Some Observations on the Reproductive Cycle of a Common Land Snail, Vallonia Pulchella. Influence of Environmental Factors[1]

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The present paper is preliminary to a more comprehensive study of the origin and development of the germ cells in *Vallonia pulchella* Müll. During the progress of the work, the animals were brought into the laboratory in considerable numbers, and there was ample opportunity for observations of egg-laying, hatching, and development, and, in connection with these, of the physiological effects of environmental factors such as cold and desiccation.

General Description of Material

Vallonia pulchella is a small land snail of wide distribution, with a smooth helicoid shell averaging a little more than 2.0 mm. in diameter. It may or may not be found associated with the ribbed species, *V. costata.* Large populations are found typically among grass roots at the edges of lawns next to a stone or cement walk or wall. They were collected from such typical locations in Ann Arbor, Michigan, and other places, where they were found at times so thickly congregated as to be crowded against one another. They were also collected from under old boards and pieces of brick and cement around dwellings and lumber yards. V. Sterki (1910) refers to the fact that, while deforestation, drainage, etc., have tended to reduce many species of snails, in the case of *Vallonia,* which is not a forest species but found in more open country, urban conditions have favored its increase, so that "a single well-sprinkled house lawn may be populated by more individuals than were in 1000 acres of original forest." Sporadic appearances of *V. pulchella* have been reported by Clapp (1897) in Pittsburgh, where they appeared literally in millions on his front walk after a rain, and (1901) in similar numbers near a stone wall on the estate of a friend about six miles out of town, and again by Stearns (1902) in Los Angeles.

During the present study, adults were collected at all seasons of the year, and eggs and juveniles during the months of May, June, July, and August, and juveniles also in September. During August a number of mixed collections of eggs, juveniles of various sizes, and adults were made along one of the walks on the Butler University Campus at Indianapolis. In the Ann Arbor locations, large aggregations of adults were found in the late fall and early spring, and considerable numbers in the winter, but juveniles were not noted among them.

The eggs, as observed both in the natural habitats and in the laboratory, are laid singly. They are spherical in form, a little more than 0.5 mm. in diameter, and quite fill up the aperture of the shell as

[1] Contribution from the Department of Zoölogy, University of Michigan.

they are extruded. They are covered with a pure white calcareous shell and are visible through the shell of the parent animal some time before being laid. When freshly laid, they appear somewhat translucent and have a pearly lustre but become darker and more opaque with age. They are affixed by mucous material to the under surfaces of bits of wood or dead leaves, or to grass roots or moss if present, or they may be inserted into the interstices of loose clumps of soil. These locations reflect the habits of the animals, which apparently do not burrow to any great extent but seek cover under surface objects. Even in winter they are found at the surface beneath the snow in an inactive condition.

Collecting and Culture Methods

Collections of individuals from one of the typical locations in Ann Arbor were brought in from time to time during the late fall, winter, and early spring. At the same time this typical site was watched for the appearance of eggs. The animals were inactive when brought in but soon became active under the usual laboratory conditions. The collections were kept in covered finger bowls with a little soil and a bit of dead leaf or decaying wood brought along from the natural location. A little fresh lettuce was also supplied occasionally. These laboratory cultures were kept moist by sprinkling every day or two, care being taken to avoid saturation, since under too moist conditions the animals would crawl up on the sides and covers of the dishes and remain inactive.

Eggs appeared freely in these mass cultures in a short time, usually after not more than a week. They were not found in any abundance in the out-of-door locations until May 21.

Production of Eggs by Isolated Individuals

In order to make a more detailed study of egg laying, the number of eggs produced by individual animals, and the time intervals involved, eighteen individuals from a collection made on April 24 were isolated in separate stender dishes with food, moisture, and temperature conditions as described above. These cultures were maintained over a period of several months, during which time records were kept of the number of eggs produced by each individual. Five of these individual records, quite typical of the group as a whole, are given below in Table I. It did not prove feasible to make daily records during the entire period, but this was done part of the time. Portions of the table are blocked off calling attention to the tendency for egg laying to occur at approximately 24-hour intervals when conditions were favorable. However, inspection of the table reveals that the interval may frequently be less. One exact record (not shown) was obtained of a 23-hour interval and another of 14 hours and 35 minutes.

It will be recalled that in the out-of-door location eggs were not found in any abundance until about May 21. By that time the eighteen laboratory individuals had laid a total of 322 eggs.

