INDUCTION OF SEXUAL REPRODUCTION AND RESTING EGG PRODUCTION IN BRACHIONUS PLICATILIS REARED IN SEA WATER

Esther (wajc) LUBZENS, Rachel FISHLER & Viviane BERDUGO-WHITE

Israel Oceanographic & Limnological Research, Tel-Shikmona, P.O.B. 8030 Haifa, Israel

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Abstract

Brachionus plicatilis raised in our laboratory in sea water reproduces asexually even under high crowding conditions (at least 40 individuals per ml). Amictic females were induced to produce mictic females, males and resting eggs by reducing the concentration of the sea water culture medium. Mictic females and males appeared predominantly among the progeny produced by the amictic females during 4 days following their transfer into 25% sea water. Resting eggs appeared first 5-12 days after the onset of the experiment. Following the disappearance of males, the culture consisted of amictic females.

Resting eggs produced by the method described above may be preserved for at least three months at -14°C or by desiccation at room temperature. Under the appropriate experimental conditions, resting eggs hatch into amictic females. Since *B. plicatilis* is one of the most commonly used food sources of fish larvae in aquaculture, the methods reported here may offer an easy and versatile way of preserving rotifer culture stock to be used on demand.

Introduction

One of the main aims in growing zooplankton for fish hatcheries is to become independent of the immediate success or failure of the zooplankton culture.

The rotifer *Brachionus plicatilis* is one of the most commonly used organisms in aquaculture, serving as an important food source for fish larvae. An adequate supply of food for fish larvae depends, therefore, on a successful culture of rotifers. One of the possible ways of ensuring a good supply of rotifers would be the building up of reserve stocks and their use when the demand rises. This goal can possible be achieved by: (a) finding methods for inducing rotifers to produce resting eggs in large quantities; (b) finding ways for preserving and hatching the resting eggs.

It has been previously reported that changes in environmental conditions, such as an increase in crowding, cold-shocks, decrease in food quantity and changes in photoperiod (see review by Gilbert, 1974) may induce production of males. Ito (1960) reported that *B. plicatilis* collected from eel ponds (Cl 2-3‰) was induced to produce large numbers of resting eggs when transferred from high chlorinity (18‰) culture media into lower chlorinity media.

Brachionus plicatilis was raised in our laboratory for the past seven years in sea water (38‰) and fed on a variety of marine species of algae. Under these conditions, rotifers reproduced asexually even under high crowding conditions (40 or more individuals per ml). Induction of sexual reproduction was achieved by reducing the salinity of the sea water culture medium. Large numbers of resting eggs could be thus produced and various methods for their preservation and hatching were tested under laboratory conditions.

Material and methods

Brachionus plicatilis was collected in the summer of 1972 from sea water fish ponds near Dor, Israel. The rotifers were since then bred in filtered (0.45 μm) sea water and fed on various species of marine algae. Rotifers used for the experiments reported here were fed on *Chlorella stigmatophora*. Sea water dilutions were carried out with double distilled water.

The appearance of females carrying three or more eggs, mictic females, males and resting eggs was followed in three experimental set-ups:

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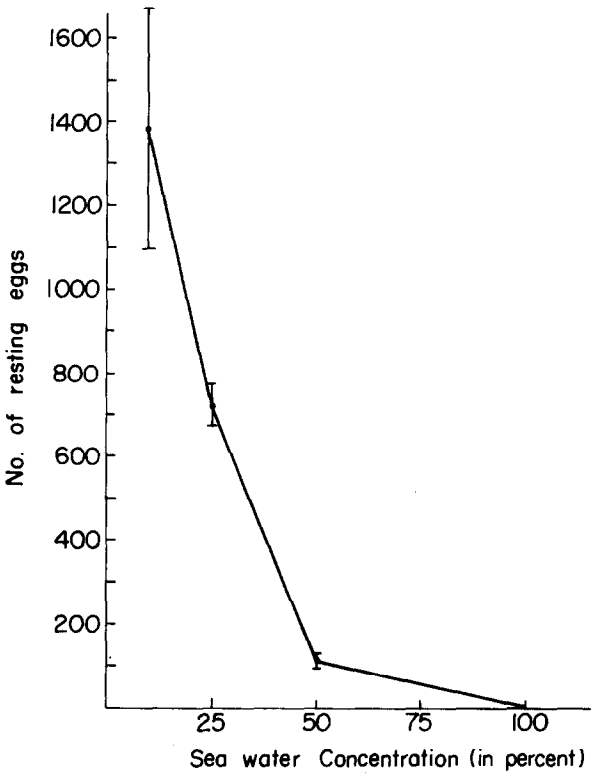

Fig. 1. The number of resting eggs (Mean ± S.E.M.) produced by 50 amictic *B. plicatilis* females, three weeks after their transfer into various sea water concentrations.

