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FOREWORD

In spite of our advanced state of civilization, we still endow numbers with special significance. For example, the number 50 has taken on an importance only second to 100. The fiftieth year of any institution must be celebrated with elaborate ceremonies even if the 25th or the thirtieth have been passed by unnoticed. The editor of STERKIANA is no exception to the rule and as the fateful fiftieth number loomed nearer and nearer, he felt that the most appropriate way to celebrate the occasion would be to publish an especially significant paper by someone whose reputation was of such proportions that some of its brilliance would rub off on these pages.

Ordinarily, such a paper is hard to find and it is often a command performance by busy people who can do no better than to summarize some of their earlier work and emphasize its significance. In our case, events fortunately did not take that course and by a happy conjuncture, a paper of some size, by two authors of considerable reputation, happened to be ready and available for publication.

It would be tedious for the reader to repeat here the long list of works which have made Henry van der Schalie and Elmer Berry leaders in their field, and the long succession of malacologists who received their graduate training with them. The mere listing of their names as authors of the following paper is enough indication of its importance. The editor is delighted to present to the readers of STERKIANA this fiftieth number by two of his special friends of very long standing whom it is a pleasure to honor in this way.

A. L.

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ABSTRACT

The effects of temperature on the biology of freshwater snails were studied using two lymnaeids *(Lymnaea stagnalis* and *L. emarginata)*, three planorbids *(Helisoma trivolvis, H. anceps,* and *H. campanulatum)*, and one physid *(Physa gyrina)*--all pulmonate 'pond' snails; only one gill-breathing operculate *(Amnicola limosa)* was tested. Both growth and egg-laying were measured in several temperatures ranging from 6°C to 36°C. The gonad development was determined through serial paraffin sections which were stained with haematoxylon and eosin; reproduction was measured in terms of egg-laying. The data show that the lymnaeids grow best at 22°C and above. In contrast, the planorbids tend to grow better under warmer conditions (about 25°C); however, when 30°C is reached growth may appear better but reproduction is inhibited. The physids tolerate the widest range, sometimes conditions warmer than 30°C, although at this temperature reproduction is also inhibited. The one operculate, *Amnicola limosa*, studied showed a preference for cool conditions and was more like the lymnaeids in temperature responses.

Each of the seven species of animals studied was stressed in aquaria with water maintained at 6o, 12°, 18°, 24°, 30°, and 34° or 36°C. The range varied with the groups as indicated but none could be cultured much below 12°C and none would reproduce when temperatures exceeded 30°C. Growth was usually better in 'warm' water but advantages in growth were generally offset by a lack of gonad development.

Mollusks, like other animals cited, are very sensitive to ambient temperatures. Even small changes are shown to be important in their influence on the environmental area affected. Studies are encouraged to determine the effects of temperature changes well in advance of projected developments such as the use of natural waters for cooling reactors, e.g.--'improvements' that could adversely affect the flora and fauna of the surrounding region.

SECTION I. CONCLUSIONS

1. Mollusks in nature make up much of the biomass; optimal temperatures are shown to be important requisites both for reproduction and growth in this group.

2. This temperature-stressing study demonstrated three reproductive patterns among the seven species of aquatic snails tested:

(a) the lymnaeids reproduce and thrive best in cool (19° to 22°C) conditions;

(b) the planorbids require warmer water (22° to 25° C);

(c) the physids are highly tolerant and can maintain themselves in a much wider temperature range (12° to 30°C).

The gill-breathing operculate snail (as opposed to the pulmonate above), *Amnicola limosa* showed a preference for 'cool' conditions similar to that of *Lymnaea stagnalis*.

3. Sensitivity of aquatic mollusks to temperature is surprisingly narrow. Although an increase in size might be interpreted as optimum for the well-being of a given species, the present study demonstrated that gonad development is arrested even in slightly higher temperatures. Raising the optimum temperature as little as 5° C was demonstrated to cause disastrous effects among the sensitive species.

4. The circumpolar snail, *Lymnaea stagnalis*, no longer inhabits southern Michigan lakes, but occurs 150 miles further north. Its disappearance is probably due to the slightly higher temperatures in lakes in recent decades, sufficient to cause critical breeding failures.

5. Physids showed wide tolerance to heat fluctuations. This capacity explains their wide distribution and dominance under adverse conditions, e.g., sewage disposal facilities (where they clog sieves), thermal springs, etc.

6. In 'plop tests' it appeared that pulmonate snails withstood sudden changes of higher temperature better than sudden chilling. More studies on behavior due to changing temperatures are projected.

7. Techniques for culturing these particular snails have been reasonably well developed. The pulmonates, especially, could serve as important tools for studying not only the role of temperature but other environmental factors as well. The cycle for *Lymnaea stagnalis* from egg to adult is 4 months; for the other snails studied a 2-month period is required.

8. For most invertebrates studied, the normal functional range lies somewhere between 12° and 30°C; environmental changes that fall to either side of those limits are apt to be destructive to the fauna.

SECTION II. RECOMMENDATIONS

Every effort should be made to maintain the original, normal and natural situations in the environment and to preserve as much as possible the existing ecological conditions. The dangers, as pointed out by Cairns (1968) in his article, 'We're in hot water,' can no longer be ignored. The recommendations that follow essentially substantiate what he has already stressed so well based on his wide field and laboratory experience. After outlining the way power plants operate, he stated:

> 'At present we are throwing most of this heat away, as we do with many other waste products, and it is capable of polluting our environment just as surely as do sewage, industrial waste and agricultural waste.'

Figures given on the amounts of heated waste water that will return to a river, estuary, ocean or lake are astounding. The elimination of species will depend on their sensitivity, and we have now just begun to measure how sensitive some of them are. The problem is compounded because the higher temperatures in water make less dissolved oxygen available to the aquatic animals. In view of these conditions, field survey work deserves far more encouragement than it is receiving. Such studies should be both qualitative and quantitative and should serve to predict the probable changes heated waters would produce.

It is obvious that much more laboratory work is needed to assay the effects of temperature on a variety of biotic groups that will be affected. The prospective changes caused by elevated temperatures have also been outlined by Cairns (1968: 190) as: 'death through direct effects of heat; internal functional aberrations (reduced oxygen, disruption of food supply decreased resistance to toxic substances); interference with spawning or other critical activities in the life cycle; competitive replacement by more tolerant species as a result of the above physiological effects.' These several effects were clearly evident in the experiments carried out and cited in this report. For example, while the snails grew well in warmer than usual water, they often failed to reproduce. The acclimatization shown could remove the normal occupants such as lymnaeids and allow the establishment of conditions that might favor planorbids like *Biomphalaria*, which can serve as intermediate hosts for schistosomiasis. More studies are needed especially with the operculate, gill-breathing snails for which there was not sufficient time in this project.

When field conditions have been measured and the controlled laboratory studies have been undertaken, sites should be monitored to assay the changes in the 'biological, chemical and physical effects' brought about by the source of (in this case) heat pollution. Some groups lend themselves better for such monitoring studies, and mollusks, without question, are among the best. Unfortunately, they have not had nearly the attention they deserve. Not only are they widespread and common but, in terms of biomass, they are far superior to insects which have been considered as indices of heat pollution. Yet, too little basic work has been done with mollusks which are often ideal as tools for assaying environmental conditions. It is now possible to maintain a number of the common species in culture. Some groups, such as the mussels, already are established by M.M. Ellis (1937) as important in studies for the 'detection and measurement of stream pollution.' The role of many mol-

lusks for determining the ecology during the past, the present and the future deserves far more attention than it has hitherto received. Studies of immediate interest involve:

(1) the use of mussels as natural filtration agents in streams where they may serve in programs designed to alleviate eutrophication;

(2) the potential use of mussels as monitors for measuring the amount and the time of atomic fall-out as was demonstrated by Nelson (1964) at Oak Ridge;

(3) projecting tolerances to temperature, as well as other factors, as determined in the laboratory better to understand conditions during interglacial periods when mollusk deposits were among the most prominent among the animals preserved.

Specific recommendations include:

1) More 'plop' tests are needed to understand the levels of tolerance to sudden temperature changes among such a large and prominent group as the mollusks.

2) Too little is known, as yet, about the effects of temperature on the gill-breathing operculate snails (such as the pleurocerids, viviparids, amnicolids, etc.) that often pave the bottom of streams and lakes. The success of such investigations will depend on the ability to maintain these animals in culture to make the necessary tests.

3) It has been established that trematode infections are often regulated by the temperatures to which their mollusk hosts are subjected. These factors directly control some of the serious human diseases such as blood fluke (schistosomiasis) and 'swimmer's itch' (schistosome dermatitis). While studies have been initiated in our laboratory in this field, more work is needed to determine the effects of temperature on the development of the parasites in their intermediate hosts.

4) If additional heat should enter rivers and lakes to produce 'blooms' of algae, such natural filtration agents as the freshwater mussels, which often are found in large populations in the affected area, should be tested to determine whether they could effectively serve in this capacity.

5) Since it has been shown that certain species of mollusks are definitely heat sensitive, it should be possible to use some of those groups for testing tolerance in areas suspected of being subjected to 'heat pollution' of 'calefaction.'

6) More studies are needed on the 'fingernail clams' (Sphaeriidae), a widespread and common group in the benthic fauna; they are also important in the food chain (fish food, etc.). Their life histories are virtually unknown, let alone their temperature tolerances.

7) More detailed knowledge of the role of temperature on the biota should be obtained to understand fully the nature of such problems today as heat pollution. It also could provide the information needed by the paleoecologist to help in interpreting the type of climate present in various geological horizons. Most of the biogeographical interpretation of the Great Lakes region in its geologic past will depend on some careful delineation of the temperature tolerances in several of the widely distributed mollusk groups.

8) The behavior patterns of mollusks are closely related to ambient temperatures; it is necessary, therefore, not only to ascertain their ability to withstand sudden changes (as in the 'plop' tests) but to learn how they orient themselves under normal and abnormal temperature stress. A neglected area of research is that of the effect of thermal stress on behavior; on the rate of change of activity patterns (i.e., movement, respiration, feeding), alterations in copulatory and egg-laying, and changes in horizontal and vertical migrations. Especially needed are studies dealing with the effects of short-term (i. e. hours) and long-term (i. e., days) thermal stress on behavior, as well as studies comparing the effects of fluctuating as opposed to constant elevated temperatures.

SECTION III. INTRODUCTION

While there are many references indicating that temperature plays an important role in the biology of animals, it is often difficult to find specific information dealing with its influence on species or species groups. As pointed out by Cairns (1972:831), many good references to papers on the 'effects of increased temperatures in the aquatic environment' are published in Raney and Menzel (1969) on fishes, and in Holdaway *et al.*, (1969). While information relating to field situations is of a rather general kind, there are several basic studies showing the importance of temperature in nature. The earliest references were those of Hogg (1854) and Semper (1881) who studied field conditions for *Lymnaea stagnalis*. Both R. M. DeWitt (1954) in Michigan and W. F. DeWit (1955) in the Netherlands recognized the importance of field temperatures in their studies with species belonging to the Physidae. McNeil (1959, 1960, 1961 and 1963) studied winter survival of both *Lymnaea* and *Physa* snails in the Columbia drainage system in the state of Washington. Schiff (1963, 1966), Sturrock (1966) and Jobin (1970), considered the role of temperature in the bionomics of the planorbid snails that serve as intermediate hosts to the blood parasite which causes the disease, schistosomiasis, in Africa and Puerto Rico. It was clearly shown from their field studies of these tropical species that there is a tendency for planorbids to thrive better in warmer water.

The most definitive and earliest basic temperature study in the laboratory with a planorbid species, *Biomphalaria* (formerly *Australorbis) glabrata,* was that of Standen (1952). Michelson (1961), also working with *B. glabrata,* discovered that 30°C was the optimal temperature for maximum growth, but in that range reproduction was inhibited. Schiff (1963, 1967) discovered that *Bulinus (Physopsis) globosus,* which also belongs to the Planorbidae, can just maintain itself at 18°C but that its optimum temperature is 25°C. Sturrock's (1966) laboratory investigations with *B. pfeifferi* led him to the belief that the intermediate host snails for *S. mansoni,* when exposed in nature to temperatures 'in excess of 28–30°C for several months on end' cannot persist in such habitats.

Chernin (1967) compared *B. glabrata* and *Bulinus truncatus* with *Lymnaea palustris* in a thermal gradient. Whereas the former 'were generally found in moderately warm loci, *L. palustris* tended to accumulate in the relatively cool portions of the gradient, and *Bulinus truncatus* distributed themselves fairly uniformly throughout the gradient.' Jobin (1970) studied *B. glabrata* living in three farm ponds in Puerto Rico and found that optimum temperature for oviposition was 25°C 'with oviposition diminishing to zero as the temperatures approach 20°C and 30°C.'

The best summary statement dealing with 'acclimation in molluscs' appears to be that by Segal (1961) who stated:

> 'We now know that growth rates, and other rate functions of poikilotherms from different environments, do not differ as much as expected from the temperature differences between environments. Poikilotherms, which are passive conformers to the environmental temperature, show compensatory changes in growth rates and metabolic rates in response to the temperatures encountered in different latitudes (Fig. 1), seasons (Fig. 2), and microgeographic areas (Fig. 3).'

As indicated by the several references cited above, the majority of the studies which have been made, particularly with species in the family Planorbidae, are restricted to the tropics, e.g., *Biomphala-*

ria pfeifferi, B. glabrata, Bulinus truncatus, etc. Comparatively little is known regarding the effect of temperature in temperate regions in spite of the fact that the planorbid species in the Great Lakes areas aremore numerous and diversified than those found in the tropics. These species are ofparticular importance in that they constitute a major food item of fish. Thermal pollution has become a most serious factor in the Great Lakes region because of the number of thermal reactors installed in lakes and rivers in order to generate electricity. More are being planned for installation in the near future. What its effect will be on the molluscan fauna has not been known.

The objective of the present study was to measure the influence of temperature, ranging between 6°C and 36°C, on the growth and reproduction of both pulmonate (pond) snails and operculate (gill-breathing) snails.

SECTION IV. MATERIALS AND METHODS

All of the cultures were placed in 8-gallon aquaria filled with circulating spring water at room temperature(about 25°C). Small chips of crushed limestone were added to produce stronger shells. By means of a continuous flow system coming from a chiller the water in each tank was gradually heated to the desired temperature (ranging between 6°C and 34° or 36°C) with heat probes controlled by electronic monitors.

The equipment used for maintaining constant temperatures (Figure 1) consisted of a large fiberglass storage tank holding 140 gallons of spring water. This water was cooled to 4°C by a cooling unit or chiller inserted in a 55-gallon, insulated drum. A submersible water pump in the drum supplied a continuous supply of water to the fiberglass storage tank, with an 'overflow' elbow to prevent flooding. The chilled water ran as a continuous gravity flow into the several 8-gallon aquaria. The enlarged section in the diagram (Figure 1) shows: aheat probe and the thermoregulator connected to the heat-regulating relay box; the drain-hole at the rear of the aquarium to permit overflow water to return to the chiller by a gravity drain pipe; the compressed air tube supplying continuous bubbling of air to the aquarium; the floating C° thermometer; and the protective plastic screens to prevent snails from 'going down the drain' at one end and from being burned by the heat probe at the other end. The aquaria were encased in boxes made of styrofoam and covered with glass lids, each with a corner cut to admit the several tubes. Fluorescent lights were regulated to encourage algal growth which could serve as supplemental snail food.

Oxygen determinations were made with Oxygen Meter Model 54 (Yellow Springs Instrument Co., Inc.) as indicated in the tables; pH was measured and tabulated (using a meter made by Analytical Measurements, Inc.). The oxygen and pH were always at a sufficiently high level to maintain good tolerance levels. The water kept in circulation was from the Arbor Springs Water Company supply and was of a reasonably well-balanced grade as shown in the following table.

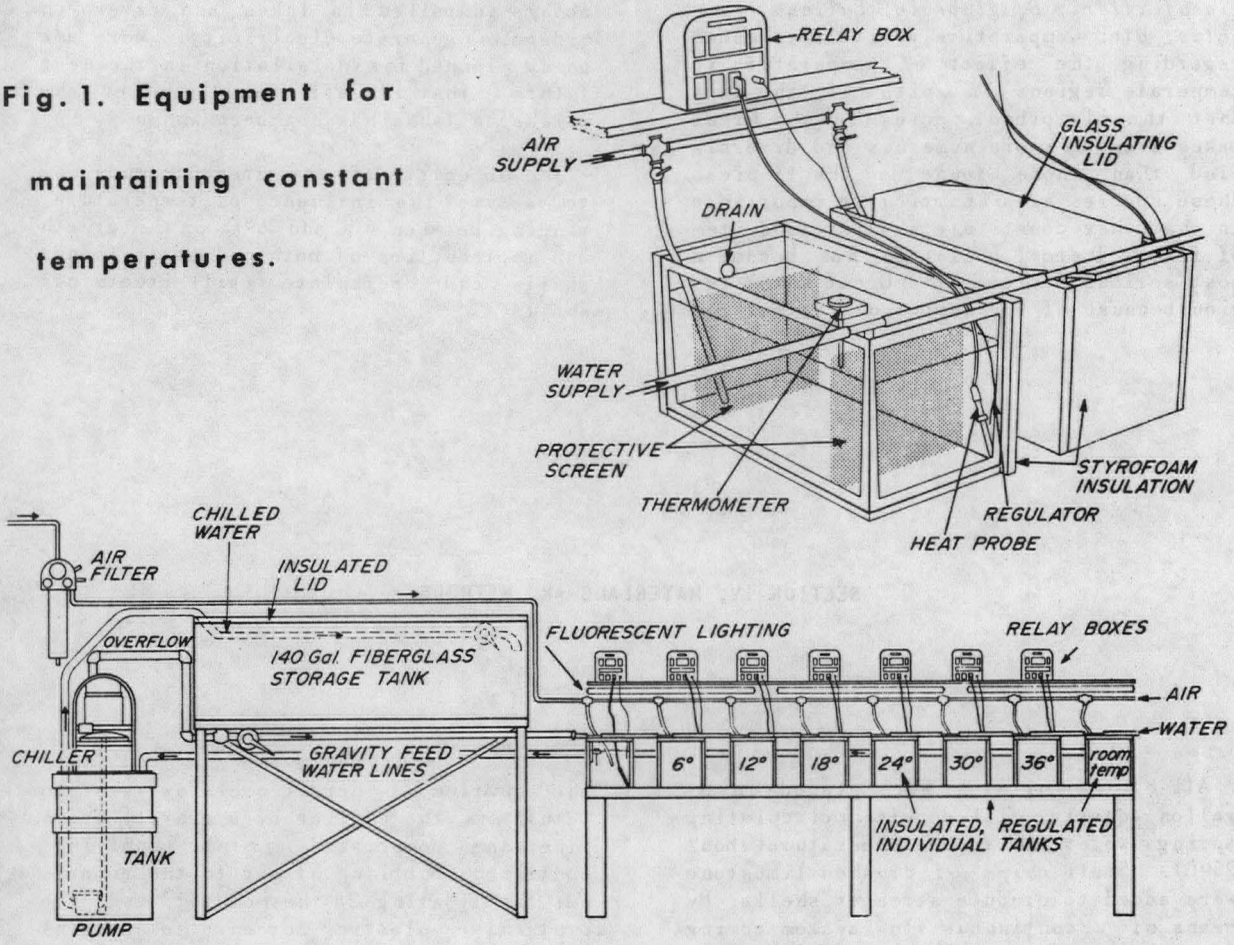


Fig. 1. Equipment for maintaining constant temperatures.

ANALYSIS OF ARBOR SPRING WATER

	Parts per Million	Grains per Gallon
Total Solids (Residue on evaporation)	481.0	28.13
Calcium (Ca)	110.0	6.43
Alkalinity in terms of $CaCO_3$		
Normal carbonate	11.7	0.68
Bicarbonate	289.2	16.91
Hardness in terms of $CaCO_3$	394.8	23.9
Chlorides (Cl)	40.0	2.34
Iron (Fe)	0.06	0.003
Sulphates (SO_4)	90.9	5.32
Sodium (Na)	26.2	1.53
pH	7.95 (not in ppm)	

A theoretically possible combination of the salts present is as follows:

Sodium Chloride	65.9	3.85
Sodium Sulphate	0.9	0.053
Magnesium Sulphate	50.1	2.93
Calcium Sulphate	70.8	4.14
Magnesium Carbonate	66.2	3.87
Calcium Carbonate	222.7	13.02

The snails were laboratory reared and all shells smaller than 16 mm were measured with ocular micrometers; larger snail sizes were determined with a ruler under the dissecting microscope. Data obtained was not on growth alone but also on egg-laying (followed by serial sectioning for cytological studies), sites of egg deposition, number of eggs laid, size of clutches, and even viability when it was possible to determine.

The first tests were usually made at 6°C intervals, namely: 6°, 12°, 18°, 24°, 30° and 34°C, using room temperature as control; these were followed by tests at 2°C intervals within what appeared to be the likely tolerated ranges. In studying reproduction, adjustments were made to determine the percentage of snails remaining (X%); the number of eggs laid during the period was then nultiplied by 100% - X% and added to the sum.

In addition to the egg outtput, reproduction was studied using microscopic gonad sections. At the end of an experiment the animals from each aquarium were relaxed in sodium nembutal, killed and fixed in Bouin's fluid, sectioned at 10 μ using the paraffin technique, and finally stained with Harris haematoxylon and an eosin counterstain. The microphotographs were made at X 24, X 70 and X 400.

The oxygen figures in the tables are often too high in terms of what would be expected at the given temperatures. This condition is undoubtedly due to the continuous bubbling of air in the tanks and the method of sampling. It should be realized that the air content of the water is not given to show accurate oxygen levels but rather to indicate that the animals were maintained in conditions with sufficient oxygen so as not to create special problems that might be brought about through poor aeration.

SECTION V. PULMONATE AND OPERCULATE SNAILS TESTED

Lymnaea stagnalis L.

This large, circumpolar species is one of the most extensively studied mollusks. It is not surprising that Hogg (1854), studying its development and growth but a century ago, considered temperature only as it related to the development of the eggs. Some basic and pioneering work by Semper (1881) was concerned largely with the dwarfing or stunting of these snails when raised under 'crowded' conditions, but he did indicate that temperature was decisive in its influence when it reached either of its extremes. One of the first attempts to measure the influence of temperature was a study by Bachrach and Cardot (1924), whose interests were mainly along the lines of development. They observed growth in temperatures between 7.5°C and 33°C in ten steps about 2°C apart. Their optimum was 23°C, but since 'development did not continue to completion' they set the optimum at 21°C. In a series of studies on *Lymnaea stagnalis* in Michigan, Crabb (1929) emphasized the effects on growth of crowding, food, pollution and 'media',' stating (1929: 52):'Temperature and light are undoubtedly factors in the growth of this snail, but our preliminary work does not indicate the quantity or the quality which might be tolerated.'

Cheatum (1934) published one of the most informative papers dealing with the effects of temperature on snail behavior in nature. He indicated that the 9 species of pulmonates (pond snails) inhabiting the shores of Douglas Lake in northern Michigan near Cheboygan had an annual migratory cycle which was seasonally regulated and showed that the animals (4 lymnaeids, 3 planorbids and 2 physids) adjusted to the temperature changes by moving to deeper water in the fall, remaining at deeper levels in the winter, and coming back on the shores with the approach of spring and summer. As shown by laboratory studies, this migration pattern appeared to be regulated to the need for 'breathing' surface air in the process of filling the pulmonary chamber when, with increased meta-

bolism at higher temperatures, there was less oxygen available for cutaneous respiration. His laboratory data indicated that in water with 1.7 cc of oxygen per liter, the animals used surface air three times oftener than when the water contained 6.4 cc per liter.

A number of questions remain unanswered. Are there critical temperatures that trigger migration? Do the several groups and species respond in the same way? What are some of the depths to which the animals descend? When the animals are said to be 'active' in deeper water during the winter periods, what is meant by active? Are there differences with respect to movements depending on the size of the animals involved? Studies have been made, and are now being prepared for publication, of observations on one of these snails, *Lymnaea emarginata* (also more recently called *L. catascopium)* which has remained on the shoals of Lake Ann, north of Interlochen, Michigan. For the past two summers, Clampitt has been studying the distribution and migration of the snails on the shores of Douglas Lake. His studies will provide substantial information on the movement of those snails.

Forbes and Crampton (1942) studied *Lymnaea palustris* from two sites--one at Croton, New York, the other a mill-pond in Newton, Connecticut. These animals were also studied experimentally in the laboratory. They concluded that:

> 'Variation in *Lymnaea palustris* is exhibited in fertility, rate of growth, size, and longevity. Individual, family and group differences recur in successive generations in spite of the essential uniformity of the laboratory conditions in culture; hence their genetic causation seems probable.'

In this study the role of temperature was not assessed although, as will be noted later, it plays an important role in the dynamics of the snail population.

Cort, McMullen, Oliver and Brackett (1940), in a series of studies relating to schistosome dermatitis (swimmer's itch) in northern Michigan, especially in the vicinity of the University of Michigan Biological Station, relate both the development of the key (then) snail host, *Lymnaea* (called *Stagnicola* by them) *emarginata angulata,* as well as the occurrence of the infectious cercariae to seasonal conditions. Temperatures are not given but it is evident from their observations that the heat budgets in the region play an important part. They stated (1940:64):

> 'Our records show that reports of dermatitis first begin to come in late in June and that the worst outbreaks usually occur in early July especially during and immediately after spells of hot weather.'

Both the development of the snail hosts and the fate of the trematode parasites they carry are closely allied to the seasonal temperature changes. The dynamics of these temperature-controlled patterns as observed in nature are in need of far more study than hitherto given.

Fraser (1946) studied the embryology of the reproductive tract of *Lymnaea stagnalis.* He was careful to maintain a temperature of 22°C to 25°C in the jars in which the eggs were developing. At temperatures that averaged 23°C it took the eggs between 13 and 15 days to hatch. These temperatures will be referred to later since they are close to the optimum needed in the development of this snail. Vaughn (1953), who like Fraser worked with L. E. Noland st the University of Wisconsin, studied the effects of temperature on both growth and hatching. He discovered that *L. stagnalis* eggs will hatch within a range of 9.9°C to 28°C; at 16°C it takes the eggs 59 days to hatch. At the higher temperatures, particularly when it reached 27.5°C, the egg masses exhibited unequal development, and when the temperature reached 32°C, development was extremely unequal and all the embryos 'were dead in 10 days.' Vaughn cites the work of Imai (1937) on *Lymnaea japonica* in which the hatching time took about 8 days at 28°C and 16 days at 18°C. Imai observed that at lower temperatures the snails tended to become large while in warm conditions larger amounts of energy were expended and the snails tended to remain smaller.