For ten of the eighteen individuals, records like those above were obtained extending through May, June, and July. The average number of eggs produced during this period was 53, the individual records varying from 30 to 69. Egg laying continued into August, but these records are not included.

TABLE I.—Individual Records of Egg-laying

Date of Record	No. Eggs Found D.	E.	J.	K.	N.	Date of Record	No. Eggs Found D.	J.	K.	N.	Date of Record	No. Eggs Found D.	J.	K.	N.
April 27	1				2	May 22	3	1	1		July 1	1		1	
April 28			2	1	1	May 23	1	1	1	2	July 3	1	1		2
April 29		1	1		1	May 28	8		3	2	July 4		1	1	
April 30		1			1	June 4	9	8		6	July 6	1	3	1	2
May 1	3	1	3	3	2	June 5	1	1		2	July 7	1	1		1
May 2	1	1			1	June 6	1	2		2	July 8		1		
May 3	1	1	2	1	3	June 14		2			July 9	1	1	1	1
May 4	1	1			1	June 15	9			2	July 10	1	1	1	1
May 5			2			June 22		6	1		July 11	1		1	1
May 7	2	1	1	1	3	June 23				1	July 12		1	1	
May 10	5	2	2	4	5	June 25			2		July 13	1		1	1
May 11		1	1	2		June 26				1	July 30	1		1	1
May 12					4	June 27	1	1		1					
May 15	4		4			June 28	1								
May 19	5	3			3	June 29	1	3		1					

Observations of Growth and Development

The embryo, when dissected out of a recently laid egg, consists of a small delicate ball of vacuolated cells a mere fraction the size of the entire egg. But at the time of hatching, which occurs in the laboratory in about twelve days, the young animal has filled up the egg shell and has attained its characteristic form and completed a whorl of its body shell. The little animal is delicately transparent, and the beating of the heart can be easily observed. It moves about actively after hatching.

In order to test whether individuals reared in isolation would reproduce and to get an idea of the rate of growth of the juveniles and the time required to reach sexual maturity, about two dozen juveniles produced in the laboratory by the group of eighteen individuals mentioned above were reared in separate stender dishes from the time of hatching. For 15 of these it was possible to obtain growth records at intervals of about a week up to and including the formation of the peristome and the beginning of reproduction. Samples of these records are given in Table II. As shown by the table, growth was fairly steady, the average gain in width being about 0.2 mm. per week. A little more rapid gains are shown at times when fresh food materials were supplied. In a number of instances when culture dishes were allowed to become dry, the animals became inactive and failed to gain, but they made normal gains after favorable conditions were restored.

TABLE II—Growth Records of Isolated Juveniles

Date	B-1	D-6	E-4	F-1	Remarks
May 12	....	....	....	0.7 mm.	
May 13	0.6 mm.	....	....	....	Just hatched.
May 18	....	0.7 mm.	....	....	
May 20	0.8 mm.	....	....	1.0 mm.	
May 21	....	....	0.7 mm.	....	
May 25	....	0.9 mm.	....	....	
May 26	....	....	....	1.2 mm.	
May 27	1.1 mm.	....	....	....	
May 28	....	....	0.9 mm.	....	
June 2	1.2 mm.	1.1 mm.	1.1 mm.	1.7 mm.	New substrate
June 10	1.6 mm.	1.7 mm.	1.6 mm.	1.8 mm.	
June 24	....	....	....	....	New substrate
June 25	1.7 mm.	2.1 mm.	1.7 mm.	1.9 mm.	Peristome formed in D-6 and F-1.
June 29	....	1st and 2nd eggs	....	1st egg	
July 1	....	3rd egg	....	2nd egg	
July 2	1.9 mm.	....	2.0 mm.	....	Peristome not yet formed in B-1. Present in E-4.
July 6	....	4th egg	....	3rd egg	
July 9	2.1 mm.	5th egg	1st egg	4th egg	Peristome formed in B-1.
July 15	....	6th, 7th, 8th eggs	....	....	
July 17	1st and 2nd eggs	....	2nd, 3rd, 4th eggs	....	
July 19	3rd egg	9th egg	5th and 6th eggs	....	

On the average in this group, egg-laying began in a week or less after the formation of the peristome was completed. The average time between hatching and maturity for the fifteen specimens was 59 days, and the average diameter at the time of first oviposition was 2.0 mm. These figures must be regarded as approximate, since it was not always possible to record exact dates of either egg-laying or hatching.

