Effect of salinity during resting egg formation and hatching on descendent reproduction in the rotifer *Brachionus rotundiformis* Tschugunoff

Hee-Jin Kim and Atsushi Hagiwara*

Graduate School of Science and Technology, Nagasaki University, Bunkyo 1-14, Nagasaki, Japan

*Author for correspondence

Tel & Fax: 81-95-819-2830, E-mail: hagiwara@nagasaki-u.ac.jp

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Abstract

This study investigated the effect of experienced salinity during resting egg formation and hatching on the descendents’ reproduction, and on the resting egg hatching in the monogonont rotifer *Brachionus rotundiformis* Tschugunoff. The study was divided into two parts. First, the resting eggs formed at 17 psu were incubated at four salinities (8, 11, 22 and 33 psu) and then the hatchlings were cultured at the same salinities as incubated salinities. Second, the resting eggs formed at the four salinities were incubated at the same salinities as formed salinities, and the hatchlings were cultured at 33 psu. The resting eggs formed at a salinity of 17 psu and incubated at four different salinities, showed a higher percent of hatching at lower salinities (8 and 11 psu). When resting eggs incubated at the same salinities as at formation (8, 11, 22 and 33 psu), the eggs showed no significant differences in a total hatching rate among treatments (83.3-86.7%). There was no significant difference in population growth when hatchlings from resting eggs experienced no salinity changes. While, hatchlings at 8 psu was comparatively inactive in the population growth at 33 psu caused by serious salinity increase. In sexual reproduction, rotifers showed different patterns associated with the experienced salinity during resting egg hatching. This study shows that the experienced salinity during resting egg formation is optimal one for egg hatching. Moreover, the incubated salinity during resting egg hatching strongly affects the descendents’ sexual reproduction than the experienced salinity during egg formation.
Introduction

The euryhaline monogonont rotifer *Brachionus rotundiformis* Tschugunoff is commonly distributed in tropical and subtropical estuaries (Segers, 1995). The dominant feature of the estuarine environment is salinity fluctuation by the following factors: the tide; Coriolis effect; and results of seasonal changes in evaporation (Nybakken, 2001; Chanson and Trevethan, 2006). Moreover, the seasons in the tropics and subtropics are dominated by the movement of the tropical rain belt and results in a dry season and a wet season, which are other factors influencing the salinity variation in these areas (Ridd and Stieglitz, 2002). Thus, salinity variation in the tropics and subtropics is more pronounced than in other regions, and estuarine animals in these areas must have the capability to adapt to salinity variation and maintain the ionic balance of body fluids, in accordance with environmental salinities (Calder and Maýal, 1997; García-Roger et al., 2008; Dumont, 1983; Nybakken, 2001; Saraswathy and Nair, 1974). Salinity plays an important regulatory role in the reproductive system of monogonont rotifers (Cabrera et al., 2005; Hagiwara et al., 1988, 1989; Hino and Hirano, 1988; Lubzens et al., 1985a; Pozuelo and Lubián, 1993). Thus, seasonal changes of salinity in the tropical and subtropical estuaries are directly related to the reproduction of monogonont rotifers.

The monogonont rotifer *B. rotundiformis* exhibits cyclic heterogony and alternating asexual (amictic) reproduction with sexual (mictic) reproduction. The rotifers show a punctuated sexual reproduction pattern which is the optimal strategy in their habitats to endure harsh environmental changes (Dumont, 1983; Schröder, 2005; Ricci, 2001). Hagiwara et al. (1995) reported salinity effects on reproduction in various *B. rotundiformis* strains. They found that Fiji and Thai strains showed active asexual and sexual reproduction at lower salinities, while rotifers showed a huge variation of reproduction parameters even when the same clonal rotifers were cultured at the same salinity (Hagiwara et al., 1988, 1989, 1995). Thus, we assumed that other factors such as experienced salinity during resting egg formation and hatching, affect reproduction of descendent
rotifer populations. The resting egg pool of a natural population contains eggs produced at different times of the year, under different environmental conditions (García-Roger et al., 2005, 2006). These parental reproductive features from experienced environmental salinity are transferred to the next generation (Hino and Hirano, 1985, 1988).

