Distribution and Natural History of the Snail Pomatiopsis cincinnatiensis (Lea)

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Distribution and Natural History of the Snail Pomatiopsis cincinnatiensis (Lea)¹

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ABSTRACT: The regional and local distribution of *Pomatiopsis cincinnationsis*, a snail inhabiting river banks, was studied to determine some of the factors limiting its range. Soil texture was not a factor. The frequent flooding of streams and/or the considerable silt and clay deposition on their banks are detrimental to both the snails and their eggs, particularly the latter. Streams subjected to such conditions did not sustain *P. cincinnationsis*. When present, snails occur in a narrow zone on the banks of rivers. Experiments indicate that this zonation results from the preference shown by this species for a relatively narrow range of substrate moisture (approximately 70% of saturation); other factors studied were not effective in this regard.

The eggs of *P. cincinnatiensis* are laid usually between mid-May and late June; the young hatch from early August to late September. Most of the population hatch in early August and may reach sexual maturity before going into hibernation in late October. Some copulation occurs between these individuals, but no successful egg development can occur so late in the fall. By mid-May of the following spring, about a month after the snails emerge from hibernation, copulation reaches a peak. It is clear that *P. cincinnatiensis* is an annual species, in which almost all the adult population dies off between mid-August and mid-October. The sex ratios of the newly hatched snails ranged from 2 to 4 females per male; among the adults the ratio was from 1.5 to 1.8 females per male. A greater survival of the males was indicated. No difference was found in the diurnal and nocturnal behavior of *P. cincinnatiensis*.

INTRODUCTION

Six years of field and laboratory work with *Pomatiopsis cincinnatiensis*, a North American prosobranch snail related to *Oncomelania*, the intermediate host of *Schistosoma japonicum* in the Pacific area, have led us to believe that this species is in many ways different from another common local species, *Pomatiopsis lapidaria*, as well as from the four nominal species of *Oncomelania*. Information bearing on the comparative similarities and differences between these snails will appear in another publication (van der Schalie and Getz, in press). In this report we review the regional and local distribution as well as the natural history of *Pomatiopsis cincinnatiensis*. Although information of a similar nature was previously published concerning this species (van der Schalie and Dundee, 1955; van der Schalie and Walter, 1957), new data are now available so that it is possible to examine the conclusions of the earlier studies more critically.

Acknowledgments.—Bonifacio Dazo and George Davis were generous in assisting with certain phases of this study. Mrs. Anne Gismann examined the

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manuscript critically and assisted in its preparation. Mrs. Stanlee Lonsdale made most of the graphs and drawings, William Brudon the remainder.

Geographical Distribution known range

While *Pomatiopsis cincinnatiensis*, a snail species living on river banks, is at present relatively abundant in southeastern Michigan and northwestern Ohio (van der Schalie and Dundee, 1955), its general range is not well known. The scattered records from Indiana, Illinois, Iowa, Kentucky, Michigan, Ohio and Virginia indicate that the species was, at least at one time, rather widely distributed throughout the upper Mississippi drainage and lower Great Lakes region. To obtain a better understanding of its present distribution, as well as an indication of its former range, surveys were made of several streams in Ohio, Illinois and Michigan.

It was found most expedient to check streams wherever roads crossed them. At such sites a close examination was made of those banks which appeared to have the proper habitat conditions for *P. cincinnatiensis*, i.e., a fine-textured and sufficiently moist substrate. The banks were examined for about a hundred yards both above and below the bridge. Soil samples were taken at most stations and observations of the general conditions of the bank and stream were recorded.

P. cincinnationsis was not found in Illinois. It was discovered, however, in a few new localities in Michigan and in fourteen new ones in Ohio (Fig. 1). Unfortunately, streams in the latter state have been

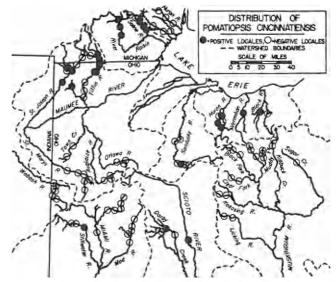


Fig. 1.—Recent distribution of *Pomatiopsis cincinnatiensis* in Ohio and southeastern Michigan (mostly new localities). Negative localities are those sites at which this species was not found.

greatly disturbed by man. Many were heavily polluted with sewage and industrial wastes and others had been dredged to clear and straighten the banks (primarily in the headwaters); the lower reaches were all rather heavily silted. Extensive farming in that state causes a rapid runoff of rain water, producing flood conditions in the streams during the year. Such high waters not only render the banks unsuitable as habitats for P. cincinnations during and after the floods, but also deposit an unfavorable layer of silt and clay on the portion of the banks occupied by these snails. The combined effects of pollution, soil particles in the water, and the scouring of the water itself, all undoubtedly act adversely upon these snail populations. Such conditions are particularly harmful to the eggs since not only would many be washed away, but the pollution would tend to reduce the hatching potential among those remaining on the bank. The layer of silt and clay deposited on the bank dries out to form a hard layer over the surface. Eggs so covered are not likely to hatch. If they did, the young would be unable to survive.

The survey indicated that *P. cincinnatiensis* was able to maintain itself only in the headwaters of the streams examined (or their small tributaries) where there was little pollution and where the banks of the river were not subjected to frequent flooding. Only a few and very sparse colonies were found in some of the streams that were greatly disturbed. These collections indicate that this species may have been more abundant at one time but that it is now becoming exterminated in the streams suffering from human disturbances.

Based on a consideration of the known colonies of P. cincinnationsis, the species was probably formerly present in all of the major drainages in Ohio (Fig. 1). It was not found in the Muskingum River drainage during the present study, but this stream has been greatly disturbed. The upper regions of some of the tributaries appear to have suitable bank characters and soil types, particularly the Killbuck and Mochian (Muddy Fork), but P. cincinnationsis was not found in them. The streams in this region have suitable banks for only five miles, approximately, of their course. The rapid runoff of water from the surrounding agricultural areas brings about a sudden increase in stream size as one progresses down river from the headwaters. Consequently, they soon become too large to have the type of banks upon which P. cincinnationsis is normally found; this development leaves only a short portion of the streams where this species might occur. In Ohio the best colonies were found in the Vermilion and Black rivers, and the northern tributaries of the Maumee, which are much less disturbed than are those to the south.

In the Saline River in Michigan, P. cincinnationsis was found much further upstream (a colony was found above Milan) than was previously reported (van der Schalie and Dundee, 1955). Because there was an old, and presumably erroneous, record from the Grand River, a fairly thorough check was undertaken of the Huron, Grand, and Kalamazoo rivers, as well as several smaller streams emptying into

14 (2-51)

Negative localities

tive and negative fo	r Pomati	opsis cincinnat	iensis in Ohio ar	nd M ichigan	
	No. of		Soil texture		_
	sites	Sand	Silt	Clay	
Positive localities	24	62 (39-79)	26 (13-42)	12 (7-24)	

57 (12-90)

54

29 (6-56)

Table I.—Soil texture (average and extreme percentages) at sites posi-

western Lake Erie. P. cincinnationsis was not found in any of those streams.

