

Does temperature affect dimorphic reproduction in benthic foraminifera? A culture experiment on *Rosalina leei*

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The possible effects of environmental factors on the dimorphic mode of reproduction in benthic foraminifera have been reported in the literature. Published observations based on field samples have indicated an inverse relationship between the ratio of microspheric/megalospheric forms and temperature or salinity. These findings imply their potential use in palaeoclimatic studies. Two experiments were conducted on the benthic foraminifera *Rosalina leei* (Hedley and Wakefield) collected from Vineyard Sound near Woods Hole, Massachusetts, USA. In the first experiment, a large number of specimens were maintained at four different temperatures (5, 15, 20 and 25°C) and observations were made on the frequency of pairing (an indication of sexual activity). The percentage of pairs formed was directly related to the temperature, i.e. 0 pairs at 5°C to 19.3% pairs at 25°C. In the second experiment, paired and unpaired specimens were maintained separately at three different temperatures (15, 20 and 25°C). Nearly one-half of the unpaired specimens reproduced at all three temperatures, while none of the paired specimens reproduced. Type and frequency of feeding were common in all the experiments. We surmised that dimorphic reproduction in *R. leei* requires a different environment.

STUDIES on the life cycle or differential mode of reproduction in benthic foraminifera have been of utmost importance and they have been investigated by many workers¹⁻⁶. Boltovskoy and Wright⁷ in a review, indicated that microspheric and megalospheric generations in foraminifera might have different environmental requirements. Later, attempts were made to correlate dimorphic test ratios with climatic changes, suggesting their utility in palaeoclimatic applications⁸⁻¹². However, Nigam and Khare¹¹ suggested that this approach must be tested through laboratory experiments. Therefore, we have attempted to study *Rosalina leei*¹³ species of benthic foraminifera in laboratory culture to see whether microspheric and megalospheric forms required different environmental conditions. The selection of *Rosalina* species was based on its availability in field collections and that it is known to have a dimorphic life cycle^{14,15}.

First, a preliminary survey was made for collection of benthic foraminifera from sea grasses and surface sediment samples from three locations near Woods Hole, Massachusetts, USA, viz. (i) Vineyard Sound at the shore lab facility of WHOI, (ii) Buzzard Bay at the Marine Biological Laboratory and (iii) Woods Hole (near Redfield Laboratory). Based on the richness of the foraminiferal fauna, the first location was selected as a permanent station for sample collection. The species *R. leei* was selected for future experiments. Foraminiferal specimens were obtained by vigorous shaking of *Sargassum* into a plastic bucket filled with sea water. The residue was passed through a 63 µm opening sieve. The > 63 µm fraction was kept in a jar filled with sea water and the specimens were sorted out with a pipette. Filtered (0.22 µm Millipore filtered) sea water was used as culture medium and changed every alternate day.

A mixture of three microalgae was cultured separately and used as food (every alternate day) for living specimens of foraminifera. The following species (for which stock cultures were available at Woods Hole Oceanographic Institution) were cultured: (i) *Isochrysis galbana* [clone T-ISO]; (ii) *Chlorella capsula* [clone FLA-E]; and (iii) *Minutocellus polymorphus* [clone Minuto]. All the species were cultured in F-2 medium.

With the objective to check the effect of temperature on inducing pairing of two gamonts by their ventral side for sexual reproduction¹⁶, the picked specimen were kept in one large jar at four different temperatures (5, 15, 20 and 25°C). The percentage of specimens making pairs has been calculated in each case.

In another experiment, a large number of specimens were kept in a jar at 20°C. Paired and unpaired specimens were picked after a week and kept one each in six well-sterilized culture dishes. A total of 36 pairs and 36 single specimens were used in this experiment. Mixed prey (one drop) was added every alternate day. This experiment was conducted at three different temperatures, i.e. 15, 20 and 25°C. The objective was to study differences in sexual and asexual reproduction.

When a large number of specimens was kept in a container, many of them formed pair. The experiment conducted (Table 1) to study the effect of temperature on their behaviour indicated that an increase in temperature

Table 1. Effect of temperature on frequency of pairing (an indication of sexual activity)

Temperature (°C)	No. of specimens kept in experiment	No. of pairs after a week	Pairs (%)
5	61	0	0
15	39	6	15.3
20	64	11	17.0
25	26	5	19.3

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Table 2. Effect of temperature on single specimens and paired specimens

Temperature (°C)	Single specimen		Paired specimen		
	No. of specimens	No. of reproduced specimens	No. of pairs	No. of separated pairs	No. of reproduced pairs
15	12	5	12	2	0
20	12	5	12	4	0
25	12	6	12	5	0

resulted in more specimens forming pairs and thus represents increased tendency for sexual reproduction.

When paired and unpaired specimens were kept in individual culture dishes at temperatures of 15, 20 and 25°C, none of the paired specimens reproduced (Table 2). In addition, when kept for a prolonged period, paired specimens showed a tendency to separate at higher temperature, indicating death without reproduction. Whereas, in similar conditions, reproduction was observed at all the temperatures in single specimens (Table 2). In this case, a single specimen produced as many as 39 young ones.

We surmise that dimorphic reproduction in benthic foraminifera (at least in the case of *R. leei* species) requires different environmental factors to complete the life cycle. An increase in temperature resulted in an increase in tendency for sexual reproduction. However, we are yet to obtain optimum environmental conditions, (including type of food) in which paired specimens can reproduce. The study suggests the need for more experiments with different benthic foraminiferal species at different temperatures with varieties of prey.

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