SOME OBSERVATIONS ON THE GROWTH OF *PERINGIA ULVAE* (PENNANT) 1777 IN THE LABORATORY.

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(With 3 text-figures.)

INTRODUCTION.

DURING the study of the effect of Trematode parasites on the growth of *Peringia ulvae*, an attempt had been made to estimate the age of individual snails by some external feature. Great variation was recorded in the size, shell colour, shell shape and shell texture of this mollusc (Rothschild 1938). It seemed probable that this variation was linked with environmental factors, thus rendering the size of the snails an unsuitable criterion for estimating their age. The following experiments were undertaken with the hope of obtaining some definite data on this subject, and to prove whether, in the laboratory at any rate, some slight differences in environment produced any marked contrast in the growth rate, shell colour, etc., of the snails. It was also thought that, simultaneously, the growth of specimens with Trematode infections might be compared with those free from parasites.

A number of specimens of *P. ulvae* ranging from 0·6 mm. to 5·3 mm. in length were collected at random from two different habitats, the mud-flats of St. John's Lake and a permanent pool in the saltings at "Egypt," Plymouth (see Map, Hartly & Spooner, 1938). A certain number of each were placed in finger bowls and test tubes respectively, and their growth compared by measurements taken periodically.

TECHNIQUE.

The snails were collected by placing scoops of mud in dishes and allowing the specimens to crawl out. Any infected individuals were included purely accidentally, as they were not isolated before the commencement of the experiment in order to determine whether they were parasitized.

The glass test tubes and finger bowls in which they were placed were filled to within two inches of the top with a mixture of half sea and half fresh water. The snails were provided with a very plentiful supply of *ulva*—a seaweed upon which they frequently feed in nature. The water in both types of vessel was changed once a month. All were kept at room temperature upon a wooden shelf in the laboratory. The positions of individual bowls and tubes on the shelf, as well as all bowls and tubes *en bloc*, were changed from time to time.

From two to six specimens were placed in each bowl, and from two to four in the tubes. Under these conditions the snails appeared healthy and quite active. The survival rate was in fact remarkably high,[1] only four snails dying out of a total of 152.

Measurements were taken once a month from December 1937 to December 1938 with the aid of a micrometer. The larger size groups were measured,

[1] In this connexion it is worth noting that in brevet jars containing several hundred *P. ulvae* —even if the water is changed daily and the food supply is unlimited—the mortality is high, perhaps more than 50 per cent. of the snails die off within six months.

less accurately, with a ruler marked in quarter millimetres. In the case of the subsidiary tube experiment, which is considered separately, measurements were made at long irregular intervals over a period of three years.

The Growth of *P. ulvae* in Bowls and Tubes

It is, of course, well known that several species of fresh-water *Limnaea* are dwarfed if kept in small vessels. The exact cause of this dwarfing is evidently not properly understood. The result of the experiment with *P. ulvae* shows clearly that specimens from both habitats grew considerably less when kept in tubes than in bowls. Measurements are given below in Tables I and II.

