THE EGG-LAYING HABITS OF *POMATIOPSIS CINCINNATIENSIS* (LEA)

By

HENRY VAN DER SCHALIE
AND
HAROLD J. WALTER

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THE EGG-LAYING HABITS OF *POMATIOPSIS CINCINNATIENSIS* (LEA)[1]

Henry van der Schalie and Harold J. Walter
Museum of Zoology, University of Michigan

Introduction

Although the eggs of *Pomatiopsis cincinnatiensis* (Lea) had not been described previously, Berry and Rue (1948, p. 18) stated that those of *P. lapidaria* (Say) "were 1.2 mm. in diameter, laid singly on sandy loam, coated with a sandy husk." Studies of the biology of species of *Oncomelania* have shown that their eggs are basically similar in structure and in ecological requirements to those of *Pomatiopsis*. Abbott (1946) and McMullen (1947) described the egg of *Oncomelania quadrasi* (Moellen.) in the Philippines; Fu-Ching Li (1935) and Vogel (1948) reported on the structure of the egg of *O. hupensis* Gledler from China; whereas Sugiura (1933) and Richie *et al* (1951) described those of *O. nosophora* Robson in Japan. All observers stress that the eggs are coated with mud, sand, or snail faecal material so that they tend to blend almost perfectly with the surrounding soil; that they are deposited separately; and that they usually are well-concealed.

The geographic distribution, seasonal population structure, mating habits, and general ecology of *Pomatiopsis cincinnatiensis* (Lea) were treated in a recent publication (van der Schalie and Dundee, 1955). No information on egg-laying habits of this species under field conditions was obtained during that study and efforts to find the eggs in the field proved fruitless. Some description was given of eggs laid under laboratory conditions, however. These were found to be deposited separately on soil, or partly buried in it; they were individually coated with a tough and resilient husk of earthy material making them almost visually undetectable. This paper deals with the egg-laying habits of *P. cincinnatiensis*, particularly in the field situation, and it contributes further information on the nature of the egg. In the structure of its egg and in its general biology *Pomatiopsis* is very similar to the species of *Oncomelania* which serve as intermediate hosts of schistosomiasis in the Orient. A knowledge of the biology of *P. cincinnatiensis*, and its reproductive stages in particular, may have practical application to problems in the control of this disease.

The authors wish to thank Dee S. Dundee for her assistance in several phases of this study.

Materials and Methods

The previous investigation indicated an annual life cycle for *P. cincinnatiensis* and gave some information as to when eggs of this species were most likely to be found in the field. Consequently, the field work was arranged to cover the period from early May into August of 1955; this period embraced the reported interval between the observed maximum

[1]This work was carried out under the sponsorship of the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and was supported by the Office of the Surgeon General, Department of the Army.

frequency of mating and the appearance of young in abundance. The two field stations previously established for ecological work were used. Both are located on the banks of the Raisin River in southeastern Michigan: one adjacent to a pasture about one mile northwest of Clinton in Washtenaw County; the other along a wooded flood-plain about four miles southeast of Tecumseh in Lenawee County. As in previous years, the adult snails were abundant at both localities; over the period of study they were concentrated largely within a narrow band along the banks.

Eggs were recovered in the laboratory from samples of soil cut from the surface of the stream-bank habitat. Each sample was obtained by impressing a metal ring into the bank until it was flush with the surface and then the so-formed soil cake was cut free. These discoidal cakes were three and one-half inches in diameter and one-half inch thick.

The samples were taken so as to make transects of the habitat which would intersect the concentration of snails. Each transect consisted of a linear series of samples, spaced one inch apart and running at right angles to the stream margin. The samples were subsequently placed in water and reduced to a soupy consistency; this process required several hours of soaking and frequent agitation. The resulting mixture was then strained through sieves of ten- and twenty-mesh per inch. The latter retained the snail eggs while the larger mesh sorted out larger *Pomatiopsis*, grass, etc. From time to time, a sieve of finer mesh was used to check the possible loss of eggs through the No. 20 screen. The eggs and various organisms present were separated from the residual detritus with the aid of a dissecting microscope. All samples, when taken, were wrapped in paper in order to prevent escape of any organisms; they were treated as carefully as possible during all stages of processing.

The Tecumseh station was sampled on each date, and most of the data were obtained there. At any one place the presence of *Pomatiopsis* was the only indication of the possible presence of eggs. Since the snails were about equally abundant over much of the study area, choice of sites was essentially arbitrary. Sampling was generally restricted, however, to banks of moderate height; the steeper banks and certain other situations proved very difficult to transect. Sections of bank with a high degree of uniformity for any considerable length were not available. The better areas usually permitted the taking of only a few transects spaced in such a way as to insure that the snail populations would not be unduly disturbed. One limited area selected early in the program eventually became greatly modified by erosion and was abandoned in mid-season. Sampling was then largely transferred to another segment of bank from which transects were obtained on five dates; the latter section was comparatively uniform in most characters over a ten-yard distance. These transects (six) were irregularly spaced, mostly over a yard apart. A second transect of each date was usually taken in a somewhat different type of situation in a different area, and sometimes at the other station. The transects at the Tecumseh station were spread over a distance of around five hundred yards. No order was followed in selecting sites in any area.

The most convenient environmental feature to which the samples in a transect could be spatially related was the downslope boundary of the rooted vegetation that covered most of the banks. Although the snail concentrations occupied only a small fraction of the width of the habitat, the remainder of the snails were diffusely spread from the top of the banks and down to about a foot below the vegetative boundary. The total width of the area occupied varied from about three to five feet, largely depending on the width of the zone of vegetation. In an attempt to retain a close spacing of samples it generally proved impractical to make complete transects of such broad habitats. Most transects however fell short of the extreme upslope and downslope limits of the populations by only a few inches. Accordingly, they were quite varied in length. High

TABLE I.

