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**STUDIES ON SCHISTOSOMIASIS  
IN THE LOWER MEKONG BASIN:  
THE AQUATIC ECOLOGY AND MOLLUSCICIDE  
SENSITIVITY OF *LITHOGLYPHOPSIS APERTA***

Submitted to the Committee for the Coordination  
of Investigations in the Lower Mekong Basin

by

The University of Lowell  
Lowell, Massachusetts, U.S.A.

27 June 1976

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## INTRODUCTION

### Background of the Study

At its 67th (special) session, held November 6 through 11, 1974, in Vientiane, the Mekong Committee approved an integrated approach to schistosomiasis control activities in the Lower Mekong Basin which included three aspects: (1) Technician training and epidemiological surveillance to obtain base-line data; (2) Control measures for early implementation including improvements in the town water supply and latrines, health education, and environmental snail control by engineering improvements to the town shore line; and (3) Testing for molluscicide sensitivity and other studies relating to possible additional control measures including snail control by molluscicides, snail life history studies, and academic training for riparian personnel.

The Committee suggested that part (3) could be performed under a contract with the University of Lowell (formerly, Lowell Technological Institute) with a subcontract to the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Following established procedures, the relevant contracts were developed. The Work Plan, annexed to the prime Lowell Contract, was approved by the Committee at its 69th session in New Delhi, February 26 to March 7, 1975. It was the original intent, subject to availability of funds, to continue the contracts on an annual basis up to a total of three years. Events intervened, however, and in December, 1975, an amendment was signed by both contracting parties agreeing to limit the period of the project to a total of 18 months with a termination date of June 27, 1976. All items in the Work Plan which originally were projected for a three-year period were to be pursued by the contractor, but with a reduction in scope in line with reduced work time.

Under the terms of the Work Plan, the University of Lowell agreed, in part, to undertake the following studies:

I Population Dynamics. Additional base-line data would be collected on year-round aspects of natural snail biology including, if possible, data on the fate of the Mekong hydrobiid snail fauna at difficult collecting times (e.g. during periods of high water).

II Life Cycle Studies of L. aperta. An attempt would

be made to complete the life cycle of this snail under controlled laboratory conditions with the aim of providing perennial supplies of the snail for cyclical maintenance of the Mekong Schistosoma.

III Molluscicide Testing. Tests would be carried out on the sensitivity of Lithoglyphopsis aperta toward conventional and novel molluscicides. Among the former were included copper sulphate, sodium pentachlorophenate, Bayluscide (Niclosamide), N-tritylmorpholine (Frescon), and yurimin. The latter included the organotin compound tri-butyltin oxide (TBT0) dispersed in slow-release rubber pellets.

IV Incrimination of Other Mekong Snail Species in the Transmission of Schistosomiasis. Snails sympatric with L. aperta would be tested as potential transmitters.

The present final report incorporates the results of field studies made during the latter part of the dry season of 1975 (May) and the entire collecting season of 1976 (March through May), as well as laboratory studies conducted at the Faculty of Tropical Medicing, Bangkok, Thailand, and the Museum of Zoology, University of Michigan, Ann Arbor, Michigan, U. S. A.

The conclusions drawn are based on necessarily incomplete data and must be viewed conservatively.

## FINDINGS

### I Population Dynamics of Lithoglyphopsis Aperta

1. L. aperta snails were found and collected without difficulty in Thai areas of the Mekong and Mun Rivers.
2. Two sites near the town of Khemmarat (Ubon Ratchathani Province) supplied all the alpha and gamma snails used in the present study.
3. These sites differed physically only in minor respects, yet produced significantly different numbers of snails.
4. Variation in physical parameters, with the exception of water velocity, may not play an important role in population dynamics.
5. Some fluctuation in chemical components of the water occurred during the period of the study but there is a question whether this was enough to affect distribution of snails.
6. The sudden appearance of large numbers of baby snails in the early part of the year, where a few days before there had been none, suggested that the high water period may be survived in the egg stage.
7. Major elements of the mollusk population sympatric with L. aperta at the two Khemmarat sites A and B included species of Lacunopsis, Jullienia, Hydrorissoia and Hubendickia. The genera Manningiella and Paraprososthenia, although known to occur in this section of the river, were not represented in the present collections.
8. Minor elements of the mollusk population, as represented by the small numbers collected, included Pachydrobia (Hydrobiidae), Stenothyra (Stenothyridae), Clea (Buccinidae) and small bivalves, chiefly the fresh water mussel, Limnoperna.
9. Female L. aperta were approximately twice as numerous as males during the middle and late periods of the dry season, suggestive of a rapidly maturing population with early male die-off.
10. Site A was more productive of alpha snails and site B was more productive of gamma snails.
11. Site B supported larger absolute numbers of snails than did site A.

12. Beta snails, collected only at Ban Hin Laht, were found in large numbers at new localities on the Mun River in Thailand.

13. Beta snails were three times more susceptible to infection with miracidia than were gamma, and about 20 times more susceptible than alpha snails.

14. The presence of beta L. aperta in the Mun River may constitute a health threat in Thailand within the framework of future water management schemes (cf., the Pak Mun Dam) where the snails may be associated with an increase in riverine human populations. The "egg" hypothesis was strengthened by the finding of large numbers of eggs of beta L. aperta in the Mun River a week in advance of finding many baby snails, but no adults of this race in the same area.

## II Maintenance of Lithoglyphopsis Aperta in the Laboratory

15. The three races were successfully maintained in Petri dishes of 9 centimeter diameter, in the presence of silt or sand, fixed diatoms for food, and artificial (Fluorescent) light.

16. An initial high mortality of snails in Petri dishes in June and July coincided with capture and transfer. A second peak of high mortality occurred after the egg laying period in January and February.

17. In Petri dishes, eggs of alpha were laid between September and April, but eggs laid after November failed to hatch. Eggs of beta snails were laid between September and April, but few hatched from eggs laid after December. Gamma snails did not lay eggs in Petri dishes.

18. F<sub>1</sub> alpha snails reached mature size in about 18 weeks, compared with about 20 weeks for beta snails.

19. When snails were reared in round bottles, they were more active and growth was accelerated. However, observations of mortality, egg laying and development were more difficult to make.

20. In nature, alpha and gamma L. aperta reached sexual maturity at 6 weeks and full growth at 10 weeks. Under laboratory conditions, the snails needed about twice as much time to reach the same degree of development.

## III Molluscicide Testing

21. Five conventional molluscicides (Bayluscide, copper sulphate, Frescon, sodium pentachlorophenate and Yurimin) and

one slow-release molluscicide (tributyltin oxide) were tested against wild caught alpha and gamma snails in the field laboratory at Khemmarat. LC<sub>50</sub> and LC<sub>90</sub> values, 95% confidence limits, and slope functions were calculated.

22. Among the conventional molluscicides, Frescon (N-tritylmorpholine) and Bayluscide (Niclosamide) seemed to be most effective against L. aperta, although Yurimin, NaPCP and copper sulphate also showed reasonable effectiveness.

23. LC<sub>50</sub> values for L. aperta and Oncomelania nosophora (hydrobiid vector of schistosomiasis in Japan) did not differ greatly with Bayluscide, Frescon, or NaPCP, but the LC<sub>50</sub> with Yurimin was much lower for L. aperta than for O. nosophora.

24. Lethal concentrations of Bayluscide for the aquatic pulmonate vectors (Biomphalaria glabrata and B. pfeifferi) were lower than for L. aperta and O. nosophora.

25. Lethal concentrations of Frescon for L. aperta and B. glabrata were similar.

26. Lethal concentrations of TBTO for alpha and gamma snails decreased as the soaking time of the rubber pellets increased. LC<sub>50</sub> and LC<sub>90</sub> were reached at day 1 and 3 respectively for alpha snails, and at day 2 for gamma snails.

27. The gamma race snails were slightly more susceptible than alpha snails to the highest concentration of TBTO tested (5.12 ppm). At the lowest concentration tested (0.01 ppm), gamma snails were killed in one half to one third of the time needed to kill alpha snails.

#### IV Incrimination of Other Snails as Hosts of the Mekong Schistosome

28. Six genera and possibly 12 species of snails sympatric with L. aperta at the Khemmarat sites failed to release cercariae after being suitably exposed to miracidia of the Mekong Schistosoma. These species were thus considered unable to transmit schistosomiasis.

29. Although the attempted incrimination of other hydrobiid species merits continued effort (the above numbers represent only about 13% of all the hydrobiid species reported from the Lower Mekong Basin) it now appears unlikely that species other than L. aperta will prove to be efficient carriers of schistosomiasis.

30. With present information, control efforts at Khong Island should continue to focus on control of L. aperta and other snails can probably be ignored.

## RECOMMENDATIONS

1. Security conditions on the Mekong River prevented the exploration of many areas that could eventually prove to be productive of schistosome bearing snails. At present, such snails have been studied principally at Khemmarat, Khong Chiam (Ban Dan), and Khong Island in the Mekong River, and at Phibun Mangsahan and Kaeng Ta Tai in the Mun River. In the future, under more normal conditions, expanded efforts to locate and characterize all other L. aperta sites in the Mekong and Mun Rivers should be made.

2. The original plan to study the natural life cycle of L. aperta around the calendar year should eventually be completed, possibly utilizing the help and facilities of the Royal Thai Naval Station at Khemmarat, both as a work site and for protection of equipment.

3. Strong evidence now supports the "egg hypothesis," i.e., that the transmitting L. aperta snails survive periods of high water primarily in the egg stage. The evidence is as yet indirect. Direct evidence could be derived from stone trapping throughout the year.

4. Beta L. aperta have been found in two sites on the Mun River: Phibun Mangsahan and Kaeng Ta Tai. Predictably, other localities along this large tributary will be found. In view of plans to develop the Mun River at its confluence with the Mekong (Pak Mun Dam), the need to expand the search for this potentially dangerous snail species becomes evident.

5. According to available evidence, L. aperta is found only in the Mekong and Mun Rivers. It is not found in lakes or ponds and could not survive in such locations even if deliberately introduced. This point is, however, based on field observations, not experimental evidence. Since beta L. aperta have now been collected close to the confluence of the Mun and Lam Dom Noi (and by extension, the Sirindhorn Dam) a field experiment should be devised to test the ability of L. aperta (of all three races) to survive in enclosed or impounded waters on the Korat Plateau. Such an experiment could be carried out in waters adjacent to the Mun or Mekong Rivers so as to minimize the danger of accidental spread to new areas.

6. Future field work should be designed to make as much use as possible of such non-endemic areas as Khemmarat and Ban Dan in order to minimize risk. At the same time, it is necessary to note that there is no proof that schistosomiasis does not occur in these areas but, only that cases



have not come to light. In view of this gap, it is urged that in depth epidemiological surveys be conducted and that case finding studies be pursued in all areas where biological studies are planned.

7. With regard to use of molluscicides to control snail populations, the sensitivity of L. aperta to conventional as well as novel molluscicides tends to resemble that for classical transmitters of schistosomiasis (Oncomelania, Biomphalaria, Bulinus). Thus, costs of conventional mollusciciding operations in the Mekong River may not differ from those in similar localities elsewhere in the world.

8. In the river, where there is detectable current, L. aperta will probably be best controlled by the use of TBTO in elastomeric formulations, in the forms of strings or sheets. Rubber pellets maintained adequate levels of chemical under experimental conditions but are likely to be covered by sand or silt in nature. Another possibility may be to paint rocks and embankments with a rubber-base paint containing tributyltinfluoride (TBTF), an approach that has proved successful in marine antifouling operations for barnacles but, field trials will be required in advance of application.

9. Where L. aperta occurs in pockets of quiet water with little current, such as can be found in the main transmission site at Khong Island, conventional molluscicides could be used with appropriate field testing. The chemical of choice would be Bayluscide which is little affected by the high pH and other physico-chemical conditions of the Mekong. Cheaper chemicals may lose effectiveness too quickly; copper sulphate is adversely affected by high pH; sodium pentachlorophenate is quickly destroyed in the presence of direct sunlight. Of course, such considerations could be offset by repeated application of chemical.

10. With regard to other molluscan hosts of the Mekong Schistosoma, none tested has shown signs of being any sort of threat. This includes primarily the sympatric hydrobiids. It would be useful to test the susceptibility of unrelated snails such as the pond pulmonates but, such an experiment would not have a high priority in any list of research projects still to be done.

11. If eradication is envisaged, eggs as well as adults must be killed. There is no evidence that molluscicides are toxic to eggs of L. aperta but, such evidence has not been systematically sought (Bayluscide is effective against eggs of Oncomelania, Biomphalaria, and Bulinus). Since egg laying may be a fairly continuous process, reaching a peak at the end of the dry season (there is a suggestion of two generations during each low water period), applications of chemical would

have to begin early and continue late. It will not be possible to achieve quick eradication of L. aperta in any focus. Criteria of effectiveness will have to be sought by snail surveys in the following season.

12. With regard to host parasite relations, it is recommended to initiate a study on the effects of miracidial numbers at the time of infection on cercarial output of the snail. This should be done varying such parameters as salinity, pH, and turbidity.

RESULTS OF FIELD AND LABORATORY  
STUDIES

## I Population Dynamics of Lithoglyphopsis Aperta

A temporary snail laboratory was established in March, 1975 in a rented house in Khemmarat, Ubon Ratchathani Province, Thailand, on the right bank of the Mekong River. Good collecting sites for transmitting and other types of snails were to be found at short distances to the east (Thai side) and the west (Lao side). Field staff took up residence in the house in April, 1975 and rented a boat and the services of its boatman on a full-time basis.

Collections of alpha race L. aperta were made from islands in the middle of the river, 4 km. east of Khemmarat, at a site called Bang Koey, (also named site A in this report).

In previous years, large collections of gamma race L. aperta had been made close to the left bank shore (Lao side) of the Mekong about 5 km. west of Khemmarat. In 1975, visits to this side were risky and in 1976, the area could not be visited at all. An alternative gamma site was sought and eventually found near the town of ban Khi Lek on the Thai side of the river, about 5 km. east of Khemmarat. This site, called Bung Kong, is also named site B in the present report. Sites A and B are identified in the map, Figure 1.

Collections were brought to the field laboratory and established in aerated aquariums. Only the L. aperta sites were sampled and only species sympatric with L. aperta are considered in this report. For example, the sand dwelling Stenothyra which predominate in most areas in the Mekong River are not truly sympatric with L. aperta and the small numbers recovered in this study must have been accidental. The genus Manningiella, found elsewhere near Khemmarat, was not collected at either of the two sites mentioned above.

### 1. Description of the Collecting Sites

Site A. A series of temporary islands in the Mekong River, east of Khemmarat, called Bang Koey (see map, Figure 1). Snails were found on stones in 60-130 cm depth. Fluctuations in water level are shown in Table 1. Stones measuring between approximately 400 and 1,000 square centimeters in surface area were brought up and all snails collected. Early in March (March 4) the stones bore clusters of snail eggs and filamentous green algae as well as snails. All surfaces of the stones were found to support snail populations but, Lithoglyphopsis aperta were found only on the under surfaces. Snail eggs were also deposited on all surfaces. The eggs were of different sizes and were deposited singly in the clusters that are typical of aquatic Hydrobiidae. Each egg was protected by a capsule composed of silt particles. Colors of the various types of eggs ranged from black and gray

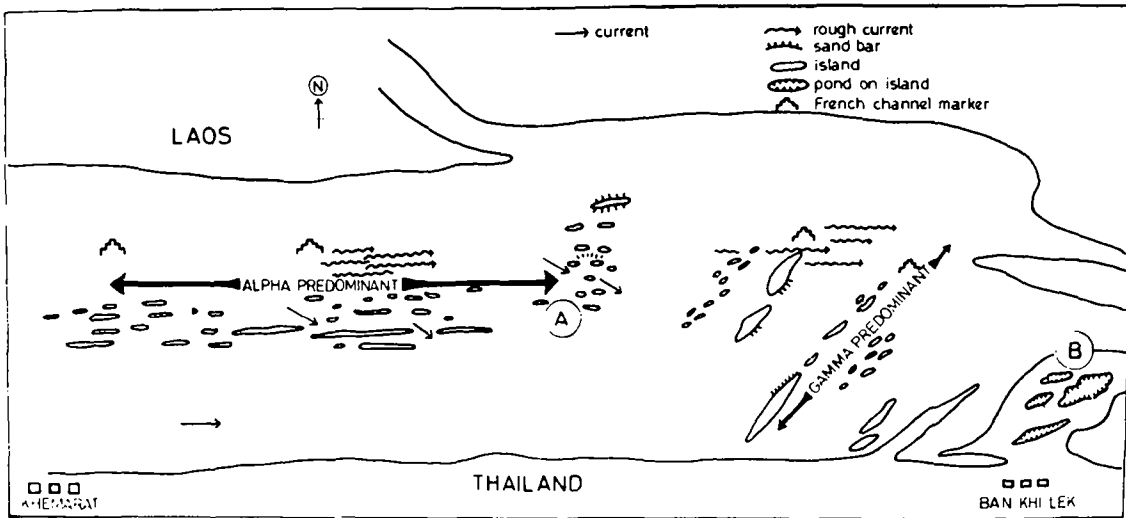


Fig. 1.--Collecting sites A and B in the Mekong River between Khemmarat and Ban Khi Lek.

Table 1 Fluctuations in water depth between March 4 and May 13, 1976, at collecting sites A (Bang Koey) and B (Bung Kong) in the Mekong River (in centimeters).

<u>Week</u>	<u>Site A</u>	<u>Site B</u>
1	60-70	
2		70-90
3	60-70	
4		60-80
5	50-60	
6		60-70
7	60-70	
8		60-80
9	70-90	
10		80-100
11	70-130	

to reddish brown and yellow. Laboratory observations later determined which of these various types of eggs had been laid by L. aperta (see below) but no attempt was made to determine the specific origin of all egg types.

During the 5th week (April 1), the water level at collecting site A subsided by half a foot. During the 7th week (April 15), the level rose again by about 4 inches. At this time, there seemed to be a reduction in the quantity of filamentous green algae at the collecting site. Sand appeared at the edges of the small islands. The stones then appeared clean, although a few still bore traces of algae. Most snails were found clustering beneath the stones at this time.

Site B. Near the right bank of the Mekong River at Ban Khi Lek, a series of islets emergent during low water periods and connecting obliquely with the shore, called Bung Kong (see map, Figure 1). Stones under water supported a luxuriant growth of filamentous green algae. During the 2nd week (March 11), snail eggs of various sizes and colors were discovered deposited on the undersurfaces of stones. By the 4th week (March 25), the water level had come down 3 inches. Stones thus exposed were then covered with thick mats of dried algae. Underwater, snails were found crawling on stone surfaces beneath the layers of green algae. As the water level fell with the advancing dry season, more and more stones near the edge of the water were left dry with drying mats of algae covering them. During the 8th week (April 22), the water level rose 4 inches, carrying away much of the dead algae. At this time, most snails were found on the sides and upper surfaces of still submerged stones; the undersurfaces of the stones were black and muddy.

Differences between sites A and B. In spite of their relative proximity, separated by only about 1 kilometer on the same river, sites A and B differed from each other with respect to water depth (Table 1, Figure 2), water velocity (Table 2), total mollusk populations (Tables 3, 4 and Figure 3), and relative abundance of alpha and gamma race L. aperta.

Site B was generally more productive of mollusks, particularly hydrobiids, than site A, although initial collections at site B were somewhat smaller than site A. Given the method of collecting with its capacity for human error, the fact that the sizes of the weekly collections remained well with a degree of magnitude of each other attests to the stability of the populations sampled and suggests that no catastrophe occurred to these snails during the periods of field study that might have influenced the interpretation of results.

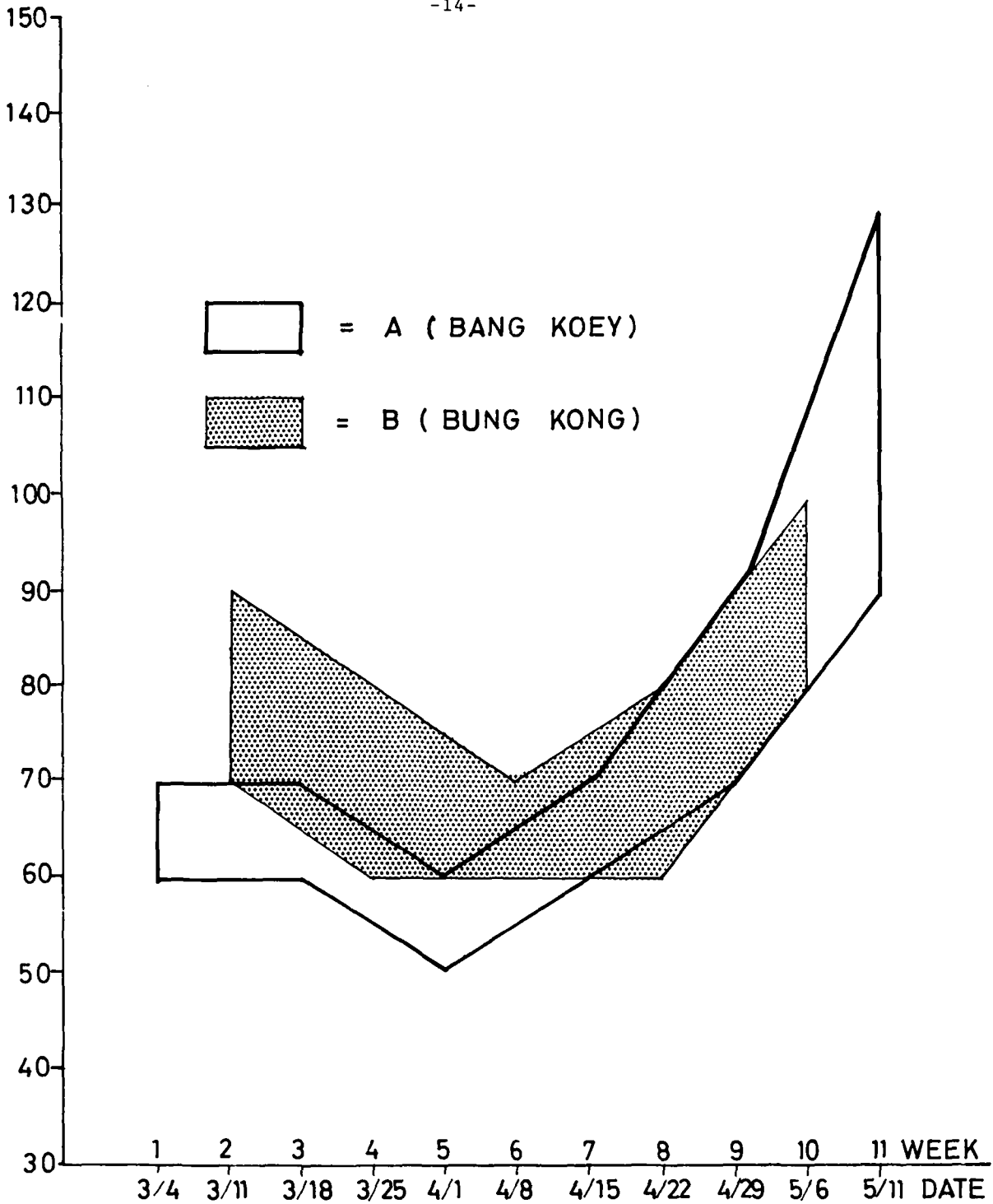


Fig. 2.--Fluctuations in water depth from March 4 to May 13, 1976, at collecting sites A and B in the Mekong River (in centimeters).



Table 2 Water velocity in the Mekong River at sites A (Bang Koey) and B (Bung Kong) in 1976, in feet per second.

Date	Site A (1) Surface	Site B (2) Surface	Bottom
3-11	-	4.57	0.91
3-18	2.00	-	-
3-25	2.08	4.39	0.73
4-1	2.08	-	-
4-8	-	1.30	0.71
4-15	2.50	-	-
4-22	-	1.41	0.61
4-29	2.56	-	-
5-6	-	1.46	1.06
5-13	2.78	-	-

(1) Measured by calculating the time necessary for a plastic float to move 10 feet downstream.

(2) Measured by a Price type AA Current Meter (Arline Precision Instruments, Inc., Baltimore) calibrated by the U. S. Bureau of Standards according to the formula:  $V = 2.218N + 0.022$ , where  $N$  = revolutions of wheel per second and  $V$  = velocity in feet per second.

Table 3 Mollusk collections at site A (Bang Koey) in the Mekong River from March 4 to May 13, 1976.

Groups	Date: No. of Weeks: Snails	3-4-76 1st week		3-18-76 3rd week		4-1-76 5th week		4-15-76 7th week		4-29-76 9th week		5-13-76 11th week	
		#	%	#	%	#	%	#	%	#	%	#	%
A. Predominant hydrobiids	Hydrorissoia	276	8.58	190	12.90	103	4.78	12	0.43	6	0.18	0	0.00
	Hubendickia	1,382	42.96	41	2.78	228	10.59	278	10.08	649	19.20	1,935	67.28
	Lacunopsis	647	20.11	440	29.87	315	14.63	419	15.19	236	6.98	71	2.47
	L. aperta-alpha	640	19.89	640	43.45	1,331	61.82	1,776	64.37	2,073	61.33	568	19.75
	L. aperta-gamma	5	0.16	107	7.26	86	4.00	165	5.98	312	9.23	237	8.24
B. Other hydrobiids	Jullienia	216	6.72	21	1.43	22	1.02	27	0.98	26	0.77	3	0.10
	Pachydrobia	2	0.06	0	0.00	1	0.05	4	0.14	30	0.89	32	1.11
C. Other mollusks	Stenothyra	47	1.46	12	0.81	61	2.83	62	2.25	46	1.36	28	0.98
	Clea	0	0.00	2	0.14	1	0.05	2	0.07	0	0.00	0	0.00
	Bivalves	2	0.06	20	1.36	5	0.23	14	0.51	2	0.06	2	0.07
	TOTALS	3,217	100.00	1,473	100.00	2,153	100.00	2,759	100.00	3,380	100.00	2,876	100.00

Table 4 Mollusk collections at site B (Bung Kong) in the Mekong River from March 11 to May 6, 1976.

Groups	No. of Weeks:	Date:	3-11-76		3-25-76		4-8-76		4-22-76		5-6-76	
			2nd week		4th week		6th week		8th week		10th week	
			#	%	#	%	#	%	#	%	#	%
A. Predominant hydrobiids	Hydrorissoia	1,289	48.97	785	30.46	732	22.00	212	6.27	35	0.70	
	Hubendickia	1	0.04	261	10.13	226	6.79	11	0.33	70	1.40	
	Lacunopsis	793	30.13	710	27.55	1,122	33.71	1,498	44.33	714	14.23	
	<u>L. aperta</u> -alpha	242	9.20	338	13.12	237	7.12	93	2.75	76	1.51	
	<u>L. aperta</u> -gamma	166	6.31	411	15.95	639	19.20	1,381	40.87	4,024	80.21	
B. Other hydrobiids	Jullienia	6	0.23	5	0.19	0	0.00	0	0.00	0	0.00	
	Pachydrobia	4	0.15	6	0.23	306	9.20	152	4.50	80	1.59	
C. Other mollusks	Stenothyra	38	1.44	4	0.16	26	0.78	24	0.71	11	0.22	
	Clea	0	0.00	0	0.00	1	0.03	0	0.00	0	0.00	
	Bivalves	93	3.53	57	2.21	39	1.17	8	0.24	7	0.14	
TOTALS		2,632	100.00	2,577	100.00	3,328	100.00	3,379	100.00	5,017	100.00	

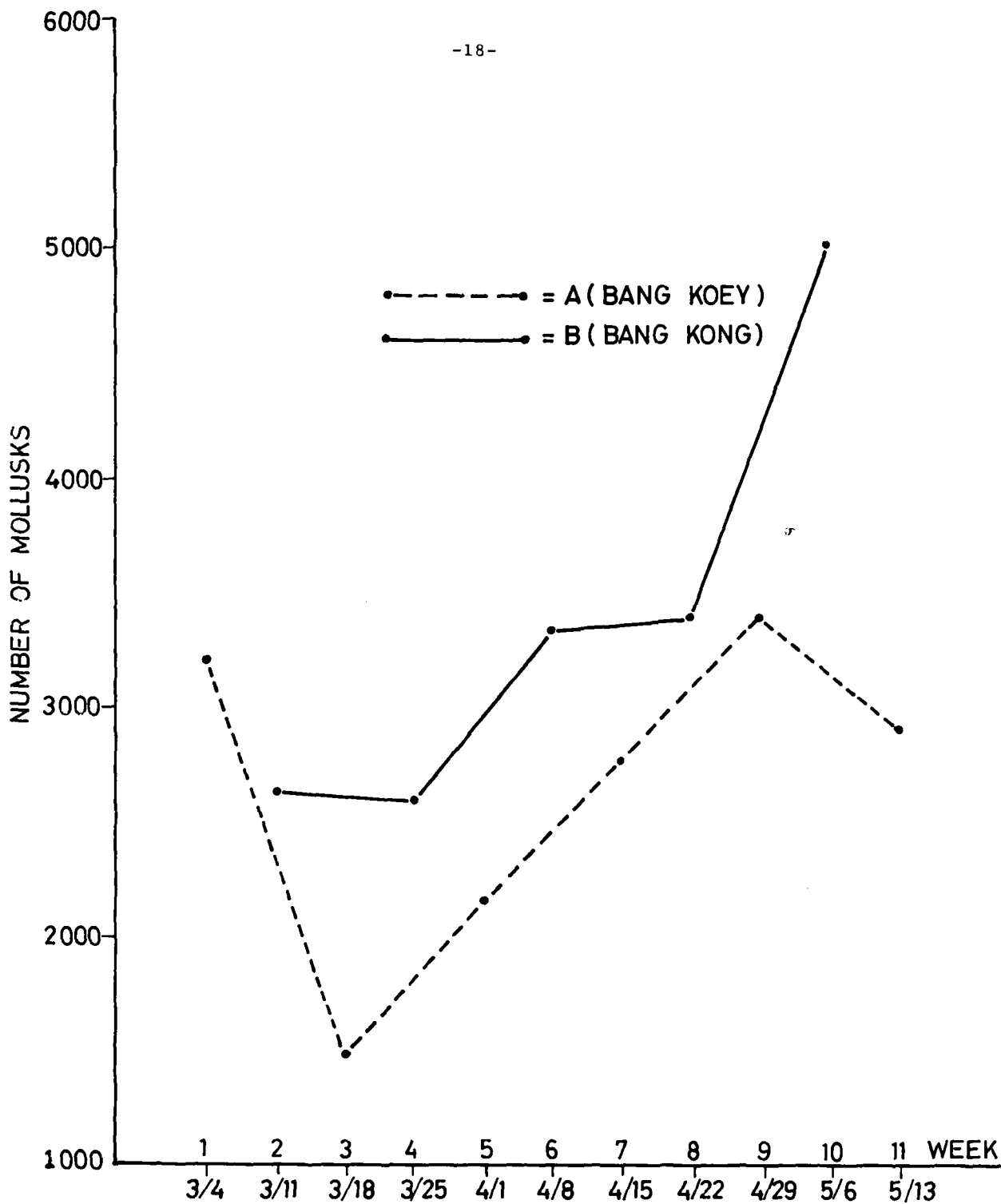


Fig. 3.--Total numbers of mollusks collected at sites A and B in the Mekong River from March 4 to May 13, 1976.

## 2. Time of Collections and Field Work

Field work commenced in April, 1975 when funds became available and continued for 4 weeks to the beginning of June. At this time, the work was forcibly interrupted by high water and bad collecting conditions.

In 1976, the field laboratory at Khemmarat was re-established in February, and collections began again the first week in March when water levels had subsided sufficiently to permit hand collecting. At this time, problems of security dictated a change in collecting methods. Fishermen were hired to collect stones from the Bang Koey site, replacing the two technicians who had done this work in 1975. Each rock was placed in a separate bucket and was brought to the temporary laboratory in Khemmarat for separation and identification. Molluscicide studies were done in the laboratory. Work continued for a period of 12 weeks and was suspended after the third week of May because of the early onset of the wet season and the rising water.

## 3. Physical and Chemical Parameters of the Water

Physical and chemical parameters were observed and recorded regularly during the period of study. Normally, these were conducted weekly when the field work was in progress. At other times they were done monthly.

### Physical Conditions

1. Temperature. Air and water temperatures were recorded at 10 A.M. on the days that water analyses were conducted (Table 5). The air temperature usually was between 26° - 34°C; however, in summer the air temperature often reached 38°C. In 1976, there was an abnormal cold spell in March when the temperature dropped to 23°C. Unfortunately, records of air and water temperature during the past few years were not available for comparison but, in general, air temperatures in this region rarely drop below 20°C.

The water temperatures were measured at 5 cm. below the surface. During the study period, water temperatures ranged between 24° - 28°C. The temperature dropped to 21°C one week in March, 1976 (Table 5).

2. Turbidity. In dry season the water in the river was low and clear. It gradually became turbid as the rain started to fall. From June, in association with the heavy rains, the current was fast and the turbidity rose to 300 - 400 F.T.U. (Table 5). At this time, the water was rising rapidly and, despite many attempts, snails could no longer be collected.

Table 5 Analyses of water from the habitats of L. aperta.

Location	Date	Temp. °C		pH	Turbidity (F.T.U.)	Dis O <sub>2</sub> (mg/L)	mg/liter							Total Hardness (ppm)	
		Water	Air				Mg	Ca	Mg/Ca	Cu	NaCl	Cl	SO <sub>4</sub>		CaCO <sub>3</sub>
Khemmarat	12-3-75	-	-	8.40	10	7.0	20	70	1:3.5	0.00	23.10	14.00	13	19.74	90
Khemmarat	11-4-75	32.0	36.0	8.36	18	7.0	25	75	1:3.0	0.02	18.15	11.00	15	15.51	100
Ban Khi Lek	12-4-75	36.8	38.0	8.37	8	7.5	25	73	1:2.9	0.00	20.63	12.50	9	17.63	98
Bang Koev	14-4-75	32.0	38.0	8.20	5	6.0	25	80	1:3.2	0.00	21.45	13.00	13	18.33	105
Bang Koev	21-4-75	31.0	34.8	8.39	5	7.0	25	75	1:3.0	0.00	18.15	11.00	8	15.51	100
Ban Khi Lek	22-4-75	30.5	33.0	8.48	6	7.5	22	80	1:3.1	0.00	18.98	11.50	15	16.22	102
Ban Khi Lek	7-5-75	32.5	33.5	8.30	105	6.5	23	75	1:3.3	0.00	18.15	11.00	6	15.51	98
Bang Koev	5-5-75	34.5	34.0	8.48	2	7.0	21	80	1:3.8	0.00	20.63	12.50	4	17.63	101
Bang Koev	19-5-75	29.8	30.5	8.35	25	7.0	25	70	1:2.8	0.00	18.98	11.50	12	16.22	95
Ban Khi Lek	21-5-75	30.0	31.5	8.30	42	7.0	25	65	1:2.6	0.00	16.50	10.00	9	14.10	90
Hauy Na Wang	2-6-75	28.0	28.0	8.00	59	4.0	15	55	1:3.6	0.00	17.33	10.50	10	14.81	70
Ban Khi Lek	2-6-75	28.0	28.8	8.50	15	7.5	7	28	1:4.0	0.00	3.30	2.00	3	2.80	35
Khemmarat	12-6-75	28.2	26.0	8.00	130	6.0	12	48	1:4.0	0.00	9.08	5.50	17	7.76	60
Khemmarat	29-7-75	26.8	26.6	8.40	300	6.0	15	55	1:3.7	0.00	6.60	4.00	2	5.64	70
Ban Khi Lek	29-7-75	28.0	28.5	9.55	430	6.0	18	50	1:2.8	0.00	7.43	4.50	0	6.34	68
Ban Khi Lek	2-9-75	28.5	31.0	8.10	130	5.5	15	35	1:2.4	0.00	6.60	4.00	0	5.64	50
Bung Kong	3-9-75	27.0	28.0	8.00	128	4.5	12	38	1:3.1	0.00	6.27	3.75	0	5.36	50
Khemmarat	3-9-75	26.0	26.0	8.20	135	4.0	14	36	1:2.3	0.00	4.95	3.00	2	4.23	50
Khemmarat	1-10-75	25.5	25.5	8.50	200	6.0	13	40	1:3.1	0.00	4.95	3.00	2.5	4.23	53

Table 5 (Continued)

Location	Date	Temp. °C		pH	Turbidity (F.T.U.)	Dis. O <sub>2</sub> (mg/L)	mg/liter						Total Hardness (ppm)		
		Water	Air				Mg	Ca	Mg/Ca	Cu	NaCl	Cl		SO <sub>4</sub>	CaCO <sub>3</sub>
Ban Khi Lek	1-10-75	28.5	29.5	7.70	135	6.5	14	41	1:2.9	0	5.28	3.25	2.0	4.51	55
Bung Kong	1-10-75	28.5	29.5	8.10	150	5.5	15	40	1:2.6	0	4.13	2.50	3.5	3.53	55
Ban Khi Lek	13-2-76	26.5	27.0	8.52	4	7.0	25	70	1:2.8	0	16.50	10.00	5.0	14.10	95
Bang Koei	4-3-76	24.5	26.0	8.10	9	7.5	27	65	1:2.5	0	18.15	11.00	9.0	15.51	95
Bung Kong	11-3-76	24.0	24.0	8.60	20	7.5	25	65	1:2.6	0	21.45	13.00	12.0	18.33	90
Bang Koei	18-3-76	21.0	23.0	8.10	12	6.5	25	70	1:2.8	0	23.10	14.00	14.0	19.74	95
Bung Kong	22-3-76	30.0	34.0	8.30	10	7.5	26	70	1:2.7	0	23.10	14.00	17.0	19.74	96
Bung Kong	25-3-76	28.0	29.0	8.20	9	7.0	22	70	1:3.2	0	24.75	15.00	13.0	21.15	92
Bang Koei	29-3-76	25.0	26.0	8.10	5	7.0	24	71	1:2.9	0	25.58	15.00	17.0	21.86	95
Bang Koei	1-4-76	25.0	26.0	8.10	4	6.5	26	68	1:2.6	0	20.63	12.50	17.0	17.63	94
Bung Kong	6-4-76	26.5	27.0	8.20	11	7.0	25	70	1:2.8	0	26.40	16.00	16.0	22.56	95
Bung Kong	8-4-76	29.0	30.0	8.20	5	6.0	23	68	1:2.9	0	19.80	12.00	14.0	16.92	91
Bang Koei	13-4-76	26.0	27.0	8.65	15	8.0	23	60	1:2.6	0	24.75	15.00	15.0	21.15	83
Bang Koei	16-4-76	34.5	35.5	8.00	6	7.0	28	70	1:2.8	0	24.75	15.00	18.0	21.15	98

Diurnal changes in temperature and water turbidity were measured from May to October 1975 (Table 6). There was little outstanding variation. The temperature at night was a few degrees lower than during the day.

The data collected are only suggestive since the time of study was short. However, it appears that variation in physical parameters with the exception of water velocity and level may not play an important role in snail population dynamics.

#### Chemical Factors

Determination of chemical components of the water in the L. aperta sites was conducted by a Hach Portable Water Kit (Hach Chemical Company, Ames, Iowa, U.S.A.). Analysis was made of the chemical components of ecological significance including magnesium, calcium, copper, sodium chloride, sulphate, calcium carbonate, pH and total hardness (Table 5).

Analyses with the Hach Kit were not highly accurate because the kit is only for rough field determinations. The data are presented with this reservation.

Water analysis during the study months showed some seasonal fluctuation in the chemical components especially during the low and high water periods (Table 5). However, the fluctuation apparently was not so great as to play a role in the ecology of the snails. For example, the salinity in the dry season was around 18 - 25 mg/litre NaCl and around 3 - 7 mg/litre NaCl in the rainy season. Though the fluctuation of salinity seems to coincide with the hatching and development of baby snails, it is doubtful that these small changes in salinity could be a significant factor in snail population dynamics.

Analysis of the mineral ions at sites A and B had shown that river water contained sufficient amounts of such minerals as are generally recognized as necessary components of snail growth. Calcium, for example, normally required for the formation of shell, was present at the level of 28 - 80 mg/litre.

Observations of pH and total hardness of the river water showed that the river water was regularly alkaline and soft. At all times of the year the pH was never below 7 and the total hardness was never above 110 ppm (as CaCO<sub>3</sub>).

Other studies have shown that pH and water hardness influence the performance of molluscicides or other pesticides (Harrison, 1960; WHO, 1965). For example, hard water with



Table 6 Diurnal variation of temperature, turbidity and pH of water in Mekong River during May through October, 1975.

Date	Time of Determination	Temp. °C		Turbidity (F.T.U.)	pH
		Air	Water		
May 21, 1975	6 A.M.	26.0	29.0	N.D.	N.D.
	10 A.M.	31.1	30.0	42	8.3
	12 P.M.	27.0	30.0	N.D.	N.D.
June 4, 1975	6 A.M.	24.8	28.5	N.D.	N.D.
	10 A.M.	28.8	28.0	15	8.5
	12 P.M.	26.0	29.0	N.D.	N.D.
June 11, 1975	6 A.M.	25.8	28.2	N.D.	8.5
	10 A.M.	26.0	28.2	130	8.4
	12 P.M.	26.2	28.5	N.D.	8.0
September 2, 1975	6 A.M.	24.8	26.0	130	8.2
	10 A.M.	31.0	28.5	130	8.1
	12 P.M.	24.5	26.0	160	8.15
October 1, 1975	6 A.M.	24.0	28.0	200	8.5
	10 A.M.	25.5	25.5	200	8.5
	12 P.M.	24.5	28.8	190	8.4

F.T.U. = Formazin Turbidity Units.

a pH above 7 decreases the solubility of copper sulphate. In contrast, the solubility of Bayluscide (niclosamide) increases in alkaline water and sharply falls off if the pH is below 7. The requirement of hard water for Bayluscide is considered to be a serious disadvantage in snail control in Africa (Meyling et al., 1962). Since longitudinal observations on water chemistry of the L. aperta habitats revealed no great difference in the pH and total hardness, these data obtained could be used as a guide line for snail control in future.

#### 4. Fate of the Snails During High Water Periods

During the period of high water, no hydrobiids and few mollusks of any kind could be collected in the Mekong River because of dangerous conditions. In order to complete a picture of the natural life cycle of the schistosome transmitting snails, some explanation of their fate during the high water period is needed. In this connection the following facts are pertinent:

In the early part of the dry seasons of 1975 and 1976 (in January or February), rocks collected at known snail sites were covered with typical hydrobiid eggs but, adults, with the exception of Hubendickia and a few Lacunopsis, were rare or absent. This was true in all parts of the Mekong and Mun Rivers that were explored. A week or so later (in early March), rocks in the same areas, increasingly exposed to the surface by dropping water levels, were found to be teeming with snails. These sudden, large populations could not have been "missed" by collectors during the previous weeks. The snails were newcomers and were, moreover, always all juveniles.

During the previous season, it had been difficult to collect adult snails in late May or early June, a few weeks after the water level began to rise, although many eggs were present. It could be postulated that rising water killed snails which could not move vertically in order to stay in their preferred niche. We found, however, that Lithoglyphopsis aperta could be collected at Khong Island in 2 or 3 meters in mid-June (1973, 1974) and the specimens seemed to be healthy and survived transfer to the laboratory. seemingly, these snails would have been swept away by increasingly rapid current and this was undoubtedly the fate of some. On the other hand, experimental studies showed that snails underwent a significant die-off after laying eggs in the laboratory, where the environmental stress was conspicuously absent. It is assumed that the annual egg-laying and the annual die-off were associated phenomena and there is no reason to think that this was not as true under natural conditions as in the laboratory.

## 5. Species of Mollusks Collected During the Study

Species sympatric with Lithoglyphopsis aperta at sites A and B included species of Lacunopsis, Jullienia, Hydrorissoia and Hubendickia. There were also small numbers of Pachydrobia, Stenothyra (Stenothyridae), Clea (Buccinidae) and small bivalves, chiefly Corbicula and Limnoperna.

The largest absolute numbers of mollusks found at any one collecting period occurred at site B. The bulk of these (during the last two collecting weeks) were L. aperta, gamma race (40.87% of the entire collection during the 8th week and 80.21% during the 10th week).

The various snails are discussed by genus below:

Hydrorissoia. Very large numbers of primarily H. elongata were collected at site B in week 2 (Figure 4). Populations dwindled steadily during the entire collecting period at both sites. Hydrorissoia regularly formed a larger percentage of the total bulk of mollusks collected at site B (where they were dominant early in the season) than at site A (where they did not appear to be dominant). This genus is attractive to miracidia of the Mekong Schistosoma, according to the Smithsonian Institution (1974).

Hubendickia. The collections included H. siamensis and H. tuberculata. Large numbers were taken at site A early and late in the collecting season, suggesting that two generations had occurred, with relatively rapid maturation of intervening stages (Figure 5). Relatively reduced numbers were found at site B. According to Brandt (1974), species of this genus were not accepted by miracidia of the Mekong Schistosoma; however, the Smithsonian report (1974) states that infection of Hubendickia was experimentally observed although no cercariae were produced.

Lacunopsis. Collections at the Khemmarat sites included L. conica, L. coronata, L. Fischerpiettei, L. globosa, L. massiei and L. sphaerica. Numbers remained elevated and relatively constant at site A, with the appearance of a small peak during week 4, (Figure 6). At site B, smaller absolute numbers were collected, dwindling notably by week 11. Lacunopsis formed a major portion of the hydrobiid populations at both sites throughout most of the 11 week collecting period. They cannot transmit the schistosome cycle (see below).

Lithoglyphopsis aperta, alpha race. This is the large, globose form originally described by Temcharoen (1971) as L. aperta. In March, this snail comprised approximately

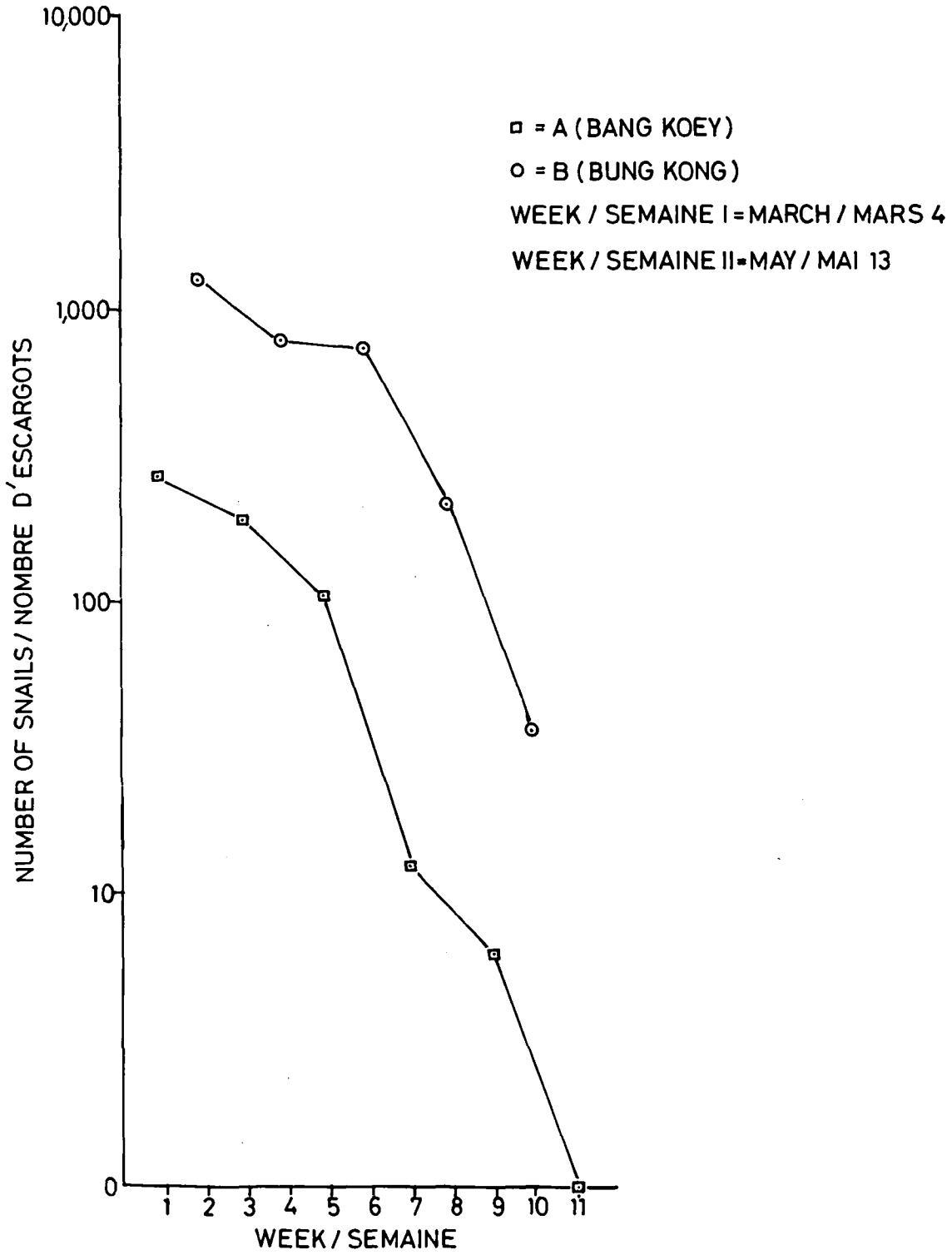


Fig. 4.--Numbers of Hydrorisssoia collected at two sites on the Mekong River, 1976.

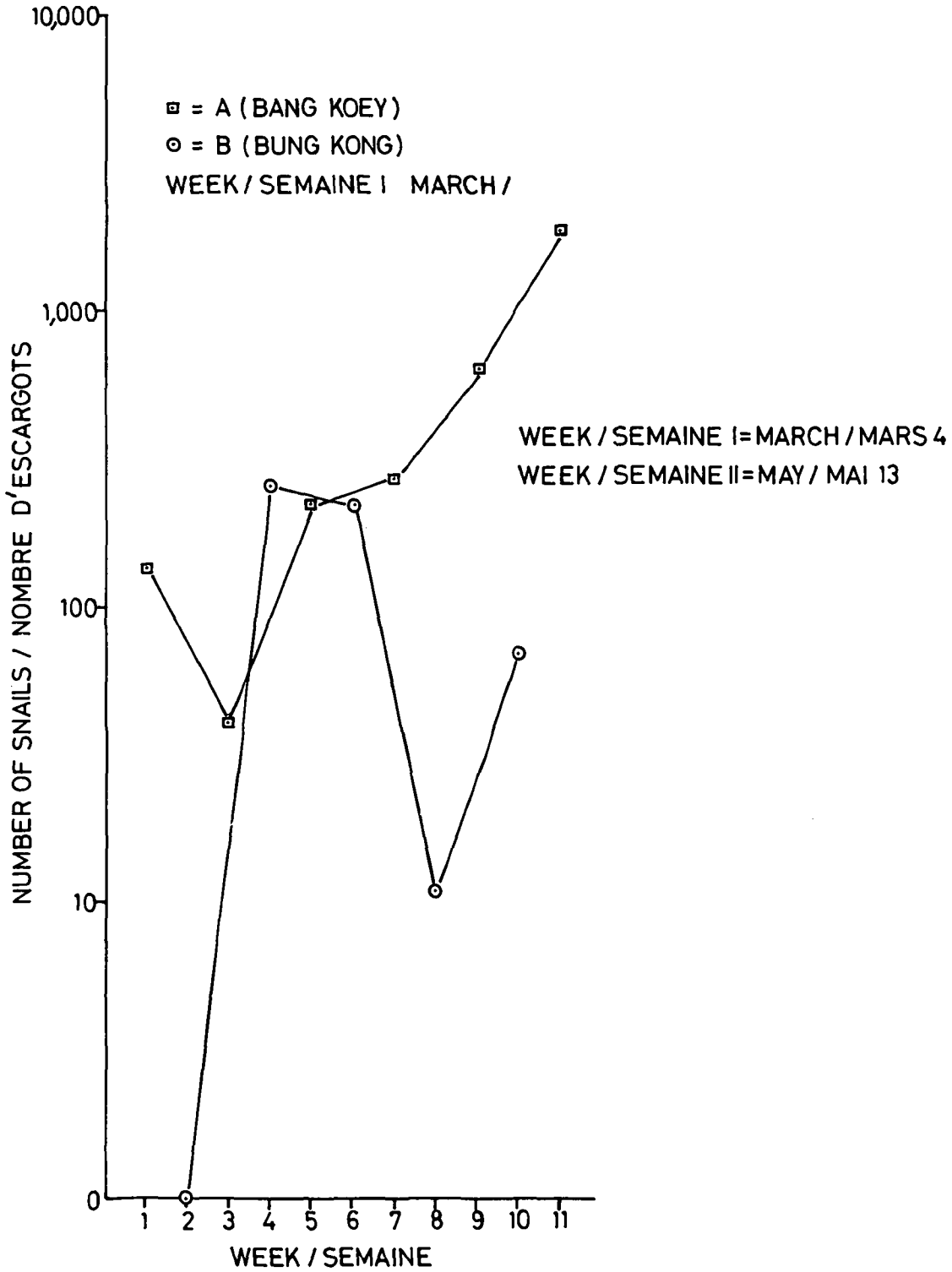


Fig. 5.--Numbers of Hubendickia collected at two sites on the Mekong River, 1976.