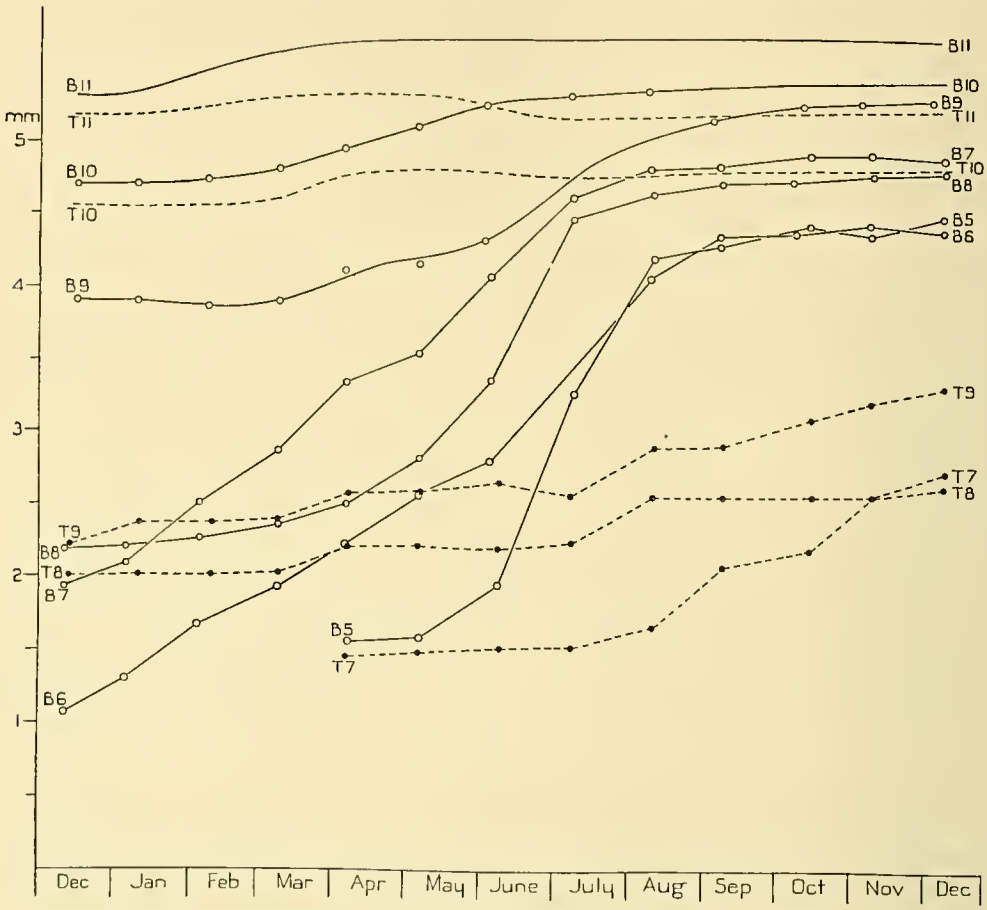
TABLE II.

GROWTH OF INFECTED AND UNINFECTED SPECIMENS COMPARED.

(*Numbers in brackets indicate number of specimens.*)

Locality, Bowl and Tube No.	Mean Initial Size (Dec. 1937)	Final Size Uninfected	Final Size Infected	Sex of Infected Snails
St. John's Lake :				
Bowl *3* . .	0·67 (2)	4·45 (2)	—	—
Bowl *5* . .	0·79 (4)	4·76 (3)	5·70 (1)	♀
Bowl *4* . .	0·87 (4)	5·11 (3)	6·11 (1)	♂
Bowl *2* . .	0·91 (2)	4·46 (2)	—	—
Bowl *13* . .	1·44 (4)	5·19 (4)	—	—
Bowl *10* . .	1·71 (4)	5·29 (3)	6·22 (1)	♂
Bowl *14* . .	1·93 (4)	5·35 (4)	—	—
Bowl *12* . .	1·95 (3)	4·86 (2)	4·64 (1)	♂
Bowl *11* . .	2·13 (4)	5·35 (4)	—	—
Bowl *18* . .	3·75 (4)	4·99 (2)	5·44 (2)	♂♀
Bowl *18a* . .	4·19 (4)	4·97 (3)	5·21 (1)	♂
Bowl *19* . .	4·63 (4)	4·87 (3)	6·85 (1)	♂
Tube *7* . .	1·32 (3)	2·33 (3)	—	—
Tube *15* . .	1·63 (4)	2·69 (4)	—	—
Tube *16* . .	2·13 (4)	2·73 (4)	—	—
Tube *17* . .	2·94 (4)	3·94 (3)	4·95 (1)	♀
Egypt Saltings :				
Bowl *20* . .	1·09 (3)	4·43 (3)	—	—
Bowl *21* . .	1·76 (3)	5·35 (3)	—	—
Bowl *26* . .	1·87 (5)	4·97 (5)	—	—
Bowl *22* . .	2·07 (3)	4·60 (3)	—	—
Bowl *23* . .	2·11 (4)	5·35 (4)	—	—
Bowl *27* . .	2·53 (5)	5·37 (4)	Suspected of Infection 5·93 (1)	♀
Bowl *28* . .	3·92 (6)	5·23 (5)	5·60 (1)	♂
Bowl *29* . .	4·71 (6)	5·39 (6)	—	—
Tube *24* . .	2·02 (3)	2·61 (3)	—	—
Tube *25* . .	2·24 (4)	3·28 (4)	—	—