The studies on growth undertaken by Vaughn (1953) were somewhat similar to those reported here. He used the shell

length as his measure. Nine cultures of 50 snails each (measuring between 6 and 15 mm at the beginning of the experiment) were maintained at the following temperatures: 3.4°, 6.9°, 11.5°, 15.7°, 20.1°, 24.2°, 28.1°, 32° and 36°C. The control was 24.1°C. Lettuce was given to them for food. Since Holm (1946) had determined that snails 25-30 mm in length were sexually mature, Vaughn terminated his experiments after 6 weeks when the snails had doubled in size. After the first week the snails in the 23° and 36°C tanks succumbed; those ator below 6.9°C failed to develop. He stated that 'biological zero' for this snail was around 11°C. Vaughn (1953:224) concluded:

> 'Even though the growth was more rapid at 24.2°C than at lower temperatures, it was evident in both jars run at that temperature that the mortality was somewhat higher than at 15.7° and 20.1°C. The mortality figures suggest that the optimum temperature for the growth and development of *Lymnaea stagnalis appressa* lies somewhere between 16° and 20°C.'

It is also of interest that Belehradek (in Vaughn, 1953: 225) listed 12°C as the biological zero of *Lymnaea*.

Studies on the life history and growth of a smaller Lymnaeid *(Lymnaea humilis)* were published by McCraw (1961). His observations were largely concerned with the animals in the field where he found that 'little growth occurred from the months of December to March when both the air and water temperatures were below 10°C. Since the initiation of both growth and oviposition was controlled by temperature there were no egg masses 'until April when the water and air temperatures were 13° and 20°C respectively.' Also, a study of field conditions undertaken by McNeil (1963) to test the winter survival of *Lymnaea palustris nuttalliana* in irrigation canals of the Columbia Basin of Washington indicated that about a fourth of them survived winter in the out-of-doors between late October and late March; he also reported that 'the large-sized snails survived better than small snails.'

For several years intensive studies of many kinds have been undertaken with *Lymnaea stagnalis* in the biological laboratory of the Free University in Amsterdam. In a study by Joosse (1964) on the dorsal bodies and neurosecretory cells of the cerebral ganglia, he found (p. 95) that:

> 'The ovotestes showed a periodicity of the spermatogenetic activity parallel with that of the dorsal cells and bodies. Starting in March spermatogenesis was most active in April. The majority of the ripe sperm-cells appeared in the ovotestes in May. The percentage distribution of the three types of female sex-cells (early oocytes, oocytes and degenerating oocytes) showed no change throughout the year.'

It is clear that there is an important temperature-controlling mechanism at work in nature which determines the reproductive patterns and which is under the control of annual heat budgets.

In a recent study, Foster (1971) observed the winter movements (vagility) of *Lymnaea bulimoides*, the intermediate host of *Fasciola hepatica*, in Oregon. That this snail, like many Lymnaeids, is able to tolerate cold temperatures was shown (p. 66) in its ability 'to survive in the laboratory at a water temperature of 5°C for more than three months with or even without food.' The difficulties these animals experience, as will be shown later, are greater with higher temperatures than with lower since, as Segal (1961) has stressed, the animals can, under known circumstances, become acclimated to their surroundings.

The first tests (see Table 1 and Figure 2) using *Lymnaea stagnalis* were started in January, 1969, when 30 young laboratory-reared specimens ranging in size from 4.4 to 8 mm were placed in each of 7 aqua-

mm to 8 mm were placed in each of 7 aquaria. At this time the water in each tank was at room temperature; on each successive day the temperature was changed by 6°C until the aquaria were established at 6°, 12°, 18°, 24°, 30° and 36°C, respectively. The pH ranged from 7.8 to 8.2; the dissolved oxygen was 6 ppm at the highest temperature and 12.29 at the lowest, i. e., 6°C.

At the cold end (6°C), 13 of the 30 snails died while those remaining frequent-

TABLE 1. Summary of data for Lymnaea stagnalis at 6°C intervals, studied for 84 days (1/6/69 - 3/31/69); 30 specimens per tank. (Experiment 1)

TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
		Total change ± 2 $s_{\bar{x}}$	Survival	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
°C	ppm	in mm	%			
8.4 ± 0.6	9.8 ± 0.7	4.1 ± 0.9	63.3	--	--	--
12.1 ± 0.2	9.1 ± 0.6	10.1 ± 1.3	60.0	--	--	--
17.9 ± 0.3	8.1 ± 0.7	21.4 ± 1.5	73.3	58	29/758	
24.1 ± 0.2	7.1 ± 0.5	20.2 ± 4.5	16.7	--	--	--
30.1 ± 0.4	6.1 ± 0.2	5.8 (3)	10.0	--	--	--
36.	no survival					
22.2 ± 0.7 (room)	7.2 ± 0.6	17.8 ± 2.2	86.7	68	1/30	

*3-4 weeks old at day 0; average length, 5.3 mm.

ed the lettuce but grew little, averaging only 0.7 mm in the first month. In the 12°C aquarium, 20 (or 60%) survived and they doubled their size (an average increase of 4 to 8 mm). The 18°C group had good survival; in the first month their average size increase was between 5.8 and 11.1 mm. At this latter temperature the animals were also much more active than in the colder tanks. In the aquarium kept at 24°C the snails tended to crawl out of the water, indicating distress; they did grow better and increased in size between 5.4 and 12.4 mm. Eight of the 30 snails disappeared during the first month in the 30°C tank; the snails were frequently found out of water and growth was again somewhat inhibited with the increase in size between 5 and 9 mm. At 36°C all of the snails died within a month; growth increase was about 1 mm and the snails usually left the water. Since that temperature was critical, replicates of the 36°C were undertaken.

Two replicate series were established in 8-gallon tanks maintained at 36°C; the 30 snails in one were between 6 and 8 mm in length. All 30 specimens in the latter tank died within 6 days; among those in the former, 3 of the 30 lasted for 14 days but grew only 1 mm in that two-week period. It must be concluded that 36°C is above the critical maximum temperature for *Lymnaea stagnalis*.

As previously stated, Vaughn also found, in the process of maintaining this species, that 32°C and 36°C cannot be tolerated. The data compiled here (Table 1 and Figure 2 indicate, as also shown by earlier investigators, that the optimal temperature for maintaining *Lymnaea stagnalis* is around 18°C or 20°C. The summary of the data in Experiment 1 (from the series run for 84 days at 6°C intervals ranging from about 8° to 36°C) shows that growth was good at 18°C and 24°C. However, reproduction needs to be considered as well.

The distribution of *Lymnaea stagnalis* is circumpolar. Map 1 shows its distribution in Michigan, where the snails are subjected to prolonged periods of freezing when they overwinter off the shoals and in the deeper waters of the lakes or impoundments they inhabit. It was anticipated that during cold-stressing they would survive very well. For example, two-thirds of

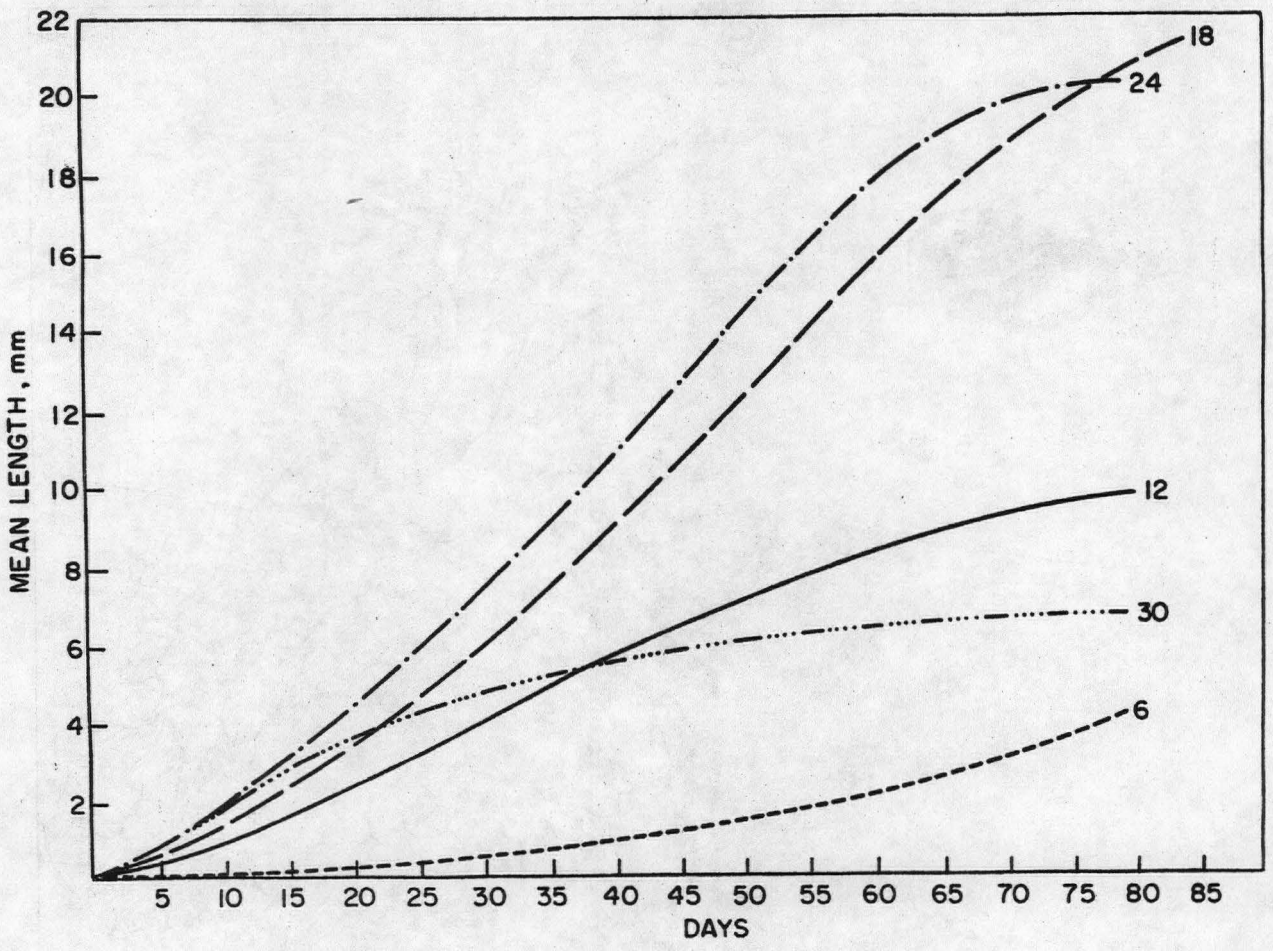
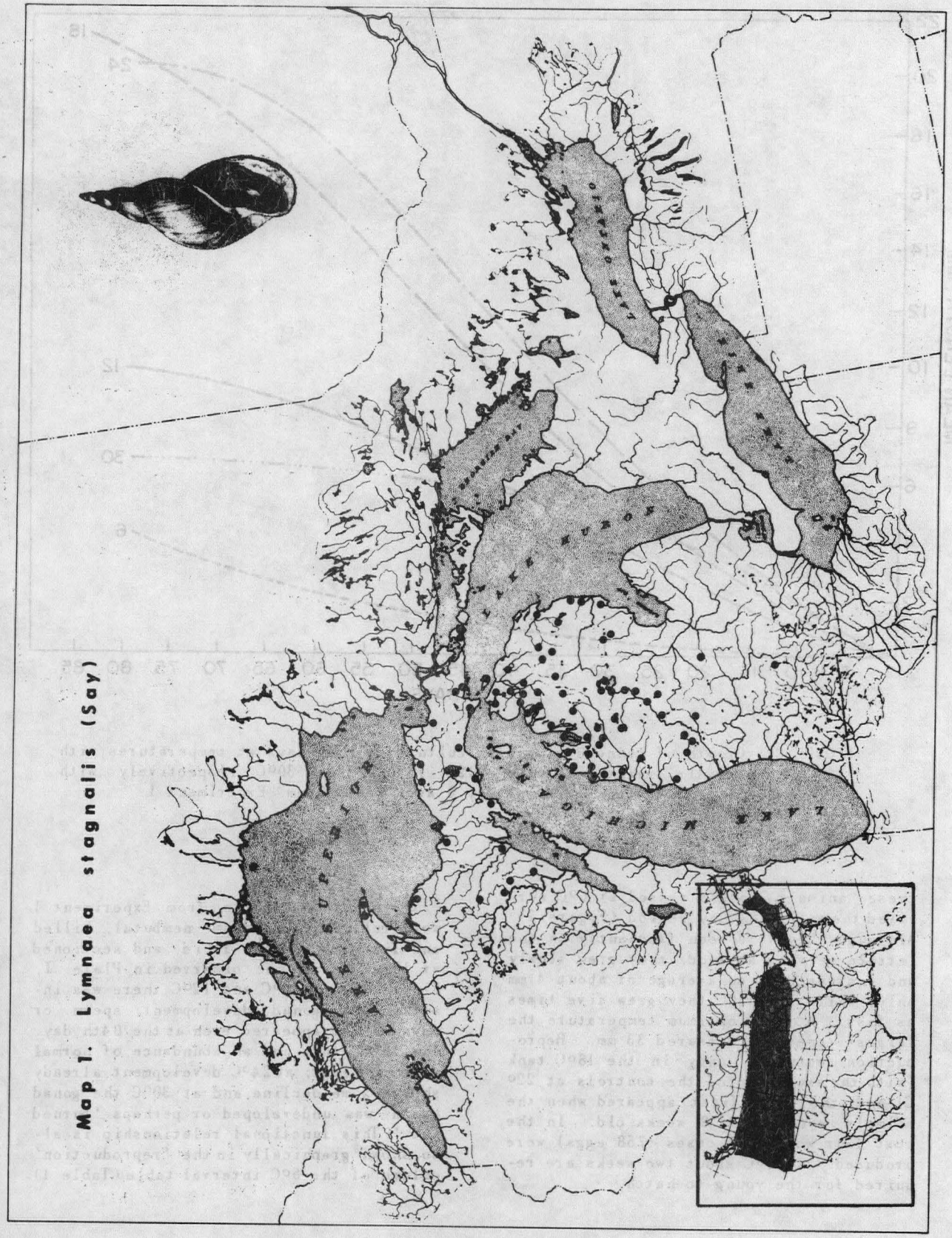


FIGURE 2. Growth of *Lymnaea stagnalis* cultured for 84 days at temperatures with 6°C intervals maintained at 6°, 12°, 18°, 24° and 30°C, respectively with 30 specimens per tank. Size at day 0 averaged 5.3 mm. Experiment 1.

these animals cold-stressed at 6°C survived the entire 84-day period (Figure 2). Although they were seen frequently on the lettuce serving as food, they grew slowly and increased on an average of about 4 mm only, while at 18°C they grew five times as fast. At this optimum temperature the largest specimen measured 33 mm. Reproduction occurred only in the 18°C tank (with the exception of the controls at 22° C) and the eggs first appeared when the animals were about 8 weeks old. In the next four weeks 29 cases (758 eggs) were produced. At 18°C about two weeks are required for the young to hatch.

The surviving snails from Experiment 1 were relaxed in sodium nembutal, killed and fixed in Bouin's fluid, and sectioned at 10 μ. As can be observed in Plate I, Figures 1-5, at 6°C and 12°C there was insufficient gonad development; sperm or eggs had not appeared even at the 84th day. The 18°C group had an abundance of normal sperm and eggs; at 24°C development already showed some decline, and at 30°C the gonad again was undeveloped or perhaps 'burned out.' This functional relationship is also shown graphically in the 'reproduction' column of the 6°C interval table (Table 1).



Map 1. Lymnaea stagnalis (Say)

To determine more accurately the optimum temperature for the growth and reproduction of *Lymnaea stagnalis*, another set of observations (Experiment 3) were made. These tanks were maintained in the same way as those at the 6°C intervals, but in this case a series of six aquaria were maintained at 2°C intervals, as follows: 14°, 16°, 18°, 20°, 22°, and 24°C; the control was about 25°C. In this set (see Table 2; Figure 3) it appears that 20.4°C is somewhat closer to the optimum since 80% of the snails survived the 111 days in culture at that temperature, while only about half, 47.7%, survived at 18°C in this series.

Data were also obtained on other aspects of the reproductive process. The average number of egg cases produced, as well as the number of eggs, was tallied. While this number varied considerably from day to day (Figure 4) it can be observed that the largest number appear between the 70th and 110th day with peak production among the 18° to 22°C groups. When viewed in a cumulative way (Figure 5), the 18° to 22°C again appear best; the control, or room temperature, group may have been subject to somewhat warmer conditions (about 26°C) and as a consequence produced fewer eggs. The graph (Figure 6), showing survival as well as cumulative number of eggs laid, indicates that this snail survives much better at 20°C and tends to produce more offspring at that temperature. If the data are graphed on a cumulative and adjusted basis (Figure 5) the 18°C and 22°C totals look best, but the high zone in production is shown more clearly (Figure 7) when the total number of eggs produced are tallied. As for survival (Figure 8), it was best at 20°C but was close to 50% in the range between 14° and 25°C.

From these analyses it is evident that *Lymnaea stagnalis* is quite sensitive to temperature, especially as it responds in its reproductive period to the higher range (above 24°C). The observations on the development of egg cases were based on collections from each tank made once a week. The eggs were measured under a dissecting microscope and their size and development evaluated.

In Experiment 3 (run at 2°C intervals), egg production in the 20°C tank began on the 64th day when the animals were about

TABLE 2. Summary of data for Lymnaea stagnalis at 2°C intervals, studied for 111 days (6/10/69 through 9/29/69); 30 specimens per tank. (Experiment 3)

TEMPERATURE	OXYGEN	GROWTH*					REPRODUCTION				
°C	ppm	Aver. Size Day 0 mm	Average Size Day 111 mm	Total Change ± 2 $s_{\bar{x}}$ mm	No. of Snails on Day 111	Survival %	1st Day of Egg-Laying No.	Aver. Size Start of Egg-Laying mm	Total Days of Egg-Lay. No.	Tot. Cases / Tot. Eggs No.	Egg Viability %
14.3 ± 0.3	7.5 ± 0.1	2.8	28.9	26.1 ± 0.9	12	40.0	92	26.0	19	10/314	98.7
16.3 ± 0.6	7.1 ± 0.1	2.8	30.6	27.8 ± 1.8	19	63.3	85	25.8	26	46/1561	100.0
18.4 ± 0.5	6.8 ± 0.2	2.8	32.9	30.1 ± 2.0	14	47.7	69	24.8	42	60/2402	99.7
20.4 ± 0.3	6.3 ± 0.1	3.0	30.3	27.3 ± 1.3	24	80.0	64	21.2	47	90/3024	99.8
22.2 ± 0.2	6.2 ± 0.1	2.6	31.4	28.8 ± 1.9	13	43.3	69	23.6	42	75/3166	99.9
24.6 ± 0.3	5.8 ± 0.2	2.7	31.6	29.0 ± 1.4	21	70.0	73	26.6	38	75/2770	97.0
25.6 ± 0.3 (room)	5.7 ± 0.1	2.9	30.5	28.3 ± 1.2	22	73.0	74	27.0	37	42/1405	99.4

*6 days old at beginning of experiment.

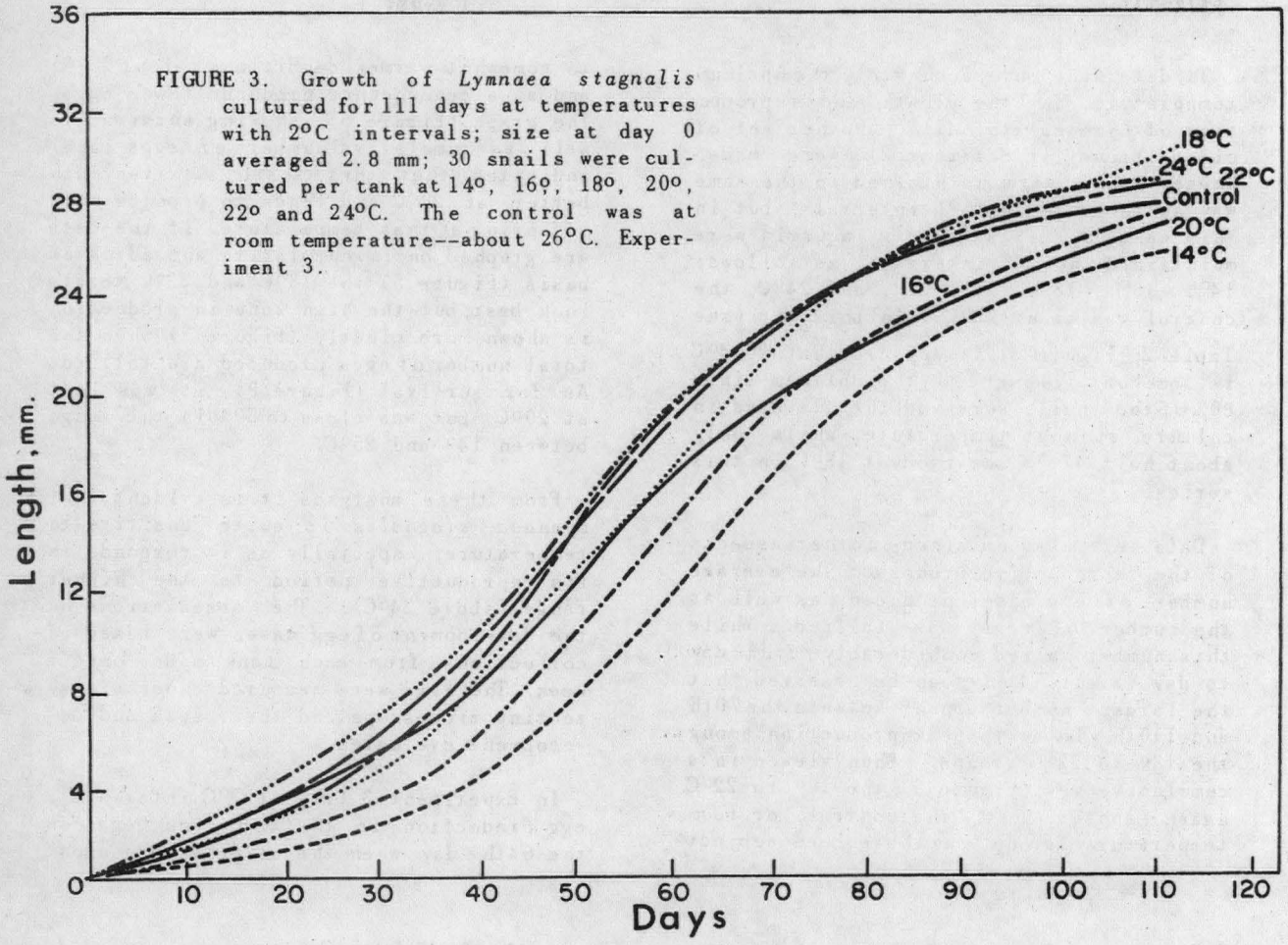


FIGURE 3. Growth of *Lymnaea stagnalis* cultured for 111 days at temperatures with 2°C intervals; size at day 0 averaged 2.8 mm; 30 snails were cultured per tank at 14°, 16°, 18°, 20°, 22° and 24°C. The control was at room temperature--about 26°C. Experiment 3.

TABLE 3. Summary of data, Experiments 1 and 3, for Lymnaea stagnalis; 30 specimens per tank.

1/6/69-3/31/69 by 6°C intervals - 84 days Experiment 1								6/10/69-9/29/69 by 2°C intervals - 111 days Experiment 3							
	Growth*		Reproduction						Growth*		Reproduction				
Aver. Temp. °C	Final Size mm	Total Growth ± 2S$_{\bar{x}}$ mm	# Day Laying Began	Laying Size mm	Tot.Cases / Tot.Eggs #	Survival Snail %	Survival Egg %	Aver. Temp. °C	Final Size mm	Total Growth ± 2S$_{\bar{x}}$ mm	# Day Laying Began	Laying Size mm	Tot.Cases / Tot.Eggs #	Survival Snail %	Survival Egg %
9.3	9.2	4.1 ± 0.9 (19)*1	--	--	--	63.3	--	13.8	28.9	26.1 ± 0.9 (12)	92	26.0	10 / 314	40.0	98.9
14.4	14.8	10.1 ± 1.3 (18)	--	--	--	60.0	--	16.5	30.6	27.8 ± 1.8 (19)	85	25.8	46 / 1561	63.3	100.
18.3	27.2	21.4 ± 1.5 (22)	58	19.7	29 / 758	73.3		18.5	32.9	30.1 ± 2.0 (14)	69	24.8	60 / 2402	47.7	99.7
24.1	25.6	20.2 ± 4.5 (5)	--	--	--	16.7	--	20.4	30.3	27.3 ± 1.3 (24)	64	20.5	90 / 3024	80.0	99.8
29.3	10.8	5.8 (3)	--	--	--	10.0	--	22.1	31.4	28.8 ± 1.9 (13)	69	24.8	75 / 3166	43.3	99.9
30.0	no	survivors						24.7	31.6	29.0 ± 1.4 (21)	73	26.6	75 / 2770	70.0	97.0
22.2 (room)	23.4	17.8 ± 2.2 (26)	68	21.8	1 / 30	86.7	--	25.6 (room)	30.5	28.3 ± 1.2 (22)	74	27.0	42 / 1405	73.0	99.4

*Beginning ages: 6°C interval - 3 to 4 weeks; 2°C intervals - 1 week.

*1Number of snails at end of experiment.