Distribution of Eggs In All Transects

Date	Transect Number	Number of Eggs Recovered, Recorded for Each Sample in its Original Relative Position in the Transect (Upslope Direction is to the Right)	Total Eggs in Each Transect
May 20	Tr. 1+	X 0 0 1| 0 *11* 1 1 *10* 3 3 1 2 1 1	34
	Tr. 2#	X X 0 3 2 1 3 3 X	12
May 26	Tr. 1*	X 22 20 21| 25 *32* 4 0 1 0 X X X	125
	Tr. 2*	X 13 10 4| 5 *8* 3 11 *19* 3 2 0 0 0 0	74
June 4	Tr. 1#	0 0 0 0| 3 16 *38* 4 9 *13* 11 5 X X	99
	Tr. 2#	0 0 0 0 0 0 0 0 8 X	8
June 27	Tr. 1*	0 2 3 0| 15 *150* 56 3 *18* 6 X X X	253
	Tr. 2+	0 2 3 4| 2 *96* 14 7 4 *66* 31 10 5 X	231
July 24	Tr. 1*	X X X X| 8 *51* 1 5 0 0 0 0	65
	Tr. 2#	X X X 3| 3 6 4 0 0 0 0 9 7 X	36
Aug. 6	Tr. 1*	X X X X| 11 *42* 12 4 0 1 0 1 X	70
	Tr. 2#	X X 1 2| *17* 2 1 1 0 0 X X	24
Aug. 16	Tr. 1*	X 4 5 0| 3 *22* 2 2 2 X X	42
	Tr. 2#	X 2 4 8 5 8 X X 3 X	30

\+ From Clinton Station
\# From atypical banks, Tecumseh Station
* From typical bank, Tecumseh Station
X Samples lacking
Dashed vertical line shows position of vegetation boundary, where definite

waters prevented downslope sampling on two dates (July 24 and August 6). The visible characteristics of each transect site were usually recorded in detail, with measurements relating the sample to the dimensions and contour of the bank; laboratory notes were kept on the nature of the soil samples. All of the field observations were made during the daytime. In conjunction with the field study new information was obtained on oviposition under laboratory conditions.

Spatial Pattern of Oviposition

Eggs of *Pomatiopsis cincinnatiensis* were recovered from all of the transects and from most of the samples (Table I). The total number recovered was about eleven hundred. This figure includes a small fraction of specimens that were identifiable only as fragmented husks after washing the samples (see Fig. 1). Some husks were possibly of eggs that had died or hatched, but most were clearly of those in late developmental stages;

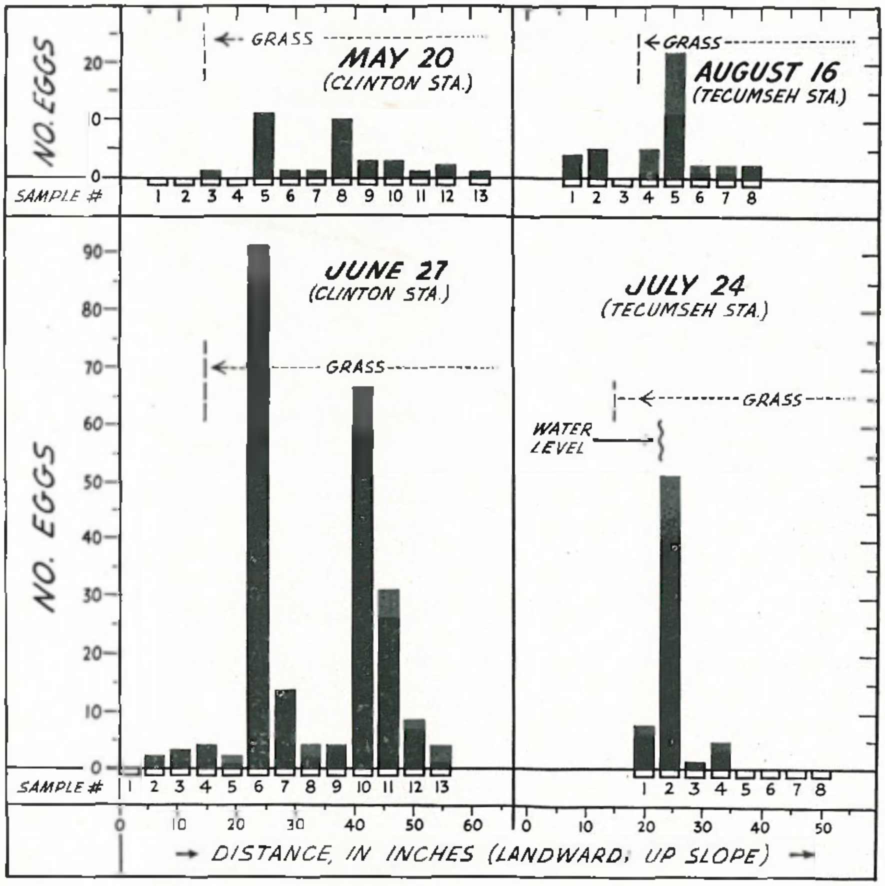

FIGURE 1: Distribution of eggs within four transects, indicating seasonal trends of abundance and zonal concentrations. There is some spatial distortion as each bank site represented was of a different convexity. Dotted parts of bars represent eggs recovered as broken husks. Compare Table I.

a similar number of shelled embryos were generally found in the screening residues. Few, if any, eggs appear to have been lost in the recovery process. The consistent recovery of eggs from all of the sites sampled indicates that the distribution of eggs along the stream was highly continuous from early May to mid-August. It was evidently uninterrupted on a scale of inches for lateral distances measured in yards or rods.

