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FINAL REPORT

ON

BIOLOGICAL CONTROL OF SCHISTOSOMIASIS-TRANSMITTING SNAILS IN SOUTHEAST ASIA

SUBMITTED TO

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BY

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I. BACKGROUND

The award of the project entitled "Biological Control of Schistosomiasis-transmitting Snails in Southeast Asia " was presented by His Excellency, Mr. John Gunther Dean, Ambassador of the United States of America to Thailand, to Professor Dr. Natth Bhamarapravati, Rector of Mahidol University, at the Rockefeller Foundation Conference Room, Faculty of Science, Mahidol University, on 5 March 1984. The first payment of the grant was made available to the Faculty of Science in early April 1984. Five reports had been submitted, i.e. the sixth-month report of year one, the annual report of year one, the sixth-month report of year two, the annual report of year two, and the sixth-month report of year three. This is the final report of the project covering the period from April 1984 to May 1987.

The total three-year budget was $150,000, of which $94,100 and $55,900 were allo cated to Mahidol University and University of California, respectively.

II. PROJECT OBJECTIVE

The overall objective of this research project was as follows:

1. to study the efficacy of biological control of snails transmitting schistosomiasis in Southeast Asia using biological agents, and

2. to study genetic variability of snails transmitting schistosomiasis in Southeast Asia in relation to degrees of parasite compatibility and susceptibility.

III EXPERIMENTAL GOALS

1. Laboratory experiments. These studies included the biological aspects of snail agents and insects and their efficacy against the target snail species. The life histories of snail agents and insects were studied and various doses of biological agents and target species were tested. This phase of study took approximately 16 months.

2. Simulated field experiments. Biological agents and target species were tested in simulated field habitats. This phase of study took approximately 8 months.

3. Genetic and susceptibility studies. Genetic variability of snails transmitting schistosomiasis in relation to degrees of parasite compatibility and susceptibility was studied under laboratory conditions. This phase of study took approximately 3 years.

4. Small scale field experiments. Pending the results of "goals" one and two as delineated above, a small scale field trial was initiated. These experiments included only local target species. This last stage took approximately one year.

IV RESEARCH FINDINGS

A. Biological control of snails

1. Mollusks

Four species of snails from Thailand were used to test their efficacy against target snails; these were *Brotia costula costula* (Prosobranchia : Thiaridae), *Tarebia (Thiara) granifera* (Prosobranchia : Thiaridae), *Melanoides tuberculata* (Prosobranchia : Thiaridae) and *Pila ampullacea* (Prosobranchia : Ampullaridae).

1.1 Biology

1.1.1 *Brotia costula costula*

B.c.costula is the largest representative of the family in Thailand. They occur in Southeast Asia, in countries such as Thailand, Laos, Cambodia, Burma and India. They can be found in rivers and mountain creeks with running water, rarely in still water (Brandt, 1974).

B.c. costula is a very large snail, the largest of the family Thiaridae. Adults may vary in shell length from 5.5 - 7.4 cm. Figure 1 shows the photograph of adult *B.c. costula* collected from the field. The shell is elongately turreted, solid to thick, covered with a dark brownish or olive-brown periderm. The apex is generally truncate. The shell is sculptured with numerous spiral grooves which are weaker on the upper half of the body whorl. The animal ribs are more or less strongly developed. The shells are brown with 1-3

dark brown spiral bands. The operculum is almost circular, with 5 whorls. The animal is dark grey with orange or yellow pigment spots (Brandt, 1974).

B.c. costula is ovoviviparous and parthenogenetic. Males consist about 3% of the population. They are non-functional and the male reproductive organs seem to have no functional gonads. The females have many small, conical embryonic shells in the brood pouch. The young are reared in the brood pouch and are usually 0.2-0.3 cm long when born. Adult size or maturity is reached within 18-24 months. Several young are produced daily during the dry season (December-April) and gradually decreased in number. *B.c. costula* feeds on diatoms, algae and detritus and does not harm growing vegetation. They prefer the flowing freshwater streams with the temperature around 25 ^{o}C.

B.c. costula shares many of the characteristics with *T. granifera* and lives in the same type of habitat. It is considered to be another potential biological control agent. However, one species of *Brotia, B. asperata* had been reported to be the intermediate host of the human lung fluke, *Paragonimus westermani* in the Philippines (Cabrera and Vajrasthira, 1973).

1.1.2 *Tarebia (Thiara) granifera*

T. granifera is found in abundance in almost all provinces in Thailand. They occur in Southeast Asia, Southern China, India, Sri Lanka, Indonesia,

Philippines and numerous Western Pacific Islands. It is common in lakes, ponds, rivers, canals and creeks (Brandt, 1974).

T. granifera is a moderate-sized snails. Adults may vary from 1.5 - 3 cm. in shell length, with the common size of 2.5 cm. (Fig. 2). The shell is elongated, brownish or olive-colored, sculptured with several spiral rows of beads or blunt tubercles. The shell apex is pointed but often eroded. The body whorl is large and measures about half the length of the shell. The operculum is thin, opaque and blackish brown in color. The animal is grey with yellow and blackish pigmentation (Tucker Abbot, 1952).

T. granifera is ovoviviparous and parthenogenetic. Males are non-functional. The females have subhaemocoelic brood pouch. The young are reared in the brood pouch and is 0.2-0.3 cm. when born. The young reach maturity within 12-18 months. Several young are produced daily and the snail is capable of maintaining very high densities in a habitat over long periods of time. *T. granifera* feeds on diatoms, algae and detritus and does not harm growing vegetation.

T. granifera can act as the first intermediate host of the human lung fluke, *P. westermani*. However, man contracts paragonimiasis only incidentally by eating the raw or undercooked second intermediate host (various species of freshwater crustaceans) of *P. westermani*. However, this need not necessarily detract *T. granifera* from its usefulness as

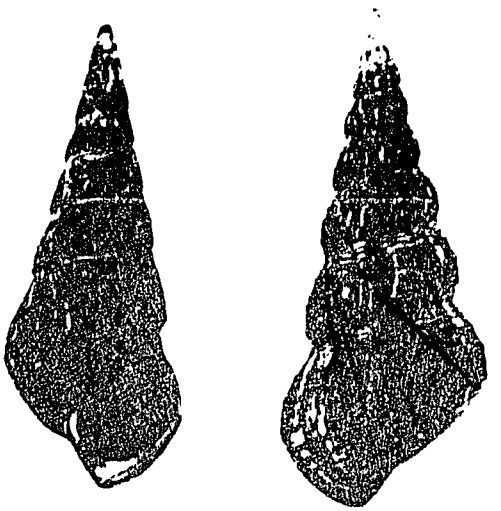


Fig. 1. *Brotia costula costula* (as ig)

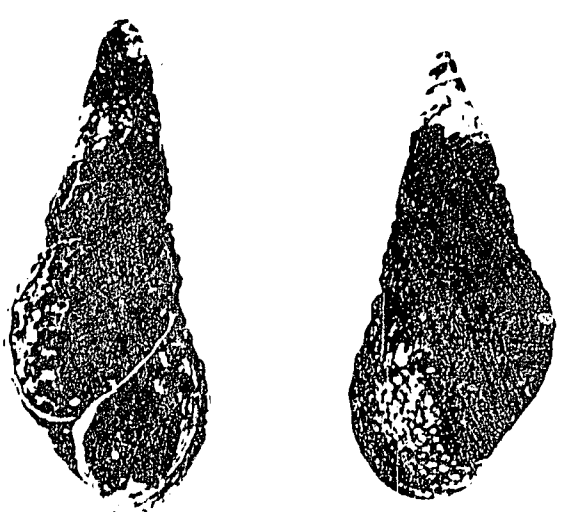


Fig. 2. *Tarebia granifera* (x2.5)

an effective biocontrol agent of snails. *T. granifera* has been reported to be an effective biocontrol agent for *Biomphalaria glabrata* in Puerto Rico (Hairston *et al.*, 1975), St. Lucia (WHO document, 1981). Laracuente *et al.* (1979) had done a preliminary study which showed that various species of aquatic snails could be used as decoys to intercept schistosome miracidia and prevented them from reaching their snail intermediate host. *T. granifera* has shown to be one of the slightly effective decoy for *Schistosoma mansoni* miracidia infecting *B. glabrata* especially in the laboratory, i.e. caused slight reduction in the proportion of *B. glabrata* infected by miracidia of *S. mansoni*.

1.1.3 *Melanoides tuberculata*

M. tuberculata has a very wide geographical range, from Eastern Europe through Africa and Asia to the West Pacific Islands and Australia. This species is found in all provinces of Thailand in abundance. Its habitats range from lakes, ponds, rivers, brooks, mountain creeks and are abundant in the tidal areas (Brandt, 1974).

M. tuberculata is a small snail, with the average size of adult ranging from 1.5-2.0 cm. (Fig. 3). The shell is moderately thick, elongate, covered with a brownish, yellowish or olive periderm; and sculptured with many narrow spiral ridges which are often crossed by obtuse ribs. The animal itself is dark grey with yellowish pigment spots.

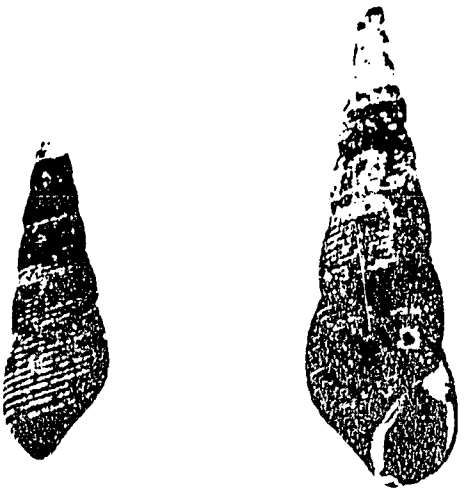


Fig. 3. *Melanoides tuberculata* (x2)

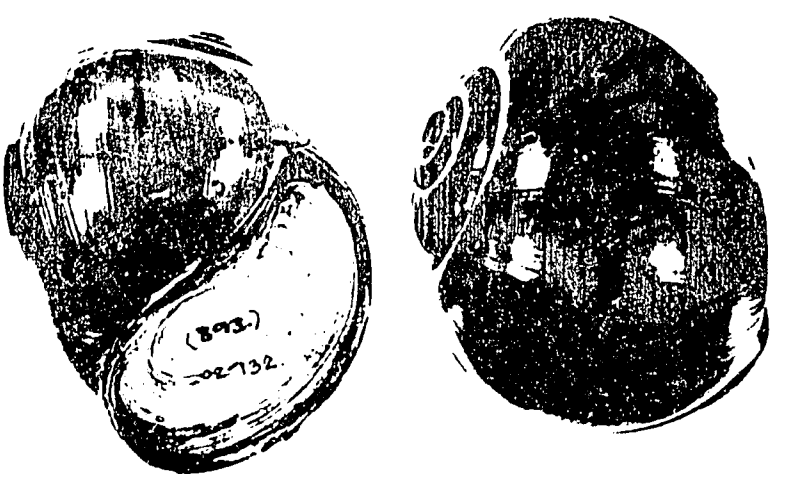


Fig. 4. *Pila ampullacea* (as is)

M. tuberculata is ovoviviparous and parthenogenetic. The males contain reproductive organs without functional gonads. The young snails are reared in the brood pouch of the female and are 0.8 mm. long when born. Maturity is reached within 6-7 months. Young snails are produced daily but the number is much fewer than those produced by *T. granifera* or *B.c. costula*. *M. tuberculata* also feeds on diatoms, algae and detritus and does not harm growing vegetation.

M. tuberculata has been reported to act as intermediate host for *P. westermani*. However, no naturally infected snails have been found in Thailand. *M. tuberculata* has been reported to be the biological control agent of *B. glabrata* on the island of Martinique, West Indies (Pointier, personal communication).

1.1.4 *Pila ampullacea*

P. ampullacea occurs widely in Southeast Asia such as Thailand, Malaysia, Indonesia, Borneo, Laos, Cambodia and Vietnam. In Thailand, it is found in almost all provinces, inhabitats in ponds, rivers, lakes and creeks (Brandt, 1974).

P. ampullacea is a very large snail, with the shell length ranges from 6-10 cm. Figure 4 shows the photograph of adult *P. ampullacea* with the shell length of 6 cm. The shell is subglobose with low conical, or almost flat spire. The body whorl is large and inflated. Periderm is unicolored

of olive-green or with greenish or brown spiral bands. The operculum is thick, with greyish white nacre on the inner surface.

P. ampullacea is oviparous. The eggs are laid in white mass attached to mud or rock surface. The young reaches maturity within 12-18 months. *P. ampullacea* harbors metacercariae of *Echinostoma ilocanum*, serves as important intermediate host for the rat's lung worm *Angiostrongylus cantonensis*. People can be infected by eating raw or uncooked meat of *P. ampullacea*. However, it can be considered as a potential biological agent due to its large size and its ability to feed on wide range of food such as algae, detritus and fish food.

1.2 Cultivation of the biological agents (mollusks).

The four species of snails *B.c. costula*, *T. granifera*, *M. tuberculata* and *P. ampullacea* were cultured in the laboratory of the Center for Applied Malacology and Entomology. In general, these snails were cultured with the same technique. *B.c. costula* were collected from Kanchanaburi Province, Northeast Thailand, while *T. granifera*, *M. tuberculata* and *P. ampullacea* were collected from Kasetsart University, Bangkok, Thailand.

The aquaria for maintenance of the four species of snails were round glass aquaria with the diameter of 24 cm and with the height of 18 cm. They were equipped with aeration pumps, plastic tubes and air stones. Snail cultures

were maintained under 8 h of 40-watt cool fluorescent lamps per day.

Aged tap water which had been left standing for at least 2 days, was used for rearing snails. The room and water temperatures were maintained at 27°-28°C. Water levels in snail containers, aeration, water and room temperatures were checked daily.

All four species of snails required substrate such as mud or sand. For *B.c. costula*, the fine sand brought from the snail collecting site was used as snail substrate. The sand had been put in the oven and baked for 4 h at 160°C before use. The thick layer of sand of about 1 in deep was laid in the glass aquaria and then filled with aged tap water up to 1/2 of its height. Twenty adult snails were introduced into each aquarium. The snails were fed with ground lettuce leaves.

For *T. granifera* and *M. tuberculata*, dried mud powder which had been put in the oven at 160°C for 4 h was used as snail substrate. The mud powder was put into an aquarium just to fill its bottom (about 1 cm thick). The container was then filled with aged tap water up to 2/3 of its height. Thirty adult snails were introduced into each aquarium. The snails were fed with ground lettuce leaves.

For *P. ampullacea*, rich brown top soil collected from rice fields or river bank was used to make substrate on the

bottom of the aquarium in the form of a mud slope of about 5-6 cm in height. A few pieces of rocks were placed on the mud slope to serve as egg-laying sites for the snails. The container was filled with aged tap water approximately 2 cm. above the top of the mud slope. Ten adult snails were introduced into each aquarium. The snails were fed with fish food and fresh lettuce leaves.

1.3 Growth rates of snail agents

B. c. costula, T. granifera and *M. tuberculata* are ovoviviparous (bear young). Approximately 1-2 days after the introduction of adult snails to the breeding cultures, the young snails emerged and they were removed to another culture to prevent the aquarium from being overcrowded. The young snails were fed with ground lettuce leaves supplemented with diatoms. The shell lengths of the young snails were measured once a week. These measurements provided data necessary for the growth rates of the snails. The results of the experiments are shown in Tables 1-4 and Figures 5-8.

For *B.c. costula*, the newly emerged young snails were 0.25 cm in shell length. The mean shell growth per month was 0.2 cm (Table 1 and Fig. 6). It took 18 months for *B.c. costula* to become reproductively mature with the size of 3.82 cm in shell length.

For *T. granifera*, the newly emerged young snails were 0.2 cm in shell length. The mean shell growth per month was 0.14 cm (Table 2 and Fig. 6). After 12 months, the snails

TABLE 1

Growth table for *Brotia costula costula.*

Month	Ave. shell length per week (cm.)	Ave. shell growth per week (cm.)
1	0.29	0.16
2	0.45	0.18
3	0.63	0.28
4	0.91	0.21
5	1.20	0.38
6	1.58	0.03
7	1.61	0.30
8	1.91	0.19
9	2.10	0.16
10	2.26	0.07
11	2.33	0.25
12	2.58	0.19
13	2.77	0.25
14	3.02	0.18
15	3.20	0.22
16	3.42	0.18
17	3.60	0.22
18	3.82	

Mean shell growth per month = 0.20 cm.

TABLE 2

Growth table for *Tarebia granifera*.

Month	Ave. shell length per week (cm.)	Ave. shell growth per week (cm.)
1	0.30	0.31
2	0.61	0.14
3	0.75	0.07
4	0.82	0.14
5	0.96	0.02
6	1.08	0.05
7	1.13	0.28
8	1.41	0.15
9	1.56	0.05
10	1.61	0.09
11	1.70	0.19
12	1.89	

Mean shell growth per month = 0.14 cm.

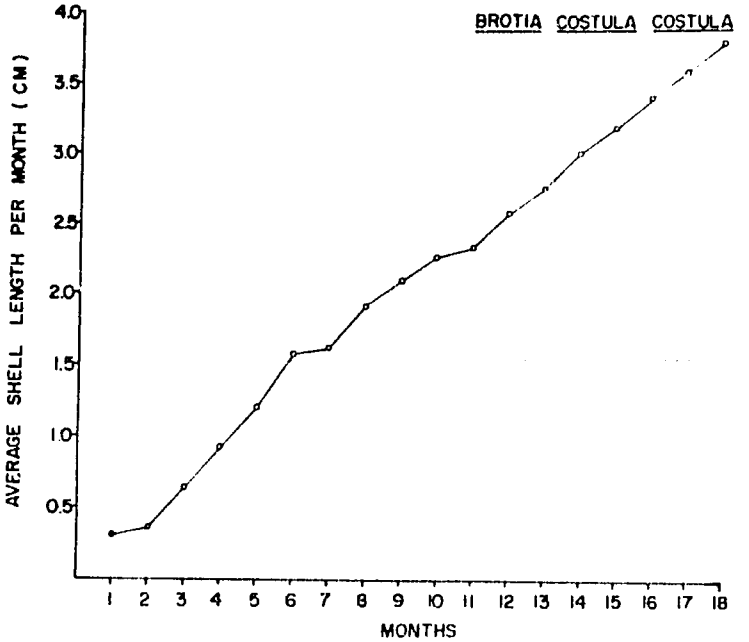


Fig. 5. Growth table for *Brotia costula costula*

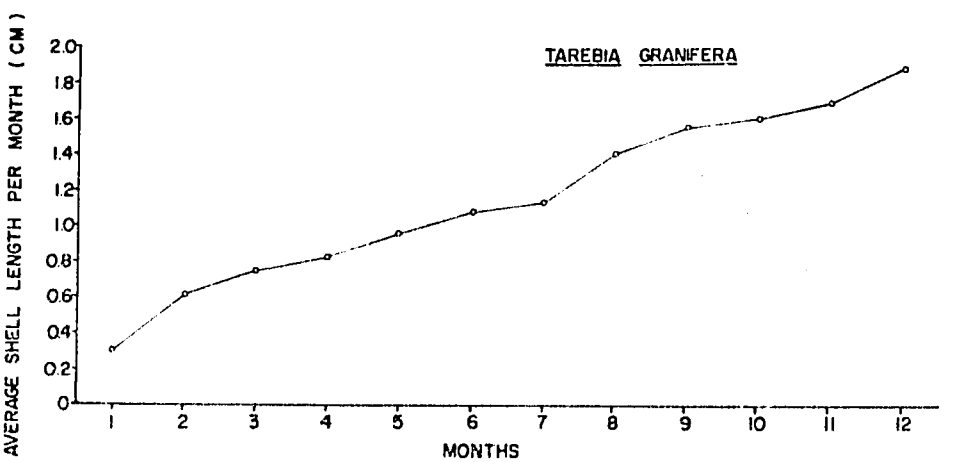


Fig. 6. Growth table for *Tarebia granifera*

had reached maturity at the size of 1.89 cm in shell length and started to lay young snails.

For *M. tuberculata*, the newly emerged young snails were 0.8 mm in shell length, and the full grown snails were 12.8-13 mm long. The mean shell growth per month was 0.5 mm. and it had taken the young snails 25 weeks to become fully mature and start to reproduce (Table 3 and Fig. 7).

For *P. ampullacea* which are oviparous (bear eggs), the white egg masses were laid on rocks or attached to the inner side of the aquaria. The eggs hatched and the young snails emerged in 12-14 days. The mean shell growth per month was 0.28 cm, and the full grown snails in shell length, after 16 months, were 4.58 cm (Table 4 and Fig.8).

1.4 Experimental work on biological control of mollusks in the laboratory

Various numbers of mollusk biological agents and target species were tested to determine the efficacy of the agents in terms of competition, predation or suppression of reproductive potential of the target species.

The four species of snail biological control agents were *Brotia costula costula*, *Tarebia (Thiara) granifera*, *Melanoides tuberculata*, and *Pila ampullacea*

Snails which were used as target species were as follows:

TABLE 3

Growth table for *Melanoides tuberculata*.

Week	Ave. shell length per week (mm.)	Ave. shell growth per week (mm.)
1	0.8	
2	1.2	0.4
3	1.5	0.3
4	1.8	0.3
5	2.2	0.4
6	2.5	0.3
7	3.0	0.5
8	3.6	0.6
9	4.0	0.4
10	4.5	0.5
11	5.1	0.6
12	5.9	0.8
13	6.5	0.6
14	7.0	0.5
15	7.5	0.5
16	8.0	0.5
17	8.5	0.6
18	9.0	0.5
19	9.5	0.5
20	10.0	0.4
21	10.3	0.4
22	11.0	0.7
23	11.5	0.6
24	12.0	0.5
25	12.8	0.8

Mean shell growth per week = 0.5 mm.

TABLE 4

Growth table for *Pila ampullacea*.

Month	Ave. shell length per month (cm.)	Ave. shell growth per week (cm.)
1	0.42	0.33
2	0.75	0.36
3	1.11	0.31
4	1.42	0.44
5	1.86	0.45
6	2.31	0.73
7	3.04	0.30
8	3.34	0.14
9	3.48	0.34
10	3.82	0.34
11	4.16	0.23
12	4.39	0.02
13	4.41	0.08
14	4.49	0.05
15	4.54	0.04
16	4.58	

Mean shell growth per month = 0.28 cm.

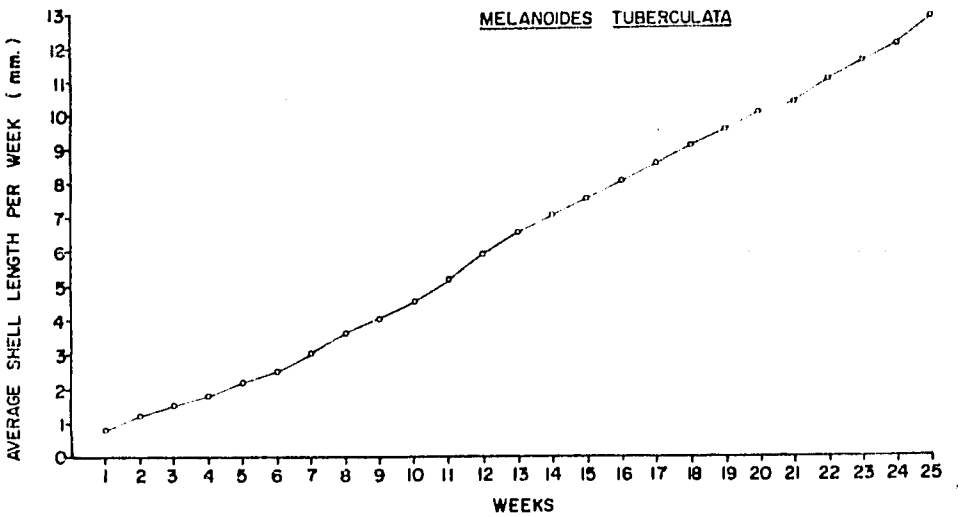


Fig. 7. Growth table for *Melanoides tuberculata*

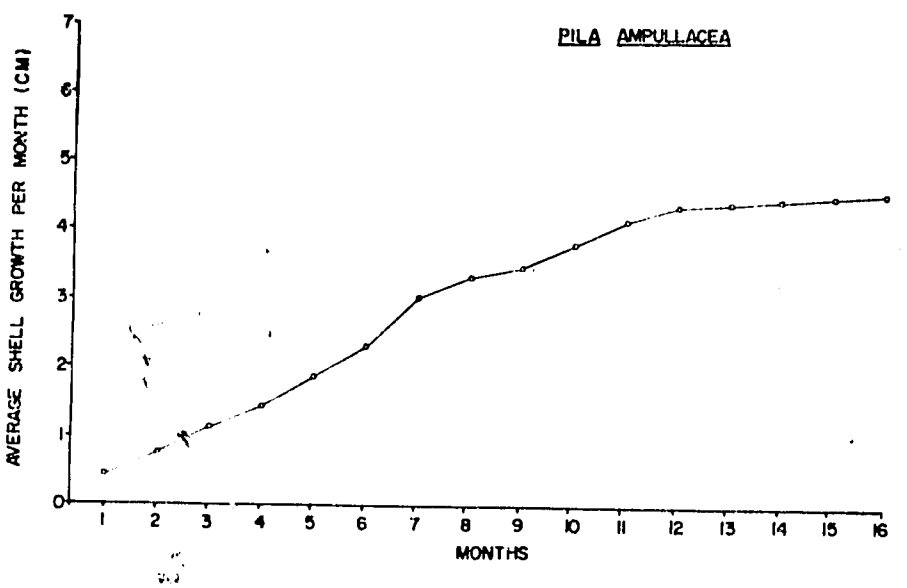


Fig. 8. Growth table for *Pila ampullacea*

(a) *Tricula aperta* - intermediate host of *Schistosoma mekongi* in Mekong delta (Fig. 9).

(b) *Robertsiella kaporensis* - intermediate host of *S. japonicum* in Malaysia (Fig. 10).

(c) *Oncomelania hupensis quadrasi* - intermediate host of *S. japonicum* in the Philippines (Fig. 11).

(d) *Biomphalaria glabrata* - intermediate host of *S. mansoni* in Puerto Rico (Fig. 12).

(e) *Bithynia siamensis goniomphalos* - first intermediate host of *Opisthorchis viverrini* in Thailand (Fig. 13).

(f) *Radix rubiginosa* - intermediate host of *S. incognitum* (cause dermatitis in man) (Fig. 14).

(g) *Indoplanorbis exustus* - intermediate host of *S. spindale* (cause dermatitis in man) (Fig. 15).

These target snail species have been successfully cultured in the Center's laboratory for 6 years.

The 24-cm diameter glass aquaria were used with the water depths of 15 cm. (Figs. 16,17). The ratio between biological agent and target species was as follows:

Control agent	Target species
0	40
10	30
20	20
30	10
40	0

Control agents were *T. granifera* and *M. tuberculata*

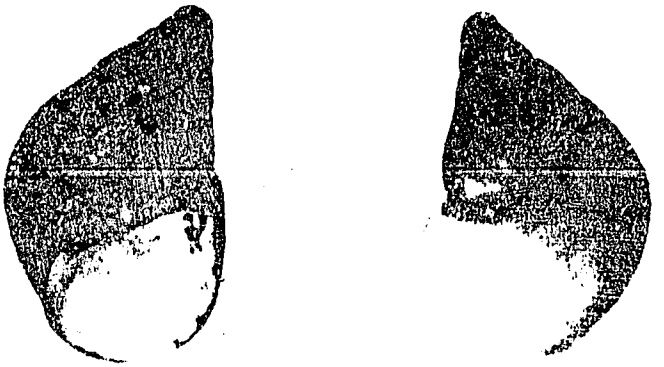


Fig. 9. *Tricula aperta* (beta race) (x16)

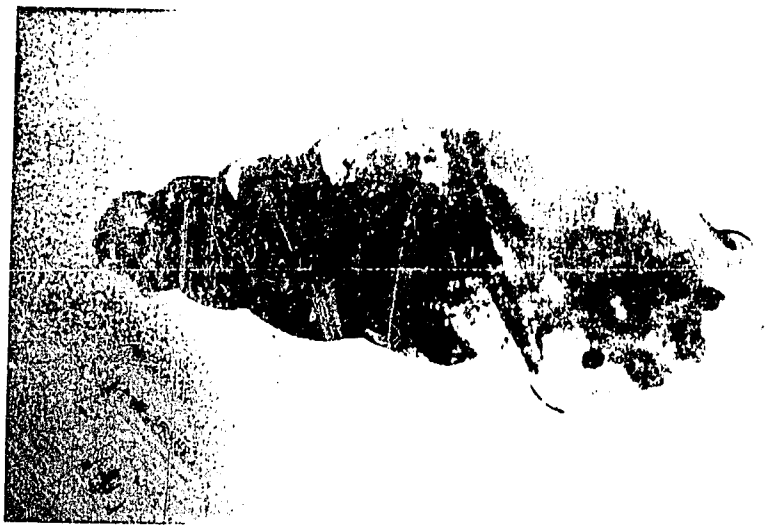


Fig. 10. *Robertsiella kaporensis* (x32)

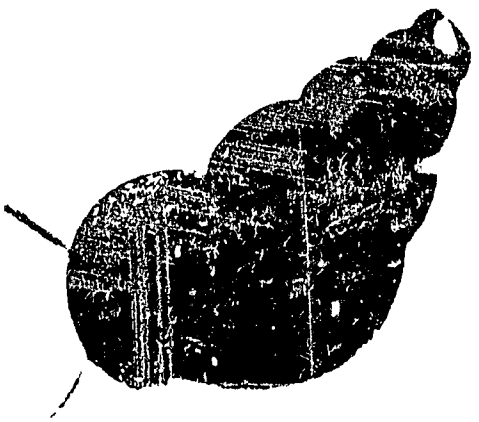


Fig.11. *Oncomelania hupensis quadrasi* (x17)

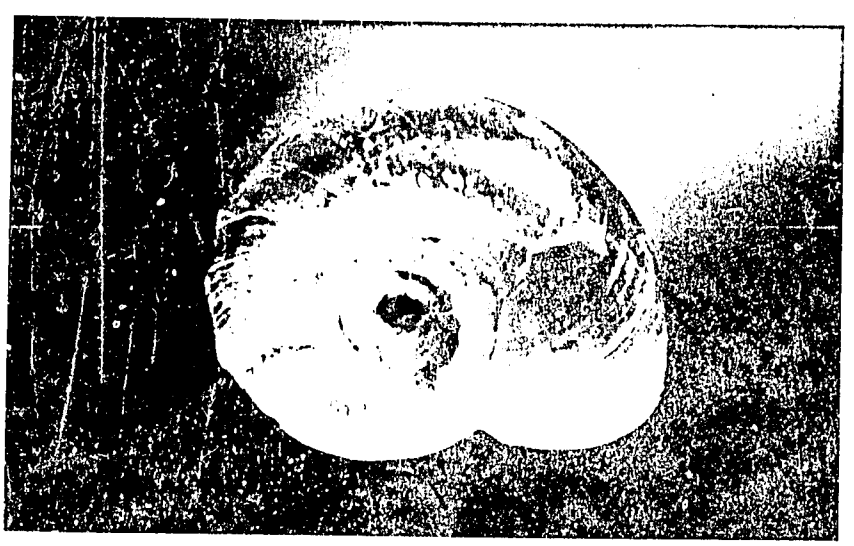


Fig. 12. *Biomphalaria glabrata* (x4.5)

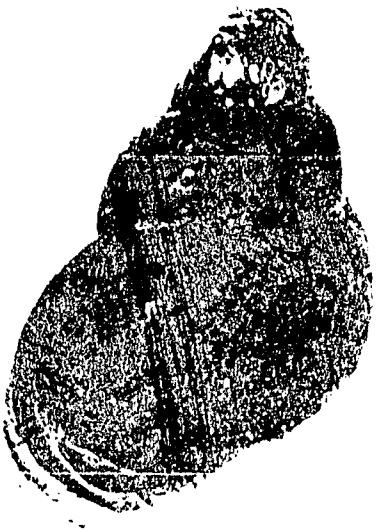


Fig 13. *Bithynia siamensis goniomphalos* (x12)

Fig. 14. *Radix rubiginosa* (x8)

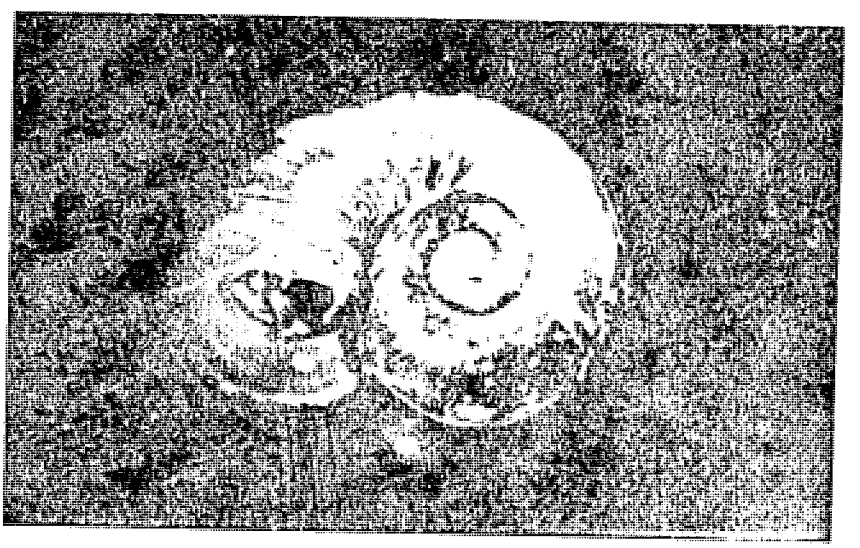


Fig. 15. *Indoplanorbis exustus* (x7)

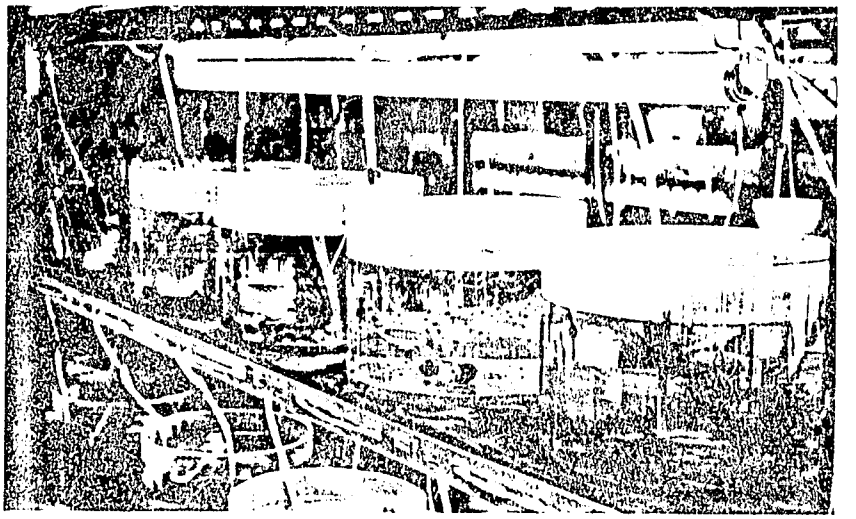


Fig. 16. The glass aquaria for testing biological control effects of target snails in the laboratory.