It will be seen that as a rule specimens kept in bowls, measuring under 2·6 mm. at the commencement of the experiment, had increased at the end of a twelve-month period to a mean length of about 5 mm., whereas tubed snails attained a mean of only approximately 3 mm. Even though snails with an initial measurement of over 3 mm. do not grow as rapidly as the smaller speci-



TEXT-FIG. 221.—GROWTH-RATE OF *P. ULVAE* FROM EGYPT SALTINGS. IN BOWLS (———) AND TUBES (·····)

Measurements at monthly intervals from December 1937 to December 1938.

B5 :	2 bowls, 7 specimens	T7,	3 specimens
B6 :	1 bowl, 3 ,,	T8,	3 ,,
B7 :	3 bowls, 11 ,,	T9,	4 ,,
B8 :	2 bowls, 12 ,,	T10,	4 ,,
B9 :	1 bowl, 6 ,,	T11,	4 ,,
B10 :	1 bowl, 6 ,,		
B11 :	1 bowl, 6 ,,		

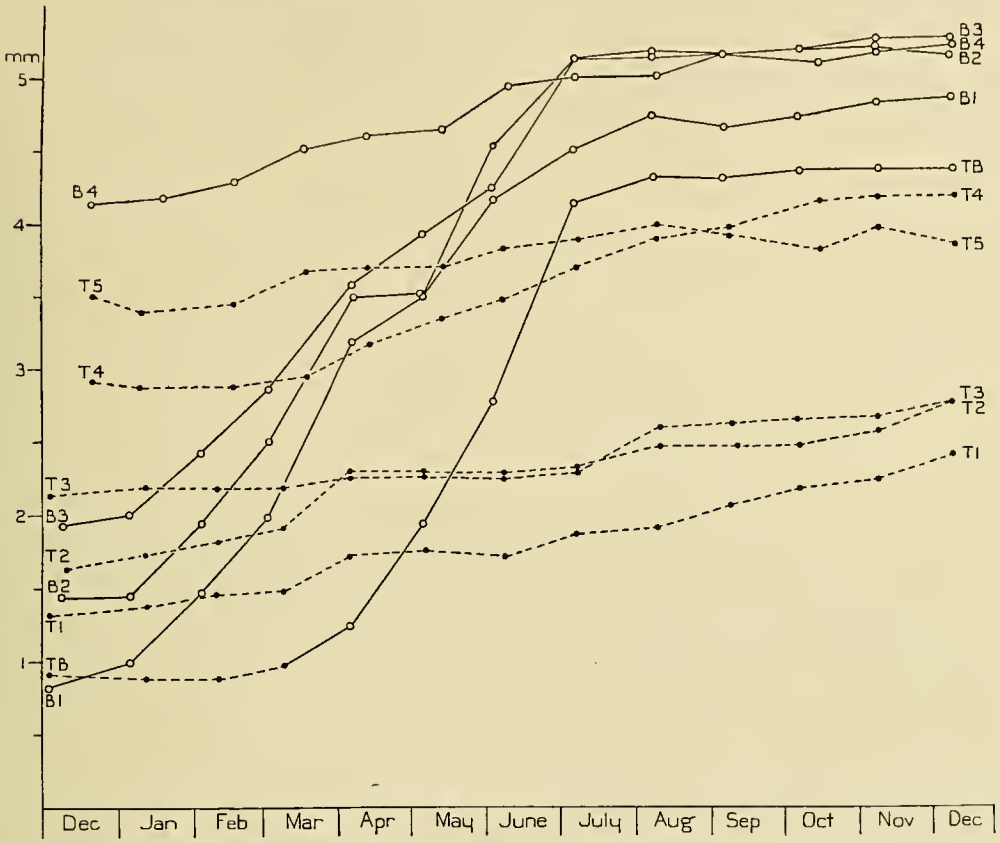
Graphs B9, B10, B11, T10, T11 are smoothed freehand curves (see text).

mens, the contrast between the growth rate of specimens in bowls and tubes remained constant. A comparison, for example, of bowls Nos. 10, 11, 18 and 19 with tubes Nos. 15, 16, 17*a* and 31 show that the average increment of snails from the bowls is three to four times as great as that of the tubed snails.