Throughout most of the extensive area sampled at the Tecumseh station, the *Pomatiopsis* snail distribution showed only occasional breaks of several yards or more. It can be assumed that the lateral egg distribution generally coincided with that of the snails. On the much shorter distance of bank studied at the Clinton station, the distributional pattern was clearly similar. There the two transect sites were ten yards apart, with the snails distributed continuously between them. Numerous soil samples were obtained from a number of widely spaced localities several miles upstream of the Tecumseh station prior to the transect program (May 12); these yielded numerous eggs, demonstrating a similar distributional continuity over long distances between the stations.

The distribution of the eggs between the stream margin and the flood plain above it also proved to be extremely broad and continuous (Table I, and Figs. 1, 3). At several transect sites in two limited sampling areas, the distribution was quite discontinuous or restricted in this direction, but quite localized special circumstances were involved. These are regarded as "atypical" and will be considered in a later section dealing with environmental conditions. Nearly all transects appeared to fall somewhat short of covering the width of the distribution. Eggs were spread continuously, again on a scale of inches, from the approximate tops of some of the widest banks down to and well beyond the downslope vegetation boundary. With some local variation, this condition existed at least as early as May 20 and as late as June 27. Later, there seemed to be a tendency toward narrowing of the distribution from the upslope direction.

As late as August 16, however, the distribution was still found to be very continuous across nearly the whole three-foot width of at least one bank. There seems to have been considerable variation in the streamward extension of ovipositing. The data concerned are fragmentary, however, and the situation may have been obscured by erosion or sedimentation. In two instances where transects were adequate, the apparent streamward limit was about 13 inches downslope of the vegetation boundary (June 27 transects); this coincided with the apparent extreme limit of the snail distribution. In some other places the egg distribution evidently extended some distance further streamward (May 26 transects). The total area of oviposition described above was approximately coextensive with the distribution of the snails.

Within the broad limits just delineated a conspicuous spatial pattern of oviposition was discovered which was strikingly similar at both stations. In most transects one or two samples contained a particularly large number of eggs. Those samples clearly represented two zonal egg-concentrations, which continued along the banks throughout most of the study area. Both concentrations occurred within the vegetational area of the banks and they were about five inches or less in width. The lower one generally occurred between five and ten inches upslope from the vegetation boundary. Along the ten-yard segment of uniform bank at the Tecumseh station, this zone was found to fall between 20 and 27 inches (vertically) above the stream's minimal level at the six different points sampled. Since these loci represent dates over the whole sampling period, these data show that there was no significant seasonal displacement of the zone. On another but lower bank (Transect 2, June 27) this zone was only about a foot above the low-water level, whereas on an "atypical" bank higher than these (Transect 1, June 4), it was 30 inches or more above that reference level.

At the two remaining "typical" sites, of moderate height, the vertical displacement was between those extremes. Despite such variations, the upslope concentration of eggs almost precisely paralleled the lower. They were almost exactly ten inches apart as measured over the slope, except in one case associated with special circumstances (Transect 2, June 27).[2] There was no evidence of a seasonal change in this spacing. These zones evidently represented peaks of concentration within two separate broader zones which were rather diffuse and overlapped irregularly. Together, the latter nearly covered the full width of the vegetational area.

Both narrow zonal concentrations were already recognizable on the earliest sampling date, May 20. They may have been developed better than the transect results indicate, as will be explained later in reference to environmental conditions. Both persisted at least until late June, when the lower one became strikingly developed. At one site, eggs in this zone reached a concentration of at least 17 to the square inch (Transect 1, June 27). The upper concentration generally showed a relatively small increase in egg-numbers during that same period, and sometime before July 24 it disappeared. The lower concentration persisted and dominated the pattern through August 16, the final sampling date. Although the broader upper zone became unrecognizable some eggs occurred at that general level throughout the period of study.

A third but diffuse zone of ovipositing also occurred on the downslope exposed area of the banks where it was set off by a tendency for the distribution to be broken in the vicinity of the vegetation boundary. Whenever observed, the zone of snail concentration occurred within this zone, usually toward its upper edge. This egg zone also appeared early in the season and lasted through the end of the sampling period. Whereas it generally seemed to be a minor zone, it contained large numbers of eggs in the spring. The latter development was found only in the transects of May 26 which were within a few yards of each other in fairly similar situations (Table I). A comparison of the distribution patterns in these transects gives the impression that egg-laying temporarily "spilled over" from the usual zones into this lowest one. A local shifting is similarly suggested by the data from the June 27 sites. While nearly equal numbers of eggs had been laid at both places, ovipositing activity seems to have been transferred from the major lower zone to the upper in the one instance (Transect 2). There also seemed to be considerable shifting in the egg-numbers within the broader zones around the rather static upslope concentration. The factors responsible for the development of the pattern of egg-distribution described here have not been determined definitely. The environmental conditions under which the pattern was found suggest possibilities regarding its origin, however. These matters will be considered later.

Seasonal Abundance of Eggs

The average number of eggs of *P. cincinnatiensis* recovered over the season was about eight per sample or about one per square inch of soil taken from the habitat (Table I). Definite seasonal trends in abundance were found (Fig. 1). Although the data allowed only a crude estimate

[2]In Figure 1 this transect may be misleading in several respects; refer to Table I, Figure 3, and the later section on environmental conditions.

for certain dates, those most critical for a general appraisal of seasonal abundance (*i.e.*, both transects of June 27) appeared particularly complete and reliable. The definite spatial pattern of egg-distribution aided in this appraisal. It served to indicate the probable scale of abundance beyond the ends of those transects that had fallen short of the limits of the distribution. This loss was judged to have been generally insignificant. Information bearing on the environmental conditions at each site gave some indication of the relative reliability of the results from the different transects. In the discussion that follows, the evaluation of the data in particular instances can be observed by referring to Table I. These conditions are further clarified in the discussion of the environment which follows. The results from transects taken at "atypical" sites are largely ignored here.

