J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1988), 27: 93—99

Embryonic Development of Amictic Eggs of a Rotifer *Brachiounus plicatilis*

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Received October 3, 1988

Abstract Since the euryhaline rotifer *Brachionus plicatilis* is widely used as a food organism in aquaculture, a lot of information concerning the biology of this species has been accumulated, but no information is available on the developmental stages of the amictic eggs. In the present work, some observations were made on the development of the parthenogenetic or amictic eggs of *B. plicatilis* reared under a constant environmental conditions (temperature: 25°C, salinity: 25‰, photoperiod: 12D–12L, food: *Chlorella* sp.). The average time for development is ca. 20h. Based on the morphological characteristics of embryos, the process of the development is classified into 19 stages, each of which is pictured, figured and described. The pattern of early cleavage in *B. plicatilis* is similar to the previous descriptions in other rotifers.

INTRODUCTION

The rotifer, *Brachionus plicatilis*, which once was considered as a pest in Japanese eel culture ponds (ITO, 1955), nowadays is widely used as a food source for larvae and fingerlings of many species of prawn and fish in rearing techniques (WATANABE *et al.*, 1983; KUNGUANKIJ, 1985; LIAO, 1985). Many studies have been done with this rotifer because of its importance in aquaculture. Recently, the preservation on this rotifer has been studied and one of the methods attempted is cryopreservation (KING *et al.*, 1983; OKAMOTO *et al.*, 1987). KING *et al.* (op. cit.), suggested that the age of *B. plicatilis* is critical for the success of its cryopreservation. Our preliminary experiments (unpublished data) indicate that the difference in developmental stage of the amictic eggs must also be taken into account for the accomplishment of cryopreservation of this species. However, none is available regarding the developmental stages of the amictic eggs of *B. plicatilis*. On the other hand, information related with the embryonic development of rotifers is generally scarse (SALENSKY, 1872; JENNINGS, 1896; TANNREUTHER, 1920; de BEAUCHAMP, 1956; PRAY, 1965; PLASOTA and PLASOTA, 1980). Therefore, the present work has been done in order to get some information about the development of the amictic eggs of *B. plicatilis*, which is basic to the practical use of its cryopreservation. Light microscopic observations have been made and each developmental stage is pictured, figured and described.

MATERIALS AND METHODS

The *Brachionus plicatilis* (Nagasaki strain) used in the present work were derived from the culture which has been maintained in laboratory conditions since 1969 and kindly supplied by Kazutsugu HIRAYAMA, Nagasaki University. Stock culture was maintained under a constant environmental conditions (temperature: 25°C, salinity: 25‰, photoperiod: 12D–12L, food:

Chlorella sp.). Egg-carrying amictic females were transferred using a micropipette from the stock culture into wells of a CORNING 25820 multiwell plate containing 1ml of *Chlorella* sp. During 24h of incubation, neonate females were produced and they were individually placed into new wells with fresh medium, and 24h later the individuals with fully developed ova were transferred onto a glass depression slides containing 1ml of *Chlorella* sp. Each slide was places into a Petri dish to avoid evaporation.

The individuals with developed ova were observed under a light microscope at every 30m interval until the first egg was laid. Then this egg was subsequently observed at 2h interval, but during the last 2h before hatching the observations were done more frequently (30m interval). At each observation, the development of embryos was photographed, and a part of the medium was replaced with a fresh one. Freehand drawings were made to simplify the photographs.

RESULTS

The average time for development of the first amictic egg of *B. plicatilis* from laying to hatching under the conditions of the present study was 19.91h (S.D.±0.63h, n=10).

Based on the morphological difference of embryos, the stage of development was classified into 19 categories. Some morphological criteria for each developmental stage are shown in Table 1. The number of cells could be counted in early cleavage stages up to stage 6 (8 cells), beyond which it was imposible to count, and stages were classified based on the shape and/or appearance of embryo.

