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STUDIES ON *SCHISTOSOMA JAPONICUM* INFECTION IN THE PHILIPPINES *

2. The Molluscan Host

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SYNOPSIS

This study of the molluscan host *(Oncomelania quadrasi)* of bilharziasis in the Philippines is divided into two parts, the first dealing with the biology of the host and the second with the interrelationship between the host and *Schistosoma japonicum.*

In the first part, the snail's distribution and habitat are considered in some detail, and then field and laboratory studies on its behaviour and activity are reported on. A section on the life history of *O. quadrasi* covers its growth, reproduction, egg-laying and survival. This is followed by a study of the population dynamics of the snail.

In the second part, the laboratory procedures used for infecting snails and for obtaining cercariae are described, and the finding that more female than male snails are seen infected is discussed. A section is devoted to the effect of infection on the reproduction, growth and longevity of the snail, and an account is given of cercarial output, of the distribution of cercariae in a snail colony and of their presence downstream from snail colonies. The final section deals with natural fluctuations in snail infection rates, which appear to show a cycle related to rainfall.

BIOLOGY OF THE INTERMEDIATE HOST

Distribution

General geographical distribution

Oncomelania quadrasi is apparently confined to the Philippine Islands. It was first described by Mollendorff (see Bartsch, 1936) on specimens

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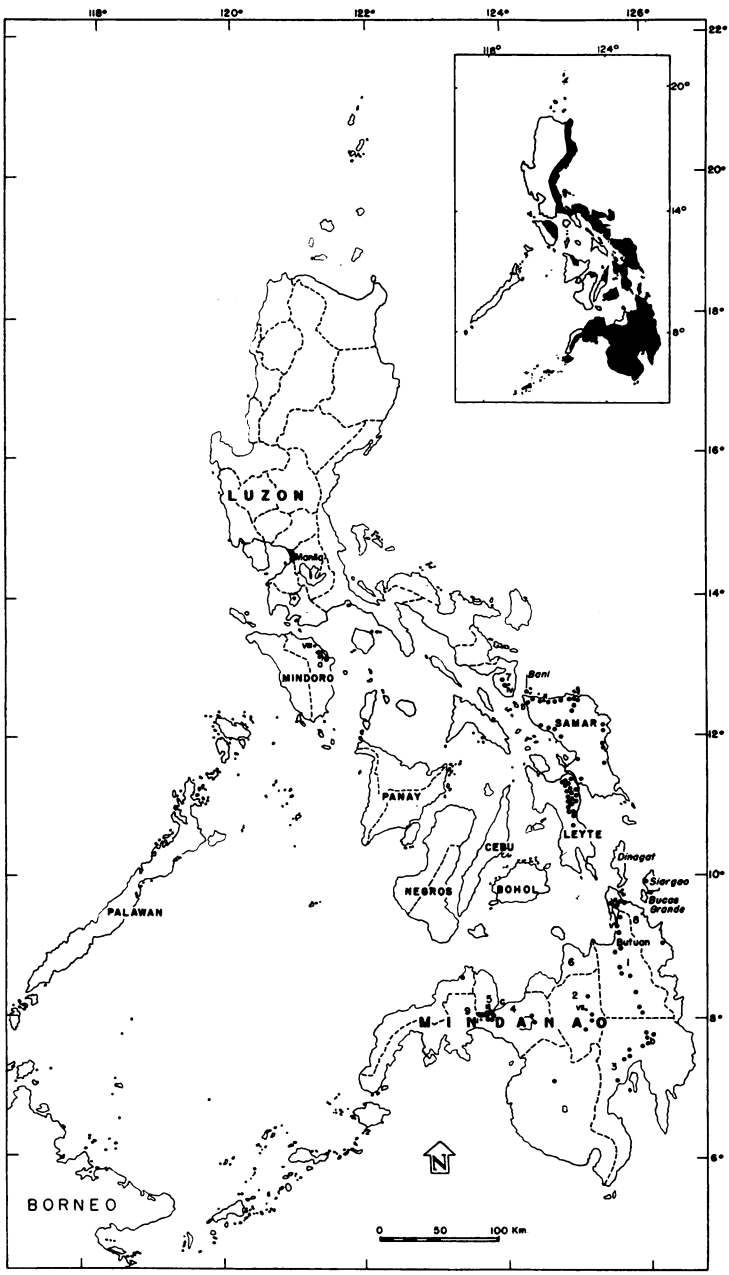
from Surigao Province in Mindanao, and first recognized as the intermediate host of *Schistosoma japonicum* by Tubangui on the basis of observations made at Palo, Leyte, in 1932. The subsequent history of information concerning its range is completely bound up with that of the parasite. With certain minor exceptions, its known distribution is that described by Pesigan (1953). The snail is known to occur over most of Mindanao and Samar Islands, eastern Leyte, and in relatively small areas in extreme south-eastern Luzon and eastern Mindoro (Fig. 1). It has also been found on the minute island of Bani off the north-west coast of Samar, and on Siargao Island, north-east of Mindanao. The only probable range extensions will be along the east coast of Mindanao, including the adjacent islands of Dinagat and Bucas Grande. None of these areas has been surveyed. The reasons why other areas are not suspect are two. The first is the correlation between the distribution of the snail and the annual rainfall pattern; Tubangui & Pasco (1941) and Pesigan (1948, 1953) have pointed out that the snail is confined to areas that get no annual dry season. Second, the areas with this rainfall pattern, aside from those listed, are so located that foci endemic for bilharziasis would probably have come to the attention of health authorities by now, or else they have been surveyed thoroughly for the presence of the disease. The most interesting confirmation of the theory that the pattern of rainfall limits the distribution of *O. quadrasi* is the case of the north coast of Mindanao, where a large area, including almost the entire province of Misamis Oriental, has a marked dry season, and is conspicuously lacking in *O. quadrasi*, although the snail is found adjacent to this area in every direction.

Local distribution

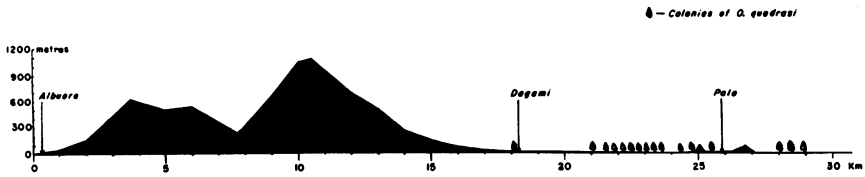
Within the endemic islands, the general distribution of the snail is related to local topography. Although most often found near sea level, the snail is known to occur at elevations up to nearly 900 m in Bukidnon Province in Mindanao. At whatever elevation, the outstanding characteristic of snail-inhabited regions is their flatness. This feature makes for retention of water, a point of obvious importance to an animal with an aquatic stage in its life-history. The point is well illustrated by descriptions of the two areas where the details of snail distribution are known best—Leyte and Sorsogon. In Leyte, *O. quadrasi* is confined to approximately the north-eastern fourth of the island. A profile drawn through Palo along a NE-SW line (Fig. 2) illustrates the local topography. The snail colonies that are known to occur along this line are also shown. The south-western half of the profile cuts across rugged mountainous terrain, and these mountains extend virtually to the sea, leaving no coastal plain at all. North-east of the mountains, the land is so flat that the difference in elevation is less than 30 m in more than 19 km. This flat area is broken near the coast by

FIG. 1. AREAS OF ENDEMIC BILHARZIASIS IN THE PHILIPPINES



Bilharziasis endemic areas are indicated by black spots.
Provinces: 1, Agusan; 2, Bukidnon; 3, Davao; 4, Lanao; 5, Misamis Occidental; 6, Misamis Oriental; 7, Sorsogon; 8, Surigao; 9, Zamboanga del Sur.
The areas shown in black on the inset map have no dry season.

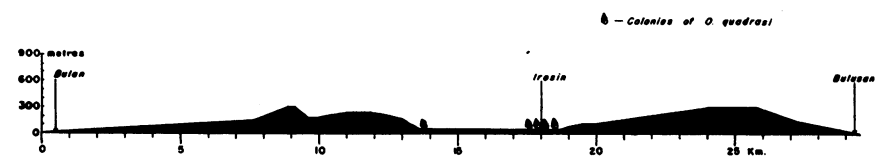
FIG. 2. PROFILE OF LEYTE, SHOWING RELATION BETWEEN TOPOGRAPHY AND DISTRIBUTION OF *ONCOMELANIA QUADRASI*



a series of low mountains, but these rise abruptly from the surrounding plain, thus having only a small effect upon the general level. Snails are found throughout this flat north-eastern plain, as indicated in the figure. No *O. quadrasi* are found in any other part of Leyte, although a number of surveys have been made. There is one flat area in western Leyte that might provide an exception to the observation that it is the flat areas that harbour snails. The area north-west of Ormoc (Pagsangahan plain), while not as flat as eastern Leyte, still appears suitable to provide some snail habitats. Climate may provide the answer to the problem posed. Although officially classified as having no real dry season, Ormoc nevertheless has an average of several consecutive months that have less rain than the driest month in Tacloban.

Sorgoson Province, at the extreme south-eastern tip of Luzon, provides a somewhat different picture. The province consists mainly of a peninsula connected with the rest of the island by a narrow strip of land. The peninsula, which is less than 20 km across, is mountainous, with peaks up to 1650 m in elevation. But the central part is typically flat and low and is drained by the northward-flowing Caloscos river. In the municipality of Irosin, the valley of this stream is approximately 3 km wide, but as the river leaves Irosin and flows through the adjacent municipality of Juban, the mountains encroach and the valley narrows to less than 1 km in width. All of the snail colonies are located in the valley, most of them where it is widest and flattest. A profile across the province (Fig. 3) going along a NE-SW line through Irosin shows the situation graphically. The concentration of snail colonies at the edges of the valley may be due to the large

FIG. 3. PROFILE OF SORSOGON PROVINCE, SHOWING RELATION BETWEEN TOPOGRAPHY AND DISTRIBUTION OF *ONCOMELANIA QUADRASI*



number of springs and seepage areas at the foot of the mountains, which have not been intensively farmed, in contrast to the intensive rice-farming that is done on the valley floor.

Habitats of *Oncomelania quadrasi*

General features

A number of descriptions of snail habitats appear in the literature on bilharziasis (Tubangui, 1932; Tubangui & Pasco, 1941; Avery, 1946; Abbott, 1946, 1948; McMullen, 1947; McMullen et al., 1954). None of these descriptions includes the habitats in Sorsogon Province, and only the last-cited authors gave any thought to the problem of the original habitat of *O. quadrasi*. A systematic description seems in order here, even though it includes some information already in the literature.

It is the wet places, of course, that actually harbour snails. These places are of many different types, but they can be grouped into a small number of general categories, as follows.

Flood-plain forests and swamps. These apparently represent the most extensive original habitat of the snail. Because the land is desirable for growing lowland rice, these forests are gradually destroyed, and it is only under the pioneering conditions in certain parts of Mindanao that we can find the habitat in an undisturbed condition. The largest snail habitat yet discovered covers the entire Manat River swamp in Davao Province. It is approximately 20 km long by at least 2-3 km wide. At its northern end, a new road is under construction. The Manat River at this point is contained within banks, and the forest can be entered readily. Snails were found on the forest floor in places that seemed scarcely lower than the general ground level, and that contained no water at the time that the place was observed. Elsewhere, as at a trail-crossing near the centre of the swamp, the river fingers out and has no defined channel, covering a large part of the forest with water. Snails were found all along this trail from the point of its entrance into the swamp onwards for at least 2 km.

The sample description could be repeated with minor variations for many other places in Mindanao. As mentioned above, the land is desirable for rice-farming, and all such habitats seen are under agricultural encroachment.

Rice-fields. The above discussion brings up the subject of the fate of snails under the changing conditions described. In Mindanao, it is not common to find *O. quadrasi* in rice-fields, and in the localities where this happens, the fields are either newly cut out of swamps, or else have been abandoned for one year or more. This is in striking contrast to Leyte,

where the largest snail habitat is the Cogon-Anahaway rice-field, which covers an estimated 12 km². The difference can only be accounted for on the basis of the primitive agriculture practised in eastern Leyte. The routine method in Mindanao seems to be to plough the land wherever absence of roots permits, and to perform at least some weeding during the growing season of the rice.

Thus, as rice-fields are pushed into a snail-inhabited swamp, the snails are able to continue living for a time, the length of which depends upon the intensity of cultivation. We are fortunate in having some record of the events in one case, that of the rice-fields at Managok, Malaybalay, Bukidnon Province. During 1952, a unit of the Division of Schistosomiasis of the Philippines Department of Health was stationed near by, and they reported that the rice-fields harboured snails. In September 1955, the place was resurveyed, and an intensive search revealed that snails remained in only one field but were abundant in the adjacent road ditch. The exception was a field that had been abandoned for two years. According to residents, the place was first farmed in 1948, and we may conclude that four years of the type of farming practised there are not sufficient to eliminate *Oncomelania*, but that after seven years, none remain. The reversion of one field after two fallow years shows that conditions are otherwise suitable for snails.

Streams. Throughout all the areas inhabited by *O. quadrasi*, there occur meandering, sluggish streams, densely populated with snails. The typical picture seen is a stream bed 3-15 m wide, at a level one metre or more below that of the surrounding country. The stream bed is flat, clogged with vegetation, and very soft. In spite of the vegetation, there is usually a moderate flow of water. The amount of flow and the absence of a defined channel constitute a paradox. The explanation seems to lie at least partly in the habits of the people and of their domestic animals. Several species of fish, particularly the mudfish (*Ophicephalus* sp.), inhabit these streams and are widely sought for food. The method used in catching them is to dike off or dam a part of the stream, and then to wade in and capture the fish by hand. The repeated temporary damming up of the streams prevents the water from making anything like a permanent channel. In addition, the streams are very widely used as wallowing sites for the water buffalo (carabao), the principal work-animal of the Philippines. The wallows are temporary, and many different sites may be used by the same animal. This wallowing has the same effect as the fishing—it forces the water away from any defined channel, softens the whole stream bed, and by making the flow more sluggish promotes the growth of semi-aquatic grasses and other plants, which in turn impede the water still further.

Small swamps. These areas are much more like the streams in appearance than they are like the large flood-plain swamps. The principal dif-

ferences from the streams are their small size and the lesser flow of water through them. Like the streams, they are fished and used as carabao wallows. These small swamps, which we have called " pockets " in our records, are always located at the foot of rather high and steep banks, and the sources of their water are seepages and springs emerging below the banks.

In many places, but particularly in the Sorsogon area, spring outlets may support snails without forming a well-defined swampy area. Often the banks around such a spring, though rather steep, and even sandy, are continuously wet from seeping water. Such places may be very small. Omagom spring in Irosin, Sorsogon, forms an *Oncomelania* habitat whose maximum dimensions are 10×2 m. It is particularly these small swamps that have given such a vivid impression of discontinuity in snail distribution and have led us and others to refer to individual snail-inhabited areas as " colonies ".

Road ditches and borrow-pits. In the construction of roads in lowland areas, it is often necessary to do extensive filling in order to raise the road bed above ordinary high-water levels. For economic reasons, the source of the fill-dirt cannot be far removed, and the most common practice seems to be to take it from the immediately adjacent land. The result of this is that a rather large ditch is left on either side of the road. These ditches remain wet because of their low level, and in many places provide good habitats for *O. quadrasi*.

Discussion. Not every snail colony seen can be fitted exactly into the categories described above, as many of them are really combinations of types. The classification is intended mainly as an aid in description.

One interesting feature of these snail colonies is their permanence. Not only have all those that we have followed constantly since early 1953 continued to maintain themselves, but there is good evidence of much longer continuity. The swamp behind MacArthur (formerly Tarragona), Leyte, is discussed by Sullivan & Ferguson (1946) as a snail habitat, and there is every reason to suppose that the snails found by Tubangui in 1932 came from either Binog or Gacao streams—both flourishing colonies at present. The indications are that the factors making for suitability do not ordinarily change over a period up to some tens of years. The example of the road ditches, however, is enough to warn us that it is possible to alter places so that snails can breed in them.

A consideration of all habitats of *O. quadrasi* reveals some striking differences. The extremes of conditions would appear to be the gravel-bottomed springs and seeps in Sorgoson, the moist and not waterlogged forest floor in Davao, and the Cogon-Anahaway rice-field in Leyte. These areas are so different that any features common to all of them would appear more or less to define the requirements of the snail.

One characteristic appears to be that they never dry out for any length of time. Drying is prevented by constant seepage of underground water in Sorsogon, by the dense forest cover in Davao, and by the flat topography and clay subsoil in the rice-field. Therefore, the right amount of water proper to the slope and soil would appear to be the most important requirement for *O. quadrasi.*

As seen above, any further disturbance of the rice-field through more intensive cultivation would probably eliminate snails there, just as removal of the forest cover would permit the ground to dry enough to make that place unsuitable. We conclude, tentatively at least, that a relatively undisturbed situation is necessary for snails to thrive. These two features would thus appear to make the major differences between the places that are and those that are not suitable for snails, and the obvious implication is that control measures should be designed with them in mind.

Physical and chemical components

The analysis of snail habitats presented above, while quite satisfactory in general, becomes inadequate when detailed surveys are made within a single area. The reason for this is that there are many places that look suitable for *Oncomelania*, but do not harbour them. Such places apparently have the necessary moisture, topography, and vegetation. Some of these negative areas can apparently be explained by the fact that the water is stagnant and foul. This is particularly evident in the case of the large swamp behind the town of MacArthur, Leyte. In some parts, the water has a moderate flow, and such places support snails, whereas other parts of the swamp, where there is a foul smell to the habitat, have none.

Even after discarding the stagnant areas, however, there still remains a residue of apparently favourable places that have no snails, and in a few cases, these are actually connected with snail colonies. It was this observation of apparently suitable but negative habitats that led to the hope that a detailed chemical analysis of such places would provide a clue to some easily controllable requirement of the snail.

One of our first efforts, then, was directed towards analysing the soil and water of such places, and comparing the results with those from snail colonies. As a matter of fact, Tubangui & Pasco (1941) had done this, and preliminary results had already been obtained by the original WHO advisory team, through co-operation with the Division of Soil Survey. Both sets of workers concluded that although the approach was hopeful, the results were somewhat inconclusive. As can be seen from Table I, our results were equally inconclusive. There is complete overlap in every feature tested, Nevertheless, we took advantage of the opportunity to have our results checked independently when a party of soil technologists from the Bureau of Soil Conservation came to Palo to make a detailed survey of

TABLE I. RESULTS OF SOIL AND WATER ANALYSES IN *ONCOMELANIA QUADRASI* HABITATS AND IN SIMILAR PLACES WITHOUT *O. QUADRASI* *

Chemical	34 snail colonies		8 areas without snails	
	Range of values (p.p.m.)	Average (p.p.m.)	Range of values (p.p.m.)	Average (p.p.m.)
NO_3	0.8–20	5.9	0.8–4.0	1.65
P	12.5–100	90.8	75–100	93.3
K	45–185	90.4	50–110	49.37
Ca	250–5000	1286.8	500–1000	781.25
NH_3	0.5–2.5	0.85	0.5–2.5	0.75
Mg	0.5–5.0	3.5	2.5–25	6.25
Mn	0.5–5.0	3.5	0.5–12.5	3.8
Al	0.5–50	23	12.5–25	23.4
NO_2	1–5	1.5	1–1	1
Fe	0.5–25	15	0.5–25	94.4
SO_4	50–250	85.2	50–250	81.25
C1	25–100	51.0	25–100	43.75
pH (soil)	4.6–7.2	6.6	5.4–7.2	6.35
pH** (water)	6.2–7.8	7.2	6.2–7.8	7.4

*** Soil analysis performed by LaMotte colorimetric method; water pH values determined with Beckman electronic pH meter.**

**** 50 habitats, 35 negative areas.**

soil types in the Palo-Tacloban irrigation district. At our request, they made two parallel series of samplings in snail habitats and similar-appearing snail-free areas, in as wide a variety of soil types and subtypes as there were in Palo. Their results of the chemical analysis and the classification of the soil types are given in Table II. Of the 12 soil subtypes found, only one failed to appear in both snail-inhabited and snail-free areas, and we are certain that this was an accident of sampling due to our having neglected to select a negative area, rather than to our failure to find one.

The chemical analysis gave the same general results as those obtained in our own work. We attribute the discrepancies in the results for iron, magnesium and manganese to one of two factors: we used a rapid field technique less accurate than theirs, and we took only the surface soil for our analysis, whereas the soil technologists used a coring device that included part of the subsoil. We regard it as conclusively demonstrated that soil chemistry has nothing to do with the distribution of *Oncomelania* in the Palo area.

TABLE II. RESULTS OF CHEMICAL ANALYSIS OF SOIL SAMPLES FROM SNAIL COLONIES AND SNAIL-FREE AREAS IN PALO, LEYTE (12 SEPTEMBER–29 OCTOBER 1955) *

Sample No.	Soil type **	Location	pH value	Available constituents (p.p.m.)							
				NH_3	NO_3	P	K	Ca	Mg	Mn	Fe
Samples from snail colonies											
4	Pawing SL	Kilot stream	5.00	10	trace	9	trace	3000	290	800	300
6	San Manuel SL	Cogon zone along road to San Joaquin	5.30	10	trace	33	8	4000	580	600	362
9	Palo FSL	Takurañga zone	4.30	10	trace	trace	30	1500	210	100	232
11	Palo Clay	Lower Gacao stream	5.30	10	trace	12	trace	4000	550	800	140
13	Palo SCL	Cogon Anahaway	4.90	10	trace	10	trace	4000	350	1240	177
15	San Manuel sl	Cogon Anahaway	4.70	10	2	3	trace	2700	270	400	206
16	Palo CL	Stream at boundary of Cogon-Gacao	4.70	10	trace	12	82	4000	430	680	140
18	Tacloban Clay	Binangkawan tributary No. 2	5.40	10	trace	trace	8	3200	480	46	380
19	San Manuel FSL	Stream in Malirong	4.70	10	trace	58	trace	3200	250	680	186
21	San Manuel SCL	Naliwatan creek	4.70	25	2	18	68	3200	250	800	265
22	San Manuel sl	Upper Malirong river	4.80	25	trace	26	68	2600	320	800	239
23	San Manuel sl	Curahao lake	4.50	10	trace	trace	18	2700	380	920	169
Samples from snail-free areas											
1	San Manuel FSL	Near Campitic canal	4.80	10	trace	19	42	4000	350	1520	564
2	Palo CL	Near Campitic canal	4.70	10	trace	15	trace	4000	430	680	150
3	Pawing SL	Along road to Pawing	4.40	10	trace	8	8	4000	480	920	150
5	Pawing SL	Barrio Candahug stream	4.90	10	trace	8	18	1500	270	180	100
7	San Manuel SL	Barrio Cogon	3.90	10	trace	20	20	4000	750	100	256
8	Palo FSL	Lower Takurañga main stream	4.10	10	trace	55	30	900	320	200	210
10	Palo SCL	Upper Takurañga main stream	4.80	10	trace	33	30	3700	550	2280	126
12	Palo SCL	Cogon Anahaway	5.10	10	trace	26	282	3200	380	640	161
14	San Manuel sl	Cogon Anahaway	4.80	10	trace	3	trace	4000	350	100	126
17	Tacloban Clay	Binangkawan tributary No. 1	6.40	10	trace	trace	trace	4300	480	100	30
20	San Manuel SCL	Corner Malirong river	4.40	25	2	29	8	1500	180	920	196
24	San Manuel sl	Near Curahao lake	4.60	10	trace	34	220	1800	350	76	126

* Chemical analysis performed by the soil laboratory of the Bureau of Soil Conservation, DANR

** Soil type: SL = sandy loam; FSL = fine sandy loam; SCL = silty clay loam; sl = silt loam; CL = clay loam.

When our preliminary data on soil analysis failed to provide any leads explaining the details of snail distribution, we turned our attention to the possible significance of fluctuations in water levels. Six snail colonies and four likely-looking areas without snails were investigated. Marked stakes were placed in each and the water level recorded daily for periods up to 702 days. As the results (Table III) show, there are no regular differences between the positive and negative areas.

From the ecological and evolutionary standpoint, these results are not surprising. A species, in order to maintain itself, must be adapted to its whole environment, and it is the components of the whole environment, interacting in innumerable different ways, that determine the survival of the species. Ecological results have indicated that the biological part of the environment is ordinarily more important than the physico-chemical

TABLE III. CHANGES IN WATER LEVEL IN SIX SNAIL COLONIES AND FOUR SIMILAR AREAS WITHOUT SNAILS IN PALO, LEYTE

Snail colony	Total days observed	Total range of water level (cm)	Averages for days with changing levels (cm)	
			average daily rise	average daily fall
A Kilot	702	107	5.80	6.88
B Binog	689	97	9.82	6.97
C Gacao	689	104	8.88	6.49
D Agoong	289	179	14.85	8.31
E Juber	289	77	7.03	5.57
F Naliwatan	289	65	4.82	3.99
Average		104.83	8.17	6.60
Negative areas				
W Marasbaras	700	92	7.92	4.95
X Utap	686	94	10.13	6.88
Y Baras	256	85	4.88	3.72
Z Purisima	256	145	11.99	10.69
Average		104.0	8.99	6.14

TABLE IV. ECOLOGICAL DISTRIBUTION OF *ONCOMELANIA QUADRASI* IN BINOG COLONY, PALO, LEYTE, BASED ON A SERIES OF FOUR TRANSECTS

Distance from stream bank (metres)	Number of samples	Average number of live snails	Average physical condition								
			soil water (% wet weight)	depth of water (cm)	pH of water	turbidity (p.p.m. SiO_2)	water temperature (°C)	soil temperature (°C)	air temperature (°C)	relative humidity (%)	light intensity (foot candles)
0	8	0.38	45.64	0.63	7.37	15.5	29.5	27.8	29.8	73.0	382
0.5	8	6.38	54.48	0.00	—	—	—	27.8	30.2	73.5	1141
1.0	8	1.75	59.66	0.63	7.69	8.8	29.8	27.8	29.8	73.2	1286
1.5	8	2.63	61.01	3.25	7.52	13.0	28.6	27.7	29.7	72.8	1284
2.0	8	2.13	63.34	2.75	7.61	14.1	29.3	27.7	29.9	72.6	2756
2.5	7	4.43	60.39	3.57	7.67	12.1	29.3	27.3	30.4	72.3	2370
3.0	6	1.33	62.56	6.33	7.63	5.3	28.3	27.5	30.1	71.7	3342

part. To select an example from the field of public health, it was once thought that *Anopheles atroparvus*, a malaria vector in Europe, could only breed in brackish water, an obvious restriction due to chemicals. It was later found that this was not true, and that the apparent restriction was due to the fact that other species of *Anopheles*, better adapted to fresh water than *A. atroparvus*, prevented it from entering fresh-water habitats through competition (Hackett, 1937). Although there are no other species of *Oncomelania* in the Philippines, it might be of value to investigate the local gastropod fauna and the association of the various species with *O. quadrasi*.

