

Studies on the Regeneration in Macrostomum orthostylum (BRAUN) (Turbellaria: Macrostromida)

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ABSTRACT

The regeneration process was observed in the laboratory cultured macrostomid turbellarian, *Macrostomum orthostylum*. Recovery of cut portions was very fast at the anterior region as compared to the posterior region. The average regeneration rate observed was 0.069 mm/day. However, regeneration was not observed in the region anterior to the cerebral ganglion.

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Information available on the regeneration in free-living Turbellaria indicates that acoelid turbellarians have limited power of regeneration (Ghild, 1907; Peebles 1913 and Keil 1929). In the case of allococeles and macrostomid, Ruhl (1927) has reported that the former are incapable of any regeneration and the latter whilst incapable of a sexual reproduction, can regenerate, particularly towards the tail portion.

Keeping in view the paucity of information on the regeneration in *M. orthostylum* (BRAUN), the present studies was undertaken and results are reported here.

MATERIALS AND METHODS

A culture of *Macrostomum orthostylum* was maintained in the laboratory in glass beakers following the method described by Shirgur (Shirgur 1980). For the present study, healthy adult specimens of uniform

size, were isolated from the culture stock. The animals were placed on a microscopic slide with a drop of water and allowed to extend. A cut was made at desired part of the body of animal by a sharp razor. Sometimes due to the fast movement of the animal, it was not possible to make a cut at the desired part of the body and thus, animals were placed on a cavity slide in a drop of water, and then a drop of PHCl_3 was added by a fine dropper. Movement of the animal was subsequently slowed down, making a cut at the desired part easier.

Cut portions were immediately transferred into separate glass cavity block (2 ml capacity) having culture media of 9‰ salinity. Observations on the cut portion were carried out every day under a low power dissecting microscope. The figures, of different developmental stages of the cut portion, were made using a camera lucida, while all measurements were taken by a micrometer.

RESULTS AND DISCUSSION

Macrostomum orthostylum, an euryhaline turbellarian, recorded first time from Indian water (Shirgur 1980) and cultured in the laboratory for nutritional requirements of hatchery level prawn seed production (Shirgur and Ingole 1983). It has been observed in the mass culture of *M. orthostylum* that sometimes the tail portion of the animal gets detached in

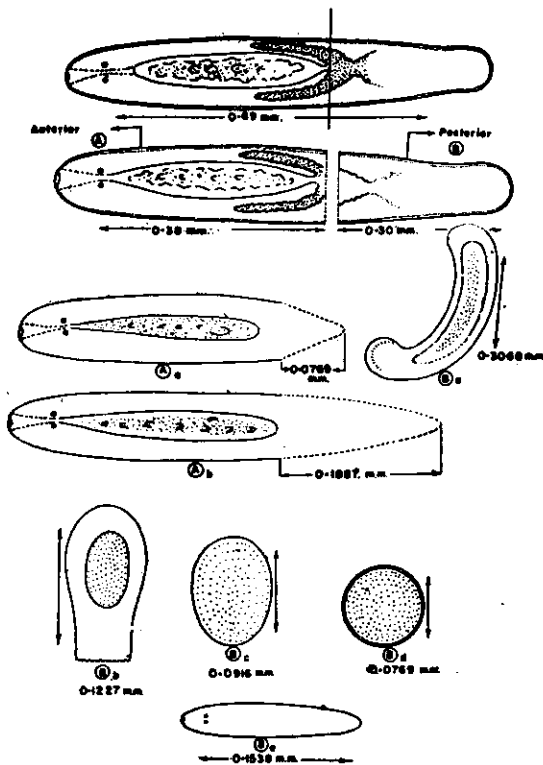


Fig. 1 Epimorphosis and morphallaxis regeneration
 1A Anterior cut portion
 1Aa Anterior portion regenerating its lost portion
 1Ab Anterior portion completely regenerated
Fig. 1B Posterior cut portion
 1Ba Posterior cut portion changed into curve shape
 1Bb Curve shape changed into cap like structure
 1Bc Cut portion transformed into spherical shape
 1Bd Egg-shaped cut portion
 1Be Newly hatched young one

the course of laying of subitaneous eggs and regenerates to a new young animal. Specimens below 0.4 mm length were immature and did not show morphallaxis type of regeneration. Therefore, only the adult specimens measuring more than 0.4 mm, which were found to undergo

