

Occurrence of Resting Eggs in *Macrostomum orthostylum* (M. Braun, 1885) (Turbellaria : Macrostomida)

By BABAN S. INGOLE

Biological Oceanography Division National Institute of Oceanography,
Dona Paula, Goa (India)

With 2 Figures and 1 Table

(Eingegangen am 24. September 1986)

Key words; resting eggs, salinity, temperature, *Macrostomum orthostylum*, Turbellaria

Abstract

An euryhaline macrostomid Turbellaria, *Macrostomum orthostylum* (Braun, 1885) laid two different types of eggs, subitaneous and resting, each of which produced in response to different environmental conditions especially temperature and salinity. Individually reared specimen produced only subitaneous eggs whereas, resting eggs were produced after mating. Production of resting eggs increased with increasing salinity, and the greatest production of resting eggs (66%) was in the salinity range of 18–21‰. Hatching of resting eggs was influenced by temperature and highest (55%) hatching success was achieved at 27°C, with a minimum incubation period (18.5 days). The relationship between the hatching time (days) and temperature (°C) was calculated as $D = -0.5530(T-15.0) - 1.3249$.

Introduction

Free-living Turbellaria are known to lay two kinds of eggs which differ in morphology and physiology (HYMAN 1951). The type of eggs produced depends upon environmental conditions. Subitaneous (non-resting) eggs are produced throughout the life span and increase population size under normal environmental conditions. Dormant (resting) eggs are produced during adverse environmental conditions and ensure survival of the population over unfavourable periods.

The occurrence of both types of eggs in the life cycle of rhabdocoelid turbellarians has been reported by several workers (LUTHER 1963, KAESTNER 1965, YOUNG 1975, INGOLE 1982, SURGUR and INGOLE 1983). However there is still a lack of information on the environmental factors which influence the type of egg produced and which control their hatching.

This paper reports on studies of egg-laying by *Macrostomum orthostylum* (Braun) an euryhaline macrostomid species which has been reported from different localities in Europe, East Africa, North America and India (PAPI 1951, LUTHER 1960, YOUNG 1976, SURGUR 1980). Particularly, observations were made on 1. the production of subitaneous and resting eggs in different salinities, and 2. the effect of temperature on the hatching of resting eggs.

Materials and Techniques

General Culture Technique

Egg-bearing specimens of *M. orthostylum* were collected originally from the brackish-water fish farm at Kakinada, India, and cultures were maintained at the Marine Biological Research Station (M.B.R.S.) at Ratnagiri, India (SHIRGUR 1980). Live specimens were obtained from the M.B.R.S., Ratnagiri in 1981 and sub-cultures were established in the Aquaculture Laboratory of N.I.O., Goa. The methods used to culture the turbellarians involved the 'phased fertilization' technique, which has been described in detail by INGOLE (1982) and SHIRGUR et al. (1984).

Production of resting eggs

Mature specimens of *M. orthostylum* carrying eggs in their tail portion were introduced into separate cavity blocks containing water of 15–21‰ salinity. Eggs were laid after 6–8 days, and 50% of them were incubated, without drying, in a fresh culture medium of 9‰ salinity. Hatchability of the eggs was observed under a constant temperature ($24^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and photoperiod (12 h D: 12 h r L). The remaining 50% were carefully placed on glass slides and allowed to dry at room temperature ($24 \pm 1^{\circ}\text{C}$). After a desiccation period of 30 days, 10% of the eggs (dried) were cultured in water of 9‰ salinity using the same temperature and photoperiod as above. The experiment was repeated twice.

Egg laying in different salinities

SHIRGUR (1980) and INGOLE (1982) have reported that euryhaline *M. orthostylum* can reproduce in water salinities ranging from 0 to 21‰ though the optimum value for massculture is 8–10‰. In the present study, the egg-laying process was observed at different salinities within the range of 0–21‰ (Tab. 1). Animals were kept in glass petri-dishes of 5 ml capacity, and four petri-dishes were used at each of the salinities. The experiments were conducted at $24 \pm 1^{\circ}\text{C}$ with a photoperiod of 12 h r D: 12 h r L. Two series of experiments were conducted. In the first, single mature specimens of uniform size and age, were introduced into each petri-dish and observations were made daily on egg production, and any eggs produced were isolated from the dishes and counted. Observations continued till the death of the animal. 50% of the eggs produced were placed directly into water of an optimum salinity of 9‰, 50% were dried at $24 \pm 1^{\circ}\text{C}$ on glass slides for 30 days before introduction to the optimum salinity. All eggs were observed for hatching.