Distribution of snails within the habitat

Like practically all other species of animals, *O. quadrasi* is distributed neither evenly nor randomly over its habitats. This fact increases the complexity of sampling and statistical analysis, as will be seen in the section of the present paper dealing with population dynamics. Our concern here is with factors influencing distribution within a colony. The site of the observations on horizontal distribution was Binog colony in Barrio Gacao, Palo. It is a colony fitting well the description of streams given above. Four transects were made across the colony, with samples taken at 50-cm intervals along each transect. Sampling was by the tube method, described in a later section. At each sampling site, we recorded the depth, temperature, pH, and turbidity of the water, and the temperature and relative humidity of the air within 5 cm of the surface. Light readings were also made at the surface at each site.

A Beckman pH meter, model N-2, a Hellige turbidimeter, and an ordinary thermometer were used for the water readings. A Serdex direct-reading hygrometer and a Metraphot light-meter calibrated in foot-candles by comparison with a Weston Master II were used for humidity and light, respectively.

Although there was no clearly defined channel, the water was deepest halfway between the banks, near both of which there was a zone of mud not covered by water at all. The vegetation was densest at the edges of the colony and thinned out towards the middle. Snails were most numerous near the banks (Table IV), and their density seemed related inversely to light intensity and depth of water (Tables V and VI). All these observations were made in mid-morning (9.00-11.00 a.m.) in clear weather.

On a number of occasions in the field, snails have been seen climbing to surprising heights on suitable vegetation. Specimens have been observed more than 2 m above the mud on the stems and leaves of "palawan" *(Cyrtosperma merkusii)*. The specimens were not active at any time that they were observed, and appeared to be quite dry. Laboratory snails have a similar tendency to climb. The glass sides of aquaria often contain many snails that are dry and seem unable to descend—a fact that has been noted

TABLE V. RELATION BETWEEN DEPTH OF WATER AND DENSITY OF *O. QUADRASI* IN BINOG STREAM

Depth of water (cm)	Number of samples	Average number of live snails per sample
0– 0.9	25	3.8
1.0– 2.9	13	2.8
3.0– 5.9	9	0.7
6.0–15.0	7	0.6

TABLE VI. RELATION BETWEEN LIGHT INTENSITY AND NUMBER OF *O. QUADRASI* IN BINOG STREAM

Light intensity (foot candles)	Number of samples	Average number of live snails per sample
3–150	11	5.7
175–250	10	4.0
300–500	11	2.1
800–9600	21	2.0

before. The field observations suggest that the same thing occurs in nature. In the field, rain or wind would soon shake these "trapped" snails back onto the mud of the habitat, but in the laboratory this must be done regularly by the observer, or the snails will die in the exposed position. More will be said about the function of climbing later. The general observation prompted us to make further studies on the vertical distribution of snails in a colony. In order to obtain sufficient numbers, we chose Naliwatan stream in Barrio Malirong, Palo, a colony where snail densities have been consistently high. To simplify the vertical classes, we selected sampling sites that were not covered with water. The habitat was divided vertically into four zones: below the surface of the mud, on the surface of the mud, up to 5 cm above the surface, and more than 5 cm above the surface. Previous observations had shown the existence of an upward movement of snails at night, and this study was done at 8.30 a.m. in order to obtain as average conditions as were feasible. The results (Table VII) show that although climbing is common, most of such activity is rather restricted in height, as only 3.4% of the total were found higher than 5 cm above the mud. Of more interest are the differences in sex ratio at different levels. Above the surface, males and females were about equal in numbers, but on and in mud females predominated. This observation may well be related

TABLE VII. VERTICAL DISTRIBUTION OF *O. QUADRASI* IN NALIWATAN CREEK *

Snail position	Total collected		Sex		Sex ratio (M : F)
	number	%	male	female	
Above 5 cm	10	3.4	5	5	1 : 1
0–5 cm	92	31.1	49	43	1 : 0.88
On surface	118	39.9	53	65	1 : 1.23
In mud	76	25.6	27	49	1 : 1.84
Total	296	100.00	134	162	1 : 1.21

*** Data taken from 10 ring and tube samples taken from 8.30 a.m. to 9.45 a.m., 2 August 1955.**

to the fact that nearly all the snails found above 5 cm were adults, whereas the young snails were mostly on and in the mud.

Behaviour and Activity of *O. quadrasi*

Field studies

An understanding of the ecology of any species is incomplete without information about its habits—what it does in nature, and how it reacts to various common stimuli. At the same time, general descriptions are less satisfying scientifically than those that are expressed in such a way that numerical comparisons are possible. Such descriptions are briefly given by Abbott (1946) and McMullen (1947). In order to be certain that we were not failing to observe some form of activity, we carried out our observations over continuous 24-hour periods, and for more accurate comparisons, we used a regular system of classifying the information as it was being gathered. Not all forms of behaviour could be watched in the field, and for those that could not (specifically, feeding and egg-laying) we necessarily confined our observations to the laboratory. The activities that were observable in the field were copulation, movement and climbing.

After a number of preliminary trials, both during the day and at night, the following techniques were adapted. For observing movement, three different sites in the colony were selected. At each, a square metal frame 18×18 cm was carefully set in place on the ground and left during the whole 24 hours of observation. Each frame was ruled on all four sides into 2-cm units. With the help of similarly ruled sticks, the position of each snail

TABLE VIII. SUMMARY OF 24-HOUR ACTIVITY OF *O. QUADRASI* AT AGOONG COLONY, IN FOUR QUARTERLY OBSERVATIONS, APRIL 1955-FEBRUARY 1956

	Quarterly observations	10.00 a.m.	2.00 p.m.	6.00 p.m.	10.00 p.m.	2.00 a.m.	6.00 a.m.
				Movement			
Number of snails in view (total for 3 counts)	1-2 April 1955	44	45	56	62	60	55
	19-20 July 1955	25	26	23	29	32	33
	11-12 Oct. 1955	18	19	21	23	19	19
	7-8 Feb. 1956	20	25	32	33	31	23
Total average		27	29	33	37	36	33
Average movement (cm) per snail per 10 minutes	1-2 April 1955	0.525	0.439	0.602	0.408	0.532	0.534
	19-20 July 1955	0.556	0.160	0.665	0.781	0.760	0.618
	11-12 Oct. 1955	0.472	0.471	0.803	0.935	0.437	0.726
	7-8 Feb. 1956	0.693	0.629	0.673	0.916	0.543	0.716
Total average		0.562	0.425	0.686	0.760	0.568	0.649
Percentage of snails climbing on vegetation	1-2 April 1955	28	21	26	29	32	14
	19-20 July 1955	64	44	74	79	81	73
	11-12 Oct. 1955	50	53	43	48	53	63
	7-8 Feb. 1956	35	56	66	70	77	70
Total average		44	44	52	57	61	55
Average height (cm) of snails on vegetation	1-2 April 1955	1.26	1.54	1.32	1.47	2.68	1.19
	19-20 July 1955	1.52	1.35	1.61	1.46	0.75	1.07
	11-12 Oct. 1955	1.39	1.48	5.06	3.30	4.27	2.38
	7-8 Feb. 1956	0.96	0.76	1.54	2.81	3.62	3.73
Total average		1.28	1.28	2.38	2.26	2.83	2.09
				Copulation			
Number of snails observed	1-2 April 1955	44	45	56	62	60	55
	19-20 July 1955	195	298	492	524	379	340
	11-12 Oct. 1955	215	744	428	260	281	378
	7-8 Feb. 1956	252	226	220	264	261	373
Total		706	1313	1196	1110	981	1146
Number of snails copulating	1-2 April 1955	0	0	0	4	0	4
	19-20 July 1955	4	2	10	8	6	4
	11-12 Oct. 1955	6	32	14	12	14	4
	7-8 Feb. 1956	2	0	10	22	6	14
Total		12	34	34	46	26	26
Percentage of snails copulating	1-2 April 1955	0	0	0	6.5	0	7.3
	19-20 July 1955	2.1	0.7	2.0	1.5	1.6	1.2
	11-12 Oct. 1955	2.8	4.3	3.3	4.6	5.0	1.1
	7-8 Feb. 1956	0.8	0	4.5	8.3	2.3	3.8
Total average		1.4	1.3	2.5	5.2	2.2	3.4

could be recorded on a mimeographed grid of the same size as the observation frames. In order to follow the movements of individuals more accurately, we selected sites of low density, in which a frame contained 10 or fewer snails, and repeated the observations twice at 10-minute intervals. The minimum straight-line distances necessary to produce the observed changes in position were taken as an index of snail movement. This procedure of repeated observations at each of the three frames was done six times during the 24 hours at 4-hour intervals, starting at 10.00 a.m. In addition to horizontal movement, the height above ground was recorded for all snails climbing on the vegetation.

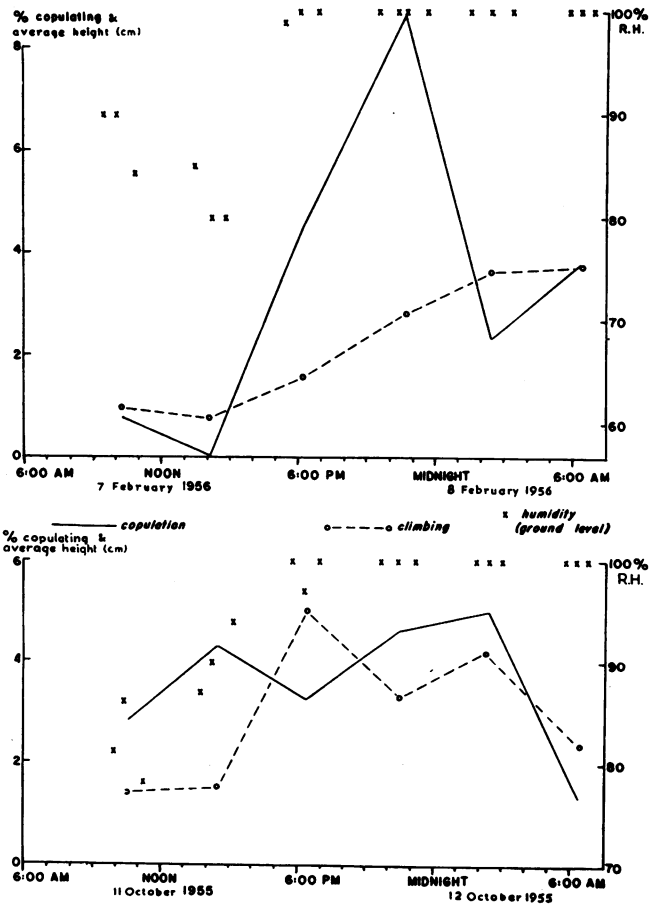
During the first overnight study, it became apparent that not enough snails were under observation to register accurately the proportion *in copula*. In the succeeding studies three separate quadrats, 50-cm square, were staked out and the proportion of copulating snails recorded every four hours. Because of the disturbance involved in examining such a large area minutely, these quadrats were moved after each observation. During the last three overnight studies, weather records were taken at three heights —the surface, 20 cm above the surface, and 1 m above. Ambient air temperatures and humidities were recorded at ground level near each observation frame while snail movements were being recorded.

Four of these studies, spaced at approximately quarterly intervals, were performed in 1955 and 1956. The results are summarized in Table VIII. The similarities in the results are more striking than are the differences among them. It is clear that snails are active at all times of the day and night. Both the amount and the kind of activity do change during a 24-hour period, however. All forms of activity, but especially copulation, are greater at night. Rate of horizontal movement changes least among the activities measured. Mid-afternoon is the time of least activity. The difference between afternoon and morning would appear even greater had not an accident of weather upset the figures for 11-12 October. At around noon of the 11th, there was a light rain, and the sky remained cloudy until after dark. For this reason, instead of becoming hotter and drier than the morning, the afternoon became wetter and there was no rise in temperature. Both copulation and climbing responded to the change in weather. Fig. 4 and Fig. 5 show the differences in activity pattern between the day with a rainy afternoon and one when the afternoon remained clear (7 February 1956).

Laboratory studies

The influence of physical factors on movement. It is clear that snails respond to weather changes, but it is not possible from field observation to be sure of the relative importance of the four factors of humidity, temperature, light and atmospheric pressure. The first three change regularly over the 24 hours, and any or all of them could therefore cause the observed

FIG. 4. RELATION BETWEEN RELATIVE HUMIDITY AND CLIMBING AND COPULATION IN SNAILS

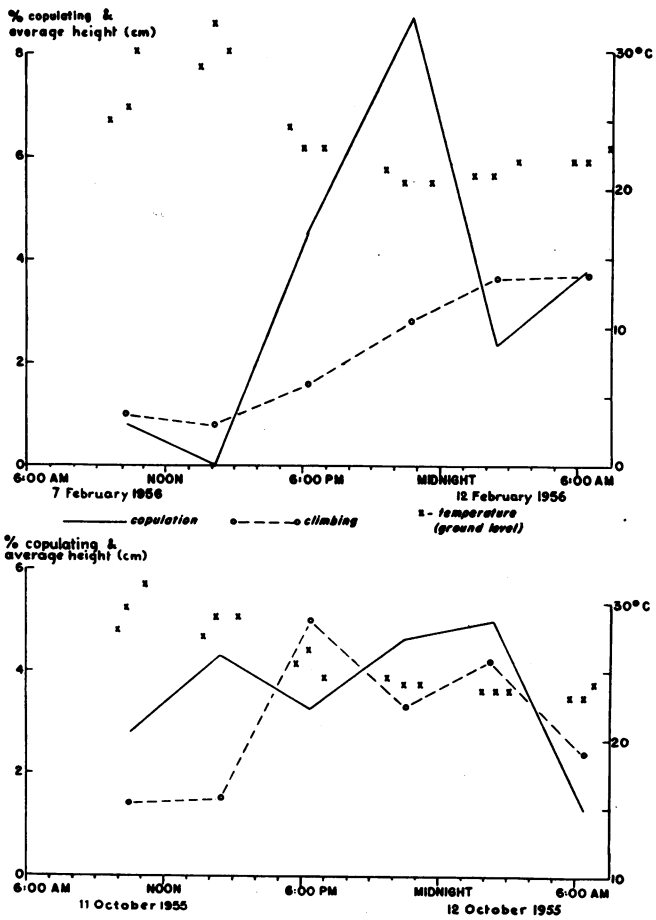


Note the earlier increase in activity on the date when the humidity rose during the afternoon.

changes in snail activity. Since all these factors could be controlled in the laboratory, several series of experiments were performed to test the effect of each on the rate of movement of snails of both sexes. The experiments were done using two glass desiccators, one for control and one for experimental changes.

For humidity experiments, the control desiccator had the base filled with water, giving a relative humidity of 90%-95% under working conditions. The experimental desiccator contained silica gel. In the amount used, this gave a relative humidity of 35%-45% under working conditions.

FIG. 5. RELATION BETWEEN AIR TEMPERATURE AND CLIMBING AND COPULATION IN SNAILS



Humidities were determined with a Serdex direct-reading hygrometer, which fits conveniently inside the desiccator.

For light experiments, each desiccator contained water. The control was placed under a shelf behind black cloth, and the experimental desiccator was placed in various situations for different light intensities. Behind the black cloth, the light intensity was measured as 0.5 foot-candle. Experiments were done at 20, 800, and 10 000 foot-candles.

For temperature experiments, the control contained water at room temperature (27°-30°C). Temperature was adjusted up or down by adding ice-water or warm water to the experimental desiccator. A range of

temperatures from 20°C to 35°C was thus obtained. This is approximately the maximum range of air temperatures in Leyte.

Experiments on pressure were done by opening the pet-cock in the desiccator lid and sucking out the air. Experimental pressures down to an equivalent of about 711 mm of mercury could be obtained in this manner. Pressure was determined with an aneroid barometer placed inside the desiccator.

Snail movement was determined by placing one snail of each sex at the centre of a 14-cm Petri dish, and allowing one such dish to remain in each desiccator for 10 minutes. As the snails crawled over the dry glass, they left a mucus track which was easily revealed by shaking talc around in the Petri dish after removal of the snails. The exact distance travelled could then be measured with dividers.

A series of not less than five trials was performed for each change in conditions, in order to average out the considerable variation between performances of individual snails. For each series different snails from a common stock were used for each trial, because we found that movement was affected not only by the conditions of the experiment, but also by conditions to which the snails had been exposed previously.

The results of these experiments are given in Tables IX-XIII and Fig. 6 and 7. All factors tested had a significant effect upon the activity of snails. A decrease in humidity resulted in reduced movement. Increase in temperature and light increased movement, and it is interesting to note that the effect was more pronounced for females than for males.

TABLE IX. EFFECT OF ATMOSPHERIC MOISTURE ON MOVEMENT OF *O. QUADRASI*

Relative humidity	Sex	Distance crawled per 10-minute trial (cm)										Average
90 %–95 % (control)	F	7.0	0	13.5	2.0	8.5	1.2	3.5	1.4	1.3	8.5	4.69
	M	0	11.5	0	0	6.5	0.3	3.5	0.4	1.3	0.3	2.38
35 %–45 %	F	0	2.7	0	0	2.5	0	0.9	0.5	0.8	0.4	0.78
	M	0	7.5	1.5	0	0	0	0	0.5	0	0.2	0.97

A consideration of the results of the laboratory experiments seems to provide an explanation for the field observations. The reaction to light is clearly one of avoidance, as is easily demonstrated in the laboratory. Snails invariably crawl away from a strong light source placed at the side of the container. During the day, the progress of the sun across the sky exposes many snails to its direct rays, and under these conditions they can be seen

TABLE X. EFFECT OF TEMPERATURE ON MOVEMENT OF *O. QUADRASI*

Temperature (°C)	Sex	Distance crawled per 10-minute trial (cm)										Average
20.0	F	3.3	3.4	5.6	5.0	4.3	5.3	0	7.5	0.9	2.4	3.77
	M	3.3	4.9	5.6	2.7	1.3	2.3	0	7.2	6.3	1.5	3.51
27.0	F	0	13.6	2.6	5.5	7.1	3.3	4.4	6.5	9.1	4.8	5.69
	M	3.0	0	6.7	3.2	3.6	3.2	4.4	6.6	7.2	9.6	4.72
30.5	F	2.4	8.4	0	8.8	8.0	6.3	16.2	4.9	6.5	13.0	7.45
	M	2.0	8.1	8.5	7.2	3.0	1.7	13.6	9.2	6.5	6.0	6.58
35.0	F	16.2	14.0	6.3	12.1	5.4	5.2	6.1	6.4	6.1	9.1	8.69
	M	10.9	8.8	6.3	6.7	5.4	5.2	7.7	1.1	13.0	4.8	6.99

moving rapidly into the nearest shade. The effect would probably be sufficient to produce considerable movement during the day as shade positions change. There would be some reinforcement of this effect by temperature

TABLE XI. EFFECT OF LIGHT OF DIFFERENT INTENSITIES ON MOVEMENT OF *O. QUADRASI*

Light (in foot-candles)	Sex	Distance crawled per 10-minute trial (cm)										Average
0.5 (control)	F	11.4	8.6	16.5	0	0	10.4	0.8	0	0	1.0	0	4.43
	M	0	8.8	4.1	0	8.4	11.2	1.6	0	0	0	3.6	3.43
20	F	17.0	13.2	9.5	13.6	16.1	10.8	1.4	0	0	0	6.4	8.00
	M	0	7.5	20.4	6.6	12.1	10.8	9.4	0	6.2	0	0	6.64
0.5 (control)	F	3.6	0	0	0.9	3.3							1.56
	M	6.9	7.6	2.6	5.1	8.1							6.06
800	F	9.6	8.6	7.1	6.1	10.4							8.36
	M	17.6	6.6	6.5	4.0	9.0							8.74
0.5 (control)	F	0	0	0	8.0	9.0	0	2.8	4.4	6.3	5.6	0	3.28
	M	9.6	9.6	3.2	8.5	9.8	0	5.6	7.3	6.4	1.9	7.7	6.33
10 000	F	20.7	14.6	36.0	9.1	11.6	16.4	21.0	9.7	19.3	13.8	10.8	16.64
	M	15.3	8.5	0.8	4.2	14.3	15.6	17.6	14.0	8.4	24.0	5.6	11.66

TABLE XII. VARIANCE ANALYSIS OF LIGHT EXPERIMENTS FOR 0.5, 20.0 AND 10 000 FOOT CANDLES*

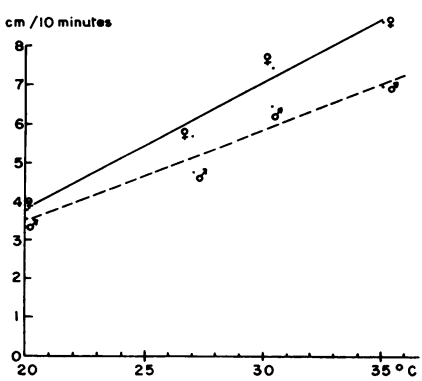
Source of variance	Degrees of freedom	Sum of squares	Mean square	F	P
Light intensities	2	7 155	3 578	19.42	< 0.005
Sexes	1	78	78	< 1	
Interaction between light and sexes	2	919	460	2.49	< 0.05
Residual	82	15 109	184		
Total	87	23 262			

* Angular transformation for binomial distribution used, calculating each value as the percentage of the observed maximum (36 cm per 10 minutes).

TABLE XIII. EFFECT OF REDUCED ATMOSPHERIC PRESSURE ON MOVEMENT OF *O. QUADRASI*

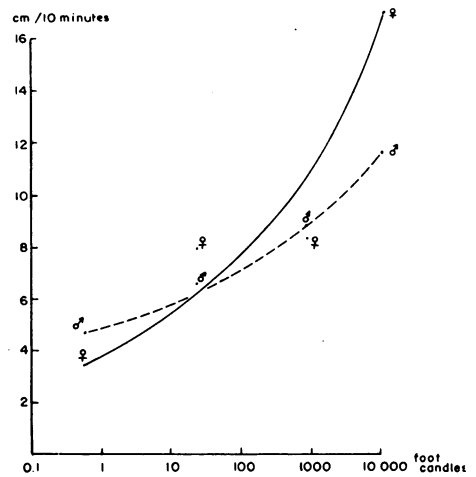
Pressure (mm Hg)	Sex	Distance crawled per 10-minute trial (cm)							Average
759 (control)	F	4.9	0	7.2	7.1	1.1	10.4	3.6	4.90
	M	8.4	0	0	6.3	1.8	10.3	0	3.83
711	F	6.6	0.8	11.2	12.6	7.6	7.5	10.3	8.09
	M	12.8	0	0	7.2	18.7	11.3	8.4	8.34

FIG. 6. RELATION BETWEEN AIR TEMPERATURE AND RATE OF CRAWLING IN MALE AND FEMALE *O. QUADRASI*



increases, although these are probably not as great on the surface of the mud as those observed in air. During the day, climbing would be inhibited because of the lower humidity above the mud and also because of the light from the sky. At night, increasing humidity would encourage movement, especially climbing and copulation, because of the increased exposure of soft parts to air. Thus, the effect of temperature and light during the day seems almost to balance the effect

FIG. 7. RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF CRAWLING IN MALE AND FEMALE *O. QUADRASI*



of humidity at night as far as horizontal movement is concerned, and what is left is the effect of humidity on climbing at night.

The diurnal cycle of egg-laying. The act of egg-laying has never been observed by us, but in order to find out something about the egg-laying habits of *O. quadrasi*, the following experiment was performed. Two hundred adult female snails were taken from the field and one hundred placed in each of two deep, white, enamel pans. Each pan contained mud and a selected number of strips of coconut husk. At 7.00 p.m. and 7.00 a.m. for each of five successive days, the strips of coconut husk were removed and replaced by others. The ones removed were then examined for eggs. The results are shown in Table XIV. Nearly three times as many eggs were laid at night as during the day.

Inasmuch as most eggs are laid above the water, the advantages of egg-laying at night are obvious. The lower temperature, higher humidity and absence of sunlight would all contribute to the protection of the female snail during the process of egg-laying.

The diurnal cycle of feeding. Attempts to discover whether faeces are the source of the material used in the egg-covering led to the employment of substances of a different colour from the mud and detritus upon which snails normally feed. Both charcoal and filter-paper proved satisfactory, as snails ingesting them produced black and white faeces, respectively. When removed to a situation suitable for egg-laying, however, the faeces always became brown within a few hours, and before any eggs were laid.

TABLE XIV. NUMBER OF EGGS LAID DURING DAY AND NIGHT BY TWO GROUPS OF 100 FEMALE SNAILS EACH (PANS 1 AND 2)

Date	Day count *		Night count **		Total (200 snails)
	pan 1	pan 2	pan 1	pan 2	
17-18 October			45	34	79 †
18-19 October	5	10	32	25	72
19-20 October	9	4	28	21	62
20-21 October	19	15	31	24	89
21-22 October	7	5	8	14	34
22-23 October	24	10	28	17	79
Total	64	44	172	135	
12-hour average	12.8	8.8	28.7	22.5	

* Day count covers 12 hours from 7.00 a.m. to 7.00 p.m.
** Night count covers 12 hours from 7.00 p.m. to 7.00 a.m.
† Of total of 100 snails

This last observation provided an opportunity to discover if there were any variations in the rate of feeding at different times of day.

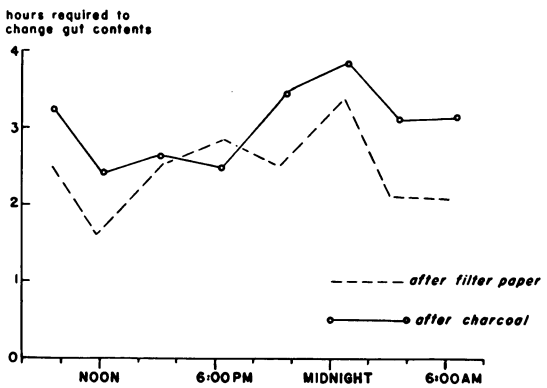
Ten snails, five of each sex, were removed from charcoal, and similarly ten from filter-paper, every three hours, starting at 8.00 a.m. They were

TABLE XV. MEAN NUMBER OF HOURS REQUIRED TO CHANGE GUT CONTENTS OF *O. QUADRASI*, STARTING AT DIFFERENT TIMES OF THE DAY

Time of isolation after feeding	Filter paper			Charcoal		
	female	male	both sexes	female	male	both sexes
8.00 a.m.	2.35	2.70	2.53	3.75	2.75	3.25
11.00 a.m.	1.80	1.35	1.58	2.35	2.45	2.40
2.00 p.m.	2.51	2.50	2.31	2.30	2.95	2.63
5.00 p.m.	2.75	2.90	2.83	2.50	2.40	2.45
8.00 p.m.	2.35	2.65	2.50	3.85	3.10	3.48
11.00 p.m.	3.85	2.90	3.38	4.45	3.25	3.85
2.00 a.m.	2.10	2.15	2.13	2.90	3.30	3.10
5.00 a.m.	2.15	2.00	2.08	3.65	2.60	3.13
Average	2.44	2.39	2.41	3.22	2.85	3.03

placed in small aquaria with mud and detritus, and examined hourly to determine the colour of their faeces. The time required for each to start excreting brown faeces was regarded as an index of the feeding-rate, as it represents the time required for a complete change of gut-contents. The results are shown in Table XV and Fig. 8. Statistical analysis of the data

FIG. 8. CHANGES IN RATE OF FEEDING OF *O. QUADRASI* EXPRESSED AS TIME REQUIRED TO CHANGE GUT CONTENTS



(Table XVI) reveals that the observed differences between times of day are significant, as is the difference between snails that had received charcoal and those that had received filter-paper. The latter difference is probably due to the fact that the filter-paper in the gut becomes stained by the new food, whereas the charcoal is not affected. This has resulted in significantly longer records for the charcoal-fed snails.

