Clonal variation in reproductive response to temperature by a potential bulking control agent, *Lecane inermis* (Rotifera)

Edyta Fiałkowski, Wioleta Kocerba, Agnieszka Pajdak-Stós, Beata Klimek and Janusz Fyda

**ABSTRACT**

The novel idea of using rotifers *Lecane inermis* (Rotifera, Monogononta) as a tool to overcome activated sludge bulking generates an on-going need to study rotifer biology. The results of biological research on rotifers can serve to improve the method so that it can be most effective when applied in treatment plants. The aim of this study was to test the effect of temperature on four selected rotifer clones originating from different treatment plants. The rate of population development from a single individual (parthenogenetic female) during a 10-day experiment was measured at three temperatures: 8, 15 and 20 °C. The temperatures used reflect the annual temperature distribution in the majority of municipal wastewater treatment plants in the temperate zone. The growth rate coefficient (r) and doubling time (tD) were calculated for each clone. Under the most favourable conditions (20 °C), r values varied between 0.41 and 0.47 d and doubling time between 1.5 and 1.7 d. At a temperature of 15 °C, the doubling time was approximately two times longer (2.5–3.4 d). The strongest intraspecific variations were observed at the lowest temperature of 8 °C. At this temperature, one of the clones almost failed to proliferate, and another exhibited a doubling time of 7.9 d. The doubling times were a few times greater for the remaining two clones (60 d for Lk1, 33.3 d for Lk4). These results could be very useful in predicting the chances that the rotifers would survive in a biological reactor in a wastewater treatment plant at the temperatures used in these reactors.

**Key words** | activated sludge, intraspecific variation, rotifers, sludge age, temperature

**INTRODUCTION**

The excessive proliferation of filamentous bacteria causes bulking in activated sludge systems. Despite extensive research, this problem remains unsolved. There are still many plants where bulking control methods fail or systems for which troubleshooting procedures are too expensive to be implemented (Martins *et al.* 2004; Tsang *et al.* 2006). Moreover, up-to-date control methods for bulking in activated sludge do not limit the production of excessive sludge. The addition of chemicals could improve the settlement characteristics of the sludge. However, they could also increase the production of excessive sludge dry mass (Ødegaard 1998).

An alternative method of bulking control is to use the natural enemies of filamentous bacteria as a biological tool to limit their growth. We have obtained promising results from our preliminary studies on rotifers *Lecane inermis* as consumers of filamentous bacteria (Fiałkowska & Pajdak-Stós 2008). An additional advantage of this approach is that excessive sludge production can be reduced considerably if sufficiently effective consumers are used (Rensink & Rulkens 1997). As Lipinski & Tunnacliffe (2003) showed bdelloid rotifers are able to significantly reduce biomass production in wastewater treatment plants.

However, it is worth noting that these experiments were carried out at a temperature of 20 °C (Fiałkowska & Pajdak-Stós 2008). In municipal wastewater treatment plants, the probability of occurrence of filamentous bacteria in activated sludge increases with decreasing temperature. The greatest problems with bulking and foaming occur
when the temperature of the sludge in the reactor drops below 15 °C (Eikelboom 2000). In most cases, these problems are caused by overproliferation of the most troublesome bacteria, *Microthrix parvicella* and *Nostocoida limicola* (Eikelboom et al. 1998).

The key factor for sustaining a population of microorganisms in activated sludge is the population doubling time. This time should be sufficiently short to prevent the organisms from being washed out of the system. Thus, it is extremely important to test how temperature could modify the doubling time of rotifers. Results of previous studies of *L. inermis* have indicated that the lifespan was considerably shorter at temperatures exceeding 20 °C, whereas the number of eggs laid and the reproductive rate were highest at temperatures ranging between 21 and 29 °C (Edmondson 1946; Perez-Legaspi & Rico-Martinez 1998). In rotifers, temperature is well known to be one of the main factors affecting life-history parameters. These parameters include birth rate, growth rate and duration of embryonic development (Herzig 1983; Galkovskaja 1987; Stelzer 2002).

We examined the effect of three temperatures, 8, 15 and 20 °C, on the reproduction of four clones of the rotifer *Lecane inermis*. The values chosen reflect the temperature distribution in the majority of municipal wastewater treatment plants in the temperate zone.

### MATERIALS AND METHODS

Four clonal populations of the monogonont rotifer *Lecane inermis* were used in the experiments. The clones were isolated from different wastewater treatment plants in Poland. The Lk1 clone, cultured in the laboratory for three years, was previously used in experiments in which the rotifers were shown to be effective in controlling the growth of filamentous bacteria (Fiałkowska & Pajdak-Stós 2008). Two other strains, Lk2 and Lk3, were isolated from one of the largest treatment plants located in southern Poland. The fourth strain, Lk4, was isolated from another wastewater treatment plant in southern Poland. All strains were obtained from single individuals reared separately in wells of tissue culture test plates (Cell Wells™ Corning) filled with Żywiec brand mineral water and 25 μL of 3.0‰ molasses solution (Greenland Technologia products for EM-1 proliferation) as a medium. In each well of a 24-well tissue culture test plates. The rotifers were fed on bacteria that proliferated on the oat grains. The same techniques were used to maintain each culture: rotifers were grown in Petri dishes kept in darkness at a temperature of 20 °C.

