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Effect of algal diet and temperature on the embryonic development time of the rotifer *Brachionus plicatilis* in culture

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Abstract

The embryonic development times of two strains of *Brachionus plicatilis* (Bs and S-1) cultured on three different algal diets (*Nannochloris oculata, N. maculata* and *Nannochloropsis gaditana*), have been determined at 20 °C, 25 °C and 30 °C. As expected, the embryonic development times decreased with increasing temperature in all cases. However, embryos from adults fed on *N. gaditana* tended to develop more slowly than those of individuals fed on the other algal species. Mean egg volume was also affected by diet, larger eggs being produced by females fed on *N. gaditana.* No obvious relationship between egg size and temperature was detected.

Two principal factors seemed to affect the embryonic development time. The first was temperature which acts through its well known effect on metabolic rates. The second was maternal diet which probably affects development time through its effect on yolk content, as reflected in the size of the egg.

Introduction

It is well known that embryonic development time in rotifers, as in other zooplankton, is highly correlated with temperature [for recent review see Duncan, 1983; Herzig, 1983]. However, there was no evidence that other factors could affect this process, until recent studies detected a possible influence of food quantity [Walz, 1983] and quality [Yúfera & Pascual, 1984] in *Brachionus* spp.

The literature reports a relationship between several parameters involved in the developmental process, particularly between embryonic development time, egg volume and maternal size [Pourriot, 1973; Duncan, 1983]. Some authors have also reported that food type may influence the size of females in cultures [Yúfera, 1982; Snell & Carrillo, 1984]. This suggests a possible association between food and embryonic development time.

In the present work, the influence of diet on the embryonic development time of rotifers in mass culture has been studied and compared to the effect of temperature. Variations in egg volume under different conditions have also been examined, with particular reference to the relationship between egg size and development time.

Materials and methods

Two strains of *Brachionus plicatilis* (strains Bs and S-1) were used for the experiments. The characteristics of both strains, and the culture methods for algae and rotifers, are described by Yúfera [1982] and Yúfera *et al.* [1983]. The algal species used as food were: *Nannochloris oculata* (Cambridge, 251/6), *Nannochloris maculata* (Cambridge, 251/13) and *Nannochloropsis gaditana* (Cádiz, B-3).

The embryonic development time of the rotifers was determined at 20 °C, 25 °C and 30 °C for each algal species. Females were removed from 1 litre culture flasks (after acclimatization to the experimental food and temperature) during the exponential growth phase. The animals were placed in small petri dishes with $\simeq 5$ ml of algal suspension at a concentration of $50-100$ μg d.w. ml^{-1}. The algae were also taken from cultures in the exponential phase of growth. Cultures were examined at half hourly intervals during the critical periods before laying and hatching. Thus, each determination has an error of ± 0.5 hours. Embryo development time was determined 20 times for each set of culture conditions.

Size of females [following the method of Yúfera, 1982] and egg volume ($0.524 \times$ length $\times$ width^2). were measured with an ocular micrometer with 2 μm precision.

Results

Figure 1 shows the relationship between embryonic development time and temperature for each alga tested. The data has been fitted to an exponential regression (see Table 1). Both *Brachionus* strains showed the expected decrease in embryonic development time with increasing temperature, but the values were different for each strain. Embryos from strain S-1 consistently developed at a slower rate than those from strain Bs, for any given temperature. In addition, populations fed on *Nannochloropsis gaditana* tended to have longer development times than those fed on *Nannochloris oculata* and *N. maculata*. This was true of both rotifer strains over all temperatures tested, except for strain Bs at 20 °C and strain S-1 at 30 °C.