Amictic females hatched from resting eggs (stem female) are more vulnerable to environmental conditions and easily get reproduction characteristics from experienced environmental condition (Hagiwara et al., 2005; Gilbert, 2003). These reproduction features can be inherited by their offspring through the maternal (stem female’s) cytoplasm (Hagiwara et al., 2005; Gilbert, 2003). As sporadic salinity varies considerably in tropical and subtropical estuaries, rotifer populations in these habitats could experience salinity variation during resting egg formation and hatching. For resting egg hatching, external conditions during not only resting egg incubation, but also resting egg formation are important influence factors (Gilbert, 1980; Hagiwara and Hino, 1989; Lubzen et al., 1985b). Both experienced salinity during resting egg formation and hatching affect not only resting egg hatching but also rotifer descendents’ reproduction. However, no studies have investigated what level of salinity most strongly affects descendent reproduction and resting egg hatching when salinities are varied at different life stages. We investigated whether experienced salinity during different stages (resting egg formation and hatching) affect descendents’ reproduction and resting egg hatching in the rotifer B. rotundiformis Tshugunoff.
Method

The Italian rotifer, *Brachionus rotundiformis* Tshugunoff, was used in the current study. We divided the study into two parts: (*i*) the effect of exposure to differing salinity levels during resting egg hatching and subsequent hatchling culture; (*ii*) the effect of exposure to differing salinity levels during resting egg formation and hatching (see Fig. 1). All the resting eggs used in the present study were preserved at 4.0 ± 0.2°C in total darkness for three months after formation. The resting eggs were produced under a regimen of daily feeding of $0.24 \times 10^6$ *Tetraselmis tetrathele* cells/mL at 30.0 ± 0.5°C in both experiments.

Experiment 1

The first experiment investigated the effect of exposure to different salinity levels during resting egg hatching and subsequent hatchling culture on the pattern of population growth and sexual reproduction among descendant rotifers. Forty resting eggs formed at 17 psu (practical salinity unit) were incubated in a single well of six-well polystyrene microplate containing 5 mL of either 8, 11, 22 or 33 psu water and food suspension of *T. tetrathele* ($0.24 \times 10^6$ cells/mL). Each treatment was replicated six times, and was incubated at 30.0 ± 0.5°C under continuous light (3000 lx) without aeration. The number of new hatchlings was counted once a day for each of the six replicates. Counted hatchlings were removed from their well for counting of new hatchlings continued for three consecutive days (a total of 18 counts of new hatchlings in each treatment). The total hatching rate per treatment was calculated as the mean of 18 counts made of three days. The hatchlings were inoculated at 1 ind./mL to one of 12 screw-capped bottles (three replicates for each of four treatments) containing 25 mL water of the same salinity as during resting egg hatching (8, 11, 22 or 33 psu). They were cultured at 30±0.5°C on *T. tetrathele* ($0.24 \times 10^6$ cells/mL; same amount added daily) without aeration in total darkness for seven days. All culture
media were prepared by diluting natural seawater with milli-Q water (Millipore 0.2 μm) followed by GF/C filtration and sterilization (121°C, 15 min). The number of each of four types females (list 4 types), and the number of males, were recorded daily. Females were classified into four types: females of unknown reproduction status without eggs (?♀); female-producing amictic females (F♀); male-producing mictic females (M♀); and resting egg-producing mictic females (R♀). Females without eggs (?♀) included immature females, post-reproductive females and non-spawning adult females (Hagiwara et al., 1988; Sudzuki, 1964). The population growth (total number of female individuals/day) and the two sexual reproduction parameters, percent mixis and fertilization were calculated by the following three equations (Hagiwara et al., 1988):

Population growth rate (r): \(\ln (N_t/N_0) / t\)

Mixis (%): [(M♀+R♀)/(F♀+M♀+R♀)]X100

Fertilization (%): [(R♀)/(M♀+R♀)]X100

where t is the culture days, and N₀ and Nₜ are the number of all the types of female rotifers on Day 0 and t, respectively. Resting eggs were harvested from Day 4 to the end of the culture. The average data of triplicates were used to calculate the reproduction parameters.