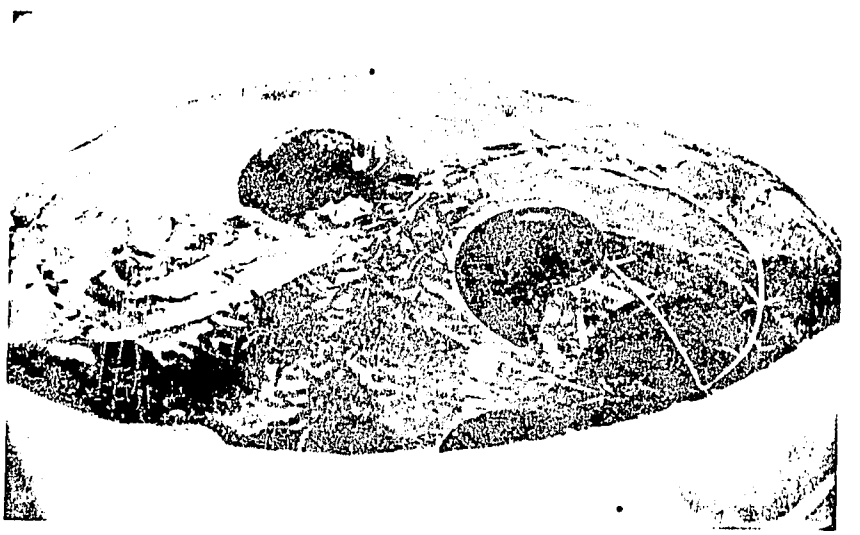


Fig. 17. The high magnification picture showing control agent, *Pila ampullacea* in the glass aquarium.

For *B.c. costula* and *P. ampullacea* being control agents, the following ratio had been used.

Control agents	Target species
0	20
5	15
10	10
15	5
20	0

The general procedure, observation on mortality rates of both control and target species was done daily. The original snails were marked with finger nail polish so that the offspring could be distinguished from them. The number and shell growth of offspring were counted and measured once every two weeks.

The following lists of control and target species of snails on the biological control study were completed in the laboratory.

Control agent	Target species
B.c.costula	*T. aperta*
	R. Kaporensis
	O.h. quadrasi
T. granifera	*B. glabrata*
	B.s. goniomphalus
M. tuberculata	*R. rubiginosa*
	I. exustus

P. ampullacea *O.h. quadrasi*

B. glabrata

1.4.1 *Brotia costula costula*

For the biological control experiments with *B.c. costula* as the control agent, we used young *B.c. costula* of 3-4 cm in size (the adult *B.c. costula* was 6-7 cm) due to the very small size of the target species. Thus, there would be no reproductivity of *B.c. costula* during the whole experimental period.

The biological control studies between *B.c. costula* and *T. aperta* (Table 5, Fig. 18), *R. kaporensis* (Table 6, Fig. 19), and *O.h. quadrasi* (Table 7, Fig 20) revealed very similar results. There seemed to be no interference between the control and target species. This may be due to the differences in habitats of the control and target species. Although *B.c. costula*, the control agent, and *T. aperta* and *R. kaporensis* are aquatic and usually found in flowing water, they are very different in microhabitats. *B.c. costula*. in nature, are found embedding themselves in the sand substrate along the river or canal. *T. aperta* are usually attached themselves to the rock surfaces with abundant diatoms. *R. kaporensis* are found attaching to the roots of certain plants along the stream. Under the laboratory conditions, in the glass aquaria, it had been observed that *B.c. costula*, the control agent usually were at the bottom embedding in the sand substrate, while *T. aperta* were on the rock surfaces and

TABLE 5

Biological control study between *Brotia costula costula* (control) and *Tricula aperta* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Ta	Bcc	Ta	Bcc	Ta	Bcc	Ta	Bcc	Ta
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	20	0	15	5	10	10	5	14	0	19
6	20	0	15	5	10	6	5	14	0	18
8	20	0	15	5	10	6	5	14	0	18
10	20	0	15	4	9	4	5	13	0	18
12	20	0	15	4	9	4	5	13	0	18
14	20	0	15	3	9	4	5	12	0	15
16	19	0	14	3	9	3	5	12	0	13

Bcc= *Brotia costula costula*

Ta = *Tricula aperta* (beta race)

Ratio of *B.c. costula* : *T. aperta*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

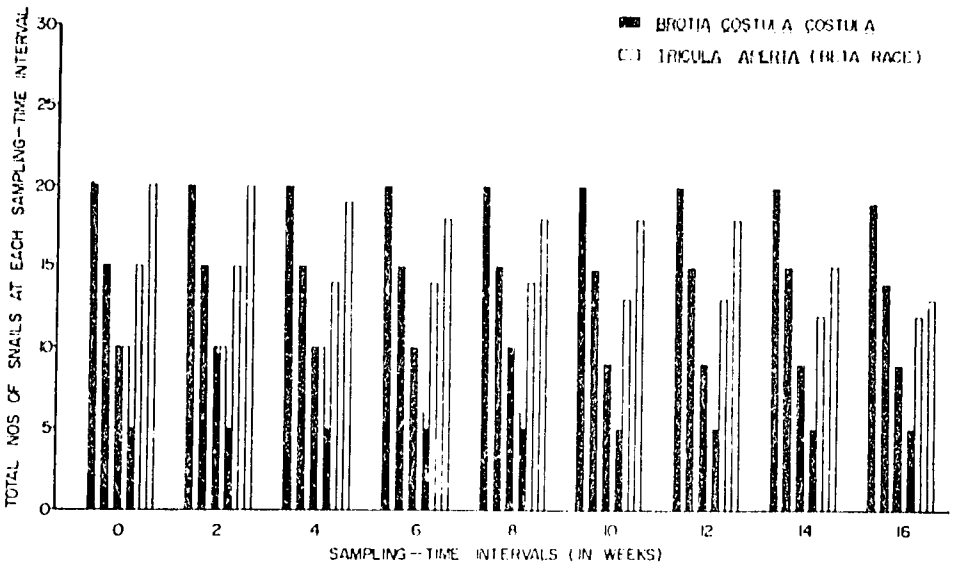


Fig. 18. Biological control study between *B.c. costula* (control) and *T. aperta* (target) under laboratory condition.

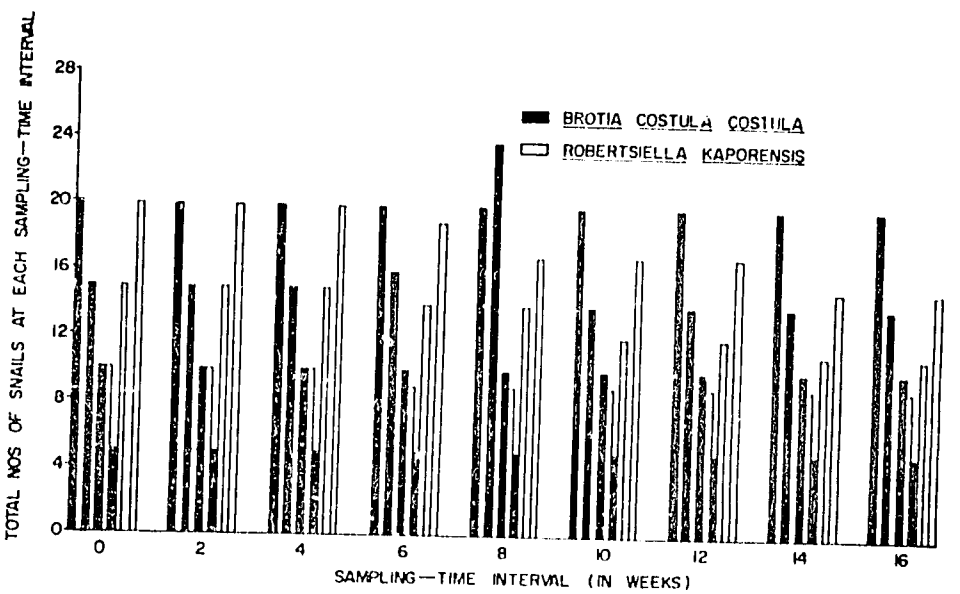


Fig.19. Biological control study between *B.c. costula* (control and *R. kaporensis* (target) under laboratory condition.

TABLE 6

Biological control study between *Brotia costula costula* (control) and *Robertsiella kaporensis* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Rk	Bcc	Rk	Bcc	Rk	Bcc	Rk	Bcc	Rk
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	20	0	15	5	10	10	5	15	0	20
6	20	0	16	5	10	9	5	14	0	19
8	20	0	24	4	10	9	5	14	0	17
10	20	0	14	4	10	9	5	12	0	17
12	20	0	14	4	10	9	5	12	0	17
14	20	0	14	4	10	9	5	11	0	15
16	20	0	14	4	10	9	5	11	0	15

Bcc = *Brotia costula costula*

Rk = *Robertsiella kaporensis*

Ratio of *B.c. costula* : *R. kaporensis*

1 = 20 : 0

2 = 15 : 5

3 = 10 : 10

4 = 5 : 15

5 = 0 : 20

TABLR 7

Biological control study between *Brotia costula costula* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Oq	Bcc	Oq	Bcc	Oq	Bcc	Oq	Bcc	Oq
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	20	0	15	5	10	10	5	15	0	20
6	20	0	15	5	10	10	5	15	0	20
8	20	0	15	5	10	10	5	15	0	20
10	20	0	15	4	10	10	5	15	0	20
12	20	0	14	3	10	9	5	15	0	20
14	20	0	14	3	10	9	5	15	0	20
16	20	0	14	3	10	9	5	15	0	20

Bcc= *Brotia costula costula*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *B.c. costula* : *O.h. quadrasi*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

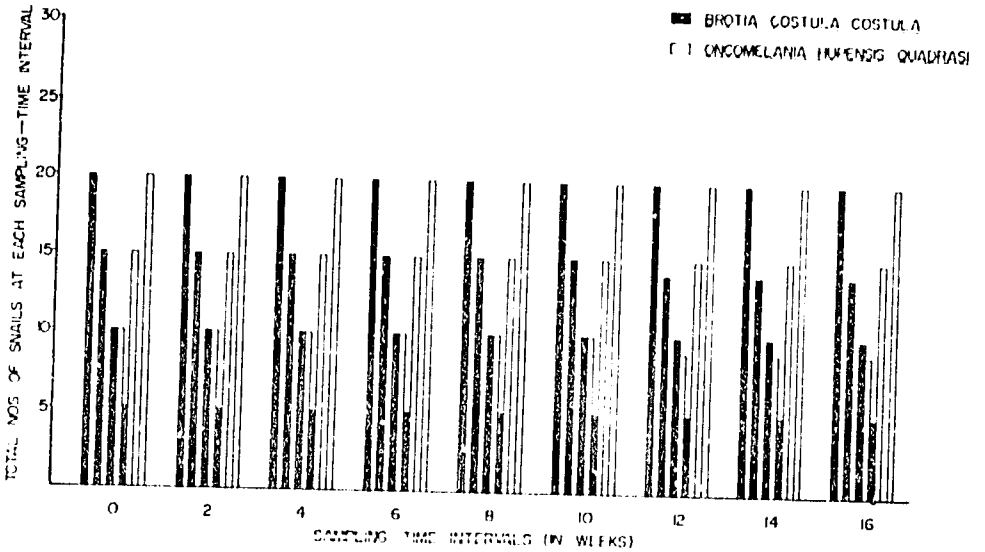


Fig. 20. Biological control study between *B.c. costula* (control) and *O.h. quadrasi* (target) under laboratory condition.

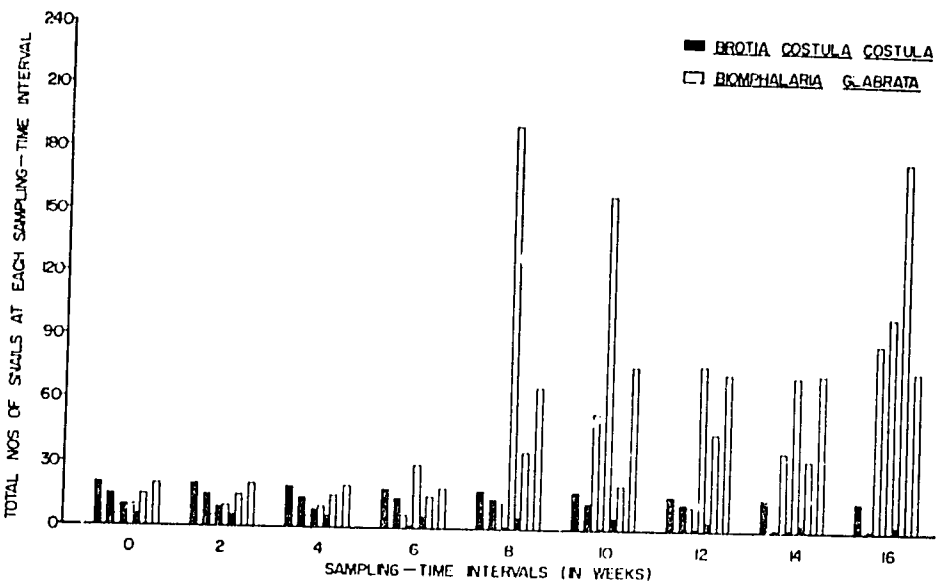


Fig. 21. Biological control study between *B.c. costula* (control) and *B. glabrata* (target) under laboratory condition.

R. kaporensis were attached to leaves or on the sides of the aquaria. As for *O.h. quadrasi*, they live in entirely different habitats from *B.c. costula*, because they are amphibious in nature. *O.h. quadrasi* were found to attach themselves to the damp filter paper which lined the sides of the aquaria.

The biological control study between *B.c. costula* and *B. glabrata* (Pulmonata : Planorbidae) revealed the following results (Table 8, Fig. 21). During the first six weeks, no sign of competition was observed between the control and target species except that the control species, *B.c. costula* started to decrease in number. At the same time, the target species, *B. glabrata* started to lay large numbers of egg masses. Between 6-8 weeks, the eggs were hatched and the young snails started to increase in large numbers. Within 16 months, the number of the target in each container was larger than that of the control species.

The biological control study between *B.c. costula* and *B.s. goniomphalos* (Table 9, Fig. 22) showed that there appeared to be no competition between the control and target species. However, only the container of *B.s. goniomphalos* alone produced few offsprings, while those with *B.c. costula* did not produce any offspring.

The biological control studies between *B.c. costula*, being the control agent, and *R. rubiginosa* (Table 10, Fig. 23) being the target species, had revealed that *R. rubiginosa*

TABLE 8

Biological control study between *Brotia costula costula* (control) and *Biomphalaria glabrata* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Bg	Bcc	Bg	Bcc	Bg	Bcc	Bg	Bcc	Bg
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	9	10	5	15	0	20
4	19	0	14	5	8	10	5	15	0	19
6	18	0	14	6	1	29	5	15	0	19
8	17	0	14	12	1	192	5	39	0	67
10	17	0	12	55	1	159	5	21	0	77
12	16	0	12	11	1	78	5	46	0	74
14	15	0	1	39	1	73	3	34	0	74
16	14	0	1	89	0	102	3	177	0	76

Bcc= *Brotia costula costula*

Bg = *Biomphalaria glabrata*

Ratio of *B.c. costula : B. glabrata*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 9

Biological control study between *Brotia costula costula* (control) and *Bithynia siamensis goniomphalos*(target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg
0	20	0	15	5	10	10	5	15	0	20
2	20	0	14	5	10	10	5	15	0	20
4	20	0	13	5	10	10	5	15	0	20
6	20	0	13	5	10	10	5	15	0	20
8	20	0	13	4	9	9	5	14	0	17
10	20	0	13	4	8	7	5	14	0	23
12	20	0	12	4	7	7	5	14	0	23
14	20	0	12	4	7	7	5	14	0	27
16	20	0	11	4	7	5	5	14	0	25

Bcc= *Brotia costula costula*

Bsg= *Bithynia siamensis goniomphalos*

Ratio of *B.c. costula* : *B.s. goniomphalos*

1 = 20 : 0

2 = 15 : 5

3 = 10 : 10

4 = 5 : 15

5 = 0 : 20

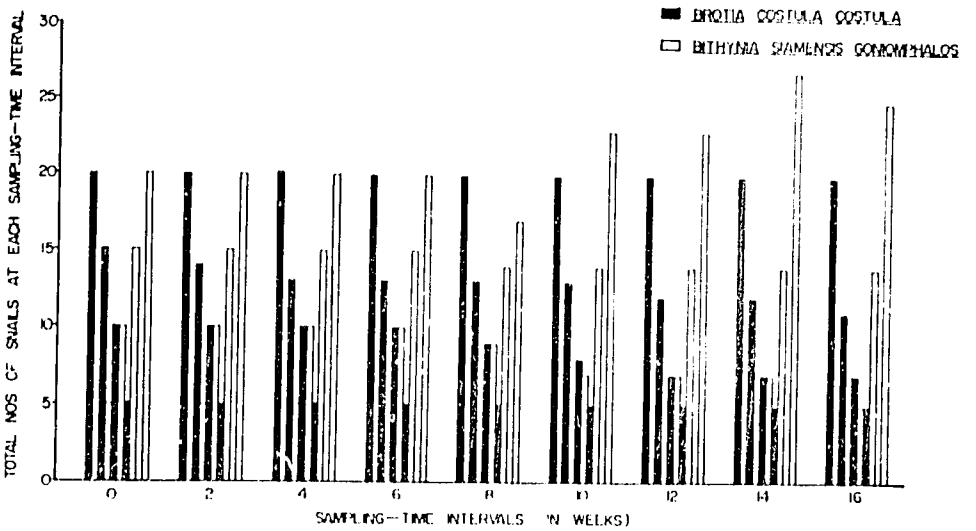


Fig. 22. Biological control study between *B.c. costula* (control) and *B.s. goniomphalos* (target) under laboratory condition.

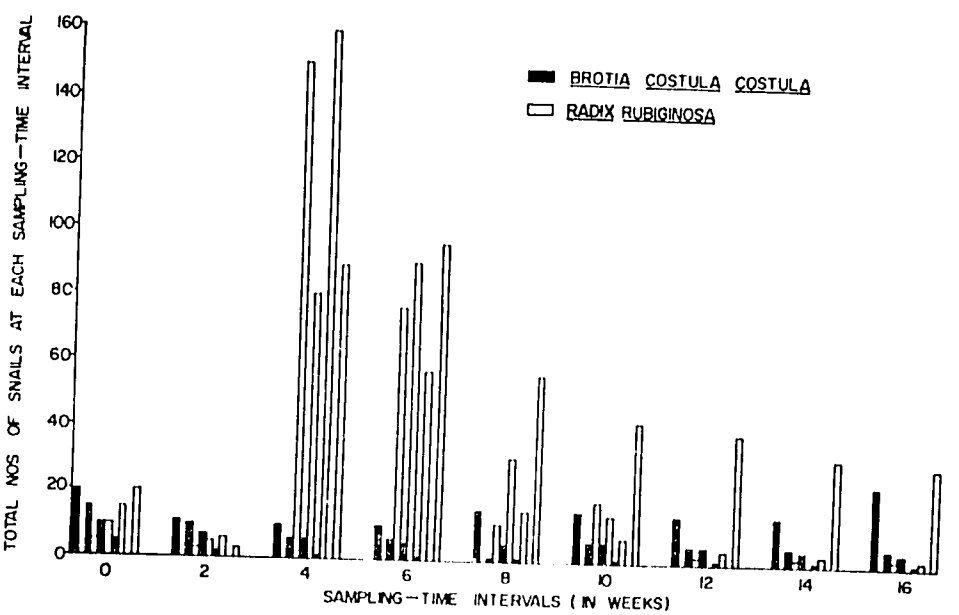


Fig. 23. Biological control study between *B.c. costula* (control) and *R. rubiginosa* (target) under laboratory condition.

TABLE 10

Biological control study between *Brotia costula costula* (control) and *Radix rubiginosa* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Rr	Bcc	Rr	Bcc	Rr	Bcc	Rr	Bcc	Rr
0	20	0	15	5	10	10	5	15	0	20
2	18	0	10	3	7	5	2	6	0	3
4	18	0	6	150	6	80	1	160	0	90
6	17	0	6	76	5	90	1	57	0	96
8	15	0	1	11	5	31	1	15	0	56
10	15	0	6	18	5	14	1	7	0	42
12	14	0	5	2	5	0	1	4	0	39
14	14	0	5	2	4	0	1	3	0	32
16	24	0	5	2	4	0	1	2	0	30

Bcc = *Brotia costula costula*

Rr = *adix rubiginosa*

Ratio of *B.c. costula* : *R. rubiginosa*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

living together with the control agent, *B. c. costula* or alone behaved in similar fashion, i.e. the adult snails would start to lay large number of eggs and then died off. The offspring of *R. rubiginosa* would grow to a certain size, then started to die off after 6 weeks. At the end of 16 weeks, there were only a few number of *R. rubiginosa remained.*

The biological control study between *B.c. costula* and *I. exustus* revealed very similar result as that between *B.c. costula* and *B. glabrata* (Table 11, Fig. 24), except that the numbers of offspring produced by *I. exustus* were fewer than those produced by *B. glabrata.*

It is apparent that we could not draw any conclusive results from these experiments since we used snails too young to reproduce. However, *B.c. costula* in nature or raised in cement tanks with sand substrate could produce several young daily. These experiments were repeated using the field simulated conditions. Larger number and full-grown snails were used and the experimental period was much longer than that in the laboratory.

From the results, it is apparent that the pulmonate snails (*I. exustus, B. glabrata*) are more difficult to control than the prosobranch snails. This is due to the hermaphroditic nature of pulmonates, their ability to lay large numbers of eggs daily and shorter period of time to become mature. *B.c. costula*, even though could produce large numbers of young, requires 1-2 years to become fully mature

TABLE II

Biological control study between *Brotia costula costula* (control) and *Indoplanorbis exustus* (target).

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Ie	Bcc	Ie	Bcc	Ie	Bcc	Ie	Bcc	Ie
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	4	10	9	5	14	0	19
4	20	0	15	10	9	11	5	15	0	55
6	20	0	15	2	9	10	5	11	0	19
8	20	0	15	9	7	7	4	14	0	25
10	20	0	15	9	7	7	4	39	0	57
12	20	0	13	11	5	8	3	23	0	26
14	20	0	13	11	5	8	3	23	0	26
16	20	0	11	34	4	8	3	6	0	50

Bcc= *Brotia costula costula*

Ie = *Indoplanorbis exustus*

Ratio of *B.c. costula : I. exustus*

1 = 20 : 0

2 = 15 : 5

3 = 10 : 10

4 = 5 : 15

5 = 0 : 20

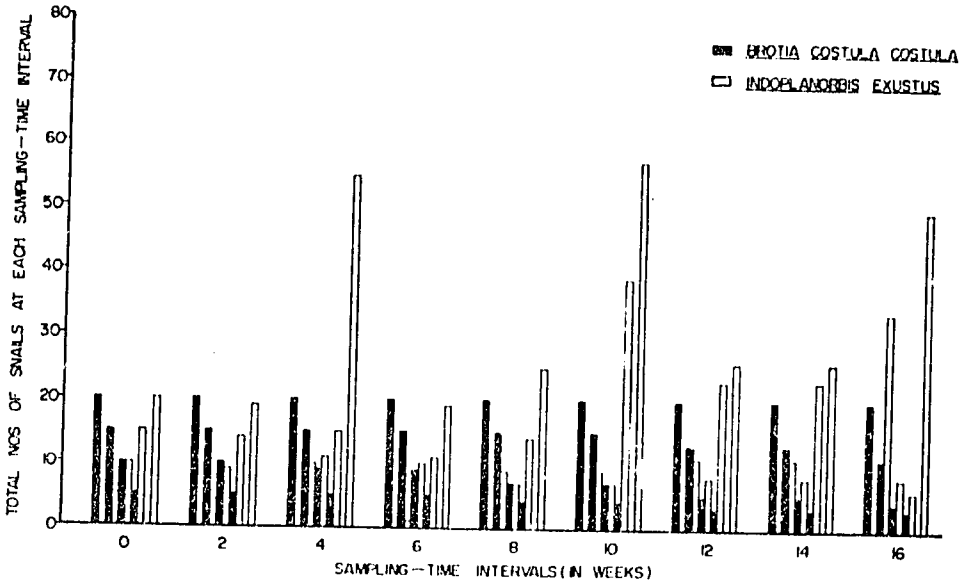


Fig. 24. Biological control study between *B.c. costula* (control) and *I. exustus* (target) under laboratory condition

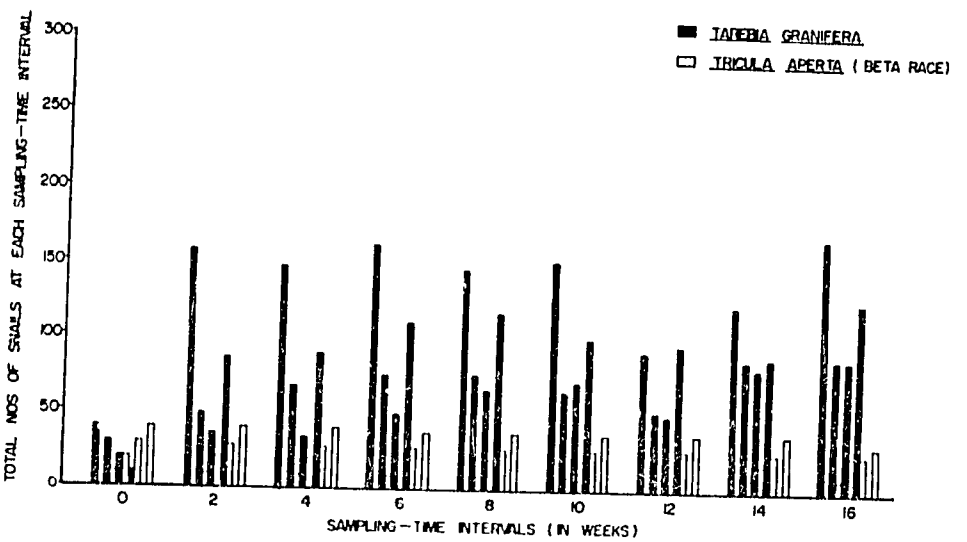


Fig. 25. Biological control study between *T. granifera* (control) and *T. aperta* (target) under laboratory condition.

due to the very large size of the snails. In addition, there is a difference in the habitats of the control and target snails. The pulmonate snails float on the surface of water while *B.c. costula* remain at the bottom of the container most of the time.

1.4.2 *Tarebia granifera*

The biological control study between *T. granifera,* being the control agent, and *T. aperta,* being the target species revealed the following results (Table 12, Fig. 25). The number of *T. granifera* has remarkable increased upto the sixth week, then decreased and remained contant until the end of the experiment. The target species, *T. aperta* did not have any eggs. Within 16 weeks, there was larger number of control species, *T. granifera* over the target species, *T. aperta.*

The biological control study between *T. granifera,* being the control agent, and *R. kaporensis*, being the target species revealed very similar results to those with *T. aperta* (Table 13, Fig. 26) in the aforementioned paragraph. The number of *T. granifera* fluctuated during the whole experiment, but still remained in much larger number over the target species, *R. kaporensis*, at the end of the experiment.

The biological control study between *T. granifera,* being the control agent, and *O.h. quadrasi*, being the target species, showed that, during the first 6 weeks, *T. granifera* increased remarkably in number due to the ovoviviparity of

TABLE 12

Biological control study between *Tarebia granifera* (control) and *Tricula aperta* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Ta	Tg	Ta	Tg	Ta	Tg	Ta	Tg	Ta
0	40	0	30	10	20	20	10	30	0	40
2	158	0	49	9	36	20	86	28	0	40
4	147	0	67	9	34	20	89	27	0	40
6	162	0	75	9	49	20	109	27	0	37
8	146	0	76	9	65	20	117	27	0	37
10	152	0	65	9	71	20	100	27	0	37
12	92	0	52	9	49	18	96	27	0	37
14	123	0	86	9	81	18	88	25	0	37
16	168	0	88	9	87	15	126	25	0	31

Tg = *Tarebia granifera*

Ta = *Tricula aperta* (beta race)

Raito of *T. granifera* : *T. aperta*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 13

Biological control study between *Tarebia granifera* (control) and *Robertsiella kaporensis* (target)

Week	Number of snails									
	1		2		3		4		5	
	Tg	Rk	Tg	Rk	Tg	Rk	Tg	Rk	Tg	Rk
0	40	0	30	10	20	20	10	30	0	40
2	95	0	61	9	37	20	20	29	0	40
4	135	0	75	9	43	20	26	22	0	40
6	130	0	78	9	26	20	34	22	0	40
8	98	0	49	9	23	11	50	17	0	37
10	82	0	40	9	20	11	52	17	0	37
12	76	0	32	9	20	10	48	17	0	37
14	84	0	23	3	22	10	42	13	0	37
16	81	0	27	3	20	9	39	11	0	30

Tg = *Tarebia granifera*

Rk = *Robertsiella kaporensis*

Ratio of *T. granifera* : *R. kaporensis*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

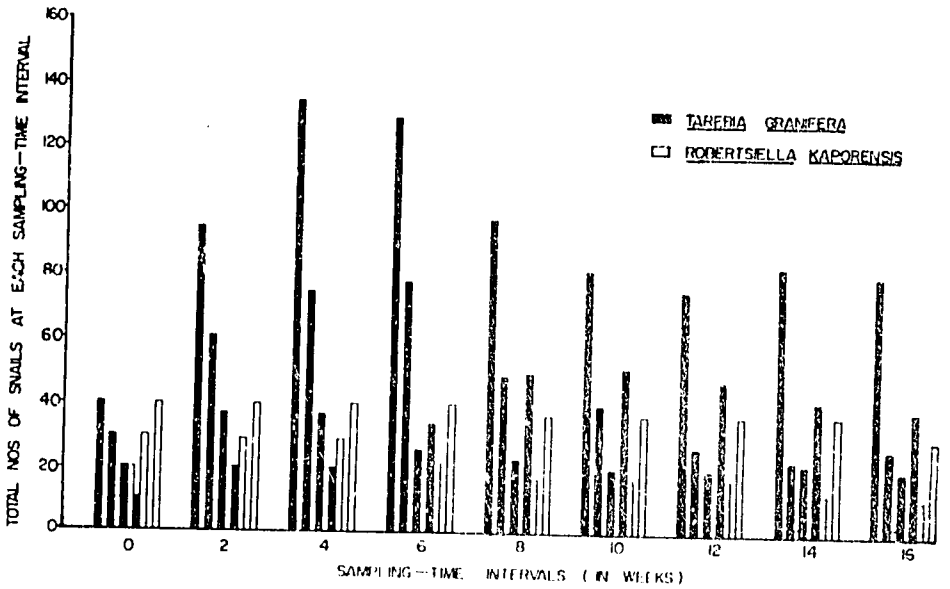


Fig. 26. Biological control study between *T. granifera* (control) and *R. kaporensis* (target) under laboratory condition.

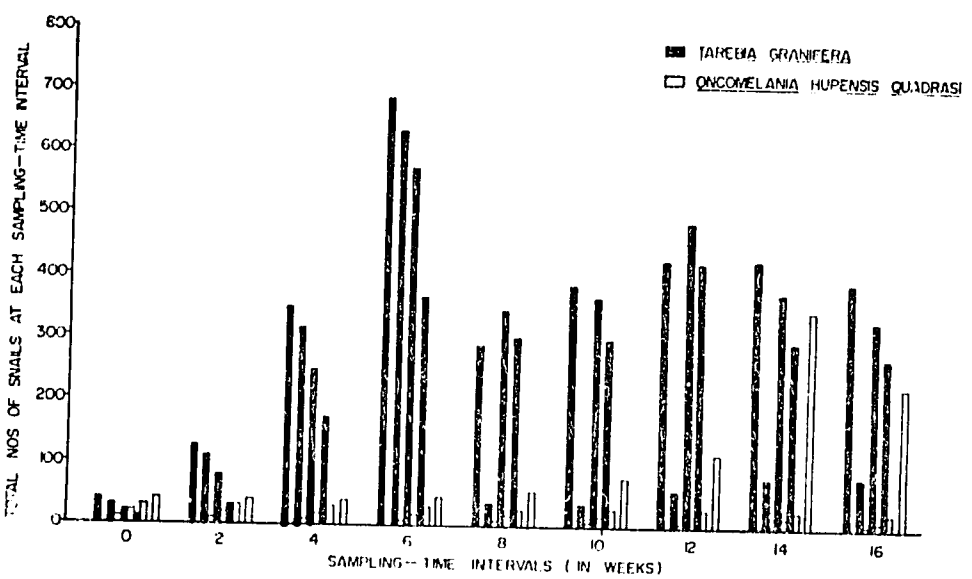


Fig. 27. Biological control study between *T. granifera* (control) and *O.h. quadrasi* (target) under laboratory condition.

the snails (Table 14, Fig. 27). After the sixth week, the number of young *T. granifera* has dropped which may be due to the overcrowding of snails in one container. While the target species *O.h. quadrasi* alone reproduced a large number of offsprings, those living with *T. granifera* did not reproduce and the mortality rates seemed to increase. Within 16 weeks from the beginning of the experiment, there was a larger number of control species, *T. granifera* over the target species, *O.h. quadrasi*.

The biological control studies between *T. granifera*, being the control agent, and *B. glabrata* being the target species, showed that *T. granifera* could not control *B. glabrata* (Table 15 and Fig. 28). *B. glabrata* have been reared in the laboratory for a number of years and they are certainly well adapted in terms of mortality and numbers of offspring. It usually takes *B. glabrata* 3-4 months to become fully mature, while it takes *T. granifera* 12 months to reach maturity.

The biological control study between *T. granifera*, being the control agent and, *B.s. goniomphalos*, being the target species, showed that, during the first six weeks, the number of *T. granifera* increased remarkably, decreased after the sixth week and remained relatively constant until the end of the experiment (Table 16, Fig. 29). Container with *B.s. goniomphalos* alone yielded few young snails while those with *T. granifera* and *B.s. goniomphalos* did not yield any young

TABLE 14

Biological control study between *Tarebia granifera* (control) and *Oncomelania hupensis quadrasi* (target)

Week	Number of snails									
	1		2		3		4		5	
	Tg	Oq	Tg	Oq	Tg	Oq	Tg	Oq	Tg	Oq
0	40	0	30	10	20	20	10	30	0	40
2	127	0	109	9	73	18	28	30	0	40
4	351	0	314	9	248	17	172	30	0	38
6	689	0	634	8	573	17	364	28	0	44
8	287	0	34	7	369	17	301	26	0	55
10	388	0	34	6	369	15	301	26	0	77
12	430	0	57	5	489	15	424	26	0	117
14	430	0	77	5	379	15	297	25	0	350
16	394	0	79	5	329	10	270	23	0	224

Tg = *Tarebia granifera*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *T. granifera* : *O.h. quadrasi*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 15

Biological control study between *Tarebia granifera* (control) and *Biomphalaria glabrata* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Bg	Tg	Bg	Tg	Bg	Tg	Bg	Tg	Bg
0	40	0	30	10	20	20	10	30	0	40
2	40	0	30	10	20	20	10	30	0	40
4	90	0	29	40	20	25	2	38	0	52
6	643	0	0	94	0	106	0	82	0	76
8	324	0	0	151	0	306	0	255	0	134
10	77	0	0	70	0	114	0	77	0	108
12	89	0	0	65	0	102	0	86	0	96
14	95	0	0	63	0	96	0	93	0	87
16	102	0	0	58	0	94	0	101	0	75

Tg = *Tarebia granifera*

Bg = *Biomphalaria glabrata*

Ratio of *T. granifera* : *B. glabrata*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 16

Biological control study between *Tarebia granifera* (control) and *Bithynia siamensis goniomphalos* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Bsg	Tg	Bsg	Tg	Bsg	Tg	Bsg	Tg	Bsg
0	40	0	30	10	20	20	10	30	0	40
2	287	0	211	10	117	20	63	30	0	40
4	388	0	286	10	157	20	97	29	0	46
6	430	0	332	10	170	20	144	12	0	47
8	394	0	153	10	184	19	139	11	0	47
10	279	0	97	10	187	19	120	10	0	47
12	357	0	89	10	173	19	125	7	0	47
14	282	0	98	10	166	18	149	7	0	40
16	305	0	108	10	152	18	160	7	0	40

Tg = *Tarebia granifera*

Bsg= *Bithynia siamensis goniomphalos*

Ratio of *T. granifera* : *B.s. goniomphalos*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

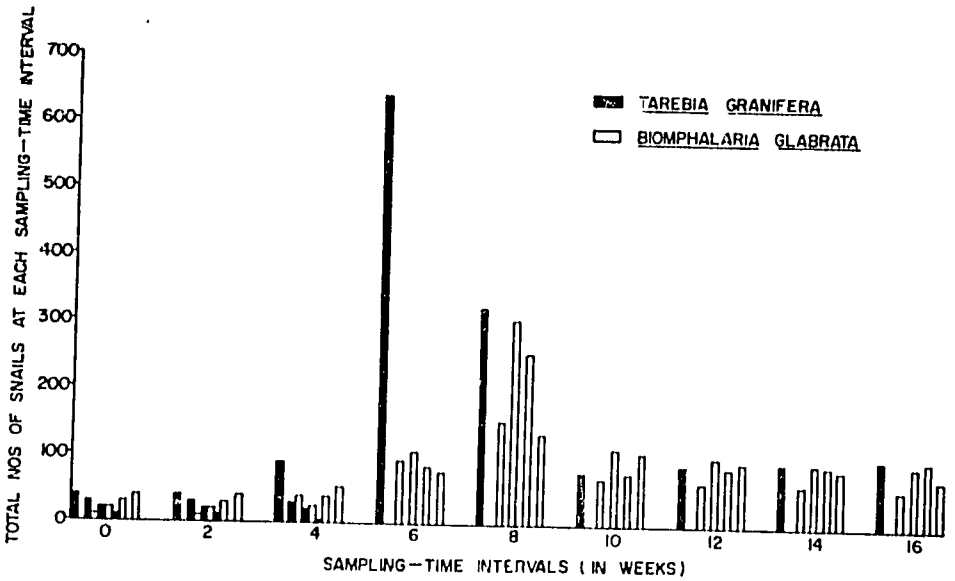


Fig. 28. Biological control study between *T. granifera* (control) and *B. glabrata* (target) under laboratory condition.

