Nippon Suisan Gakkaishi 54(4), 577–580 (1988)

PN-ABH-023
ISN 56579

Comparison of Resting Egg Formation Among Five Japanese Stocks of the Rotifer *Brachionus plicatilis*[*1,2]

Atsushi Hagiwara,[*3] Akinori Hino,[*4] and Reijiro Hirano[*4]

(Received September 30, 1987)

Resting egg formation was compared among five stocks of the marine rotifer *Brachionus plicatilis*. The three large type (L-type) and two small type (S-type) stocks were collected in Japan.

The number of resting eggs produced by 10,000 rotifers at 25°C and 8‰ Cl', was: 3680 (L-type University of Tokyo stock), 2270 (S-type Mie Prefecture stock), 1480 (L-type Mie Prefecture stock), 1.7 (S-type Lake Hamana stock) and 0 (L-type Yashima stock). These results were supported by data on the appearance rate of mictic females and the fecundity of resting egg producing females.

Results of the current study suggest that the selection of rotifer stock is crucial to mass production of resting eggs.

The process of bisexual reproduction resulting in resting egg formation and the characteristics of resting eggs of monogonont rotifers have been reviewed by several authors.[1~3] An interesting aspect of their life history, from a culturist's point of view, is the production of resting eggs. It has been suggested that rotifer resting eggs in the form of preserved seeds may be useful for marine fish larval rearing.[4~7] Only one trial, however, has been reported.[7] The major difficulty in conducting such trials is the limited number of resting eggs that are produced. Because of a lack of fundamental information, culturists cannot take full advantage of rotifer biology to maximize resting egg production.

Rotifer resting egg production is influenced by both external and internal factors (reviewed by Pourriot and Snell[3]). Previous studies on *B. plicatilis*[5,7,8] suggested that optimal environmental conditions for resting egg production are low temperatures and salinities (external factors). Hino and Hirano[10] reported a range of mictic female appearance rates among strains (clones) of a University of Tokyo stock (internal factor).

It is probable that rotifer stocks reared in different facilities or originating from geographically separate areas will exhibit different bisexual reproductive patterns. For example, the University of Tokyo stock showed active resting egg production,[7] while the University of Nagasaki stock produced few mictic females.[11] A third stock, collected in Florida in 1976, did not produce resting eggs, though it produced males.[12]

In this study, we compared the rate of resting egg production among five rotifer stocks collected in Japan. The fecundity of each rotifer stock was estimated and, in one stock, the effect of temperature on mictic female production was investigated.

Material and Methods

The origins and symbols of three large type (L-type) stocks and two small type (S-type) stocks are listed in Table 1. Sudzuki[13] identified the three L-type stocks as *Brachionus plicatilis typicus* and the two S-type stocks as *B. plicatilis rotundiformis*. The Y-L stock has repeatedly been cultured from resting eggs produced at low temperatures (10–15°C).[8] We received this stock as resting eggs. The M-S, M-L and T-L stocks were reared at 25°C in our laboratory for one, two and ten years, respectively. The cultures progressed repeatedly through resting egg production, hatching and rearing. Resting eggs of the H-S stock were collected from the lake bottom

*1 Studies on the Formation and Hatching of Fertilized Eggs of the Rotifer *Brachionus plicatilis*—III. (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—III).

*2 An outline of this report was presented at the spring meeting of the Japanese Society of Scientific Fisheries, Tokyo, April, 1985.

*3 Oceanic Institute, Makapuu Point, Waimanalo, Hawaii 96795, U.S.A. (萩原篤志：ハワイ海洋研究所).

*4 Department of Fisheries, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo 113, Japan. (日野明徳，平野礼次郎：東京大学農学部水産学科).

and were kept at 5°C and OL: 24D for three years.

A culture of each stock was prepared using 20 stem mothers (S♀) newly hatched from resting eggs (for definitions of female type symbols, see Hagiwara *et al.*[2]). Therefore, each stock was composed of 20 strains. Temperature and chlorinity were maintained at 25 ± 1°C and 8‰ Cl'. These levels have been shown to stimulate active bisexual reproduction and accelerate population growth for the T-L stock.[7] Photoperiod was OL: 24D. Throughout the culture period, the rotifers were fed *Tetraselmis tetrathele* at a daily feeding density of 5 × 10⁵ cells/m*l* after adding centrifuged algae. Sterilized seawater, diluted by distilled water, was used as the culture medium. Each stock was precultured in 500 m*l* of the medium for 15–20 days. This has been demonstrated to be a sufficient time for the production of mictic females.[8] Inoculation of 50 individuals/m*l* every other day maintained exponential rotifer growth.

Following preculture, 2,000 young females (?♀), which were not carrying eggs, were identified and removed. These were inoculated into 200 m*l* of culture medium, at an initial density of 10/m*l*. Two such cultures were prepared for each of the five stocks and reared until their density reached 150/m*l*.