Experiment 2

The second experiment investigated the effect of experienced salinity during resting egg formation and hatching on the pattern of three reproduction parameters (population growth rate, percent mixis and fertilization) in the descendent rotifers (see Fig. 1). Resting eggs formed at four salinities (8, 11, 22 and 33 psu) were hatched in six-well polystyrene microplates. Forty resting eggs were placed in a microplate well containing 5 mL of water at one of four salinities and food as described above. Each treatment of hatchlings were inoculated into 33 psu salinity water and cultured for seven days in 12 screw-capped bottles (three replicates for each of four treatments).
Individual culture

The individual cultures were performed to investigate the causes for the differences of the reproduction parameters in the first experiment. Twenty-four hatchlings from resting eggs formed at 17 psu were incubated at one of four salinities (8, 11, 22 and 33 psu). At 24 hours after incubation, hatchlings (≤2h post-hatch) were individually allocated to 24-well polystyrene microplates containing 2 mL of the same salinity water and food as the first experiment. We observed the rotifers every 8 hours, and recorded the number of offspring and condition of the maternal females three times a day. After the last daily observation, rotifers were transferred to another well containing fresh water and food. In order to estimate a trend of offspring production, “four parameters Weibull regression equation” was used.

Statistical analysis

Analysis of variance (ANOVA) was used to evaluate the effect of salinity on the population growth rate, sexual reproduction parameters and the number of offspring. When significant effects were found, the Bonferroni/Dunn multiple comparison test was performed to see if salinity variation affected descendent reproduction parameters. In the case of resting egg hatching, the Tukey-Kramer multiple comparison test was performed after the ANOVA test. All of the statistical analysis in the present study was tested by Statview version 5.0 software (SAS Institute, Inc., USA).
Results

Resting egg hatching

In the first experiment illustrated in Fig. 1, resting eggs formed at 17 psu started to hatch one day after incubation (Day 1), at all four salinities (8, 11, 22 and 33 psu). Fifty percent hatching was reached on Day 2 at 8 and 11 psu but not until Day 3 at 22 and 33 psu (Fig. 2a). The hatching rate was significantly higher at 8 and 11 psu (62.9 and 64.6%, respectively) than at 22 and 33 psu (48.3 and 40.0%, respectively; Tukey-Kramer test, \( p < 0.05 \)).

In the second experiment (Fig. 1), resting eggs were both formed and incubated at one of four salinities (8, 11, 22 or 33 psu). There was no significant difference in the total percent of hatching (83.3–86.7%) among treatments (ANOVA test, \( p > 0.05 \)). However, differences in temporal pattern of hatching were observed (Fig. 2b). Over 50% of hatching was achieved at 8 and 11 psu on Day 1 (78.3% and 80.0%, respectively), while less than 50% of hatching at 22 and 33 psu occurred on Day 2 (46.7 and 25.0%, respectively).

Batch culture

In the first experiment (Table 1; Fig 3a), the rotifers from resting eggs formed at 17 psu showed no significant differences (1.19±0.05 - 1.31±0.01/day) in population growth on Day 4 (ANOVA test, \( p > 0.05 \)). In the second experiment (Table 2; Fig 3b), the hatchlings from resting eggs formed at 11, 22 and 33 psu showed no significant differences; 0.88 ± 0.02 - 0.99 ± 0.01/day in population growth on Day 6, except the hatchlings at 8 psu (0.58 ± 0.10/day; Tukey-Kramer test, \( p < 0.05 \)).

In the sexual reproduction, the rotifers from resting eggs formed at same salinity (17 psu) showed different pattern of sexual reproduction (Experiment 1; see Table 1). Higher percent of
mixis and fertilization were shown at 11 and at 8 psu, respectively. Two types of mictic females comprising male, and resting egg-producing, females, initially appeared on Day 1 and Day 2, respectively (Fig. 3a), and the highest rate of mixis and fertilization occurred on Day 2 and Day 4, respectively at all salinities (Fig. 4a). The highest numbers of resting eggs were produced at 8 and 11 psu (18.2 and 17.8 eggs/mL, respectively), while rotifers were significantly less productive at 22 and 33 psu (8.4 and 2.6 eggs/mL, respectively; Bonferroni/Dunn test, \( p < 0.05 \); Fig. 5a).

The rotifers from resting egg hatching at different four salinities were cultured at the same salinity (33 psu, second experiment), they maintained at 22 and 33 psu during resting egg incubation showed the highest number of male-producing females (Fig. 3b). However, the highest number of males (11.5 ind./mL) was occurred by the rotifer population from resting egg hatching at 11 psu. Rotifers maintained at 8 psu pre-hatch were comparatively hindered in both types of reproduction; they showed the lowest population growth rate (\( r = 0.58 \pm 0.10/\text{day} \); Table 2) and the slowest induction rate of mixis and fertilization on Day 4 and Day 6, respectively (Fig 4b). Rotifers treatments from 22 and 33 psu were inactive in the sexual reproduction with mixis (1.2 ± 0.1 and 1.4 ± 0.5 %, respectively) and fertilization (57.2 ± 6.4 and 67.2 ± 6.7 %, respectively; Table 2). The rotifers from resting egg hatching at 11 psu formed the highest number of resting eggs (24.3 ± 3.5 eggs/mL), followed by 33, 8 and 22 psu (16.6 ± 5.2, 9.9 ± 5.0 and 8.9 ± 0.6 eggs/mL, respectively; Bonferroni/Dunn test, \( p < 0.05 \); Fig. 5b).