Not only did the above group of juveniles reared in isolation lay eggs as shown in the table, but some of these eggs placed in individual stender dishes hatched in the normal manner, giving rise to juveniles of the third generation, which in turn laid viable eggs of the fourth generation. Thus it was possible to construct a calendar covering portions of four generations as in Table III.

TABLE III.—Record of Four Generations of *Vallonia pulchella* Müll.

Line	Total days	Parent	When mature	Egg	Date of laying	Hatched	Generation	Days
I	146	D	Coll. 4/24	D-6	5/6	5/17-18	2	53
		D-6	6/29	D-6-b	7/1	7/12	3	62
		D-6-b	8/30-9/1	D-6-b-1	8/30-9/1	9/13-19	4	....
II	147	F	Coll. 4/24	F-1	5/1	5/12	2	59
		F-1	6/29	F-1-a	6/29	7/11	3	62
		F-1-a	8/30-9/1	F-1-a-1	8/30-9/1	9/13-19	4	....

Environmental Factors Affecting Egg-laying, Development, and Survival

The fact that egg-laying took place in the laboratory in winter and early spring, but not until considerably later out of doors, suggested that the higher and more uniform temperature and moisture in the laboratory favored egg-laying, since the substrate for the laboratory specimens was essentially like that of the natural habitat. As shown in Table I, animals collected April 24 were prepared to lay eggs in a short time if suitable conditions were provided. To test the inhibiting effects of low temperature and desiccation, a part of this collection was divided into three experimental groups; one group was placed in individual stender dishes in a refrigerator at temperatures of 12°-15° C., the second group was placed in dry vials with cotton stoppers, at laboratory temperatures of 21° to 22° C., and the third group in dry vials in the refrigerator. At weekly intervals one individual was removed from each of the above groups and restored to favorable conditions of temperature and moisture and the time noted when egg-laying began. The last two groups were necessarily deprived of food as well as moisture during the experimental periods since the animals cannot feed while in the inactive aestivating condition. A few specimens were also kept for periods of one to three weeks in stender dishes provided with moist filter paper but no soil or food.

Besides these adult groups, a few groups of eggs and juveniles were tested for their resistance to varying periods of cold and dryness.

The inhibiting effects of low temperatures and desiccation and the recovery from these effects are shown in Tables IV-VII. The animals in the first group were not immobilized by the refrigerator temperatures used but were often found slightly active and feeding when the dishes were taken out for inspection. Nor did refrigeration completely inhibit egg-laying, as shown in Table IV. However, only 13 eggs were recorded laid at refrigerator temperature by the group as a whole over a period of about three months, as compared to hundreds laid by the group represented by Table I, which was considered as a control group. No record was obtained of the hatching of any eggs in the refrigerator.

In the case of the animals kept in dry vials, no egg-laying took place during the inactive aestivating period, but as shown by Tables V and VI, oviposition began in a few days after favorable conditions of moisture, temperature, and food were restored. The recovery interval

TABLE IV.—Effects of Refrigeration on Egg-laying

Individuals	Refrigeration Period	Restored to Lab. Temp.	Egg-laying Begun at Normal Rate	Recovery Interval
AA	7 days	May 1	May 2	1 day
AB	16 days	May 10	May 12	2 days
AD	22 days	May 16	May 17	1 day
AF	30 days	May 24	May 28	4 days
AG*	40 days	June 3	June 6	3 days
AH	45 days	June 8	Inactive after June 28	No eggs
AI	68 days	June 28	June 29	1 day
AP*	81 days	July 11	July 15-18	4-7 days
AS*	89 days	July 19	July 20	1 day
AT*	96 days	July 26	July 27	1 day
AX*	103 days	Aug. 2	Aug. 3	1 day

*Laid 1-3 eggs in refrigerator before restoration to laboratory temperature.

TABLE V.—Effects of Desiccation on Egg-laying

Individuals	Aestivation Period	Moisture etc. Restored	Egg-laying Begun	Recovery Interval
BA	7 days	May 1	May 6	5 days
BB	16 days	May 10	May 12	2 days
BD	22 days	May 16	Became inactive	No Record
BF	30 days	May 24	May 27	3 days
BG	40 days	June 3	June 6	3 days
BH	45 days	June 8	June 12	4 days
BI	68 days	June 28	July 7*	9 days*
BJ	75 days	July 5	July 8	3 days
BK	81 days	July 11	July 17	6 days
BL	89 days	July 19	July 23	4 days
BM	96 days	July 26	Became inactive	No record
BN	103 days	Aug. 2	Became inactive	No record
BO	110 days	Aug. 9	Became inactive	No record
BQ	117 days	Aug. 16	Aug. 21	5 days

*Observation was delayed in this instance. The interval was probably less.

