwashed with water fluidization alone. Therefore, to completely remove naidids in BAC filters, increasing the rounds of backwashing was needed.

The instantaneous growth rate is commonly used in naidid reproduction studies (Löhlein 1999). However, it is very difficult for this method to quantify the effects of environmental conditions and food on naidid reproduction. In this study, t calculated using the life table method was 5.22–22.96 d, compared with the 4.8–14.7 d and 5.7–15.5 d under lab and field conditions respectively which were calculated by the instantaneous growth rate method. Considering the differences in the experimental set-up, the present results were close to those of previous studies, indicating the life table method was suitable for investigating naidid reproduction and provided more information than the instantaneous growth rate method.

When the temperature reached 23 °C, further increases of temperature induce no significant changes of reproduction parameters. It is interesting that the average results at 25 °C are equal to the ones at 30 °C and not to the ones at 23 °C. At first sight, it was not expected that the average results at 25 °C would be more similar to the ones at 30 °C and not to the ones at 23 °C. This is easily explained by the fact that there are no reproduction differences between 23 °C and 30 °C, as the results showed. Moreover, working with living organisms there are always some natural oscillations. Nevertheless, once the temperature reaches 23 °C, it is necessary to check naidid density regularly to collect information regarding population dynamics, which enable effective responses to naidid pollution breakouts.

The reproduction parameters at the initial DO of 5 mg/L had no significant difference compared with those of 3 mg/L, indicating naidids have a strong adaptive capacity to low DO conditions. This could have occurred because naidids are benthic organisms. The DO level at the bottom of BAC filters has been reported as high as 2–3 mg/L (Feng *et al.* 2012); thus, naidid reproduction may occur throughout the BAC filter. A previous study showed although naidids have strong tolerance for low oxygen conditions, they still show strong aerotaxis (Beaudet *et al.* 2000). In the current study, we also found that a high level of initial DO favored rapid naidid reproduction.

Under simulation conditions, naidids could reproduce asexually, providing direct evidence of naidid outbreaks induced by worm reproduction in the BAC filter. It has been widely reported that the backwashing cycle of BAC filters usually takes more than 7 days, sometimes reaching 28 days (Laurent *et al.* 2003; Persson *et al.* 2006; Velten *et al.* 2011). Assuming the backwashing cycle was 20 days and the naidid population doubling time t was 5 days, the naidid population density would increase by a factor of 16 within one backwashing cycle, indicating that the naidid outbreak could be inhibited only if a removal rate of 93.8% could be obtained by backwashing. However, naidids are generally firmly attached to the surface of active carbon particles (Christensen *et al.* 2011); thus, high removal rates are hard to achieve (Beaudet *et al.* 2000; Weeks *et al.* 2007). Naidid detection results from the effluent and backwashing water of the BAC filters also indicated the limited removal efficiency of backwashing (Figure 1). However, the naidid population doubling time t can be increased by temperature and other factors. When the temperature was low (e.g., <16 °C), t was longer than 22.96 days, and it was almost impossible for naidids to propagate excessively in the BAC filters. Therefore, naidid contamination in the BAC filters is seasonal, as shown in Figure 1. Once the backwashing cycle of the BAC filters lasts longer than the naidid population doubling time t, naidid contamination will probably occur. In this case, controlling the filtration cycle length to be shorter than the population doubling time t may be a promising strategy for preventing naidids' presence in the effluent of BAC filters.

CONCLUSIONS

Naidid pollution in the Bijiashan Water Plant was found to occur seasonally and to be induced by the excessive

propagation of naidids in the BAC filters. One round of BAC filter backwashing has a limited naidid removal effect. When the temperature was between 16 °C and 30 °C and initial DO between 3 and 8 mg/L, naidids could reproduce asexually, with higher temperature and DO being associated with higher reproduction rates. The simulation experiment in BAC filters indicated that naidids can reproduce asexually in BAC filters, and the population doubling time was 12.6 days, which provides direct evidence of excessive naidid propagation in BAC filters.

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