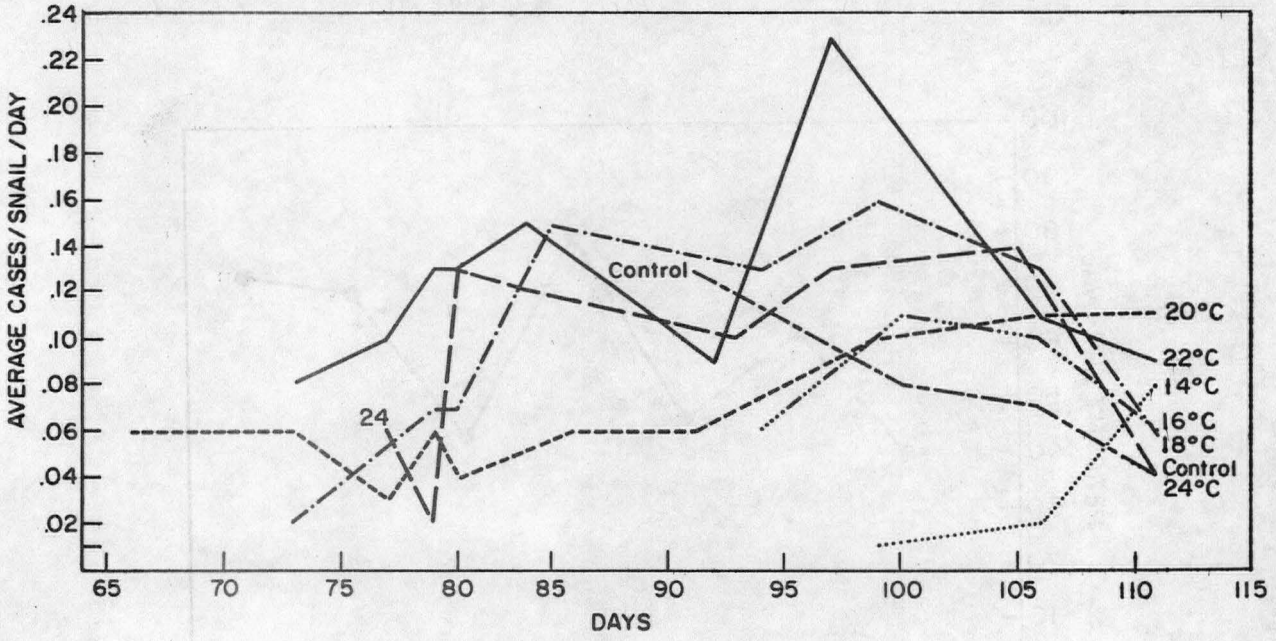


Figure 4. The average number of egg cases produced per snail per day by Lymnaea stagnalis during a culture period of 111 days; 30 snails per tank maintained in 7 aquaria at the following temperatures: 14°, 16°, 18°, 20°, 22° and 24°C; the control was at room temperature (about 26°C). Experiment 3.

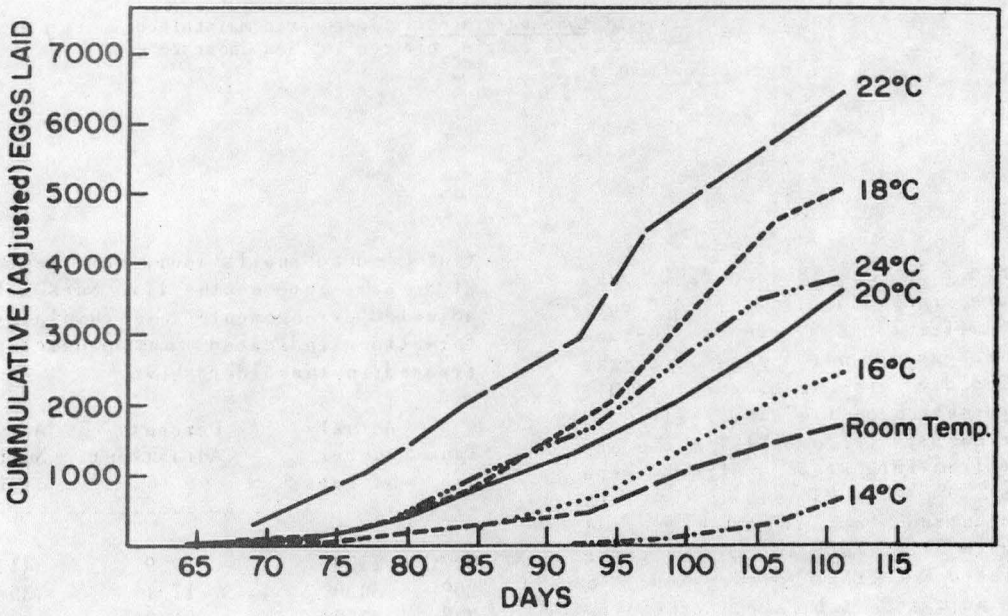


Figure 5. Estimated number of eggs laid by Lymnaea stagnalis when calculated on 100% survival with 30 snails per tank maintained in aquaria at the following temperatures: 14°, 16°, 18°, 20°, 22°, and 24°C; controls were at room temperature (about 26°C). Experiment 3.

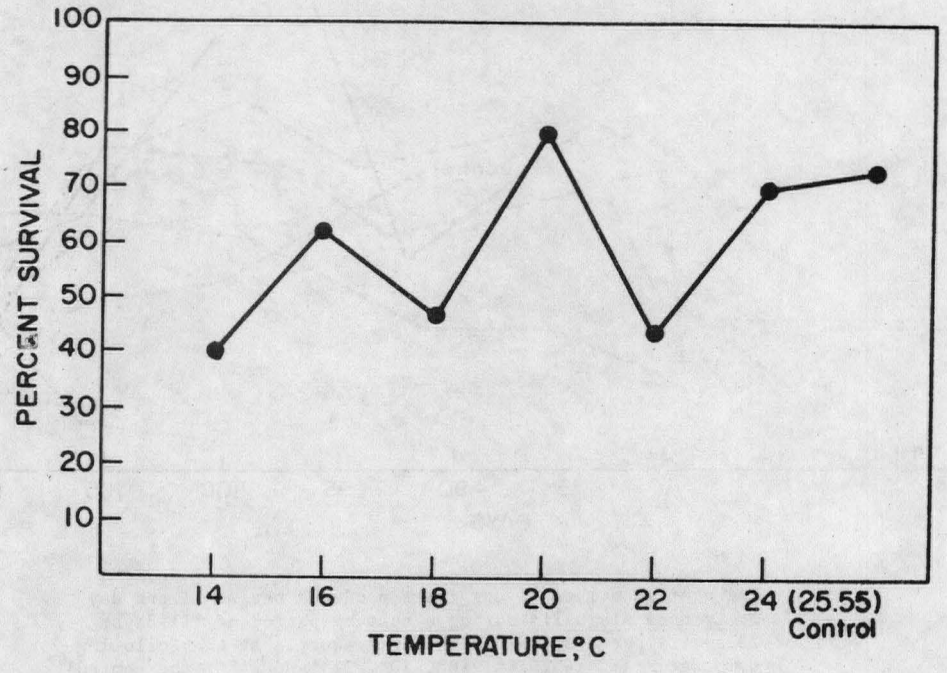


Figure 6. Survival of Lymnaea stagnalis in aquaria maintained at 2°C intervals ranging between 14° and about 26°C. Experiment 3.

10 weeks old. In the 18° and 22°C tanks they appeared in 69 days, in the 24°C control group after 73 days. In the 16° and 14°C groups eggs did not appear until the 85th and 92nd day, respectively (Figure 9). At the termination of the experiment, the cumulative numbers (Figure 9), in the order of size from largest to smallest, were as follows: 20°, 22°, 24° and 18°C. This ranking occurred both in the adjusted graph (Figure 5) as well as in the tanks when tabulated as growth progressed. To make the adjusted tabulation, the percentage of the snails remaining (x%) was determined in terms of the incremental laying period. The number of eggs laid during this period was multiplied by 100% - x% and added to the sum which assumes that the dead snails would have reproduced at the same rate as the live ones. In the adjusted arrangement, the cumulative information indicated that production decreased in the order shown:

Tank	Actual Number of Eggs	Percent Adjustment	Adjusted Number of Eggs
22°C	2170	29.9	4118
20°	3030	17.1	3548
24°	2858	21.9	3484
18°	2409	36.5	3288
16°	1516	26.4	1973
Control	1413	- 4.6	1349
14°	318	41.7	451

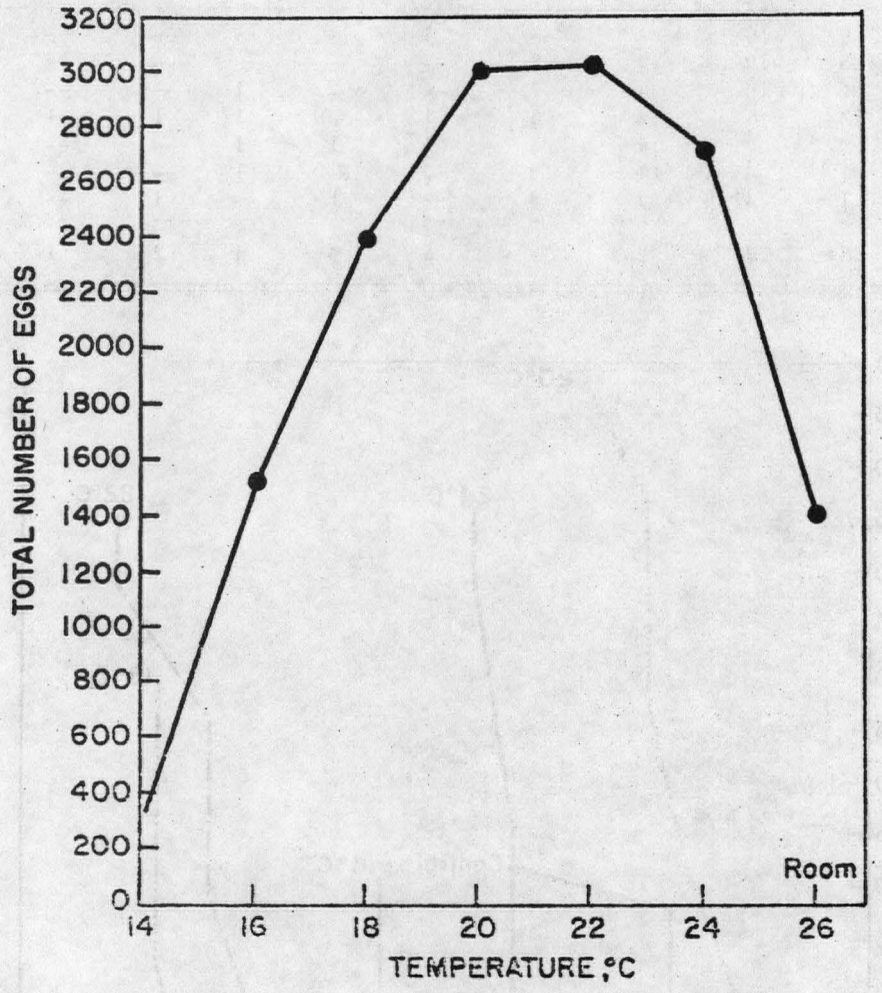


FIGURE 7. Total number of eggs produced by *Lymnaea stagnalis* cultured for 111 days in aquaria maintained at intervals of 2°C ranging between 14° and 26°C. (Experiment 3).

In making these adjustments it was noted that the average number of eggs per case did increase slightly as the snails became older although with this rise there were fluctuations in number per case.

Viability in all tanks remained high. It is possible that in early stages viability may not always be determined accurately and that embryo mortality may come at later stages. However, as shown in Figure 8, there was a very high rate (almost 90%) of survival of the embryos.

Egg cases were found in all parts of the tanks. Their distribution can best be summarized in the following table.

Tank	Float	Side	Bottom	Filter	Lettuce	Hose	Screen	Dish	Wrong Side	Wrong Bottom	Shell
Control	14	9	16	--	--	1	--	--	--	--	--
24°C	21	25	12	10	5	--	--	--	--	--	1
22°	23	22	10	6	7	--	2	1	--	--	--
20°	35	14	18	14	5	1	--	1	1	1	--
18°	37	3	8	5	3	1	1	1	--	--	--
16°	15	1	4	10	12	2	--	1	--	--	--
14°		1	4	2	3	--	1	--	1	--	--
	---	--	--	--	--	--	--	--	--	--	--
TOTAL	145	75	72	72	35	5	4	4	2	1	1

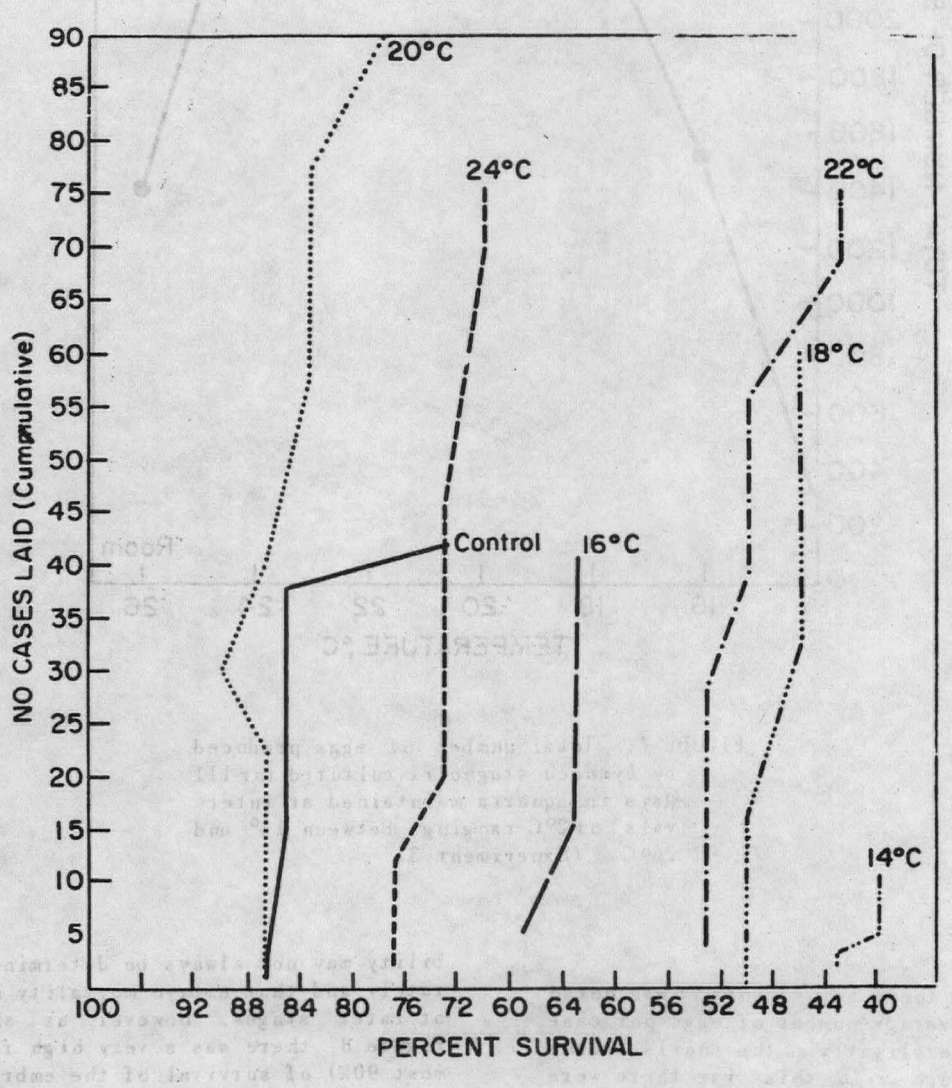


Figure 8. The number of egg cases laid by Lymnaea stagnalis in terms of the percentage of snails that survived in tanks at the following temperatures: 14°, 16°, 18°, 20°, 22°, 24°C; the control was about 26°C. Experiment 3.

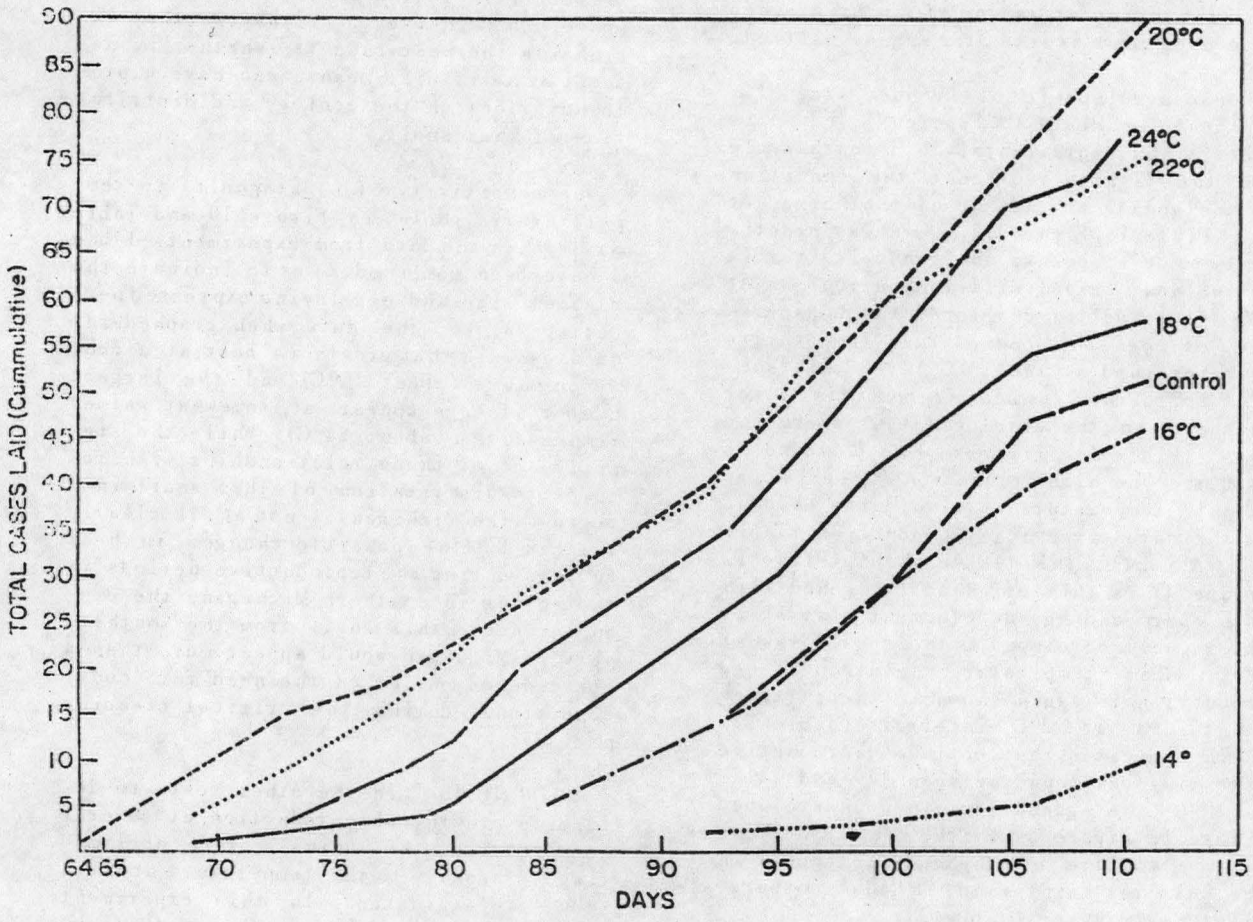


Figure 9. Total number of egg cases of *Lymnaea stagnalis* produced in 111 days when aquaria, each with 30 snails, were maintained at 2 C intervals, as follows: 14°, 16°, 18°, 20°, 22° and 24°C. The control was room temperature estimated at 26°C. The data on which the cumulative figures were based are: (Experiment 3)

Control	73%	22 snails
24°C	70%	21 "
22°	43%	13 "
20°	80%	24 "
18°	47%	14 "
16°	63%	19 "
14°	40%	12 "

It is evident that the floats used to hold the thermometers and the aquaria sides were used most as sites for egg deposition.

Gonad development of *Lymnaea stagnalis* is shown in Plate I, Figures 1 - 5. This species has approximately a 4-month cycle and the figures represent the condition of the gonads at the end of that time. At 6°C (Plate I, Figure 1) there was practically no development; the gonad is visible but without sexual differentiation. At 12°C it is better developed but no appearance of eggs or sperm. When temperature is maintained at the 18°C level this species approaches its best reproductive level as shown in the development of sperm and eggs (Plate I; Figures 3' (X24) and 3b (X400). The high production of viable eggs at this temperature is also quite evident in the data given for reproduction (Table 3). At 24°C the tissue shown (Plate I, Figure 4) is that of an active gonad with both sperm and egg development, but it is not as well developed as the tissue was at 18°C. This temperature relation to egg production is again shown in the cultures maintained at 2°C intervals (Table 2), which indicates that optimum reproductive potential was found between 18° and 24°C. At 30°C the gonad remained undeveloped (Plate I, Figure 5) which was also shown by the failure of any eggs to appear in the cultures maintained in that comparatively warm water (Table 3).

These observations on the relation of temperature to gonad development have a ready application to the history of the distribution of *Lymnaea stagnalis* in Michigan. The distribution map (Map 1 shows clearly that *L. stagnalis* at present is found about 200 miles north of the Ann Arbor region. Yet, some 40 or 50 years ago this snail was common in lakes of southern Michigan. Studies by investigators in Ann Arbor (including George R. LaRue and E. D. Crabb) used this snail from stocks common in local lakes (Third Sister Lake and others) for studies in parasitology, genetics and functional anatomy. The studies on the sensitivity of this snail to temperatures above 25°C serve to emphasize that increasing the heat budgets, whether by natural or artificial means, can have a profound effect on the ecology and distribution of this snail.

The sensitivity of *L. stagnalis* to temperature is shown in Figure 10 and Table 3 in which the data from Experiments 1 and 3 have been combined so as to indicate the maximum size and egg-laying expressed percentage-wise. The data when graphed indicate again that growth is best at a cool temperature (about 18°C) and the largest number of eggs appear at somewhat warmer temperatures (about 24°C). While the significance of these relationships with respect to distribution of this snail in a region like Michigan is not at all clearly related to the possible changes in heat budgets during the reproductive periods in the spring in southern Michigan, the disappearance of this snail from the southern part of Michigan would appear more than a coincidence in view of the need for 'cool' conditions during the critical breeding periods.

'PLOP TESTS.' In the other tests to determine growth and reproduction at several temperatures, the animals were acclimatized gradually to the temperature at which they were evaluated. In this experiment the young were taken from the stock tanks at room temperature and placed directly in the water at the test temperatures. The results (Table 4; Figures 11, 12 and 13) when *Lymnaea stagnalis* were maintained at 6°, 12°, 20° and 30°C, indicate good tolerance at colder and the normal, natural temperatures; they rapidly disappeared in the warm (30°C) water. Again it is evident that *L. stagnalis* cannot tolerate warm conditions, and that its distribution (as shown in Map 1) in northern Michigan is probably conditioned by the annual heat budgets in that region.

TABLE 4. Summary of data for Lymnaea stagnalis "plop test" (animals subjected to sudden changes of temperature in tanks from room temperature), studied for 21 days (4/1/71 to 4/22/71), 30 specimens per tank.

TEMPERATURE °C	NUMBER OF SNAILS AT DAY 21	SURVIVAL %	AVERAGE SIZE* AT DAY 0 mm	AVERAGE SIZE AT DAY 21 mm	CHANGE IN SIZE FROM DAY 0 mm	OXYGEN ppm
6.35 ± 0.03	25	83.3%	2.76 mm	3.06 mm	0.30 mm	17.42 ± 0.33
12.45 ± 0.14	25	83.3%	2.82 mm	5.47 mm	2.65 mm	14.44 ± 0.13
20.64 ± 0.39	28	93.3%	2.64 mm	8.11 mm	5.47 mm	11.50 ± 0.49
30.98 ± 0.07	0	0.0%**	2.67 mm	--**	--**	8.12 ± 0.01

*Age at Day 0: 3-14 days.

**31°C tank: 3 snails (10%) alive at day 14, with average size of 3.85 mm (change in size 1.18 mm).

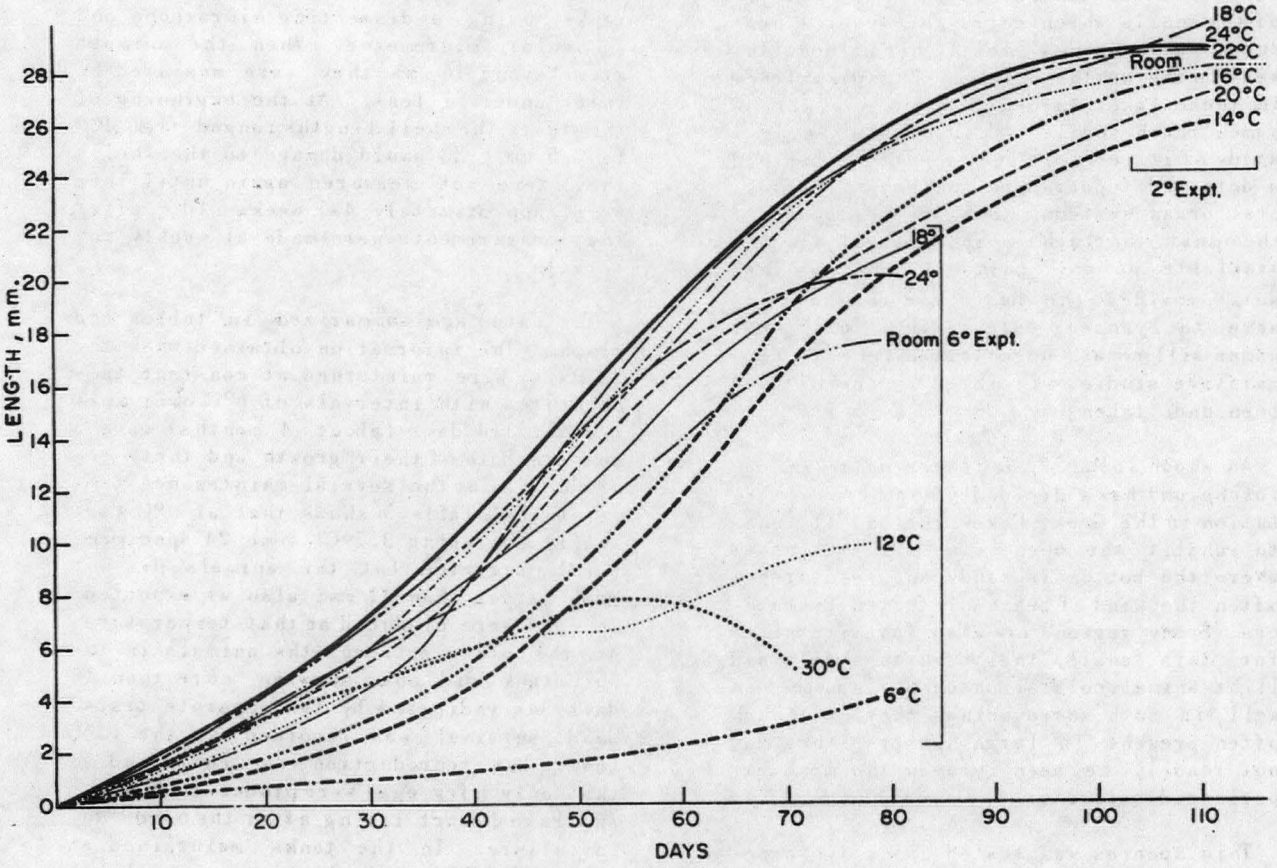


FIGURE 10. Data obtained from Experiments 1 and 3, as given in Table 3, for *Lymnaea stagnalis*, and plotted to show overlapping areas. Optimum temperature is between 18°C and 24°C with better survival in colder temperatures.