The first cleavage occurs 30 to 40m after being laid. This cleavage results in two very unequal parts (Plate I.2; Fig. I.3), which are rounded later (Plate I.3; Fig. I.4). The four cell stage (Plate I.4; Fig. I.5) was observed 45 to 55m after being laid. The foot is evident 8.5 to 10.5h after being laid (Plate I.13; Fig. I.15, 16), and the foot can be observed with its two pointed toes (Plate I.15, 16; Fig. I.19, 20) 11.0 to 11.5h after being laid. The movements of the corona begin after 13.5 to 15.5h of incubation (Plate I.17; Fig. I.21), and the eye is evident after 15.5 to 16.5h of being laid (Plate I.19; Fig. I.23). The cilia on the corona can be observed 17.0 to 17.5h after being laid (Plate I.20; Fig. I.24), a short time thereafter the eye is more conspicuous and the mastax becames very active (Plate I.21, 22; Fig. I.26, 26). Thirty minutes to one hour before hatching, the egg shows a triangular shape, and the embryo expands, and neonate is born.

DISCUSSION

YÚFERA (1987) reported that embryonic development time in two strains of *B. plicatilis* was influenced by temperature, genotype and diet. Our data given here (19.91±0.63h) is similar to his results obtained with Bs strain reared with *Nannochloropsis gaditana* as food and at 25°C of temperature. However, our results were obtained by using only the first eggs of maternal rotifers, because in this way the possible influence of maternal age on the egg developmant time was avoided. PIAVAUX (1970) has observed that the duration of development in some rotifers is affected by the age of the maternal females.

The first cleavage which occurs 30 to 40m after being laid in the egg of *B. plicatilis*, has been reported to occur at around 30m after deposition in *Monostyla cornuta* (PRAY, 1965). De BEAUCHAMP (1956) reported that the amictic egg of *Ploesoma hudsoni* develops from deposition to 4 cells stage in about one hour, and we observed that it occurs at about 55m in the egg of *B. plicatilis*. On the other hand, the observations reported here agree with those of TANNREUTHER (1920) who pointed out that in the egg of *Asplanchna ebbesbornii*, at the completion

Table 1. Some morphological characteristics of each developmental stage of amictic eggs of *B. plicatilis*.

Stage	Observation note	Time after being laid (h: m)	Corresponding figures
1	just laid (with dense yolk granules)	0:02–0:15	Plate I.1; Fig. I.1
2	with small space between yolk and shell	0:15–0:30	Fig. I.2
3	first cleavage	0:30–0:40	Plate I.2; Fig. I.3
4	2 cells	0:40–0:50	Plate I.3; Fig. I.4
5	4 cells	0:45–0:55	Plate I.4; Fig. I.5
6	8 cells	1:00–1:30	Plate I.5; Fig. I.6, 7
7	X number of cells	2:15–3:00	Plate I.6; Fig. I.8
8	invagination almost around the embryo (beginning)	3:00–4:15	Plate I.7; Fig. I.9
	(completed)	4:15–5:00	Plate I.8; Fig. I.10
9	strong invagination	5:00–6:30	Plate I.9; Fig. I.11
10	embryo folding (beginning)	6:30–7:00	Plate I.10; Fig. I.12
	(completed)	7:00–8:30	Plate I.11, 12; Fig. I.13, 14
11	foot evident	8:30–10:30	Plate I.13; Fig. I.15, 16
12	symetrical embryo	10:30–11:00	Plate I.14; Fig. I.17, 18
13	foot at its maximum development	11:00–11:30	Plate I.15, 16; Fig. I.19, 20
14	beginning of corona movements	11:30–13:30	Plate I.17; Fig. I.21
15	active movements	13:30–15:30	Plate I.18; Fig. I.22
16	eye (red pigmented body) recognizable	15:30–16:30	Plate I.19; Fig. I.23
17	cilla of the corona evident	17:00–17:30	Plate I.20; Fig. I.24
18	eye becomming more red and bigger, mastax very active	17:30–19:00	Plate I.21,22; Fig. I.25, 26
19	just before hatching (with triangular shape)	19:00–20:00	Plate I.23,24; Fig. I.27, 28

of the eight-cell stage, the embryo often assumes the shape of the one-cell condition and the cleavage furrows are scarcely distinguishable. Therefore, the pattern of early cleavage in *B. plicatilis* is similar to previous descriptions of this process in other rotifers. In general, the information presented here coinsides with that given by SALENSKY (1872) on the egg development of *Brachionus urceolaris*, even though this species is a freshwater one and *B. plicatilis* is an euryhaline one.

Probably, the duration of each developmental stage given here is also variable depending on the temperature, strain, food, and maternal age, but the time course of the development obtained could be considered as a typical one. The information on the embryonic developmen-

tal stages given could be used to define the age of embryo (as well as physiological state) in future experiments on cryopreservation of amictic eggs of *B. plicatilis*.