Individual culture

Among individual cultures associated with first experiment, rotifers showed no significant differences in lifespan (5.1 - 6.6 days) or the number of offspring produced per amictic female (17.1 - 22.0 ind.; ANOVA test, \( p > 0.05 \); Table 3). However, the temporal pattern of offspring production varied among treatments (Fig. 6). Rotifers cultured at 8 and 11 psu produced a larger number of offspring in the early phase of population growth (see Day 1, Fig 6) and showed faster
decline in offspring production. On the other hand, rotifers cultured at 22 and 33 psu produced lower numbers of offspring at Day 1 and a slower decline in offspring production. All of the rotifer treatments showed the peak of offspring production on Day 2 (Fig. 6).
Discussion

A dominant feature of tropical and subtropical estuaries is the seasonal fluctuation of salinity, which makes these areas a particularly stressful and rigorous habitat. For organisms to survive and successfully colonize this area, they must possess certain physiological tolerances (Calder and Mañal, 1997; Dumont, 1983; Nybakken, 2001; Saraswathy and Nair, 1974). The estuarine rotifer *Brachionus rotundiformis* Tshugunoff is capable of tolerating salinity variation like many other euryhaline zooplanktons (Assavaaree et al., 2003). We hypothesized that resting egg formation and hatching associated with seasonal changes of salinity could affect descendental sexual reproduction. In order to test this hypothesis, culture conditions (i.e., temperature and food) were adjusted to induce active sexual reproduction of *B. rotundiformis* according to previous studies (Assavaaree et al., 2003; Hagiwara et al., 1989, 1995; Hirayama and Rumengan, 1993). In the presence of abundant and high quality food and an optimal temperature for rotifer population growth, the rotifers in the current study showed strong population growth compared to a natural population (<0.5 ind/mL, Snell et al., 2001). However, natural zooplankton populations, including rotifers, also show sporadic high population densities, for example, local blooms in response to strong nutrient inflow (Nybakken, 2001). Moreover, Carmona et al. (1995) found that *B. rotundiformis* has periodic population blooms in nature and that rotifer population densities are highly variable throughout the annual cycle, ranging from 3 to 16,700 ind/L.

In the current study, population growth (*r*) showed no significant difference when rotifers were cultured at the same salinities as at hatching (8, 11, 22 and 33 psu, see Table 1). Compared to *B. plicatilis* which shows significant differences of the population growth rate in relation to culture salinity variation (Hagiwara, 1994; Hagiwara et al., 1989; Hino and Hirano, 1985; Lubzens et al., 1985a; Park and Hur, 1996; Pourriot and Snell, 1983), the salinity endurance of *B. rotundiformis* is stronger than *B. plicatilis*. On the other hand, *B. rotundiformis* showed decreasing population density when they experienced serious salinity variation (in the second experiment; Table 2).
When the hatchlings at 8 psu were cultured at 33 psu, only 25% of them survived on Day 1, and the population growth was slow because of serious salinity increase. This obstacle of population growth (salinity increase) should affect the sexual reproduction, because sexual reproduction occurs when parthenogenetic reproduction; population growth, actively occurs (Gilbert, 1963; 2003; Lubzens et al., 1985b; Hagiwara et al., 1988, 1989, 1997; Hino and Hirano, 1976; Snell, 1987; Ricci, 2001).

