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Cocoon Production and Development in the Turbellarian Procotyla fluviatilis

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COCOON PRODUCTION AND DEVELOPMENT

IN THE TURBELLARIAN PROCOTYLA FLUVIATILIS

(TITLE)

BY

TERRY M. WUNDERLE

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THESIS

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COCOON PRODUCTION AND DEVELOPMENT

IN THE TURBELLARIAN *PROCOTYLA FLUVIATILIS*

Presented by

TERRY M. WUNDERLE

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is acceptable.

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INTRODUCTION

Procotyla fluviatilis Leidy is a dendrocoelid turbellarian distributed over the northeastern part of North America. It has been described from a number of collections in Illinois (Woodworth, 1897; Hyman, 1928; Wunderle, 1965), but these records suggest its distribution in the state is scattered. Wunderle (1965) described a dense, growing population of *P. fluviatilis* in a spring-fed, fish-free drainage ditch located in Mason County, Illinois. This population is still flourishing, and the specimens used in the present investigation were obtained from it.

Although there have been a number of studies on the general biology and morphology of *P. fluviatilis*, apparently none have been published on its reproductive behavior, cocoon production, or hatching and emergence of young. In view of the scarcity of information concerning the reproduction of *Procotyla fluviatilis*, the present investigation was undertaken in an attempt to clarify some aspects of the reproductive cycle based on examination of specimens from the population described by Wunderle (1965) in Mason County, Illinois.

LITERATURE REVIEW

Procotyla fluviatilis Leidy has been described by Hyman (1928) as a large milkwhite or creamy flatworm with a maximum length of 20 mm. and width of 5 mm. The middle third of the truncate anterior margin is occupied by an adhesive organ or sucker with several pairs of eyes a short distance behind the adhesive organ. *P. fluviatilis* is known to eat only live prey, consisting of daphnids, amphipods, and isopods (Pennak, 1953).

While no definitive work dealing with the worldwide distribution of Turbellaria is available, a number of more restrictive papers are accessible. The turbellarians of Lake Titicaca were described by Beauchamp (1939) in a publication that reviews the free-living platyhelminths of the world. An earlier report by Halles (1879) catalogs the flatworms found in northern France. The most recent work on the distribution of European worms is that of Luther (1961), in which the Turbellaria, especially triclads, are reviewed. Among his many papers on the invertebrates of South America, Marcus (1951) has included at least one on the natural history of triclads.

The Turbellaria of Asia are elucidated in a monograph of the class by Okugawa (1953), but major emphasis is placed on the region of Japan.

The distribution of Tricladida in North America is discussed in the papers of Stringer (1909), Higley (1918), and, of course, Hyman (1928, 1951). Morgan (1939) presents a superficial, but readable, general account of North American flatworms.

The family Dendrocoelidae, in which Procotyla is placed, occurs abundantly in Europe and Asia (Hyman, 1951), but is poorly exemplified in America. Sorecelis has a species in Oklahoma caves (Hyman, 1937), but Procotyla is the only commonly occurring genus. This worm inhabits fish-free ponds, springs, and streams of northeastern North America (Kenk, 1944).

Collection of P. fluviatilis in Illinois has been infrequent. It has been collected at Havana, (Woodworth, 1897), Cary (Hyman, 1928), and by Wunderle (1965) at Mason City, Illinois.

Several studies have been made of the biology and histology of P. fluviatilis; but little is known concerning details of their reproductive behavior, especially copulation, cocoon laying, hatchability of cocoons, and their seasonal reproductive cycle. Hyman (1928) was concerned with the external and internal anatomy, histology and taxonomy; and presented a limited discussion of habits, habitat, and the distribution of Procotyla fluviatilis. Kenk (1944), in his monograph of the triclads of Michigan, discussed the internal and external anatomy, the distribution, and to a limited extent, the ecology of P. fluviatilis. Morgan (1930) described the cocoon as being "about the size of a mustard seed, shiny, chestnut brown, and raised on a tiny stalk." Kenk (1944) questioned the possibility of the stalk, but Anderson (1951) verified the presence of the tiny stalk on the cocoon and discussed the rearing of fourteen cocoons until they hatched. He also described the young worms as being similar to the adults in appearance and movement. DeWitt (1953) also described the cocoons and helped to clarify the presence of a stalk on the cocoon. Hyman (1928) reported that P. fluviatilis became sexually mature from September to January and that the specimens taken from January to August always possessed degenerate sex organs. Curtis and Schulz (1940) reported that worms collected early in the period from February 1 to June 1 were all sexually mature, but later there was an increasing number of smaller worms without reproductive organs. They concluded that these were juvenile worms from eggs of the current season and not the result of asexual reproduction by fission. It was the opinion of these investigators that regenerative ability of Procotyla is poor.

