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CONTENTS

PAGE

YUNG-SAN LIANG -- CULTIVATION OF <i>BULINUS</i> (<i>PHYSOPSIS</i>), <i>GLOBOSUS</i> (MORELET) AND <i>BIOMPHALARIA PFEIFFERI</i> <i>PFEIFFERI</i> (KRAUSS), SNAIL HOSTS OF SCHISTO- SOMIASIS (Continued from Sterkiana No. 53)	1
JAMES X. CORGAN -- FURTHER NOTES ON PSEUDOGASTROPODS	28
BOOKS RECEIVED	28
REGINA BROWN -- RESOURCES FOR MALACOLOGICAL RESEARCH IN ORTON MEMORIAL LIBRARY OF GEOLOGY	29

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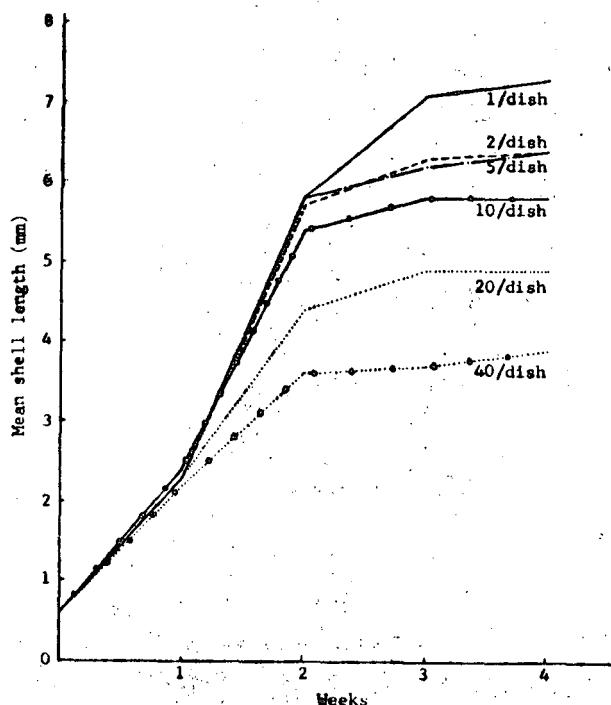


Fig. 18. Effect of crowding on mean shell length of *B. pfeifferi* reared for 4 weeks

in 525° C-heated steamed extract. Silica was highest in 525° C-heated steamed extract, less in steamed extract, and lowest in stirred extract. Sulphate was highest in 525° C-heated steamed extract with 630 ppm ($=680 + 580/2$) and was about the same between stirred extract and steamed extract with 360 ppm and 350 ppm ($=360 + 340/2$), respectively. The different values for chloride, magnesium hardness, nitrate and nitrite nitrogen, pH, and phosphate appeared insignificant. In conclusion, excessive amounts of calcium, silica, sulphate, or combinations of them appear to be harmful to snail growth.

Part IV. Formulation of Synthetic Mud

Previous studies showed that mud, collected from a local natural habitat of amphibious *Pomatiopsis cincinnatiensis* snails, was suitable for growing a variety of blue-green algae which in turn, supported good growth of *Bulinus globosus* and *Biomphalaria pfeifferi* snails (as indicated in Part I and Part II). This same mud was also excellent for growing a unialgal culture of *Nostoc muscorum* which was even better than the mixture of blue-green

algae for supporting growth of *Biomphalaria pfeifferi* snails (Part III).

Culture methods like these might be difficult to reproduce by those not having access to this particular type of mud, or a suitable type of mud might not be available locally if the habitat were polluted or destroyed. Therefore, the following methods were designed in an attempt to develop a synthetic mud to be substituted for the natural product.

The formulation of the various types of synthetic muds was accomplished essentially on the basis of trial and error (see Table 7 for formulae) and involved altogether 12 experiments. Some components justified for use in the earlier formulae were eliminated later because they had not shown beneficial properties. For each formula, 3 Petri dishes (Experiments 1 through 5) or 4 Petri dishes (Experiments 6 through 12) were established. Usually *Nostoc muscorum* was inoculated into the cultures immediately after the dishes were prepared. Five snails were added to each Petri dish 5 to 16 days after algal inoculation. As a rule, Petri dish preparations were not replaced during the course of these experiments. Measurements were made weekly over a 1 to 5 week period.

Experiment 1

Petri dishes were made according to the formulae shown in Table 7. Three days after algal inoculation, all the dishes emitted a strong, sour 'rotten fish' odor, apparently caused by putrefaction of the fish meal and fermentation of the glucose. Snails were, therefore, not introduced into these dishes.

Experiment 2

The amount of fish meal used in the formulae was cut to half that used in Experiment 1, and glucose was omitted. The amounts of oyster shell and bone meal used were also each reduced to half that in Experiment 1; KNO_3 was also omitted. Snails were added five days after algal inoculation and cultured for five weeks; the results are shown in Table 39. To the end of the fourth week, survivorship in the Petri dishes with distilled water as overlay (Formulae 1 and 3) was better than those with artificial water as overlay (Formulae 2 and 4). This difference, however, became insignificant by the end of the fifth week.

To the end of the second week Petri dishes with distilled water used in making paste (Formulae 1 and 2) supported shell growth better than those with BBMSU used for paste (Formulae

Table 38. Analyses of kinds of mud extracts used in Experiments 1 and 2

Tests	Kinds of mud extracts					
	Steamed extract		Stirred extract		525°C-heated steamed extract	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Alkalinity (as ppm CaCO ₃)						
Carbonate	0.0	0.0	*	0.0	0.0	0.0
Bicarbonate	65.0	75.0	*	155.0	50.0	40.0
Chloride (as ppm Cl)	45.0	50.0	*	45.0	55.0	45.0
Copper (ppm)	0.0	-	*	-	0.0	-
Hardness (as ppm CaCO ₃)						
Total	440.0	560.0	*	640.0	960.0	840.0
Calcium	405.0	440.0	*	540.0	890.0	800.0
Magnesium	35.0	120.0	*	100.0	70.0	40.0
Iron (ppm)	0.0	-	*	-	0.0	-
Magnesium (ppm)	0.0	-	*	-	0.0	-
Nitrate & nitrite nitrogen (as ppm nitrogen)	0.080	0.063	*	0.034	0.076	0.030
pH value	7.15	7.55	*	7.40	7.25	6.95
Total phosphate	0.27	0.48	*	0.23	0.52	0.25
Silica (ppm)	52.0	50.0	*	34.0	80.0	60.0
Sulphate (ppm)	360.0	340.0	*	360.0	680.0	580.0

* = Not applicable; '-' = Not tested.

3 and 4). However, this relationship was reversed at the end of the third week. By the end of the fifth week, shell length was about the same among snails in Formulae 1 through 3, with means of 5.0, 5.2, and 5.1 mm, respectively; in Formula 4 the mean shell length was as high as 6.2 mm. Although the differences in mortality might have eventually affected the values for shell lengths among the survivors, there was an indication that Petri dishes with artificial water as overlay supported better shell growth than those with distilled water as overlay. Egg-laying occurred only in the Formula 4 Petri dishes, where altogether 32 eggs were laid at the end of the third week and 99 at the end of the fourth.

In conclusion, a workable synthetic mud was formulated. In order to culture snails to the end of the second week, either Formula 1 or Formula 2 with distilled water for pasting is recommended. To culture snails up to the end of the fifth week and for egg-production, Formula 4, BBMSU for pasting and artificial water as overlay, is recommended. On the whole, for shell growth and egg production, artificial water was always superior to distilled water as overlay.

Experiment 3

The amounts of oyster shell, bone meal and fish meal used were the same as in Experiment 1; charcoal was increased to 3 gm., and KNO₃ was added. The amount of agar used was reduced to 0.5 gm. Snails were introduced nine days after algal inoculation and were cultured for six weeks. The results are shown in Table 40.

No snails died in Formula 1 throughout

the entire course of the experiment; in Formula 2, two out of 15 snails died. The difference in survivorship between these two groups may not be meaningful.

To the end of the fourth week, Formula 2 supported better growth than Formula 1, and the mean shell length was 6.2 mm versus 5.7 mm. This difference between the two formulae became insignificant by the end of the sixth week, when the shell length values were 6.5 mm and 6.4 mm, respectively. With Formula 2 egg-laying first occurred at the end of the fourth week; with Formula 1 it did not occur until the end of the fifth week. In both cases, the elapsed time for egg-laying after the snails hatched was considerably longer than observed among snails cultured previously with natural mud (see Table 33).

In conclusion, artificial water was again superior to distilled water as overlay for egg production. The artificial water was also superior to distilled water in producing shell growth at least up to the end of the fourth week.

Experiment 4

The solid components used in the formulae were similar to those used in Experiment 2 except that bone meal was omitted and the amount of agar was reduced to 0.3 gm. Snails were introduced 13 days after algal inoculation and were cultured for one week. The results are shown in Table 41. Survivorship in Formulae 1 and 2 was about the same, with 93.3% (14/15) and 100.0% (15/15) surviving; in both Formulae 3 and 4 with 80.0% (12/15) survived. The rate of growth with all of the formulae was about the same, with a mean shell length in the range of 1.1 to 1.7 mm, which was con-

Table 39. Effect of synthetic muds (in Expt. 2) on survivorship, shell length (\bar{x}) (mm) and fecundity of *B. pfeifferi* reared for 5 weeks *

Formulae **	Dish No.	Weeks														
		1			2			3			4			5		
		S	SL	E	S	SL	E	S	SL	E	S	SL	E	S	SL	E
1 Dis + Dis	1	5	2.5	0	5	4.7	0	5	4.7	0	5	4.9	0	5	5.0	0
	2	5	2.4	0	5	4.1	0	4	5.1	0	4	5.2	0	4	5.2	0
	3	5	2.7	0	5	4.4	0	5	4.5	0	5	4.6	0	4	4.7	0
	TM	15	2.5	0	15	4.4	0	14	4.8	0	14	4.9	0	13	5.0	0
2 Dis + Art	1	3	1.8	0	2	3.2	0	1	5.9	0	1	6.8	0	1	7.1	0
	2	5	2.8	0	5	4.3	0	5	4.3	0	5	4.5	0	5	4.6	0
	3	5	2.9	0	5	4.8	0	4	5.5	0	4	5.6	0	3	5.7	0
	TM	13	2.6	0	12	4.3	0	10	4.9	0	10	5.2	0	9	5.2	0
3 BBMSU + Dis	1	5	2.2	0	5	4.3	0	5	4.9	0	5	4.9	0	3	4.9	0
	2	5	2.0	0	5	4.2	0	5	5.1	0	5	5.2	0	3	5.2	0
	3	5	2.2	0	5	3.6	0	5	5.0	0	5	5.1	0	4	5.2	0
	TM	15	2.1	0	15	4.0	0	15	5.0	0	15	5.1	0	10	5.1	0
4 BBMSU + Art	1	3	1.6	0	3	3.5	0	2	5.9	22	2	6.4	27	2	6.6	0
	2	5	2.4	0	5	4.3	0	5	5.8	10	5	5.9	25	5	6.1	0
	3	4	1.7	0	4	3.0	0	4	5.5	0	4	5.9	47	4	6.0	0
	TM	12	2.0	0	12	3.9	0	11	5.7	32	11	6.0	99	11	6.2	0

S = No. of survivors; SL = Shell length; E = Total eggs; TM = Total and mean; * Snails were introduced 5 days after algal inoculation; ** Prototype = Clay 100 + Shell 0.5 + Charcoal 2 + Agar 2 + Bone 0.5 + Fish 0.5 (see Table 7), 1st liquid used for paste and 2nd for overlay.

Table 40. Effect of synthetic muds (in Expt. 3) on survivorship, shell length (\bar{x}) (mm) and fecundity of *B. pfeifferi* reared for 6 weeks *

Formulae **	Dish No.	Weeks																	
		1			2			3			4			5			6		
		S	SL	E	S	SL	E	S	SL	E	S	SL	E	S	SL	E	S	SL	E
1 Dis + Dis	1	5	1.8	0	5	3.3	0	5	4.8	0	5	5.6	0	5	6.0	37	5	6.4	99
	2	5	1.9	0	5	3.3	0	5	4.7	0	5	5.9	0	5	6.2	43	5	6.3	86
	3	5	1.9	0	5	3.4	0	5	4.7	0	5	5.7	0	5	6.2	85	5	6.5	127
	TM	15	1.9	0	15	3.3	0	15	4.7	0	15	5.7	0	15	6.1	165	15	6.4	312
2 Dis + Art	1	5	2.5	0	5	4.6	0	5	5.4	0	5	5.9	0	5	5.9	40	5	6.1	48
	2	3	2.0	0	3	4.0	0	3	5.2	0	3	6.2	0	3	6.5	0	3	6.9	7
	3	5	2.5	0	5	4.7	0	5	6.0	0	5	6.5	107	5	6.5	16	5	6.6	7
	TM	13	2.4	0	13	4.5	0	13	5.6	0	13	6.2	107	13	6.3	56	13	6.5	62

S = No. of survivors; SL = Shell length; E = Total eggs; TM = Total and mean; * Snails were introduced 9 days after algal inoculation; ** Prototype = Clay 100 + Shell 1 + Charcoal 3 + Agar 0.5 + Bone 1 + Fish 1 + KNO_3 0.05 (see Table 7), 1st liquid used for paste and 2nd for overlay.

siderably smaller than found in Experiment 2, where the value over the same period was between 2.0 and 2.6 mm (see Table 39). Omitting bone meal or reducing agar, or the combination, might account for this unsuccessful attempt. However, it was certain that using modified artificial water I for pasting caused more mortality (as in Formulae 3 and 4) than when distilled water was used (as in Formulae 1 and 2).

