SCHNEIDER, W. C. 1946. Intracellular distribution of enzymes. I. The distribution of succinic dehydrogenase, cytochrome oxidase, adenosinetriphosphatase, and phosphorus compounds in normal rat tissues. Jour. Biol. Chem., 165: 585–593.

SEN, P. B. 1930. A method of locating urease within tissue by a microchemical method. Indian Jour. Med. Res., 18: 79–82.

STERN, A. H., and MIRSKY, A. E. 1952. Some enzymes of isolated cell nuclei. Nature, 169: 128–129.

SULLIVAN, W. D. 1950. Distribution of alkaline phosphatase in *Colpidium campylum*. Trans. Amer. Micr. Soc., 69: 267–271.

WEISZ, P. B. 1949. Phosphatase in normal and reorganizing stentors. Biol. Bull., 97: 108–110.

WILLMER, E. N. 1942. The localization of phosphatase in cells in tissue cultures. Jour. Exper. Biol., 19: 11–13.

ZORZOLI, A., and STOWELL, R. E. 1947. Comparison of the distribution of an hexosediphosphatase and glycerophosphatase in different tissues. Anat. Rec., 97: 495–503.

DEVELOPMENTAL OBSERVATIONS, THE EGG CAPSULE AND SEXUAL MATURITY OF THE LAND PLANARIAN *RHYNCHODEMUS SYLVATICUS*

ROBERT E. OGREN

Ursinus College, Collegeville, Pa.

Prolonged observation of *Rhynchodemus sylvaticus* (Leidy) in culture one summer provided new and interesting information, extending our knowledge of its biology. Egg capsules were observed and a mature specimen was sectioned, thus supplementing previous papers on ecological and physiological observations (Ogren 1953, 1956).

Successful survival was obtained in a petri dish containing coarse sand, moistened with pond water. Black soil and pebbles were added at one side. A glass slide, supported on the sand surface provided a place of refuge other than the dish cover. The container was placed in a large moist chamber containing several folds of paper towels. The planarians frequently left the inner container but were readily found among the moist towels. At least once a week fresh liver was placed overnight on the sand. By this method two planarians were maintained successfully for 140 days.

For observation of planarians a moist filter paper or paper towel was employed as a surface. All handling of the worms was done by means of a small metal spatula made from a large needle heated, hammered flat and thin, then filed so as to be one mm. wide. The planarians were gently lifted from the substrate by placing the spatula under the middle part of the body. The triclad made contact with the spatula and was carried to another location.

Collection and Distribution

During a visit to Lakewood, N. Y. on Chautauqua Lake specimens of *Rhynchodemus sylvaticus* were collected alive June 9–15, 1954. After several attempts were made, four were successfully transported to the writer's college laboratory. Triclads were found also in dark, shaded soil of an old orchard on Regent Street, Jamestown, N. Y. A portable ice box was employed to maintain favorable temperature conditions.

Specimens of *Rhynchodemus sylvaticus* were collected also from a flower bed containing largely flox and lilies. The soil, classified as Homer Silt Loam by Mr. Robert R. Finley, Soil Scientist, U. S. Soil Conservation Service, Jamestown, N. Y., was high in organic matter and slightly alkaline. It was underlaid by gravel on top of shale and clay. The area had been disturbed and filled in at one time. There were many dead roots in the soil as well as abundant planarian passageways. Blocks of soil with clusters of flox and lilies were brought back to the laboratory in a wooden box 8 x 15 x 6 inches. Each soil sample was 7 x 7 x 5 inches. During the next month triclads were observed on six different occasions and three of these were collected. The maximum number of worms observed at one time was three on a total soil area of 0.68 square feet. The population was 4.5 worms per square foot, a figure similar to one reported recently (Ogren, 1953). The above evidence indicated that these land planarians can be transported and disseminated with potted plants and soil around perennials, which may thus explain their sporadic distribution. A growing population is now established in the writer's yard.

