

NOTES ON BREEDING, GROWTH AND JUVENILE REGENERATION IN *PERIONYX SANSIBARICUS* MICHAELSEN 1891 (OLIGOCHAETA).

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Breeding.—This Indian species has now been bred in the laboratory. Worms apparently may be fully sexual, as indicated by a marked spermatozoal iridescence (visible through body wall of anesthetized specimens) on male funnels, from July 15 onwards. In the laboratory however first deposition of cocoons was observed only on July 31. Cocoons have been deposited, but only sparingly, in the laboratory during August-October, much more freely in November-March. No cocoons were deposited in the laboratory during April but in a cellar, where temperature remained between 70-75° F., cocoons were deposited until April 20. There was no deposition of cocoons during the remainder of April and the whole of May, even in the cellar (temperature between 75-80° F.). Cocoons have been found in soil of areas inhabited by the species only to end of February. Copulation has not been observed.

Cocoons.—Cocoons are of the usual form, rather spindle-shaped, 4-7 mm. long, portion distended with albumen 2-3 mm. long and 1 mm. thick at middle. Case itself has a light brown or yellowish brown colour.

Cocoon deposition terminated by anesthesia and/or operation.—On several occasions worms that had deposited cocoons during the previous night were anesthetized with chlorotone and the first five to ten segments removed. Although all such excisions are well anterior to the clitellum, no cocoons were ever deposited after operation.

Duration of development and hatching.—From cocoons deposited during night of December 10, in the cold season, worms first hatched out on December 24. Other cocoons hatched during the next four days. In embryos of cocoons deposited during night of March 7, in much warmer weather, circulating blood was first observed on the fifteenth. Worms hatched out March 16-19.

In the period between first and last hatching, in both series, no albumen was recognizable in viable cocoons, which were usually open at one end. In periods between first and last hatchings, on numerous occasions, individual worms were seen protruding the head to the exterior, to varying distances, and apparently exploring the substratum. No worm emerged completely from the cocoon while under observation, without some slight assistance. Withdrawal of worm into cocoon, after partial emergence, may have been a reaction to the rather strong light. So far as is known, all worms emerged from the cocoons during the night time. Only one worm emerged from each cocoon.

Many of the cocoons deposited in the laboratory did not give rise to young worms. Best percentages of hatching were obtained in December-January.

Just hatched juveniles.—Just hatched worms are 6-10 mm. long, 0.5-0.75 mm. thick. Pigmentation may be quite unrecognizable (1), or as follows; slight and recognizable only in first sixteen to twenty segments (several), recognizable behind xx only in region of mid-dorsal muscle band (several), i-x heavily pigmented, xi-xxi slightly pigmented (1). In a late-hatching worm with 82 segments, i-lxxiii are pigmented.

Number of setigerous segments, in 35 specimens, is 53-82 as follows:—53 (1), 61 (1), 64 (1), 66 (1), 67 (1), 68 (1), 69 (2), 70 (3), 71 (2), 72 (2), 73 (5), 74 (4), 75 (4), 76 (2), 77 (3), 78 (1), 82 (1). At the posterior end of each worm there is always a large, white growth zone, anteriorly with every small, closely crowded rudiments of segments marked off by rudimentary intersegmental furrows, setae and nephropores either lacking or unrecognizable. Posteriorly even the rudimentary intersegmental furrows are lacking, though there is usually space for a number. The small size, close crowding and (more posteriorly) vagueness of, what appear to be rudiments of intersegmental furrows render it impossible to determine with certainty the number of actual segments. Hence only setigerous segments, *i.e.*, those on which setae are actually recognizable, were counted. If recognizable rudiments of segments were counted the number would in each case still be much less than a hundred. The smooth posteriormost zone, in which no rudiments of segmental furrows are recognizable, may occasionally be unusually long. The long growth zone apparently indicates a region of rapid production of new tissues and differentiation of new segments, as compared with a much smaller and less obvious growth zone of larger specimens where new tissues are produced much more slowly and segments may be differentiated only one at a time.