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シオミズツボワムシの胚発生過程

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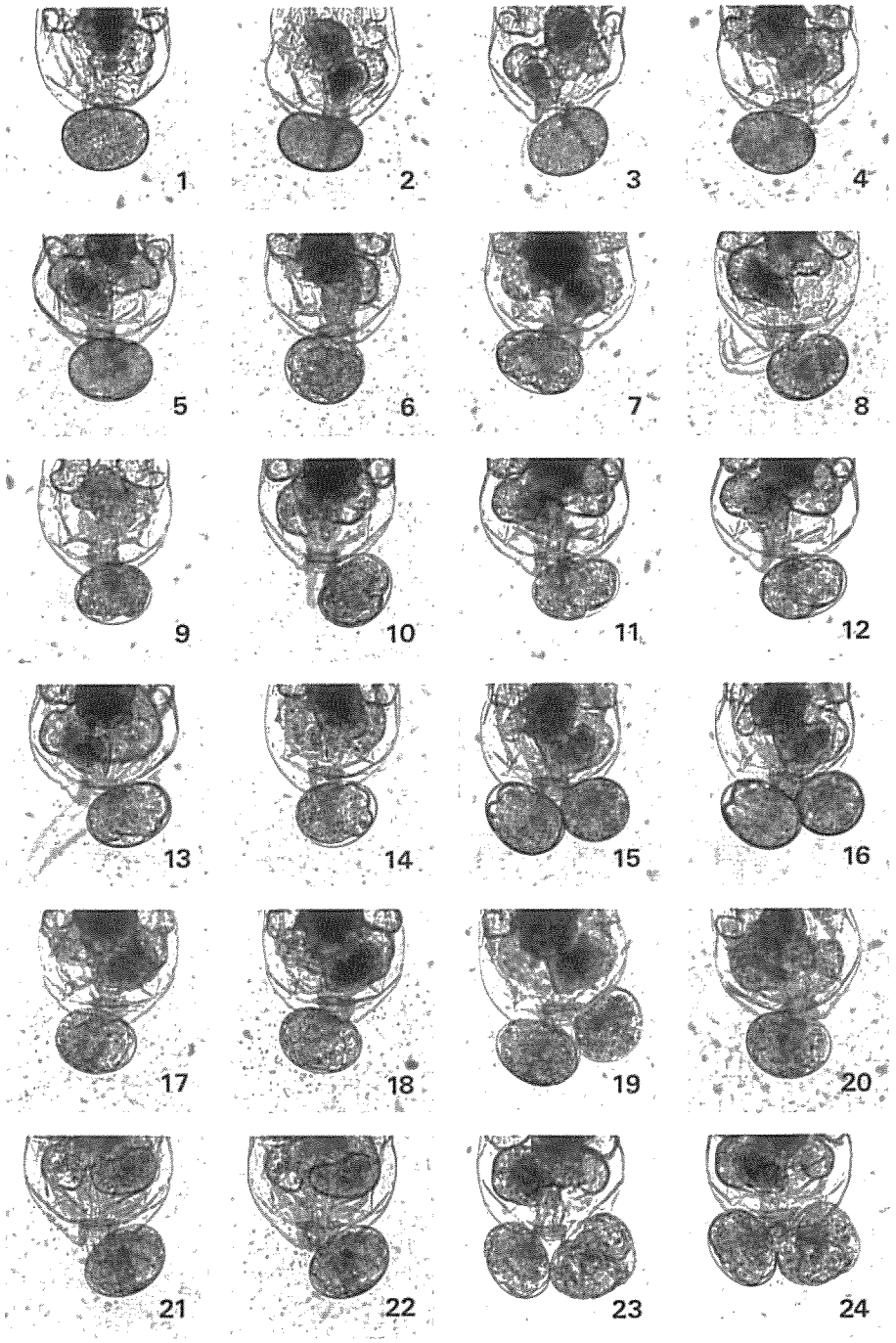
筆者らはシオミズツボワムシ諸株の増殖と保存に関する一連の研究を行っているが，その中で供試卵の発生段階の明確な記載の必要が生じたことから，その胚発生の経過について詳細な観察を行った。歴代培養したいわゆる長崎株の単性生殖雌卵からの孵化個体を供試材料とし，水温25℃，塩分25‰，明暗各12時間の条件下で，海産クロレラを餌料として個別飼育を行い，それらの産出した単性生殖雌卵（親個体に懸着）の胚発生過程を顕微鏡下で連続的に観察した結果，

