Hydrobiologia **313/314**: 373–375, 1995.
J. Ejsmont-Karabin & R. M. Pontin (eds), Rotifera VII.

Desiccation survival of the eggs of the rotifer *Adineta vaga* (Davis, 1873)

Aydin Örstan
13348 Cloverdale Place, Germantown, MD 20874, USA

Key words: bdelloid rotifers, anhydrobiosis, parthenogenesis

Abstract

Age at the time of drying affects the desiccation survival of the embryos of the bdelloid rotifer *Adineta vaga*. Although the embryos younger than 24 hours do not survive desiccation, up to 71% and 6% of the embryos at least 45 hours old survive desiccation for 2 and 10 days, respectively.

Introduction

Desiccation survival of the eggs of bdelloid rotifers has been studied rarely. Although some rotiferologists had believed since early in this century that the eggs of bdelloids could survive drying (Murray, 1908; Dobers, 1915; Bryce, 1922), supportive data were not available until Ricci *et al.* (1987) showed that the eggs of *Macrotrachela quadricornifera* Milne, 1886 survived drying for 4 days. Here, I present data on the desiccation survival of the eggs of another bdelloid, *Adineta vaga* Davis, 1873.

Methods and materials

The rotifers were from a clone cultured since November 1990. The clone was established with a rotifer from a rain gutter around a roof in Saline, Michigan, USA. I have given some information on the morphology, reproduction and embryology of this clone elsewhere (Örstan, 1992). The rotifers were cultured individually or in groups in glass culture slides kept in plastic boxes either at controlled room temperature or in a water bath. The experiments were at 22 ± 1 °C. The rotifers, kept in the dark most of the time, were exposed to direct light only during brief periods of observation. The following culture media were used. White rice extract (WRE) was prepared by boiling for 5 minutes 4 grains of white (milled) rice in 25 ml of distilled water. The grains were discarded after boiling. Brown rice extract (BRE) was prepared similarly using brown rice. Boiled soil extract (BSE) is the supernatant obtained by boiling for 5 minutes 0.35 g of garden soil in 30 ml of distilled water. Several batches of WRE and BRE but one batch of BSE were used throughout the experiments.

For the purposes of this study, the term 'drying' denotes the period during which the visible traces of water evaporated from a sample and the term 'desiccation' denotes the following period during which the sample was kept in a dry state. The eggs of *A. vaga* hatch in about 44 to 56 hours at 22 ± 1 °C. Eggs to be dried were collected within 1 hour of oviposition and incubated in distilled water in culture slides until a desired age. Additionally, eggs of unknown ages were collected and incubated for at least 45 hours. The eggs that were at least 45 hours old were examined microscopically and only those with developed embryos, indicated by the easily visible trophi, were dried. To dry an egg, as much water as possible was removed with a pipet until a thin film was left around the egg, which was allowed to evaporate in about 2 minutes. This is dubbed the 'quick drying' process. Following quick drying, dried eggs were placed in a desiccator containing $CaSO_4$ (Indicating Drierite, W. A. Hammond Drierite Co., Xenia, OH, USA). After the desiccation period, the eggs were rehydrated with distilled water and observed periodically for several days. Dead embryos appear coagulated and darker than live ones and often shrink (Örstan, 1992). If an egg hatched following rehydration, culture medium was added and

Table 1. Desiccation survival of the quickly dried eggs of *A. vaga* (A = embryo age at drying; D = desiccation time; N_e = number of eggs; N_h = number of eggs hatched; N_a = number of rotifers alive 24 hours after hatching; NA = not available).

Period	Medium	A	D	N_e	N_h	N_a
Winter 1992[1]	WRE	controls		228	226 (99%)	NA
		≤24 h	2 d	31[3]	0	–
		45±1 h	2 d	28	21	20 (71%)
Fall 1992[1]	WRE	controls		323	311 (96%)	NA
		≥45 h	2 d	10	2	1 (10%)
		45±1 h	10 d	17	0	–
Fall 1993[2]	WRE	controls		139	139 (100%)	NA
		≤24 h	2 d	17[4]	0	–
		≥45 h	2 d	30	21	20 (67%)
		≥45 h	10 d	62	10	4 (6%)
Winter 1994[2]	WRE	controls		76	76 (100%)	NA
		≥45 h	2 d	40	12	3 (8%)
	BRE	controls		155	147 (95%)	NA
		<12 h	2 h	22	0	–
		≤21 h	2 d	50	0	–
		20–44 h	2 d	47	5	5 (11%)
		≥45 h	2 d	30	24	20 (67%)
		≥45 h	10 d	60	2	0
	BSE	controls		7	7 (100%)	NA
		≥45 h	2 d	17	7	4 (24%)