TABLE VI.—Effects of Combined Refrigeration and Desiccation on Egg-laying

Individuals	Period of Refrigeration & Aestivation	Restored to Moisture Lab. Temp. etc.	Egg-laying Begun	Recovery Interval
CA	7 days	May 1	May 7	6 days
CB	16 days	May 10	May 15	5 days
CD	22 days	May 16	May 19	3 days
CF	30 days	May 24	May 27	3 days
CG	40 days	June 3	June 6	3 days
CH	45 days	June 8	Became inactive	No record
CI	68 days	June 28	Record uncertain	
CJ	75 days	July 5	Became inactive	No record
CM	81 days	July 11	July 18	7 days
CN	89 days	July 19	July 26	8 days
CO	96 days	July 26	Aug. 3	8 days
CP	103 days	Aug. 2	Became inactive	No record
CQ	110 days	Aug. 9	Aug. 12-15	3-6 days

TABLE VII.—Environmental Effects on Eggs and Juveniles

Eggs		Hatching	Juveniles		
Exper. Period	No. Individuals		Exper. Period	No. Individuals	Recovery
A. Effects of Refrigeration					
15 days	Group 1—14	+	8 days	5	+
	Group 2—10	—	7 days	3	+
7 days	22	+	2 days	8	+
B. Effects of Desiccation					
15 days	14	—	61 days	10	—
7 days	15	—	14 days	7 small	— One Doubtful
2 days	7	—		6 larger	+
			8 days	3	+
			7 days	5	+
			2 days	8	+
C. Effects of Refrigeration + Desiccation					
15 days	12	—	8 days	5	—
8 days	7	—	7 days	3	—
7 days	14	—	2 days	5 small	—
2 days	5	—		3 larger	?

apparently does not differ greatly whether the experimental period has been long or short, and the animals recover about as well from a combination of refrigeration and desiccation as from desiccation alone. In part, the importance of the recovery interval seems to be to permit a period of feeding. The few animals provided with moisture but no food or soil did not deposit any eggs under those conditions but in several cases laid eggs at the normal rate when food and soil were added.

In Table VII, the symbols + and — indicate whether any eggs in a given group hatched or whether any juveniles recovered activity after experimental treatment. The eggs proved fragile and difficult to handle after drying, and there was some breakage, loss of specimens, and difficulty in making exact observations in each case. Because of the limited nature of the experiments, the results should perhaps be regarded as merely suggestive; but they indicate that the eggs are very susceptible to desiccation when exposed. It will be recalled that in nature they are deposited in protected places where moisture is retained and that the juveniles are not hatched until the body shell has developed to take over the protection hitherto provided by the egg shell. The juveniles are apparently more resistant to desiccation than the eggs but less so than the adults. Younger juveniles are less resistant than older ones.

In the case of eggs which hatched after undergoing a period of refrigeration, it should be mentioned that hatching was delayed for a period approximately equal to the refrigeration period.

Discussion

The above experiments indicate that reproduction in *Vallonia pulchella* may involve parthenogenetic development or some type of self-fertilization, since it was found that individuals reared in isolation produced eggs which were viable and hatched in the normal manner for several successive generations. Literature records several instances of a similar kind among snails. Crabb (1927) found that, in the case of *Lymnaea stagnalis*, "individuals raised from isolated eggs and reared in strict isolation reproduce as abundantly as do those in mass cultures" and quotes Colton (1922) as having recorded self-fertilization in *L. columella* for 47 generations.

Self-fertilization has been suggested by Boycott (1917) and Steenburg (1918) for *Acanthinula aculeata*, a species believed to be closely related to *Vallonia*, since they found the distal portions of the male ducts absent in many cases. Boycott, in discussing this phenomenon, inclines to the view that the aphallic state is due to the simplification of structure which is necessarily associated with diminution in absolute size.

Watson (1919) dissected 98 specimens of *Vallonia*, including some *V. costata*, some *V. pulchella*, and some *V. excentrica* collected in both spring and fall, and found male organs present only in three specimens of *V. costata*. In the remaining 95 they were entirely absent.

Reproduction by isolated *Vallonia* as found in the present study is in harmony with these anatomical observations. It is hoped in the further study to correlate these evidences with histological and cytological findings.