The rotifer *B. rotundiformis* showed differing sexual parameters (i.e., mixis, fertilization and resting egg formation) associated with the experienced salinity during resting egg hatching. Changes of salinity gradients in tropical and subtropical estuaries typically reflect seasonal variations in evaporation (dry season), fresh-water flow (wet season), or both. A mixis pattern of rotifers reproduction would be the optimal strategy in these habitats because rotifers are limited by the effect of salinity fluctuation and decreasing temperatures (Carmona et al., 1995; Dumont, 1983; King and Serra, 1998; Ricci, 2001; Serra et al., 1998). Those changes during the resting egg formation and incubation are known to affect the reproduction in the parental rotifer population, and these characteristics are transmitted to next generations (Gilbert, 2002; Hino and Hirano, 1976, 1985, 1988; Hagiwara et al., 2005; Stelzer, 2005). The stem females which are diploid amictic females from resting egg hatching are difficult to distinguish from asexually produced amictic females by their morphology alone. On the other hand, they are more vulnerable to environmental conditions than amictic females in the parthenogenetic life cycle. The reproduction characteristics which stem females get from environmental condition are transferred to descendant by their cytoplasm (Gilbert, 2003; Hagiwara et al., 2005). In the first experiment, *B. rotundiformis* showed differences in the pattern of sexual reproduction associated with the type of salinity exposure from the hatching to the culture, although all of the resting eggs were formed at the same salinity (17 psu) (Table 1, Figs. 3a, 4a, 5a). In the second experiment, all the rotifers were cultured at the same salinity (33 psu), while they showed different patterns in the sexual reproduction associated with the level of salinity exposure from resting egg formation to
hatching (Table 2, Figs. 3b, 4b, 5b). According to these results, the level of experienced salinity during resting egg hatching more actively affect descendant sexual reproduction because of the specific characteristics of stem females.

In the individual culture, there were no differences in the number of offspring, and lifespan, associated with salinity variation (Table 3). However, temporal patterns of population growth differed with culture salinity. The rotifers at 8 and 11 psu produced higher number of offspring in the early phase of life span than at 22 and 33 psu (Fig. 6). The inducer of sexual reproduction is the population density in the early phase of population growth (Gilbert, 1963; 2003; Lubzens et al., 1985b; Hagiwara et al., 1988, 1989, 1997; Hino and Hirano, 1976; Snell, 1987; Ricci, 2001). Moreover, the fate of offspring oocyte (amictic or mictic) is determined by maternal population density during oogenesis (Gilbert, 2007). Thus, the induction of sexual reproduction was active at 8 and 11 psu in the experiment 1. The rotifers showed active population growth at lower salinities (8 and 11 psu) in the early phase of population growth, and this population growth pattern affects sexual reproduction.

When the hatchlings at 22 psu were cultured at 33 psu, they formed the lowest number of resting eggs (see Fig. 5b), even if they showed no significant difference in the rate of mixis and fertilization compared to the rotifers experiencing no salinity change; hatchlings at 33 psu were cultured at 33 psu (Table 2, Fig. 4b). Hagiwara et al. (1989) suggested that salinity might be another factor which directly or indirectly regulates the fertilizing capacity of males. In this experiment, the salinity increase obstructed the reproductivity of female rotifers, and high salinity affect the fertilizability of male rotifers. Thus, the rotifers at 33 psu from resting egg hatching at 22 psu produced the lowest number of resting eggs because of reductions in both female and male sexual reproductivity.

Gilbert (1980) reported that the resting eggs show the highest hatching rate when eggs are incubated in the same conditions (i.e., temperature and salinity) as formation. Moreover, García-Roger et al. (2008) found that resting egg hatching was the most successful when eggs from field
sediments were incubated at the same salinity as in collecting area. In the present study, resting eggs formed at 17 psu showed significant differences in hatching rate associated with incubated salinity (Fig. 2a). However, the resting eggs were incubated at same salinity as formation salinity, all treatments showed same hatching rate. Moreover, the hatching rate was higher than when eggs were incubated at different salinities from formation salinity (Fig. 2a, b). Thus, the present study also showed that the salinity during resting egg formation is appropriate for egg hatching.

The current study emphasizes that the experience of salinity variation in the different developmental stages affect the descendant sexual reproduction. The experienced salinity during resting egg hatching has a stronger effect on the descendents’ sexual reproduction than during resting egg formation. In tropical and subtropical estuaries, the females from resting egg hatching (stem females) experience salinity variation, and effectively respond to environmental changes. After then their reproduction patterns which are acquired from environmental conditions are transferred to descendant. This feature enables rotifer populations continue to reproduce in the presence of fluctuating environment.
Acknowledgments

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References


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Nausitora hedleyi Schepman (Bivalvia-Teredinidae). Hydrobiol., 44(4), 397-411.


Table 1. Population growth rate ($r$) and two sexual reproduction parameters: mixis and fertilization rate when the rotifer *B. rotundiformis* was cultured at the same salinity as at hatching (8, 11, 22 and 33 psu). The indicated salinity is at the resting egg hatching and rotifer culture.