1）産出された卵の孵化までに要する時間は，約20時間で一定していた。
2）胚の発生段階は，卵の外部から識別される形態的特徴にしたがって19期に分けられた。
3）産出時から各期に至るまでの経過時間，ならびに各期の形態的特徴を図示・記載すると共に，顕微鏡写真により示した。

EXPLANATIONS OF PLATE I AND FIG. I

Plate I. Photographs 1–24. *Brachionus plicatilis*. Amictic egg development (see Table 1). Photos 15, 16, refer to the eggs of the left side. Photo 15 anterior view, Photo 16 posterior view. Photos 23 and 24, refer to the egg of the right side.

Plate I.



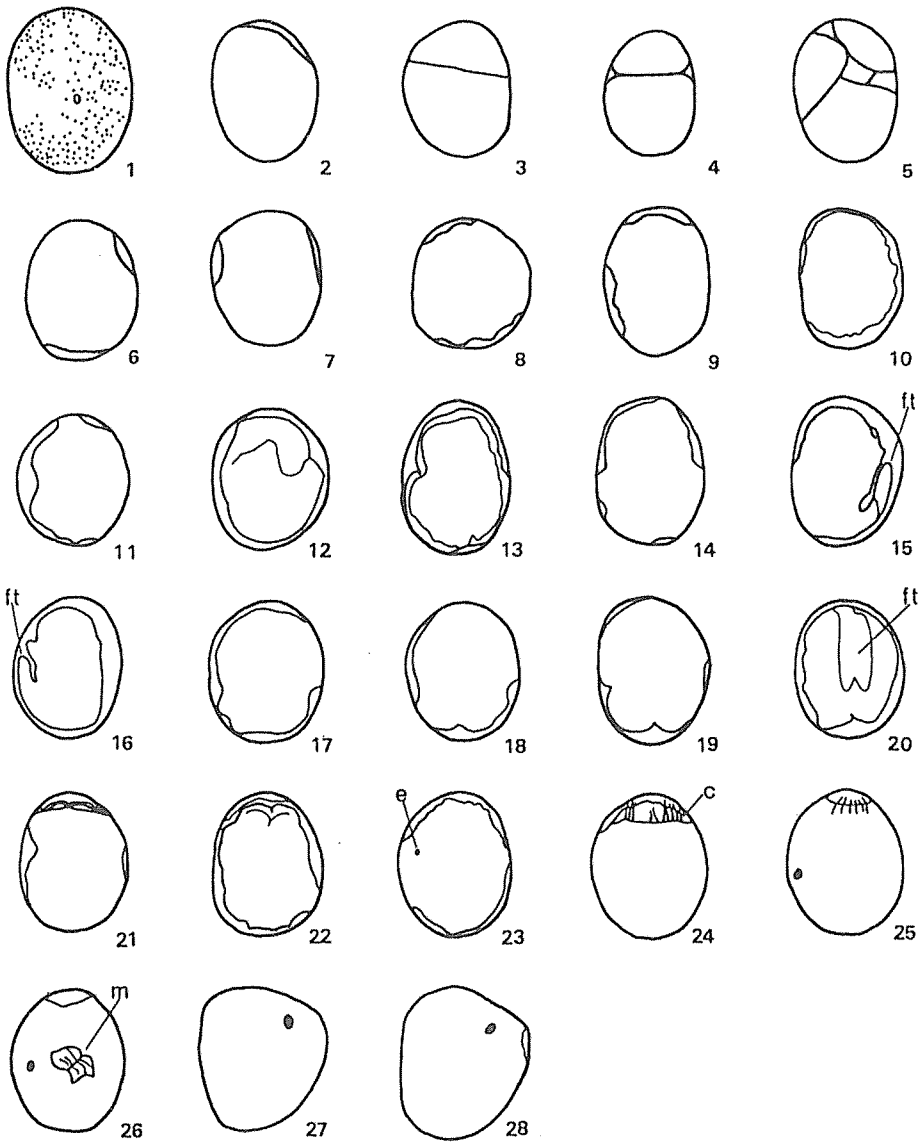


Fig. I.

Fig. I. Figures 1–28. *Brachionus plicatilis*. Amictic egg development (see Table 1). c:cilia; e:eye; ft: foot; m:mastax.