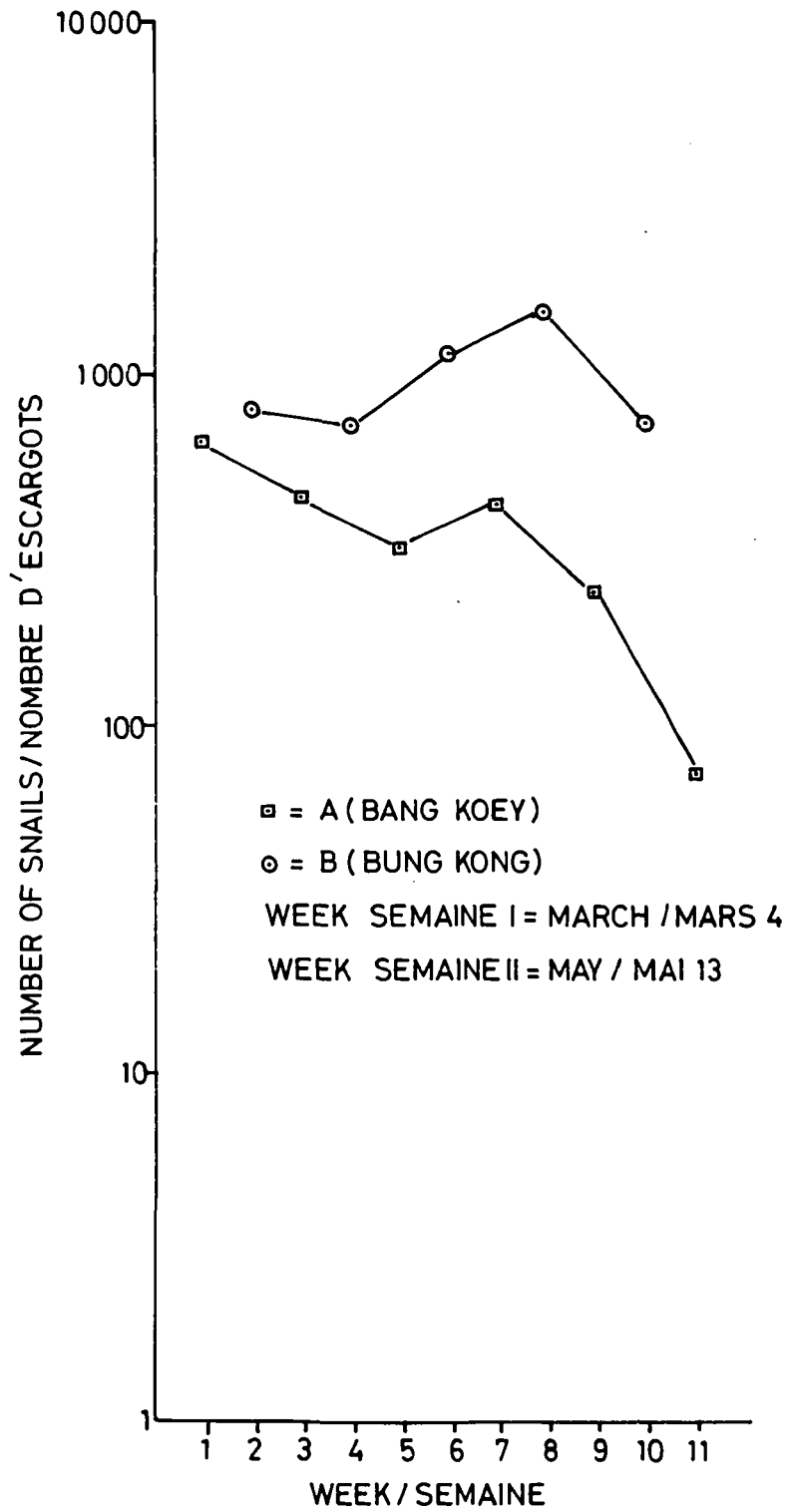


Fig. 6.--Numbers of Lacunopsis collected at two sites on the Mekong River, 1976.

20% to 40% of total collections at site A. By the 7th week (April 15), they constituted 64.37% of all snails at site A but, thereafter, declined in numbers (Figure 7). The data was not sufficient to know if there were two generations of snails during the collecting season. At site B only small numbers of alpha were collected. They reached a small peak in the 4th week (March 25), whereas at site A, a distinct peak was not reached until the 9th week (April 29).

The alpha race is moderately susceptible to the Mekong Schistosoma (see below) and is capable of carrying the cycle through to completion.

Lithoglyphopsis aperta, gamma race. Site A supported a relatively reduced population. Two distinct, but small, peaks were seen in numbers, the first in the 3rd week (March 18) and the second in the 9th week (April 29), (Figure 8). It is believed that this was evidence of two generations during the one dry season. At site B, numbers of gamma increased steadily until they comprised more than 80% of the total collection in the 11th week (May 13). Gamma race L. aperta are the actual transmitters of human schistosomiasis at Khong Island, about 200 km further south. Growth of gamma was slow compared with alpha; throughout the collecting period, the ratio of young (smaller than 2 mm) to old (larger than 2 mm) shells remained in the vicinity of 2:1. Site B is considered a "gamma" site, supporting very large numbers of gamma by the middle of May, both with relation to the numbers of sympatric alpha (Table 7) and to the total mollusk population (Tables 3 & 4).

Jullienia. Members of this genus were collected at site A in large numbers only during the 1st week (March 4), (Figure 9). Thereafter, numbers declined markedly and may have represented accidental introduction of individuals from other nearby niches. Only occasional specimens were collected at site B, and none after the 6th week (April 8). Brandt (1974) reported that three species of Jullienia (J. harmandi, J. nucula, and J. rolfbrandti) were not attractive to miracidia of the Mekong schistosome. This genus, then, does not appear to have any biomedical importance in the Mekong River.

Pachydrobia. Only insignificant numbers of Pachydrobia were collected at site A during 1976. At site B, a small increase in population was noted during the 6th and 8th weeks (April 8 to 22), (Figure 10). This genus is attractive to miracidia of the Mekong Schistosoma. Lo et al. (1971) reported that sporocysts developed beyond 24 hours in P. bavayi from Khong Island, although cercariae were not produced. Pachydrobia appears to be a good decoy snail for miracidia.

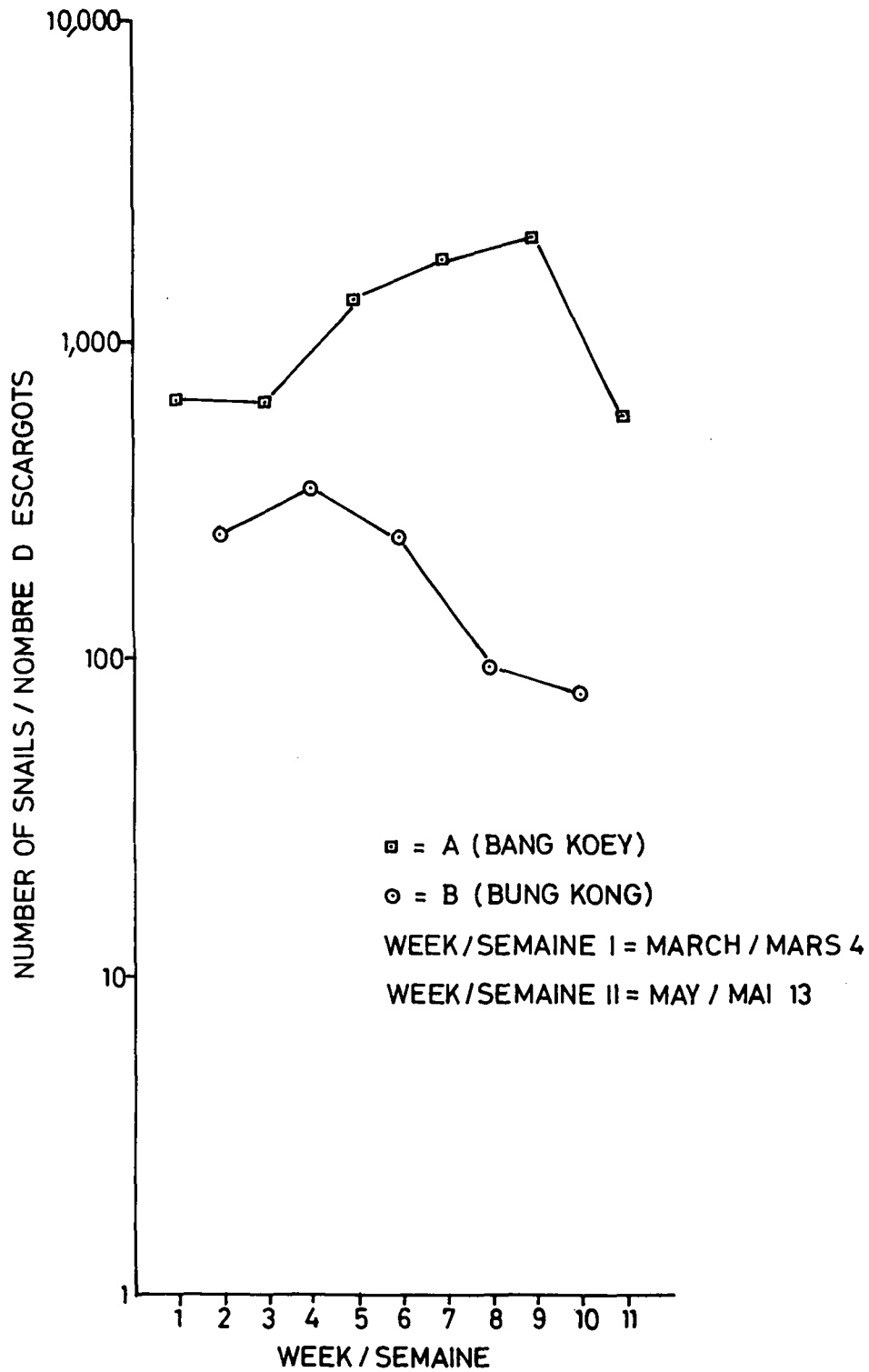


Fig. 7.--Numbers of *Lithoglyphopsis aperta*, alpha race, collected at two sites on the Mekong River, 1976.



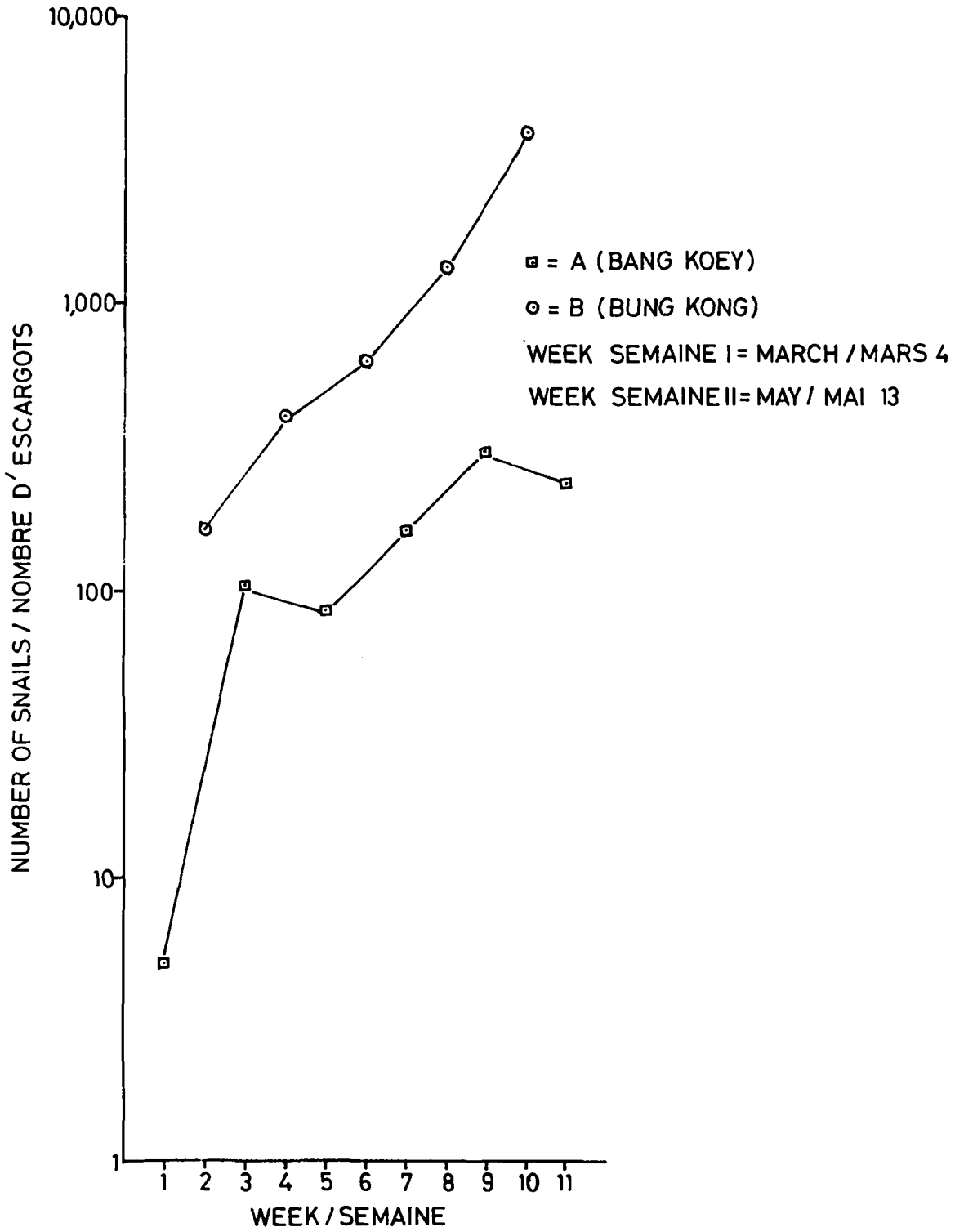


Fig. 8.--Numbers of *Lithoglyphopsis aperta*, gamma race, collected at two sites on the Mekong River, 1976.

Table 7 Ratio of alpha and gamma snails of all sizes collected at sites A & B on the Mekong River during 11 weeks in 1976.

Site A (considered an "alpha" site)

<u>Date</u>	<u>Alpha</u>	<u>%</u>	<u>Gamma</u>	<u>%</u>	<u>Total</u>
3-4	640	99.2	5	0.3	645
3-18	640	85.7	107	14.3	747
4-1	1,338	94.0	86	6.0	1,424
4-15	1,776	91.5	165	8.5	1,941
4-29	2,073	86.6	321	13.4	2,394
5-13	568	70.6	237	29.4	805

Site B (considered a "gamma" site)

<u>Date</u>	<u>Alpha</u>	<u>%</u>	<u>Gamma</u>	<u>%</u>	<u>Total</u>
3-11	242	59.3	166	40.7	408
3-25	338	45.1	411	54.9	749
4-8	237	27.1	639	72.9	876
4-22	93	6.3	1,381	93.7	1,474
5-6	76	1.9	4,024	98.1	4,100

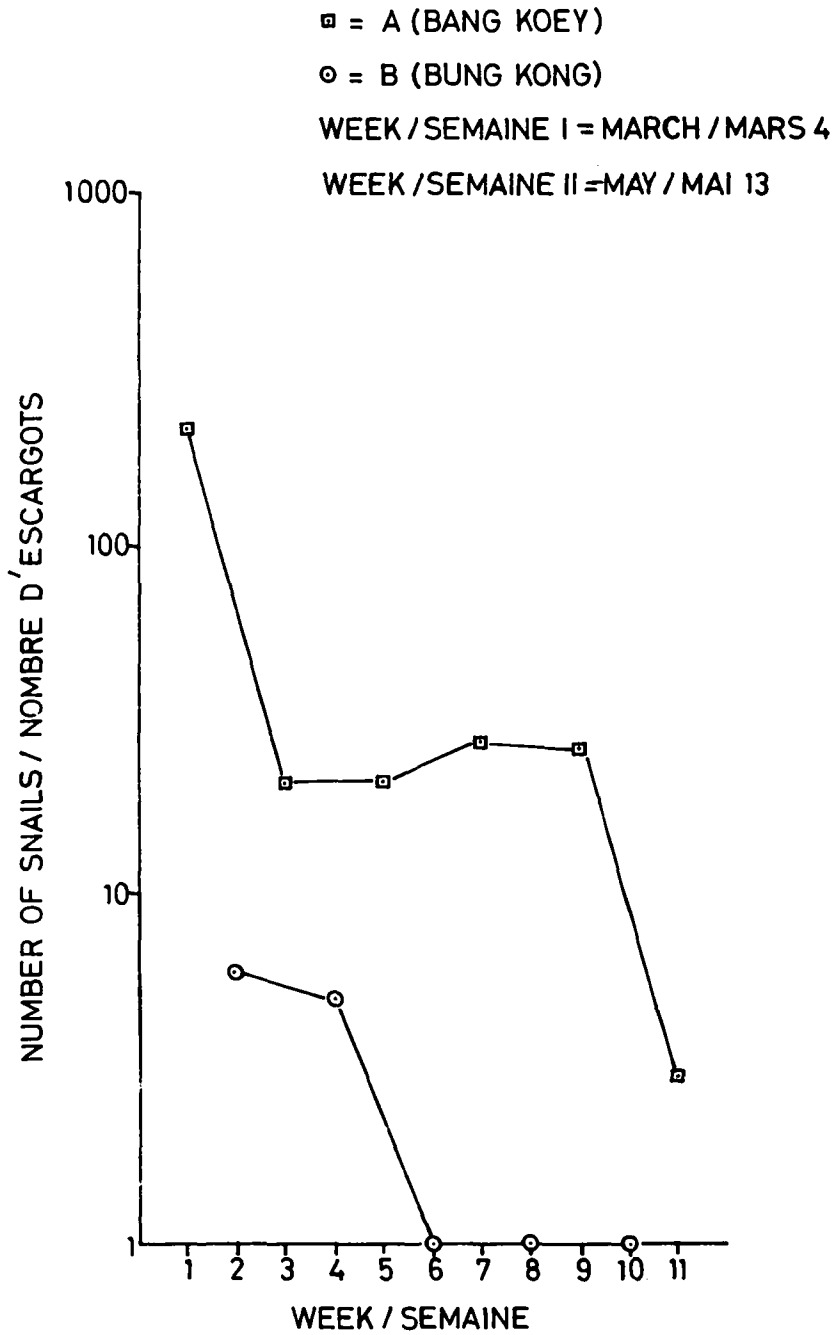


Fig. 9.--Numbers of Jullienia collected at two sites on the Mekong River, 1976.

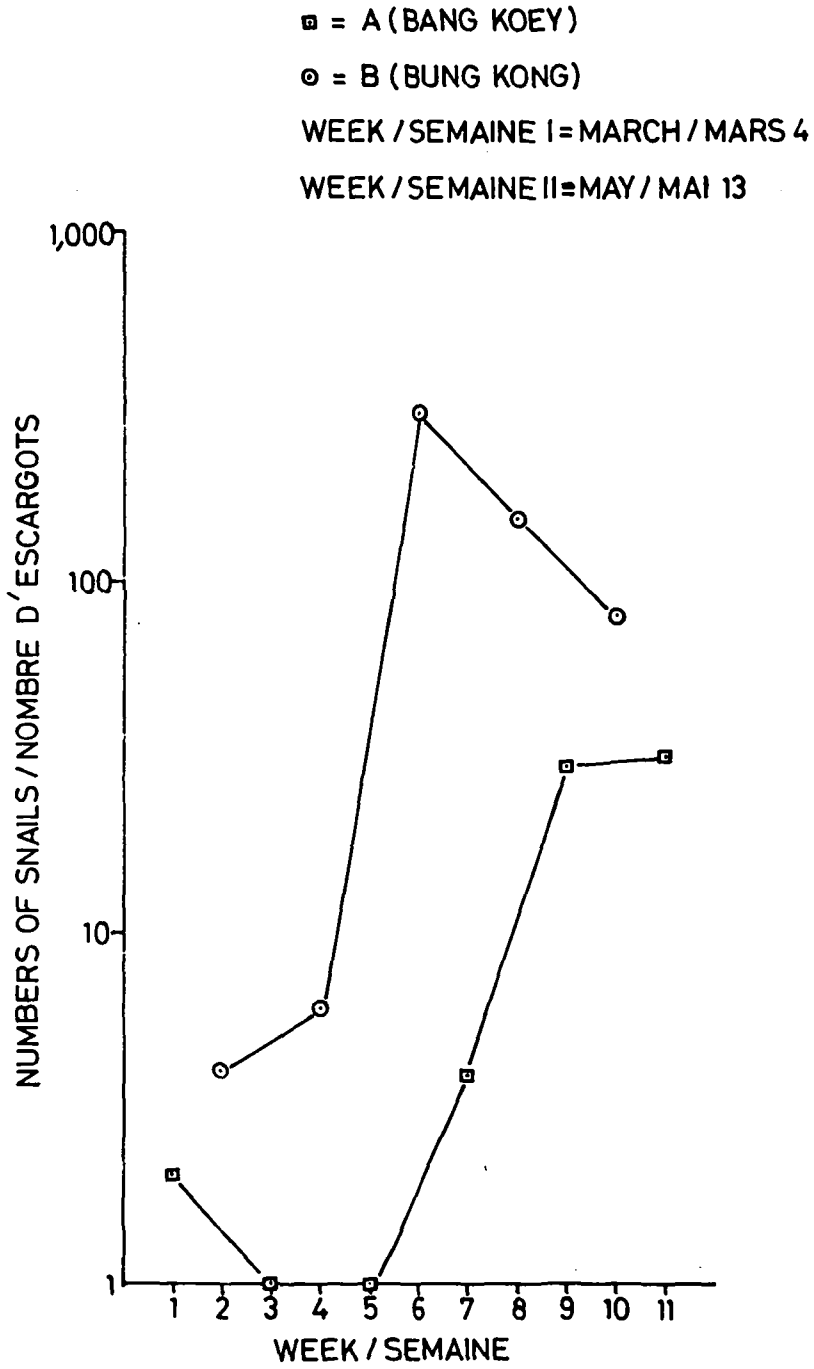


Fig. 10.--Numbers of *Pachydrobia* collected at two sites on the Mekong River, 1976.

Stenothyra. These ubiquitous snails appear to be accidental in the L. aperta habitat, although they are dominant in sandy areas, (Figure 11). They are not attractive to miracidia and do not seem to have any biomedical importance.

Clea. C. jullieni is most commonly found on muddy sand. It was rarely encountered at sites A and B which were characterized by large numbers of stones. This is a carnivorous snail which possibly hunts for hydrobiids away from its natural areas. It has no known biomedical importance.

Bivalves. The Asiatic clam, Corbicula, and the freshwater mussel, Limnoperna, were encountered at both sites but, more frequently at site B, (Figure 12). Neither is known to have any biomedical importance.

#### 6. Sex Ratio of L. Aperta

Females of alpha and gamma L. aperta tended to be somewhat more numerous than males (Tables 8, 9, 10 & 11), although the ratio was reversed for alpha L. aperta at site B during three successive days in June, 1975 (Table 10). In general, an even sex ratio is characteristic of a young mollusk population; females become more numerous as the population ages, males tending to die early (Fretter and Graham, 1964). Another possibility, that of sex reversal in older L. aperta populations with males absorbing the verge and developing functional ovaries, has not been observed in culture.

#### 7. Ratio of "alpha" to "gamma" L. Aperta

Site A was considered to be an "alpha" locality and site B a "gamma" locality for making field collections.

In the case of alpha snails, site A produced only "small" snails early in March (week 1) and eventually (by week 9) large numbers of predominately "large" snails (Table 12, Figures 13 & 15). Gamma snails, on the other hand, were collected at site A in relatively reduced numbers throughout the collecting period and "large" specimens were taken (in very small numbers) only during and after the 7th week (Table 12, Figures 14 & 15).

Site B was initially productive of a few alpha snails but, numbers fell off rapidly during the 8th and 10th weeks when the few recovered were mostly "large" (Table 12, Figures 13 & 16). Gamma snails, however, were steadily present at site B, although all were "small" specimens during the 2nd and 4th weeks, and the percentage of "small" snails remained high throughout the season, the population showing an explosive increase in absolute numbers during the 8th and 10th weeks (Table 12, Figures 14 & 16).

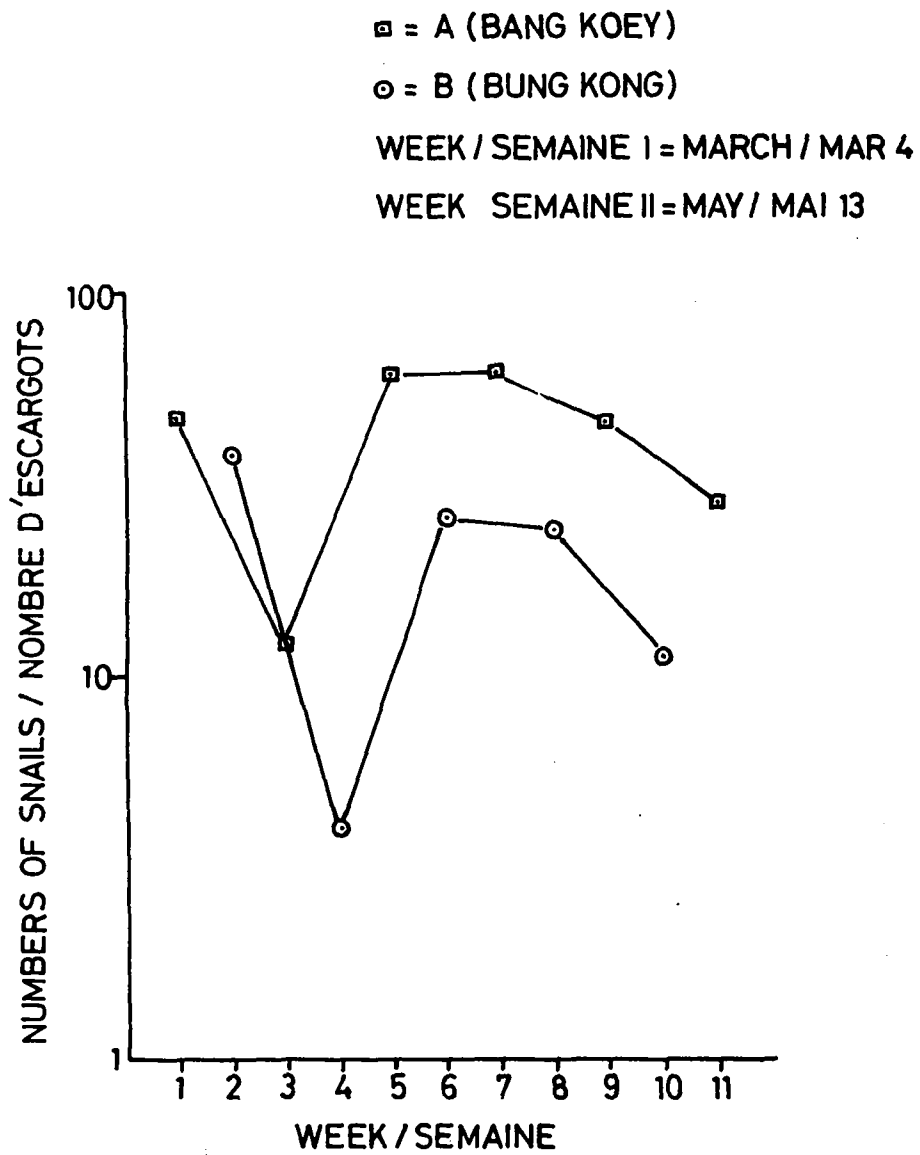


Fig. 11.--Numbers of Stenothyra collected at two sites on the Mekong River, 1976.

▣ = A (BANG KOEY)

⊙ = B (BUNG KONG)

WEEK / SEMAINE I = MARCH / MARS 4

WEEK SEMAINE II = MAY / MAI 13

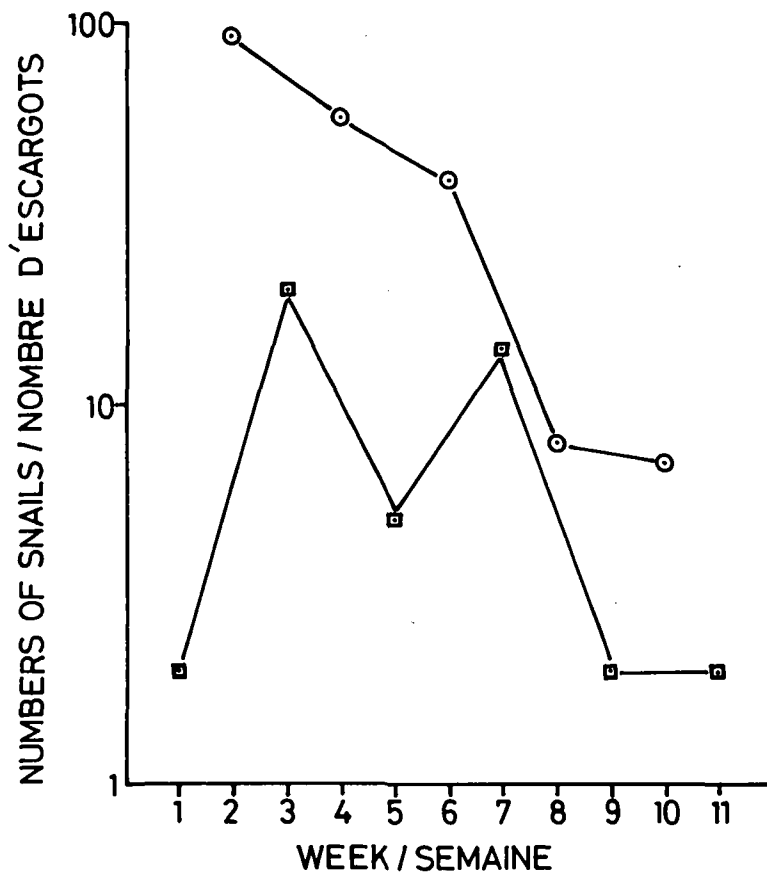


Fig. 12.--Numbers of bivalves collected at two sites on the Mekong River, 1976.

Table 8 Sex ratio of alpha L. aperta from site A (Bang Koey) in 1975.

Date	Total	Males	Females	Ratio Male:Female
3-5-75	47	18	29	1:1.61
4-5-75	53	15	38	1:2.53
7-5-75	104	42	62	1:1.48
17-5-75	132	61	71	1:1.16
25-5-75	9	3	6	1:2.00
26-5-75	151	83	68	1:0.82
	496	222	274	1:1.23

Range Male:Female = 1:0.82 -- 1:2.53



Table 9 Sex ratio of gamma L. aperta from site A (Bang Koey) in 1975.

Date	Total	Males	Females	Ratio Male:Female
17-5-75	49	16	33	1:2.06
26-5-75	10	5	5	1:1.00
	59	21	38	1:1.80

Table 10 Sex ratio of alpha L. aperta from site B (Bung Kong) in 1975.

Date	Total	Males	Females	Ratio Male:Female
25-5-75	53	25	28	1:1.12
1-6-75	266	119	147	1:1.24
2-6-75	257	119	138	1:1.16
3-6-75	30	12	18	1:1.50
4-6-75	255	131	124	1:0.95
5-6-75	146	88	58	1:0.66
6-6-75	340	174	166	1:0.95
7-6-75	442	201	241	1:1.20
8-6-75	341	137	204	1:1.49
9-6-75	592	273	319	1:1.17
10-6-75	594	300	294	1:0.98
11-6-75	174	74	100	1:1.35
12-6-75	152	76	76	1:1.00
13-6-75	448	206	242	1:1.17
14-6-75	1,228	603	625	1:1.04
	5,318	2,538	2,780	1:1.10

Range Male:Female = 1:0.66 -- 1:1.35

Table 11 Sex ratio of alpha L. aperta from sites A and B in 1976, based on 100 snails.

Date	Site	Males	Females	Ratio Male:Female
15-4-76	A	46	54	1:1.17
22-4-76	B	41	59	1:1.44
29-4-76	A	42	58	1:1.38
6-5-76	B	40	60	1:1.5
13-5-76	A	45	55	1:1.22

Table 12 Ratio of large ( $\geq 2$ mm) to small ( $< 2$ mm) *L. aperta*, alpha and gamma races, collected at two sites (A & B) in the Mekong River from March 4 to May 13, 1976.

ALPHA: Site A (Bang Koey)

<u>Date</u>	<u>Large</u>	<u>%</u>	<u>Small</u>	<u>%</u>	<u>Total</u>
3-4	0	0.0	640	100.0	640
3-18	78	12.2	562	87.8	640
4-1	497	37.1	841	62.9	1,338
4-15	1,478	83.2	298	16.8	1,776
4-29	1,992	96.1	81	3.9	2,073
5-13	512	90.1	56	9.9	568

ALPHA: Site B (Bung Kong)

<u>Date</u>	<u>Large</u>	<u>%</u>	<u>Small</u>	<u>%</u>	<u>Total</u>
3-11	0	0.0	242	100.0	242
3-25	100	29.6	238	70.4	338
4-8	120	50.6	117	49.4	237
4-22	86	92.5	7	7.5	93
5-6	73	96.1	3	3.9	76

GAMMA: Site A (Bang Koey)

<u>Date</u>	<u>Large</u>	<u>%</u>	<u>Small</u>	<u>%</u>	<u>Total</u>
3-4	0	0.0	5	100.0	5
3-18	0	0.0	107	100.0	107
4-1	0	0.0	86	100.0	86
4-15	53	32.1	112	67.9	165
4-29	98	30.5	223	69.5	321
5-13	86	36.3	151	63.7	237

GAMMA: Site B (Bung Kong)

<u>Date</u>	<u>Large</u>	<u>%</u>	<u>Small</u>	<u>%</u>	<u>Total</u>
3-11	0	0.0	166	100.0	166
3-25	0	0.0	411	100.0	411
4-8	203	31.8	436	68.2	639
4-22	485	35.1	896	64.9	1,381
5-6	182	4.5	3,842	95.5	4,024

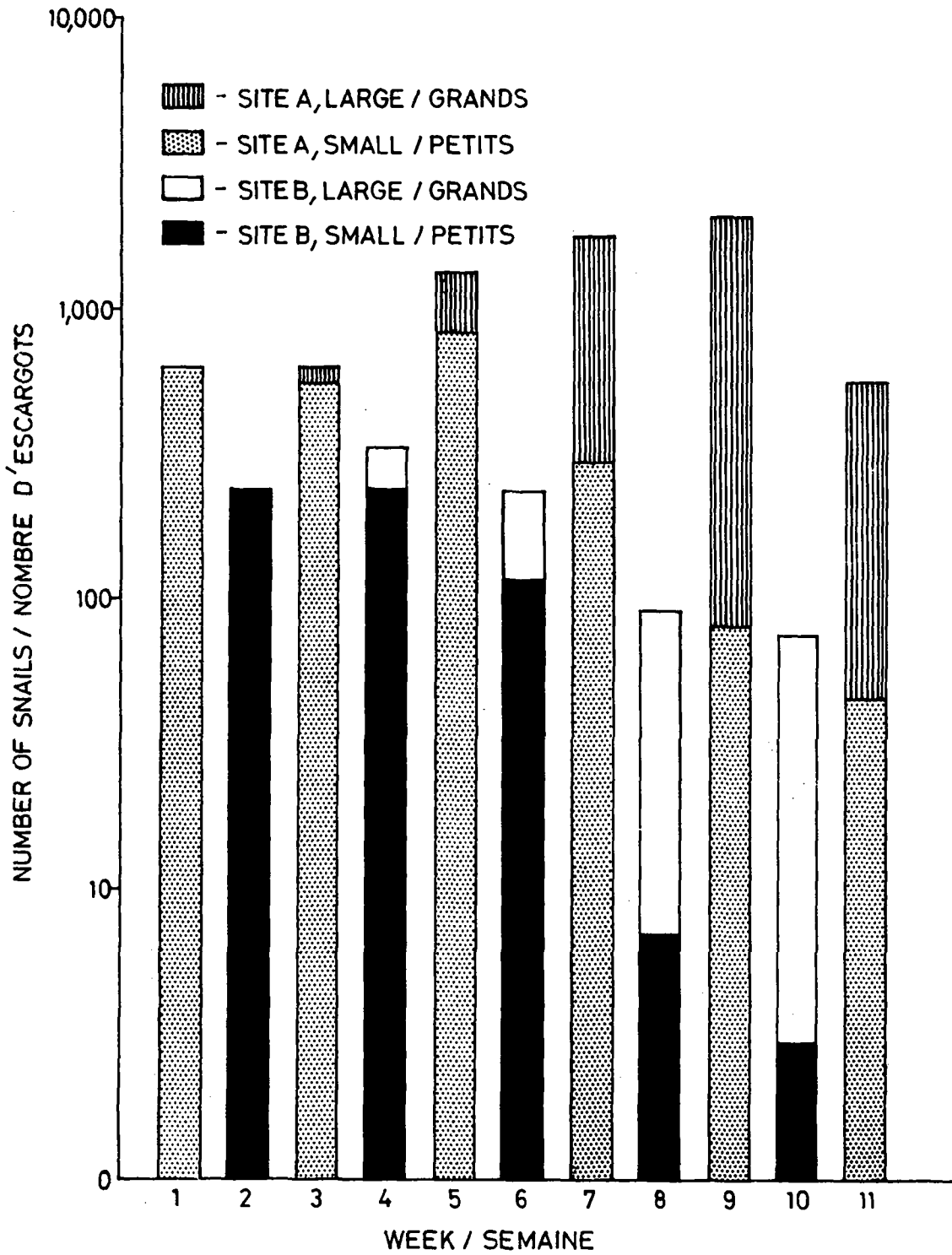


Fig. 13.--Comparison of numbers of large and small alpha race *L. aperta* at two sites on the Mekong River, 1976.

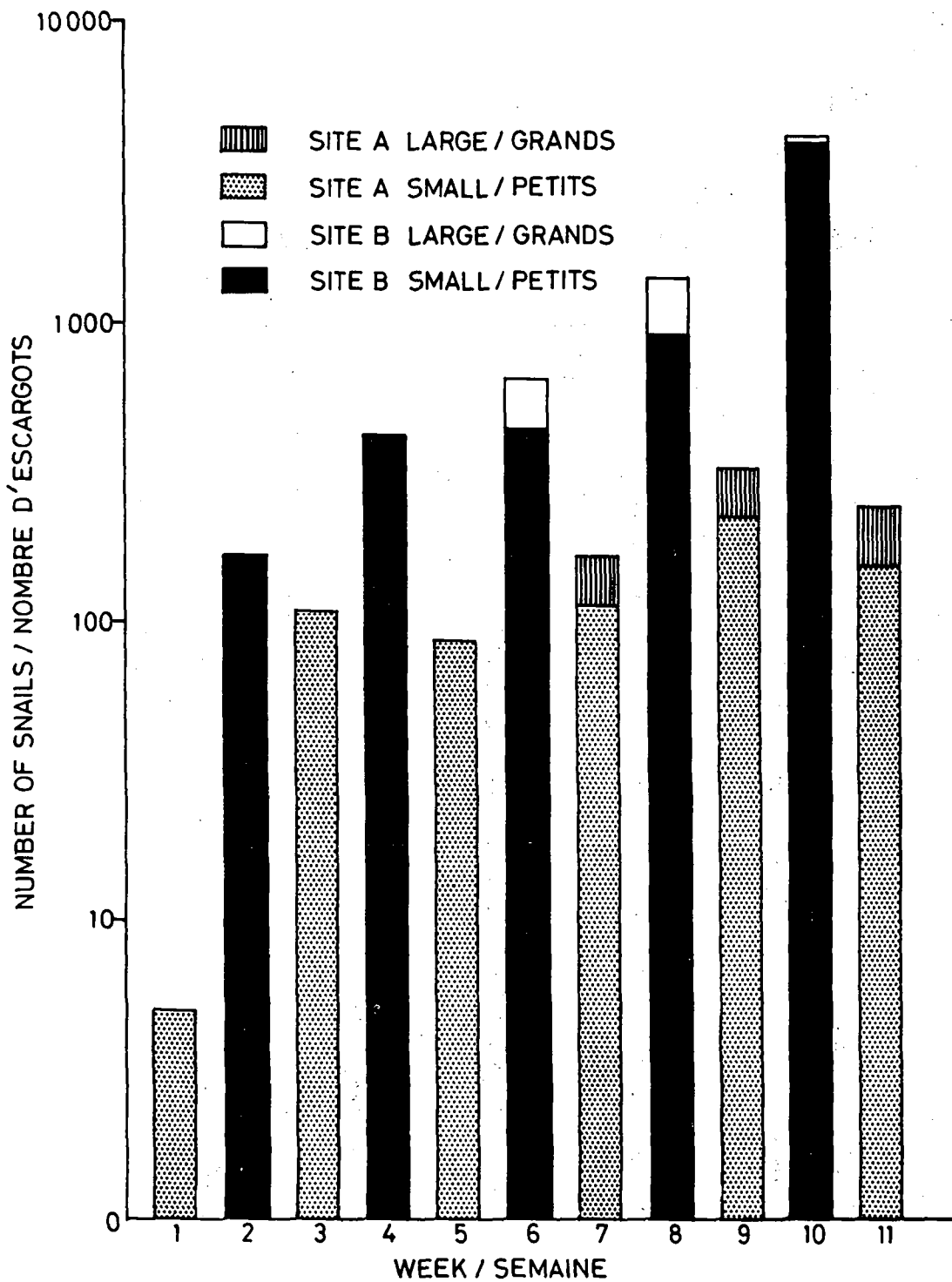


Fig. 14.--Comparison of numbers of large and small gamma race *L. aperta* at two sites on the Mekong River, 1976.

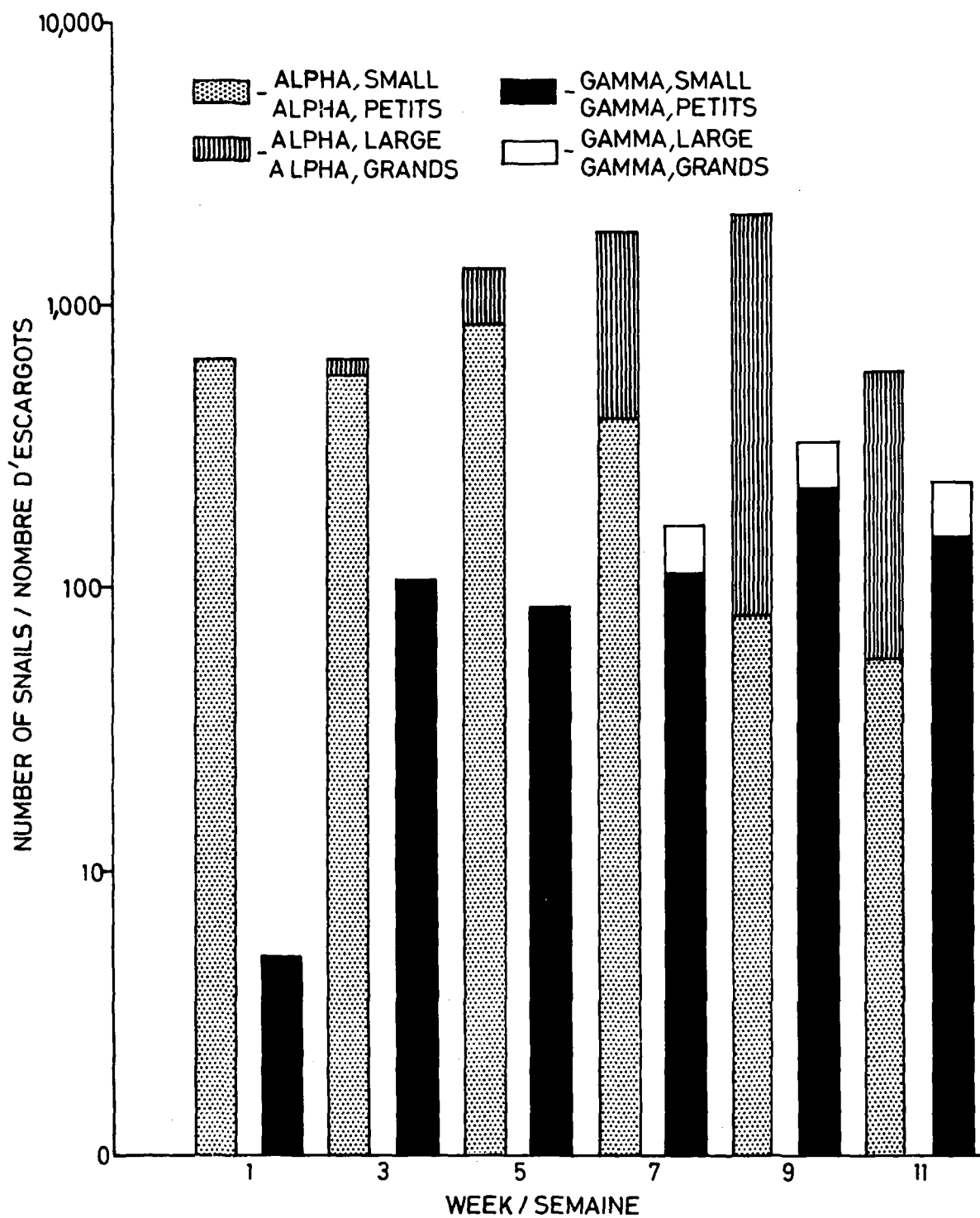


Fig. 15.--Comparison of numbers of alpha and gamma race *L. aperta* at site A (Bang Koey) on the Mekong River, 1976.

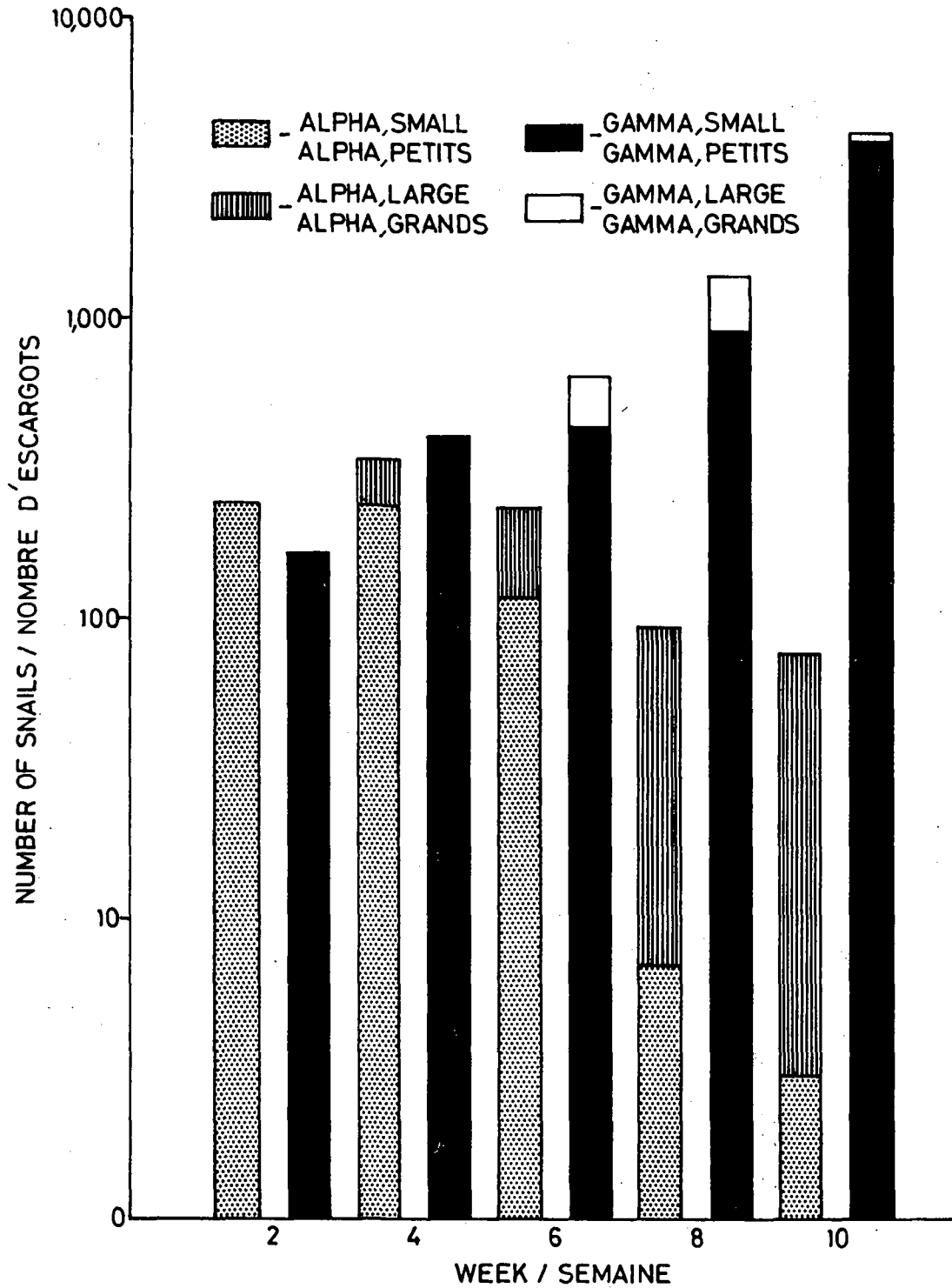


Fig. 16.--Comparison of numbers of alpha and gamma race *L. aperta* at site B (Bung Khong) on the Mekong River, 1976.



No explanation for these differences was evident from the available information. The two sites, so markedly different with regard to the kind of L. aperta in residence, were separated physically by only about 5 km of direct distance on an otherwise apparently homogeneous river.

One point should be emphasized. It was assumed that the reduction in numbers of "small" snails and increase in numbers of "large" snails would coincide with maturation of snails. "Large" was defined as greater than 2 mm and "small" was less than 2 mm. But it is remembered that alpha race L. aperta are, by definition, intrinsically larger than gamma race (Davis et al., 1976). Thus, the figures may be misleading to the extent that only size was used as an indicator of sexual maturity. A repetition of the measurements, using a better criterion for maturity, might reveal a larger percentage of mature gamma individuals at both sites, but particularly at site B where gamma predominated. It is difficult to believe that the population was composed primarily of immature gamma snails toward the end of the growing season, when environmental dictates would demand mature, egg-laying females in abundance.

#### 8. Population Densities of L. Aperta

Regardless of race or age, site B supported larger populations of L. aperta than did site A.

Population density was expressed as the area on a stone (in square centimeters) available to each individual snail, counting all snails on 10 stones selected at random (Table 13, Figure 17).

Populations at site A of both races of L. aperta were approximately as dense at week 11 as at week 1, although there was a noteworthy increase in numbers (and consequent decrease in available crawling space) during weeks 5 through 9.

Populations of L. aperta at site B, on the other hand, showed a progressive increase in total numbers of both races with the greatest number of snails being observed at the time of the last field collection (week 10).

Thus, there was a distinct difference in the two sites as regards their suitability as niches for L. aperta. This observation supports the conclusion of the previous paragraphs, but provides no explanation. So far as is known, the number of stones was not greater at site B.

#### 9. Beta Race L. Aperta

Three races of Lithoglyphopsis aperta bearing the

Table 13 Population densities of L. aperta at sites A & B, based on an average of 10 stones (in cm<sup>2</sup>/snail).