Lymnaea emarginata Say

Lymnaea emarginata Say (recently called *Lymnaea catascopium* Say by H. J. Walter, 1969) was studied quite intensively 30 years ago in northern Michigan at the University of Michigan Biological Station by those interested in its role as one of the most important intermediate hosts of schistosome dermatitis, or 'swimmer's itch.' Based on the need for controlling infestations on beaches inhabited by this snail, the Michigan Stream Control Commission published a bulletin in 1939 entitled 'Water Itch' that has been widely used in the Great Lakes Region. At that time, *L. emarginata* was considered the most harmful of the snails which carry the several non-human schistosomes (mostly bird blood flukes) in the northern lakes. Its prevalence in these lakes earned for it the common name 'beach snail.' H. J. Walter made a study of its ecology and recently published a detailed topographic anatomy of its several organ systems, thus providing one of the most thorough morphological studies available on any pulmonate snail. This work provided the basis for changing the name to *Lymnaea catascopium*, but these names will remain uncertain until more comparative studies of related lymnaeids have been undertaken.

As shown in Map 2, *L. emarginata (L. catascopium)* has a decidedly northern distribution in the Great Lakes region. It tends to inhabit the open, wind-swept beaches where the bottom is sandy and weed-free—often the kind of beach preferred by bathers. Stony regions are also favorite sites for this snail; their white shells and light animal coloration camouflage them so well in such surroundings that, although often present in large numbers, they may not readily be seen by even the most experienced collector.

This species was tested using two experiments; in Experiment 4 the animals were stressed for 121 days at temperatures with 6°C intervals between; in Experiment 5 they were maintained at 2°C intervals over a period of 77 days. As confirmed by the data in these studies, it is evident that *Lymnaea emarginata*, like *L. stagnalis* (also a northern snail) prefers temperatures ranging between 18°C and 24°C, definitely on the cool side.

The first test (Experiment 4) with *Lymnaea emarginata* was started July 3, 1969, and involved 6 tanks each containing 30 specimens which were maintained at 6°, 12°, 18°, 24°, 30° and 36°C, respectively. A control was kept at room temperature (about 25 C). The snails used were about two weeks old, and their shell length was measured just prior to being put in the tanks using a dissecting microscope and an ocular micrometer. When the animals grew beyond 16 mm they were measured by ruler under a lens. At the beginning of the tests the shell lengths ranged from 1.9 to 3.5 mm. To avoid damage to the shells they were not measured again until they were approximately 4-5 weeks old; after that measurements were made at weekly intervals.

The data are summarized in tables and graphs. The information obtained when the animals were maintained at constant temperatures with intervals of 6°C over a period of 110 days (about 4 months) gave a measure both of their growth and their reproduction at the several maintenance temperatures. Table 5 shows that at 6°C (actually averaging 8.7°C) some 24 specimens (77%) survived but the animals did not grow larger than 11 mm; also, as expected, no eggs were produced at that temperature. At the other extreme, the animals in the 36°C tank did not survive more than 18 days, as indicated by two separate tests. Good survival was recorded at the 12°C level but reproduction was curtailed so that only a few eggs were produced when the snails did start laying after the 63rd day in culture. In the tanks maintained at 18°C and 24°C survival was not good but the snails that did live grew to normal size, averaging 15 mm and 17 mm, respectively. Figure 14, which plots survival in the form of a graph, shows that survival

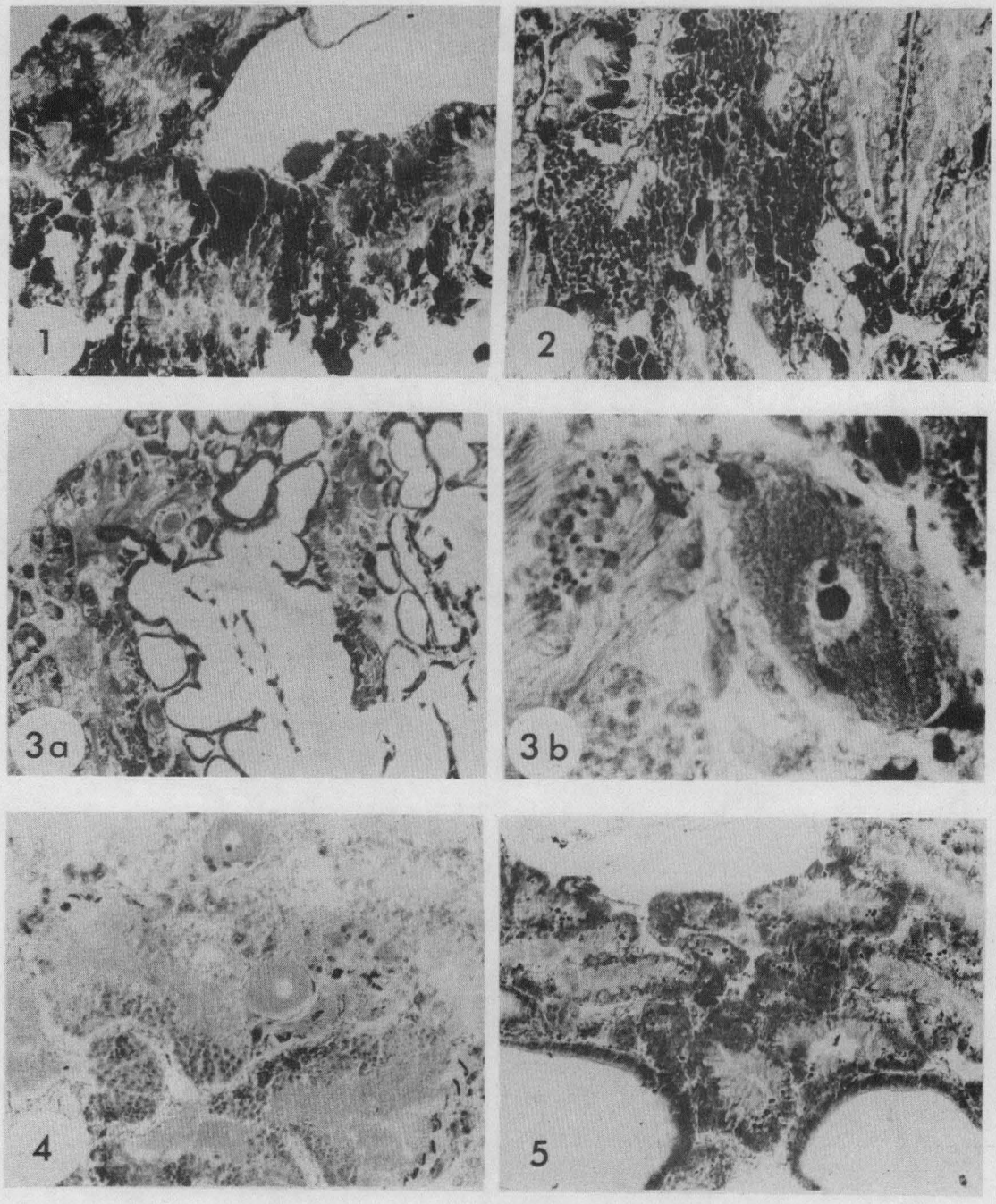


PLATE I. Gonad tissue sections from Lymnaea stagnalis cultured for 84 days at different temperatures.

Fig. 1. 6°C (X70)
Fig. 2. 12°C (X70)
Fig. 3a. 18°C (X24.5)
Fig. 3b. 18°C (X400)
Fig. 4. 24°C (X70)
Fig. 5. 30°C (X70)

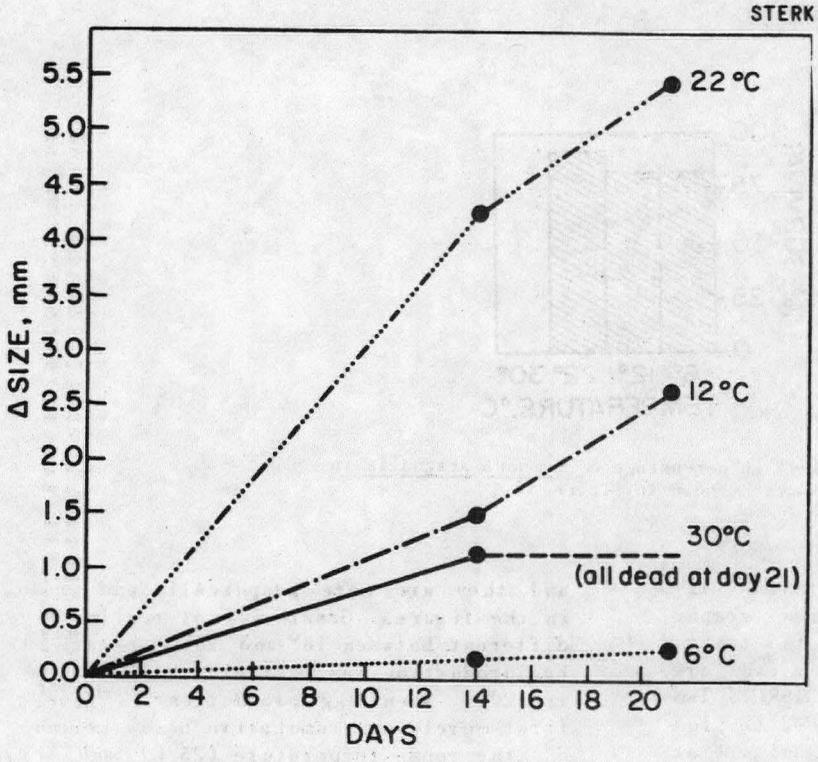


Figure 11. Growth of 30 Lymnaea stagnalis placed directly into tanks with water at four different temperatures ("plop test"): 6°, 12°, 22° and 30°C. Those at 30°C survived only 21 days compared to the normal life span of at least 4 months.

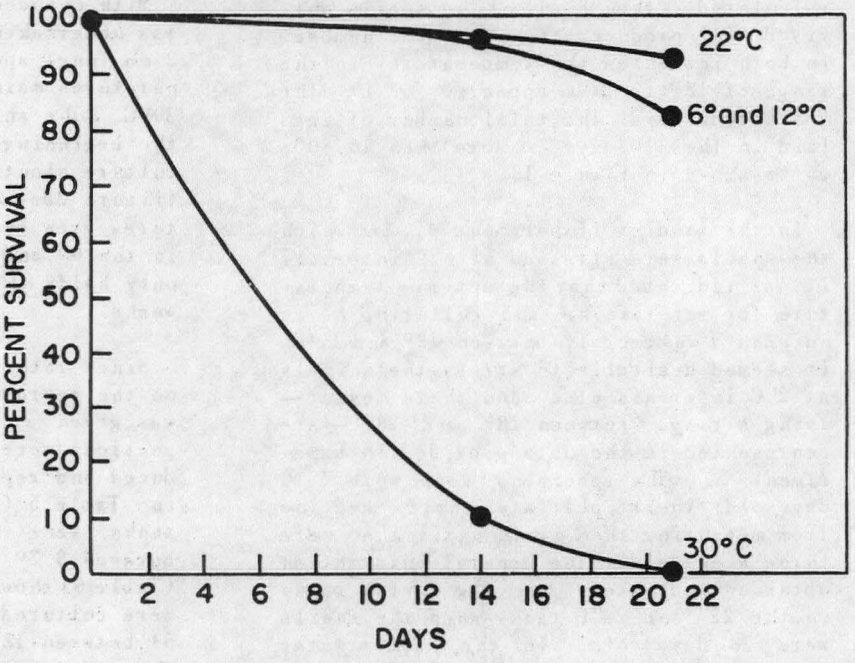


Figure 12. Survival of Lymnaea stagnalis in "plop test" when 30 specimens were maintained for 21 days in tanks at 6°, 12°, 22° and 30°C.

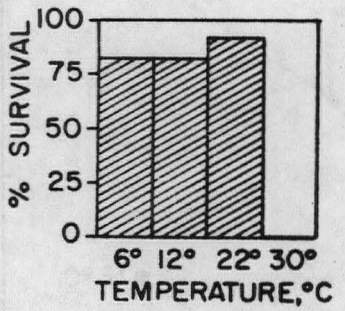


Figure 13. Survival in percentage of Lymnaea stagnalis in "plop test" shown in Figure 12.

tends to be best in the cooler ranges (12° to 18 C). From Figure 15, which graphs growth and recovery for each of the tanks, it is clear that growth and survival are best in the cool range (roughly 18°C). Two graphs (Figures 16 and 17) are used to plot the egg cases produced during culture at the four temperatures the animals were maintained; Figure 16 gives the total number of cases produced by the surviving snails; Figure 17a, b, c, d and e is the 'adjusted' total when the production is calculated as though all of the snails survived and produced the potential number. In both instances the temperatures in the range of 18° to 24°C appeared to be the most productive. The total number of eggs laid in the 110 days was more than 16,000, as is shown in Figure 18.

In the studies (Experiment 4) in which the snails were stressed at 6°C intervals it was indicated that the optimum temperature for maintaining and culturing *L. emarginata* was roughly between 18° and 25°C. It seemed desirable to stress the animals at 2°C intervals also, and these tests,—using a range between 18° and 28°C—are represented in the data provided in Experiment 5. The specimens used were 7-10 days old; their delicate shells kept us from measuring them again until they were three weeks old. The general information obtained indicated that egg-laying began in the 22° and 24°C tanks when the snails were 28 days old. In the warmer water (26° and 28°C) mortality was comparatively high with almost half of the 30 snails decimated. These data are shown in Table 6 and they are more graphically indicated in the figures. Growth was not really very different between 18° and 28°C (Figure 19). Egg production was best around 24°C (Figure 20). When egg production is viewed first merely on a cumulative basis (Figure 21) the room temperature (25°C) and the 24°C temperatures look best, but if they are calculated on the basis of 100% survival (Figure 22) the 26°C temperature is better.

With *Lymnaea emarginata*, a 'plop test' was undertaken by placing 30 young (about 2 mm long) specimens in tanks with temperatures maintained at 6°, 12°, 22° and 30°C. The animals were 4-14 days old at the beginning of the test and remained in culture about 3 weeks (Table 7). In growth (Figure 23a) those at the warmer temperatures grew best; the survival (Figure 23b) in the 6° and 30°C tanks was poor with only half of those tested alive after 3 weeks.

Since rather comprehensive information on the reproduction of *Lymnaea emarginata* was given in terms of egg-laying. gonad sections were not made. Data on eggs produced and reproductive potential are found in Table 5 (Experiment 4) in which the tanks were maintained at 6°C intervals between 8.7° and 34.6°C; another series (Table 6) shows egg output when these snails were cultured at 2°C intervals (Experiment 5) between 18° and 28°C. Reproduction, as shown for both series, indicates that *L. emarginata* is similar to *L. stagnalis* in that even at 13°C, although it took more

than 60 days to produce eggs, a surprising number of egg-cases were deposited in this cool water and the eggs had an unusually high viability. At 18°C production was better, as it was also in the 24°C tanks. Beyond that, i. e., at 30° and 34°C, conditions were too warm and no eggs were produced. In the series run at 2°C intervals (Table 6) a more comprehensive survey of egg-laying appears when the range was between 18° and 28°C; again, the best cultures appeared in the 18° to 26°C range; above the latter temperature fewer egg masses were produced and the eggs are shown to be less viable. For all practical purposes, *L. emarginata* and *L. stagnalis* are very similar in their northern distribution pattern and their preference for waters on the cooler side, i.e., below 26°C and even as low as 14°C.

Although the tissues were prepared for cutting and staining, there was not sufficient time allotted in this project to prepare gonad sections. This work will be done later; hopefully, these and other studies can be continued.

TABLE 5. Summary of data for Lymnaea emarginata at 6°C intervals, studied for 121 days (7/2/69 through 10/29/69), 30 specimens per tank. Experiment 4.

TEMPERATURE	OXYGEN	GROWTH*				REPRODUCTION								
		Aver. Size Day 0	Aver. Size Day 111	Total Change ± 2 s x	Survival	Start of Laying			Day 121					
						Day	Snails	Size	Total Cases / Total Eggs	Cases/ Day	Eggs/ Case	Eggs/ Day	Egg Viability
°C	ppm			mm	%	No.	No.	mm	No.	No.	No.	No.	%
8.7 ± 0.5	9.0*1	2.4	11.0	8.6 ± 0.7	76.6	--	--	--		--	--	--	--
13.0 ± 1.0*2	8.1	2.6	13.6	11.0 ± 0.7	90.0	63	27	12.0	32*3 / 525	0.68	16.4	11.2	99.6
18.2 ± 0.7*2	7.5	2.4	15.1	12.7 ± 0.9	60.0	42	23	10.0	178 / 1957	2.3	11.0	24.8	97.4
24.6 ± 0.4	6.4	2.7	17.4	14.7 ± 2.3	16.7	36	5	10.4	154 / 3694	1.8	24.0	43.5	97.9
29.8 ± 0.1	5.9	2.7	13.8	11.1 (3)*4	10.0	63	4	13.4	20 / 0	0.43	0	0	0
34.6*5	--	--	--	--	--	--	--	--	--	--	--	--	--
25.4 ± 0.8 (room)	6.3	2.4	20.2	17.8 ± 1.2	56.7	34	21	10.3	522 / 11,582	6.0	22.2	133.0	94.3

*At beginning of experiment, animals were 3-4 weeks old.

*1Only one reading for dissolved oxygen - meter not working.

*2Difficulties encountered with heaters, relay boxes and controls.

*3No more eggs laid after Day 110.

*4Number of snails left at end of experiment too few for calculation of standard deviations.

*5Started two different groups: (1) all dead before measuring at 18 days;
(2) 2 snails alive at 7 days and all dead at 14 days.

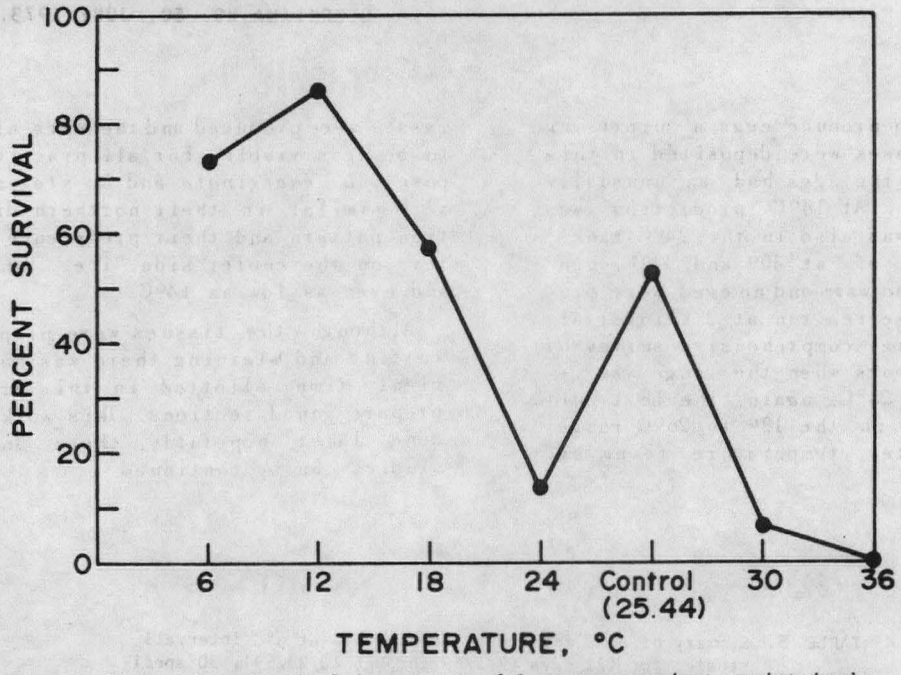


Figure 14. Survival in percent of Lymnaea emarginata maintained at 2° temperature intervals between 0° and 36°C. The trend is toward fewer surviving at the warmer temperatures. Experiment 4.

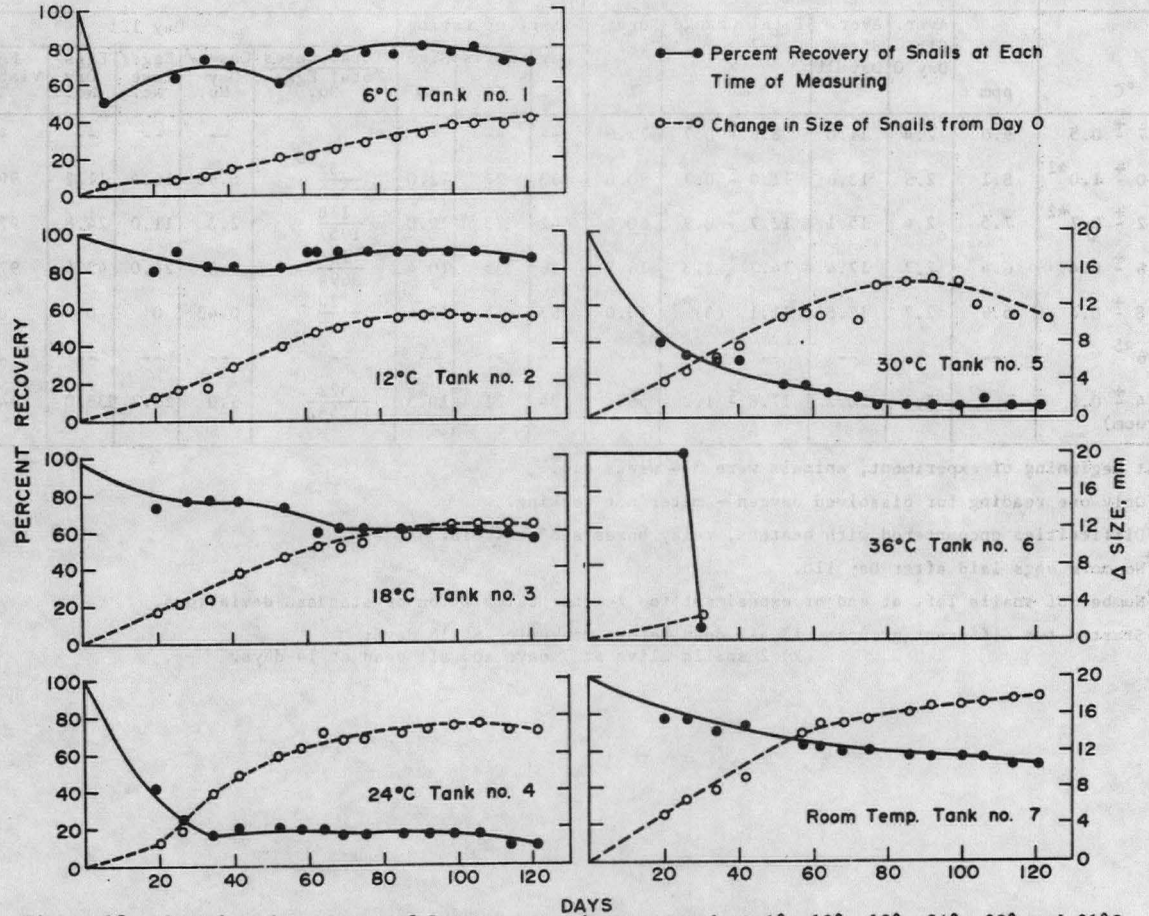
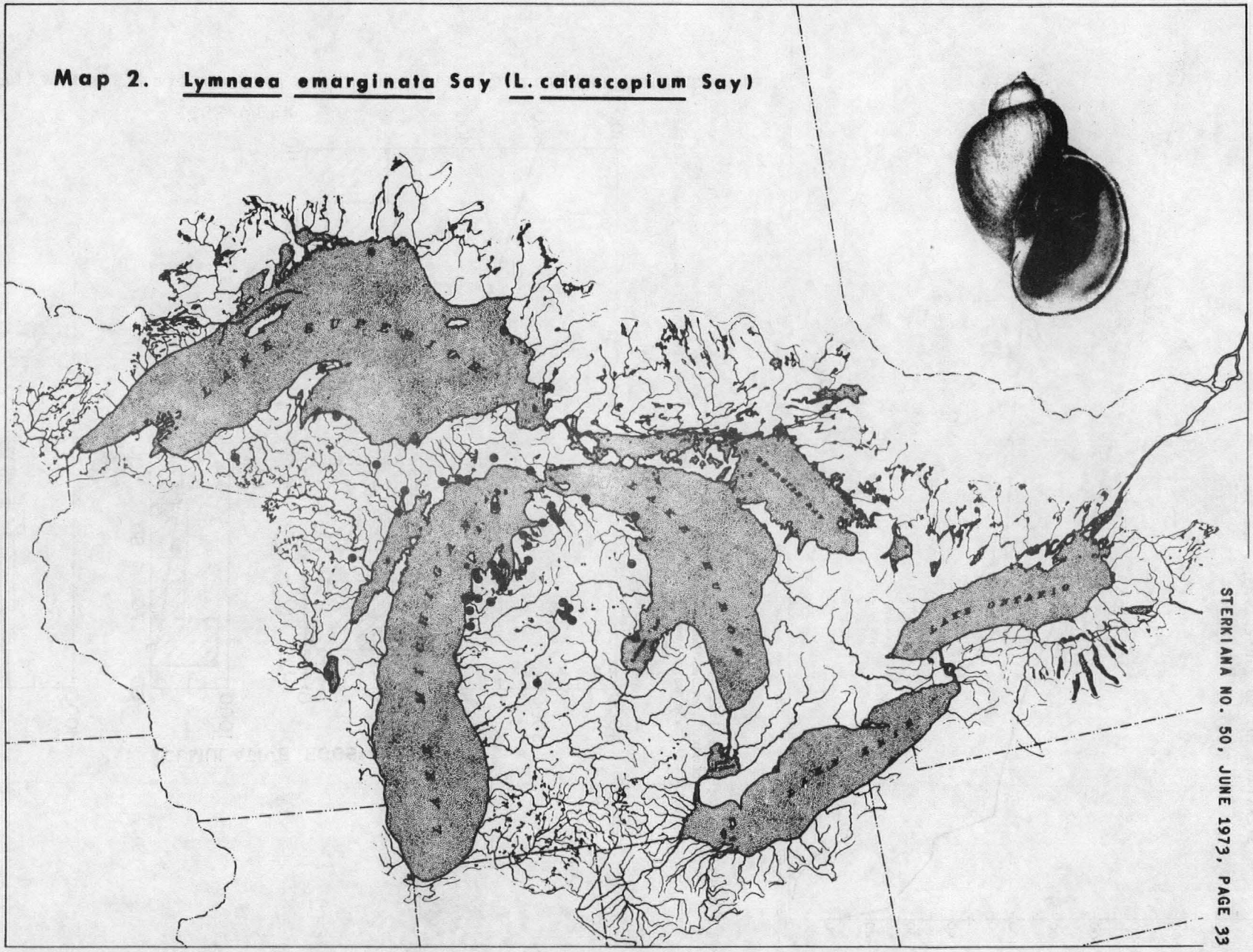


Figure 15. Growth and recovery of Lymnaea emarginata reared at 6°, 12°, 18°, 24°, 30° and 36°C for 121 days; control at room temperature (25°C). Experiment 4.