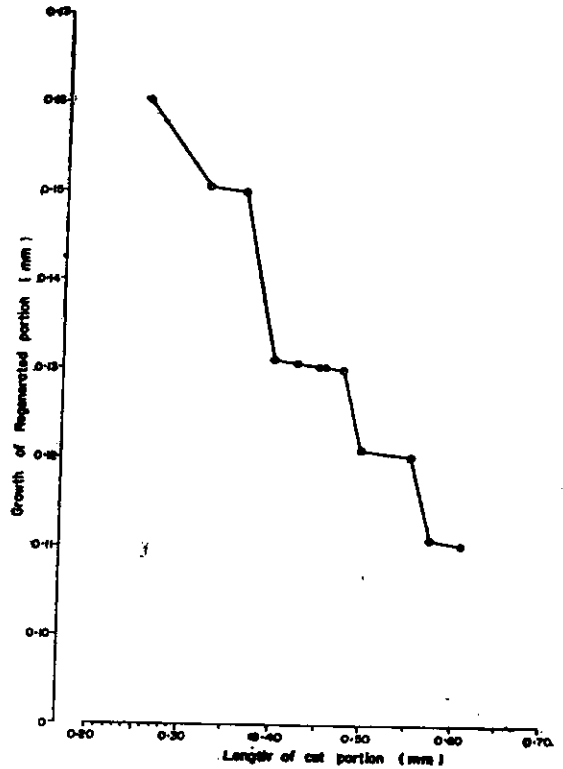


Fig. 2 Growth of regenerated portion

epimorphosis and morphallaxis regeneration, selected in the present study.

Process of Regeneration

The animal measuring 0.69 mm (Fig. 1) was cut into two portions — an anterior portion of 0.38 mm and a posterior portion of 0.30 mm (Fig. 1, A & B.)

The regeneration of the lost portion at the anterior end was epimorphosis type. Growth in tissue mass was very fast resulting in the formation of complete animal after five days (Fig. 1A a; and 1A b).

To study the regeneration of posterior portion, the cut part was kept in a separate cavity block in fertilized culture media, where it showed circular movements