Experiment 5

The solid components used in the formulae were the same as those in Experiment 3 but in some the amounts used differed. Charcoal was increased from 3 to 4 gm., fish meal was increased from 1 to 1.5 gm. and agar was reduced from 0.5 to 0.3 gm.; altogether 4 formulae were prepared. All Petri dishes in all formulae emitted a rotten fish smell 3 days after

Table 41. Effect of synthetic muds (in Expt. 4) on survivorship and shell length (\bar{x}) (mm) of *B. pfeifferi* reared for 1 week *

Formulae **	Dish No.	Week 1	
		No. of survivors	Shell length
1 Dis + Dis	1	4	1.3
	2	5	1.3
	3	5	1.3
	Total & Mean	14	1.3
2 Dis + Art	1	5	1.6
	2	5	1.6
	3	5	1.7
	Total & Mean	15	1.6
3 Mod Art 1 + Dis	1	5	1.0
	2	3	1.1
	3	4	1.2
	Total & Mean	12	1.1
4 Mod Art 1 + Art	1	4	1.6
	2	4	1.8
	3	4	1.7
	Total & Mean	12	1.7

* Snails were introduced 12 days after algal inoculation; ** Prototype = Clay 100 + Shell 0.5 + Charcoal 2 + Agar 0.3 + Fish 0.5 (see Table 7), 1st liquid used for paste and 2nd for overlay.

algal inoculation. Since some spotty algal growth was found in all dishes, snails were introduced five days after algal inoculation. All of those snails were dead when examined one week after they were introduced. The failure of this experiment was probably due to the excessive amount of fish meal used in the formulae.

Experiment 6

This experiment was designed to test whether a synthetic mud without fishmeal could support growth of snails; and whether kaolin or white sand could be substituted for pottery clay as a substrate for synthetic mud. Formula 1 was prepared so that all solid components were exactly the same as the ones found in Experiment 5, except that KNO_3 was not added. In Formula 2, fish meal was omitted. In Formulae 3 and 4, kaolin and white sand respectively were substituted for pottery clay; the alga was inoculated as usual. In Formula 4, strong rotten fish smell was evident three days after algal inoculation; no algal growth was observed. In Formula 1 and Formula 3, although the rotten fish smell was present three days after algal inoculation, spotty algal

Table 42. Effect of synthetic muds (in Expt. 6) on survivorship, shell length (\bar{x}) (mm) and fecundity of *B. pfeifferi* reared for 3 weeks *

Formulae **	Dish No.	Weeks											
		1			2			3					
		S	SL	E	S	SL	E	S	SL	E			
1 Prototype	1	0											
	2	0											
	3	0											
	4	0											
	TM	0											
2 No fish	1	5	2.1	0	5	4.4	0	5	5.5	8			
	2	5	1.9	0	5	3.9	0	5	5.3	12			
	3	5	1.9	0	5	3.8	0	5	5.8	37			
	4	5	1.8	0	5	3.9	0	5	5.5	28			
	TM	20	1.9	0	20	4.0	0	20	5.5	85			
3 Kaolin 100 gm in place of Clay 100 gm	1	0											
	2	0											
	3	0											
	4	0											
	TM	0											
4 Sand 140 gm in place of Clay 100 gm	1	#											
	2	#											
	3	#											
	4	#											
	TM	#											

S = No. of survivors; SL = Shell length; E = Total eggs; TM = Total and mean; * Snails were introduced 12 days after algal inoculation; ** Prototype = Clay 100 + Shell 1 + Charcoal 4 + Agar 0.3 + Bone 1 + Fish 1.5 (see Table 7), in all formulae, dist. water used for paste and mod. art. water III for overlay; # All 4 dishes putrefied 3 days after algal inoculation, therefore, no snails were introduced.

growth was still observed. In Formula 2, the algae grew nicely. Snails were, consequently, introduced 12 days after the algal inoculation to Formulae 1 through 3; they were cultured for three weeks with weekly examinations. The results are shown in Table 42.

In Formulae 1 and 3, all snails died by the end of the first week; in Formula 2, no snails died throughout the entire course of the experiment.

Growth of snails in Formula 2 was fairly good and was comparable to that in Formula 4 of Experiment 2 (see Table 39) and Formula 2 of Experiment 3 (see Table 40). At the end of the third week, the mean shell length was 5.5 mm. Egg-laying started at the end of the third week, which was, again, comparable to Formula 4 of Experiment 2.

To conclude: (a) synthetic mud without fish meal supported growth and egg production at least as well as the synthetic mud with fish meal; (b) the suitability of kaolin or white sand as substitute for pottery clay was not conclusively determined in present experiment since Formula 1, which supposedly served as control, was found not able to support

Table 43. Effect of synthetic muds (in Expt. 9) on survivorship and shell length (X) (mm) of *B. pfeifferi* reared for 3 weeks *

Formulae **	Dish No.	Weeks					
		1		2		3	
		S	SL	S	SL	S	SL
1 Prototype	1	2	3.3	2	6.1	2	6.1
	2	4	3.1	4	4.0	4	4.0
	3	3	3.2	3	4.5	3	4.5
	4	3	2.8	3	3.4	3	3.5
	Total & Mean	12	3.1	12	4.3	12	4.4
2 No clay	1	0					
	2	0					
	3	2	1.7	0			
	4	0					
	Total & Mean	2	1.7	0			

S = No. of survivors; SL = Shell length; * Algae were inoculated and snails were introduced on the same day, more algae were inoculated 4 and 7 days later; ** Prototype = Clay 100 + Shell 2 + Charcoal 3 + Agar 0.5 (see Table 7), in both formulae, art. water used for paste and overlay.

growth because of the excessive amounts of fish meal; and (c) in Formula 4 the fish meal apparently putrefied more rapidly in the presence of sand.

Experiment 7 and Experiment 8

Survival was very erratic, with numerous deaths in formulae which were satisfactory in previous experiments. The reasons for this mortality are not known.

Experiment 9

The solid components were pottery clay, oyster shell, charcoal and agar. In order to determine the value of pottery clay, other than to serve as a substrate for algal growth, two formulae were prepared having the same constituents with the exception that Formula 2 lacked pottery clay. After introducing snails into the four Petri dishes in each of the two formulae an excessive amount of alga was added to all of the Petri dishes on days 0, 4 and 7 after snail introduction to insure immediate availability of algal food for all snails in the first week of their life. The

Table 44. Effect of synthetic muds (in Expt. 10) on survivorship, shell length (X) (mm) and fecundity of *B. pfeifferi* reared for 3 weeks

Algae introduced on day	Formulae **	Dish No.	Weeks								
			1			2			3		
			S	SL	E	S	SL	E	S	SL	E
-14	1 Prototype (Art + Art)	1	5	3.0	0	5	5.5	48	5	5.6	119
		2	5	3.0	0	5	5.6	16	5	5.7	66
		3	5	3.1	0	5	6.0	57	5	6.0	108
		4	5	3.0	0	#					
		TM	20	3.0	0	15	5.7	121	15	5.8	293
	2 Prototype (BBM + Art)	1	5	2.2	0	#					
		2	5	2.0	0	#					
		3	5	2.0	0	#					
		4	4	2.3	0	#					
		TM	19	2.1	0	#					
	3 No clay (Art + Art)	1	0								
		2	0								
		3	0								
		4	0								
		TM	0								
	4 No clay (BBM + Art)	1	0								
2		0									
3		0									
4		0									
TM		0									

S = No. of survivors; SL = Shell length; E = Total eggs; TM = Total and mean; * Day 0 refers to the day snails were introduced; ** Prototype = Clay 100 + Shell 1 + Charcoal 3 + Agar 0.5 (see Table 7), 1st liquid used for paste and 2nd for overlay; # = Snails killed by accident.

snails were cultured for three weeks and the results are shown in Table 43.

Survivorship was good in Formula 1 in which 60% (12/20) of the snails survived throughout the 3-week period. In Formula 2, only 10% (2/20) survived to the end of the first week; and none to the end of the second week. The rate of growth was good in Formula 1, with a mean shell length of 3.1 mm at the end of the first week; this value was almost twice that in Formula 2, in which the mean shell length in the same period was only 1.7 mm.

This experiment indicated that the pottery clay was essential in Formula 1 for the growth of snails for reasons other than supporting the growth of algae. It was also evident that snails could be cultured in the absence of bone meal.

Experiment 10

The basic formula in this experiment was derived from Formula 1 of Experiment 9, but the amount of oyster shell used was reduced to 1 gm. Altogether 4 formulae, two with pottery

Table 44. Continued

Algae in-oculated on day #	Formulae **	Dish No.	Weeks								
			1			2			3		
			S	SL	E	S	SL	E	S	SL	E
0, 1, 2, 3 & 4	1 Prototype (Art + Art)	1	3	2.6	0	3	4.8	0	3	4.9	0
		2	5	2.5	0	5	3.9	0	5	3.9	0
		3	5	2.6	0	5	4.4	0	5	4.4	0
		4	5	2.8	0	5	4.8	0	5	4.8	0
		TM	18	2.6	0	18	4.4	0	18	4.5	0
	2 Prototype (BBM + Art)	1	3	2.3	0	3	4.1	0	3	4.8	14
		2	5	2.2	0	5	3.7	0	5	3.7	0
		3	4	2.4	0	4	4.7	0	4	5.1	14
		4	4	2.2	0	3	5.1	0	3	5.4	34
		TM	16	2.3	0	15	4.3	0	15	4.7	62
	3 No clay (Art + Art)	1	4	1.5	0	0					
		2	3	1.5	0	1	1.7	0	1	1.8	0
		3	1	1.5	0	0					
		4	1	1.7	0	0					
		TM	9	1.5	0	1	1.7	0	1	1.8	0
	4 No clay (BBM + Art)	1	0								
2		1	1.2	0	1	1.7	0	1	1.7	0	
3		1	1.2	0	0						
4		0									
TM		2	1.2	0	1	1.7	0	1	1.7	0	

clay and two without pottery clay, were prepared. For each formula four Petri dishes, totaling 16 dishes, were made; algae were then inoculated. Snails were introduced 14 days later (designated hereafter as '-14 day group'). On the day of snail introduction (day 0), duplicates of the above four formulae, each still with four Petri dishes totaling 16 dishes, were made. Into these 16 dishes the snails were also introduced immediately after algal suspension, in excessive amount, was inoculated (designated hereafter as '0-1-2-3-4 group'). Snails in both the -14 group and the 0-1-2-3-4 group were cultured for three weeks. Meanwhile, more algal suspension, still in excessive amount, was repeatedly added to the 0-1-2-3-4 group on days 1, 2, 3 and 4 after the introduction of snails; the results are shown in Table 44.

Survivorship was good at the end of the first week in Formula 1 and Formula 2 of both the -14 and the 0-1-2-3-4 group, and continued to be so until the end of the third week. Unfortunately, dish 4 in Formula 1 and all of the dishes in Formula 2, all in -14 group, were accidentally destroyed. On the other hand, the survival in Formula 3 and Formula 4, regardless of whether they were from the -14 group or from the 0-1-2-3-4 group, was very poor. No snails were alive at the end of the first week in Formula 3 or Formula 4 of the -14 group. In the 0-1-2-3-4 group, only 10% (2/20 in Formula 4) to 45% (9/20), in Formula 3) of the snails survived to the end of the first week; at the end of the third week, only 5% (1/20) in each formula were alive.

Table 45. Effect of synthetic muds (in Expt. 11) on survivorship of *B. pfeifferi* reared for 4 days *

Formulae **	Dish No.	Days			
		1	2	3	4
1 Prototype	1	4	4	4	0
	2	5	5	5	0
	3	5	5	4	0
	4	5	5	5	0
	Total	19	19	18	0
2 No charcoal	1	0			
	2	0			
	3	0			
	4	0			
	Total	0			

* Algae were inoculated and snails were introduced on the same day, more algae were inoculated 1, 2, 3 and 4 days later;

** Prototype = Shell 1 + Charcoal 3 + Agar 0.5 (see Table 7), in both formulae, art. water used for paste and overlay.

Using the available data, the rate of body growth was best in Formula 1 of the -14 group, which had a mean shell length of 5.8 mm at the end of the third week. The growth in both Formula 1 and Formula 2 of the 0-1-2-3-4 group was about the same for the same period of time, giving a mean shell length of 4.5 mm and 4.7 mm, respectively. The growth in both Formula 3 and Formula 4 of the 0-1-2-3-4 group was rather poor, giving only 1.8 mm and 1.7 mm, respectively in the mean shell length for the same three-week period.

Egg-laying was first observed in Formula 1 of the -14 group at the end of the second week. This time interval was, so far, the shortest ever observed for *Biomphalaria pfeifferi* snails with either natural or synthetic mud. At the end of the third week egg-laying was also found in the Formula 2 of the 0-1-2-3-4 group.

To summarize: (a) the conclusions from Experiment 9 were confirmed, i.e., of the materials tested pottery clay was essential for the growth of snails and the snails could be cultured in a synthetic mud without the addition of bone meal; (b) inoculating algae before introducing snails was not a necessity, but it was better for snail growth (compare Formula 1 of the -14 group with Formula 1 of the 0-1-2-3-4 group); and (c) with everything else equal between 2 formulae, the use of BBM for mixing (Formula 2 of the 0-1-2-3-4 group) resulted in better body growth and egg production than the use of artificial water (Formula 1 of the 0-1-2-3-4 group).