The analysis of the many soil samples examined indicated that types of soil probably are not a limiting factor in the northern extension of the range of P. cincinnationsis (Table I). The soil texture was rather sandy in some streams, but in others it was almost identical with that on river banks in which the species is abundant. The Grand River (except for the Sandstone Creek tributary), in particular, had banks that appeared to be favorable. The main river has been heavily polluted from Jackson downstream; above Jackson it may be too small. Although it is possible that this species did occur in the Grand River drainage prior to the human disturbances of the river, it is doubtful that any colonies now exist on its banks.

In a survey of southern Illinois, we checked the following localities, which were, however, all negative for P. cincinnationsis: Mud Creek, 17 mi S Crescent City, Iroquois Co.; Vermilion R., 5 mi W Paxton, Ford Co.; Sangamon R., 17 mi W Champaign, Piatt Co.; Salt Creek, 5 mi E Clinton, De Witt Co.; Sugar Creek, 8 mi W Lincoln, Logan Co.; Quiver Creek, 6 mi N Havana, Mason Co.; "small stream," 1 mi N Jacksonville, Morgan Co.; Apple Creek, 12 mi N Greenfield, Greene Co.; Macoupin Creek, 5 mi S Chesterfield, Macoupin Co.; Little Shoal Creek, 1 mi N Greenville, Bond Co.; Crooked Greek, 2 mi E Salem, Marion Co.; Grooked Greek, 3 mi S Salem, Marion Co.; Casey Fork, 1 mi E Mt. Vernon, Jefferson Co.; East Muddy River, 4 mi W Mt. Vernon, Jefferson Co.; "small stream," 4 mi S Coulterville, Randolph Co.; "small stream," 2 mi E Eden, Randolph Co.; Beaucoup River, 2 mi E Pinckneyville, Perry Co.; "small stream," 5 mi NW Lesser, Perry Co.; Dutchmans Greek, 3 mi W Vienna, Johnson Co.; "small stream," 2 mi N Dixon Springs, Pope Co.; South Fork, Saline River, 6 mi S Harrisburg, Saline Co.; Fox River, 7 mi S Mt. Erie, Wayne Co.; Elm River, 5 mi W Mt. Erie, Wayne Co.; Big Muddy Creek, 2 mi NE Sailor Springs, Clay Co.; East Fork, 2 mi E Coffeen, Montgomery Co.; Kaskaskia River, 1 mi E Shelbyville, Shelby Co.; Little Wahash, 3 mi W Mattoon, Coles Co.; Embarrass River, 3 mi NW Oakland, Coles Co.

All of these rivers were also greatly disturbed; silting was conspicuous. The survey was made between 18 and 29 August, 1959. A heavy rain in the region about ten days before the trip produced flooding in most of the streams. All rivers were back to their normal level when the survey began. Their banks, however, were completely plastered with a 1 to 2 cm layer of silt and clay. The period after the rains was hot and sunny and, as a result, the mud was baked hard. Since these floods came during the period in which the young would normally be hatching (van der Schalie and Walter, 1957), it was

apparent that it would be difficult for *P. cincinnatiensis* to maintain itself under such conditions.

The widely scattered records available on this species, and the observations of habitat changes on the banks of rivers in relatively recent times lead to the conclusion that before the arrival of the white man, P. cincinnationsis was distributed throughout Ohio, Indiana, Illinois, eastern Iowa, and southern Michigan. Whether the Kentucky and Virginia records available in the Museum of Zoology (University of Michigan) represent relict populations, or whether the species also occurred throughout those regions until fairly recent times is not known. The information as to its former range in Illinois is somewhat tenuous since only one recent colony has been located there. The single museum record from adjacent extreme southwestern Indiana and the observations that the streams in this area appear at one time to have had suitable banks for P. cincinnationsis indicate that the species may have occurred in central and southern Illinois before the clearing of the land. It is evident that one may never acquire an understanding of its distribution prior to settlement.

INFLUENCE OF SOIL TEXTURE

Field studies.—As indicated below, when given a choice, P. cincinnationsis will select a finer textured substrate, i.e., one with less sand. In the regional survey, soil samples were taken at most of the sites visited to determine the influence of soil texture (percentages of sand, silt and clay) on the distribution of P. cincinnationsis. In the case of some streams the samples were taken at regular intervals along their course so as to correlate the composition of the soil with the presence or absence of snail colonies at those particular sites. In three such drainages, the Raisin, Saline and St. Joseph rivers, P. cincinnationsis was absent in the headwaters while colonies were abundant below. Series of soil samples were taken in those rivers, therefore, in both the negative upper and positive lower regions.

The soil samples were obtained by pressing a 16-ounce ointment can into the bank in a zone that appeared to be typical at that site. A modified Bouycous method was used to determine the texture of the soil samples. They were air-dried in the laboratory for at least three weeks and then rolled and sieved through a 2 mm mesh sieve. A 51 gram subsample (equivalent to 50 grams of oven-dried soil) was placed in a 200 ml flask with approximately 150 ml of water and 10 ml of "calgon" dispersing agent. The flasks were shaken and then allowed to slake for 15 minutes; they were then placed on a shaker table for 20 minutes. The contents of a flask were placed in a sedimentation cylinder, water was added to the appropriate level and the cylinder was then shaken vigorously for one minute. An hydrometer reading was taken 40 seconds after cessation of shaking and again two hours later. Temperatures were also recorded at each reading and the hydrometer values corrected accordingly (0.2 units added or subtracted for each 1F above or below 67F, respectively). The corrected

hydrometer readings give the actual grams of soil particles left in suspension. At the 40 second reading the sand particles have settled out; both the silt and clay fractions are still in suspension. After two hours, only the clay fraction remains in suspension. It is possible, therefore, to calculate the percentages of sand, silt and clay in the original sample.

In the Raisin River the soil becomes more sandy above Manchester (78 to 88% sand as compared to 40 to 69% below that town). P. cincinnatiensis was not found above Manchester (the positive locality shown by van der Schalie and Dundee, 1955, resulted from an error in the preparation of the map). This species was found, however, on banks of other streams in which the soil had a sand content as high as 79 per cent. Since sand of at least that percentage is not a limiting factor elsewhere, it appears doubtful that soil texture limits the distribution of this snail in the Raisin River. There was also no correlation between soil texture and snail distribution in either the Saline or St. Joseph rivers. The banks of the Saline, and to a certain extent those of the St. Joseph (Maumee drainage), have been greatly disturbed. The resulting changes in the banks probably are the primary reason for the absence of this snail in the headwaters of those two streams.

Over-all comparisons of the soil texture of positive and negative localities indicate that the present geographic distribution is not controlled by soil texture (Table I). Many streams in which no colonies of this snail were found had soils less sandy than other areas in which

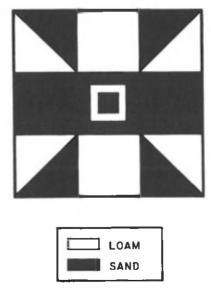


Fig. 2.—Arrangement of soil types in the clay dish used in determining substrate texture preferences. In another series of experiments, clay was substituted for the sand.