Information on egg abundance is given in figure 2. These data serve for the making of this diagrammatic model illustrating possible seasonal relationships between abundance of eggs, the cumulative factor, rates of oviposition, hatching, and the abundance of snails produced as young-of-the-year. It is intended as a broad and partly hypothetical reconstruction of these relationships. Calculated estimates of egg-numbers shown in the model are related to an area of definite size. This area is given as an uninterrupted strip of typical habitat, comparable in length and width to a sample-transect five feet long; it is assumed to represent nearly optimal conditions. The estimates are based on the number of eggs recovered per unit of sample area and on spatial distribution curves derived from the data as graphed in figure 1. It is assumed that the variation in the width of the habitat did not greatly affect these estimates.

As the model indicates, eggs were rather abundant by May 12. A general idea of abundance was obtained by extensive random sampling on that date. By June 27 the number of eggs had greatly increased and there were then about 450 eggs in the designated transect of the habitat. This figure probably represents the approximate seasonal maximum. A marked decline occurred by July 24 and the reduction continued through the final sampling date, August 16. On the latter date the abundance was roughly the same as in early May which was about one-sixth of that on June 27. The total number of eggs laid in the given section over the season was about 600. Apparently half of them were laid in June. Although the spring onset of laying was not established it probably began in April. Some freshly laid eggs were recovered on all sampling dates, suggesting that ovipositing continued throughout the period of study. On the final sampling date about half of the few eggs found were at or near the hatching stage. Since some of the remainder found on that date were in early developmental stages, it is likely that all eggs did not hatch by September (see below). Since only a few female snails were taken in the transects, very high individual egg-production is indicated. The proportion of adult snails to eggs shown in figure 3 is roughly indicative of this relationship which otherwise was unmeasured.

Although seasonal abundance is related to the length of the incubation period of eggs under field conditions, this factor as yet is unknown. There are records, however, to indicate the time required for eggs to hatch in the laboratory. van der Schalie and Dundee (*l.c.*) previously reported that eggs hatched in five to seven weeks at an average temperature of about 18 degrees C. More recent records on some 50 eggs incubated under similar conditions showed that 80 percent hatched in the fifth week; some the seventh week and others the fourth. This is a remarkably long and variable incubation time. Whereas this time factor may be different in the field, it is probably quite lengthy, and it would have had a marked influence on the seasonal abundance of eggs. Assuming that incubation time was about the same in the field, abundance during the first month of

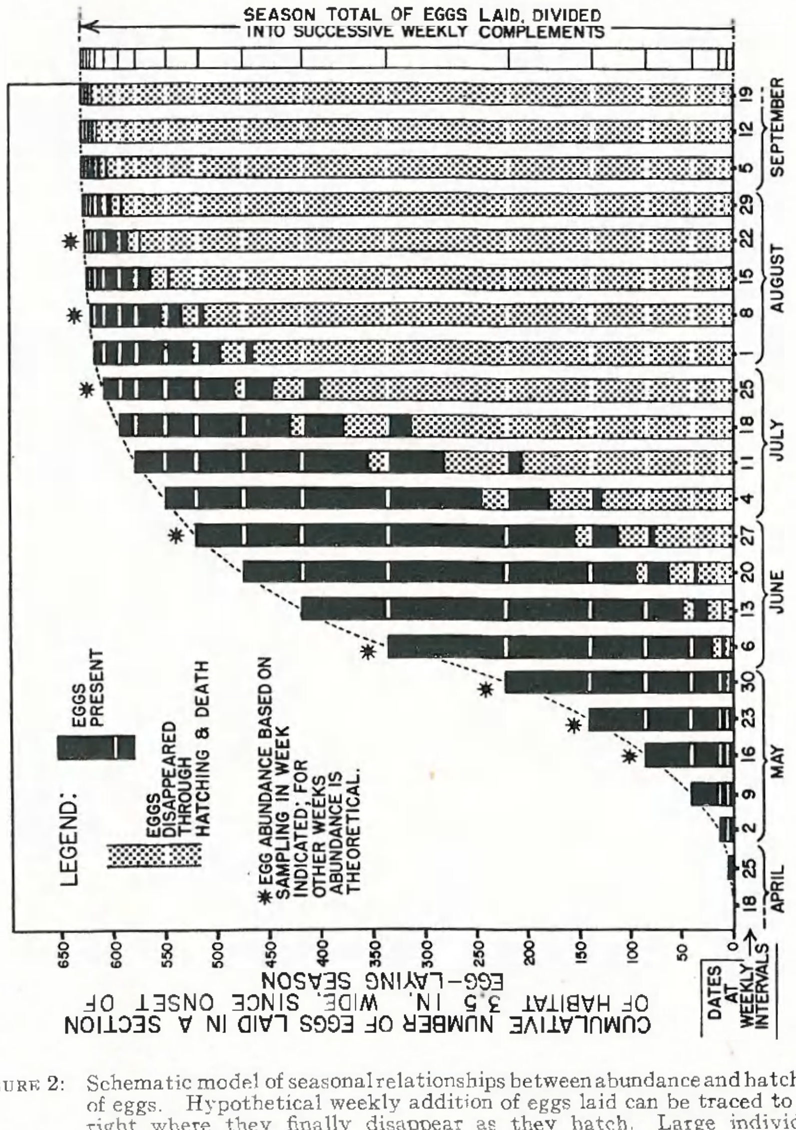

FIGURE 2: Schematic model of seasonal relationships between abundance and hatching of eggs. Hypothetical weekly addition of eggs laid can be traced to the right where they finally disappear as they hatch. Large individual variation in incubation time (five to seven weeks) is indicated. The total length of black in a bar at a given date represents a measure of egg abundance.