We measured the rate at which populations developed from single individuals. To check the fecundity of individual females during their lifetime, the experiment lasted 10 days, one day longer than the mean lifetime of amictic females of *Lecane inermis* (Miller 1951). One rotifer was transferred into each well of a 24-well tissue culture test plates. The rotifers were of approximately the same age because they were chosen from hatching eggs separated from the cultures one day previously. Each well contained 1 mL of Żywiec brand mineral water and 25 μL of 3.0‰ molasses solution (Greenland Technologia products for EM-1 proliferation) as a medium. During the experiment, we checked the influence of the three temperatures 8, 15 and 20 °C. The plates containing rotifers were kept in Sanyo MLR-350 environmental test chambers. Twelve replicates (wells) were used for each strain and temperature. The number of living and dead rotifers and the number of eggs were counted directly in the wells with the help of an inverted microscope working at a total magnification of 100x. The counts were repeated every 24 h. The values of the population growth rate coefficient (r) and doubling time (tD) are of particular interest for the use of rotifers in treatment plants. These values were calculated as described in James & Dias (1985).

To investigate possible differences between clones in the number of live rotifers and eggs relative to temperature, factorial ANOVA and post hoc unequal N HSD tests were used to analyse data from the wells in which the females survived until the 10th day.

### RESULTS

The results of our experiment show a strong influence of temperature on the proliferation of rotifers. At the lowest temperature of 8 °C, the number of living rotifers was very low throughout the experiment, and no differences were found between clones (Figure 1). At 15 °C, the number of living rotifers was very low, and the number of rotifers increased very slowly during the experiment. However, slight differences were observed between clones: on the last day of the experiment, the highest number of rotifers was observed in clones Lk2 and Lk3. The differences between clones were most apparent at the highest temperature. In each clone, the number of rotifers started to increase between the 4th and the 5th day. Except in clone Lk2, the increase continued until the very end of the experiment. The highest number of living rotifers was exhibited by the Lk3 clone and exceeded 100 individuals per ml. At a
The greatest increase in the number of living rotifers occurred between the 7th and the 9th day (Figure 1). Accordingly, the $r$ coefficient was calculated for each clone between the 7th and the 9th day to determine the clone having the highest growth rate (Table 1). Surprisingly, at 8°C, clone Lk2 proved to have the highest growth rate. At 15 and 20°C, the values of $r$ were very similar in all clones (Table 1). The $r$ coefficient values calculated for each clone between the 1st and the 10th day showed low intraspecific variation. The $r$ values for the four rotifer clones varied between 0.01 and 0.09 at 8°C, between 0.22 and 0.28 at 15°C and between 0.41 and 0.47 at 20°C.

The doubling time calculated for observations between the 1st and the 10th day at 8°C was the shortest for the Lk2 clone (7.94 d), whereas the longest doubling time was observed for the Lk1 clone (60 d). The minimum doubling time at 15°C was observed for Lk3 (2.48 d). At 20°C, the doubling times were similar for all clones and exhibited values between 1.48 and 1.73 (Table 2).

The experiment also showed that the temperature affects the number of eggs laid and that the strength of this influence varies among rotifer clones. At the lowest temperature, solitary eggs were detected in all clones. The differences between clones were visible at 15°C (Figure 2). In clones Lk1 and Lk4, the number of eggs began to increase on the 7th day and did not exceed 20. In clones Lk2 and Lk3, the increase also started on the 7th day, but the number of eggs at the end of the experiment was greater than 20. The differences among clones were most pronounced at a temperature of 20°C. Clone Lk3 differed significantly from the other three clones (unequal N HSD test, $p < 0.001$).

### Table 1

<table>
<thead>
<tr>
<th>Temp</th>
<th>Lk1</th>
<th>Lk2</th>
<th>Lk3</th>
<th>Lk4</th>
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<tbody>
<tr>
<td>8°C</td>
<td>0.32 ± 0.18</td>
<td>0.03 ± 0.1</td>
<td>0.03 ± 0.1</td>
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<tr>
<td>15°C</td>
<td>0.39 ± 0.16</td>
<td>0.39 ± 0.1</td>
<td>0.42 ± 0.12</td>
<td>0.59 ± 0.24</td>
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<tr>
<td>20°C</td>
<td>0.56 ± 0.14</td>
<td>0.53 ± 0.08</td>
<td>0.54 ± 0.09</td>
<td>0.51 ± 0.07</td>
</tr>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Temp</th>
<th>Lk1</th>
<th>Lk2</th>
<th>Lk3</th>
<th>Lk4</th>
</tr>
</thead>
<tbody>
<tr>
<td>8°C</td>
<td>0.01 ± 0.03</td>
<td>0.09 ± 0.03</td>
<td>0.03 ± 0.04</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>15°C</td>
<td>0.22 ± 0.06</td>
<td>0.25 ± 0.06</td>
<td>0.28 ± 0.02</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>20°C</td>
<td>0.41 ± 0.06</td>
<td>0.44 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>0.43 ± 0.02</td>
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<table>
<thead>
<tr>
<th>Temp</th>
<th>Doubling time (tD)</th>
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<tbody>
<tr>
<td>8°C</td>
<td>60</td>
</tr>
<tr>
<td>15°C</td>
<td>5.34</td>
</tr>
<tr>
<td>20°C</td>
<td>1.73</td>
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**Figure 1** The change in the number of living rotifers from each experimental clone at the temperatures of 8°C (♦), 15°C (▪) and 20°C (▴) throughout the experiment.
The change in the number of eggs laid by rotifers from each experimental clone at the temperatures of 8 °C (♦), 15 °C (▪) and 20 °C (▴) throughout the experiment.