Map 2. Lymnaea emarginata Say (L. catascopium Say)
LAKE SUPERIOR
LAKE MICHIGAN
LAKE HURON
GEORGIAN BAY
LAKE ERIE
LAKE ONTARIO

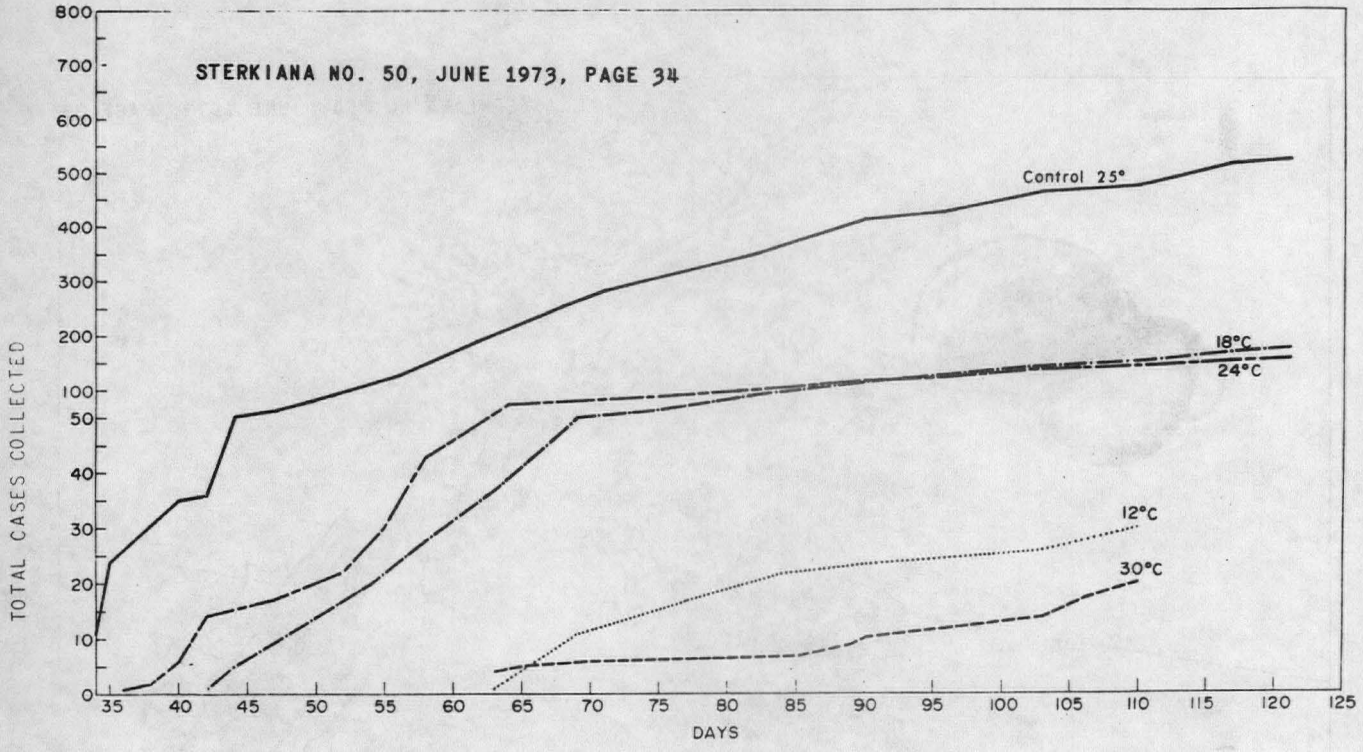
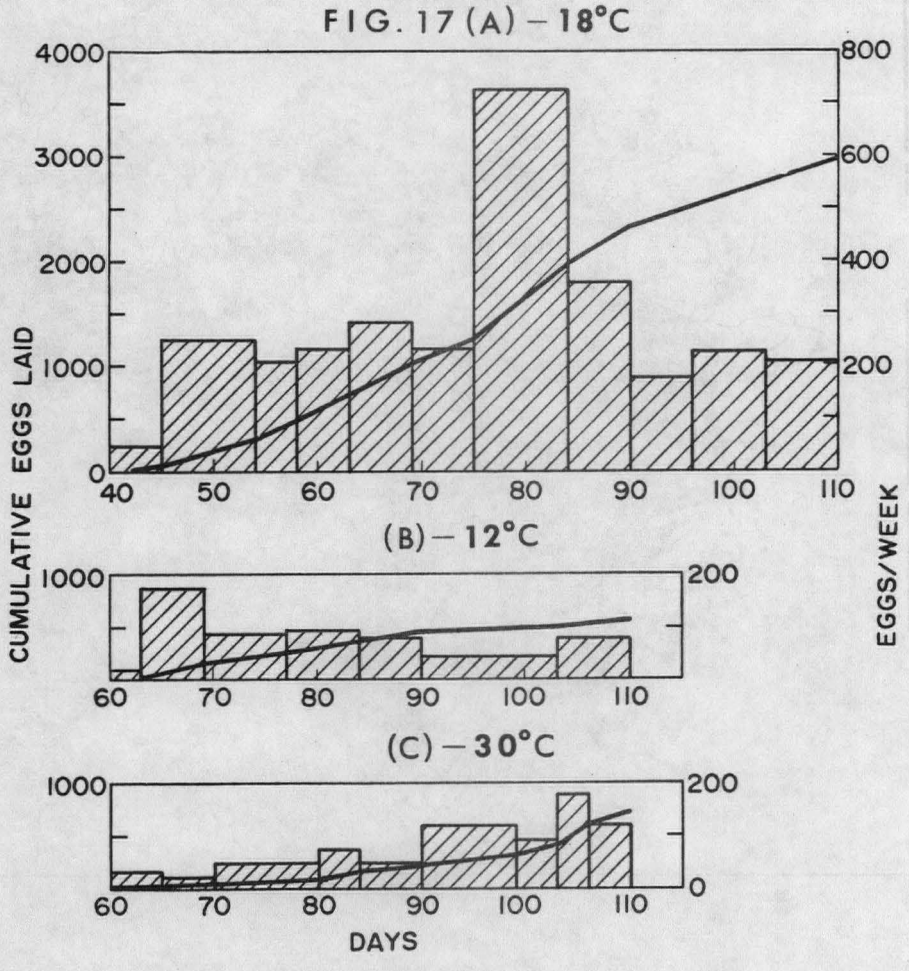


Figure 16. Egg cases laid (cumulative) by Lymnaea emarginata maintained at 12°, 18°, 24° and 30°C, and the control temperature; egg-laying between 18° and 24°C extended over a 90-day period. In both cold and warm conditions it began late and was greatly reduced. Experiment 4.



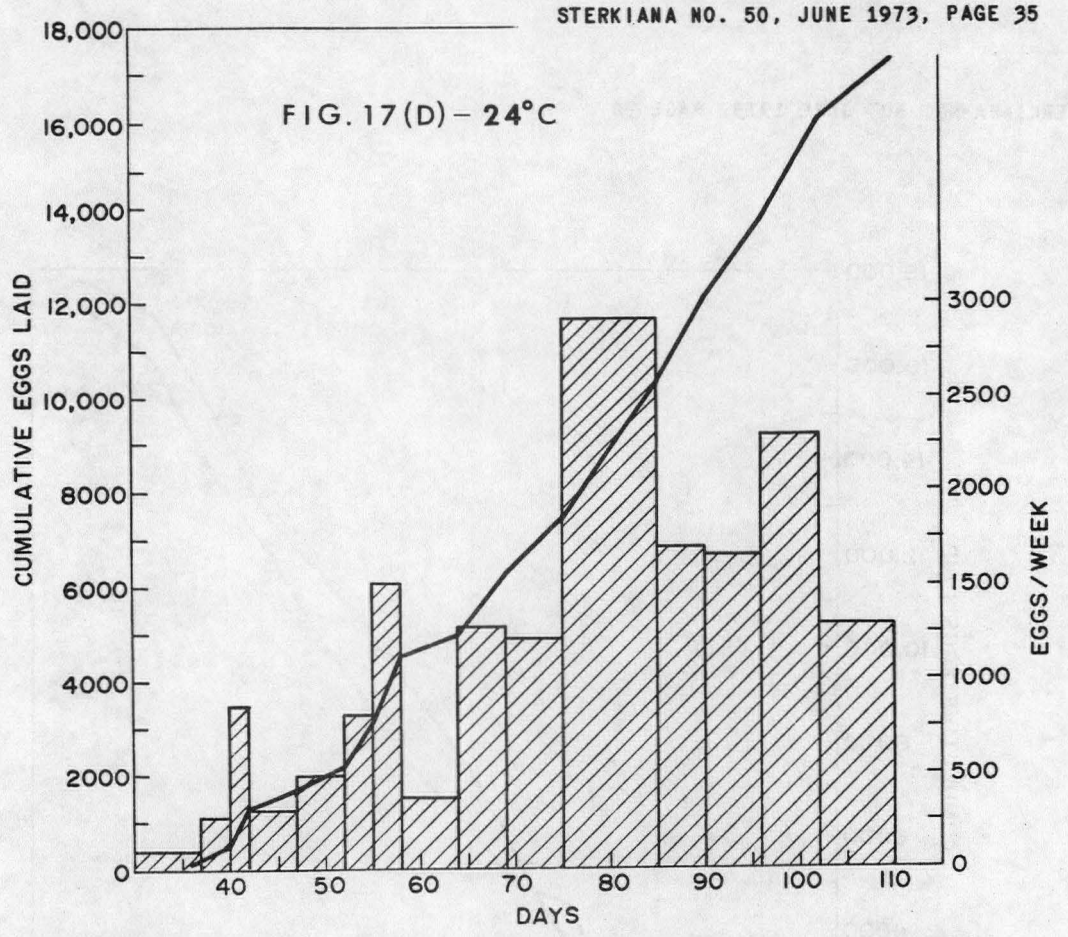


FIG. 17 (D) – 24°C
CUMULATIVE EGGS LAID
EGGS/WEEK
DAYS
18,000
16,000
14,000
12,000
10,000
8000
6000
4000
2000
0
3000
2500
2000
1500
1000
500
40
50
60
70
80
90
100
110

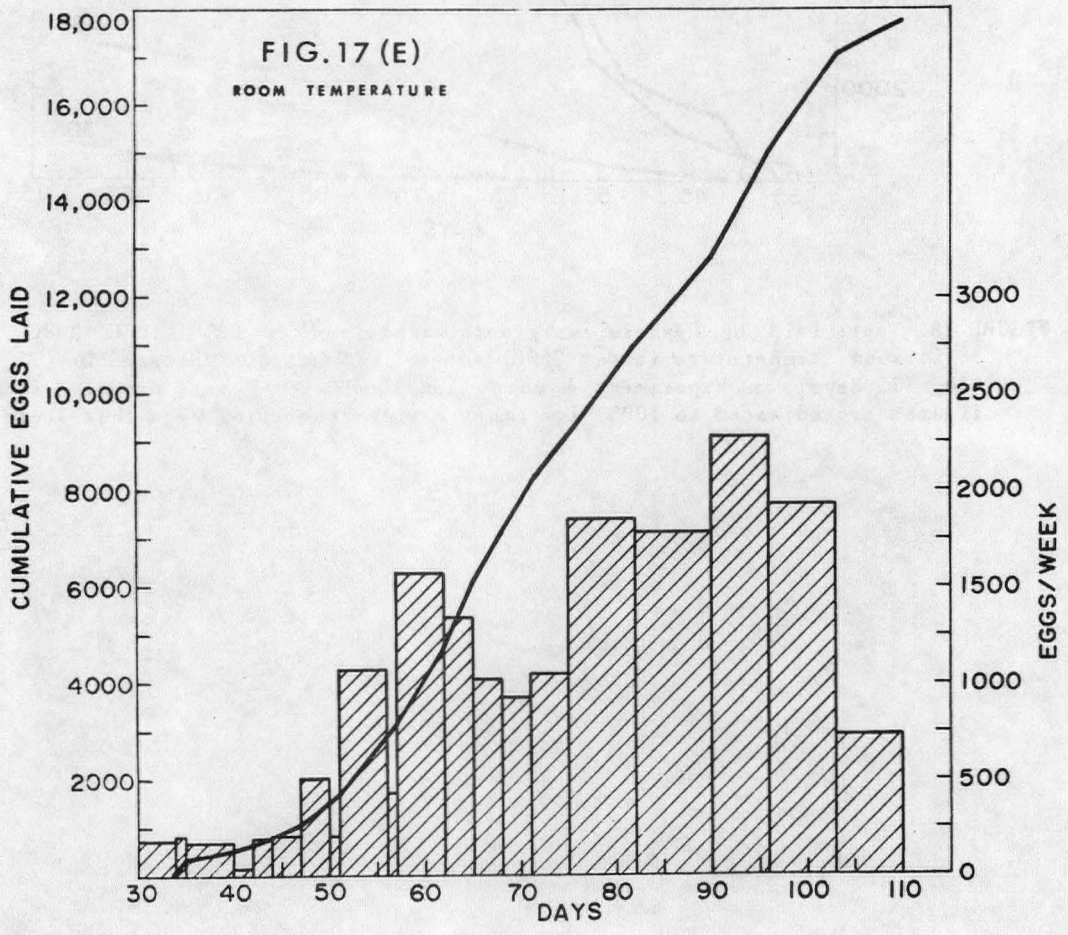


FIG. 17 (E)
ROOM TEMPERATURE
CUMULATIVE EGGS LAID
EGGS/WEEK
DAYS
18,000
16,000
14,000
12,000
10,000
8000
6000
4000
2000
0
3000
2500
2000
1500
1000
500
30
40
50
60
70
80
90
100
110

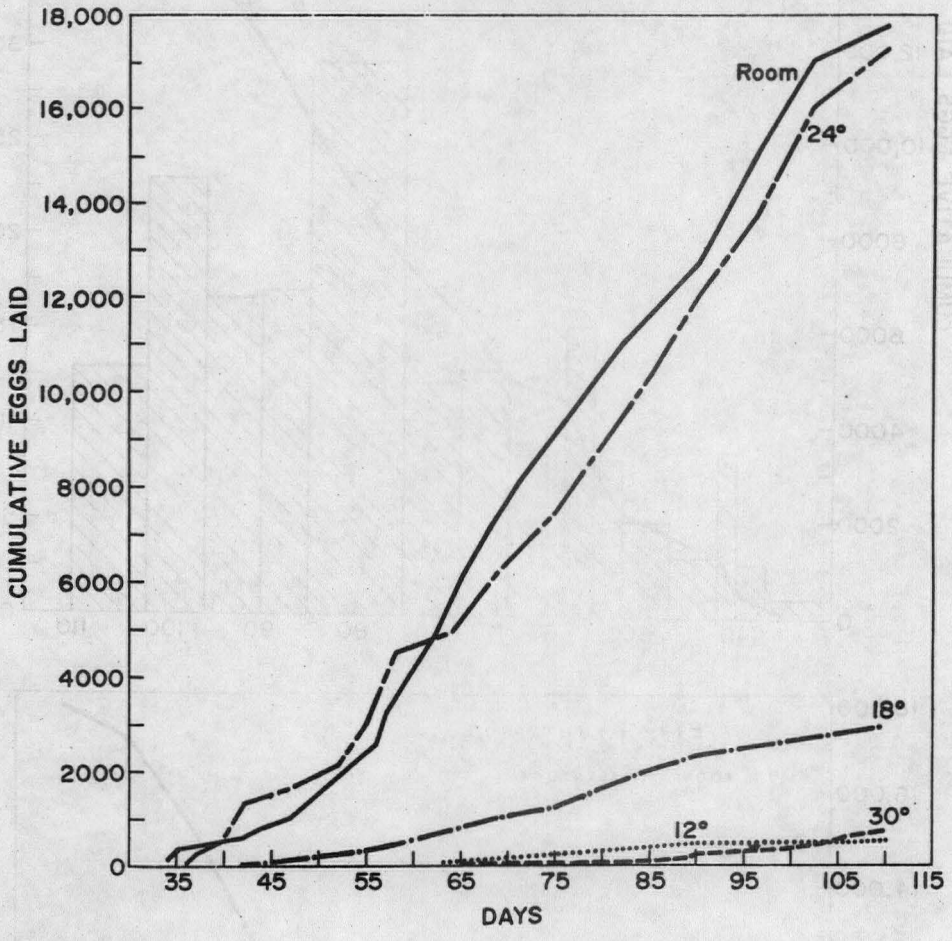


FIGURE 18. Eggs laid by *Lymnaea emarginata* maintained at 12°C, 18°C, 24°C and 30°C; room temperature (about 25°C) served as control. Between Day 35 and 110 (75 days) in Experiment 4 more than 16,000 eggs were produced (these figures are adjusted to 100%, i.e., they are the number of eggs that would be

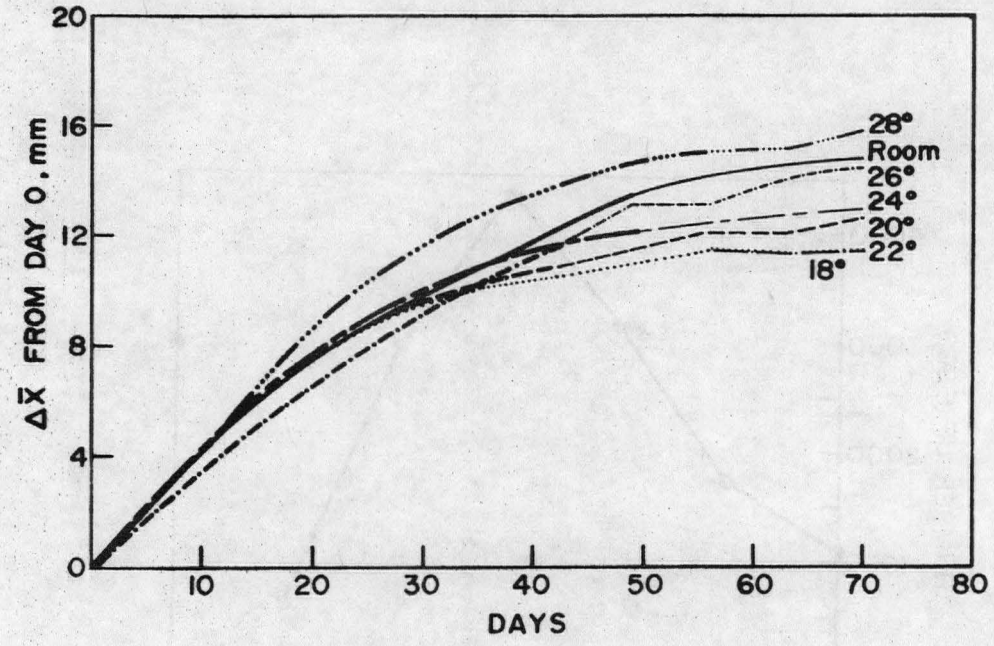


Figure 19. Growth of Lymnaea emarginata maintained at 2°C intervals for 70 days. Experiment 5.

TABLE 6. Summary of data for Lymnaea emarginata at 2° intervals, studied for 77 days (12/9/69 through 2/24/70), 30 specimens per tank. (Experiment 5)

TEMPERATURE °C	OXYGEN ppm	GROWTH*		REPRODUCTION		
		Total change in mm ± 2 $s_{\bar{x}}$	Survival %	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
18.6 ± 0.4	9.0 ± 0.2	11.9 ± 0.8	70.0	35	105/1137	98.7
20.4 ± 0.2	8.3 ± 0.2	13.0 ± 0.5	73.3	28	184/1744	98.4
22.2 ± 0.3	8.1 ± 0.2	12.2 ± 0.9	53.3	28	288/3162	97.1
23.9 ± 0.2	7.6 ± 0.2	13.1 ± 0.5	76.6	28	335/4343	97.4
26.0 ± 0.9	7.3 ± 0.2	14.8 ± 1.2	23.3	28	182/2907	93.5
27.9 ± 0.3	6.6 ± 1.1	16.3 ± 1.1	33.3	28	76/560	83.4
26.9 ± 0.4 (Control)	6.9 ± 0.4	15.3 ± 0.5	86.7	21	308/2953	80.1

*At beginning of experiment, animals were 7-10 old, 2.7 mm average length.

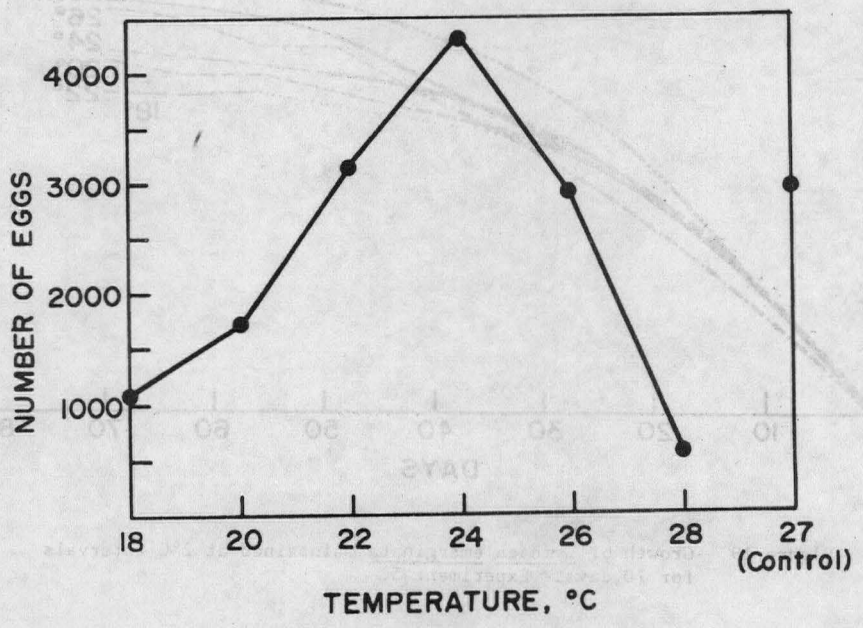


Figure 20. Eggs produced by Lymnaea emarginata at 2° intervals between 18° and 28°C; peak production appeared at 24°C. Experiment 5.

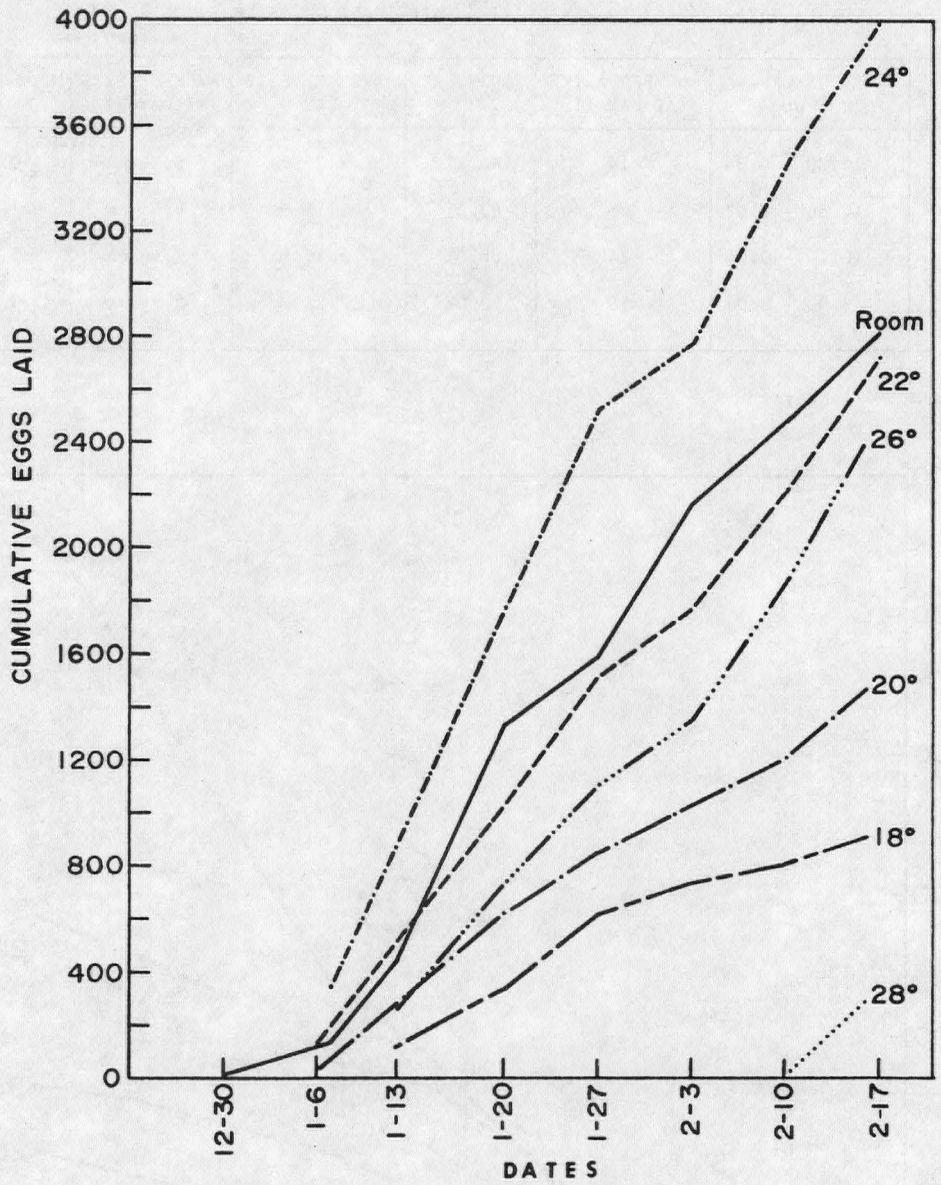


Figure 21. Cumulative (not adjusted to 100% survival) eggs produced by Lymnaea emarginata maintained at 2°C intervals between 18° and 28°C; room temperature (about 25°C) served as control. Experiment 5.

TABLE 7. Summary of data for Lymnaea emarginata "plop test" (animals subjected to sudden changes of temperature in tanks from room temperature), studied for 21 days (5/6/71 to 5/27/71), 30 specimens per tank.