Specimens

Observation of the ventral surface of the triclads moving on the under side of a glass, showed the presence well-forward of one pair of purple-brown ovaries. By serial sections a double row of six or more pairs of pigmented testes was determined extending just posterior to the pharynx. The sensory tracts were evident as non-pigmented lines on the ventro-lateral aspect of the proboscidiform anterior.

Changes were observed in color and pattern. When first collected the specimens were nearly black but became increasingly lighter during the period of culture. It was observed that color was lighter also during movement. There was also some variation at times in the prominence of the saddle, which during motion appeared as a pale spot. In their final days the dorsal bands disappeared and the worms presented a mottled appearance.

Length, Testes, and Reproductive Maturity

Original lengths of the three worms were respectively 12, 18, 18 mm. On the 14th day they were 18, 19, 22, on the 17th day, 20, 22, 22 mm. long in motion, whereas by 51 days they were all 16 mm., and at the end of 140 days the two remaining triclads were four mm. and 10 mm. long. These specimens were all killed and preserved. The first triclad, 16 mm. long, was fixed when it contained an egg capsule. The other two were fixed, after their size decrease was less than one-half, and subsequently sectioned. Both worms presumably had formed several egg capsules. The four mm. specimen showed no sign of differentiated reproductive complex.

Study of the 10 mm. specimen showed a nearly mature reproductive complex (Fig. 1). One pair of ovaries; anterior testes distributed as follows: six pre-pharyngeal, one pair at level of ovary, three pairs post-pharyngeal (numbers one, two and five), a distribution indicating others were present originally. Testes were spent and contained only a few sperm, although mitotic figures were evident.

The significance of this degenerative decrease in size is taxonomic, for a "mature" worm collected in nature may not represent the mature length of the species. Since these changes occur late in the life cycle, mature specimens collected in the fall may not represent the original maturation length. This explains how 10 mm. specimens of Leidy and Walton could be mature but below average length. In all specimens sectioned the male antrum was regularly shorter and smaller than that of the female. This differs from Hyman's (1943) description. In the specimen with the egg capsule, however, the male antrum was considerably enlarged (Fig. 2), muscular, and more nearly similar to the type specimens.

The wall of the sperm duct was very muscular and protruded into the antrum in the form of a penis papilla (shown at top of Fig. 2). This has not been reported previously, and Hyman (1954) makes the absence of a penis papilla a character of the genus *Rhynchodemus*. Pantin (1950) figured the beginning of a penis papilla, however, in *Rhynchodemus bilineatus* (Metsch.).

Careful study of the reproductive system showed: one pair of anterior ovaries, six pairs pre-pharyngeal testes, one pair just anterior to ovaries, and six to eight pairs post pharyngeal testes, the last pair just in front of the male antrum. Since this was the gonad complement of a mature individual, the number of testes here indicated the degree of sexual maturity. There was also a relationship between the fully developed male antrum and number of testes. Worms with a small non-muscular male antrum lacked a penis papilla and had only three or four pharyngeal testes. A very poorly differentiated male antrum was associated with only a few pre-pharyngeal and no post-pharyngeal testes.

Graff (1907, p. 3011) indicated that in land planarians the maturation of the male antrum was accompanied by increased muscularization and the eventual appearance of a prominent penis papilla. A smaller projection also occurred in the mature female antrum. The specimen with egg capsule confirmed this (Fig. 2). Therefore, the reproductive complex in *Rhynchodemus* appeared most mature in worms 16–22 mm. long which had laid one or more egg capsules. Perhaps only under these conditions is a mature male antrum present. The reproductive complex tended to be protogynous.

Measurement of the distance from the pharynx to the genital pore (p-g distance) in sectioned specimens of various sizes showed that this region grew longer during maturation. Immature specimens (10 mm.) with only three post pharyngeal testes had a p-g distance of 1.42 mm., larger specimens (11–12 mm.); with small male antrum, distances of 1.70 mm. The 16 mm. specimen with an egg capsule had a p-g distance of 2.14 mm. The pharynx to genital pore distance, the number of post pharyngeal testes, and the degree of maturity of the male antrum were indicative of the degree of sexual maturity in this series of specimens.