<u>Week</u>	<u>Date</u>	<u>Site A</u> <u>(Bang Koey)</u>	<u>Site B</u> <u>(Bung Kong)</u>
1	3-4	10.7	-
2	3-11	-	17.0
3	3-18	9.8	-
4	3-25	-	10.2
5	4-1	4.6	-
6	4-8	-	9.6
7	4-15	3.8	-
8	4-22	-	5.5
9	4-29	3.1	-
10	5-6	-	1.9
11	5-13	9.1	-

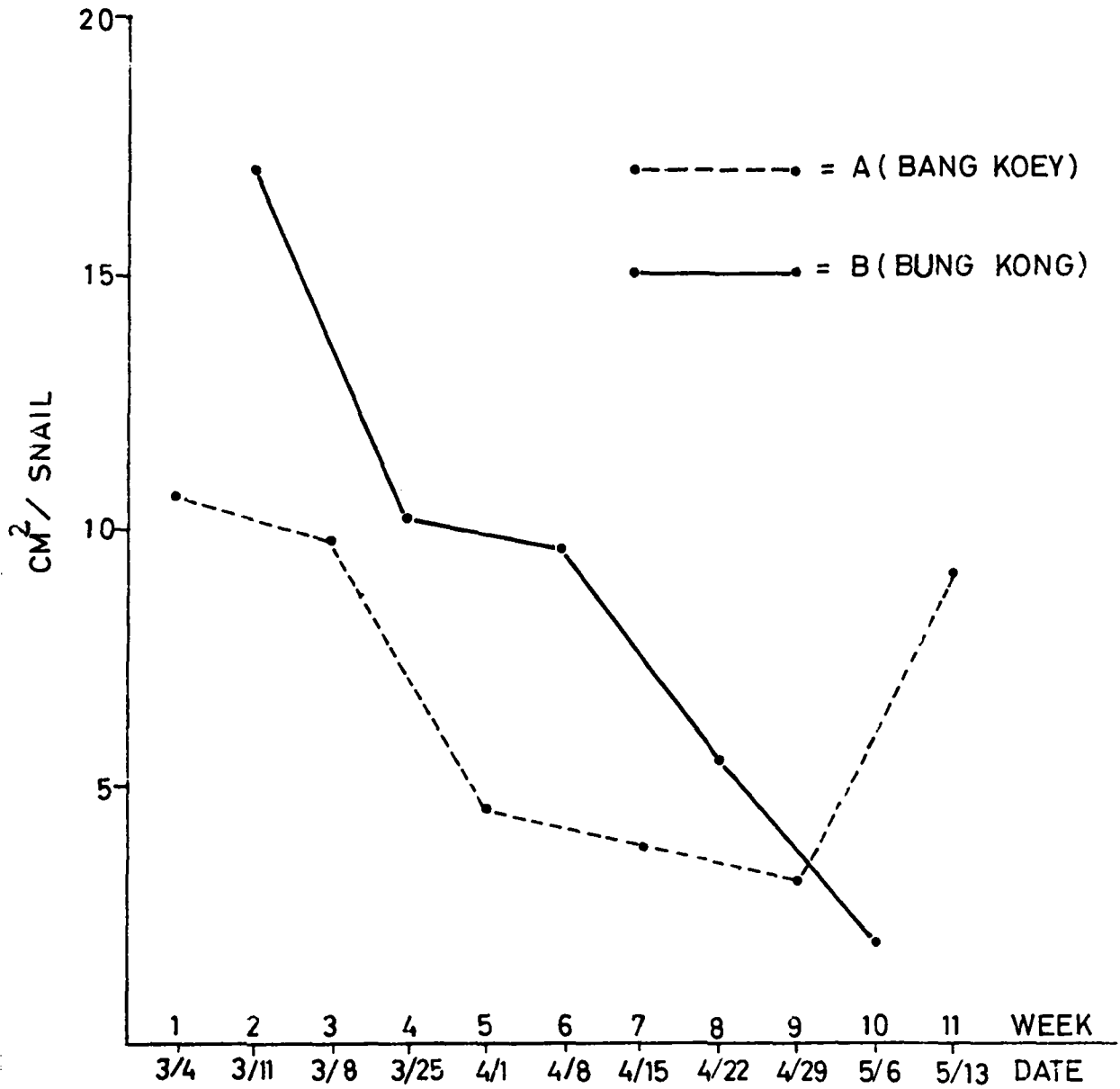


Fig. 17.--Population density of *L. aperta* (mixed races) at sites A and B on the Mekong River, 1976.

identifying tags alpha, beta and gamma have been described (Smithsonian Institution, 1974; Davis et al., 1976). Alpha and gamma, which occurred abundantly in the Mekong River at the sites near the Khemmarat field laboratory, are the principal subjects of the present report. However, some advances were recorded with regard to beta.

In the Smithsonian Report (1974), beta snails were reported as occurring in only one detected location in the Mun River of Thailand. They were found under stones and on sticks near the town of Ban Sai Mun, east of Phibun Mangsahan during a few days of late February to early March, 1974. The locality was just upstream from a stretch of white water rapids. Snails were collected in the main river at this site, as well as in rock pools left by splashing and by receding water. Their water was typically cool, well aerated and free of obvious gross impurities. Beta snails were not collected in early February, 1974 at this site, although, other genera (Hydrorissioia, Pachydrobia, Paraprososthenia) were fairly abundant. The fact that beta snails could be found here in large numbers, a week or so after none at all were present, lends strength to the hypotheses that these snails survive the high water period as a population in the egg stage, possibly in a state of suspended development. It is easy to conceive that such a state could be sustained by low temperatures and/or relative anoxia.

Laboratory studies have confirmed that beta snails are relatively more susceptible to infection by miracidia of the Mekong Schistosoma than either alpha or gamma. Under similar exposure conditions, 58.5% of adult beta snails could be infected, compared with 2.7% alpha and approximately 20% gamma (Sornmani, 1976). If efficiency is equated only with susceptibility (ignoring questions of age, viability or concomitant disease in the snails), this means that beta snails were 3 times more efficient than gamma snails (the natural transmitters), and about 20 times more efficient than alpha snails.

In early March, 1976, a new locality for beta snails was discovered in the lower Mun River east of its confluence with the Lam Dom Noi. This new locality lies within a few kilometers of the mouth of the Mun. The discovery suggests that other places in the Mun will be found to harbor this snail.

Thus, the possibility exists that beta snails could threaten public health along the lower Mun River where water development schemes may coincide with future population increases. At present, the river east of Ubon Ratchathani is sparsely settled. The few communities are small and tend to stretch out along the shore. Although there is a

suspicion that schistosomiasis may already have been introduced into this area (Desowitz et al., 1967), case finding has yet to be done here on a systematic basis. Nevertheless, it must be assumed that: (1) development schemes may bring increased numbers of people to this riverine habitation, and (2) the Pak Mun Dam will alter ecological conditions in the lower Mun in such a way as to affect the seasonal numbers of beta L. aperta. If the effect proves favorable to the snail, then a major health problem due to schistosomiasis could develop in this part of Thailand. It seems unlikely, however, that the snail will be transferred to other Thai waters associated with the Mun River, although the possibility of introducing beta snails into the Mekong River or into its other large tributaries, such as the Xe Banghiang or Xe Don in Laos, or the Tonle Kong, Tonle Son or Tonle Srepok in Cambodia (or the fact of their present establishment) needs exploration.

#### 10. The "Egg" Hypothesis

The rainy season coincides with a rapid change in conditions in the river. Within a week or two, the river changes from a gentle stream to a raging torrent. A flow rate of less than 2,000 m<sup>3</sup>/sec in the last week of May can increase to 10,000 m<sup>3</sup>/sec by the first week of June, can double in July and, at peak flow in September, can exceed 30,000 m<sup>3</sup>/sec. At this time, the water is filled with detritus of every kind and size, including whole tree trunks, to venture into the current is extremely dangerous and is avoided by all.

Under the circumstances it is understandable that few investigations of the river bottom sites of alpha and gamma were made during the high water months. From past experience, however, we know that hydrobiid snail populations dwindle rapidly to zero as the water comes up. The advent of the rains then terminates the life cycle of the snails in nature. That the snails are intrinsically capable of a longer life, is shown by their survival in the laboratory for many months after the onset of the rains.

The annual water cycle peaks in September and then reverses, by December one can often find a snail or two in the river, possibly survivors from the previous May. The preferred collecting sites, i.e., those suitable for hand collecting, do not become fully exposed until February but, by this time, hydrobiids of certain species have already completely colonized the stones. The rapidity with which they do this is remarkable and sustains the following hypothesis regarding snail survival.

Prior to the onset of the rains, the last act of the female snail is to lay large numbers of eggs. The presence

of eggs on the stones at this time has been confirmed by direct observation. According to the hypothesis, eggs are able to persist in a more or less unchanged state, protected as they are on the undersurface of their stones, throughout the period of high water. Possibly, the slowing down of development is mediated by environmental changes in such parameters as light intensity, temperature or oxygen tension. Hypothetically, the trigger to resume development would be the reversal of these physical parameters with lowering water levels in November and December.

Strengthening the egg hypothesis is the fact that, as early in the collecting season as the middle of February, large numbers of hydrobiid eggs can be found on stones, at known collecting sites, although no adult snails are in evidence. A week later, the same stones support enormous populations of immature snails which had clearly hatched from the eggs.

## II Maintenance of L. Aperta in the Laboratory

L. aperta of three races are maintained in the laboratory. Four techniques for breeding were tried:

1. Petri dish (9 cm. dia.)
2. Glass bottle aquarium
3. Glass bowl
4. Stainless steel tray

Snails were kept with sex ratio in the same proportion as in nature (see below). Each culture was regularly fed diatoms. Records were kept on egg deposition and mortality.

### I. Petri Dish Cultures

Nine cm. Petri dishes were thoroughly cleaned with conditioned water and autoclaved (15 lbs. for 15 min.). They were then filled with diatom suspension and left under the fluorescent light for 5 to 7 days to allow maximal growth of diatoms.

Snails of all three races were grown in the Petri dishes. Each dish contained 6 females and 4 males in 30 cc. of conditioned water (Figure 18). Cultures were observed regularly for the activity of the snail, growth, mortality, egg laying, pH and temperature. During the first few months of culture, according to the availability of field snails, dead snails were replaced with living ones. If rotifers, ciliates or algae appeared in the cultures, the snails were transferred to new cultures immediately. Otherwise, the water in the Petri dishes was changed once a week.

Diatoms (Navicula spp.), isolated from the natural



Fig. 18.--Snail Rearing in Petri dishes.

habitat, were cultured (Figure 19), and used as snail food. Two months after placing the snails in the Petri dishes, about 0.4 gm. of a sterile (autoclaved) mixture of silt and sand was added to the culture dishes to promote growth. In the presence of silt and sand, the snails were more active and produced more fecal pellets, suggesting an improved metabolism.

The presence of rotifers and ciliates in the cultures may be considered one factor in the high mortality of snails. These microscopic pests were seen to irritate the snails, making them withdraw into their shells. Populations of rotifers and ciliates were controlled by leaving snails in 1:10,000 Tc. merthiolate for 24 hours and then transferring them to new dishes.

Overgrowth with blue-green algae was inhibited by partially reducing direct sunlight in the culture room.

a. Appearance. Petri dish cultures with not more than 10 snails per dish seem to be favorable for rearing adult L. aperta when close observation is required. It is easy to observe activity, mortality, egg laying and development of eggs under the dissecting microscope. However, it was tedious work; the culture dishes had to be replaced almost every week, sometimes sooner, if the rotifers and ciliates increased. Under normal conditions, after one week, there were many snail fecal pellets and only a few rotifers in each plate. If the light was too strong, green algae would appear, sometimes trapping the snails with their hyphae. The appearance of rotifers, ciliates, algae and too many fecal pellets were used as indicators of when to change the culture plate. They were also changed when and if diatoms became greatly reduced.

The cultures were observed every week and dead snails were replaced by wild caught ones, if available. Routinely, pH and temperature of cultures were measured every week. During the rearing period, pH was between 7.4 and 8.1, and the water and room temperatures were between 25.4° C to 29.9° C and 26.0° C and 30.2° C, respectively.

b. Mortality. The mortality rates appear in Table 14. They are not cumulative. There were two peaks of high mortality, the initial one during June and July, and the second during January and February.

Since the high initial mortality coincided with snail capture and transfer to culture, it is not clear whether it was also synchronized with the season. The second peak of high mortality was observed in January to March, that is, after the egg laying period (October to December). In nature, a large die-off occurs after egg



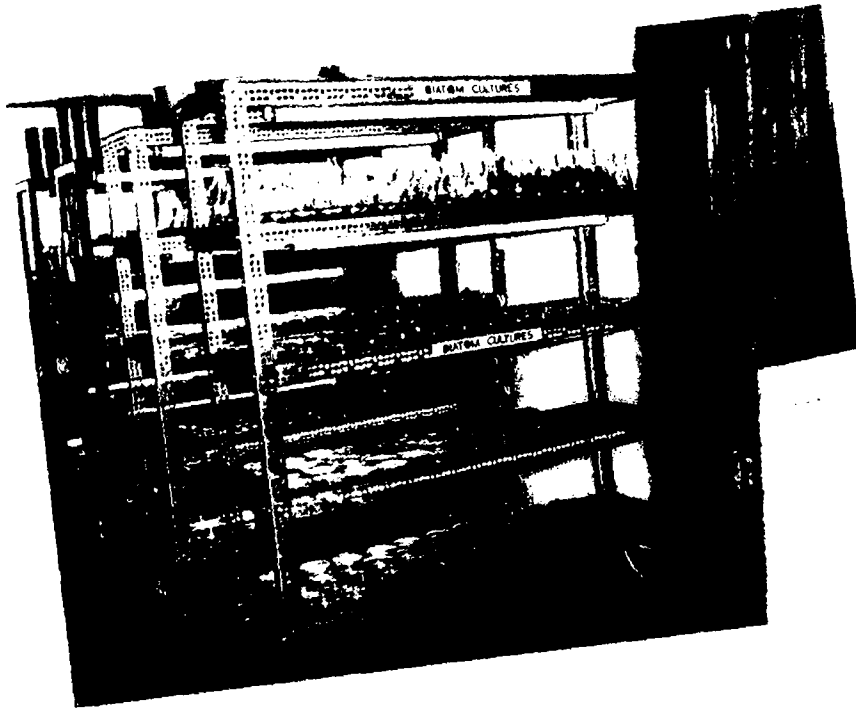


Fig. 19.--Culture of diatoms for snail food in the laboratory.

Table 14 Monthly mortality rates of *L. aperta* cultured in 9 cm. Petri dishes (from May 1975 to May 1976).

Races	Mortality of snails per month														
	May			June			July			August			September		
	INS	No.	%	INS	No.	%	INS	No.	%	INS	No.	%	INS	No.	%
Alpha	300	23	7.67	300	57	19.00	300	76*	25.33	300	8	2.67	292	14	4.79
Beta	300	7	2.33	300	9	3.00	300	124*	41.33	150	8	5.33	142	1	0.70
Gamma	-	-	-	-	-	-	-	-	-	40	0	0.00	40	0	0.00

Races	October			November			December			January			February		
	INS	No.	%	INS	No.	%	INS	No.	%	INS	No.	%	INS	No.	%
Alpha	278	18	6.47	260	22	8.46	238	28	11.76	210	33	15.71	177	56	31.64
Beta	141	3	2.13	138	5	3.62	133	4	3.01	129	9	6.98	120	28	23.33
Gamma	40	2	5.00	38	4	10.53	34	4	11.76	30	10	33.33	20	5	25.00

Races	March			April			May		
	INS	No.	%	INS	No.	%	INS	No.	%
Alpha	121	79	65.29	42	25	59.52	17		
Beta	92	40	43.48	52	14	26.92	38		
Gamma	15	7	46.67	8	1	12.50	7		

INS = initial no. of snails.

No. = number of dead snails.

\* = high rates of mortality due to adding merthiolate in dishes to kill ostracods.

pH range: 7.4 - 8.1

Temperature range: water 25.4 - 29.0°C  
room 26.0 - 30.2°C

Each Petri dish contained 4 male and 6 female snails.

laying due to obvious extrinsic factors (sudden rise in water level and thus disappearance of food) and possible to intrinsic factors.

c. Egg Production. In September, i.e., 4 months after placing the snails in Petri dishes, eggs were found attached to the bottom of the dishes. Only eggs of alpha and beta races were initially found in the cultures.

In dishes without silt or sand, eggs were all transparent (Figure 20). Contrariwise, they were covered with thick yellowish-brown granules if laid in the presence of silt and sand (Figures 21 & 22). The outer wall was soft and spongy with clear, colorless fluid and a yellowish embryo inside. The embryo darkened and became actively mobile as development progressed.

Eggs laid in the natural habitat were always found covered with a yellowish-brown coat, similar in appearance to eggs laid in the laboratory in the presence of sand and silt.

Transparent eggs were never found in nature. It is postulated that normally, *L. aperta* produces transparent eggs encased in a soft transparent membrane, to which minute silt particles attach, if present, to make a protective coat.

In Petri dish cultures, the beta race produced more eggs than the other two races (Table 15, Figures 23 & 24). The high egg production periods were from October to March for the beta race and, from October to November for the alpha race. Based on the number of females, beta snails laid as many as 9.59 eggs/female per month, while alpha snails produced only 2.5 eggs/female per month (Table 16, Figure 25). A large percentage of eggs ceased development a few days after being laid. The few which survived the first few weeks continued development until a baby snail became visible inside the shell (Figure 26).

Those eggs which were arrested in development during the first few weeks failed to develop further, even when kept for many months.

No attempt was made to study the relationship between egg production, the longevity of eggs and the hatchability of the baby snails, because the objective of the present study was to culture snails under laboratory conditions. The results of the laboratory work did not coincide with the natural breeding season since most of the eggs in nature hatched out during the dry season (March and April).



Fig. 20.--Egg of L. aperta, beta race, one day old.

Note: the transparency of the newly laid egg.

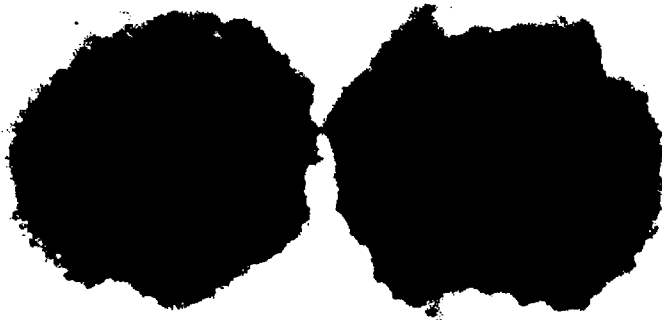


Fig. 21.--Eggs of L. aperta as seen in nature.

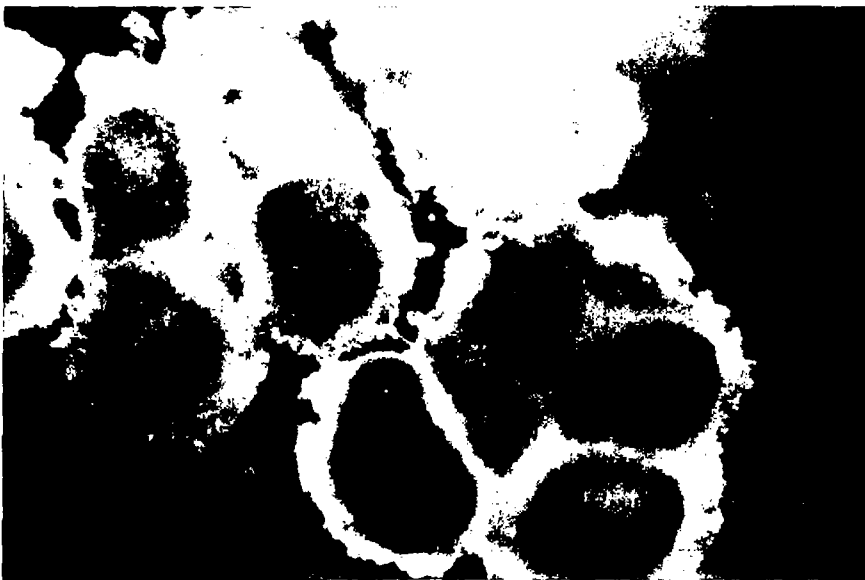


Fig. 22.--A cluster of eggs of L. aperta detached from rock.

Table 15 Monthly production of eggs and percentages of hatching of 3 races of *L. aperta* maintained in 9 cm. Petri dishes from May 1975 to May 1976

Races	Monthly production of eggs and percentages of egg hatching of <i>L. aperta</i>														
	May			June			July			August			September		
	E	No.	%	E	No.	%	E	No.	%	E	No.	%	E	No.	%
Alpha	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	60*	2	3.33
Beta	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	155*	50	32.26
Gamma	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00
Races	October			November			December			January			February		
	E	No.	%	E	No.	%	E	No.	%	E	No.	%	E	No.	%
Alpha	430	24	5.58	214	11	5.14	42	0	0.00	63	0	0.00	79	0	0.00
Beta	522	10	1.92	720	115	15.98	815	20	2.46	380	11	2.89	324	11	3.40
Gamma	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00	0	0	0.00
Races	March			April			May								
	E	No.	%	E	No.	%	E	No.	%						
Alpha	7	0	0.00	8	0	0.00	0								
Beta	300	9	3.00	83	0	0.00	0								
Gamma	0	0	0.00	0	0	0.00	0								

E = Number of eggs produced.

No. = Number of baby snails hatched.

\* = Adult snails started to produce eggs on September 9, 1975.

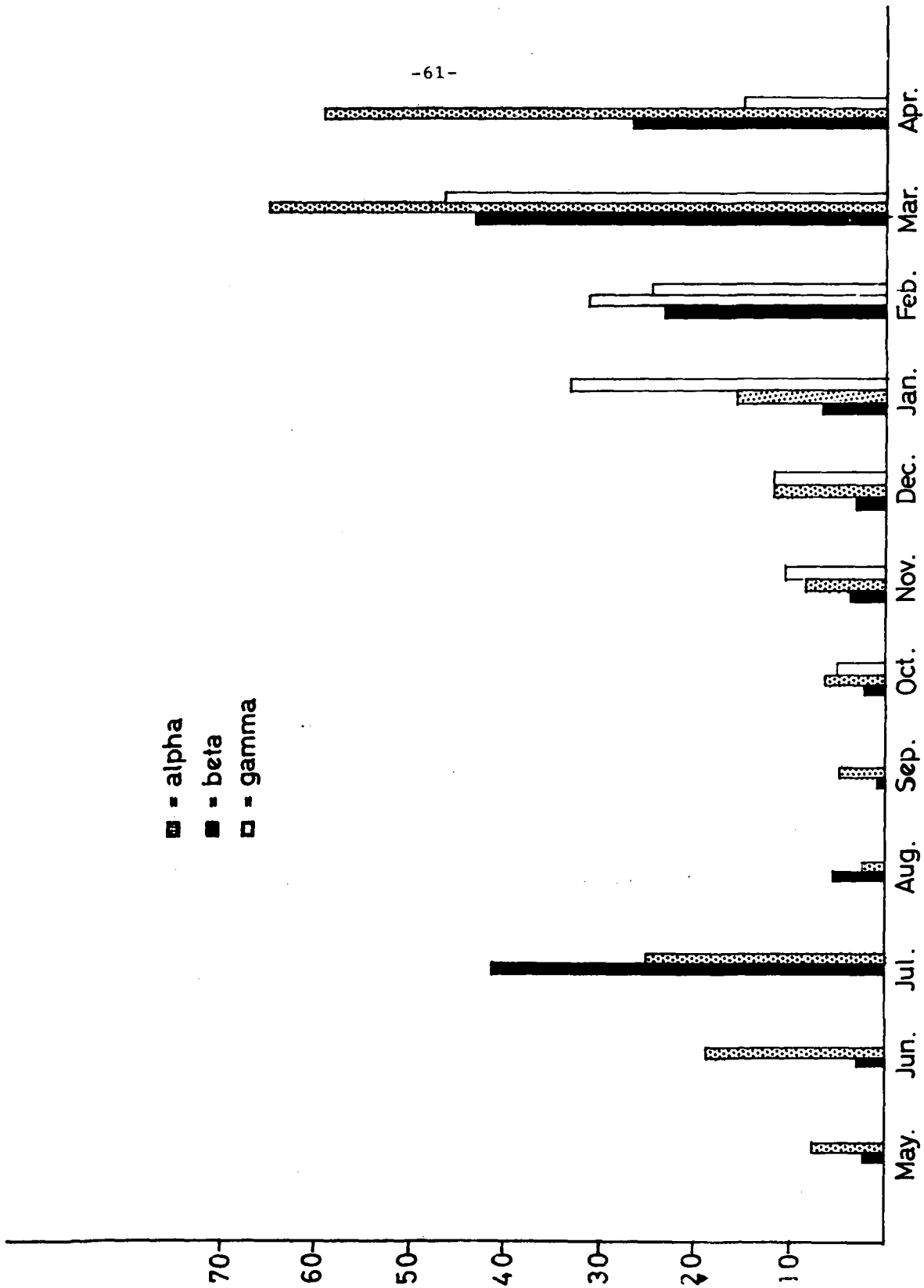


Fig. 23.--Monthly production of eggs of three races of *L. aperta*, reared in Petri dishes, May 1975 to May 1977.

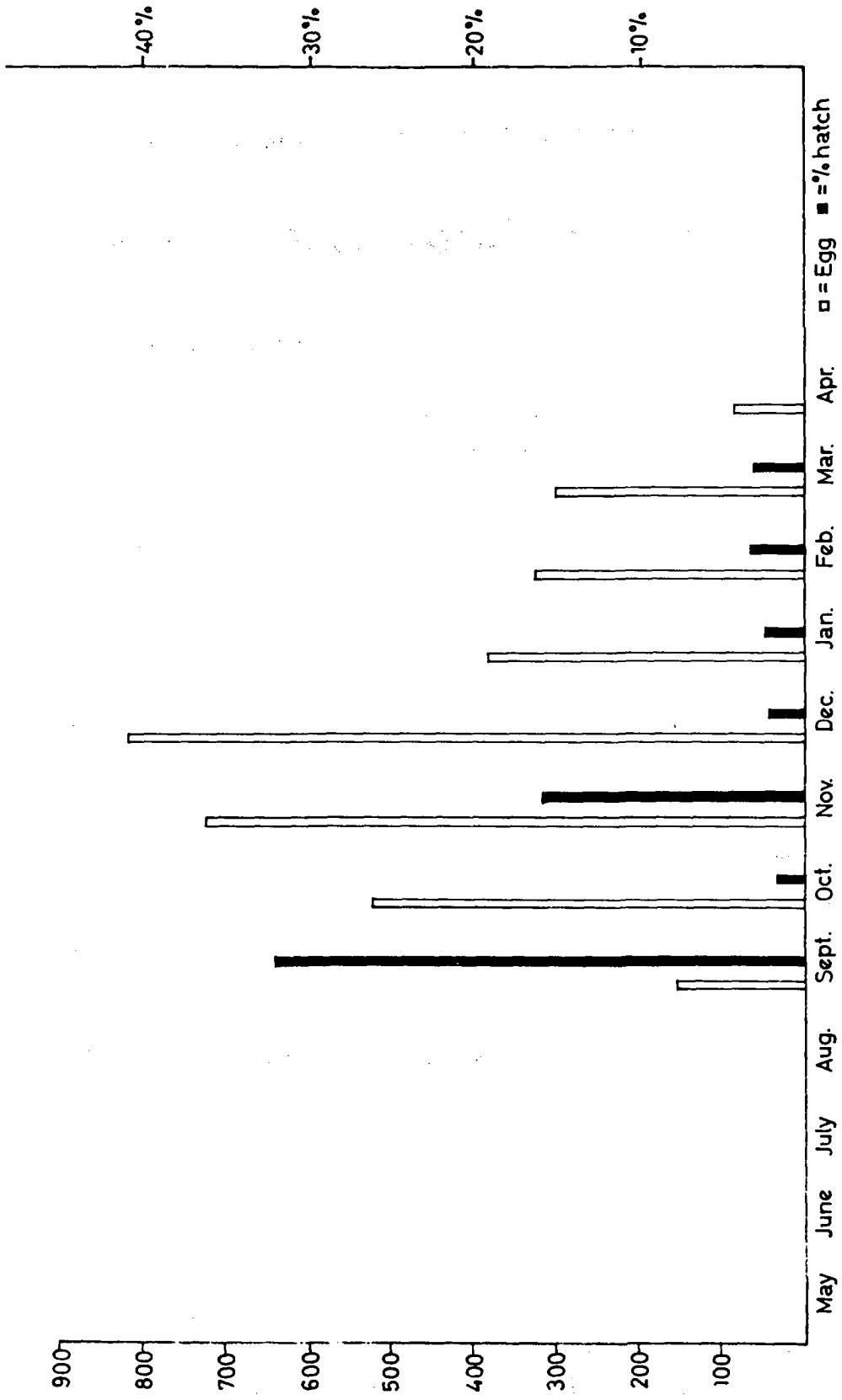


Fig. 24. --Relationship of egg production to hatching of beta race *L. apertus*, September 1975 to April 1976.



Table 16 Monthly production of eggs and percentage of hatching of alpha and beta *L. aperta* during September 1975 to March 1976.

Races	Monthly Egg Production							
	September				October			
	No. female	No. egg collected	Egg female	No. egg hatched	No. female	No. egg collected	Egg female	No. egg hatched
Alpha	180	60	0.33	2 (3.3%)	172	430	2.50	24 (5.6%)
Beta	90	155	1.72	50 (32.3%)	89	522	5.87	10 (1.9%)
Races	November				December			
	No. female	No. egg collected	Egg female	No. egg hatched	No. female	No. egg collected	Egg female	No. egg hatched
	Alpha	159	214	1.35	11 (5.1%)	146	42	0.29
Beta	88	720	8.18	115 (15.9%)	85	815	9.59	20 (2.5%)
Races	January				February			
	No. female	No. egg collected	Egg female	No. egg hatched	No. female	No. egg collected	Egg female	No. egg hatched
	Alpha	128	63	0.50	0 (0.0%)	106	79	0.75
Beta	83	380	4.58	11 (2.9%)	79	324	4.10	11 (3.4%)
Races	March							
	No. female	No. egg collected	Egg female	No. egg hatched				
	Alpha	71	7	0.10	0 (0.0%)			
Beta	63	300	4.76	9 (3.0%)				



Fig. 25.--Comparison of egg production of alpha and beta *L. aperta* from September 1975 to March 1976.



Fig. 26.--Egg of alpha L. aperta on the 13th day showing developing embryo.

d. Egg Size. *L. aperta* eggs, alpha and beta race, were measured in the transparent stage (using a Wild compound microscope and ocular micrometer). They were spherical and measured 0.576 - 0.704 mm. (average 0.627 mm.) and 0.448 - 0.544 mm. (average 0.500 mm.) respectively, (Table 17).

e. F<sub>1</sub> Generation Snails. Eggs were harvested each month from the rearing dishes. They were transferred into other Petri dishes containing conditioned water and were regularly observed for development under the dissecting microscope. Almost 4 weeks were needed for an egg to mature. Only a few eggs covered with their silt coat were able to hatch by themselves; most were helped out by cracking open the egg coat with a dissecting needle. On hatching, newborn snails had shells with 1 1/2 - 2 whorls. They moved and fed actively. These baby snails were kept in Petri dishes containing conditioned water, sand and silt and fed with diatoms. The growth of baby snails was observed under the dissecting microscope. Measurements of the length and width of the body and aperture and counts of the number of whorls were done weekly for 24 weeks (Tables 18 & 19 and Figures 27 & 28).

In the first week of life, the baby snails had 1 1/2 - 2 whorls; the average length and width of alpha and beta snails were 0.61 x 0.49 mm. and 0.49 x 0.40 mm., respectively. The average dimensions of the aperture were 0.43 x 0.28 mm. for alpha snails and 0.35 x 0.22 mm. for beta snails. Under laboratory conditions, both races required 19 to 20 weeks to grow to 6 whorls (Table 20). After 6 whorls, further development of whorls or in their size were not apparent. The full grown alpha and beta snails were 4.34 x 2.86 mm. (L x W), 3.01 x 2.26 mm. (aperture) and 3.79 x 2.18 mm. (L x W), 2.40 x 1.47 mm. (aperture), respectively.

Apparently snails were not happy in the Petri dishes after becoming adult, as their activities, such as movement and defecation decreased.

Further attempts to provide a better culture technique by transferring the adult (F<sub>1</sub>) snails into larger containers, such as, glass bowls and bottles is now in progress.

f. Mortality of F<sub>1</sub> Generation Snails (baby snails). Observations were made for 25 weeks after the baby snails hatched out. Mortality of baby snails was highest during the first week of life in both alpha and beta races (Table 21). It should be noted that, in addition to natural causes, the deaths of the baby snails might have arisen from the mechanical effort of measuring their sizes. In the later weeks, the mortality was much lower as the snails grew bigger and easier to handle.

Table 17 Size of transparent L. aperta eggs laid in Petri dish cultures.

No. of eggs	Size of egg (mm.)	
	alpha race	beta race
1	0.704	0.544
2	0.608	0.544
3	0.640	0.512
4	0.608	0.480
5	0.640	0.480
6	0.608	0.544
7	0.640	0.512
8	0.608	0.544
9	0.608	0.480
10	0.608	0.480
11	0.608	0.480
12	0.576	0.448
13	0.640	0.480
14	0.640	0.512
15	0.704	0.480
16	0.672	0.480
17	0.608	0.480
18	0.608	0.480
19	0.608	0.512
20	0.608	0.544
average	0.6272	0.5008
range	0.576-0.704mm.	0.448-0.544mm.

Table 18 Mean measurements (in mm) of alpha L. aperta for 24 weeks after hatching.

Measurement time (in weeks)	No. of snail measurement	Mean measurements (in mm) of <u>L. aperta</u>			
		Body		Aperture	
		length	width	length	width
1	25	0.61	0.49	0.43	0.28
2	21	0.71	0.51	0.46	0.28
3	20	0.95	0.61	0.57	0.36
4	20	1.17	0.74	0.70	0.43
5	19	1.38	0.82	0.81	0.50
6	18	1.62	0.88	0.86	0.56
7	18	1.83	1.04	0.97	0.64
8	18	2.11	1.10	1.12	0.65
9	18	2.21	1.21	1.21	0.73
10	17	2.45	1.33	1.41	0.82
11	15	2.59	1.41	1.43	0.84
12	15	2.87	1.48	1.60	0.90
13	13	3.02	1.63	1.72	1.01
14	13	3.38	1.89	2.06	1.28
15	10	3.62	2.05	2.34	1.42
16	10	3.82	2.25	2.61	1.55
17	10	3.95	2.31	2.70	1.70
18	10	4.11	2.41	2.80	1.92
19	9	4.20	2.43	2.88	1.95
20	9	4.24	2.45	2.89	1.98
21	9	4.28	2.46	2.93	2.06
22	9	4.34	2.51	2.97	2.06
23	9	4.34	2.69	2.99	2.16
24	9	4.34	2.86	3.01	2.26

Table 19 Mean measurements (in mm) of beta L. aperta for 22 weeks after hatching.

Measurement time (in weeks)	No. of snail measurement	Mean measurements (in mm) of <u>L. aperta</u>			
		Body		Aperture	
		length	width	length	width
1	25	0.49	0.40	0.35	0.22
2	25	0.68	0.49	0.46	0.28
3	25	0.81	0.56	0.54	0.37
4	25	1.07	0.69	0.71	0.39
5	25	1.27	0.80	0.81	0.44
6	25	1.45	0.91	0.99	0.51
7	25	1.70	1.07	1.06	0.58
8	25	1.84	1.16	1.17	0.68
9	25	1.95	1.25	1.25	0.68
10	25	2.19	1.31	1.43	0.74
11	25	2.44	1.46	1.62	0.81
12	25	2.58	1.55	1.68	0.87
13	25	2.75	1.65	1.78	1.02
14	25	2.97	1.74	1.94	1.02
15	25	3.06	1.78	1.98	1.06
16	25	3.18	1.84	2.02	1.10
17	25	3.30	1.95	2.11	1.17
18	25	3.47	2.03	2.18	1.31
19	25	3.65	2.11	2.32	1.36
20	25	3.79	2.18	2.40	1.47
21	25	3.79	2.18	2.40	1.47
22	25	3.79	2.18	2.40	1.47

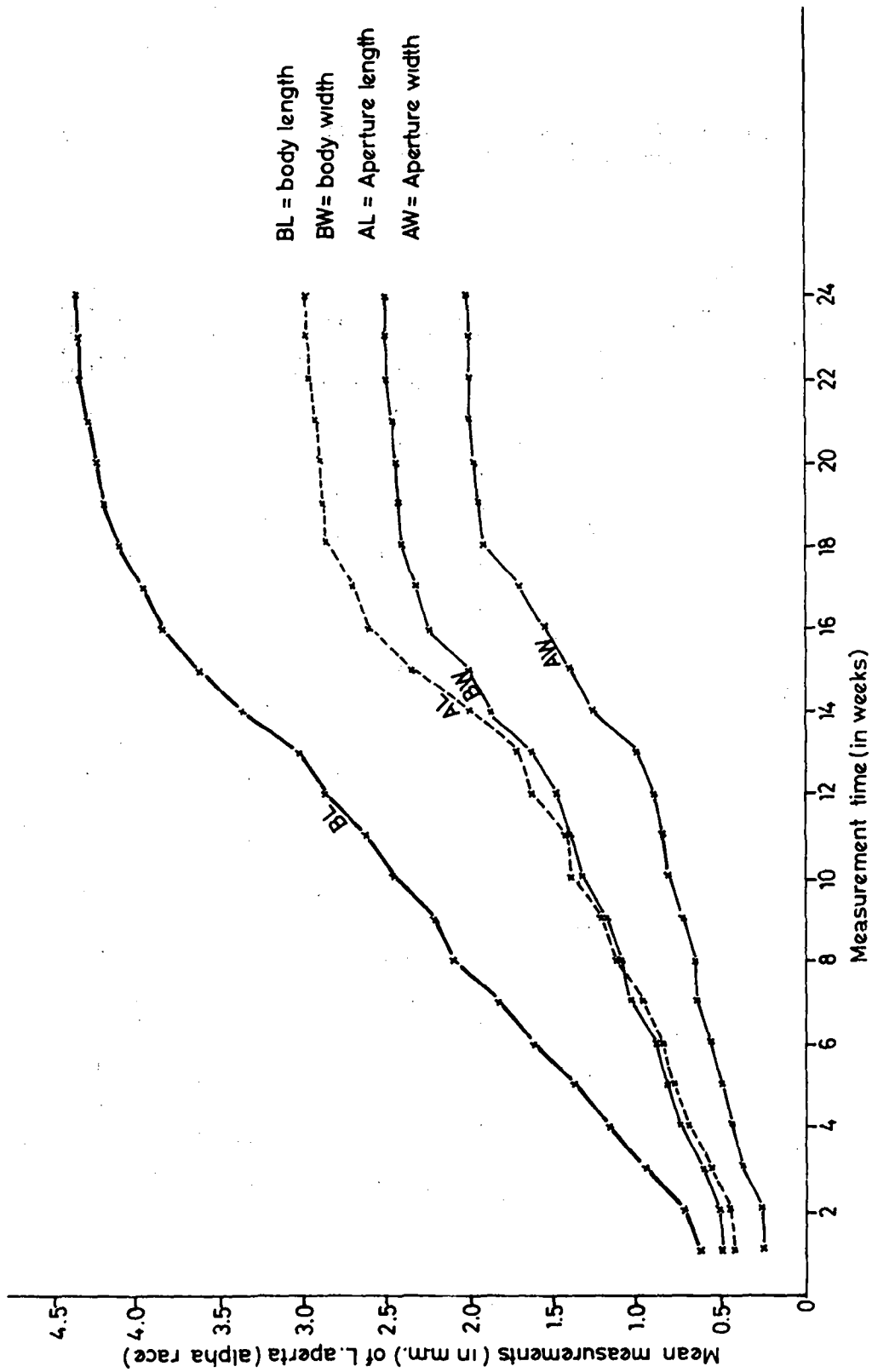


Fig. 27.--Mean measurements of alpha L. aperta from time of hatching to age of 24 weeks.



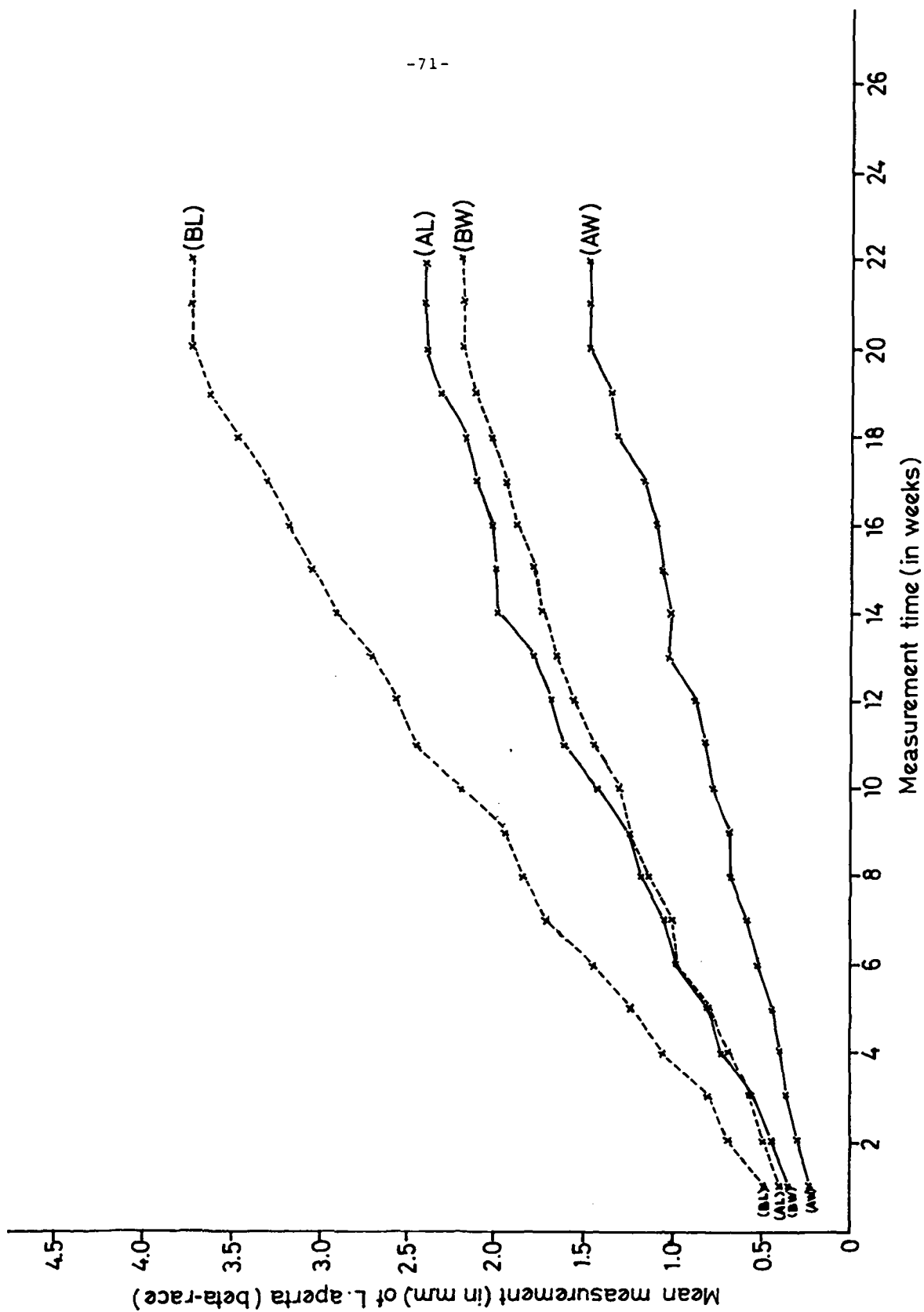


Fig. 28.--Mean measurements of beta *L. aperta* from time of hatching to age of 22 weeks.

Table 20 Development of whorls in F<sub>1</sub> baby snails.

Week	Alpha race			Beta race		
	No. snails observed	range	majority	No. snails observed	range	majority
1	25	2.00-2.50	2.0 (13)	25	1.50-3.00	2.00 (12)
2	21	2.00-2.50	2.5 (12)	25	2.25-3.25	2.50 (11)
3	20	2.25-3.50	3.0 (7)	25	2.50-3.50	3.00 (12)
4	20	2.50-3.50	3.5 (7)	25	2.50-4.00	3.50 (10)
5	19	2.50-4.00	3.5 (8)	25	3.00-4.00	3.50 (12)
6	18	2.75-4.25	4.0 (5)	25	3.00-4.50	4.00 (16)
7	18	2.75-4.75	4.0 (6)	25	3.50-5.00	4.00 (10)
8	18	2.75-5.00	4.0 (9)	25	4.00-5.00	4.50 (11)
9	18	3.50-5.00	4.0 (10)	25	4.00-5.00	4.50 (17)
10	17	3.50-5.00	4.5 (8)	25	4.50-5.50	5.00 (11)
11	15	4.00-5.00	4.5 (11)	25	4.50-5.50	5.00 (12)
12	15	4.00-5.00	5.0 (8)	25	4.50-5.75	5.00 (12)
13	13	4.00-5.00	5.0 (11)	25	5.00-6.00	5.50 (12)
14	13	5.00-5.50	5.0 (6)	25	5.00-6.00	5.75 (15)
15	10	5.00-5.50	5, 5.5(4)	25	5.00-6.00	5.75 (15)
16	10	5.00-5.50	5.5 (9)	25	5.00-6.00	5.75 (18)
17	10	5.50	5.5 (10)	25	5.50-6.00	6.00 (14)
18	10	5.50-6.00	6.0 (6)	25	5.75-6.00	6.00 (18)
19	10	5.50-6.00	6.0 (6)	25	6.00	6.00 (25)
20	9	6.00	6.0 (9)	25	6.00	6.00 (25)
21	9	6.00	6.0 (9)	25	6.00	6.00 (25)
22	9	6.00	6.0 (9)	25	6.00	6.00 (25)
23	9	6.00	6.0 (9)	25	6.00	6.00 (25)
24	9	6.00	6.0 (9)	25	6.00	6.00 (25)

( ) = No. of snails in the majority group.

Table 21 Weekly mortality of F<sub>1</sub> baby alpha and beta L. aperta cultured in Petri dishes for 25 weeks.

Week	Mortality of <u>L. aperta</u>					
	Alpha race			Beta race		
	INS	No.	%	INS	No.	%
1	37	14	37.83	104	21	20.19
2	23	1	4.34	83	12	14.45
3	22	0	0.00	71	6	8.45
4	22	2	9.09	65	0	0.00
5	20	1	5.00	65	11	16.92
6	19	0	0.00	54	2	3.70
7	19	0	0.00	52	1	1.92
8	19	0	0.00	51	3	5.88
9	19	1	0.00	48	1	2.08
10	18	2	11.11	47	0	0.00
11	16	0	0.00	47	1	2.17
12	16	2	12.50	46	0	0.00
13	14	0	0.00	46	0	0.00
14	14	3	21.42	46	0	0.00
15	11	0	0.00	46	0	0.00
16	11	0	0.00	46	0	0.00
17	11	0	0.00	45	1	2.22
18	11	2	18.18	45	0	0.00
19	9	0	0.00	45	0	0.00
20	9	0	0.00	45	6	15.38
21	9	0	0.00	39	4	11.42
22	9	0	0.00	35	2	6.06
23	9	0	0.00	33	0	0.00
24	9	0	0.00	33	2	6.45
25	8	1	11.11	31	3	9.67

INS = initial number of snails.

No. = number of dead snails.

A correlation could not be established between the time of high mortality and the races of the snail, since there was only one lot available for study.

## 2. Bottle Cultures

Two types of bottles were employed: small, round bottles (3 1/2" x 3 3/4") and large, round bottles (4 1/2" x 8"), (Figures 29 & 30).

Each bottle contained small rocks or stones from the Mekong River and was preconditioned with diatoms before the snails were introduced. Water levels were maintained at 2.5" in the small bottles, and 6" in the large ones. Twenty snails (12 females and 6 males) were placed in each small bottle and 40 snails (24 females and 16 males) in each large one. Diatoms were regularly added as food. All cultures were exposed to 8 hours of natural light daily, however, since the growth of green algae under these conditions was rapid, we were forced to change the cultures often. Light exposure, and thus, unwanted algae, could be reduced by blocking the direct daylight with sheets of black paper.

All three races of L. aperta were maintained under these conditions. Water levels, pH, and temperature were maintained and recorded regularly. Egg laying and snail mortality were recorded.

a. Appearance of the Culture. Conditions in the bottles seemed to be more favorable than those in Petri dishes; snails were more active, grew faster and changes of culture were required less often. Unless there were algae, ciliates or rotifers, the cultures remained unchanged. However, observations of mortality, egg laying, development of snails and populations of ciliates or rotifers were more difficult to make.

Attempts to study the growth rate of snails by microscopic measurement failed because many snails died after the first few trials.

b. Mortality Rates (Table 22). As an indicator of the success of rearing, observations on mortality rates were made on a monthly basis except during the egg laying period (September to October), when snails could not be taken out for counting. Manipulation always disturbed the females about to lay eggs and arrested development of the eggs already laid.

During the first 10 months, the mortality rate was seen to increase from October onward, i.e., after the egg laying period. This phenomenon was also seen in Petri



Fig. 29.--Snail rearing in large bottles.

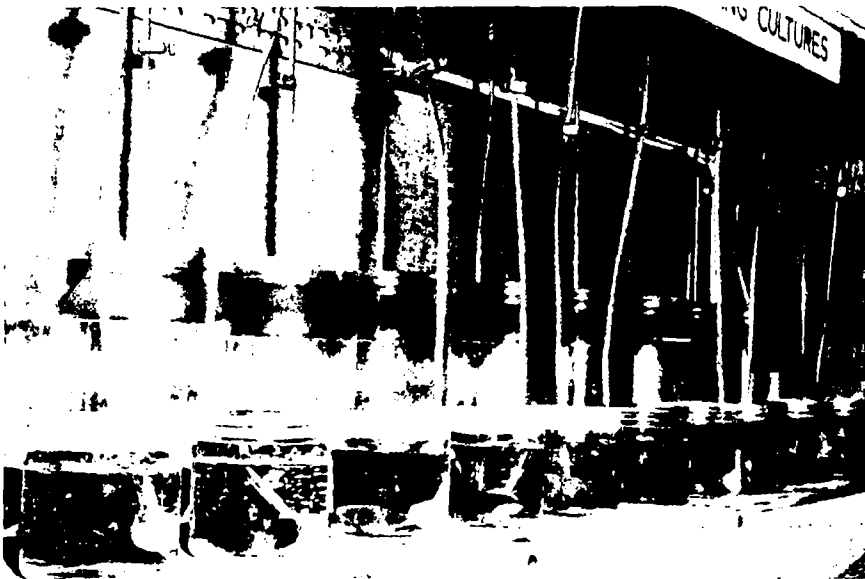


Fig. 30.--Snail rearing in small bottles.

Table 22 Mortality of 3 races of *L. aperta* reared in large and small bottles during May 1975 to February 1976.

	May			June			July			August			September		
	I	M	%	I	M	%	I	M	%	I	M	%	I	M	%
<u>Large bottle</u>															
alpha	400	0		408	45	11.07	364	50	13.73	398	ND		ND	ND	
beta	200	0		200	29	14.50	175	61	34.86	200	25	12.50	171	29	16.96
gamma	120	0		120	23	19.17	99	23	23.23	120	19	15.83	96	15	15.63
<u>Small bottle</u>															
alpha	200	4	2.00	200	26	13.00	200	37	18.50	197	ND		ND	ND	
beta	200	0		200	52	26.00	184	45	24.46	200	ND		180	53	29.44
gamma	200	0		60	2	3.33	58	5	8.62	60	8	13.33	56	13	23.21
	October			November			December			January			February		
	I	M	%	I	M	%	I	M	%	I	M	%	I	M	%
<u>Large bottle</u>															
alpha	382	80	20.94	247	52	21.05	163	66	40.49	121	60	49.59	58	22	37.93
beta	144	ND		ND	ND		ND	ND		ND	ND		ND	ND	
gamma	87	13	14.94	74	13	17.57	61	13	21.31	48	2	4.17	39	4	10.26
<u>Small bottle</u>															
alpha	207	70	33.82	111	31	27.93	85	48	56.47	35	18	51.43	16	5	31.25
beta	ND	ND		ND	ND		102	22	21.57	9	1	11.11	7	1	14.29
gamma	42	ND		ND	ND		31	15	48.39	12	6	50.00	8	2	25.00

ND = Not done.  
 I = Initial number of snails in each bottle.  
 M = Mortality in each bottle.

dish cultures, suggesting that it may be their nature for snails to die after laying eggs.

c. Egg Production. Eggs were first observed in bottle cultures in September 1975. Eggs were laid on stones and on the sides of the bottle. Morphology was the same as described above. These eggs were left alone and observed for development and hatching. No attempt was made to measure them or detach them from their sites. Only eggs of the alpha race were observed in bottle cultures.

d. F<sub>1</sub> Snails. Six weeks after their first appearance, young F<sub>1</sub> snails with 2 to 2 1/2 shell whorls hatched out. Among 26 eggs, 9 snails hatched by themselves and the others were assisted by breaking the shell coat with a dissecting needle.

It was evident from the previous year that the snails preferred larger and more open spaces. New culture methods were tested at the beginning of the new breeding season (1976). Glass bowls, (8" diameter) (Figure 31) and stainless steel trays (15 x 10 1/2 x 2 1/4") (Figure 32), were used together with the Petri dishes and large bottles.

Rearing was started in March and April 1976. All containers were implanted with diatoms and filled with conditioned water, silt and small stones except the Petri dishes. Snails were observed for activity, mortality, egg laying and young snails using the same procedure as the previous year. Preliminary results appear in Tables 23, 24, 25 & 26.

### 3. The Development of L. Aperta in Nature

Attempts to study the development of L. aperta in nature were made. Studies were conducted at two localities on the Mekong River near Khemmarat where large populations of alpha and gamma races occurred (see above). The beta race, which is limited to the Mun River, could not be studied because of small numbers.

The study was carried out as follows:

a. The Growth of L. Aperta in Nature. The growth of L. aperta, alpha and gamma races, was studied at Khemmarat in the same area where the population dynamic study was conducted (see above).

The snails were collected every two weeks. From each collection, 100 snails were sexed and measured with an ocular micrometer under a dissecting microscope. The length and width of the body and the aperture were recorded (Table 27, Figures 33 & 34).



Fig. 31.--Snail rearing in glass bowls.





Fig. 32.--Snail rearing in stainless steel trays.

Table 23 Weekly mortality rates for *L. aperta* cultured in glass bowls (diameter 8"; water level, 4") from March 22, 1976 to May 31, 1976.

Races		Mortality of snails per week																	
		March			April														
		Week of culture																	
1st		2nd		3rd		4th		5th		6th		7th		8th		9th			
INS	M	INS	M	INS	M	INS	M	INS	M	INS	M	INS	M	INS	M	INS	M		
Alpha*	400	1	0.25	400	3	0.50	400	0	0.00	400	0	0.00	400	0	0.00	400	98	24.50	
Beta**	400	0	0.00	400	32	8.00	400	0	0.00	400	0	0.00	400	0	0.00	400	148	37.00	
Gamma***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.25	

Races		May																	
		Week of Culture																	
		6th		7th		8th		9th											
INS	M	INS	M	INS	M	INS	M	INS	M	INS	M	INS	M	INS	M	INS	M		
Alpha*	400	0	0.00	400	1	0.25	399	9	2.25	390	1	0.25	390	1	0.25	390	1	0.25	
Beta**	280	0	0.00	400	162	57.85	118	14	11.86	104	53	50.96	104	53	50.96	104	53	50.96	
Gamma***	400	0	0.00	400	0	0.00	400	0	0.00	400	0	0.00	400	0	0.00	400	1	0.25	

INS = initial number of snails.  
M = number of dead snails.  
\* = started on 22 March 1976.  
\*\* = started on 27 March 1976.  
\*\*\* = started on 16 April 1976.