“Do earthworms grow by adding segments?” This question has been answered by Sun and Pratt (1931), after study of the Lumbricid *Eisenia foetida*, apparently in the negative, with the suggestion that juveniles have a larger mean number of segments than adults. This latter is of course entirely possible in view of the known frequency of autotomy¹ posteriorly.

Number of segments in adult individuals of *P. sansibaricus* may be as many as 200 and clitellate specimens with normal posterior portions usually have much more than a hundred segments. The largest, just-hatched juvenile has only 82 setigerous segments. Each juvenile obviously has a large growth zone, in which rapid production of new segments is under way. It may however be possible that all of the juveniles hatched in the laboratory emerged prematurely. To check on that possibility, on several occasions, number of juveniles were collected from natural habitats and segmental counts were made on those of such small size as to indicate recent hatching. Results obtained are much the same as from juveniles hatched in the laboratory. Larger juveniles and presumably older juveniles may also have a large zone of rapid growth, as in the following series. (1) Length 14-25 mm,

¹This is used as a convenient single word to indicate loss of a portion of the body whether by action of the worm itself or by action of some unknown external agent.

diameter 1-1.75 mm, number of segments 85-133. (2) Length 26-35 mm, diameter 2-2.5 mm, number of segments 118-153. In many of those specimens with no large zone of rapid growth these are indications of autotomy, with or without regeneration of a short or very short tail portion.

All the evidence available, accordingly, seems to show that in *P. sansibaricus* at least, worms continue to grow by "adding segments", after hatching.

Furthermore, clitellate and fully sexual specimens of *P. sansibaricus* without any indication of posterior autotomy and regeneration, but with a large, white zone of growth, have occasionally been found. Apparently then, in this species, an occasional individual may become sexual before growth in length has ended, and before segmental differentiation or formation has been completed.

Autotomy.—During the very first week after hatching, three out of eleven juveniles autotomized short tail portions. One of the three developed a short anal bud but no regeneration by the other two was recognizable during the remainder of the time the specimens were under observation.

Juvenile regeneration.—As previously shown (Gates, 1944), *P. sansibaricus* has an unusually high regenerative capacity for an earthworm. As in all previous work on regeneration in earthworms, the experiments were on mature specimens. Do very young juveniles, just hatched out from the cocoon, have a similar regenerative capacity? A single experiment has been tried.

On December 26, three juveniles which had emerged from cocoons during the previous night, were cut approximately into equal halves according to length, without anesthetic. During the next three weeks one anterior and one posterior substrate died. Each of the other two anterior portions (of 23 and 27 segments) developed a small white tail regenerate with terminal anus but no metameric differentiation. Each of the posterior halves (of 37 and 44 setigerous segments) regenerated a head. One head regenerate had no externally recognizable metameric differentiation. The second was marked off into nine segments but setae and nephropores, as well as pigment, were unrecognizable.

SUMMARY.

Cocoons have been deposited in the laboratory from July 31 to April 1, in a cooler cellar to April 20. Worms hatched in 14-18 days in December, in 9-12 days in March. Just hatched juveniles have only 53-82 segments and a large growth zone from which new segments are differentiated to a total of 200. An occasional individual may become sexually mature while rapid formation and differentiation of segments is still continuing. 27% of one series autotomized posteriorly within first week after hatching. Anterior substrates of just-hatched juveniles can regenerate a tail from 23/24 posteriorly. Posterior substrates of 37 or more setigerous segments can regenerate a head.

REFERENCES.

- Gates, G. E. 1944. Regenerative capacity in *Perionyx sansibaricus* Michaelsen 1891. *Current Sci.* XIII, p.16.
- Sun, K. H., and Pratt, K. C. 1931. Do earthworms grow by adding segments ? *Am. Nat.* LXV, pp. 31-48.