Formation of the Egg Capsule

Observations indicated early capsule development required three or

four days and that feeding provided the stimulus. In six cases the capsule appeared on the third day after the last beef liver meal and in another the capsule appeared two days after a snail-blood meal. The first visible indications of capsule-formation were elongation and an evident enlargement in the post pharyngeal region. Four days after the post pharyngeal enlargement appeared in one triclad, the egg capsule was visible as a cream-colored swelling. As the enlargement became more prominent the region appeared light-colored as the result of stretching of the body wall. The capsule area continued to enlarge until it measured 1.2–1.5 mm. in diameter. The triclad (Fig. 3) at this time was very quiet and moved with difficulty. The capsule was deposited about eight hours later. Before deposition the color of the enlargement changed from cream to orange. During deposition the capsule was forced from the reproductive antrum and cemented to some object upside down or upright on the sand. In one case the triclad took a position beneath a glass slide and left the capsule conveniently cemented there (Fig. 4). The freshly deposited egg capsule was orange, became cherry red after 12 hours of exposure to air, and turned black in 24 hours. During a period of 140 days observation (June-September) three worms deposited nine capsules, apparently three each. A mature 16 mm. triclad, collected June 23, 1955 and placed in a culture dish, had a capsule enlargement. One month later, July 23, five tiny triclades three to five mm. long were collected from the dish. Another capsule observed was kept wet to facilitate proper respiratory exchange by being placed between folds of wet paper toweling in a covered petri dish. The capsule was deposited July 9, 1956, swelled slightly and split on August 7. The total time was 29–30 days at tem-

---

DESCRIPTION OF FIGURES

All drawings were made with the aid of camera lucida. For Figs. 1, 2, 5–9 each scale division represents 10 microns. For Figs. 3, 4 each scale division represents 1 millimeter.

FIG. 1. Reproductive complex, post mature regressing specimen (RC–5.), ×50.

FIG. 2. Mature reproductive complex of specimen (RC–3) containing egg capsule oblique view, gonopore not shown. Male antrum above has considerable muscularization represented by black fibers of the penis papilla. An expansion of the sperm duct serves as a vesicle holding sperm. The female papilla below contains many granules in epithelium and groups of terminations of the eosinophil glands. The intestine is shown on the left. Isolated eosinophil cells are scattered in antrum epithelium, ×50.

FIG. 3. Mature land planarian with egg capsule. Considerable anastomosing of dorsal pigmented bands.

FIG. 4. Egg capsule on underside of glass slide. Black in life.

FIG. 5. Vitelline cell, living, from egg capsule in dilute Ringer's solution, neutral red. The small globules were colorless vitelline droplets. The large spheres were stained orange, ×475.

FIG. 6. Vitelline cell, living, neutral red, nucleus shown on the left. The large sphere at right was orange and contained small actively moving granules and larger globules, ×475.

FIG. 7. Section through an egg capsule showing vitelline cells and wall. Inner vitelline layer (black) was orange, paler outer layer was stained faint blue. Aniline-blue orange G, ×475.

FIG. 8. Eosinophil cell in epithelium of reproductive antrum. The numerous red stained granules nearly obscured the nucleus. Hematoxylin and eosin, ×475.

FIG. 9. Eosinophil cell in epithelium of reproductive antrum. Contents of cell appear forced out. Hematoxylin and eosin, ×475.