Four snails from tube 6 were changed to a bowl in April 1938. During this four-month period in the tube the mean size of the snails had increased by only

0·05 mm. Snails of a similar initial size in a bowl increased by an average of 1·02 mm. during the same period. After the change to a bowl three specimens (the fourth died) rapidly increased in size, eventually reaching a mean of 4·38 mm. in December 1938.

In text-figs. 221 and 222 the curves marked B—representing the average of



Text-Fig. 222.—Growth-rate of *P. ulvae* from St. John's Lake. In Bowls (———) and Tubes (.....).

Measurements at monthly intervals from December 1937 to December 1938.

B1 : 4 bowls, 13 specimens	T1, 3 specimens
B2 : 1 bowl, 4 ,,	T2, 4 ,,
B3 : 4 bowls, 15 ,,	T3, 4 ,,
B4 : 3 bowls, 12 ,,	T4, 4 ,,
TB, tube and bowl, 3 ,,	T5, 4 ,,

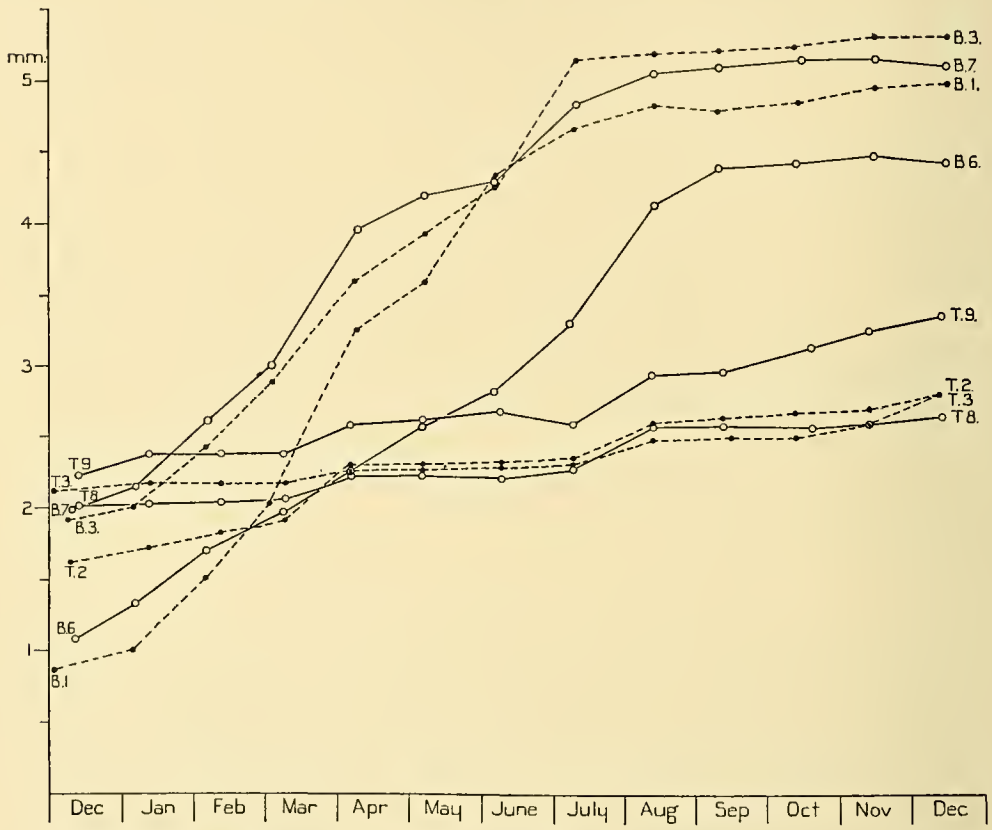
a number of bowls containing specimens of approximately the same initial size —are compared with specimens from tubes (T). In order to simplify the diagram, smoothed freehand curves have been given for specimens of 4 mm. and over (which were measured with the aid of a ruler only). Many of these large individuals grew very little during the twelve months and the fluctuations in the measurements, which are noted in the Tables, can probably be accounted for by chipping of the spires.