METHODS AND MATERIALS

Collections of Flatworms

Flatworms used in this investigation were collected from a drainage ditch located in Sec. 29, 30, 31; T. 19 N., R. 5 W., Mason County, Illinois. The spring-fed drainage ditch is 1.8 miles long, has its origin as ground water seepage, and empties into Salt Creek. Water movement is very slow; and this promotes growth of quantities of aquatic vegetation including *Lemna minor*, *Ceratophyllum demersum*, *Bidens cernua*, *B. cernua*, *B. coronata*, *Polygonum fluitans*, and *Nasturtium officinale*.

Flatworms were collected by removing with a scalpel, from the undersides of objects submerged in the ditch, and placed in a quart jar containing water from the habitat. Duration of collection period was from August, 1967, through May, 1968. Collections were made every three or four weeks during August through December. January through March collections were made every six to fourteen days.

Culture Method and Material

Adults were taken into the laboratory where fifteen to twenty each were placed in 500 ml. beakers containing water from the ditch. The beakers were held in a refrigerator at a temperature of 10°C. Live prey, such as copepods and daphnids were obtained, using a plankton net, and introduced into the laboratory colonies as food. Evaporating water from the beakers was replaced with water from the collection site. Cultures of adult worms were observed daily to note behavior and the appearance of cocoons. When cocoons appeared, each was removed and placed in a marked culture dish or test tube and kept at a determined constant temperature (1°C., 10°C., or 20°C.). Cocoons were kept at different temperatures to study the effects of such on incubation time. Twenty cocoons were measured with a micrometer. During February and March the water temperature of the collecting site was measured once a week. Some cocoons were kept at 10°C. to correspond with the upper range of the natural habitat temperature; other cocoons were kept at 1°C. to correspond with the lower range of temperature. Cocoons were maintained at 20°C. to test the hypothesis that increased temperature would shorten the incubation period required for hatching. Twenty degrees Centigrade was selected because it had been observed that this was the maximum temperature at which adults thrive (Young, 1912). Observations were made daily on the cocoons to establish the time of hatching and to tabulate the number of young produced per cocoon. Attempts were made to feed newly hatched juvenile worms on ground bits of *Nais sp.* and/or by placing them in water which contained *Paramecium sp.* Neither of these methods was successful.

Different methods were tried to initiate cocoon production. These methods revolved around the concept that cocoon production is triggered by changes in environmental temperature. Cultures of adults were placed in a refrigerator at 1°C. for fourteen days, then transferred to 15°C. Other cultures were kept at 20°C. for seven days and were then transferred to 5°C.

In summary: Data as indicated above were collected and the following parameters analyzed:

1. Number of cocoons produced per adult worm.
2. The seasonal disposition and longevity of cocoons.

3. Percent hatch of cocoons.

4. Average number of young per cocoon hatched.

5. Influence of temperature on rate of cocoon development.

RESULTS

Ditch Temperature

During February and March the water temperature of the collecting site was measured and found to vary from 1°C. to 10°C. (Average: 7°C.)

Copulation

Five pairs of flatworms were observed copulating. One pair were specimens that had been collected on January 21, 1968, and maintained in culture with other worms at 1°C. in the refrigerator. Copulation, which lasted nearly two hours, was observed seven days after collection. The other four pairs of worms were observed in copula at the time of collection. One pair was included in a collection obtained on February 24, 1968; one pair on March 3, 1968; and two pair on March 9, 1968.

Character of Cocoons

The cocoons were round, very shiny, and chestnut brown in color. They were about the size of a mustard seed and were attached to the sides of the culture dish by a short white stalk which had the appearance of mucus.

Measurements were taken of twenty cocoons. The average diameter of the cocoons was 152 microns. The largest cocoon was 207 microns in diameter while the smallest was 84 microns in diameter.

Production of Cocoons

A total of seventy-four cocoons was collected and kept under laboratory conditions in a refrigerator (Table I). The first cocoon was seen on January 24, and the last on March 15. This was the only time when the cocoons were seen throughout the research period. Seven cocoons were produced in January, thirty-three in February, and thirty-four in March. There was a minimum cocoon production from January 24 to February 14. From February 14 to March 14, there was a marked increase in production which peaked around March 7. After March 14, there was a rapid decline in production.

During the period in which cocoons were collected, twenty sexually mature adult worms were observed to have a well-formed, light greyish-brown cocoon in their genital apparatus. It was noted that these cocoons were deposited within eight hours after appearing in the genitalia. Within two hours after deposition, the cocoon took on the characteristic dark mahogany color mentioned by Morgan (1939). It was noted that after the worm deposited the cocoon on the side of the container, it usually remained over the cocoon for three to six hours. This behavior seemed related to the rapidity in which cocoons darkened. It was never observed that an adult laid more than one cocoon.