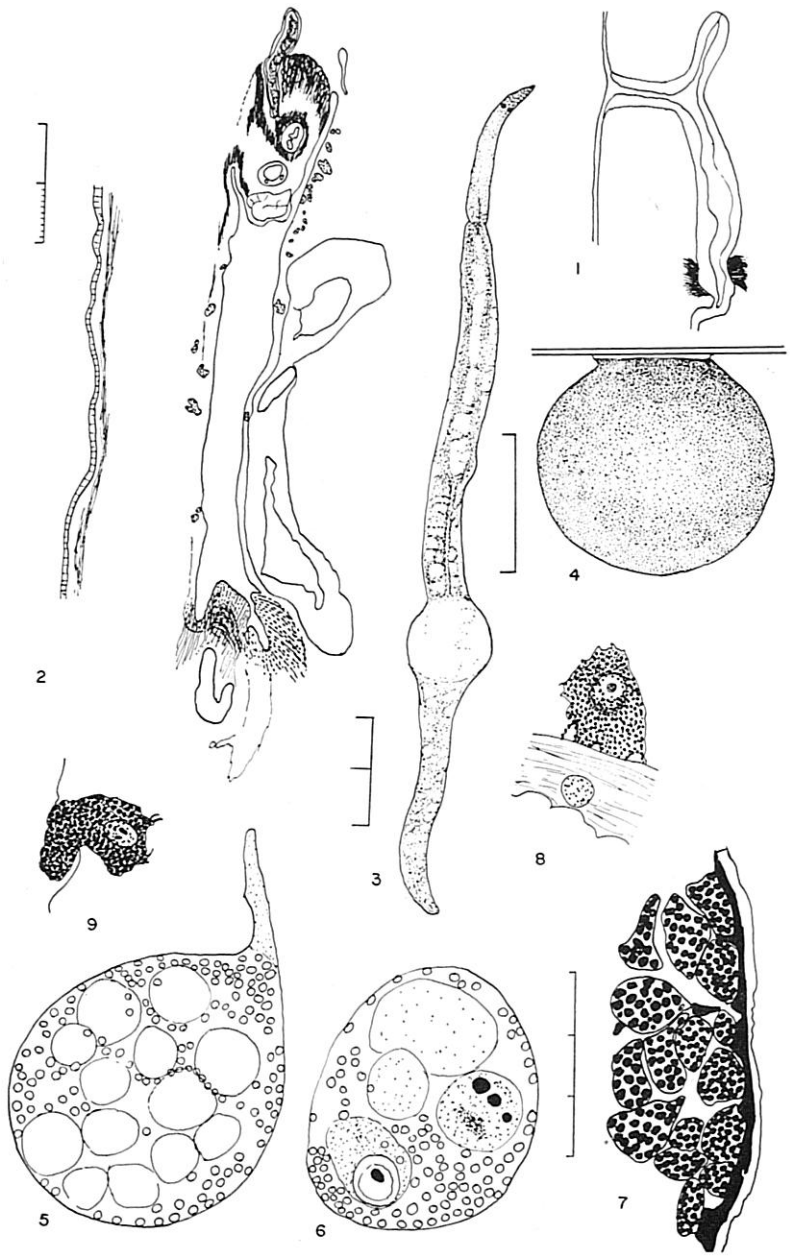

PLATE I

peratures of 18–20 degrees C. It produced four light chocolate-colored triclads three to four mm. long, depending on the degree of contraction. Their dorsal surfaces and anterior tips were slightly darker in pigmentation but the dorsal longitudinal bands were distinct. Hence it appears that four or five triclads may develop from one capsule in 30 days.

Description of Egg Capsule

Two of the capsules were opened for study. One was fixed and used for serial sections, but three were destroyed by nematodes and mold in the culture dish and three accidentally dried in refrigeration. The black, opaque capsules were spherical, resembling a small seed with one surface cemented to the substrate (Fig. 4). The diameters were 1.2 and 1.5 mm. respectively. The surface was hard, elastic and irregular with wavy markings. When pierced by a sharp needle the capsule cracked and was easily torn, the pieces, however, retaining their curvature.