Table 24 Weekly mortality rate of *L. aperta* cultured in stainless steel trays from April 5 to May 31, 1976.

Mortality of snails per week												
April												
Races	1st			2nd			3rd			4th		
	INS	M	%	INS	M	%	INS	M	%	INS	M	%
Alpha*	400	20	5.00	400	6	1.50	400	12	3.00	400	12	3.00
Beta**	400	47	11.75	400	24	6.00	400	47	11.75	400	36	9.00
Gamma***	-	-	-	-	-	-	400	0	0.00	400	0	0.00

Mortality of snails per week												
May												
Races	5th			6th			7th					
	INS	M	%	INS	M	%	INS	M	%			
Alpha*	388	14	3.60	374	19	4.70	355	4	1.09			
Beta**	364	39	10.70	325	84	26.66	241	35	15.15			
Gamma***	400	0	0.00	400	4	1.00	396	2	0.50			

INS = initial number of snails.  
M = number of dead snails.  
\* = started on 5 April 1976.  
\*\* = started on 5 April 1976.  
\*\*\* = started on 16 April 1976.

Table 25 Weekly mortality rate of L. aperta cultured in 9 cm. Petri dishes from March 22 to May 31, 1976.

Races		Mortality of snails per week																	
		March			April														
		Week of culture																	
1st		2nd			3rd			4th			5th								
INS	M	%	INS	M	%	INS	M	%	INS	M	%	INS	M	%					
Alpha*	100	4	4	100	5	5	100	2	2	100	2	2	100	5	5				
Beta**	-	-	-	100	1	1	100	0	0	100	0	0	100	0	0				
Gamma																			

Races		Mortality of snails per week														
		6th			7th			8th			9th					
		INS	M	%	INS	M	%	INS	M	%	INS	M	%			
Alpha*	100	0	0	100	0	0	100	0	0	100	0	0				
Beta**	100	0	0	100	0	0	100	0	0	100	0	0				
Gamma***	100	3	3	100	0	0	100	1	1							

INS = initial number of snails.  
M = number of dead snails.  
\* = started on 22 March 1976.  
\*\* = started on 26 March 1976.  
\*\*\* = started on 27 April 1976.

Table 26 Monthly mortality rates for L. aperta reared in large bottles from March 22 to May 31, 1976.

Races	Mortality of snails per month								
	March			April			May		
	INS	M	%	INS	M	%	INS	M	%
Alpha*	400	0	0.00	400	167	41.75	400	94	23.50
Beta**	400	10	3.20	400	176	44.00	224	181	80.80
Gamma***	-	-	-	400	6	1.50	400	114	28.50

INS = initial number of snails.

\* = started on 22 March 1976.

\*\* = started on 26 March 1976.

\*\*\* = started on 16 April 1976.

Table 27 Mean measurement of 100 alpha and gamma L. aperta collected during March to May 1976.

Mean measurements (mm) of <u>L. aperta</u> (100 snails)								
alpha race					gamma race			
Week	Body		Aperture		Body		Aperture	
	length	width	length	width	length	width	length	width
0	1.09	0.67	0.74	0.50	0.89	0.51	0.59	0.34
2	1.37	0.82	0.91	0.58	1.29	0.74	0.86	0.48
4	1.94	1.18	1.28	0.82	1.62	0.94	1.04	0.60
6	2.78	1.68	1.88	1.09	2.37	1.40	1.49	0.86
8	3.55	2.09	2.46	1.59	2.76	1.62	1.69	0.99
10	3.92	2.19	2.73	1.98	-	-	-	-

UI = unidentifiable

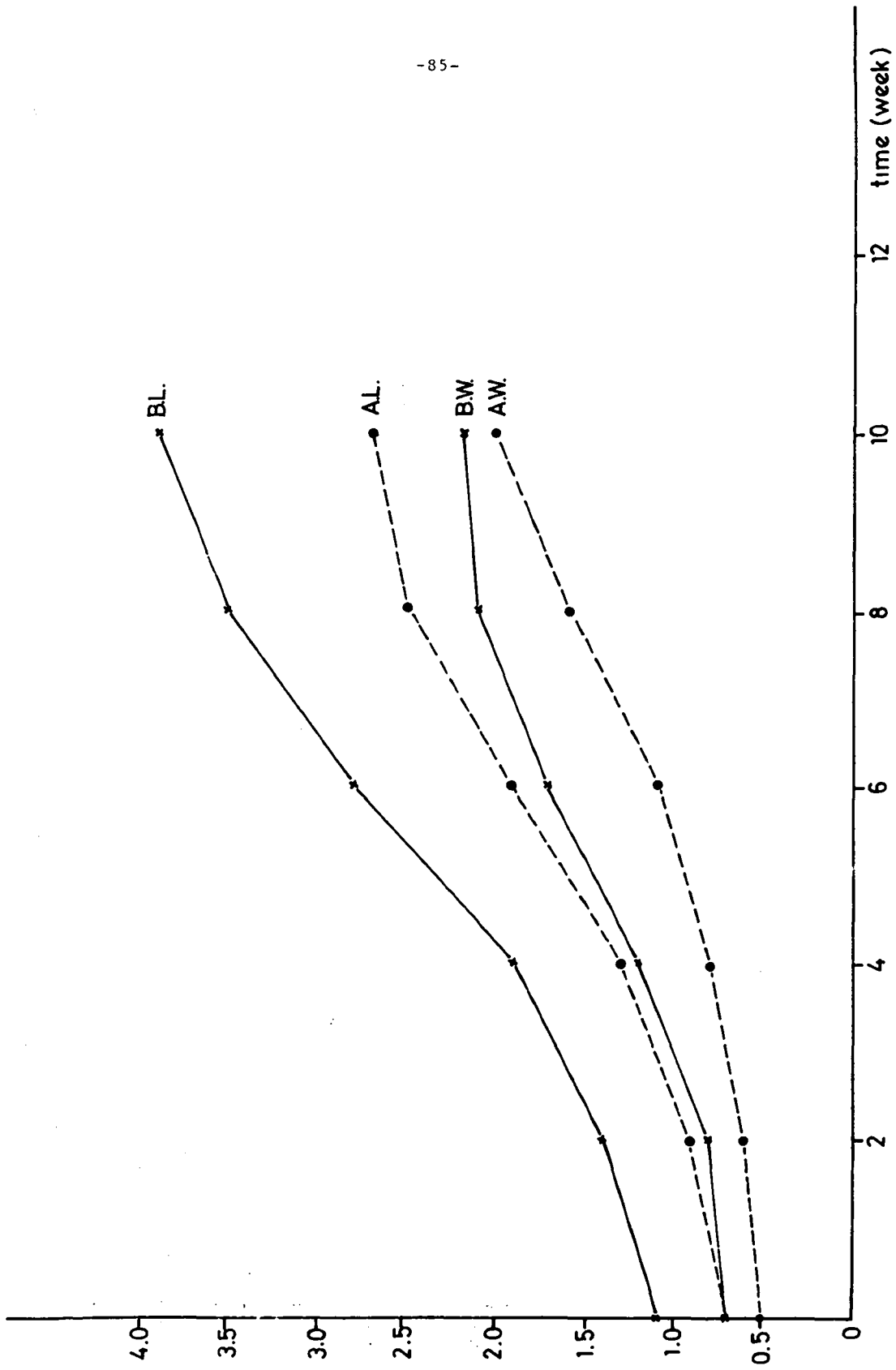


FIG. 33.--Growth of alpha *L. aperta* in nature.

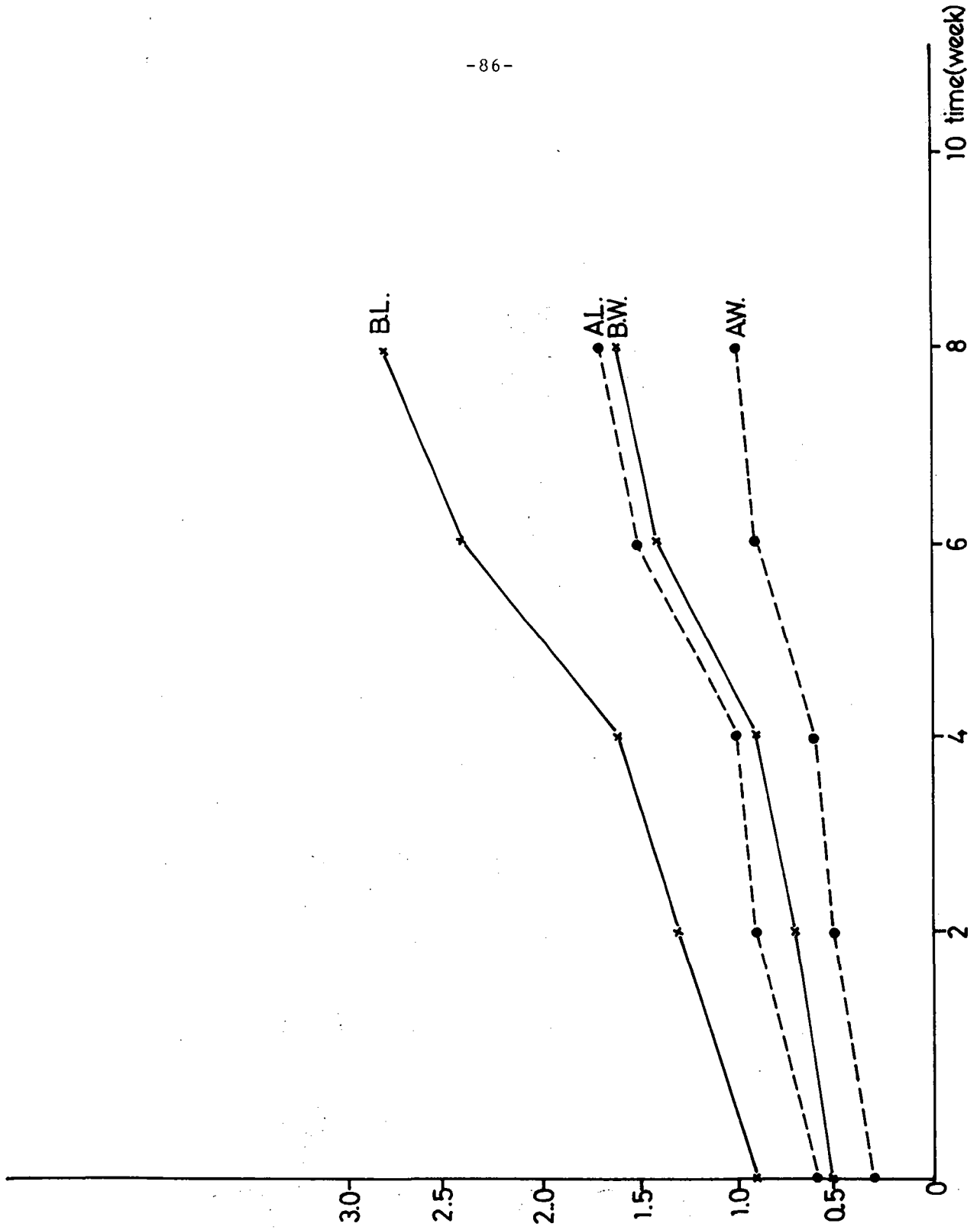


Fig. 34.--Growth of *gamma L. aperta* in nature.



Taking the development of distinguishable sex organs as the criterion of snail maturity, the alpha race in nature became adult when they were 2.78 mm. in length and 1.68 mm. in width, the corresponding size of aperture being 1.88 x 1.09 mm.

The gamma race was similar.

From consecutive studies of size, it was assumed that in nature, L. aperta matured within 6 weeks of hatching.

Unfortunately, observations on the development of sex organs was not done with baby snails in the laboratory but, it is presumed that under laboratory conditions, the snail needed at least 12 weeks to become mature.

Then the growth rates of snails in nature were compared with those in the laboratory, it was apparent that laboratory snails needed almost twice as much time as snails in nature to reach the same size (Figures 35 & 36).

b. Morphology of L. Aperta Eggs in Nature. In the dry season, rocks in the Mekong River were generally covered with a deposit of many varieties of snail eggs. Some were covered with blackish particles, others with yellow or red. No eggs with transparent gelatinous membranes, as seen in the laboratory, were found in nature. Most, if not all, of the eggs were not newly laid, since in the very early period of low level of water (late February), adult snails were hardly seen but, numerous baby L. aperta snails were found crawling on the same rocks.

The identification of alpha race L. aperta eggs in nature was done by collecting rocks with eggs on which many baby L. aperta snails were also to be found. All snails were washed off and the rocks were kept in aquariums with aerated river water. The eggs on the rocks were regularly observed until the baby snails hatched out. These snails were maintained in the aquarium until they could be identified.

The L. aperta eggs were hemispherical in shape and fixed to the under surface of the rock (Figures 37 & 38). They were covered with a thick, fine granular envelope of reddish-yellow silt or sand. The size and color of L. aperta eggs varied. Measurements were made of 20 eggs (Table 28).

Eggs of L. aperta in nature had an average length and width of 0.752 and 0.569 mm. (range: 0.504 - 0.90 x 0.482 - 0.684 mm.).

Upon breaking the silty coat, a clear membranous egg with a yellow or dark embryo was seen. This appeared

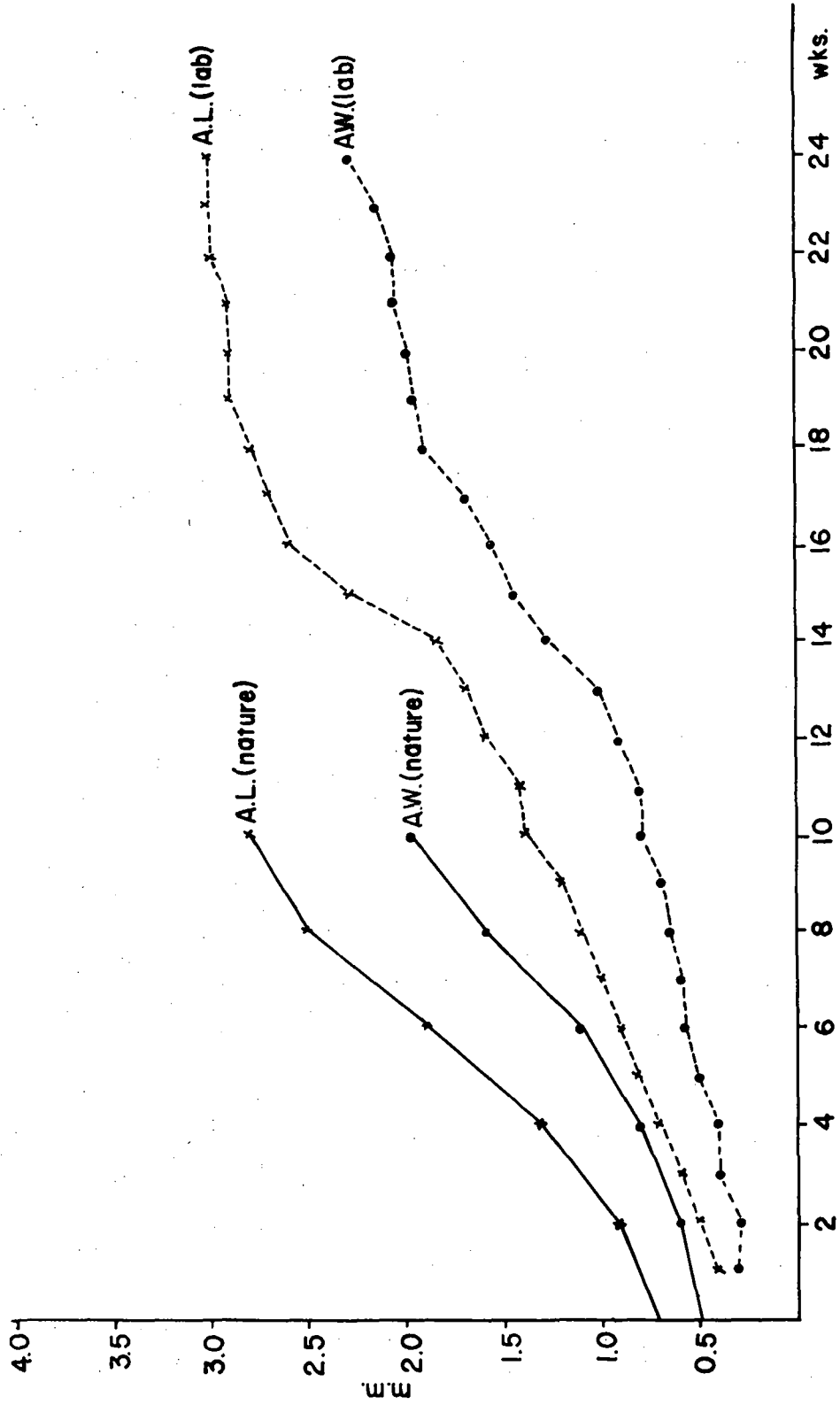


Fig. 35.--Comparison of the growth of alpha L. aperta in nature and in the laboratory; body measurements.

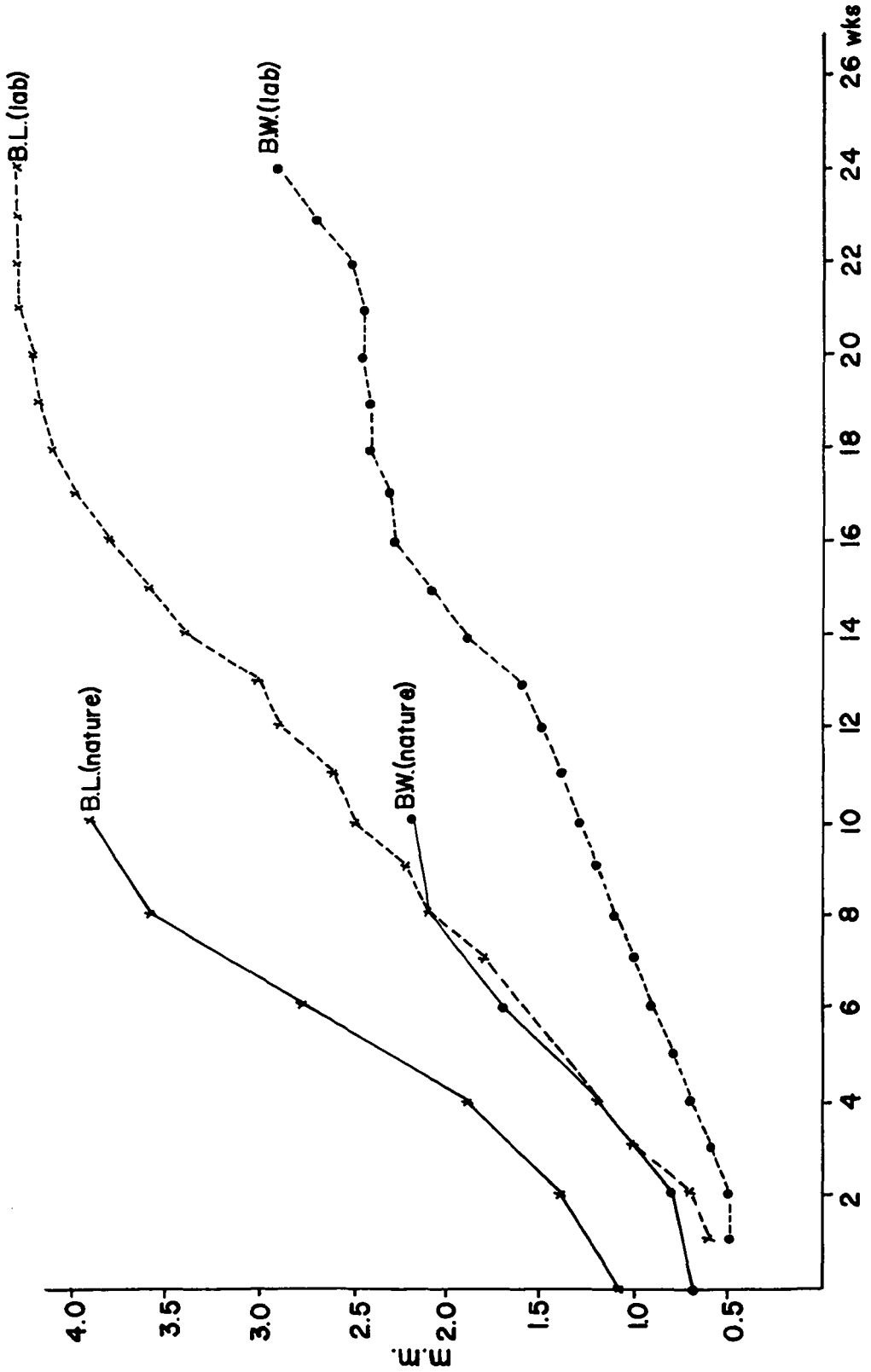


Fig. 36.--Comparison of the growth of alpha L. aperta in nature and in the laboratory; aperture measurements.



Fig. 37.--Rock from the Mekong River near  
Khemmarat bearing L. aperta and eggs.

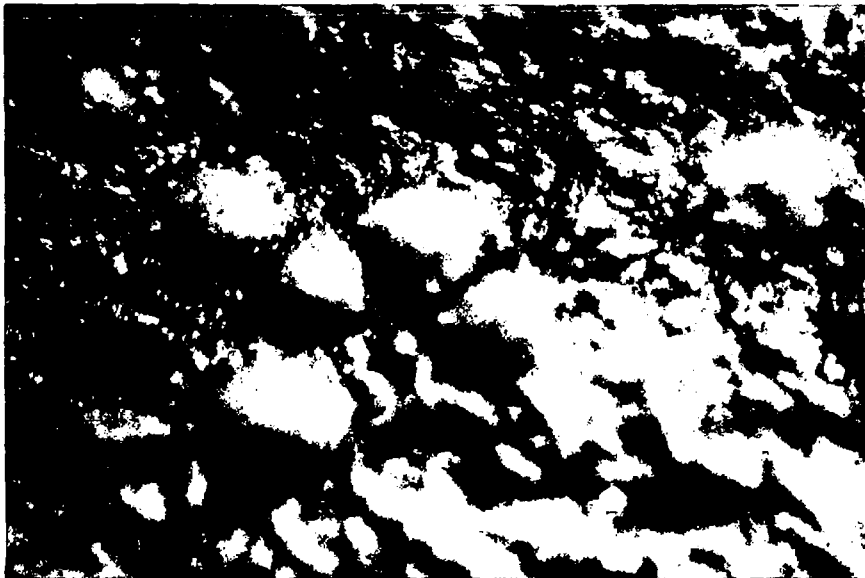


Fig. 38.--Eggs of L. aperta on rock from the  
Mekong River.

Table 28 Size of alpha L. aperta eggs in nature, measuring eggs deposited on rock surfaces.

Number	Length (mm.)	Width (mm.)
1	0.756	0.504
2	0.576	0.432
3	0.684	0.684
4	0.900	0.648
5	0.612	0.504
6	0.684	0.540
7	0.720	0.576
8	0.864	0.576
9	0.504	0.504
10	0.612	0.612
11	0.756	0.576
12	0.648	0.648
13	0.720	0.648
14	0.792	0.720
15	0.684	0.504
16	0.900	0.504
17	0.684	0.504
18	0.756	0.540
19	0.756	0.540
20	0.684	0.612
Average	0.752	0.569
Range	0.504-0.900	0.432-0.684

similar to the eggs produced in the laboratory. The embryo inside was in constant motion if the egg was viable.

### III Molluscicide Testing

#### 1. Materials and Methods

A field laboratory was established in Khemmarat, Ubon-ratchatani Province. At Khemmarat, large numbers of alpha and gamma race Lithoglyphopsis aperta were taken from stones on islands situated along the bank or in the center of the Mekong River. Snails were maintained in river water aquaria in the field laboratory for no longer than 48 hours before testing with certain molluscicides. The snail collecting sites were not endemic for Schistosoma japonicum and the snails collected from these sites were also shown to be free from any other schistosome infection. Attempts were made to test young and adult L. aperta of both races with each molluscicide; however, all tests could not be completed owing to limitations of time and availability of snails. In particular, L. aperta snails could be collected during only 4 months of the year, the low water period, from February to May.

A protocol to study the effects of certain molluscicides under controlled laboratory conditions was established as follows:

#### A. Conventional Molluscicides

##### 24 - Hour Exposure Lethal Concentration

Molluscicide + snails  
Molluscicide + silt + snails

##### Time Concentration Relationships

Molluscicide + snails

Exposure time: 1 hour  
Exposure time: 6 hours  
Exposure time: 12 hours  
Exposure time: 24 hours (repeat 1. a.)

Recovery period for snails was 48 hours.

##### Slow Release Molluscicide

##### Soaking Time Intervals of Molluscicide

Tributyltin oxide (TBTO) pellets were weighed to give the required concentrations. Prior to snail exposures, the weighed pellets were soaked in water simultaneously for

1, 2, 3, 4, 8, 16 and 32 days. Snails were exposed to TBTO solutions (after pellets were removed) for 24 hours, and were then maintained in a recovery chamber for 72 hours.

#### Continuous Exposures of Snails

TBTO pellets were weighed to give the required concentrations, and were placed in water. Snails were then exposed to these TBTO concentrations. Snail mortality was checked and recorded every 24 hours starting at day one and terminating at day thirty-three.

The apparatus for testing molluscicides consisted of wooden frames, nylon tubes and screws assembled together as shown in Figure 39. Snails were exposed to selected molluscicides in 1.5 liter plastic bags. Before conducting an experiment, plastic bags were placed into nylon tubes, with the open ends of the former folding over the open tops of the latter. The open ends of plastic bags were held tightly to the open tops of nylon tubes with rubber bands. The height of each plastic bag was adjusted to hold about 1 liter of tested solution. To prevent snails from crawling out, the top of each plastic bag was covered with a glass sheet (Figures 40 & 41).

For each molluscicide, a few trials with a wide range of concentrations were performed in order to observe the range of snail mortality. Subsequently, two trials in a narrower range were performed in order to obtain lethal concentrations for 50% (LC<sub>50</sub>) and 90% (LC<sub>90</sub>) of snails. Thirty snails were used for testing each concentration. In some experiments, 100 grams of silt were added to each of the test solutions.

To calculate the LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and slope functions, the methods of Litchfield and Wilcoxon (1949) were used.

## 2. Results

a. Bayluscide (Niclosamide, 25% Emulsifiable Concentrate, 6076). A summary of LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and slope functions is given in Table 29 and Figures 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52 & 53. Raw data are given in Appendix II.

There was a decrease in lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) as the exposure time increased.

The lethal concentrations for young snails were slightly higher than for adult snails.

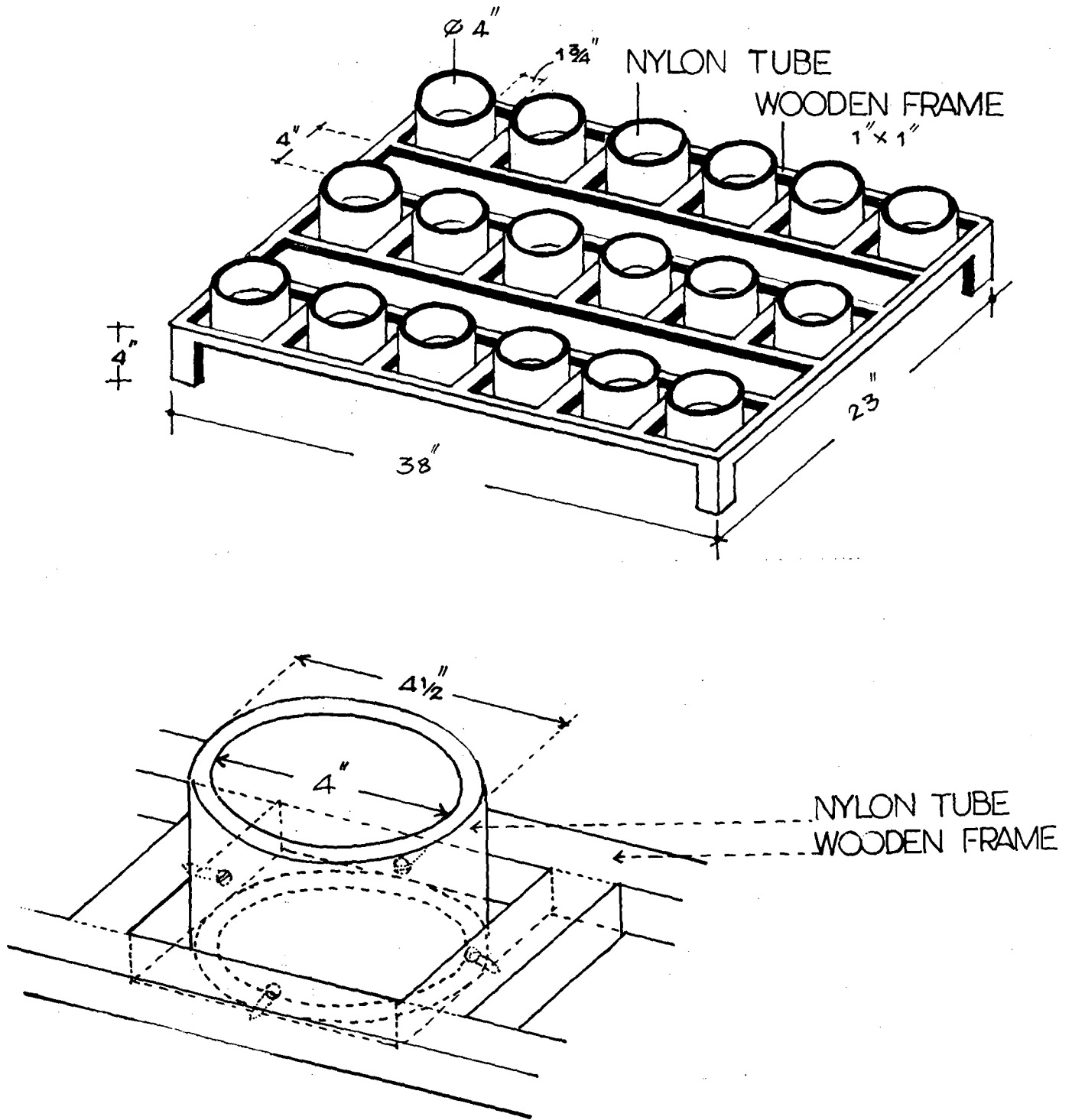


Fig. 39.--Diagram of apparatus for testing molluscicides.



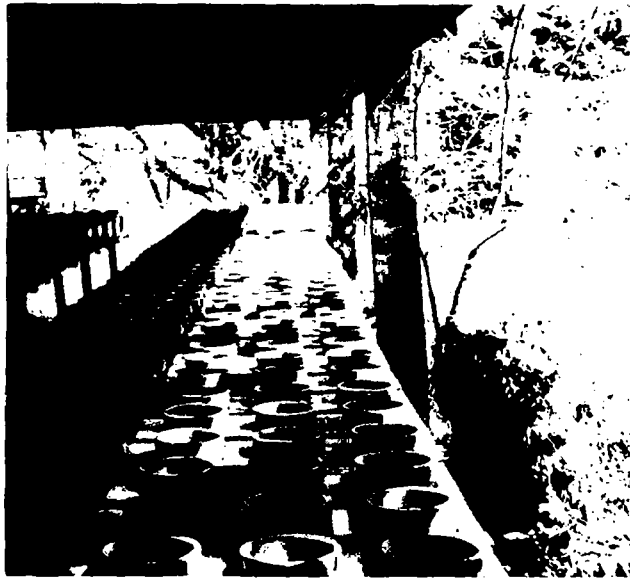


Fig. 40.--Molluscicide testing apparatus in place in the field laboratory.



Fig. 41.--Close-up view of molluscicide testing apparatus, showing units with filled plastic bags and glass plates in position.

Table 29 Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) in parts per million (ppm) with 95% confidence limits and slope functions of two races of *L. aperta* exposed to Niclosamide, emulsified concentrate, 25% a.i. (Bayuscide) at intervals of 6, 12 and 24 hours.

Race of <i>L. aperta</i>	Exposure time (in hours)	Mean mortality of <i>L. aperta</i> (in ppm)				Slope function	
		LC <sub>50</sub> (95% confidence limit)		LC <sub>90</sub> (95% confidence limit)		Young	Adult
		Young	Adult	Young	Adult		
Alpha	6	ND	0.200(0.170-0.240)	ND	0.320(0.260-0.390)	ND	1.46
	12	ND	0.102(0.080-0.130)	ND	0.230(0.170-0.300)	ND	1.93
	24	0.136(0.120-0.160)	0.096(0.080-0.110)	0.250(0.220-0.290)	0.125(0.110-0.140)	1.60	1.28
Gamma	24S	ND	0.080(0.070-0.090)	ND	0.116(0.100-0.140)	ND	1.36
	6	0.159(0.130-0.200)	0.260(0.240-0.290)	0.420(0.320-0.550)	0.400(0.350-0.460)	2.10	1.41
	12	0.102(0.080-0.130)	0.128(0.110-0.150)	0.222(0.180-0.280)	0.232(0.180-0.300)	1.77	1.62
	24	0.077(0.060-0.100)	0.083(0.070-0.100)	0.136(0.100-0.180)	0.122(0.100-0.150)	1.60	1.42
	24S	0.079(0.060-0.100)	ND	0.141(0.110-0.180)	ND	1.58	ND

24S = silt was added in experimental solutions and exposure time was 24 hours.

ND = not done.

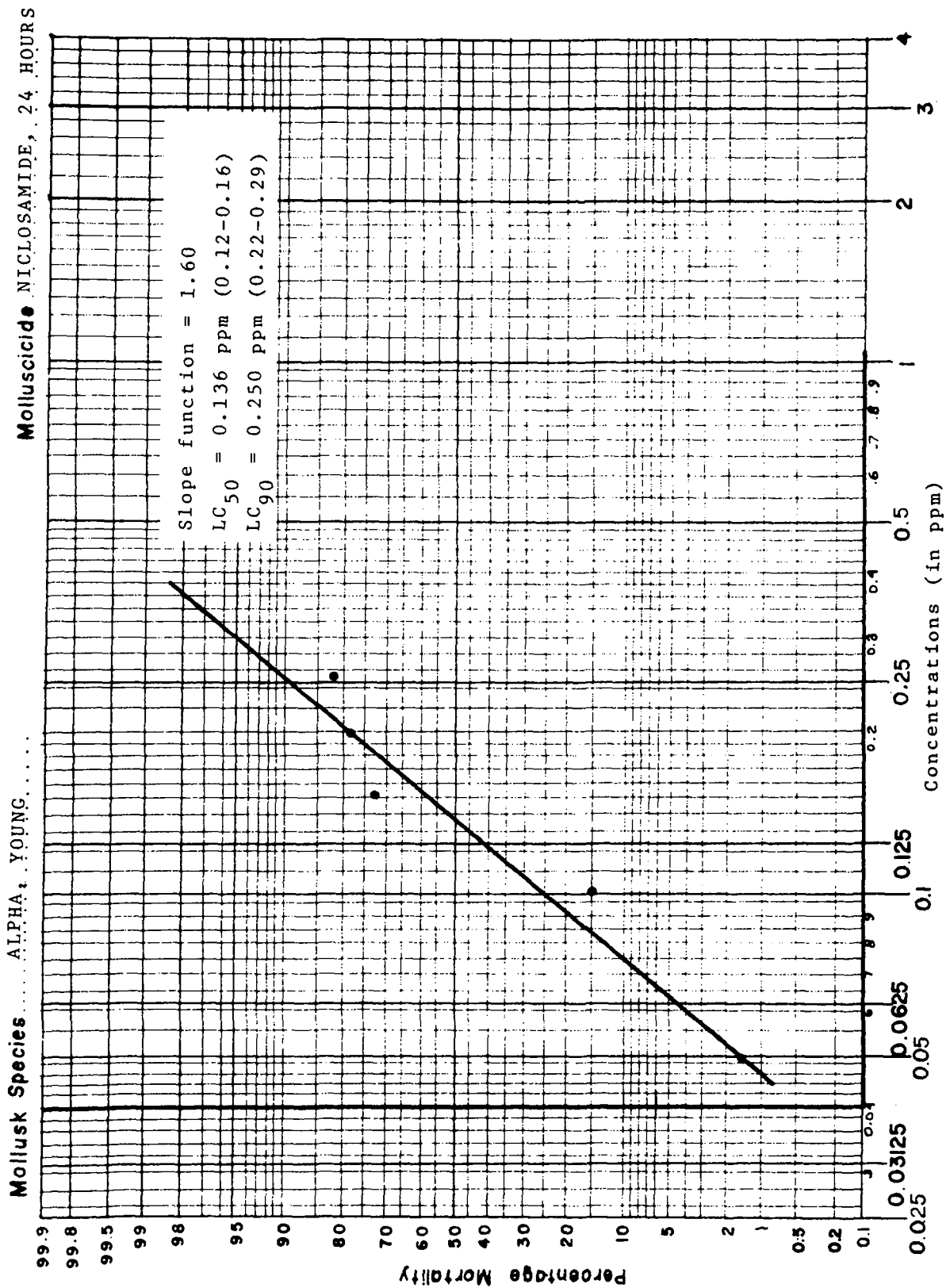


Fig. 42.--Mortality of young alpha L. aperta exposed to Niclosamide for 24 hours.

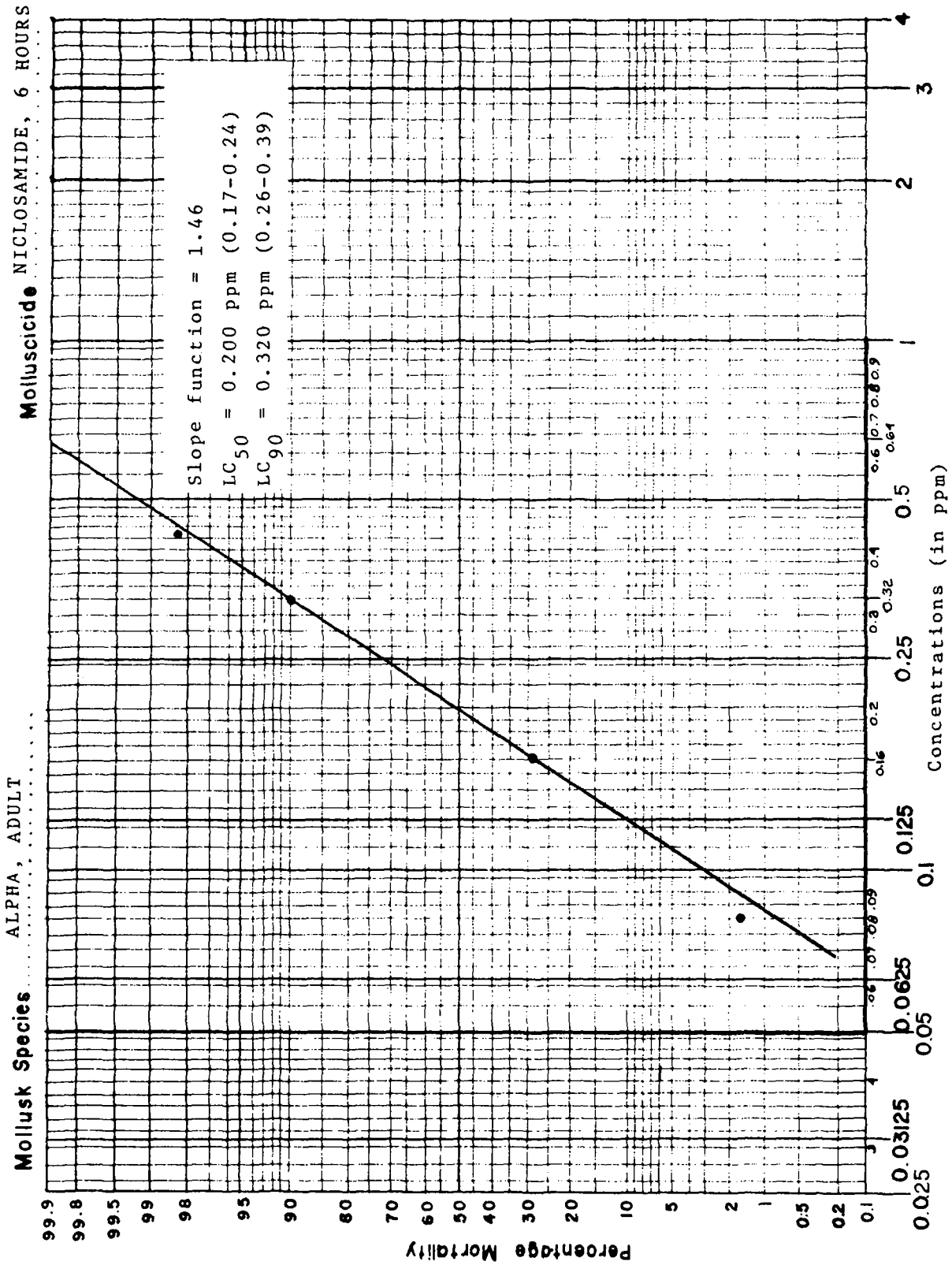


Fig. 43.--Mortality of adult alpha L. aperta exposed to Niclosamide for 6 hours.

MOLLUSK SPECIES ALPHA, ADULT ..... MOLLUSCIDIC MICLOSANIDE, 12 HOURS

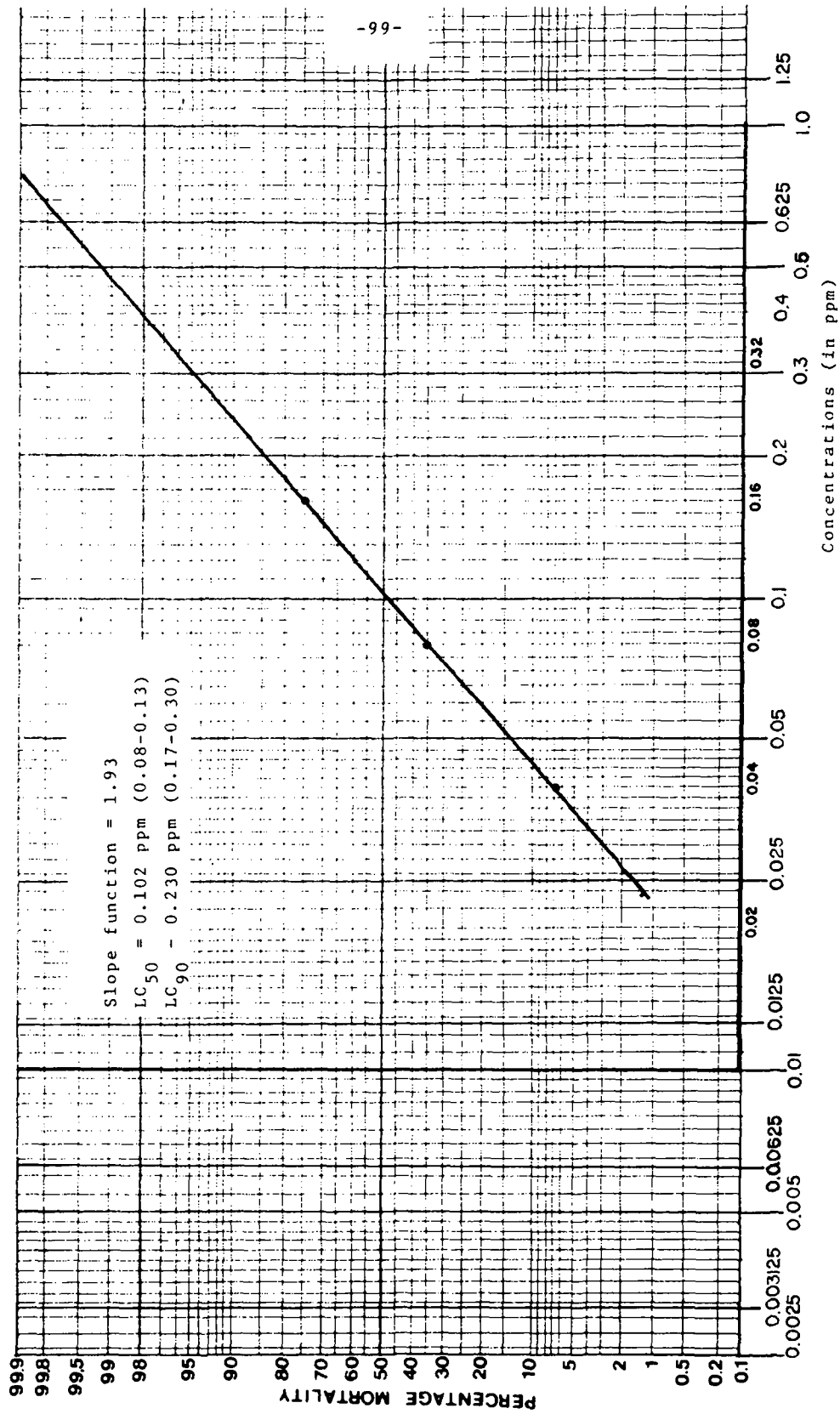


Fig. 44.--Mortality of adult alpha *L. aperta* exposed to Niclosamide for 12 hours.

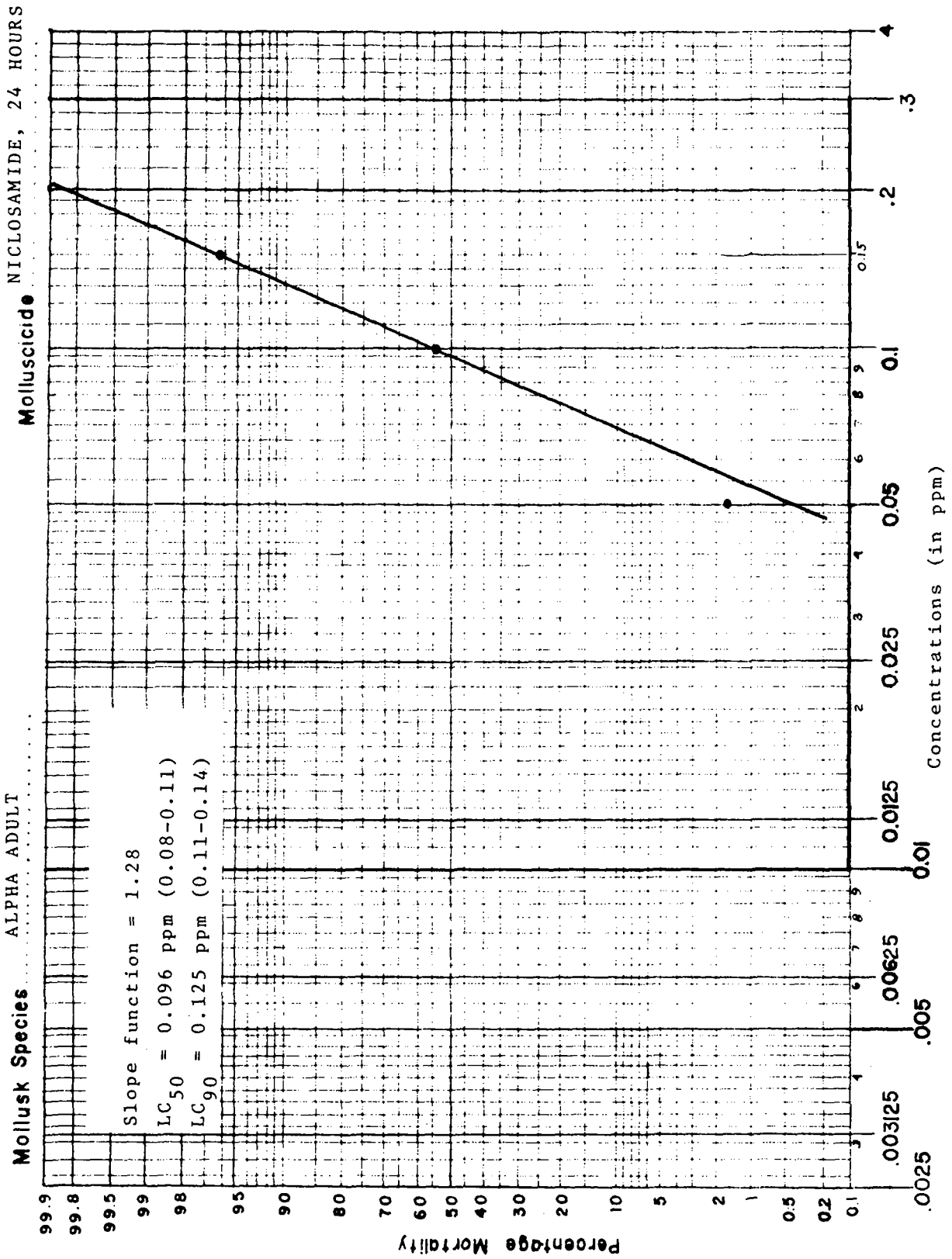


Fig. 45.--Mortality of adult alpha L. aperta exposed to Niclosamide for 24 hours.

Mollusc Species ALPHA, ADULT  
Molluscicide NICLOSAMIDE, 24 HOURS + SILT

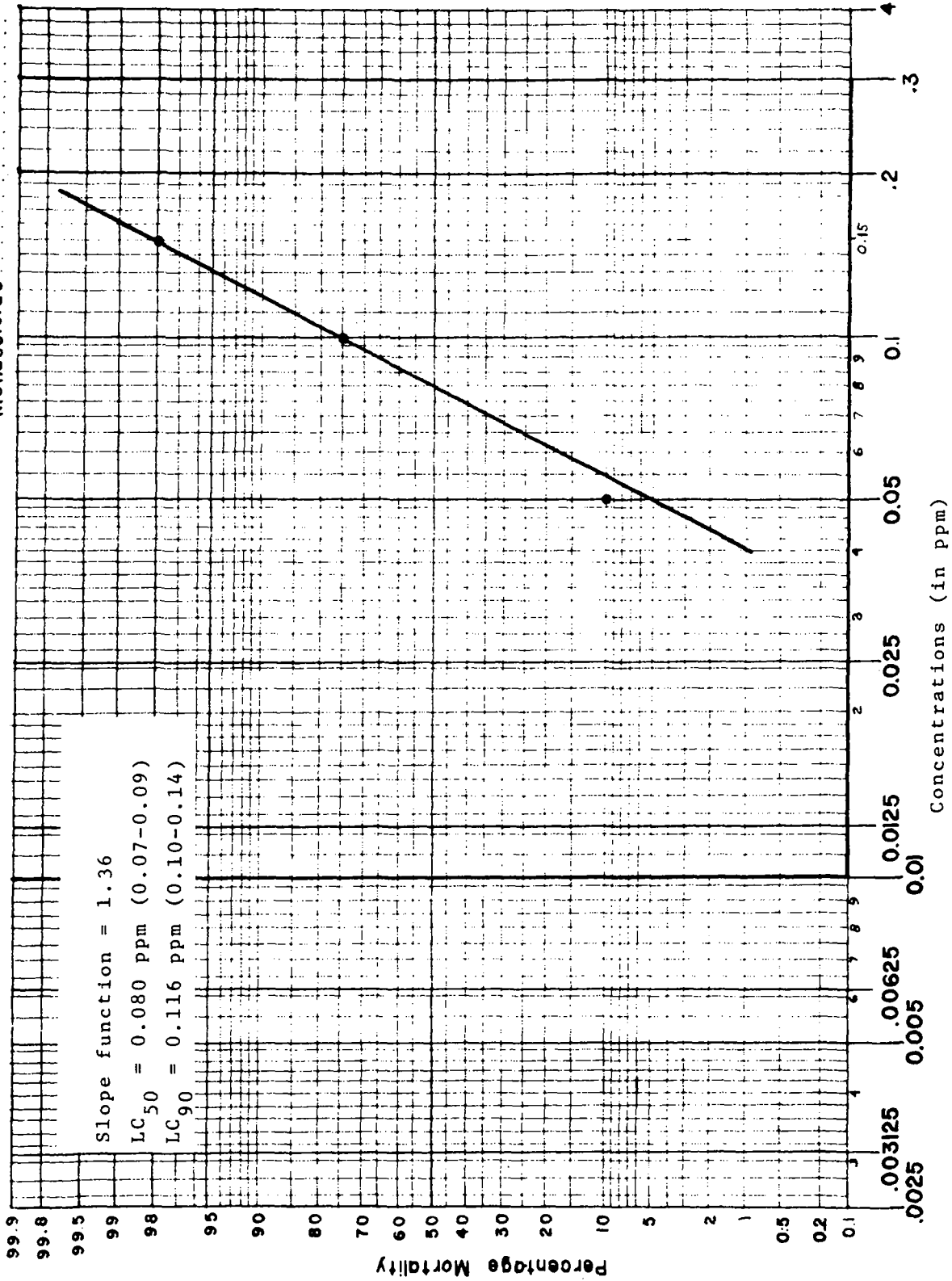


Fig. 46.--Mortality of adult alpha L. aperta exposed to Niclosamide plus silt for 24 hours.