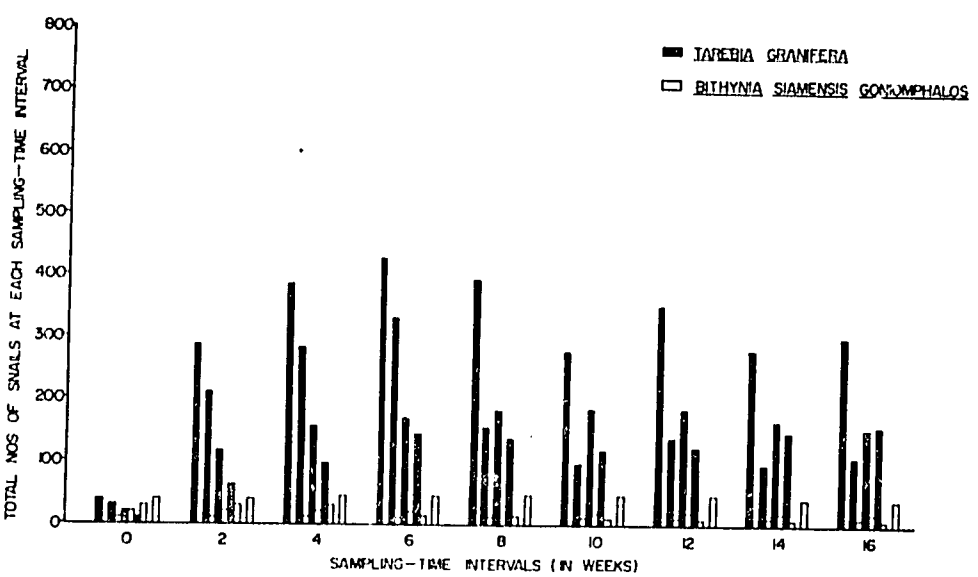


Fig. 29. Biological control study between *T. granifera* (control) and *B.s. goniomphalos* (target) under laboratory condition.

B.s. goniomphalos. Within 16 weeks from the beginning of the experiment, there was a larger number of control species, *T. granifera* over the target species, *B.s. goniomphalos*.

The biological control study between *T. granifera* and *R. rubiginosa* revealed the following results (Table 17, Fig. 30). *R. rubiginosa*, when put into container, they usually started laying large numbers of eggs, and then the adult snails died. The eggs would hatch into young snails until they reached a certain size, then they started to die off. However, *R. rubiginosa* living together with *T. granifera* did not leave any offspring. It could be that *T. granifera* has exerted some control over *R. rubiginosa*, especially on the reproductive ability of the target snails.

The biological control study between *T. granifera*, being the control agent, and *I. exustus*, being the target species, revealed the following results (Table 18, Fig. 31). During the first four weeks, the number of control agent, *T. granifera* increased remarkably. After ten weeks, the number of *T. granifera* dropped considerably and almost died out at the end of the experiment. The number of target speices, *I. exustus* also increased up to the sixth week, then started to decline and remained constant except for the container of 10 *T. granifera* and 30 *I. exustus*, in which *I. exustus* were all dead after ten weeks. There was a larger number of the target species, *I. exustus*, over the control species, *T. granifera*.

TABLE 17

Biological control study between *Tarebia granifera* (control) and *Radix rubiginosa*(target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Rr	Tg	Rr	Tg	Rr	Tg	Rr	Tg	Rr
0	40	0	30	10	20	20	10	30	0	40
2	40	0	30	3	17	14	9	12	0	6
4	90	0	55	0	13	4	6	0	0	180
6	450	0	38	0	20	1	63	0	0	182
8	324	0	81	0	62	1	24	1	0	112
10	77	0	137	0	84	0	31	0	0	100
12	76	0	168	0	95	0	16	0	0	94
14	84	0	97	0	88	0	11	0	0	72
16	81	0	53	0	84	0	15	0	0	70

Tg = *Tar bia granifera*

Rr = *Radix rubiginosa*

Ratio of *T. granifera* : *R. rubiginosa*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 18

Biological control study between *Tarebia granifera* (control) and *Indoplanorbis exustus* (target).

Week	Number of snails									
	1		2		3		4		5	
	Tg	Ie	Tg	Ie	Tg	Ie	Tg	Ie	Tg	Ie
0	40	0	30	10	20	20	10	30	0	40
2	270	0	255	10	151	20	47	30	0	40
4	351	0	253	9	214	20	61	30	0	39
6	309	0	200	64	40	61	29	60	0	66
8	159	0	122	48	55	46	12	20	0	43
10	56	0	47	47	24	22	14	0	0	43
12	52	0	14	48	18	29	16	0	0	43
14	32	0	18	20	6	45	8	0	0	43
16	26	0	24	42	6	43	2	0	0	54

Tg = *Tarebia granifera*

Ie = *Indoplanorbis exustus*

Ratio of *T. granifera* : *I. exustus*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

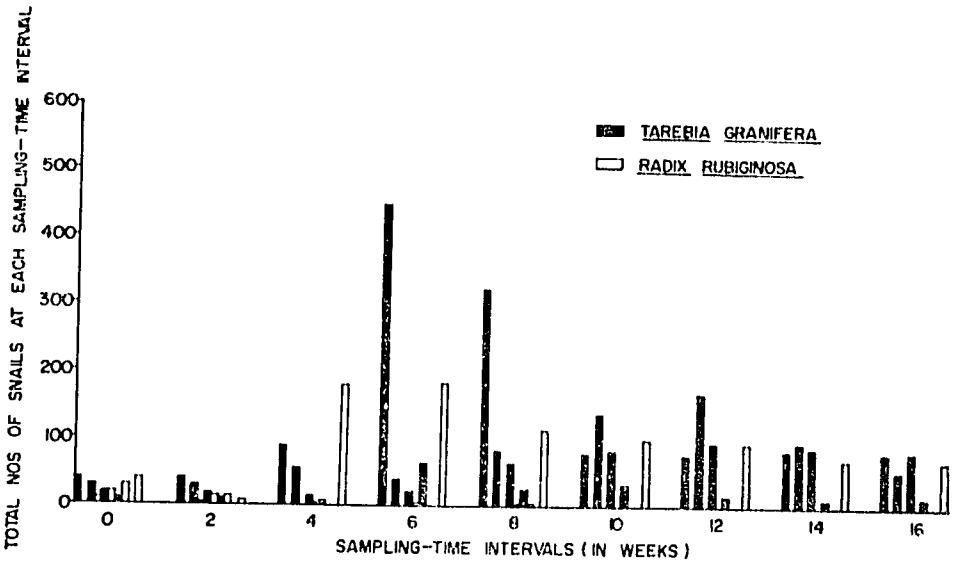


Fig. 30. Biological control study between *T. granifera* (control) and *R. rubiginosa* (target) under laboratory condition.

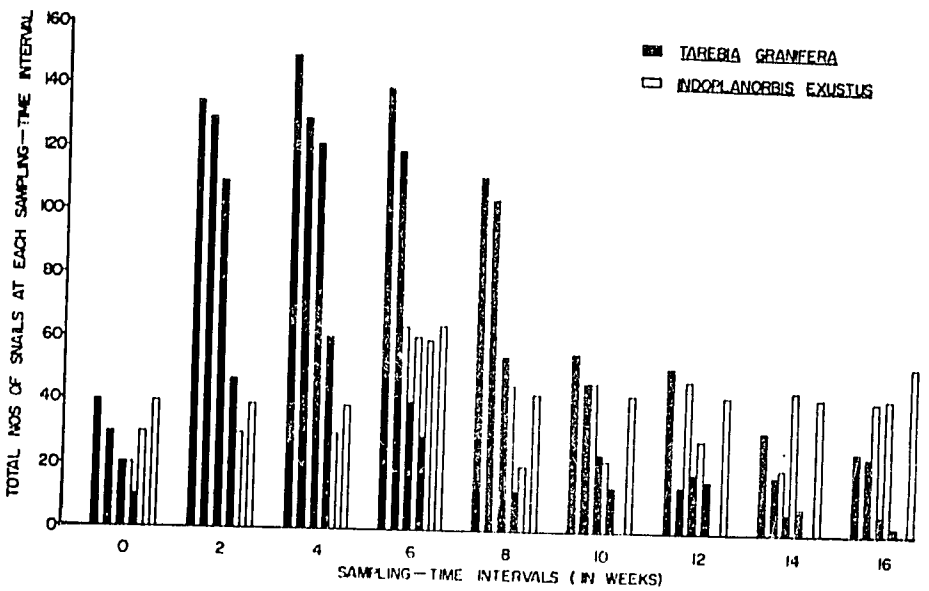


Fig. 31. Biological control study between *T. granifera* (control) and *I. exustus* (target) under laboratory condition.

Based on the results obtained from these experiments, it is apparent that the pulmonate snails are more difficult to control than the prosobranch snails since the pulmonates are hermaphrodite and could lay large numbers of egg masses while the prosobranchs have separate sexes. *T. granifera* is very difficult to colonize in the laboratory. They usually could produce a large number of young for a certain period of time but the young snails have very high mortality rates. This may be due to the limitation of space or overcrowding of snails. It should be noted that in the experiments between *T. granifera* and the target prosobranch snails (*O.h. quadrasi*, *B.s. goniomphalos*), the target species living together with the control did not reproduce any offsprings. There may be a suppression in reproductivity exerted on the target species by the control species, *T. granifera*. This phenomenon was investigated in the field simulated experiments, in which a larger number of snails was used and a longer period of time was required.

1.4.3 *Melanoides tuberculata*

The biological control study between *M. tuberculata* and *T. aperta* revealed the following results (Table 19, Fig. 32). The number of *M. tuberculata* has gradually increased upto the tenth week, then decreased or fluctuated in some containers until the end of the experiment. The number of *T. aperta* was more or less constant during the whole experiment. The number of control species, *M. tuberculata* was larger than that of *T. aperta*

TABLE 19

Biological control study between *Melanoides tuberculata* (control) and *Tricula aperta* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Ta	Mt	Ta	Mt	Ta	Mt	Ta	Mt	Ta
0	40	0	30	10	20	20	10	30	0	40
2	55	0	53	8	37	20	9	27	0	40
4	46	0	51	8	50	20	22	27	0	40
6	44	0	50	8	67	20	24	27	0	38
8	31	0	47	8	71	20	23	27	0	38
10	129	0	63	8	83	19	30	27	0	38
12	116	0	49	8	84	19	33	27	0	37
14	122	0	46	8	104	19	33	24	0	37
16	117	0	44	8	120	19	41	23	0	37

Mt = *Melanoides tuberculata*

Ta = *Tricula aperta* (beta race)

Ratio of *M. tuberculata* : *T. aperta*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

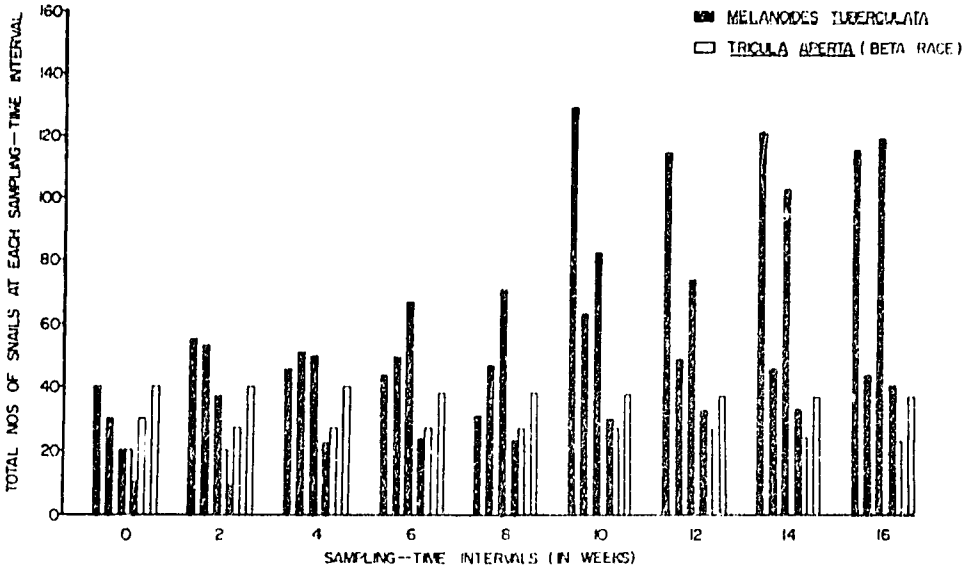


Fig. 32. Biological control study between *M. tuberculata* (control) and *T. aperta* (target) under laboratory condition.

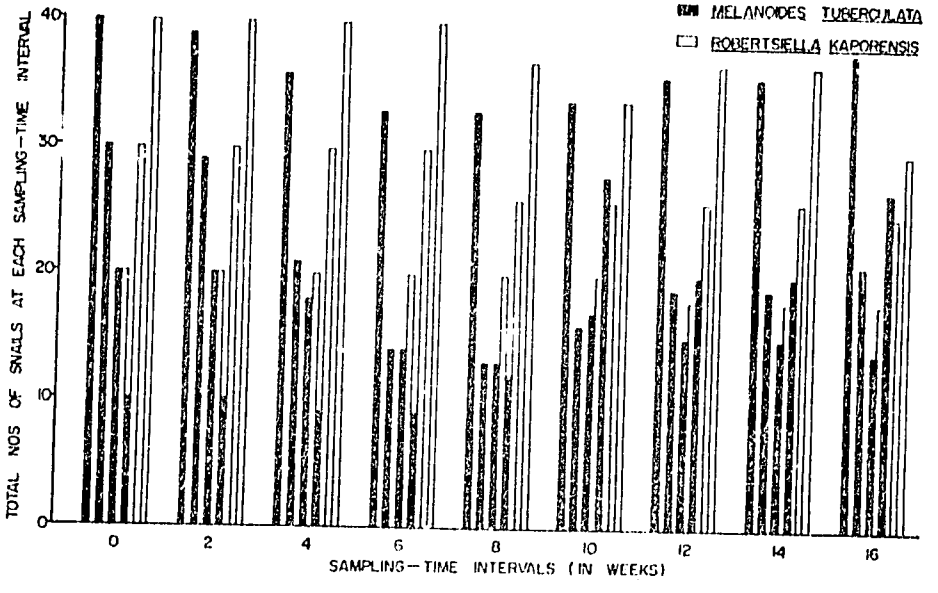


Fig. 33. Biological control study between *M. tuberculata* (control) and *R. kaporensis* (target) under laboratory condition.

after 16 weeks.

The biological control study between *M. tuberculata* and *R. kaporensis* revealed the following results (Table 20, Fig. 33). Neither the control nor target species had produced any offsprings. During the whole experiment, no sign of competition or interaction was observed.

The biological control study between *M. tuberculata*, being the control species and *O.h. quadrasi*, being the target species, revealed the following results (Table 21, Fig. 34). During the first six weeks, there was a great increase in numbers of offsprings of *M. tuberculata*. After that, the number dropped slightly and remained about the same until the end of the experiment. Only the container with *O.h. quadrasi* alone produced offspring while those with *M. tuberculata* did not reproduce. Within 16 weeks, there was a larger number of the control species, *M. tuberculata* over the target species, *O.h. quadrasi*.

The biological control studies between *tuberculata*, being the control agent, and *B. glabrata* (Tal 22, Fig. 35), *B.s. goniomphalos* (Table 23, Fig. 36), *R. rubiginosa* (Table 24, Fig. 37), *I. exustus* (Table 25 and Fig. 38), being the target species could be concluded that *M. tuberculata could not control the planorbid snails, B. glabrata* and *I. exustus*. They apparently had some control over *R. rubiginosa*. For *B.s. goniomphalos*, even though the target snails seemed to be decreased in numbers, but the control container of the target

TABLE 20

Biological control study between *Melanoides tuberculata* (control) and *Robertsiella kaporensis* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Rk	Mt	Rk	Mt	Rk	Mt	Rk	Mt	Rk
0	40	0	30	10	20	20	10	30	0	40
2	39	0	29	10	10	20	10	30	0	40
4	36	0	21	10	18	20	9	30	0	40
6	33	0	14	6	14	20	9	30	0	40
8	33	0	13	6	13	20	12	26	0	37
10	34	0	16	6	17	20	28	26	0	37
12	36	0	19	6	15	18	20	26	0	37
14	36	0	19	6	15	18	20	26	0	37
16	38	0	21	4	14	18	27	25	0	30

Mt = *Melanoides tuberculata*

Rk = *Robertsiella kaporensis*

Ratio of *M. tuberculata: R. kaporensis*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 21

Biological control study between *Melanoides tuberculata* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Oq	Mt	Oq	Mt	Oq	Mt	Oq	Mt	Oq
0	40	0	30	10	20	20	10	30	0	40
2	113	0	82	10	89	20	67	30	0	39
4	186	0	138	10	137	20	100	29	0	42
6	187	0	100	10	115	18	112	29	0	49
8	150	0	105	9	122	15	114	25	0	50
10	142	0	80	8	104	13	111	21	0	43
12	141	0	74	7	108	13	111	18	0	39
14	137	0	130	7	102	12	111	16	0	44
16	133	0	124	7	100	12	148	16	0	40

Mt = *Melanoides tuberculata*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *M. tuberculata* : *O.h. quadrasi*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

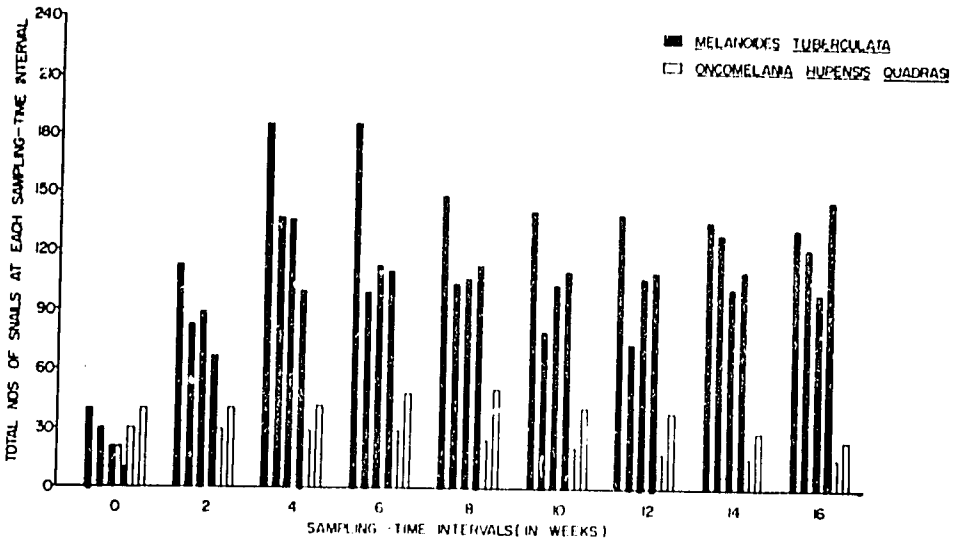


Fig. 34. Biological control study between *M. tuberculata* (control) and *O.h. quadrasi* (target) under laboratory condition.

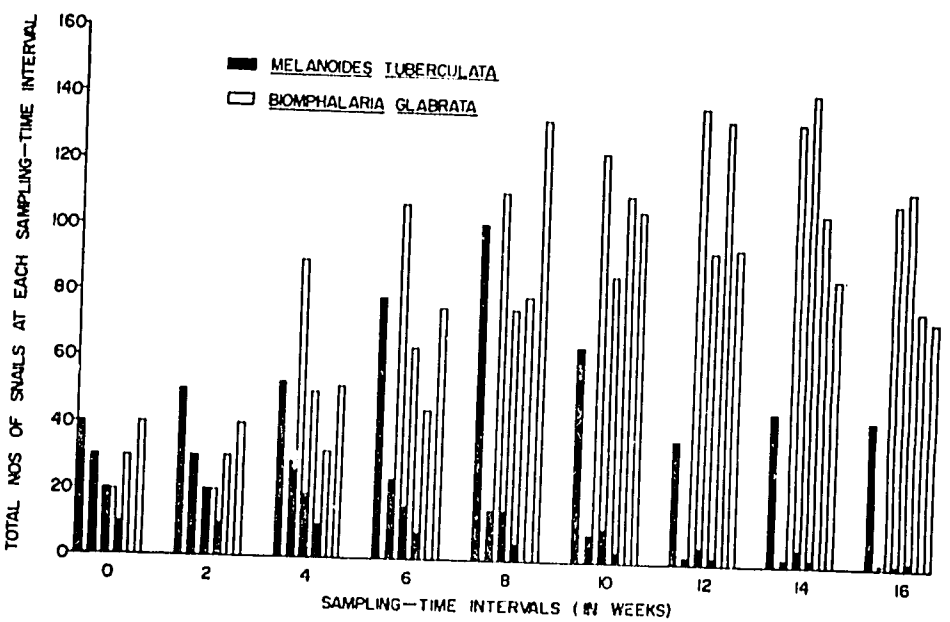


Fig. 35. Biological control study between *M. tuberculata* (control) and *B. glabrata* (target) under laboratory condition.

TABLE 22

Biological control study between *Melanoides tuberculata* (control) and *Biomphalaria glabrata* (target)

Week	Number of snails									
	1		2		3		4		5	
	Mt	Bg	Mt	Bg	Mt	Bg	Mt	Bg	Mt	Bg
0	40	0	30	10	20	20	10	30	0	40
2	50	0	30	10	20	20	10	30	0	40
4	53	0	30	90	19	50	10	32	0	52
6	79	0	24	108	15	64	8	45	0	76
8	102	0	15	112	15	76	5	80	0	134
10	65	0	8	125	10	87	3	112	0	108
12	37	0	2	139	5	95	2	135	0	96
14	46	0	2	135	5	144	2	107	0	87
16	44	0	1	111	1	115	2	78	0	75

Mt = *Melanoides tuberculata*

Bg = *Biomphalaria glabrata*

Ratio of *M. tuberculata* : *B. glabrata*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40.

TABLE 23

Biological control study between *Melanoides tuberculata* (control) and *Bithynia siamensis goniomphalos* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Bsg	Mt	Bsg	Mt	Bsg	Mt	Bsg	Mt	Bsg
0	40	0	30	10	20	20	10	30	0	40
2	42	0	30	10	20	20	10	30	0	40
4	53	0	39	6	23	16	17	22	0	29
6	79	0	63	6	39	11	65	17	0	25
8	102	0	55	5	32	10	60	15	0	22
10	65	0	54	5	36	10	65	15	0	20
12	37	0	53	5	38	10	72	13	0	20
14	46	0	30	4	42	8	37	13	0	11
16	44	0	36	4	52	8	54	13	0	20

Mt = *Melanoides tuberculata*

Bsg = *Bithynia siamensis goniomphalos*

Ratio of *M. tuberculata* : *B.s. goniomphalos*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

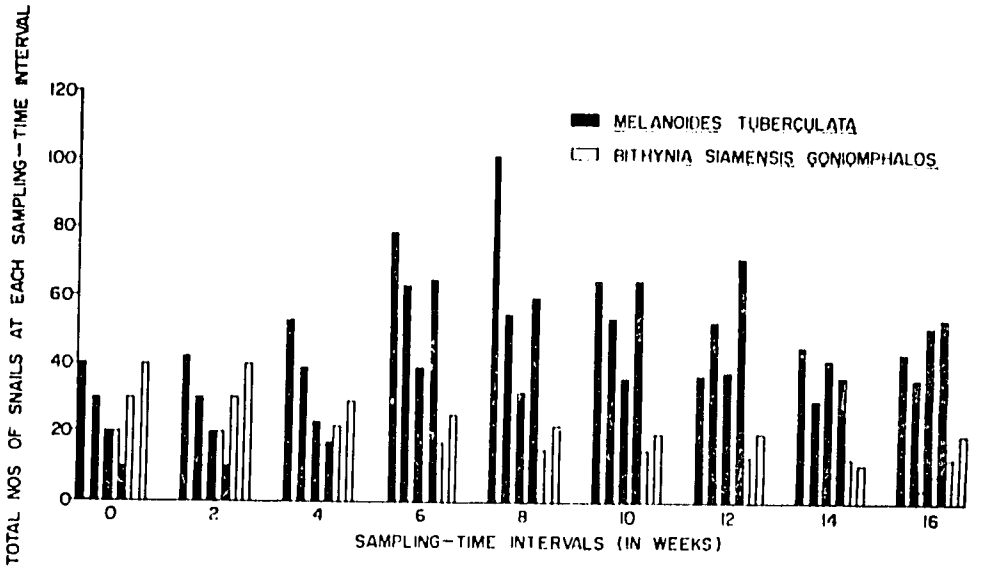


Fig. 36. Biological control study between *M. tuberculata* (control) and *B.s. goniomphalos* (target) under laboratory condition.

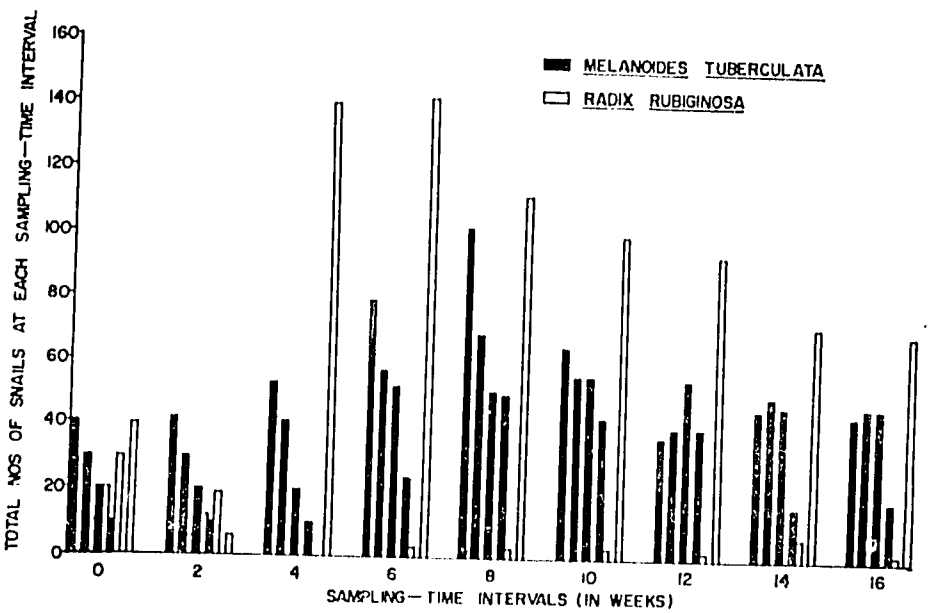


Fig. 37. Biological control study between *M. tuberculata* (control) and *R. rubiginosa* (target) under laboratory condition.

TABLE 24

Biological control study between *Melanoides tuberculata* (control) and *Radix rubiginosa* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Rr	Mt	Rr	Mt	Rr	Mt	Rr	Mt	Rr
0	40	0	30	10	20	20	10	30	0	40
2	42	0	30	6	20	12	10	19	0	6
4	53	0	41	0	20	0	10	0	0	140
6	79	0	57	0	52	0	24	3	0	142
8	102	0	69	0	51	0	50	3	0	112
10	65	0	56	0	56	0	43	3	0	100
12	37	0	40	0	55	0	40	2	0	94
14	46	0	50	0	47	0	16	7	0	72
16	44	0	47	0	47	0	18	2	0	70

Mt = *Melanoides tuberculata*

Rr = *Radix rubiginosa*

Raito of *M. tuberculata* : *R. rubiginosa*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 25

Biological control study between *Melanoides tuberculata* (control) and *Indoplanorbis exustus* (target).

Week	Number of snails									
	1		2		3		4		5	
	Mt	Ie	Mt	Ie	Mt	Ie	Mt	Ie	Mt	Ie
0	40	0	30	10	20	20	10	30	0	40
2	42	0	30	10	12	50	10	30	0	40
4	53	0	41	77	13	50	10	64	0	131
6	79	0	30	26	4	46	2	150	0	43
8	102	0	37	92	3	84	2	81	0	60
10	65	0	35	45	3	55	2	56	0	60
12	37	0	32	58	2	50	2	18	0	27
14	46	0	31	26	2	130	2	103	0	63
16	44	0	35	253	2	363	2	230	0	123

Mt = *Malanoides tuberculata*

Ie = *Indoplanorbis exustus*

Ratio of *M. tuberculata* : *I. exustus*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

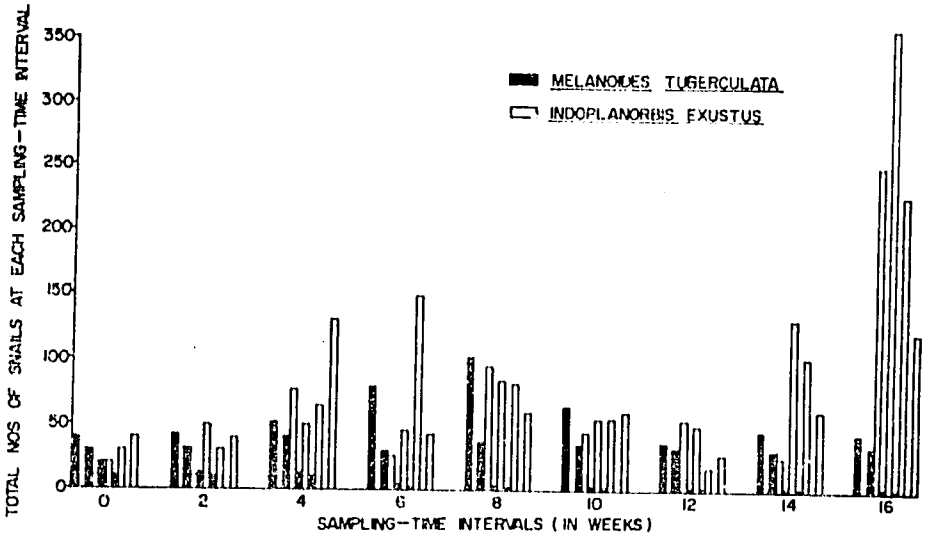


Fig. 38. Biological control study between *M. tuberculata* (control) and *I. exustus* (target) under laboratory condition.

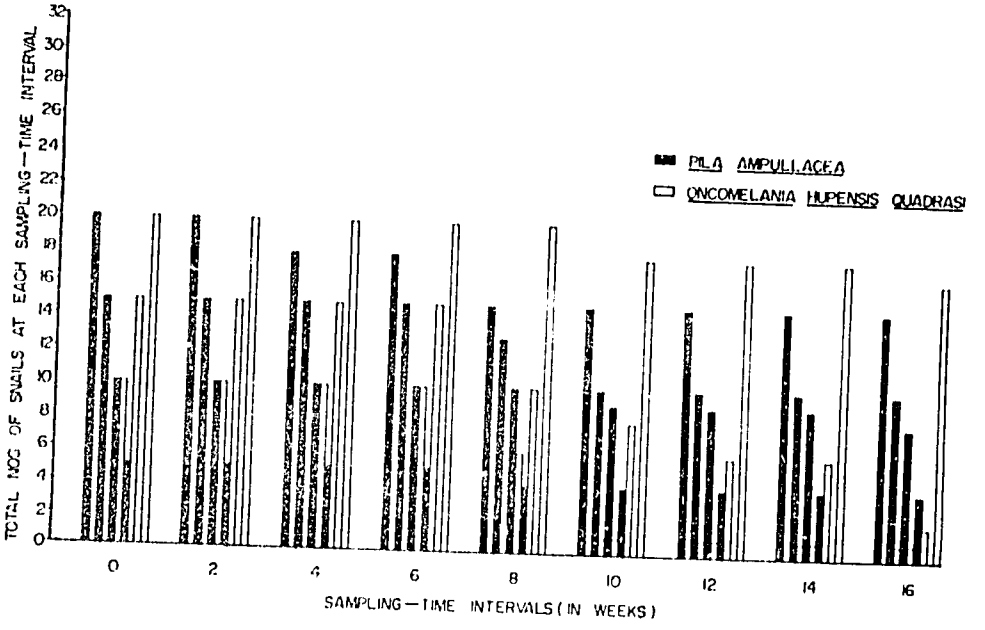


Fig. 39. Biological control study between *P. ampullacea* (control) and *O.h. quadrasi* (target) under laboratory condition.

snails also behaved in similar fashion. Hence, *M. tuberculata* and *B.s. goniomphalos* seemed to have no reaction with one another.

1.4.4 *Pila ampullacea*

For the biological control experiments with *P. ampullacea* as the control agent, young *P. ampullacea* (3-4 cm in size) were used. Hence, there would be no reproductivity of *P. ampullacea* during the whole experiment.

The biological control study between *P. ampullacea* and *O.h. quadrasi* revealed the following results (Table 26, Fig. 39). *P. ampullacea* appeared to have lower mortality rate than *O.h. quadrasi*. *O.h. quadrasi* did not produce any offspring. This may be due to the very low number of snails which was insufficient to produce any eggs.

The biological control study between *P. ampullacea*, being the control agent, and *B. glabrata*, being the target species, appeared very promising (Table 27 and Fig.40). Even though *B. glabrata* did not die off completely, but they appeared to be under control of *P. ampullacea*. The effect of *P. ampullacea* on *B. glabrata* could be as an accidental predator on the egg masses of *B. glabrata*. Further study especially using the simulated field conditions was investigated.

TABLE 26

Biological control study between *Pila ampullacea* (control) and *Oncomelania hupensis quadrasi* (target).

Week	Number of snails									
	1		2		3		4		5	
	Pa	Oq	Pa	Oq	Pa	Oq	Pa	Oq	Pa	Oq
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	18	0	15	5	10	10	5	15	0	20
6	18	0	15	5	10	10	5	15	0	20
8	15	0	13	3	10	6	4	10	0	20
10	15	0	10	1	9	1	4	8	0	18
12	15	0	10	1	9	1	4	6	0	18
14	15	0	10	1	9	1	4	6	0	18
16	15	0	10	1	8	1	4	2	0	17

Pa = *Pila ampullacea*

Oq = *Oncomelania hupensis quadrasi*

Ratio of *P. ampullacea* : *O.h. quadrasi*

1 = 40 : 0

2 = 30 : 10

3 = 20 : 20

4 = 10 : 30

5 = 0 : 40

TABLE 27

Biological control study between *Pila ampullacea* (control) and *Biomphalaria glabrata* (target).