The capsules were opened in dilute Amphibian Ringer's solution. Inside was a white viscous, jelly-like mass. Microscopic examination showed this to be composed of thousands of vitelline cells but ova and spermatozoa were not identified, and the capsules were considered sterile. The living vitelline cells were irregular and had the appearance of Figs. 5, 6. The peripheral cytoplasm contained countless vitelline globules beneath the transparent membrane and there was a large nucleus at one side. The vitelline droplets failed to stain with neutral red *in vivo* but they became yellow in sections with van Gieson's connective tissue stain. In living cells freshly stained with neutral red there appeared several large red to orange globules (Figs. 5, 6). These contained granules in Brownian movement and occasionally smaller dark red spheres. The granules stained green with Janus Green-B and dark purple with toluidine blue. With the latter dye *in vivo* the granules, which may represent mitochondrial bodies, clumped together as rods and irregular masses.

The capsule wall was composed of two layers (Fig. 7). The thick outer layer did not stain with eosin or orange-G counter stain, but colored slightly with aniline blue. The inner layer resulted from the fusion of vitelline droplets and stained with orange-G, and hematoxylin. The cortical vitelline cells were closely applied to the inner layer. Masses of loose vitelline substance were found between the cells in the capsule. The origin of the outer capsule membrane was from the secretions of eosinophilous glands in the antrum. (Graff, 1917; Hyman, 1951). The specimen containing an egg capsule had a much-stretched antrum epithelium. In some places it appeared only as a thin membrane beneath the intestine (Fig. 2). Eosinophil granules were especially concentrated in the prominence of the female antrum and filled the epithelium here and in adjacent regions. Large eosinophilous gland cells (Figs. 8, 9) were located in the epithelium of the male antrum and extended toward the female antrum (Fig. 2). These cells were so full of coarse eosinophil granules that the nucleus was obscured and the cell appeared as an irregular mass of solid material. The epithelium of the female antrum contained countless eosinophil granules from flask-shaped gland cells clustered around this region.

Regeneration

Rhynchodemus was able to regenerate anterior protions including the eyes, and the last millimeter of the posterior region. Extensive experiments might prove rewarding from a comparative viewpoint.

Discussion

The egg capsules of triclad *Turbellaria*, land planaria in particular, tend to be large, spherical and often colored, containing hundreds of vitelline cells whose numerous droplets contribute to the formation of the capsule wall (Bresslau, 1928). Published descriptions of egg capsules among the triclad order Terricola are not numerous for the most important families, Geoplanidae, Bipalidae, and Rhynchodemidae (Hyman, *l.c.*). An extensive review was given by Graff (1917).

Description of the egg capsule of *Geoplana notocelis* was made by Carlé (1935), including the embryonic development of the pharynx. The capsule of this large planarian was spherical, eight to nine mm. in diameter. It was whitish when laid, changing slowly to a reddish yellow-dark brown. In about 20 days six to eight small worms had left the capsule. Egg capsules in a group of 15–20, of *Bipalium adventitium* Hyman, 1943 were observed and described by Hyman (1954). The capsules, attached to the underside of a stone, were light grey, with one embryo.

Descriptions and photographs of the egg capsule and early embryology of the European land planarian, *Orthodemis* (=*Rhynchodemus*) *terestris* (Müller) Hyman, 1954 were published by Carlé (*l.c.*). Capsules were laid at night in the soil. During their development the enlargement of the posterior of the worm was evident. About 12 hours later the capsule left the atrium and was deposited as a light brown sphere. Color changes from whitish, to yellow, to brown were observed. The similarity of the above to *Rhynchodemus sylvaticus* is evident.

The color changes of the covering present one of the interesting features of these egg capsules. Stephenson (1947) indicated that the shell material of trematodes formed from vitelline droplets was a sclero-protein. The brown color of these capsules was due to quinone tanning and oxygen was a factor in increasing degree of coloration. A similar situation appears possible in the case of *Rhynchodemus*.