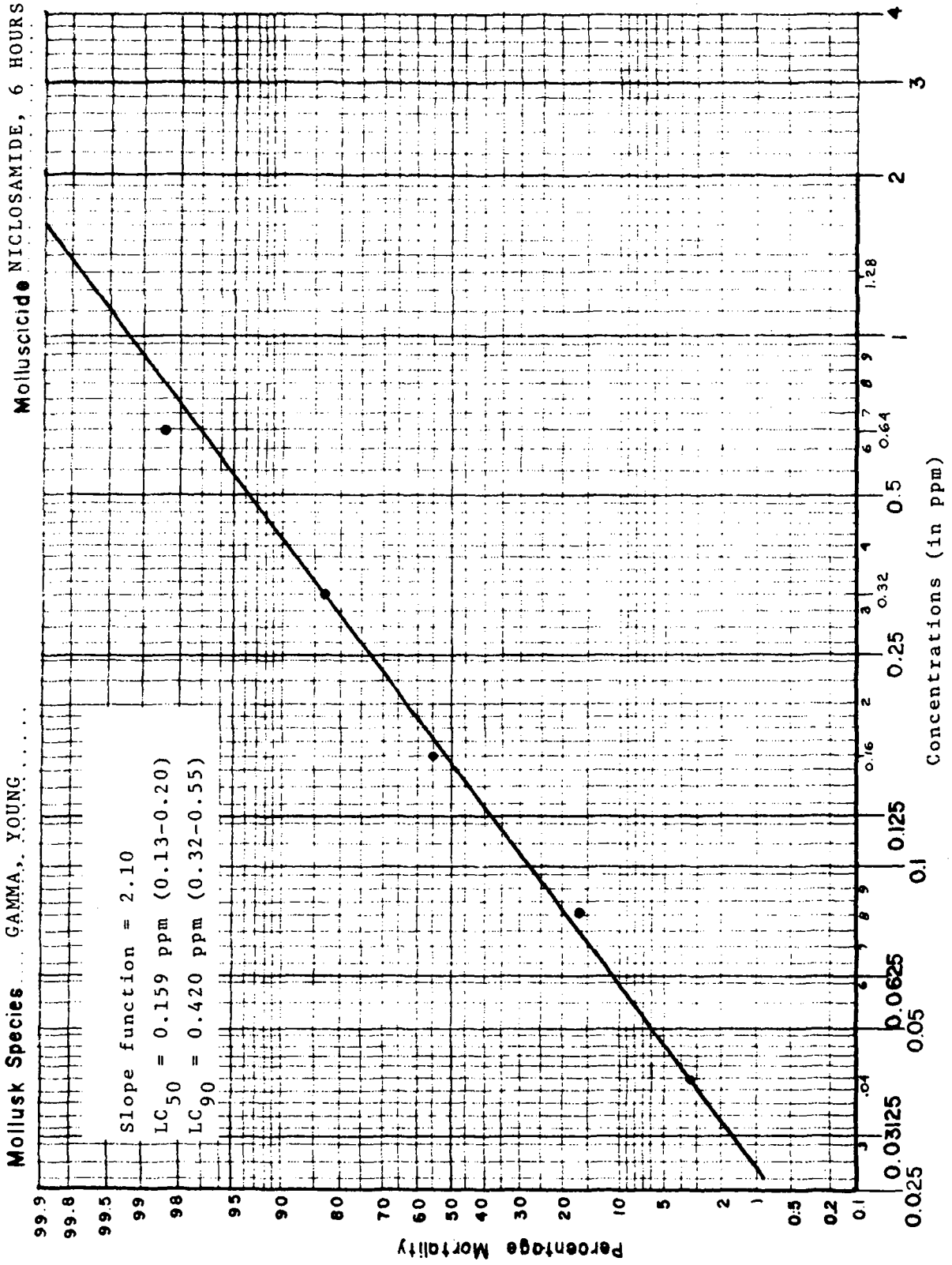


Fig. 47.--Mortality of young gamma L. aperta exposed to Niclosamide for 6 hours.



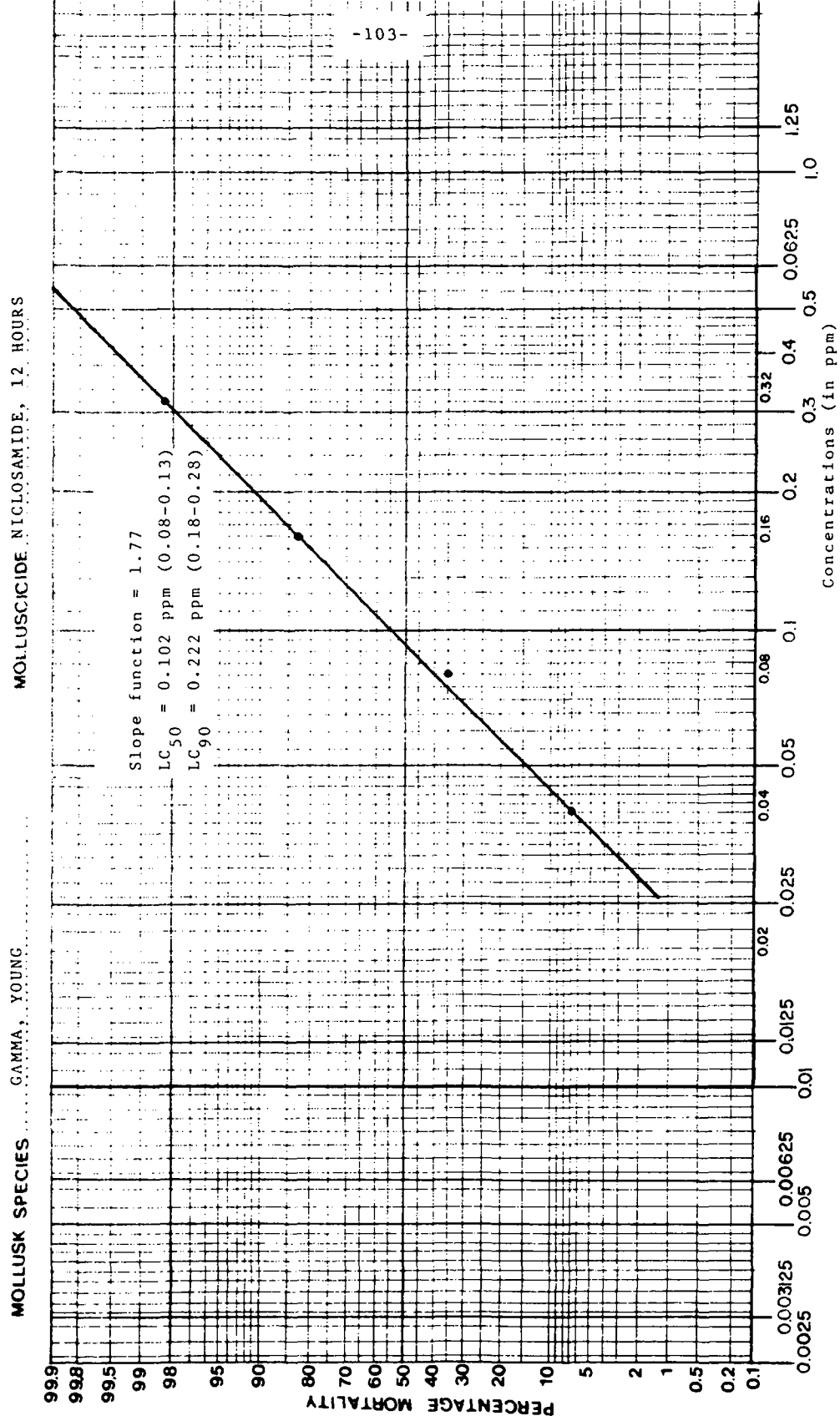


Fig. 48.--Mortality of young gamma L. aperta exposed to Niclosamide for 12 hours.

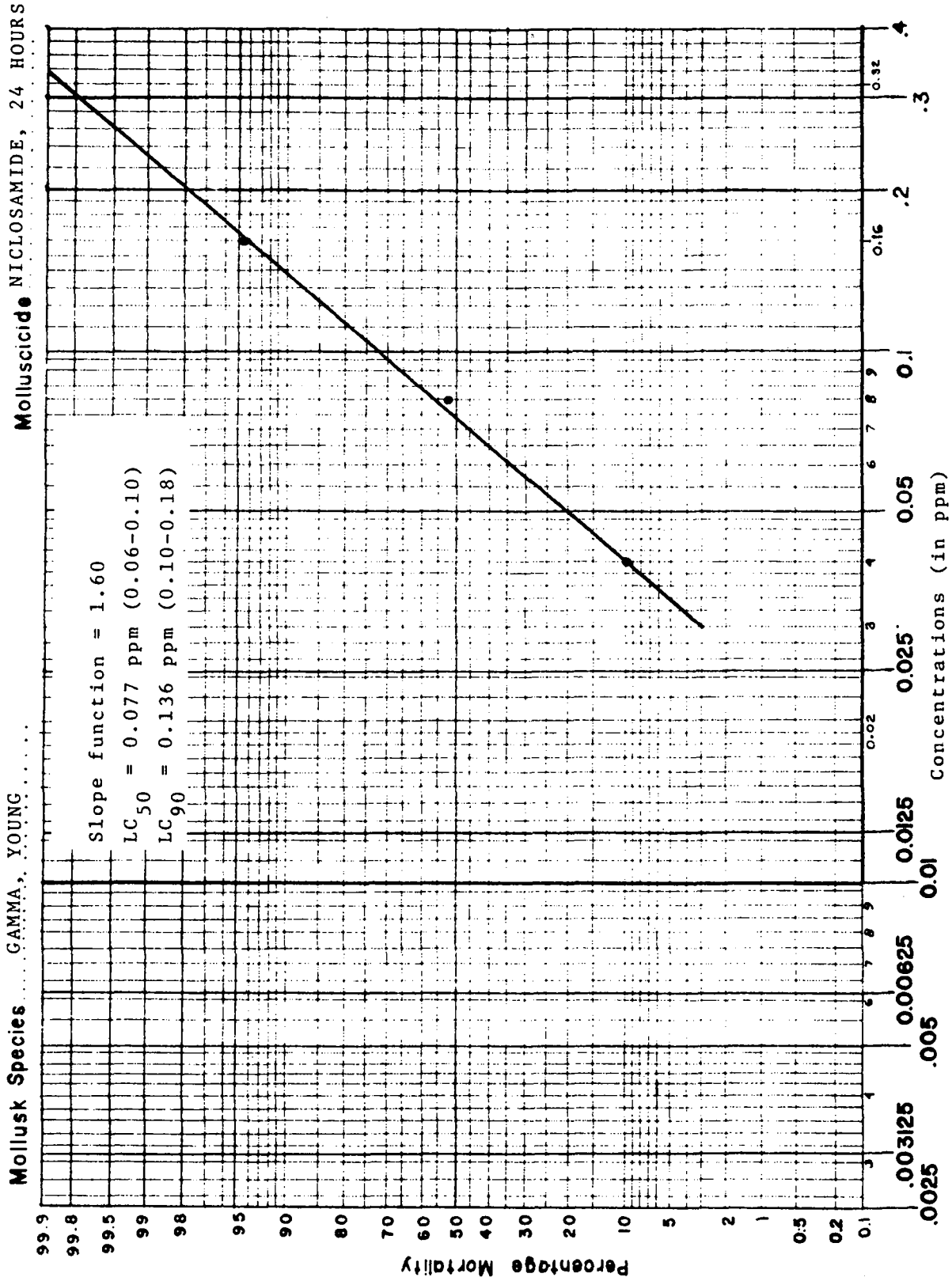


Fig. 49.--Mortality of young gamma L. aperta exposed to Niclosamide for 24 hours.

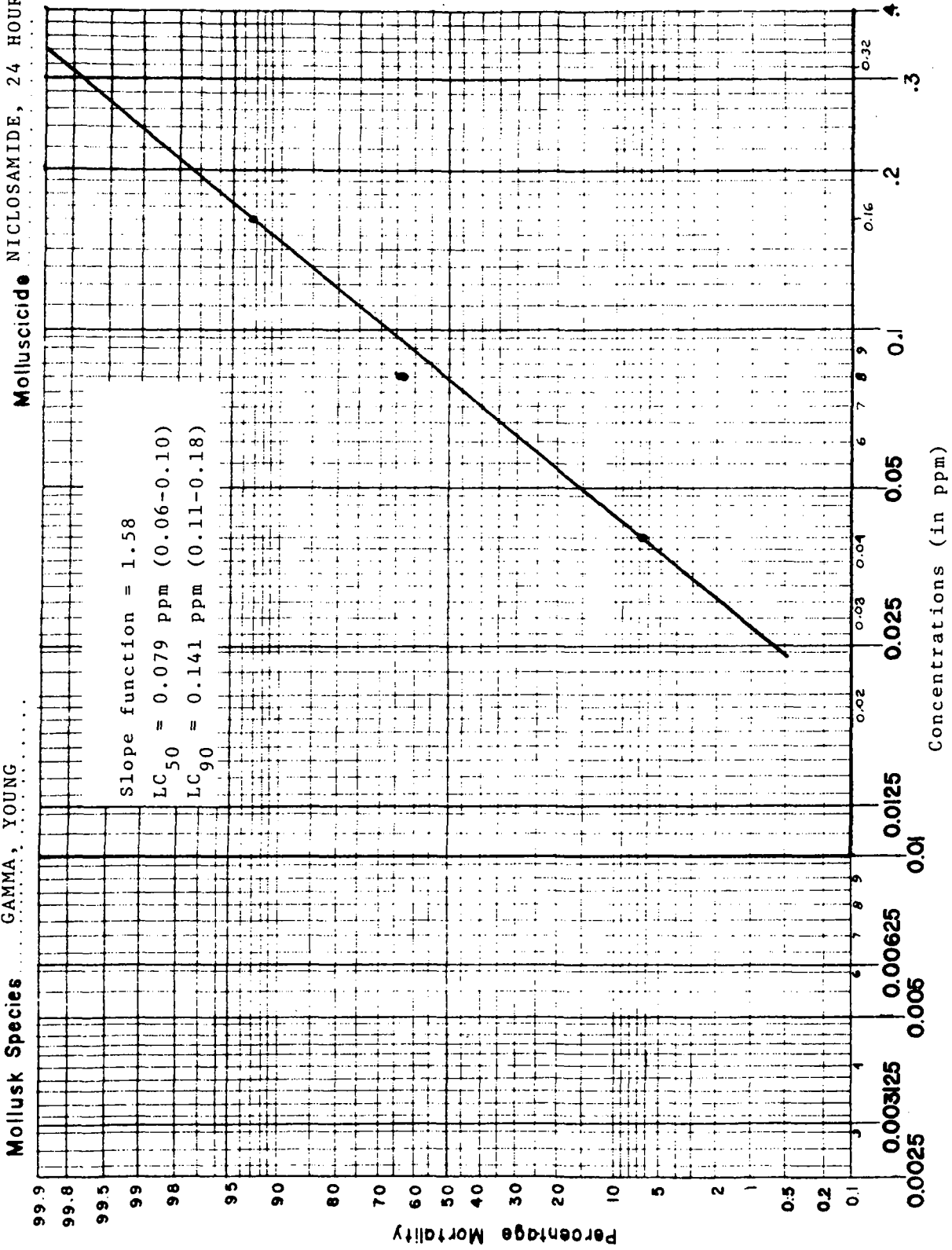


Fig. 50.--Mortality of young gamma *L. aperta* exposed to Niclosamide plus silt for 24 hours.

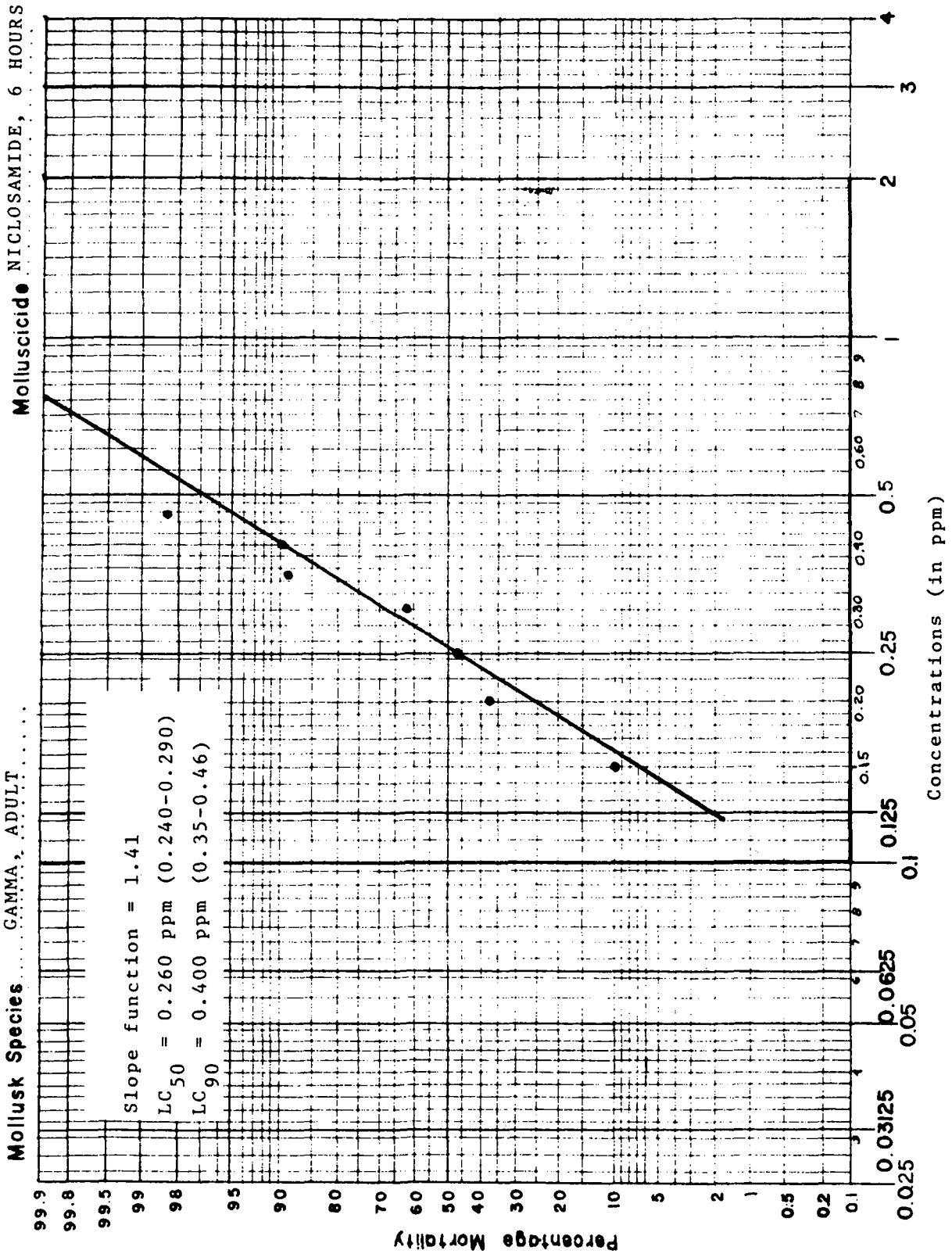


Fig. 51.--Mortality of adult gamma L. aperta exposed to Niclosamide for 6 hours.

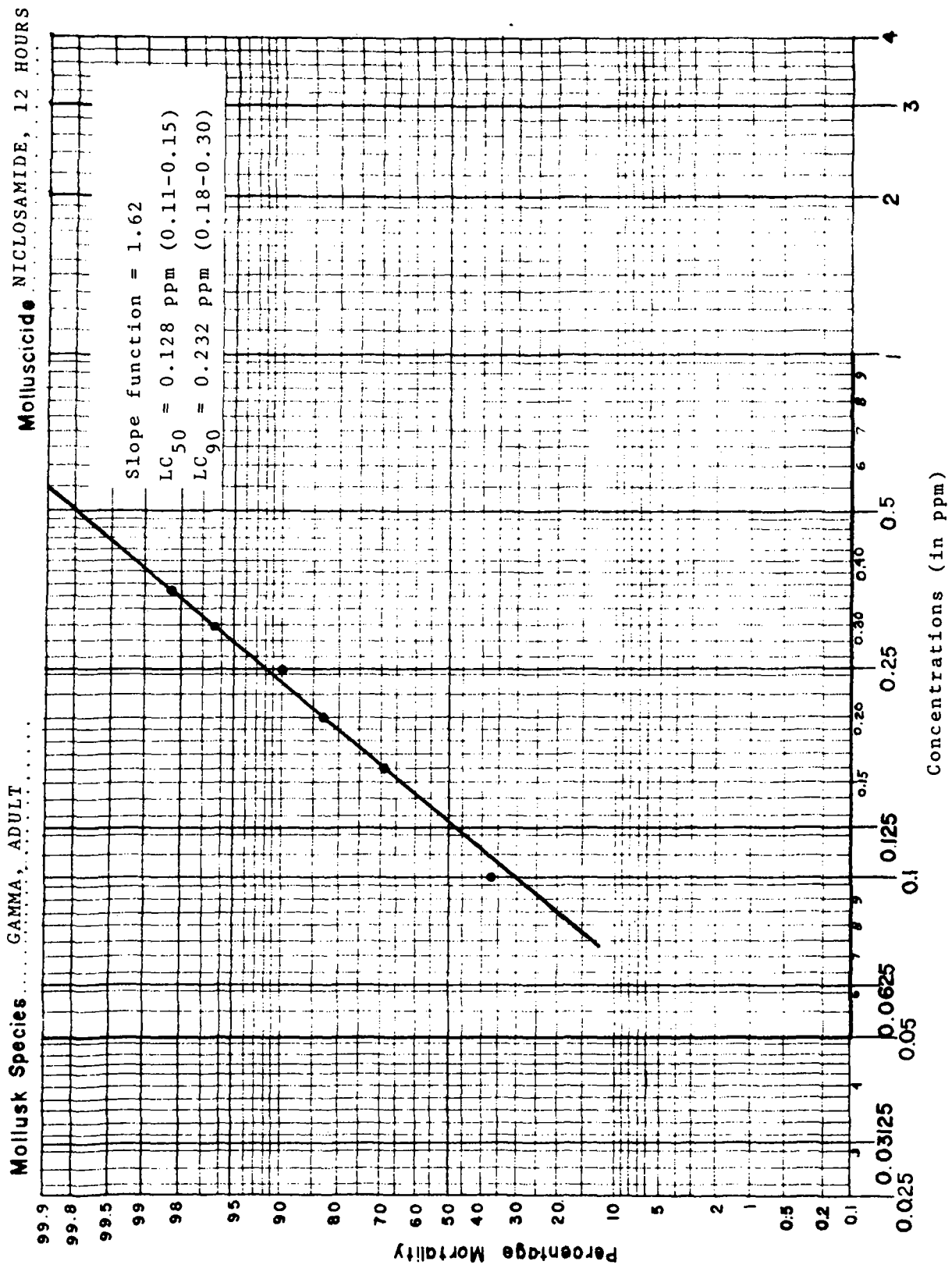


Fig. 52.--Mortality of adult gamma L. aperta exposed to Niclosamide for 12 hours.

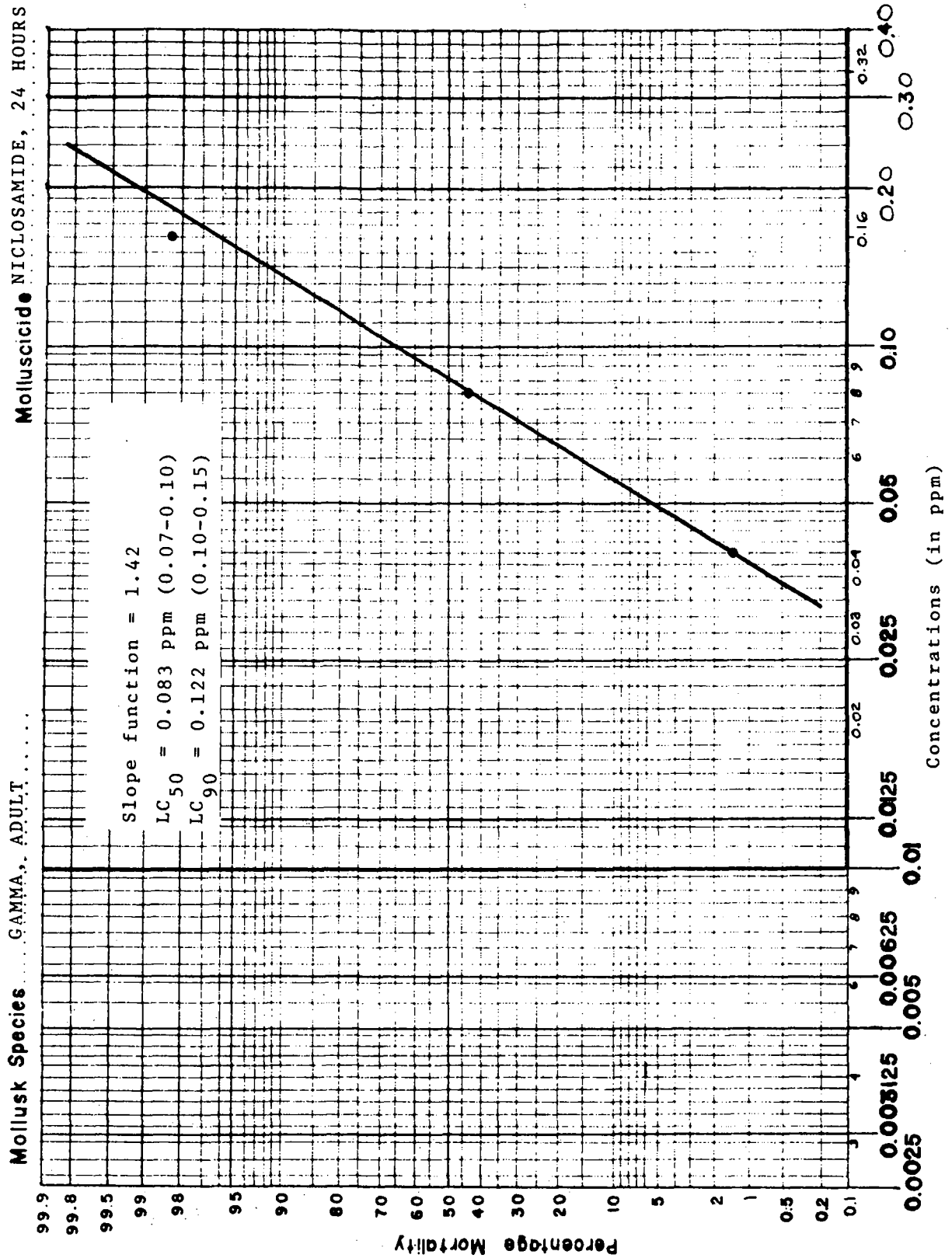


Fig. 53.--Mortality of adult gamma L. aperta exposed to Niclosamide for 24 hours.

The lethal concentrations of snails exposed to molluscicide plus silt did not differ from those of snails exposed to molluscicide alone.

The lethal concentrations for alpha and gamma races did not differ significantly.

b. Copper Sulphate. A summary of LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and slope functions is given in Table 30 and Figures 54, 55, 56, 57, 58, 59 & 60. Raw data are given in Appendix III.

There was a decrease in lethal concentrations as the exposure time increased.

The lethal concentrations for young snails were slightly lower than for adult snails.

The lethal concentrations for snails exposed to molluscicide plus silt were much higher than for snails exposed to the molluscicide alone. This indicated that silt absorbed copper sulphate.

The lethal concentrations for alpha and gamma races did not differ significantly.

c. Frescon (Tritylmorpholine, FX 28, 16.5% Active Ingredient). A summary of LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and slope functions is given in Table 31 and Figures 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73 and 74. Raw data are given in Appendix IV.

There was a decrease in lethal concentrations as the exposure time increased.

The lethal concentrations for young snails were higher than adult snails.

The lethal concentrations for snails exposed in molluscicide plus silt did not differ much from those of snails exposed to the molluscicide alone.

The lethal concentrations for alpha and gamma races did not differ significantly.

d. Sodium Pentachlorophenate (NaPCP, 90% Active Ingredient Granules) A summary of LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and a slope function is given in Table 32 and Figure 75. Raw data are given in Appendix V.

Table 30 Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) in parts per million (ppm) with 95% confidence limits and slope functions of two races of *L. aperta* exposed to copper sulphate at intervals of 6, 12 and 24 hours.

Race of <i>L. aperta</i>	Exposure time (in hours)	LC <sub>50</sub> (95% confidence limit)		Mean mortality of <i>L. aperta</i> (in ppm)		LC <sub>90</sub> (95% confidence limit)		Slope function	
		Young		Adult		Young		Adult	
		Young	Adult	Young	Adult	Young	Adult	Young	Adult
Alpha	6	ND	4.680(3.490-6.270)	ND	13.200(9.360-18.610)	ND	2.26	ND	2.26
	12	ND	3.400(2.520-4.590)	ND	7.300(5.250-10.150)	ND	1.79	ND	1.79
	24	0.630(0.500-0.790)	0.730(0.570-0.940)	1.420(1.060-1.900)	2.830(2.020-3.960)	1.92	2.42	1.92	2.42
	24S	ND	4.100(3.110-5.410)	ND	10.900(7.900-15.040)	ND	2.15	ND	2.15
Gamma	6	ND	ND	ND	ND	ND	ND	ND	ND
	12	ND	ND	ND	ND	ND	ND	ND	ND
	24	0.800(0.620-1.040)	ND	1.850(1.390-2.460)	ND	1.67	ND	1.67	ND
	24S	3.190(2.280-4.470)	ND	10.500(6.560-16.800)	ND	2.56	ND	2.56	ND

24S = silt was added in experimental solutions and exposure time was 24 hours.

ND = not done.



Mollusc Species... ALPHA, YOUNG

Molluscicide COPPER SULPHATE, 24 HOURS

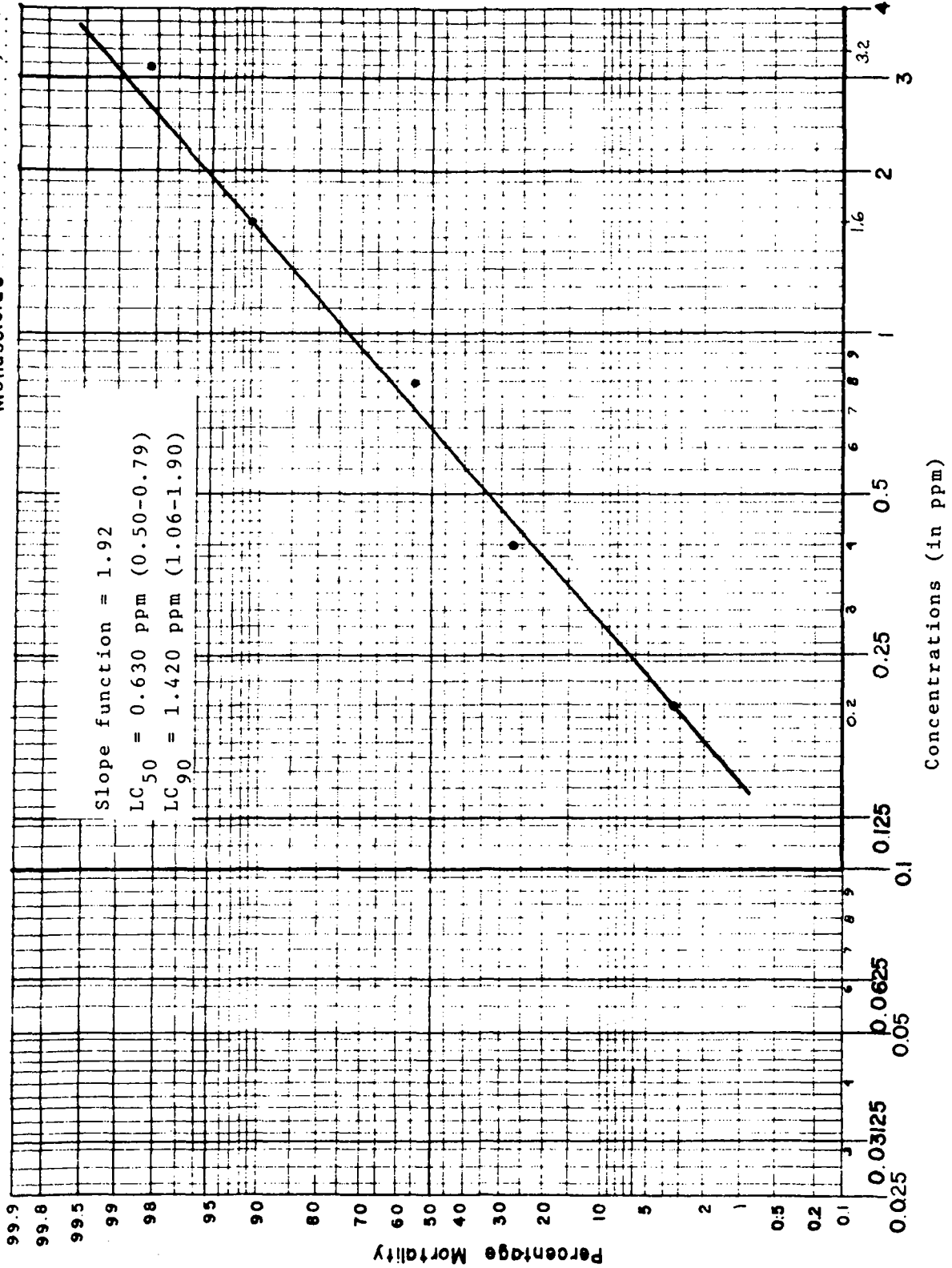


Fig. 54.--Mortality of young alpha L. aperta exposed to copper sulphate for 24 hours.

MOLLUSK SPECIES ..... ALPHA, ADULT ..... MOLLUSCICIDE COPPER SULPHATE, 6 HOURS

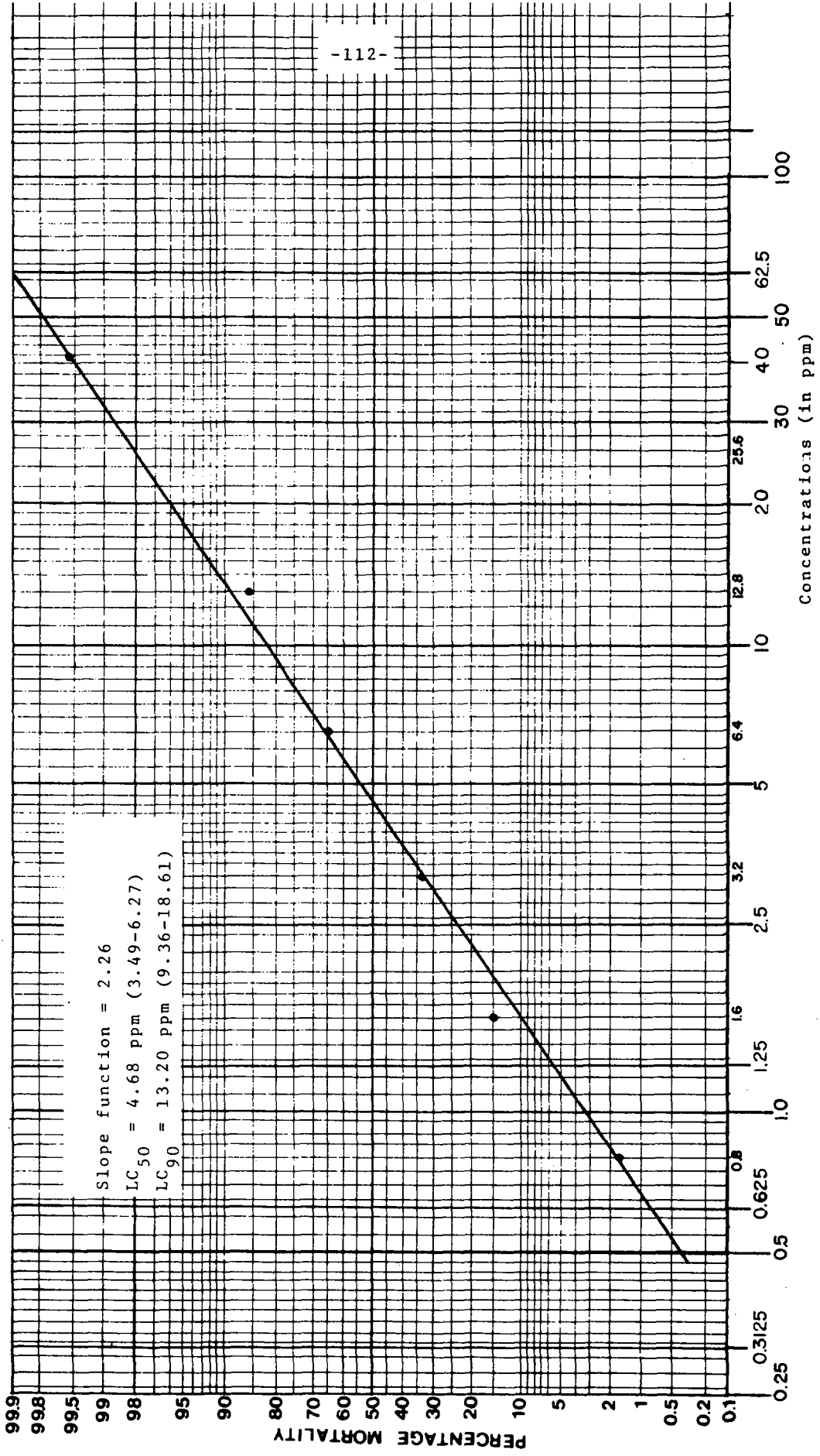


Fig. 55.--Mortality of adult alpha L. aperta exposed to copper sulphate for 6 hours.

Molluscicide COPPER SULPHATE, 12 HOURS

Mollusk Species ALPHA, ADULT

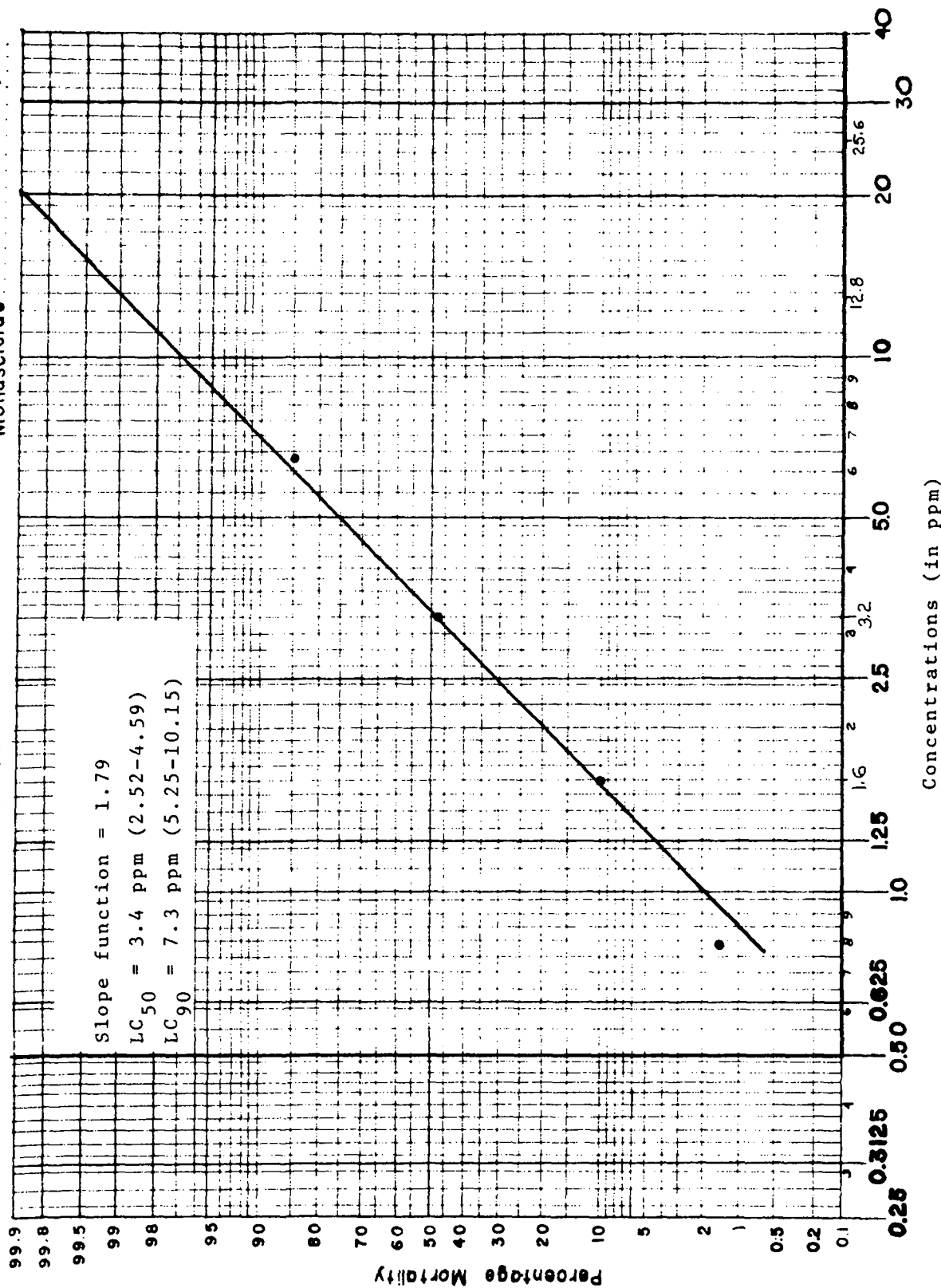


Fig. 56.--Mortality of adult alpha L. aperta exposed to copper sulphate for 12 hours.

MOLLUSK SPECIES ALPHA, ADULT ..... MOLLUSCICIDE COPPER SULPHATE, 24 HOURS

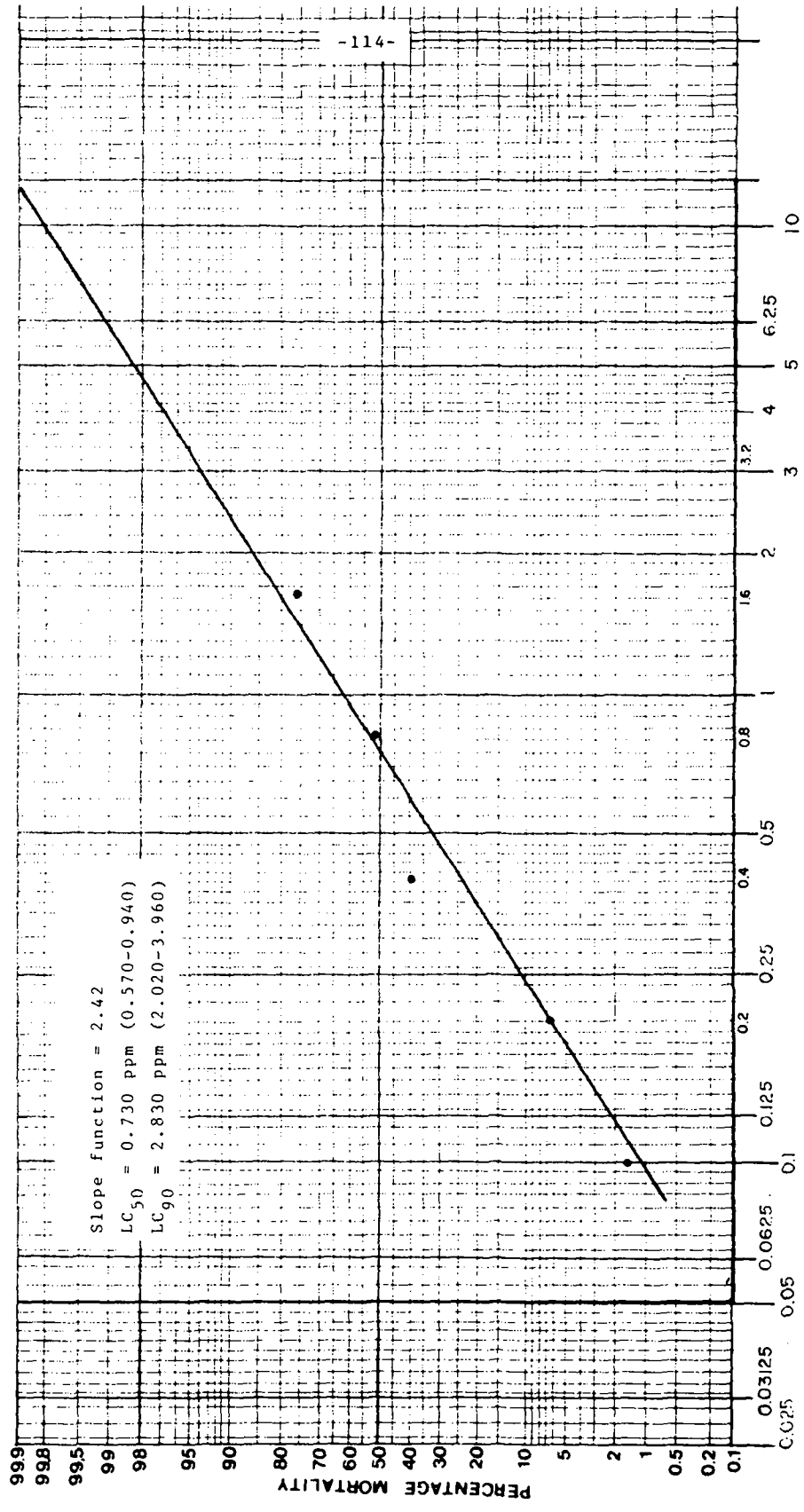


Fig. 57.--Mortality of adult alpha L. aperta exposed to copper sulphate for 24 hours.

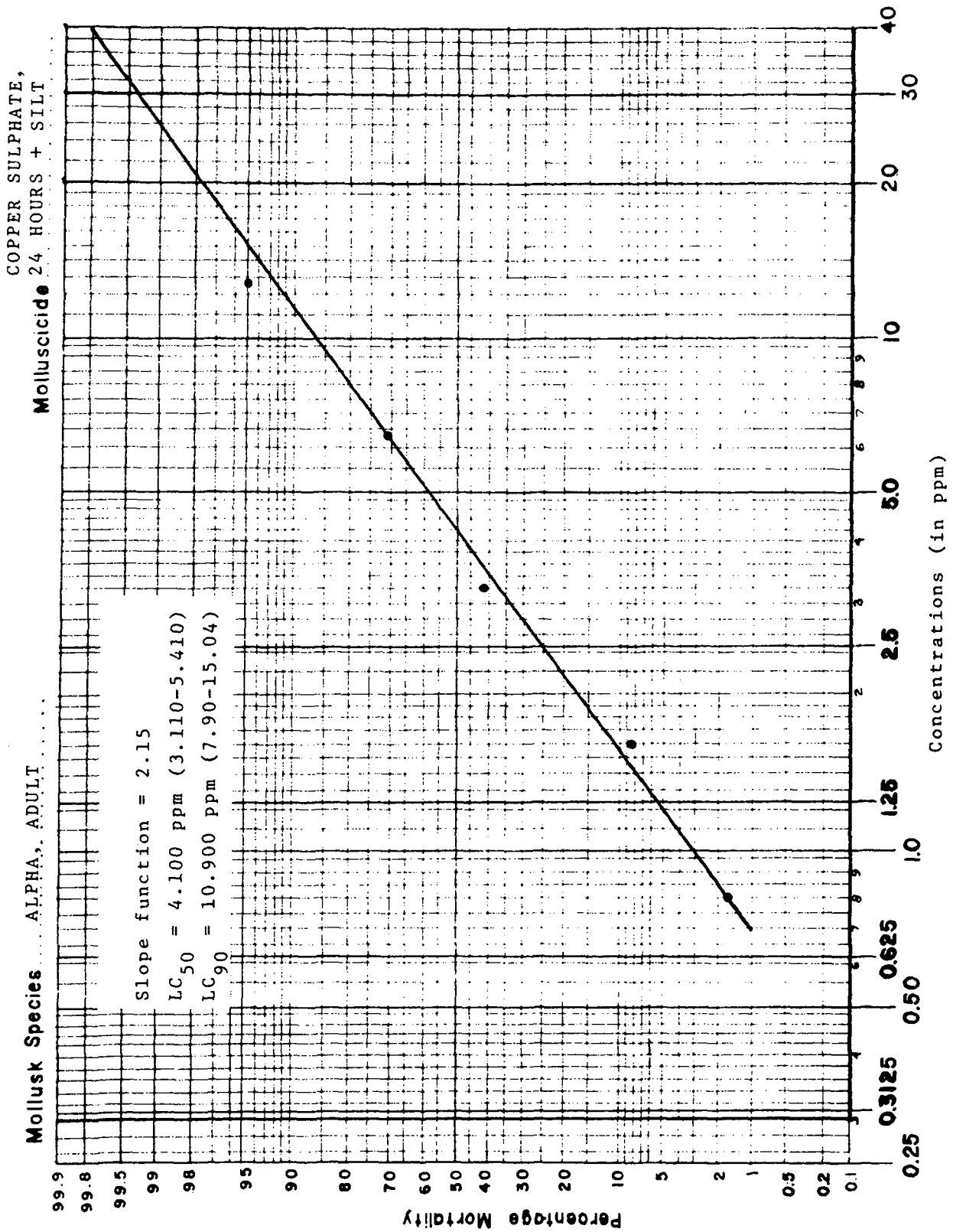


Fig. 58.--Mortality of adult alpha *L. aperta* exposed to copper sulphate plus silt for 24 hours.

Mollusc Species GAMMA, YOUNG Molluscicide COPPER SULPHATE, 24 HOURS

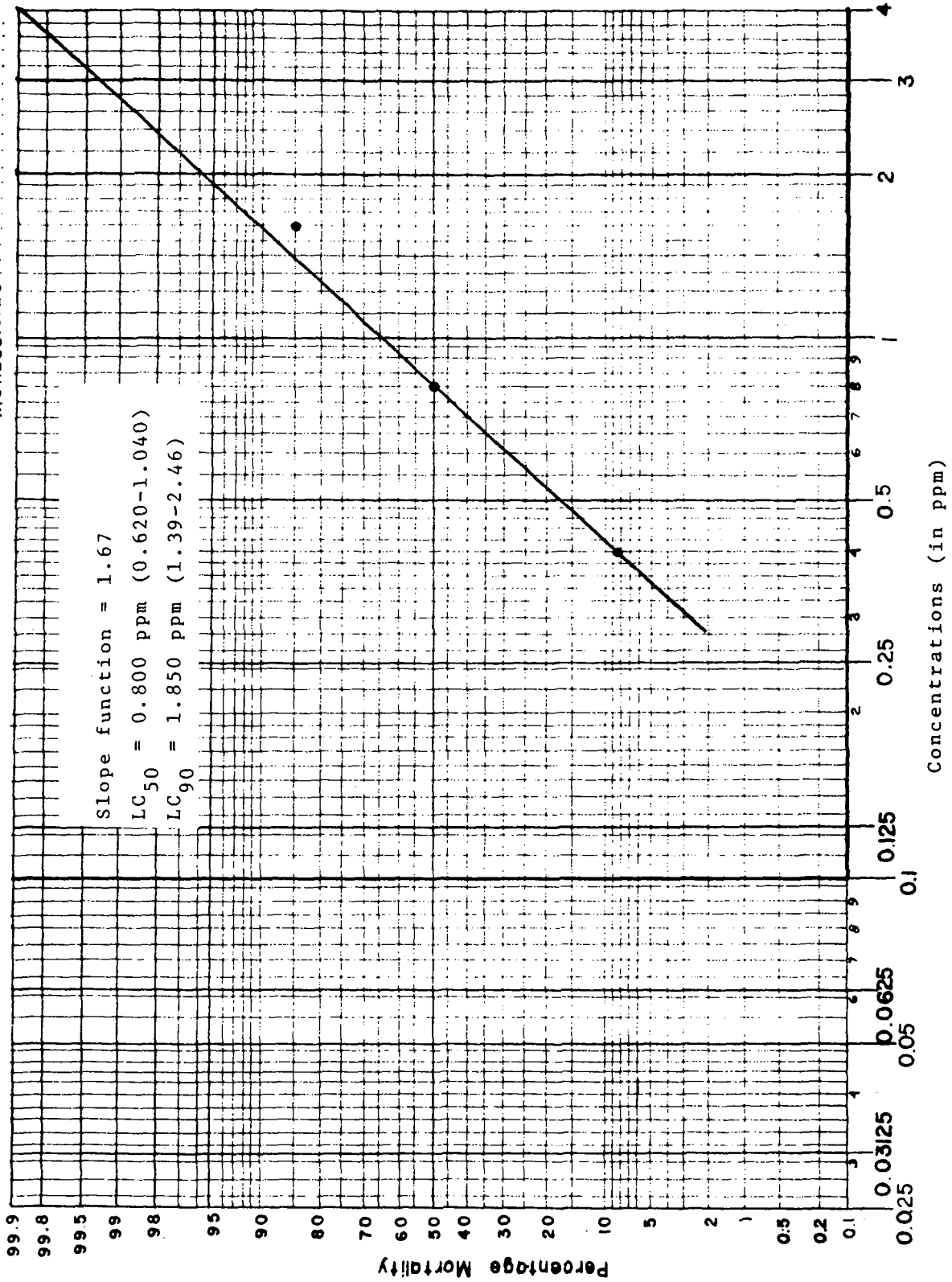


Fig. 59.--Mortality of young gamma L. aperta exposed to copper sulphate for 24 hours.