**DISCUSSION**

Rotifers of the species *Lecane inermis* have been found to be efficient at limiting the number of filamentous bacteria in activated sludge (Fiałkowska & Pajdak-Stós 2008). Temperature and food are among the most important factors influencing the growth and development of rotifers (Perez-Legaspi & Rico-Martinez 1998). Therefore, it is of vital importance to know how temperature affects the reproduction of different clones of rotifers that are to be used as a potential biological agent to control bulking.

Developmental periods length in a poikilotherm are closely related to temperature. According to Weltzien and co-authors, the duration of developmental periods increases in lower and decreases in higher temperatures (Weltzien et al. 1999). Within a tolerable temperature range, an organism can carry out normal activities, although the ambient temperature exerts a major influence on the pace of various biological processes (Fry 1947). The intrinsic rate of population increase is a comprehensive parameter including age-specific survival and fecundity, age at maturity and reproductive interval (Ma et al. 2010). Thus, the intrinsic rate of population increase is an especially useful parameter for predicting population growth at different temperatures. Rotifer populations exhibit a complication in that two types of eggs may be laid, mictic and amictic (Miller 1931). However, the clones used in the experiments described in this paper have never produced males or amictic eggs during laboratory cultivation. The effect of temperature on the intrinsic rate of population increase of rotifers has been shown to depend on genotype and population (Ricci 1991; Perez-Legaspi & Rico-Martinez 1998; Tao et al. 2008), but it may also result from plasticity through indirect effects of the changes in body size and egg size (Stelzer 2002). In a laboratory experiment, Ma et al. (2010) have shown that eight geographic populations of *Brachionus calyciforus* from Chinese regions characterised by a range of mean annual temperatures differed in such population parameters as the intrinsic rate of population increase or the proportion of sexual offspring when cultured at 18, 23 and 28 °C. Miracle & Serra (1989) have reviewed the effects of temperature on rotifer population dynamics. In their review, they...
underline the clear relationship between temperature and $r$ values. They have shown that $r$ increases exponentially in relation to temperature and that the slope of the response depends on the genotype. Intraspecific differences in the population growth coefficient in relation to temperature were confirmed by our experiments (Table 2).

Considered as a potential biological tool to control bulking, the most desirable species/clones of rotifers are those that can be efficient enough to overcome sludge bulking under given temperature conditions. In the present study, the doubling time and growth rate values were similar for all clones investigated at temperatures of 15 and 20°C. Information about the intrinsic growth rate and the doubling time of a certain organism exceeds the sludge age, then the organism will be washed out of the system along with the excess sludge. It is interesting to note that at the lowest temperature of 8°C, the highest value of the $r$-coefficient was attained by the Lk2 clone (Tables 1 and 2). Surprisingly, the doubling time for this clone, calculated over the 10 days of the experiment, was much less than the doubling times found for the other clones. Indeed, it was eight times less than the doubling time found for the Lk1 clone. This result suggests that it is possible to select clones of *Lecane inermis* that show better adaptation to lower temperatures. If an appropriate inoculum of such rotifers is transferred to the reactor before the winter, then their population will have a chance to survive. The population may even have a chance to grow if a longer sludge age is used.

Slacedek (1983) has emphasised that rotifers are especially likely to occur in treatment plants having longer retention times because the reproductive rate in rotifers is relatively slow (days or weeks). For this same reason, they cannot persist in plants having shorter retention times. Our results show that *Lecane inermis* is one of the most fecund species of rotifers. Its population is able to double its abundance at a temperature of 20°C within approximately a day and a half and within four days at 15°C (or slightly longer, depending on the clone). Because the minimum sludge age that is usually applied in a system using nutrient removal is 7 days during the summer and 10 days during the winter, we can predict that when an appropriate inoculum of rotifers is transferred to the biological reactor they would be able not only to survive but also to proliferate. Our results might be helpful in predicting how the population of rotifers would react to changes in temperature, giving the WWTP operators a chance to control the sludge age so that the number of the consumers of filamentous bacteria being washed-out is significantly reduced.

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**REFERENCES**


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