TABLE II.—Substrate texture preference shown by *Pomatiopsis cincinnatiensis* (location of snails 24 hours after being placed on experimental design shown in Fig. 2)

		Number of snails	
Trial number	Total	On loam	On sand
1	39	30	9
2	47	30	17
3	48	31	17
4	52	36	16
5	44	32	12
	-		_
Total	230	159	71

they were abundant. It appears, therefore, that factors other than soil type are influencing its distribution.

Experimental studies.—Soil texture preferences: In order to test the possibility that P. cincinnatiensis may select or show a preference for certain kinds of soil, loam (from the banks inhabited by P. cincinnatiensis) and sand (from banks in which they did not occur) were arranged in a clay dish on a water table in a pattern as shown in Figure 2. The animals were placed in the center of the dish and the numbers present on each soil type were recorded 24 hours later. Five such tests indicated that there was a definite preference for loam as compared with sand (Table II). A similar experiment was conducted using loam and clay (from Illinois river banks where the snails were not found). Three trials showed that clay was definitely preferred to loam (Table III), in spite of the fact that these snails were not found on such a substrate in Illinois. Evidently, P. cincinnatiensis shows a preference for a finer textured substrate.

Movement: Since P. cincinnations has a peculiar loping type of locomotion, it seemed possible that locomotion might perhaps be somewhat impeded on a sandy type of substrate. To determine this possibility, ten petri dishes were filled with moist loam and ten with moist sand. By pressing the top of a jar against the substrate, a circle approximately 2.5 inches in diameter was formed in each dish. One snail was placed in the center of each circle and the time required for it to reach the edge was recorded. Ten observations were made on each substrate with each snail.

Table III.—Substrate texture preference shown by *Pomatiopsis cincinnationsis* (location of snails as in Table II)

		Number of snai	ls
Trial number	Total	On clay	On loam
1	25	20	5
2	25	24	1
3	25	25	0
		_	-
Total	75	69	6

The snails appeared to move faster, although not significantly so, on sand than on loam. Since it was shown that they tended to avoid sand, it is possible that this greater movement was in response to some unfavorable stimulus. At any rate, these observations suggest that movement is not impeded by a sandy substrate. The manner of locomotion of several individuals on both sand and loam was examined under a dissecting microscope. The sand did not appear to impede their movement.

Local Distribution influence of soil moisture

Preferences.-Field studies: The zonation observed throughout several seasons of the year on the banks appeared to be in response to a moisture gradient. The significance of this factor seemed important, thus some studies were designed to obtain a better measure of the moisture differences in the habitats. On October 12, 1960, a transect was established on the bank of the River Raisin four miles below Tecumseh, Michigan ("Tecumseh Station"). This site appeared to have a typical soil moisture profile and it contained a large number of snails. The transect, 50 cm wide and 120 cm high, was marked off on the bank to form 12 horizontal zones, 10 cm wide. The number of snails in each zone was then recorded. To measure the degree of moisture in the upper layer of the ground, two soil samples were taken from each of these horizontal zones, using round ointment cans (6.7 cm in diameter and 1.6 cm deep); each sample was taken approximately 5 to 10 cm from the edge of the transect. A sample was collected by placing the can on the soil (open end down), cutting around it with a spatula (to a depth of about 2 to 3 cm) and gently pressing the can into the soil. When the bottom of the can was flush with the ground surface, the spatula was slipped under it and a plug of soil removed. The excess soil was sliced off level with the edge of the can so that a sample was obtained with little disturbance to the original soil structure. The can was then closed and the position of the sample recorded.

The amount of water in each sample was determined by comparing wet and dry weights; the latter was obtained by oven-drying the sample at 100 C for two days. To obtain the percentages of saturation of the original field samples the amount of water required to saturate each sample was determined. Distilled water was added to the soil in each can until a film remained on the surface. The samples were left for an hour; more water was added then, if necessary, and the samples inverted on a 2 mm mesh screen until no more water drained out. Each sample was shaken slightly before being turned upright to insure that no water was trapped around the edges. The amount of water contained in each sample was again determined by the gravimetric method mentioned above. From the dry weight it appeared that little soil had been lost (less than 0.1 g per sample) in the handling, nor did the structure of the soil appear to be altered.

A comparison of the moisture gradient with the zonation of the

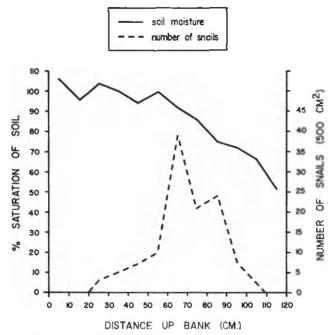


Fig. 3.—Comparison of substrate moisture and distribution of *Pomatiopsis cincinnatiensis* on a bank of the River Raisin, 12 October 1960.

snails on the bank indicated that the snails were distributed on soils with a moisture content 75 to 92 per cent of saturation (Fig. 3). This value is somewhat higher than that observed in the laboratory experiments (see below). The differences may be due in part to the failure of the field method to measure the moisture conditions at the very surface of the soil, i.e., the layer most important in regard to the zonation of these snails. As moisture evaporates from the surface, this portion becomes drier than the soil below the surface. Even though the plugs of earth used in this study were about as thin as feasible for accuracy in determining moisture values, they were still too deep to give the precise percentages of saturation at the surface.

Experimental studies: Comparison of substrate moisture preferences between young and adult *P. cincinnatiensis* was recently published (van der Schalie and Getz, 1961). The substrate used in those experiments consisted of pads of paper toweling. While that medium provided suitable comparisons among the different size groups of this species, it seemed desirable, also, to obtain substrate moisture preferences using soil as the substratum. This more recent series of moisture gradients was established in a metal box (30 x 25% x 21/4 in) separated into ten (21/2 x 25% x 2 in) compartments. All the compartments were filled with soil of a uniform texture produced by screening

it through a 0.4 mm mesh sieve. The amount of moisture needed to saturate completely the soil of a compartment of the box was determined by filling a pressed paper box of the same size as a compartment with a similarly textured soil and saturating that soil with distilled water. Various decreasing series of substrate moisture could then be established and maintained in these compartments. The snails were given freedom in selecting any of the compartments.

In the first series of experiments a saturation gradient of 100 to 10 per cent was established. The saturations down through 40 per cent were made by pouring distilled water directly into the compartments, since it had been found experimentally that the water required for these percentages of saturation soaked completely through the soil. The saturations of 30 per cent and below required a mixing of the soil and the water in a bowl (with an allowance for evaporation); these soils were then placed immediately in the proper compartments of the box.