Of more importance is the difference between times of day. The results indicate that snails feed more rapidly during the day than at night. This means that we must continue to specify what kinds of activity are being observed in our 24-hour studies.

The times given should not be regarded as absolute rates for exchange of gut-contents under normal conditions, because the natural food is probably more attractive than the artificial food. The reversal of the above experiment—namely, moving snails from mud to charcoal or filter-paper—provides an upper limit to the natural rate of feeding. This experiment, which was started at 11.30 p.m., gave average rates of exchange of gut-contents of 9.93 hours (mud to charcoal), and 10.45 hours (mud to filter-paper). The probable limits for rate of exchange of gut-contents under natural conditions are thus about 3 and 10 hours. Returning to a general consideration of 24-hour activities, those that relate to reproduction (copulation and egg-laying) are more pronounced at night, whereas feeding is

TABLE XVI. COMPLETE ANALYSIS OF VARIANCE OF DATA ON RATE OF FEEDING OF *O. QUADRASI* (LOGARITHMIC TRANSFORMATION)

Sources of variance	Degrees of freedom	Sum of squares	Mean square (variance)	F	P
Between times	7	30.4562	4.3509	3.66	<0.005
Between prior foods	1	15.2705	15.2705	12.86	<0.005
Between sexes	1	1.759	1.759	1.48	>0.05
Interaction between times and foods	7	8.09290	1.156	—	>0.05
Interaction between times and sex	7	8.34295	1.19185	1.004	>0.05
Interaction between sex and foods	1	0.9896	0.9896	—	>0.05
Interaction between time, sex and foods	7	4.51205	0.64458	—	>0.05
Residual	127	150.8009	1.187		
Total	158	220.2241			

Conclusions:

1. Rate of feeding is faster during the day than during the night.

2. Prior foods significantly affect the observer's judgement. The fact that it was easy for the soil to stain the filter paper may account for the apparent differences in the rate of food passing through the gut.

3. Sexes do not feed at different rates.

4. The factors of sex, time and prior foods are independent of each other as far as their influence on feeding is concerned.

more pronounced during the day. The factors influencing movement and climbing have been discussed above. On the basis of our present knowledge about the activity cycle and the frequency of copulation, we advance the hypothesis that climbing is related to reproductive activity, and probably represents mate-seeking.

Life History of *O. quadrasi*

Growth

McMullen (1947) was the first investigator to make observations on the rate of growth of *O. quadrasi*. He reported on the basis of field observations that snails grew approximately 0.25 mm per week. He made bi-weekly collections and by measuring all snails collected was able to follow apparent broods over several weeks by comparing the modes of the successive size-distribution curves.

In our work, we have used several different methods of studying growth. For field studies, McMullen's technique is superior to any other, and we

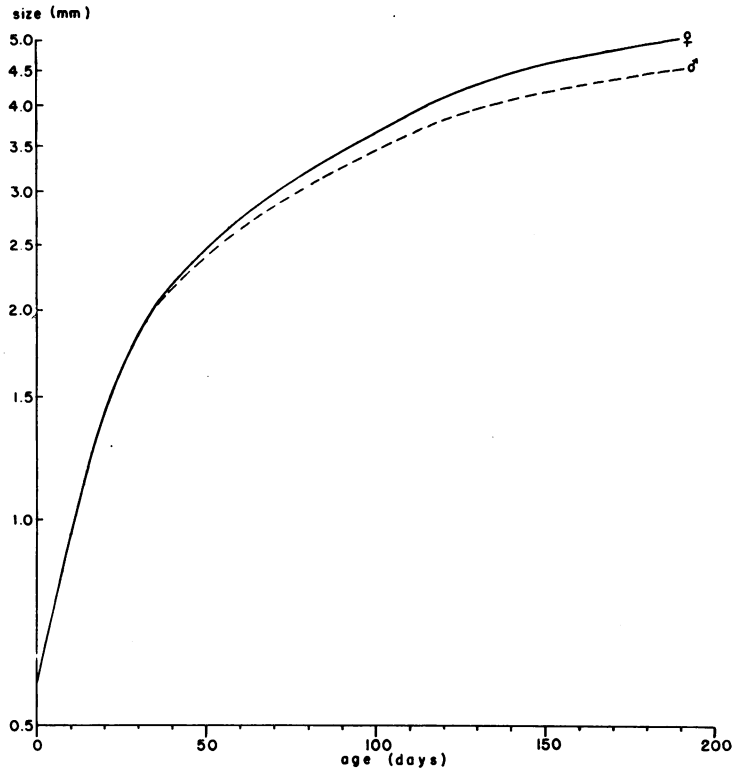
have applied it to a very large amount of field data accumulated in bi-weekly collections in ten snail colonies over a period of one year (July1953-July 1954). In general, our findings confirm McMullen's over the part of the growth curve that he observed. After the snails reach maturity, they grow at slower and slower rates, requiring an estimated two weeks to grow 0.1 mm after reaching an age of six months, at which time males are 4.5-mm long and females 5.0 mm. Above these sizes, we were unable to obtain reliable data, because only a small proportion (less than 10%) of the collectable snails reach these sizes. Very small snails are also too rare in field collections for their rate of growth to be estimated by this technique.

We have made a great many observations in the laboratory on growth rates under various conditions, such as size and type of aquarium, amount of isolation, etc. In one large-scale experiment, we used two types of aquaria, one a standard glass model with metal frame, the other a local product made of clay. Snails grew much faster in the former, from an average of 2.2 mm to 3.9 mm in 42 days (113 snails), than in the latter (2.1-2.4 mm in 36 days for 390 snails). Field data fall between these figures, but closer to those for the glass aquaria.

Incidentally, the snails in the glass aquaria grew much more slowly after the first 42 days, adding only about 0.2 mm to their average size in the next 60 days. These figures still do not give growth rates for the period immediately after hatching. Several different sets of observations were used to obtain the necessary data. In the first, 20 newly hatched snails, starting from an average size of 0.53 mm at hatching, attained an average size of 2.01 mm after 22 days—the most rapid growth that we have observed. These were snails, raised in a large aquarium, whose sex could not yet be determined. It is difficult to determine the sex of snails less than 2.0 mm in length, and in order to obtain the growth rates by sexes, we tried isolating specimens into small aquaria after hatching. The results were disappointing, as under these conditions both sexes grew slower than the average for snails of undetermined sex in large aquaria. Sixteen females reached an average length of 3.1 mm 105 days after hatching, and 14 males an average length of 2.9 mm after 100 days. The most rapid growth was in a female that reached a length of 4.0 mm in 76 days. The best growth of a male was to 2.4 mm. in 60 days.

From all our data combined, we have drawn growth curves (Fig. 9) and made a table (Table XVII) that represent our best estimate of the average state of affairs. It will be seen that there is little if any difference between growth rates of the two sexes up to about 35 days, at which time the snails are slightly larger than 2.0 mm. After that age, males grow less rapidly than females. We have used a logarithmic scale for the growth curve because it emphasizes the rate rather than the amount of growth. The reason for doing this is that the same amount of growth means much more early in life than late in life. A growth of 0.1 mm represents nearly a 20%

FIG. 9. GROWTH CURVES FOR MALE AND FEMALE *O. QUADRASI* *



* Data from both field and laboratory studies are included

increase to a newly hatched snail, but for a female six months old it is only a 2% increase. In our figure, the slope of the curve is a direct representation of the rate of growth in terms of the size that the snails have attained, and we can see at a glance that the growth rate decreases throughout life.

The size of snails at hatching has varied from around 0.5 mm to 0.7 mm, with an average of 0.577 mm for one series of 52 snails. This is slightly larger than the 0.5 mm reported by McMullen and the 0.49 mm by E. D. Wagner.[a]

The value of obtaining an accurate curve can be appreciated best when we realize that size is the only practical method of determining the age of field snails, and it is the age distribution that we must have before we can make any estimates of ability to survive under field conditions.

[a] In the 1955 *Annual Progress Report to the Commission on Parasitic Diseases* of the US Armed Forces Epidemiological Board, Contract Da-49-007-MD-307.

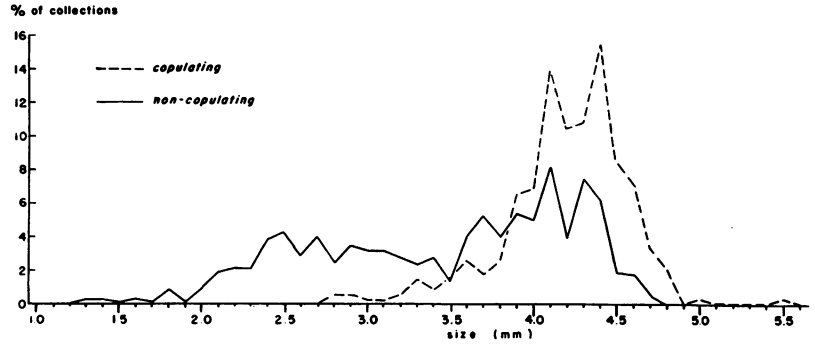
TABLE XVII. SIZES OF *O. QUADRASI* SNAILS AND THEIR CORRESPONDING AGE IN DAYS BASED ON GROWTH CURVE DERIVED FROM LABORATORY AND FIELD DATA

Size (mm)	Age (days)		Size (mm)	Age (days)	
	female	male		female	male
1.0	11	11	3.1	77	82.5
1.1	14	14	3.2	81	87
1.2	16	16	3.3	86	92
1.3	18	18	3.4	90	97
1.4	20	20	3.5	94	102
1.5	22	22	3.6	98	108
1.6	24	24	3.7	102	114
1.7	26.5	26.5	3.8	105.5	119.5
1.8	29	29	3.9	110.5	126.5
1.9	31	31	4.0	114	136
2.0	33.5	33.5	4.1	118	144
2.1	36	36	4.2	124.5	152
2.2	40.5	42	4.3	130	162
2.3	44	46	4.4	136.5	172.5
2.4	47	50	4.5	145.5	185
2.5	52	55	4.6	153	
2.6	55.5	59	4.7	161	
2.7	60	64	4.8	168	
2.8	64	69	4.9	175	
2.9	68	73.5	5.0	189	
3.0	72.5	78			

Reproduction

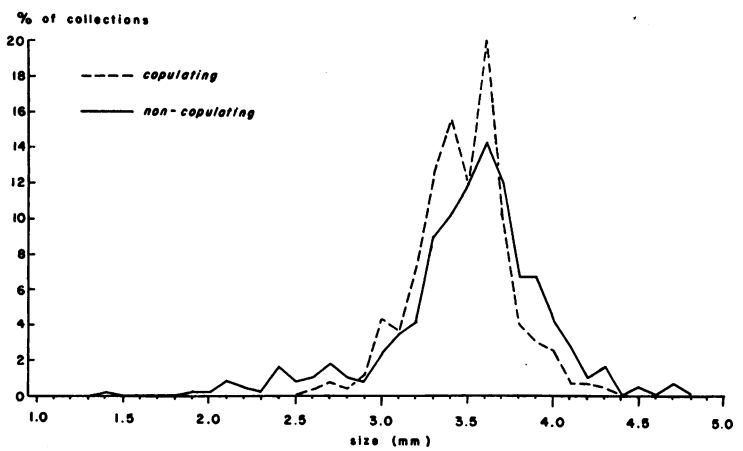
Age at maturity. In order to discover the age at which snails begin to reproduce, a large number of copulating pairs was collected in the field. Although some collecting errors resulted from the difficulty of being certain that two snails are copulating, size-distribution curves of copulating snails can be drawn and compared (Fig. 10 and 11) with similar curves of non-copulating snails collected in the same colony by exhaustive collecting of randomly placed small samples (" ring " method described on page 521). The female snails taken *in copula* were considerably larger on the average than non-copulating females, a fact which suggests strongly that there is a more or less definite age at which they become mature. Male snails, however, showed no such clear separation into two size groups. The

FIG. 10. SIZE DISTRIBUTION OF COPULATING AND NON-COPULATING FEMALE *O. QUADRASI* COLLECTED IN GACAO STREAM



narrower range of sizes of copulating males could easily be due to the smaller total number of snails in the group (278, as compared with 489 non-copulating males). It is true that almost none of the copulating males was less than 3.0 mm long. The roughly estimated age of maturity of male snails, then, would be 78 days.

FIG. 11. SIZE DISTRIBUTION OF COPULATING AND NON-COPULATING MALE *O. QUADRASI* COLLECTED IN GACAO STREAM



Since practically no female with a length of less than 3.0 mm was taken *in copula*, and very few were below 3.9 mm, there remained the question whether they could produce viable young. Accordingly, from the above group that appeared to be *in copula*, female snails of four different sizes were isolated from further contact with males. The numbers are small, but the results are clear. The first group, 11 snails smaller than 3.4 mm in length,

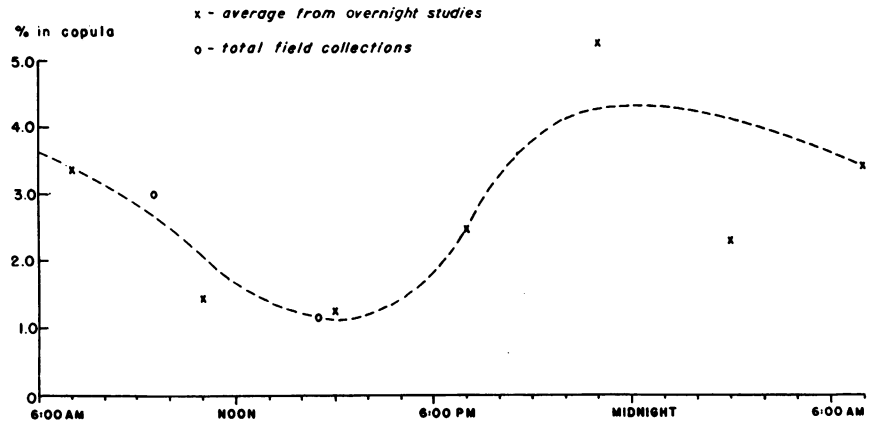
was isolated on 23 February 1955 and laid no eggs at all until 14 April, 50 days later, by which time the smallest was 3.6 mm long and the average length slightly over 3.9 mm. The egg that was laid on that date failed to hatch, as did all 16 others that were laid up to 21 May. The next eggs were laid on 30 May, or 96 days after isolation, but eggs laid that day and subsequently hatched normally.

The second group, consisting of three female snails that measured 3.5 mm in length, was isolated at the same time. They laid their first hatchable eggs on 4 April, or 40 days after isolation. Two snails in the third group, measuring 3.7 mm, and four in the fourth group, measuring 3.8 mm, laid hatchable eggs five days after isolation.

From the above data two things are clear. First, successful copulation and actual production of hatchable eggs are entirely different entities, and may be greatly separated in time. Second, the laboratory conditions under which the snails were kept influenced the amount of time required for them to reach a condition where the laying of viable eggs was possible. This condition is not the attainment of any given size, since the snails were big enough to lay viable eggs at the time when they first began to lay non-hatchable ones. Taking this point into consideration, we conclude that egg-laying probably begins in nature at a size somewhat smaller than the observed 3.7 mm but probably not much before the snails are 3.5 mm long. From our growth figures, this would be at an age of 90-94 days.

Copulation. The influence of time of day and of weather on the rate of copulation in *O. quadrasi* have been discussed briefly above. Besides the overnight studies, we have a large amount of data on the proportion of snails *in copula* in daytime collections made in five colonies over a period of one year (July 1953-June 1954). Most of these collections were made during the morning hours from 8.00 a.m. to 11.00 a.m., but enough were made in the afternoon from 1.00 p.m. to 4.00 p.m. to give a satisfactory comparison. Of 128 416 snails observed in mid-morning, 3858, almost exactly 3.0%, were *in copula*, whereas 106 of 9085, or 1.17%, were *in copula* in the afternoon. Considering that the morning observations of our overnight studies were made later in the day than these, the two sets of averages agree rather well, and we have combined all available data to produce the estimated average copulation rates over a period of 24 hours shown in Fig. 12. In a number of observations, both in the laboratory and the field, the duration of copulation has varied from 25 minutes to more than two hours, with an average between 60 and 70 minutes, which is also the duration most often observed. If we assume an average duration of one hour, and sum the successive proportions over the 24 hours, we find that 70% of the collectable snails copulate within that period. This is approximately the proportion of adults obtained in routine collections and we conclude that snails copulate once a day.

FIG. 12. CHANGES IN PROPORTION OF COPULATING SNAILS OVER 24 HOURS



It is difficult to reconcile this estimate with the observation that a female is able to lay viable eggs more than three months after isolation from males —an observation that is in agreement with Ritchie's (1955) on *O. nosophora.* The ability to store sperm, either in the reproductive tract or as resting nuclei in the oocytes, is of great selective significance, because a single female could then start a new colony if transported to a favourable habitat, or if there were few survivors from a catastrophe. It is probable that this ability is not often utilized in nature, and the two phenomena of frequency of copulation and ability to continue egg-laying after isolation are not as contradictory as might appear at first glance. At any rate, when the sexes are separated for two days, a large proportion of individuals will copulate immediately they are brought together—an observation that has often been made, and one which suggests that the estimate of copulation once in 24 hours may be completely accurate.

In addition to the information on the relative amount of copulation at different times of day, the data can be analysed for seasonal differences and the effect of weather. Only morning figures were used in these analyses.

Table XVIII shows that definite seasonal trends occur, with a low in June and a high in September. This observation is reinforced by the data from the four overnight studies, in which the same seasonal differences are apparent, although a different year was observed. For the overnight studies, the figures represent the unweighted averages of the six successive observations. They are mostly higher than the morning figures for 1953-54, probably because they contain the night copulation rates, which, as we have shown, are higher than those for the day. There is a suggestion of an influence of weather on the rates for different months, since June is the

TABLE XVIII. SEASONAL VARIATION IN COPULATION RATES OF *O. QUADRASI*

	1953–54 *			1955–56 **
	total snails collected	total *in copula*	percentage *in copula*	average percentage *in copula*
April	8 498	174	2.05	2.29
May	1 502	16	1.07	—
June	1 323	6	0.45	—
July	5 514	102	1.85	1.51
August	9 045	338	3.74	—
September	9 962	456	4.58	—
October	11 915	386	3.24	3.55
November	5 991	228	3.81	—
December	4 942	84	1.70	—
January	9 720	276	2.84	—
February	11 963	278	2.32	3.36
March	6 060	250	4.13	—

* Data for 1953-54 represent morning collections from five colonies in Palo.
** Data for 1955-56 represent average rates from 24-hour activity studies.

middle of the drier season and also the period of maximum light, while September coincides with the beginning of increased rainfall. The weather was recorded for each of the 1953-54 observations, and Table XIX shows

TABLE XIX. INFLUENCE OF WEATHER ON COPULATION OF *O. QUADRASI* DURING THE HOURS 8.00 A.M.—12.00 NOON

Weather	Total snails observed	Number *in copula*	Percentage *in copula*
Clear	67 285	1 866	2.77
Clear after rain	23 878	854	3.58
Cloudy	34 502	1 080	3.13
Heavy rain	2 750	58	2.11

the influence of four different kinds of weather on the proportion of snails seen *in copula*. The influence of heavy rain in reducing copulation may account for the secondary low in December, which is the month of maximum rainfall.

Egg-laying

The eggs of *O. quadrasi* were first discovered by Abbott (1946), but, as far as we know, no serious attempts were made to estimate the rate of egg-laying quantitatively until B. W. Halstead & E. D. Wagner in 1954 [a] made observations on them under laboratory conditions in California. They reported a maximum rate of 0.34 eggs per female per day. Repeated experiments in the laboratory in Leyte have essentially confirmed their observations. It is not always possible to obtain sustained egg-laying under laboratory conditions, especially those so arranged that all the eggs can be found, but we have one such set of observations that continued over a period of three months without appreciable diminution of rate. This is in agreement with Ritchie's (1955) observation on *O. nosophora*. Thirteen female snails collected in the field while *in copula*, were isolated and followed for a maximum period of three months. Not all of them survived the whole period, but the observation covered 843 snail days. A total of 308 eggs was counted during the period, and if we correct for the six out of 102 observations when the eggs could not be counted, the average production was 0.39 eggs per snail per day. The average time between layings was 8.26 days, but this figure is misleading because of a few large numbers. More than half of the times between layings were less than five days, the commonest interval being four days. For the 68 observations when the counted eggs were known to have been laid within 24 hours, the average clutch was 2.3 eggs, with one being the commonest number. In round numbers, the figure of two eggs every five days is a good estimate of a female snail's output.

Other observations, carried out over short times, agree with this estimated rate. If we examine the totals in Table XIV, we see that the 200 snails laid 370 eggs in five days (7.00 p.m., 17 October to 7.00 p.m., 22 October) —an average of 1.85 per snail. Not all experiments gave these rates. Many times the rates were substantially lower, as in the case of the experiment on changing water-levels described below, where the rate varied between 0.12 and 0.25 eggs per female per day (Table XX).

The beginning of maturity does not mean the immediate assumption of the maximum rate of egg-laying, as the following experiment shows.

Three sets of field snails, consisting of 22 females and 20 males, were isolated in separate aquaria. The females were selected by size, 3.5 mm,

[a] In the 1954 *Annual Progress Report to the Commission on Parasitic Diseases* of the US Armed Forces Epidemiological Board, Contract Da-49-007-MD-307.

TABLE XX. EFFECT OF FLUCTUATING WATER LEVEL ON EGG-LAYING HABITS OF *O. QUADRASI* (240 FEMALES, 200 MALES)

Week	Number of eggs laid in falling water level	Number of eggs laid in rising water level
1	—	—
2	—	—
3		420
4	199	—
5	—	226
6	186	—
7	—	234
8	201	—
9	—	320
10	220	—
Total	786	1459

4.0 mm and 4.5 mm being the sizes chosen. At approximately weekly intervals, the aquaria were searched minutely for eggs, and about one month after the start the snails were measured again. Fig. 13 shows the changes in rate of egg-laying for 45 days after the start of the experiment. The smaller snails laid none at all until after the twelfth day, and only after a month did the rate approach that for the larger snails. By that time, they had grown to an average length of 4.1 mm. The egg-laying rates in this experiment were the highest that we have observed, and this was probably due to exceptionally favourable conditions, especially after the initial lag shown by all three sets of snails. Growth rates obtained also indicate the good conditions, equalling field rates for the snails starting at 3.5 mm and 4.5 mm and exceeding field rates for those starting at 4.0 mm.

Although copulation is necessary for young to be produced, female snails do lay unfertilized eggs under conditions that preclude copulation. These eggs usually differ from fertilized ones in having imperfect soil jackets. The coating is thin or even absent on top, so that the egg itself can frequently be seen. There is never an embryo in it, of course. That this phenomenon is not due to an abnormality of the individual snail is shown by the following observations. Thirty female field snails, all shorter than 2.5 mm, were isolated from males. It was assumed that since they were at least 0.4 mm shorter than any seen copulating, they could not have copulated before isolation. After a period of 94 days, the first eggs appeared in the aqua-terrarium. Nine days later, the average size of the snails was 3.55 mm.

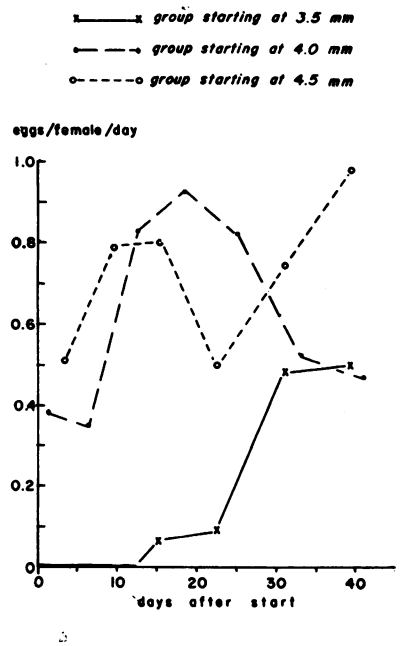


FIG. 13. CHANGES IN RATE OF EGG-LAYING BY SNAILS OF THREE DIFFERENT SIZES

These eggs, none of which ever hatched, continued to be laid at an increasing rate up to the 173rd day after isolation, by which time only five snails remained alive, and they were laying eggs at the normal rate of 0.4 eggs per snail per day. At this time, three of the snails were placed with males. One of these pairs, in which the female measured 4.3 mm in length when mated, produced hatchable eggs after an additional 62 days and continued to do so thereafter. Another pair, with the female 4.5 mm long, produced hatchable eggs 81 days after being placed together. The third pair, with the female 4.9 mm long, produced no hatchable eggs up to 135 days.

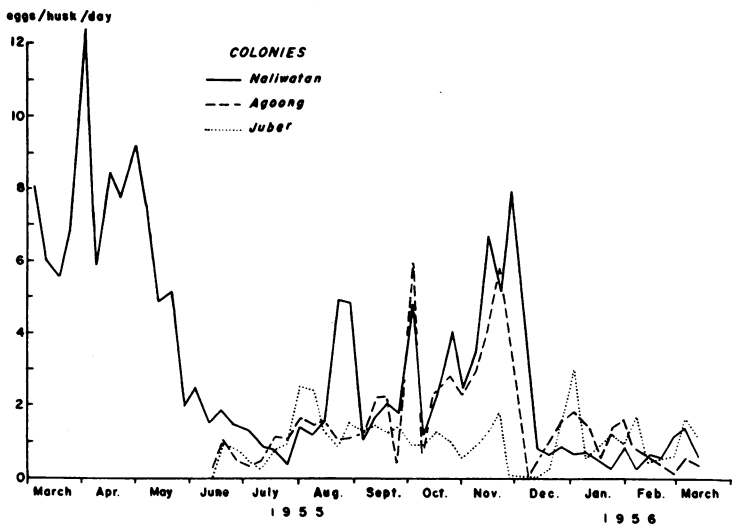
The observed delay between the time the sexes are united and the appearance of hatchable eggs does not always take place. A group of three large female snails, which had been isolated since hatching, and which had been observed to lay unfertilized eggs, were placed with males on 3 February 1956. The eggs were not counted, but on 2 March, 28 days later, a young snail was recovered. This young snail measured 1.7 mm in length, and since we estimate the average age of such snails as 26 days, the egg that produced it must have been laid almost immediately after the females were mated. Even so, the incubation period must have been minimal and the growth rate maximal in this case.

The eggs of *O. quadrasi* are relatively easy to find in the field, and one might suppose that the determination of egg-laying rates under natural conditions would be a simple task. It turns out that it is complicated by a great many factors. The most important of these is the question of sampling. The eggs are apparently only laid on solid objects, and the differing sizes and the non-random distribution of these objects are such as to make systematic or random sampling an inaccurate method of obtaining egg-counts. The same factors, of course, preclude the deliberate selection of sample sites, because the eggs are much more easily found on some objects than on others, and in grassy areas it is nearly impossible to locate them at all.