Week	Number of snails									
	1		2		3		4		5	
	Pa	Bg	Pa	Bg	Pa	Bg	Pa	Bg	Pa	Bg
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	14	0	20
4	20	0	15	5	10	10	5	14	0	99
6	18	0	14	5	10	10	5	14	0	108
8	18	0	14	5	6	24	5	32	0	151
10	17	0	13	5	6	19	5	14	0	74
12	17	0	10	4	5	19	4	15	0	67
14	16	0	10	3	4	20	4	12	0	66
16	15	0	10	3	4	15	4	12	0	56

Pa = *Pila ampullacea*

Bg = *Biomphalaria glabrata*

Ratio of *P. ampullacea* : *B. glabrata*

1 = 20 : 0

2 = 15 : 5

3 = 10 : 10

4 = 5 : 15

5 = 0 : 20

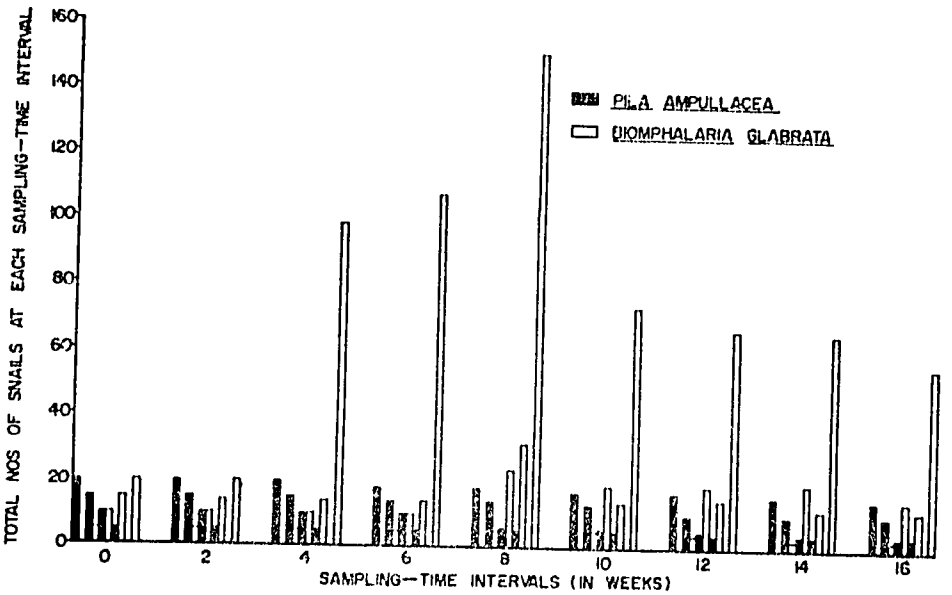


Fig. 40. Biological control study between *P. ampullacea* (control) and *B. glabrata* (target) under laboratory condition.

Fig. 41. Cement tanks used for testing biological control effects of target snails under simulated field conditions.

1.6 Experimental work on biological control of molluska under simulated field conditions

For simulated field conditions, the testing was performed outside the laboratory, at a small field station. Habitats simulating standing waters were constructed in such a way that the experimental agents and target species were not able to escape.

The testings were done in cement tanks with the diameter of 80 cm and the height of 35 cm. The tanks were aerated and furnished with water plants and rocks in order to simulate the snails' habitats (Fig.41). The ratio between biological agent and target species was as follows:

Control agent	Target species
0	100
50	50
100	0

Control agents were *T. granifera* and *M. tuberculata.*

For *B.c. costula* and *P. ampullacea* being control agents, the following ratio was used.

Control agent	Target species
0	50
25	25
50	0

The target species were:

B. glabrata

I. exustus

R. rubiginosa

T. aperta

O.h. quadrasi

B.s. goniomphalos

The general procedure, observation on mortality rates of both control and target species was done weekly while the counting of the snail offspring was done biweekly. The original snails were marked with finger nail polish so that the offspring could be distinguished from them.

The experiments were completed for the total period of 8 months. The results of the experiments are shown in Tables 28-51 and Figures 42-65.

1.5.1 *Brotia costula costula*

Effect on *Biomphalaria glabrata*

The result of the biological control study between *B.c. costula* (control) and *B. glabrata* (target) is shown in Table 28 and Fig. 42. The experimental tank contained equal numbers of *B.c. costula* and *B. glabrata* (25:25). During the first 2-4 months, the target snails started to multiply to a large number, while the control snails did not produce any offspring. After 5 weeks, the number of target species, *B. glabrata* had reduced due to

TABLE 28

Biological control study between *Brotia costula costula* (control) and *Biomphalaria glabrata* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Bg	Bcc	Bg	Bcc	Bg
0	50	0	25	25	0	50
2	1278	0	25	25	0	50
4	1680	0	24	23	0	47
6	1483	0	23	76	0	495
8	985	0	23	242	0	176
10	975	0	23	276	0	95
12	743	0	22	302	0	180
14	723	0	22	336	0	137
16	748	0	20	394	0	173
18	748	0	20	461	0	65
20	736	0	20	223	0	125
22	706	0	20	256	0	367
24	732	0	19	206	0	507
26	543	0	19	277	0	468
28	529	0	19	275	0	392
30	638	0	18	130	0	368
32	321	0	17	155	0	342

Bcc = *Brotia costula costula*
Bg = *Biomphalaria glabrata*

Ratio of B.c. costula : B. glabrata

1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

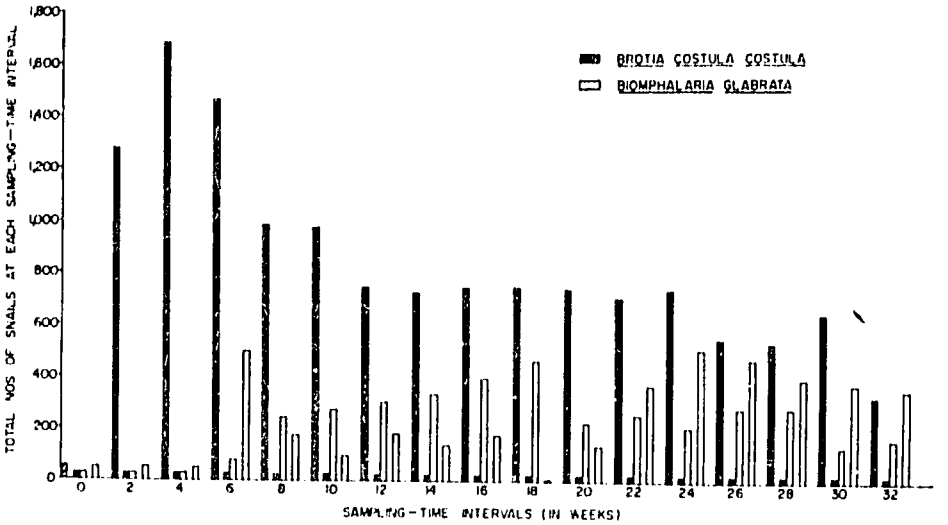


Fig. 42. Biological control study between *B.c. costula* (control) and *B. glabrata* (target) under simulated field condition.

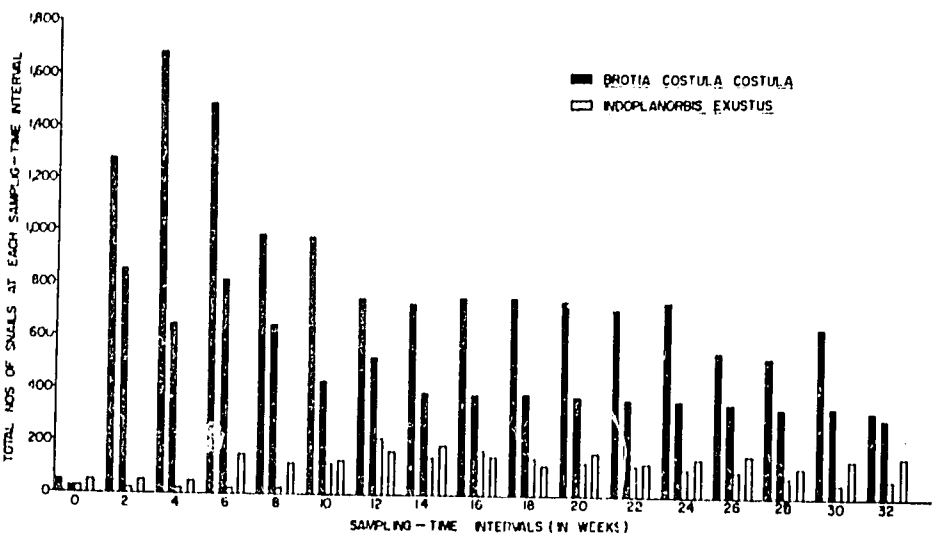


Fig. 43. Biological control study between *B.c. costula* (control) and *I. exustus* (target) under simulated field condition.

overcrowding, and remained relatively constant until the end of 8 month period. It was further observed, however, that the control agent, *B.c. costula* started to multiply after 10 months, producing a large number of offspring. Hence, it appeared that reproduction of *B.c. costuta* could be seasonal under the simulated field condition. No competition interactions were indicated between *B.c. costula* and *B. glabrata*. Both control and target snails maintained separately were mostly alive with an increase in their numbers. It can be concluded that *B.c. costula* was not an effective biological control agent for *B. glabrata*.

Effect on *Indoplanorbis exustus*

The result of the biological control study between *B.c. costula* (control) and *I. exustus* (target) is shown in Table 29 and Fig. 43. The experimental tank contained 25 *B.c. costula* and 25 *I. exustus*. Two weeks after the initiation of culture, *B.c. costula* started to multiply to a very large number due to their high reproductive rate. After 2 months, the target species, *I. exustus* had grown up to sexual maturity and begun to breed. A month later, adult snails of both species started to die until there were no adult snails left in the experimental tank. There was no sign of competition observed between the control agent and the target species. However, the number of the control offspring had always been greater than that of the target species. By the end of 8 months, the number of *B.c. costula* offspring was almost five times the number of *I. exustus*

TABLE 29

Biological control study between *Brotia costula costula* (control) and *Indoplanorbis exustus*(target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Ie	Bcc	Ie	Bcc	Ie
0	50	0	25	25	0	50
2	1278	0	875	21	0	50
4	1680	0	645	20	0	48
6	1483	0	804	20	0	152
8	985	0	642	22	0	115
10	975	0	429	116	0	128
12	743	0	522	215	0	166
14	723	0	387	142	0	190
16	748	0	378	169	0	149
18	748	0	383	142	0	114
20	736	0	372	124	0	166
22	706	0	364	118	0	128
24	732	0	360	105	0	142
26	543	0	349	98	0	160
28	529	0	338	79	0	112
30	638	0	337	51	0	142
32	321	0	297	69	0	151

Bcc = *Brotia costula costula*
Ie = *Indoplanorbis exustus*

Ratio of	*B.c. costula*	:	*I. exustus*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

offspring. Both control and target snails maintained separately were mostly alive with an increase in their numbers. The result has indicated that the control species, *B. c. costula* had more reproductive potential and relatively lower mortality rate than the target species, *I. exustus*

Effect on *Radix rubiginosa*

The result of the biological control study between *B.c. costula* (control) and *R. rubiginosa* (target) is shown in Table 30 and Fig. 44. The experimental tank contained 25 *B.c. costula* and 25 *R. rubiginosa*. Three to four weeks after the initiation of culture, *B.c. costula* began to multiply. Due to overcrowding, the young *B.c. costula* was reduced in number until becoming relatively stable by the end of 5 months. It took *R. rubiginosa* in the experimental tank 2 months to multiply, but the number of offspring produced was not as large as that of *B.c. costula*. At the end of the seventh month, the young snails of *R. rubiginosa* started to die off, while those of *B.c. costula* remained relatively stable. By the end of 8-month period, the number of *B.c. costula* snails was almost tenfold the number of *R. rubiginosa*. The control snails, *B.c. costula* maintained alone were mostly alive with an increase in their numbers. Adult snails of the target species, *R. rubiginosa* mostly died but left a number of young snails behind. The experiment indicated the superior reproductive ability of *B.c. costula* over *R. rubiginosa*. In addition, *B. c. costula*

TABLE 30

Biological control study between *Brotia costula costula* (control) and *Radix rubiginosa* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Rr	Bcc	Rr	Bcc	Rr
0	50	0	25	25	0	50
2	1278	0	25	25	0	45
4	1680	0	707	23	0	37
6	1483	0	980	21	0	37
8	985	0	648	21	0	37
10	975	0	411	78	0	123
12	743	0	476	230	0	199
14	723	0	285	69	0	147
16	748	0	371	128	0	188
18	748	0	341	226	0	262
20	736	0	341	226	0	197
22	706	0	212	155	0	130
24	732	0	238	153	0	95
26	543	0	219	101	0	68
28	529	0	138	63	0	148
30	638	0	133	32	0	81
32	321	0	122	18	0	90

Bcc = *Brotia costula costula*
Rr = *Radix rubiginosa*

Ratio of *B.c. costula* : *R. rubiginosa*
1 = 50 : 0
2 = 25 : 25
3 = 0 : 50

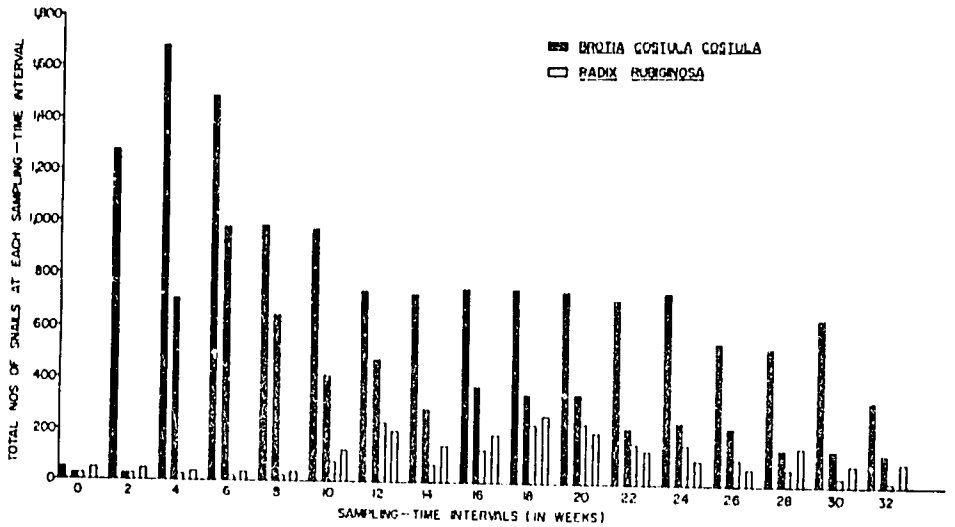


Fig. 44. Biological control study between *B.c. costula* (control) and *R. rubiginosa* (target) under simulated field condition.

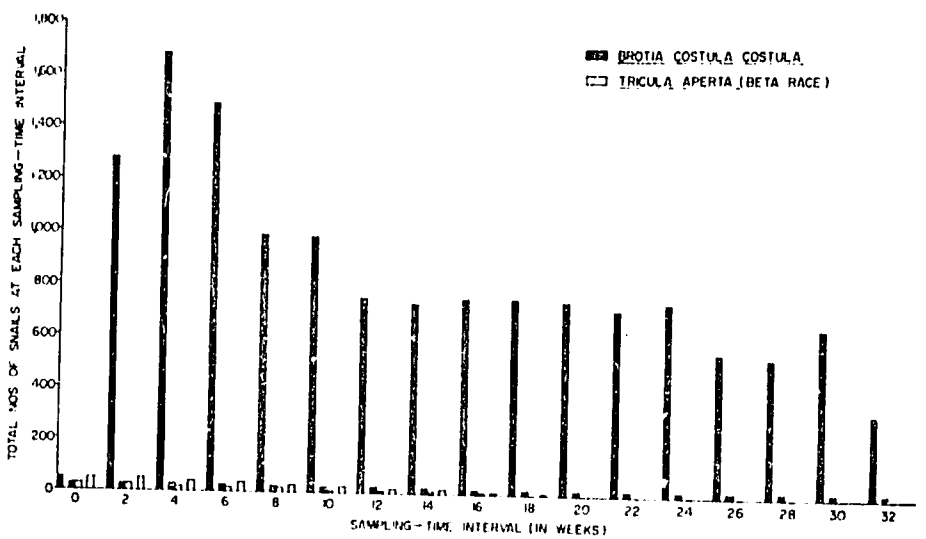


Fig. 45. Biological control study between *B.c. costula* (control) and *T. aperta* (target) under simulated field condition.

young snails seemed to have better survival rate than those of *R. rubiginosa* due to their hard shells and opercula and their ability to withstand adversed environmental condition better than *R. rubiginosa*. Owing to the differences in their habitat preferences, i.e. *B.c. costula* usually stayed under water in the sand, while *R. rubiginosa* were mostly at the water surface, there seemed to be no interaction between these two species of snails.

Effect on *Tricula aperta*

The result of the biological control study between *B.c. costula* (control) and *T. aperta* (target) is shown in Table 31 and Fig. 45. *B.c. costula* maintained together with *T. aperta* did not produce any young snails. This is due to the fact that when the experimental culture was initiated, *B.c. costula* were not in the breeding season. *T. aperta* in the experimental tank and in the control tank behaved in similar fashion, i.e. they had very high mortality and no reproduction occurred.

In nature, *B.c. costula* and *T. aperta* are found in different habitats. *B.c. costula* are found in mostly slow-running water embedded under the sand. *T. aperta* are found in the river with strong current attached themselves to the underside of the rocks and the water is quite cool most of the year. *B. c. costula* could survive better under the simulated field condition than *T. aperta*. However, the two species of snails did not seem to interact with one another

TABLE 31

Biological control study between *Brotia costula costula* (control) and *Tricula aperta*(target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Ta	Bcc	Ta	Bcc	Ta
0	50	0	25	25	0	50
2	1278	0	25	25	0	50
4	1680	0	25	18	0	40
6	1483	0	24	17	0	36
8	985	0	24	14	0	28
10	975	0	23	10	0	24
12	743	0	22	8	0	19
14	723	0	22	6	0	16
16	748	0	20	3	0	9
18	748	0	20	0	0	5
20	736	0	20	0	0	0
22	706	0	20	0	0	0
24	732	0	19	0	0	0
26	543	0	19	0	0	0
28	529	0	19	0	0	0
30	638	0	18	0	0	0
32	321	0	18	0	0	0

Bcc = *Brotia costula costula*
Ta = *Tricula aperta*

Ratio of *B.c. costula* : *T. aperta*

1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

as observed during the whole experimental period.

Effect on *Oncomelania hupensis quadrasi*

The result of the biological control study between *B.c. costula* (control) and *O.h. quadrasi* (target) is shown in Table 32 and Fig. 46. The data presented is much similar to that of *B. c. costula* and *T. aperta*. *B.c. costula* and *O. h. quadrasi* live in different habitats. *B. c. costula* are aquatic and *O.h. quadrasi* are amphibious in nature. While *B.c. costula* survived and reproduced very well in the cement tank, *O.h. quadrasi* could not and they all died after 4 months. Despite the superior reproductive and survival rates of *B.c. costula* over *O.h. quadrasi*, they did not seem to interact with one another during the experimental period.

Effect on *Bithynia siamensis goniomphalos*

The result of the biological control study between *B.c. costula* (control) and *B.s. goniomphalos* (target) is shown in Table 33 and Fig. 47. Similar to the experimental work on *B.c. costula* and *T. aperta*, the experimental culture of *B.c. costula* and *B.s. goniomphalos* was initiated when *B.c. costula* were not in the breeding season. Neither *B.c. costula* nor *B.s. goniomphalos* reproduced but the former seemed to have a better survival rate than the latter. It is worth noted that if *B.c. costula* culture was initiated during their breeding season, the number of *B.c. costula* would be far greater than that of *B.s.*

TABLE 32

Biological control study between *Brotia costula costula* (control) and *Oncomelania hupensis quadrasi*(target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Oq	Bcc	Oq	Bcc	Oq
0	50	0	25	25	0	50
2	1278	0	25	25	0	40
4	1680	0	25	18	0	32
6	1483	0	113	10	0	30
8	985	0	106	6	0	29
10	975	0	163	6	0	19
12	743	0	181	2	0	16
14	723	0	137	2	0	15
16	748	0	141	2	0	12
18	748	0	121	1	0	11
20	736	0	112	0	0	11
22	706	0	119	0	0	11
24	732	0	127	0	0	11
26	543	0	141	0	0	11
28	529	0	148	0	0	11
30	638	0	145	0	0	10
32	321	0	137	0	0	10

Bcc = *Brotia costula costula*
Oq = *Oncomelania hupensis quadrasi*

Ratio of *B.c. costula* : *O.h. quadrasi*
1 = 50 : 0
2 = 25 : 25
3 = 0 : 50

TABLE 33

Biological control study between *Brotia costula costula* (control) and *Bithynia siamensis goniomphalos* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg
0	50	0	25	25	0	50
2	1278	0	25	25	0	50
4	1680	0	25	25	0	48
6	1483	0	25	25	0	48
8	985	0	22	20	0	44
10	975	0	20	20	0	38
12	743	0	20	17	0	26
14	723	0	20	14	0	24
16	748	0	20	12	0	24
18	748	0	20	8	0	21
20	736	0	19	4	0	17
22	706	0	19	2	0	13
24	732	0	19	2	0	12
26	543	0	18	2	0	12
28	529	0	18	1	0	11
30	638	0	18	1	0	10
32	321	0	18	0	0	10

Bcc = *Brotia costula costula*
Bsg = *Bithynia siamensis goniompalos*

Ratio of *B.c. costula* : *B.s. goniomphalos*

1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

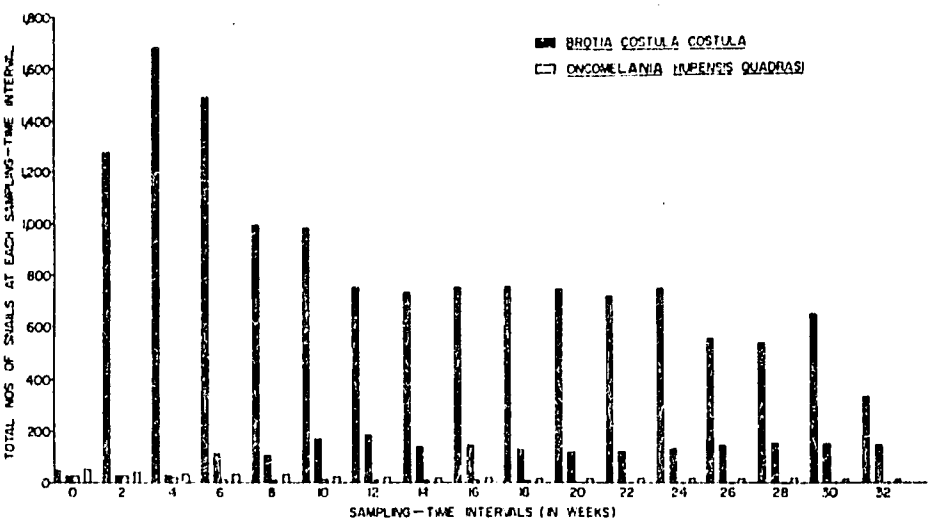


Fig. 46. Biological control study between *B.c. costula* (control) and *O.h. quadrasi* (target) under simulated field condition.

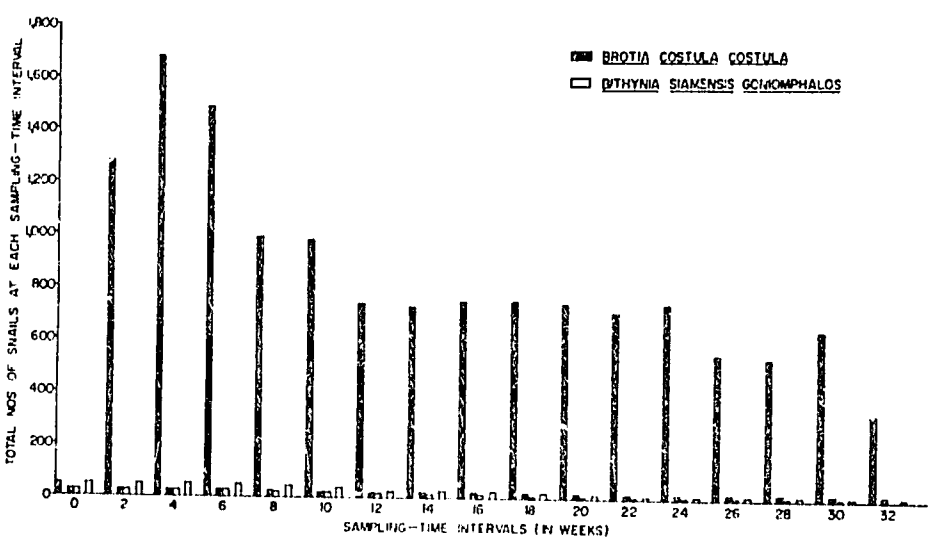


Fig. 47. Biological control study between *B.c. costula* (control) and *B.s. goniomphalos* (target) under simulated field condition.

goniomphalos due to their ovoviviparity and better survival rate.

It can be concluded that *B.c. costula* appear to have some possibility as an effective competitor in certain type of habitat. The major factor favoring *B.c. costula* as bio-control agent appear to be superior reproduction and relatively low mortality rate. They seem to be able to control the population of pulmonate snails, *I. exustus* and *R. rubiginosa*. However, this is not the case for *B. glabrata*. There was no interference observed between *B.c. costula* and *T. aperta* and *O.h. quadrasi* due to the different preferences in habitats.

1.5.2 *Tarebia granifera*

Effect on *Biomphalaria glabrata*

The result of the biological control study between *T. granifera* (control) and *B. glabrata* (target) is shown in Table 34 and Fig. 48. After 2 months, the control and target snails maintained together started to multiply producing a large number of offspring. However, the number of young *T. granifera* was much lower than that of *B. glabrata*, all through the experimental period. The result obtained here was similar to that of the experiment done under the laboratory condition. So, it is apparent that under either laboratory or simulated field condition, *T. granifera* could not control the population of *B. glabrata*.

TABLE 34

Biological control study between *Tarebia granifera* (control) and *Biomphalaria glabrata* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Bg	Tg	Bg	Tg	Bg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	92	0	48	50	0	990
8	390	0	43	48	0	353
10	650	0	279	567	0	189
12	667	0	128	769	0	361
14	327	0	614	1510	0	273
16	444	0	471	987	0	345
18	390	0	317	832	0	131
20	396	0	205	448	0	251
22	219	0	186	430	0	734
24	216	0	163	344	0	1014
26	246	0	122	350	0	936
28	189	0	96	322	0	784
30	190	0	75	342	0	736
32	259	0	52	350	0	684

Tg = *Tarebia granifera*
Bg = *Biomphalaria glabrata*

Ratio of		*T. granifera*	:	*B. glabrata*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

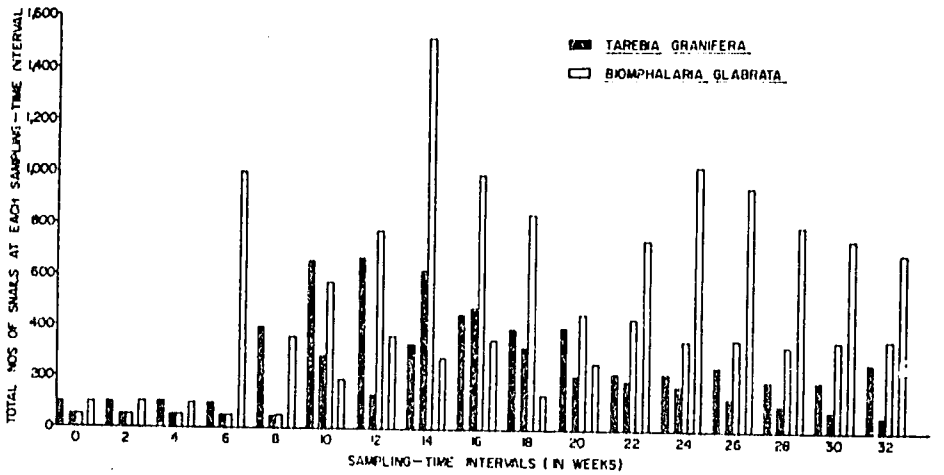


Fig. 48. Biological control study between *T. granifera* (control) and *B. glabrata* (target) under simulated field condition.

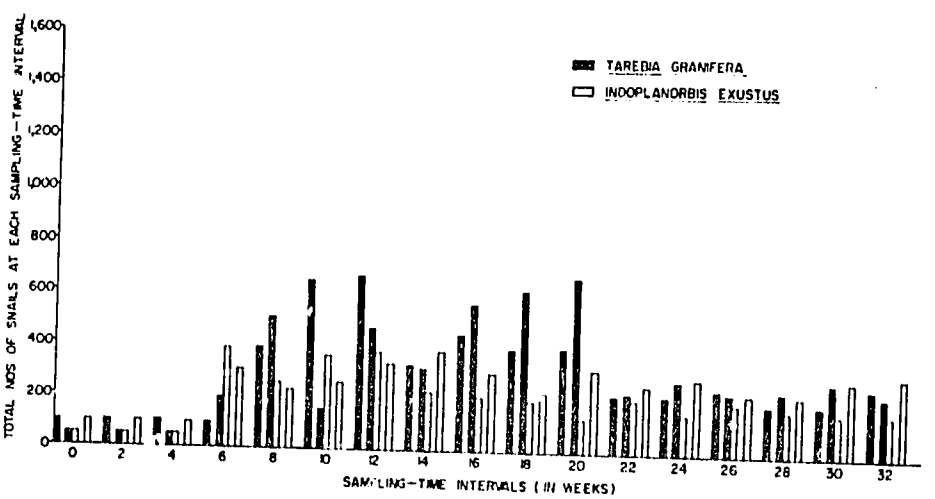


Fig. 49. Biological control study between *T. granifera* (control) and *I. exustus* (target) under simulated field condition.

B. glabrata had been long established and well adapted to the laboratory and simulated field conditions much better than *T. granifera*. Both control and targer snails maintained separately were mostly alive with an increase in their numbers.

Effect on *Indoplanorbis exustus*

The result of the biological control study between *T. granifera* (control) and *I. exustus* (target) is shown in Table 35 and Fig. 49. Six weeks after the initiation of culture, both control and target snail species maintained together or alone started to multiply. On the sixth week, the number of young produced by *I. exustus* exceeded that produced by *T. granifera*. During the next 2-5 months, there were more *T. granifera* snails than *I. exustus* snails. After that, the numbers of the two species became slightly different, with more *T. granifera* than *I. exustus*. At the end of the eighth month, there were more *T. granifera* than *I. exustus*. Both control and target snails maintained separately were mostly alive with an increase in their numbers. The result indicated that the control snails, *T. granifera* seemed to have more reproductive potential and relatively lower mortality rate than the target species, *I. exustus*.

TABLE 35

Biological control study between *Tarebia granifera* (control) and *Indoplanorbis exustus* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Ie	Tg	Ie	Tg	Ie
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	92	0	196	386	0	303
8	390	0	502	257	0	229
10	650	0	150	361	0	256
12	667	0	465	374	0	332
14	327	0	315	221	0	380
16	444	0	560	202	0	297
18	390	0	616	191	0	227
20	396	0	664	127	0	312
22	219	0	223	201	0	259
24	216	0	277	149	0	286
26	246	0	230	191	0	230
28	189	0	238	168	0	224
30	190	0	278	159	0	284
32	259	0	229	159	0	301

Tg = *Tarebia granifera*
Ie = *Indoplanorbis exustus*

Ratio of		*T. granifera*	:	*I. exustus*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

Effect on *Radix rubiginosa*

The result of the biological control study betwen *T. granifera* (control) and *R. rubiginosa* (target) is shown in Table 36 and Fig. 50. During the first two months, the target snails, *R. rubiginosa* maintained together with *T. granifera* started to multiply. A few weeks later, all the adult snails of *R. rubiginosa* died, only young snails were present but the number was decreasing gradually. After 4-5 months, there was no *R. rubiginosa* present in the experimental tank. On the contrary, *T. granifera* maintained together with *R. rubiginosa* started to multiply after 2 months, and they still maintained the relatively constant number of young during the whole experiment. The control species maintained alone were mostly alive with an increase in their number. All adult target snails maintained alone died, only young snails were present. The result indicated the superior reproductive potential and lower mortality rate of *T. granifera* over the target snails, *R. rubiginosa*.

Effects on prosobranch snails (*Tricula aperta*, *Oncomelania hupensis quadrasi*, and *Bithynia siamensis goniomphalos*).

The results of the biological control studies between *T. granifera* (control) and the target species, *T. aperta*, *O.h. quadrasi* and *B.s. goniomphalos* are shown in Tables 37-39 and Figures 51-53, respectively. All the prosobranch target species behaved in similar fashion. The target snails

TABLE 36

Biological control study between *Tarebia granifera* (control) and *Radix rubiginosa* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Rr	Tg	Rr	Tg	Rr
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	42	14	0	100
6	92	0	42	36	0	73
8	390	0	161	151	0	73
10	650	0	142	93	0	180
12	667	0	152	52	0	194
14	327	0	155	28	0	196
16	444	0	177	12	0	523
18	390	0	184	0	0	394
20	396	0	210	0	0	334
22	219	0	191	0	0	261
24	216	0	185	0	0	191
26	246	0	169	0	0	136
28	189	0	162	0	0	296
30	190	0	166	0	0	162
32	259	0	174	0	0	181

Tg = *Tarebia granifera*
Rr = *Radix rubiginosa*

Ratio of		*T. granifera*	:	*R. rubiginosa*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

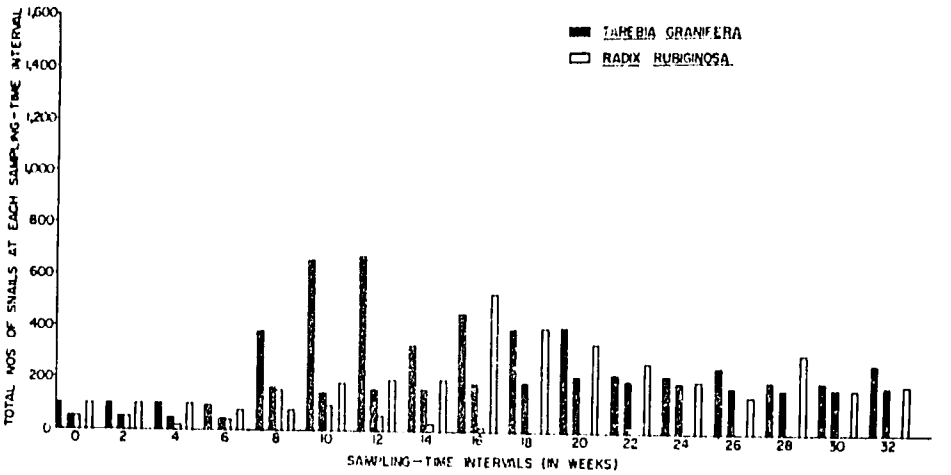


Fig. 50. Biological control study between *T. granifera* (control) and *R. rubiginosa* (target) under simulated field condition.