<table>
<thead>
<tr>
<th>Salinity (psu)</th>
<th>Population growth (/day)</th>
<th>Mixis (%)</th>
<th>Fertilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.19 ± 0.08</td>
<td>15.8 ± 6.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>74.1 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>1.19 ± 0.05</td>
<td>23.9 ± 11.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.9 ± 7.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>22</td>
<td>1.25 ± 0.03</td>
<td>11.7 ± 2.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>63.9 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>33</td>
<td>1.31 ± 0.01</td>
<td>6.3 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.9 ± 11.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Values in the same column with different superscript letters are significantly different (a>b>c, Bonferroni/Dunn test, n=3, $p<0.05$).
Table 2. Population growth ($r$) and two sexual reproduction parameters: mixis and fertilization when the rotifer *B. rotundiformis* was cultured at 33 psu after hatching at four salinities (8, 11, 22, and 33 psu). The indicated salinity is at the resting egg formation and hatching.

<table>
<thead>
<tr>
<th>Salinity (psu)</th>
<th>Population growth ( /day)</th>
<th>Mixis (%)</th>
<th>Fertilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>$0.58 \pm 0.10^b$</td>
<td>$3.3 \pm 1.7^{ab}$</td>
<td>$53.9 \pm 13.4^b$</td>
</tr>
<tr>
<td>11</td>
<td>$0.88 \pm 0.02^a$</td>
<td>$4.8 \pm 1.2^a$</td>
<td>$86.5 \pm 4.0^a$</td>
</tr>
<tr>
<td>22</td>
<td>$0.99 \pm 0.01^a$</td>
<td>$1.2 \pm 0.1^b$</td>
<td>$57.2 \pm 6.4^b$</td>
</tr>
<tr>
<td>33</td>
<td>$0.98 \pm 0.03^a$</td>
<td>$1.4 \pm 0.5^b$</td>
<td>$67.2 \pm 6.7^{ab}$</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation. Values in the same column with different superscript letters are significantly different (a>b, Bonferroni/Dunn test, n=3, $p<0.05$).

Table 3. Lifespan and the number of offspring when the rotifer *B. rotundiformis* was cultured at four salinities (8, 11, 22 and 33 psu).

<table>
<thead>
<tr>
<th>Salinity (psu)</th>
<th>Life span (day)</th>
<th>No. of offspring (/amictic female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>$5.5 \pm 1.4$</td>
<td>$22.0 \pm 6.2$</td>
</tr>
<tr>
<td>11</td>
<td>$5.1 \pm 1.8$</td>
<td>$18.0 \pm 11.0$</td>
</tr>
<tr>
<td>22</td>
<td>$5.4 \pm 2.1$</td>
<td>$17.2 \pm 7.6$</td>
</tr>
<tr>
<td>33</td>
<td>$6.6 \pm 3.2$</td>
<td>$17.1 \pm 8.5$</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.
Fig. 1. Experimental design for two experiments in which parental females ($\varphi^p$), are exposed to differing salinities.
Fig. 2. The variable pattern of resting egg hatching at four salinities. The average of six replicates was plotted to illustrate (a) the percent of hatching of resting eggs formed at 17 psu and incubated at different salinities (8, 11, 22, and 33 psu) and (b) of resting eggs incubated at the same salinity at which they were formed (8, 11, 22, and 33 psu). Lower-case letters indicate significant differences (a>b, Tukey-Kramer test, \( P<0.05 \)).
Fig. 3. Population growth curves of amictic and two types of mictic females (the male and resting egg-producing females) and males. (a) Results for rotifers cultured at the same salinities as at hatching (8, 11, 22 and 33 psu), and (b) shows results for rotifers that were cultured at 33 psu, after they hatched at four salinities.
Fig. 4. The pattern showing reproduction parameters for rotifers cultured (a) at the same salinities as at hatching (8, 11, 22 and 33 psu) and (b) for rotifers that were cultured at 33 psu after hatching at four salinities.
Fig. 5. The number of resting eggs formed by rotifers cultured (a) at the same salinities as at hatching (8, 11, 22, and 33 psu) and (b) for rotifers that were cultured at 33 psu after hatching at four salinities. Lower-case letters and vertical lines describe significant differences and standard deviations, respectively (a>b, Bonferroni/Dunn test, $P<0.05$).
Fig. 6. The number of offspring produced daily during a lifetime of single amictic female rotifers, at four salinities in Experiment 1. The trend lines were drawn by four parameters Weibull regression equation.