# シオミズツボワムシ(L,S型)の飼育水温と耐久卵大量形成について


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# Resting Egg Formation of the L- and S-type Rotifer *Brachionus plicatilis* under Different Water Temperature*[1,2]

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Rotifer resting eggs can potentially be preserved in a manner similar to *Artemia* cysts and used as live feed in larval rearing. The potential for producing resting eggs from the most desirable rotifer stocks, the L-type (Japanese stock) and the S-type (Hawaiian stock), at different temperatures was investigated. Rotifers from these stocks were reared in 1000 *l* tanks and fed *Tetraselmis tetrathele*. Salinities were controlled at optimum levels for resting egg production (8 ppt and 32 ppt for L- and S-type, respectively). At culture temperatures ranging from 23.1 to 30.6°C, the L-type rotifers produced more resting eggs at the lowest temperature (23.1°C) and the S-type produced more at a higher temperature (28.2 and 30.6°C). The relationship of resting egg production to temperature is expressed by a logistic curve. The highest total number of resting eggs was 17.8 million in 8 days at 23°C for L-type rotifers and 101.5 million in 10 days at 28°C for S-type rotifers. The maximum number of resting eggs produced was 3,400 and 14,000 per 10,000 L-type and S-type rotifers respectively. The maximum number of resting eggs produced per $1\times10^8$ cells of *T. tetrathele* was 2,270 and 11,100 for the L- and S-types respectively.

The marine rotifer *Brachionus plicatilis* has been used as a live food by aquaculturists since the initial work on this species was performed.[1)]

Major constraints to the rotifer's use in aquaculture are the considerable amounts of manpower and extensive facilities required for culturing phytoplankton as rotifer food. To minimize rotifer culture constraints, many studies have been conducted to improve culture techniques.[2-10)] Other studies have suggested that the resting eggs of *B. plicatilis* could be an on-demand source of feed similar to the cysts of brine shrimp.[11-15)] Resting eggs, the products of rotifer bisexual reproduction could be produced as a by-product of the hatchery off-season for use during spawning season.

Rotifer bisexual reproduction is affected by internal and external factors.[13,16)] L-type and S-type rotifers respond differently to some environmental factors. Conditions suitable for resting egg production have been determined only under small-scale experimental conditions. Only little large-scale production information has been available.[11,17)] Imamura *et al.*[11)] cultured L-type *B. plicatilis* in 4 $m^3$ tanks and obtained a maximum harvest of 30 million resting eggs at temperatures between 23 and 30°C. Kokura *et al.*[17)] harvested 24 million resting eggs from a 40 $m^3$ tank. Until now, no study had been conducted to evaluate the maximal resting egg production of either L-type or S-type rotifers under optimal conditions. In this study, preselected strains*[5] of L-type and S-type rotifers were cultured in their respective optimal salinity conditions and fed on *Tetraselmis tetrathele*. The production of resting eggs at different times of the year was compared in terms of water temperature, rotifer density, and the total amount of algae consumed.

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*1 Studies on the Formation and Hatching of Fertilized Eggs of the Rotifer *Brachionus plicatilis*-V. (シオミズツボワムシ受精卵の形成および孵化生態に関する研究—V)

*2 An outline of this report was presented at the autumn meeting of the Japanese Society of Scientific Fisheries, Hakodate, October, 1987.

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*5 In the current paper, rotifer strain means an assemblage of rotifers that are genetically identical by apomixis. This is equivalent to "clone" termed by G. Bell (The Masterpiece of Nature, University of California Press, Berkeley, 1982).

## Materials and Methods

*Selection of Rotifer Stock*

L-type rotifers from the University of Tokyo stock and S-type rotifers from the Oceanic Institute stock were selected for this study based on highest rates of resting egg formation as compared to other available stocks. Each stock was preserved as a resting egg form. Strains hatched from these eggs in each stock are genetically different and may have different reproductive features.

From each stock, 30 randomly chosen strains were evaluated in preliminary rearing trials to identify the one with the highest rate of resting egg production. Only this strain was selected for the study. Culture salinities were set at the optimal range (8 ppt for L-type and 32 ppt for S-type rotifers), as has been determined.[18,19]