1. Fifty or 2000 rotifers were transferred from 100% sea water (SW) into beakers containing 100 ml and 2 litres of diluted sea water, respectively. The number of females carrying three or more eggs and the number of resting eggs were counted at intervals for up to three weeks from the onset of the experiment (see Figs. 1, 2 and 4).

2. Ten amictic females were transferred from 100% SW culture into small petri dishes containing 2 ml of 25% SW culture medium. These adult amictic females were transferred daily into new petri dishes, leaving their progeny behind in the old culture dish. The progeny produced during the first day following the transfer was termed 1st day progeny, the progeny produced during the second day after the transfer was termed 2nd day progeny, etc. This procedure was replicated 8 or 10 times in Experiments 1 and 2, respectively, as reported in Table 1. The appearance of mictic females, males and resting eggs was recorded at daily intervals.

3. Same as in (2) above, except that single amictic females were transferred. The appearance of mictic females, males and resting eggs was followed in females that carried no eggs or one egg at the time of transfer (Experiments 3 and 4, respectively, in Table 1) or on females that carried two or three eggs 24 hours after the transfer (Experiments 5 and 6, respectively, in Table 1). Observations on the appearance of males (Fig. 3) were carried out by recording the presence of males in the petri dishes containing the original transferred amictic females and those of their progeny from 1-8 days after the transfer (1st to 8th day progeny).

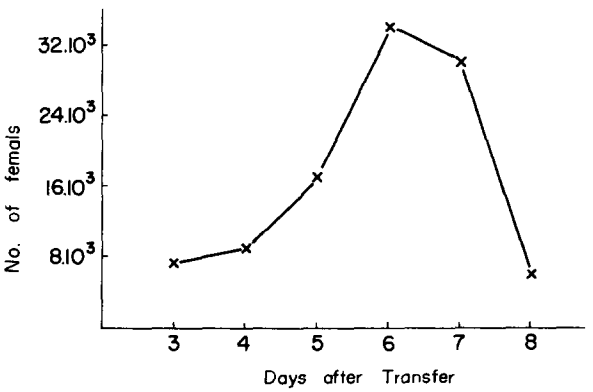

Fig. 2. The average number of females carrying three or more eggs, produced during 8 days by 2000 amictic females transferred into 25% sea water.

Counted batches of resting eggs were frozen in distilled water (-14°C) or left to dry at room temperature and kept in a desiccator. Resting eggs were hatched at room temperature in 100% SW containing *C. stigmatophora* and were followed at intervals for three weeks.

Results and discussion

Production of resting eggs

B. plicatilis raised in our laboratory for several years in sea water (38‰) was induced to produce resting eggs by reducing the concentration of sea water in the culture medium. The number of resting eggs produced increased with the decrease in concentration (Fig. 1). Attempts to culture these rotifers in media containing less than 10% SW were unsuccessful. Ito (1960) obtained maximal numbers of resting eggs in cultures of 7.3‰ chlorinity. The induction of resting egg production, in our laboratory, was independent of the density of the transferred rotifers (Table 1). In addition, feeding rotifers reared in 100% SW with *C. stigmatophora* previously transferred into 25%

Table 1. The time of appearance of the first mictic females, males and resting eggs, after transferring amictic females from 100% sea water into 2 ml of 25% sea water culture medium

	Experiment no.	No. of replicates	Mictic females Time (days)	Mictic females No. of observations	Males Time (days)	Males No. of observations	Resting eggs Time (days)	Resting eggs No. of observations
Females transferred in groups of 10 individuals into petri dishes (30°C)	1	8	2	2	2	2	5	3
	2	10	2	5	3	5	5	3
Females transferred singly into petri dishes* (24-26°C)	3	8	4	2	4	2	11	1
	4	10	7	3	7	3	12	2
	5	10	5	2	5	2	-	-
	6	10	4	1	5	1	-	-

*In Experiment 3 and Experiment 4, females carried no eggs or one egg, respectively, at the time of transfer. In Experiment 5 and Experiment 6, females carried two or three eggs, respectively, 24 hours after their transfer into 25% sea water.

SW for extended periods of time did not induce sexual reproduction (unpublished results). Rotifers also produced resting eggs, although in smaller numbers, if fed on *Platymonas* and *Phaeodactylum* in diluted sea water media. Most probably, *Chlorella* tolerated lower salinities better than the other two algal species.