the oviposition season would then be reflected as a simple additive increase. Since in all instances the intervals between the collecting dates was less than five weeks, the eggs laid in a given week would be represented in samples taken in successive transects. This "carry-over" tends to establish continuity between collections. These accumulations are schematically shown in figure 3. The relationships illustrated give some indication of the number of eggs laid during successive collections. In this way, it is possible to indicate the possible rates of egg-laying diagrammatically. The weekly accumulations of eggs shown are theoretical; they were derived from relationships inherent in the graphed data. The relatively wide variation in the incubation time complicates the attempt to indicate digrammatically the progressive reduction in the weekly complements through hatching. The smoothed curve does not necessarily imply that oviposition was uninterrupted. In drawing the curve the higher egg counts were generally favored. Since such maximum numbers were found in the same uniform section of bank at the Tecumseh station they should be most directly comparable. The data prior to June 27 are the least satisfactory because many of the samples during that period came from "atypical" situations and the results were quite variable. Since eggs may have been destroyed by erosion and sedimentation in the lowest parts of the habitat, perhaps the scale of abundance was greater than indicated here.

The model (Fig. 2) gives the relative seasonal abundance of young *Pomatiopsis* snails in the field, although counts were not made consistently. Mortality rates are unknown. It was evident that the total number of eggs, including empty shells found, obviously greatly exceeded the number of young. Thus, a high mortality in the egg seems indicated. Consequently, the scale of abundance for the young as shown may be greatly exaggerated. van der Schalie and Dundee (*l.c.*) reported that young snails were first observed in July but by August they appeared in large numbers. This model tends to verify those observations except that the events reported here occurred earlier and appeared to be more regular. In this study, eggs near the hatching stage were recovered early in June and on all subsequent sampling dates. By June 27 some young were seen in the field, but they were found only by a careful search. Young snails, common on July 24, were very abundant on August 6 when many had grown considerably. Unusually high seasonal temperatures during this period probably accelerated egg-development. Some discrepancy, however, may be due to observational difficulties which was emphasized in the course of this work. Upon hatching, the young snails were thinly dispersed over the whole width of the habitat and most of them occurred in the vegetation areas where they were virtually undetectable. As the season progressed they migrated downslope and became concentrated in a zone on the smooth exposed soil below the vegetation boundary. This tendency reached a peak in August when, by rapid growth, many young had grown conspicuously larger (van der Schalie and Dundee, *l.c.*). At that time, probably, the large majority of snails are readily observable. All these circumstances tend to exaggerate the apparent lateness of hatching and the suddenness of the numerical increase of young snails.

The Egg and the Environment

The sampling methods employed depended largely on the size of the egg of *P. cincinnatiensis* and the resiliency and toughness of its husk. Eggs, with their husks, were about 1.3 mm. in diameter. Although generally quite spherical they were often rather lop-sided because of non-uniformity in the thickness of the husk. Measurements from several collections showed a range of 1.1 mm. to 1.5 mm.; the variation was due largely to differences in the thickness of the husk. The eggs without their husk were about 0.9 mm. in diameter. In a previous report, van der

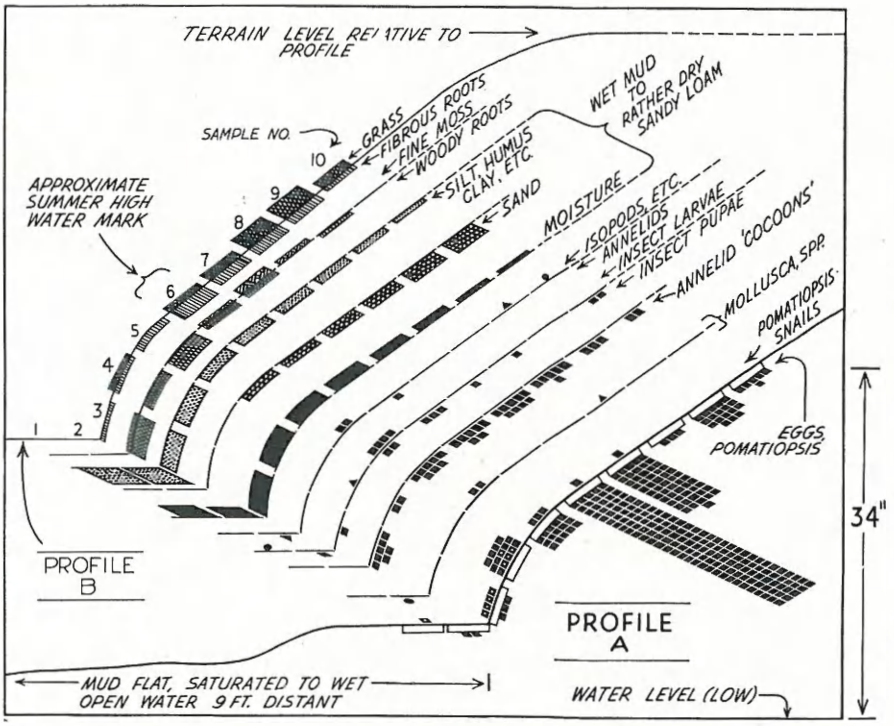

Figure 3: The zonation of eggs in relation to environmental features as revealed by a typical transect (June 27, Tecumseh station). Animal constituents in the samples are graphed in the series of duplicate profiles (A to B) at the site. Each egg or animal is represented by one symbol; the area of the symbols for the other constituents is a gross indication of their relative quantity. The dotted squares represent adult female snails.