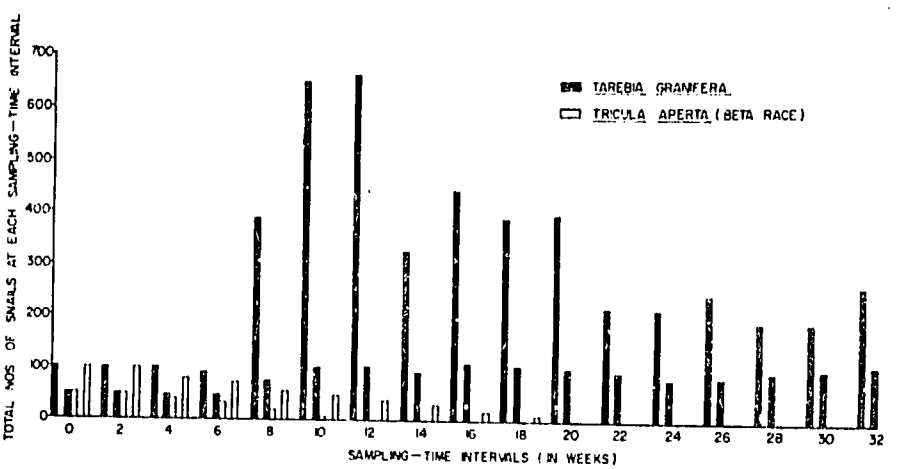


Fig. 51. Biological control study between *T. granifera* (control) and *T. aperta* (target) under simulated field condition.

TABLE 37

Biological control study between *Tarebia granifera* (control) and *Tricula aperta* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Ta	Tg	Ta	Tg	Ta
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	48	41	0	80
6	92	0	48	32	0	72
8	390	0	77	18	0	56
10	650	0	102	6	0	48
12	667	0	102	0	0	38
14	327	0	94	0	0	32
16	444	0	112	0	0	18
18	390	0	105	0	0	10
20	396	0	99	0	0	0
22	219	0	95	0	0	0
24	216	0	81	0	0	0
26	246	0	84	0	0	0
28	189	0	95	0	0	0
30	190	0	100	0	0	0
32	259	0	109	0	0	0

Tg = *Tarebia granifera*
Ta = *Tricula aperta*

Ratio of	*T. granifera*	:	*T. aperta*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 38

Biological control study between *Tarebia granifera* (control) and *Oncomelania hupensis quadrasi* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Oq	Tg	Oq	Tg	Oq
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	49	48	0	63
6	92	0	47	42	0	60
8	390	0	45	34	0	41
10	650	0	186	21	0	38
12	667	0	187	21	0	32
14	327	0	168	12	0	30
16	444	0	205	11	0	24
18	390	0	149	11	0	20
20	396	0	172	11	0	20
22	219	0	128	10	0	14
24	216	0	101	9	0	14
26	246	0	170	9	0	14
28	189	0	188	6	0	14
30	190	0	181	6	0	14
32	259	0	179	6	0	14

Tg = *Tarebia granifera*
Oq = *Oncomelania hupensis quadrasi*

Ratio of	*T. granifera*	:	*O.h. quadrasi*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 39

Biological control study between *Tarebia granifera* (control) and *Bithynia sinmensis goniomphalos* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Bsg	Tg	Bsg	Tg	Bsg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	92	0	62	50	0	87
8	390	0	351	43	0	56
10	650	0	447	45	0	51
12	667	0	339	33	0	48
14	327	0	146	28	0	48
16	444	0	193	16	0	42
18	390	0	154	10	0	34
20	396	0	136	5	0	26
22	219	0	171	5	0	24
24	216	0	179	5	0	24
26	246	0	478	3	0	22
28	189	0	363	3	0	22
30	190	0	468	2	0	20
32	259	0	629	0	0	20

Tg = *Tarebia granifera*
Bsg = *Bithynia sinmensis goniomphalos*

Ratio of	*T. granifera*	:	*B.s. goniomphalos*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

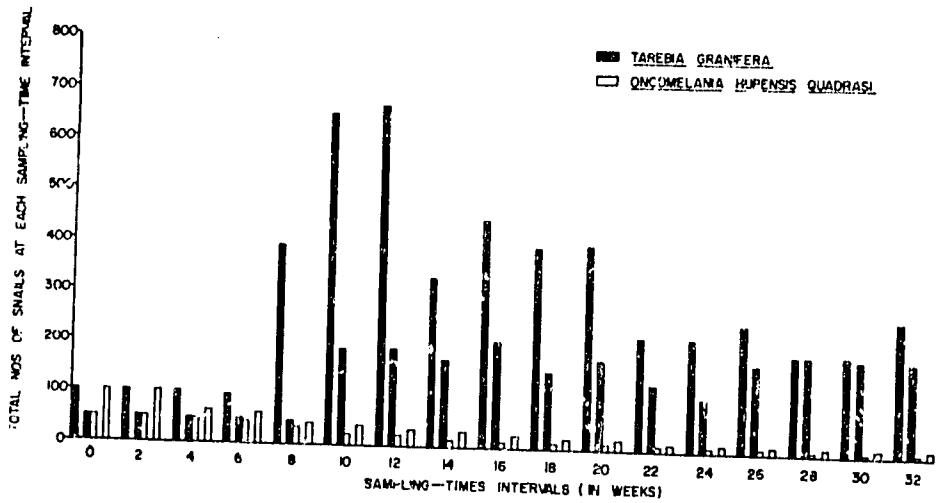


Fig. 52. Biological control study between *T. granifera* (control) and *O.h. quadrasi* (target) under simulated field condition.

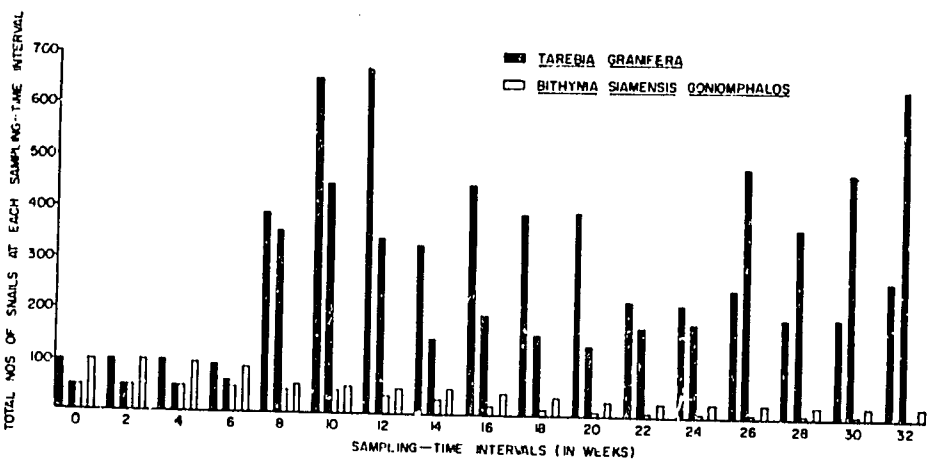


Fig. 53. Biological control study between *T. granifera* (control) and *B.s. goniomphalos* (target) under simulated field condition.

maintained together with control snails had very high mortality rate and no reproduction occurred. Since the target snail species maintained alone behaved in the same way, it might be concluded that no interaction exist between the control and the three species of target snails, *T. aperta*, *O.h. quadrasi* and *B.s. goniomphalos*. For *T. aperta* and *O.h. quadrasi*, this could be explained on the basis of different habitat preferences.

It can be concluded that *T. granifera* appear to have some possibility as an effective competitor for *I. exustus* and *R. rubiginosa*, the pulmonate snails, i.e. superior reproductive potential and low mortality rate. This is not the case with the other pulmonate, *B. glabrata*. There seemed to be no interference between *T. granifera* and *T. aperta*, *O.h. quadrasi* or *B.s. goniomphalos*.

1.5.3 *Melanoides tuberculata*

Effect on *Biomphalaria glabrata*

The result of the biological control study between *M. tuberculata* (control) and *B. glabrata* (target) is shown in Table 40 and Fig. 54. In the experimental tank containing 50 *M. tuberculata* snails and 50 *B. glabrata* snails after one month, both species of snails began to multiply. The quantity of young snails of both species fluctuated, but they appeared to have similar reproduction and mortality rates. At the end of 8 months, the numbers of both control and target snails were almost the same. Both control and

TABLE 40

Biological control study between *Melanoides tuberculata* (control) and *Biomphalaria glabrata* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Bg	Mt	Bg	Mt	Bg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	236	0	76	125	0	990
8	286	0	131	48	0	353
10	223	0	68	140	0	189
12	312	0	165	324	0	361
14	384	0	162	288	0	273
16	514	0	590	568	0	345
18	656	0	775	433	0	131
20	504	0	675	186	0	251
22	264	0	606	497	0	734
24	246	0	511	321	0	1014
26	267	0	199	227	0	936
28	300	0	154	226	0	784
30	662	0	210	193	0	736
32	643	0	184	162	0	684

Mt = *Melanoides tuberculata*
Bg = *Biomphalaria glabrata*

Ratio of	*M. tuberculata*	:	*B. glabrata*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

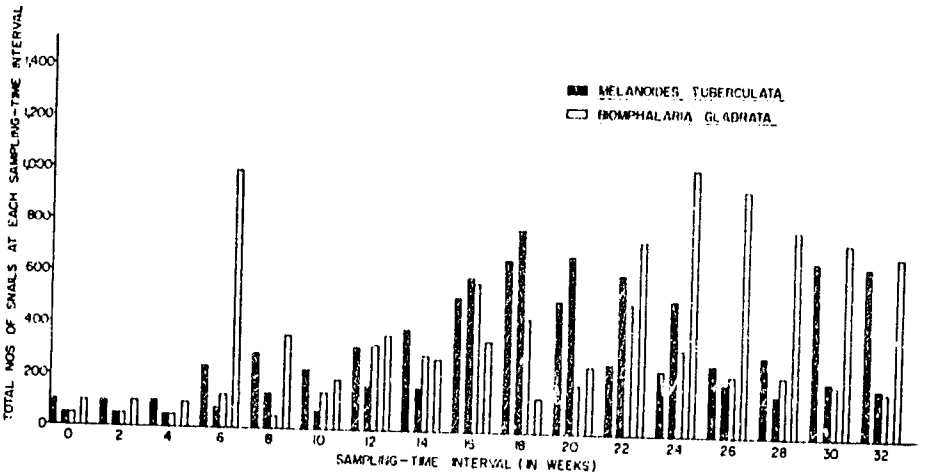


Fig. 54. Biological control study between *M. tuberculata* (control) and *B. glabrata* (target) under simulated field condition.

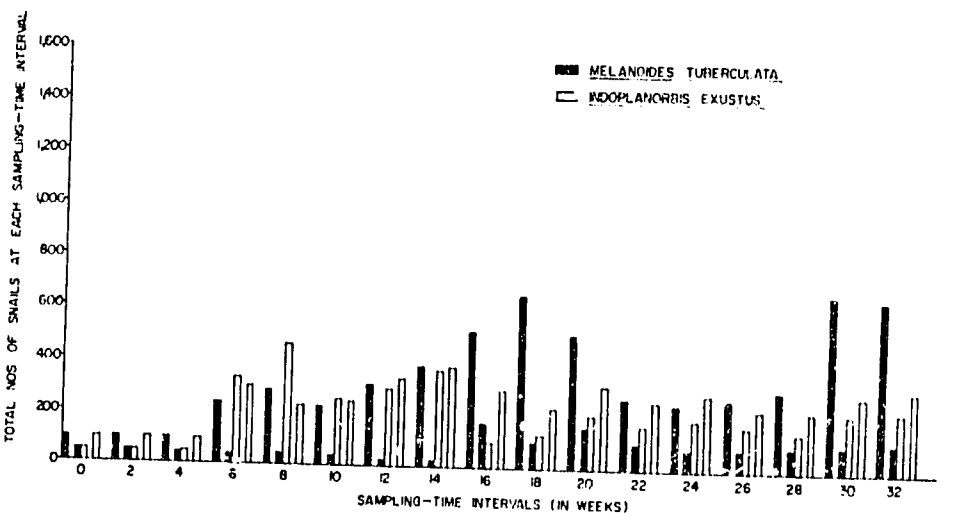


Fig. 55. Biological control study between *M. tuberculata* (control) and *I. exustus* (target) under simulated field condition.

target snails maintained separately were mostly alive with an increase in their numbers. It can be concluded that *M. tuberculata* might not be a suitable bio-control agent for *B. glabrata*.

Effect on *Indoplanorbis exustus*

The result of the biological control study between *M. tuberculata* (control) and *I. exustus* (target) is shown in Table 41 and Fig. 55. In the experimental tank containing both control and target species, *M. tuberculata* started to multiply much later than *I. exustus*. Six weeks after the initiation of culture, *I. exustus* already laid egg masses and produced offspring, while it took *M. tuberculata* almost 4 months to start producing offspring. In addition, the number of offspring produced by *M. tuberculata* was less than that by *I. exustus*. Both control and target snails maintained separately were mostly alive with an increase in their numbers. It can be concluded that *M. tuberculata* might not be a suitable bio-control agent against *I. exustus* due to their lower reproduction and high mortality rates than the target species.

Effect on *Radix rubiginosa*

The result of the biological control study between *M. tuberculata* (control) and *R. rubiginosa* (target) is shown in Table 42 and Fig. 56. After 6 weeks, *M. tuberculata* in the same experimental tank as *R. rubiginosa* started to multiply

TABLE 41

Biological control study between *Melanoides tuberculata* (control) and *Indoplanorbis exustus* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Ie	Mt	Ie	Mt	Ie
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	43	49	0	95
6	236	0	40	333	0	303
8	286	0	40	460	0	229
10	223	0	38	262	0	256
12	312	0	25	297	0	332
14	384	0	23	368	0	380
16	514	0	167	97	0	297
13	656	0	93	127	0	227
20	504	0	154	202	0	312
22	264	0	96	161	0	259
24	246	0	73	188	0	286
26	267	0	78	162	0	230
28	300	0	85	146	0	224
30	662	0	92	215	0	284
32	643	0	105	226	0	301

Mt = *Melanoides tuberculata*
Ie = *Indoplanorbis exustus*

Ratio of	*M. tuberculata*	:	*I. exustus*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 42

Biological control study between *Melanoides tuberculata* (control) and *Radix rubiginosa* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Rr	Mt	Rr	Mt	Rr
0	100	0	50	50	0	100
2	100	0	48	43	0	100
4	100	0	48	43	0	100
6	236	0	136	35	0	73
8	286	0	190	23	0	73
10	223	0	223	12	0	180
12	312	0	298	8	0	194
14	384	0	286	8	0	196
16	514	0	202	12	0	523
18	656	0	195	12	0	394
20	504	0	184	19	0	334
22	264	0	192	14	0	261
24	246	0	188	12	0	191
26	267	0	176	12	0	136
28	300	0	183	10	0	296
30	662	0	175	10	0	162
32	643	0	187	10	0	181

Mt = *Melanoides tuberculata*
Rr = *Radix rubiginosa*

Ratio of	*M. tuberculata*	:	*R. rubiginosa*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

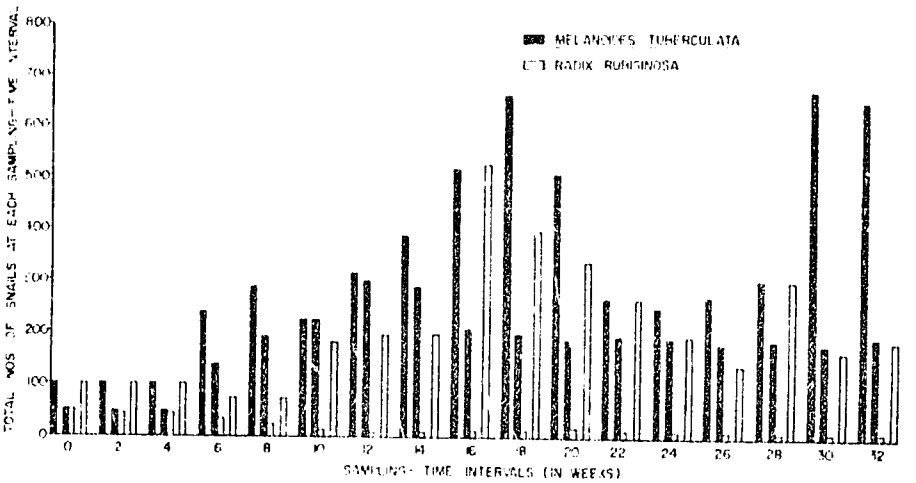


Fig. 56. Biological control study between *M. tuberculata* (control) and *R. rubiginosa* (target) under simulated field condition.

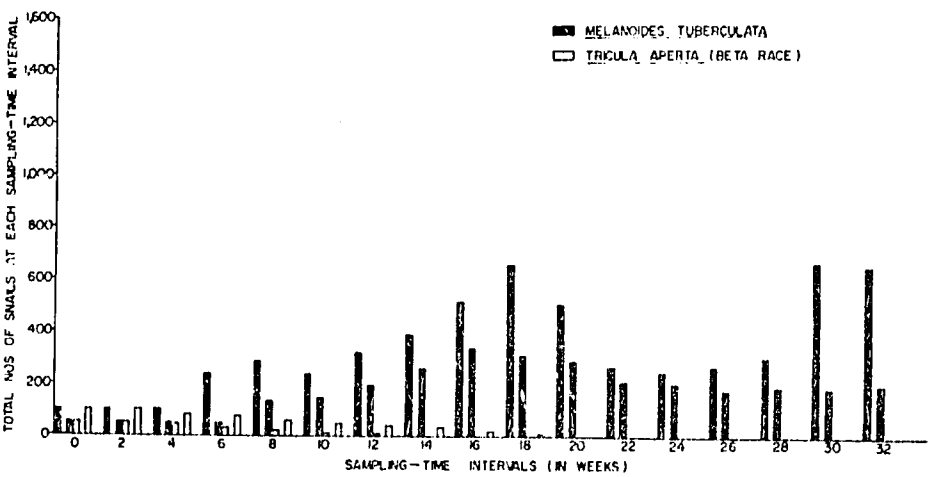


Fig. 57. Biological control study between *M. tuberculata* (control) and *T. aperta* (target) under simulated field condition.

and the number of young snails remained relatively constant until the end of 8-month period. On the contrary, *R. rubiginosa* produced very few egg masses and they started to die off. At the end of the 8-month period, the number of *R. rubiginosa* was drastically reduced. The control snails maintained alone were mostly alive with an increase in their number. The adult target snails maintained alone all died but left a number of young snails behind. It is apparent that *R. rubiginosa* had lower growth and egg laying rates when in competition with *M. tuberculata* than when maintained alone. Even though no interference was observed between the control and target species due to the different preferences of habitats, *M. tuberculata* still showed greater adaptability, hardiness and rapid multiplication.

Effect on *Tricula aperta* and *Oncomelania hupensis quadrasi*

The results of the biological control study between *M. tuberculata* (control) and *T. aperta* (target) is shown in Table 43 and Fig. 57; and between *M. tuberculata* (control) and *O.h. quadrasi* (target) is shown in Table 44 and Fig. 58. After 8 months, the results were as follows : (1) The target snails, *T. aperta* and *O.h. quadrasi* started to die off after one month. By the end of the eighth month, there were no *T. aperta* snails left while there were only a few *O.h. quadrasi* snails present. (2) The target snails did not reproduce. (3) The control snails, *M. tuberculata* both maintained alone or together with target species were present in large numbers.

TABLE 43

Biological control study between *Melanoides tuberculata* (control) and *Tricula aperta* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Ta	Mt	Ta	Mt	Ta
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	49	42	0	80
6	236	0	49	30	0	72
8	286	0	131	18	0	56
10	223	0	145	10	0	48
12	312	0	194	5	0	38
14	384	0	258	0	0	32
16	514	0	334	0	0	18
13	656	0	308	0	0	10
20	504	0	288	0	0	0
22	264	0	205	0	0	0
24	246	0	200	0	0	0
26	267	0	174	0	0	0
28	300	0	185	0	0	0
30	662	0	181	0	0	0
32	643	0	193	0	0	0

Mt = *Melanoides tuberculata*
Ta = *Tricula aperta*

Ratio of	*M. tuberculata*	:	*T. aperta*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 44

Biological control study between *Melanoides tuberculata* (control) and *Oncomelania hupensis quadrasi* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Oq	Mt	Oq	Mt	Oq
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	38	0	63
6	236	0	277	26	0	60
8	286	0	378	26	0	41
10	223	0	627	23	0	38
12	312	0	801	19	0	32
14	384	0	1428	15	0	30
16	514	0	825	15	0	24
18	656	0	748	15	0	20
20	504	0	636	15	0	20
22	264	0	498	15	0	14
24	246	0	580	14	0	14
26	267	0	677	14	0	14
28	300	0	722	13	0	14
30	662	0	537	10	0	14
32	643	0	526	10	0	14

Mt = *Melanoides tuberculata*
Oq = *Oncomelania hupensis quadrasi*

Ratio of	*M. tuberculata*	:	*O.h. quadrasi*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

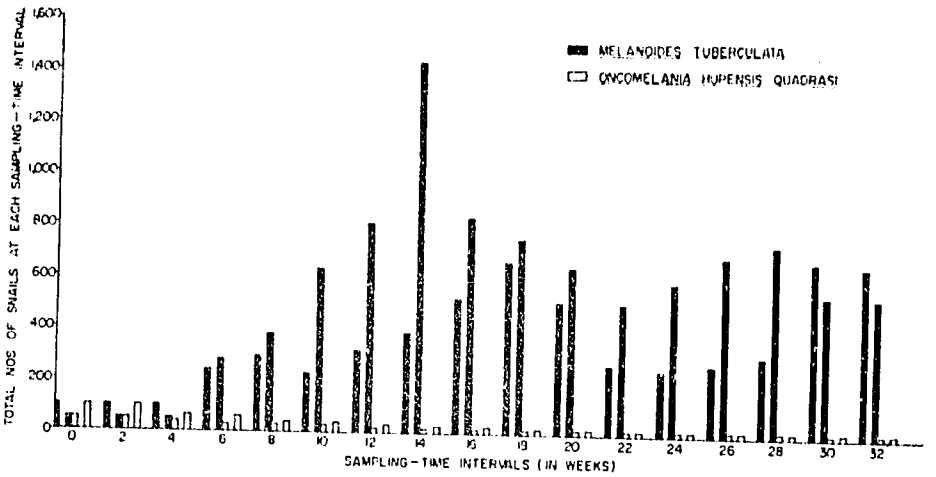


Fig. 58. Biological control study between *M. tuberculata* (control) and *O.h. quadrasi* (target) under simulated field condition.

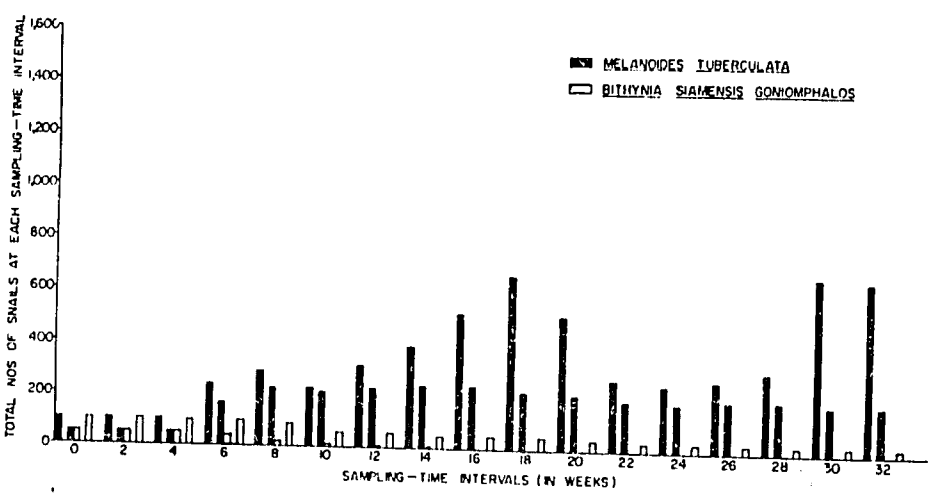


Fig. 59. Biological control study between *M. tuberculata* (control) and *B.s. goniomphalos* (target) under simulated field condition.

It was observed during the two experiments that no competition interaction between the control and the two target species was indicated due to the difference in their habitats. The target snails maintained alone behaved in similar fashion as those maintained with the control snails.

Effect on *bithynia siamensis goniomphalos*

The result of the biological control study between *M. tuberculata* (control) and *B.s. goniomphalos* (target) is shown in Table 45 and Fig. 59. In the experimental tank which contained 50 *M. tuberculata* snails and 50 *B.s. goniomphalos* snails, six weeks after the initiation of culture, *M. tuberculata* started to multiply in relatively large number, while *B.s. goniomphalos* was decreased drastically. At the end of 4 months, no *B.s. goniomphalos* snails present in the experimental tank. The control snails maintained alone were mostly alive with an increase in their number. The target species maintained alone decreased drastically in number until there were only a few target snails present at the end of the 8-month period. No concrete conclusion could be drawn from this experiment since both *B.s. goniomphalos* maintained alone and with *M. tuberculata* behaved in rather similar fashion. It should be noted, however, that the control agent, *M. tuberculata* are generally very hardy and have a much higher reproductive potential than the target species, *B.s. goniomphalos*.

TABLE 46

Biological control study between *Melanoides tuberculata* (control) and *Bithynia siamensis goniomphalos* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Bsg	Mt	Bsg	Mt	Bsg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	236	0	165	40	0	95
8	286	0	221	16	0	87
10	223	0	208	8	0	56
12	312	0	225	2	0	51
14	384	0	238	2	0	48
16	514	0	238	0	0	48
18	356	0	215	0	0	42
20	504	0	204	0	0	34
22	264	0	186	0	0	26
24	246	0	178	0	0	24
26	267	0	189	0	0	24
28	300	0	192	0	0	22
30	662	0	174	0	0	22
32	643	0	179	0	0	20

Mt = *Melanoides tuberculata*
Bsg = *Bithynia siamensis goniomphalos*

Ratio of		*M. tuberculata*	:	*B.s. goniomphalos*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

It can be concluded that *M. tuberculata* appear to have some possibility as an effective competition in certain type of habitat. It is not an effective bio-control agent for *B. glabrata* and *I. exustus*, but they seemed to have some interference with the other pulmonate, *R. rubiginosa* in terms of superior reproduction and relatively low mortality rate. There seemed to be no interference between *M. tuberculata* and *T. aperta* or *O.h. quadrasi* due to the differences in habitats. For *B.s. goniomphalos*, the conclusion could not be drawn but *M. tuberculata* certainly had superior reproductive potential and lower mortality rate than *B.s. goniomphalos*.

1.5.4 *Pila ampullacea*

Effects on pulmonate snails (*Biomphalaria glabrata*, *Indoplanorbis exustus* and *Radix rubiginosa*).

The results of the biological control studies between *P. ampullacea* (control) and *B. glabrata*, *I. exustus* and *R. rubiginosa* (targets) are shown in Tables 46-48 and Figures 60-62, respectively. The competition between *P. ampullacea* and the three target species of snails had been demonstrated experimentally under the simulated field condition. The three target species, *B. glabrata* (Table 46, Fig. 61), *I. exustus* (Table 47, Fig.61) and *R. rubiginosa* (Table 48, Fig. 62) behaved in similar fashion when maintained with *P. ampullacea*, the control agent. During the first month, the target species started to lay egg masses. A few weeks later, the first sign of competition had appeared :

TABLE 46

Biological control study between *Pila ampullacea* (control) and *Biomphalaria glabrata* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Bg	Pa	Bg	Pa	Bg
0	50	0	25	25	0	100
2	50	0	25	20	0	100
4	48	0	25	20	0	95
6	48	0	25	20	0	990
8	48	0	25	18	0	353
10	44	0	25	9	0	189
12	40	0	25	9	0	361
14	40	0	23	9	0	273
16	38	0	23	9	0	345
18	38	0	22	9	0	131
20	38	0	22	9	0	251
22	38	0	22	9	0	734
24	38	0	22	9	0	1014
26	36	0	22	8	0	936
28	36	0	22	8	0	784
30	36	0	21	7	0	736
32	36	0	21	7	0	684

Pa = *Pila ampullacea*
Bg = *Biomphalaria glabrata*

Ratio of		*P. ampullacea*	:	*B. glabrata*
1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

TABLE 47

Biological control study between *Pila ampullacea* (control) and *Indoplanorbis exustus* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Ie	Pa	Ie	Pa	Ie
0	50	0	25	25	0	50
2	50	0	25	25	0	50
4	48	0	25	12	0	48
6	48	0	25	7	0	152
8	48	0	25	7	0	115
10	44	0	25	7	0	128
12	40	0	25	6	0	166
14	40	0	25	6	0	190
16	38	0	23	6	0	149
18	38	0	23	2	0	114
20	38	0	23	2	0	166
22	38	0	23	2	0	128
24	38	0	20	2	0	142
26	36	0	20	2	0	160
28	36	0	20	2	0	112
30	36	0	20	2	0	142
32	36	0	20	2	0	151

Pa = *Pila ampullacea*
Ie = *Indoplanorbis exustus*

Ratio of	*P. ampullacea*	:	*I. exustus*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 48

Biological control study between *Pila ampullacea* (control) and *Radix rubiginosa* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Rr	Pa	Rr	Pa	Rr
0	50	0	25	25	0	50
2	50	0	25	25	0	45
4	48	0	24	17	0	37
6	48	0	24	11	0	37
8	48	0	24	6	0	37
10	44	0	24	0	0	123
12	40	0	24	0	0	199
14	40	0	23	0	0	147
16	38	0	23	0	0	188
18	38	0	23	0	0	262
20	38	0	22	0	0	197
22	38	0	22	0	0	130
24	38	0	22	0	0	95
26	36	0	21	0	0	68
28	36	0	20	0	0	148
30	36	0	18	0	0	81
32	36	0	18	0	0	90

Pa = *Pila ampullacea*
Rr = *Radix rubiginosa*

Ratio of		*P. ampullacea*	:	*R. rubiginosa*
1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

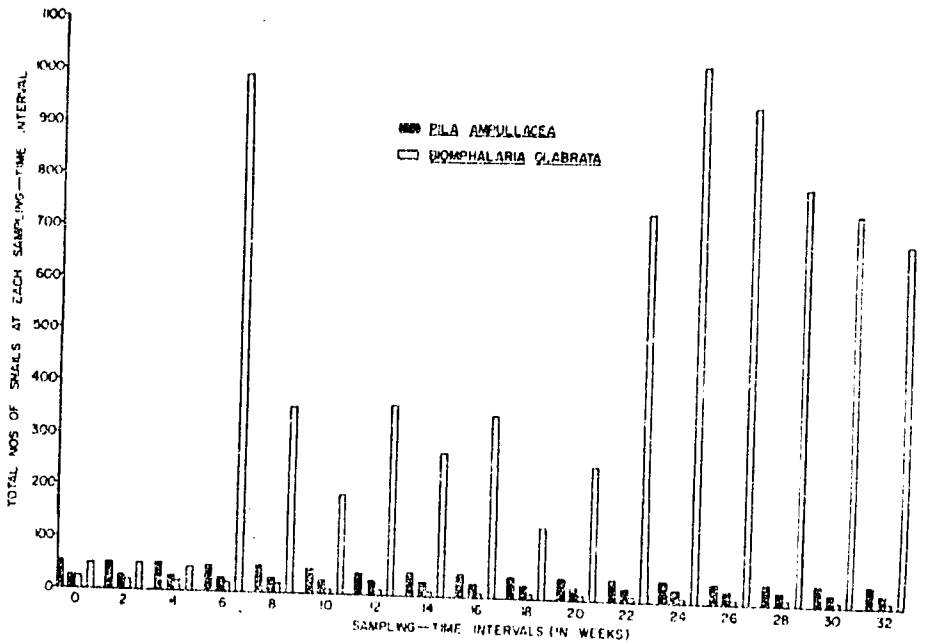


Fig. 60. Biological control study between *P. ampullacea* (control) and *B. glabrata* (target) under simulated field condition.

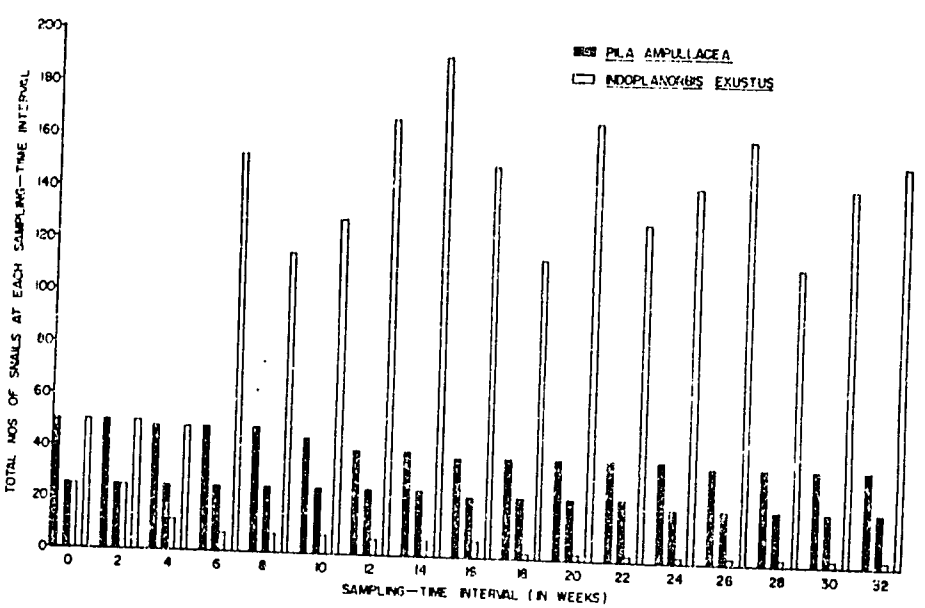


Fig. 61. Biological control study between *P. ampullacea* (control) and *I. exustus* (target) under simulated field condition.

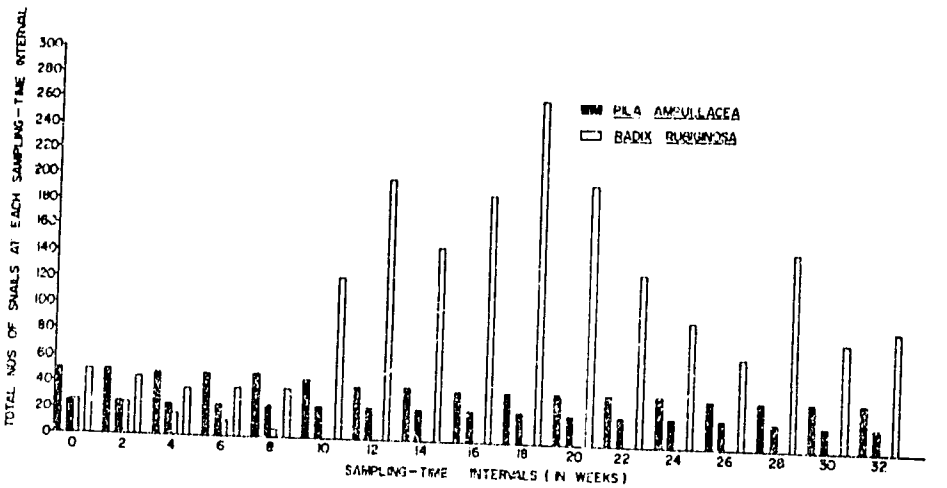


Fig. 62. Biological control study between *P. ampullacea* (control) and *R. rubiginosa* (target) under simulated field condition.