[1] Most of the eggs were from rotifers that were less than 8 days old.
[2] Eggs were from rotifers of undetermined ages.
[3] 22 eggs were less than 1 hour old and 9 eggs were about 24 hours old.
[4] 5 eggs were less than 1 hour old and 12 eggs were about 24 hours old.

the rotifer was kept for at least one day. Since *A. vaga* begins to lay eggs at an age of 2 or 3 days, those that die before then would not contribute reproductively to the survival of their population. Therefore, only those rotifers that lived for at least 24 hours after hatching were counted as survivors. Control eggs were incubated without drying (Table 1). Although exact figures are not available, almost all of the rotifers that hatched from the control eggs survived for more than 24 hours. Rotifers were also quickly dried using the method described above. Dried rotifers were kept over $CaSO_4$ and rehydrated with either distilled water or WRE. The rotifers that were alive 24 hours after rehydration were counted as survivors.

Results and discussion

Bdelloids survive long periods of desiccation (from days to years) if several conditions are met before and during drying, including that they be dried slowly (Jacobs, 1909). In my experiments, 37% (24 of 65), 41% (31 of 76) and 12% (18 of 156) of quickly dried young (less than 1 day old) *A. vaga* survived 10, 20 and 60 minutes of desiccation, respectively. And 18% (18 of 99) of quickly dried old *A. vaga* (more than 1 day old) survived 60 minutes of desiccation. But none of the 69 quickly dried young rotifers survived 2 days of desiccation.

In contrast, Table 1 indicates that quickly dried eggs of *A. vaga* may survive desiccation for up to 10 days. The data also indicate that the ages of embryos at the time of drying strongly influence their desiccation survival. For example, based on data obtained with BRE in the winter of 1994, the eggs that are younger than 24 hours do not survive quick drying even when they are desiccated for only 2 hours. But 11% of the eggs 20 to 44 hours old and 67% of the eggs that are at least 45 hours old survive desiccation for 2 days. The statistical comparison of the last two sets of data, using a contingency table, indicates that the difference is significant ($\chi^2 = 23.9$, $P < 0.005$). Also, based on

data from the fall of 1993, up to 6% of the eggs that are at least 45 hours old may survive desiccation for 10 days.

However, the results obtained with older embryos over a two year period were variable. The data from the winter of 1994 suggest that the culture medium may be partially responsible for the observed variations. The statistical comparison of the data for 45-hour old eggs dried for 2 days, using a contingency table, indicates that the difference between the hatching of eggs produced using WRE and BRE is significant ($\chi^2 = 24.4$, $P<0.005$). But the difference between the hatching of eggs produced using BRE and BSE is less significant ($\chi^2 = 6.5$, $P<0.025$). Taking into account the variability of the results obtained with WRE over a two year period, at this point it is not clear if any dietary component influences the desiccation survival of the embryos.

I did not notice any structural differences between the eggs that survived desiccation and those that did not. The egg shell does not prevent drying of the embryo, for the shell, which is almost completely filled by the fully hydrated embryo, quickly collapses in air, indicating water loss from the embryo. It remains to be determined how age influences the desiccation survival of the embryos of *A. vaga* and why older embryos are better able to survive quick drying than are rotifers. Two pieces of evidence suggest that in other bdelloids embryo age may not affect desiccation survival. First, Dobers (1915), without specifying any species or presenting data, stated that the bdelloid eggs dried for 13 or 14 months continued to develop further after rehydration, regardless of how far the embryos had developed before drying. Second, Ricci *et al.* (1987) reported that 19% of the eggs of *M. quadricornifera* survived drying for 4 days. These eggs, which normally hatched in 4 days (Ricci *et al.*, 1987), were less than 24 hours old when they were dried (C. Ricci, pers. com.).

Many of the rotifers that hatched from the eggs that survived 2 or 10 days of desiccation grew and reproduced normally. Since the quickly dried rotifers barely survive for more than an hour, desiccation survival of the eggs may be an essential factor in the long term survival of the populations of *A. vaga* that inhabit microhabitats subject to quick drying.

References

Bryce, D., 1922. On some rotifera from Spitsbergen. J. Quekett Micros. Club Ser. 2, 14: 305–332.

Dobers, E., 1915. Über die Biologie der Bdelloidea. Int. Revue ges. Hydrobiol. Suppl. 7: 1–127.

Jacobs, M. H., 1909. The effects of desiccation on the rotifer *Philodina roseola*. J. exp. Zool. 6: 207–263.

Murray, J., 1908. Some African rotifers. J. r. microsc. Soc. 665–670.

Örstan, A., 1992. Toxicity of acrylamide derivatives to embryos of the rotifer *Adineta vaga*. Bull. envir. Contam. Toxicol. 48: 901–906.

Ricci, C., L. Vaghi & M. L. Manzini, 1987. Desiccation of rotifers (*Macrotrachela quadricornifera*): survival and reproduction. Ecology 68: 1488–1494.