Schalie and Dundee (*l.c.*) gave a much smaller size (0.75 mm.) for some eggs laid in the laboratory. The husks were always of the same color and texture as the surrounding soil and they were typically light brown and studded with irregular varicolored sand grains. Those occurring in fine dark mud were dark and smooth while a few found in a yellow, clay soil were quite yellow. Most of the eggs were unattached to objects and they appeared free in the residues after the samples were washed. Occasionally, one was firmly bound to grass roots or to an elongate sand particle which projected like a tail. In the densest concentrations they were sometimes found paired and so attached by their confluent husks as to form a "dumbbell." The egg tended to lose its resilience toward the end of the incubation period; the husks became friable or flaccid by the time of hatching.

Eggs were abundant in situations illustrated in figure 3 which shows the profile of the chief sampling area at the Tecumseh station and illustrates the approximate average curvature and height of that section of bank. This area was somewhat lower than most of the banks in the vicinity of that station and it had a more gradual slope. The sampling area at the Clinton station was even lower and had even a more gradual slope. It was unusual in having a broken contour in part of the area, where there was a low shelf upslope having a vertical face (Transect 2, June 27).[3] Both of these low productive areas were particularly similar in soil type, vegetation, and in having extensive mud flats along their bases. In those areas the dominant vegetation was grass, but nettle (*Urtica*) and poison ivy (*Rhus*) were common. The surface soil, from the top of the banks to the downslope vegetation boundary, was a light-colored sandy loam generally about a half-inch thick. The soil was interlaced with a dense mat of grass roots and the loam was easily removed from the roots when the samples were washed. A firm clay was consistently found below that layer; it was highly cohesive and was very difficult to disintegrate by washing. The mud flats consisted of a fine black sediment that was evidently rich in organic detritus. Disturbance of the deeper layers often produced a strong odor of decomposition. This sediment extended upslope to where it thinly overlapped the loam about at the edge of the lower vegetation boundary. Both areas were largely exposed to sunlight. The Tecumseh station was more shaded upslope by bordering trees, while the Clinton area had taller and denser grass.

The steeper banks at the Tecumseh station were up to six feet high; sometimes they approached the vertical, or were concave in profile because of weathering. Many of these areas were well-shaded by the adjacent forest. There was little or no herbaceous vegetation in such habitats, but dense growths of giant ragweed (*Ambrosia*) were present in some (Transect 2, August 6). Exposed networks of thick, woody roots covered most of the surface of some of these areas; they were heavily coated with fine sediment about up to the level of the general vegetation boundary. Loam covered most of these banks also, but it was usually comparatively dark, dry, and crumbly (Transect 2, August 6). In some places clay was exposed in abundance along the base of the banks, where the upslope loam formed hardly more than a thin film ("atypical," Transect 2, August 16). Similar conditions occurred at the most frequently sampled "atypical" location (five transects). Seepage and runoff from an adjacent bluff were apparent on that bank, which was quite moist, although open to the sun. The steep lower slope, eroded by the stream, consisted of bare clay; the elevated crown of the bank was largely covered with dense grass and an irregularly thin loamy layer. Some of the samples from within the grass consisted almost wholly of clay, as did nearly all those taken on the exposed areas; eggs were absent in them. In all the other samples that

[3]The spatial relations of the transect samples here are especially distorted as represented in Table I and figure 1: a sample from under the shelf, and one from the vertical face above, were equidistant from the stream margin, rather than as shown.

yielded eggs loam was evident. One transect revealed two egg-concentrations, characteristic of the typical distribution pattern, within the vegetation. These concentrations, while having the typical ten-inch spacing, were several inches further from the streamward vegetational margin than was found in any other instance.

Specimens of adult *P. cincinnatiensis* occupied the diverse habitats described above, although they seemed somewhat less abundant and sometimes absent in the most heavily shaded areas. Unstable conditions probably account for the variable or low numbers of eggs found in some of the areas described. In two instances poor samples were taken on lower "typical" banks where trampling on the sites seemed to be involved. These sites also seemed rather dry because of sparse grass and the full exposure to the sun (Transect 1, May 20; Transect 1, July 24). Moisture in relation to the density of vegetation or to other factors appears to be involved in some of the local variations in egg distribution as noted above. It may be implicated in the variant of the pattern found at the Clinton station (Transect 2, June 27). In this instance the upslope peak of egg-concentration was displaced distinctly landward in relation to the lower one and it occurred under the low overhanging shelf in a moist shaded pocket where it was unusually well-developed. The abundant, tall grass at that level may have been a contributing factor. In spite of these variables the over-all distribution of the eggs indicates that ovipositing was largely unaffected by a variety of enviromental conditions. Eggs were abundant in dense vegetation, in sandy loam, in well-shaded areas, and on vertical slopes, as well as in soil fully exposed to sunlight, in fine mud and on horizontal surfaces.

While the major egg concentrations showed considerable vertical displacement with extremes in bank contour, as previously described, they tended to remain horizontal within a fair range of variation in contour. This arrangement was also true for the banded way the snails were concentrated during the day. They also showed parallel displacements. The concentrations of snails generally coincided with the edge of the shade cast by the vegetation; however, both snail and egg zones tended to remain horizontal even where there was considerable variation in density of the vegetation or where the latter's boundary was quite sinuous. The snails showed a tendency to descend on to exposed mud flats, and also were unusually common well upslope on the moist "atypical" bank. It is not known how this zonation is affected by different weather conditions, or whether there is a daily cyclic change. It is possible that ovipositing occurs largely at night.