Table 46. Effect of synthetic muds (in Expt. 12) on survivorship, shell length (SL) (mm) and fecundity of *B. pfeifferi* reared for 4 weeks *

Formulae **	#	Dish No.	Weeks											
			1			2			3			4		
			S	SL	E	S	SL	E	S	SL	E	S	SL	E
1 Prototype	3+	1	5	2.6	0	4	5.5	0	4	6.2	121	4	6.4	0
		2	5	3.4	0	5	5.0	0	4	5.7	0	4	5.7	0
		3	5	2.8	0	5	5.3	0	5	5.9	0	5	5.9	0
		4	5	2.0	0	5	5.3	0	5	5.5	39	5	5.5	0
		TM	20	2.9	0	19	5.3	0	17	5.8	160	18	5.8	0
2 No charcoal	3+	1	5	2.9	0	5	5.1	21	5	5.1	16	5	5.1	0
		2	5	2.6	0	5	5.0	0	5	5.2	27	4	5.4	0
		3	5	2.6	0	5	4.6	0	5	5.5	0	5	5.6	0
		4	5	2.8	0	5	4.7	1	5	4.9	9	5	4.9	0
		TM	20	2.7	0	20	4.9	22	5	5.1	52	19	5.2	0
3 No shell	0	1	5	1.5	0	4	1.7	0	0					
		2	5	1.6	0	5	1.6	0	1	4.0	0	0		
		3	5	2.0	0	5	2.0	0	3	2.2	0	0		
		4	5	1.9	0	3	1.9	0	2	1.9	0	0		
		TM	20	1.7	0	17	1.8	0	6	2.4	0	0		
4 Clay and agar only	0	1	0											
		2	0											
		3	0											
		4	0											
		TM	0											
5 No clay	2+	1	3	1.2	0	2	1.7	0	0					
		2	0											
		3	1	1.3	0	0								
		4	4	1.4	0	2	2.0	0	0					
		TM	8	1.3	0	4	1.9	0	0					

S = No. of survivors; SL = Shell length; E = Total eggs; TM = Total and mean; * Snails were introduced 16 days after algal inoculation; ** Prototype = Clay 100 + Shell 1 + Charcoal 3 + Agar 0.5 (see Table 7), in all formulae, art. water used for paste and overlay unless otherwise indicated; # = Growth of algae on day 0 (= the day snails introduced).

Experiment 11

From Experiments 9 and 10 it was concluded that formulae without pottery clay did not support snail growth. As a preliminary test for the experiment that followed (Experiment 12 below), the role of charcoal in the formulae was tested without pottery, clay. Formula 1 contained charcoal, and Formula 2 did not. The algal suspension, in excessive amount, was inoculated to both formulae on days 0, 1, 2, 3 and 4 after the snails were introduced. Snails were checked daily. The results are shown in Table 45.

In Formula 1 most snails survived to the end of the third day and all were dead at the end of the fourth day. With Formula 2 all snails were dead at the end of the first day. It is likely that the charcoal absorbs toxic substances from the formula.

Table 46. Continued

Formulae **	#	Dish No.	Weeks											
			1			2			3			4		
			S	SL	E	S	SL	E	S	SL	E	S	SL	E
6 Shell and agar only	2+	1	4	1.5	0	3	2.0	0	1	2.5	0	0		
		2	0											
		3	0											
		4	0											
		TM	4	1.5	0	3	2.0	0	1	2.5	0	0		
7 Charcoal and agar only	0	1	4	1.4	0	0								
		2	0											
		3	0											
		4	1	1.5	0	0								
		TM	5	1.4	0	0								
8 Agar only	2+	1	2	1.1	0	0								
		2	0											
		3	0											
		4	2	1.2	0	0								
		TM	4	1.1	0	0								
9 Prototype (BBM + Art)	3+	1	5	2.2	0	5	4.1	0	5	5.5	102	5	5.7	121
		2	5	2.0	0	4	3.6	0	4	4.7	7	4	4.9	0
		3	5	1.7	0	4	1.9	0	3	2.2	0	2	2.6	0
		4	5	1.8	0	4	2.0	0	2	2.3	0	1	2.6	0
		TM	20	1.9	0	17	3.0	0	14	4.1	109	12	4.7	121
10 Natural mud (Dis + Dis)	3+	1	5	2.8	0	5	6.6	0	5	7.2	169	5	7.2	11
		2	5	2.7	0	5	7.0	0	5	8.0	85	5	8.0	0
		3	5	3.4	0	5	7.0	0	5	8.3	0	5	8.5	0
		4	5	3.3	0	5	7.3	0	5	7.6	137	5	7.6	0
		TM	20	3.0	0	20	7.0	0	20	7.8	391	20	7.8	11

Experiment 12

Formula 1 of Experiment 10, in which algae were inoculated 14 days before snail introduction, was found best for supporting both growth and egg production among all of the formulae tested in the previous 11 experiments. Therefore, in this experiment, this formula was used as a prototype. Based on this prototype, altogether 8 formulae (1 through 8), were designed in such a way as to determine the roles of pottery clay, oyster shell and charcoal. Formula 9 was designed to compare the efficiencies of artificial water and BBM, when both were used for mixing. Formula 10, in which natural mud was used in place of synthetic mud, served as control. In Experiment 10 it had been shown that prior algal inoculation was better than inoculation immediately after introduction of the snails. In this experiment, therefore, algae were inoculated 16

Table 47. Summary of synthetic mud formulae which support good growth of *B. pfeifferi*

Expt. No.	Formula No.	Synthetic muds										Snails				
		Solid (gm)								Liquid		S. (%) at wk 2	SL (mm) at wk		Oviposi. at wk	
		Clay	Shell	Charcoal	Agar	Bone	Fish	KNO ₃	Paste (70 ml)	Overlay (240 ml)	2		3	2	3	
2	1	100	0.5	2	2	0.5	0.5	-	Dis	Dis	100	4.4	4.8	-	-	
	2	100	0.5	2	2	0.5	0.5	-	Dis	Art	80	4.3	4.9	-	-	
	3	100	0.5	2	2	0.5	0.5	-	BBMSU	Dis	100	4.0	5.0	-	-	
	4	100	0.5	2	2	0.5	0.5	-	BBMSU	Art	80	3.9	5.7	-	+	
3	1	100	1	3	0.5	1	1	0.05	Dis	Dis	100	3.3	4.7	-	-	
	2	100	1	3	0.5	1	1	0.05	Dis	Art	87	4.5	5.6	-	-	
6	2	100	1	4	0.3	1	-	-	Dis	Mod Art III	100	4.0	5.5	-	+	
9	1	100	2	3	0.5	-	-	-	Art	Art	60	4.3	4.4	-	-	
10	A-1	100	1	3	0.5	-	-	-	Art	Art	75	5.7	5.8	+	+	
	B-1	100	1	3	0.5	-	-	-	Art	Art	90	4.4	4.5	-	-	
	B-2	100	1	3	0.5	-	-	-	BBM	Art	75	4.3	4.7	-	+	
12	1	100	1	3	0.5	-	-	-	Art	Art	95	5.3	5.8	-	+	
	2	100	1	-	0.5	-	-	-	Art	Art	100	4.9	5.1	+	+	
	9	100	1	3	0.5	-	-	-	BBM	Art	85	3.0	4.1	-	+	

S = Survivorship; SL = Shell length; A = -14 group; B = 0-1-2-3-4 group.

days before snail introduction; the snails were cultured for four weeks and the results are shown in Table 46.

Survival was good in Formulae 1, 2, 9 and 10, with all snails alive at the end of the first week; at the end of the fourth week, the survival was 90% (18/20), 95% (19/20), 60% (12/20) and 100% (20/20), respectively. The rate of growth was also good in these formulae, with mean shell lengths of 5.8 mm, 5.2 mm, 4.7 mm and 7.8 mm, respectively, at the end of the fourth week. The poor survival and growth in Formulae 3 through 8 indicate that elimination of oyster shell or pottery clay was detrimental.

The algal growth in the present experiment was closely related to snail growth. In other words, luxuriant algal growth was correlated with better snail growth.

Egg-laying was first observed in Formula 2 at the end of the second week and it was of the same magnitude as found in one of the formulae in Experiment 10 (see Table 44, Formula 1 of -14 group). By the end of the third week, egg-laying had started in Formulae 1, 9 and 10.

From this experiment the following conclusions are made: (a) synthetic mud, composed of pottery clay, oyster shell, charcoal and agar and mixed with artificial water to form a paste and using the same kind of water as overlay (as Formula 1) was found suitable for

snail growth and egg-laying; (b) omission of charcoal from the formula did not affect the rate of growth to a great extent (compare Formulae 1 and 2), in fact the start of egg-laying might be enhanced by the absence of charcoal; (c) omitting oyster shell was harmful to both survival and rate of shell growth (compare Formulae 1 and 3); (d) pottery clay and agar alone did not support the snails (compare Formulae 1 and 4); (e) formulae without pottery clay did not sustain the snails (Formulae 5 through 8); and (f) for mixing, artificial water was superior to BBM in supporting the survival and rate of growth, but the BBM was, in turn, superior to artificial water in terms of fecundity of snails (compare Formulae 1 and 9).

For convenience the formulae in which the survivorship of the snails was 60% or more and the mean shell length was 3.0 mm or more at the end of the second week are listed in Table 47. Altogether 14 formulae were involved. Formula A-1 of Experiment 10 (and the identical Formula 1 of Experiment 12) and Formula 2 of Experiment 12 were found most promising.

DISCUSSION

Part I. The Effect of Crowding on Survivorship, Growth and Fecundity

Although the growth and fecundity of schistosome snail vectors have been studied by many workers, suitable methods for culturing *Bulinus globosus* and *Biomphalaria pfeifferi*, both important African intermediate vectors, are difficult to find. There are only a few workers who have studied the growth and fecundity of these two species of snails intensively (Shiff, 1964a, 1964b; Sturrock, B.M., 1966 and Sturrock, R.F., 1966). A few other workers have found these snails rather difficult to maintain in laboratory cultures (Jennings *et al.*, 1970 and Lo, 1972). Methods undertaken with the use of trays now indicate that those snails can quite easily be cultured in the laboratory provided certain care is exercised.

1. *Bulinus globosus*

As judged by survivorship, the present tray method appears to be promising. With four different population densities, *i.e.*, 5-, 10-, and 40-snails per tray, there was 80% to 100% survivorship over an eleven-week period (Table 8). These values are 20 to 40% higher than the highest value obtained by Shiff (1964a) who cultured the same species of snails in large tanks with six snails or less per tank. While more detailed comparison with his work will be discussed later (Part II), it suffices to state that this tray method stimulates earlier and greater egg production and the snails have larger shells.

With respect to body growth, there was a definite trend toward reduced growth with an increase in individual variation of shell size as the snails became more crowded (Table 8 and Fig. 2). At the same time fecundity was also adversely affected under conditions of crowding (Table 9 and Fig. 3). Shiff (1964b) indicated that *Bulinus globosus* snails at a density of one snail per liter of water showed optimum body growth. The optimum number of snails in the present investigation depended on the avowed purpose of the culture.

For obtaining good survivorship and body growth, the method using five snails per tray during the later weeks in culture is recommended, since survivorship was highest and the shell length was largest. In practice, one can start 40 newly hatched snails in a tray culture; at the end of the second week the snails (about 2.4 mm long) can be placed in 4 trays with 10 snails per tray. After the fourth week these snails (now about 5.5 mm) can be

rehoused in eight trays with five snails per tray. Following the sixth week, the snails (about 8.2 mm) would then be mature.

To obtain good egg-laying, the method using 10 snails per tray during the later weeks is recommended. While it was shown that the number of eggs laid per snail in 10 snails per tray was smaller than in trays with five snails per tray, the total yield of eggs in the former was considerably greater. A density of 20 snails per tray or 40 snails per tray is not recommended either for growing snails or for egg production. Most eggs were laid on or under lettuce leaves (Table 10); the significance of this pattern will be discussed in Part II.

2. *Biomphalaria pfeifferi*

The tray method was excellent for culturing this species also. After the initial rearrangement of the cultures by the end of the second week none of the snails died during the entire period. As with *Bulinus globosus*, reduced growth rate and increased individual size variation were observed as the snails became more crowded (Table 11 and Fig. 4). With this same species Sodeman (1970) reported that stunting did not affect the start of egg production in any of the populations he tested with 7, 14, 35 and 55 snails per tank. In the present study, however, the start of egg-laying in the group with 40 snails per tray occurred one week later than the remaining groups, with 5, 10 and 20 snails per tray (Table 12). The differences observed were probably due to the larger size of the tank used by Sodeman; it was big enough not to become a growth limiting factor. The tray used in the present study was relatively small and it became a limiting factor with 40 snails in it. The small number of eggs produced also showed that adverse conditions existed because of crowding (Table 12 and Fig. 5).

Again the optimum number of snails to be accommodated in one tray depends on the purpose of the culture. If the snails are to be grown faster and to a larger size they should be cultured using five snails per tray at the very beginning of the culture, since density effects appear as early as the end of the second week. Although in these cultures growth rate decreased as density increased, body growth with either the 10-, 20- or 40-snail groups still surpassed the size obtained by Sturrock, B.M. (1966) and Sturrock, R.F. (1966).

For purposes of obtaining more eggs, the

snails should be cultured at a density of ten snails per tray. It might be argued that, since all of the energy available in a tray was about the same, the total yield of eggs from one tray should be similar to another regardless of the number of snails present. Results shown in Table 12 indicate that this was not the case. A simple multiplication shows that the total numbers of eggs produced in trays with five snails per tray were 10,360 (2,072 X 5); 10 snails, 12,820 (1,282 X 10); 20 snails, 10,960 (548 X 20); and 40 snails, 9,000 (225 X 40). One might argue that these calculations did not consider the differences in the time when egg-laying started (between the third and fourth week for 5-, 10- and 20-snail groups, but the fourth and fifth week for the 40-snail group). Recalculating and counting from the fifth week showed that the results were essentially the same, i.e., 10,200 (2,040 X 5), 12,680 (1,268 X 10), 10,880 (544 X 20) and 9,000 (225 X 40), respectively. Consequently there was no question but that a density of 10 snails per tray was best for egg production; the 20-snail group was next, followed by the 5-snail group; and the 40-snail group showed the smallest in egg production. In this instance most eggs were laid on the walls of the trays (Table 13), and the significance of this observation is discussed in Part II.

Najarjan (1960b) in his study of *Bulinus truncatus* found that differences in the volume of water per snail did not affect the mean number of eggs per egg-mass laid. However, Chernin and Michelson (1957a) found that with *Biomphalaria glabrata* the more crowded the snails the fewer the eggs per egg-mass. In the present study with *Biomphalaria pfeifferi* it was also observed that the more crowded the snails in a tray, the smaller the mean number of eggs per egg-mass (Table 14 and Fig. 6).