As far as the present study goes, it does not give evidence of any very definite periodicity or anything very complicated about the reproductive cycle but indicates rather that egg-laying may take place whenever the animal is in a good state of nutrition and external conditions are favorable. Egg production is somewhat limited at best because of the large size of the eggs in proportion to the size of the animal, which necessitates their being laid singly at a rather slow rate. Temperature and moisture conditions impose further limitations. It would be a disadvantage if eggs were laid under dry conditions, as they are very susceptible to desiccation, or during cold weather, because the retarding effect on hatching would prolong their exposure to other hazards.

Along with the inhibiting effects of these factors, we have noted the ability of the animals to recover from them quickly. We have observed that specimens collected in the fall and winter began laying eggs after a short time in the laboratory. Individuals collected in the spring, ready to lay eggs in a short time, were retarded experimentally for periods of one week to three months by cold and dry conditions, but egg-laying began in a few days when favorable conditions were provided. This opportunism in egg-laying appears to be of considerable adaptive significance in enabling the animals to take advantage of favorable conditions when they occur.

It is hoped to make studies of other factors such as nutrition as they affect egg-laying. The fact that the animals begin to lay eggs usually in less than a week after the peristome is formed, i. e., when the shell is completed (Table II), might suggest that mineral materials taken in with the food, used hitherto for adding to the shell, at this time become available for the formation of the egg shells.

We can see from these studies how reproduction in *Vallonia* would be favored by urban conditions as emphasized by Sterki, previously quoted. Egg-laying could take place during a much greater part of the summer season on a regularly sprinkled lawn than in regions favored only by natural rainfall. We can interpret the sporadic appearances recorded by Clapp and Stearns and the large aggregations found during the present study in a similar way. It has been suggested that these large aggregations may be due to migrations inward to some protected favorable location from surrounding territory, but it seems more likely that they may represent the piling up of populations under somewhat artificially favorable conditions.

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Summary

1. Specimens of *Vallonia pulchella* Müll. brought into the laboratory in fall, winter, and early spring began laying eggs in from a few

days to a week or two. Eggs were not found in any considerable numbers until the middle of May. They were collected from time to time throughout the summer.

2. The eggs are laid singly, and under optimum conditions in the laboratory one egg is laid each day and sometimes more.

3. Hatching occurs in the laboratory in about 12 days. The juveniles at the time of hatching are well-developed and quite active. One whorl of the shell is completed, and they measure about 0.6 mm. in diameter.

4. Juveniles reared in isolation from the egg showed a steady growth under favorable conditions of about 0.2 mm. per week (diameter of the shell) and reached adult size in an average of 59 days. They began laying eggs usually in less than a week after the formation of the peristome. The average diameter at this time was 2.0 mm., the normal adult size.

5. Individuals reared in isolation for several succeeding generations reproduced.

6. Refrigeration and desiccation have retarding and inhibiting effects on egg-laying and development, but the animals recover rapidly from these effects. Refrigeration is less inhibiting than desiccation. The eggs are very sensitive to desiccating influences. In nature they are deposited in protected places. Juveniles are less susceptible to desiccation than the eggs. With age the juveniles become progressively more resistant to unfavorable conditions.

7. In general, this study shows that *Vallonia* is able to reproduce without cross-fertilization, suggesting either parthenogenesis or some form of self-fertilization, and that the reproductive cycle is strongly influenced by environmental factors.

Literature Cited

Boycott, A. E., 1917. The genitalia of Acanthinula aculeata. Proc. Malac. Soc. Lond. 12:221-226.

Clapp, G. H., 1897. Vallonia pulchella in Pittsburgh. Naut. 10:143. 1901. Vallonia pulchella. Naut. 14:130.

Crabb, Edward D., 1927. The fertilization process in the snail Lymnaea stagnalis appressa Say. Biol. Bull. 53:67-91.

Stearns, Robert E. C., 1900. Vallonia pulchella Müll. in Los Angeles and elsewhere in California. Naut. 14:65-67.

Steenburg, C. M., 1918. Anatomie des Acanthinula et des Vallonia. Les organes genitaux. Vidensk. Meddel. fra Dansk Naturh. Foren. 69:1-15.

Sterki, V., 1910. Civilization and snails. Naut. 24:99.

Watson, H., 1921. The affinities of Pyramidula, Patulastra, Acanthinula, and Vallonia. Proc. Malac. Soc. London. 14:6-11.