The tests were made by placing 20 snails on the soil of each compartment. The top of the box was covered with a plastic sheet ("Saran Wrap") to maintain a proper humidity; a glass plate was placed over the latter. The entire box was covered with an opaque cloth to eliminate any possible influence of light. The box was left undisturbed for a day before the snails in each section were counted. After several trials it was clear that the snails avoided the sections with a moisture saturation of 90 to 100 per cent. On the other hand, the snails in the lower part of the range (the 10 and 20 per cent saturation compartments) were unable to move. Those snails that did move into the 20 per cent saturated compartments also became inactive. Even the snails in the 30 and 40 per cent compartments experienced difficulty in moving, especially in the former. This situation was partly due to the looseness of the soil (a result of the mixing of these soils with water). The snails on the loose soils exuded a large quantity of slime and many individuals eventually became bogged down. These experiments indicate that the snails showed a preference for a substrate with a moisture ranging between 50 and 80 per cent of saturation.

With these experiments as a guide, another series of tests were made using a range of saturation between 50 and 80 per cent. In order to eliminate the difficulties previously encountered due to loose soil, the whole box was twice filled with distilled water and dried in an oven for four days. This treatment produced a reasonably solid soil surface with the particles adhering more firmly. With the reconditioned soil surfaces just described, the compartments were then moistened in the following descending and ascending sequence of percentages: 100+, 80, 70, 60, 50, 50, 60, 70, 80, and 100+. The two compartments at each end were kept supersaturated so as to have a film of water over the soil surface at all times. Evaporation from these sections maintained a relatively high humidity throughout the box and helped to cut down the evaporation from the other compartments. After 20 snails had been placed in each of the compartments the box was again

covered as previously indicated. The distribution of the snails was recorded after two days. Two trials of this kind (Fig. 4) showed that the snails largely avoided soils 80 per cent saturated and definitely preferred a substrate saturation of approximately 70 per cent. More snails were recorded on the 50 and 60 per cent saturated compartments than were found on the 80 per cent saturated soil.

While the field data indicated a substrate moisture preference higher than that of the experimental studies (75 to 92 per cent compared to 70 per cent), these data are in relatively close agreement. The surface of the banks is probably slightly drier than the data of the field studies indicate; this difference may account for the discrepancy noted. However, it is clear that *P. cincinnatiensis* is found only in a fairly narrow range of soil moisture. This factor may be one of primary importance by way of restricting this species to a narrow zone on the banks of rivers.

Slope of bank and width of zonation: There is a correlation between the slope of a bank and its soil moisture gradient; the less the degree of slope, the more of the capillary fringe zone will be exposed. In this zone only a certain percentage of the soil interspaces are filled with water and therefore the soil moisture content is somewhat below saturation. If the snails selected some portion of the capillary fringe, one would expect a much wider zonation on banks with a lesser amount of slope than on those approaching the vertical. This would

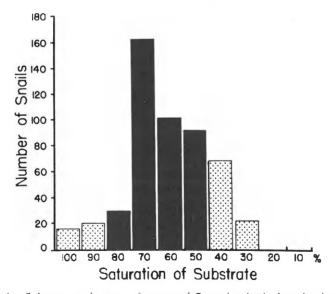


Fig. 4.—Substrate moisture preferences of *Pomatiopsis cincinnatiensis*. Solid bars represent data from an experiment utilizing a moisture range of 50 to 80 per cent of saturation; the stippled bars are indications of tendencies observed on a moisture gradient of 10 to 100 per cent of saturation.

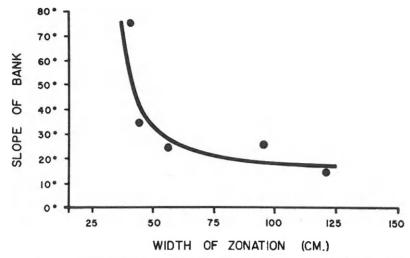


Fig. 5.—Average width of zonation of *Pomatiopsis cincinnationsis* (from five different counts) in comparison to slope of the river bank at five localities on the River Raisin.

be evident particularly on banks in which the upper areas are high enough to be well above the capillary fringe and thus too dry to support *P. cincinnatiensis*.

As part of another study (van der Schalie and Getz, 1961) five transects were established on the banks of the River Raisin. The slope of these five banks ranged from 15° to 75° from the horizontal; all were high enough to be dry at the top of the transect.

Five counts were made of the distribution of snails in each transect between 16 August and 20 October 1959. It has, therefore, been possible to compare the width of zonation of the snails with the slope of the bank at these sites. The data for all the counts at each transect were totaled to give an average figure for the width of the zonation and to avoid any variations caused by rains, or other disturbances, to a particular transect.

There was a rather close correlation between the width of zonation of the snails and slope of the bank (Fig. 5); thus, the zonation was particularly narrow on the steeper banks. General observations on the width of zonation at various other places on the River Raisin also indicated that the steeper banks had the narrower zones of distribution. This evidence, therefore, suggests that soil moisture is an important factor in the local distribution of *P. cincinnatiensis*.

Distribution on the bank of a small tributary stream.—A small stream, leading from a spring, empties into the River Raisin at the Tecumseh station. At its confluence the backwater of the river forms a pool approximately 5 to 6 m wide, 10 to 20 cm deep, and 20 to 25 m long. Above this pool the stream rapidly narrows and, approximate-

ly 40 m from the outlet, it is but a small trickle. The moisture on the banks of this stream corresponds to the size of the stream, i.e., the banks are quite moist until the stream size decreases, and at the upper end they become relatively dry.

In order to obtain further information regarding the influence of moisture upon the distribution of P. cincinnatiensis, comparisons were made of the numbers of snails on the banks at given points up the tributary. Nine stations, five meters apart, were established along its banks, starting at its outlet to the river. The number of snails in two (one-half square meter) quadrats was determined for each station. The data obtained indicated that there was a steady decrease in the abundance of snails from the backwater at the outlet to the point where the stream was no more than a trickle (Fig. 6). Additional evidence was furnished, therefore, to indicate that moisture plays an important role in the local distribution of P. cincinnatiensis.

INFLUENCE OF TEMPERATURE

Studies of the temperature preferences of *P. cincinnatiensis* (van der Schalie and Getz, in press) showed that this species tended to concentrate at temperatures around 21C. Soil surface temperatures were measured on four widely separated strips demarcated up the bank of the River Raisin. The zones selected by the snails were then recorded to determine if their distribution could be explained as a response to

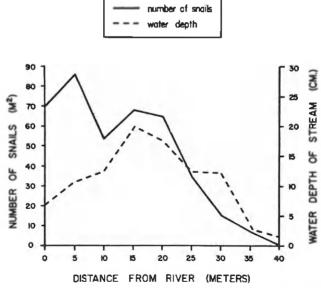


Fig. 6.—Distribution of *Pomatiopsis cincinnationsis* on the banks of a small tributary of the River Raisin, quickly narrowing at a point 20 m above its mouth.