Another complicating factor is the likelihood that water-level changes cause shifts in the sites at which the majority of eggs are laid, as Halstead

& Wagner (referred to above) have shown that most of them are laid above the water—an observation that we have confirmed. This would make repeated collections in the same area unreliable, and changing the collecting site would be worse, as it would add the problem of differing snail densities. We have attempted to avoid some of these difficulties by introducing marked pieces of coconut husk as " standard " egg-laying sites. At least five of these were attached to permanent stakes in each colony under study, and removed to the laboratory at five-day intervals so that the eggs could be removed and counted; then they were replaced at the same sites. We have records of the average number of eggs recovered per husk per day in one colony over a period of one year, and in two other colonies for nine months each. These are shown in Fig. 14.

FIG. 14. RECOVERY OF EGGS FROM COCONUT HUSKS SET IN THREE COLONIES OF *O. QUADRASI*



There are some interesting similarities between the histories for the three colonies, a fact that suggests that the seasonal differences observed are real. The recorded differences, though, are much greater than are the observed changes in snail density (see the discussion of population dynamics, page 523), a combination of circumstances that could only be true if egg-laying and mortality were correlated. It seems likely that this may actually be the case (as is discussed under population dynamics), but we are not sure whether the changes in our index of reproduction are due to changes in rate of egg-laying or to changes in survival of young snails. Wagner, in

the report cited above (see footnote on page 508), reported major fluctuations in egg-production that were apparently not related to factors external to the experiments.

In the laboratory, we have attempted to discover the influence of changing water-levels on the egg-laying habits of *O. quadrasi.* A large glass aquarium was stocked with 240 females and 200 males. Egg-laying sites were provided in the form of a number of coconut husks. The water-level was carefully regulated so as to rise 2.5-3.0 cm during one week, and fall the same amount the next, over a period of nine weeks. Table XX shows that nearly twice as many eggs were laid during weeks of rising water as during those when the level was falling. Inasmuch as fluctuating rather than stationary water-levels are the rule in nature, these observations may provide part of the explanation for the correlation between the reproduction index and rainfall, described below under population dynamics. The observation is in agreement with Wagner's that egg-laying was stimulated by periodic flooding.

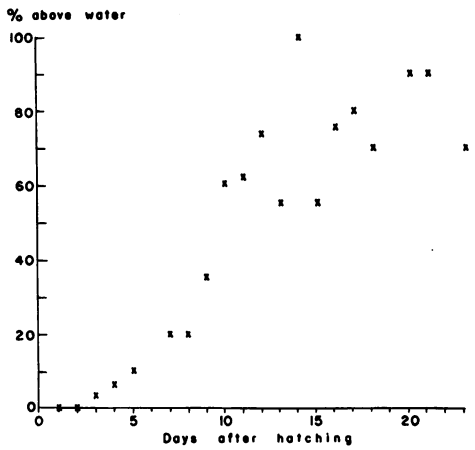
The proportion of eggs that hatch is ordinarily quite high. In our experience, disturbance of the eggs through careless removal from the substrate is the commonest cause of hatching failures. When this has been avoided, up to 96% of the eggs have hatched. The average rate in a number of series of observations was 88%. It seems unlikely that field rates would be much lower than this figure, in spite of occasional losses through disturbance or desiccation.

Survival of O. quadrasi

As has long been recognised, the newly hatched snails are aquatic and remain so for some time. Daily observations on a group of 30 young snails (Fig. 15) showed representative figures for the appearance of snails above the water-line. The data are for the proportion out of the water at 8.00 a.m. over a period of three weeks after hatching. We do not have data on the length of time that they remained above water. There were very few out during the first week, but from the ninth day on, the proportion emerging increased rapidly up to 12-14 days, after which the proportion remained practically constant at about 75%-80%. This is the same as the proportion of all snails above the mud in the field studies. Conditions must have been nearly ideal for these young snails, as there was only one death during the three weeks—by far the best survival that we have observed. This would seem to indicate that they were not stimulated to crawl out of the water at an abnormally early age, and we conclude that two weeks is the duration of the aquatic stage.

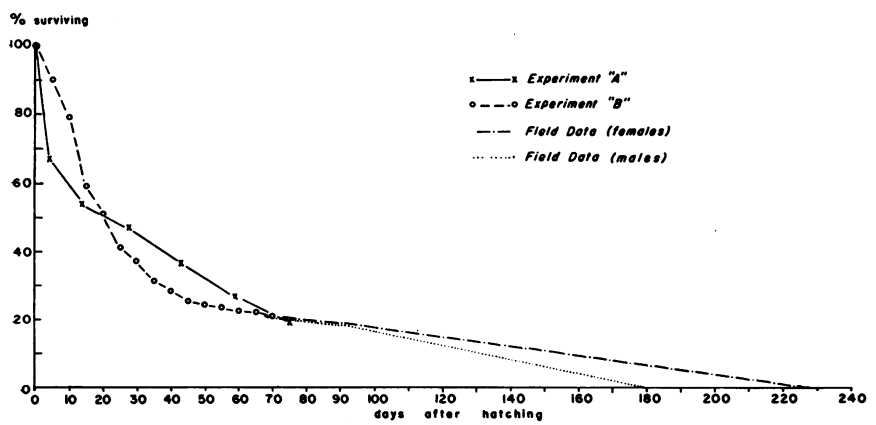
In spite of the success of this experiment, these very young snails are delicate, and mortality rates are higher among them than for any other

FIG. 15. PROPORTION OF YOUNG *O. QUADRASI* ABOVE WATER AT 8.00 A.M. DAILY FOR THREE WEEKS AFTER HATCHING



period in the life-cycle. This is illustrated in Fig. 16, which shows the results of two large-scale laboratory experiments (A and B) and of calculations based on field data. (For further details on field data, see the section on population dynamics below.) In both the experiments, nearly half the snails had died by the end of the aquatic stage. Thereafter, survival improved up through adulthood. The method of obtaining adult mortality figures is given in the following section on population dynamics. Both laboratory experiments showed a survival of about 21% at 70 days after hatching.

FIG. 16. SURVIVAL OF *O. QUADRASI* IN THE FIELD AND THE LABORATORY



The differences prior to that time were evidently due to the differing conditions under which the snails were kept. Those in experiment B were continously supplied with more water than the ones in experiment A, and this apparently resulted in better survival during the aquatic stage but somewhat poorer survival during the next month.

If, as indicated in the preceding section, the average egg production of a female is two eggs in five days and if the mean survival of adult females is 65.8 days (see page 535 et seq.), the average egg production of a female is 26 eggs during its adult life. If 88%, or about 23 of these, hatch, and if 19% of them survive to adulthood (see Fig. 16), then there would be 4.4 adults in the next generation for each adult female. In our field collections, females predominate, constituting some 58% of the total, and this would mean that there would be 2.6 adult females for each female in the preceding generation. This is obviously impossible, on the grounds of both logic and observation, since the populations are relatively stable (see below). Therefore, either fewer eggs are laid in the field than in the laboratory, or survival to adulthood is considerably worse.

We have already shown that the rate of egg-laying in the field is subject to considerable fluctuation, and it is likely that our laboratory observations represent maximal rather than average rates. It is also quite possible that the young snails in experiments A and B had better survival rates than ordinarily occur in the field. We are certain that adult snails have longer lives in the laboratory than in the field (see below).

Population Dynamics

Methods of sampling for snails and of recording data

The purpose of sampling is to determine the characteristics of a population without examining all individuals. The characteristics that are most important in ecological studies are population density and the age and sex structure of the population. For the density to be correctly estimated, areas of differing density must be represented proportionately among the samples. Thus, since our initial knowledge of relative densities is negligible, the samples must be distributed as widely as possible over the area. Therefore, a large number of small samples is preferable in general to a small number of large samples. Theoretically, then, the ideal arrangement would be a grid of evenly spaced small samples covering the entire area under consideration.

In order to meet the requirement of determining the population structure, all individuals present should be recovered from each sampling area. In sampling for small aquatic or semi-aquatic animals such as *O. quadrasi*, there are important difficulties in realizing the ideal. The first of these is the inaccessibility of some parts of the snail colony to the collectors. Even

though the water is ordinarily quite shallow, the mud may be several feet deep, making wading impossible. We have found that the collectors' efficiency can be greatly increased by the use of sportsman's hip boots, with rubberized fabric tops that can be rolled down when not needed (an important consideration in the tropics). Even so, direct collecting is hampered in areas covered by even a small depth of water and becomes impossible where the water is more than 5 cm deep. A second difficulty is in the collecting of young snails, which, as has been shown above, are less than 1 mm long when hatched. Such snails cannot be seen in the mud and debris of their habitat; and direct collecting of snails less than 2.5 mm long is not reliable, because success in finding them is strongly influenced by the character of the collecting site.

While some of the difficulties stated could be overcome by intensification of effort, it was clear that some sort of compromise had to be made in order to meet the requirement of numerous samples. We used two different collecting techniques, the choice depending upon the circumstances and the use to which the data were to be put. Our first method was direct collecting on a series of uniformly spaced sampling sites. At each site a metal ring, 13.5 cm in diameter, is dropped and all the snails inside it are picked up with forceps and placed in a small paper envelope which is identified by an appropriate numbering system. This technique, referred to throughout as ring sampling, was our routine method of collecting. It allowed us to take a large number of samples in a relatively short time, ensuring representation over all or a large part of the snail colony. We preferred to have at least 30 samples before drawing conclusions about the population. The second method used by us was devised to make more areas available to collecting and to recover a larger fraction of the young snails. In using this method, a length of brass tube 13.5 cm in diameter, is pushed into the mud for a distance of 15-30 cm, and the plug thus obtained is washed through a series of sieves with mesh-sizes decreasing from 12.7 mm to 0.833 mm in diameter. The snails are recovered from the screens of finer mesh, either directly or by examining all the contents of these screens under water in a white enamel pan. Difficulties in bringing up the plug can be overcome by inserting a hoe or army entrenching tool beneath the tube.

In six series of trials, tube sampling on the sites already collected by the ring method has added 32.2% to the number of living snails found (Table XXI). Nearly all of these are young snails less than 3 mm long.

Tube sampling is the only method yielding appreciable numbers of dead snails. Against this advantage must be balanced the extra time involved in the tube method. In our hands it takes approximately ten times as long as the ring method to obtain the snails, and this places a severe restriction upon the number of samples that can be taken. We have used tube sampling where depth of water or densely matted grasses make ring collecting impos-

TABLE XXI. NUMBER OF SNAILS ADDED TO RING SAMPLES BY TAKING TUBE SAMPLES OF SPOTS ALREADY EXHAUSTED BY RING METHOD

Colony	Number of samples	Number of living snails	
		recovered by ring	added by tube on same spot
Takurañga	4	6	0
Experimental Creek No. 1	2	1	0
Kilot	3	5	1
Binog	5	66	28
Batang	2	40	4
Naliwatan	10	220	76
Total	26	338	109

sible or unreliable, and where the age structure of the population is of primary importance to us.

In order to avoid the influence of obvious differences in snail densities, sampling sites are selected before going to the field, and we adhere rigidly to the pattern thus determined. This results in occasional groups of negative samples, but we hold that the objectivity obtained by the method more than makes up for the relatively small amount of time lost in examining the negative areas.

The actual pattern of sampling varies with a number of factors. The most common pattern is a linear series of samples, evenly spaced, within a few metres of the edge of the colony. This is especially useful if snail infection rates are the primary goal, since we have found that infection rates vary widely in different parts of the same colony and the linear series spreads the samples maximally, usually covering completely the longest dimension of the colony. This pattern is also satisfactory for determining snail densities in most situations. Where the area covered by the colony is wide, we use either a series of straight-line transects, 50 m apart with samples at 5-m or 10-m intervals, or else a grid of samples spaced 5-10 m apart—the latter in the case of our agricultural experiments. Results are always reported in mean density per sample and are readily converted to estimates for other areas, since a sample covers almost exactly 1/70 m^2. They can thus be compared with information from any other study in which densities are reported on an area basis.

When the snails are brought into the laboratory, they are arranged on examination blocks for measurement. The examination block is of clear plastic $150 \times 80 \times 4$ mm and on it are attached two strips of cellulose tape,

adhesive side up. The snails, arranged by samples, are stuck onto the tape, aperture up, and are measured under a dissecting microscope fitted with an ocular micrometer. Each snail measurement is then entered on a tally sheet, which is printed so that it automatically converts the measurement to the nearest 0.1 mm. For each sample, the records are kept in sequence, so that when the snails are removed from the tape for crushing the same sequence can be maintained, and to the recorded size can be added the sex and the stage of any infection that may be found.

Observations on density

During the period May 1954 to March 1956, a large number of density counts were made. The following figures are a summary of the mean densities obtained in 148 series of samplings in a variety of snail colonies:

Average number of snails per ring	*Number of colonies with observed density*
< 1	4
1.0 - 1.99	17
2.0 - 2.99	10
3.0 - 3.99	21
4.0 - 4.99	30
5.0 - 5.99	17
6.0 - 6.99	14
7.0 - 7.99	9
8.0 - 8.99	10
9.0 - 9.99	6
10.0 - 10.99	3
11.0 - 11.99	3
12.0 - 12.99	1
13.0 and over	3

None of these colonies had been the object of any attempt at snail control. The lowest average density was 0.86 per ring sample, and the highest was 18.12. The largest number of snails collected in one ring sample was 170, but negative samples ordinarily outnumber any other category. The distribution of snails by samples is shown in Table XXII for several different kinds of colony, and for the total of all observations in 10 colonies over a period of 18 months. This long-term study has yielded valuable information about *Oncomelania* populations. It was done in the vicinity of Barrio Malirong, Palo (Fig. 17), where the topography is sufficiently varied to give different kinds of colonies. The results of this study as far as snail densities are concerned, are summarized in Table XXIII. The variations in the number of samples taken at different times in the same colony were made necessary by the requirement of having at least 300 snails for infection rate determinations.

Statistical analysis of the data has been somewhat complicated because the raw data do not yield homogeneous variances. Bliss & Fisher (1953)

TABLE XXII. FREQUENCY DISTRIBUTION OF *O. QUADRASI* BY SAMPLES IN REPRESENTATIVE TYPES OF COLONIES AND IN SUMMATION OF 10 MALIRONG COLONIES OVER 18 MONTHS (MAY 1954-NOVEMBER 1955)

Number of snails per sample	Rice-field No. 1	Agoong Creek (South Bank): 1st recheck	Naliwatan Creek (upstream): 7th recheck	Malirong Swamp 1st recheck	Total for 10 Malirong colonies
0	17	18	4	38	2624
1	31	8	0	9	630
2	56	6	0	3	652
3	46	6	2	4	533
4	35	2	3	2	458
5	40	4	2	2	461
6	20	3	3	2	413
7	17	3	2	4	361
8	11	1	4	2	300
9	9	2	3	2	241
10	8	0	1	1	250
11	3	2	3	0	222
12	3	2	0	2	158
13	2	0	2	1	140
14	1	2	2	1	116
15	0	0	1	2	119
16	0	0	0	1	83
17	0	1	3	0	79
18	0	0	2	1	20
19	0	1	1	1	53
20	1	2	1	0	53
21	—	0	0	0	40
22	—	1	2	0	39
23	—	0	1	0	37
24	—	1	0	1	23
25	—	0	1	0	21
26	—	0	0	0	21
27	—	—	0	1	18
28	—	—	0	0	13
29	—	—	1	—	12
30	—	—	1	—	10
31	—	—	—	—	9
32	—	—	—	—	9
33	—	—	—	—	5
34	—	—	—	—	13
35	—	—	—	—	5
36	—	—	—	—	5
37	—	—	—	—	4
38	—	—	—	—	1
39	—	—	—	—	4
40 and over	—	—	—	—	15
Total	300	65	45	80	8320

have provided a satisfactory method of handling data on field populations. They show that the tendency of organisms to cluster in certain parts of their habitat can be described mathematically by the negative binomial distribution, and provide formulae for the determination of the necessary terms from the data in hand. Using this distribution, we have, in most cases, been able to obtain satisfactory normalizations, and thus to achieve the homogeneous variances that are necessary for statistical comparisons.

In analysing the data for Malirong, there is one additional complication that has been mentioned in the description of sampling techniques. This is the problem of groups of negative samples within the series of samples. Now, while some zero values are to be expected in sampling any clumped population, it is not reasonable that many such samples should occur consecutively in a linear series. The presence of such groups means that we have inadvertently crossed the boundary of the snail-infested area in

FIG. 17. MALIRONG ZONE, PALO

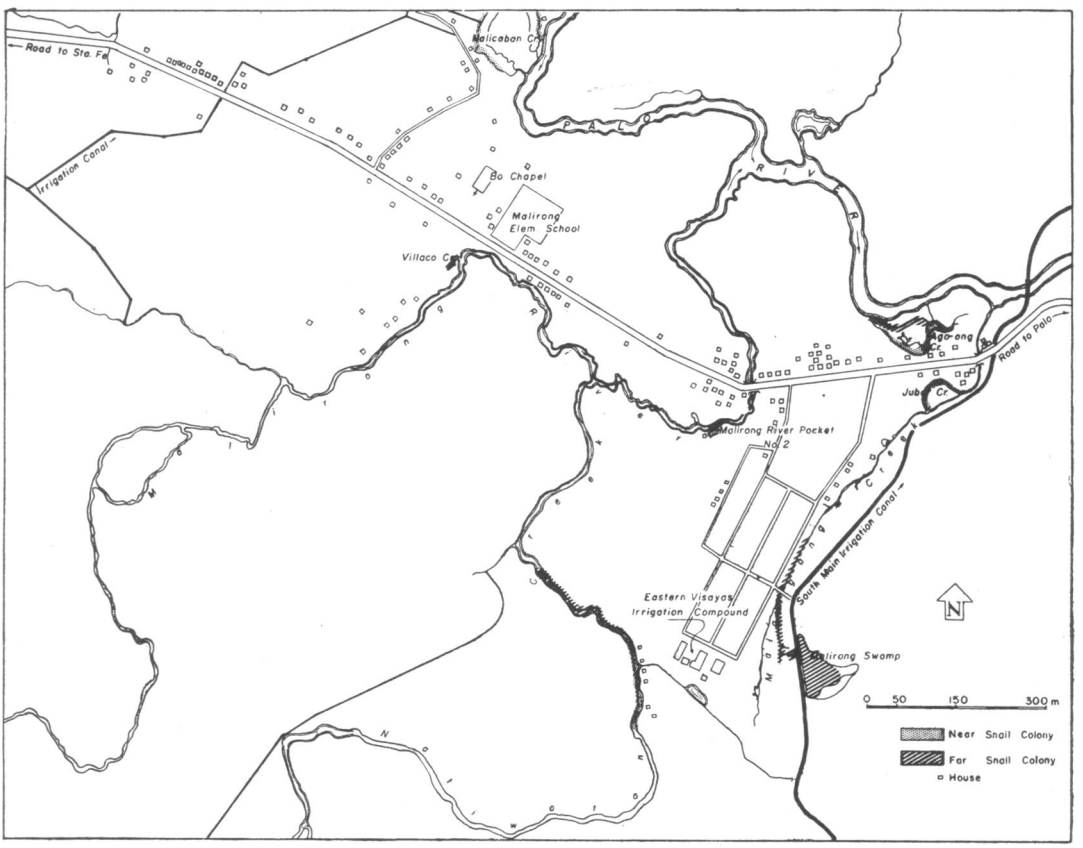


TABLE XXIII. MEAN DENSITIES OF *O. QUADRASI* IN 10 MALIRONG COLONIES (MAY 1954-NOVEMBER 1955)

Colony	1954			1955						Average
	17 May-4 June	16 Aug.-1 Sept.	18 Oct.-11 Nov.	10 Jan.-4 Feb.	22 Mar.-5 April	6 May-8 June	12 July-24 July	15 Aug.-27 Aug.	10 Oct.-9 Nov.	
Agoong South	4.81	5.03	5.92	8.26	7.81	7.21	6.86	2.29	8.80	7.00
Villaco	1.77	4.13	3.39	7.22	4.13	4.34	5.07	5.17	5.82	4.56
Naliwatan upstream	7.93	10.35	7.66	13.88	9.13	9.31	12.68	11.28	11.88	10.46
Nalicaban	1.39	3.12	3.77	4.40	3.16	3.44	4.09	4.18	3.23	3.42
Vicob-Malaigang	10.35	5.06	9.48	7.03	7.62	6.39	7.48	6.80	5.51	7.30
Pocket No. 2	4.59	11.69	6.05	8.28	8.14	8.94	5.21	4.09	3.38	6.70
Naliwatan downstream	7.11	6.37	5.33	6.39	6.88	6.35	5.25	6.27	6.47	6.27
Malirong Swamp	4.36	3.92	4.10	4.47	4.79	4.26	4.26	5.86	4.33	4.48
Agoong North	4.01	5.62	5.58	5.67	8.56	6.18	6.01	8.18	8.58	6.49
Juber	5.30	4.09	3.58	3.32	3.66	4.02	3.69	2.50	6.04	4.02
Average	5.16	5.94	5.49	6.89	6.39	6.04	6.06	6.26	6.40	6.07

TABLE XXIV. CORRECTED MEAN DENSITIES IN 10 MALIRONG COLONIES (MAY 1954-NOVEMBER 1955)

Colony	1954			1955						Average
	17 May-4 June	16 Aug.-1 Sept.	18 Oct.-11 Nov.	10 Jan.-4 Feb.	22 Mar.-5 April	6 May-8 June	12 July-24 July	15 Aug.-27 Aug.	10 Oct.-9 Nov.	
Agoong South	4.97	5.27	6.03	8.26	7.81	7.44	7.43	8.84	8.94	7.22
Villaco	3.83	7.44	6.59	9.28	5.73	6.02	6.99	6.39	8.08	6.71
Naliwatan upstream	8.00	10.35	7.66	13.88	9.34	9.98	14.28	11.81	11.88	10.80
Nalicaban	2.29	4.03	4.24	4.61	3.38	3.76	4.73	5.04	3.68	3.97
Vicob-Malaigang	14.67	5.06	10.09	7.64	7.62	7.09	8.10	7.23	5.72	8.14
Pocket No. 2	5.00	12.37	8.13	8.28	8.38	10.26	6.64	6.88	4.97	7.88
Naliwatan downstream	7.26	7.10	5.69	6.94	7.02	6.29	6.38	7.44	6.88	6.78
Malirong Swamp	4.80	5.51	4.57	4.47	6.51	5.96	5.23	5.87	4.34	5.25
Agoong North	3.92	5.62	5.61	6.05	8.56	6.18	6.77	9.23	9.03	6.78
Juber	5.30	5.68	5.11	3.89	3.95	4.70	3.91	4.07	6.48	4.79
Average	6.01	6.84	6.37	7.33	6.83	6.77	7.05	7.28	7.00	6.83

taking our line of samples. It is, as we have stated above, one of the penalties of maintaining objectivity in sampling. The excessive zero values thus accumulated upset any mathematical distribution, and after careful consideration, we decided to discard all zero samples that did not fall adjacent to a sample containing snails. This resulted in the omission of 1348 samples, and, of course, raises the mean density values accordingly. Table XXIV gives these corrected means for each of the ten colonies over nine different samplings. The distribution obtained after discarding these samples can be fitted reasonably well with the negative binomial. The formula used in normalizing the data is $\sin h^{-1}\sqrt{\frac{x+0.375}{k-0.75}}$ for each successive value of x (number of snails in a sample) (Anscombe, 1948). The constant k can be obtained in one of several ways. In the present case, the formula $k = \frac{\bar{x}^2}{s^2 - \bar{x}}$ gave a value of approximately 1, and this has been used. This transformation comes very close to giving homogeneous variances between the individual samplings, and when four of the colonies are removed, the remaining six do show variance homogeneity. The results of the variance analysis for these six colonies are given in Table XXV. If the failure to obtain

TABLE XXV. COMPLETE ANALYSIS OF VARIANCE IN MEAN DENSITY IN 6 MALIRONG COLONIES FOR 9 DIFFERENT SAMPLING DATES

Source of variance	Degrees of freedom	Sum of squares	Mean square (variance)	F	P
Between colonies	5	98.087	19.617	58.77	$<< 0.005$
Between times	8	20.570	2.571	7.70	$<< 0.005$
Interaction	40	35.338	0.8835	2.65	$<< 0.005$
Residual	4688	1564.758	0.3338		
Total	4741	1718.803			

Conclusion: All differences are highly significant; P is greatly below 0.005.

variance homogeneity is ignored, and the variance analysis is performed for all ten colonies, the results are the same as for the six (Table XXVI).

All differences tested are highly significant; in fact all go completely off the statistical tables available to us. The high significance of the interaction term becomes of special interest, because it shows that the differences between the average densities at different times are more likely due to large fluctuations in a few colonies than to changes common to all or most of

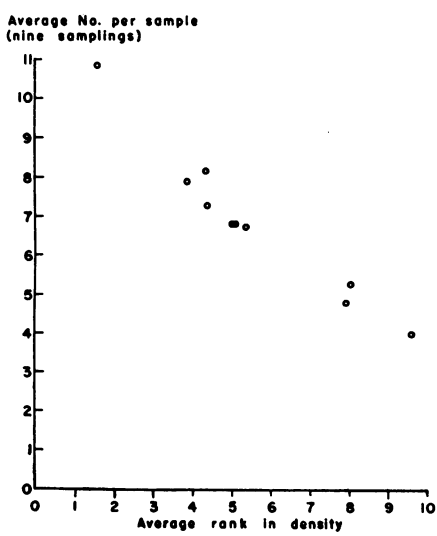
TABLE XXVI. COMPLETE ANALYSIS OF VARIANCE IN MEAN DENSITY OF 10 MALIRONG COLONIES FOR 9 DIFFERENT SAMPLING DATES*

Source of variance	Degrees of freedom	Sum of squares	Mean square (variance)	F	P
Between colonies	9	114.820	12.758	36.69	< 0.005
Between times	8	19.843	2.480	7.13	< 0.005
Interaction	72	63.385	0.880	2.53	< 0.005
Residual	6882	2393.021	0.3477		
Total	6971	2591.069			

* Variance not quite homogeneous.
Conclusion: All differences are highly significant.

the colonies. In other words, the density of snails tends to change independently in different colonies. It would thus be fruitless to search for a common cause for such changes. The differences between colonies, however, appear to have been maintained over the 18 months of this study. A different approach to this statement, though less sound statistically than the variance analysis, is to consider how well the colonies maintain their final rank in average density at the different samplings. If the colonies are ranked according to density for each sampling, and the average of these nine rankings is plotted against the final density for each colony, a linear relationship is obtained (Fig. 18).