TEMPERATURE °C	OXYGEN ppm	NUMBER ALIVE AT DAY 21	SURVIVAL %	AVERAGE SIZE AT DAY 0*	AVERAGE SIZE AT DAY 21	CHANGE IN SIZE FROM DAY 0
6.63 ± 0.10	15.30 ± 0.34	14	46.7%	2.29 mm	3.28 mm	0.99 mm
12.31 ± 0.10	14.56 ± 0.07	25	83.3%	2.18 mm	4.55 mm	2.38 mm
21.88 ± 0.06	10.14 ± 0.04	24	80.0%	2.19 mm	7.46 mm	5.27 mm
30.50 ± 0.02	8.00 ± 0.06	18	60.0%	2.27 mm	5.99 mm	3.72 mm

*Age at Day 0: 4-14 days.

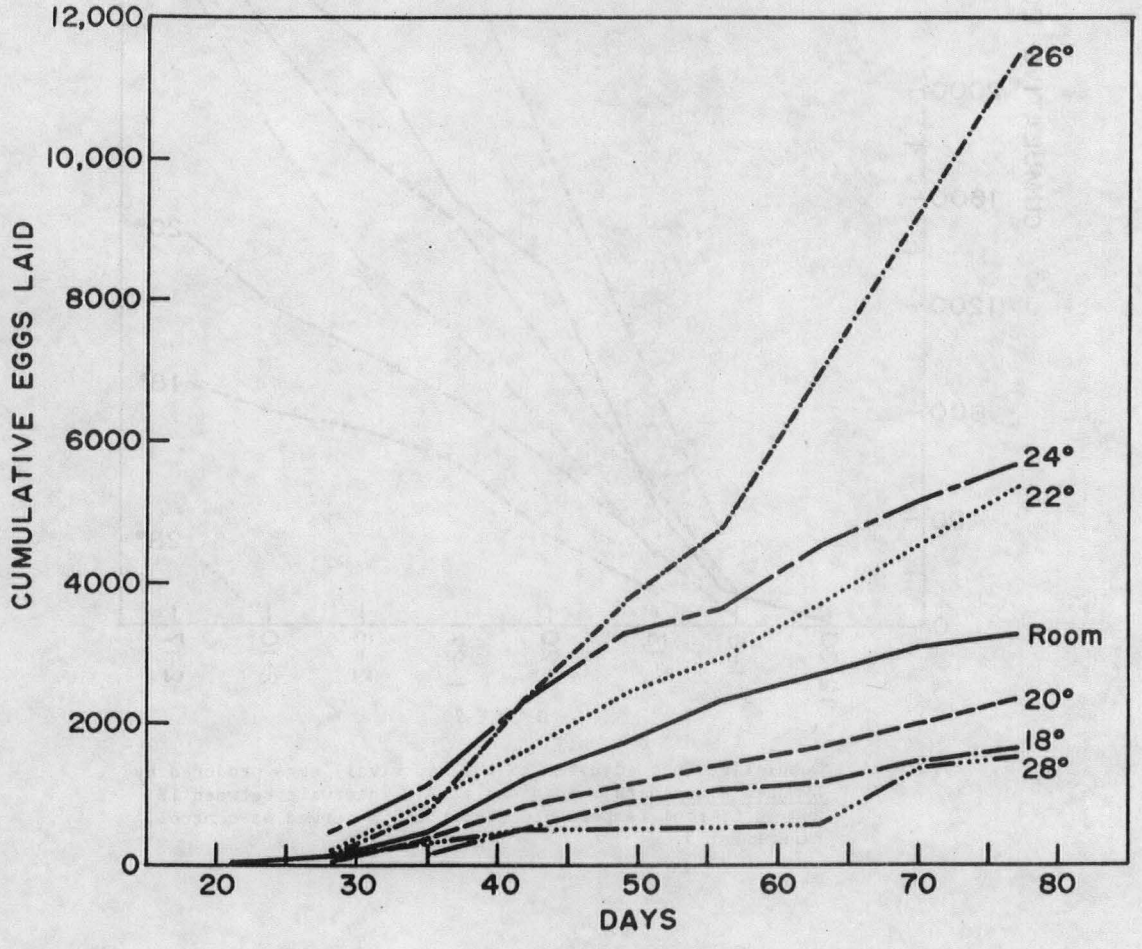


FIGURE 22. Cumulative (adjusted to 100% snail survival) number of eggs produced by *Lymnaea emarginata* maintained at 2° intervals between 18° and 28°C; room temperature (about 25 C) served as control. Experiment 5.

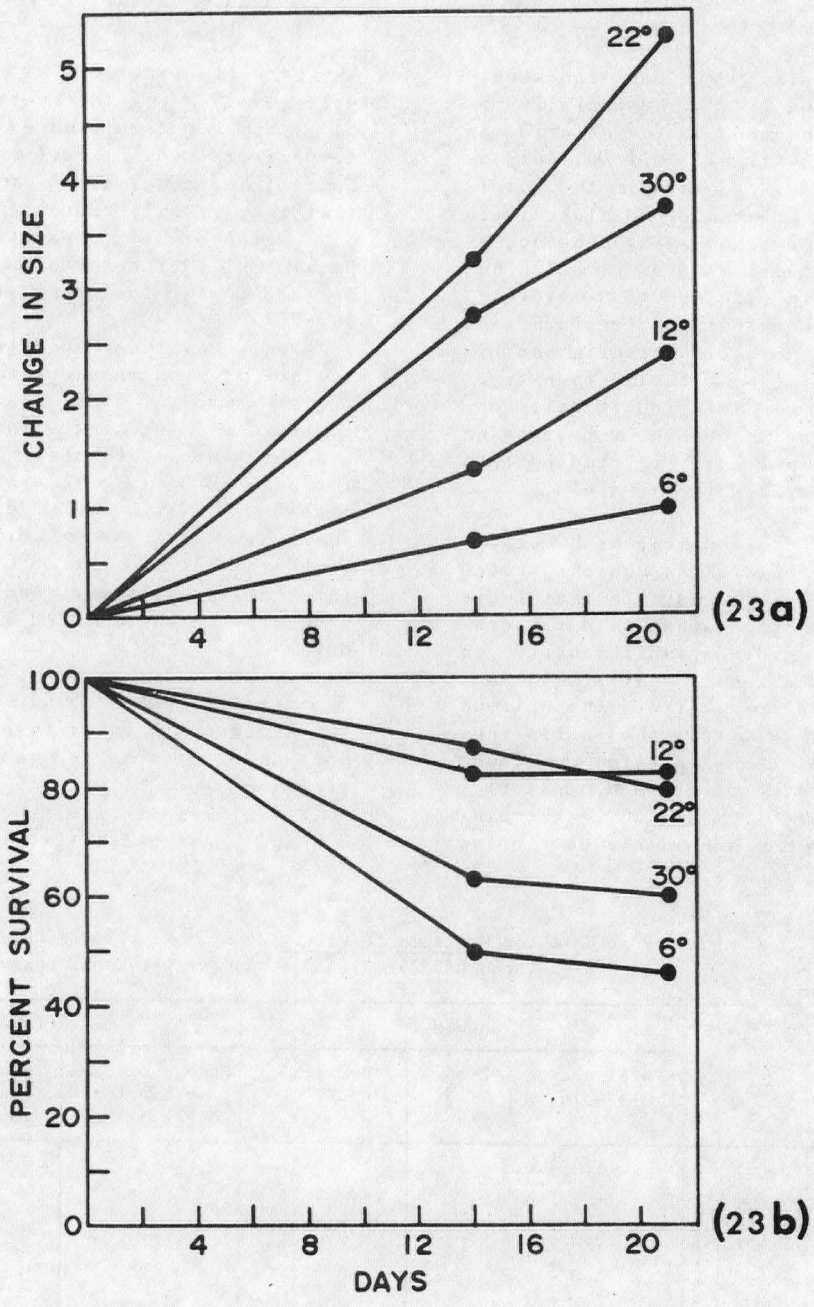


FIGURE 23. Data on 'plop test' with *Lymnaea emarginata* showing growth (23a) and survival (23b) in a 3-week period at different temperatures.

Helisoma trivolvis (Say)

Planorbid snails are abundant and widely distributed. The group is especially important since species of *Biomphalaria* (in the Americas and Africa) and *Bulinus*, or *Physopsis*, (in Africa) serve as the intermediate hosts of human blood fluke (Bilharziasis or schistosomiasis). Consequently, some useful basic studies have been undertaken to learn more about the role of temperature in the growth and reproduction of some of the more important planorbid snail intermediate hosts. The investigations referred to in this report will concern mainly those that have a bearing on the role of temperature as studied both in the laboratory and in the field.

Standen (1952:48), working with an Egyptian strain of *Schistosoma mansoni*, cited several references to indicate that there is a 'relationship of season and temperature to the incidence and intensity of schistosome infection in snails.' In some studies designed to ascertain the optimum temperature for culturing the snails (now called *Biomphalaria glabrata)* he found that the optimum temperature for that planorbid was between 26° and 28°C; the minimum necessary for adequately developing the parasite (sporocysts) was 26 C. Stirewalt (1954), using the Puerto Rican strain of *S. mansoni*, also studied the effects of temperature on this snail and its parasite. Some of her conclusions are important in that they clearly indicate the tolerance of *B. glabrata* to comparatively warm temperatures. Her results can best be summarized in the following statement (1954: 507-08):

'Even under these conditions the influence of temperature changes is obvious: at 23° to 25°C, 28% of exposed snails developed infections, 32% of the survivors. Further temperature increase to 31 to 33°C increased the number of snails which died, but of those which survived 52% developed infections. At 33° to 35°C, the death rate was prohibitive, and at 36° to 38°C all the snails died within three days.'

Stirewalt *(Ibid:* 515) in discussing the effects of temperature on the snail host and its parasite, made the following statement.

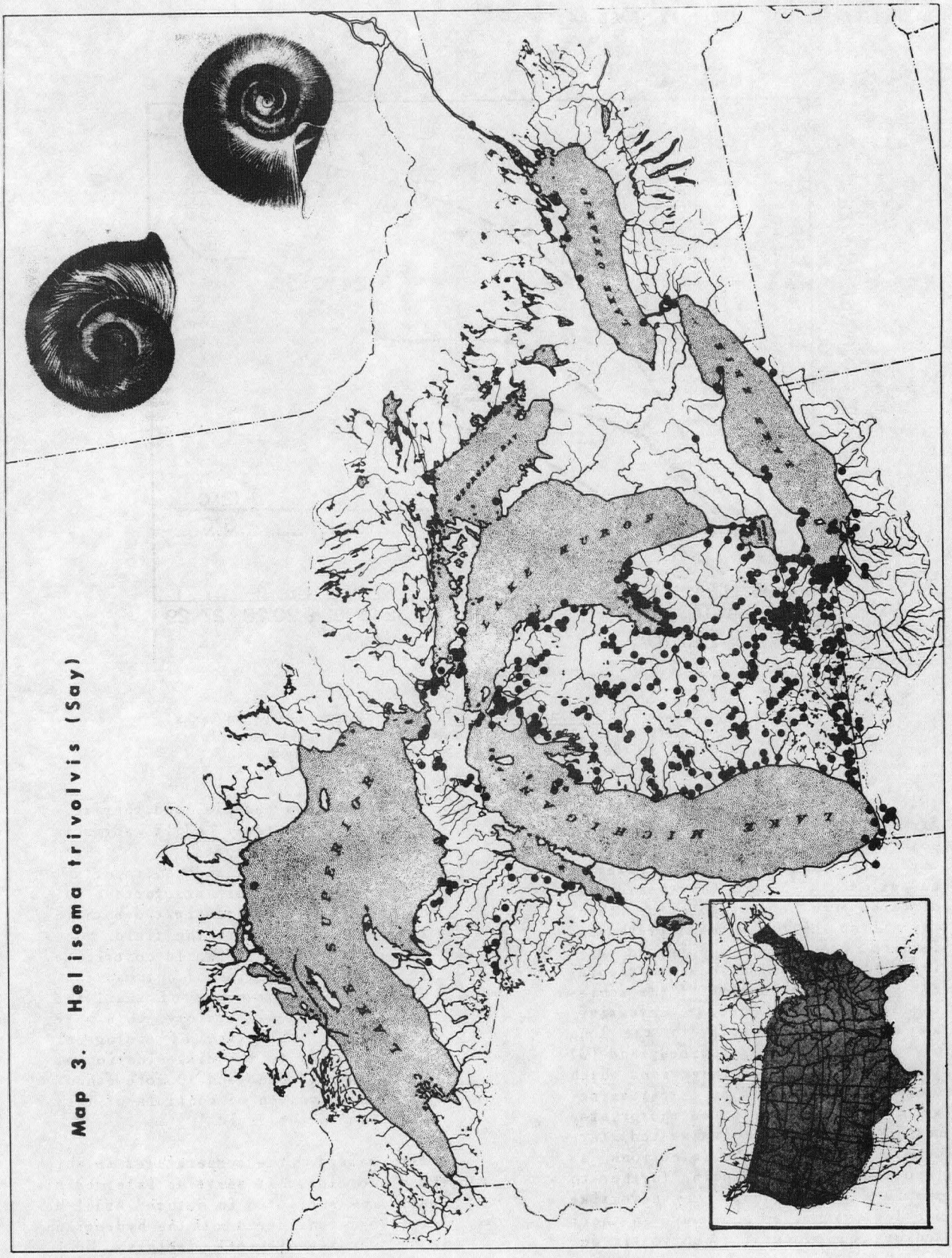
'The responsible mechanism is not known. An attractive hypothesis is

TABLE 8. Summary of data for Helisoma trivolvis at 6° intervals, studied for 51 days (4/8/69 through 5/29/69), 30 specimens per tank. (Experiment 2)

TEMPERATURE	OXYGEN	GROWTH		REPRODUCTION		
°C	ppm	Total change $\pm 2\ s_{\bar{x}}$ in mm	Survival %	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
9.9 ± 0.7	8.0 (6)*	1.0 ± 0.3	90.0	--	--	--
12.1 ± 0.1	7.7 (5)*	1.4 ± 0.3	76.7	--	--	--
18.2 ±	7.0 (3)*	2.0 ± 0.5	76.6 (31)**	--	--	--
24.0 ± 0.5	6.2 ± 0.5	5.0 ± 0.9	80.0	42	15/212	94.6
29.9 ± 0.1	5.6 ± 0.3	6.5 ± 0.8	60.0	28	21/240	97.6
35.5 ± 0.5	5.5 (4)*	2.2	0.0(36)**	--	--	--
25.2 ± 1.0 (room)	5.5 ± 0.3	4.6 ± 1.0	96.7	31	19/295	93.6

*Number insufficient for calculating standard deviation, i.e., less than 8.

**Number of days at termination of tank.



Map 3. Helisoma trivolvis (Say)

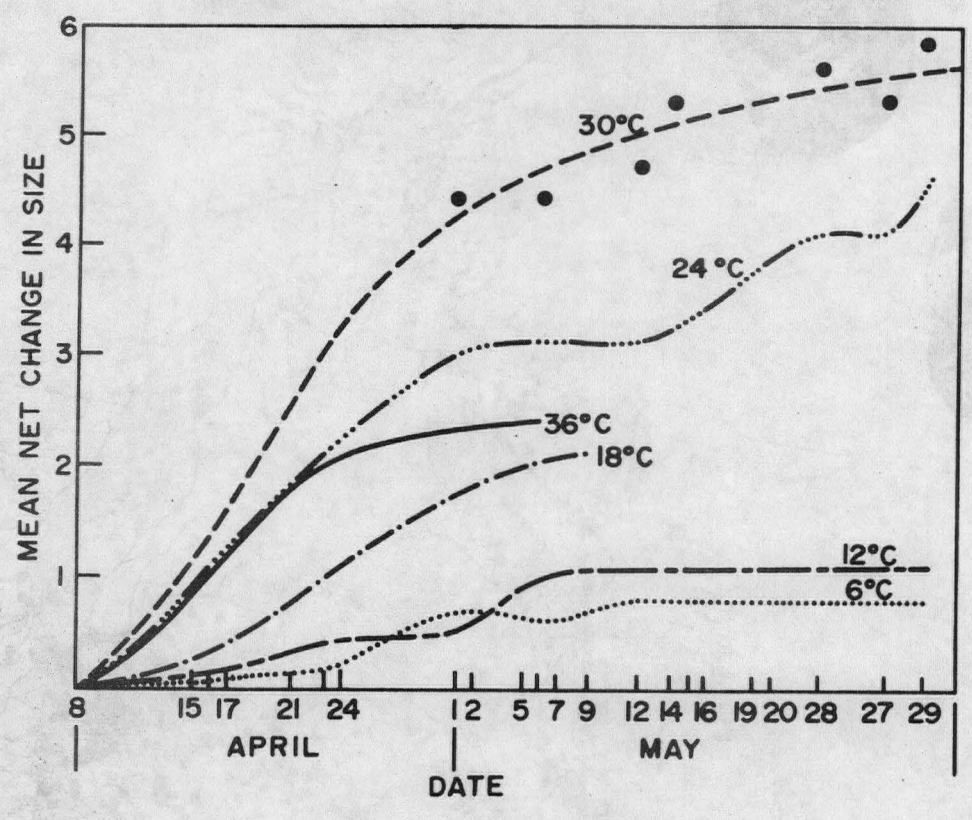


Figure 24. Growth of Helisoma trivolvis maintained for 51 days in tanks at 6°C intervals ranging from 6° to 36°C. Development is best in the warmer water (24° - 30°C). Experiment 2.

that the low temperature, transferred from the environment to the exposed poikilothermous molluscan hosts, retards the metabolism of the parasite as well as the host. Growth and cell division of the schistosomes, then, may be so inhibited that (1) the infection is completely suppressed, giving the lowered infection rate recorded; (2) the development of the schistosomes from miracidia to cercariae is retarded, resulting in the long pre-patent period described; and (3) the duration of those infections which do mature is limited as cercariae develop within the retarded sporocysts, until the latter are exhausted, terminating many of the infections, as reported. It is tempting further to surmise that perhaps the cercariae are less mature in some way, in their sensory or muscular or enzymatic development, for example, and therefore they produced fewer adult worms in susceptible mouse hosts.

'With temperature such an important environmental factor, its further effects should be investigated both in the laboratory and in the field. The results of such work should contribute to a better conception of optimal conditions for maintenance of snail and parasite in the laboratory, to a more intelligent evaluation of ecological data relative to the dissemination of human schistosomes, and to more economical application of controls of this parasite in the field.'

With regard to the temperatures to which the planorbids that serve as intermediate hosts are subjected in nature, Abdel Malek (1958) considered both the hydrographic and hydrogeological factors. He in-

dicated that in the Nile basin seasonal variations were greater in the north than in the south. The temperatures varied considerably depending on rainfall; his recorded range in various parts of the Nile (1958:698) was between 24°C in November at Kosti to 31°C in May at Khartoum.

Michelson (1960) studied the effects of temperature on the growth and reproduction of *Biomphalaria* (formerly *Australorbis) glabrata* in the laboratory. He exposed 15 snails in 3-liter pyrex battery jars to temperatures of 5°, 15°, 20°, 25°, 30° and 35°C, with 25°C as controls. He found that on the higher levels (30°C) the reproductive process failed and few eggs were produced. Dissected specimens in this 'hyperthermal' group had 'the albumin glands atrophied, abnormal in color, or entirely absent. Sections of the ovotestis in these snails showed that the female elements were poorly developed although the male elements appeared normal.' He stated (1961:72): 'Thus, although maintenance at 30°C appeared to be optimal for maximum growth, reproduction was inhibited.' At the 35°C level all the snails died within 15 days. When his snails were maintained at lower temperatures (the 'hypothermal' groups), growth and reproduction were inhibited but, since no serious tissue alterations occurred, those processes carried on in a normal way when the animals were transferred to optimal temperatures.

Shiff (1963) published a series of three papers on the biology of Rhodesian *Bulinus (Physopsis) globosus* in which he discussed: I. The influence of temperature on the intrinsic rate of natural increase; II. Factors influencing the relationship between age and growth; and III. Bionomics of a natural population existing in a temporary pool. In his summary (1963:104) he reported that his life tables used for determining the influence of temperature on 'the intrinsic rate of natural increase' indicated that the optimal temperature was 25°C; he also reported (1963: 115) that 'the snails grew most rapidly at 25°C; and when he studied a natural population that temperature also was the one at which 'egg production is highest.' Later Shiff (1966: 214), in a study of the influence of temperature on vertical movement of this snail both in the laboratory and in the field, stated: 'It has been shown that for both *Biomphalaria pfeifferi* and *Bulinus globosus* in the laboratory, the intrinsic rate of natural increase rises considerably with temperature from 18° to 22°C. Any movement of snails from cold to warmth will result in a higher rate of increase at a time of the year when the size of the population may be critical for enabling the species to survive the possible drying out of the habitat during early summer.'

Sturrock (1966) studied the influence of temperature on *Biomphalaria pfeifferi*, the intermediate host for *Schistosoma mansoni*. He observed that these snails were usually not found in the low, hot regions along the coasts of Kenya, Mozambique, the Red Sea and those of West Africa. His thesis is that temperature may be a controlling factor in the distribution of this snail. He emphasized the role played by temperature, as follows:

> 'Nevertheless, where water bodies may be expected to have temperatures in excess of 28–30°C for several months on end, as on the coastal plain of Tanzania, it is unlikely that any suitable natural habitats will be successfully colonized by *B. pfeifferi*, and it is therefore reasonable to assume that high temperatures have been and will remain, a major barrier to the colonization of such habitats.'

He found that the optimum temperature was 25°C; survival was good at 19°C but it was poor at 30°C. The maximum heat tolerated was 32°C, and under field conditions he doubted that this species of snail could survive for any extended period at temperatures much above 28°C.

Chernin (1967) studied the reaction of *Biomphalaria glabrata, Bulinus truncatus* and *Lymnaea palustris* in a thermal gradient established in a shallow water trough. He found that *B. glabrata* avoided thermal extremes but tended to select 'zones proximating 27° to 32°C.' When food was placed in a 'cool' zone these snails 'congregated' there. His observations (1967:1233), as will be shown later, tend to corroborate those reported here in that the three species studied 'differed in their distributional responses to the thermal gradient; thus, *B. glabrata* were generally found in moderately warm loci; *L. palustris* tended to accumulate in the relatively cool portions of the gradient, and *B. truncatus*

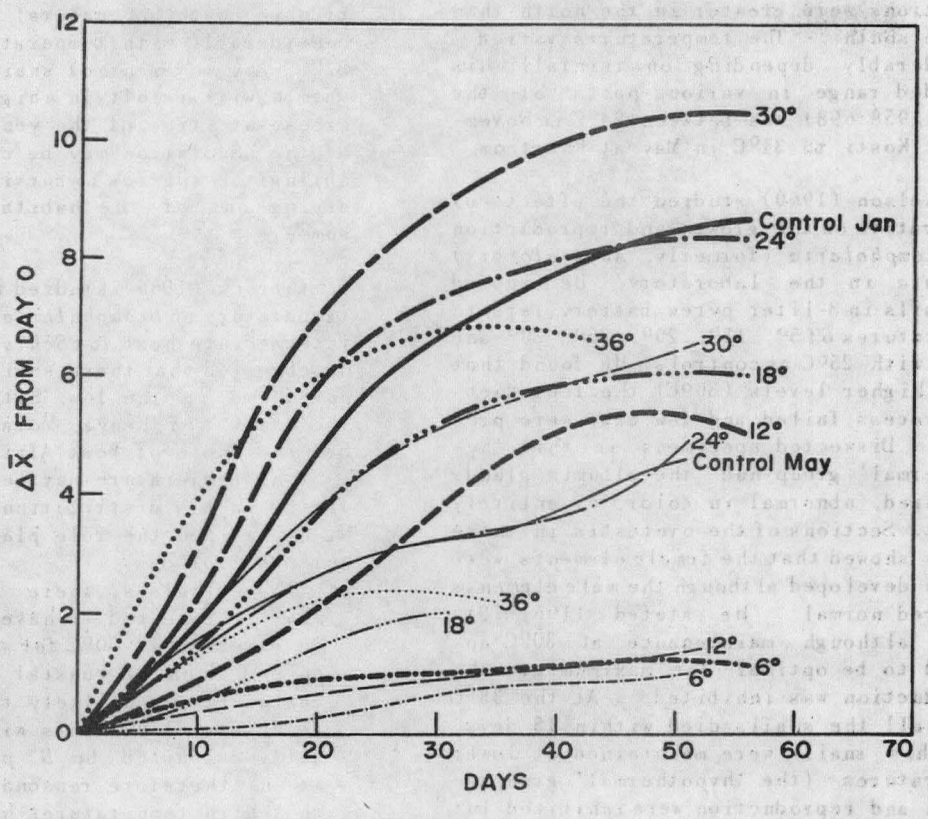


Figure 25. Two sets of growth observations made on Helisoma trivolvis are compared to indicate that the basic pattern is the same but the growth patterns differ. The optimum in each (January - heavy lines; May - light lines) is still between 24° and 30°C. Experiments 2 and 6.

distributed themselves fairly uniformly throughout the gradient. The general findings may contribute to a better understanding of some of the influences underlying the localization of discontinuity of natural colonies of snails.'

Jobin (1970) studied *Biomphalaria glabrata* in three farm ponds in Puerto Rico. Temperature was an important factor in the dynamics of the populations there and he stated (1970:1046) as follows: 'Specifically, the population declines of *B. glabrata* in Ponds B and C were related to extreme water temperatures that limited reproduction; in Pond A the temperature was 25°C to 28°C, optimum for reproduction, when the *B. glabrata* disappeared. ....' He also observed that: 'Optimum temperature for oviposition diminished to zero as the the temperatures approach 20°C or 30°C. Since the temperatures in the three ponds reached and even surpassed the extreme limits during winter and summer, temperature plays an important part in controlling the pattern of reproduction of the snail populations, although it was ignored in previous field studies.[6]' 'These observations are essentially similar to those reported by others and they will be shown later to affirm the data obtained in the following studies. When temperatures go above 30°C, as they did in Ponds B and C in Puerto Rico, reproduction is adversely affected. It is important to have more such field data to corroborate the information obtained in laboratory experiments.