*Preparation of Rotifer Food*

*Tetraselmis tetrathele* was chosen as the rotifer food because resting eggs fed on this species have comparatively higher hatching rates than those produced by rotifers fed on other algae types.[20] *T. tetrathele* was cultured in two 10 $m^3$ outdoor tanks with agricultural grade fertilizers at concentrations of 100 g ammonium sulfate, 30 g monopotassium phosphate, 15 g Fe-EDTA, and 4 g Clewat-32, a mineral complex (produced by Teikoku Kagaku, Japan) in 1000 *l* seawater. The *T. tetrathele* was harvested during its exponential growth at a density of 3 to $6\times10^5$ cells/m*l*. Algae were condensed through a continuous flow-through centrifuge and supplied to each tank. Using condensed algae eliminates the dilution of rotifer culture water with the algae medium. Exchanging the culture water has been shown to significantly reduce the mixis rate in a rotifer population.[21] Algal density (measured by hemacytometer) in the rotifer tank ranged from 3 to $10\times10^5$ cells/m*l*. The total amount used in trials varied from 5.37 to $25.53\times10^{11}$ cells.

*Rotifer Culture and Resting Egg Production*

L- and S-type rotifers were cultured separately in 1000 *l* cylindrical tanks (800 *l* in water volume) for resting egg production. Six mass culture trials of L- and four of S-type rotifers were conducted between February and August, 1987. The 1000-*l* cylindrical tanks were located inside the hatchery under natural sunlight. The temperatures were regulated by a thermostat with two 200-watt electric heaters set within 1°C of the highest ambient temperature during a culture period. The duration of each culture was about 7 to 10 days (Table 1). Water temperatures ranged from 23.1 to 30.6°C over the 6-month experimental period (Table 1). The initial stocking density of rotifer cultures was one individual/m*l*. Higher inoculation densities cause rapid population increases by parthenogenesis and may not lead to mass cyst formation through bisexual reproduction. Transferring higher density rotifer populations to new media suppresses mixis.[21] Rotifer density was monitored and recorded daily. The proportion of mictic females and amictic females was also determined daily.[18]

**Table 1.** Culture duration, water temperature, and total feeding amount of *T. tetrathele* in L- and S-type trials

| Trial | Duration (Days) | Temperature (°C) | Feeding amount ($\times10^{11}$ cells) |
|---|---|---|---|
| L-type | | | |
| I | 8 | 23.1 | 7.83 |
| II | 8 | 23.1 | 9.70 |
| III | 10 | 24.6 | 8.03 |
| IV | 9 | 26.3 | 9.30 |
| V | 8 | 29.6 | 7.55 |
| VI | 7 | 27.3 | 9.27 |
| S-type | | | |
| I | 10 | 23.5 | 9.15 |
| II | 9 | 26.3 | 5.37 |
| III | 8 | 30.6 | 7.67 |
| IV | 10 | 28.2 | 25.53 |

At the end of each culture trial, resting eggs were harvested according to methods described by Hagiwara *et al.*[18,22] The sediment on the culture tank bottoms was siphoned out and filtered through a series of screens with different mesh sizes. Resting eggs of L- and S-type rotifers were retained by 63 $\mu$m and 45 $\mu$m meshes, respectively. The total number of resting eggs produced was extrapolated from the number in a small subsample. The number of resting eggs in the subsample was counted under a stereomicroscope.

*Data Analysis*

The percentage of micitic females (or percent mixis) in the population and the fertilization rate (the number of fertilized mictic females divided by the total number of mictic females), were computed.[18] Among the trials, total amounts of algae fed and total rotifer populations were

not identical because of seasonal and other factors. To standardize the baseline, resting egg production was expressed as the number produced from 100 million cells of *T. tetrathele* fed to rotifers and from 10,000 rotifers.[18] Then the number of resting eggs produced was compared for water temperature and a regression relation for these two elements were analyzed.

## Results

Water temperature, total amount of algae provided during each trial, and trial duration are shown in Table 1. Water temperature of the different trials ranged from 23.1 to 30.6°C. The amounts of algae provided at different trials were approximately the same (except for S-type rotifer trial IV). Rotifer condition (*i. e.*, maximum density, percent mixis, and percent fertilization) and total resting egg production varied widely among rearing trials (Table 2). The maximum density of S-type rotifers in each trial decreased as water temperature increased. However, the L-type rotifer density showed no relationship to water temperature. Maximum rotifer density ranged from 35.5 to 112.7 individuals/m*l* for L-type rotifer trials and from 71 to 586 individuals/m*l* for S-type rotifer trials.