Wright (1960), working with *Bulinus forskalii*, and Webbe (1962a) with *Biomphalaria sudanica tanganyicensis* (Smith), found that the number of eggs per egg-mass increased as the parent snails became older. Similar results were obtained by Sturrock, B. M. (1966) with *Biomphalaria pfeifferi* kept individually in 250 ml of dechlorinated tap water in crystallizing dishes; the number of eggs per egg-mass increased from about 6.1 to 12.5 during the eighth to thirtieth week period. In the present study the data also show that the number of eggs per egg-mass increased as the snails aged. From the start of egg-laying up to 12 weeks in age, the number increased in the 5-snail group from 6.5 to 18.8; in the 10-snail group from 6.4 to 16.2; in the 20-snail group from 6.1 to 11.1; and in the 40-snail group from 3.9 to 8.1 (Table 14 and Fig. 7).

Part II. The Effect of Types of Waters on Survivorship, Growth and Fecundity

A. Culturing Snails in Deionized, Distilled or Spring Water

1. Survivorship and Growth

As shown in Table 15, the survivorship of *Bulinus globosus* was apparently not affected by the types of waters used since the percentage of the snails surviving at the end of the forty-fourth week was 73.3% (11/15) in all three types of waters used. The survivorship of *Biomphalaria pfeifferi* was also unaffected by the types of waters used, except that those in spring water showed lower survivorship (Table 19). As to the rate of body growth, there seemed no significant differences among snails in the types of waters tested, as shown for both the *Bulinus globosus* and the *Biomphalaria pfeifferi* groups (Tables 15 and 19). When the growth curves for both species were compared with those given by Shiff (1964b) and Sturrock, B.M. (1966) some significant differences appeared. The snails in the present study always completed logarithmic phases earlier and grew bigger than the snails reported by those investigators. Presumably in the present investigation enough substances, both organic and inorganic, were seeping from the mud mounds into the overlying water, whatever the type of water—deionized, distilled, tap or spring. Thus, the types of waters used were not crucial for the snails as far as their survivorship and body growth were concerned. Again, it was confirmed that the tray method was promising.

2. Fecundity

The fecundity of *Biomphalaria pfeifferi* and especially of *Bulinus globosus* were greatly affected by the types of waters used. As shown in Table 16 and Fig. 9, the number of eggs produced by *Bulinus globosus* in distilled water was always twice or more than that produced by those in spring water. A similar pattern, though with a less significant difference, was also observed in egg production of *Biomphalaria pfeifferi*, as shown in Table 20 and Fig. 11. Those observations imply that the spring water used was exerting some negative effects on egg production. Harrison *et al.* (1970) also found egg-laying rates with *Biomphalaria pfeifferi* were lower when they were cultured in hard waters.

While the numbers of eggs produced by *Bulinus globosus* in tap water were somewhere be-

tween those in distilled water and spring water, the number produced by *Biomphalaria pfeifferi* was highest in this same kind of tap water for egg-laying was distilled water with *Bulinus globosus* and tap water with *Biomphalaria pfeifferi*. Harrison and Schiff (1966) and also Williams (1970a, 1970b) found that the chemical composition of the water exerted a profound influence on *Bulinus globosus* and *Biomphalaria pfeifferi*. They found that *Bulinus globosus* was present in water bodies throughout the whole range of the hardness, but the populations were denser in the medium hard water; *Biomphalaria pfeifferi*, on the other hand, was restricted to medium and hard waters. The data in the present study essentially confirm the results just mentioned. When distilled water overlies the mud mound, it could be considered a medium water; tap water over the mud mound would be hard water. Spring water over the mud mound would then be 'superhard' water. Therefore, the latter was not suitable even for *Biomphalaria pfeifferi*.

The reasons for the way the hardness of the water affects the number of eggs laid are not clear. It is possible that with *Bulinus globosus* distilled water may somehow change the texture of the lettuce, the algae, or both, in such a way as to render them more easily ingested by snails, thus providing more food materials to produce eggs. On the other hand, with *Biomphalaria pfeifferi* it is possible that a change in texture of these foods as brought about by tap water was more easily ingested and thus more nutritional for egg production.

Shiff (1964a, Table 2, 25° C) reported that the number of eggs laid per *Bulinus globosus* snail was 1,032 over a 44-week period; in the present study with distilled, tap and spring waters over the 44-week period the values were 4,480, 3,391 and 2,053, respectively (Table 16). With *Biomphalaria pfeifferi*, Sturrock, R.F. (1966, Fig. 2, 25° C) reported the number of eggs laid per snail was about 930 over a 29-week period; in the present study with deionized, distilled, tap and spring waters over the same 29-week period these values were 3,949, 4,111, 4,764 and 3,455, respectively (Table 20). These results imply that the tray method provided even better conditions for reproduction.

3. Sites of egg-laying

In Part I of this dissertation it was mentioned briefly that *Bulinus globosus* laid most eggs on or under lettuce leaves when those snails were cultured with spring water; *Biomphalaria pfeifferi* laid most eggs on the walls and bottoms of the trays when they were cultured in tap water. These findings are supported by the data in Tables 17 and 21, which strongly indicate that the types of waters used significantly influenced the sites used

for egg-laying with both *Bulinus globosus* and *Biomphalaria pfeifferi* snails. In distilled or deionized water most eggs were laid on the wall and bottom of trays; in tap water only a modest number were laid on these sites, and in spring water the smallest number were deposited there.

These differences in fecundity and in the sites of egg-laying, together with the information obtained from water analyses (Table 18), all suggest that water hardness plays an important role in culturing snails, especially in their reproductive potential.

B. Culturing Snails in Trays while Alternating the Types of Waters

For some time it was known that snails maintained in aquaria had distinct preferences for certain egg-laying sites and that the egg output was lowered under unfavorable conditions (Barlow and Muench, 1951; Standen, 1951; Olivier and Haskins, 1960; Olivier *et al.*, 1962; Schiff, 1964c; and Pellegrino and Gonçalves, 1965). Coles (1970) showed that the egg-laying behavior with three species of *Biomphalaria* in laboratory was related to the environment from which these snails originated. He found that 90% of the egg-masses laid by *Biomphalaria sudanica* were deposited on floating plastic; with *Biomphalaria pfeifferi* it was only 49%; with *Biomphalaria choanomphala* all egg-masses were found on the bottom of the bowls. The *B. sudanica* came from swamp water where the egg-masses would be subjected to anaerobic conditions if they were not laid close to the surface; the *B. pfeifferi* came from a rocky stream with only small amounts of floating vegetation; and the *B. choanomphala* originated from gravel beds at the bottom of the lake, where higher plants were absent for the deposition of egg-masses.

In the present study it was found that the percentages of eggs laid on a particular site varied according to the types of waters used. Both *Bulinus globosus* and *Biomphalaria pfeifferi* laid most of their eggs on the walls and bottoms of the containers, when they were maintained in distilled water or deionized water; they laid fewest eggs on these sites when they were in spring water; in tap water the number of eggs laid on these sites varied from experiment to experiment (Tables 22, 23 and 24). Reasons for these differences are not clear. Presumably the sites for egg-laying were governed by a behavior pattern like that advocated by Standen (1949 and 1951). He noticed that snails did not lay eggs on any substance likely to decay before the normal egg incubation period is completed. For example, they oviposited on raw lettuce but not on cooked lettuce. In distilled water the low

concentration of ions caused the lettuce to decay more readily, and the snails then had no choice but to lay their eggs on the wall or bottom of the container. With spring water the high concentration of ions prevented the lettuce from decaying. Consequently, the snails had a choice and could select the sites they preferred. Since the site underneath lettuce leaves was relatively secluded, the snails seemed to prefer laying their eggs there. In tap water, the ion concentration fluctuated, presumably due to the fact that the water came from several sources, and the degree of lettuce decay thus also fluctuated. In this case, the percentages of eggs laid on the container wall and bottom also fluctuated.

C. Culturing Snails in Calcium, Magnesium, or Calcium-magnesium Water

Essentially this study repeats the work described in 'A' above except that waters were used with known amounts of the chemicals, calcium and magnesium. It appeared that neither calcium water nor magnesium water had any adverse effect on the survivorship of *Bulinus globosus* or *Biomphalaria pfeifferi* snails, since none died during the experimental periods. Similarly, neither the calcium water nor the magnesium water affected the rate of growth in *Bulinus globosus* snails (Table 25 and Fig. 12). With *Biomphalaria pfeifferi* the calcium water may have had some adverse effects as judged by the data found in Table 28 and Fig. 14.

Egg-laying, on the other hand, was strongly affected by the types of waters used with both *Bulinus globosus* (Table 26 and Fig. 13) and *Biomphalaria pfeifferi* (Table 29 and Fig. 15). In both cases, calcium water was found to be responsible for reducing egg production. Harrison *et al.* (1966) observed that high ratios of magnesium-to-calcium (12.4 to 1) significantly lowered the egg-laying potential of *Biomphalaria pfeifferi*. The magnesium water at the concentrations tested, however, did not show any adverse effect on the egg-laying of the snails in the present study.

The reasons for the reduction in egg-laying in calcium water were not clear. The possibility remains that calcium ions might somehow change the texture of the algae or lettuce, or both, so that they were less easily consumed by the snails, so that the snails in calcium water had less food for egg production.

As to the sites of egg-laying, both *Bulinus globosus* and *Biomphalaria pfeifferi* showed similar behavior patterns in that they laid fewest eggs on the container when they

were in calcium water and most of their eggs on that same site when they were in magnesium water (Tables 27 and 30). The reasons for these differences in egg-laying sites were probably similar to those given in 'B' above, i.e., calcium water somehow hardened the lettuce leaves, and the snails selected the lettuce for egg-laying. In the waters without calcium the lettuce decayed quickly, and the snails had to lay their eggs on the container walls or bottoms.

Part III. The Effect of Various Species of Algae and Various Kinds of Muds and Mud Extracts on the Growth of *Biomphalaria pfeifferi*

A. Culturing Snails in Unialgal Preparations

Very few investigators have deliberately inoculated algae into snail cultures and those who did generally used the filamentous blue-green alga *Oscillatoria* sp. This alga at times was found harmful to the snails. In the present study, with all of the blue-green algae and diatoms tested survivorship was found generally to be fair to excellent (Table 31). Such authors as Malek (1958), Gohar and El-Gindy (1962) and Webbe (1962b) considered that it was the quantity of blue-green algae and diatoms, rather than the quality that was important for conditioning the habitats of snails; also the snail density and the sizes of individuals were correlated directly with the amount of food available. In the present study it is shown that the kinds of algae used did affect the rate of growth, at least when the algae were young, as in the 10-day and 14-day groups in Table 31. From Table 31 it is clear that any one of the two pure blue-green algae, i.e., *Nostoc muscorum* or *Fischerella ambigua*, supported snail growth better than did the mixture of blue-green algae.

B. Culturing Snails in Various Kinds of Tray and Petri Dish Preparations

Wright (1960) considered that at least three factors were involved in stunting of snails—food, collisions and chemical reaction due to 'pheromones.' In the present study, although no snails were found stunted in any of the cultures tested, there were, nevertheless, significant differences among snails, as shown in Table 32 and Figures 16 and 17. The fact that the snails in the *Nostoc muscorum* cultures were larger than those found in the mixture of blue-green algae was probably due to the higher nutritional value of the former. It is also presumed that the addition of lettuce was an improvement because it might

provide additional nutrition for snails in the production of more eggs. The fact that larger snails were found in trays, as compared to Petri dishes, however, could not be interpreted as simply a food factor since no more alga or lettuce was provided in trays than in Petri dishes. It would appear that the collision and pollution factors were more likely involved in terms of the differences between trays and Petri dishes, since in the former there was 1,500 ml of water per tray with a surface area of about 600 cm² while in the latter there was only 60 ml of water per Petri dish with a surface area of only about 60 cm². Webbe (1962a, 1962b, 1965) recommended the use of mean growth curves to designate the probable age of snails collected in the field. The results of present study indicate that the size of snails should not be used indiscriminately as a criterion for estimating their age. It is quite possible that sizes of snails having the same age and collected from two localities might be very different merely because of differences in the kinds of algae they were consuming.

As to fecundity, more eggs were found in trays than in Petri dishes. Presumably, there was more space available for egg deposition when trays were used. *Nostoc muscorum* was also found better than the mixture of blue-green algae, probably because of its higher nutritional value. The effect of lettuce on the fecundity of snails, however, was not significant (Table 33). Besides, it may be suspected that the presence of lettuce in some way delays the start of oviposition, since snails in two out of three trays supplied with *Nostoc muscorum* (without lettuce) began to lay eggs after two to two and one half weeks, but none in the trays supplied with *Nostoc muscorum* with lettuce did so during the same period of time (Table 33). To conclude, lettuce enhanced body growth but inhibited egg-laying.

Pereira and Deslandes (1954) and Perlowagora-Szumlewicz (1958) found that snail size correlated better with the onset of egg-laying than with snail age. However, Ritchie *et al.* (1963a) found that the size at the onset of egg-laying varied with the growth rate, i.e., a rapidly growing snail reached a larger size before laying eggs than a snail that grew slowly. The data in the present study agree with this observation of Ritchie *et al.* For example, according to the normal growth curve, as shown in Fig. 10, the shell lengths of two-week old snails in trays with *Nostoc muscorum* with or without lettuce and in Petri dishes with *Nostoc muscorum* with or without lettuce were all large enough for oviposition; however, none laid eggs until the end of two and one half or three weeks (Tables 32 and 33).

C. Culturing Snails under Crowded Conditions

The observations here (Table 35) are similar to those found in Part I (see Table 11). Although a direct comparison between these two studies is not appropriate because of differences in their designs, it is possible to verify that present results appear far better than those obtained in Part I.

From the fact that the shell lengths at the end of the first week were about the same among all six different density groups tested, as seen in Table 35, it appeared that there were no limiting factors exerting effects on the growth of snails during the first week. During the second week there were still no limiting factors in the 1-, 2- and 5-snail groups. In the 10-, 20- and 40-snail groups, however, the alga appeared to be the limiting factor for growth of snails, since the growth rate among those groups slowed down immediately or soon after the alga was consumed. During the third week period, factor(s) other than algae were added and played a more important role in snail growth. This is based on two observations: (a) although there was still enough alga left in the two-snail group up to the end of the fourth week, and there was none left in the five-snail group at the end of the second week, the shell lengths in the two groups were almost identical; and (b) both the one-snail group and the two-snail group had enough alga until the end of the fourth week, yet the shell length in the one-snail group was considerably greater than that in the two-snail group (Table 35). One could thus speculate that either waste product(s) or pheromone-like substance(s) might be involved.