It is interesting that, apart from the different rapidity of increase in size, the specimens in bowls and tubes display certain differences in their growth curve. Thus the growth rate of the specimens in bowls falls abruptly in August

irrespective of age and size. Even those specimens of under 2 mm. which were started in April instead of December display this feature. On the other hand, the tubed snails tend to stop growing from April to July—the period of maximum growth of the snails in the bowls—but to recover with a slow steady growth subsequently.

RESEMBLANCE BETWEEN THE GROWTH OF SPECIMENS FROM MUD-FLATS (ST. JOHN'S LAKE) AND SALTINGS (EGYPT).

In nature, random collections of *P. ulvae* from the mud-flats of St. John's Lake and permanent pools from the saltings at Egypt, display certain marked



TEXT-FIG. 223.—GROWTH-RATE OF *P. ULVAE* FROM EGYPT SALTING (———) AND ST. JOHN'S LAKE (.....).

Obviously abnormal specimens with retarded growth have been omitted (one specimen from B1, and one from B7), and the aggregates modified accordingly.

differences (Rothschild 1938, Plates I & II). The former are of medium size (maximum infected specimens reaching about 5·8 mm. in length) with a medium hard, grey or reddish shell, showing fairly well-preserved spires. The latter are very large (maximum infected specimens reaching 10·5 mm. in length) with an exceptionally hard, thick, very red shell, showing blunted spires in all large individuals. In the laboratory, snails collected from these two habitats exhibited similar growth curves. These are compared in text-fig. 223, in which single specimens of obviously abnormal growth have been omitted. The two sets of

TABLE I.

GROWTH OF SPECIMENS IN BOWLS AND TUBES.

Mean length in mm. at monthly intervals.

ST. JOHN'S LAKE. BOWLS.

No. of Bowl	No. of Specimens	1937 2–10. xii	1938 4.i	1938 3.ii	1938 2.iii	1938 5.iv	1938 5.v	1938 3.vi	1938 6.vii	1938 8.viii	1938 5.ix	1938 6.x	1938 7.xi	1938 8.xii	No. of Specimens infected	Group of Cercariae
3	2	0·67	1·02	1·06	1·99	3·82	3·83	4·02	4·39	4·66	4·39	4·47	4·42	4·48	—	—
5	4	0·79	1·02	1·69	2·27	3·28	3·59	4·36	4·64	4·65	4·66	4·74	4·89	5·00	1 (♀)	Pleurolophocerca
2	3	0·86	1·00	1·26	1·68	2·86	3·31	3·42	3·71	4·17	4·04	4·20	4·25	4·28	—	—
4	4	0·87	0·91	1·30	1·89	3·00	3·40	4·55	5·03	5·28	5·28	5·29	5·35	5·36	1 (♂)	Ubiquita
13	4	1·44	1·44	1·93	2·50	3·49	3·52	4·52	5·13	5·18	5·16	5·20	5·21	5·16	—	—
10	4	1·71	1·80	2·30	3·01	3·78	4·14	4·29	5·19	5·27	5·33	5·43	5·55	5·49	1 (♂)	Ubiquita
14	4	1·93	1·95	2·02	2·48	3·72	3·93	4·72	5·29	5·24	5·31	5·24	5·38	5·32	—	—
12	3	1·95	1·98	2·59	3·02	3·58	3·59	3·85	4·57	4·67	4·71	4·74	4·76	4·81	1 (♂)	Ubiquita
11	4	2·13	2·23	2·87	2·99	3·29	3·64	4·03	5·37	5·33	5·27	5·31	5·32	5·37	—	—
		20.xii	18.i	17.ii	18.iii	13.iv	13.v	10.vi	8.vii	10.viii	7.ix	14.x	8.xi	9.xii		
18	4	3·75	3·94	3·87	4·25	4·31	4·31	4·81	4·95	4·95	5·31	5·13	5·13	5·30	2 (♀♂)	Pleurolophocerca
18a	4	4·19	4·19	4·37	4·56	4·75	4·88	5·06	4·89	5·00	5·00	5·00	5·08	5·02	1 (♂)	Ubiquita
19	4	4·63	4·56	4·63	4·75	4·75	4·75	4·98	5·14	5·14	5·23	5·23	5·36	5·37	1 (♂)	Ubiquita

ST. JOHN'S LAKE. TUBES.