Size changes, such as decrease following sexual maturity, and increase to maximum length only during egg capsule formation, indicated that length of these land planarians is only a presumption of sexual maturity. The postpharyngeal region showed great elongation during growth. There is, finally, the suggestion that this land planarian may be sexually mature only during and just following egg capsule formation. This condition and factors stimulating egg capsule formation should be analysed by further experimentation.

Literature Cited

Bresslau, E. 1928. Turbellaria. Kukenthal and Krumbach, Handbuch der Zoologie. Bd. 2, Heft 1. Berlin.

Carlé, R. 1935. Beiträge, zur Embryologie der Landplanarien, I. Zeit. Morphol. Okol. Tiere., 29: 527–558.

Graff, L. 1912–1917. Turbellaria. Teil II. Tricladida. F. Oecologie. Bronn, Klassen und Ordunungen des Tierreichs. Leipzig.

Hyman, L. H. 1943. Endemic and exotic land planarians in the United States with a discussion of the necessary changes in names in the Ryhnchodemidae. Amer. Mus. Novitates, 1241: 1–21.

1951. The Invertebrates: Platyhelminthes and Rhyncocoela. McGraw-Hill.

1954. Some land planarians of the United States and Europe with remarks on nomenclature. Amer. Mus. Novitates, 1667: 1–21.

OGREN, R. E. 1953. Ecological observations on the occurrence of *Rhynchodemus* a terrestrial turbellarian. Trans. Amer. Micros. Soc., 74: 54–60.

1956. Physiological Observations on movement and behaviour of the land planarian *Rhynchodemus sylvaticus* (Leidy). Proc. Penn. Acad. Sci., 30: 218–225.

PANTIN, C. F. A. 1950. Locomotion in British terrestrial nemertines and planarians: with a discussion on the identity of *Rhynchodemus bilineatus* (Mezznikow) in Britain and on the name *Fasciola terestris* O. F. Müller. Proc. Lnnean Soc. London, 162: 23–37.

STEPHENSON, W. 1947. Physiological and Histochemical observations on the adult liver flukes *Fasciola hepatica* L. III. Egg shell formation. Parasit., 38: 129–139.

THE ANATOMY OF THE MALE AND FEMALE REPRODUCTIVE SYSTEMS OF *ONCOMELANIA NOSOPHORA*[1]

ARIEL A. ROTH[2] AND EDWARD D. WAGNER

School of Tropical and Preventive Medicine, College of Medical Evangelists, Loma Linda, California

This investigation was conducted to facilitate certain phases of the basic biological studies being conducted in this and other laboratories on oncomelanid snails. It is hoped that such studies eventually will aid in the development of practical control measures of oriental schistosomiasis for which these snails serve as intermediate hosts. Descriptions of the gross and microscopic anatomy of the male and female reproductive systems of *Oncomelania nosophora* (Robson) are given.

The existing literature on the anatomy of *Oncomelania* is for the most part inadequate to be of value for certain types of study. Disagreement exists among the published results. An early paper on the anatomy of *O. nosophora* which is very brief is that of Nakamoto (1923). Itagaki's (1955) work is based on studies made from gross dissections and is also brief. Work done at the 406th Medical General Laboratory (1954) and which is still in progress deals largely with the muscular system and makes reference to gametogenesis. Li (1934) published his findings on the anatomy of *O. hypensis* Gredler, and Abbott (1948) on *O. quadrasi* (Mollendorf), both of these descriptions being quite brief.

MATERIALS AND METHODS

The snail used in this work was *O. nosophora* imported from Japan about one year previously and maintained as stock in the laboratory. Observations were based on gross dissections and sectioned material. Forty snails were serially sectioned in various planes and stained with

[1]This investigation was supported by a research grant E-644 from The National Microbiological Institute, of the National Institutes of Health, U. S. Public Health Service.

[2]Address: Department of Biology, Pacific Union College, Angwin, California.