Based on data given in Table 35 and Fig. 18, a practical way for culturing snails is proposed. Snails could be first cultured in Petri dishes with 40 snails per dish. At the end of the first week, they could be thinned out so that only five or 10 snails remained in each dish. At the end of the second week, the snails would have reached a size (5.4 mm or 5.8 mm) such that they could be harvested for experiments or placed in culture for egg-laying.

D. Culturing Snails in Various Kinds of Muds and Mud Extracts

Observations in the first experiment indicated that the presence of organic substances in mud (natural mud, steamed mud or 100° C-dried mud) was necessary for the normal

growth of snails regardless of whether alga was inoculated before snail introduction (Table 36, -10 group) or afterwards (Table 36, 0-3-11 group). Body growth, however, was always found to be better in the former than in the latter. In mud without organic substances (such as 525° C heated steamed mud) or an extract with insufficient amounts of organic substances (such as steamed extract), repeated addition of the alga on days 0, 3 and 11 after snail introduction was found better than adding alga only once, 10 days before snail introduction (Table 36). The second experiment (Table 37) essentially repeated the first one, but a few more preparations were tested. On the basis of the results from these two experiments and from the chemical analyses (Table 38), the following hypothesis was constructed:

Natural mud contained some organic and inorganic substances needed for algal growth, but in excess amounts it exhibited some negative effects on snail growth. Through steaming or stirring, some of these excessive substances were extracted. Consequently, it was reasonable to assume that both extracts supported algal growth and hence snail growth in part. Steamed mud or stirred mud, in turn, also appeared to contain ingredients that produced optimum growth of the alga, hence of the snails.

Similarly, the 100° C-dried mud also contained the same excessive amounts of those harmful substances as the natural mud did; therefore, it also supported snail growth to about the same magnitude as the natural mud. Through heating at 525° C, the excessive amounts of substances, as well as some other substances which may originally have been bound tightly to soil particles and were harmful to snail growth, became more water-soluble so that the snails were not able to grow at all in the Petri dishes made up with this 525° C-heated mud. Through extraction by steaming, these hypothetical harmful substances were then transferred to the 525° C-heated steamed extract. This may explain why the 525° C-heated steamed mud partially supported snail growth but the 525° C-heated steamed extract did not. When this steamed extract, which presumably contained some harmful substances, was combined with 100° C-dried mud, as in Table 37, the latter acted as a buffer to absorb some of these harmful substances. Consequently, Petri dishes with this combination only supported growth of snails moderately.

If the above hypothesis is plausible, however, it remains rather difficult to explain why the 525° C-heated steamed mud in Table 37 did not support snail growth at all, since the similar preparation in Table 36 did support the growth of snails to a certain extent. It

would also be rather difficult to explain why the Petri dishes with only half-strength 525° C-heated steamed extract, as shown in Table 37, did not support snail growth. At this dilution, the concentrations of calcium, silicon, sulphate, or a combination were about the same as, or even less than, those found in steamed extract or stirred extract (Table 38), both of which had, as previously mentioned, partially supported snail growth.

Part IV. The Formulation of Synthetic Mud

As previously mentioned, other investigators have observed the importance of algae for culturing snails. Some also tried semi-synthetic foods for this purpose. Still others tried an axenic medium. However, there have not previously been any attempts to grow algae on a formulated, synthetic mud which could serve as a medium on which to culture snails. The method reported here was accomplished essentially on the basis of trial and error. Various components, both solid and liquid, were tried. Finally, two simple formulae (Formula 1 in the -14 group in Table 44 and the identical Formula 1 in Table 46; and Formula 2 in Table 46) were most promising. The former consisted of pottery clay, oyster shell, charcoal and agar; the latter used pottery clay, oyster shell and agar. In both formulae artificial water (of known chemical compositions) was used for making a paste and as an overlay. The roles played by each component are described as follows:

1. Pottery Clay

This clay was used as a basis for the synthetic muds in all 12 experiments. In the absence of the clay none of the cultures were able to sustain snails (Tables 43, 44 and 46). The speculation that the clay was important to the snails primarily by way of supporting the algal growth, which in turn supported snail growth, was rejected since repeated addition of the alga into cultures without clay still appeared ineffective for snail growth (Tables 43 and 44). On the other hand, the clay plus agar alone also did not support the growth of the alga nor, consequently, of the snails (Compare Formula 1 and Formula 4 in Table 46). Therefore, it was assumed that the primary importance of clay was mechanical rather than nutritional, as also advocated by Pan (1958), that is, to assist in grinding ingested food.

2. Oyster Shell

This was used in all 12 experiments on the chance that it might furnish substrates

for the formation of snail shells. In the absence of oyster shell none of the cultures supported the growth of the alga nor, consequently, of the snails (compare Formula 1 and Formula 3 in Table 46). Oyster shell plus agar alone did support to a moderate degree the algal growth but was still unable to support snail growth (compare Formula 1 and Formula 6 in Table 46). It appeared very likely that oyster shell was important primarily for enhancing algal growth.

3. Charcoal

The primary purpose of adding charcoal to the synthetic mud was to absorb some of the harmful waste products produced during the culturing process; it was also used in all 12 experiments.

Wright (1960) suspected that charcoal might be responsible for the poor increase in shell size among *Bulinus forskalii* snails. In the present study with all of the other components present, the absence of charcoal was not critical. In fact, it appeared advantageous for the snails, since they laid eggs earlier (compare Formula 1 and Formula 2 in Table 46). In cultures without pottery clay, on the other hand, the absence of charcoal was harmful to the snails (compare Formula 1 and Formula 2 in Table 45; and Formula 1 and Formula 6 in Table 46). Charcoal plus agar alone did not support algal growth, hence snail growth suffered (compare Formula 1 and Formula 7 in Table 46).

The reasons for these observations are not clearcut. It may well be possible that with a high calcium content (as the charcoal was made from crushed bone) there were harmful conditions for egg production, and egg-laying was inhibited; but because of its porous structure charcoal was useful in absorbing waste product(s), and it then prolonged the life of the snails.

4. Agar

Preliminary observation indicated that pottery clay needed some sticky substance to make a more solid texture, which would be important for promoting better movement of snails, especially in the first few days of life. Accordingly, agar was added in all 12 experiments. The agar itself might also act as a source of carbohydrate food for snails. In the present study the agar alone did support a moderate degree of algal growth, but it did not support snail growth at all (compare Formula 1 and Formula 8 in Table 46). To explain the effect of agar, Stiglingh and van Eeden

explained that the agar was too slippery and caused the snails either to topple over or to slide backward as they fed. It was likely that the snails were dying from exhaustion.

5. Artificial Water

Distilled water did not contribute to algal growth. Other kinds of water were thought suitable for algal growth, but they were harmful for snail growth. The ingredients of artificial water were somewhat in between and contained substances which contributed to algal growth but were not harmful to snail growth.

While the above five components proved to be necessary in preparing the synthetic mud, some other components which seemed justified earlier in the experiments were discarded later for lack of beneficial effects.

In conclusion, the efficiency of these synthetic muds was still inferior to the natural mud, but its use opens a promising field for further study.

SUMMARY

The laboratory culture of the snail intermediate hosts *Bulinus globosus* and *Biomphalaria pfeifferi*, of African schistosomiasis, has up to now been very uncertain and difficult. This study aims to give simpler, more efficient, more dependable and more easily reproducible culture methods. Included also is information which provides a better understanding of the ecological requirements of these snails.

Part I

The optimum number of snails which can be accommodated in one tray (7.5 X 20 X 30 cm), along with the mixture of blue-green algae and lettuce which served as food, was investigated. Within the range studied crowding of snails did not affect their survivorship significantly. Growth, however, was affected by crowding. As the density increased, the sizes of snails became smaller and less uniform. Egg-laying likewise was affected by the crowding, and no eggs were laid in the most crowded groups.

The optimum number of individuals to be accommodated in one tray was determined. For best growth five snails per tray is recommended,

since the shell length was greatest at this density. In terms of egg-laying the use of 10 snails per tray is recommended, since total egg production in the 10-snail trays was highest.

Part II

The effects of deionized water, distilled water, tap water and spring water on survivorship, growth and fecundity of snails were measured. The work was undertaken using a mixture of blue-green algae and lettuce as food.

Survivorship of *Bulinus globosus* over 44 weeks was not affected by the types of waters (distilled, tap and spring) used. Likewise survivorship of *Biomphalaria pfeifferi* over 33 weeks was not affected in deionized, distilled or tap water but was reduced in spring water.

As for growth, there were no significant differences among the types of water tested in both species of snails.

Egg-laying was greatly affected by the types of waters used. With *Bulinus globosus* over the 44-week period the largest number of eggs were laid in distilled water, a moderate number in tap water and the smallest in spring water. With *Biomphalaria pfeifferi* during a 33-week period the results were slightly different: the largest number of eggs were laid in tap water; a moderate number in both deionized water and distilled water; and the smallest in spring water.

A comparison with the results of other investigations reveals that the present tray method is efficient both for snail growth and egg production.

The percentages of eggs laid on the container wall and bottom were also closely related to the kinds of waters used. Both species of snails laid the largest number of eggs on the container when kept in distilled water or deionized water; they laid the smallest number of eggs on the container when in spring water. In tap water, this egg-laying pattern was intermediate. Experimentally altering the types of waters used in the trays showed that these particular behaviors of egg-laying were definite in pattern. Differences in ion concentrations in the types of waters used were presumably responsible for the differences observed in egg-laying sites.

When three kinds of waters, calcium, magnesium and calcium-magnesium were tested, none of them had adverse effects on snail survivorship. Similarly these waters did not affect the growth of *Bulinus globosus* snails; with *Biomphalaria pfeifferi*, the calcium water may

have had some adverse effect. Egg-laying, on the other hand, was strongly affected by these three kinds of waters used. With both species of snails, data indicate that the smallest number of eggs was found in calcium water; the largest in magnesium water; and only a moderate number in calcium-magnesium water. Likewise, the percentages of eggs laid on the container wall and bottom were closely related to the kinds of waters used. Calcium ions appear to affect the number of eggs laid as well as the egg-laying sites.

Part III

The effects of various kinds of algae and various kinds of mud and mud extracts on the growth of *Biomphalaria pfeifferi* were studied. Three species of blue-green algae (*Nostoc muscorum*, *Schizothrix calcicola*, and *Fischerella ambigua*) and one species of diatom (*Nitzschia frustulum*) were isolated from the mixture of blue-green algae which had been used exclusively in Part I and Part II above. Among them, *Nostoc muscorum* and *Fischerella ambigua* were both found better than the mixture of blue-green algae for supporting the snail growth. In later experiments *Nostoc muscorum* was used exclusively, because it was very easily grown in Petri dish preparations.

The efficiencies of tray and Petri dish preparations were compared. In general, the trays supported growth better than Petri dishes except when a mixture of blue-green algae with lettuce was used as food. *Nostoc muscorum*, either alone or combined with lettuce, was always better than the mixture of blue-green algae with lettuce. The beneficial effect of additions of lettuce appeared as early as the end of the second week in trays; in Petri dishes, however, this effect was not observed until the end of the third week. To conclude, best growth was observed in trays with *Nostoc muscorum* and lettuce; second best in trays with *Nostoc muscorum* but no lettuce; third in Petri dishes with *Nostoc muscorum* but no lettuce; and poorest in trays or Petri dishes with the mixture of blue-green algae and lettuce.

The optimum number of snails to be accommodated in a Petri dish was studied with *Nostoc muscorum* as the only food. Altogether six different population densities, 1, 2, 5, 10, 20 and 40 snails per dish, were tested. None of the snails died in the first three groups over the four-week period. In the last three groups over the same time period, the survivorship ranged from 63.3 to 96.7%.

With regard to the growth of snails, the shell lengths were about the same among all six groups measured at the end of the first

week. At the end of the fourth week, however, the shell length decreased as the population size increased. The possibility of the alga and waste products as the limiting factors on snail growth is discussed.

Egg-laying took place in 1-, 2-, 5- and 10-snail groups before the end of the fourth week. No eggs were found in any of the dishes with 20 and 40 snails.

A practical way of culturing *Biomphalaria pfeifferi* in Petri dishes with *Nostoc muscorum* was developed. With this method the snails were ready for harvesting at the end of the second week.

The effects of various kinds of mud and mud extracts on snail growth were studied. Organic substances in mud, such as those found in natural mud, steamed mud and 100° C-dried mud, were found essential for normal snail growth. When these muds were used, the results were always better in the groups in which the alga was inoculated once ten days before the snails were introduced and in the groups in which the alga was repeatedly inoculated after snail introduction.

In the mud with no organic substances (such as 525° C-heated steamed mud) or extract with an insufficient amount of organic substances (such as steamed or stirred extract), repeated inoculation of the alga after snail introduction was found to be better than the inoculation of alga once 10 days before snail introduction.

The 525° C-heated mud and 525° C-heated steamed extract did not support the growth of snails at all.

It is concluded that the natural mud contained substances needed for algal growth, but

in excessive amounts there were some negative effects on snail growth. Heat treatment of the mud at 525° C appears to release harmful substances otherwise firmly bound in the soil particles.

Part IV

A formulation of synthetic mud was attempted; two simple formulae were found most promising. One is composed of pottery clay, oyster shell, charcoal and agar in the ratios of 100 : 1 : 3 : 0.5; the other is a mixture of pottery clay, oyster shell and agar in the ratios of 100 : 1 : 0.5. Both formulae use an artificial water for making the paste and as an overlay.

The roles played by each component remain speculative. In the absence of the pottery clay, none of the cultures sustained snails. Presumably the presence of clay was important to the snails in grinding ingested food. In the absence of oyster shell, none of the cultures supported algal growth nor, consequently, snail growth. The absence of charcoal was not critical provided that the three other components (pottery clay, oyster shell and agar) were all present. The main purpose of the charcoal was to absorb some of the water products accumulated in the cultures. Agar was important since it solidified the synthetic mud so as to give the snails a firm substrate, important for feeding. The artificial water provided some of the chemicals contributed to algal growth but were not harmful to snail growth.