Within the 7-to-10 day culture period, S-type rotifers produced more resting eggs than did L-type rotifers (Table 2). The maximum number of resting eggs harvested in L-type trials was 17.8 million eggs in February (Trial I) when the culture temperature was the lowest (23.1°C) of all the trials. After the temperatures increased to 26.3 to 29.6°C, between June and August (Trials IV, V, VI), 2.1 million or less eggs were harvested per trial. S-type rotifers produced less than 12 million resting eggs in Trials I (23.5°C) and II (26.3°C) and more than 85 million eggs in Trials III (30.6°C) and IV (28.2°C). Maximum harvest for the S-type was 101.5 million eggs in Trial IV, with

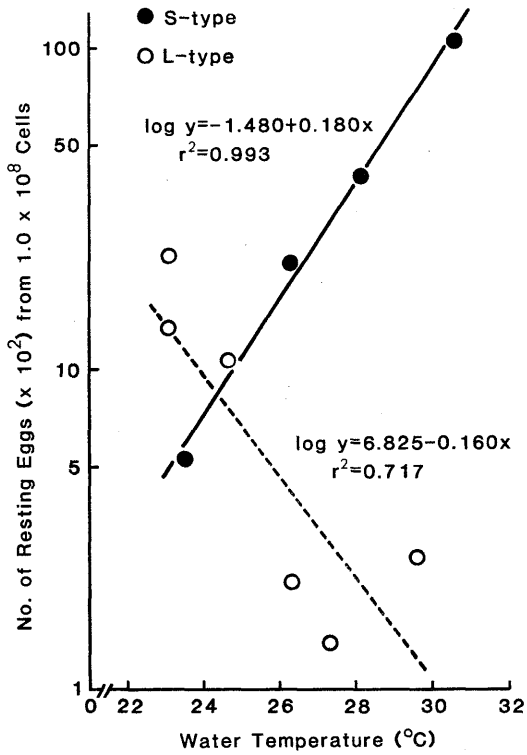


**Fig. 1.** The relationship of culture water temperature to the number of resting eggs produced from $1.0\times10^8$ cells of *T. tetrathele* for L- and S-type rotifers.

**Table 2.** Maximum rotifer density, percent mixis, percent fertilization, and number of resting eggs harvested from L- and S-type trials

| Trial | Max. Density (Individuals/m*l*) | Mixis* (%) | Fertilization* (%) | Resting Eggs ($\times10^6$) |
|---|---|---|---|---|
| L-type | | | | |
| I | 66.5 | 23.5± 5.4 | 80.6±13.2 | 17.8 |
| II | 81.3 | 30.5± 5.9 | 50.1± 9.3 | 13.1 |
| III | 78.0 | 27.9±11.7 | 67.3±23.2 | 6.7 |
| IV | 92.4 | 13.2± 3.7 | 44.9±14.8 | 2.1 |
| V | 35.5 | 22.8± 9.4 | 51.8±23.4 | 2.0 |
| VI | 112.7 | 5.3± 4.0 | 6.2±18.7 | 1.3 |
| S-type | | | | |
| I | 586.0 | 8.7± 2.0 | 50.3±11.8 | 4.8 |
| II | 288.7 | 19.6± 3.1 | 41.5± 8.7 | 11.4 |
| III | 71.0 | 75.6± 4.5 | 96.1± 2.3 | 85.2 |
| IV | 114.0 | 81.0± 3.2 | 81.4± 3.5 | 101.5 |

* Values represent mean±SD.

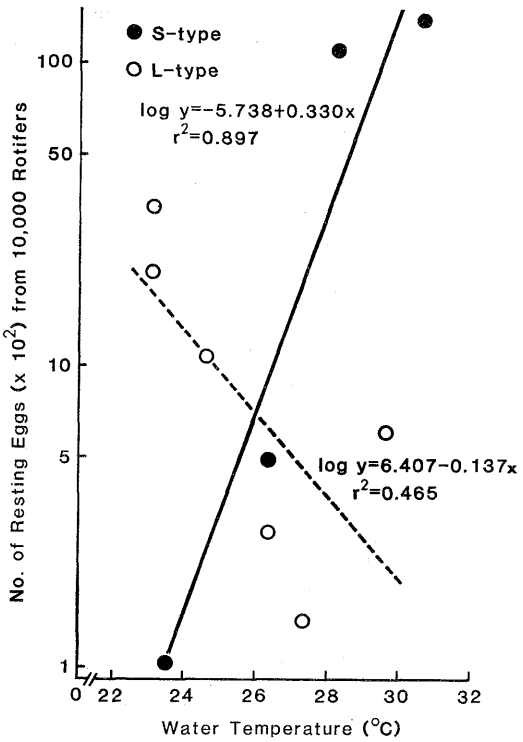


**Fig. 2.** The relationship of culture water temperature to the number of resting eggs produced from 10,000 rotifers for L- and S-type rotifers.