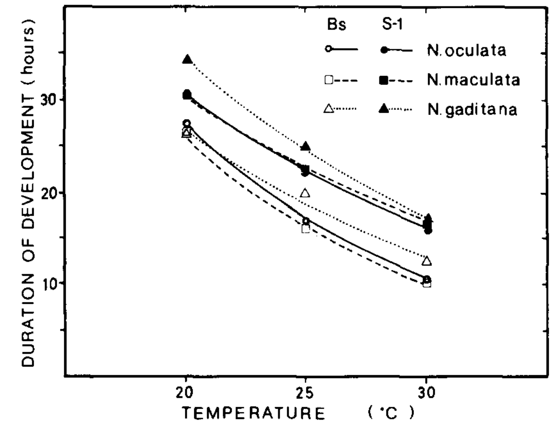

Fig. 1.. Relationship between embryonic development time and temperature for two strains of *Brachionus plicatilis* (Bs and S-1) fed on three different algal species.

An analysis of variance showed a highly significant influence of temperature, algal diet and rotifer strain ($P<0.001$) on embryonic development time (Table 2). The two-way interactions (algae $\times$ strain) and (temperature $\times$ algae) as well as the three-way interaction were significant ($P<0.001$). These data showed that embryonic development time was influenced primarily by temperature and genotype. The effect of diet was less and depended upon the genetic characteristics of the rotifers, and on their response to temperature when fed on each algae.

Temperature did not sifnificantly affect egg volume (Table 3). Only strain Bs showed any response – a reduction in egg size at 30 °C. However, for a given

Table 1. Coefficients of an exponential equation of the form $y = ae^{bx}$ relating embryonic development time to incubation temperature for two strains of *Brachionus plicatilis* (Bs and S-1) fed on three algal diets.

Alga	*Brachionus plicatilis* strain					
	Bs			S-1		
	a	b	r	a	b	r
N. oculata	181.79	−0.095	−0.994	110.74	−0.064	−0.995
N. maculata	173.13	−0.095	−0.996	98.70	−0.059	−0.991
N. gaditana	115.67	−0.073	−0.979	141.66	−0.075	−0.991

Table 2. Factorial ANOVA of the effects of temperature, algal food species, and rotifer strain on embryonic development time. Fixed model with 20 replicates.

Source	df	SS	SM	F	P
Temperature	2	13986.05	6993.03	13194.4	0.001
Algae	2	295.80	147.90	282.83	0.001
Strain	1	2667.78	2667.78	5033.55	0.001
Temperature × algae	4	55.36	13.84	26.11	0.001
Temperature × strain	2	1.70	0.85	1.60	ns
Algae × strain	2	31.80	15.90	29.94	0.001
Temperature × algae × strain	4	152.42	38.11	71.76	0.001
Error	342	181.50	0.53		