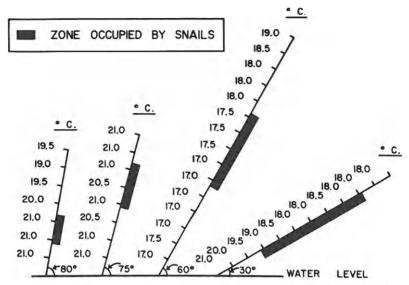


Fig. 7.—Surface temperatures at 10 cm intervals up the banks of the River Raisin at selected localities with different bank gradients, 22 August 1961. The 75° bank was exposed to sunlight and the others were shaded.

a temperature gradient. Surface temperatures were obtained by placing a glass thermometer flat on the bank and pressing it into the soil until the bulb was about half buried. The measurements were made between 1330 and 1500 on 22 August 1961, when the air temperature was about at maximum. All sites were shaded except the 75° transect (Fig. 7), which was exposed to sunlight.

A slight temperature gradient of two to three degrees was observed along the three shaded transects; two had the higher temperatures at the bottom of the bank while the opposite gradient was observed in the third (Fig. 7). There was no temperature gradient on the sunlit bank at the fourth locality. No correlation could be made between the distribution of the snails and the surface temperatures of the bank (Fig. 7). The small temperature difference observed, therefore, does not appear to be an important factor in the zonation of *P. cincinnatiensis* on river banks.

POPULATION STUDIES

From previous work it was established that a few individuals may survive for 14 to 18 months; for all practical purposes, however, *P. cincinnatiensis* is an annual species (van der Schalie and Dundee, 1955). The data available also indicated that females were both larger and more abundant than the males. However, these conclusions were based on relatively few data. Consequently, a series of 13 periodic collections were made between 10 August 1959 and 11 August 1960

at both the Tecumseh Station and the Clinton Station (River Raisin, 1 mile North of Clinton, Michigan) to obtain more complete data on growth and sex ratios. The samples were obtained with the assistance of a square frame to insure complete sampling of all size classes. The frame was placed on the bank and all the snails within it were removed. During the time that newly hatched snails were present, a reading glass was used to obtain as complete a collection as possible. If the collection did not contain at least 100 snails from one sample, the frame was shifted slightly to one side and all the snails in that area were then taken. This process was repeated until a minimum sample of 100 snails was obtained.

The snails collected were relaxed with nembutal (van der Schalie, 1953) and fixed in formalin. The shells in each series were then measured with an ocular micrometer in a dissecting microscope. After measurement, each snail was placed in a separate, numbered vial and the shell was removed with Bouin's solution. The sex of the animals was then determined by the presence or absence of the verge. It was found that the verge was present in recently hatched males (1.5 mm long). In this way it was possible to ascertain the size and sex of each snail in each series.

Growth rates.—Late in the growing season, on 17 August 1960, when it seemed safe to assume that the snails had reached their maximum growth, a series of adults was obtained from each station. They were measured to obtain an average maximum size (shell length) for each sex. The values obtained were 3.8 mm and 4.8 mm for males and females, respectively. The measurements of the snails in all the previous regular collections were then converted to a percentage of the average maximum growth to make the growth rates of the two sexes more easily comparable.

The growth rates were approximately the same at each station (Figs. 8 and 9). There did appear, however, to be a slight trend toward more rapid growth of the males at both stations. A heavy rain on the day after the collection on 20 October 1959 brought the river to the top of its banks. When the water subsided, approximately ten days later, the snails were all found to be in hibernation under dead vegetation, logs and leaf mold. Therefore, the 20 October samples represent the maximum growth of the young before they entered hibernation. At that time many of the animals had reached their full growth (Figs. 8 and 9) or very nearly so.

The spring of 1960 was rather late; the first snails did not appear on the banks before 12 April. The spring rains and high water made it impossible to obtain the first series of snails earlier than 22 April. The small difference in the size distributions between the 20 October 1959 and 22 April 1960 series indicated that little growth had occurred between the time the snails emerged from hibernation and the time of the first collection. Growth was relatively rapid after the first week of May; most specimens were nearly full grown by the first week of June.

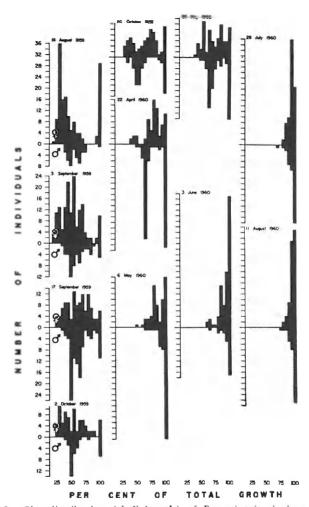


Fig. 8.—Size distribution (shell length) of *Pomatiopsis cincinnatiensis* collected at the Tecumseh Station; the measurements have been converted to a percentage of the average maximum growth attainable for each sex (4.8 and 3.8 mm, females and males, respectively). Females are shown above the line, males below.

The young appeared at approximately the same time (3 August) at both stations in the summer of 1959. However, in 1960 the young did not appear at the Tecumseh station until about one week after they were first observed at the Clinton station. The reason for the delay was not determined.

 \acute{S}_{ex} ratios and survival.—van der Schalie and Dundee (1955) found that the females outnumber the males (in the ratio of 4 to 1).

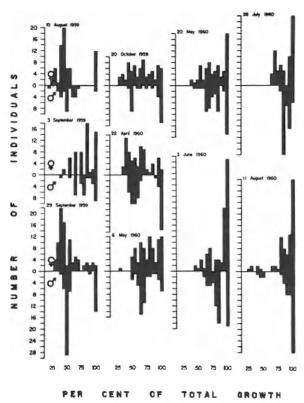


Fig. 9.—Size distribution (shell length) of *Pomatiopsis cincinnationsis* collected at the Clinton Station; the measurements have been converted to a percentage of the average maximum growth attainable for each sex (4.8 and 3.8 mm, females and males, respectively). Females are shown above the line, males below.

The present data indicate, however, somewhat less unbalanced conditions in general, and also that the ratio difference is more pronounced among the younger animals than among the more mature groups (Table IV). The sex ratios for the young of the year collected 10 and 16 August 1959 vary between two and four females per male. A combined total of all animals, half grown or less, yielded a sex ratio of 2.3 females to 1 male. On the other hand, a combined total of all animals reaching 90 per cent or more of growth yielded a sex ratio of only 1.4 females per male. Consequently, it appears that while there are considerably more young females than males, the males may have a somewhat higher survival so that the sex ratio becomes more balanced by the time the animals reach maturity. These results differ from those of van der Schalie and Dundee (op. cit.), who indicated a lesser sur-

Table IV.—Sex ratios of *Pomatiopsis cincinnationsis* as determined from a population of snails hatched in the summer of 1959

Date of	Clinton	Station	Tecumseh	Station
sample	Females	Males	Females	Males
10 Aug. 59	55	28	108	27
16 Aug 59			112	35
3 Sep. 59	33	18	144	49
17 Sep. 59			120	99
29 Sep. 59	90	58	h = v - u -	
2 Oct. 59			79	36
20 Oct. 59	74	31	83	48
22 Apr. 60	73	59	75	88
6 May 60	85	50	65	58
20 May 60	70	56	86	67
3 Jun. 60	89	64	100	29
28 Jul. 60	68	54	79	48
11 Aug 60	81	54	83	43
Totals	718	473	1134	627
Sex ratios	1.5	1	1.8	1

vival among the males. However, they did not separate the young of the year from the adults of the previous year, so comparison of survival values could not be made.