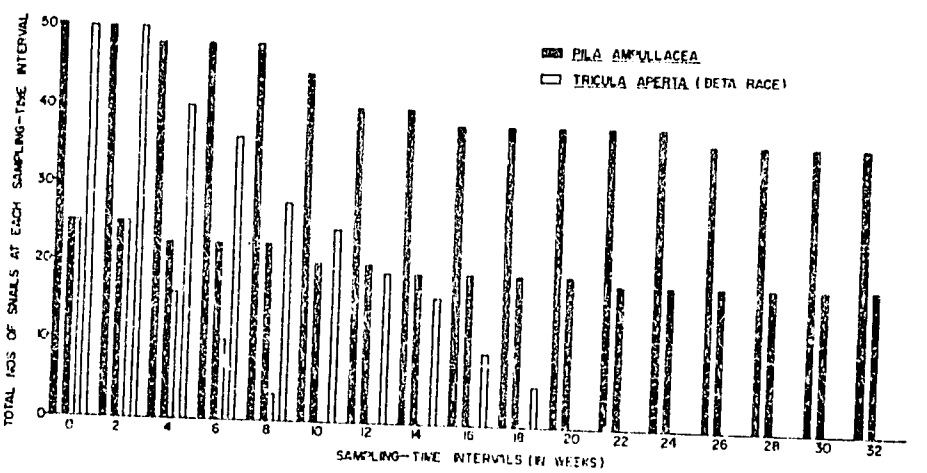


Fig. 63. Biological control study between *P. ampullacea* (control) and *T. aperta* (target) under simulated field condition.

clutches of eggs of all three target species failed to hatch, some disappeared, and the young of these snails began to die. After two months, it was noticed that all the younger snails had completely disappeared from the experimental tanks, and only a few of the original snails were still alive. In the case of *R. rubiginosa*, all of the adult snails died. By the end of the 8-month period, the numbers of the three target species were drastically reduced to the insignificant numbers for competition. On the contrary, the target species maintained alone were mostly alive with an increase in their numbers.

Based on the results obtained from these experiments, there is good reason to believe that *P. ampullacea* compete for food and cause interference with the hatching of egg masses, juvenile and adult target snails. They certainly devour egg masses of the target species causing the disappearance of the egg masses present. In addition, due to their large sizes, they may excrete certain waste products which might be lethal to the adult target snails.

Effects on prosobranch snails (*Tricula aperta*, *Oncomelania hupensis quadrasi* and *Bithynia siamensis goniomphalos*).

The results of the biological control studies between *P. ampullacea* (control) and *T. aperta*, *O.h. quadrasi* and *B.s. goniomphalos* are shown in Tables 49-51 and Figures 63-65, respectively. In the experimental tanks containing both

control and target snails together, all three target snails species had very high mortality, and did not reproduce. *T. aperta* were very sensitive to water and temperature condition and could not tolerate the simulated field condition. Hence, *T. aperta* maintained with *P. ampullacea* or alone both had high mortality rate and all died after 2-4 months (Table 49, Fig. 63). *P. ampullacea* did not seem to have any interaction with *O.h.quadrasi* (Table 50, Fig. 64). *P. ampullacea* could be found in the same natural habitat as *B.s. goniomphalos*, but the result here indicated no interaction since *B.s. goniomphalos* maintained alone did not reproduce and had a very high mortality rate (Table 51, Fig. 65).

The conclusions from the experiments on the competition between *P. ampullacea* and the pulmonate target snails species of *B. glabrata*, *I. exustus* and *R. rubiginosa* are that the competition results in a reduced growth and reproduction of the target species, and these effects are based on competition for food, physical interference between snails or predation on egg masses of the target species. On the contrary, *P. ampullacea* did not have any interaction with either *T. aperta or O.h. quadrasi*, the prosobranch snails. As for the other prosobranch snail, *B.s. goniomphalos*, no conclusion could be drawn. Further work on field-trial experiment under natural habitat should yield more conclusive results.

TABLE 49

Biological control study between *Pila ampullacea* (control) and *Tricula aperta* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Ta	Pa	Ta	Pa	Ta
0	50	0	25	25	0	50
2	50	0	25	25	0	50
4	48	0	22	16	0	40
6	46	0	22	10	0	36
8	48	0	22	3	0	28
10	44	0	20	0	0	24
12	40	0	20	0	0	19
14	40	0	19	0	0	16
16	38	0	19	0	0	9
18	38	0	19	0	0	5
20	38	0	19	0	0	0
22	38	0	18	0	0	0
24	38	0	18	0	0	0
26	36	0	18	0	0	0
28	36	0	18	0	0	0
30	36	0	18	0	0	0
32	36	0	18	0	0	0

Pa = *Pila ampullacea*
Ta = *Tricula aperta*

Ratio of	*P. ampullacea*	:	*T. aperta*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 50

Biological control study between *Pila ampullacea* (control) and *Oncomelania hupensis quadrasi* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Oq	Pa	Oq	Pa	Oq
0	50	0	25	25	0	50
2	50	0	25	25	0	40
4	48	0	25	18	0	32
6	48	0	24	10	0	30
8	48	0	22	4	0	29
10	44	0	20	0	0	19
12	40	0	20	0	0	16
14	40	0	19	0	0	15
16	38	0	19	0	0	12
18	38	0	19	0	0	11
20	38	0	19	0	0	11
22	38	0	19	0	0	11
24	38	0	19	0	0	11
26	36	0	18	0	0	11
28	36	0	18	0	0	11
30	36	0	18	0	0	10
32	36	0	18	0	0	10

Pa = *Pila ampullacea*
Oq = *Oncomelania hupensis quadrasi*

Ratio of		*P. ampullacea*	:	*O.h. quadrasi*
1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

TABLE 51

Biological control study between *Pila ampullacea* (control) and *Bithynia siamensis goniomphalos*(target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Bsg	Pa	Bsg	Pa	Bsg
0	50	0	25	25	0	50
2	50	0	25	25	0	50
4	48	0	25	25	0	48
6	48	0	25	18	0	48
8	48	0	20	12	0	44
10	44	0	20	7	0	38
12	40	0	20	0	0	26
14	40	0	20	0	0	24
16	38	0	19	0	0	24
18	38	0	19	0	0	21
20	38	0	19	0	0	17
22	38	0	18	0	0	13
24	38	0	18	0	0	12
26	36	0	18	0	0	12
28	36	0	18	0	0	11
30	36	0	18	0	0	10
32	36	0	18	0	0	10

Pa = *Pila ampullacea*
Bsg = *Bithynia siamensis goniomphalos*

Ratio of	*P. ampullacea*	:	*B.s. goniomphalos*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

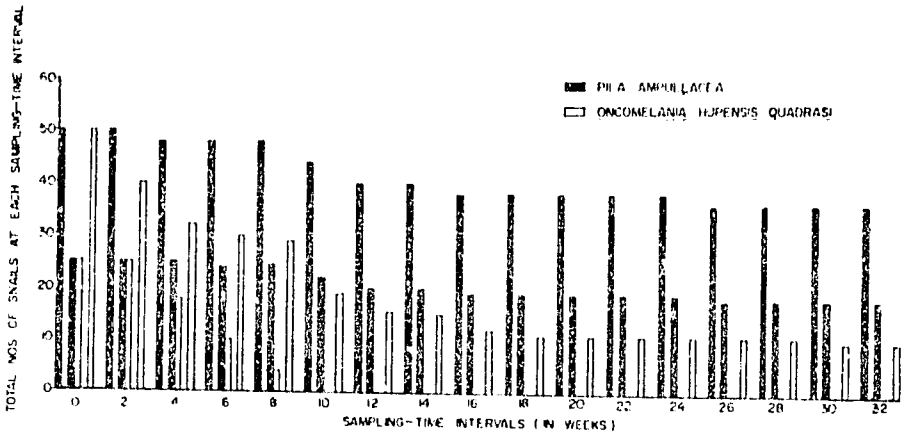


Fig. 64. Biological control study between *P. ampullacea* (control) and *O.h. quadrasi* (target) under simulated field condition.

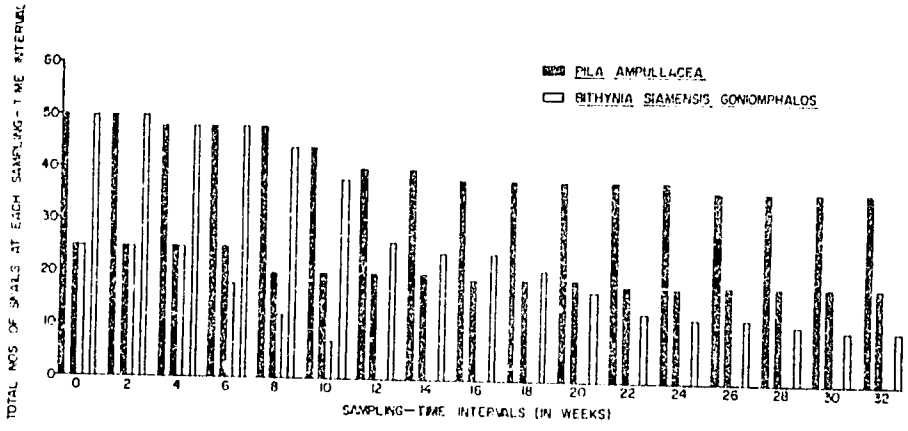


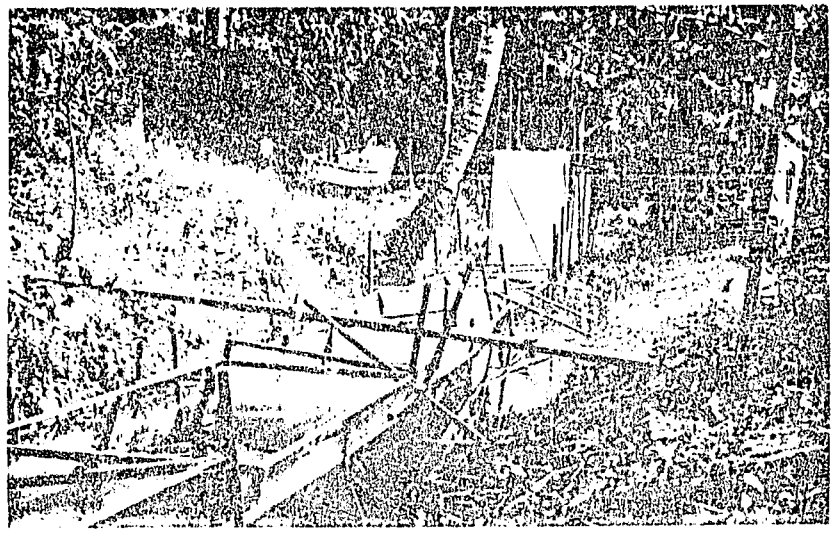
Fig. 65. Biological control study between *P. ampullacea* (control) and *B.s. goniomphalos* (target) under simulated field condition.

1.6 Experimental work on biological control of mollusks under a small scale field trial

According to the results obtained from the experimental work on biological control of mollusks in the laboratory and under simulated field conditions, we decided to choose *P. ampullacea* as the control agent for a small scale field trial experiment. *P. ampullacea* appeared to be a very suitable control agent due to their large size and their voracious habit. According to the data obtained from previous experiments, there was a reduction in growth and reproduction of the snail target species, such as *R. rubiginosa*, *I. exustus*, and *B. glabrata*. These effects were based on competition for food, physical interference between snails as predator on egg masses of the target species.

The study included only local target species of snails, such as *I. exustus*, *R. rubiginosa* and *B.s. goniomphalos*.

The small scale field trial experiment was conducted at a local field station outside Bangkok where there were natural canals. The canal selected was 15 meters long and 1 meter wide. It was divided into 7 partitions, each of the size of 2x1 squaremeters, by using the nylon net hang over the wooden frames. There were natural aquatic plants in the canal, such as water hyacinths, *Elodea* and water lettuce (Fig.66).



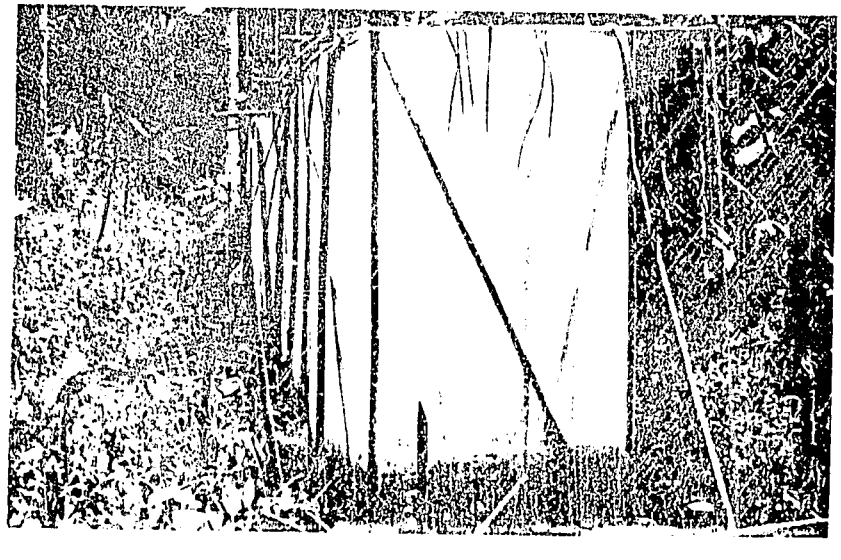


Fig.66 Natural canals used for testing biological control effects of target snails. Top : snails as control agents. Bottom : insects as control agents

The ratios between biological agent and snail target species were as follows:

First partition	100 *P. ampullacea*
	300 *I. exustus*
Second partition	100 *P. ampullacea*
	300 *R. rubiginosa*
Third partition	100 *P. ampullacea*
	300 *B.s. goniomphalos*
Fourth partition	100 *P. ampullacea*
Fifth partition	300 *I. exustus*
Sixth partition	300 *R. rubiginosa*
Seventh partition	300 *B.s. goniomphalos*

The general procedures, observation on mortality rates of both control and target species and the counting of the snail offspring were done biweekly. The original snails were marked with finger nail polish so that the offspring could be distinguished from them.

The experiments are now being under study. The results of the experiments thus far are shown in Tables 52-54.

Effects on pulmonate snails (*Indoplanorbis exustus* and *Radix rubiginosa*)

The result of the biological control study between *P. ampullacea*) (control) and *I. exustus* and *R. rubiginosa*

(target) are shown in Tables 52-53. The two target species, *I. exustus* and *R. rubiginosa* behaved in similar fashion. In the experimental partition containing 100 control snails and 300 target snails, it appeared that the target snails have decreased drastically in their numbers. The adult target snails had very high mortality rate. Even though they laid large numbers of eggs but only a few number of offsprings survived. At the end of 30th week, the number of control agent, *P. ampullacea* was 3-6 times over that of the target snails, *I. exustus* and *R. rubiginosa*. Target snails maintained alone behaved in similar fashion as that with the control agent. However, the number of offsprings produced was higher and they had better survivel rate than those maintained with *P. ampullacea*. *P. ampullacea* maintained alone were mostly alive with an increase in their numbers.

Effect on prosobranch snail (*Bithynia siamensis goniomphalos*)

The result of the biological control study between *P. ampullacea* (control) and *B.s. goniomphalos* (target) is shown in Table 54. In the experimental partition containing 100 *P. ampullacea* and 300 *B.s. goniomphalos*, the number of the target snails *B.s. goniomphalos* decreased drastically. They reproduced only a few number of offsprings. By the end of 30th week, the number of control agent, *P. ampullacea* was much higher than that of the target snail. *B.s. goniomphalos* maintained alone survived better and reproduced more than

TABLE 52

Biological control study between *Pila ampullacea* (control) and *Indoplanorbis exustus* (target) under small scale field trial.

Week	Number of snails					
	1		2		3	
	Pa	Ie	Pa	Ie	Pa	Ie
0	100	0	100	300	0	300
2	98	0	96	136	0	291
4	107	0	113	97	0	280
6	118	0	134	63	0	136
8	129	0	162	98	0	97
10	125	0	133	94	0	63
12	122	0	144	90	0	53
14	131	0	138	90	0	65
16	134	0	116	32	0	60
18	134	0	124	32	0	61
20	137	0	124	32	0	68
22	136	0	123	35	0	64
24	134	0	121	35	0	53
26	147	0	123	35	0	62
28	151	0	127	33	0	56
30	151	0	129	24	0	52

Pa = *Pila ampullacea*

Ie = *Indoplanorbis exustus*

Ratio	of	*P. ampullacea*	:	*I. exustus*
1	=	100	:	0
2	=	100	:	300
3	=	0	:	300

TABLE 53

Biological control study between *Pila ampullacea* (control) and *Radix rubiginosa* (target) under small scale field trial.

Week	Number of snails					
	1		2		3	
	Pa	Rr	Pa	Rr	Pa	Rr
0	100	0	100	300	0	300
2	98	0	98	249	0	286
4	107	0	97	201	0	252
6	118	0	154	174	0	224
8	129	0	244	92	0	130
10	125	0	202	80	0	132
12	122	0	179	68	0	133
14	131	0	191	40	0	90
16	134	0	149	35	0	90
18	134	0	156	76	0	71
20	137	0	152	38	0	68
22	136	0	152	66	0	49
24	134	0	160	54	0	118
26	147	0	151	[illegible]	0	100
28	151	0	142	34	0	96
30	151	0	139	48	0	72

Pa = *Pila ampullacea*

Rr = *Radix rubiginosa*

Ratio of *P. ampullacea* : *R. rubiginosa*

1 = 100 : 0

2 = 100 : 300

3 = 0 : 300

TABLE 54

Biological control study between *Pila ampullacea* (control) and *Bithynia siamensis goniomphalos* (target) under small scale field trial.

Week	Number of snails					
	1		2		3	
	Pa	Bsg	Pa	Bsg	Pa	Bsg
0	100	0	100	300	0	300
2	98	0	93	78	0	280
4	107	0	109	58	0	187
6	118	0	116	50	0	160
8	129	0	131	47	0	87
10	125	0	145	77	0	87
12	122	0	133	71	0	152
14	131	0	137	71	0	164
16	134	0	132	63	0	200
18	134	0	123	62	0	200
20	137	0	123	62	0	199
22	136	0	125	62	0	193
24	134	0	127	62	0	205
26	147	0	127	62	0	203
28	151	0	127	63	0	203
30	151	0	127	62	0	189

Pa = *Pila ampullacea*

Bsg = *Bithynia siamensis goniomphalos*

Ratio	of	*P. ampullacea*	:	*B.s. goniomphalos*
1	=	100	:	0
2	=	100	:	300
3	=	0	:	300

those with the control agent. *P. ampullacea* maintained alone are mostly alive with an increase in their numbers.

Based on the results obtained from these experiments, it is apparent that *P. ampullacea*, due to their large sizes, survive better than the small target snails. In addition, they may excrete certain waste products which might be lethal to the adult target snails.

This stage of experiments may take longer than one year for the population densities of the control agents and the target species will have to be studied regularly until the target species disappeared or are replaced by the snail control agents.

2. Insects

2.1 Cultivation and growth rates of sciomyzid flies.

Adults of *Sepedon plumbella* were collectd from natural habitats. A pair of male and female sciomyzid flies was reared in a 2000-ml glass jar containing a batch of rice plants. These rice plants were used as resting and oviposition sites in the breeding jars. Cotton wool was used in place of sphagnum moss to provide a moisture-retaining substrate in the breeding jars. 20 pairs were used for this study.

The adult insects were fed with 3 types of food : (1) crushed flesh of *Radix rubiginosa* snail, (2) crushed flesh of *R. rubiginosa* snail and honey bee syrup, (3) crushed flesh of *Indoplanorbis exustus*, and (4) crushed flesh of *I.*

exustus and honey bee syrup.

The results are shown in Tables 55 and 56 and Fig. 67. The life durations of eggs, larvae, pupae and adults were 2.5, 13.5, 9.0 and 33.0 days, respectively, when they were fed with crushed flesh of *R. rubiginosa* and honey bee syrup. The oviposition period was 139.5 days, and a female laid approximately 1069.8 eggs. The life span of male and female flies was 138 and 129 days, respectively (Table 55). The mean numbers of eggs laid per female fly were 489.5, 1069.8, 392.7 and 395.7, respectively, when they were fed with flesh of *R. rubiginosa* alone, with flesh of *R. rubiginosa* and honey bee syrup, with flesh of *I. exustus* alone, and with flesh of *I. exustus* and honey bee syrup (Table 56). The survival rates from egg to adult were higher in groups of flies fed with flesh of snails and honey bee syrup than those fed with flesh of snails alone (Table 56).

2.2 Experimental work on biological control of mollusks using sciomyzid fly larvae in the laboratory

Sciomyzid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4, as follows:

Ratios of sciomyzid fly larvae to snails

4:0 3:1 2:2 1:3 0:4

Numbers of sciomyzid fly larvae to snails

40:0 30:10 20:20 10:30 0:40

TABLE 55

Life history for *Sepedon plumbella*, reared with crushed flesh of *Radix rubiginosa* snail and honey bee syrup.

Developmental stages	Life duration (days) Mean ± S.D. (range)	Average size (mm) Length ± S.D. (range)	Width ± S.D. (range)
Eggs	2.5 ± 1.29 (1-4)	1.08 ± 0.09 (0.95-1.2)	0.34 ± 0.15 (0.32-0.36)
First stadium	4.5 ± 1.29 (3-6)	1.13 ± 0.08 (1.00-1.25)	0.31 ± 0.01 (0.30-0.33)
Second stadium	4.5 ± 1.29 (3-6)	3.25 ± 0.16 (3.0-3.5)	0.8 ± 0.15 (0.6-1.1)
Third stadium	4.5 ± 1.29 (2-7)	8.5 ± 0.39 (8.0-9.0)	2.25 ± 0.18 (2.0-2.5)
Pupae	9.0 ± 3.31 (4-14)	5.5 ± 0.3 (5.0-6.0)	2.25 ± 0.18 (2.0-2.5)
Adults : Female	32.0 ± 4.47 (24-39)	10.5 ± 0.25 (9.0-12.0)	1.97 ± 0.35 (1.8-2.2)
Male	34.0 ± 3.89 (28-40)	12.0 ± 1.58 (10.0-14.0)	2.14 ± 0.17 (1.90-2.35)
One complete generation	33.6 ± 2.58 (25-45)		
Pre-oviposition period	13.5 ± 5.33 (9-22)		
Oviposition period	139.5 ± 9.66 (9-270)		
Frequency of egg laying/female	53.2 ± 4.07 (20-75)		
No. of eggs/batch	15.1 ± 7.67 (3-27)		
No. of eggs/female	1069.8 ± 69.2 (128-2450)		
Adult longevity : Female	30.8 ± 3.12 (25-68)		
Male	47.4 ± 2.94 (32-60)		
Life span : Female	129 ± 7.43 (32-210)		
Male	138 ± 8.39 (32-270)		

TABLE 56

Life history for *Sepedon plumbella* reared with various types of food

Developmental stage	Types of food			
	Flesh of *R. rubiginosa*		Flesh of *I. exustus*	
	alone	with honey bee syrup	alone	with honey bee syrup
Oviposition rate of female with a complete cycle of development (mean number of eggs per female ± S.D.)				
	489.5 ± 151.25	1069.8 ± 69.2	392.75 ± 12.5	395.75 ± 32.7
Survival rate of each developmental stage, reared from eggs to adults (%)				
Eggs	100	100	100	100
First instar	71	94	65	81
Second instar	55	78	51	70
Third instar	54	64	48	67
Pupae	42	59	32	59
Adults	31	57	25	51

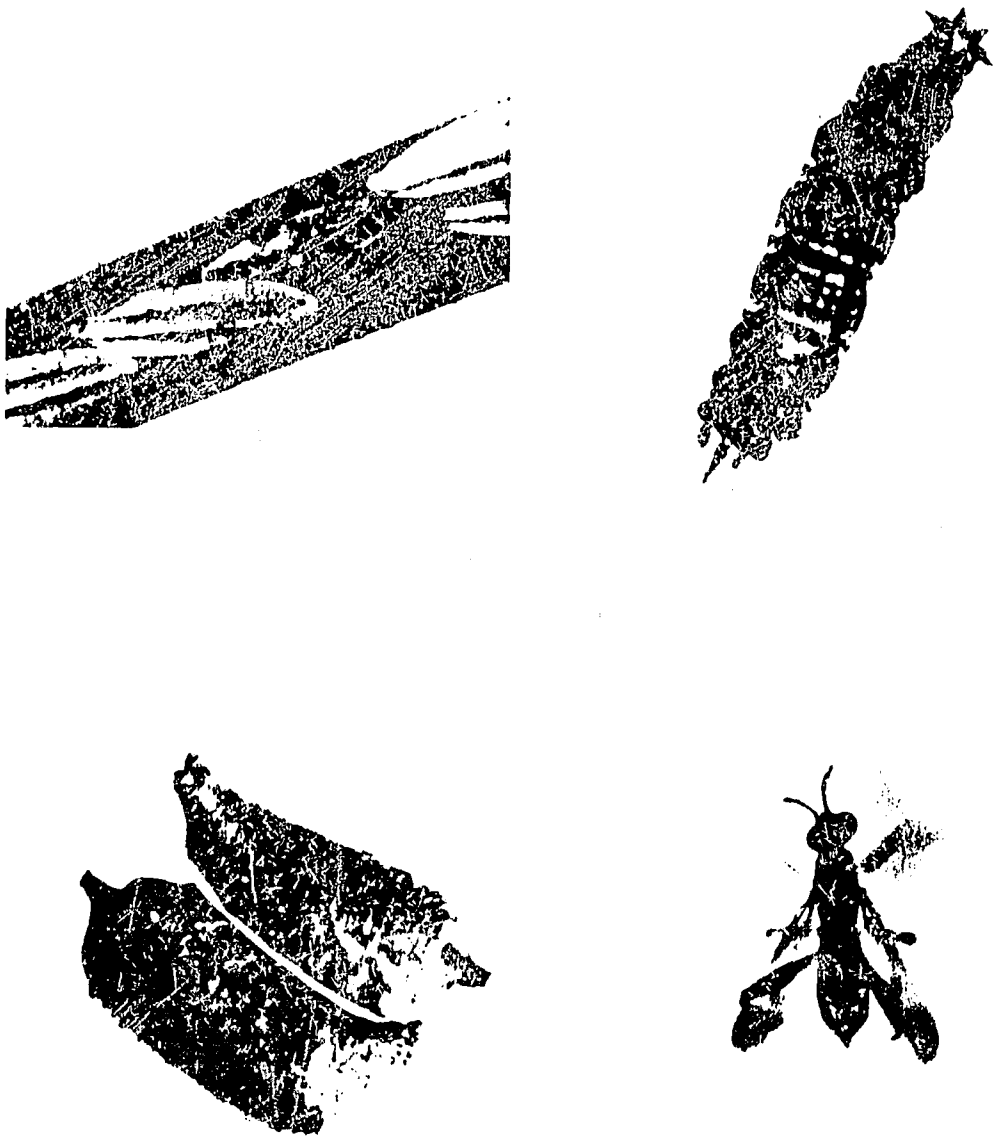


Fig.67. Showing various stages of sciomyzid fly (*Sepedon plumbella*). Eggs on rice plant, larva, pupae and adult (female).

Each experiment was replicated three times. The experiment was performed using 2000-ml glass jars.

Table 57 shows results of biological control study between sciomyzid fly larvae and snails. It can be summarized as follows: (1) the sciomyzid fly larvae were effective in controlling pulmonate snails only, (2) it was better to use the third stage sciomyzid fly larvae because its survival rate was higher than the second stage, and (3) the ratio of 3 sciomyzid fly larvae to 1 snail rendered the best result.

2.3 Experimental work on biological control of mollusks using sciomyzid fly larvae under simulated field conditions

Sciomyzid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3, and 0:4, under simulated field conditions, as follows:

Ratios of sciomyzid fly larvae to snails

4:0 3:1 2:2 1:3 0:4

Numbers of sciomyzid fly larvae to snails

400:0 300·100 200:200 100:300 0:400

The experiment was performed in cement tanks with the diameter of 80 cm and the height of 35 cm. The tanks were covered with nylon net and were aerated and furnished with water plants to simulate natural habitats (Fig. 68).

The results of biological control testings between sciomyzid fly larvae (control agent) and pulmonate and

TABLE 57

Biological control study between sciomyzid fly larvae (control) and snails (target). L = sciomyzid fly larvae, S = snails.

Snail species	Stage of sciomyzid fly larvae	Death (days)	Mortality rates (%) Ratio of sciomyzid fly larvae (L) to snail (S)									
			L : S 4 0	L : S 3 1	L : S 2 2	L : S 1 3	L : S 0 4					
Pulmonate snails												
Biomphalaria glabrata	2nd	5	27.5	-	25.0	75.0	27.5	57.5	2.5	47.5	-	0
	3rd	4	0	-	10.0	100	4.7	81.8	9.1	77.8	-	0
Bulinus (P.) abyssinicus	2nd	4	27.5	-	25.0	100	20.0	75.0	25.0	50.0	-	0
	3rd	3	0	-	0	100	0	75.0	25.0	83.3	-	0
Indoplanorbis exustus	2nd	4	10.0	-	14.0	80.0	20.0	62.5	22.5	49.1	-	0
	3rd	3	0	-	0	100	0	87.5	0	66.7	-	0
Radix rubiginosa	2nd	4	0	-	8.3	25.0	0	77.3	9	56.0	-	0
	3rd	4	25.0	-	15.0	100	2.5	87.5	0	58.3	-	0
Operculate snails												
Bithynia s. goniomphalos	2nd	7	0	-	5.0	15.0	1.5	4.0	0	2.0	-	0
	3rd	9	0	-	9.0	22.0	2.0	8.0	0	5.0	-	0
Oncomelania h. quadrasi	2nd	3	27.5	-	13.3	0	25.0	0	30.0	0	-	0
	3rd	5	6.3	-	16.7	0	20.0	0	10.0	0	-	0
Tricula aperta	2nd	3	25.0	-	23.3	0	27.5	0	30.0	0	-	0
	3rd	5	25.0	-	16.7	0	50.0	0	10.0	0	-	0
Robertsiella kaporensis	2nd	3	18.8	-	20.0	0	22.5	0	20.0	0	-	0
	3rd	5	12.5	-	13.3	0	25.0	0	15.0	0	-	0

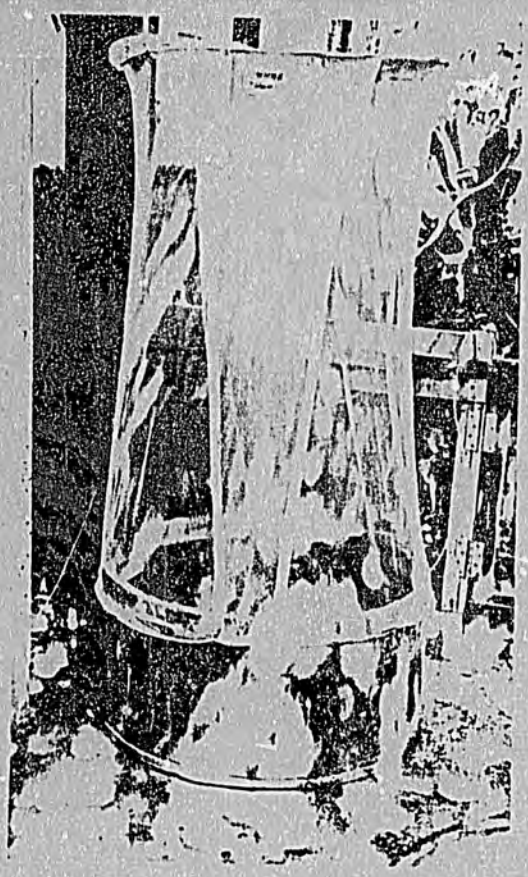


Fig.68. Left : cement tank used for testing biological control effects of target snails by sciomyzid fly larvae and lampyrid fly larvae. Right : a lampyrid fly larva attacking an operculate snail, *B.s. goniomphalos*.

operculate snails (target species) are shown in Tables 58-61.

Tables 58 and 59 show, respectively, the effects of the second and third stage larvae of sciomyzid fly *S. plumbella* on pulmonate snails. The sciomyzid fly larvae caused higher mortality rates in juvenile than in adult snails. The best result of snail control by sciomyzid fly larvae was obtained at the ratio of 1:3 (snails : sciomyzid fly larvae), i.e. from 88% to 100% for juvenile snails and from 70% to 98% for adult snails.

Table 60 and 61 show, respectively, the effects of the second and third stage larvae of sciomyzid fly *S. plumbella* on operculate snails. The sciomyzid fly larvae did not have much effect on operculate snails, possibly owing to the fact that the operculate snails possess opercula which can be used to prevent any attack from the fly larvae. In addition, the fly larvae can be killed when the snail closes its operculum.

2.4 Experimental work on biological control of mollusks using lampyrid fly larvae in the laboratory

Lampyrid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4, under laboratory conditions, as follows:

Ratios of lampyrid fly larvae to snails				
4:0	3:1	2:2	1:3	0:4
Numbers of lampyrid fly larvae to snails				
40:0	30:10	20:20	10:30	0:40

TABLE 58

Results of biological control testings between second instar larvae (L2) of sciomyzid fly (control agent) and pulmonate snails (target species) under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L2)									
	Death (days)	Sn:L2 4:0	Death (days)	Sn:L2 3:1	Death (days)	Sn:L2 2:2	Death (days)	Sn:L2 1:3	Death (days)	Sn:L2 0:4
B. glabrata										
juvenile	28	7.5:0.0	28	47.5:2.0	28	70.0:10.0	28	90.0:15.8	28	0.0:5.0
adult	28	6.0:0.0	28	42.2:2.0	28	51.0:13.0	28	70.0:17.3	28	0.0:8.5
B. (P.) abyssinicus										
juvenile	12	6.5:0.0	9	43.6:14.0	4	83.0:14.0	4	94.0:16.5	14	0.0:3.5
adult	14	5.0:0.0	10	80.9:18.0	8	72.0:16.0	5	88.0:17.8	16	0.0:4.5
I. exustus										
juvenile	21	7.5:0.0	21	46.9:14.0	21	73.5:15.0	21	90.0:9.6	21	0.0:7.25
adult	28	7.0:0.0	28	39.5:16.0	28	59.5:17.5	28	72.0:8.6	28	0.0:9.5
R. rubiginosa										
juvenile	14	9.5:0.0	14	57.1:8.0	14	81.5:0.0	14	93.0:6.9	14	0.0:5.5
adult	21	7.0:0.0	21	50.5:6.0	21	72.0:0.0	21	81.0:5.9	21	0.0:7.0

TABLE 59

Results of biological control testings between second instar larvae (L3) of sciomyzid fly (control agent) and pulmonate snails (target species) under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L3)									
	Death (days)	Sn:L3 4:0	Death (days)	Sn:L3 3:1	Death (days)	Sn:L3 2:2	Death (days)	Sn:L3 1:3	Death (days)	Sn:L3 0:4
B. glabrata										
juvenile	28	8.0:0.0	28	69.5:15.0	28	75.0:8.0	28	90.0:15.0	28	0.0:10.5
adult	28	6.0:0.0	28	55.0:12.0	28	70.0:10.0	28	88.0:12.0	28	0.0:10.0
B. (P.) abyssinicus										
juvenile	12	4.5:0.0	9	69.3:12.0	7	87.5:10.2	4	100:5.4	14	0.0:10.0
adult	14	6.3:0.0	10	72.5:9.0	8	89.5:5.6	5	98.5:5.6	16	0.0:9.5
I. exustus										
juvenile	21	6.3:0.0	21	65.5:0.0	21	68.0:12.0	21	83.0:15.0	21	0.0:10.3
adult	28	6.3:0.0	28	60.5:2.0	28	65.0:14.0	28	92.0:18.0	28	0.0:10.8
R. rubiginosa										
juvenile	14	9.8:0.0	14	68.2:15.0	14	90.5:14.2	10	97.0:10.2	14	0.0:8.7
adult	21	9.3:0.0	21	62.8:11.0	21	87.0:34.0	14	96.0:15.1	21	0.0:10.3

TABLE 60

Results of biological control testings between second instar larvae (L2) of sciomyzid fly (control agent) and pulmonate snails (target species) under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L2)									
	Death (days)	Sn:L2 4:0	Death (days)	Sn:L2 3:1	Death (days)	Sn:L2 2:2	Death (days)	Sn:L2 1:3	Death (days)	Sn:L2 0:4
B.s. goniomphalos										
juvenile	28	10.0:0.0	28	23.5:15.0	21	28.0:12.0	28	30.0:6.7	28	0.0:10.5
adult	30	11.0:0.0	30	18.0:17.0	21	15.0:15.0	30	20.0:18.6	30	0.0:10.5
O.h. quadrasi										
juvenile	18	9.0:0.0	15	5.0:25.0	8	8.0:8.5	10	15.0:20.0	18	0.0:12.0
adult	21	10.0:0.0	17	4.0:30.0	9	8.0:7.5	12	10.0:12.5	21	0.0:11.0
R. kaporensis										
juvenile	18	10.0:0.0	18	6.7:2.5	18	10.0:5.0	18	30.0:16.7	18	0.0:11.0
adult	21	10.0:0.0	21	13.3:2.5	21	15.0:10.0	21	20.0:16.7	21	0.0:9.0
T. aperta										
juvenile	27	5.0:0.0	30	6.7:10.0	27	14.0:5.0	27	21.0:6.7	27	0.0:10.0
adult	35	5.0:0.0	37	6.7:20.0	35	10.0:5.0	35	15.0:13.3	35	0.0:11.0

TABLE 61

Results of biological control testings between second instar larvae (L3) of sciomyzid fly (control agent) and pulmonate snails (target species) under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L3)									
	Death (days)	Sn:L3 4:0	Death (days)	Sn:L3 3:1	Death (days)	Sn:L3 2:2	Death (days)	Sn:L3 1:3	Death (days)	Sn:L3 0:4
B.s. goniomphalos										
juvenile	28	12.0:0.0	28	26.7:8.0	21	27.0:10.0	14	30.0:6.7	28	0.0:10.5
adult	30	14.0:0.0	30	23.3:12.0	21	24.0:12.0	14	26.0:8.6	30	0.0:8.5
O.h. quadrasi										
juvenile	18	15.0:0.0	15	16.7:30.0	8	20.0:8.5	10	20.0:23.3	18	0.0:10.5
adult	21	12.5:0.0	17	16.7:27.0	9	18.0:15.5	12	19.0:18.6	21	0.0:10.0
R. kaporensis										
juvenile	18	15.0:0.0	18	16.7:5.0	18	18.0:10.0	18	30.0:13.3	18	0.0:12.5
adult	21	12.5:0.0	21	13.3:5.0	21	14.0:5.0	21	20.0:6.7	21	0.0:12.5
T. sperta										
juvenile	27	7.4:0.0	30	10.0:36.0	27	12.0:10.0	27	18.0:15.6	27	0.0:12.0
adult	35	5.0:0.0	37	6.7:40.0	35	10.0:10.0	35	10.0:17.5	35	0.0:10.0

The experiment was performed in 2,000-ml glass jars.