		1937 2.xii	1938 11.i	1938 10.ii	1938 9.iii	1938 6.iv	1938 6.v	1938 7.vi	1938 7.vii	1938 10.viii	1938 7.ix	1938 6.x	1938 7.xi	1938 8.xii		
7	3	1·32	1·39	1·46	1·47	1·72	1·75	?	1·86	1·92	2·06	2·19	2·25	2·41	—	—
15	4	1·63	1·72	1·81	1·93	2·29	2·29	2·28	2·31	2·47	2·49	2·49	2·59	2·78		
					[illegible]	2·96	2·26	2·25	2·28	2·62	2·64	2·66	2·68	2·78	—	—
		20.xii	19.i	17.ii	18.iii	13.iv	13.v	7.vi	8.vii	10.viii	7.ix	14.x	8.xi	9.xii		
17	4	2·94	2·88	2·88	2·95	3·19	3·35	3·49	3·71	3·90	3·98	4·16	4·19	4·20	1 (♀)	Oocysta
17a	5	3·50	3·40	3·45	3·67	3·70	3·70	3·81	3·88	3·98	3·94	3·84	3·98	3·8	1 (♀)	Oocysta
17b	5	4·88	4·65	4·75	4·90	4·90	4·85	4·85	4·85	4·85	4·77	4·77	4·95	4·84	2 (♀♂)	Metentera Pleurolophocerca

EGYPT SALTINGS. BOWLS.

		1937 10–13. xii	1938 5.i	1938 4.ii	1938 8.iii	1938 5.iv	1938 5.v	1938 3.vi	1938 7.vii	1938 9.viii	1938 6.ix	1938 7.x	1938 8.xi	1938 8.xii		
20	3	1·09	1·32	1·69	1·95	2·24	2·59	2·82	3·28	4·06	4·37	4·39	4·45	4·40	—	—
21	4	1·71	1·81	2·18	2·35	2·72	2·75	3·36	4·32	4·63	4·64	4·73	4·73	4·67	—	—
26	6	1·88	1·87	1·98	2·17	2·23	?	2·86	4·04	4·29	4·39	4·41	4·45	4·47	—	—
22	3	2·07	2·18	2·59	2·94	3·39	3·88	4·29	4·49	4·50	4·56	4·58	4·62	4·58	—	—
23	4	2·11	2·34	2·85	3·40	3·96	4·12	4·64	5·07	5·25	5·27	5·36	5·35	5·34	—	—
27	6	2·56	2·59	2·58	2·61	2·78	3·07	3·68	4·94	5·01	5·06	5·07	5·09	5·15	—	—
		15.xii	10.i	9.ii	8.iii	5.iv	6.v	3.vi	8.vii	10.viii	7.ix	14.x	8.xi	9.xii		
28	6	3·92	3·92	3·89	3·92	4·14	4·17	4·34	5·05	4·81	5·16	5·27	5·26	5·30	1 (♂)	Ubiquita
29	6	4·71	4·71	4·75	4·83	4·96	5·13	5·28	5·34	5·36	5·45	5·45	5·28	5·50	—	—
30	6	5·33	5·33	5·50	5·79	5·79	5·75	5·57	5·87	5·60	5·80	5·53	5·87	5·77	—	—
		—	—	—	—	6.iv	6.v	7.vi	7.vii	10.viii	6.ix	13.x	8.xi	8.xii		
20a	3	—	—	—	—	1·56	1·58	1·97	3·80	4·60	4·62	4·75	4·76	4·76	—	—
20b	4	—	—	—	—	1·61	1·64	1·98	2·83	3·90	4·09	4·20	4·11	4·31	—	—

EGYPT SALTINGS. TUBES.