FIG. 18. RELATION BETWEEN AVERAGE DENSITY OF *O. QUADRASI* AND AVERAGE RANKING OF DENSITY OVER 9 SAMPLES



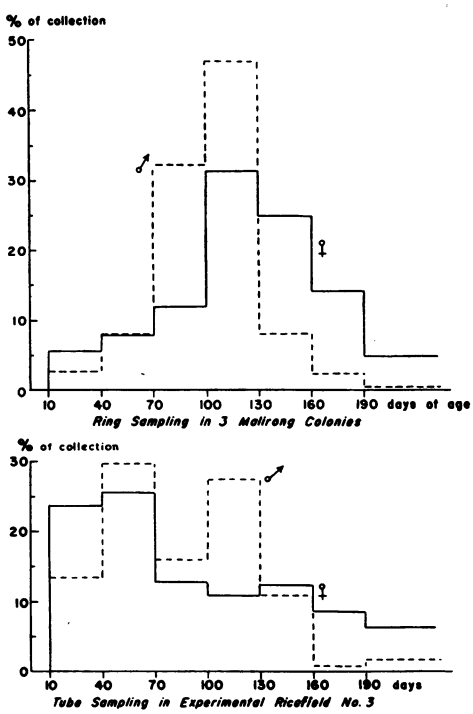
It is clear, then, that the mean density of *O. quadrasi* is a function of place, and is much less dependent upon changes in weather, which would affect all colonies alike.

Population structure

The possession of an accurate growth curve for both sexes, plus information as to the size and sex of each snail collected, enables as to analyse population dynamics in considerably more detail than is possible through

a consideration of mean densities alone. Unfortunately, very young snails cannot be collected at all, and snails are collectable in proportion to their size up to 2.0-2.5 mm. However, since males become sexually mature at 3.0 mm and females at 3.5 mm, there is always a number of collectable immature snails. Of course, tube sampling improves the collection of young snails and provides something of a standard against which to judge

FIG. 19. AGE DISTRIBUTION OF *O. QUADRASI* OBTAINED BY TWO DIFFERENT METHODS OF SAMPLING

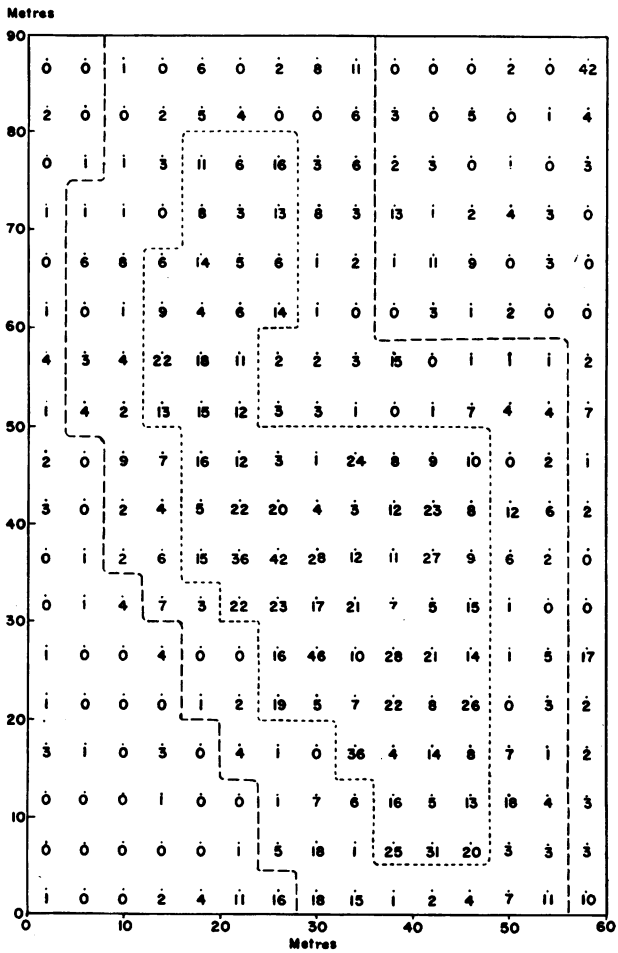


the results of ring sampling. Our most extensive set of tube samples in an undisturbed area is the 270 samples taken for baseline data in experimental rice-field No. 3. The age distribution of snails in this set of samples is shown in Fig. 19. For comparison we include the age distribution of snails collected by ring sampling in three Malirong colonies at approximately the same time. The comparison makes it clear that ring sampling has missed a large proportion of the snails less than 70 days old. Even tube sampling fails to recover snails less than 10 days old, and there is an apparent deficiency in the 10-39-day age-group—a deficiency of around 25% of all snails present aged 10 days and older. The 25% estimate is based on

a comparison of the observed age-distribution curves and the survival curve (Fig. 16). Ring sampling, then, must be only around 55%-60% effective in recovering all snails present.

Such age-distribution curves change with intensity of breeding and change in survival rates. For example the " humps " in the rice-field data at around 130 days reflect a wave of breeding in June. We shall return to this point below. What concerns us here is the observation that the size distribution is not constant for all parts of the colony. Experimental rice-field No. 3 exemplifies this phenomenon best, because the sampling is as

FIG. 20. DISTRIBUTION OF *O. QUADRASI* IN EXPERIMENTAL RICE-FIELD NO. 3 *



* The location of each tube sample and the number of living snails obtained are shown. The outlined areas represent zones of differing densities, as explained in the text.

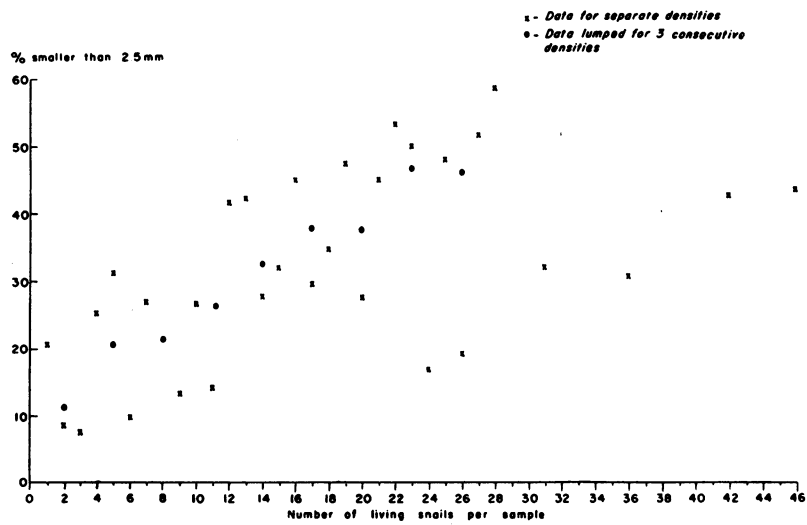
nearly ideal as we can achieve (a large number of tube samples arranged in a grid pattern). The arrangement of samples and the number of living snails recovered from each are shown in Fig. 20. There is an area of high density running diagonally across the field, and it is surrounded by zones of decreasing numbers of snails. For convenience, we have separated the field into the three areas delimited by the dotted line, the broken line, and the edge of the field. In considering the age structure of the population in these three areas, we have used the proportion of snails that fall below 2.5 mm in size, or up to about 50 days of age. The area in the middle of the field, where the density is highest (14.74 per sample), contains 38% young snails; the zone next to it, with 3.8 per sample, contains 19% young; and the remainder, with 2.38 per sample, contains 23% young. It is noted that if the seven highest samples are removed from this last group, the percentage of young becomes 14.4, and the mean density falls to 1.27 per sample.

The difference in proportion of young between the central area and the other two is not due solely to the difference in mean density. If we consider only the samples that contained the same number of snails, omitting those thickly populated samples from the dense area that were not matched in the periphery, the central area still has almost twice as many young. For example, taking only those samples with 5-12 snails, the two areas have comparable numbers of each. The central area has 28 such samples, containing a total of 231 snails, of which 29.44% are less than 2.5 mm long; in the peripheral area, the 30 samples containing 5-12 snails (total of 225 individuals) have 15.11% young.

This relation between density and proportion of young snails holds between individual samples (Fig. 21). There is a linear relation up to about 25 snails per sample. Above that figure, the relationship may not hold, but we do not have enough samples with high densities to be sure. In considering possible reasons why density and age structure of the population should be related in this way, we have given thought to several factors: (1) egg production and survival, (2) survival of young, (3) survival of adults, and (4) migration. In order to produce the observed relationship, at least two of these factors must be different in the two places, since our evidence is that population densities are essentially stable, and these would change if only one factor were different in the two areas. In a succeeding section (Fig. 22 and 23), we present evidence that adult survival is inversely related to the production and survival of young. If in the densely populated central area adult survival is relatively low and reproduction rates and survival of young are high, the resulting population will have a higher proportion of young snails than the surrounding area, where adult survival is higher and reproduction and survival of young are low.

Snail movement seems unlikely to produce significant effects upon age structure of adjacent populations. From our 24-hour studies on snail

FIG. 21. RELATION BETWEEN NUMBER OF *O. QUADRASI* PER SAMPLE AND PROPORTION OF SNAILS SMALLER THAN 2.5 MM *



* Data taken from Experimental Rice-field No. 3 before initial farming.

activity, we know that the average position of snails after 10 minutes is 0.61 cm from their original position (Table VIII). Assuming that their dispersal from any starting-point is essentially random, the time required for them to reach an average distance away will be proportional to the square of that distance. Thus, for snails to spread 61 cm, or an average distance 100 times as great as that reached in 10 minutes, would require 10 minutes $\times (100)^2$, or 100 000 minutes—about 69 days. Since only about 21 % of an initial population of snails survive this long (Fig. 16), and since 61 cm is a negligible distance in terms of the field under consideration, we reject movement of snails as a means whereby the relation between density and age structure can be maintained between parts of the colony. Oriented movements would, of course, produce greater average dispersals, but the absence of centrifugal orienting influences and the adequacy of differential survival and differential reproduction as an explanation make the hypothesis of migration unlikely.

In areas where ring sampling alone has been used, the proportion of young snails collected is much lower than the figures quoted above, but the relation between age structure and population density still holds. This has been considered in detail in Villaco colony, where we have made 12 samplings since the start of the Malirong study. The north side of this colony has consistently shown a greater density of snails than the south side. Table XXVII shows the mean density and the proportion of young snails for the two sides for each sampling. The south side never had as

TABLE XXVII. RELATION BETWEEN DENSITY AND PROPORTION OF SNAILS LESS THAN 2.5 MM. LONG FOR TWO PARTS OF VILLACO COLONY IN 12 SAMPLINGS (MAY 1954-NOVEMBER 1955)

		Villaco colony			
		north bank		south bank	
		percentage of young	average number per ring	percentage of young	average number per ring
1954	20 May	3	2.77	1	1.15
	20 Aug.	4	8.45	0	1.39
	28 Oct.	4	7.00	3	1.04
1955	4 Feb.	2	9.68	2	6.12
	3 Mar.	2	8.81	0	5.63
	5 April	7	5.40	2	3.37
	8 June	5	6.83	8	2.78
	22 July	10	8.63	1	2.84
	27 Aug.	4	8.11	1	3.24
	30 Sept.	9	7.63	16	2.04
	4 Nov.	8	7.97	6	4.48
	23 Nov.	8	5.67	4	3.98
Total		5.6	7.25	3.4	3.16

great a density as the north, and on only two occasions did it have a higher proportion of young. It is clear from these data that the differences in population structure in different parts of a colony tend to be maintained over long periods, as are differences in density.

Rather surprisingly, these relationships hold only for the different parts of a colony. When 10 different colonies are compared, the mean density and population structure are unrelated. The probable reasons for this will be discussed below.

Although it is not universal, there is a tendency for the different colonies to change in the same direction, as far as the proportion of young snails is concerned. The proportions for each colony at each sampling are shown in Table XXVIII.

TABLE XXVIII. PROPORTION OF YOUNG SNAILS IN 10 MALIRONG COLONIES FOR 10 SAMPLINGS (MAY 1954-JANUARY 1956)

Colony	1954			1955						1956	Average
	17 May-4 June	16 Aug.-1 Sept.	18 Oct.-11 Nov.	10 Jan.-4 Feb.	22 Mar.-5 April	6 May-8 June	12 July-22 July	15 Aug.-27 Aug.	10 Oct.-9 Nov.	3 Dec.-23 Jan.	
Agoong South	2	6	4	10	5	12	20	3	5	9	8.0
Villaco	2	3	4	2	4	6	7	3	7	4	4.6
Naliwatan upstream	4	3	18	8	3	3	6	1	4	13	5.8
Nalicaban	10	8	9	4	10	5	9	5	5	13	7.6
Vicob-Malaigang	5	3	3	8	5	1	5	1	0	2	3.4
Pocket No. 2	14	8	3	4	2	7	5	9	6	8	6.3
Naliwatan downstream	5	2	29	10	4	6	5	3	3	5	6.8
Malirong Swamp	3	1	1	4	9	3	4	6	4	15	5.1
Agoong North	3	12	8	6	0	8	11	4	1	11	5.8
Juber	1	2	3	2	4	7	4	3	1	9	3.4
Average	5.4	5.1	7.3	5.3	4.6	6.2	8.2	3.8	4.0	9.0	5.8

We conclude, therefore, that population structure, like density, is characteristic of a place, and that month-to-month changes in population structure may be quite large.

Reproduction rates and survival in the field

The relationship between mean density and proportion of young snails, which provides such a satisfactory explanation of differing densities within a colony, does not hold between colonies, and therefore survival of young snails cannot be the only explanation of differences in density from one colony to another. The maintenance of different population structures independently of density, then, must depend upon differences in survival rates for both young and adult snails. Both must be involved, since equal survival rates for adults and different rates for young, or *vice versa*, would result in a direct relation between population structure and density and this situation clearly does not obtain.

From our successive age-distribution curves, we can make quite accurate calculations of the mortality rates of adult snails in the 10 Malirong colonies for each interval between samplings. This is done by considering the number of adults at any sampling as a starting-point, and by reference to the growth table (Table XVII) calculating the size to which the smallest adult would have grown by the time of the next sampling. The number of snails of this expected size and larger is then subtracted from the starting number to give the total loss. The ratio between this number lost and the original number is the percentage mortality. Then, since the time intervals between samplings are not always the same, this percentage mortality is divided by the number of days between the two samplings to give the average daily mortality rate. These average daily mortality rates for all 10 colonies are shown in Table XXIX. The sexes are separated for several reasons. Males reach maturity at a smaller size and grow more slowly than females and, as the table shows, have a considerably higher mortality rate than females. With the average daily mortality rate of 0.76%, the average female *O. quadrasi* lives for 65.8 days after reaching maturity; the average adult male lives for only 47.6 days. This is the obvious explanation for the unbalanced sex ratio that we have consistently found in our field collections. This has averaged 1.4 females per male for the 10 colonies over the 18 months of observation.

It comes as something of a surprise to find such short average lives in the field, since it is rather easy to keep snails much longer in the laboratory (Ward, Travis & Rue, 1947). Laboratory snails, however, do escape a number of hazards to which field snails are subject, such as being carried away by rising water, predation, and local changes of the habitat by large animals such as wallowing carabaos.

TABLE XXIX. AVERAGE DAILY MORTALITY RATES (%) FOR ADULT *O. QUADRASI* IN 10 MALIRONG COLONIES FOR NINE INTERVALS BETWEEN SAMPLINGS (MAY 1954-JANUARY 1956)

Colony	1954			1955					1956	Average
	17 May-1 Sept.	16 Aug.-11 Nov.	18 Oct.-4 Feb.	10 Jan.-5 April	22 Mar.-8 June	6 May-22 July	12 June-27 Aug.	15 Aug.-9 Nov.	9 Nov.-23 Jan.	
	Adult females									
Agoong South	0.98	0.65	0.85	0.42	0.94	1.44	0	1.13	1.78	0.90
Malirong Swamp	0.73	0.98	0.83	0.85	0.83	0.70	0	0.91	0.68	0.76
Naliwatan upstream	0.94	1.27	0.63	0.88	0.57	0.49	0.77	0.32	1.30	0.83
Villaco Creek	0.83	1.02	0.06	0.92	0.89	0.62	0.59	0.94	1.15	0.80
Juber Creek	0.64	0.49	0.57	0	0.86	0.66	0.50	0.22	0.79	0.55
Naliwatan downstream	0.90	1.11	0.86	0.19	1.22	0.86	0.52	0.77	1.17	0.91
Vicob-Malaigang	0.99	0.30	0.86	0.60	0.88	0.64	0.33	0.48	0.72	0.72
Agoong North	1.01	1.09	1.06	0	1.09	1.22	0	0.61	1.25	0.82
Nalicaban Creek	0.91	0	0.63	0.91	0.68	0.90	0.44	1.19	1.05	0.74
Malirong Pocket No. 2	0.53	0.71	0.60	0.34	0.56	0.92	1.36	0.35	0.60	0.63
Average	0.85	0.76	0.70	0.51	0.85	0.85	0.45	0.69	1.05	0.76

	Adult males									
Agoong South	1.05	1.21	1.09	0.40	0.96	1.60	0.55	1.26	2.08	1.13
Malirong Swamp	1.01	1.31	1.00	1.06	0.66	1.26	0	1.28	1.10	1.02
Naliwatan upstream	1.00	1.32	1.01	1.12	0.78	0.51	0.77	1.36	1.41	1.06
Villaco Creek	0.76	1.21	0.77	1.21	1.15	0.80	0.95	1.35	1.60	1.12
Juber Creek	0.70	0.96	0.89	0.09	1.14	0	1.14	1.04	1.00	0.81
Naliwatan downstream	1.11	1.22	1.14	1.08	1.26	1.40	0.87	1.37	1.29	1.22
Vicob-Malaigang	0.94	1.17	1.02	1.23	0.25	1.08	0.48	1.16	0.52	0.99
Agoong North	1.01	1.33	0.99	0.34	1.16	1.21	0	1.15	1.28	0.96
Nalicaban Creek	1.05	1.30	1.07	1.03	1.08	0.99	1.61	1.69	1.18	1.18
Malirong Pocket No. 2	0.93	1.39	0.96	0.81	0.78	1.19	1.18	0.89	0.90	0.99
Average	0.96	1.24	0.99	0.84	0.92	1.00	0.76	1.26	1.24	1.05
	Total adult snails									
Agoong South	1.01	0.93	0.97	0.40	0.96	1.53	0.23	1.20	1.93	1.01
Malirong Swamp	0.86	1.13	0.91	0.94	0.77	0.93	0	1.09	0.88	0.88
Naliwatan upstream	0.96	1.29	0.82	1.09	0.65	0.50	0.77	0.95	1.35	0.94
Villaco Creek	0.79	1.10	0.41	1.04	0.98	0.70	0.77	1.14	1.36	0.94
Juber Creek	0.67	0.72	0.72	0	0.98	0.32	0.85	0.58	0.87	0.66
Naliwatan downstream	1.00	1.15	0.99	0.69	1.24	1.12	0.67	1.01	1.23	1.05
Vicob-Malaigang	0.97	0.77	0.94	0.92	0.63	0.88	0.41	0.81	0.92	0.85
Agoong North	1.01	1.22	1.03	0	1.11	1.22	0	0.90	1.26	0.88
Nalicaban Creek	0.98	0.55	0.82	0.95	0.90	0.95	1.18	1.48	1.11	0.95
Malirong Pocket No. 2	0.68	1.09	0.76	0.56	0.66	1.04	1.28	0.61	0.73	0.80
Average	0.89	1.00	0.84	0.66	0.89	0.92	0.62	0.98	1.16	0.90

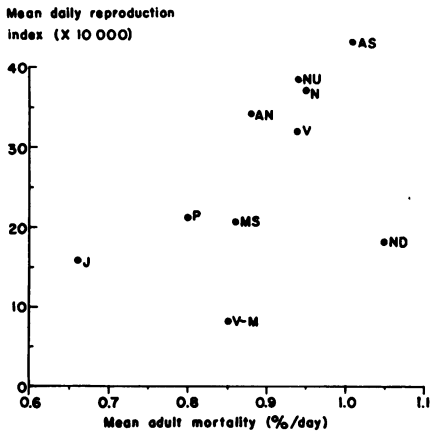
For whatever reasons, mortality rates of adult snails show considerable differences among the colonies studied. These differences are large enough to have a material effect on both density and population structure.

Reproduction and the survival of immature snails, as noted above, must also enter the picture. Neither egg-laying nor survival of young snails in the field can be determined with the accuracy of adult mortality rates. The best that we can obtain is an index of a combination of the two. This is done by dividing the number of adult female snails at one sampling into the number of young at the next. The number of young is determined by reference to the growth curve, which gives the maximum size that a young snail could have reached if the egg were laid on the day that the adult females were counted. The ratio thus obtained is divided by the number of days between samplings to obtain the mean daily reproduction index. The number of days must not be less than 55 nor more than 85, or else the collectability of the young will influence the data enough to invalidate them. This requirement restricts the number of intervals for which valid indices can be obtained. The results of these calculations are given in Table XXX. The figures are not related to mean density, but they are related to the mean daily mortality rates for adults. This relationship is shown in Fig. 22. The two colonies with an unusually high adult mortality in relation to the reproduction index are both along streams and are thus in positions to have

TABLE XXX. MEAN DAILY REPRODUCTION INDEX (x 10 000) FOR 10 MALIRONG COLONIES FOR 7 INTERVALS BETWEEN SAMPLINGS (AUGUST 1954-NOVEMBER 1955)

Colony	1954		1955					Weighted average
	16 Aug.-11 Nov.	18 Oct.-4 Feb.	10 Jan.-15 April	22 March-8 June	6 July-22 July	24 Sept.-9 Nov.	18 Nov.-23 Nov.	
Agoong South	26	72	16	61	22	43		39
Villaco	15		6.1			27		16
Naliwatan upstream	48	95	1	20		30	28	34
Nalicaban	54	36	25		16	15	55	35
Vicob-Malaigang	3	30		10	2		4.8	15
Pocket No. 2	8.5	42	9.2	12		16	40	21
Naliwatan downstream	13	33	16	23		7.6	17	29
Malirong Swamp	3	27	43	16		4.8	21	20
Agoong North	40	31	0.6	23	13		17	19
Juber	12	22			14	6.8	20	17
Average	22.25	43.11	15.73	23.57	13.40	18.77	25.35	23.16

FIG. 22. RELATION BETWEEN REPRODUCTION INDEX AND ADULT MORTALITY IN 10 COLONIES OF *O. QUADRASI*



AS = Agoong South; NU = Naliwatan upstream; N = Nalicaban; AN = Agoong North; V = Villaco; P = Pocket No. 2; MS = Malirong Swamp; ND = Naliwatan downstream; J = Juber; V-M = Vicob-Malaigang

their populations strongly affected by flowing water, which would introduce and carry away unpredictable numbers of snails. Only one of the other eight colonies is so situated, and it has more gently sloping banks and a less rapid flow.

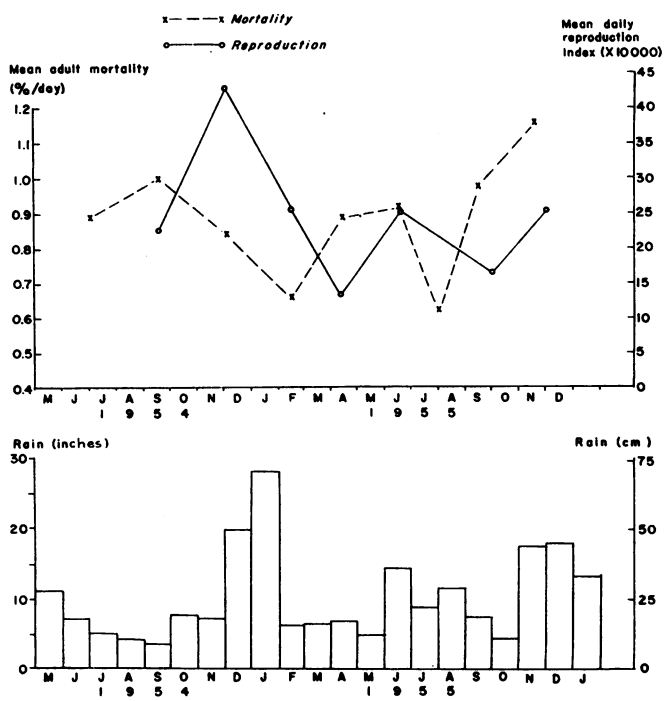
This relation between reproduction and adult mortality in different colonies provides a satisfactory explanation for the fact that these colonies fail to show a relation between mean density and population structure. If the same conditions operate to increase the number of young and limit the number of adults, and *vice versa*, the density would come into balance at a level that could be predicted if we had data on the absolute rates of production and survival of young. What we have, however, are indices that are valid only for comparison purposes.

There is some evidence that the same weather conditions affect both reproduction and adult mortality. Fig. 23 shows the changes in both compared with monthly rainfall data for the 18 months of the study. The two lines apparently follow the same trends, with reproduction lagging behind adult mortality. Rainfall seems to stimulate reproduction, but at the same time increases adult mortality. The balance between these opposing factors is evidently different for different colonies, and in this we have the most likely explanation for the fact that they do not behave alike as far as mean densities are concerned.

Potential repopulation of snail colonies

Before snail control measures can be properly evaluated, it is important to consider how long they will remain effective. Because in general practice few operations are likely to result in complete eradication of snails from a colony, and because of the possibility of reintroduction from uncontrolled areas, we have calculated the time required for surviving or reintroduced snails to repopulate a colony to various proportions of its original density. The information necessary to make these calculations has been presented in preceding sections. We know the rate of egg-laying, the rate of survival and the time required from egg-laying to maturity, and the average length of

FIG. 23. CHANGES IN AVERAGE REPRODUCTION RATES AND ADULT MORTALITY RATES FOR 10 COLONIES OF *O. QUADRASI*, AND THE RELATION OF BOTH TO MONTHLY RAINFALL



adult life of female snails. From survival rates in the laboratory, we conclude that the young snails, especially those in the aquatic stage, are most delicate and most susceptible to any sort of control measure. It follows that most or all of the survivors after control will be adult snails.

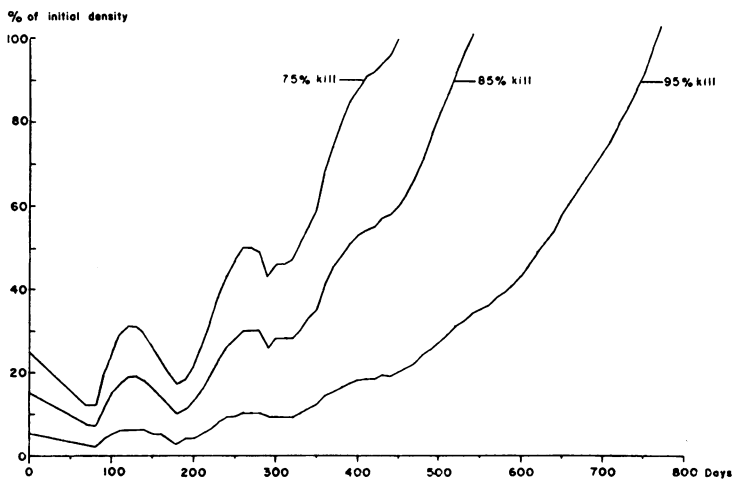
In calculating repopulation rates, it is most convenient to start with a finite population. Other studies have shown that continuous presence of males is not necessary, as females, once they are laying fertile eggs, continue to do so for three or more months, or longer than their average adult life. Therefore, we do not need to allow for continuous or repeated contact between the sexes.