The average sex ratio for all specimens (2,952) of all ages, from both stations, was 1.7 females to 1 male. This ratio is considerably more balanced than the values of 4 to 1 given by van der Schalie and Dundee (op. cit.). The recent ratios are based upon more than twice as many specimens and are probably more reliable.

ACTIVITY

Movement.—The activity of P. cincinnationsis was studied in the field to determine the factors that influence movement in its normal habitat. To obtain such data, three quadrats (8 x 13 in) were established on the banks of the River Raisin at the Clinton Station. Short pegs with nails driven into the top of each were placed at the corners of the quadrat. With these markers as anchors, a wire grid made of hardware cloth (¼-in mesh), marked off into 1-in squares, was set on the pegs across and above the bank so as to allow the observer to sight the position of the snails under the grid. The position of each snail in the quadrat was then located and plotted on sheets of ¼-in graph paper.

Two quadrats (Numbers 1 and 3) were established on the west bank of the river. They were selected so that Number 1 was on an exposed and open bank, while Number 3 was in a zone shaded by overhanging brush. Quadrat Number 2 was placed in an exposed position on the east bank of the river. A Serdex laboratory hygrometer was placed near each grid; leads from a thermister thermometer were inserted 1/8 in below the surface, at 2-in intervals up the bank at quadrat Number 1. In order to insure accuracy only five snails

were left under each quadrat; the others, as well as all those snails that were in the vicinity of each quadrat, were removed.

Two of the snails in each square were marked with yellow paint to facilitate differentiating individuals when tracing their movements. Any snail observed approaching the quadrat from the outside was removed. Study snails found crawling out of the quadrat were placed back in the center of the grid. The locations of the snails within the quadrat were plotted at hourly intervals and soil temperature and air humidity readings were taken. Soil moisture was not determined, but apparently was within the "optimum" range. The presence or absence of sunlight was also recorded. With the use of the methods described, a series of observations were made on 2 to 3 August 1960. Observations were begun 1200 on 2 August, and readings were made every hour until 0100 on 3 August. At that time a heavy rain washed most of the snails from the banks and it was necessary to discontinue the observations. Although this study was interrupted, sufficient data were obtained to indicate that there was no essential difference as between night and day (Fig. 10) in the amount of activity expressed in movement.

Since the sky was overcast throughout the entire day of 2 August, it seemed advisable to repeat the observations on a clear, sunny day. When this was done on 11 August, it was again evident that there were no differences in the amount of diurnal and nocturnal activity (Fig. 11).

These studies of the movement of *P. cincinnatiensis* during August at the height of normal activity revealed that this snail is about equally active at all times of the day and night. Also, within the ranges of temperature (17 to 24C) and humidity (50 to 100%) recorded during

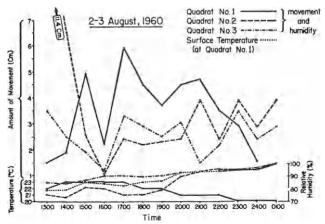


Fig. 10.—Average movement of *Pomatiopsis cincinnationsis* on the bank of the River Raisin at the Clinton Station in relation to time of day, temperature, and air humidity (overcast day).

those periods, there was no indication that either or both factors influenced the movements of this snail. Evidently the variations in the amount of sunlight on the banks also had no noticeable effect.

Dispersal.—The movement studies showed that, for its size, this species is quite active. Consequently, it seemed desirable to learn something about the tendencies of *P. cincinnatiensis* to disperse along the river banks.

A series of adult snails (150 individuals) were brought into the laboratory on the afternoon of 23 June 1961. Their shells were cleaned and dried with paper toweling, following which the upper 3 to 4 whorls were dipped into yellow paint. After the paint had dried overnight the snails (all alive) were released again at their original site on the river (Clinton station). The animals were placed on the bank in a circle about 10 cm in diameter. At intervals during the first month

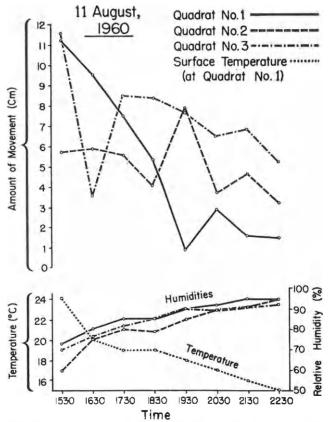


Fig. 11.—Average movement of *Pomatiopsis cincinnationsis* on the bank of the River Raisin at the Clinton Station in relation to time of day, temperature, and air humidity (sunny day).

and at the end of the second month the bank was carefully examined to determine their distribution. Until late July, the normal time for natural mortality to begin, it was possible to locate approximately half of the individuals released each time the bank was checked.

After 30 days a few snails had moved as far as 12 meters from the release point; the distribution after 60 days was essentially the same as that of the 30th day (Table V). While the experiment was in progress,

TABLE V.—Dispersal of marked Pomatiopsis cincinnationsis both upstream and downstream from release point on river bank from 3 to 60 days later

Distance in	Number of days after release										
meters from		3		6		17		30		60	
release point	U_{P}	Down	U_{p}	Down	Up	Down	Up	Down	Up	Down	
05	30	28	20	13	6	5	3	11	5	8	
.5- 1.0	6	9	7	15	8	10	8	4	3	4	
1.0- 1.5	5	1	5	6	5	5	6	1	5		
1.5- 2.0		1	6	3	5	6	4	2	4		
2.0- 2.5			3	1	3	4	6	3	4	1	
2.5- 3.0		1		2	4	1	5		3		
3.0- 3.5		p. =				3	2	1	2		
3.5- 4.0						1		1			
4.0- 4.5						2				1	
4.5- 5.0								1			
5.0- 6.0								1			
6.0- 7.0								2			
7.0-8.0								1		2	
8.0- 9.0		w r								2	
9.0-10.0										1	
10.0-11.0										2	
11.0-12.0								2			

vegetation grew down the bank to within 3.5 m upstream of the release point. It was difficult to find the snails in this vegetation; the smaller amount of upriver movement recorded may have resulted, therefore, from not finding the snails rather than from the actual absence of marked specimens 3.5 m above the release point. A heavy rain occurred between the 17th and 30th day observations; some of the snails may have been washed into the water and carried downstream. This could account, at least in part, for some of the downstream movement.

Dispersal downstream could be accomplished very rapidly by individuals washed into the river and carried far down to new localities. Upstream dispersal would depend primarily on the locomotive ability of the snails themselves or through the action of man or other animals. The young snails would have approximately two months in the fall in which to move before going into hibernation; during the spring and summer they could disperse over a period of another four months before completing their egg-laying. Based on the above figures, one could anticipate that these snails might disperse upstream on their own at a rate of approximately 15 to 20 meters per generation.