$26\times10^{11}$ cells of *T. tetrathele*, three times more than was provided for other S-type trials.

Resting eggs produced for every $1\times10^{8}$ cells of *T. tetrathele* provided at different culture temperature is illustrated in Fig. 1. Resting egg production for L-type rotifers (Y) decreased logistically as water temperature (X) increased. The relationship is expressed as:

$$\log y=6.825-0.160x\,,\quad r^2=0.717\,,$$
$$F=10.14\,,\quad P=0.03$$

In contrast, S-type rotifers produced more resting eggs (Y) at higher water temperatures (X). This relationship is expressed as:

$$\log y=-1.480+0.180x\,,\quad r^2=0.993\,,$$
$$F=289.91\,,\quad P=0.003$$

According to these formulae, the difference in the predicted resting egg production at water temperature of 23°C and 30°C was 13.2 times for L-type rotifers and 18.2 times for S-type rotifers.

The relationship to culture temperature (X) of the number of resting eggs (Y) produced from 10,000 rotifers is illustrated in Fig. 2. This figure shows a trend similar to that in Fig. 1. Regression analysis indicated a significant relationship between the two variables:

$$\log y=-5.738+0.330x\,,\quad r^2=0.897\,,$$
$$F=17.50\,,\quad P=0.05$$

for the S-type rotifers but not for the L-types.

## Discussion

In this study, the highest yields of resting eggs from a 1 m³ tank were 17.8 million L-type eggs in seven days versus 101.5 million S-type eggs in ten days. Compared with Imamura *et al.*,[11] we harvested L-type cysts four times more efficiently, probably because our cultures were conducted under more optimal external and internal conditions for inducing a high percentage of mixis in rotifer populations. The percentage of mixis in a rotifer population is positively related to the number of resting eggs produced.[18]

Ideally, one would compare resting egg production at different temperature with other external and internal factors constant. Unfortunately, circumstances did not allow us to control all such factors as the algae amount and rotifer density for the 1 m³ tanks. Rotifer population growth was affected by water temperature and was uncontrollable. In any case, the controlling rotifer density in the rearing tank may confound the actual effect of water temperature on resting egg production because differences in rotifer population change the resting egg production rate. The total algae amounts used in different trials did not greatly vary except in trial IV for S-type rotifers. Differences in rotifer population and the algae amount used among different trials, however, were considered when the resting egg production under different water temperatures was compared. We have expressed resting egg production in terms of a constant amount of rotifers or a constant amount of algae provided.[19] Doing so ameliorated the potential effects from differences in the total amount of rotifers in the tank or algae provided. The average of the feeding amounts listed in Table 1 is $8.21\pm1.35\times10^{11}$, except for one trial. However, this feeding amount is not necessarily optimal for maximal resting egg production. The ingestion rate of L-type rotifers is about 1.7 times greater than that of S-type.[23] The ingestion rate of Hawaiian S-type rotifers at 28°C was 350 to 650 cells of *T. tetrathele*/rotifer/hour.[19] At this rate approximately $11.39\times10^{11}$ and $6.7\times10^{11}$ cells are

necessary for the daily feeding of 800-*l* L-and S-type rotifer cultures, respectively, when the rotifer density is 100 individuals per m*l*. If this estimate is correct, the feeding amounts in this study were too low. At starvation or low food levels, percentage of mixis is suppressed.[24] Resting egg production may be increased by supplying more algae to the culture. Studies on preserved diet for resting egg formation is currently under progress.

Hagiwara *et al.*[18] using University of Tokyo L-type rotifer stock (different strains of the same stock used in this study), studied the rate of resting egg production at 15 and 30°C. At salinities of 7.2 ppt, 10,000 rotifers produced 2,007 eggs at 25°C and 8,300 at 15°C. By comparison, in the current study, the number of resting eggs produced from 10,000 rotifers at 23°C was 3,350. Therefore, the rotifer strains in this study performed very well. No information was available for resting egg production of S-type rotifers in any previous study. In our study, however, S-type rotifers clearly produced nore resting eggs than did L-type rotifers.

The number of resting eggs produced by L-and S-type *B. plicatilis* was related to water temperature (Fig. 1 and 2). At a temperature of 29.6°C (Trial V), the resting egg production of the L-type rotifers deviated inexplicably from the trend of the other trials. Additional trials are necessary to determine why. Resting egg production of L-type rotifers increased as temperature decreased, which is consistent with previous findings.[18,25] The mixis rate of a Florida strain S-type *B. plicatilis* increased when the temperature increased from 20 to 30°C but decreased at more than 30°C.[26] This trend is consistent with our results for S-type rotifers in the temperature range of 20 to 30°C.