Cocoon Hatching

Twenty-three cocoons were held at 10°C.; fifteen hatched. An average of 5.4 young emerged from each cocoon with a range from two to twelve young. Incubation ranged from sixteen to thirty-six days with an average of 22.5 days.

Thirty-two cocoons were held at 20°C.; twenty-one hatched. An average 4.2 young hatched from each cocoon with a range from one to ten young. Incubation ranged from thirteen to thirty-two days with an average of 17.4 days.

Nineteen cocoons were maintained at 1°C. for a period of sixty-two to thirteen days. None hatched at that temperature. Ten of the cocoons were transferred from 1°C., where eight of the ten hatched (Table II). Eight cocoons were transferred from 1°C. to 10°C. Three of the cocoons hatched (Table III).

A greater number of the cocoons laid early in the reproductive period hatched than did cocoons laid later in the reproductive period (Table IV). There was a 100 percent hatch of cocoons deposited from the period of January 24 to February 7. In the February 7 to February 21 period and in the February 21 to March 7 period, there was a 66.7 percent hatch; and, in the March 7 to March 21 period, there was a 40 percent hatch. In the February 7 to February 21 period, two of the cocoons that were laid were asymmetrical and did not hatch. Each appeared concave on one side. Many of the cocoons which did not hatch appeared to crack open; and the material, which appeared to be partially developed flatworms, protruded out the opening.

A greater number of young are produced from cocoons laid at the beginning of the cocoon reproductive period (Table IV). The only cocoons to produce ten or more young were those deposited in the January 24 to February 7 period, with the exception of one cocoon laid on March 13 which produced ten young. Four cocoons laid from January 24 to January 27 produced ten, eleven, ten, and twelve young, respectively.

The cocoons which did not hatch remained under observation until they deteriorated or aborted. The cocoons referred to as aborted were the ones which split open and a material protruded out the opening. This material was observed under a microscope and did appear to be partially developed worms. The whitish mass was composed of cells several layers thick and of several kinds.

There were nine aborted cocoons, all of which were laid after March 1. Seven of these cocoons were kept at 20°C. and aborted after an average of 12.7 days, whereas the average hatching time at this temperature is 17.4 days. The two cocoons at 10°C. aborted on the 20th and 24th days, which was about the average hatching time at this temperature (22.5 days).

DISCUSSION

Anderson (1951) reported that *Procotyla* cocoons are colorless when deposited, remaining so as long as the adult remained over it; but during the next few hours the cocoon darkened to a mahogany color. This author noted several times that, after a worm deposited a cocoon on the side of the container, it remained over the cocoon for three to six hours, as the cocoon took on the mahogany color mentioned. Cocoons took approximately twice as long to darken when the adult did not remain. Possibly a substance contributed by the parent worm aids in hastening the hardening of the cocoon wall.

Anderson (1951) also found that the size of the cocoon depended upon the size of the adult depositing the cocoon. The author did not measure the adult worms, but it was noted that the larger worms did lay larger cocoons. Specific data was difficult to collect on this aspect because approximately fifteen to twenty adult worms were kept in the same culture dish and only a limited number were actually observed depositing cocoons. As far as could be ascertained, no adult produced more than one cocoon.

Hyman (1928) reported that Procotyla fluviatilis became sexually mature from September to January and that specimens taken from January to August always presented degenerate sex organs. This assumption was based upon direct observation and the appearance of one cocoon that was laid during November, 1927. This specimen was lost before further observations could be made. Hyman's research was carried out on a population near Chicago, Illinois; but she assumed her conclusions to be true for the genus throughout the United States. For the particular specimens observed in this study, the data in Table I indicate the period of peak cocoon reproductive activity to be from February through March. Sexual maturity and cocoon production may vary with different populations and environments.

Table II shows the variation in the number of days the cocoons were kept at $1^{o}C.$ and the variation in the number of days before hatching after the temperature was changed to $20^{o}C.$ The data suggest that development of the young inside the cocoon continues but at a slower rate when kept at $1^{o}C.$ This is shown by the fact that cocoons kept at $1^{o}C.$ for a period of forty-seven to sixty days took less time to hatch at $20^{o}C.$ than did the cocoons kept at $1^{o}C.$ for a period of thirteen to thirty-three days and then transferred to $20^{o}C.$

Table III shows the variation in the number of days cocoons were kept at $1^{o}C.$ and the variation in the number of days before hatching after being changed to 10°C. While these data are very limited, the fact that a cocoon kept at 1°C. for fifty-four days took five days to hatch after being transferred to 10°C., and that a cocoon kept at 1°C. for thirteen days took twenty-four days to hatch after being transferred to 10°C., suggests that development of young in cocoons kept at 1°C. continues, but at a slower rate. It is very possible that cocoons maintained at 1°C. may hatch if allowed a long enough period to develop.