REPRODUCTION

Seasonal pattern.—Reproduction of P. cincinnationsis was studied by van der Schalie and Dundee (1955) and van der Schalie and Walter (1957). They found that egg-laying occurred mainly from late May to early July; most of the eggs hatched in August and in early September. Most copulating pairs were observed in the early spring (April to May). Growth is quite rapid so that many individuals are almost adult size before they go into hibernation. In view of these developments it seemed of interest to determine whether the gonads had matured and whether any copulation occurred before hibernation began.

Observations were made of the snails on the banks in the fall to discover copulating pairs; a few scattered pairs (all obviously young of the year) were found copulating in mid-October, 1959. A series of snails (young of the year) were collected on 20 October 1959, just a few days before they would have gone into hibernation. These animals were sectioned to determine the degree of gonad development as well as to observe whether sperm was present in the females. The gonads of these individuals were also compared with those of adult snails collected 2 August 1960. Although more detailed information on gonad development will be published elsewhere, it should be indicated here that, by late October, the gonads of the larger individuals (those that hatched early in August) were already well developed. The fact that sperm were stored in the seminal receptacles and bursa of a few females was a further indication that copulation had occurred. However, there was no indication that eggs were moving through the oviduct in any of the females. Copulation, therefore, appears to be influenced by age and physical development rather than by seasonal factors. Too few data were obtained to ascertain whether or not eggs are laid by the young of the year in the fall. Since in the latitudes where the species occurs, eggs deposited in the fall would not have time to develop before winter, any eggs laid at that season would probably not hatch. After the snails emerge from hibernation in the spring, growth resumes and the animals soon reach full growth (Figs. 8 and 9). As they reach maturity, breeding commences; copulation occurs with greatest frequency during the period from late May through June.

Daily pattern.—To obtain more information on the time of egglaying, a series of snails was collected at hourly intervals from 1030, 2 August to 0130, 3 August, 1960. These animals were immediately relaxed with nembutal and then fixed and sectioned. Particular attention was given to determine if eggs were passing through the oviduct and the lower reproductive tract ("spermathecal duct" of van der Schalie and Dundee, 1956). It was assumed that the presence of eggs in the lower tract indicated that egg-laying was occurring. The information obtained from examination of 56 serially sectioned individuals indicated that there was no daily pattern in egg-laying (Table VI).

A study of the time when copulation occurred was not made. Many

pairs were observed copulating at all times of the day; it is assumed that copulation occurs throughout the night as well, since other activities of the snails were observed to continue over a 24-hour period.

Distribution of eggs.—In a study of the zonal distribution of the eggs of P. cincinnationsis, van der Schalie and Walter (1957) indicated that there were two zones of concentrations of eggs, i.e., a large one on the lower portion of the bank and a smaller one slightly higher on the banks of the River Raisin. They concluded that this arrangement was caused by fluctuating water levels of the river due to interrupted water flow brought about by hydroelectric dams upstream.

For several years the dam above the Clinton Station has not been in operation. Consequently, a study was made of the zonation of eggs on the banks at this station to see if a double concentration of eggs still persisted. The distribution of eggs was determined along four transects (for the techniques employed, see van der Schalie and Walter, 1957) on 2 August 1960. Three of these showed no indication of a double zone of concentration (Fig. 12). The fourth transect did have a small concentration of eggs below the main concentration. Since this smaller concentration involved only one soil sample, it would appear rather to be a result of sample error than an actual double zonation of the eggs. Evidently, the double zonation previously observed no longer occurs at the Clinton Station. The facts available indicate that fluctuating water levels may have been responsible for the double zonation observed earlier at this station.

CHANGE IN RIVER CONDITIONS

For several years field work has been conducted at the Tecumseh Station. In September, 1960, a loop of the river at this station was cut off from the main course by the construction of a new bridge over the

TABLE VI.—Time of egg-laying of *Pomatiopsis cincinnationsis* as indicated by the presence of eggs in the lower reproductive tract (oviduct or spermathecal duct)

	Time of		Number of snails			
Date	sample	Examined	With eggs	Without eggs		
	1230	2	2	0		
	1330	5	5	0		
	1430	5	4	1		
	1530	6	5	1		
Aug. 2-	1630	2	2	0		
1960	1730	5	2	3		
	1830	4	3	1		
	1930	4	3	1		
	2130	5	4	1		
	2230	6	6	0		
	2330	3	3	0		
	0030	5	5	0		
	0130	4	3	1		

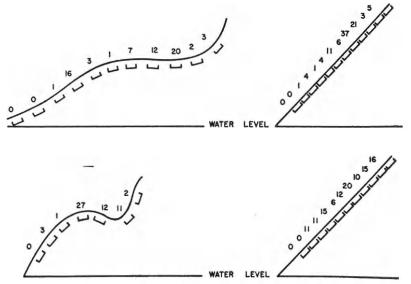


Fig. 12.—Distribution of eggs of *Pomatiopsis cincinnationsis* (numbers in a circular area of a 3½ inch diameter) at the Clinton Station, 2 August 1960.

river and the opening up of a new connecting channel. As a consequence, the banks of the dead arm in this area were isolated along with a large population of *P. cincinnatiensis*. These banks are now being studied to determine the influence of this major change upon the snail population.

While the water level in the cut-off portion of the river dropped by approximately one meter, water remained in it throughout the first year after this change. The eggs that had been laid in the spring and summer had hatched by the time the 1960 cut-off became effective, and the snail population appeared normal during the summer of 1961. Young snails began appearing in the first week of August, 1961, an indication that reproduction had not been greatly disturbed. However, a lapse of only one year is insufficient to determine the effects of the altered conditions on this snail population; this area will continue to be examined periodically.

CULTURE METHODS

In the laboratory maintenance of groups that have a relationship to medical zoology, considerable difficulty has been encountered in attempting to rear *P. cincinnatiensis* (Stunkard, 1946; van der Schalie and Dundee, 1955; van der Schalie *et al.*, in press). The information contained in this report, together with the data in a publication which compares the moisture and temperature responses of *Pomatiopsis* with those of *Oncomelania* (van der Schalie and Getz, in press), indicates

most of the important conditions that are required for success in culturing P. cincinnationsis.

The culture techniques previously employed for this species (van der Schalie and Dundee, op. cit.) have been shown to provide far too moist an environment when compared to the conditions in which these snails are found in nature. Although several standard methods of culture utilized for other snails were tried, all of them were unsuccessful because the substrate was again too wet. A new culture chamber has been designed in which the substrate retains a constant and suitable moisture content.

This vivarium is made by using a clay flowerpot, seven inches in diameter at the top and five and one-half inches high. A roll of filter paper is inserted through the hole in the bottom (Fig. 13) so that only one to two inches of the paper protrudes below the bottom of the pot. The pot is then filled with loam (tightly packed) to within an inch of the top. The filter paper "wick" is moistened and trimmed to form a solid roll of paper approximately 3/16 inches in diameter. The pot is then placed in a rack over a water table with the filter paper wick projecting into the water. To maintain air circulation, approximately half the top of the flowerpot is covered with a piece of plate glass, the remainder with screen wire. Evaporation from the chamber produces a relatively dry surface and keeps the air humidity somewhat below saturation. These conditions have proven most suitable for *P. cincinnatiensis*. If the substrate becomes too moist, the wick is trimmed to obtain a smaller diameter so that less water enters the soil in the pot.