For efficiency, the synthetic mud was still inferior to natural mud, but it does open a promising field for further study.

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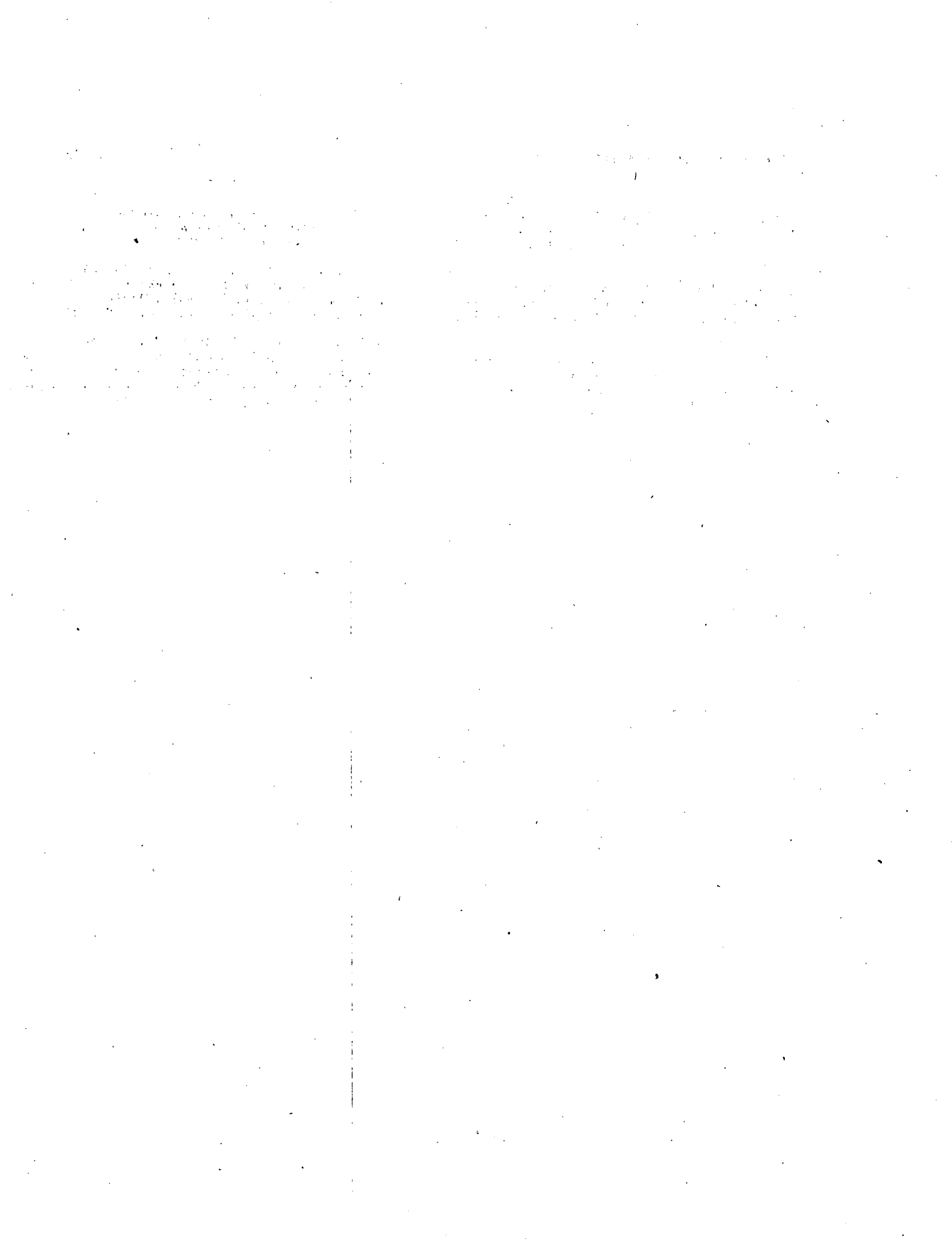


TABLE OF CONTENTS

Abstract	7		
Acknowledgements	7		
Introduction	8		
Literature review	8		
Proposed study	17		
Materials and methods	18		
1. Snails	18		
2. Methods to obtain newly hatched snails	18		
3. Water	19		
4. Algae	20		
5. Petri dish preparations for algal growth	21		
6. Lettuce	22		
7. Methods for preparing aquaria and initiating snail cultures	22		
8. Measuring survival, growth and fecundity	25		
Results	25		
Part I. Effect of crowding on survivorship, growth and fecundity	25		
1. <i>Bulinus globosus</i>	25		
2. <i>Biomphalaria pfeifferi</i>	26		
Part II. Effect of types of waters on survivorship, growth and fecundity	32		
A. Culturing snails in deionized, distilled, tap or spring water	32		
1. <i>Bulinus globosus</i>	32		
2. <i>Biomphalaria pfeifferi</i>	35		
B. Culturing snails in trays while alternating the types of waters	37		
1. <i>Bulinus globosus</i>	37		
2. <i>Biomphalaria pfeifferi</i>	38		
C. Culturing snails in calcium, magnesium or calcium-magnesium water	40		
1. <i>Bulinus globosus</i>	40		
2. <i>Biomphalaria pfeifferi</i>	42		
Part III. Effect of various species of algae and various kinds of muds and mud extracts on the growth of <i>Biomphalaria pfeifferi</i>	43		
A. Culturing snails in unialgal preparations	43		
B. Culturing snails in various kinds of tray and Petri dish preparations	45		
C. Culturing snails under crowded conditions	47		
D. Culturing snails in various kinds of mud and mud extracts	48		
Part IV. Formulation of synthetic mud	53		
Discussion	61		
Summary	67		
Literature cited	69		
		3. Analyses of calcium, magnesium and calcium-magnesium waters (spring water served as control)	19
		4. Components of modified artificial waters I, II and III	20
		5. Components of BBM and BBMSU	20
		6. Mud and extract preparations	22
		7. Synthetic mud preparations	23
		8. Effect of crowding on survivorship of <i>B. globosus</i> reared in spring water for 11 weeks	26
		9. Effect of crowding on fecundity of <i>B. globosus</i> reared in spring water for 11 weeks	27
		10. Egg-laying sites of <i>B. globosus</i> reared in spring water for 11 weeks	27
		11. Effect of crowding on shell length of <i>B. pfeifferi</i> reared in tap water for 12 weeks	28
		12. Effect of crowding on fecundity (eggs/snail) of <i>B. pfeifferi</i> reared in tap water for 12 weeks	29
		13. Egg-laying sites of <i>B. pfeifferi</i> reared in tap water for 12 weeks	30
		14. Effect of crowding on the number and the size of egg-mass of <i>B. pfeifferi</i> reared in tap water for 12 weeks	31
		15. Effect of distilled, tap and spring waters on survivorship and shell length of <i>B. globosus</i> reared for 44 weeks	32
		16. Effect of distilled, tap and spring waters on fecundity of <i>B. globosus</i> reared for 44 weeks	33
		17. Effect of distilled, tap and spring waters on egg-laying sites of <i>B. globosus</i> reared for 44 weeks	34
		18. Analyses of distilled, tap and spring waters taken from trays with 24-week old <i>B. globosus</i>	34
		19. Effect of deionized, distilled, tap and spring waters on survivorship and shell length of <i>B. pfeifferi</i> reared for 33 weeks	35
		20. Effect of deionized, distilled, tap and spring waters on fecundity of <i>B. pfeifferi</i> reared for 33 weeks	35
		21. Effect of deionized, distilled, tap and spring waters on egg-laying sites of <i>B. pfeifferi</i> reared for 33 weeks	36
		22. Effect of alternation of waters (Dis—Spr—Tap—Dis) on egg-laying sites of <i>B. globosus</i>	37
		23. Effect of alternation of waters (Dis—Tap—Spr—Dis) on egg-laying sites of <i>B. globosus</i>	38
		24. Effect of alternation of waters (Dei—Dis—Tap—Spr—Dei) on egg-laying sites of <i>B. pfeifferi</i>	39

LIST OF TABLES

1. Analyses of distilled, tap and spring waters	19
2. Components of calcium, magnesium and calcium-magnesium waters	19

25. Effect of calcium, magnesium and calcium-magnesium waters on shell length of <i>B. globosus</i> reared for 16 weeks (spring and distilled waters served as control)	40	41. Effect of synthetic muds (in Expt. 4) on survivorship and shell length of <i>B. pfeifferi</i> reared for 1 week	56
26. Effects of calcium, magnesium and calcium-magnesium waters on fecundity (eggs/snail) of <i>B. globosus</i> reared for 16 weeks (spring and distilled waters served as control)	41	42. Effect of synthetic muds (in expt. 6) on survivorship, shell length and fecundity of <i>B. pfeifferi</i> reared for 3 weeks	56
27. Effect of calcium, magnesium and calcium-magnesium waters on egg-laying sites of <i>B. globosus</i> reared for 16 weeks (spring and distilled waters served as control)	42	43. Effect of synthetic muds (in Expt. 9) on survivorship and shell length of <i>B. pfeifferi</i> reared for 3 weeks	57
28. Effect of calcium, magnesium and calcium-magnesium waters on shell length of <i>B. pfeifferi</i> reared for 16 weeks (spring and distilled waters served as control)	42	44. Effect of synthetic muds (in Expt. 10) on survivorship, shell length and fecundity of <i>B. pfeifferi</i> reared for 3 weeks	57
29. Effect of calcium, magnesium and calcium-magnesium waters on fecundity (eggs/snail) of <i>B. pfeifferi</i> reared for 16 weeks (spring and distilled waters served as control)	43	45. Effect of synthetic muds (in Expt. 11) on survivorship of <i>B. pfeifferi</i> reared for 4 days	58
30. Effect of calcium, magnesium and calcium-magnesium waters on egg-laying sites of <i>B. pfeifferi</i> reared for 16 weeks (spring and distilled waters served as control)	44	46. Effect of synthetic muds (in Expt. 12) on survivorship, shell length and fecundity of <i>B. pfeifferi</i> reared for 4 weeks	59
31. Effect of species of algae on survivorship and shell length of <i>B. pfeifferi</i> reared for 2 weeks (mixture of blue-green algae and mud served as control)	45	47. Summary of synthetic mud formulae which support good growth of <i>B. pfeifferi</i>	60
32. Effect of types of containers and food additives on survivorship and shell length of <i>B. pfeifferi</i> reared for 3 weeks	45		
33. Effect of types of containers and food additives on fecundity of <i>B. pfeifferi</i> reared for 3 weeks	46		
34. Effect of types of containers and food additives on egg-laying sites of <i>B. pfeifferi</i> reared for 3 weeks	47		
35. Effect of crowding on survivorship, shell length and fecundity of <i>B. pfeifferi</i> reared for 4 weeks	48		
36. Effect of kinds of muds and mud extracts on survivorship and shell length of <i>B. pfeifferi</i> reared for 2 weeks (Expt. 1)	51		
37. Effect of kinds of muds and mud extracts on survivorship and shell length of <i>B. pfeifferi</i> reared for 2 weeks (Expt. 2)	52		
38. Analyses of kinds of mud extracts used in Experiments 1 and 2	54		
39. Effect of synthetic muds (in Expt. 2) on survivorship, shell length and fecundity of <i>B. pfeifferi</i> reared for 5 weeks	55		
40. Effect of synthetic muds (in Expt. 3) on survivorship, shell length and fecundity of <i>B. pfeifferi</i> reared for 6 weeks	55		

LIST OF FIGURES

1. Procedures for preparing different kinds of muds and mud extracts	21
2. Effect of crowding on mean shell length of <i>B. globosus</i> reared in spring water for 11 weeks	26
3. Effect of crowding on the number of eggs (cumulated) laid per <i>B. globosus</i> reared in spring water for 11 weeks	26
4. Effect of crowding on mean shell length of <i>B. pfeifferi</i> reared in tap water for 12 weeks	28
5. Effect of crowding on the number of eggs (cumulated) laid per <i>B. pfeifferi</i> reared in tap water for 12 weeks	29
6. Effect of crowding on the size of egg-mass (=no. of eggs/egg mass) of <i>B. pfeifferi</i> reared in tap water for 12 weeks	30
7. Effect of crowding on the mean egg-mass size of <i>B. pfeifferi</i> reared in tap water for 12 weeks	32
8. Effect of distilled, tap and spring waters on mean shell length of <i>B. globosus</i> reared for 44 weeks	33
9. Effect of distilled, tap and spring waters on the number of eggs (cumulated) laid per <i>B. globosus</i> reared for 44 weeks	33
10. Effect of deionized, distilled, tap and spring waters on mean shell length of <i>B. pfeifferi</i> reared for 33 weeks	34
11. Effect of deionized, distilled, tap and spring waters on the number of eggs (cumulated) laid per <i>B. pfeifferi</i> reared for 33 weeks	36

12. Effect of calcium, magnesium and calcium-magnesium waters on mean shell length of <i>B. globosus</i> reared for 16 weeks (spring and distilled waters served as control)	41	15. Effect of calcium, magnesium and calcium-magnesium waters on the number of eggs (cumulated) laid per <i>B. pfeifferi</i> reared for 16 weeks (spring and distilled waters served as control)	46
13. Effect of calcium, magnesium and calcium-magnesium waters on the number of eggs (cumulated) laid per <i>B. globosus</i> reared for 16 weeks (spring and distilled waters served as control)	43	16. Growth differences in <i>B. pfeifferi</i> reared in various kinds of tray and Petri dish preparations for 2 weeks.	49
14. Effect of calcium, magnesium and calcium-magnesium waters on mean shell length of <i>B. pfeifferi</i> reared for 16 weeks (spring and distilled waters served as control)	43	17. Growth differences in <i>B. pfeifferi</i> reared in various kinds of tray and Petri dish preparations for 3 weeks.	50
		18. Effect of crowding on mean shell length of <i>B. pfeifferi</i> reared for 4 weeks	53

FURTHER NOTES ON PSEUDOGASTROPODS

Recently La Rocque (1973) discussed pseudogastropods: organisms that have been misidentified as gastropods and gastropods that have been incorrectly assigned to various phyla. His examples call attention to actual problems faced by those who deal with nomenclature. It seems desirable to record briefly two extreme cases: a fish that masqueraded as a gastropod (Hanna, 1925) and a group of gastropods that moved several giant steps downward in the Linnaean hierarchy and were interpreted as protozoans (Browne, 1971).