Table 1. The total number of eggs produced per animal and percentages of subitaneous (S) and resting (R) eggs produced during three consecutive reproductive phases in the life span of *Macrostromum orthostylum* at each of the salinities

Range of salinities	Total number of eggs produced		Production of two types of eggs (%) during three reproductive phases						% of all stages combined	
			I		II		III			
	S	R	S	R	S	R	S	R	S	R
0–6	13	05	83.33	16.66	66.66	33.33	66.66	33.33	72.21	27.77
7–11	16	03	85.71	14.28	85.33	14.67	83.33	16.66	84.79	15.20
12–17	12	06	60.00	40.00	80.00	20.00	57.14	42.85	65.71	34.28
18–21	10	09	50.00	50.00	71.42	28.57	33.33	66.66	51.58	48.41

In the second experimental series, a pair of mature specimens were introduced into each of the salinities (0–21‰). Again, the occurrence of egg-laying was recorded in all salinities until the death of the animals, when all resting eggs were isolated and dried for 30 days.

Effect of temperature on hatching of resting eggs

Hatching success was investigated at seven different temperatures. Experimental temperatures were obtained using thermostatically controlled water baths. Resting eggs were incubated in water of a salinity of 9‰ at each of the temperatures 15, 18, 21, 24, 27, 30°C ($\pm 1^\circ\text{C}$). At each temperature, fifty eggs were placed in batches of ten in cavity blocks containing 2 ml of the culture medium. All the eggs were exposed to room temperature (24°C) for about 24 hours before incubation.

Results

Production of resting eggs

The animals cultured at a salinity of 15–21‰ produced both subitaneous and resting eggs. During the laying of resting eggs, the posterior region of the flatworm elongated and finally ruptured (Fig. 1). The subitaneous eggs were hatched within 48 hours without drying, whereas resting eggs could not be hatched without drying. When desiccated for a period of 30 days, hatching took place in a salinity of 9‰ after 25 days at room temperature (24°C), and only one hatchling emerged from each egg.

Egg laying in different salinities

In single-animal cultures, thin-shelled subitaneous eggs were produced after 6–8 days in all salinities. Only this type of egg was produced throughout the life span with a single egg being laid at intervals of 3–4 days. The number of eggs produced was greatest in the salinity of 7–11‰ with a maximum of 94 eggs per individual being recorded.

In cultures using two animals, thin-shelled subitaneous eggs were produced and after 6–8 days resting eggs were produced individually. This is the first reproductive phase in *M. orthostylum*. After 30–35 days, thick-shelled, oval-shaped eggs were observed in all salinities, as well as subitaneous eggs. This was the second reproductive phase. The production of both types of eggs was reduced after 75 days which is the third reproductive phase.

Subitaneous and resting eggs were produced in each of the three reproductive phase. The ratio of subitaneous to resting egg was highest for all phases, at a salinity of 7–11‰. Relatively more resting eggs were produced at lower and higher salinities (Tab. 1).

Effect of temperature on the hatching of resting eggs

Hatching of resting eggs occurred over a wide range of temperatures. Hatching success was greatest at 27 to 30°C and poorest at 15 to 18°C. Hatching rate increased with increasing temperature (Fig. 2). The relationship between hatching time (days) and temperature (0°C) can be expressed, using the best fit Belehradek equation (MCLA-BEN 1966), as follows:

$$D = -0.5530 (T - 15.0)^{-1.3249}$$

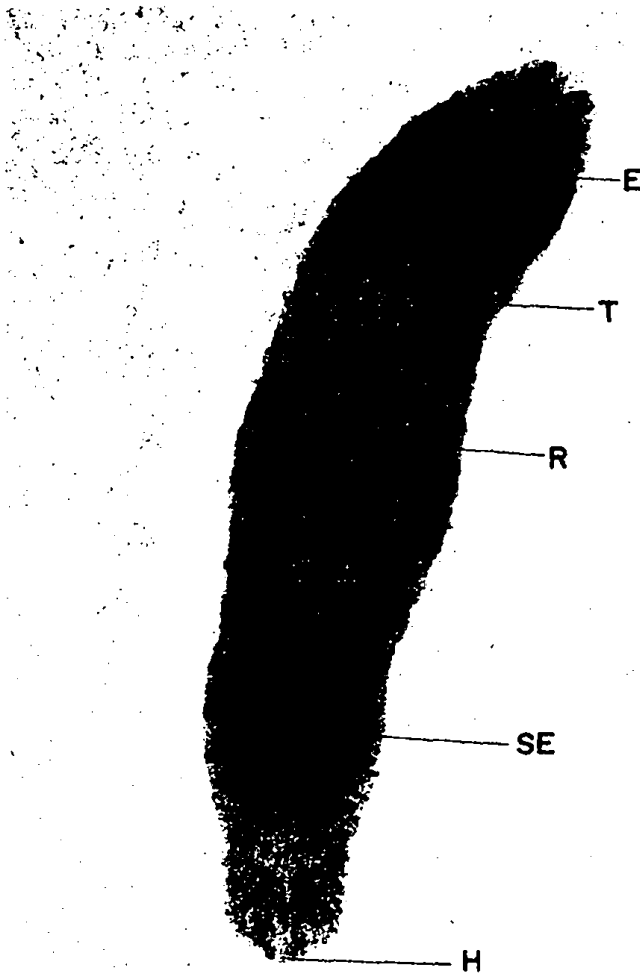


Fig. 1. *Macrostomum orthostylum* (Ventral view; L. 0.9 mm) — E, eyes; T, testes; R, rhabdites; SE, Subitaneous egg; H, Haftpapillen

Discussion

The euryhaline, hermaphrodite, turbellarian *Macrostomum orthostylum* tolerates a very wide range of salinities from 0 to 27‰ (INGOLE 1982). The present study revealed that thin-shelled subitaneous and dark, thick-shelled, dormant eggs are also produced within the salinity range of 0 to 21‰. At all salinities, the production of subitaneous eggs was found to be greater than the production of resting eggs. At an optimum salinity of 7–11‰ relatively more subitaneous eggs than resting eggs are produced than at lower or higher salinities. In other words, non-optimum salinities, low and high, stimulate the production of more resting eggs. The subitaneous eggs are similar in diameter to resting eggs (0.0610 mm). However, subitaneous eggs are thin-shelled, dark-coloured and filled with germplasm whilst resting eggs have only a compact mass in their centre.

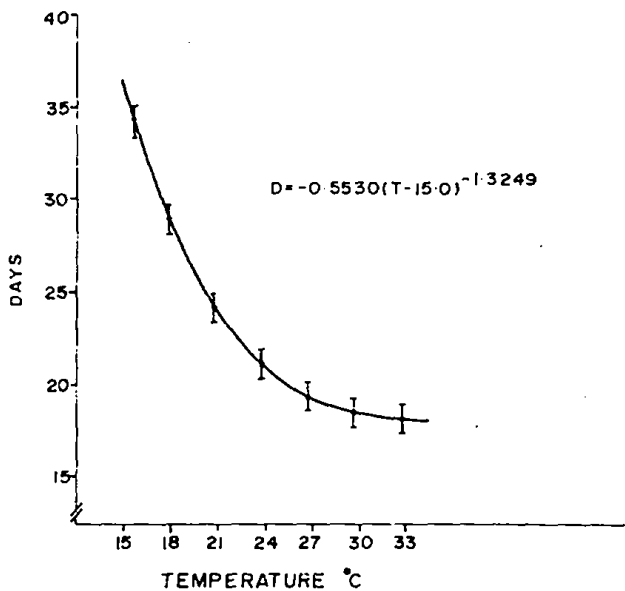


Fig. 2. Showing the relationship between hatchingtime and temperature in resting eggs of *M. orthostylum*

Individually reared specimens of *M. orthostylum* produced only subitaneous eggs throughout their life span, whilst subitaneous and resting eggs were produced by individuals when mated. Maximum number of eggs were produced in the second and third reproductive phases. KAESTNER (1965) has stated that a young *Mesostoma ehrenbergi* rapidly produced about 400 thin-shelled, subitaneous eggs but after mating the same animal produced normal hard shelled dormant eggs. Reporting on reproductive behaviour in rotifers, KING and SHELL (1977) have suggested that resting eggs were the product of true sexual reproduction. In freshwater animals, photoperiod and temperature are the most universal factors known to induce diapause (CLUTTER 1978). However, the present study revealed that salinity can also play an important role in inducing a resting stage in brackishwater Turbellaria. Temperature is one of the most important factors affecting the rate of development of resting eggs in *Macrostomum orthostylum*.