The associations of organisms found at the ovipositing sites of *P. cincinnatiensis* appeared to have an over-all amphibious character. Over broad areas of the habitat distinctly amphibious and terrestrial forms comingled. Essentially aquatic organisms occurred frequently within the ovipositing zone. The associations indicated in figure 3 are typical of those found at all sites. Among the macroscopic animal material recovered, *P. cincinnatiensis* eggs were by far the most numerous constituent in the transect samples. Lumbricid worm cocoons, however, were the greatest constituent by volume. These cocoons had a similar pattern of distribution to the snail eggs and even two diffuse zonal concentrations were recognizable at most sampling sites. Also, the downslope concentration was the larger and in several instances clearly coincided with the main peak of the snail-egg distribution. Lumbricid worms, like *Pomatiopsis* snails, were found in comparatively small numbers. Other annelids, Tubificidae and Enchytraeidae, often occurred in the mud flat samples. Several mollusks were listed by van der Schalie and Dundee (*l.c.*) from this habitat; *Deroceras* sp. and *Cionella lubrica* (Müller) should be added to the terrestrial forms previously found. *C. lubrica* was rather common upslope at both stations during the first half of the season and the eggs of both species were fairly common upslope at that time; the eggs of *Lymnaea humilis* Say occasionally appeared toward the top of lower banks. The *L. humilis* populations observed were at the stream margin but overlapped the downslope edge of the *Pomatiopsis* distribution.

Larval Diptera as a group were especially common; Chironomidae, Ceratopogonidae, Stratiomyidae, Tabanidae, and especially large Tipulidae appeared frequently. Most of them were found thoughout the vegetative areas. Larval Psychodidae were abundant on the mud flat at the Clinton station on June 27 when the odor from the mud was particularly strong, and they ranged well up into the vegetation. Terrestrial isopods and beetle larvae were spread well over the habitat. Other Coleoptera commonly found included larvae and adults of Elmidae; the adults of terrestrial running species were conspicuous in exposed areas later in the season. Most of the distinctly amphibious or semi-aquatic animals listed were much more common on the bank that was affected by seepage and especially the Dipterid groups.

Some of the associated organisms, especially the lumbricid worms and tipulid larvae, had a pronounced physical effect on the habitat. A zone of tunneling marked particularly by a broken surface and worm castings was evident in areas of sparser grass. This zone, about a foot in width, occurred along the lower vegetative boundary and overlapped the zone of maximum concentration of *Pomatiopsis* eggs. Evidently this tunneling had little effect on the egg distribution. Several transect samples were examined to determine whether this activity might have buried many eggs to some depth. When the upper and lower halves of such samples were processed separately, many eggs were found in the upper quarter-inch of soil but very few were deeper.

During the season these observations were made, there were unusual river-level fluctuations. It was an extremely dry year and during such periods the operation of hydropower dams causes great variations in the volume of flow. The dam at Tecumseh which is located between the two stations was chiefly responsible. This facility produced similar but generally smaller effects over a period of 40 years. Fluctuations occurred at both stations. For periods of time the stream flow was completely cut off; then again the stored water would be suddenly released. Information on the frequency and duration of these conditions is fragmentary. Minimal stream levels were observed as early as May 12 and as late as the final sampling date. These high and low water levels occurred several times over periods of a week or more. The low level stage apparently had a nearly constant relation to the high level with a vertical distance between them of about 20 inches. On one occasion a rise of this magnitude was observed to occur within an hour. Twice the high water level was found just overlapping the major zone of egg concentration (see Figs. 1, 3, Transect 1, July 24). At such times the snail concentrations were completely submerged.

The fluctuations in water level had little visible effect on the habitat; however, the faunal associations found may have been largely determined by these circumstances. No detrimental effects on the *Pomatiopsis* population were apparent. Only rarely were coarse drift materials present in the habitat, although Ostracoda, algal fragments, and similarly small, light objects were deposited well up in the vegetation. Sedimentation on the lower slopes appeared to be a very slow process and presumbaly did not greatly affect the distribution pattern of the eggs. It is possible that the development of this pattern may have depended directly on these stream fluctuations; this will be considered in the following section.

Ovipositing in the Laboratory

Many specimens of *P. cincinnatiensis* collected at the field stations were maintained in the laboratory. They were cultured in porous clay flower-pot saucers one inch deep, somewhat as described by van der Schalie and Dundee (*l.c.*). The bottom and the steep inside walls of these pots were coated with a smooth layer of soil about a quarter inch thick. The saucers were then set in racks at an angle (about 30 degrees) on a water table so that one edge of the bottom was in contact with the water. The soil was kept moist by capillarity and a plate glass cover was used to maintain a high humidity and to prevent the escape of snails. The soil, which was obtained from the field stations, was screened to remove all particles approaching the size of *Pomatiopsis* eggs. It remained rich in organic detritus. The sand fraction and the mud were then recombined in various proportions and used as homogeneous mixtures.

In one of two large saucers about a foot in diameter and prepared as described above, it was possible to maintain well-defined moisture gradients. Most of the soil remained a firm moist mud but a pool of rather soupy mud formed at the lowest end of the saucer. The upper walls in the most elevated half of the saucer remained rather dry. On either side of a line connecting the highest and the lowest points of the saucer, it was evident that mirror-image patterns of moisture were formed, even though the moisture was not measured.