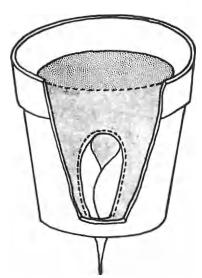


Fig. 13.—Cut-away view of the flower pot chamber used to culture *Pomatiopsis cincinnationsis*, showing position of filter paper wick which maintains proper moisture in the loam (stippled).

Adult snails placed in such a chamber in mid-June have been observed in copulation and young have been produced. However, there was some initial mortality (approximately 25%) among the adults.

It has been shown elsewhere (van der Schalie and Getz, in press) that *P. cincinnatiensis* is very susceptible to high temperatures. In its habitat, summer temperatures were found to average 20C; they seldom went above 23 or 24C over extended periods. Since the laboratory room temperature in culture work (both in the present program and that of van der Schalie and Dundee, op. cit.) averaged 27C, it is possible that too high a temperature was a factor in the mortality observed.

To investigate this point, two series of nine clay saucers (such as used previously in culturing this species, van der Schalie and Dundee, 1955) were provided with loam and moist filter paper. Ten snails were placed in each saucer. This series of vivaria were maintained at 20C and 27C, respectively, for 50 days. Almost half of those snails subjected to the higher temperature died, while less than 10 per cent of those maintained at 20C died (Table VII).

Table VII.—Survival of *Pomatiopsis cincinnationsis* maintained for 50 days at 20 C and 27 C, respectively

Saucer	20	С	27	C
number	Dead	Alive	Dead	Alive
1	0	10	7	3
2	0	10	6	4
3	0	10	6	4
4	Ü	10	2	8
5	1	9	.5	5
6	1	9	3	7
7	0	10	3	7
8	4	6	1	9
9	0	10	9	1
	-	_	_	_
Total	6	84	42	48

During the course of these tests no young were produced or eggs deposited at either temperature. As already indicated, this type of vivarium tends to be too wet for successful culturing of *P. cincinnatiensis*. Although some of the snails are able to survive such moist conditions, they apparently do not lay eggs. When they were maintained on drier substrate, as described above, they produced numerous eggs and young. The lack of success of the previous culture method is, therefore, believed to be caused by too high a temperature resulting in increased mortality among adults and too high a substrate moisture, accounting for the failure in obtaining eggs.

It is now known (van der Schalie and Getz, 1961) that young *P. cincinnationsis* require slightly more moisture than the adults. Therefore, survival of the young might be enhanced if they are provided

with more moist conditions for the first month after hatching. Experiments are in progress to determine amounts of moisture needed during early and late stages in development.

With knowledge of some of the optimum conditions required for the culture of this species, further studies are now being undertaken to determine what modifications, if any, are required for the best maintenance of *P. cincinnaticnsis* throughout its entire life cycle. There are already indications that the newer technique appears to be much more successful than any of those previously attempted.

In some of the preliminary studies, filter paper was used as a source of food. While the animals can subsist on a cellulose diet, it would seem advisable to determine the proper food for this species. Food studies have therefore also been initiated.

DISCUSSION AND CONCLUSIONS

Distribution.—The factors influencing the regional distribution of Pomatiopsis cincinnationsis have not yet been determined. Unfortunately, there are too few early records to indicate its original range. Since its present distribution has been greatly influenced by the alterations of the conditions on the river banks it inhabits, its original distribution can not be ascertained with any degree of reliability. From the records available, this snail apparently reached its northern limit in lower Michigan and its southern limit in southern Kentucky.

The tests made indicate that soil type (texture) does not appear to be a major factor in its regional distribution. It is possible that temperature may be a factor. Their tolerance to extremes of temperature and their preferences are given elsewhere (van der Schalie and Getz, in press). However, field data were not obtained to learn the significance of either of these experimental factors upon the regional distribution of *P. cincinnatiensis*.

Observations at numerous sites occupied by *P. cincinnatiensis*, as well as at those at which it does not occur, indicate that the species is found only on the banks of streams. The following type of stream seems to be required: a stream in which water can reach its banks even during the driest seasons; one that will always have a suitable zone of soil moisture; and a stream which does not cause enough bank erosion during its high water stages to wash away the snails and/or their eggs. Streams whose banks are so eroded by water action as to give the appearance of having been "graded" normally do not support *P. cincinnatiensis*. Likewise, streams with banks subjected to frequent floods and/or deposition of considerable amounts of silt and clay usually do not harbor *P. cincinnatiensis* colonies.

Their local distribution, i.e., the way in which the animals zone themselves on river banks, appears to be related primarily to moisture conditions. *P. cincinnatiensis* has been shown to have a very narrow range of moisture preference (more so than the related species, *P.*

lapidaria, which lives in moist seepage areas; van der Schalie and Getz, in press). It was also shown that the experimental moisture preference approximates the soil moisture within the zone of occurrence of these snails on the river banks. No other factor correlated as well as moisture in the determination of the zonation of the snails. Temperature did not appear to be responsible for the zonation observed. There is no apparent difference in the availability of possible food sources on the banks (George Davis, personal communication). Although air humidity may decrease on the upper portion of the bank (in direct correlation with soil moisture), the experimental data indicate that air humidity is not a primary factor in determining the position of these animals in their habitat. The air humidity in the experimental chambers was essentially the same throughout, yet the animals selected a given substrate moisture. It must be concluded. therefore, that P. cincinnationsis, which is found only on river banks and in a restricted and narrow zone on them, is so limited because it requires a definite range of substrate moisture. Only river banks (as described above) seem to afford situations in which the required moisture range is always present. Other moist situations, such as marshes, fluctuate too much in moisture content to present suitable conditions throughout the year.

Natural history.—Young P. cincinnationsis hatch in late summer or early fall and survive until the following summer. Most of the snails that hatch in early August reach maturity before going into hibernation in late October; some individuals may mate in the fall but no successful hatching of eggs can occur until spring. Although the relative growth of the males appears to be slightly greater than that of the females, their reproductive development is slower than that observed for females (van der Schalie and Getz, unpublished data). It is usually mid-May before most individuals attain sexual maturity and reproductive activity reaches a peak. Eggs may be laid from mid-May until late September. Since few individuals survive the second winter, this species has, for all practical purposes, an annual cycle.

The sex ratios of *P. cincinnatiensis* are always unbalanced in favor of the females. The percentage of females, however, is greater in the newly hatched individuals than in the adults. This evidence indicates a higher mortality rate for the females. However, direct evidence of such a difference in survival was not obtained.

Observations during both day and night failed to give any indication of difference between the nocturnal and diurnal activity of *P. cincinnatiensis*. The animals were as active during the day (on both sunny and cloudy days) as at night; likewise, egg-laying occurred throughout the day and night. Although observations were not made of copulation during the night, this activity was in evidence throughout the day and undoubtedly occurs also at night; these animals apparently maintain about the same pattern of behavior both night and day.

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