The results of biological control testings between lampyrid fly larvae (control agent) and pulmonate and operculate snails (target species) are shown, respectively, in Tables 62 and 63. The lampyrid fly larvae were more effective in killing snails than the sciomyzid fly larvae. The size of the lampyrid fly larvae was larger, and the larvae were seldom killed while attacking any operculate snails. The lampyrid fly larvae caused high mortality rates at all ratios of pulmonate snails, but were less successful with operculate snails (Figs. 68 and 69).

2.5 Experimental work on biological control of molluaks using lampyrid fly larvae under simulated field conditions

Lampyrid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4, under simulated field conditions (conditions are same as in Section 2.3), as follows:

Ratios of lampyrid fly larvae to snails

4:0	3:1	2:2	1:3	0:4

Numbers of lampyrid fly larvae to snails

200:0	150:50	100:100	50:150	0:200

The results of biological control testings between lampyrid fly larvae (control agent) and pulmonate and

TABLE 62

Results of biological control testings between lampyrid fly larvae (control agent) and pulmonate snails (target species) under laboratory conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to lampyrid fly larvae (La)									
	Death (days)	Sn:La 4:0	Death (days)	Sn:La 3:1	Death (days)	Sn:La 2:2	Death (days)	Sn:La 1:3	Death (days)	Sn:La 0:4
B. glabrata										
juvenile	14	5.0:0.0	17	86.6:0.0	14	90.0:5.0	9	100:0.0	14	0.0:10.0
adult	18	5.0:0.0	20	83.3:0.0	18	80.0:5.0	12	90.0:3.3	18	0.0:10.0
B. (P.) abyssinicus										
juvenile	14	7.5:0.0	14	83.3:0.0	12	85.0:0.0	9	90.0:3.3	14	0.0:5.0
adult	18	5.0:0.0	21	76.6:0.0	16	80.0:0.0	12	90.0:3.3	18	0.0:5.0
I. exustus										
juvenile	14	2.5:0.0	14	96.6:0.0	10	85.0:0.0	9	90.0:3.3	14	0:0.7.5
adult	18	2.5:0.0	18	93.2:0.0	15	80.0:0.0	12	80.0:6.6	18	0.0:5.0
R. rubiginosa										
juvenile	7	10.5:0.0	14	96.6:0.0	7	100:0.0	7	0.0:0.0		
adult	9	10.0:0.0	21	86.6:0.0	9	95.0:0.0	9	100:6.7	9	0.0:0.0

TABLE 63

Results of biological control testings between lampyrid fly larvae (control agent) and pulmonate snails (target species) under laboratory conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to lampyrid fly larvae (La)									
	Death (days)	Sn:La 4:0	Death (days)	Sn:La 3:1	Death (days)	Sn:La 2:2	Death (days)	Sn:La 1:3	Death (days)	Sn:La 0:4
B.s. goniomphalos										
juvenile	28	10.0:0.0	28	23.3:0.0	28	36.7:0.0	28	50.0:0.0	28	0.0:0.0
adult	35	7.5:0.0	34	19.9:10.0	35	23.3:0.0	35	40.0:13.3	35	0.0:0.0
O.h. quadrasi										
juvenile	28	9.5:0.0	28	29.0:0.0	28	35.0:0.0	28	40.0:0.0	28	0.0:0.0
adult	35	10.0:0.0	35	16.3:0.0	35	30.0:0.0	35	30.0:0.0	35	0.0:2.5
R. kaporensis										
juvenile	28	10.5:0.0	28	23.3:0.0	28	30.0:0.0	28	40.0:3.3	28	0.0:0.0
adult	35	7.5:0.0	35	16.7:10.0	35	30.0:0.0	35	30.0:3.3	35	0.0:5.0
T. aperta										
juvenile	28	7.5:0.0	28	26.6:10.0	28	30.0:0.0	28	40.0:6.6	28	0.0:0.0
adult	35	10.0:0.0	35	13.3:10.0	35	25.0:0.0	35	30.0:0.0	35	0.0:0.0

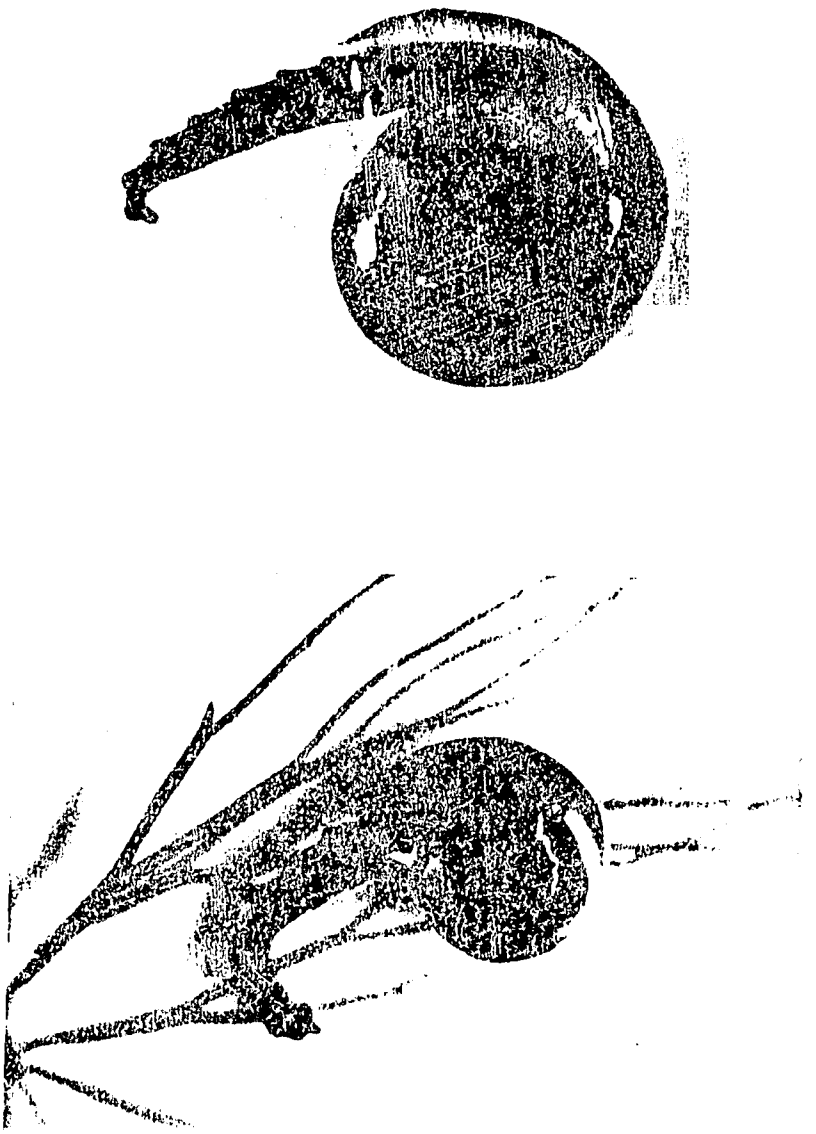


Fig.69. Lampyrid fly larva attacking *B. glabrata* (intermediate host snail of *S. mansoni*) and *I. exustus* (intermediate host snail of *S. spindale*).

operculate snails (target species) under simulated field conditions are shown in Table 64.

As shown in the laboratory, the lampyrid fly larvae were more effective in killing pulmonate snails than operculate snails. The best result of snail control by lampyrid fly larvae was obtained at the ratio of 1:3 (snails : lampyrid fly larvae).

The lampyrid fly larvae were more efficient in controlling snails than the sciomyzid fly larvae.

2.6 Experimental work on biological control of mollusks using sciomyzid fly larvae under field conditions.

Sciomyzid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 under field conditions, as follows:

Ratios of sciomyzid fly larvae to snails

4:0	3:1	2:2	1:3	0:4

Numbers of sciomyzid fly larvae to snails

400:0	300:100	200:200	100:3000	0:400

The experiment was conducted at a local field station outside Bangkok where there were natural canals. Each of the cages used measured 1 m long, 1 m wide and 2 m high. Nylon net was used to cover the wooden frame cages. Part of the cage (30 cm) was placed under the water (Fig.66).

The results of biological control testings between sciomyzid fly larvae (control agent) and pulmonate and

TABLE 64

Results of biological control testings between lampyrid fly larvae (control agent) and pulmonate snails (target species) under similated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to lampyrid fly larvae (La)									
	Death (days)	Sn:La 4:0	Death (days)	Sn:La 3:1	Death (days)	Sn:La 2:2	Death (days)	Sn:La 1:3	Death (days)	Sn:La 0:4
Pulmonate snails										
B.glabrata	12	5.0:0	12	38.9:0	12	82.0:0	12	70.0:0	12	0:0
B. (P.) abyssinicus	12	2.5:0	12	35.9:0	12	81.0:0	12	92.0:0	12	0:0
I. exustus	10	2.5:0	10	38.9:0	10	87.0:0	10	100:0	10	0:0
R. rubiginosa	10	0:0	10	40.2:0	10	93.0:0	10	100:0	10	0:0
Operculate snails										
B.s. goniomphalos	16	12.0:0	16	16.6:0	16	42.0:0	16	68.0:0	16	0:0
O.h. quadrasi	16	10.0:0	16	8.7:0	16	30.0:0	16	58.0:0	16	0:0
T. aperta	16	8.0:0	16	6.7:0	16	20.0:0	16	44.0:0	16	0:0
R. kaporensis	16	10.0:0	16	8.0:0	16	22.0:0	16	36.0:0	16	0:0

operculate snails (target species) under field conditions are shown in Table 65. At the ratio of 1:3 (snails to sciomyzid fly larvae), the best results of snail control by sciomyzid fly larvae were obtained. And at this same ratio, the mortality rate of pulmonate snails was much higher than that of operculate snails.

2.7 Experimental work on biological control of mollusks using lampyrid fly larvae under field conditions.

Lampyrid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3 and 0:4 under field conditions, as follows:

Ratios of lampyrid fly larvae to snails

4:0	3:1	2:2	1:3	0:4

Numbers of lampyrid fly larvae to snails

400:0	300:100	200:200	100:300	0:400

The experiment was performed in similar manner as that mentioned in Section 2.6.

The results of biological control testings between lampyrid fly larvae (control agent) and pulmonate and operculate snails (target species) under field conditions are shown in Table 66. The lampyrid fly larvae caused a higher mortality rate in pulmonate snails than in operculate snails. The best result of snail control by lampyrid fly larvae was obtained at the ratio of 1:3.

TABLE 65

Results of biological control testings between sciomyzid fly larvae (control agent) and pulmonate and operculate snails (target species) under field conditions.

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L2-L3)									
	Death (days)	Sn:L2 4:0	Death (days)	Sn:L2 3:1	Death (days)	Sn:L2 2:2	Death (days)	Sn:L2 1:3	Death (days)	Sn:L2 0:4
Pulmonate snails										
I. exustus	10	3.0:0	10	42.0:2.0	10	64.0:8.0	10	72.0:5.0	10	0:11.0
R. rubiginosa	10	2.0:0	10	53.0:3.0	10	72.0:15.0	10	79.0:7.0	10	0:5.0
Operculate snails										
B.s. goniomphalos	14	5.0:0	16	30.0:18.0	16	38.0:22.0	16	58.0:41.0	16	0:12.0
T. aperta	12	3.0:0	12	32.0:18.0	12	23.0:11.0	12	30.0:24.0	12	0:8.0

TABLE 66

Results of biological control testings between lampyrid fly larvae (control agent) and pulmonate and operculate snails (target species) under field conditions.

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to lampyrid fly larvae (La)									
	Death (days)	Sn:La 4:0	Death (days)	Sn:La 3:1	Death (days)	Sn:La 2:2	Death (days)	Sn:La 1:3	Death (days)	Sn:La 0:4
Pulmonate snails										
I. exusius	10	4.0:0	10	58.0:4.0	10	68.0:6.0	10	77.0:4.0	10	0:2.0
R. rubiginosa	10	3.0:0	10	61.0:4.0	10	78.0:5.0	10	83.0:5.0	10	0:0
Operculate snails										
B.s. goniomphalos	14	7.0:0	14	42.0:2.0	14	45.0:8.0	14	59.0:5.0	14	0:4.0
T. aperta	12	4.0:0	12	35.0:3.0	12	39.0:6.5	12	43.0:2.0	12	0:3.0

B. Genetics and susceptibility studies

1. Susceptibility aspect

Susceptibility of *Oncomelania hupensis quadrasi* from different geographic locations to infection with geographic strains of *Schistosoma japonicum*.

Four groups of *O.h. quadrasi* snails from different locations in the Philippines were used, i.e. from Palo, Leyte Island; from Mindoro Island; from Irosin, Luzon Island and from Mindanao Island. The snails were collected from the respective areas in the Philippines and were examined for natural schistosome infections prior to use by shedding. The negative snails were isolated and examined for cercarial shedding for another 2 months before use as breeders in the laboratory.

Snail infections were done by exposing individual laboratory-bred snails to 4-6 miracidia of *S. japonicum* of different geographic strains. Natural snail hosts were infected at the same time to serve as controls.

Three geographic strains of the parasites were available for use; the Mindoro, Leyte and Mindanao strains. The parasites were maintained in the laboratory by passage through white mice and the natural snail hosts. The mice were usually exposed to infection with 40 cercariae and were killed 6-7 weeks post infection.

The total numbers of snails for each group exposed to infection was variable because of the supply limitation.

Snails from Mindanao Island were limited due to the difficulty of obtaining snails. *S. japonicum* strain from Irosin was lacking due to the scarcity of infected snails in the area. All surviving snails were examined for cercariae 8 weeks after exposure to miracidia by shedding.

The results of the experiment are shown in Table 67. It can be concluded that strain of *S. japonicum* from Mindoro was able to develop in all geographical strains of snail host, *O.h. quadrasi*. It developed best in *O.h. quadrasi* from Irosin (28.97%), then Mindoro (20%), Mindanao (13.75%) and poorest in Leyte snails (3.5%). The strain of *S. japonicum* from Leyte was able to develop in the snails from Irosin (60.49%), Leyte (12.1%), Mindanao (8.57%) and Mindoro (7.14%). The strain of *S. japonicum* from Mindanao developed best in its own snails, *O.h. quadrasi* from Mindanao (51.43%). It developed poorly in *O.h. quadrasi* from Irosin, from Mindoro (2.86%) and from Leyte (0.90%).

2. Genetic aspect

2.1 Genetic variation in *Oncomelania hupensis hupensis* and *O.h. quadrasi*.

Oncomelania hupensis quadrasi was characterized on the basis of 7 field-collected samples representing four Philippine islands and one laboratory stock of mixed origin (Table 68). *O.h. hupensis* was characterized on the basis of a stock derived from three large samples from Guichi on the Yangtze River about 400 km west of Shanghai. The field-collected snails were maintained in our laboratory at the

TABLE 68

Samples of *Oncomelania hupensis* studied.

Sample name	Locality	Collection	N	Voucher*
O. hupensis quadrasi				
Leyte A	Vicob Ck., Palo	6.83	>1000	102
Leyte B	Mandaraq Ck., Palo	"	"	101
Leyte C	South Main Canal, Palo	"	"	103
Luzon A	San Isidro, Sorsogon	6.86	200	105
Luzon B	San Augustin, Sorsogon	"	50	106
Mindoro	Victoria	6.83	"	100
Mindoro	Mawab, Davao	8.84	500	107
Lab Stock	Palo and Victoria	'79 & ' 82	>1000	-
O. hupensis hupensis				
China A	Guichi, Anhui	4.82	>500	110
China B	guichi, Anhui	2.84	>400	-

* Voucher specimens were placed in the museum at the Center for Applied Malacology and Entomology, Mahidol University and were catalogued with the prefix MUFS-PHOO-# for Philippine samples and MUFS-CHOO-# for Chinese.

Center for Applied Malacology and Entomology, Faculty of Science, Mahidol University until they were frozen on dry ice, carried to San Diego, and held at -70 ^{o}C until analysis.

Our electrophoretic techniques are described in general terms elsewhere (Woodruff, 1985; Mulvey and Vrijenhoek, 1981). Individual snails were thawed and quickly homogenized whole in 0.1 ml of grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP,pH 7.0) with a glass rod. The homogenate was centrifuged at 10,000 g for 2 minutes and the supernatant was absorbed onto Whatman no. 3 chromatography paper wicks (3x9 mm) and inserted into 12% horizontal gels made of Sigma starch (Sigma Chemical Co., St. Louis, Missouri). electrophoresis was carried out for 15 h. by which time a bromophenol blue marker dye had migrated 100-120 mm anodally. Four to five slices were then cut from each gel and each slice was stained for a specific enzyme following standard methods (Shaw and Prasad, 1970; Harris and Hopkinson, 1977). Electrophoretic conditions for the resolution of the 21 allozymes reported here are described in Talbe 69. The esterase substrate was alpha-naphthyl acetate and the peptidase substrate was leucyl-alanine. Snails from different samples were run on each gel to facilitate comparisons: isozymes were numbered, and allozymes were assigned superscript letters a,b....., in order of decreasing anodal mobility of the primary electromorph. Commonly used enzyme abbreviations are typeset in capital letters to indicate the protein and in italics to indicate

TABLE 69

Electrophoretic buffers giving optimal resolution of proteins in *oncomelania hupensis*.

Enzyme (E.C. #)	Abbreviation	Buffer*
Acid phosphatase (3.1.3.2)	ACP	TC 2
Aspartate aminotransferase (2.6.1.1)	AAT	TBE, LiOH
Catalase (1.11.1.6)	CAT	AP
Esterase (3.1.1.1)	ES-1	TBE
	ES-3	TBE, LiOH
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6PDH	TBE, LiOH
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	GAP	AP
-Glycerophosphate dehydrogenase (1.1.1.8)	GPDH	TC-2, TC-1
Glucose phosphate isomerase (5.3.1.9)	GPI	TC-1 AP
Isocitrate dehydrogenase (1.1.1.42)	IDH-1	TC-2
	IDH-2	TC-2
Leucine aminopeptidase (3.4.11)	LAP (PEP-2)	TC-1, LiOH
Malate dehydrogenase (1.1.1.37)	MDH	TC-1
Malic enzyme (1.1.1.40)	ME	TC-2, TC-1
Mannose phosphate isomerase (5.2.1.8)	MPI	TC-2
Peptidase (3.4.11)	PEP-1	TBE, LiOH
	PEP-4	TBE
6-Phosphogluconate dehydrogenase (1.1.1.44)	PGD	AP
Phosphoglucomutase (2.7.5.1)	PGM-1	TC-1
	PGM-2	AP
Sorbitol dehydrogenase (1.1.1.14)	SorDH	TBE

* AP. 0.04 M citrate adjusted with N-(3-aminopropyl)-morpholine to pH 6.0; diluted 1:19 for gels and undiluted for electrodes (15 hr. 80 V). LiOH. Solution A: 0.03 M LiOH, 0.19 M borate, pH 8.1; Solution B: 0.008 M citrate, 0.05 M Tris, pH 8.4; 10% A plus 90% B for gel, A for electrode (15 hr. 180 V). TBE. 0.087 M Tris, 0.086 M borate, 0.001 M EDTA, pH 9.0; diluted 1:3 for gels. 0.5 M Tris, 0.065 M borate, 0.02 M EDTA, pH 8.0; undiluted for electrodes (15 hr., 80 V). TC-1 0.378 M Tris, 0.165 M citrate, pH 6.0; 13.5 ml diluted to 400 ml for gel and undiluted for electrodes (15 hr. 60 V). TC-2. 0.188 M Tris, 0.065 M citrate, pH 6.8; diluted 1:9 for gels and 1:5 for electrodes (15 hr. 150 V).

the presumed allele.

The mean number of alleles per locus, the proportion of loci polymorphic, *P* (a locus was considered polymorphic if more than one allele was detected), and the mean individual heterozygosity, $\bar{H}$ (by direct count) were calculated for each sample. Allozyme frequencies for the polymorphic loci were tested for their agreement with Hardy-Weinberg expectations for a panmictic population by X^2-test where possible and by the Fisher exact test. Population genetic structuring was studied with Wright's hierarchical F-statistics (F_{IS}, F_{ST} and F_{IT}) calculated for each locus and sample. Genetic distance coefficients (*D*) of Nei (1978) and the genetic similarity coefficient (*S*) of Rogers (1972) were calculated and clustered by the UPGMA algorithm. The above analyses were performed with the BIOSYS-1 computer program (Swofford and Selander, 1981).

Consistent and genetically interpretable results were obtained for 20-21 loci per sample. Allele frequencies are shown for all polymorphic loci in Table 70. Gel banding patterns for selected loci are shown in Figure 1. (Detailed descriptions of banding patterns and their interpretation are avaiable from the senior author).

In the snails from the Philippines, the mean number of alleles per locus (*A*) was 1.2-1.4; Chinese snails were more variable, A = 2.0-2.4. The proportion of loci polymorphic (*P*) was 0.14-0.29 in the field collected samples from the

TABLE 70

Allele frequencies for 16 polymorphic loci in 10 samples of *Oncomelania hupensis* with summary statistics of genetic variability.*

Locus/allele	Leyte			Luzon		Mindoro	Mindanao	China		
	A	B	C	A	B			Lab	A	B
Aat-a									0.92	1.00
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.08		
Acp-a	1.00	1.00	1.00	1.00	1.00	1.00	0.08	1.00		
b									0.08	0.75
c							0.92		0.92	0.10
d										0.15
Es-1-a									0.30	0.55
b									0.70	0.45
c	0.82	0.90	0.83	0.92	0.90	1.00	1.00	0.96		
d	0.18	0.10	0.17	0.08	0.10			0.04		
Es-3-a									0.02	
b									0.96	1.00
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.02	
Gap-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									1.00	1.00
Gpi-a		0.01		0.20	0.05	0.30		0.16		
b									0.08	0.10
c	1.00	0.99	1.00	0.80	0.95	0.70	1.00	0.84		
d									0.32	0.45
e									0.59	0.40
f									0.01	0.05

Locus/allele	Leyte			Luzon		Mindoro	Mindanao	China		
	A	B	C	A	B			Lab	A	B
Idh-1a	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.99	0.02	0.10
b							0.33	0.01	0.98	0.90
Idh-2a									0.02	
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00
Lep-a									0.05	0.10
b									0.55	0.40
c					0.02	0.41		0.14	0.30	0.35
d	0.99	0.97	0.96	0.99	0.98	0.40	1.00	0.73	0.09	0.15
e									0.02	
f	0.01	0.03	0.04	0.01		0.19		0.13		
Me-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									1.00	1.00
Mpi-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.56	0.70
b									0.44	0.30
Pep-4a	0.57	0.76	0.41	0.03	0.25	0.02	0.98	0.11	0.36	0.40
b	0.31	0.23	0.45	0.93	0.75	0.27	0.02	0.55	0.62	0.60
c	0.12	0.01	0.14	0.04		0.71		0.34	0.02	
Pgd-a									0.17	0.20
b	0.99	1.00	1.00	1.00	1.00	0.98	1.00	0.96	0.77	0.80
c	0.01					0.02			0.01	
d								0.04	0.02	
e									0.03	

Locus/allele	Leyte			Luzon		Mindoro	Mindanao	China		
	A	B	C	A	B			Lab	A	B
Pgm-1a	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00		
b									0.68	0.60
c									0.30	0.35
d						0.01			0.02	0.05
Pgm-2a						0.31	0.06	0.25	0.04	0.10
b	0.98	1.00	1.00	1.00	1.00	0.56	0.94	0.69	0.61	0.55
c									0.03	0.05
d	0.02					0.13		0.06	0.18	0.20
e									0.08	0.10
f									0.06	
Sordh-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.88	0.85
b								0.03	0.12	0.15
$\bar{N}$	75.9	6.1	62.2	56.2	17.4	52.5	77.5	62.2	32.4	9.6
(S.E.)	6.8	5.6	6.8	6.2	1.3	2.2	5.1	4.2	0.4	0.4
$\bar{A}$	1.3	1.2	1.2	1.2	1.2	1.4	1.2	1.5	2.4	2.0
P	23.8	19.0	14.3	19.0	19.0	28.6	19.0	38.1	66.7	52.4
$\bar{H}$	0.04	0.03	0.04	0.03	0.02	0.10	0.03	0.08	0.20	0.21

* Samples are described in Table 1; $\bar{N}$ = mean sample size (and standard error) per locus, $\bar{A}$ = mean no. alleles per locus, P = proportion of loci polymorphic (monomorphic loci : *Cat*, *G6pdh*, *Gpdh*, *Mdh-1*, *Pep-1*, excluded from this table), $\bar{H}$ = mean individual heterozygosity.

Philippines and slightly higher (P = 0.38) in the mixed origin laboratory stock. In contrast, the Chinese snails were three times as polymorphic (P = 0.52-0.67) and varied at 7 loci which were fixed or almost fixed in the Philippines. Mean individual heterozygosity also differed significantly between the Philippines and China ($\bar{H}$ = 0.02-0.10 vs 0.20-0.21).

No significant departure from panmixia was detected in the samples studied. Genotype frequencies were in close agreement with Hardy-Weinberg expectations in 35 of 38 tests (polymorphic loci/sample) involving Philippine snails. No special significance is attached to the three cases involving slight heterozygote deficiencies (*Pep-4*) in Luzon A, *Gpi* in Luzon B and *Lap* in Lab stock) as all the remaining variable loci (3-7) in each of these samples were in equilibrium. Similarly, in the case of the more variable Chinese samples, no significant departure from Hardy-Weinberg expectations was detected in 24 of 25 tests. (The remaining case, *Sordh* in China A, also involved a deficiency of heterozygotes; Fisher exact tests P = 0.048, fixation index 0.43). With only these four minor exceptions among 63 tests, we conclude that in nature these snails are outbreeding at random. This conclusion is supported by the finding that Wright's multilocus F_{IS} statistic was 0.092, a value not significantly different from zero.

Study of Table 71 shows that the five samples from

TABLE 71

Matrix of genetic similarity and distance coefficients*

Sample	1	2	3	4	5	6	7	8	9	10
1 Leyte A	*****	0.986	0.990	0.957	0.973	0.905	0.911	0.939	0.500	0.514
2 Leyte B	0.002	*****	0.980	0.955	0.972	0.902	0.921	0.936	0.492	0.506
3 Leyte C	0.001	0.005	*****	0.964	0.980	0.909	0.903	0.947	0.505	0.518
4 Luzon A	0.019	0.028	0.012	*****	0.981	0.914	0.879	0.952	0.499	0.510
5 Luzon B	0.008	0.013	0.003	0.003	*****	0.909	0.890	0.950	0.506	0.518
6 Mindoro	0.045	0.053	0.040	0.045	0.045	*****	0.842	0.951	0.506	0.523
7 Mindanao	0.057	0.051	0.064	0.097	0.077	0.118	*****	0.870	0.538	0.515
8 Labstock	0.016	0.023	0.011	0.012	0.011	0.012	0.088	*****	0.515	0.529
9 China A	0.657	0.663	0.652	0.644	0.645	0.668	0.550	0.652	*****	0.910
10 China B	0.620	0.625	0.617	0.611	0.610	0.630	0.591	0.615	0.035	*****

* Below diagonal : Nei (1978) unbiased genetic distance; above diagonal: Rogers (1972) genetic similarity.

Leyte and Luzon are practically indistinguishable at the 20 loci examined. Mindoro snails differ from those on Leyte-Luzon in having differences involving *Lap* and *Pgm-2*. Snails from Mindanao were characterized by slower *Acp* and *Idh-1* alleles but were otherwise similar to the other Philippine snails. In contrast, the Chinese snails had unique alleles at 13 of the 21 loci examined. Philippine and Chinese snails can be distinguished from one another on the basis of variation at *Aat*, *Es-1*, *Es-3*, *Gpi* and *Pgm-1*. The major differences between Chinese snails and the others are underscored by the calculated value of Wright's F_{ST} (0.64), a value significantly different from zero.

We estimated overall multilocus genetic differentiation between these samples using Nei's (1978) unbiased coefficient of genetic distance (Table 71; Roger's S values are shown for comparative purposes). Within the Philippines intersample *D* values were in the range 0.001-0.118 and *D* = 0.032. *D* between the two Chinese samples was a very similar 0.035. In marked contrast, the China-Philippines comparison yielded *D* = 0.628.

The first goal of the present study was to establish whether *Oncomelania* were variable enough to permit the preparation of a molecular or genetic phylogeny. The samples studied were found to be well suited to this type of analysis based on allozyme variation. *O. hupensis* and *O. quadrasi* differ in their levels of variability (*P* = 0.52-0.67 in the former, *P* = 0.14-0.29 in the latter, $\bar{H}$ = 0.20-0.21 vs 0.02-0.10) but neither taxon is unusual, approximate mean levels

of variation in amphimictic molluscs are $\bar{P}$ = 0.47 (range 0-1.00) and $\bar{H}$ = 0.14 (range 0-0.30) (Selander and Ochman, 1983; Nevo *et al.*, 1984). Our preliminary study has revealed up to 14 polymorphic loci per sample-- well in excess of the number needed to develop robust molecular phylogenies.

Our second goal was to explore the extent of genetic differentiation within *O. quadrasi* in the Philippines. Not surprisingly we found a strong relationship between the degree of physical isolation and the genetic distance between any two samples. Physical isolation in the case of *Oncomelania* is related to both geographic distance and saltwater barriers. Thus, the three samples collected within a few km of one another on Leyte are genetically indistinguishable. The Luzon samples, collected 140 km and three islands to the north, are very weakly differentiated from those on Leyte; map distance belies the fact that the snails of Luzon and the almost continuously distributed populations of Leyte-Samar are only separated by the shallow (135 m), narrow (<10 km) San Bernardino Strait. Mindoro snails are more differentiated from those of Luzon-Leyte having higher frequencies of certain alleles at *Lap* and *Pgm-2*. The Mindoro sample was collected 250 km from Leyte and 160 km from southern Luzon, and although Mindoro is only 20 km from central Luzon the two islands are separated by a deep and permanent saltwater strait. Mindanao is the most distinctive of the samples (with a unique *dcp*); the sample was taken 300 km south of Leyte. Today Leyte and Mindano are

separated by a 30 km wide strait with water 80 m deep. Additional samples of snails from throughout the archipelago are necessary to fully document the apparent pattern of isolation-related differentiation. In addition, future studies will need to relate the alleles found in various Philippine populations to those occurring in and possibly introduced by migratory birds from China, Taiwan and Japan. We, ourselves, plan to test the hypothesis that the Sulawesi *Oncomelania* are dervied from Mindanaoan stock.

Our final goal was to initiate a study of host-parasite coevolution between the snails of the *O. hupensis* superspecies and the trematodes referred to as *S. japonicum*. As *O. hupensis* is the sole or obligate intermediate host of *S. japonicum*, the schistosome could not have established itself in an area until after the snails were introduced. If parasite and host evolve at similar rates, we would predict that the genetic distance between source (China) and derived (Philippines) populations would be less for the parasite than for the host. This is, in fact, the case : D = 0.46 between *S. japonicum* from China and Leyte (based on 11 loci; Fletcher *et al.*, 1980) and D = 0.63 for *O. hupensis* - *O. quadrasi*. The relatively large D value between Chinese and Philippine strains of the parasite suggests that the schistosomes were introduced to the archipelago in bird-borne infected snails long before man arrived. This would explain why *S. japonicum* has an unusually wide range of mammalian hosts compared with most other schistosomes.

2.2 Genetic variation in the snail *Tricula aperta*.

Tricula aperta is the intermediate host of *S. mekongi*, the human blood fluke originally described from Khong Island in the Mekong river, Laos. George Davis studied these snails in great detail and concluded that three races could be recognised on the basis of distribution, microhabitat, and minor morphological differences. In the Mekong, alpha and gamma races co-occur, with one being found in deeper water. In the Mun River of Thailand, only the beta race was known. We studied genetic variation in samples of all three races and found that the genetics do not support this traditional taxonomy. Instead, we discovered that four sibling species may be confused under the name *T. aperta*.

We characterized samples of the alpha, gamma, and beta races at 16 allozymic loci. It appeared as if alpha and gamma races were indistinguishable from one another and both were very well-differentiated from the beta race. The multilocus genetic distance between the two groups of races was $D = 0.66$, indicating that the beta "race" was, in fact, a distinct and well-differentiated species. However, highly significant heterozygote deficiencies were apparent at many polymorphic loci in all three samples. Furthermore, there were completely unexpected significant differences in allele freguencies between males and females in each sample. The total absence of heterozygotes at some loci suggested that each of the original racial samples were in fact heterogeneous mixtures of two or more discrete species.

Regrouping the original animals according to their genotypes at selected marker loci confirmed this hypothesis.

Our results suggest that at Ban Bungkhong on the Mekong River, one cannot recognise the taxa previously called the alpha and gamma races. Instead, we find that there are two sibling species present; each species has both alpha and gamma ecophenotypes. The two species are well differentiated, D = 0.34, and there is no compelling evidence that the two taxa interbreed in nature. Similarly, at Khaeng Khao, on the Mun River, we find that what has traditionally been refered to as the beta race is in fact two well-differentiated, D = 0.22, reproductively isolated species. The newly defined species-pair in the Mekong are very different from the species-pair in the Mun river, D = 0.73.