		1937 13.xii	1938 10.i	1938 9.ii	1938 9.iii	1938 6.iv	1938 6.v	1938 7.vi	1938 7.vii	1938 10.viii	1938 7.ix	1938 13.x	1938 7.xi	1938 8.xii		
24	3	2·02	2·04	2·04	2·05	2·24	2·24	2·23	2·27	2·57	2·57	2·57	2·58	2·74	—	—
25	4	2·24	2·38	2·39	2·40	2·59	2·60	2·67	2·58	2·92	2·94	3·12	3·23	3·33	—	—
31	4	4·56	4·56	4·56	4·63	4·94	4·94	4·71	4·63	4·80	4·80	4·85	4·91	4·75	—	—
32	4	5·37	5·13	5·19	5·25	5·44	5·44	5·09	5·11	5·13	5·30	4·91	4·23	5·20	1 (♂)	Ubiquita
		—	—	—	—	6.iv	6.v	7.vi	7.vii	10.viii	6.ix	13.x	8.xi	8.xii		
20c	3	—	—	—	—	1·48	1·50	1·53	1·53	1·67	2·09	2·20	2·58	2·64	—	—

ST. JOHN'S LAKE. TUBE TO BOWL.

		1937 2.xii	1938 11.i	1938 10.ii	1938 9.iii	1938 5.iv	1938 5.v	1938 3.vi	1938 6.vii	1938 8.viii	1938 5.ix	1938 6.x	1938 7.xi	1938 8.xii		
		Tube				→Bowl								→		
6	4	0·92	0·88	0·89	0·97	1·24	1·92	2·78	4·15	4·32	4·32	4·38	4·38	4·38	—	—

curves are, in fact, remarkably alike, and it would seem safe to suppose that the differences of growth shown by *P. ulvae* in nature are not due to inherited characters, but environmental factors.

In both groups of snails the specimens measuring 1 mm. in December (which were kept in bowls) reached 4·50 mm. by the following August, and those of 1·50 mm.–2 mm. reached 5 mm. in the same period. Thus they overtake specimens which were 4 mm. in length at the start of the experiment. Any size groups which could be distinguished in December are merged into a single group by the following summer.

The shells of all snails were grey in colour at the end of the twelve-month period, and rather more friable than in normal specimens from St. John's Lake, but, on the other hand, the spires were better preserved. In those snails which had been started at 4 mm. or over, the spires were inclined to chip off or blunt.

It is interesting to find that the specimens from Egypt, which were put in bowls and tubes at 4 mm. or over, failed to grow appreciably, although in the wild they would have presumably added 2–2½ mm. to their length. They remained at about the size of maximum uninfected specimens from St. John's Lake, and also lost their red colour and thick, hard shells.

This may have been due to a sudden change of environment at a relatively late stage, but it also seems probable that the growth conditions in the laboratory are inferior to those of many of the permanent pools of the saltings, but superior in bowls, at any rate, to those pertaining on the mud-flats.

Comparison between Uninfected and Infected Specimens of *P. ulvae*.

When the growth experiment was terminated in December 1938 all the snails were dissected and examined for the presence of Trematode parasites. Twelve infected specimens were found in the material from St. John's Lake and two from the permanent pool in the saltings. A third specimen from Egypt was suspected of infection, but died a day or two before dissection.

The size of these infected snails was quite consistently larger than that of uninfected specimens as shown by the data presented below in Table II. (The final figures represent the mean of the November and December measurements. A few specimens from the bowls in which growth was abnormally low have been omitted. These were all uninfected snails.) The evidence obtained in the laboratory therefore supports the theory (Rothschild 1938) that infection with Trematode parasites causes increased growth.

Out of a total of fourteen infected snails eight proved to be males and six females. The total number of uninfected males was fifty-two, and of females sixty-six. However, some of the smallest specimens may have been males in which the penis had not yet developed. These figures are therefore inadequate for the purpose of studying the question of possible sex reversal of infected females. It is, however, worth noting that an infected male attained a length of 6·85 mm., whereas the maximum uninfected male reached only 6 mm.