MOLLUSK SPECIES ..... GAMMA, YOUNG ..... MOLLUSCIDICIDE COPPER SULPHATE, 24 HOURS + SILT

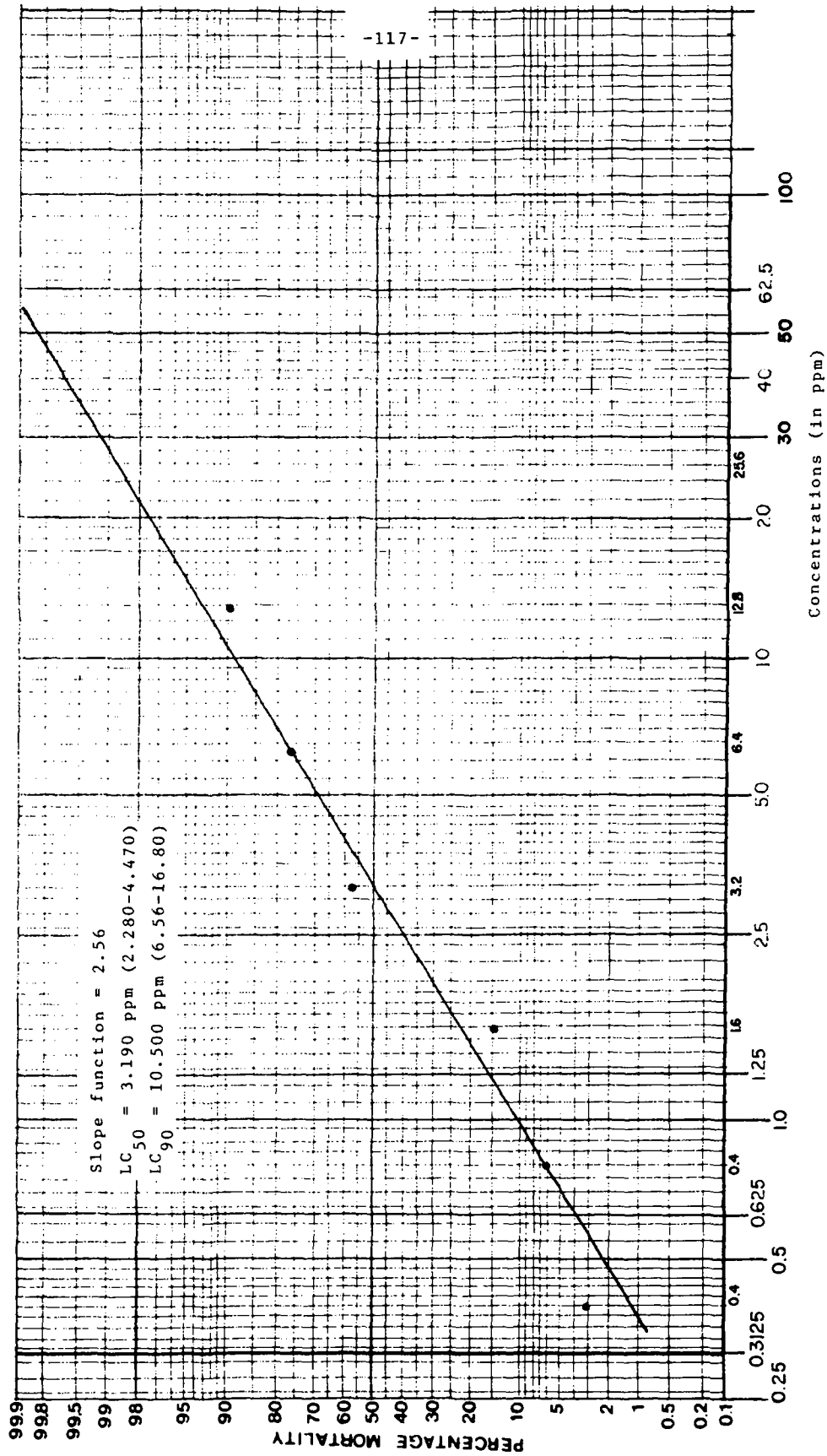


Fig. 60.--Mortality of young Gamma L. aperta exposed to copper sulphate plus silt for 24 hours.

Table 31 Lethal concentrations (LC50 and LC90) in parts per million (ppm) with 95% confidence limits and slope functions of two races of *L. aperta* exposed to Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) intervals of 1, 6, 12 and 24 hours.

Race of <i>L. aperta</i>	Exposure time (in hours)	Mean mortality of <i>L. aperta</i> (in ppm)				LC50(95% confidence limit)		LC90(95% confidence limit)		Slope function	
		LC50(95% confidence limit)		Mean mortality of <i>L. aperta</i> (in ppm)		LC50(95% confidence limit)		LC90(95% confidence limit)		Slope function	
		Young	Adult	Young	Adult	Young	Adult	Young	Adult	Young	Adult
Alpha	1	ND	0.076(0.070-0.080)	ND	0.120(0.110-0.130)	ND	0.120(0.110-0.130)	ND	1.42	ND	1.42
	6	ND	0.068(0.050-0.090)	ND	0.195(0.130-0.280)	ND	0.195(0.130-0.280)	ND	2.24	ND	2.24
	12	ND	0.062(0.050-0.080)	ND	0.139(0.110-0.180)	ND	0.139(0.110-0.180)	ND	1.84	ND	1.84
	24	0.077(0.060-0.110)	0.057(0.050-0.070)	0.380(0.240-0.600)	0.085(0.070-0.100)	0.380(0.240-0.600)	0.085(0.070-0.100)	3.50	1.35	3.50	1.35
	24S	0.085(0.060-0.120)	0.043(0.040-0.050)	0.415(0.260-0.660)	0.066(0.060-0.080)	0.415(0.260-0.660)	0.066(0.060-0.080)	3.39	1.37	3.39	1.37
Gamma	1	0.122(0.110-0.140)	ND	0.218(0.180-0.260)	ND	0.218(0.180-0.260)	ND	1.57	ND	1.57	ND
	6	0.034(0.030-0.040)	ND	0.056(0.050-0.070)	ND	0.056(0.050-0.070)	ND	1.48	ND	1.48	ND
	12	0.031(0.030-0.040)	ND	0.055(0.050-0.060)	ND	0.055(0.050-0.060)	ND	1.55	ND	1.55	ND
	24	0.057(0.040-0.080)	0.039(0.030-0.050)	0.151(0.100-0.230)	0.068(0.050-0.090)	0.151(0.100-0.230)	0.068(0.050-0.090)	2.46	1.55	2.46	1.55
	24S	0.053(0.040-0.070)	0.052(0.040-0.070)	0.113(0.080-0.160)	0.115(0.090-0.150)	0.113(0.080-0.160)	0.115(0.090-0.150)	1.82	1.90	1.82	1.90

24S = silt was added in experimental solutions and exposure time was 24 hours.

ND = not done.



MOLLUSK SPECIES ALPHA, YOUNG MOLLUSCIDICIDE FRESCON, 24 HOURS

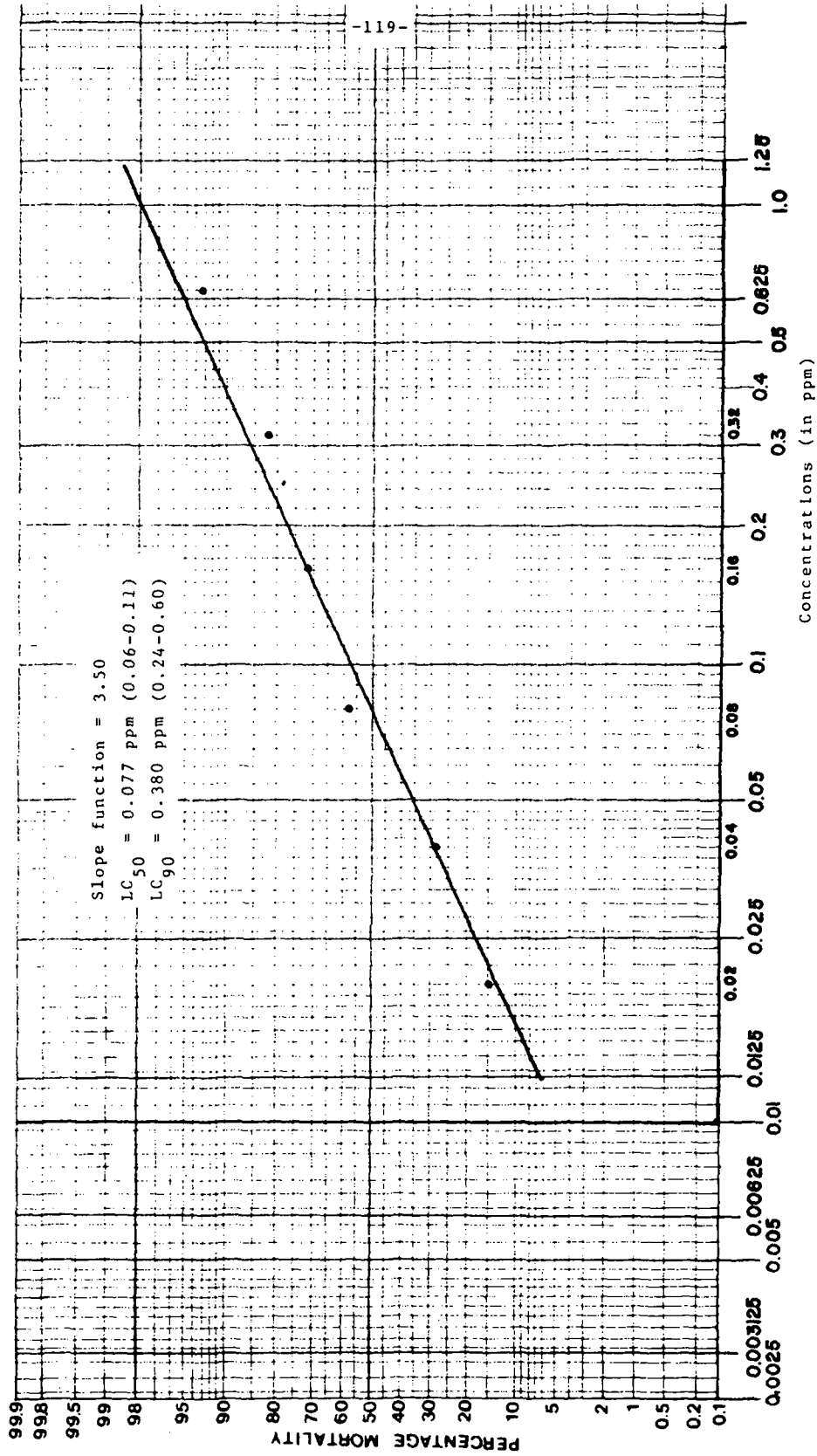


Fig. 61.--Mortality of young alpha *L. sperta* exposed to Frescon for 24 hours.

MOLLUSK SPECIES ..... ALPHA, YOUNG ..... MOLLUSCICIDE FRESCON, 24 HOURS, + SILT

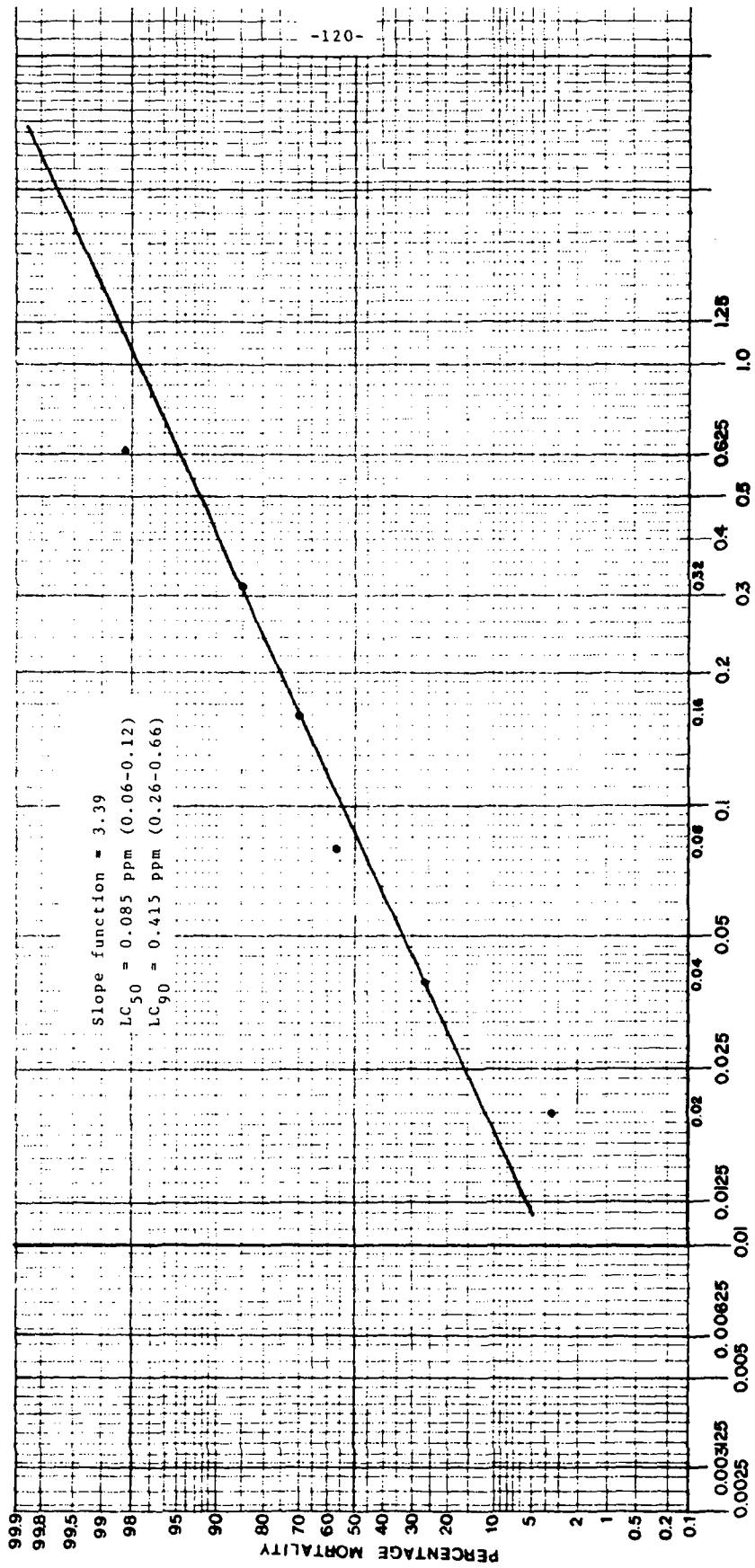


Fig. 62.--Mortality of young alpha L. aperta exposed to Frescon plus silt for 24 hours.

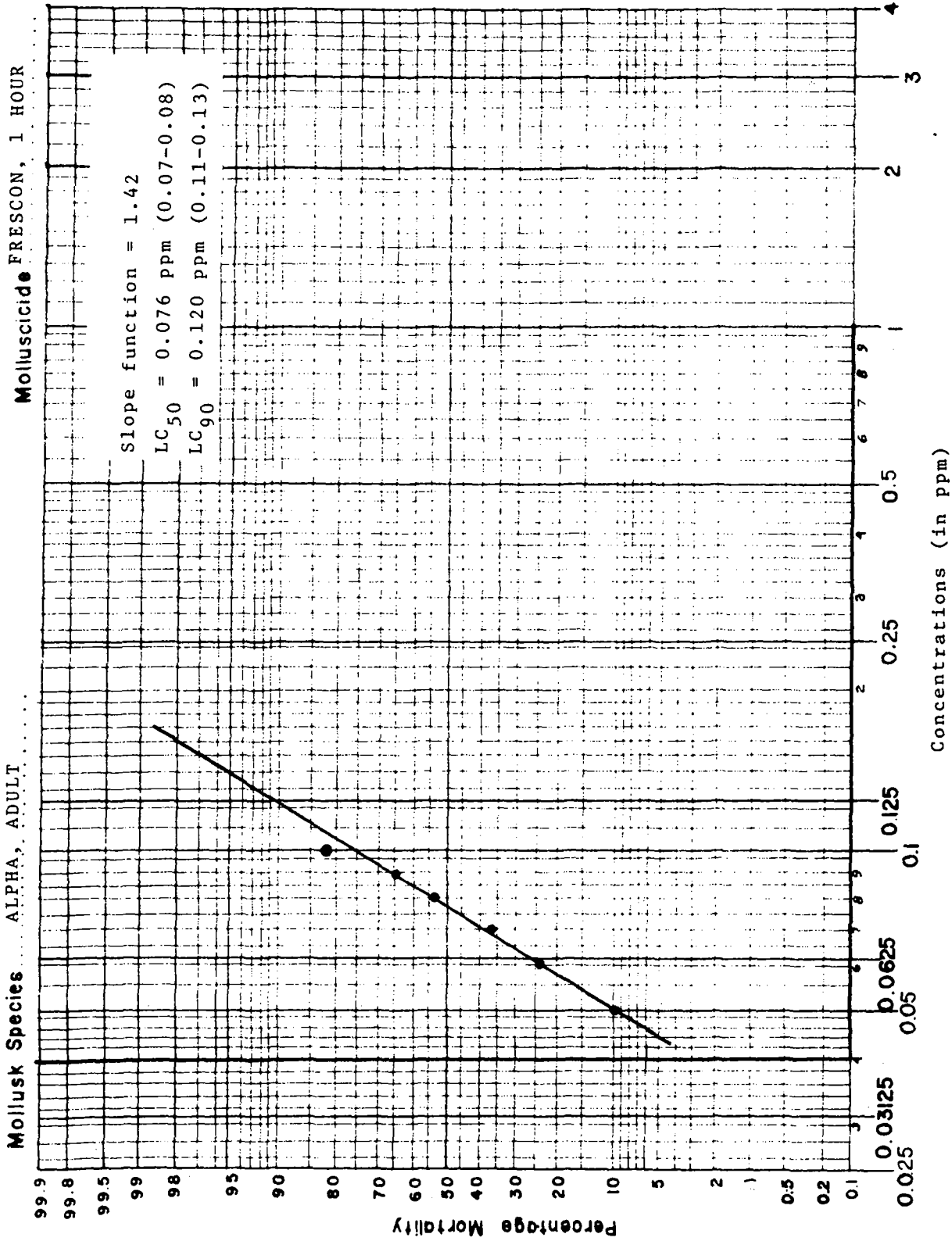


Fig. 63.--Mortality of adult alpha L. aperta exposed to Frescon for 1 hour.

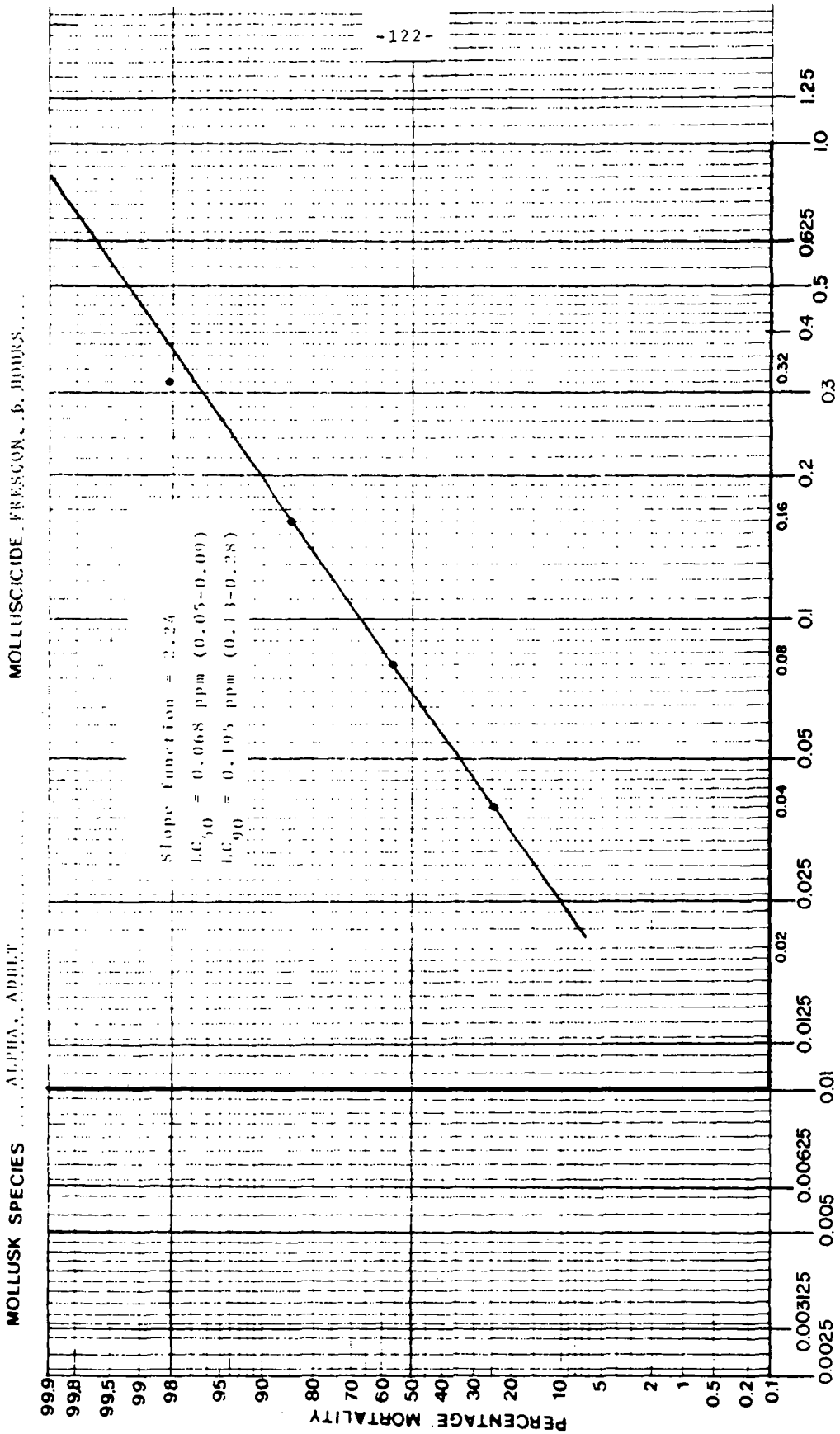


FIG. 64.--Mortality of adult alpha L. aperla exposed to Frescon for 6 hours.

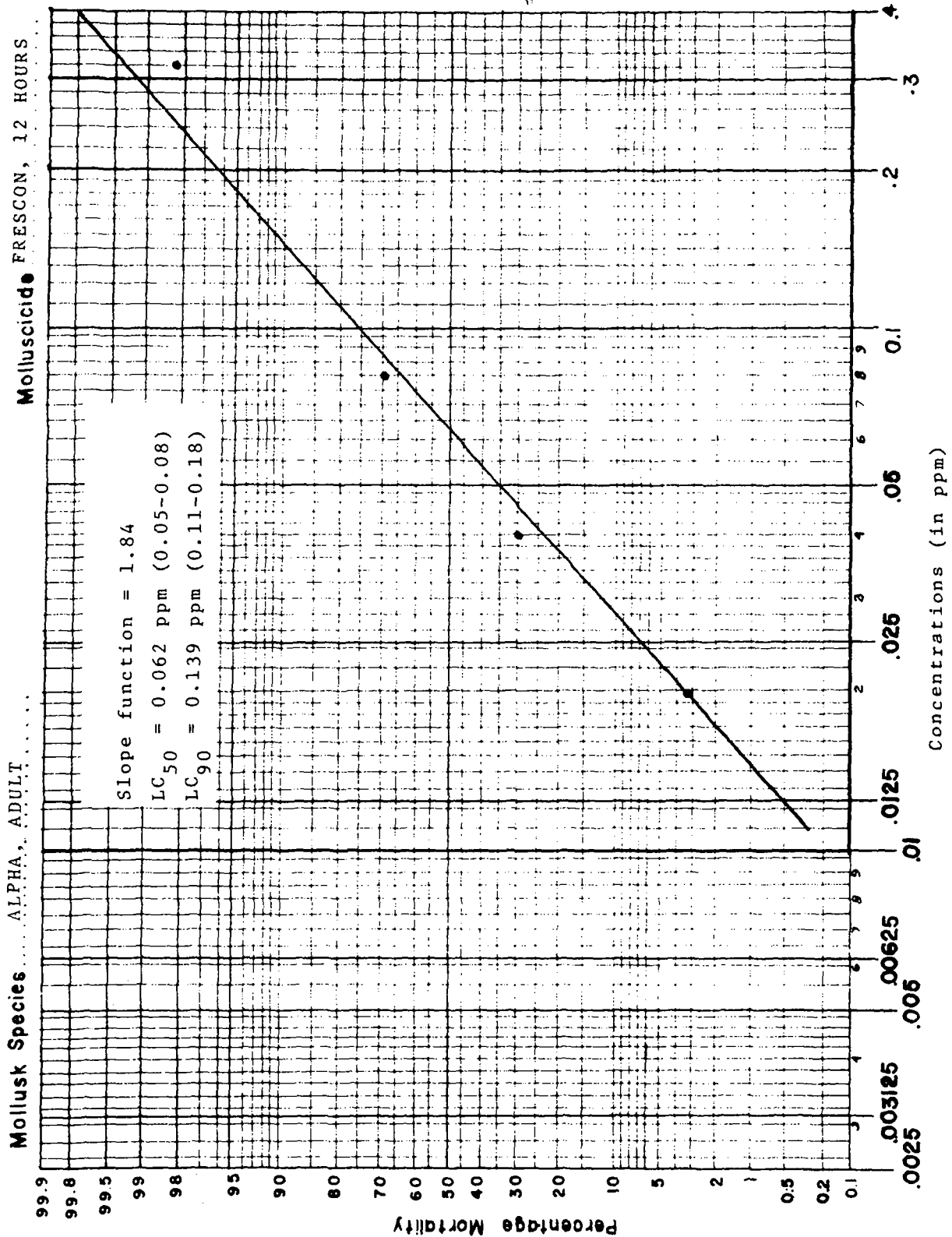


Fig. 65.-- Mortality of adult alpha L. aperta exposed to Frescon for 12 hours.

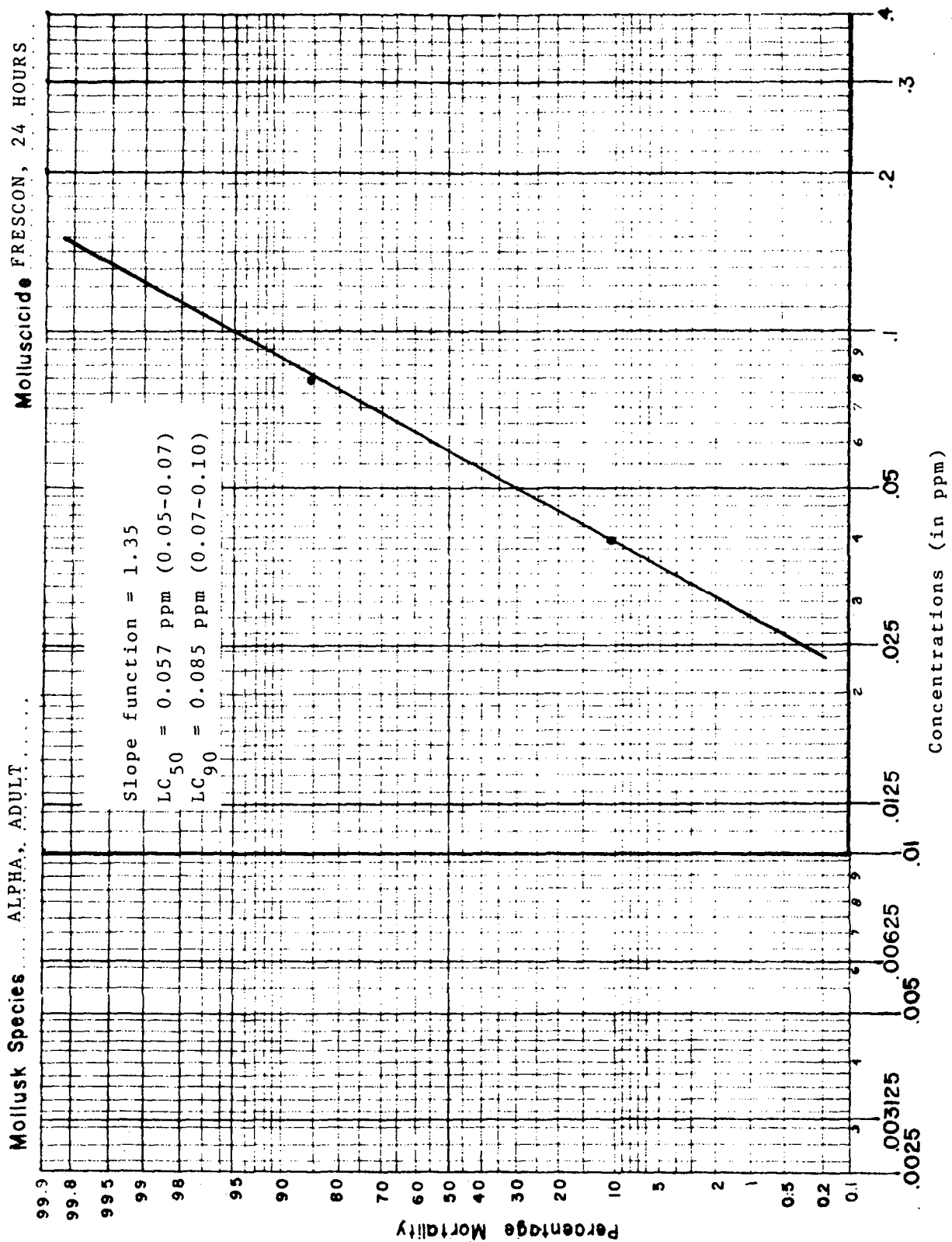


Fig. 66.--Mortality of adult alpha L. aperta exposed to Frescon for 24 hours.

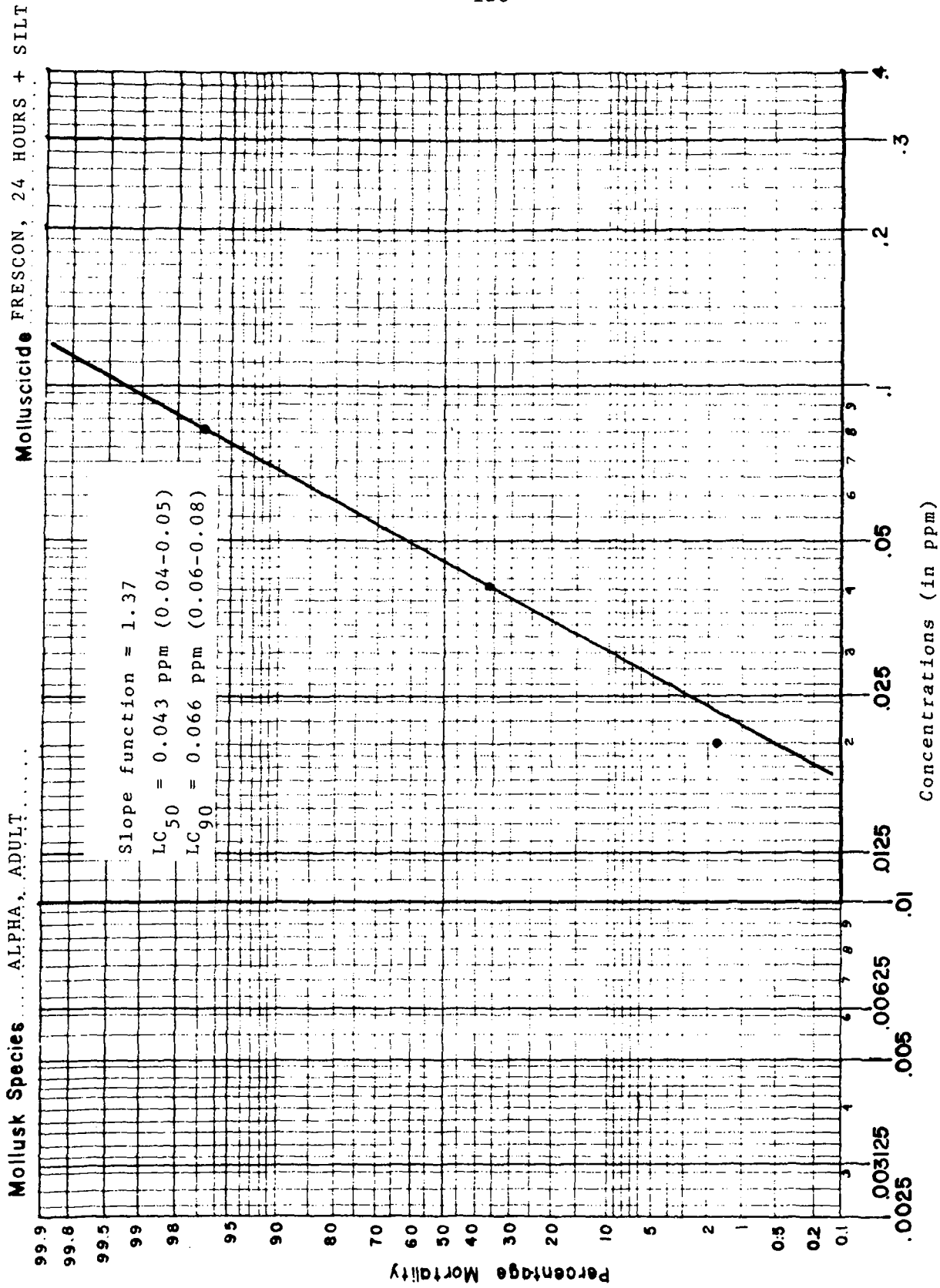


Fig. 67.--Mortality of adult alpha L. aperta exposed to Frescon plus silt for 24 hours.

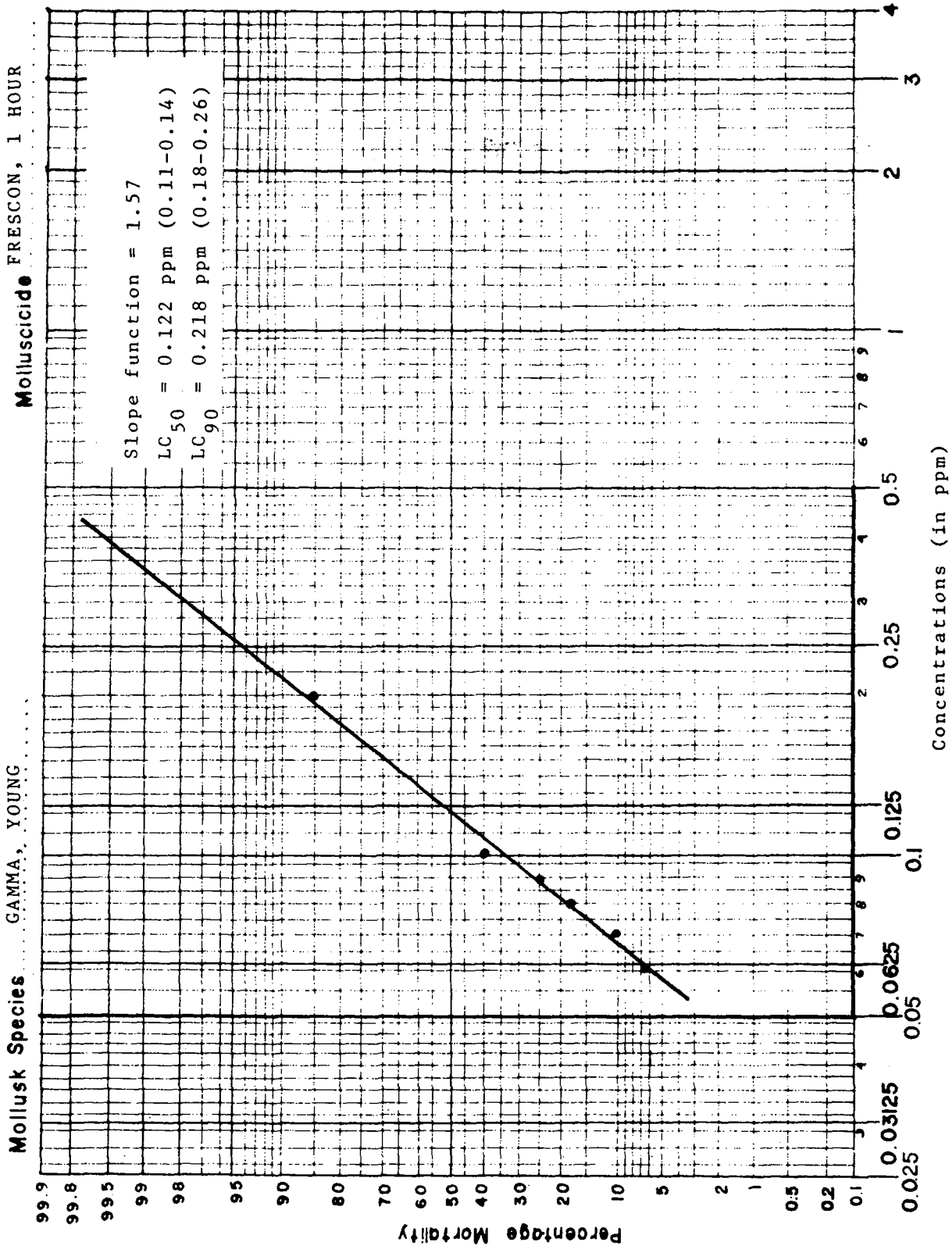


Fig. 68.--Mortality of young gamma L. aperta exposed to Frescon for 1 hour.



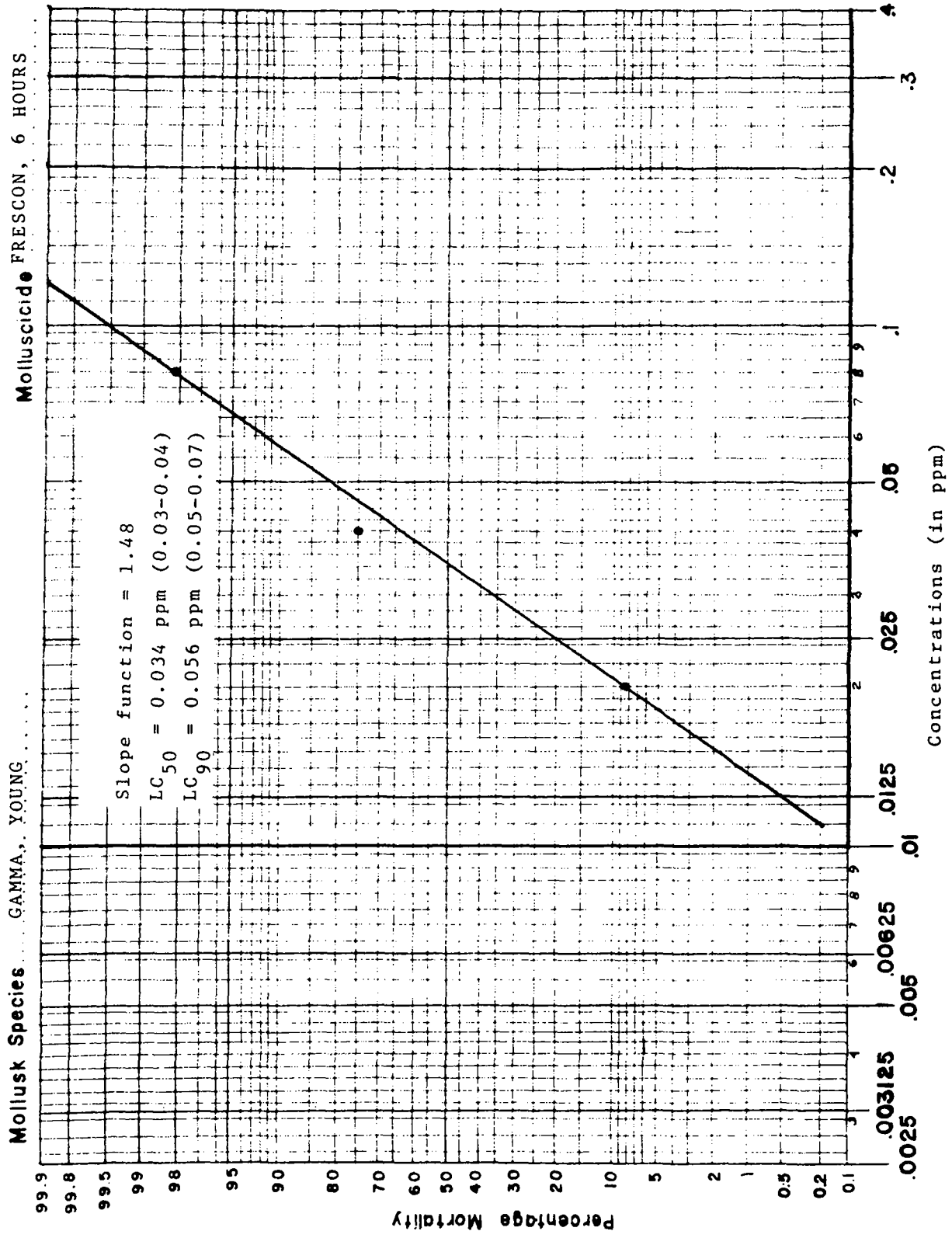


Fig. 69.--Mortality of young gamma L. aperta exposed to Frescon for 6 hours.

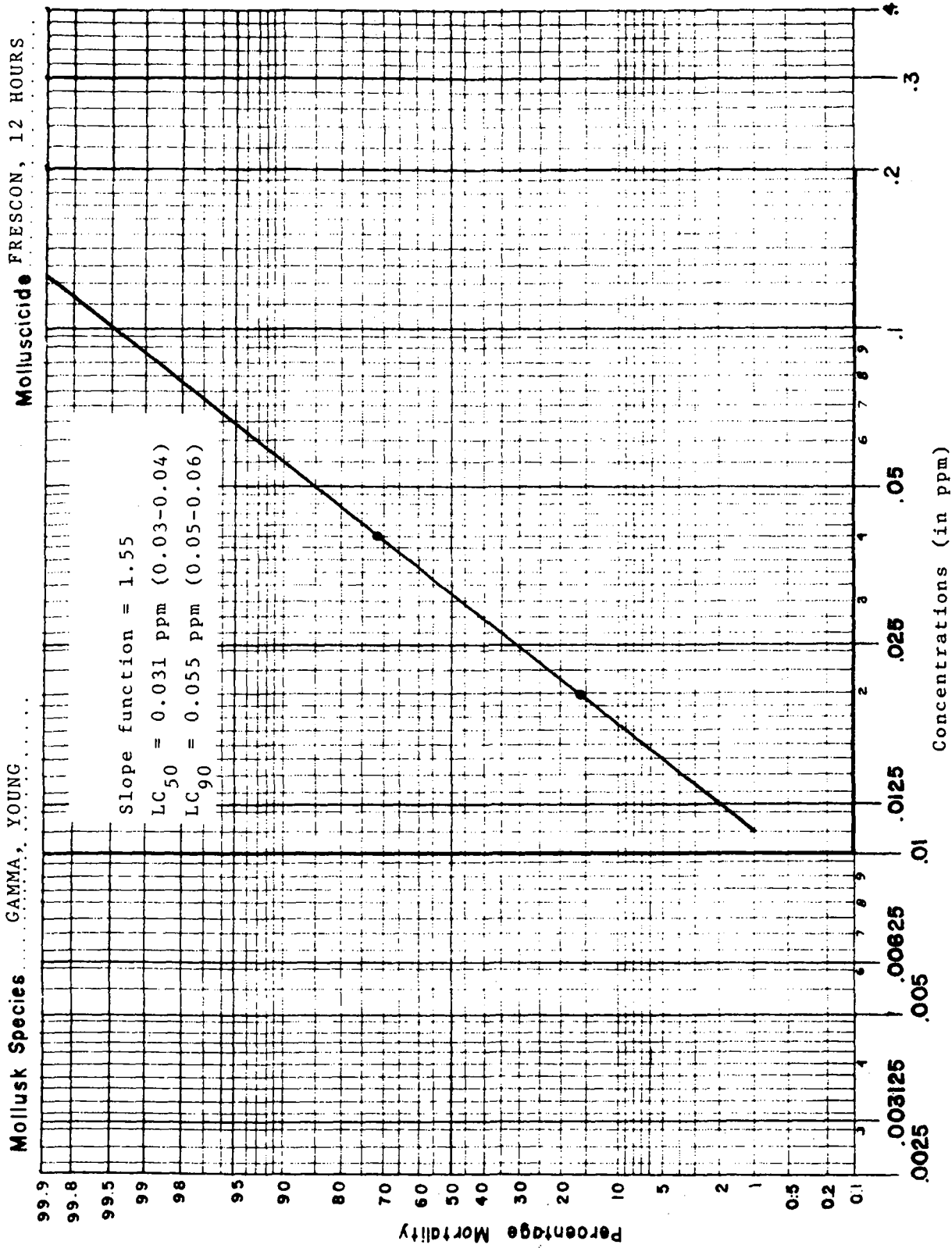


Fig. 70.--Mortality of young gamma L. aperta exposed to Frescon for 12 hours.

MOLLUSK SPECIES GAMMA, YOUNG MOLLUSCIDIDE FRESCON, 24 HOURS

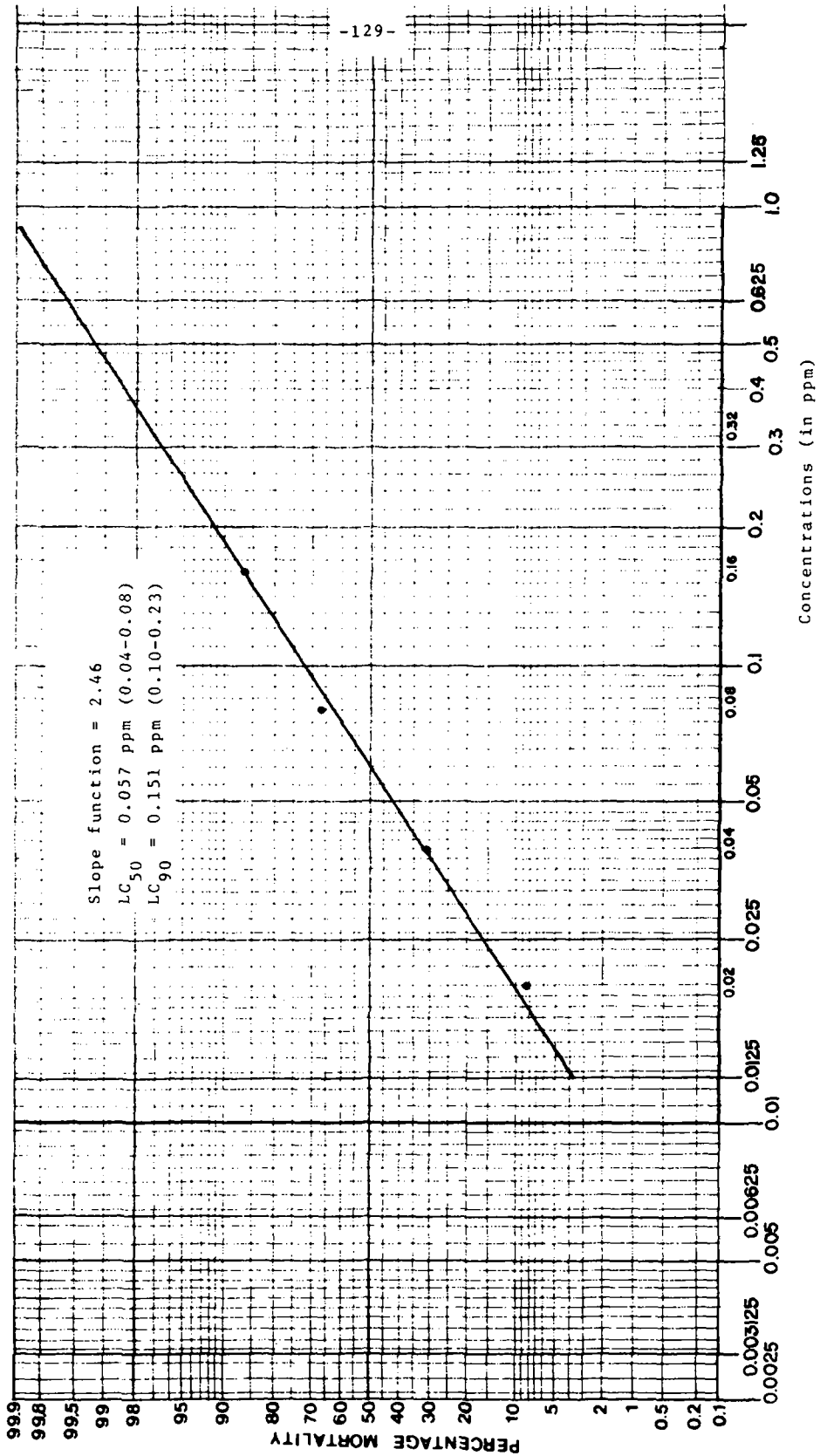


Fig. 71.--Mortality of young gamma *L. aperta* exposed to Frescon for 24 hours.

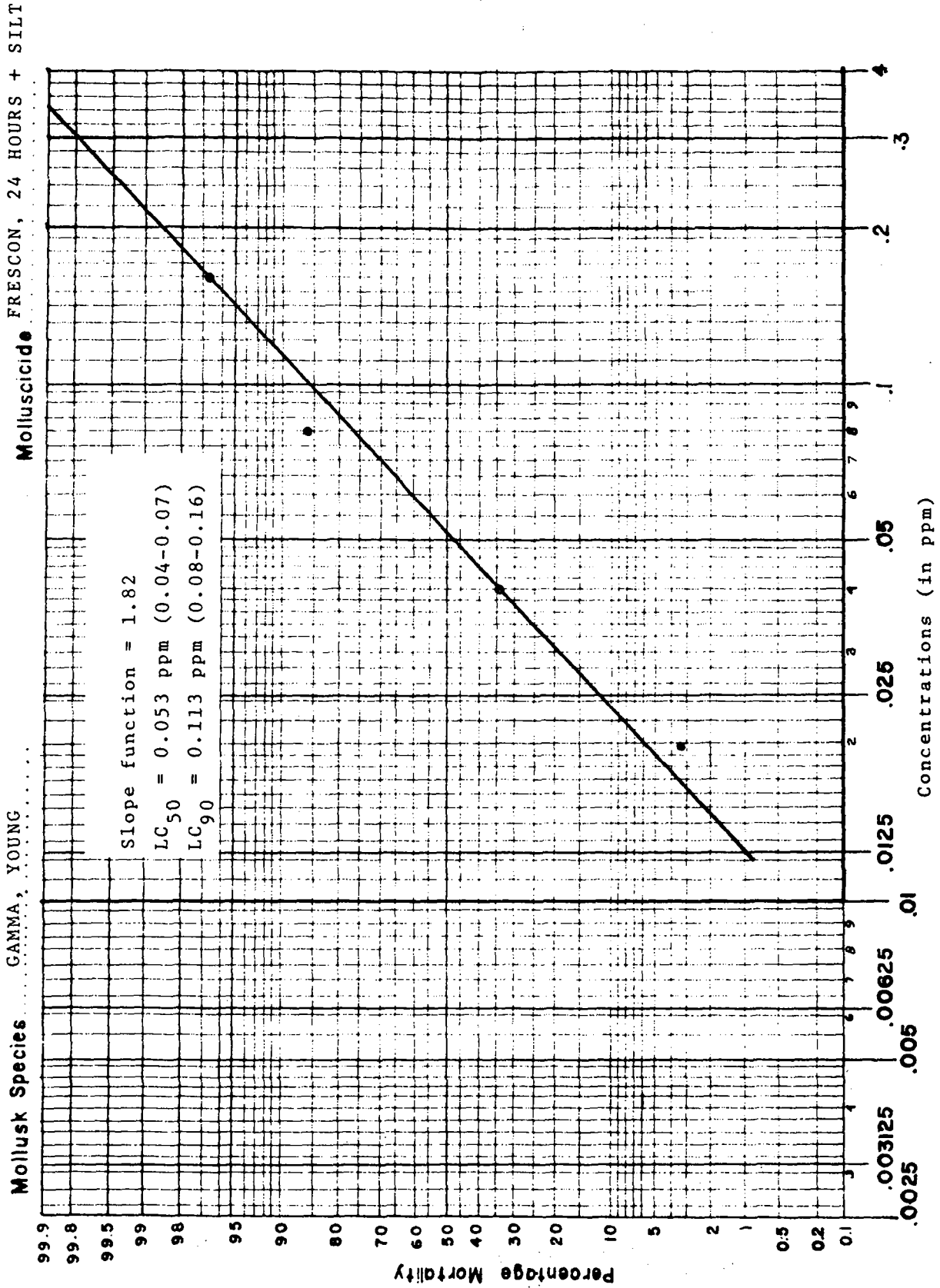


Fig. 72.--Mortality of young gamma L. aperta exposed to Frescon plus silt for 24 hours.