The fifteen cocoons which hatched after being maintained continuously at 10°C. took an average of 22.5 days to hatch. The twenty-one cocoons which hatched after continuous maintenance at 20°C. took an average of 17.4 days to hatch. Results suggest an increase in temperature shortens the developmental period of the young flatworms inside the cocoon.

This research was carried out under laboratory conditions; however, the data indicates a possibility that cocoons laid in late January and early February develop slowly until the stream temperature rises in late February and early March. The cocoons laid in late February and early March may develop in a much shorter period, due to the rising stream temperature.

SUMMARY AND CONCLUSION

An investigation was undertaken to determine some aspects of of the reproductive cycle of the dendrocoelid flatworm *Procotyla fluviatilis*. Worms were collected, during August, 1967, through May, 1968, from a population occurring in a spring at Mason County, Illinois. Collected specimens were maintained under controlled

environmental conditions, and the production, morphology, and development of cocoons studied. Attention was also given to the influence of ambient temperature on the rate of cocoon development.

Copulation was observed between five pairs of flatworms collected during the period of January 21 to March 9. Cocoons were produced during the period January 24 through March 21. Greatest cocoon productivity occurred between February 15 and March 15. After this time, production declined sharply. Only one cocoon was produced per adult.

Cocoons averaged 150 microns in diameter (range, 83-207 microns) and took on a dark mahogany color several hours after deposition. In several instances, worms were observed to remain over a cocoon for several hours after deposition. Cocoons subjected to this behavior darkened more rapidly than those that were not.

Temperature was a major influence on the development of young flatworms inside cocoons. Development proceeded in cocoons maintained at 1°C. but at a much slower rate than at higher temperatures. Cocoons kept at 10°C. averaged 22.5 days to hatching, while those maintained at 20°C. hatched in an average time of 17.4 days. An increase in ambient temperature shortened the developmental time of cocoons.

There were a greater number of young produced in cocoons deposited during the first quarter of the cocoon production period than in the following three quarters. An average eight young were hatched from cocoons deposited during January 24 through February 7, while only four young were hatched from cocoons deposited between February 8 and March 21.

Previous studies on the reproductive biology of *P. fluviatilis* have been done in the laboratory. Observations under such conditions do not necessarily reflect the natural situation. Consequently, several statements concerning the reproductive season of the worm have been made without substantial data to prove the points. The cultures for this research were taken from the stream to the laboratory and, in some incidences, there was a 9°C. temperature change. This change could have initiated cocoon production. Does a rise in temperature in the stream cause reproduction to occur naturally, or are there other factors affecting this? Limited observation by the author has shown that copulation in the stream does occur during late February and early March. The reproductive season of this species cannot be generalized for the entire range. One area may have different temperatures, pH, and food supply available which may regulate reproduction. The reproduction of this flatworm needs to be investigated in the natural habitat in an attempt to collect data which can yield valid conclusions.

Table 1.--Production of cocoons by *P. fluviatilis*.

Weekly Period	Number Cocoons Observed	Number Adults Present	Productivity per 100 Adults
January 24-January 31	7	75	9.34
February 1-February 7	1	75	1.33
February 8-February 14	1	75	1.33
February 15-February 21	14	75	18.65
February 22-February 28	12	45	26.67
March 1-March 7	24	50	48.00
March 8-March 14	14	55	45.41
March 15-March 21	1	55	1.82

Table 2.--Variation in hatching time of *P. fluviatilis* cocoons after being transferred from 1°C. to 20°C.

Number of Days Each Cocoon Kept at 1°C.	Number of Days at 20°C. Before Hatching
60	6
59	5
58	4
56	5
47	7
44	*
43	*
33	12
17	16
13	16

* Cocoon did not hatch.

Table 3.--Variation in hatching time of _P. fluviatilis_ after being transferred from 1°C. to 10°C.

Number of Days Each Cocoon Kept at 1°C.	Number of Days at 10°C. Before Hatching
62	*
55	*
55	*
54	5
47	11
44	*
36	*
13	24

* Cocoon did not hatch.

Table 4.--Percent hatch of P. fluviatilis cocoons and average number of young flatworms per hatched cocoons during biweekly periods.

Biweekly Periods	Cocoons Deposited	Cocoons Hatched		Average young per Hatched Cocoon
		#	%	
January 24-February 7	7	7	100.0	8.00
February 8-February 21	15	10	66.7	4.60
February 22-March 7	36	24	66.7	3.16
March 8-March 21	15	6	40.0	5.16

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