Subsamples of 5–10 m*l* were observed once every 24 h. The number of amictic females (♀♀), male producing mictic females (♂♀), resting egg producing mictic females (D♀) and males (♂) were counted. Resting eggs released from D♀ were collected and counted. Since D♀ of the M-S stock keep their eggs inside the lorica,[10] the eggs were counted after the death of D♀. ?♀ from all stocks, which are young and have not begun egg production, were separated into 15-m*l* petri dishes and cultured. The following day, female types were defined from the shape and size of their eggs. Data was collected until the population exceeded 150 individuals/m*l*. The rates of mictic female appearance and resting egg formation for a given stock were calculated as an average of two cultures.[7]

Twenty D♀ found among ?♀ in each stock were transferred individually to 0.2-m*l* test tubes. This individual culture technique[9] was employed to estimate fecundities for each stock.

Stock Y-L was reared in 5 temperatures (30, 25, 20, 15 and 10°C) to observe this stock's response to temperature. Random samples of 200 ?♀ from each temperature were removed during exponential growth and female types determined.

Results

The culture period (number of days to reach 150 female individuals/m*l*) for each stock is shown in Table 1. In gereral, L-type rotifers have a longer culture period than S-type rotifers. In the T-L stock, total female density reached 150/m*l* on the ninth day. After Day 6, between 500 and 1000 resting eggs were produced per day. The appearance rate of mictic females increased with rotifer density, but decreased when density exceeded 100/m*l*. Similar patterns were observed in both the M-L and M-S stocks.

In contrast, few mictic females and few males appeared in the Y-L and H-S stocks. Culture Y-L produced no resting eggs and culture H-S produced only 26. The population growth rate in these two stocks was high because most females were ♀♀. Density reached 150/m*l* on Day 4 in the H-S stock and on Day 5 in the Y-L stock.

The mictic female appearance rate among total females in the five stocks is presented in Table 2. The M-S stock demonstrated the highest rate at 59.1‰. This was followed by the T-L stock at 39.1‰ and the M-L stock at 18.1‰. The rates from the Y-L and H-S stocks were two orders of magnitude lower at 0.3 and 0.7‰, respectively. Table 2 also shows the rate of resting egg formation produced by 10,000 individuals in each stock.

Table 1. Culture period of each stock

Stock	Culture period (days)
T-L (University of Tokyo L-type)	9
M-L (Mie Prefecture L-type)	7
Y-L (Yashima Station L-type)	5
H-S (Lake Hamana S-type)	4
M-S (Mie Prefecture S-type)	5

Culture period equals days required till female density exceeds 150 individuals/m*l*.

Table 2. Rate of mictic female appearance and resting egg formation in 5 stocks

Stock	Mictic Female (‰)	Rate of resting egg formation
T-L	39.0	3680
M-L	18.1	1480
Y-L	0.3	0
H-S	0.7	1.7
M-S	59.1	2270

The resting egg formation rate is the number of eggs produced by 10,000 rotifers in batch culture.

Table 3. Fecundity of D♀ in 3 stocks

Stock	Fecundity (No. of Individuals) Mean±standard error
T-L	2.30±1.13 (20)
M-L	2.04±0.66 (26)
M-S	1.09±0.29 (200)

Table 4. Effect of temperature on the appearance of mictic females (%) in the Y-L stock

Temperature (°C)	Y-L
30	0
25	0
20	0
15	0
10	20.0

The T-L stock had the highest rate at 3680, followed by the M-S stock at 2270 and the M-L stock at 1480. The H-S stock was three orders of magnitude lower at 1.7. The value was 0 in the Y-L stock.

The fecundity of D♀ in the T-L, M-L and M-S stocks is shown in Table 3. In the H-S and Y-L stocks, no D♀ were found among the ?♀ isolated from mass culture. The average values were 2.30 and 2.04 in the T-L and M-L stocks, respectively, but only half that (1.09) in the M-S stock. The D♀ of the M-S stock retain resting eggs in their lorica. Ninety-one percent of the D♀ (182 of 200) of this stock formed one egg and 9% (18 of 200) formed two eggs.

Table 4 shows the appearance rate of mictic females in the Y-L stock in five temperatures. Although no mictic females appeared in temperatures between 15–30 C, 20% of the ?♀ (40 of 200) became mictic females at 10 C.

Discussion

The primary factors involved in bisexual reproduction are the mictic female appearance rate (Table 2) and the fecundity of D♀ (Table 3). A comparative analysis of the results shows that although the M-S stock demonstrated the highest mictic female production rate, this stock has the lowest fecundity of D♀. Okauchi *et al.*[14] stated that these D♀ do not release their resting eggs. From their observations, the authors suspect that lorica volume is the limiting factor, and that only two resting eggs can be produced per female. Table 2 shows that the T-L stock had the highest resting egg formation rate. This stock also had the second highest mictic female appearance rate (after the M-S stock) and the highest D♀ fecundity.