If we start by assuming that 100 female snails survive the control measure, the area upon which these snails are located can be adjusted for different degrees of success of the control measure. For example, if we assume an initial density of five snails per ring sample, from our population structures (see page 528 and Fig. 19) we calculate that there would be 150 adult females per square metre; and 100 adult females remaining on the same area would represent only a one-third (33%) reduction of adults.

If we assume that only 100 remain on 5 m^2, the reduction of adults would amount to 86.7%. These would represent considerably higher killing rates for all collectable snails, and much higher rates for all snails present, since we have assumed above that only adults would remain. The 100 adult females remaining would probably be accompanied by 82 adult males, leaving 182 out of the initial collectable population of 350 per square metre. Instead of 33% and 86.7% then, our estimated success would be 48% and 90%, respectively, of the collectable population. On the basis of the estimated total population, the rates would be 78% and 95.6%. In our succeeding calculations, we have only considered the population collectable by the ring method, since these are the actual figures that we obtain. This means that in calculating repopulation rates, we must allow the last generation to reach an age of about 60 days, at which time they are more than 2.5 mm long and therefore all collectable by the ring method.

One factor that we cannot estimate is the influence of density upon survival rates. The survival rates that we have observed in field and laboratory are those that hold when the population is more or less in balance—that is, when densities are somewhere near the maximum that the habitat can support. It seems reasonable to suppose that these survival rates are considerably lower than those that would be found in snails widely scattered in a favourable environment. Without direct observation, it is not possible to predict these survival rates, but we can make various assumptions, and thus obtain some idea of what to expect. The first assumption, of course, is that survival rates will remain as we have observed them under field conditions. Calculated repopulation curves following 75%, 85% and

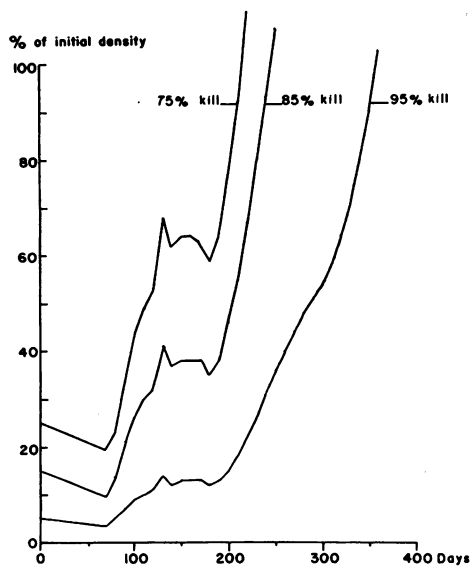
FIG. 24. CALCULATED REPOPULATION CURVES OF *O. QUADRASI*, BASED ON OBSERVED SURVIVAL AND REPRODUCTION RATES



95% killings are shown in Fig. 24. The rates are slower than they should be as indicated by previous experience in the Philippines and by other workers elsewhere (see below), and this is probably due to the above-noted likelihood of an increase in survival rates under conditions of low population densities.

It is even more unrealistic to assume perfect survival, but, for comparison, under such conditions the population would recover its original density from a 75% killing in 90 days, from 85% killing in 105 days, and from 95% in 175 days. We may consider these two assumptions as the extremes of what is likely to occur. A somewhat more reasonable assumption would be that shortly after the control measures survival would be around 50% better than that observed under normal conditions. The calculated recoveries are plotted in Fig. 25. It should be pointed out that the

FIG. 25. CALCULATED REPOPULATION CURVES OF *O. QUADRASI*, BASED ON OBSERVED REPRODUCTION RATES AND SURVIVAL 50% BETTER THAN OBSERVED

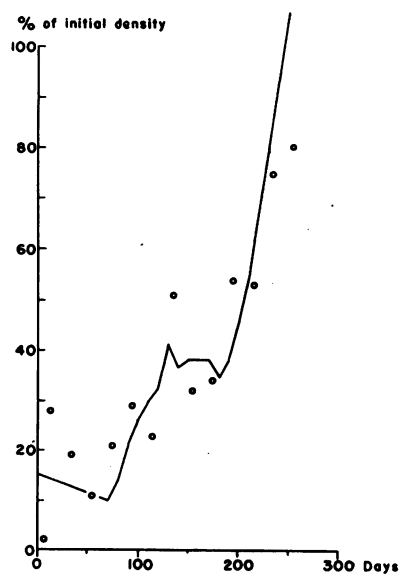


survival rate will probably be reduced as recovery approaches 100%. We have not allowed for this, because in practice control measures would need to be applied again before recovery had proceeded so far.

In both sets of curves, the effect of assuming that only adults survive is seen in the waves that reflect succeeding generations of snails. The initial decline represents the mortality of adults that takes place before the first generation of young grow large enough to become collectable.

It was of considerable interest to us, after making these calculations, to compare them with observations on actual control measures. Because our attempted control measures have all involved radical changes in the habitat, the data that we have obtained are not applicable. However, during the years 1950-52 the Division of Schistosomiasis carried out extensive campaigns of snail control, using the newer molluscicides, sodium pentachlorophenate and DN-1. The former is a herbicide, and so alters the habitat temporarily, but since the dead vegetation was not removed, conditions were suitable for snails as soon as the immediate effect of the chemical was dissipated. The data from these campaigns are available to us, and we have selected those from Sorsogon Province as being the most complete. Of the 118 colonies known to exist in that province, 111 were subjected to molluscicide operations during 1950-52. Unfortunately, snail densities both before and after treatment were recorded as estimated by the technicians, and absolute values are not available. The estimates are low, and we have used the upper limit of the range in calculating the proportion of the original density present at different follow-up times after applying the chemicals. In all, 328 separate molluscicidings were carried out on the 111 colonies.

FIG. 26. AVERAGE OBSERVED RECOVERY OF *O. QUADRASI* COLONIES IN SORSOGON PROVINCE COMPARED WITH THEORETICAL REPOPULATION FROM AN INITIAL KILL OF 85% AS SHOWN IN FIG. 25



Of the 328, some 55% were sufficiently successful (as judged by the first post-treatment follow-up) for us to use in calculating the repopulation rates. It is difficult to obtain exact figures on the initial success of these operations, not only because the snail densities were recorded as estimates, but also because for two or three weeks after application of the chemical dead or dying snails were recorded as having survived. This factor results in an apparent steep decline in density during the first weeks after treatment. We estimate that the 179 treatments used for our calculations killed between 80% and 90% of the collectable snails. The average history of these colonies after treatment is shown in Fig. 26. The predicted history, following a kill of 85% and with a survival rate 50% better than that observed in undisturbed colonies, fits the observed points remarkably

well. The observed points show the same initial decline (steeper than predicted because of the inclusion of dead and dying snails), the same wave of first generation young, and the same final sharp rise as the calculated curve. This provides good independent confirmation of our statements about the life cycle of *O. quadrasi*, especially reproduction rates and length of life.

Thus confirmed by observation, the calculated curves in Fig. 25 are of great value in deciding among possible control measures. It is obvious that any method, such as the use of molluscicides, that leaves the habitat suitable for repopulation will have to be repeated at fairly frequent intervals. We do not have enough information to state the permissible density to which the colonies can be allowed to return, but, to ensure interruption of disease transmission, this permissible density can scarcely be greater than 20%-30% of normal. To maintain even these densities, the curves tell us that the chemical would need to be applied before the peak of the first generation of young, or at intervals of about 120 days—three times a year.

It follows from the above presentation that prevention of breeding is more important than success in killing the snails that are present. The potential for repopulation can only be attacked through radical alteration of the habitat, making further breeding impossible or reducing it to the point where it does not keep pace with normal mortality rates. Under these circumstances, the population of snails would disappear, even though the initial effect of the control measure might appear slight. Moreover, once the original changes in the habitat have been made, maintenance costs should be slight compared to the cost of repeated application of molluscicide. If the land thus changed could then be put into agricultural production, the repeated disturbance of farming might well hasten the disappearance of snails and further reduce the cost of maintenance.

INTERRELATIONSHIP BETWEEN *SCHISTOSOMA JAPONICUM* AND ITS MOLLUSCAN HOST

The fact that the control project[a] was established in a highly endemic area, where detailed field and laboratory studies could be carried on over a period of more than two years, has given us a unique opportunity to make long-term investigations of a fundamental nature. These investigations have included studies on the effect of the parasite on the snail's reproduction, growth, and longevity, and on the history of the parasitism in the snail, as well as studies on seasonal changes in infection rates in the intermediate host. We have also investigated points in the interrelationship

[a] Details of control measures will be given in another paper to be published later.

of parasite and snail host that did not require prolonged studies, but which seemed from the literature to require further elucidation.

Early workers, especially Faust & Meleney (1924), had described thoroughly the development of the parasite in the snail. The annual cycle of snail infection rates and its probable cause was described briefly by McMullen (1947); and Hunter et al. (1947) reported on the susceptibility of *O. quadrasi* to infection and the mortality rates of infected snails. Bauman, Bennett & Ingalls (1948) described the diurnal cycle of emergence of cercariae.

Laboratory Procedure for Infecting Snails

The method employed for infecting snails in the laboratory consists of putting the snails and miracidia together in a drop of water on a watch-glass. The disappearance of the miracidia after a lapse of time, usually 20-30 minutes, is taken as an indication that penetration was successful. As a speedy process for infecting a large series of snails, this procedure may be practicable, although some accuracy is sacrificed. But for studying details associated with the process, we designed another procedure, as follows.

Active young snails measuring at least 2.0-2.5 mm are selected from our hatchery. The sex of the snail is determined by using the Wong & Wagner method (1954) whereby the snails are submerged in water; this stimulates them to come out of the shell faster, so that the absence or presence of the penis can be confirmed.

Fresh miracidia hatched from the eggs are collected individually with the aid of a micropipette and transferred with as little water as possible to a watch-glass containing the snail. All that happens to the miracidia and snail thereafter is watched continuously under a dissecting microscope and observations recorded.

It was observed that various organs of the snails were attacked by the miracidia—namely, the foot region, just above the eyebrow, proboscis, tentacles, etc.—and oftentimes they would swim straight into the shell and disappear. Occasionally they would appear to be stimulated by some substance emanating from the snail. Some were noted to attach and detach themselves from the snail many times until they became weak and died. Those snails which were successfully penetrated, however, were kept in aqua-terraria several weeks until cercaria were expected to mature and to be shed from the snail. By this time, each snail was placed in an individual container so that proper counting of the cercariae could be made daily until the snail died. Occasionally, when the snail was already weak, it was crushed to examine all stages in it.

Using this technique we have obtained cercariae from 44% of snails penetrated by one miracidium and 75% of snails penetrated by five miracidia.

Analysis of Infection Rate by Sex

In examining our field data we noted that more female snails were infected than males (see Table XXXI). From the great number of snails observed the differences appear important. The general infection rate obtained for males was 4.04%, while that for females was 5.16%. The difference is even more remarkable in the case of the collection from Takurañga stream, where no male snail was found infected out of a total of 815; while among the females, 12 out of 1056, or 1.13%, were infected. Statistical analysis of these figures showed that this difference in infection rates was not due to chance.

TABLE XXXI. ANALYSIS OF INFECTION RATES IN VARIOUS COLONIES IN PALO COLLECTED FROM JULY 1953 TO MARCH 1956

Colony	Sex	Total collected	Number infected	Percentage infected
Takurañga	Male	815	0	—
	Female	1 056	12	1.13
Binog	Male	3 026	46	1.52
	Female	3 895	121	3.11
Gacao	Male	4 972	35	0.70
	Female	5 762	63	1.09
Kilot	Male	920	10	1.08
	Female	1 112	21	1.89
10 Malirong colonies	Male	24 465	1 292	5.28
	Female	33 036	2 304	6.97
	Male	34 198	1 383	4.04
	Female	48 861	2 521	5.16
Total	Male + Female	83 059	3 904	4.70

This difference must be due to either physiological or ecological factors, and we have considered the following possible explanations:

(*a*) males might be more resistant to penetration by the miracidia than females;

(*b*) miracidia do not develop to cercariae as well in males as in females;

(*c*) miracidia are more attracted to females than to males;

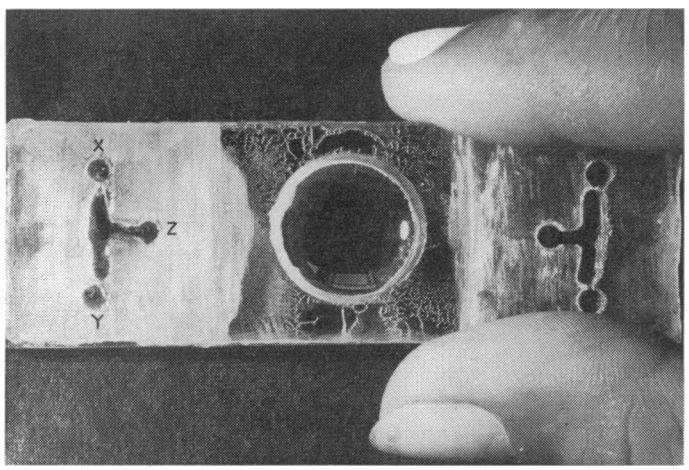
(*d*) males may have different habits from females, i.e., the males may be out of the water more of the time and hence not exposed so frequently as females.

In the laboratory, we have records for 62 snails infected with a single miracidium; it took about nine weeks before mature cercariae were liberated, and the normal range is from 53 to 70 days. Unusual cases required as long as 109 days. These differences may be due to the ease or difficulty with which the miracidium penetrates the snail, to reaction of the host, or to ecological factors affecting the snails. The data are as follows:

	Number of snails successfully penetrated	*Number of snails infected*	*Percentage of snails infected*
Male snails	25	9	36
Female snails	37	18	49
Total snails	62	27	44

We have devised a method by which to test this further. Using plastic material, we designed a T or Y maze as shown in Fig. 27. The apparatus is made up of two pieces of plastic glued together. The upper piece is cut, drilled and sanded, the sides of the grooves being slanted to allow proper observations of snails and miracidium. The lower piece is plain, exactly similar to the ordinary glass slide and of the same size as the upper piece.

FIG. 27. APPARATUS FOR TESTING REACTION OF MIRACIDIA TO MALE AND FEMALE SNAILS



The whole is as big as the ordinary microscope slide except for its greater thickness. A male and a female snail are placed in the appropriates lots, marked X and Y in the figure; the miracidium released from the point marked Z may be seen in the microscope field and watched continuously under 15× magnification.

Laboratory-hatched snails about 2.0 mm in size were used in these experiments. As soon as the miracidium had reached the junction or parting

of the T, the direction first taken by the miracidium, whether to the male or to the female snail, was noted; records were also made as to the first snail touched and the one successfully penetrated. In order to rule out the possible influence of light and other factors, the T mazes on each of the two ends of the apparatus were used alternately and a male and a female snail was placed in the same slot alternately.

This procedure was performed in 151 trials in all. For the first 108 trials, the miracidia turned 55 times towards the observer's right and 53 times to the left, which indicates that neither the construction of the maze nor its orientation has any effect on the behaviour and direction taken by the miracidia.

Each trial was observed for about 20 minutes and it was noted that not all the snails touched by the miracidia were actually penetrated. As shown in the following figures, not all penetration attempts were successful:

	Number of trials	
	male preferred	*female preferred*
First turn in T maze	80	71
First touched	61	59
First penetration attempted	48	42
Successfully penetrated	22	28

From the results above, it appears that the miracidium has no definite preference for female snails. The shift to the female side among those successfully penetrated might be due to physiological differences, but in view of the small numbers involved, it is also possible that it was due to chance.

The question arose whether the miracidia could detect at all the presence of a snail in the maze and so trials were made with a snail in one slot, and the other slot left empty. A total of 98 trials were made. The position of the snail with reference to the observer's right or left was similarly varied to eliminate any possible preference for a particular direction by the miracidium. In the tabulation below, it is shown that the miracidium turned away from the snail as frequently as towards it. As soon as the miracidium was released from its starting-point, it was observed to make a rapid aimless movement as though it could detect the presence of a snail somewhere near it. Its inability, however, to perceive the direction of the source of flow of this stimulus (probably some mucoid secretion) may be seen from the results obtained:

	Number of turns	
	towards snail	*away from snail*
For male snail	26	22
For female snail	22	26
Total	48	48

The snails that were seen to have been successfully penetrated by miracidia were kept and from time to time were allowed or forced to shed cercariae. The results were somewhat discouraging in view of the high

mortalities observed. After two months 50% of the snails were dead, and only nine were alive after six months. Of those that were found to contain the parasite, four were males and three were females.

In view of the fact that most of the snails that died were in a state of decomposition when discovered, the presence of sporocysts or any other stages of the parasite could not be determined. It is felt that we failed to detect some of those that did take the infection, and for this reason the following figures showing the results of the infection experiments in the T or Y maze might be misleading:

	Number of snails successfully penetrated	*Number of snails infected*	*Percentage of snails infected*
Male snails	9	4	44
Female snails	7	3	43
Total snails	16	7	44

Our data on successful penetration and percentage take, however, are valid and may explain the discrepancy observed in the field data. On page 548, penetration was seen to be successful in 45.83% and 66.67% of the males and females, respectively, on which attempts were made. With these results and the observation that percentage take amounted to 36.00% and 48.65% of the males and females, respectively, in those where the miracidium was seen to penetrate (see the tabulation on page 547), it is then possible to calculate the number of male and female snails that would be infected out of a group of 200 snails representing equal numbers for each sex. Allotting one penetration attempt for each snail, the calculation is as follows:

In males: $100 \times 0.4583 \times 0.3600 = 16.49$.
In females: $100 \times 0.6667 \times 0.4865 = 32.43$.

Based on the ratio of the final figures obtained, the predominance of infected female snails in the field is easy to explain.

We have listed above four possibilities that may help to explain the disparity in infection rates. Since it has been shown that the miracidium has no clear preference for either sex, and since studies so far show no great dissimilarity of habits between male and female snails, it is held that the other two possibilities are true. Further confirmation of our assumptions may only be made by a careful study of prepared slides of infected snails.

Effect of *S. japonicum* Infection on the Snail

Effect on reproduction

So far, the pathology of the infected snail has not been thoroughly explained. Some have claimed that the sporocysts develop in the region of the liver and through pressure and irritation cause liver damage which

eventually leads to the death of the snail. Others contend, however, that it is in the gonads that the sporocysts develop and consequently affect the reproduction of the snail. So far, in pursuing our own studies we have done no more than to bear these observations in mind. In the several hundreds of our routine examinations of infected snails, efforts were made to determine various stages of the parasite and their location with respect to the organs, but in view of the difficulty of identifying very early stages of the developing parasite, we undoubtedly failed to record all that were infected. In those where there were advanced stages present, most of the sporocysts were found coiled through the liver, whose tissues showed varying degrees of damage. Since the gonads are comparatively small and located not far from the liver, it was difficult to ascertain if they were likewise affected. This is especially true during routine examination of " crushed " snails. We have therefore begun to study serial sections of infected snails in order to ascertain details microscopically.

Meantime, however, we have designed experiments to determine the number and hatchability of eggs produced by both infected and non-infected female snails.

In this study, 45 mature female snails collected from the field (average size 3.7 mm and above) were confirmed to be infected by forced shedding of cercariae in the laboratory and were placed in an aqua-terrarium with solid objects, such as strips of coconut husks, on which they normally lay eggs in the field. From time to time, eggs were collected, placed in suitable hatcheries, and observed for viability rates. A similar number of non-infected female snails were maintained in another aqua-terrarium as controls.

The results obtained (see Table XXXII) indicate that fewer eggs are laid by infected snails, and hatchability rates are similarly poorer, as compared with non-infected snails. Since they were kept under identical conditions throughout the experiment, the results leave no doubt that reproduction is impaired by the parasitism. However, since some viable eggs were produced, the results can be better explained by a generally lower vitality of infected snails than by direct destruction of the gonad.

If these laboratory observations are indicative of field conditions, then we might expect a lower proportion of young snails among highly infected colonies. As mentioned above, there are a number of factors that might affect egg production and survival of young, so that it will be impossible to account for all changes in proportion of young in terms of fluctuation in infection rates. When the data from all 10 colonies in Malirong were summarized for the 18 months they were studied, the proportion of young was found to be inversely related to the infection rates, i.e., the highly infected colonies tended to show lesser proportions of young snails than did the colonies with lower infection rates (Fig. 28).

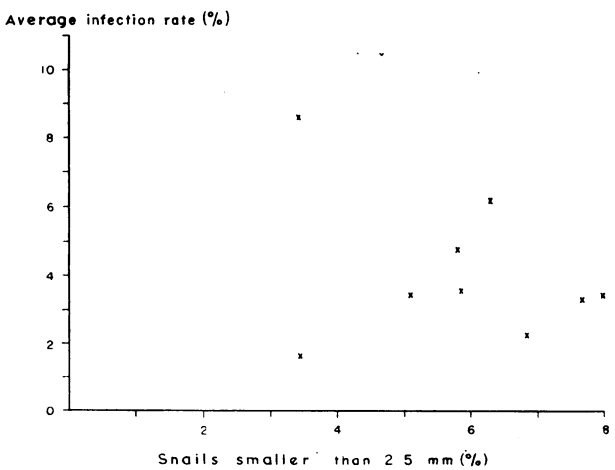
However, when the infection rates were compared to the proportion of young in individual colonies by dates, the relation was not so striking,

TABLE XXXII. COMPARATIVE STUDY OF EGG-LAYING AND HATCHABILITY RATES OF INFECTED AND NON-INFECTED SNAILS

Date of observation	Infected			Uninfected		
	number of snails	number of eggs	number of young snails recovered	number of snails	number of eggs	number of young snails recovered
31 Jan.	31	0	0	31	15	0
6 Feb.*	45	8	0	45	59	0
13 Feb.	41	11	0	42	86	0
20 Feb.	30	7	4	40	112	50
27 Feb.	23	6	6	37	34	73
5 March	19	12	0	36	28	36
12 March	16	7	8	31	22	27
18 March	10	8	11	28	36	48
22 March	—	—	—	—	—	19
Total	215	59	29	290	392	253
Hatchability			49.2 %			64.5 %
Number of days followed up		50			54	
Average number of eggs per female per day		0.0055			0.027	

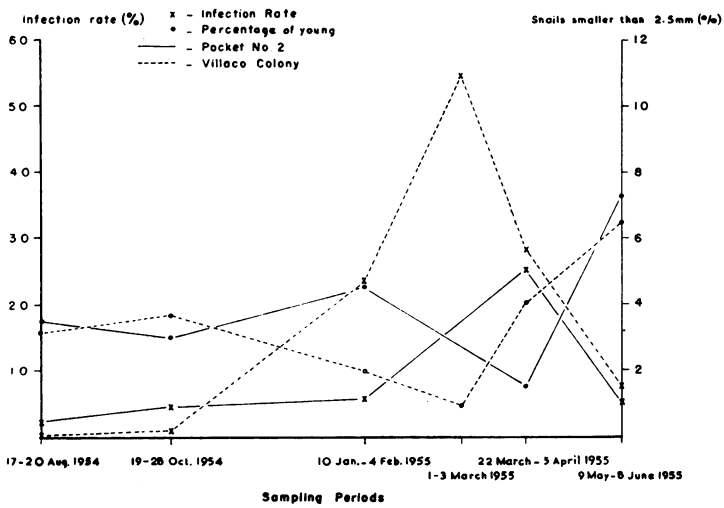
* 14 more female snails added to each group.

FIG. 28. RELATION BETWEEN AVERAGE INFECTION RATE AND PROPORTION OF SNAILS SMALLER THAN 2.5 MM



especially where the infection rates were less than 10%, as is frequently observed in nature. In colonies where great variations in infection rates occurred, however, the proportion of young was found to be affected (Fig. 29).

FIG. 29. RELATION BETWEEN CHANGES IN INFECTION RATES AND PROPORTION OF SNAILS SMALLER THAN 2.5 MM, IN VILLACO COLONY AND MALIRONG RIVER POCKET NO. 2



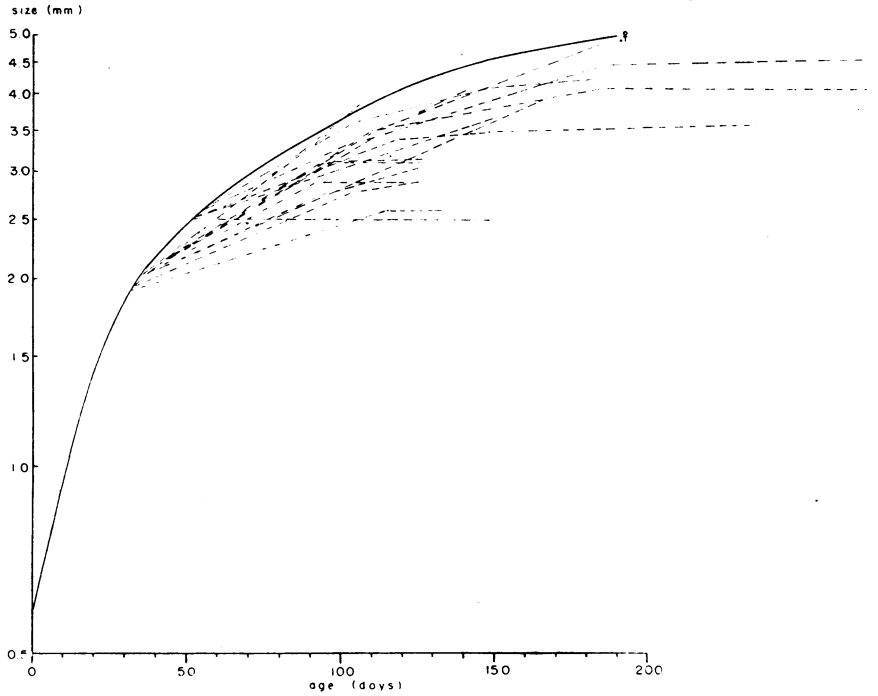
Effect on growth

Like reproduction, the growth rate is reduced among infected snails. We have a large number of snails that were measured at the time they were exposed and several times at various intervals thereafter. Since our growth curve has been derived from a large number of uninfected laboratory-bred snails and from field data in colonies where the infection rate was low, we feel that it represents a satisfactory average growth rate of uninfected snails. Using this growth curve as a basis, we plotted the measured growth rates of each infected snail, keeping the sexes separate. All showed retarded growths, and the effect was more severe among the younger snails. The curves followed by the infected snails were similar in both sexes (Fig. 30).