We must now confirm these results and recharacterize the genetically defined taxa morphologically and anatomically. If there are really four sibling species present, then the epidemiology of Mekong schistosomiasis will have to be re-examined as it is not clear whether one or both species in the Mekong transmit *S. mekongi*.

2.3 genetic relationships among snails of the subfamily Triculini.

Tricula aperta, *T. bollingi*, and *Robertsiella kaporensis* are, respectively, the intermediate host snails of the blood flukes *S. mekongi*, *S. sinensium*, and *S.* sp. (undescribed species infecting man in peninsular Malaysia -

taxonomic description being prepared collaboratively with Greer and Ow-Yang). As part of our on-going study of host-parasite coevolution, we compared these three species genetically. As the radiation of these snail species and genera was hypothesised by George Davis to have been fairly recent, we did not expect to find great genetic differences between the three taxa. We found just the opposite. First, as reported above, "*T. aperta*" appears to be two very well differentiated groups of species pairs. These four species, in turn, share almost no alleles at 16 loci with *T. bollingi*, D = 2.8, and none with *R. kaporensis* (D approaches infinity). These results suggest that the alleged rapid cladogenesis in this subfamily was accompanied by unusually rapid genetic differentiation. Our results are highly relevent to the problems of schistosome transmission as it is widely believed that schistosomes evolve more slowly than their intermediate hosts. This might explain why Asian schistosomes are capable of infecting so few species of snails.

While this result is not immediately applicable to disease control, a better understanding of the way parasites and hosts have chased one another in space and time provides a basis for the development of sounder biological and genetic control techniques.

2.4 Genetic variation in three *Schistosoma* species from the Philippines, Laos and Peninsula Malaysia

Four strains of *S. japonicum* from the Philippine islands of Leyte, Luzon and Mindoro were examined. All were cycled through *O. h. quadrasi* snails and Swiss albino outbred mice. LEYTE LAB was collected in 1980 with 20-30 infected snails collected from several sites near Palo, Leyte, and supplemented with schistosomes from about 20 additional infected snails collected in the same area in 1982. LEYTE FIELD was collected in 1984 with about 10 infected snails and passed through mice once. MINDORO was collected in 1984 with about 50 infected snails collected in Victoria, Mindoro, and passaged through mice once. LUZON was collected in 1984 with schistosomes from 3-4 infected snails collected at Irosin, near Sorsogon, in southern Luzon island and passed through mice once.

One strain of *S. mekongi* was examined : MEKONGI. Originally collected in *T. aperta* snails at Khong Island, Laos, in 1971 and maintained initially at the Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University. This strain has been cycled in Dr. Upatham's laboratory since 1979.

One strain of the Malaysian schistosome was examined: MALAYSIA. Collected in 1982 with about 10 infected *R. kaporensis* snails collected near Kuala Koyan, Pahang state, northern Peninsular Malaysia and passaged through

Swiss albino mice.

All schistosomes were cultured at the Center for Applied Malacology and Entomology (Department of Biology, Faculty of Science, Mahidol Unviersity, Bangkok). Adult worms were recovered by perfusion and dissection of anesthetized infected mice and were briefly washed in distilled water before being frozen at -30 ^{o}C.

Horizontal starch gel electrophoresis was performed to separate each animal's structural gene products and histochemical staining techniques resolved specific allozyme patterns. Techniques were very similar to those described elsewhere (Fletcher *et al.*, 1981) except that we used 12.5% gels made of Sigma starch (Sigma Chemical Co., St. Louis, Missouri). Prior to electrophoresis, the adult worms were thawed, quickly separated by sex, and homogenized individually in 7 ul (females) or 10 ul (males) of chilled distilled water. The homogenate was immediately absorbed onto a single 10 x 3 mm tab of chromatography paper and inserted in the gel. The electrophoretic buffer and stain recipes are given elsewhere except that superoxidase dehydrogenase (SOD) was resolved on gel slices stained for malate dehydrogenase (MDH), diaphorase (DIA) was resolved using the technique of Harris and Hopkins (1977) and the peptidase (PEP) appeared with a leucylglycylglycine substrate. We employed a discontinuous Poulik buffer (pH 8.2/8.7) to resolve aldolase (ALD, E.C. 4.1.2.13), DIA (1.6.2.2), glucose-6-phosphate dehydrogenase (G6PD,

1.1.1.49), hexokinase (HK, 2.7.1.1) and phosphoglucomutase (PGM, 2.7.5.1), a discontinuous citrate with aminopropylmorpholine buffer (pH 6.4/7.4) to resolve adenylate kinase (AK, 2.7.4.3), mannose phosphate isomerase (MPI, 5.3.1.9) and PEP (3.4.1.3), and a continuous citrate with aminopropylmorpholine buffer (pH 7.0) for glucose phosphate isomerase (GPI, 5.3.1.9, formerly called phosphoglucose isomerase), MDH (1.1.1.37) and SOD (1.15.1.1).

The number of individuals electrophoresed and the mean and range of the number of individuals scored per locus were: LEYTE LAB 397, 90 (32-151), LEYTE FIELD 69,32 (18-69), MINDORO 360, 85 (13-155), LUZON 4, 1.5 (1-2), MEKONGI 29, 16 (11-24) MALAYSIA 211, 48 (7-121). Schistosomes of different strains were run on the same gel to facilitate comparison of electromorphs. Allozymes were designated a,b,c in order of decreasing electrophoretic mobility and by the use of the same abbreviations employed to represent the enzymes, set in lower case and italicized. Unbiased estimates of genetic distance between the various strains were calculated using NEI's revised formula (Nei, 1978) and the BIOSYS computer program (Swofford and Selander, 1981).

Consistent and interpretable electrophoresis patterns were obtained for 11 enzymes representing 11 structural gene loci. Patterns for *Dia* and *Pep* in Asian schistosomes are described here for the first time: DIA resolved as a single sharply defined band of activity migrating anodally, PEP

resolved as series of 2-3 intense evenly spaced bands migrating anodally. The presumption is that each of these enzymes is coded as a single monomorphic locus. All other enzymes gave patterns similar to those reported previously except as noted. MDH-1 resolved as a single band of activity in all strains; this band varied in relative mobility between taxa. When MDH-1 is denatured it breaks down to give a 3-banded pattern sometimes indistinguishable from the patterns of heterozygotes for dimeric allozymes. We are convinced, however, that these *Mdh-1* pseudo-heterozygotes are actually denatured homozygotes as affected individual schistosomes also showed blurred patterns for PEP, MPI and GPI and generally no activity for AK.

Our genetic results, pooled where appropriate with those previously published, are summarized in Table 72. The proportion of loci polymorphic (P) and the mean individual heterozygosity per locus ($\overline{H}$) were both zero in all four strains of *S. japonicum*, in *S. mekongi* and in the Malaysian schistosome.

Four strains of *S. japonicum* from the Philippines were examined. The LEYTE LAB and LEYTE FIELD strains were identical at all 11 loci. The LUZON strain is not well characterized (based on number of loci and number of worms), but was identical to the LEYTE strains at all 8 loci examined. In contrast, the strain from MINDORO was weakly differentiated from the others being fixed for a different allele at *G6pd* and at *Pep*. *S. mekongi* apparently shares a

Gpi allele with Philippine *S. japonicum* but otherwise the two taxa differ at 11 out of 12 (92%) loci. The Malaysian schistosome also differs from *S. japonicum* at the same 11 loci; it shares 5 of these 11 allozymes with *S. mekongi*.

We have combined these results to produce composite samples representing *S. japonicum* from Leyte, *S. mekongi*, and the Malaysian schistosome. The resulting genetic data may then be compared with our samples representing *S. japonicum* from the Philippine islands of Luzon and Mindoro, and with previously described samples from Japan, China and Formosa (Table 72).

The lack of allozymic variation in the 15 strains of Asian schistosomes investigated for 9-18 loci is surprising. A survey (Nevo *et al.*, 1984) of variation in a thousand species of organisms showed $\bar{P}$ = 0.284 and $\bar{H}$ = 0.07 and, among 361 invertebrates, $\bar{P}$ = 0.375 and $\bar{H}$ = 0.100. We expected to find this much variation in our freshly established strains of *S. japonicum* and the Malaysian schistosome. Our previous study of 22 strains of *S. mansoni* showed $\bar{P}$ = 0.13 (0-0.33) and $\bar{H}$ = 0.04 (0-0.08) (Fletcher *et al.*, 1981).

The composite characterizations of *S. japonicum* (Leyte), *S. mekongi* and the Malaysian schistosome involve 18, 15 and 14 loci, respectively. These numbers of loci are just adequate to give reasonable estimates of actual variability (Nei, 1975). Although it would be preferable to increase the number of loci involved to about 24 which is hard to do with

Table 72

Variation at 18 loci in four Asian *Schistosoma* species. The total number of schistosomes found to have each particular locus/allele is shown.

Locus/		*Schistosoma japonicum*						*S. mekongi*	*S.*sp.
allele		Leyte	Luzon	Mindoro	China	Formosa	Japan	Laos	Malaysia
Acp	*a*	79			22	40	59	-	
	b	-			-	-	-	26	
Ak	*a*	62	2	58				-	-
	b	-	-	-				-	34
	c	-	-	-				34	-
Ald	*a*	128		13	-	-	59	-	
	b	-		-	22	40	-	-	
	c	-		-	-	-	-	26	
Dia	*a*	104	1	79					23
Ga3pd	*a*	79			22	40	59	-	
	b	-			-	-	-	26	
Gdh	*a*	79			22	40	59	26	
G6pd	*a*	-	-	45	-	-	-	-	-
	b	159	1	-	-	-	-	-	-
	c	-	-	-	22	40	59	45	23
Gpi	*a*	-	-	-	22	-	-	-	-
	b	249	4	155	-	40	59	-	-
	c	-	-	-	-	-	-	37	21

Hk	*a*	200	1	91	22	40	59	–	–
	b	–	–	–	–	–	–	65	–
	c	–	–	–	–	–	–	–	85
Ldh	*a*	79			22	40	59	–	6
	b	–			–	–	–	33	–
Mdh-1	*a*	262	4	136	22	40	59	–	–
	b	–	–	–	–	–	–	41	56
Mdh-2	*a*	62		96	22	40	59		–
	b	–		–	–	–	–		26
Me	*a*	3						–	–
	b	–						–	4
	c	–						2	–
Mpi	*a*	93		96				–	–
	b	–		–				16	–
	c	–		–				–	76
Pep	*a*	–	–	–					74
	b	–	–	138					–
	c	141	2	–					–
Pgd	*a*	7						–	–
	b	–						12	12
Pgm	*a*	–	–	–	–	–	–	70	78
	b	–	–	–	22	–	–	–	–
	c	229	1	105	–	40	59	–	–
Sod	*a*	34		30				–	–
	b	–		–				1	2

limited numbers of small animals. Nevertheless, the loci examined include enzymes with a variety of different metabolic functions, and it is reasonable to expect that our single gel electrophoretic technique detects about 80% of the effective number of alleles present.

The failure to detect genetic variation in *S. japonicum* and the Malaysian schistosome is probably not attributable to the small size of our samples. In each case, the effective initial population size and the mean number of individuals scored per locus (>40) is adequate. In fact, it has been found that a sample size of 8-12 individuals will yield an estimate of $\bar{H}$ within 1% of the value obtained from a very large sample size (Gorman and Renzi, 1979). Such loci are among the most variable in other groups of animals. Although this may be so we have included all seven polymorphic loci reported in *S. mansoni* in our sample of enzymes. Furthermore, as our characterization of *S. japonicum* (Leyte) involves 4 sequential samples of 62-262 worms, we would expect to have detected any additional alleles present at frequencies of above 0.01. Similarly, although our two sequential samples of the Malaysian schistosome from Koyan are smaller, they are adequate to detect alleles at frequencies of > 0.05.

We expected the Asian schistosomes to be as variable as African *S. mansoni*. *S. japonicum* has a lineage extending back about 10 million years, is sexually reproducing,

inhabits a wide geographic range, has coevolved with a genetically variable snail and has a diversity of mammalian hosts. Species with such characteristics might be expected to show moderate levels of protein variation. Although our previous failure to detect variation in laboratory strains of *S. japonicum* and *S. mekongi* may have been due to genetic drift and artificial selection, and this cannot completely account for the situation in the Leyte or Koyan strains. Obviously, the initial sampling of a natural population of schistosomes based on very few snails can result in significant genetic drift in the establishment of the captive strain. Similarly, artificial selection for survival under laboratory conditions (abnormal final host, intermediate host from a different geographic area, short generation time, etc.) can alter the genetics of subsequent generations. The extent to which such genetic drift and artificial selection account for the lack of variability in our strains cannot be assessed. Nevertheless, the first generation sample of *S. japonicum* from Mindoro was fully expected to show some variability as it was founded with cercariae from about 50 infected snails. Similarly, the LEYTE FIELD and MALAYSIA strains were also first generation laboratory schistosomes and were derived from samples of about 10 naturally infected snails. Nei *et al.*(1975) have shown that even 10 founders, and rapid population expansion following a founder event, would prevent significant reduction in original $\overline{P}$ and $\overline{H}$ values. given the initial effective population sizes of these strains, we had a high probability of detecting

variation if it existed. We conclude that these best characterized strains are close to isogenic, and that the populations they represent probably have very low levels of allozyme variability. Additional research with carefully established strains based on even large numbers of infected snails is now required.

As noted above, *S. japonicum* from Palo, Leyte has now been characterized on the basis of 18 monomorphic loci. We compared this population with fresh samples from Luzon and Mindoro Islands. The data for LUZON are very limited (Table 72) but indicates $D = 0$ between this sample and LEYTE. The lack of differentiation is not surprising as the sites are only 200 km apart and the parasite is found on the intervening island of Samar. Land bridges connected Luzon, Samar and Leyte during the Pleistocene when sea levels fell below-140m. In contrast, we found the MINDORO strain to be moderately differentiated from LEYTE, D=0.29, on the basis of 12 loci (Table 73). This difference stems from the fact that the two areas are characterized by different fixed alleles for *G6pd* and *Pep*. Such a difference may have evolved if the schistosomes in the two areas had been isolated from one another for long enough for the two mutations to have become fixed. Unfortunately, we still lack basic information on the antiquity of *S. japonicum* in the Philippines and on its historical pattern of dispersal through the six islands where it now occurs. Relative isolation of the population on Mindoro is a possibility, however, as this large volcanic

island to the west of Luzon is a true oceanic island separated from others by a deep (>200m) permanent water gap. Only additional sampling from throughout the range of *S. japonicum* in the philippines will allow us to interpret the genetic distance reported here. It is important to note, however, that *D* = 0.29 is a moderately high value for an intraspecific D and larger than those reported previously for the still inadequately documented China-Formosa and Formosa-Japan comparisons (Table 73). One survey of over 7,000 estimates of *D* involving conspecific populations of other organisms revealed that only 2% of the *D* values were greater than 0.10 (Thorpe, 1983).

The suggestion in Table 73 that MINDORO is more closely related to JAPAN than to LEYTE is probably erroneous as the *D* values are based on only 7 loci. It is worth remembering, however, that Japanese troops moved through the Philippines 40 years ago, and that some associated movement of schistosomes may have occurred. Although the previously published data on *S. japonicum* from Japan, Formosa and China are included in Talbe 73, discussion of the evolutionary and taxonomic significance of the higher *D* value (0.45, for 11 loci) associated with CHINA-LEYTE is still premature. Additional data on Chinese schistosomes are required, however, before recognition of separate species within the *S. japonicum* species complex would be justified.

Table 73

Genetic distance (*D*), standard error of *D* and the number of loci examined between various pairs of strains of Asian schistosomes.

Strain	LEYTE	MINDORO	CHINA	FORMOSA	JAPAN	MEKONGI	MALAYSIA
S. japonicum							
LEYTE	-	0.288	0.452	0.201	0.102	2.708	1.946
		.167	.228	.142	.135	.966	.655
		12	11	11	11	15	14
MINDORO		-	1.099	0.405	0.182	∞	1.705
			.535	.267	.169	N.A.	.640
			7	7	7	9	11
CHINA			-	0.201	0.405	1.705	1.099
				.142	.213	.671	.535
				11	11	10	7
FORMOSA				-	0.182	1.705	1.099
					.135	.671	.535
					11	10	7
JAPAN					-	1.792	1.099
						.707	.535
						10	7
S. mekongi						-	0.606
							.275
							11
Malaysian schistosome							-

The present study of *S. japonicum* and *S. mekongi* confirms the conclusion of two previous studies that these parasites are very well differentiated from one another. Interspecific *D*'s based on 10 or more loci are 1.7-2.7 - very high values for congeneric species. Using Nei's moderate calibration of the protein-electrophoretic molecular clock with a *D* value of 1.0 equivalent to about 5 million years, we conclude that the divergence between the *S. japonicum* and *S. mekongi* lineages occurred on the order of 10 million years ago. Davis (1980) has presented detailed arguments based on historical biogeography and intermediate host snail systematics to reach a similar Miocene conclusion. According to this scenario, the Asian schistosomes represented today by *S. mekongi* adapted to life in the aquatic snails of the subfamily Triculinae when the latter radiated in the upper Mekong River 12 ± 4 million years ago. At that time, the proto-*Oncomelania* were evolving an amphibious habit and spreading down the Yangtze River valley of China and subsequently dispersing to the off-shore islands. *S. japonicum* has coevolved with *Oncomelania hupensis* as the latter diverged into a number of genetically distinct geographic species and subspecies. In the absence of appropriate fossil evidence, this plausible scenario is hard to assess. At the very least the genetic data will have underestimated the time since divergence as *D* is greater than 1.0. Indeed, if a *D* value of 1.0 is equivalent to 18 million years (rather than 5 million used above) as is suggested by albumin immunology (Cross, 1984; Avise and Aquadro, 1982),

then the proto-*japonicum* and proto-*mekongi* lineages would have split about 38 million years ago. An oligocene speciation event would have preceded the collision of the Indian plate with Asia and the diversification of the host snails. This seems unlikely, and so we must suspect that the particular loci in our survey cause our molecular clock to run faster than one based on albumin.

Our most interesting result concerns the relationship between the Malaysian schistosome and the other Asian schistosomes. This *Schistosoma* sp. was first reported in 1973 among the Orang Asli people of peninsular Malaysia and Greer and collaborators subsequently found that it cycled through *Robertseilla kaporensis*, aquatic snails closely related to *Tricula* (Greer *et al.*, 1980). Based on non-genetic criteria, the Malaysian schistosome has been referred to as *S. japonicum* or as *S. japonicum*-like. However, in the first report on its geneticsm, Yong *et al.* (1985) found it was more like *S. mekongi* than *S. japonicum*. Here we confirm and quantify this result. We estimate the genetic distance between the Malaysian schistosome and *S. mekongi* is D = 0.606 and less than one third of the distance to *S. japonicum* (D = 1.7-1.9). These estimates were based on 11 and 11-14 loci, respectively, and the Malaysian-*mekongi* distance with a standard error of 0.138 is, therefore, quite significant.

We summarize our preliminary phylogenetic findings in a composite dendrogram (Fig. 70) constructed using the

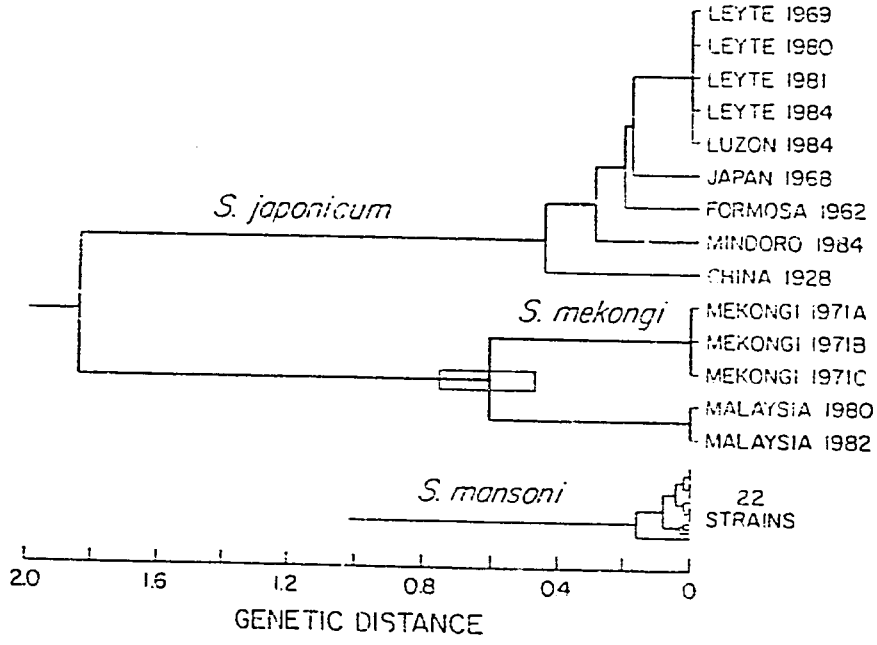


Figure 70 Dendrogram showing the relationships of some Asian schistosome strains, generated according to UPGMA cluster analysis and based on 11-15 loci (thick lines) or 7-10 loci (thin lines). For comparative purposes, a schematic outline of the tree for S. mansoni (18 loci) is shown below.

unweighted pair-group clustering method and the data in Table 73. For comparative purposes, we include a tree for *S. mansoni* derived by calculation of the genetic distances from data published previously. We conclude that protein electrophoresis is ideally suited to resolving the relationships among the more closely related schistosomes. This report has the following medical and public health consequences. First, the possible medical correlates of the increasingly complex taxonomic situation merit more attention than they have received. It is reasonable to expect the different species and species groups of schistosomes to differ in their epidemiology, pathology and response to various treatments. Second, our continued failure to find intrastrain variation in the Asian schistosomes has important ramifications. Although isogenic strains are ideal for some investigations (immunological experiments and sources for monoclonal antibodies), single isogenic strains may be inappropriate models for wide ranging species. Development of broadly applicable disease diagnosis, treatment and control techniques involving vaccines and drugs may be more difficult in view of this pattern of marked geographic variation. There is, after all, apparently much more regional differentiation within *S. japoncium*, as presently defined, than within *Homo sapiens*.

2.5 Genetic variation in the Chinese and the Philippines *S. japonicum*

The first genetic evidence suggesting that the Asian anthropophilic human blood flukes presently refered to as *S. japonicum* may actually comprise a group of sibling species was provided by Fletcher *et al.* (1980). In a study providing genetic evidence that *S. mekongi* is a separate species from *S. japonicum*, they compared laboratory strains of *S. japonicum* originally from China, Taiwan, Japan, and the Philippines and found the Chinese strain to be the most distinct. Multilocus indices of genetic differentiation for the Chinese-Philippines comparison were surprisingly high for a conspecific comparison (e.g. Nei's D = 0.45, Fletcher *et al.*, 1980); a comparable study of variation between 22 strains of *S. mansoni* yielded D = 0.06, Dmax = 0.24 (Fletcher *et al.*, 1981). This preliminary study was inadequate, however, as only 10 loci were studied and as the Chinese worms, laboratory descendents of Vogel's 1928 Kofu Province collection, were possibly unrepresentative of naturally occurring schistosomes. The purpose of the present study is to compile comparative data for a recently established Chinese strain. We find that *S. japonicum* strains from China and the Philippines are even more differentiated from one another that suspected.

Approximately 40 infected *O.h. hupensis* (natural intermediate host snail) were collected in February, 1984 from Guichi, Anhui Province, Peoples' Republic of China (on

the Yangtze River, approximately 400 km west of Shanghai), and 50 infected *O.h. quadrasi* were collected in 1984 from Victoria, Mindoro, Philippines. Cercariae of each strain were cycled through Swiss Webster mice once. All schistosomes were cultured at the Center for Applied Malacology and Entomology, Department of Biology, Faculty of Science, Mahidol University, Bangkok. After perfusion of the adult parasites from the mice and tissue preparation, starch gel electrophoresis was performed in San Diego according to techniques described in Fletcher *et al.* (1980). We resolved GAP (E.C. 1.2.1.12) and MDH-1 (E.C. 1.1.1.37) using continuous tris-citrate (pH 7.0) buffer; G6PD (E.C. 1.1.1.49), HK (E.C. 2.7.1.1) and ME (E.C. 1.1.1.40) using citrate with aminopropylmorpholine (pH 6.0) buffer; MDH-2 (E.C. 1.1.1.37), ACP (E.C. 3.1.3.2), PGM (E.C. 2.7.5.1) and PGD (E.C. 1.1.1.49) using tris-malate (pH 7.4) buffer; PEP-lgg (leucylglycylglycine substrate) (E.C. 3.4.1.3), MPI (E.C. 5.2.1.8), and GPI (E.C. 5.3.1.9) using citrate with aminopropylmorpholine (pH 7.0) buffer; ALD (E.C. 4.1.2.13), GDH (E.C. 1.4.1.3), LDH (E.C. 1.1.1.27) were resolved only by Flectcher *et al.* (1980). We here report results for 58 individual male worms from the mainland of China and 66 males from the Philippines. For technical reasons associated with the size of the schistosomes, we scored an average of 10 individuals per locus. To ensure reliability of the results, we ran all procedures twice. The proportion of polymorphic loci (P) and the mean individual heterozygosity per locus ($\overline{H}$)

are zero for both strains. These results are in concordance with Fletcher *et al.* (1980). Overall genetic difference between the two strains can be estimated using an unbiased multilocus genetic distance: Nei's genetic distance (*D*) (1978). Between *S. japonicum* from the mainland of China and *S. japonicum* from Mindoro, Philippines, Nei's *D* is 0.575 with one standard error of 0.220 (standard error calculated as described in Nei *et al.*, 1985). These values were used to prepare a dendrogram of genetic relatedness between the two strains (Fig. 71). This distance is slightly larger than reproted by Fletcher *et al.* (1980) for the Vogel's Chinese *S. japonicum* and a Philippine strain from Leyte. Comparing our results with those of Fletcher *et al.* (1980), we confirmed that 9 loci were identical in both geographic regions: *Gdh, G6pd, Hk, Ldh, Mdh-1* (anodal), *Mdh-2* (cathodal), *Me, Pep-1* (anodal), *Pep-2* (cathodal). Four newly resolved isozymes (ALD, MPI, PGD, PGI) were fixed for different alleles in the Chinese and Philippine strains. We were also able to differentiate between Chinese and Philippine *S. japonicum* at *Acp* and *Gap* as these electromorphs were clearer than reported by Fletcher *et al.* (1980). We were unable to confirm the very slight difference at the *Pgm* locus mentioned by Fletcher *et al.* (1980), and thus believe both strains share the same PGM allele.

The lack of intrastrain genetic variability observed could not be attributed to small sample size as over 50 individual from 40 infected snails were used; simulations

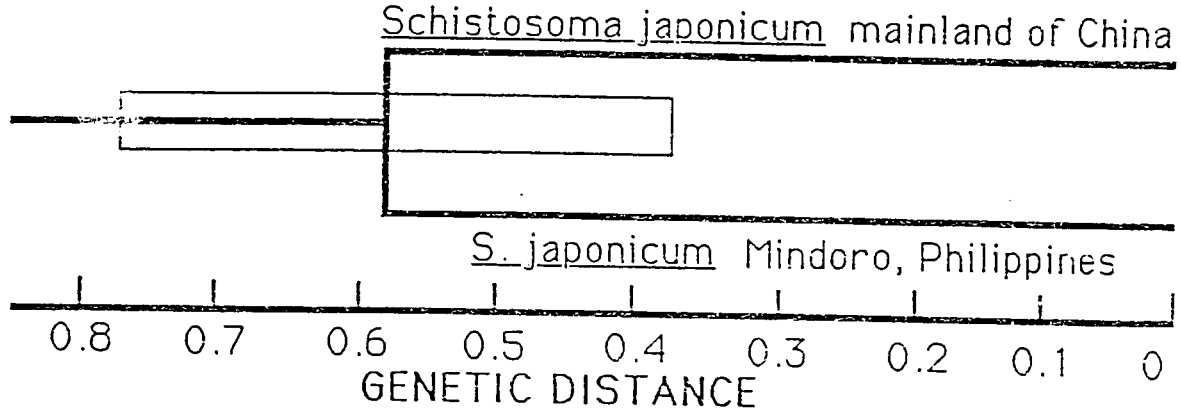


Figure 71 Phenogram based on Nei's genetic distance (1978).

show 8-12 individuals will give a reasonable estimate of $\overline{H}$ (Gorman and Renzi, 1979). Alternatively, the lack of genetic variablity could be due to the types of allozymes examined, which did not include nonspecific esterases and oxidases known to be highly variable. However, we were able to resolve all seven of the polymorphic loci found in comparable studies of the congeneric *S. mansoni* by Fletcher *et al.* (1981); this suggests that the loci examined were appropriate to detect protein polymorphism. One possibility, under investigation in our laboratory, is the *S. japoncium* may reproduce parthenogenically.

Until proof of asexual reproduction in these strains is obtained, we will follow all previous researchers and continue to assume that dioecious *S. japonicum* is sexually reproducing, and that Nei's *D* is an appropriate measure of genetic divergence. In order to interpret a Nei's genetic distance of 0.575 ± 0.220 between Chinese and Philippine *S. japonicum*, we may compare this value to other interstrain values reported for this species (<0.03 Fletcher *et al.*, 1981) and to genetic distances in other organisms. Ayala's (1983) survey of a wide range of organisms shows genetic distances (*D*) between conspecific populations are typically less than 0.06, and Thorpe (1983) found only 2% of conspecific population comparisons exceed *D* = 0.10. If *Schistosoma* are "typical" in their rate of amino acid substitutions and speciate with typical amounts of genetic change, the reported *D* value between the two strains of *S.*

japonicum exceeds the known values for conspecific populations.

The geographic populations of *S. japonicum* are also differentiated by intermediate host compatibility. The inability of Chinese *S. japonicum* from Shanghai to develop in *O.h. quadrasi*, the snai host from the Philippines, was experimentally shown by DeWitt (1954). Yuan *et al* (1984) confirmed DeWitt's results and reported a markedly reduced infectivity rate of *S. japonicum* from the Philippines in *O.h. hupensis* from China. In addition to host specificity and allozyme differences these strains can be distinguished from one another morphologically. Sobhon *et al* (1986) found differences in the types and distribution of papillae and the shape of the ridges on various parts of the adult body surface. These results further support the observed genetic divergence between the two strains.

The evidence indicates that "*S. japonicum*" may actually comprise a superspecies complex with several component allopatrically distributed semispecies, each of which will show some geographic variation. Such a taxonomic revision is premature, however, until comparable genetic data are available for worms from Japan, Taiwan and additional sites in China, and until all other relevant lines of evidence have been considered. Nevertheless, those working on diagnosis, treatment and control techniques (involving antibodies, vaccines and drugs) for schistosomiasis should be aware that the Chinese and Philippine strains have diverged at over 35% of their structural gene loci.

C. Summary

The biological control of mollusks by snail and insect control agents was conducted in 3 phases: (1) experimental work in the laboratory, (2) experimental work under simulated field conditions, and (3) experimental work under a small scale field trial.

From the experimental work on biological control of mollusks by snail control agents in the laboratory, it can be concluded that the biological control agents, *Brotia costula costula*, *Tarebia granifera*, and *Melanoides tuberculata*, even though they had higher reproductive potential than the target pulmonate snails, *Biomphalaria glabrata*, *Indoplanorbis exustus* and *Radix rubiginosa*, but they had higher mortality rates and required much longer time for the young snails to become mature, did not seem to be the appropriate control agents for the pulmonate snails. As for the prosobranch target snails, *Tricula aperta*, *Robertsiella kaporensis*, *Oncomelania hupensis quadrasi*, and *Bithynia siamensis goniomphalos*, there seemed to be no interference between the control and target snails due to different preferences of habitats. However, the three species of control agents certainly had high reproductive and survival rates than the target species. *Pila ampullacea*, the other biological control agent, appeared to be effective control agent for *B. glabrata*, the pulmonate snail. The effect of *P. ampullacea*

on *B. glabrata* could be an accidental predator on the egg masses of *B. glabrata* in the laboratory.

The biological control study between sciomyzid fly larvae and the snails in the laboratory revealed that the third stage sciomyzid fly larvae were effective control agent for pulmonate target snails as previously mentioned; the ratio of 3 sciomyzid fly larvae to 1 snail rendered the best result.

The experimental work on biological control of mollusks by snail control agents under simulated field conditions was performed at a small field station for 8 months. The results had shown that *B.c. costula*, *T. granifera* were effective control agents for *I. exustus* and *R. rubiginosa* due to the higher reproductive and survival rates. *M. tuberculata* seemed to be effective for *R. rubiginosa*, but not for *I. exustus*. These three species of control agents could not control *B. glabrata*. It could be explained that *B. glabrata* had been long established and well adapted to cultivation than the control agents. However, *P. ampullacea* was effective in controlling the population of all three target species of pulmonate snails including *B. glabrata*. The competition resulted in a reduced growth and reproduction of the target species, and these effects were based on competition for food, physical interference between snails or predation on egg masses of the target species. All four species of control agents did not seem to have any

interference with the prosobranch target snails, *T. aperta*, *O.h. quadrasi* and *B.s. goniomphalos*.

The experimental work on biological control of mollusks by insects under simulated field conditions revealed that the sciomyzid fly larvae were effective control agents for the pulmonate snails in the ratio of 1:3 (snail: sciomyzid fly larvae). They did not have much effect on operculate snails due to the closing of operculum of snails when disturbed. The lampyrid fly larvae, as the control agent, were more effective in killing pulmonate and operculate snails than the sciomyzid fly larvae, but were more successful with pulmonate than with operculate snails. Unlike the sciomyzid fly larvae,the lampyrid fly larvae were seldom killed while attacking operculate snails.

The experimental work on the biological control of mollusks by snails under a small scale field trial was performed in natural canals outside Bangkok. *P. ampullacea* was chosen as control agent against the local species of target snails, *I. exustus*, *R. rubiginosa* and *B.s. goniomphalos*. Under natural habitats, *P. ampullacea* appeared to be effective in killing snails and to have better survival rates than the small target snails due to their large size and the voracious habit. At the end of eighth month, the numbers of target snails decreased drastically. Even though the target snails laid large numbers of eggs, only a few number of offspring survived. This stage of experiment is

still in progress and may take longer than one year. The population densities of the control agents and the target species will have to be studied regularly until the target species disappeared or are replaced by the snail control agents.

The experimental work on the biological control of molluska by insects under a small scale field trial showed that lampyrid fly larvae were more effective as control agent in killing both pulmonate and operculate snails than sciomyzid fly larvae. The larvae, especially those of sciomyzid fly, caused higher mortality in pulmonate than in operculate snails.

The study on susceptibility of *Oncomelania hupensis quadrasi* from different geographical locations to infection with geographic strains of *Schistosoma japonicum* revealed that the strain of *S. japonicum* from Mindoro was able to develop in all geographical strains of snail hosts, *O.h. quadrasi*, with the best development in *O.h. quadrasi* from Irosin, then Mindoro and Mindanao, and poorest in Leyte snails. *S. japonicum* from Leyte developed best in Irosin, and poorly in snails from Leyte, Mindanao and Mindoro, respectively. *S. japonicum* from Mindanao developed best in snails from Mindanao, and very poorly in snails from Irosin, Mindoro and Leyte.

Using genetic techniques, we discovered that the major Asian human blood fluke, *S. japonicum*, and its intermediate

host snails, *O. hupensis* are both actually groups of previously unrecognized sibling species. We found that Chinese and Philippine populations of both parasite and snail belong to different species. We also found that 4 species of snails may presently be confused under the name *Tricula aperta*; which of these species are really significant intermediate hosts of *S. mekongi* will have to be re-established. Finally, the newly discovered anthropophilic schistosome from Peninsular Malaysia was shown to be a new species related to *S. mekongi* rathan than *S. japonicum*.

The systematic revision of these biomedically important animals will facilitate their control and the improved treatment and prevention of schistosomiasis.

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