Helisoma trivolvis (Say) is among the most widespread of the freshwater pulmonates in North America (see Map 3). It is a more tolerant species of heat and pollution, so that it is not uncommon to find

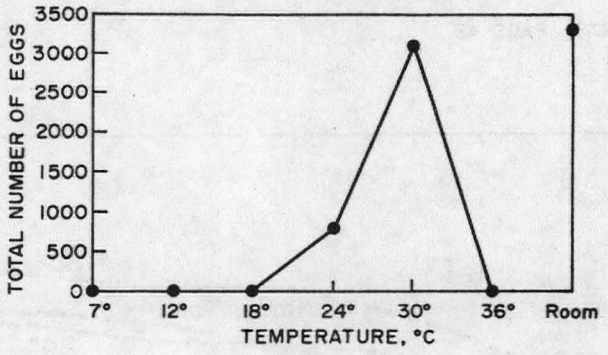


Figure 26. Egg production of Helisoma trivolvis maintained in six tanks at temperatures ranging between 7° and 36°C. Highest production occurred between 24° and 30°C. Experiment 6.

TABLE 9. Summary of data for Helisoma trivolvis at 6° intervals, studied for 77 days (12/15/69 through 3/2/70), 30 specimens per tank. (Experiment 6)*

TEMPERATURE	OXYGEN	GROWTH**		REPRODUCTION		
°C	ppm	Total change ± 2 $s_{\bar{x}}$ in mm	Survival %	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
7.6 ± 0.3	11.7 ± 0.3	1.1 ± 0.2	53.3	--	--	--
12.7 ± 0.5	9.8 ± 0.3	5.2 ± 0.6	76.7	--	--	--
18.3 ± 0.4	8.9 ± 0.3	6.4 ± 0.8	60.0	--	--	--
24.0 ± 0.3	7.5 ± 0.3	9.2 ± 0.9	46.7	35	50 / 799	93.5
29.9 ± 0.1	6.5 ± 0.2	11.2 ± 0.5	60.0	21	209 / 3109	86.2
35.6 ± 0.2	5.7 ± 0.4	6.6 ± 1.0	(all dead, 42 days)			
24.5 ± 0.5	7.3 ± 0.3	9.6 ± 0.6	73.3	35	202 / 3361	94.0

*Repeat of Experiment 2 because of insufficient data.

**At beginning of experiment, animals were 7-10 days old, 0.9 mm average diameter.

it in areas where heat and eutrophication may have eliminated Lymnaeid snails. In the tests used in stressing this snail it is clear that as a planorbid it tolerates warm conditions better than most lymnaeid snails do. Its range from Alaska to Georgia and Maine to California implies that it lives under conditions that subject it to a wide range of tolerance.

Experiment 2. The snails used were cultured from stocks obtained from Bass Lake, Livingston County, Michigan; several generations were cultured before the animals were subjected to the different temperatures used in the experiments. The first tests were conducted with young specimens stressed at six temperatures between 9.9° and 25°C (Table 8) with the aquaria maintained at 10°, 12°, 18°, 24°, 30° and 35°5 C., respectively; the control was at room temperature-about 25°C. Thirty young snails averaging 3.6 mm were cultured in each of the seven aquaria. The animals

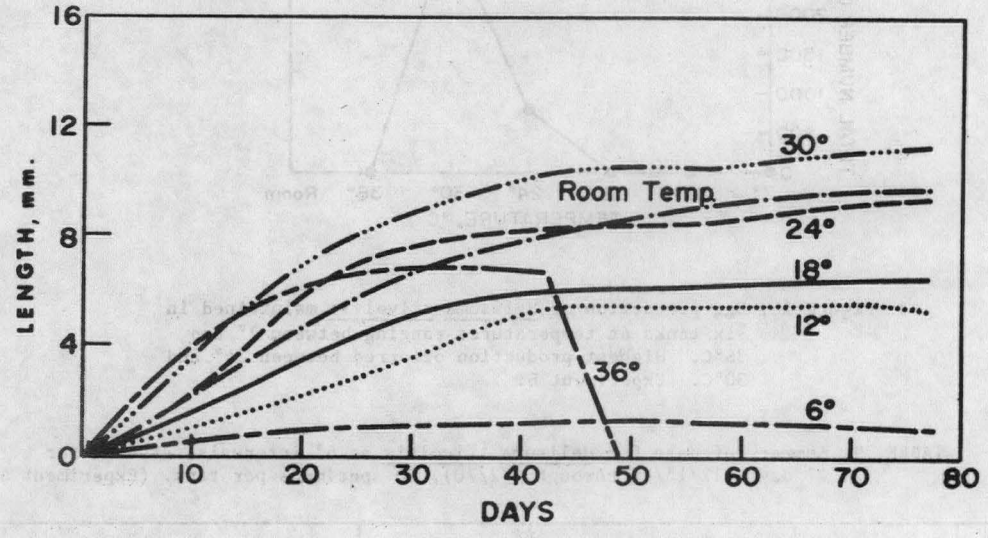


Figure 27. Growth of Helisoma trivolvis maintained for 77 days in 6 tanks at 6° intervals; room temperature (25°C) was used as a control. Note that growth was best between 24° and 30°C; survival in hot water (36°C) was surprisingly good. Experiment 6.

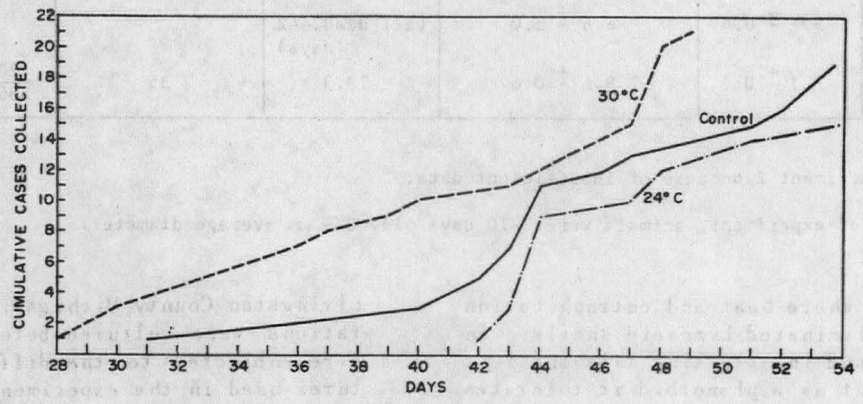


Figure 28. Helisoma trivolvis: cumulative egg-cases collected when snails were maintained in tanks at 6°C intervals between 6° and 36°C. The three temperatures represented show that warm conditions not only cause laying to begin early (the 28th day) but also stimulate the largest amount. Control temperature about 25°C. Experiment 6.

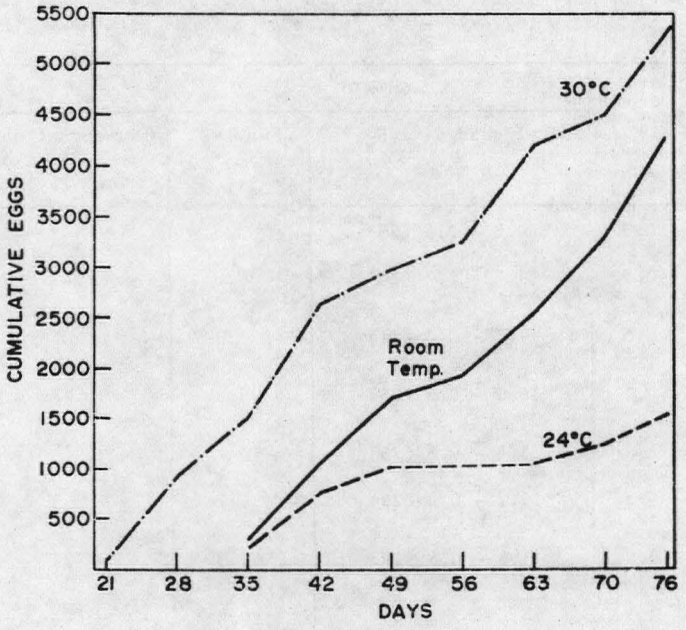


Figure 29. Cumulative eggs produced by Helisoma trivolvis from those tanks out of the six ranging in temperature from 6° to 36°C that had the best egg production. The control at room temperature was about 26°C. The warmer conditions favor high egg production. Experiment 6.

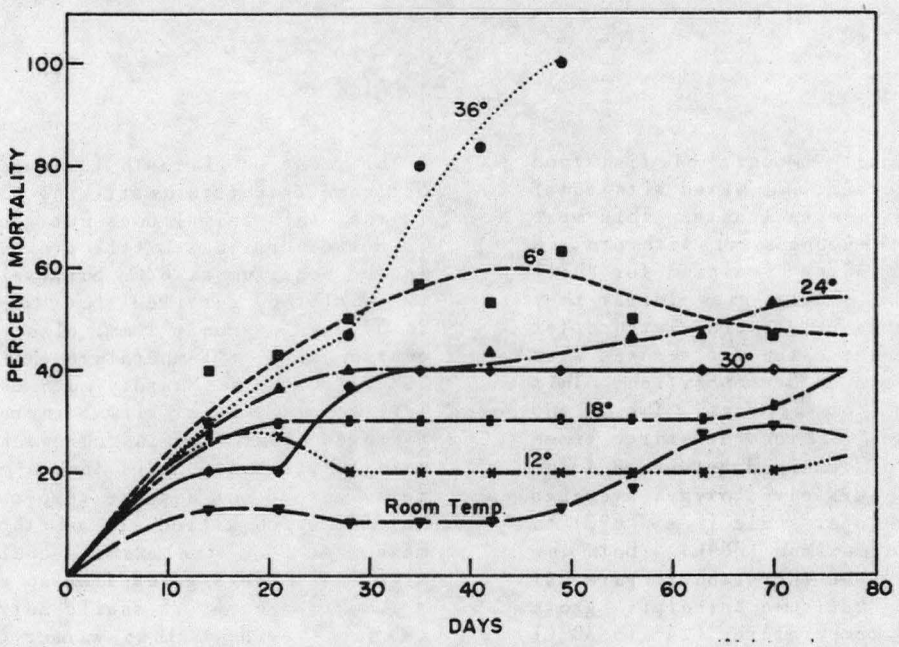


Figure 30. Mortality of Helisoma trivolvis maintained for 77 days in tanks at 6°C intervals, ranging between 6° and 36°C. Low mortality was especially noticeable at the cold (6°C) and at the hot (36°C) ends. Experiment 6.

TABLE 10. Summary of data for Helisoma trivolvis at 2° intervals, studied for 77 days (6/24/70 - 9/9/70), 30 specimens per tank. (Experiment 12)

TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
°C	ppm	Total change ± 2 $S_{\bar{x}}$ in mm	Survival %	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
20.7 ± 0.4	10.3 ± 0.5	9.3 ± 0.5 (25)**	83.3	42	98 / 2193	78.0
23.0 ± 0.4	10.0 ± 0.5	9.7 ± 0.2 (24)	80.0	49	228 / 5163	83.8
24.4 ± 0.4	9.4 ± 0.5	9.7 ± 0.3 (22)	73.3	35	261 / 4561	83.9
25.7 ± 0.2	8.7 ± 0.7	10.6 ± 0.3 (28)	93.3	28	353 / 6671	75.8
27.1 ± 0.8	9.0	5.3 ± 0.8 (28)***	0	--	--	--
29.6 ± 0.4	7.9 ± 0.5	11.2 ± 0.3 (26)	86.7	35	250 / 4246	60.0
30.6 ± 0.6	7.5 ± 0.6	11.6 ± 0.6 (19)	63.3	28	158 / 2252	62.9
32.4 ± 0.8	7.5 ± 0.5	14.0 (6)	20.0	--	--	--
23.6 ± 0.5 (room)	9.3 ± 0.4	11.0 ± 0.3 (26)	86.7	28	199 / 4055	84.8

*At beginning of experiment, animals were 1-8 days old.

**Number of snails surviving at end of experiment.

***Number of snails on 21st day; all died on 27th day from overheating.

were given small amounts of fish food ground to a powder and mixed with equal portions of calcium carbonate. This mixture supplied the young snails with protein and also gave a source of calcium for shell growth. As the snails grew larger they were given pieces of romaine lettuce for food. Lettuce not eaten was removed when there were signs of decomposition. This experiment ran for 51 days. The pH and dissolved oxygen were recorded three times per week; the pH remained between 8.1 and 8.4, while the dissolved oxygen averaged 7.7 ppm at the lower scale (i.e., 6°C) to 5.1 ppm at the maximum (36°C). Both the table (Table 8) and the graph (Figure 24) indicate that *Helisoma trivolvis* grows best at higher temperatures (24° to 30°C) and that it does rather poorly at lower temperatures at which the *Lymnaea stagnalis* and *L. emarginata* did best (18° and 20°C).

The relation of growth to temperature in *Helisoma trivolvis* is striking (Figure 24) in that this animal does not grow well in colder conditions. Little growth occurred in the aquarium at 6°C. Survival was good (27 out of 30 survived) but their growth in 51 days was only 1 mm; also, in the aquarium with a temperature that averaged 12°C, growth was hardly much better. At 18°C there was a slight increase which averaged about 2 mm in four weeks. It was unfortunate that in this tank a monitoring relay burned out and the temperature rose to 42°C which killed all of those specimens. At 24 C the animals doubled their size in 11 weeks, going from an average of 4.1 mm to 8.6 mm; 25 snails survived. It is quite evident that warmer conditions enhance the possibilities for culturing this snail. If data are graphed (Figure 25), comparing the two sets of cultures run in January and in May (heavy lines

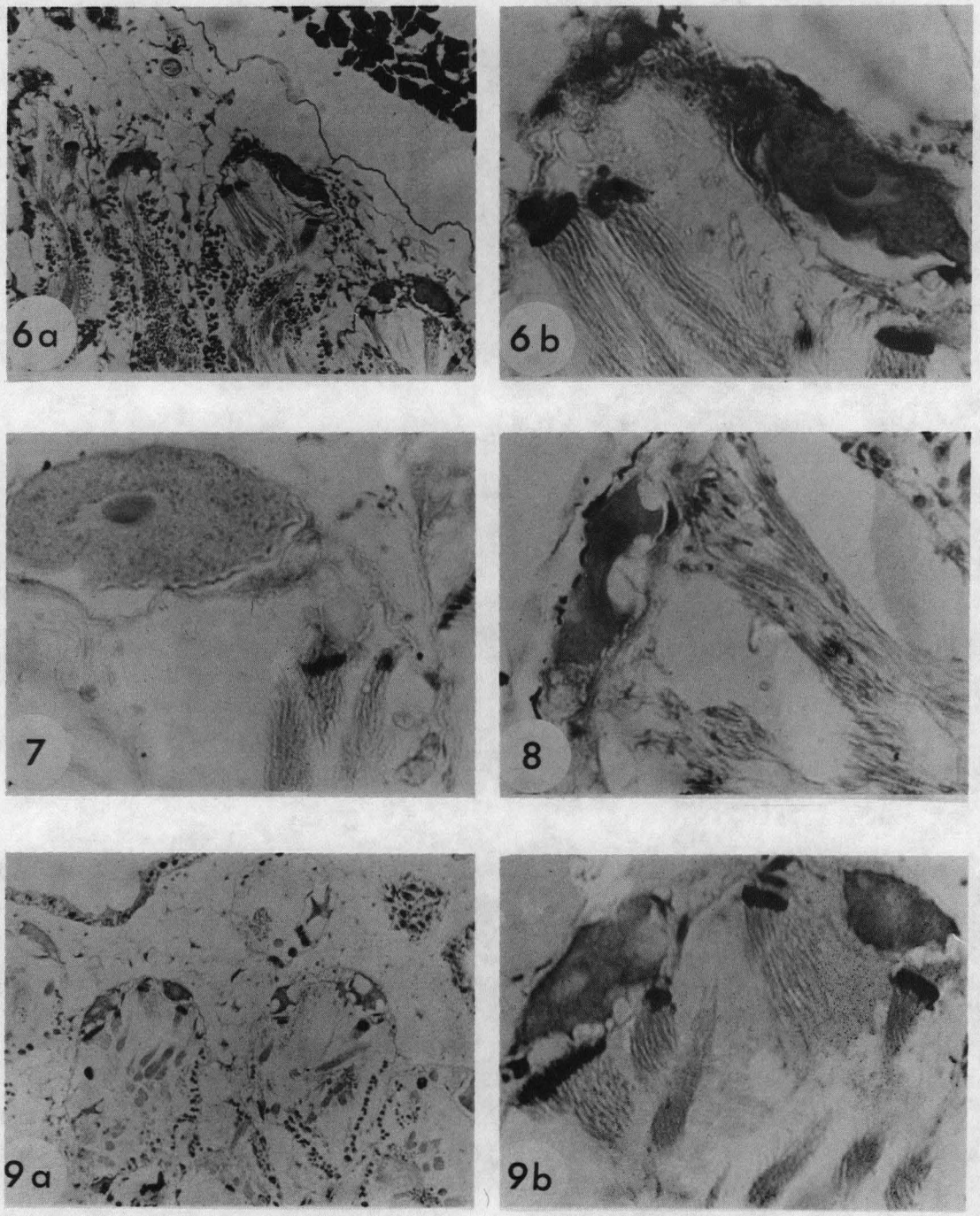


PLATE II. Gonad tissue sections from Helisoma trivolvis cultured for 77 days at different temperatures.

Figure 6a. 20°C (X70)
Figure 6b. 20°C (X400)
Figure 7. 22°C (X400)
Figure 8. Room temp. (X400)
Figure 9a. 24°C (X70)
Figure 9b. 24°C (X400)

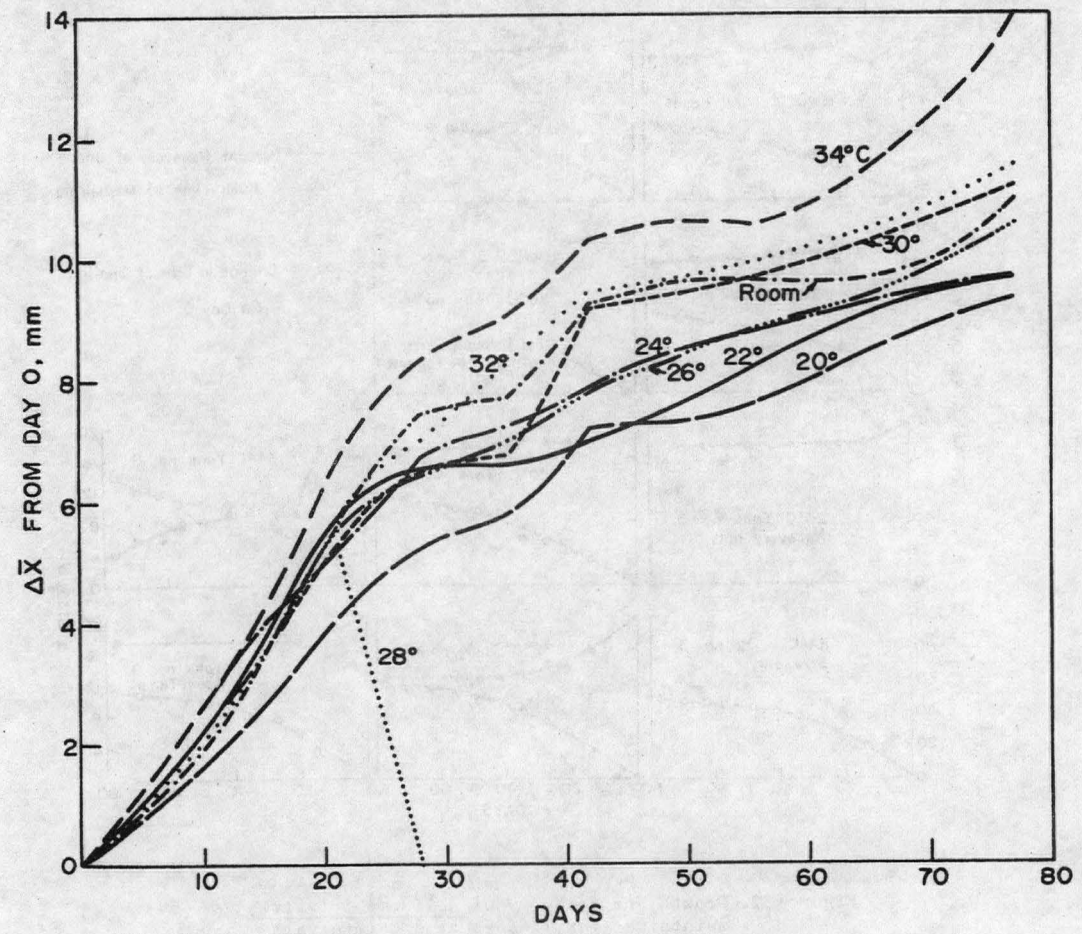


FIGURE 31. Growth of *Helisoma trivolvis* maintained for 77 days in tanks at 2°C intervals ranging from 20° through 34°C. Development was best at the warmer temperatures; the 28°C was lost due to overheating of a faulty probe. Experiment 12.

versus light lines on the graph), the pattern is essentially the same although the profiles are different; i. e., growth is poor at low temperatures, good in the warm range (24° to 30°C) but above 30°C conditions become intolerable. These results are also shown to be similar in Experiment 6.

Experiment 6. In the previous tests (Experiment 2) the 28°C tank overheated due to a faulty instrument. The results in this experiment with the temperatures at 6°C intervals are similar to those in Experiment 2 but they are more complete. As can be observed (Table 9), only half (53.3%) of the snails survived in the cold (7.6°C) water and those in the 12°C and 18°C tanks grew poorly. It was also seen that no reproduction occurred until the animals were maintained in temperatures at 24°C or above. Egg-production was good (Figure 26) among animals maintained at 30°C but the viability of the eggs at that temperature was considerably lower than that of eggs maintained at 24°C. The graph (Figure 27) also indicates that some specimens survived as long as 42 days in the very warm water (36°C), but in that temperature extreme no reproduction took

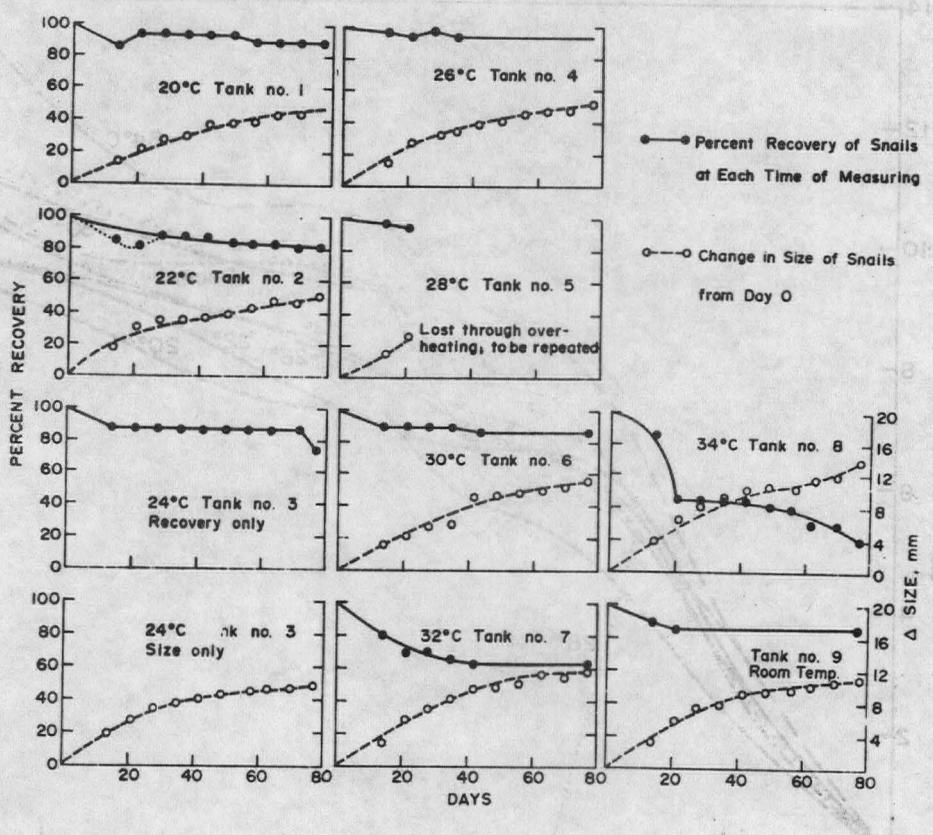
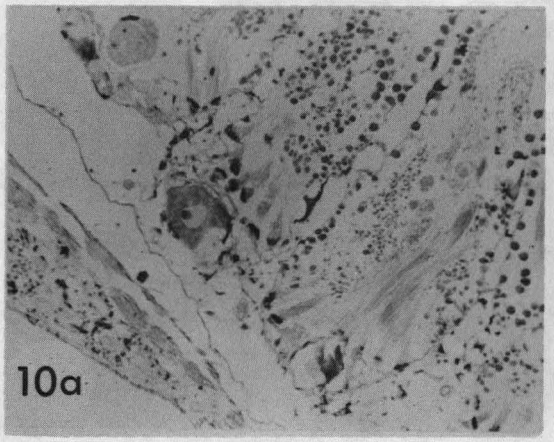
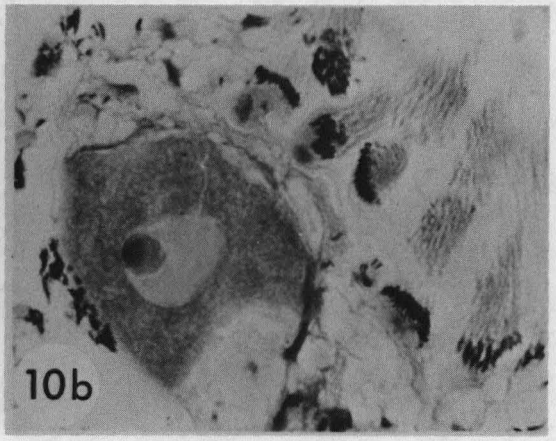


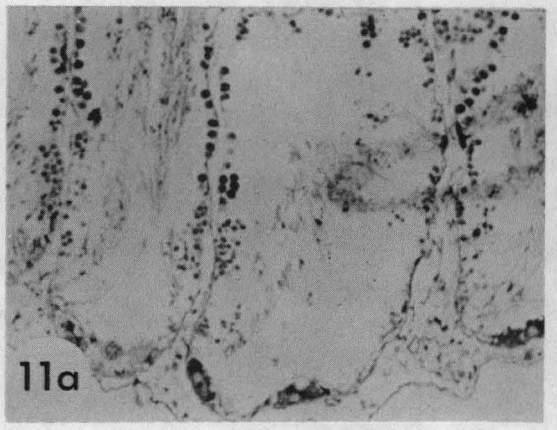
Figure 32. Growth and recovery of Helisoma trivolvis maintained for 77 days at 2°C intervals between 20° and 34°C; the control was at room temperature (about 25°C). Note that survival is good up to 30°C; also, that throughout the series growth was fairly uniform in all of the tanks. Experiment 12.