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REFERENCES

BROWNE, R. G. (1971) Misassignment of Silurian gastropod protoconch casts to taxa of Foraminifera. -- *Jour. Paleont.* 45(1): 141

HANNA, G. D. (1925) *Zalophancylus*, a fish vertebra, not a mollusk. -- *Nautilus* 39 (1): 18-19

La ROCQUE, A. (1973) Pseudogastropods. -- *Sterkiana* 51: 10

BOOKS RECEIVED

SHOUP, Charles S. (1974) A Bibliography of the Zoology of Tennessee and the Tennessee Valley Region. -- U. S. Atomic Energy Commission. Office of Information Services. Technical Information Center. iv, 251 p.

As its title indicates, this scholarly work lists published papers on the animals of the Tennessee Valley region from Protozoa to Mammalia, preceded by a section of 278 items on Ecology, General Zoology and Management. The items are numbered and arranged alphabetically by authors under each section. The Mollusca are well represented (Nos. 460-787) and present a useful introduction to the literature of this interesting part of the country. In addition, malacologists interested in the relationships of Mollusca to other groups of invertebrates will find much useful data under practically every one of the sections. After leafing through this work, one cannot help wishing that we had comparable compilations for every part of the country or, better still, for every part of the world.

A. L.

RESOURCES FOR MALACOLOGICAL RESEARCH IN ORTON MEMORIAL LIBRARY OF GEOLOGY

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Orton Hall, on the campus of the Ohio State University, Columbus, is a dramatic, appropriate setting for a geological library. The Richardsonian Romanesque structure is constructed of forty varieties of Ohio building stone, arranged in stratigraphic order. Red sandstone around the windows, doors, and chimneys tower and Berea Sandstone on the first and second floors represent the Mississippian age. The basement is constructed of Springfield Dolomite and Dayton Limestone (Silurian) and Columbus Limestone (Devonian) forms the steps leading to the main entrance. Red sandstone heads of extinct animals encircle the top of the chimneys tower. The grounds surrounding the building are enhanced with various concretions and a thirty-ton erratic boulder of anorthosite of Pleistocene origin.

Edward Orton, professor of geology and the first president of the University, conceived the idea for the unique building. As a fitting memorial to his father, Edward Orton, Jr. established and furnished a library of geological literature off the main foyer, adjacent to the Orton Geological Museum. Paintings of Ohio state geologists and geological landscapes complement the warm oak paneling and cork tile floors in the main reading room.

Since its formal dedication in 1920, Orton Library's collection has grown to 42,500 books, 477 periodical titles, and 43,700 maps. Many of its first editions are from the Ortons' personal libraries. The Library is a depository for all Ohio Geological Survey publications, not only those published by the Survey, but also those publications sent to that organization on a gift or exchange basis by other state geological surveys, surveys of foreign countries, and numerous geological organizations.

Although the Library's holdings encompass the entire range of geological research, from the traditional fields of mineralogy and paleontology to lunar geology and environmental pollution, one of its strengths is molluscan literature. It is this segment of the collection we wish to discuss. A guide to information sources (bibliographies and other reference tools) prefaces a list of serial publications that publish molluscan research. Finally, we mention the general treatises and regional studies in the Orton Memorial Library collection.

GUIDE TO INFORMATION SOURCES

There are both primary and secondary sources of information in a geological library. To obtain primary source literature (e.g., books, reports, serial articles), the researcher frequently consults general bibliographies, current and retrospective:

NICKLES, John M. (1923-1924) *Geologic literature of North America, 1785-1918*. (U.S.G.S. Bulletin 746 and 747) Washington, D.C., Govt. Prtg. Office, 2 vols.

U.S. GEOLOGICAL SURVEY (1923-1970) *Bibliography of North American geology*. Washington, Govt. Prtg. Office.

Annual; issued as U.S.G.S. Bulletins, covers the period 1919-1970. Cites publications 'concerning the geology of the North American continent, Greenland, the West Indies and adjacent islands, Hawaii, Guam, and other island possessions but not the trust territories. Articles by American authors published in foreign journals are cited if they deal with North American localities or are of a general

nature ... Articles on North America by foreign authors are included regardless of place of publication.' Arranged alphabetically by author with a subject index. Complements the Bibliography and index of geology exclusive of North America until the latter changed its name to Bibliography and index of geology in 1969 and began world-wide coverage. Ceased publication with 1970 volume. See Bibliography and index of geology after that date.

GEOLOGICAL SOCIETY OF AMERICA (1934-) Bibliography and index of geology. Boulder, Colo.

Monthly; world-wide in scope. Each issue is divided into 21 intradisciplinary categories and the citations within each category are arranged alphabetically by authors. Descriptors or key words accompany many of the citations. The code at the end of each citation represents a unique accession number in the American Geological Institute's GEO-REF, a computerized, indexed reference file. There is also a subject index for each monthly issue and annual compilation.

ZOOLOGICAL RECORD (1864-) London, Zoological Society of London.

Annual; contains roughly 15,000 references a year from world-wide literature. Issued in sections (e.g., Mollusca), each with a subject index.

SPECIALIZED BIBLIOGRAPHIES ARE ANOTHER SOURCE OF LITERATURE:

BOYLE, Cornelius Breckinridge (1893) A catalogue and bibliography of North American Mesozoic Invertebrata. (U.S.G.S. Bulletin 102, Washington, Govt. Prtg. Office, 315 p.)

BRANSON, CARL COLTON (1948) Bibliographic Index of Permian Invertebrates. (Geological Society of America. Memoir 26) New York, 1049 p.

HAAS, Otto (1958) Recent literature on Mesozoic ammonites. (Jour. Paleont. 32 (3):624-635).

KEEN, Angeline Myra (1937) An abridged checklist and bibliography of West North American marine Mollusca. Stanford, Calif., Stanford Univ. Press; London, H. Milford, Oxford University Press. 87 p.

MARCOU, John Belknap (1885) Bibliography of publications relating to the collection of fossil invertebrates in the United States National Museum, including complete lists of the writings of Fielding B. Meek, Charles A. White and Charles D. Walcott. (Bibliographies of American naturalists, 3; U.S. National Museum Bull. 30) Washington, D.C. 333 p.

RICHARDS, Horace Gardiner, comp. (1968) Catalogue of invertebrate fossil types at the Academy of Natural Sciences of Philadelphia. (Academy of Nat. Sci. Phila., Spec. Publ. no. 8) Philadelphia, Acad. Nat. Sci. 222p.

VOKES, Harold E. (1967) Genera of the Bivalvia—a systematic and bibliographic catalogue. (Bull. Amer. Paleontology, v. 51, no. 232, p. 111-394)

WELLER, Stuart (1898) A bibliographic index of North American Carboniferous invertebrates. (U.S.G.S. Bull. 153) 653 p.

WHITE, Charles Abiathar (1878) Bibliography of North American invertebrate Paleontology, being a report upon the publications that have hitherto been made upon the invertebrate paleontology of North America, including the West Indies and Greenland. By C. A. White and H. Alleyne Nicholson. (U.S.G.S. of the Territories. Misc. Publ. no. 10) 132 p.

YOCHELSON, Ellis L. (1967) A bibliographic index of North American Late Paleozoic Hyolitha, Amphineura, Scaphopoda, and Gastropoda, by E. L. Yochelson and Burnett W. Saunders. (U.S.G.S. Bull. 1210) 271 p.

IN ADDITION TO BIBLIOGRAPHIC SERIALS AND COMPILATIONS, ONE MUST BE ACQUAINTED WITH OTHER REFERENCE SOURCES IN THE LIBRARY:

ATLASES

HALLAM, A., ed. (1973) Atlas of palaeobiogeography. Amsterdam, New York, Elsevier, 531 p.

JOLEAUD, Léonce (1939) Atlas de paléobiogéographie. Paris, Lechevalier, 40 p.

VERNIOURY, René (1970) Atlas de paléontologie des invertébrés. Genève, Georg. 223 p.

BIOGRAPHICAL DIRECTORIES

AMERICAN MALACOLOGISTS, 1973-74 (1973) Editor-in-chief: R. Tucker Abbott. Falls Church, Va. 494 p.

AMERICAN MEN AND WOMEN OF SCIENCE. (1971) 12th ed., New York, Bowker, 6 vols.

LAMBRECHT, K. (1938) Palaeontologi. Catalogus bio-bibliographicus, by K. Lambrecht, W. and A. Quenstedt. (Fossilium Catalogus I: Animalia, pars 72, 495 p.)

WESTERMANN, G.E.G., comp. (1968) Directory of palaeontologists of the world (excluding Soviet Union and continental China) 2d ed. Sponsored by the International Palaeontological Union. Hamilton, Ontario, Canada, McMaster University. 250 p.

CATALOGS AND COLLECTIONS

ANDERSON, Ernest Masson (1936) Catalogue of types and figured specimens of fossils in the Geological Survey collections now exhibited in the Royal Scottish Museum, Edinburgh. London, H.M. Stat. Off. 77p.

BRITISH MUSEUM (Natural History) Department of Geology (1886) A guide to the exhibition galleries of the Department of Geology and Palaeontology in the British Museum (Natural History) 4th ed. London, Printed by order of the Trustees. 117 p.

---- (1888-1951) Catalogue of the fossil Cephalopoda in the British Museum (Natural History) by Arthur H. Foord. London, Printed by order of the Trustees. 5 vols.

---- (1891) Systematic list of the Frederick E. Edwards collection of British Oligocene and Eocene Mollusca in the British Museum (Natural

History), with references to the type-specimens from similar horizons contained in other collections belonging to the Geological Department of the Museum. By Richard Bullen Newton. London, Printed by order of the Trustees. 365 p.

---- (1907) A guide to the fossil invertebrate animals in the Department of Geology and Palaeontology in the British Museum (Natural History). London, Printed by order of the Trustees. 182 p.

---- (1923) A guide to the exhibition galleries of the Department of Geology and Palaeontology. London, Printed by order of the Trustees. 64 p.

---- (1938) A catalogue of the ammonites of the Liassic family Liparoceratidae in the British Museum (Natural History) by L. F. Spath. London, Printed by order of the Trustees. 191 p.

BRITISH MUSEUM (Natural History) Department of Zoology (1913) Catalogue of the British species of *Pisidium* (Recent and fossil) in the collections of the British Museum (Natural History). London, Printed by order of the Trustees. 144 p.

CANADA, GEOLOGICAL SURVEY (1960-) Catalogue of type invertebrate fossils of the Geological Survey of Canada, by Thomas E. Bolton, Ottawa, Queen's Printer.

DOUVILLÉ, Robert (1915) Études sur les Cosmocératidés des collections de l'École nationale supérieure des Mines et de quelques autres collections publiques ou privées. (France. Service de la carte géologique de la France. Mémoires pour servir à l'explication de la carte géologique détaillée de la France) Paris, Imprimerie Nationale. 75 p.

HANZAWA, Shōshirō, comp. (1961) Catalogue of type-specimens of fossils in Japan. Compiled by Shōshirō Hanzawa, Kiyoshi Asano, and Fuyuji Takai. Tokyo, Palaeontological Society of Japan. 422 p.

KEEN, Angeline Myra (1939) Illustrated key to West North American pelecypod genera, by A. Myra Keen and Don L. Frizzell. Stanford, Calif., Stanford University Press; London, H. Milford, Oxford University Press. 28 p.

MANTELL, Gideon Algernon (1851) Petrifications and their teachings: or, A hand-book to the Gallery of Organic Remains of the British Museum. London, H. G. Bohn. 496 p.

MILLER, Samuel Almond (1877) The American Palaeozoic fossils: a catalogue of the genera and species, with names of authors, dates, places of publication, groups of rocks in which found, and the etymology and signification of the words, and an introduction devoted to the stratigraphical geology of the Palaeozoic rocks. Cincinnati, Ohio, The Author, 253 p.

STENZEL, Henryk Bronislaw (no date) Type Invertebrate fossils of North America (Eocene, Paleocene, Oligocene). Prepared by H.B. Stenzel and F. E. Turner. Austin, Texas, Bureau of Economic Geology. Cards 1-148.

TURNER, Ruth Dixon (1966) A survey and illustrated catalogue of the Teredinidae (Mollusca: Bivalvia). Cambridge, Mass., Museum of Comparative Zoology, Harvard University. 265 p.

TYPE INVERTEBRATE FOSSILS OF NORTH AMERICA (DEVONIAN) (1936-1938) Philadelphia, Wagner Free Institute of Science. 3 vols.

WARD, Henry Augustus (1866) Catalogue of casts of fossils, from the principal museums of Europe and America, with short descriptions and illustrations. Rochester, New York, Benton and Andrews, 228 p.

WILLARD, Bradford (1966) The Harvey Bassler collection of Peruvian fossils. Bethlehem, Pa., Lehigh University. 255 p.

WOOD, WILLIAM (1856) Index Testaceologicus, an illustrated catalogue of British and foreign shells, containing about 2800 figures accurately coloured after nature. A new and entirely revised edition, with ancient and modern appellations, synonyms, localities, etc., by Sylvanus Hanley. London, Willis and Sotheman. 234 p.

WOODWARD, Arthur Smith (1908) Illustrations of type specimens of Inferior Oolite ammonites in the Sowerby collection. London, printed for the Palaeontographical Society. 33 p.

Catalogs are also published in periodicals, as the following examples:

CHAPPARS, Michael Stephen (1936) Catalog of the type specimens of fossils in the University of Cincinnati Museum. (Ohio Jour. Sci., v. 36, no. 1, p. 1-45).