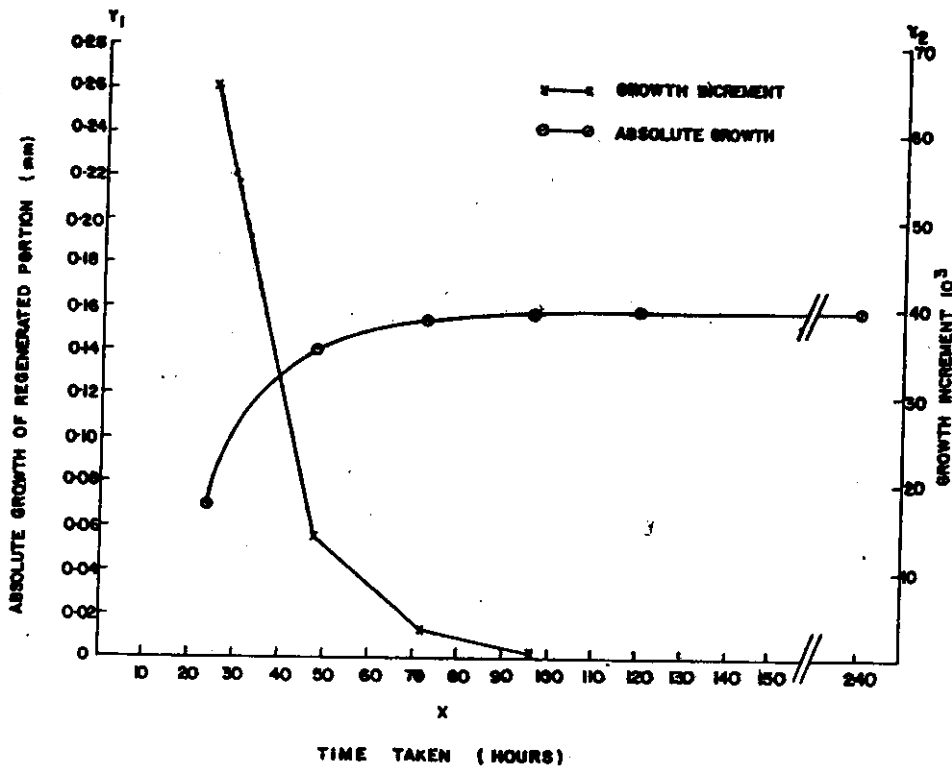


Fig. 3 Absolute growth and growth increment of regenerated tail portion.

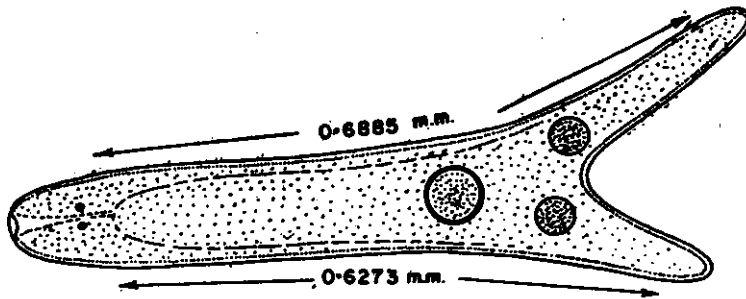


Fig. 4 Animal with bifurcated tail portion

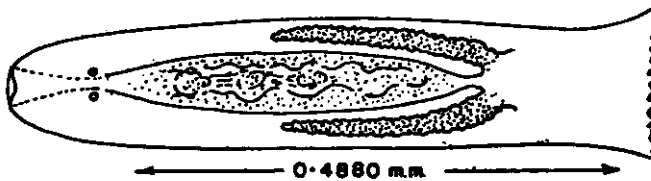


Fig. 5 Animal with severed tail portion

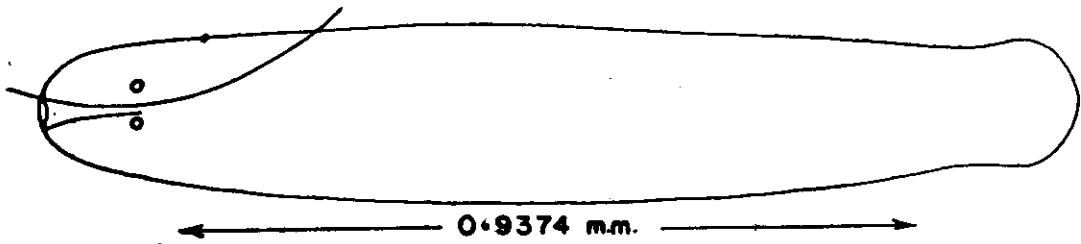


Fig. 6 Cut showing removal of eye.

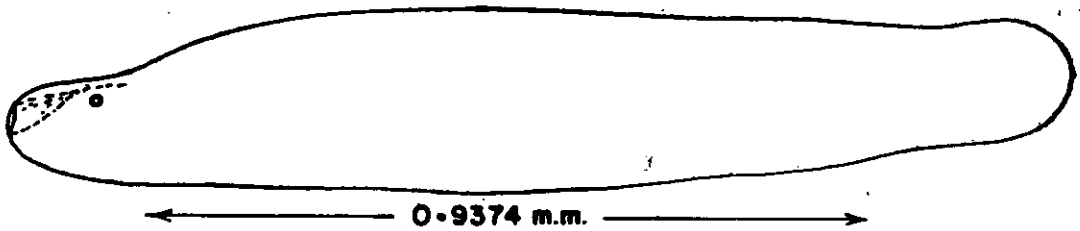


Fig. 7 Regeneration at head region.