Finally, it is pertinent to stress that these flatworms have potential as live food for the larval stages of commercially-important prawns and fishes reared in mass cultures. Transport of "seeding" material is easy due to the occurrence of resting eggs in the life-cycle.

Acknowledgement

I am most grateful to Dr. G. A. SHIRGUR, Research Officer and Head, Tarapurwale Marine Biological Research Station, Bombay, for guidance during this study. I would like to thank the Director, National Institute of Oceanography for facilities and Drs. T. S. S. RAO, A. H. PARULEKAR and ANIL CHATTERJI, Biological Oceanography Division for encouragement. Thanks are also due to Dr. J. O. YOUNG, Dept. of Zoology, Liverpool University, Liverpool (England), for critically reviewing the manuscript.

References

- CLUTTER, M. E.: In: (Ed.): Dormancy and Development Arrest. New York: Academic Press 1978.
- HYMAN, L. H.: In: (Ed.): The Invertebrates. Vol. 2. Platyhelminthes and Rhynchocoela, the acelomate Bilateria. New York: McGraw-Hill Book Co. 1951.
- INGOLE, B. S.: In: On standardisations of phased fertilisation technique for mass culture of Macrostromid turbellarian *Macrostromum orthostylum* (M. Braun, 1885) and Harpacticoid copepod *Amphiascoides subdebilis* (Willey, 1935). M.Sc. Thesis, Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri (Maharashtra : India) 1982.
- KAESTNER, A.: In: (Ed.): Invertebrate Zoology. Inter Science Publishers. Vol. 1. New York: John Wiley & Sons 1965, 162.
- KING, C. E., and T. W. SHELL: Sexual recombination in rotifers. *Heredity* **39** (1977) 357—360.
- LUTHER, A.: Die Turbellarien Ostfennoskandiens. I. Acoela, Catenulida, Macrostromida, Lecithoepitheliata, Prolecithophora, und Proseriata. *Fauna fenn.* **7** (1960) 1—55.
- : Die Turbellarian Ostfennoskandiens: 4. *Neorhabdocoela*, 2. *Fauna fenn.* **16** (1963) 1—163.
- MCLAREN, I. A.: Predicting development rate of copepod eggs. *Biol. Bull.* **131** (1966) 457—469.
- PAPI, F.: Ricerche Sui Turbellaria Macrostromidae. *Archo Zool. ital.* **36** (1951) 289—340.
- SHIRGUR, G. A.: Observations on mass culture of a euryhaline macrostromid turbellarian *Macrostromum orthostylum* (Braun, 1885) in relation to reproductive behaviour under optimum salinity and temperature. *Symp. on Coastal Aquaculture Cochin* Jan. 1980.
- , and B. S. INGOLE: Continued observations on reproductive behaviour of a species of *Macrostromum orthostylum*, turbellarian, correlated to fecundity and different feeding conditions under varying salinities. *Nat. Symp. on Shrimp Seed Production and Hatchery Management, Cochin*, Jan. 1983.
- , D. V. GADGE, B. S. INGOLE, A. NAIK and G. PANDARIKAR: Phased fertilization for culturing copepods. *Second International Conference on Copopods, Ottawa, Canada, 13—17, Aug. 1984.*
- YOUNG, J. O.: The population dynamics of *Phaenocora typhlops* (Vejdovsky) (Turbellaria : Neorhabdocoela) living in a pond. *J. Anim. Ecol.* **44** (1975) 251—262.
- : Systematic studies on limnic *Macrostromium* species (Turbellaria, Macrostromida) from East Africa. *Zool. Scripta* **5** (1976) 49—60.

BABAN S. INGOLE, Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa-403 004 (India)