Temperature optima for rotifer population growth rates of L- and S-type rotifers are different.[27] The L-type optimum was 25 to 30°C and the S-type optimum 30 to 35°C. In Japan, L- and S-type rotifers are often found in the same culture tanks, but the different population ratios vary seasonally. L-type rotifers dominated a culture from April to June and from November to January when the water temperature was less than 20°C.[28] When the temperature exceeded 20°C (from July to October), S-type rotifers dominated. This suggested that S-type *B. plicatilis* has a higher temperature optimum than the L-type, both in sexual and asexual reproduction. For maximum yield, therefore, L-type rotifer resting egg production should be conducted during the cool season and S-type egg production in the warm season. With regard to salinity, the S-type rotifer is more euryhaline and has a higher salinity optimum for inducing mixis than does the L-type rotifer.[19]

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## References

1) T. Ito: *Rep. Fac. Fisheries, Pref. Univ. Mie*, **3**, 708–740 (1960).
2) T. Watanabe, C. Kitajima, and S. Fujita: *Aquaculture*, **34**, 115–143 (1983).
3) K. Hirayama: *Hydrobiologia*, **147**, 269–270 (1987).
4) K. Hirayama, I. Maruyama, and T. Maeda: *Hydrobiologia*, **186/187**, 39–42 (1989).
5) K. Fukusho: in "Symposium Internacional De Aquaculture", Coquimbo, Chile, 1983, pp. 361–374.
6) S. Fujita: in "The rotifer *Brachionus plicatilis*-Biology and mass culture", Koseisha Koseikaku, Tokyo, 1983, pp. 9–21.
7) T. Kafuku and H. Ikenoue: in "Modern methods of aquaculture in Japan", Kodansha Ltd., Tokyo and Elsevier, Amsterdam, 1983, pp. 209–211.
8) C. Kitajima: in "Fish nutrition and diets", Koseisha Koseikaku, Tokyo, 1985, pp. 75–88.
9) E. Lubzens: *Hydrobiologia*, **147**, 245–255 (1987).
10) T. Watanabe: in "Intensive fish farming", BSP professional, Oxford, 1988, pp. 239–267.
11) S. Imamura, S. Ashidate, and H. Tojo: *Saibaigiken*, **8**, 53–61 (1979).
12) E. Lubzens: *European Maric. Soc. Spec. Publ.*, **6**, 163–179 (1981).
13) A. Hino: in "The rotifer *Brachionus plicatilis*-Biology and mass culture", Koseisha Koseikaku, Tokyo, 1983, pp. 22–34.
14) T. W. Snell: *World Maricul. Soc.*, **16**, 484–497 (1985).
15) T. W. Snell and F. H. Hoff: *Aquaculture Magazine*, **14**, 41–45 (1988).
16) R. Pourriot and T. W. Snell: *Hydrobiologia*, **104**, 213–224 (1983).

17) T. Kokura, T. Ogawa, and C. Kitajima: *Bull. Nagasaki Prefectural Inst. Fish.*, **8**, 163–164 (1982).
18) A. Hagiwara, A. Hino, and R. Hirano: *Nippon Suisan Gakkaishi*, **54**, 569–575 (1988).
19) A. Hagiwara, C.-S. Lee, G. Miyamoto, and A. Hino: *Marine Biology*, **103**, 327–332 (1989).
20) A. Hagiwara and A. Hino: *Nippon Suisan Gakkaishi*, **56**, 1965–1971 (1990).
21) A. Hino and R. Hirano: *Nippon Suisan Gakkaishi*, **43**, 1147–1155 (1976).
22) A. Hagiwara, A. Hino, and R. Hirano: *Nippon Suisan Gakkaishi*, **54**, 577–580 (1988).
23) S. Yamasaki and H. Hirata: *Suisan Zoshoku*, **34**, 137–140 (1986).
24) T. W. Snell and E. M. Boyer: *J. Exp. Mar. Ecol.*, **124**, 73–85 (1988).
25) A. Hino and R. Hirano: *Nippon Suisan Gakkaishi*, **50**, 1481–1485 (1984).
26) T. W. Snell: *Marine Biology*, **92**, 157–162 (1986).
27) S. Ito, H. Sakamoto, M. Hori, and K. Hirayama: *Bull. Fac. Fish. Nagasaki Univ.*, **51**, 9–16 (1981).
28) T. Kokura, T. Ogawa, and C. Kitajima: *Bull. Nagasaki Pref. Inst. Fish.*, **8**, 147–152 (1982).

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