Effect on longevity

As mentioned above, a great many of the snails in the maze experiment that were kept for observation died before they were able to produce cercariae. This observation and several other similar ones prompted us to compare longevity of infected and non-infected snails. Of a group that

FIG. 30. GROWTH OF 16 LABORATORY-INFECTED FEMALE *O. QUADRASI* COMPARED WITH NORMAL GROWTH CURVE



The unbroken line represents the normal growth curve.

was followed for 20 weeks (see Table XXXIII), mortality rates among the infected snails exposed to one miracidium each was noted to be high. Starting with 79 snails each in an exposed and an unexposed lot, the number

TABLE XXXIII. RESULTS OF LONGEVITY STUDIES AMONG INFECTED AND NON-INFECTED SNAILS OF AN AVERAGE LENGTH WHEN INFECTED OF 2.7 MM

Time after infection.	Exposed snails living				Unexposed snails living	
	total		infected			
	number	%	number	%	number	%
1 day	79	100	54	100	79	100
6 weeks	55	70	33	61	70	87
10 weeks	41	52	22	41	61	77
15 weeks	30	38	12	22	57	72
20 weeks	24	30	9	17	47	59

of unexposed snails (47) living at the end of the observation period was almost twice that of the exposed snails (24). Crushing of the exposed group, however, revealed that 15 had failed to become infected. Assuming that the non-infected snails survived equally well in both the aquarium containing exposed snails and that with unexposed snails, this would mean that 25 of 79 snails exposed to one miracidium each failed to become infected. Hence, the infection rate was 54/79, or 68.35%. Of the 54 successfully infected snails, only 17 lived long enough to produce cercariae, and only nine of these survived to the end of the experiment. The most likely survival rate, then, would be 9/54, or 16.6%, as compared with 59.5% in the unexposed controls. Expected numbers of live infected snails for each earlier period could then be calculated.

TABLE XXXIV. RESULTS OF LONGEVITY STUDIES AMONG INFECTED AND NON-INFECTED SNAILS OF AN AVERAGE LENGTH WHEN INFECTED OF 2.0 MM

Time after infection	Infected snails				Uninfected snails	
	Group A		Group B			
	number living	percentage living	number living	percentage living	number living	percentage living
1 day	42	100	44	100	42	100
4 weeks					40	95
5 weeks	17	40				
7 weeks			11	25		
9 weeks	10	24			38	90
11 weeks	9	21			38	90
12 weeks			6	14		
14 weeks			5	11		

In the above experiment, it must be mentioned that we used snails with an average length of 2.7 mm. When the mortalities of this batch of snails were compared with those from another experiment where younger snails (average 2.0 mm.) were used, a striking difference was noted (see Table XXXIV and Fig. 31 and 32). This suggests that younger snails die of the infection more rapidly than older ones. Comparing these curves in turn with that of the summarized field data (for all collectable sizes of non-infected and some infected snails), the longevity curve is seen to fall between that of the control and that of the exposed snails, suggesting clearly that survival in the laboratory is very much better than in the field. We have no way of knowing exact mortality rates of infected snails in the field.

FIG. 31. EFFECT OF INFECTION ON SURVIVAL OF SNAILS INFECTED WHEN 2.7 MM LONG

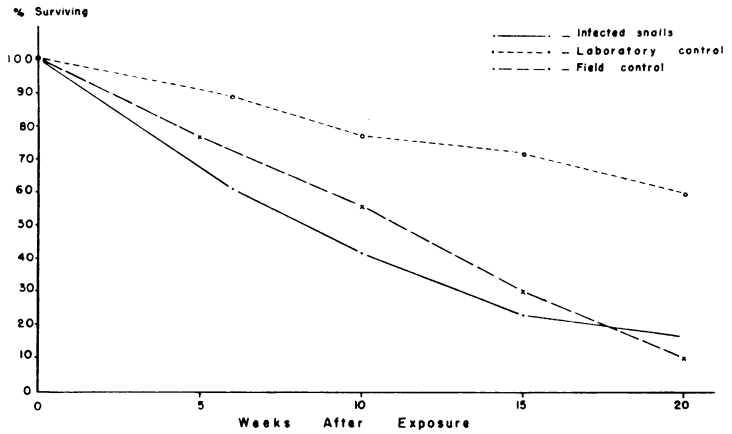
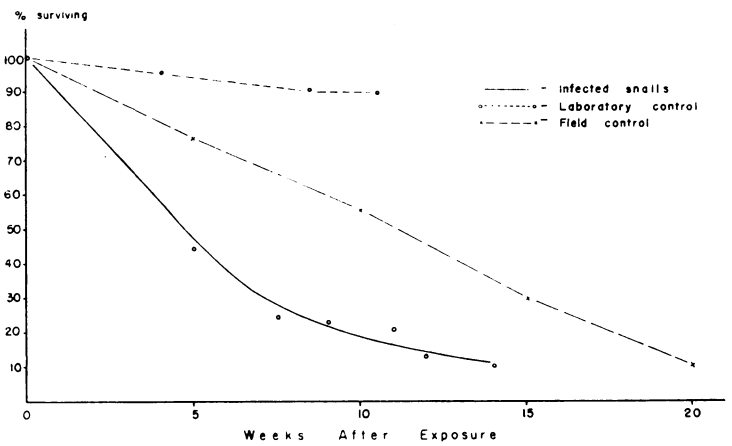


FIG. 32. EFFECT OF INFECTION ON SURVIVAL OF SNAILS INFECTED WHEN 2.0 MM LONG



Daily Cercarial Shedding Cycle

Cercaria-shedding has been reported, on the basis of experiments done in the laboratory, to be cyclic in nature (Bauman, Bennett & Ingalls, 1948). Whether this is also true in the field was not known. Our recent acquisition of a millipore-filtering apparatus[a] has enabled us to make direct observation on cercariae in natural waters. Before such a study was begun, how-

[a] Hydrosol unit, 50-mm, stainless steel, using millipore filter, 47-mm, Type HA black grid without nutrient pad (supplied by Bio-Micro Filtration Instrument Inc., San Gabriel, Calif., USA) attached to a vacuum air-pump, two-stage Welch Duo-Seal, No. 1410, with 1/3 horsepower motor (supplied by W. M. Welch Manufacturing Co., Chicago, Ill., USA).

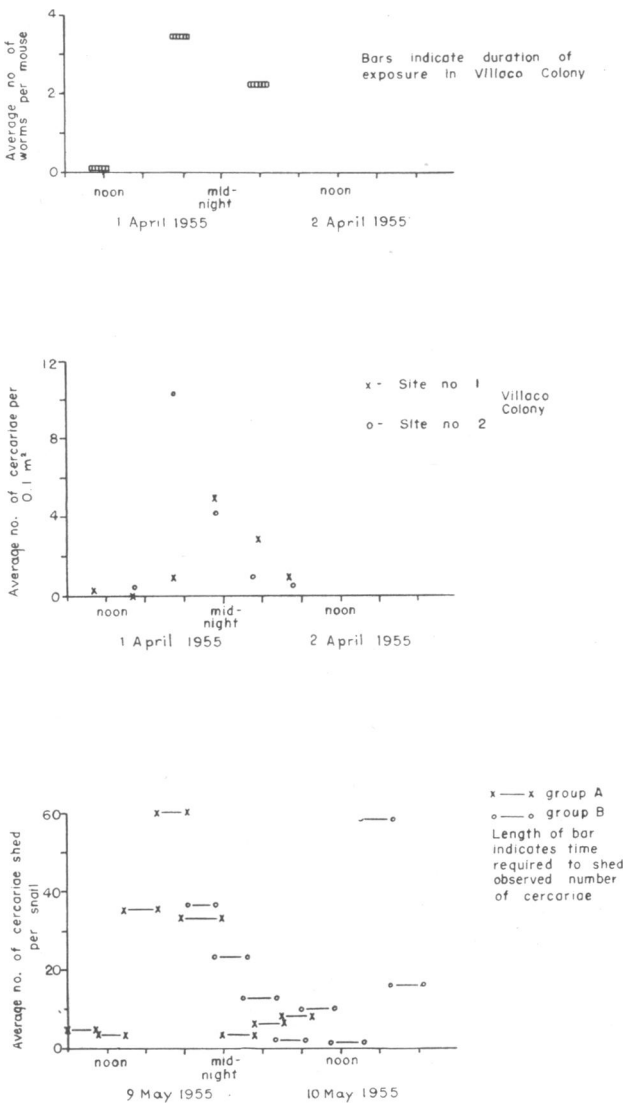
ever, laboratory trials were made by filtering a known number of cercariae in the tap-water. This was necessary because cercariae caught on the filter and observed by reflected light are different in appearance from those seen with transmitted light. Also, most of them lose their characteristic forked tail in the process. Using the above technique in the laboratory, cercariae were easily recovered at a density of 1 per litre.

Inasmuch as cercariae observed in the laboratory are concentrated on the surface film, if not confined there, we decided to skim the water in a snail colony, so as to obtain as high a concentration as possible. A practical sample was found to be 1 litre of water, skimmed so as to include approximately the top centimetre, which would cover an area of about 0.1 m^2 of surface. In order to obtain the largest number possible we selected the Villaco colony, with an over-all snail infection rate of 27.55%, for field observation. Preliminary trials were made in the most heavily infected part of the colony, in a place where the water was still and clear. The snail infection rate in this particular place was approximately 44.0%. At 10.30 a.m. on 23 March 1955, three 0.1-m^2 samples yielded 5, 5 and 2 cercariae respectively. This was encouraging and we decided to undertake a whole 24-hour study. This was done on 1-2 April 1955. Unfortunately,

TABLE XXXV. NUMBER OF CERCARIAE OBTAINED BY FILTERING WATER FROM VILLACO COLONY AT DIFFERENT TIMES OF DAY (1-2 APRIL 1955)

	11.00 a.m.	2.45 p.m.	7.00 p.m.	11.00 p.m.	3.00 a.m.	7.00 a.m.
Sampling site 1:						
Sample 1	0	0	1	5	3	1
Sample 2	1	0				
Sample 3	0					
Total cercariae	1	0	1	5	3	1
Average number of cercariae per 0.1 m^2	0.33	0	1.0	5.0	3.0	1.0
Sampling site 2:						
Sample 1		0	9	6	1	0
Sample 2		0	14	1	1	1
Sample 3		1	2	3	0	0
Sample 4			17	7	2	1
Total cercariae		1	42	17	4	2
Average number of cercariae per 0.1 m^2		0.33	10.5	4.25	1.0	0.5

FIG. 33. PERIODICITY OF CERCARIAL EMERGENCE AS INDICATED BY THREE INDEPENDENT TECHNIQUES



the original sampling location was spoiled, and two alternate sites were selected. Site No. 1 was in the small stream that serves as an outlet for the area; site No. 2 was in still, clear water opposite the original site. The snail infection rate at site No. 2 was later found to be only 11.7%. Water samples were taken at four-hour intervals, starting at 11.00 a.m. The results are given in Table XXXV and Fig. 33. Peak densities were observed

at 11.00 p.m. at site No. 1, and one observation period earlier at site No. 2. In both cases, the peaks are well defined. Both sites showed minimal concentration of cercariae at 3.00 p.m.

As a check upon the direct cercarial counts, groups of five mice were exposed just below site No. 1 at three different times, 11.00 a.m.-12.30 p.m., 7.00-8.30 p.m., and 3.00-4.30 a.m. One mouse in each of the last two sets died a week after exposure. The final infection rate in the surviving mice are given in Table XXXVI and Fig. 33. They confirm the direct count in a highly satisfying manner.

TABLE XXXVI. RECOVERY OF ADULT *S. JAPONICUM* FROM MICE EXPOSED AT THREE DIFFERENT TIMES OF DAY (VILLACO COLONY, 1-2 APRIL 1955)

Mouse	Number of flukes recovered		
	from 11.00 a.m. to 12.30 p.m.	from 7.00 p.m. to 8.30 p.m.	from 3.00 a.m. to 4.30 a.m.
A	0	0	3
B	0	4	2
C	0	3	2
D	0	7	2
E	1	died	died
Total flukes recovered	1	14	9
Average per mouse	0.20	3.50	2.25

The periodicity of cercarial densities in the field is thus established from two independent sources.

On 19 July, this experiment was repeated. This time, the snail infection rate had gone down to 6.09%, and this provided us with an opportunity to determine what the corresponding density of cercariae would be. We also included a similar study in Naliwatan colony for purposes of comparing the results.

A peak of density at 10.00 p.m. was again noted (Table XXXVII), which was comparable to the similar peak obtained in site No. 1 in the April study (Table XXXV). It is striking to note further that the average cercarial density was correspondingly decreased. This marked relation is noticeable in all observation periods. The peak of maximum abundance was noted in Naliwatan stream from 6.30 p.m. to 7.00 p.m., or one observation period earlier than Villaco. It is interesting to note that the peaks are well defined and minimal concentrations were observed in the afternoon at 3.00 p.m.

TABLE XXXVII. AVERAGE NUMBER OF CERCARIAE PER LITRE OBTAINED BY FILTERING WATER IN VILLACO AND NALIWATAN COLONIES AT DIFFERENT TIMES OF DAY (19-20 JULY 1955)

Date	Colony	10.30 a.m.–11.00 a.m.	2.30 p.m.–2.45 p.m.	6.30 p.m.–7.00 p.m.	10.00 p.m.–11.00 p.m.	2.00 a.m.–3.00 a.m.	6.00 a.m.–7.00 a.m.
1-2 April 1955	Villaco: site 1 (infection rate 27.88 %)	0.33	0	1.0	5.0	3.0	1.0
	Villaco: site 2 (infection rate 11.7 %)	not sampled	0.33	10.5	4.25	1.0	0.5
19-20 July 1955	Villaco (infection rate 6.09 %)	0.33	0	not sampled	1.0	0.67	0
	Naliwatan (infection rate 9.45 %)	0	0	1.67	0.67	0.67	0.67

An opportunity to discover whether rainfall affects the appearance of cercariae in natural water was provided on 4 April, when 1.21 inches (30.7 mm) of rain fell between 11.30 a.m. and 1.30 p.m. Because the water was somewhat cloudy after the rain, only two 0.1-m^2 samples could be filtered. Those two samples, taken from site No. 1, yielded 4 cercariae and 1 cercaria, respectively, whereas those taken on 1 April at the same time and location were negative for cercariae. We thus have considerable evidence that the danger of exposure in natural waters is influenced by at least three factors—snail infection rates, time of day, and weather conditions.

The discovery that cercariae are most abundant in the field during the early part of the night and the casual observation that more cercariae were obtained in the laboratory if the snails were kept in water overnight, presented some difficulties because of the findings of various investigators that darkness inhibits shedding (Gumble et al., 1953). In order to discover whether it is the time of day or the time since immersion of dry snails that causes the observed shedding rates, the following experiment was performed.

A large series of field snails from the Villaco colony was dried in the usual manner, and divided into two groups of 130 each. The snails in the first group were immersed in water in individual test-tubes at 8.00 a.m.; those in the second group were similarly immersed at 8.00 p.m. Each group was followed at three-hour intervals for 24 hours, and the number of cercariae shed by each positive snail was recorded for each period. The results, as shown in Table XXXVIII and Fig. 33 are unequivocal. The group immersed at 8.00 a.m. gave a single peak of shedding from 5.00 p.m. to 8.00 p.m. ; the group immersed at 8.00 p.m. gave two well-defined peaks, one immediately after immersion and another from 2.00 p.m. to

TABLE XXXVIII. RESULTS OF SHEDDING OF CERCARIAE BY TWO GROUPS OF TIMES OF DAY

Time periods for Group A immersed at 8.00 a.m.	8–11 a.m.	11 a.m.–2 p.m.	2–5 p.m.	5–8 p.m.	8 p.m.–12 midnight	12 p.m.–3 a.m.
Total cercariae shed	123	86	893	1517	827	90
Average per snail	4.92	3.44	35.72	60.68	33.08	3.60
Time periods for Group B immersed at 8.00 p.m.					8–11 p.m.	11 p.m.–2 a.m.
Total cercariae shed					660	413
Average per snail					36.67	22.94

5.00 p.m. on the following day. Shedding is thus clearly related to time of day, and prolonged exposure to light seems to be necessary.

Techniques for Obtaining Cercariae

In laboratory experiments requiring large numbers of naturally liberated cercariae at one time, the desirability of developing an efficient shedding technique is obvious. Our experience in handling laboratory-infected snails shows that cercariae are shed in considerable numbers during the first 10-20 days of shedding and that the peak of production is reached 20-40 days after they begin to be liberated. After this time, shedding becomes more or less intermittent and fewer cercariae are liberated each time. It seems then that after this initial shedding, some time is required before an appreciable number can mature and accumulate in the snail. In view of this, it would seem that prevention of the release of cercariae for several days would be an appropriate method of obtaining large numbers.

In one of the several trials made, snails from highly infected colonies were transferred to a Petri dish containing dry soil. After a varying number of days in this dry condition, the snails were transferred to individual test-tubes containing 5 ml of aquarium water. The following morning the water was examined under a stereoscopic microscope. The snails that did not shed were crushed and examined for cercariae that had not come out. A complete result of the experiment is shown in Table XXXIX and Fig. 34.

From the above figures, it is clear that the efficiency of shedding increases with the number of days of previous drying. Because many of the snails die after five days' drying, and since it is often desired to keep snails for other purposes after they have been confirmed to be infected, we adopted a four-day drying period to precede shedding attempts.

In another experiment described above, the well-defined peaks of

FIELD SNAILS DRIED FOR FOUR DAYS AND IMMERSED IN WATER AT DIFFERENT (VILLACO COLONY)

3–6 a.m.	6–9 a.m.					Time periods for Group A immersed at 8.00 a.m.
172	209					Total cercariae shed
6.88	8.36					Average per snail
2–5 a.m.	5–8 a.m.	8–11 a.m.	11 a.m.–2 p.m.	2–5 p.m.	5–8 p.m.	Time periods for Group B immersed at 8.00 p.m.
243	35	177	1	1060	182	Total cercariae shed
13.50	1.94	9.83	0.06	58.89	15.83	Average per snail

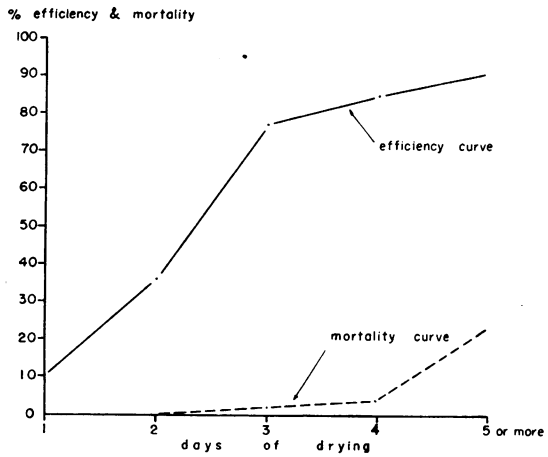
cercarial abundance from late afternoon to early evenings for two groups of infected snails immersed in water at different times of day showed clearly that shedding is related to time of day. Of the physical factors that change over a 24-hour period, light is most likely to stimulate shedding, in spite of various statements in the literature that it was not effective (Bauman, Bennett & Ingalls, 1948). The periodicity of shedding, which was not altered by the time of placing the snails in water, suggested that it was perhaps the duration of exposure to light that brought about the release of cercariae. We had previously attempted forcing the emergence of cercariae by 30 minutes' exposure of infected snails to direct sunlight, but without any noticeable success.

Six hundred highly-infected snails from Villaco Creek were divided into four groups of 150 snails each. Two groups (A and B) were dried for four days while the other two groups (C and D) were placed on moist soil.

TABLE XXXIX. RELATION BETWEEN LENGTH OF DRYING PERIOD AND EFFICIENCY OF CERCARIAL SHEDDING

Length of previous drying	Number of snails used	Total confirmed infected with mature cercariae	Snails shedding cercariae		Snail mortality	
			number	%	number	%
1 day	150	10	1	10	0	—
2 days	150	11	4	36	0	—
3 days	250	26	20	77	5	2.0
4 days	480	47	40	85	15	3.1
5 days	400	11	10	91	94	23.5

FIG. 34. RELATION BETWEEN LENGTH OF DRYING PERIOD AND EFFICIENCY OF CERCARIAL SHEDDING



Starting from the evening of the fourth day, one group from each of these (A and C) was exposed for 12 hours 30 cm below a 14-watt fluorescent lamp. At this height, it was found that the lighted lamp did not cause any increase in temperature on the surface below it. Room temperature during the experiment varied from 27°C to 30°C. The following day, all four groups were immersed in water, each snail in an individual container. All readings for cercariae shed were made before noon.

A week later, the reversed trial was performed ; Groups A and D were interchanged, as were B and C. The same procedure as in the first experiment was followed.

After these observations, all snails that did not shed were crushed, the snails bearing sheddable cercariae were counted, and the efficiency of each shedding technique was determined. Table XL shows that a greater percentage of the positive snails shed during the first experiment than during the second, indicating that some of the snails had exhausted their sheddable cercariae, and nothing but immature stages were recovered upon dissection. From all techniques tried, the one with four days of drying and 12 hours of light seemed to be the most efficient, having the greatest proportion of shedding snails and the greatest number of cercariae released per snail. Comparing techniques using 12 hours of light against four days' drying, the former may be seen to be effective in causing a greater number of infected snails to shed. The latter, however, yields more cercariae per shedding snail. Of striking interest is the group where no cercariae were shed when no stimulation either by drying or exposure to light was made. When the same group of snails, however, was dried and lighted, 35.9% of the snails with mature cercariae liberated them.

TABLE XL. COMPARATIVE EFFICIENCY OF VARIOUS CERCARIAL SHEDDING TECHNIQUES

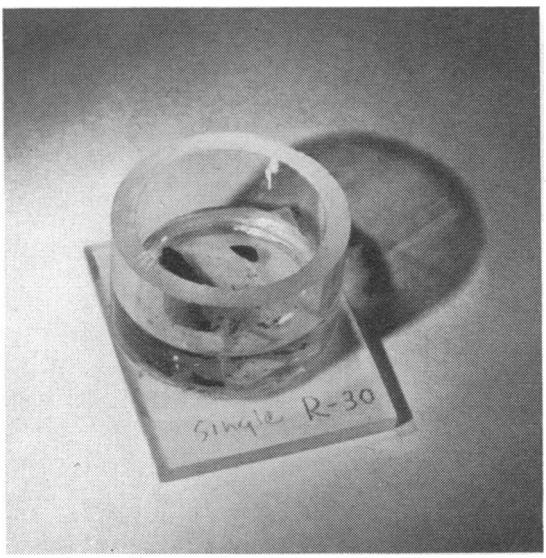
Technique	Group	Total	Snails shedding		Cercariae shed		Snail mortality	
			number	%	total	average per shedding snail	number	%
			1st experiment					
4 days' drying + 12 hours' light	A	47	42	89	5390	128.33	14	9.3
4 days' drying	B	49	13	27	1290	99.23	14	9.3
12 hours' light	C	48	35	73	2138	6.11	3	2.0
Control	D	39	0	—	—	—	2	1.3
			2nd experiment					
4 days' drying + 12 hours' light	D	39	14	36	2300	164.29	Not determined	
4 days' drying	C	48	4	8	80	20	Not determined	
12 hours' light	B	47	8	17	320	40	Not determined	
Control	A	46	0	—	—	—	Not determined	

On the basis of these observations, there can be no doubt that drying and exposure to light both stimulate shedding. As seen from Tables XXXIX and XL, four days' drying causes a high mortality rate among the snails. Since each technique offers certain advantages and disadvantages, the choice of the techniques to be used would depend largely on individual circumstances. We have made use of the light and drying technique to obtain before noon an average of 90 cercariae per snail, when the latter were laboratory-raised and exposed to one miracidium each, and 125 cercariae per field snail. In cases where the survival of the snails is important, the use of light alone has produced sufficient numbers of cercariae for most of our laboratory experiments.

Daily Cercarial Output

Heretofore, knowledge about the number of cercariae shed daily by a single *O. quadrasi* and the total number shed during its whole life-span has been fragmentary and inconclusive. The information is not easy to obtain because, in the first place, this requires a long-term investigation and opportunities are not readily available to most workers. In the second place, the technique of raising snails individually for prolonged periods has not been satisfactory. Our recent success in rearing snails in small plastic

FIG. 35. PLASTIC CONTAINER USED AS AQUA-TERRARIUM FOR *O. QUADRASI*



containers (Fig. 35) with enough debris, food and water proved to be very valuable in carrying out this investigation.

Using freshly-hatched miracidia, laboratory-bred snails measuring from 2.0-2.5 mm were infected artificially in small watch-glasses. Since not all penetration attempts result in infection, only those snails which the miracidium was seen to penetrate were retained for observation.

Previous experience has shown that snails start to liberate cercariae around seven weeks after infection. For this study, snails were isolated 30 days after infection in the plastic aqua-terraria, in which they were watched daily for the appearance of cercariae.

After examination each day, the water in these containers was changed. We used ordinary tap-water (pH 7.2-7.9). Bauman, Bennett & Ingalls, in 1948, showed that the pH of the water was a critical factor in the natural release of cercariae and that pH 7.6 was optimum. Since the little chlorination being done to the water supply was not detectable in our laboratory, we did not experience any difficulty from the use of tap-water.

During the period when this experiment was conducted (April 1955-March 1956), the snails were kept in the project snail-house, where the temperature varied from 90°F (32.2°C) during the day to 60°F (15.6°C) at night. This is about the range obtainable in the field during this period.

We have data for 46 snails that were infected with one miracidium each and 19 that were seen to be penetrated by 2-5 miracidia each. A perusal

TABLE XLI. COMPARATIVE RESULTS IN CERCARIAL OUTPUT IN SINGLY AND MULTIPLY INFECTED SNAILS

	Singly infected	Multiply infected
Shortest period from infection to first shedding	42 days	45 days
Average period from infection to first shedding	62.39 days	64.21 days
Average number of cercariae per snail-day	2.56	2.36
Average shedding period	32.11 days	66.53 days
Average number of cercariae per snail per day during shedding period	15.07	8.44
Total cercariae shed (average per snail)	232.30	279.37
Longest duration of daily continuous shedding	15 days	13 days

of the figures in Table XLI shows no apparent difference between the two groups of infected snails. Both appear to start liberating cercariae at about the same time and the average numbers shed per snail-day seem to be practically the same. Since these snails were not infected on the same day but over a period of many days, the figures obtained at different times were arranged according to date. The emergence of cercariae was not related to date, nor was there any hint that success in infection was related to season.

When the daily cercarial outputs of the batches of snails infected on the same date were grouped, so that the number of cercariae released every day might be compared, the pattern of shedding was seen to be obviously more related to the day of infection than to the first day of shedding. To summarize the data, therefore, we have assembled the results starting from day of infection. As seen in Table XLII, there is a rapid build-up in the number of cercariae released in both groups and the peak is reached after 40 days. Thereafter, there is a decline, with the group infected with multiple miracidia lagging behind by about 20 days each time (Fig. 36). This difference is not very noticeable until after the 89th day. In order to test the significance of this difference, the data for both singly and multiply infected snails have been divided into two groups (A and B) in Table XLII, representing the early and latter part of the shedding, respectively. The χ^2 test in A for the proportion of snails releasing cercariae showed no significant difference between the singles and multiples. In B, however, the difference is very significant. Group C shows later figures for multiply infected snails.