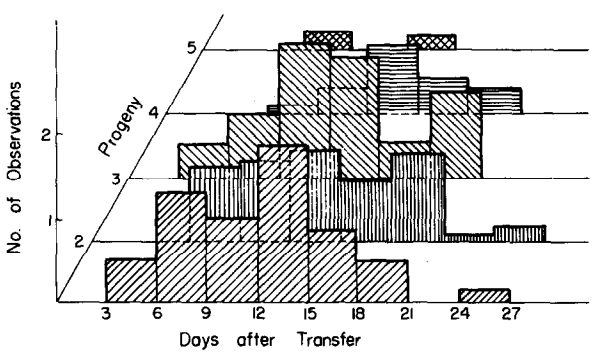

Fig. 3. A histogram showing the average number of petri dishes in which males were observed in the progeny produced by individual amictic females during the first 5 days following their transfer from 100% to 25% sea water. Results were averaged for four different experiments and normalized for the number of replicates and number of observation periods made in each experiment.

The events leading to the production of resting eggs involved the appearance of large numbers of females carrying three or more eggs shortly after the amictic females were transferred from 100% SW into 25% SW (Fig. 2). These females were seldom observed in 100% SW cultures. Females carrying three or more large eggs (130 μ × 85 μ in diameter) gave rise to amictic or mictic females, while those carrying several small eggs (90 μ × 75 μ in

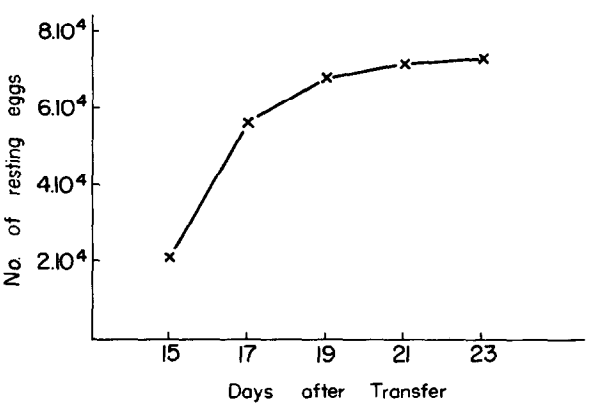

Fig. 4. The average number of resting eggs produced during 23 days by 2000 amictic females transferred into 25% sea water.

Table 2. The effect of the preservation method on hatching of resting eggs.

Method	Duration of preservation (weeks)	No. of eggs preserved	Hatching (%)	
Freezing -14°C	1	100	81±6	(3)
	2	100	70	(2)
	3	100	81	(2)
	4	166	78±13	(6)
	6	100	75	(2)
	8	200	87	(3)
	12	200	85	(1)
	16	200	50	(2)
Desiccation	2	200	100	(2)
	3	200	80	(1)
Desiccation and sonication	25	200	100	(1)

diameter) produced only male offspring. Mictic females were also produced by amictic females carrying one or two large eggs (Table 1). The first mictic females carrying small eggs appeared 2-7 days after the transfer into 25% SW was made (Table 1). This was closely followed by the appearance of males and later by that of resting eggs.

Males appeared among the progeny of between 90-100% of the females transferred into 25% SW. This was revealed by transferring single females into petri dishes containing 25% SW (see Material and methods). Most of the males appeared in the progeny of eggs that were formed by the amictic females during 1-4 days after their transfer into 25% SW (progeny 1-4 in Fig. 3). The number of newly produced males increased for 15 days and then decreased with their eventual complete disappearance. Similarly the number of resting eggs produced increased for up to 19 days (Fig. 4). After the disappearance of the males, the rotifer culture in 25% SW consisted of amictic females that carried one or two large eggs.

Although the factors leading to the production of males by amictic females transferred into 25% SW are yet unknown, they are transferred to the progeny produced closely after the transfer is made and later disappear.

Hatching of resting eggs

In contrast to the report of Ito (1960), resting eggs produced in 25% SW did not hatch readily in media containing sea water of reduced concentrations. This permitted the harvesting of large numbers of resting eggs from the bottom of the breeding vessel.

Resting eggs may be preserved for at least 12 weeks by freezing at -10°C without signifcant loss of viability (Table 2). Eggs dried and kept desiccated at room temperature retained their viability for up to 3 weeks. Hatching of desiccated eggs preserved for 25 weeks was facilitated by sonification at low energies.

Summary

Large numbers of resting eggs of the rotifer *Brachionus plicatilis* were obtained by transferring amictic females from 100% sea water into 25% sea water culture medium. This resulted from the appearance of males in the progeny produced, by almost all the transferred amictic females, during the first four days following the transfer. The induction of sexual reproduction was mainy due to the change in the salinity of the culture medium. Increasing or decreasing the density of rotifers reared in various sea water concentrations or changing the algal species fed to the rotifers had no effect on this phenomenon.

The resting eggs produced were preserved for 12 weeks at -14°C or for up to 3 weeks in desiccated form at room temperature, without loss of viability.

The methods reported here may offer an easy and versatile way of preserving rotifer culture stocks to be used on demand in fish hatcheries.

References

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