In the many smaller pots (four to six inches in diameter) such definite moisture gradients could not be established and some of these readily over-saturated. Bits of filter paper were the only food provided and the snails ingested it with mud. Other snails were maintained in saucers without soil and they were fed filter paper with vegetal fragments from the habitat. The vivaria were unequally lighted; one-half of each dish was generally subjected to indirect daylight, while the other half remained in deep shadow. In all conditions males and females were kept together.

From early May until late summer hundreds of eggs were laid in these vivaria. In all observed respects, they were identical with those found in the field. The snails survived best in the larger vivaria, in which the great majority of eggs were laid. No eggs were laid on the bare surfaces of saucers, on paper, vegetal fragments, or on sand, all of which were kept moist. Concurrently, some eggs were laid in rather sandy moist soils, whereas the large remainder were deposited in a firm mud containing very little sand. In one case a large vivarium was provided only with a bottom layer of sandy soil and some 60 adult females and fewer males were placed in it on May 1. For the first five days, no eggs were deposited, and then the walls of the saucer were plastered with a less sandy mixture. About 20 eggs were laid in the next two days, all in the newer material, and during the next five weeks 70 more appeared. The most productive vivarium was the large one in which best-defined moisture gradients were established. A fine mud was used containing but very little sand. Fifty-five adult female *Pomatiopsis* housed with fewer males produced about 300 eggs during the spring and summer. These eggs were laid in a definite zonational pattern, which obviously paralleled the bilaterally symmetrical pattern of moisture gradients. The great majority of the eggs were crowded into a zone on the base of the almost vertical wall, where there was an intermediate moisture content. The distribution was interrupted in the mid-line of the dish both at the highest and driest point and the lowest and wettest. A very few eggs were scattered across the broad open part of the dish. A count of the eggs showed almost identical numbers in either half of the saucer. The unevenness of the lighting had had no effect on this pattern, as appeared to be the result in the other vivaria. There was some apparent preference for ovipositing on steep surfaces, or perhaps an avoidance of broad open spaces.

Although ovipositing was frequent in the laboratory and the animals were under close scrutiny, the egg-laying process was never observed in its entirety. On several occasions snails were observed at some apparent stage of the act but in only two cases was egg-laying confirmed. A reconstruction of this process from these observations is given here. These observations required use of a dissecting microscope. An individual about to deposit an egg seems to search actively for an acceptable site. It moves about hesitatingly over the steep mud surfaces and from time to time stops to test a spot with a probing action of the proboscis and a subtle digging motion of the foot. When a suitable site is found, the snail's foot is worked somewhat into the soil and the proboscis begins to dig a groove. The snail may orient itself vertically, with its head uppermost, or in some other direction. The proboscis is swung down and back to the anterior edge of the foot; it touches the mud and is then vigorously swung forward while the odontophore is forcefully thrust out. This scooping action is immediately repeated. Mud and sand accumulates rapidly on the anterior surface of the proboscis and on the anterior edge of the groove being dug. Within a few minutes a hollow hemispherical dome of soil forms, which extends up from the end of the groove and masks the snail's head up to its eyes. The head and proboscis continue to move vigorously in a rotating action under the dome. The exit of the egg from the snail's body was not observed but at about this stage it somehow appears under the anterior end of the foot where it is manipulated in a rolling action. The egg is so large that it is covered by the snail's foot with difficulty. It may slip into view at the edge of the groove from time to time, when it is seen to be quite clean and transparent. Faecal pellets appear in a row along the pedal groove and seem to be slowly worked into the dome. The egg may then be hidden from view for some minutes and shortly the snail becomes quiescent. Moving to one side, the snail leaves the dome intact and departs. The egg, with its fully-formed husk, can then be found more than half-buried in the anterior end of the groove with its top just flush with the edge of the depression and overhung by the hollow dome. It can be seen only by the closest inspection. Immediately after deposition

8. The faunal associations in the habitat consisted of terrestrial, amphibious and aquatic organisms commingled over a rather wide area. Lumbricid worm cocoons were the greatest animal constituent by volume in the soil samples and appeared in a diffuse distributional pattern similar to that of the *Pomatiopsis* eggs.

9. Hydropower dams produced great stream fluctuations throughout this study area. These variations occurred repeatedly and rapidly; there was a rather constant vertical relation between high and low water levels. The high level sometimes coincided with the major zone of egg concentration and the snails themselves were submerged.

10. Many *P. cincinnatiensis* were maintained in vivaria in the laboratory where they were provided with various moist substrata, including soil taken from the field stations. Hundreds of eggs were laid in firm, fine mud, a few were laid on very sandy mud; none were laid on sand or other surfaces. In one vivarium the striking zonal pattern of ovipositing that developed appeared to be closely correlated with a zonal moisture pattern.

11. Observations of the egg-laying process indicated that the snail in a vivarium seems to search actively for an egg-laying site; it then proceeds to construct a "nest" consisting of a shallow groove in the soil with a domed roof of soil overhanging one end of it. The egg is about half-buried under the dome so that it fails to project above the edges of the groove. The snail's proboscis, radula, head, and foot are used vigorously in this process. Nearly every egg laid in vivaria could be located with the unaided eye by the dome projecting above the smoothed soil surface. Even under magnification, neither the "nest" nor the egg were ever seen on the surface in a field habitat; evidently the dome readily collapses under field conditions, completing the camouflage of the egg.

12. Soils with a certain moisture-holding quality, firmness, and cohesiveness may be essential to *P. cincinnatiensis* for the ovipositing process. Such qualities seem to be provided by the clay and fine organic detritus in the habitat. It is likely that moisture is a primary factor. It is possible that the stream fluctuations noted were largely responsible for the double zonation of eggs and provided the conditions necessary for ovipositing at most levels along the egg-laying zone of the bank.

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