These results are interesting and allow some assumptions that may

TABLE XLII. HISTORY OF THE 46 SINGLE AND 19 MULTIPLE INFECTIONS IN THE SNAIL HOST

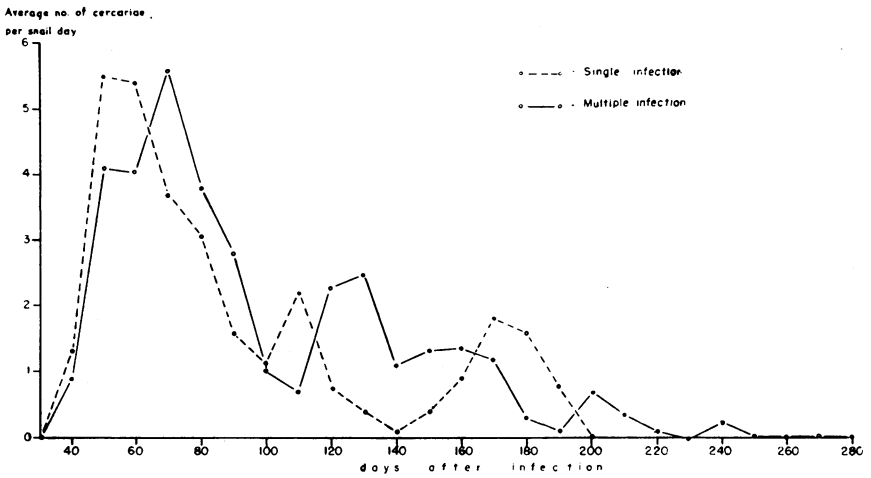
Number of days after infection	Single infections		Multiple infections	
	average number of cercariae per snail-day	percentage of snails shedding	average number of cercariae per snail-day	percentage of snails shedding
40	1.298	5.4	0.915	5.3
50	5.550	25.0	4.105	14.7
60	5.409	32.2	4.095	27.4
70	3.784	31.2	5.584	33.7
A 80	3.077	19.3	3.830	23.4
90	1.580	9.1	2.844	15.6
100	1.134	14.2	1.000	13.1
110	2.227	20.0	0.713	9.3
120	0.743	14.9	2.305	22.9
130	0.377	2.8	2.550	20.0
140	0.127	4.5	1.109	13.0
150	0.380	3.0	1.288	12.5
B 160	0.939	3.7	1.324	14.7
170	1.729	8.6	1.233	9.3
180	1.593	14.8	0.297	5.5
190	0.774	9.7	0.100	3.3
200	0*	0	0.733	6.7
210			0.367	6.7
220			0.100	3.3
230			0	0
C 240			0.25	5.0
250			0	0
260			0	0
270			0	0
280			0**	0

* 4 snails still living at 15 March 1956.
** 1 snail still living at 15 March 1956.

explain the figures observed in terms of the development of the parasite in the intermediate host.

Assuming that, when more than one miracidium entered the snail host, there followed a crowding among the miracidia and that this resulted in a

FIG. 36. COMPARISON BETWEEN AVERAGE NUMBERS OF CERCARIAE SHED PER SNAIL DAY BY SINGLY INFECTED AND BY MULTIPLY INFECTED SNAILS

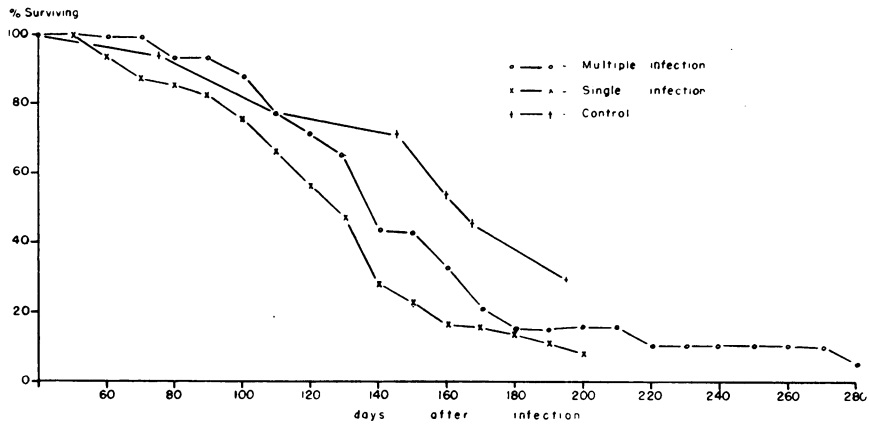


delay in contrast with those where only one miracidium was developing, it follows that, in the latter, bigger sporocysts develop whose cercariae mature almost at the same time and are thereafter shed continuously. In the other group, however, with limited room in the snail resulting in competition among the miracidia, smaller sporocysts are produced and maturity of the cercariae is delayed. Our best evidence to support these assumptions lies in the number of cercariae shed per day during the entire shedding period (see Table XLI). The singly infected snails shed twice as many (15.07) cercariae per shedding day as those with multiple infections (8.44). Since the total number shed per snail is not very different between the two groups, the apparent discrepancy would have to be compensated for by the singly infected shedding half as often as the multiply infected. Consequently, the pattern of shedding is influenced by the number of miracidia used, but the total number of cercariae is not, because opposing factors cancel each other.

A curious fact emerging from this experiment is that multiply infected snails had a better survival rate than singly infected ones (Fig. 37). It is conceivable that the postulated slower growth of several parasites allows the host to develop some resistance, whereas rapid development of a single parasite in a vital area has a more harmful effect. Histological investigations on this point have been started.

We have attempted to answer the question whether those that we were calling multiple infections were really so. It has been our experience that not all penetrations resulted in infections, and hence there might actually be some single infections among the " multiples ". The only way this could

FIG. 37. SURVIVAL OF SINGLY AND MULTIPLY INFECTED SNAILS



be tested was to infect mice with these cercariae to determine if two sexes were present. Even this method is not entirely conclusive, as a single sex found may be the result of multiple infections with several miracidia of the same sex.

A limited experiment was done using five snails with multiple infections, obtained by observing the penetration of each snail with about three miracidia. Using 1-8 cercariae from each of these snails, repeated 3-6 times, we infected one mouse with the cercariae from each snail.

Of the five mice infected, one was lost accidentally. Two of the remaining mice had flukes of both sexes, while the other two had all males. These results are nevertheless encouraging, since even with 50% production of the two sexes, there is evidence that the snails took more than one infection. The likelihood that some would be all-male infections can be computed from our data on experimental infections with single miracidia, where there is evidently a preponderance of male infections. Of 53 such infections, 39 gave male cercariae, and only 14 gave females. The probability of drawing three male cercariae from an effectively infinite pool containing the two sexes in the ratio of 14 females to 39 males would be p^3, where p is the proportion of males. Substituting $p = \frac{39}{53}$, we obtain a probability of close to 0.4 for exclusively male infections. There is thus no reason to reject the conclusion that real multiple infections were obtained.

Distribution of Cercariae in a Colony

From our periodic sampling of various snail colonies in Malirong, it was noted that different parts of the colony tended to have different infection rates, and this condition was maintained with little variation for nearly

24 months. Evidently, this can be associated with the differing ecological patterns present, especially nearness to defaecation sites. Since this would seem to show that some parts of the colony could be more dangerous than others, as far as exposure to the disease is concerned, we thought it wise to check this by determining the density of cercariae at different portions of the colony.

The Villaco colony provided us with a good site for this study. The colony has consistently shown much higher infection rates along the south bank than along the north. While it had been observed earlier that the current may randomize the distribution of cercariae in the water, this effect was apparently minimal in the case of Villaco colony. The main sources of water of the colony are the spring and underground seeps coming from the foot of the bank that delimits the colony on the north side; the water drains along a poorly defined channel towards the Malirong river. On the south bank, the current is relatively faster. At times when the river is extraordinarily high, water may be seen flowing back into the colony. When these observations were made, however, the height of the water in the river was normally low. In general the current in the colony was sluggish. Since a routine snail sampling was done a few days before, we were in a position to tell which parts of the colony had high infection rates and which had low. Thus we selected a total of eight sites from the north and south banks, representing differing infection rates. At 7.00 p.m., the time of maximum abundance of cercariae, one litre of water was taken from each of the eight sites. The water was brought to the laboratory and filtered

TABLE XLIII. RELATION BETWEEN CERCARIAL DENSITY IN THE WATER AND NUMBER OF INFECTED SNAILS COLLECTED NEAR BY (VILLACO COLONY, 29 SEPTEMBER–11 OCTOBER 1955)

Water-sample number	Number of cercariae per litre (11 October)	Number of infected snails in 5 tube samples* (12-15 October)	Number of infected snails in 5 rings nearest to water samples * (28-30 September)
1	0	0	1
2	0	1	0
3	2	0	0
4	4	5	8
5	0	0	1
6	2	0	1
7	1	0	2
8	0	0	0

* Number includes those with mature infections only.

through the millipore filter, and the cercariae were counted in the manner described above.

Within the next few days, five tube samples were taken from the immediate location of each water sample.

Table XLIII gives the number of cercariae recovered from each water sample and the number of infected snails in the five tube samples. In addition, the number of infected snails found in the five rings nearest each water sample at the time of the preceding recheck, 28-30 September, is given. Although the relationship is far from perfect, there is some correspondence of values. Failure to obtain a better one is probably due to the fact that only one water sample was taken at each site, and to the influence of water current on the distribution of cercariae. Hence we decided to repeat this study, taking more samples at fewer sites.

On 7 February 1956, we filtered three litres of water from each of three different locations, where previous samplings had shown differing densities of infected snails. Again, in order to obtain maximum numbers of cercariae, the water samples were taken at 8.30 p.m. On the next day, seven tube samples were taken in the immediate vicinity of each site and the snails obtained were crushed to determine the number of those with mature infections. An examination of the data (Table XLIV) shows a marked

TABLE XLIV. RELATION BETWEEN CERCARIAL DENSITY IN THE WATER AND NUMBER OF INFECTED SNAILS COLLECTED NEAR BY (VILLACO COLONY, 7-10 FEBRUARY 1956)

Location	Water-sample number	Number of cercariae recovered	Number of infected snails * (total for 7 tube samples)
North bank, 30 m from outlet	1	21	
	2	19	
	3	8	
		(Average: 16)	Total: 16
North bank, 5 m from outlet	1	1	
	2	6	
	3	8	
		(Average: 5)	Total: 2
South bank, 9 m from outlet	1	1	
	2	6	
	3	2	
		(Average: 3)	Total: 14

* Number includes those with mature infections only.

discrepancy in the figures from the sampling site on the south bank. At the point where this sample was taken, the flow of water was quite strong, in contrast to the two sites on the north side, where the flow of water was very slight or absent. On the north side, however, the relation between the densities of cercariae and infected snails is clear. Evidently, the larger flow of water at the south-side site was sufficient to dilute the cercariae that were present. In a similar study done previously, one sample taken at the same place on the south side also showed a relatively low number of cercariae.

Presence of Cercariae Downstream from Snail Colonies

During the military campaign on Leyte Island in 1945, about 1700 members of the United States ground forces contracted bilharziasis, and nearly half of these belonged to the engineering battalion. Sullivan & Ferguson (1946) have presented good evidence that many of them were exposed during bridge-construction work at places considerably removed from snail colonies. Since their survey of the area disclosed that the snails were located in the tributaries that join the river upstream, it would imply that they got the disease through either (*a*) the cercariae that travelled or were carried downstream, or (*b*) the cercariae that were released from the infected snails washed down from the snail colonies. For the moment, to single out which of these two possibilities was the more likely is not easy. However, it is known that the cercaria is shorter-lived than the snail. Sugiura (quoted by Ritchie, 1955) pointed out that no cercaria may be found alive after falling from a waterfall 2 m high. But this nevertheless is no guarantee that they were not able to travel far enough. The only way this could be tested experimentally was to install a snail trap across the river and catch all snails without harming the cercariae. On a number of days when we tried to trap snails in the Malirong main irrigation canal, we were able to obtain infected snails among those collected. Nevertheless, small as the canal was, it was difficult to divert all the water to the trap, and we estimate that at least an equal volume of water had bypassed the trap. Because of this experience, it is doubtful whether we could be successful in trapping all snails in a river to prove the above possibilities. At any rate, the danger of exposure to waters below snail areas remains, and for the moment our attention is directed to this problem.

In the endemic areas we observed that the people actually spend more time in the river than in the snail colonies. Because of this, we were prompted to attempt to discover the relative dangers of exposures in a colony and in a site on a river somewhat removed from the breeding-areas of the snails. Since the rivers are always too turbid for us to use the millipore filter, we resorted to experimental exposures of laboratory mice. We have shown earlier that three factors influence the number of cercariae present in

natural waters: infection rates in snails, time of day, and weather. Because of the dilution factor, we felt that we should perform the experiment under conditions of maximum cercarial density, and the evening of 30 January 1956 provided these conditions, since infection rates were high (5.51%) and it had rained heavily all day (2.13 inches (54.1 mm) in 24 hours). Fifteen mice were exposed from 7.00 p.m. to 9.00 p.m. Five were placed in Villaco colony as controls and ten were exposed in the Malirong river just above the highway bridge, at a site 150 m downstream from the nearest colony—a very small one.

Two floating cages were specially designed, so that only 0.5-1.0 cm of water flowed through them. One cage was kept in mid stream and one near the bank. Five mice were placed in each.

After a period from 32-44 days, the mice were sacrificed and all flukes were recovered by dissection. The numbers of flukes recovered are shown in Table XLV.

The number of flukes recovered from the mice exposed in the colony was 25 to 30 times as great as we had obtained before, and the river produced virtually equal numbers. It is noted, moreover, that mice exposed in mid stream yielded more flukes that those near the bank. The reason for this is that the current was swifter there and more water passed through the cage.

In another trial we considered exposures in the morning. During the previous 24-hour period it had rained 0.72 inches (18.3 mm), and the volume of the water in the river was further increased by the dumping of excess water from the Tikba dam. Although the dilution factor resulting from the latter and the time of the day were against the likelihood of obtaining maximum cercarial density in the river, the infection rates of the Malirong colonies were higher (10.38%) than at the time of the previous experiment.

Using the same technique, five mice were exposed for two hours, from 9.00 a.m. to 11.00 a.m., at each of the three sites—namely, Villaco colony, Malirong river and Palo river. This time we exposed only one group of mice in the middle of the Malirong river and another in the Palo river at the portion near Kanbanwa, separated from the nearest colony (Agoong) by a waterfall 1.5 m high and at least a kilometre of moderately flowing river. For clarity, it may be mentioned that at the site of exposure in the Malirong river (which is a tributary of the Palo river) the river was about 5 m wide, while in the Palo river it was 3-4 times as wide. To be sure that no snails were breeding on the banks of the Palo river, a thorough survey was conducted and the results were negative. As in the previous experiment we maintained a control group of five mice at the Villaco colony.

After a month, we sacrificed the mice. The flukes recovered are indicated in Table XLVI.

To give the best explanation of the results obtained, a description of the contributing factors in each site is necessary. In Villaco colony, a higher average fluke recovery resulted from the morning exposure. Since

TABLE XLV. NUMBER OF *S. JAPONICUM* FLUKES RECOVERED FROM MICE EXPOSED IN VILLACO COLONY AND IN MALIRONG RIVER 150 YARDS DOWNSTREAM FROM NEAREST SNAIL COLONY (7.00-9.00 P.M., 30 JANUARY 1956)

	Villaco colony						Malirong river											
							near bank						mid-stream					
	mouse no.						mouse no.						mouse no.					
	1	2	3	4	5	average	1	2	3	4	5	average	1	2	3	4	5	average
Number of copulating pairs	42	50	21	29	52	38.8	36	40	33	17	25	30.2	50	51	70	32	33	47.2
Number of separate males	6	7	9	15	10	9.4	1	8	0	4	0	2.6	0	8	5	0	1	3.2
Number of separate females	7	7	9	8	10	8.2	1	7	0	4	1	2.6	0	0	0	0	0	0
Number separate, not sexed	0	8	7	0	1	3.2	0	1	0	4	6	2.2	2	8	2	0	6	3.6
Total flukes recovered	97	122	67	80	125	98.2	74	96	66	46	57	67.8	102	118	147	64	73	100.8

TABLE XLVI. NUMBER OF *S. JAPONICUM* FLUKES RECOVERED FROM MICE EXPOSED IN VILLACO COLONY AND AT SITES IN MALIRONG AND PALO RIVERS DOWNSTREAM FROM SNAIL COLONIES (9.00-11.00 A.M., 10 MARCH 1956)

	Villaco colony						Malirong river						Palo river					
	mouse no.						mouse no.						mouse no.					
	1	2	3	4	5	average	1	2	3	4	5	average	1	2	3	4	5	average
Number of copulating pairs	36	45	59	106	35	56.2	0	0	0	0	0	0	0	0	0	0	1	0.2
Number of separate males	8	5	17	6	3	7.8	1	0	0	0	0	0.2	0	0	0	0	0	0
Number of separate females	1	0	17	2	3	4.6	0	0	0	0	0	0	0	0	0	0	0	0
Number separate, not sexed	24	4	0	1	7	7.2	0	0	0	0	0	0	0	0	0	0	0	0
Total flukes recovered	105	99	152	221	83	132.0	1	0	0	0	0	0.2	0	0	0	0	2	0.4

TABLE XLVII. SNAIL INFECTION RATES TAKEN BEFORE LATRINE CAMPAIGN (MAY 1954-

Place	Before latrine campaign	Rechecks after					
		1954		1955			
	17 May-4 June	16 Aug.-1 Sept.	18 Oct.-11 Nov.	10 Jan.-4 Feb.	22 March-5 April	6 May-8 June	12 July-22 July
" Near " colonies:							
1. Malirong River Pocket No. 2	3.55	2,99	3.78	4.83	25.44	6.39	1.37
2. Agoong Creek (South bank)	0.86	2.45	1.82	2.05	6.87	2.77	2.02
3. Naliwatan upstream	4.83	5.79	2.03	1.60	12.41	9.05	9.46
4. Juber Creek	4.71	1.96	0.85	8.77	9.77	16.58	6.12
5. Nalicaban Creek	1.02	0.17	0	2.76	7.65	3.21	2.28
Average by snails	3.04	2.59	1.47	3.51	12.72	6.80	4.29
Average by colonies	2.99	2.67	1.70	4.00	12.43	7.60	4.25
" Far " colonies:							
1. Agoong Creek (North bank)	0	0.89	1.79	1.76	3.36	0.81	0.62
2. Malirong Swamp No. 1	1.49	1.59	5.00	5.51	5.80	3.69	1.85
3. Villaco Creek	0.29	0.25	0.62	26.76	27.92	7.59	6.09
4. Naliwatan downstream	0.28	3.09	1.47	1.89	6.27	1.62	1.61
5. Vicob-Malaigang Creek	0.38	1.41	1.27	4.74	2.36	0.31	0.26
Average by snails	0.48	1.21	1.76	14.67	11.64	3.75	3.01
Average by colonies	0.49	1.45	2.03	8.13	9.14	2.80	2.09

the infection rate determined a few days before was higher than during the first trial, and the back-flow of water from the Malirong river had prevented the exit of cercariae from the colony, a greater number of cercariae became available to infect the mice.

On the other hand, the Malirong river exposures yielded a total of only one fluke as against 339 during the previous experiment. On the basis of the back-flow of water in colonies similar to that of Villaco, the scarcity of cercariae in the river may be expected. Moreover, the density of cercariae was further diluted by the water spilled from the Tikba dam, which raised the water level by at least 0.5 m. Hence only one of the five mice exposed was infected.

Most surprising in this experiment is the recovery of a pair of flukes from the mice exposed in the Palo river. Because of the enormous dilution in the river, plus the slim chance of cercariae surviving the drop down the waterfall, we were sceptical about getting any fluke at all. Most likely, however, the cercaria was released from an infected snail that was carried downstream from the colonies.

IN MALIRONG ZONE AND AT 13 LATER RECHECKS, USING RING METHOD MARCH 1956)

latrine campaign							
1955					1956		Place
15 Aug.-27 Aug.	24 Sept.-30 Sept.	10 Oct.-9 Nov.	18 Nov.-23 Nov.	3 Dec.-23 Jan.	23 Jan.-9 Feb.	15 Feb.-12 March	
							" Near " colonies:
1.30		1.95		2.35		38.95	1. Malirong River Pocket No. 2
2.04	5.02	7.92		2.80	15.50	10.83	2. Agoong Creek (South bank)
6.69	8.26	8.61	9.54	10.23	12.62	3.68	3. Naliwatan upstream
8.15		15.07		12.16		18.68	4. Juber Creek
4.77		1.95		8.50		8.09	5. Nalicaban Creek
4.53	6.22	7.11	9.54	7.14	14.11	16.58	Average by snails
4.59	6.64	7.10	9.54	7.21	14.06	16.05	Average by colonies
							" Far " colonies:
6.43		10.06		4.04		3.40	1. Agoong Creek (North bank)
0.67	2.85	4.55	4.85	4.52	4.40	5.90	2. Malirong Swamp No. 1
9.99	5.03	4.33	2.33	4.46	22.09	12.96	3. Villaco Creek
0		0.88		3.95		2.42	4. Naliwatan downstream
0.87		2.64		1.99		3.69	5. Vicob-Malaigang Creek
5.33	4.42	5.16	3.06	3.94	16.71	7.61	Average by snails
3.59	3.94	4.49	3.59	3.79	13.25	5.67	Average by colonies

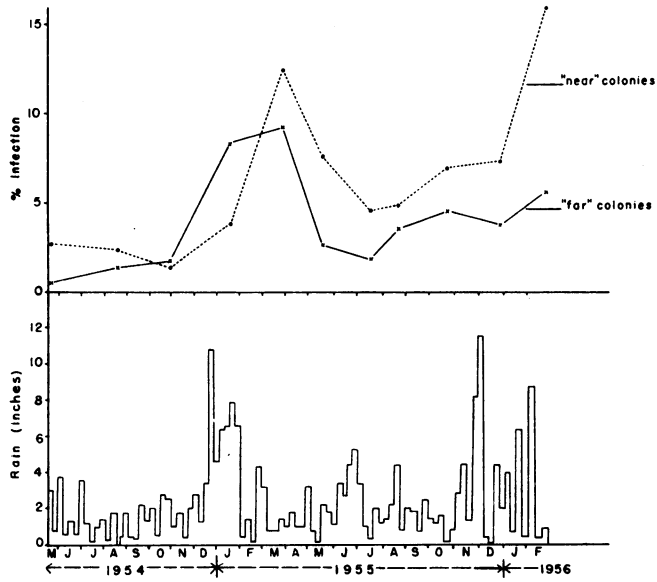
From the results thus far obtained, there seems to be no doubt that the Palo river can also be dangerous as far as exposure to bilharziasis is concerned, and by inference no fresh water downstream from any snail colony can ever be considered safe.

Natural Fluctuations in Snail Infection Rates

In addition to the population data discussed above, the studies on the snail colonies in Malirong have provided abundant data on the course of infection rates and also on the distribution of infected snails in the 10 colonies. The data on infection rates for the past 21-month period are in Table XLVII and Fig. 38. Although there are many points worthy of discussion, we shall concentrate on one point here. This is the apparent cycle of average infection rates over the period of observation.

Rates rose from July 1954 to March 1955 and fell from March 1955 to July 1955; there was a rise from July 1955 to March 1956. The July-October drop for the " near " colonies (i.e., those near houses) for 1954 is

FIG. 38. RELATION BETWEEN RAINFALL AND SNAIL INFECTION RATES IN 5 COLONIES NEAR HOUSES AND 5 COLONIES FAR FROM HOUSES IN MALIRONG



attributable to partial success of the first campaign for the construction and use of pit latrines, which will be discussed in a later paper. The rise for 1955 is more rapid than that for the corresponding periods for 1954 and 1956. This is essentially the cycle for 1946 noted by McMullen (1947).

An examination of the weekly rainfall totals for the whole period provides a likely explanation of the larger changes in infection rates. There were three periods when rainfall remained especially high for a month or more. These were 19 December 1954 through 29 January 1955 (when the rain averaged one inch (25 mm) per day), 4 June through 9 July 1955, and 4 November 1955 through 16 February 1956, when the average was at least one-half inch (13 mm) per day. Periods with most significant increases in infection rates occurred in early March, late August and December 1955 and late February 1956. Each period showing an increase in infection rate has been predicted by us by counting eight weeks forward each from the onset of the heavy rains and the start of the days with lesser rainfall. The time lag of eight weeks corresponds to the incubation period of the infection in the snail, and we conclude that excessive rain facilitates infection in the snails. Although the average rainfall per day was less (0.33 inches or 8.4 mm) during the period 19 December 1955 through 29 January 1956 than for the same period the previous year, about the same general average infection rate was noted. This might be explained by the fact that the rain

started earlier in 1955 (in November), thus providing more opportunity for the infection in snails to build up. Heavy rain did not start until the middle of December in 1954.

There are two ways in which heavy rain may increase infection rates in snails. The first is through the washing into the colony of faeces deposited on the bank before they can dry up or be consumed by animal scavengers, and the second is through the covering of more snails with water, thus providing more opportunities for the miracidia to come in contact with the snails. June is not always a rainy month, and the fact that a moderately rainy June (1955) was followed by an increase in infection rates appears to indicate that other climatic factors, such as light and temperature, are not important in determining the annual cycle of infection rates. It is further true that dry periods have been followed by decreases.

RÉSUMÉ

Oncomelania quadrasi est le seul vecteur connu de *Schistosoma japonicum* aux Philippines. Ce mollusque se rencontre dans les régions des îles dont le relief est peu accentué et où n'existe pas de saison sèche. Un cours d'eau dont la pente et le débit sont adéquats, un sol de texture favorable lui assurent des gîtes propices. Les auteurs décrivent en détail la biologie du mollusque, d'après des observations dans la nature et au laboratoire. L'accouplement est maximum en septembre et minimum en juin. Dans la nature, les changements de densité des populations de mollusques sont faibles et sont liés plutôt à l'habitat qu'aux saisons. Les mesures de lutte fondées sur la réduction de la densité de population des mollusques deviennent rapidement inefficaces, car le taux de survie augmente en fonction du dépeuplement. Une réduction de la densité de 75-95% est compensée en quelques mois. C'est pourquoi les mesures de lutte qui ne modifient pas profondément les conditions de l'habitat, telles que l'emploi de molluscicides, n'ont qu'une valeur éphémère et doivent être fréquemment répétées.

L'infection par les schistosomes affecte les mollusques, ralentit leur croissance et diminue le taux de reproduction. On ne connaît pas d'exemple de guérison d'un mollusque infecté. Les femelles sont infectées en plus forte proportion que les mâles. Les mollusques infectés éliminent environ 5 cercaires en 2 jours. L'élimination débute en moyenne 63 jours après l'infection. Elle se produit généralement dans la soirée, entre 18 et 23 heures, alors que le mollusque a été exposé durant une douzaine d'heures à une lumière d'intensité moyenne. Les eaux en aval d'un gîte doivent être considérées comme virtuellement infectantes. On a pu infecter des souris avec de l'eau récoltée 1 km en aval d'un gîte. Le taux d'infection des mollusques présente une périodicité annuelle. Les maximums s'observent huit semaines environ après les fortes chutes de pluie. Cette période de huit semaines correspond au temps d'incubation de l'infection. Les auteurs ont conclu que les pluies facilitaient l'infection des mollusques, en élevant le niveau de l'eau, ce qui favorise la pénétration d'un plus grand nombre de miracidies dans les mollusques, et en entraînant dans les gîtes des matières fécales infectées déposées sur les bords des cours d'eau.

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