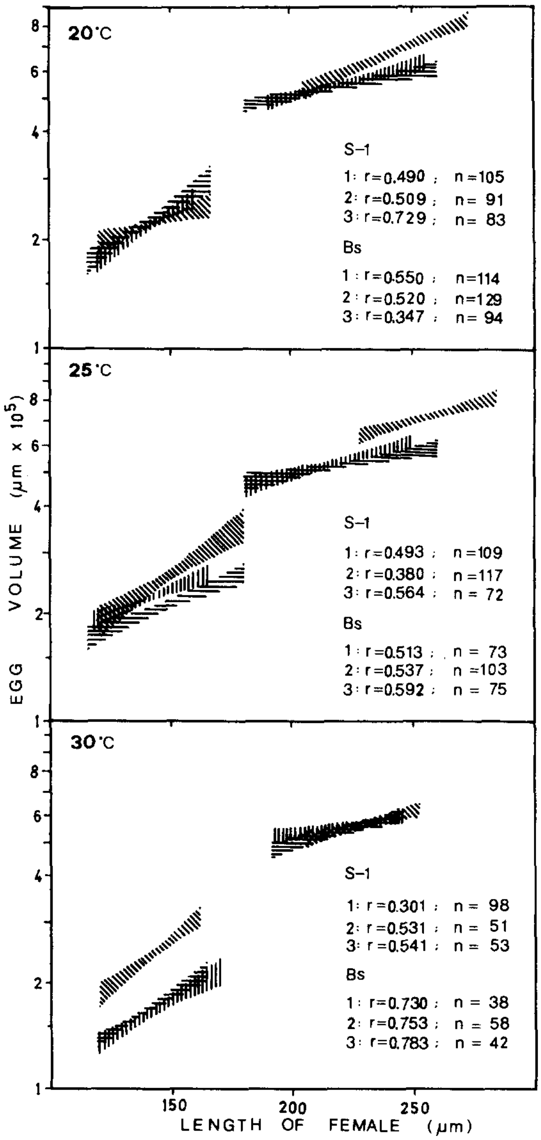

Fig. 2.. Relationship between egg volume and length of the mother individual. Shaded areas indicate 95% confidence intervals for animals fed *Nannochloris oculata* (vertical shading), *Nannochloris maculata* (horizontal shading) and *Nannochloropsis gaditana* (oblique shading). For each temperature the regressions shown on the right correspond to strain S-1, on the left to strain Bs.

temperature, egg volume did vary with diet. Animals fed *N. gaditana* tended to produce larger eggs. Figure 2 shows the relation between egg volume and maternal size. The correlation coefficients found (with the logarithmic transformed egg volumes) were significant in all cases ($P < 0.001$; except in strain S-1 fed on *N. oculata* at 30 °C: $P < 0.005$). However, the correlation coefficients were low (0.3 to 0.8) and only partially accounted for variability in egg volume.

Discussion

The results confirm that diet can affect the egg development time of *B. plicatilis* in mass culture. In this study, a change of diet to *N. gaditana* had a similar effect on embryonic development rate as a decrease in temperature of about 2 °C. This may be due to a nutritional deficiency since rotifers show limited ingestion rate with this alga [Yúfera & Pascual, 1985].

Table 3. Egg volume ± S.D. ($\mu m^3 \times 10^3$) and egg development time ± S.D. (hours) under three different food conditions and three incubation temperatures.

Strain:	Bs			S-1		
Temperature:	20	25	30	20	25	30
Egg volume						
N. oculata	219 ± 33	220 ± 24	166 ± 21	556 ± 65	545 ± 69	565 ± 50
N. maculata	227 ± 39	210 ± 40	164 ± 21	539 ± 65	530 ± 62	535 ± 49
N. gaditana	223 ± 28	258 ± 52	244 ± 37	691 ± 110	708 ± 78	572 ± 62
Development time						
N. oculata	27.4 ± 1.3	16.9 ± 0.6	10.6 ± 0.5	30.6 ± 0.6	22.2 ± 0.7	16.1 ± 0.5
N. maculata	26.0 ± 0.7	16.2 ± 0.6	10.1 ± 0.4	30.2 ± 0.7	22.5 ± 0.3	16.7 ± 0.9
N. gaditana	25.8 ± 1.0	19.8 ± 0.8	12.4 ± 0.5	34.2 ± 0.8	25.0 ± 0.9	16.9 ± 0.8

In contrast to results obtained by Pourriot [1973] for *Brachionus calyciflorus*, the present study did not show a clear relationship between egg volume and temperature. Further experiments over a wider range of temperature would be necessary to confirm this. However, the results did show that larger eggs and longer embryonic development times were associated with algal diets inducing lower egg per female ratios [Yúfera & Pascual, 1984]. This is similar to the findings of Wals [1983] who reported a good correlation between clutch size and egg volume in *Brachionus angularis*. Although the relationship between egg volume and development time is clear, the nature of this relationship is not entirely understood.

In summary, two main factors seem to affect embryonic development time in rotifers. The first, and probably the most important factor, is temperature which influences egg development rate through its effect on metabolic rates. The second is egg volume (i.e. yolk content) which is determined by the feeding conditions of the mother.

Acknowledgements

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