FLEMING, C. A. (1966) Marwick's illustrations of New Zealand shells, with a checklist of New Zealand Cenozoic Mollusca. (New Zealand. Department of Science and Industrial Research. Bull. 173, 456 p.

GRANT, Ulysses Simpson, IV (1931) Catalogue of the marine Pliocene and Pleistocene Mollusca of California and adjacent regions, by U.S. Grant and Hoyt Rodney Gale. (San Diego Society of Natural History. Memoir, v. 1, 1036 p.)

PURNELL, Louis R. (1968) Catalog of the type specimens of invertebrate fossils. Part I: Palaeozoic Cephalopoda. (U.S. National Museum. Bull. 262) Wash., D. C., Govt. Prtg Off. 198 p.

FOSSIL INDEXES

Fossilium Catalogus. I. Animalia. (1913-) 's-Gravenhage, W. Junk. Pars 1-

A comprehensive series; each part is on a separate subject, authored by a specialist.

GRABAU, Amadeus William (1909-1910) North American Index Fossils, Invertebrates, by Amadeus W. Grabau and Hervey Woodburn Shimer. New York, A. G. Seiler. 2 vols.

JOINT COMMITTEE ON INVERTEBRATE PALEONTOLOGY (1953-) Treatise on invertebrate paleontology. Edited by Raymond C. Moore. Boulder, Colo., Geological Society of America and Lawrence, Kansas, University of Kansas Press. In Progress.

A series on the major groups of invertebrate animals. Parts are divided according to phylum, and the orders, classes, and genera in each phylum are described, with references to the literature. Of interest to malacologists:

Part I - Mollusca 1 (Mollusca general features, Scaphopoda, Amphineura, Monoplacophora, Gastropoda general features, Archaeogastropoda, mainly Paleozoic Caenogastropoda and Opisthobranchia) 1960. 351 p., 1732 figs.

Part J - Mollusca 2 (Gastropoda, Streptoneura exclusive of Archaeogastropoda, Euthyneura) In preparation.

Part K - Mollusca 3 (Cephalopoda general features, Endoceratoidea, Actinoceratoidea, Nautiloidea, Bactritoidea) 1964. 519 p., 2382 figs.

Part L - Mollusca 4 (Ammonoidea) 1957. 490 p., 3800 figs. Revision in preparation.

Part M - Mollusca 5 (Coleoidea) In preparation.

Part N - Mollusca 6 (Bivalvia) 3 vols. v. 1 and 2: 1969, 952 p., 6198 figs.; v. 3: 1971, 272 p., 742 figs.

SHIMER, Hervey Woodburn (1944) Index fossils of North America; a new work based on the complete revision and reillustration of Grabau and Shimer's North American Index Fossils. (Technology Press, Mass. Inst. of Technology) New York, Wiley; London, Chapman and Hall. 837 p.

HANDBOOKS AND MANUALS

BEERBOWER, James Richard (1971) Field guide to fossils. (Earth Science Curriculum Project. ESCP pamphlet series, PS-4) Boston, Houghton Mifflin. 54 p.

CAMP, Charles Lewis (1937) Methods in paleontology, by Charles L. Camp and G. Dallas Hanna. Berkeley, Univ. of California Press, 153 p.

CHENU, Jean Charles (1859-1862) Manuel de conchyliologie et de paléontologie conchyliologique. Paris, V. Masson. 2 vols.

FISCHER, Paul Henri (1887) Manuel de conchyliologie et de paléontologie conchyliologique; ou, Histoire naturelle des mollusques vivants et fossiles. Paris, F. Savy. 1391 p.

HÖLDER, Helmut (1964) Jura. (Handbuch der stratigraphischen Geologie, Bd. 4) Stuttgart, F. Enke. 603 p.

KIRKALDY, John F. (1967) Fossils in colour. London, Blandford Press. 223 p.

KUMMEL, Bernhard (1965) Handbook of paleontological techniques. Prepared under the auspices of the Paleontological Society. Edited by B. Kummel and David Raup. San Francisco, W. H. Freeman. 852 p.

MIDDLEMISS, Frank Alexander (1968) A guide to invertebrate fossils. (Hutchinson biological monographs) London, Hutchinson, 128 p.

NEAVE, Ernest (1928) Stratigraphical palaeontology; a manual for students and field

geologists. London, Macmillan. 525 p. (2d ed., revised and enlarged, published by Clarendon Press, Oxford, Eng., 1955, 806 p.)

ORLOV, Yu. A., ed. (1962-) Fundamentals of paleontology. Osnovy Paleontologii. A manual for paleontologists and geologists of the USSR. Translated by the Israel Program for Scientific Translations staff. Jerusalem, published for the National Science Foundation by IPST.

PHILIPPI, Rudolph Amandus (1853) Handbuch der Conchyliologie und Malacozologie. Halle, E. Anton. 548 p.

PRATT, Henry Sherring (1935) A manual of the common invertebrate animals, exclusive of insects. Revised ed. New York, Macmillan. 854 p.

TRYON, George Washington (1885-1935) Manual of Conchology; structural and systematic, with illustrations of the species. Second series: Pulmonata. Philadelphia. Published by the Conchological Department, Academy of Natural Sciences of Philadelphia. 28 vols.

WEBB, Walter Freeman (1935) A handbook for shell collectors; illustrations and descriptions of 1100 species of Mollusca. Rochester, N.Y., St. Petersburg, Fla., The Author? 146 p.

WOODWARD, Samuel Pickworth (1851-1856) A manual of the Mollusca; or, Rudimentary treatise of Recent and fossil shells. London, J. Weale. 510 p. (Reprint of 4th ed. (1880) publ. by C. Lockwood, London, 1890, 628 p.)

ZITTEL, Karl Alfred Ritter von (1880-1893) Handbuch der Palaeontologie. München und Leipzig, R. Oldenbourg. 2 vols. in 5.

NOMENCLATORS

BULLETIN OF ZOOLOGICAL NOMENCLATURE (1943-) London, Printed by order of the International Commission on Zoological Nomenclature and sold by the International Trust for Zoological Nomenclature.

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE (1961) Code international de nomenclature zoologique, adopté par le XVe Congrès International de Zoologie. Editorial Committee: N. R. Stoll, chairman, and others. London, Published for the International Commission on Zoological Nomenclature by the International Trust for Zoological Nomenclature. 176 p.

NEAVE, Sheffield Airey, ed. (1939-1940) Nomenclator zoologicus; a list of the names of genera and subgenera in zoology from the tenth edition of Linnaeus, 1758, to the end of 1935. Zoological Society of London. 4 vols.

SERIAL PUBLICATIONS

Serial publications are an excellent source of information covering retrospective and current studies in molluscan research. We have previously mentioned bibliographic serials under 'Guide to Information Sources.' In addi-

tion, there are serials of a general or specialized nature in the Orton Library collection. They are issued by governmental agencies (such as geological surveys), associations and scientific societies, universities and other learned institutions, museums, book publishers and private sources as numbered series, monographs, proceedings, and field trip guidebooks. In the following selected list of serials, those titles frequently containing research of interest to malacologists are designated with an asterisk.

GOVERNMENTAL AGENCIES

UNITED STATES

U.S. Geological Survey, Washington, D. C.
(See also publications issued prior to 1879 under its former name 'U. S. Geological and Geographical Survey of the Territories.')

- * Annual Report
- * Bulletin
- * Journal of Research of the U.S. Geological Survey
- * Monograph
- * Professional Paper

State geological surveys regularly publish results of regional research in their annual reports, bulletins, circulars, reports of investigations, etc. The publications are indexed, as are those of the U.S.G.S., in the U.S.G.S.'s Bibliography of North American Geology (discontinued after 1970) and the Geological Society of America's Bibliography and Index of Geology (a monthly publication, worldwide in scope). The surveys will supply a list of their publications upon request.

FOREIGN

Geological surveys or other governmental agencies outside the U.S. issue their publications at irregular intervals. The one notable exception is the Geological Survey of Canada. One may find these publications cited in the aforementioned *Bibliography and index to Geology*.

Attention will be called to the most significant papers published by these agencies in a future publication now in preparation.

ASSOCIATIONS AND SCIENTIFIC SOCIETIES

UNITED STATES

- Academy of Natural Sciences of Philadelphia
 - * Notulae Naturae
 - * Proceedings
 - * Monographs
 - Special Publications
- Academy of Sciences of St. Louis
 - * Transactions
- American Association for the Advancement of Science, Washington, D.C.
 - Proceedings
 - Science

- American Association of Museums, New York
 - The Museum News
- American Association of Petroleum Geologists, Tulsa
 - Bulletin
- American Geographical Society, New York
 - The Geographical Review
- American Geological Institute, Washington, D.C.
 - * Akademiia Nauk SSR. Doklady: Earth Sciences sections. (English trans.)
 - * International Geology Review
 - * Paleontological Journal (Translation of the Russian Paleontologicheskii Zhurnal)
- American Malacological Union, Havertown, Pa.
 - * The Nautilus
- American Philosophical Society, Philadelphia
 - Proceedings
 - Transactions
- Association of Geology Teachers, Fort Collins, Colo.
 - Journal of Geological Education.
- Boston Society of Natural History
 - * Proceedings
- Buffalo Society of Natural Sciences
 - Bulletin
- California Academy of Sciences, San Francisco
 - Proceedings
- Cincinnati Society of Natural History
 - Journal
- Connecticut Academy of Arts and Sciences, New Haven
 - Transactions
- Des Moines Academy of Science
 - Bulletin
- Florida Academy of Sciences, Gainesville
 - Quarterly Journal
- Geological Society of America, Boulder, Colo.
 - * Bulletin
 - * Memoir
 - Proceedings (Ceased publication after 1968)
 - * Special Paper
- Gulf Coast Association of Geological Societies, Houston
 - Transactions
- Indiana Academy of Science, Indianapolis
 - Proceedings
- Iowa Academy of Sciences, Des Moines
 - Proceedings
- Michigan Academy of Science, Arts and Letters
 - Papers
- Paleontological Society, Berkeley, Calif.
 - * Journal of Paleontology
- San Diego Society of Natural History
 - * Memoirs
 - * Transactions
- Sigma Gamma Epsilon, Norman, Okla.
 - The Compass

- Smithsonian Institution, Washington, D.C.
 * Annual Report
 * Atoll Research Bulletin
 * Miscellaneous Collections
 * Smithsonian Contributions to Zoology
 * Smithsonian Contributions to Paleobiology
 Society of Economic Paleontologists and Mineralogists, Tulsa, Okla.
 * Journal of Paleontology
 Washington Academy of Sciences, Washington, D.C.
 Journal
 West Virginia Academy of Science, Morgantown, W. Va.
 Proceedings
- MIDDLE AMERICA**
- MEXICO**
 Sociedad Geológica Mexicana, México, D.F.
 Boletín
- JAMAICA**
 Geological Society of Jamaica, Kingston
 Journal (Geonotes)
- SOUTH AMERICA**
- ARGENTINA**
 Asociación Geológica Argentina, Buenos Aires
 Revista
 Asociación Paleontológica Argentina, Buenos Aires
 * Ameghiniana
- PERU**
 Sociedad Geológica del Peru, Lima
 Boletín
- EUROPE**
- AUSTRIA**
 Geologische Gesellschaft in Wien
 Mitteilungen
- BELGIUM**
 Association pour l'Étude de la Paléontologie et de la Stratigraphie houillères, Bruxelles
 * Publication
 Société Belge de Géologie, de Paléontologie et d'Hydrologie, Bruxelles
 * Bulletin
- BULGARIA**
 Bulgarska Akademiia na Naukite, Sofia. Geologicheski Institut
 Trudove vurkhu geologiiata na Bulgariia. Seriiia paleontologiia
- CZECHOSLOVAKIA**
 Slovenská Akadémia vied a Umení, Bratislava
 Geologický sborník
- DENMARK**
 Denmark. Kommissionen for Videnskabelige Undersøgelser i Grønland, Copenhagen
 Meddelelser om Grønland
- ENGLAND**
 British Antarctic Survey, London
 Bulletin
 East Midlands Geological Society, Nottingham
 The Mercian Geologist
- Falkland Islands Dependencies Survey, London
 Scientific Reports
 Geologists' Association, London
 Proceedings
 Geological Society of London
 Journal
 Transactions
 Liverpool Geological Society
 Geological Journal (Published jointly with Manchester Geological Association)
- Mineralogical Society, London
 The Mineralogical Magazine and Journal of the Mineralogical Society
 Palaeontographical Society, London
 * Publications
 Palaeontological Association, London
 * Palaeontology
 * Special papers in Palaeontology
 Ray Society, London
 Publications
 Royal Society of London
 Philosophical Transactions. Series B: Biological Sciences
 Yorkshire Geological Society, Leeds
 Proceedings
- FRANCE**
 Société Géologique de France, Paris
 * Bulletin
 Compte rendu sommaire des séances
 * Mémoires, Paléontologie
 Société Géologique du Nord, Lille
 Annales
 Société Nationale des Pétroles d'Aquitaine. Centre de Recherches de Pau
 Bulletin
- GERMANY**
 Deutsche Geologische Gesellschaft, Berlin
 Zeitschrift
 Deutsche Gesellschaft für Geologische Wissenschaften, Berlin
 Paläontologische Abhandlungen. Abt. A: Paläozoologie
 Deutsche Quartärvereinigung, Hannover
 Eiszeitalter und Gegenwart (Published in Öhringen, Württ.)
 Geologische Vereinigung, Koblenz
 Geologische Rundschau (Published in Stuttgart)
 Naturforschende Gesellschaft zu Freiburg i. B.
 Berichte
- HUNGARY**
 Magyar Földtani Tarsulat Folyóirata, Budapest
 Földtani közlöny
- IRELAND**
 Geological Society of Dublin
 Journal
- ITALY**
 Accademia Nazionale dei Lincei, Rome.
 Classe di Scienze Fisiche, Matematiche e Naturali
 Memorie (Fisica, chimica, geologia, paleontologia e mineralogia (Atti))

- Società di Naturalisti in Napoli
Bolletino
Società Geologica Italiana, Rome
Bolletino
- NETHERLANDS
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