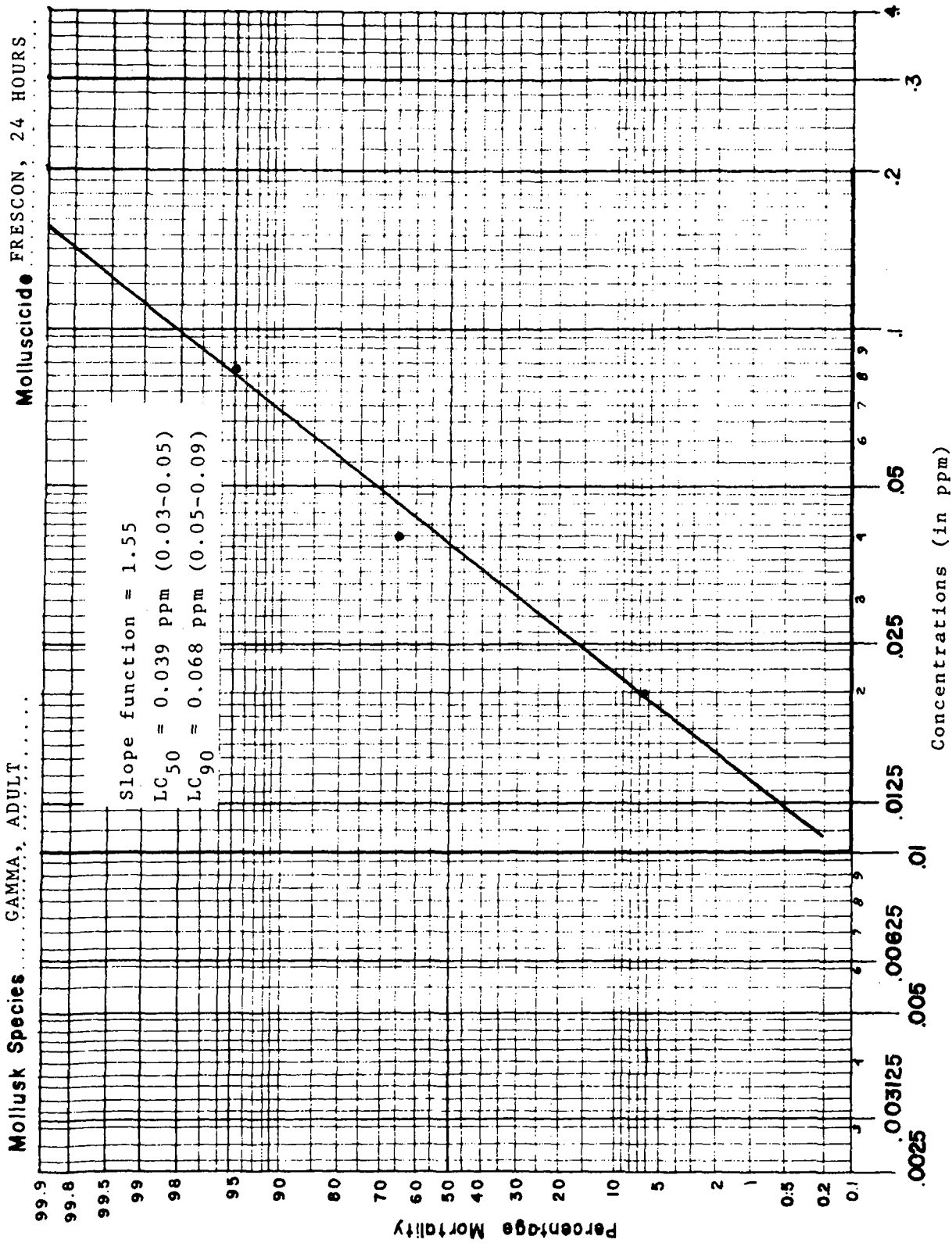


Fig. 73.--Mortality of adult gamma L. aperta exposed to Frescon for 24 hours.

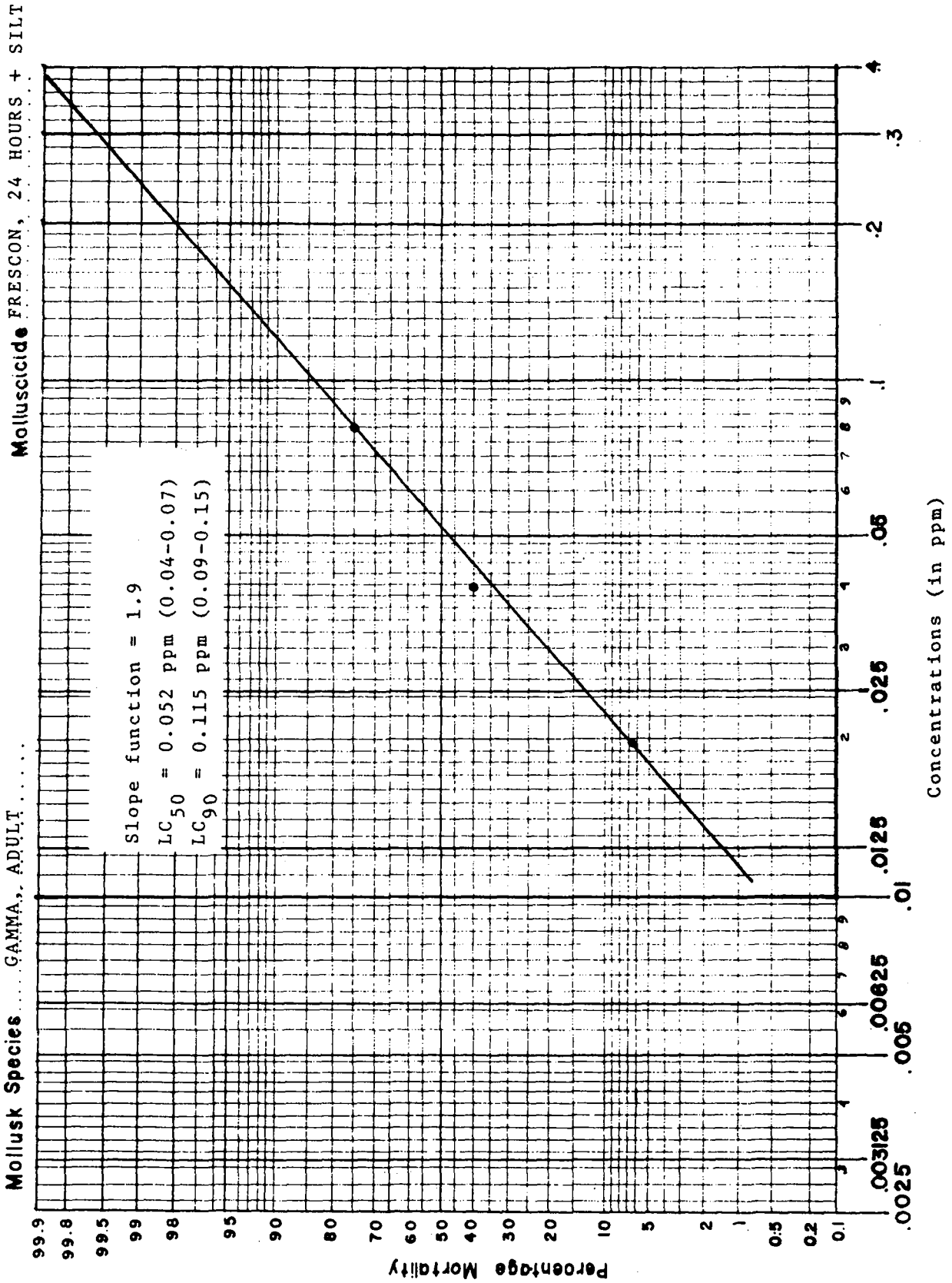


Fig. 74.--Mortality of adult gamma *L. aperta* exposed to Frescon plus silt for 24 hours.

Table 32 Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) in parts per million (ppm) with 95% confidence limits and slope function of adult *L. aperta* exposed to sodium pentachlorophenate (NaPCP), 90% granules, for 24 hours.

Race of <i>L. aperta</i>	Exposure time (in hours)	Mean mortality of <i>L. aperta</i> (in ppm)		Slope Function
		LC <sub>50</sub> (95% confidence limit)	LC <sub>90</sub> (95% confidence limit)	
Alpha	24	0.725 (0.650-0.810)	1.120 (0.940-1.330)	1.38

SODIUM PENTACHLOROPHENATE,  
Molluscicide 24 HOURS

Mollusk Species ALPHA, ADULT

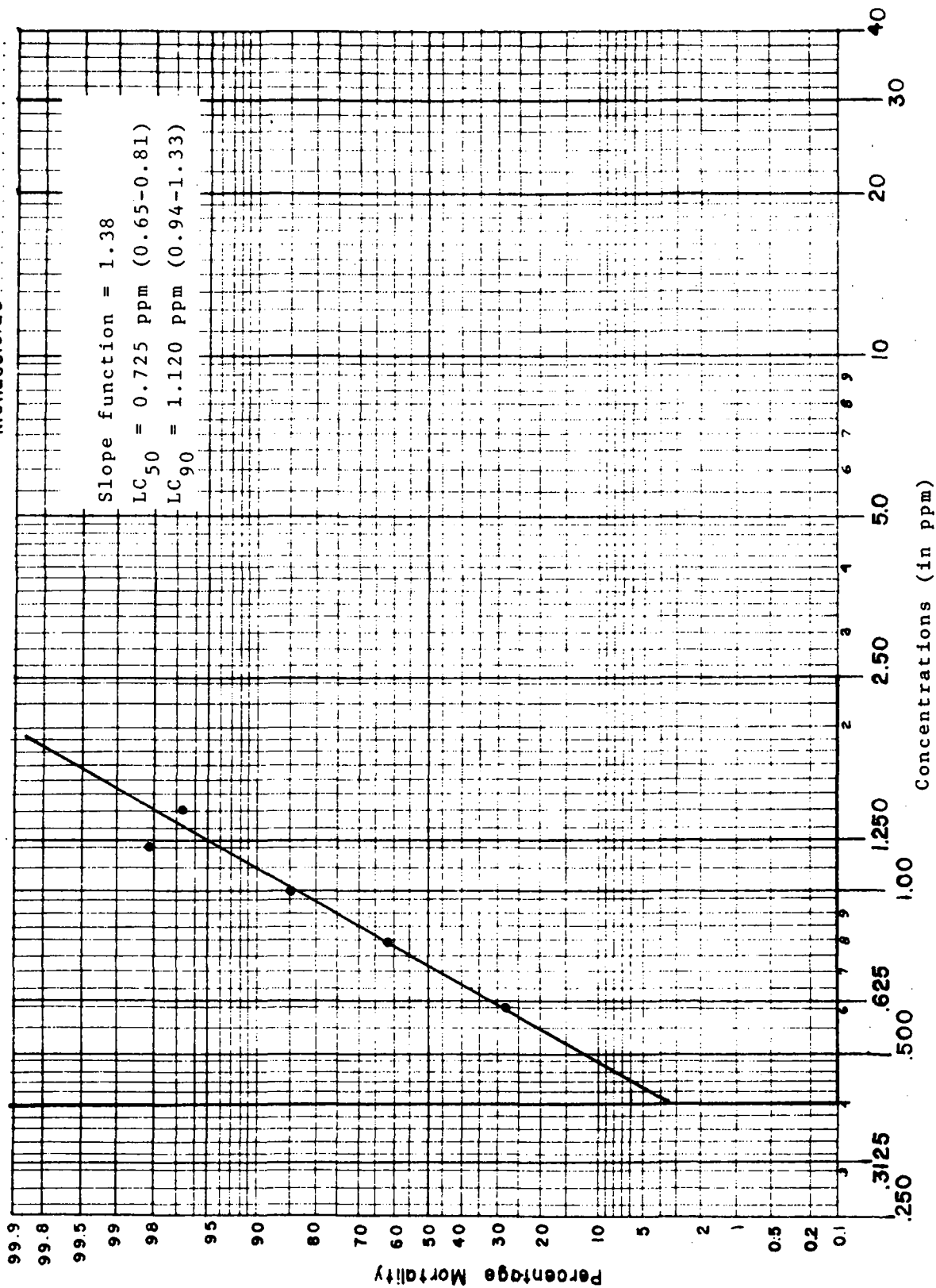


Fig. 75.--Mortality of adult alpha L. aperta exposed to sodium pentachlorophenate for 24 hours.



For this particular molluscicide, only the alpha race was tested. The LC<sub>50</sub> and LC<sub>90</sub> were 0.725 ppm and 1.120 ppm, respectively.

e. Yurimin P-99 (5% Active Ingredient Granules).  
A summary of LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and slope functions is given in Table 33 and Figures 76, 77, 78 & 79. Raw data are given in Appendix VI.

There was a decrease in lethal concentrations as the exposure time increased.

For this molluscicide, only young gamma race and adult alpha race were tested. Therefore, a comparison in lethal concentrations between young and adult within the same race could not be made.

#### B. Slow Release Molluscicide

##### Tributyltin oxide (TBTO), 5.8% Active Ingredient Pellets.

##### Experiment I: Soaking Time Intervals

A summary of LC<sub>50</sub> and LC<sub>90</sub>, 95% confidence limits, and slope functions is given in Table 34 and Figures 80, 81, 82, 83, 84 & 85. Raw data are given in Appendix VII.

There was a decrease in lethal concentrations as the soaking time increased.

LC<sub>50</sub> and LC<sub>90</sub> values were reached at day 1 and 3, respectively, for alpha race snails, and at day 2 for gamma race snails.

##### Experiment II: Continuous Exposures of L. Aperta

Summaries of data are given in Tables 35, 36 & 37 for young alpha race, adult alpha race, and adult gamma race, respectively. The raw data appear in Appendix VIII.

At 5.12 ppm (the highest concentration tested) LC<sub>50</sub> and LC<sub>100</sub> were less than 24 hours and 1 to 3 days respectively, for alpha race snails, and were less than 24 hours and 2 days, respectively, for gamma race snails. At 0.01 ppm (the lowest concentration tested) the LC<sub>50</sub> and LC<sub>100</sub> were 8 to 12 and more than 33 days, respectively, for alpha race snails, and 5 and 10 days, respectively, for gamma race snails.

Table 33 Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) in parts per million (ppm) with 95% confidence limits and slope functions of two races of *L. aperta* exposed to Yurimin P-99, granules, 5% a.i., at 24 and 48 hours.

Race of <i>L. aperta</i>	Exposure time (in hours)	LC <sub>50</sub> (95% confidence limit)		Mean mortality of <i>L. aperta</i> (in ppm)		LC <sub>90</sub> (95% confidence limit)		Slope function	
		Young	Adult	Young	Adult	Young	Adult	Young	Adult
Alpha	24	ND	0.111 (0.080-0.150)	ND	0.227 (0.140-0.370)	ND	1.79	ND	1.79
	48	ND	0.084 (0.070-0.100)	ND	0.125 (0.100-0.160)	ND	1.41	ND	1.41
Gamma	24	0.075 (0.070-0.080)	ND	0.099 (0.090-0.110)	ND	ND	1.27	1.27	ND
	48	0.0625 (0.060-0.070)	ND	0.0825 (0.080-0.090)	ND	ND	1.23	1.23	ND

ND = not done.

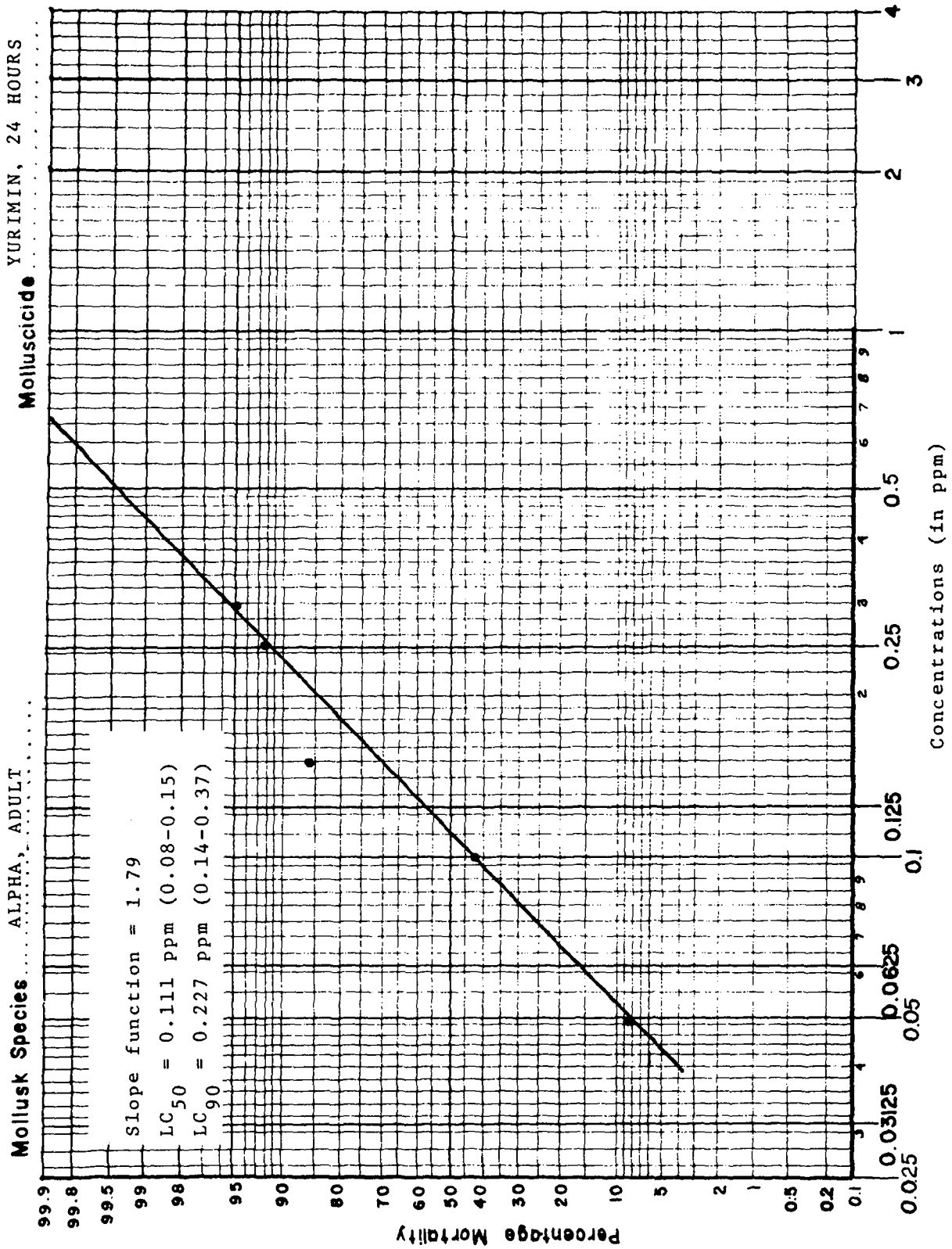


Fig. 76.--Mortality of adult alpha *L. aperta* exposed to Yurimin for 24 hours.

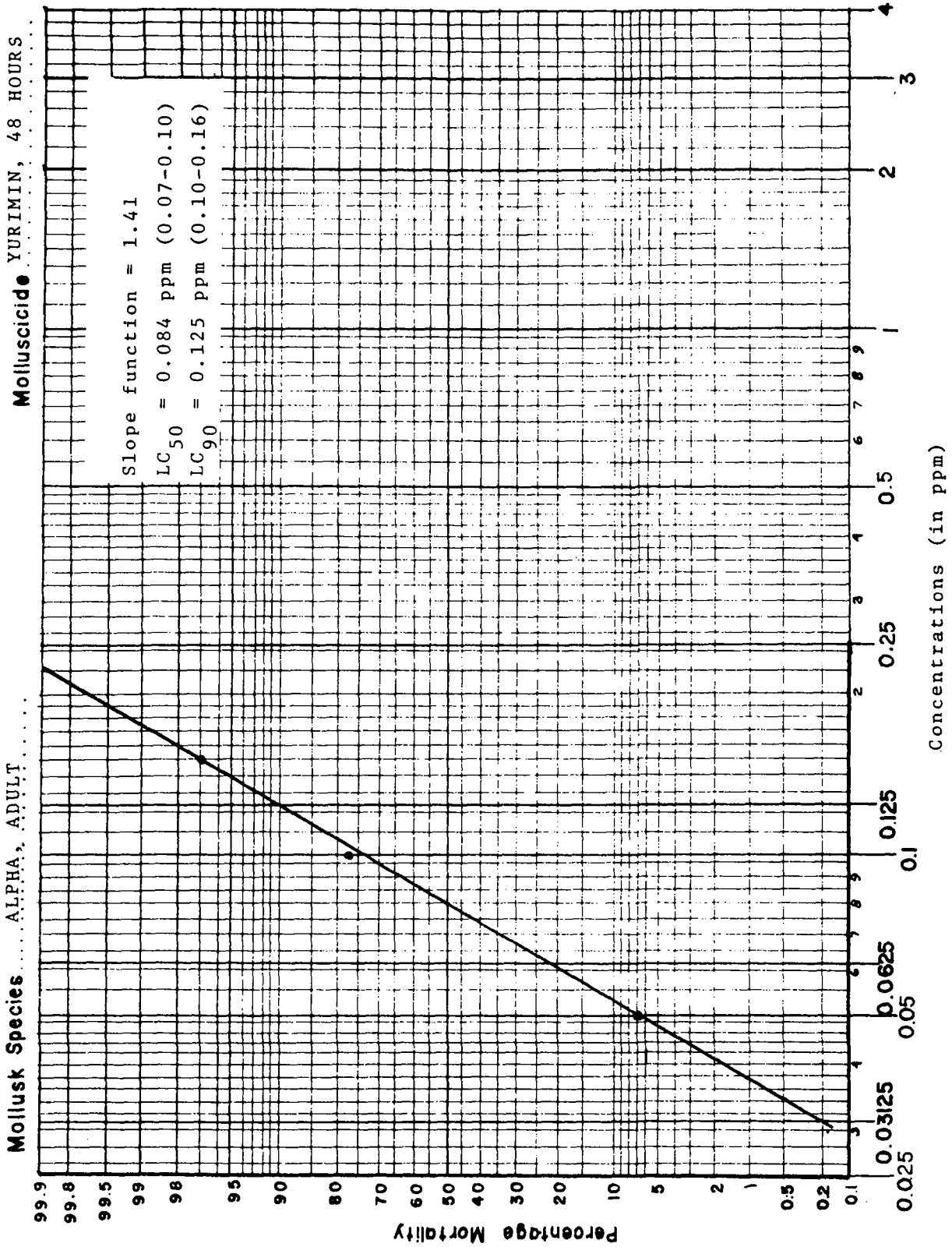


Fig. 77.--Mortality of adult alpha L. aperta exposed to Yurimin for 48 hours.

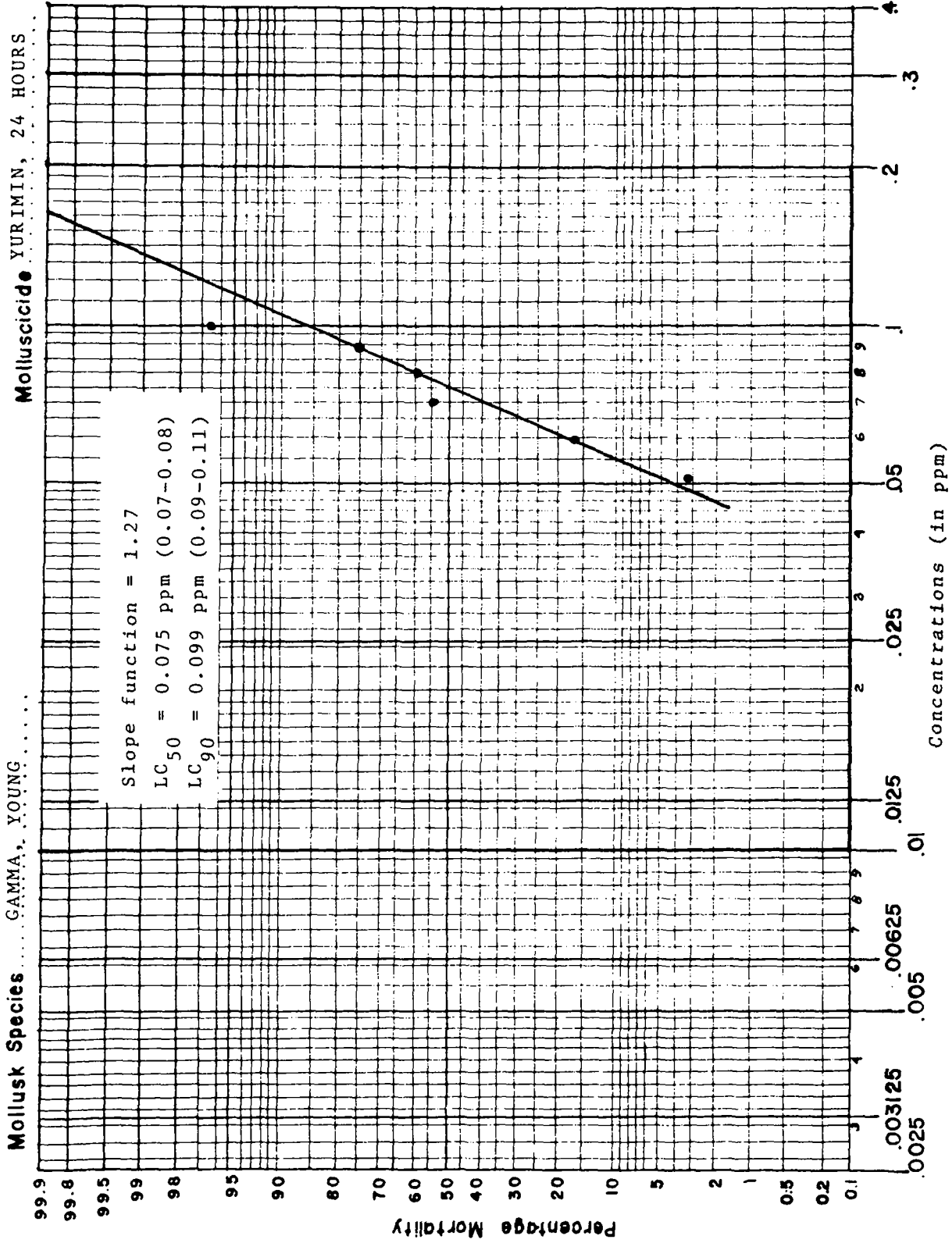


Fig. 78.--Mortality of young gamma L. aperta exposed to Yurimin for 24 hours.

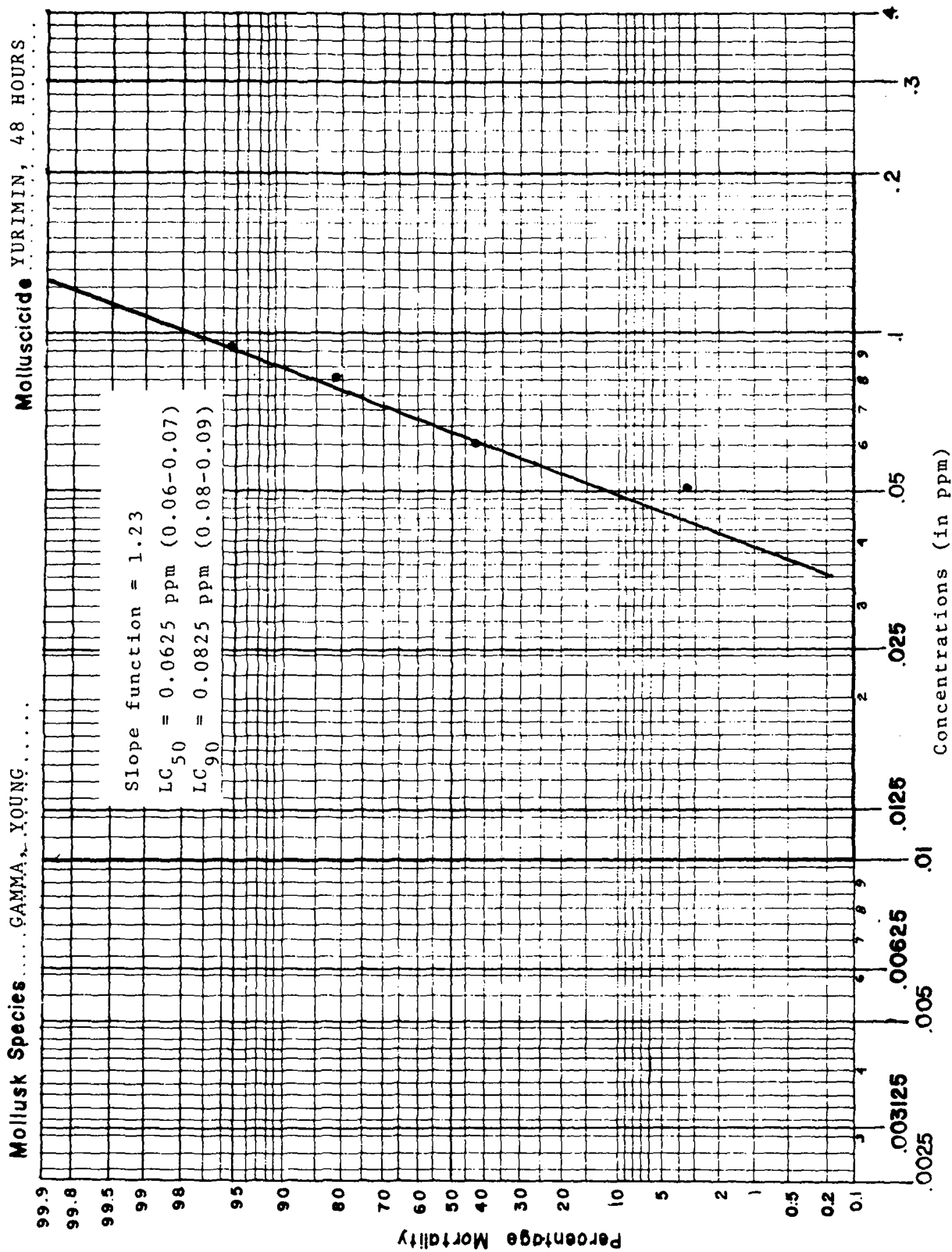


Fig. 79.--Mortality of young gamma *L. aperta* exposed to Yurimin for 48 hours.

Table 34 Lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) in parts per million (ppm) with 95% confidence limits and slope functions of two races of *L. aperta* exposed to Tributyltin oxide (TBTO), pellets, 5.8% a.i. (BioMet SRM) at soaking time intervals of 1, 2, 3, 4, 8, 16 and 32 days.

Race of <i>L. aperta</i>	Soaking time (in days)	Mean mortality of <i>L. aperta</i> (in ppm)		Slope function
		LC <sub>50</sub> (95% confidence limit)	LC <sub>90</sub> (95% confidence limit)	
Alpha	1	2.320		
	2	1.280		
	3	1.200	NC	NN
	4	1.280	(0.89-1.62)	(3.41-7.68)
	8	1.000	(0.98-1.68)	(2.88-6.13)
	16	0.980	(0.75-1.34)	(2.74-5.00)
Gamma	32	0.600	(0.67-1.43)	(2.96-8.86)
	1	1.800	(0.43-0.83)	(2.11-5.47)
	2	1.150	NF	NN
	3	0.970	(1.29-2.52)	(3.82-6.16)
	8	0.215	(0.91-1.46)	(2.94-5.44)
	16	0.530	(0.69-1.37)	(2.19-4.98)
	32	0.640	(0.13-0.35)	(0.58-2.27)
			(0.41-0.68)	(1.23-2.27)
			(0.46-0.88)	(1.00-2.07)
				2.57
				2.61
				2.58
				3.76
				2.43
				1.88

NC = not computable.  
 NF = mortality of snails tested was lower than 50% at the concentrations exposed.  
 NN = mortality of snails tested was lower than 90% at the concentrations exposed.

MOLLUSK SPECIES ALPHA..... MOLLUSCIDICIDE TRIBUTYLTIINOXIDE.....

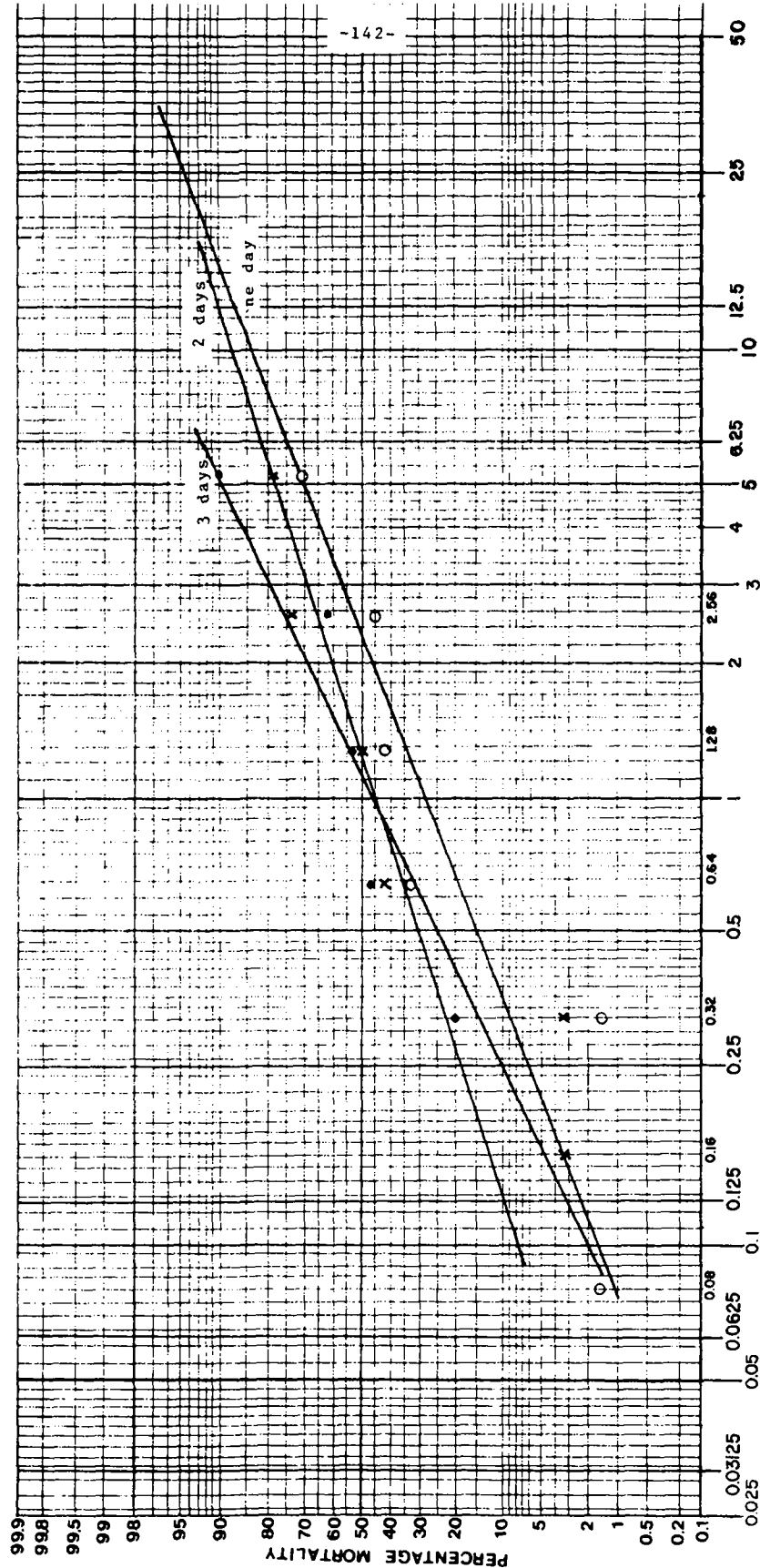


Fig. 80.--Mortality of alpha *L. aperta* exposed to TBTO for 1, 2 and 3 days.



MOLLUSK SPECIES ..... ALPHA

MOLLUSCIDICIDE TRIBUTYL TIN OXIDE

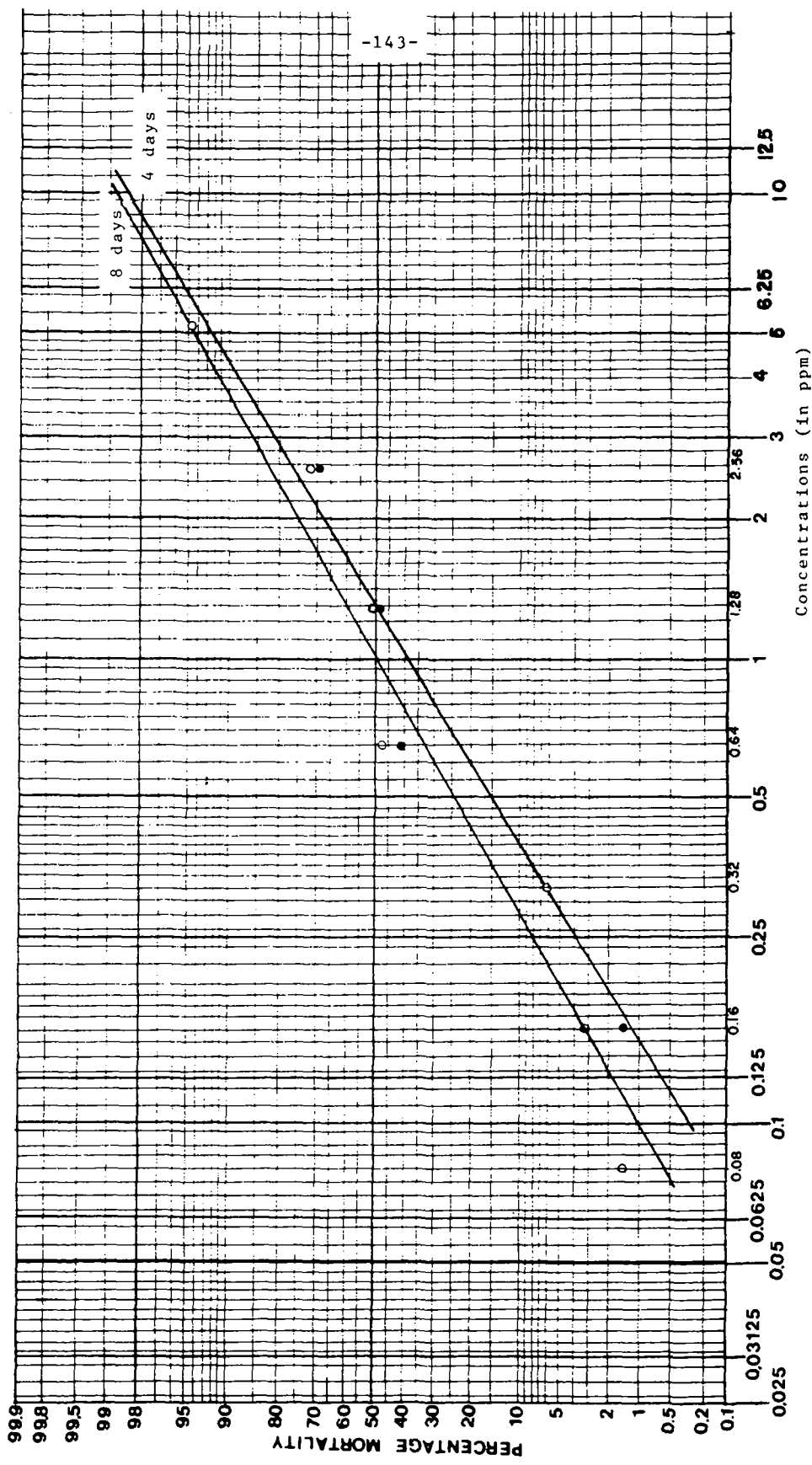


Fig. 81.--Mortality of alpha L. apurta exposed to TBTO for 4 and 8 days.

MOLLUSK SPECIES ..... ALPHA..... MOLLUSCIDIDE TRIBUTYLTIINOXIDE....

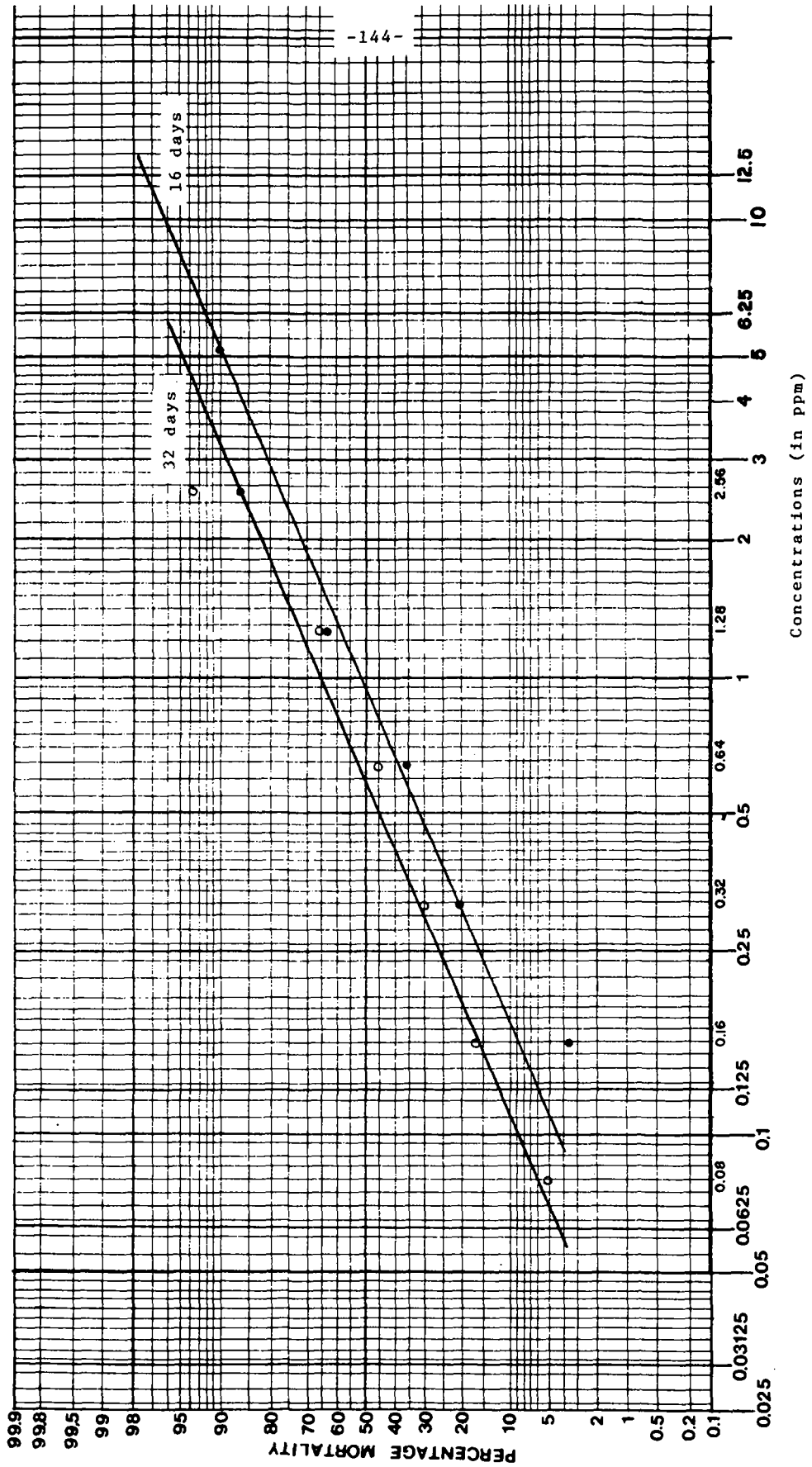


Fig. 82.--Mortality of alpha *L. sperta* exposed to TBTO for 16 and 32 days.

MOLLUSK SPECIES ..... GAMMA ..... MOLLUSCICIDE TRIBUTYLTHIOXIDE...

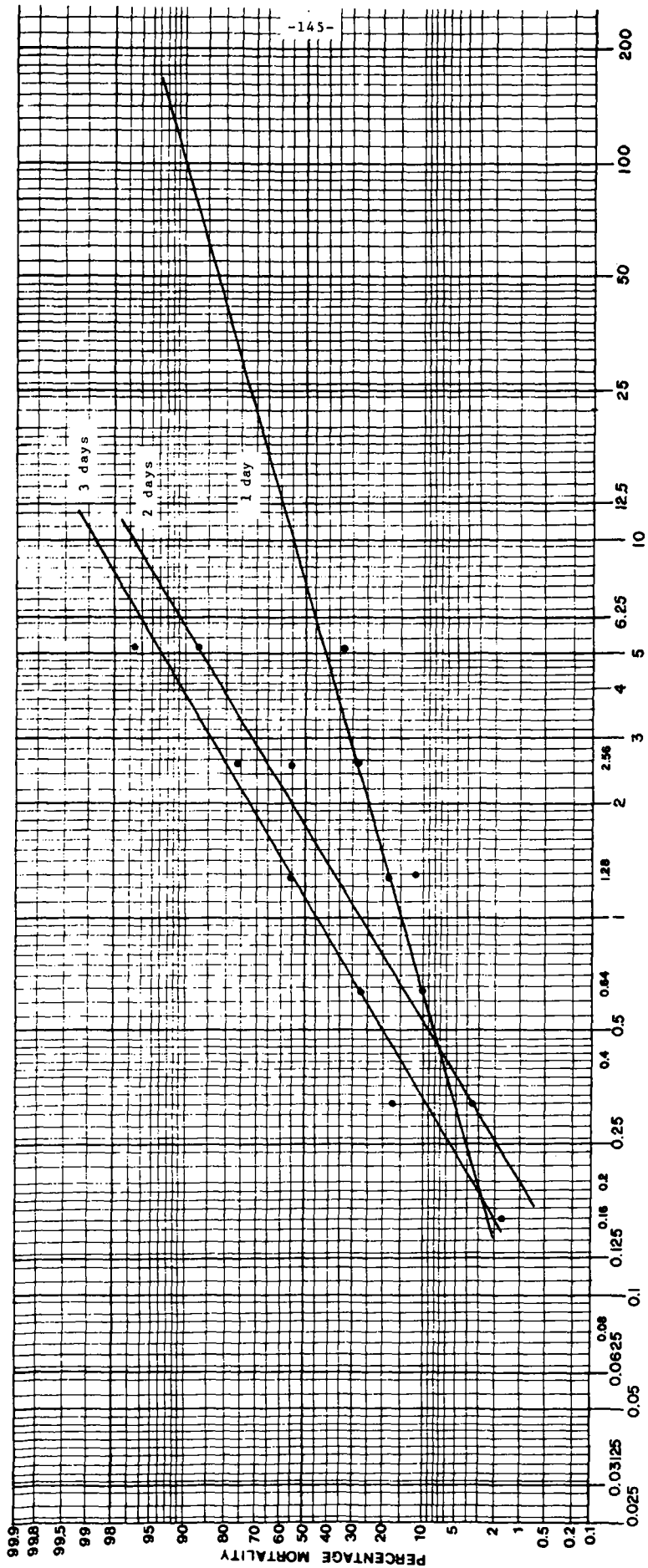


Fig. 83.--Mortality of gamma *L. sperta* exposed to TBTO for 1, 2 and 3 days.

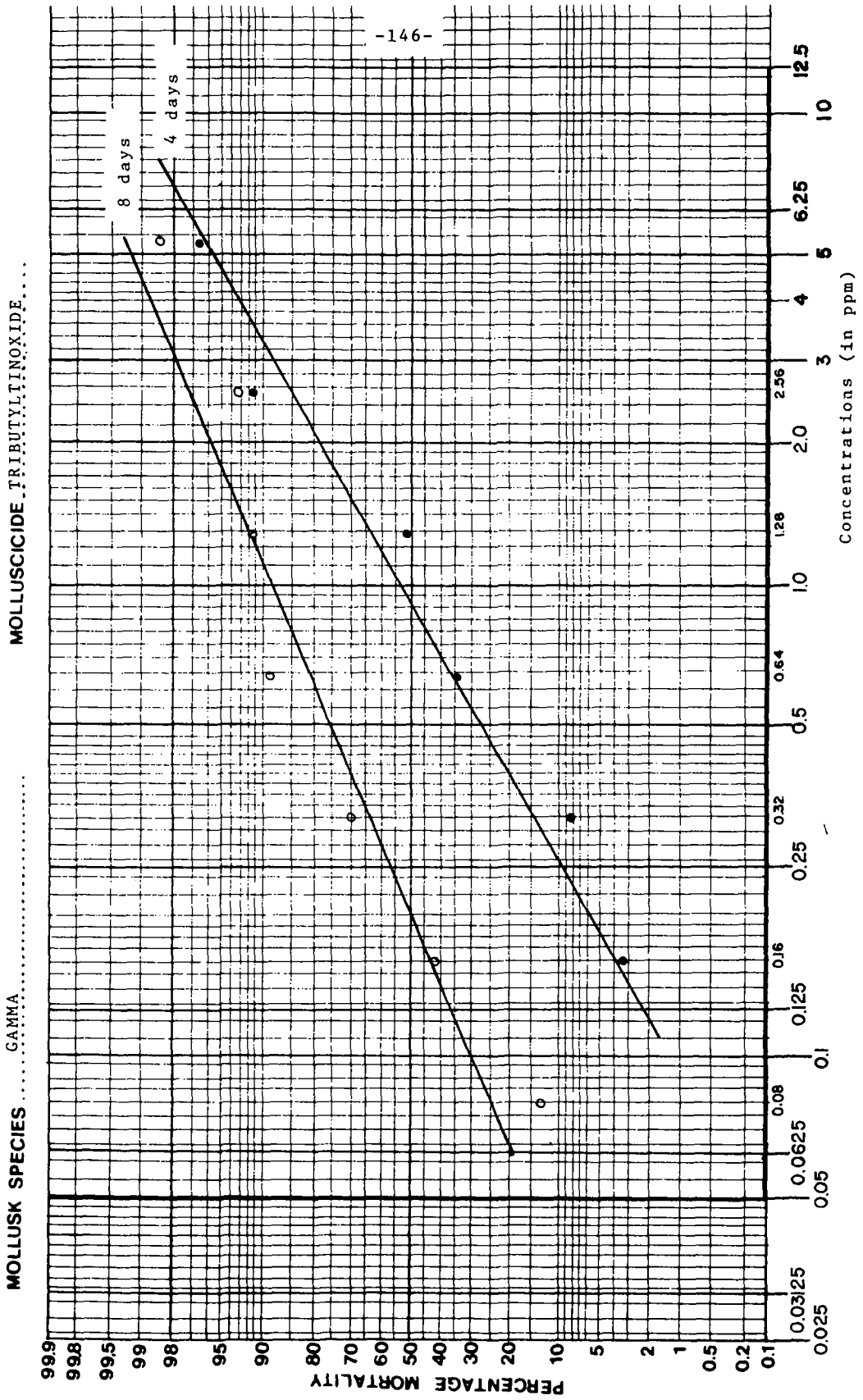


Fig. 84.--Mortality of gamma L. aperta exposed to TBTO for 4 and 8 days.

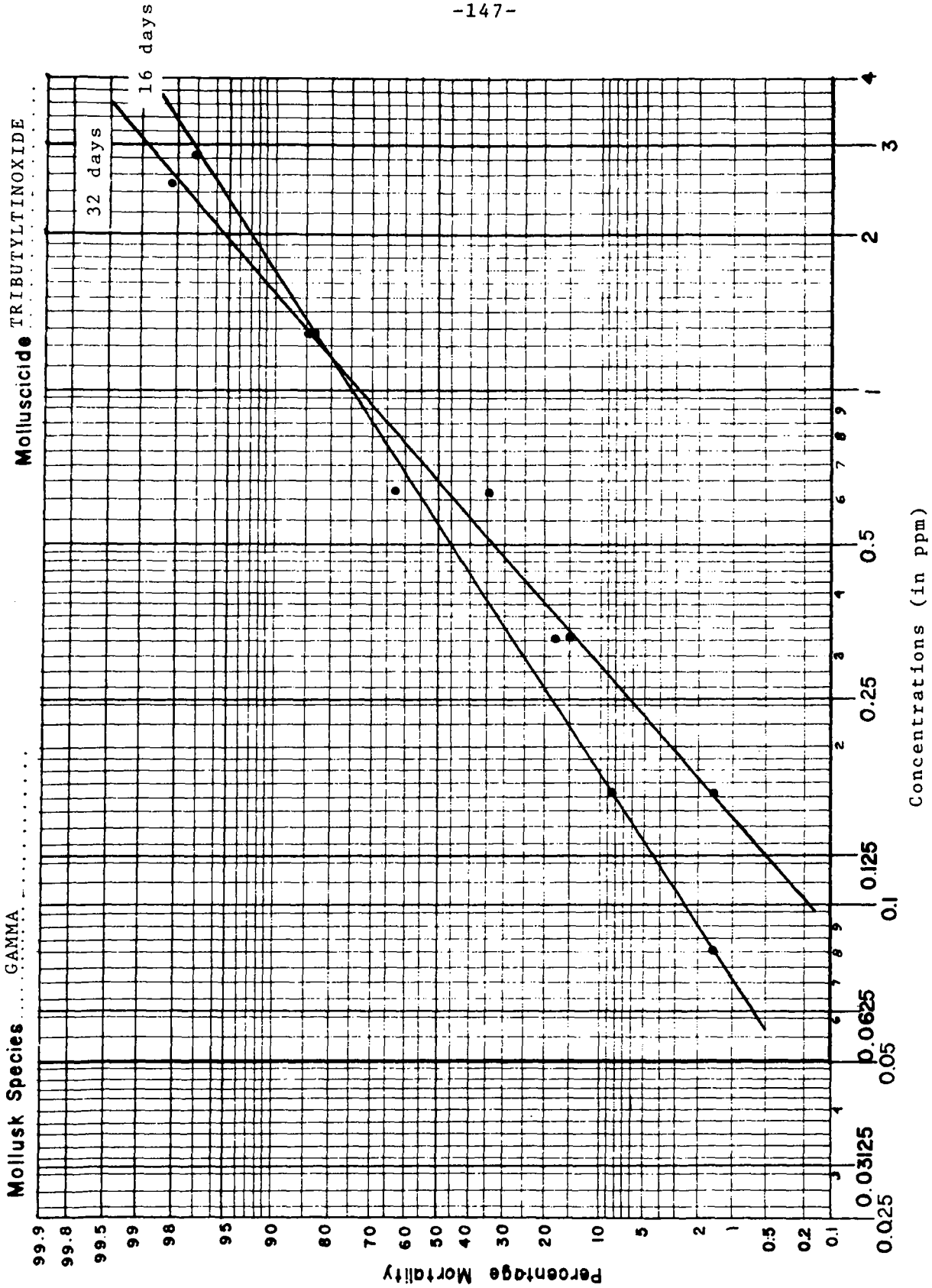


Fig. 85.--Mortality of gamma L. aperta exposed to TBTO for 16 and 32 days.