In the Y-L stock, mictic females appeared only at 10°C (Table 4). Previous studies on the T-L stock[7,15] suggested that the appearance of mictic females gradually increased with decreases in temperature. This was also observed with the M-L stock in the laboratory (unpublished data). Hino and Hirano[16] reported that exposure of a rotifer population to various environmental parameters significantly influences mictic female production in the derived strain. Differences in culture history among the stocks studied here may be one factor which causes variations in resting egg production. The Y-L stock has been cultured exclusively during the winter. And the appearance of mictic females only at low temperatures is consistent with Hino and Hirano's hypothesis. The mechanism of action, however, is unknown.

Because of their size and availability, rotifers are presently the food of choice for fish larvae at first feeding. *B. plicatilis* exhibit variability in lorica shape and size.[5,17,18] Variations in rotifer size can be utilized to correspond to variations in mouth size observed among in fish larvae at initial feeding.[19–21] For these reasons, information on increasing the production of both S- and L-type resting eggs is important. Resting egg production among different rotifer types varies in response to similar environmental stimuli. When salinity varies, Hawaiian S-type rotifers respond in a manner opposite to that of the L-type rotifers used in the current study (Hagiwara *et al.* in preparation).

In order to achieve the highest production of resting eggs, studies need to be designed to select the best strain in the preferred stock. In the current study, both external and internal parameters were shown to have a pronounced effect on resting egg production. It is suggested that by controlling culture histories and inbreeding favorable stocks, resting egg harvests increase.[10,22] Further investigation is required to enable culturists to manipulate rotifer stocks to select those better suited for use as live feed.

Acknowledgments

Portions of this research were supported by a Grant in Aid to Scientific Research from the Ministry of Education, Japan and by the U. S. Agency for International Development (Dan-4161-A-00-4055-00). The authors wish to thank K.

Fukusho, M. Okauchi and A. Nishiki for providing rotifer stocks, C.-S. Lee, C. Tamaru and D. Shiraishi for their review and comments, and A. Belanger for preparation of the manuscript.

References

1) J. Gilbert: *Trans. Amer. Microsc. Soc.*, **93**, 490–513 (1974).
2) A. Hino: *Saibaigiken*, **10**, 109–123 (1981). (In Japanese.)
3) R. Pourrito and T. Snell: *Hydrobiologia*, **104**, 213–224 (1983).
4) A. Hino and R. Hirano: *Nippon Suisan Gakkaishi*, **42**, 1147–1155 (1976).
5) E. Lubzens: *European Maricult. Soc., Spec. Publ.*, **6**, 163–179 (1981).
6) E. Lubzens: *Hydrobiologia*, **147**, 245–255 (1987).
7) A. Hagiwara, A. Hino, and R. Hirano: *Nippon Suisan Gakkaishi*, **54**, 569–575 (1988).
8) S. Inamura, M. Ashidate, and H. Tojo: *Saibaigiken*, **8**, 53–61 (1979). (In Japanese.)
9) E. Lubzens, R. Fishler, and V. Berdugo-White: *Hydrobiologia*, **73**, 55–58 (1980)
10) A. Hino and R. Hirano: *Nippon Suisan Gakkaishi*, **43**, 1357–1363 (1977).
11) K. Hirayama and S. Ogawa: *Nippon Suisan Gakkaishi*, **38**, 1207–1214 (1972).
12) T. Snell: *Mar. Biol.*, **92**, 157–162 (1986).
13) M. Sudzuki: *Hydrobiologia*, **147**, 45–48 (1987).
14) T. Okauchi and K. Fukusho: *Nippon Suisan Gakkaishi*, **51**, 1907 (1985).
15) A. Hino and R. Hirano: *Nippon Suisan Gakkaishi*, **50**, 1481–1485 (1984).
16) A. Hino and R. Hirano: *Nippon Suisan Gakkaishi*, **51**, 511–514 (1985).
17) S. Ito, H. Sakamoto, M. Hori, and K. Hirayama: *Bull. Fac. Fish. Nagasaki University*, No. 51, 9–16 (1981). (In Japanese with English summary.)
18) K. Fukusho: in "*Brachionus plicatilis*, its biology and mass culture", Koseisha koseikaku, Tokyo, 1983, pp. 35–51. (In Japanese.)
19) A. Shirota: *Nippon Suisan Gakkaishi*, **36**, 353–368 (1976).
20) Y. Tsukashima, N. Yoshida, C. Kitajima, S. Matsumura, and C. Besch III. *The Aquiculture*, **30**, 202–210 (1983). (In Japanese with English summary.)
21) C. Kitajima: in "Fish nutrition and diets", Koseisha Koseikaku, Tokyo, 1985, pp. 75–88. (In Japanese.)
22) A. Hagiwara: Ecological study on fertilized eggs of the rotifer *Brachionus plicatilis*, Doctoral thesis, University of Tokyo, 1986, Tokyo, 154 pp. (In Japanese).