As in nature, the penis of all infected snails showed abnormalities in size and shape.

Comparison in Growth Rate of Males and Females.

Although the figures at our disposal are not very large there is sufficient data to show the average growth rate of uninfected females during the period

of this experiment was higher than that of uninfected males. A comparison of both sexes is made in Table III. (Tubes in which there was only very little or no growth are omitted.) This agrees with the evidence obtained by examining samples of *P. ulvae* from various habitats and tends to confirm the suggestion that in this species the larger size of the females is due to increased growth rate rather than a longer life (Rothschild 1938).

TABLE III.

GROWTH OF MALES AND FEMALES (UNINFECTED).

(*Figures in brackets indicate number of specimens.*)

Locality and Bowl and Tube No.	Initial Length	Mean Final Length of Males	Mean Final Length of Females
Egypt : Tubes 20*c*, 24, 25 . . .	1·90–2·60	3·25 (*5*)	2·91 (*3*)
St. John's Lake : Tubes 17, 17*a*, 17*b* .	2·50–5·00	3·91 (*3*)	4·32 (*7*)
Egypt : Bowls 20, 20*a*, 20*b* . . .	under 1·25	4·24 (*4*)	4·58 (*6*)
St. John's Lake : Bowls 2, 3, 4, 5, 6 .	0·65–0·95	4·56 (*6*)	4·85 (*5*)
Egypt : Bowls 21, 22, 23, 26, 27 . .	1·55–2·77	4·52 (*10*)	5·61 (*9*)
St. John's Lake : Bowls 10, 11, 12, 13, 14	1·37–2·27	4·97 (*6*)	5·39 (*11*)
Egypt : Bowls 28, 29, 30 . . .	3·50–5·50	5·21 (*6*)	5·67 (*10*)

THE SUBSIDIARY TUBE EXPERIMENT.

From April 1936 to March 1939 nine specimens of *P. ulvae* collected from St. John's Lake were kept in tubes and measured at several months interval. One snail died in December 1937. The individual measurements are given in Table IV. It will be seen that the snails grew most rapidly during the first twelve months and from thereafter only increased very slowly, but steadily, in size for eighteen months. During the final six months of the experiment only two snails increased at all, and then only by 0·10 mm. Three specimens remained stationary and three became reduced in length, probably by chipping off the spires. It would therefore appear likely that under these conditions the snails reach their limit of growth by the end of the third year.

TABLE IV.

SUBSIDIARY TUBE EXPERIMENT.

Tube No.	April 1936	Oct. 1936	May 1937	Dec. 1937	March 1938	Oct. 1938	March 1939
1 . . .	0·6	2·0	2·2	3·2	3·2	4·1	4·1
2 . . .	0·6	2·1	2·8	3·3	3·5	4·2	4·2
3 . . .	0·6	2·7	3·2	3·9	4·1	4·4	4·2
4 . . .	1·2	3·0	3·4	4·0	4·2	4·5	4·6
5 . . .	1·2	3·2	3·7	4·2	4·3	4·9	5·0
6 . . .	1·9	3·5	3·8	4·0	4·2	4·7	4·6
7 . . .	1·9	3·8	4·0	4·0	4·3	4·8	4·8
8 . . .	1·9	3·8	4·1	4·4	4·5	4·9	4·8

Summary.

1. A comparison is made between the growth rate of *P. ulvae*, collected from two different habitats and kept in the laboratory in (*a*) glass bowls, (*b*) glass tubes.

2. The snails kept in bowls grow considerably faster than snails kept in tubes ; their size is consequently no criterion of their age.

3. The growth curve is different in specimens kept in bowls and in tubes.

4. When reared in the laboratory, specimens collected from the mud-flats and saltings, which in nature differ from each other in size, shell shape, shell colour and shell texture, show no such variation. These differences are therefore thought to be due to environmental rather than genetic factors.

5. Snails parasitized by Trematodes grow faster than uninfected specimens.

6. The uninfected females grow faster than the uninfected males.

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