at first and then finally settled at the bottom of the cavity block. One end of the portion was attached to the bottom and undulation of the body occurred. The type of regeneration in this portion was totally morphallaxis type. At first, the cut portion drummed to a curve-shaped structure measuring 0.3068 mm in length (Fig. 1B a). At the end of 4th day of observations, the curve-shaped portion changed to a cap like structure at the distal end with a length of 0.1227 mm (Fig. 1B b). However, no change was observed on the 5th and 6th days. The entire portion was transformed into a egg shaped structure having diameter of 0.0916 mm, at the end of 7th day (Fig. 1B c). At the end of 8th day, the shape of the cut portion became exactly similar to that of a subitaneous egg with a diameter of 0.0765 mm (Fig. 1B d) and then a single young animal was hatched out at the end of 11th day of

observation (Fig. 1B c.) Discussing the similar type of regeneration in other species Hyman (1951) and Pascolini (1968) have reported that a piece of moderate size from any level forms a complete new worm. While discussing the process of cell totipotency in *Digesia gonocephalla*, Gremigni (1972) has reported that a single cell of somatic tissue develops a complete organisms.

Absolute growth and growth increment of the regenerated tail portion.

It was observed that an anterior portion normally regenerated its lost portion within five days some observations were made making cuts at different body line towards the anterior end. The pattern of regeneration was observed in twelve successive experiments and the average rate of regeneration calculate. In the case of an anterior cut portion of 0.25 mm in length, the length recovery

of the regenerated portion was 0.16 mm at the end of 120 hours (Fig. 2). In the third experiment of the series, in a cut portion of 0.45 mm length; the regenerated part was 0.13 mm, while 0.11 mm in the case of a 0.60 mm cut portion, at the end of 120 hours.

Absolute growth was 0.07 mm after 24-hour interval and 0.14 mm after a 48-hour interval. At the intervals of 72 and 96 hours absolute growth were 0.15 mm and 0.16 mm respectively, while at the end of 120 hours an average growth of 0.16 mm was observed which remained constant subsequently. Average growth rate was 0.069 mm/day, whereas the growth increments calculated from 12 experiments were 0.0681 mm, 0.0139 mm, 0.0037 mm and 0.0035 mm at the end of 24, 48, 72 and 96 hours, respectively (Fig. 3). It was found that the growth rate of the regenerated portion was faster at first (0.24 hours) in the anterior region. Fast growth rate of this portion may be due to the presence of extraordinary high number of undifferentiated cells in the parenchyma. Germigni and Banchetti (1972) have also reported the presence of extraordinary cells in the parenchyma of *Dugesia gonocephala*. Fast growth increment indicates the high activity of regenerating cells within a determined period. It is also reported in *Dugesia mediterranea* and *Dugesia tigrina* that the rapid accumulation of neoblast cells at the beginning of regeneration and a decrease after 2-3 days (Baguna and Romero, 1981).

Bifurcation at tail portion (Fig 4)

The bifurcation of a tail portion was

usually noticed in the case of an individual which sustained a severed tail portion during the release of large subitaneous egg (Fig. 5). Further observations on such animals show that they reproduce normal hatchlings (without biaxial tail). However, some observations were also made in case of the selected adult specimens by making cuts in the tail region. It was observed that their tails regenerated into normal form, without bifurcation.

Observations at the head region

Cuts were made at the anterior end to observe the power and pattern of regeneration of structures in the head region. A cut just posterior to the eyes, was observed to replace its lost portion within few days. However, when a portion of head with one eye of the animal was detached (Fig. 6), it was observed that the animal grew to a normal size without further replacement of detached eye (Fig. 7). This is further supported by Peebles (1913) finding, where he has reported that the portion removed anterior to the cerebral ganglion replaced perfectly but without replacing the cerebral ganglia and stytocyst.

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