Table 35 Lethal concentrations (LC<sub>50</sub> and LC<sub>100</sub>), in days, of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (BioMet SRM) for young, alpha L. aperta after exposure times from day 1 to day 33.

Concentrations (in ppm)	Mortality of young, alpha race of <u>L. aperta</u>	
	LC <sub>50</sub> (in days)	LC <sub>100</sub> (in days)
5.12	less than 1 day	3 days
2.56	within day 3	3 days
1.28	within day 3	3 days
0.64	within day 4	6 days
0.32	within day 3	6 days
0.16	5 days	7 days
0.08	within day 7	10 days
0.04	within day 7	more than 33 days
0.02	8 days	more than 33 days
0.01	12 days	more than 33 days

Table 36 Lethal concentrations (LC<sub>50</sub> and LC<sub>100</sub>) in days, of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (BioMet SRM) for adult alpha L. aperta after exposure times from day 1 to day 33.

Concentrations (in ppm)	Mortality of alpha race of <u>L. aperta</u>	
	LC <sub>50</sub> (in days)	LC <sub>100</sub> (in days)
5.12	less than 1 day	1 day
2.56	less than 1 day	2 days
1.28	less than 1 day	3 days
0.64	less than 1 day	3 days
0.32	less than 1 day	3 days
0.16	within day 3	4 days
0.08	within day 4	5 days
0.04	within day 3	5 days
0.02	within day 4	7 days
0.01	8 days	more than 33 days

Table 37 Lethal concentrations (LC<sub>50</sub> and LC<sub>100</sub>), in days, of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (BioMet SRM) for adult gamma L. aperta after exposure times from day 1 to day 10.

Concentrations (in ppm)	Mortality of gamma race of <u>L. aperta</u>	
	LC <sub>50</sub> (in days)	LC <sub>100</sub> (in days)
5.12	less than 1 day	2 days
2.56	less than 1 day	2 days
1.28	less than 1 day	2 days
0.64	within day 2	4 days
0.32	within day 2	4 days
0.16	within day 2	4 days
0.08	within day 2	5 days
0.04	within day 2	5 days
0.02	within day 2	9 days
0.01	within day 5	10 days



3. Comparison of the Effectiveness of Various Conventional Molluscicides on L. Aperta and Other Human Schistosome Transmitting Snails

Among the conventional molluscicides, N-tritylmorpholine and Niclosamide seem to be most effective against Lithoglyphopsis snails. Yurimin, sodium pentachlorophenate, and copper sulphate also show reasonable effectiveness against Lithoglyphopsis snails (Table 38). Yasuraoka (1968) reported that the LC<sub>50</sub> for Oncomelania nosophora was 6.3 ppm with Yurimin P-99 and 0.24 - 0.59 ppm with sodium pentachlorophenate. With sodium pentachlorophenate, the LC<sub>50</sub> for L. aperta and O. nosophora did not differ greatly; but with Yurimin P-99, it did differ significantly: the LC<sub>50</sub> of L. aperta was lower (Table 39). With Niclosamide, the LC<sub>50</sub> for L. aperta and O. nosophora also did not differ significantly. The data in Table 39 indicate that the LC<sub>50</sub> and LC<sub>90</sub> of Niclosamide for Biomphalaria glabrata and Biomphalaria pfeiferi were lower than those for L. aperta and O. nosophora. With regard to Tritylmorpholine (FX 28), the LC<sub>50</sub> and LC<sub>90</sub> for L. aperta and Biomphalaria glabrata were in the same ranges.

4. Recommendations for Use of Molluscicides

Field trials should be carried out at Khong Island using Bayluscide and slow release Tributyltin oxide (TBTO).

Bayluscide is effective not only against snails but also against snail eggs (Oncomelania, Biomphalaria, Bulinus). Its molluscicidal efficacy is not affected by light or pH. Unfortunately, Bayluscide kills fish and other aquatic fauna of the same concentrations required to kill snails. Due to the large dilution factor in the Mekong River, it would not seem practical or economical to control snails with Bayluscide or any conventional molluscicides. Nevertheless, Bayluscide could still be of use in treating small pockets of water along the bank of, or on islands, in the river.

Slow release TBTO may be the most practical molluscicide for controlling snails at Khong Island. It has been incorporated into rubber and manufactured in different formulations such as rubber pellets, rubber sheets, rubber strings, and paints. These formulations will release the molluscicide slowly at a concentration calculated to kill only the target snails. Therefore, slow release formulations may offer an approach to schistosomiasis control without the hazards of environmental contamination.

Slow release TBTO has been tested in the field against schistosome transmitting snails in Rhodesia (Shiff, 1975), in Brazil (Castleton, 1974), and in St. Lucia (Upatham, 1975).

Table 38 Comparison of lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) in ppm for L. aperta exposed to various conventional molluscicides for 24 hours.

Molluscicide	Race of <i>L. aperta</i>	Age of <i>L. aperta</i>	Mean mortality of <i>L. aperta</i>	
			LC <sub>50</sub> (in ppm)	LC <sub>90</sub> (in ppm)
Niclosamide	alpha	young	0.136	0.250
		adult	0.096	0.125
	gamma	young	0.077	0.136
		adult	0.083	0.122
Copper sulphate	alpha	young	0.630	1.420
		adult	0.730	2.830
	gamma	young	0.800	1.850
N-tritylmorpholine	alpha	young	0.077	0.380
		adult	0.057	0.085
	gamma	young	0.057	0.151
		adult	0.039	0.068
NaPCP	alpha	adult	0.725	1.120
Yurimin	alpha	adult	0.111	0.227
	gamma	young	0.063	0.083

Table 39 Comparison of lethal concentrations (LC50 and LC90) in ppm for some human schistosome transmitting snails exposed to various molluscicides for 24 hours.

Molluscicide	Lithoglyphopsis aperta (a)		Oncomelania nosophora (b)		Biomphalaria glabrata (c)		Biomphalaria pfeifferi (d)		Bulinus (Physopsis) (e)	
	LC50	LC90	LC50	LC90	LC50	LC90	LC50	LC90	LC50	LC90
Niclosamide	0.083-	0.122-	0.07-	-	0.045	0.055 <sup>(c1)</sup>	0.055	0.061	0.071	0.110
	0.096	0.125	0.19 <sup>(b1)</sup>							
Tritylmorpholine (FX 28)	0.039-	0.068-			0.042	0.070 <sup>(c1)</sup>				
	0.057	0.085			0.0					
Yurimin	0.111	0.227	6.3 <sup>(b2)</sup>	-						
NaPCP	0.725	1.120	0.24 <sup>(b2)</sup>	-	0.400	1.200 <sup>(c2)</sup>				
			0.59							

- (a) = Vector of the Mekong Schistosoma japonicum (the present study).
- (b) = Vector of S. japonicum in Japan (b<sub>1</sub>: Hosaka et al, 1969; b<sub>2</sub> Yasuraoka et al, 1968).
- (c) = Vector of S. mansoni in St. Lucia and Puerto Rico (c<sub>1</sub>: Research and Control Department Fourth Report 1970-1971; c<sub>2</sub>: Ritchie et al, 1963).
- (d) = Vector of S. mansoni in Rhodesia (Schiff et al, 1970).
- (e) = Vector of S. haematobium in Rhodesia (Schiff et al, 1970)

At Khong Island, it is recommended that field trials be carried out using rubber strings and/or sheets and paint formulations. Rubber strings and/or sheets and painted rocks, and/or other objects could be set up along the transmission site and at the human water contact points. Rubber pellets could not be used at Khong Island because they would soon be buried underneath the mud and lose their molluscicidal efficacy.

#### IV Incrimination of Other Snails as Intermediate Hosts of the Mekong Schistosome

Apart from L. aperta, no other snails are yet known to serve as intermediate hosts. Attempts to implicate others were made by collecting snails from the Mekong River and exposing them to miracidia of the Mekong schistosome.

##### 1. Methods and Materials

Snails. Insofar as time and mollusk populations were available, approximately 6 genera and 12 species were tested. Snails of the same size of each species were collected from the same habitat. In each collection, one hundred snails were selected at random, crushed and examined under the microscope for naturally occurring larval stages of schistosomes. The remainder of each collection was used in the ensuing experiments. No "wild" sporocysts or other stages of any schistosome were found in the crushed snails.

Exposure to Miracidia. Miracidia used in the tests were obtained from infected dog feces. Only freshly hatched, active miracidia were used. Snails were individually exposed to 5 miracidia in a plastic cup (1" diam.). They were left exposed for 3 hours before they were transferred to the aquarium.

The first experiment was done in 1975 in which all the snails were kept in the laboratory and fed with cultured diatoms. The second lot was exposed in 1976 and the snails were kept in earthenware dishes at the field station and fed diatoms from river rocks.

Snails were regularly checked for mortality; those which were weak or died were crushed and examined for sporocysts.

##### 2. Results

Owing to difficulty in maintaining the snails in the laboratory, only 5 species in the first lot of Mekong snails could be tested (Table 40). However, many, including the control group, died before crushing. The rest were crushed

Table 40 Exposure of Mekong snails other than L. aperta to miracidia of the Mekong schistosome, 1975, in the laboratory, Bangkok.

Species of snail (a)	No. snails	Area of origin	Date of exposure	No. dead snails before crushing	No. snails crushed (b)	Result
<u>Hydroissoia elongata</u> (= <u>H. hospitalis</u> )	100	Mekong River (Khemmarat)	3-18-75	69	31	negative
<u>Lacunopsis coronata</u> <u>L. globosa</u> (mixed) (c)	191	Mekong River (Khemmarat)	3-19-75	180	11	negative
<u>Lacunopsis jullieni</u> (d)	200	Mekong River (Khemmarat)	4-4-75	152	48	negative
<u>Lacunopsis coronata</u>	100	Mekong River (Khemmarat)	4-4-75	91	9	negative

- (a) each snail was exposed individually to 5 miracidia of the Mekong schistosome.  
 (b) snails were examined 80 days after the exposure.  
 (c) included some immature Lacunopsis, possibly L. sphaerica.  
 (d) included some immature, possibly L. massiei.

80 days after exposure and none were found to be positive.

The second lot was exposed at the field station using river water and rocks for maintaining the snails in earthenware dishes. By this technique the mortality rate was low in every group. About 10 species of Mekong snails were tested (Table 41). All were crushed and examined 50 days after exposure except the weak and dead snails. No sporocysts or other parasitic stages were found in any of these snails.

On the basis of these data, it seems improbable that any of the common, predominant hydrobiid snails sympatric with L. aperta can serve as intermediate hosts of the Mekong schistosome. However, there are still other untested species of snails in the Mekong River. Moreover, different species predominate at different times of the year, and in different habitats. The attempted incrimination of other snails as intermediate hosts of the Mekong schistosome merits continued investigation.

Table 41 Exposure of Mekong snails other than *L. aperta* to miracidia of the Mekong schistosome, 1976, in the field laboratory, Khemmarat.

Species of snail (a)	No. snails	Area of origin	Date of exposure	No. dead snails before crushing	No. snails crushed (b)	Result
<i>Hubendickia siamensis</i>	100	Mun River (Phibun)	3-8-76	8	92	negative
<i>Hubendickia siamensis</i>	100	Mun River Phibun	3-10-76	2	98	negative
<i>Hubendickia coronata</i>	200	Mekong River (Khemmarat)	3-16-76	26	174	negative
<i>H. tuberculata</i> (mixed)						
<i>Manningiella polita</i>	200	Mekong River (Khemmarat)	3-17-76	14	186	negative
<i>Jullienia harmandi</i>	200	Mekong River (Khemmarat)	3-23-76	32	168	negative
<i>Lacunopsis conica</i>	100	Mekong River (Khemmarat)	3-24-76	17	83	negative
<i>Lacunopsis</i> sp. (c)	200	Mekong River (Khemmarat)	4-4-76	25	175	negative
<i>Stenothyra hybocystoides</i>	200	Mekong River (Khemmarat)	4-5-76	32	168	negative
<i>Lacunopsis conica</i>	100	Mekong River (Khemmarat)	4-7-76	24	76	negative
<i>Manningiella expansa</i>	200	Mekong River (Khemmarat)	4-9-76	31	169	negative

(a) each snail was exposed individually to 5 miracidia of the Mekong schistosome.

(b) snails were examined 50 days after the exposure.

(c) young snails, probably *L. fischerpiettei*.

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APPENDIX I

DENSITY OF L. APERTA ON STONES

Table 1. Density of L. aperta on stones.  
Site A.

No. of Stone	No. of <u>L. aperta</u>			Surface area (cm <sup>2</sup> )	cm <sup>2</sup> / Snail
	alpha	gamma	Total		
Bang Koev, 1st week (4 March)					
1	11	0	11	450	40.9
2	16	0	16	576	36.0
3	0	0	0	980	0.0
4	14	0	14	726	51.9
5	357	0	357	962	2.7
6	13	0	13	609	46.9
7	8	0	8	825	103.1
8	113	0	113	450	4.0
9	8	0	8	704	88.0
10	100	5	105	638	6.1
Total/ Mean	640	5	645	6,290	10.7
Bang Koev 3rd week (18 March)					
1	17	5	22	768	34.9
2	86	9	95	1,044	11.0
3	36	6	42	713	17.0
4	66	39	105	936	8.9
5	21	2	23	672	29.2
6	88	11	99	432	4.4
7	66	4	70	780	11.1
8	46	3	49	572	11.7
9	150	22	172	910	5.3
10	64	6	70	522	7.5
Total/ Mean	640	107	747	7,349	9.8
Bang Koev, 5th week (1 April)					
1	70	1	71	432	6.1
2	156	5	161	999	6.2
3	248	12	260	682	2.6
4	29	3	32	918	28.7
5	78	12	90	624	6.9
6	274	35	309	580	1.9
7	123	2	125	572	4.6
8	140	12	152	736	4.8
9	139	2	141	567	4.0
10	74	2	76	456	6.0
Total/ Mean	1,331	86	1,417	6,566	4.6

Table 1 (Cont'd)

No. of Stone	No. of <i>L. aperta</i>			Surface area (cm <sup>2</sup> )	cm <sup>2</sup> / Snail
	alpha	gamma	Total		
Bang Koey, 7th week (15 April)					
1	170	25	195	972	5.0
2	98	2	100	1,015	10.2
3	223	1	224	713	3.2
4	258	33	291	988	3.4
5	341	33	374	696	1.9
6	121	1	122	580	4.8
7	159	14	173	696	4.0
8	75	36	111	520	4.7
9	178	8	186	621	3.3
10	153	12	165	616	3.7
Total/ Mean	1,776	165	1,941	7,417	3.8
Bang Koey, 9th week (29 April)					
1	41	24	65	644	9.9
2	59	34	93	825	8.9
3	63	4	67	598	8.9
4	245	7	252	770	3.1
5	111	15	126	667	5.3
6	167	5	172	713	4.1
7	454	0	454	720	1.6
8	124	10	134	682	5.1
9	388	56	444	1,073	2.4
10	421	157	578	672	1.2
Total/ Mean	2,073	312	2,385	7,364	3.1
Bang Koey, 11th week (13 May)					
1	108	4	112	560	5.0
2	34	0	34	640	8.8
3	9	19	28	792	28.3
4	21	66	87	748	8.6
5	74	39	113	925	8.2
6	7	16	23	851	37.0
7	134	7	141	567	4.0
8	129	43	172	792	4.6
9	29	38	67	504	7.5
10	23	5	28	972	34.7
Total/ Mean	568	237	805	7,351	9.1

Table 1 (Cont'd)

Site B.

No. of Stone	No. of <i>L. aperta</i>			Surface area (cm <sup>2</sup> )	cm <sup>2</sup> / Snail
	alpha	gamma	Total		
Bung Kong, week 2 (11 March)					
1	14	17	31	450	14.5
2	37	30	67	576	8.6
3	43	18	61	980	16.1
4	25	6	31	726	23.4
5	16	15	31	962	31.0
6	31	11	42	609	14.5
7	32	16	48	825	17.2
8	22	38	60	450	7.5
9	9	9	18	704	39.1
10	13	6	19	638	33.6
Total/ Mean	242	166	408	6,920	17.0
Bung Kong, week 4 (25 March)					
1	32	62	94	828	8.8
2	25	78	103	609	5.9
3	56	65	121	432	3.6
4	47	19	66	986	14.9
5	79	52	131	635	4.8
6	13	16	29	990	34.1
7	19	20	39	682	17.5
8	35	21	56	936	16.7
9	19	50	69	1,080	15.7
10	13	28	41	494	12.1
Total/ Mean	338	411	749	7,672	10.2
Bung Kong, week 6 (8 April)					
1	25	40	65	759	11.7
2	46	42	88	1,160	13.2
3	19	121	140	805	5.8
4	75	105	180	986	5.5
5	1	26	27	972	36.0
6	4	55	59	630	10.7
7	12	82	94	888	9.5
8	6	103	109	850	7.8
9	35	41	76	525	6.9
10	14	24	38	850	22.4
Total/ Mean	237	639	876	8,425	9.6

Table 1 (Cont'd)

Site B.

No. of Stone	No. of <i>L. aperta</i>			Surface area (cm <sup>2</sup> )	cm <sup>2</sup> / Snail
	alpha	gamma	Total		
Bung Kong, week 8 (22 April)					
1	2	78	80	851	10.6
2	21	128	149	598	4.0
3	2	200	202	693	3.4
4	1	90	91	980	10.8
5	27	132	159	962	6.1
6	18	223	241	900	3.7
7	3	70	73	696	9.5
8	13	168	181	864	4.8
9	2	209	211	651	3.1
10	4	83	87	891	10.2
Total/ Mean	93	1,381	1,474	8,086	5.5
Bung Kong, week 10 (6 May)					
1	11	1,050	1,061	912	0.9
2	7	293	300	504	1.7
3	2	221	223	992	4.4
4	8	675	683	783	1.1
5	7	241	248	644	2.6
6	0	250	250	910	3.6
7	3	304	307	744	2.4
8	4	413	417	651	1.6
9	29	308	337	891	2.6
10	5	269	274	667	2.4
Total/ Mean	76	4,024	4,100	7,698	1.9

APPENDIX II



Table 2. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to young, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.05	0/30	1/30	0.0	3.3	1.6
0.10	1/30	8/30	3.3	26.7	15.0
0.15	21/30	22/30	70.0	73.3	71.6
0.20	22/30	25/30	73.3	83.3	78.3
0.25	24/30	25/30	80.0	83.3	81.6
0.30	30/30	30/30	100.0	100.0	100.0
0.35	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 3. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 6 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	1/30	0/30	3.3	0.0	1.6
0.16	9/30	8/30	30.0	26.7	28.3
0.32	28/30	26/30	93.3	86.7	90.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 4. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 12 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	2/30	2/30	6.7	6.7	6.7
0.08	8/30	13/30	26.7	43.3	35.0
0.16	25/30	20/30	83.3	66.7	75.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 5. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.05	1/30	0/30	3.3	0.0	1.6
0.10	19/30	15/30	63.3	50.0	56.6
0.15	29/30	29/30	96.7	96.7	96.7
0.20	30/30	30/30	100.0	100.0	100.0
0.25	30/30	30/30	100.0	100.0	100.0
0.30	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 6. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, alpha race of Lithoglyphopsis aperta, exposure in water and silt 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.05	3/30	0/30	10.0	0.0	5.0
0.10	21/30	26/30	70.0	86.7	78.3
0.15	30/30	29/30	100.0	96.7	98.3
0.20	30/30	30/30	100.0	100.0	100.0
0.25	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 7. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 6 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	1/30	1/30	3.3	3.3	3.3
0.08	5/30	5/30	16.7	16.7	16.7
0.16	15/30	18/30	50.0	60.0	55.0
0.32	25/30	25/30	83.3	83.3	83.3
0.64	29/30	30/30	96.7	100.0	98.3
Control	0/30	0/30	0.0	0.0	0.0

Table 8. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 12 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	2/30	2/30	6.7	6.7	6.7
0.08	10/30	11/30	33.3	36.7	35.0
0.16	25/30	25/30	83.3	83.3	83.3
0.32	30/30	29/30	100.0	96.7	98.3
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 9. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	4/30	2/30	13.3	6.7	10.0
0.08	13/30	18/30	43.3	60.0	51.6
0.16	28/30	29/30	93.3	96.7	95.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 10. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to young, gamma race of Lithoglyphopsis aperta, exposed in water and silt for 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	2/30	2/30	6.7	6.7	6.7
0.08	19/30	19/30	63.3	63.3	63.3
0.16	28/30	28/30	93.3	93.3	93.3
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 11. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, gamma race of Lithoglyphopsis aperta at the exposure time of 6 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.10	0/30	0/30	0.0	0.0	0.0
0.15	5/30	1/30	16.7	3.3	10.0
0.20	12/30	11/30	40.0	36.7	38.3
0.25	15/30	14/30	50.0	46.7	48.3
0.30	18/30	19/30	60.0	63.3	61.6
0.35	26/30	27/30	86.7	90.0	88.3
0.40	27/30	27/30	90.0	90.0	90.0
0.45	29/30	30/30	96.7	100.0	98.3
0.50	30/30	30/30	100.0	100.0	100.0
0.55	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 12. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, gamma race of Lithoglyphopsis aperta at the exposure time of 12 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.10	9/30	13/30	30.0	43.3	36.6
0.15	18/30	20/30	60.0	66.7	63.3
0.20	25/30	25/30	83.3	83.3	83.3
0.25	27/30	27/30	90.0	90.0	90.0
0.30	28/30	30/30	93.3	100.0	96.6
0.35	29/30	30/30	96.7	100.0	98.3
0.40	30/30	30/30	100.0	100.0	100.0
0.45	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 13. Effect of various concentrations of Niclosamide, E.c., 25% a.i. (Bayluscide) to adult, gamma race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	1/30	0/30	3.3	0.0	1.6
0.08	11/30	16/30	36.7	53.3	45.0
0.16	30/30	29/30	100.0	96.7	98.3
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

APPENDIX III

Table 15. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to young, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
	0.1	0/30	0/30	0.0	
0.2	0/30	2/30	0.0	6.7	3.3
0.4	6/30	11/30	20.0	36.7	28.3
0.8	13/30	20/30	43.3	66.7	55.0
1.6	28/30	27/30	93.3	90.0	91.6
3.2	30/30	29/30	100.0	96.7	98.3
6.4	30/30	30/30	100.0	100.0	100.0
12.8	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 16. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 6 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
	0.1	0/30	0/30	0.0	
0.2	0/30	0/30	0.0	0.0	0.0
0.4	0/30	0/30	0.0	0.0	0.0
0.8	0/30	1/30	0.0	3.3	1.6
1.6	5/30	4/30	16.7	13.3	15.0
3.2	10/30	10/30	33.3	33.3	33.3
6.4	21/30	18/30	70.0	60.0	65.0
12.8	28/30	24/30	93.3	90.0	86.6
25.6	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0



Table 17. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 12 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.1	0/30	0/30	0.0	0.0	0.0
0.2	0/30	0/30	0.0	0.0	0.0
0.4	0/30	0/30	0.0	0.0	0.0
0.8	1/30	0/30	3.3	0.0	1.6
1.6	2/30	4/30	6.7	13.3	10.0
3.2	14/30	14/30	46.7	46.7	46.7
6.4	28/30	23/30	93.3	76.7	85.0
12.8	30/30	30/30	100.0	100.0	100.0
25.6	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 18. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.1	1/30	0/30	3.3	0.0	1.6
0.2	3/30	1/30	10.0	3.3	6.6
0.4	11/30	13/30	36.7	43.3	40.0
0.8	12/30	20/30	40.0	66.7	53.3
1.6	21/30	25/30	70.0	83.3	76.6
3.2	28/30	29/30	93.3	96.7	95.0
6.4	30/30	30/30	100.0	100.0	100.0
12.8	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 19. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to adult, alpha race of Lithoglyphopsis aperta exposed in water and silt for 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.1	0/30	0/30	0.0	0.0	0.0
0.2	0/30	0/30	0.0	0.0	0.0
0.4	0/30	0/30	0.0	0.0	0.0
0.8	1/30	0/30	3.3	0.0	1.6
1.6	4/30	1/30	13.3	3.3	8.3
3.2	13/30	12/30	43.3	40.0	41.6
6.4	22/30	21/30	73.3	70.0	71.6
12.8	28/30	29/30	93.3	96.7	95.0
25.6	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 20. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.1	0/30	0/30	0.0	0.0	0.0
0.2	0/30	0/30	0.0	0.0	0.0
0.4	2/30	3/30	6.7	10.0	8.3
0.8	15/30	15/30	50.0	50.0	50.0
1.6	27/30	24/30	90.0	80.0	85.0
3.2	30/30	30/30	100.0	100.0	100.0
6.4	30/30	30/30	100.0	100.0	100.0
12.8	30/30	30/30	100.0	100.0	100.0
25.6	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 21. Effect of various concentrations of Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to young, gamma race of Lithoglyphopsis aperta, exposed in water and silt for 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.1	0/30	0/30	0.0	0.0	0.0
0.2	0/30	0/30	0.0	0.0	0.0
0.4	1/30	1/30	3.3	3.3	3.3
0.8	3/30	1/30	10.0	3.3	6.6
1.6	5/30	4/30	16.7	13.7	15.0
3.2	20/30	15/30	66.7	50.0	58.3
6.4	24/30	22/30	80.0	73.3	76.6
12.8	26/30	28/30	86.7	93.3	90.0
25.6	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

APPENDIX IV

Table 23. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	5/30	4/30	16.7	13.3	15.0
0.04	8/30	9/30	26.7	30.0	28.3
0.08	18/30	17/30	60.0	56.7	58.3
0.16	23/30	20/30	76.7	66.7	71.7
0.32	25/30	24/30	83.3	80.0	81.6
0.64	28/30	28/30	93.3	93.3	93.3
1.28	30/30	30/30	100.0	100.0	100.0
2.56	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 24. Effect of various concentration of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, alpha race of Lithoglyphopsis aperta, exposed in water and silt for 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	1/30	1/30	3.3	3.3	3.3
0.04	7/30	9/30	23.3	30.0	26.6
0.08	16/30	18/30	53.3	60.0	56.6
0.16	20/30	22/30	66.7	73.3	70.0
0.32	26/30	25/30	86.7	83.3	85.0
0.64	30/30	29/30	100.0	96.7	98.3
1.28	30/30	30/30	100.0	100.0	100.0
2.56	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 25. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of one hour.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.05	3/30	3/30	10.0	10.0	10.0
0.06	6/30	9/30	20.0	30.0	25.0
0.07	8/30	15/30	26.7	50.0	38.3
0.08	14/30	20/30	46.7	66.7	56.5
0.09	16/30	23/30	53.3	76.7	65.0
0.10	24/30	26/30	80.0	86.7	83.3
0.20	30/30	30/30	100.0	100.0	100.0
0.40	30/30	30/30	100.0	100.0	100.0
0.80	30/30	30/30	100.0	100.0	100.0
Control	0/30 0/30	1/30 0/30	0.0 0.0	3.3 0.0	1.7 0.0

Table 26. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 6 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.1	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	6/30	9/30	20.0	30.0	25.0
0.08	17/30	17/30	56.7	56.7	56.7
0.16	27/30	24/30	90.0	80.0	85.0
0.32	30/30	29/30	100.0	96.7	98.3
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 27. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 12 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	2/30	0/30	6.7	0.0	3.3
0.04	11/30	7/30	36.7	23.3	30.0
0.08	23/30	19/30	76.7	63.3	70.0
0.16	28/30	28/30	93.3	93.3	93.3
0.32	30/30	29/30	100.0	96.7	98.3
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 28. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	4/30	3/30	13.3	10.0	11.6
0.08	27/30	25/30	90.0	83.3	86.6
0.16	30/30	30/30	100.0	100.0	100.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 29. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult, alpha race of Lithoglyphopsis aperta, exposed in water and silt for 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	1/30	0/30	3.3	0.0	1.6
0.04	11/30	10/30	36.7	33.3	35.0
0.08	29/30	29/30	96.7	96.7	96.7
0.16	30/30	30/30	100.0	100.0	100.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 30. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, gamma race of Lithoglyphopsis aperta at the exposure time of one hour.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.05	0/30	0/30	0.0	0.0	0.0
0.06	2/30	2/30	6.7	6.7	6.7
0.07	3/30	3/30	10.0	10.0	10.0
0.08	6/30	5/30	20.0	16.7	18.3
0.09	5/30	10/30	16.7	33.3	25.0
0.10	10/30	14/30	33.3	46.7	40.0
0.20	26/30	24/30	86.7	83.3	85.0
0.40	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0



Table 31. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 6 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	3/30	2/30	10.0	6.7	8.3
0.04	24/30	21/30	80.0	70.0	75.0
0.08	30/30	29/30	100.0	96.7	98.3
0.16	30/30	30/30	100.0	100.0	100.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 32. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 12 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	5/30	5/30	16.7	16.7	16.7
0.04	24/30	19/30	80.0	63.3	71.6
0.08	30/30	30/30	100.0	100.0	100.0
0.16	30/30	30/30	100.0	100.0	100.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 33. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, gamma race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	3/30	2/30	10.0	6.7	8.3
0.04	11/30	8/30	36.7	26.7	31.7
0.08	18/30	22/30	60.0	73.3	66.6
0.16	27/30	25/30	90.0	83.3	86.6
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
1.28	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 34. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to young, gamma race of Lithoglyphopsis aperta, exposed in water and silt for 24 hours.

Concentrations (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	1/30	1/30	3.3	3.3	3.3
0.04	11/30	9/30	36.7	30.0	33.3
0.08	27/30	25/30	90.0	83.3	86.6
0.16	29/30	29/30	96.7	96.7	96.7
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 35. Effects of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult, gamma race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	2/30	2/30	6.7	6.7	6.7
0.04	19/30	20/30	63.3	66.7	65.0
0.08	28/30	29/30	93.3	96.7	95.0
0.16	30/30	30/30	100.0	100.0	100.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 36. Effect of various concentrations of Tritylmorpholine, FX 28, 16.5% a.i. (Frescon) to adult gamma race of Lithoglyphopsis aperta, exposed in water and silt for 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	3/30	1/30	10.0	3.3	6.7
0.04	14/30	10/30	46.7	33.3	40.0
0.08	22/30	23/30	73.3	76.7	75.0
0.16	30/30	30/30	100.0	100.0	100.0
0.32	30/30	30/30	100.0	100.0	100.0
0.64	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

APPENDIX V

Table 38. Effect of various concentrations of Sodium pentachlorophenate (NaPCP), 90% a.i. granules, to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.6	5/30	12/30	16.7	40.0	28.4
0.8	15/30	22/30	50.0	74.0	63.3
1.0	25/30	26/30	83.3	86.7	85.0
1.2	30/30	29/30	100.0	96.7	98.3
1.4	29/30	29/30	96.7	96.7	96.7
1.6	30/30	30/30	100.0	100.0	100.0
1.8	30/30	30/30	100.0	100.0	100.0
2.0	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

APPENDIX VI

Table 40. Effect of various concentrations of Yurimin, P. 99, granules, 5% a.i. to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.05	2/30	3/30	6.7	10.0	8.3
0.10	16/30	10/30	53.3	33.3	43.3
0.15	25/30	27/30	83.3	90.0	86.6
0.20	28/30	27/30	93.3	90.0	91.6
0.25	26/30	29/30	86.7	96.7	91.7
0.30	27/30	30/30	90.0	100.0	95.0
0.35	30/30	30/30	100.0	100.0	100.0
0.40	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 41. Effect of various concentrations of Yurimin, P. 99, granules, 5% a.i. to adult, alpha race of Lithoglyphopsis aperta at the exposure time of 48 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.05	2/30	2/30	6.7	6.7	6.7
0.10	24/30	22/30	80.0	73.3	76.6
0.15	29/30	29/30	96.7	96.7	96.7
0.20	30/30	30/30	100.0	100.0	100.0
0.25	30/30	30/30	100.0	100.0	100.0
0.30	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 42. Effect of various concentrations of Yurimin, P. 99, granules, 5% a.i. to young, gamma race of Lithoglyphopsis aperta at the exposure time of 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
	0.05	1/30	1/30	3.3	
0.06	5/30	7/30	16.7	23.3	20.0
0.07	14/30	19/30	46.7	63.3	55.0
0.08	17/30	20/30	56.7	66.7	61.7
0.09	22/30	25/30	73.3	83.3	78.3
0.10	28/30	30/30	93.3	100.0	96.6
0.11	30/30	30/30	100.0	100.0	100.0
0.12	30/30	30/30	100.0	100.0	100.0
0.13	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 43. Effect of various concentrations of Yurimin, P. 99, granules, 5% a.i. to young, gamma race of Lithoglyphopsis aperta at the exposure time of 48 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
	0.5	0/30	2/30	0.0	
0.06	11/30	14/30	36.7	46.7	41.7
0.07	22/30	20/30	73.3	66.7	70.0
0.08	24/30	24/30	80.0	80.0	80.0
0.09	28/30	29/30	93.3	96.7	95.0
0.10	30/30	30/30	100.0	100.0	100.0
0.11	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0



APPENDIX VII

Table 45. Response of young, alpha race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltinoxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 1-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	1/30	0/30	3.3	0.0	1.6
0.16	2/30	0/30	6.7	0.0	3.3
0.32	0/30	1/30	0.0	3.3	1.6
0.64	11/30	9/30	36.7	30.0	33.3
1.28	13/30	12/30	43.3	40.0	41.6
2.56	14/30	13/30	46.7	43.3	45.0
5.12	20/30	22/30	66.7	73.3	70.0
Control	0/30	0/30	0.0	0.0	0.0

Table 46. Response of young, alpha race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltinoxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 2-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	0/30	0/30	0.0	0.0	0.0
0.32	1/30	1/30	3.3	3.3	3.3
0.64	14/30	12/30	46.7	40.0	43.3
1.28	15/30	15/30	50.0	50.0	50.0
2.56	23/30	21/30	76.7	70.0	73.3
5.12	27/30	20/30	90.0	66.7	78.3
Control	0/30	0/30	0.0	0.0	0.0

Table 47. Response of young, alpha race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltinoxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 3-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	1/30	0/30	3.3	0.0	1.6
0.08	0/30	0/30	0.0	0.0	0.0
0.16	2/30	0/30	6.7	0.0	3.3
0.32	6/30	6/30	20.0	20.0	20.0
0.64	14/30	14/30	46.7	46.7	46.7
1.28	14/30	18/30	46.7	60.0	53.3
2.56	17/30	20/30	56.7	66.7	61.7
5.12	27/30	27/30	90.0	90.0	90.0
Control	0/30	0/30	0.0	0.0	0.0

Table 48. Response of young, alpha race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltinoxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 4-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed		Rep. I	Rep. II	
	Rep. I	Rep. II			
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	1/30	0/30	3.3	0.0	1.6
0.32	3/30	1/30	10.0	3.3	6.6
0.64	11/30	14/30	36.7	46.7	41.7
1.28	13/30	17/30	43.3	56.7	50.0
2.56	18/30	24/30	60.0	80.0	70.0
5.12	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 49. Response of young, alpha race of *Lithoglyphopsis aperta* to various concentrations (in ppm) of Tributyltin oxide (TBT0), pellets, 5.8% a.i. (Bio Met SRM), at 8-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	1/30	0.0	3.3	1.6
0.16	0/30	2/30	0.0	6.7	3.3
0.32	2/30	2/30	6.7	6.7	6.7
0.64	21/30	8/30	70.0	26.7	48.3
1.28	18/30	13/30	60.0	43.3	51.6
2.56	22/30	21/30	73.3	70.0	71.6
5.12	27/30	30/30	90/0	100.0	95.0
Control	0/30	0/30	0.0	0.0	0.0

Table 50. Response of young, alpha race of *Lithoglyphopsis aperta* to various concentrations (in ppm) of Tributyltin oxide (TBT0), pellets, 5.8% a.i. (Bio Met SRM), at 16-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	1/30	1/30	3.3	3.3	3.3
0.32	8/30	4/30	26.7	13.3	20.0
0.64	14/30	7/30	46.7	23.3	35.0
1.28	18/30	20/30	60.0	66.7	63.3
2.56	24/30	28/30	80.0	93.3	86.6
5.12	25/30	29/30	83.3	96.7	90.0
Control	0/30	0/30	0.0	0.0	0.0

Table 51. Response of young, alpha race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 32-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	1/30	2/30	3.3	6.7	5.0
0.16	4/30	6/30	13.3	20.0	16.6
0.32	10/30	8/30	33.3	26.7	30.0
0.64	15/30	12/30	50.0	40.0	45.0
1.28	21/30	18/30	70.0	60.0	65.0
2.56	28/30	28/30	93.3	93.3	93.3
5.12	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 52. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 1-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails		Percentage mortality		Mean percentage mortality
	No. of snails exposed				
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	0/30	0/30	0.0	0.0	0.0
0.32	0/30	0/30	0.0	0.0	0.0
0.64	2/30	4/30	6.7	13.3	10.0
1.28	6/30	5/30	20.0	16.7	18.3
2.56	9/30	7/30	30.0	23.3	26.6
5.12	10/30	10/30	33.3	33.3	33.3
Control	0/30	0/30	0.0	0.0	0.0

Table 53. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBTO), 5.8% a.i. (Bio Met SRM), at 2-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
	0.01	0/30	0/30	0.0	
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	0/30	0/30	0.0	0.0	0.0
0.32	1/30	1/30	3.3	3.3	3.3
0.64	5/30	11/30	16.7	36.7	26.7
1.28	5/30	2/30	16.7	6.7	11.7
2.56	15/30	18/30	50.0	60.0	55.0
5.12	27/30	25/30	90.0	83.3	86.6
Control	0/30	0/30	0.0	0.0	0.0

Table 54. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 3-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
	0.01	0/30	0/30	0.0	
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	1/30	0/30	3.3	0.0	1.6
0.32	5/30	5/30	16.7	16.7	16.7
0.64	5/30	11/30	16.7	36.7	26.7
1.28	11/30	22/30	36.7	73.3	55.0
2.56	21/30	24/30	70.0	80.0	75.0
5.12	28/30	30/30	93.3	100.0	96.6
Control	0/30	0/30	0.0	0.0	0.0

Table 55. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 4-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	1/30	1/30	3.3	3.3	3.3
0.32	2/30	3/30	6.7	10.0	8.3
0.64	8/30	13/30	26.7	43.3	35.0
1.28	8/30	23/30	26.7	76.7	51.7
2.56	27/30	28/30	90.0	93.3	91.6
5.12	29/30	29/30	96.7	96.7	96.7
Control	0/30	0/30	0.0	0.0	0.0

Table 56. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM), at 8-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	8/30	0/30	26.7	0.0	13.3
0.16	12/30	13/30	40.0	43.3	41.6
0.32	21/30	21/30	70.0	70.0	70.0
0.64	26/30	27/30	86.7	90.0	88.3
1.28	28/30	27/30	93.3	90.0	91.6
2.56	29/30	27/30	96.7	90.0	93.3
5.12	30/30	29/30	100.0	96.7	98.3
Control	0/30	0/30	0.0	0.0	0.0

Table 57. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBT), pellets, 5.8% a.i. (Bio Met SRM), at 16-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	1/30	0/30	3.3	0.0	1.6
0.16	2/30	3/30	6.7	10.0	8.3
0.32	5/30	6/30	16.7	20.0	18.3
0.64	20/30	18/30	66.7	60.0	63.3
1.28	26/30	24/30	86.7	80.0	83.3
2.56	30/30	29/30	100.0	96.7	98.3
5.12	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0

Table 58. Response of young, gamma race of Lithoglyphopsis aperta to various concentrations (in ppm) of Tributyltin oxide (TBT), pellets, 5.8% a.i. (Bio Met SRM), at 32-day soaking time. The exposure time was 24 hours.

Concentration (in ppm)	No. of dead snails No. of snails exposed		Percentage mortality		Mean percentage mortality
	Rep. I	Rep. II	Rep. I	Rep. II	
0.01	0/30	0/30	0.0	0.0	0.0
0.02	0/30	0/30	0.0	0.0	0.0
0.04	0/30	0/30	0.0	0.0	0.0
0.08	0/30	0/30	0.0	0.0	0.0
0.16	1/30	0/30	3.3	0.0	1.6
0.32	4/30	5/30	13.3	16.7	15.0
0.64	12/30	9/30	40.0	30.0	35.0
1.28	27/30	24/30	90.0	80.0	85.0
2.56	30/30	30/30	100.0	100.0	100.0
5.12	30/30	30/30	100.0	100.0	100.0
Control	0/30	0/30	0.0	0.0	0.0



APPENDIX VIII

Table 60. Showing response of young, alpha race of *Lithoglyphopsis aperta* to various concentrations of Tributyltin oxide (TBTO), pellets, 5.8% a.i., (Bio Met SRM) after exposure times from day 1 to day 33.

Exposure time (in days)	Control		Concentration of TBTO (in ppm)									
			0.01		0.02		0.04		0.08		0.16	
Day 1	0/30 0.0	0/30 <sup>a</sup> 0.0 <sup>b</sup> 0.0 <sup>c</sup>	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	1/30 3.3	0/30 0.0	2/30 6.7	0/30 0.0
Day 2	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	1/30 3.3	0/30 0.0	2/30 6.7	0/30 0.0
Day 3	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	1/30 3.3	0/30 0.0	2/30 6.7	0/30 0.0
Day 4	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	5/30 16.7	8/30 26.7	2/30 6.7	5/30 16.7	1/30 3.3	2/30 6.7	3/30 10.0	4/30 13.3
Day 5	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	5/30 16.7	10/30 33.3	4/30 13.3	7/30 23.3	9/30 30.0	14/30 46.7	17/30 56.7	14/30 46.7
Day 6	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	5/30 16.7	10/30 33.3	7/30 23.3	7/30 23.3	10/30 33.3	17/30 56.7	26/30 86.7	17/30 56.7
Day 7	0/30 0.0	0/30 0.0	0/30 0.0	5/30 16.7	9/30 30.0	12/30 40.0	11/30 36.7	23/30 76.7	18/30 60.0	25/30 83.3	30/30 100.0	30/30 100.0
				8.3	35.0		56.7		71.6		100.0	
Exposure time (in days)	Control		Concentration of TBTO (in ppm)									
			0.32		0.64		1.28		2.56		5.12	
Day 1	0/30 0.0	0/30 <sup>a</sup> 0.0 <sup>b</sup> 0.0	0/30 0.0	2/30 6.7	1/30 3.3	3/30 10.0	13/30 43.3	6/30 20.0	4/30 13.3	13/30 43.3	14/30 46.7	19/30 63.3
Day 2	0/30 0.0	0/30 0.0	0/30 0.0	2/30 6.7	1/30 3.3	3/30 10.0	17/30 56.7	6/30 20.0	4/30 13.3	21/30 70.0	14/30 46.7	19/30 63.3
Day 3	0/30 0.0	0/30 0.0	29/30 96.7	20/30 66.7	6/30 20.0	9/30 30.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0
Day 4	0/30 0.0	0/30 0.0	29/30 96.7	22/30 73.3	29/30 96.7	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0
Day 5	0/30 0.0	0/30 0.0	30/30 100.0	29/30 96.7	29/30 96.7	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0
Day 6	0/30 0.0	0/30 0.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0
Day 7	0/30 0.0	0/30 0.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0

a = number of dead snails/number of snails tested  
 b = percentage mortality  
 c = mean percentage mortality

Table 60 (Cont'd)

Exposure time (in days)	Control	Concentrations of TBTO (in ppm)																																									
		0.01		0.02		0.04		0.08		0.16																																	
Day 8	0/30 0/30 0.0 0.0 0.0	0/30 9/30 0.0 30.0 15.0	13/30 17/30 43.3 56.7 50.0	15/30 24/30 50.0 80.0 65.0	24/30 27/30 80.0 90.0 85.0	30/30 30/30 100.0 100.0 100.0	Day 9	0/30 0/30 0.0 0.0 0.0	0/30 11/30 0.0 36.7 18.3	13/30 18/30 43.3 60.0 51.6	22/30 26/30 73.3 86.7 80.0	24/30 29/30 80.0 96.7 88.3	30/30 30/30 100.0 100.0 100.0	Day 10	0/30 0/30 0.0 0.0 0.0	0/30 12/30 0.0 40.0 20.0	15/30 19/30 50.0 63.3 56.6	22/30 29/30 73.3 96.7 85.0	30/30 30/30 100.0 100.0 100.0	Day 11	0/30 0/30 0.0 0.0 0.0	10/30 18/30 33.3 60.0 46.6	18/30 28/30 60.0 93.3 76.6	27/30 30/30 90.0 100.0 95.0	30/30 30/30 100.0 100.0 100.0	Day 12	0/30 0/30 0.0 0.0 0.0	10/30 20/30 33.3 66.7 50.0	18/30 29/30 60.0 96.7 78.3	27/30 30/30 90.0 100.0 95.0	30/30 30/30 100.0 100.0 100.0	Day 13	0/30 0/30 0.0 0.0 0.0	10/30 20/30 33.3 66.7 50.0	18/30 29/30 60.0 96.7 78.3	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	Day 17	0/30 0/30 0.0 0.0 0.0	10/30 20/30 33.3 66.7 50.0	21/30 29/30 70.0 96.7 83.3	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0
Exposure time (in days)	Control	Concentrations of TBTO (in ppm)																																									
		0.32		0.64		1.28		2.56		5.12																																	
Day 8	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 9	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 10	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 11	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 12	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 13	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 17	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	

Table 60 (Cont'd)

Exposure time (in days)	Control	Concentrations of TBTP (in ppm)																																							
		0.01		0.02		0.04		0.08		0.16																															
Day 18	0/30 0/30 0.0 0.0 0.0	11/30 21/30 36.7 70.0 53.3	22/30 30/30 73.3 100.0 86.6	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 19	0/30 0/30 0.0 0.0 0.0	12/30 23/30 40.0 76.7 58.3	22/30 30/30 73.3 100.0 86.6	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 21	0/30 0/30 0.0 0.0 0.0	12/30 23/30 40.0 76.7 58.3	23/30 30/30 76.7 100.0 88.3	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 26	0/30 0/30 0.0 0.0 0.0	12/30 23/30 40.0 76.7 58.3	27/30 30/30 90.0 100.0 95.0	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 31	0/30 0/30 0.0 0.0 0.0	15/30 24/30 50.0 80.0 65.0	27/30 30/30 90.0 100.0 95.0	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 33	0/30 4/30 0.0 13.3 6.7	16.30 27/30 53.3 90.0 71.7	27/30 30/30 90.0 100.0 95.0	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0

Exposure time (in days)	Control	Concentrations of TBTP (in ppm)																																			
		0.32		0.64		1.28		2.56		5.12																											
Day 18	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 19	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 21	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 26	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 31	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	Day 33	0/30 4/30 0.0 13.3 6.7	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0

Table 62. Showing response of adult, alpha race of *Lithoglyphopsis aperta* to various concentrations of Tributyl tin oxide (TBTO), pellets, 5.8% a.i., (Bio Met SRM) after exposure times from day 1 to day 33.

Exposure time (in days)	Control		Concentrations of TBTO (in ppm)										
			0.01		0.02		0.04		0.08		0.16		
Day 1	0/30 0.0 0.0 <sup>c</sup>	0/30 <sup>a</sup> 0.0 <sup>b</sup>	0/30 0.0 0.0	0/30 0.0	0/30 0.0	0/30 0.0	0/30 0.0	3/30 10.0	0/30 0.0	3/30 10.0	2/30 6.7	3/30 10.0	6/30 20.0
Day 2	0/30 0.0 0.0	0/30 0.0	1/30 3.3 1.6	0/30 0.0	3/30 10.0 8.3	2/30 6.7	11/30 36.7 21.7	2/30 6.7	7/30 23.3	5/30 16.7	20.0	3/30 10.0	9/30 30.0
Day 3	0/30 0.0 0.0	0/30 0.0	3/30 10.0 5.0	0/30 0.0	11/30 36.7 25.0	4/30 13.3	27/30 90.0 73.3	17/30 56.7	9/30 30.0	10/30 33.3	31.6	11/30 36.7	25/30 83.3
Day 4	0/30 0.0 0.0	0/30 0.0	4/30 13.3 10.0	2/30 6.7	25/30 83.3 61.6	12/30 40.0	28/30 93.3 81.6	21/30 70.0	22/30 73.3	19/30 63.3	68.3	30/30 100.0	30/30 100.0
Day 5	0/30 0.0 0.0	0/30 0.0	6/30 20.0 26.6	10/30 33.3	29/30 96.7 98.3	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 6	0/30 0.0 0.0	0/30 0.0	8/30 26.7 31.7	11/30 36.7	29/30 96.7 98.3	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 7	0/30 0.0 0.0	0/30 0.0	11/30 36.7 40.0	13/30 43.3	30/30 100.0 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Exposure time (in days)	Control		Concentrations of TBTO (in ppm)										
			0.32		0.64		1.28		2.56		5.12		
Day 1	0/30 0.0 0.0 <sup>c</sup>	0/30 <sup>a</sup> 0.0 <sup>b</sup>	18/30 60.0 66.6	22/30 73.3	21/30 70.0 75.0	24/30 80.0	25/30 83.3 86.6	27/30 90.3	29/30 96.7	39/30 96.7	96.7	30/30 100.0	30/30 100.0
Day 2	0/30 0.0 0.0	0/30 0.0	25/30 83.3 83.3	25/30 83.3	21/30 70.0 85.0	30/30 100.0	27/30 90.0 95.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 3	0/30 0.0 0.0	0/30 0.0	30/30 100.0 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 4	0/30 0.0 0.0	0/30 0.0	30/30 100.0 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 5	0/30 0.0 0.0	0/30 0.0	30/30 100.0 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 6	0/30 0.0 0.0	0/30 0.0	30/30 100.0 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0
Day 7	0/30 0.0 0.0	0/30 0.0	30/30 100.0 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	30/30 100.0	100.0	30/30 100.0	30/30 100.0

a = number of dead snails/number of snails tested  
 b = percentage mortality  
 c = mean percentage mortality





Table 64. Showing response of adult, gamma race of *Lithoglyphopsis aperta* to various concentrations of Tributyl tin oxide (TBTO), pellets, 5.8% a.i. (Bio Met SRM) after exposure times from day 1 to day 10.

Exposure times (in days)	Control	Concentrations of TBTO (in ppm)									
		0.01		0.02		0.04		0.08		0.16	
Day 1	0/30 0/30 <sup>a</sup> 0.0 0.0 <sup>b</sup> 0.0 <sup>c</sup>	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0
Day 2	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	0/30 0/30 0.0 0.0 0.0	22/30 20/30 73.3 66.7 70.0	25/30 23/30 83.3 76.7 80.0	13/30 21/30 43.3 70.0 56.6	29/30 29/30 96.7 96.7 96.7				
Day 3	0/30 0/30 0.0 0.0 0.0	3/30 3/30 10.0 10.0 10.0	26/30 28/30 86.7 93.3 90.0	27/30 28/30 90.0 93.3 91.6	21/30 27/30 70.0 90.0 80.0	30/30 20/30 100.0 96.7 98.3					
Day 4	0/30 0/30 0.0 0.0 0.0	7/30 10/30 23.3 33.3 28.3	26/30 29/30 86.7 96.7 91.7	30/30 29/30 100.0 96.7 98.3	28/30 30/30 93.3 100.0 96.6	30/30 30/30 100.0 100.0 100.0					
Day 5	0/30 0/30 0.0 0.0 0.0	17/30 19/30 56.7 63.3 60.0	27/30 30/30 90.0 100.0 95.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 6	0/30 0/30 0.0 0.0 0.0	19/30 25/30 63.3 83.3 73.3	29/30 30/30 96.7 100.0 98.3	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 7	0/30 0/30 0.0 0.0 0.0	22/30 26/30 73.3 86.7 80.0	29/30 30/30 96.7 100.0 98.3	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Exposure time (in days)	Control	Concentrations of TBTO (in ppm)									
		0.32		0.64		1.28		2.56		5.12	
Day 1	0/30 0/30 <sup>a</sup> 0.0 0.0 <sup>b</sup> 0.0 <sup>c</sup>	0/30 0/30 0.0 0.0 0.0	9/30 11/30 30.0 36.7 33.3	21/30 12/30 70.0 40.0 55.0	25/30 21/30 83.3 70.0 76.6	27/30 22/30 90.0 73.3 81.6					
Day 2	0/30 0/30 0.0 0.0 0.0	23/30 23/30 76.7 76.7 76.7	28/30 27/30 93.3 90.0 91.6	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 3	0/30 0/30 0.0 0.0 0.0	28/30 29/30 93.3 96.7 95.0	29/30 29/30 96.7 96.7 96.7	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 4	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 5	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 6	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					
Day 7	0/30 0/30 0.0 0.0 0.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0	30/30 30/30 100.0 100.0 100.0					

a = number of dead snails/number of snails tested  
 b = percentage mortality  
 c = mean percentage mortality