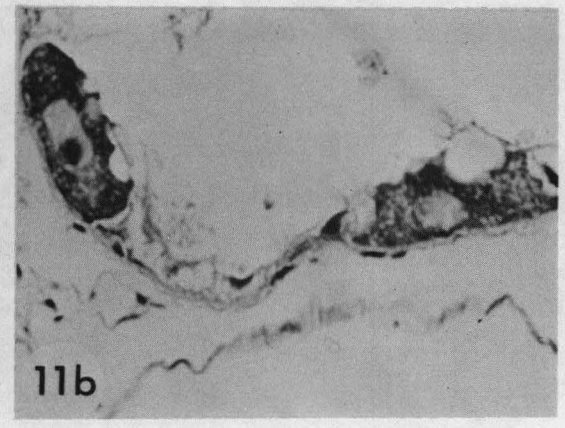
place. Data shown in Figures 28 and 29 again indicate that the optimum temperature appears to be between 24° and 30°C. When mortality was plotted on a percentage basis (Figure 30) it is apparent that *Helisoma trivolvis* does not survive either in the extreme cold (6°C) nor in the hot (36°C) range; however, between 12° and 30°C mortality is shown to be relatively low tending to be better under warm conditions. Since *H. trivolvis* appears to prefer warm conditions, tests were undertaken to maintain them at 2°C intervals ranging between 20° and 34°C to determine optimum conditions, maximum growth and egg production. (See Experiment 12).

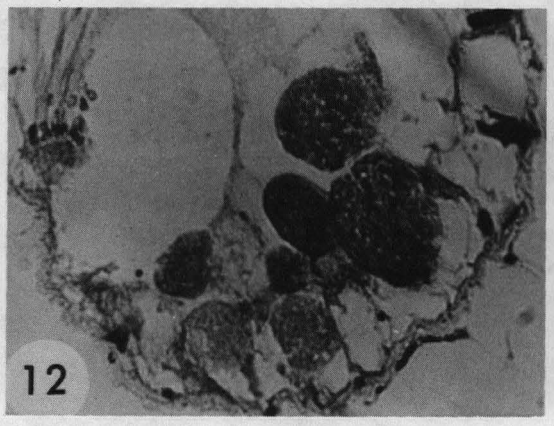
Experiment 12. In the course of the studies involved with stressing the *Helisoma trivolvis* at 6°C intervals (Experiments 2 and 6), growth and egg production were found to be best in the range between 24° and 30°C. As a consequence, another set of observations were made with the range between 20° and 32°C, using tanks maintained at 2°C intervals. A tube failure in the control of the 28°C tank ended that one on the 27th day due to overheating. The data on growth and survival are summarized in Table 10 and Figure 31. It can be seen that growth and survival within the normal ranges, i. e., between 20° and 30°C, were reasonably good (Figure 32).











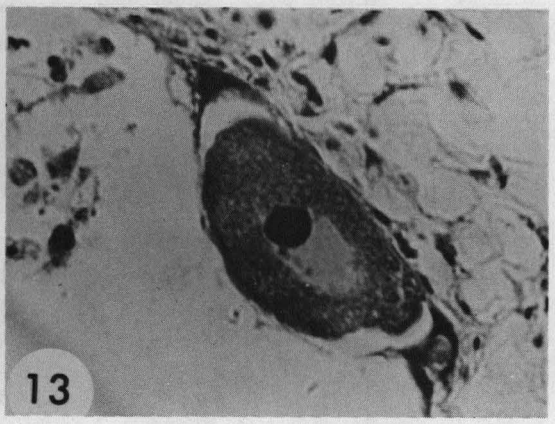


PLATE III. Gonad tissue sections from Helisoma trivolvis cultured for 77 days at different temperatures.

Figure 10a. 26°C (X70)
Figure 10b. 26°C (X400)
Figure 11a. 30°C (X70)
Figure 11b. 30°C (X400)
Figure 12. 32°C (X400)
Figure 13. 34°C (X400)

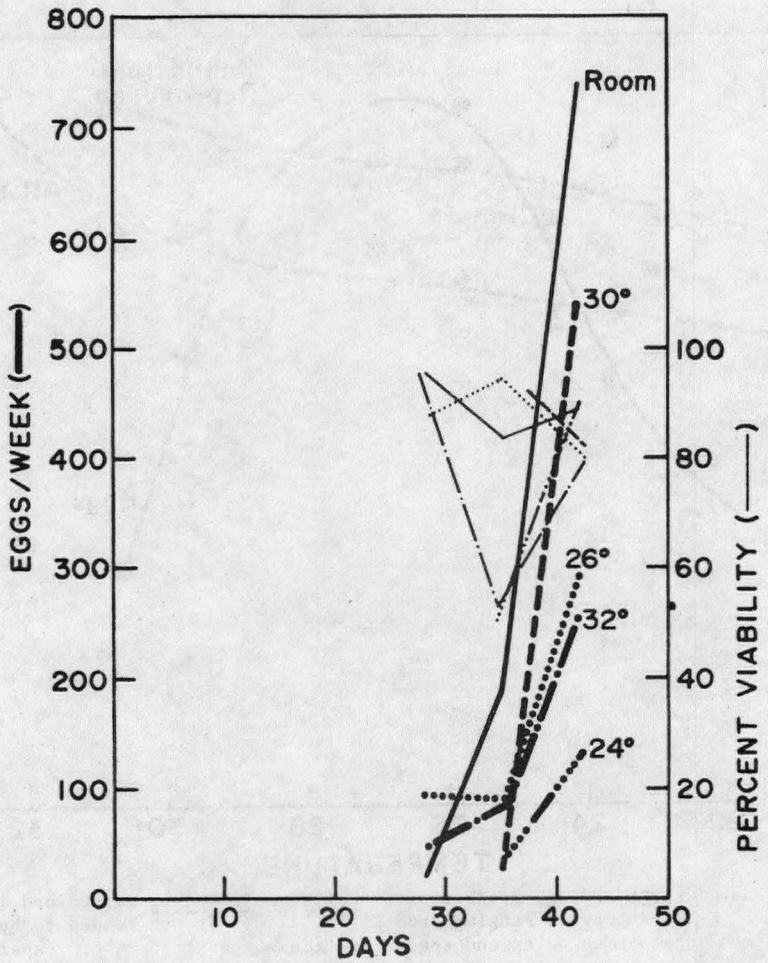


Figure 34. Egg production of Helisoma trivolvis on a weekly basis (after the 4th week). Largest yields were in warm water (room temperature or about 25°C to 30°C). Experiment 12.

In reproductive potential (Figure 33) the greatest egg output occurs around 26°C. When growth and reproduction, in terms of eggs laid, are compared as between the snails that reproduced and those that may have produced eggs on a cumulative basis the results are similar. Again, growth is best in warm conditions and maximum egg production occurred in the 26°C range. It was determined that eggs appeared after the 4th week and that viability was highest in the warmer water (26° to 30°C) (See Figure 34).

A 'plop test' was carried out with *Helisoma trivolvis* to test the effect of a sudden change in temperature on the growth of the snails. Ninety young snails between 1 and 14 days old were measured and placed in aquaria at 6°, 12°, and 30°C temperatures. Thirty snails were placed in each test aquarium and they were taken from a stock supply at room temperature (about 25°C) without any previous acclimatization. Since excessive handling interferes with growth and survival, these snails were not measured until the 14th day. By the 7th

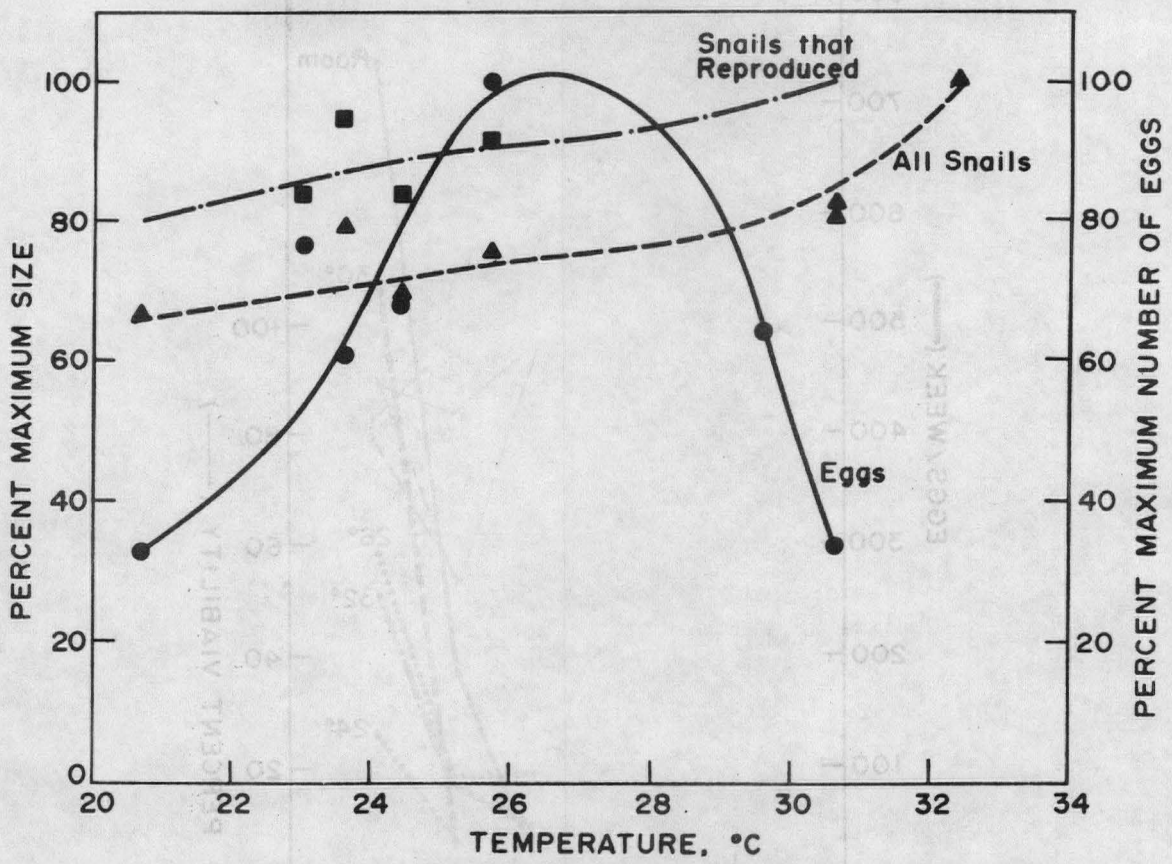
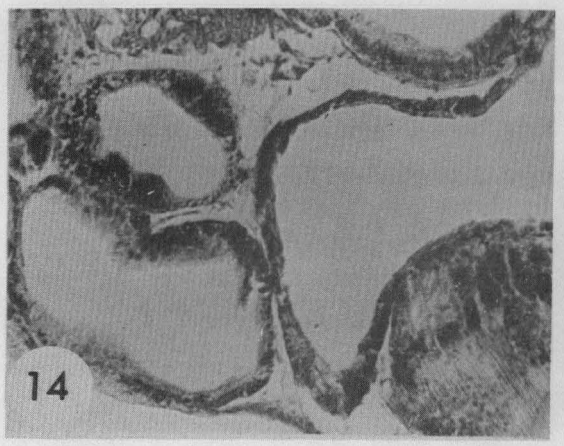


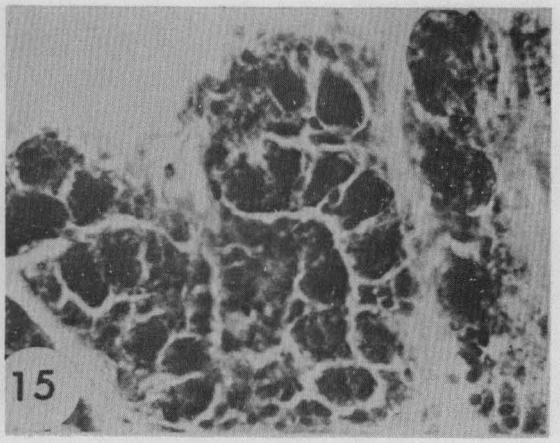
Figure 33. Growth and egg production of Helisoma trivolvis maintained in tanks at 2°C intervals ranging from 20° to 32°C. Growth tended to be better in the warmer water and egg production was best at 26°C. Experiment 12.

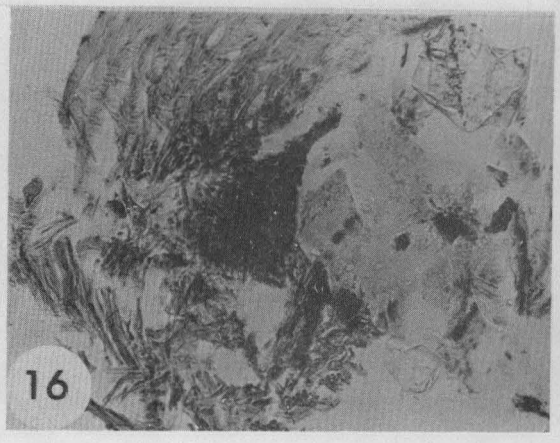
TABLE 11. Summary of data for *Helisoma trivolvis* 'Plop test' (animals subjected to sudden changes of temperature in tanks from room temperature), studied for 14 days (1/29/71 to 2/12/71), 30 specimens per tank.

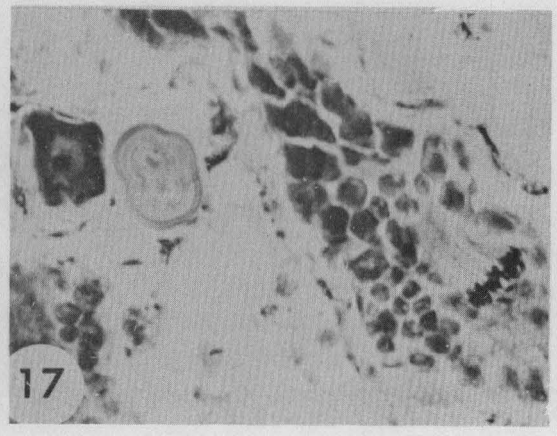
TEMPERATURE °C	NUMBER OF SNAILS AT DAY 14	SURVIVAL %	AVERAGE SIZE AT* DAY 0	AVERAGE SIZE AT DAY 14	CHANGE IN SIZE FROM DAY 0	OXYGEN ppm
7.59 - 0.52	3	10%	0.8420 mm	1.2766 mm	0.4340	17.20
12.40 - 2.05	0	0%	0.7663 mm	--	--	14.50
29.40 - 1.09	3	10%	0.7793 mm	4.4500 mm	3.6710	8.53

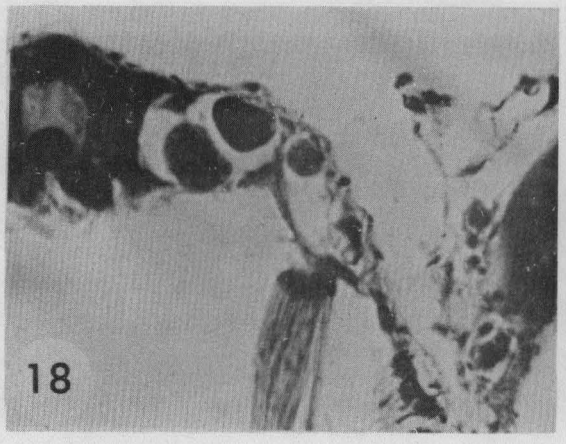
* Age at Day 0: 1-14 days.











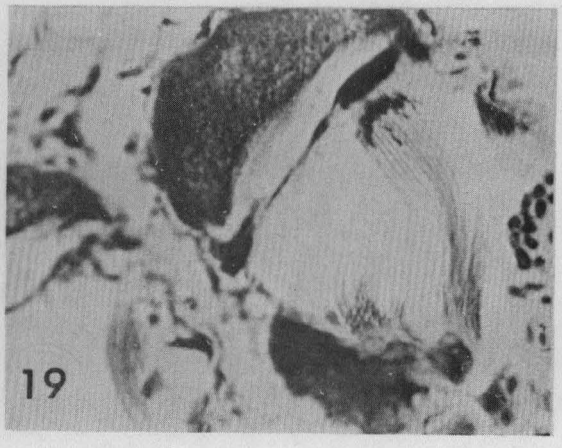


PLATE IV. Gonad tissue sections from Helisoma trivolvis cultured for 51 days at different temperatures.

Figure 14. 6°C (X70)
Figure 15. 12°C (X400)
Figure 16. 18°C (X70)
Figure 17. 24°C (X400)
Figure 18. Room temp. (X400)
Figure 19. 30°C (X400)

day the number of snails surviving was already less than half the number started. On the 14th day only 3 snails remained alive (see Table 11) in the 6° and 30°C aquaria; no live specimens were found in the 12°C aquarium. In growth, the 30°C group increased by only 3.7 mm. More tests of this kind are needed and they have been projected for the future.

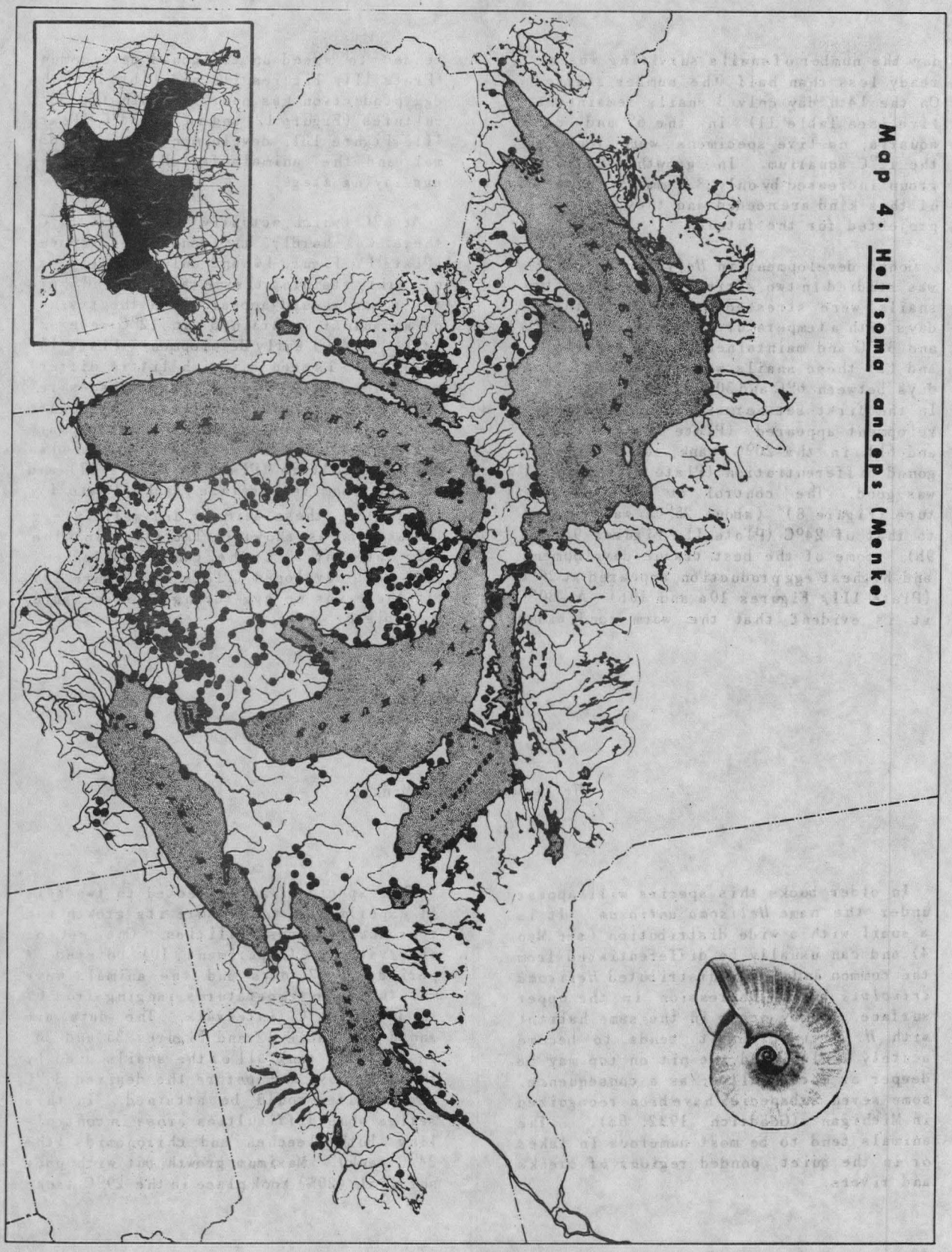
Gonad development in *Helisoma trivolvis* was studied in two series in which (1) the snails were stressed in cultures for 77 days with a temperature range between 20°C and 34°C and maintained at 2°C intervals; and (2) these snails were cultured for 51 days between 6°C and 30°C at 6°C intervals. In the first set normal egg and sperm development appeared (Plate II, Figures 6a and 6b) in the 20°C tank; also, at 22°C gonad differentiation (Plate II, Figure 7) was good. The control at room temperature (Figure 8) (about 25°C) was similar to that of 24°C (Plate II, Figures 9a and 9b). Some of the best tissue development and highest egg production appeared at 26°C (Plate III, Figures 10a and 10b). At 30°C it is evident that the warm conditions tended to speed up the gonad development (Plate III, Figures 11a and 11b) but the egg production was poor. In both the 32°C cultures (Figure 12) and in the 34°C (Plate III, Figure 13), development was not normal and the animals failed to reach the egg-laying stage.

At 6°C (which actually ran about 10°C) there was hardly any gonad development (Plate IV, Figure 14) and only a small mass of darkly staining tissue was observed where the gonad should appear. When the tissues of an animal maintained at 12°C were enlarged (X400) early development (Plate IV, Figure 15) is seen but with little differentiation. Also, at 18°C the tissues remained poorly developed (Plate IV, Figure 16) and eggs and sperm did not appear. The first good differentiation appeared in the 24°C culture (Plste IV, Figure 17) and in the room temperature tank (Plate IV, Figure 18); these animals did well in egg production as shown in Table 8. In the warm water at 30°C the gonad was reasonably well developed (Plate IV, Figure 19) but there was no egg-laying as seen again in Table 8.

Helisoma anceps (Menke)

In older books this species will appear under the name *Helisoma antrosum*. It is a snail with a wide distribution (see Map 4) and can usually be differentiated from the common and widely distributed *Helisoma trivolvis* by the depression in the upper surface. It may occur in the same habitat with *H. trivolvis*. It tends to become acutely keeled, and the pit on top may be deeper or more shallow; as a consequence, some seven subspecies have been recognized in Michigan (Goodrich, 1932: 63). The animals tend to be most numerous in lakes or in the quiet, ponded regions of creeks and rivers.

This species was subjected to two sets of experiments to measure its growth and reproductive possibilities. One set of observations (Experiment 10) covered a period of 77 days and the animals were maintained in temperatures ranging from 6° to 36°C at 6°C intervals. The data are shown in Table 12 and Figures 35 and 36. In the 34°C tank all of the snails died by the 27th day and before the desired 36°C temperature could be attained. In this series some difficulties arose in controlling both leeches and chironomids (the 24°C tank). Maximum growth but with poor survival (20%) took place in the 29°C tank;



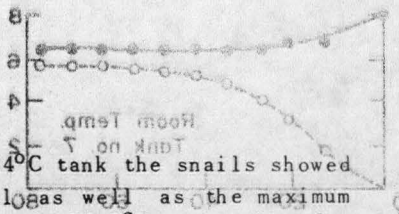
Map 4. Helisoma anceps (Menke)

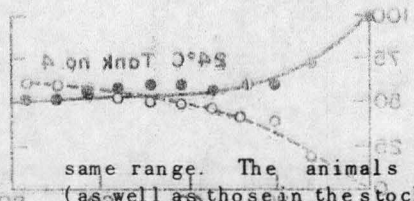
TABLE 12. Summary of data for Helisoma anceps at 6°C intervals, studied for 77 days (4/1/70 - 6/16/70); 30 specimens per tank. (Experiment 10)

TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
		Total change $\pm$ 2 $S_{\bar{x}}$	Survival	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability
°C	ppm	in mm	%			%
7.3 ± 0.2	14.6 ± 0.3	0.85 ± 0.33(13)*1	43.3	--	--	--
13.6 ± 0.5	12.7 ± 0.4	4.86 ± 0.67(12)	40.0	70	1/7	42.9
18.7 ± 0.4	10.7 ± 0.2	4.92 ± 0.52(14)	46.7	70	33/211	80.1
24.1 ± 0.3	9.3 ± 0.2	4.87 ± 0.46(16)	53.3	56	70/444	87.8
29.3 ± 0.3	7.7 ± 0.4	6.91 ± 1.12(6)	20.0	--	--	--
34.0 ± 1.0	7.0 ± 0.4	3.04 ± 0.49(7)	(all dead, 27 days)	--	--	--
24.9 ± 0.4 (room)	8.3 ± 0.4	5.77 ± 0.30(24)	80.0	56	119/1,064	77.2

*1-14 days old at day 0; average diameter 1.16 mm.

*1 Number of snails surviving at end of experiment.





however, in the 24°C tank the snails showed maximum survival as well as the maximum egg production. At 18.7°C, eggs showed a cloudy appearance, perhaps due to a fungus. The single egg case produced at 13.6°C had only 3 of the 7 eggs viable. The fact that any eggs at all were produced was attributed to a heater malfunction which permitted a rise in temperature of from .4° to 3.4°C for a few days. It was of interest to find that egg production was reasonably good at 18°C, reflecting, perhaps, an innate ability in this snail which is widespread in the northern parts of Michigan to adjust to relatively cold conditions. Additional tests are reported in Experiment 14 which measured responses to temperatures at 2°C intervals between 16° and 28°C.

The information obtained in this 2°C interval study (see Table 13, Figures 37 and 38) indicates that *H. anceps* grows better in the warmer conditions (26° and 28°C) and that it produces young better in that same range. The animals in this series (as well as those in the stock tanks) showed poor survival, presumably due to the presence of chironomids. While the greatest change in growth occurred at room temperature (8.63 mm), only 3 snails were alive at the end of the experiment. Growth was 7 mm in the warmer conditions, which is in keeping with the results obtained in the 6°C set of observations.

'Plop test' with *Helisoma anceps* (Table 14). That this species is more tolerant of warm conditions than of cold is shown in the results when animals living in room temperature tanks (24°C approximately) were suddenly introduced to tanks maintained at 6°, 12°, 22° and 30°C, respectively. At the lower ranges (6° and 12°C) there was virtually no growth during the 21 days of stressing (Figure 39), but at 12°C survival was reasonably good. In the 22°C tank the survival was very good and growth was best. At 30°C growth was good but at that temperature the survival was again

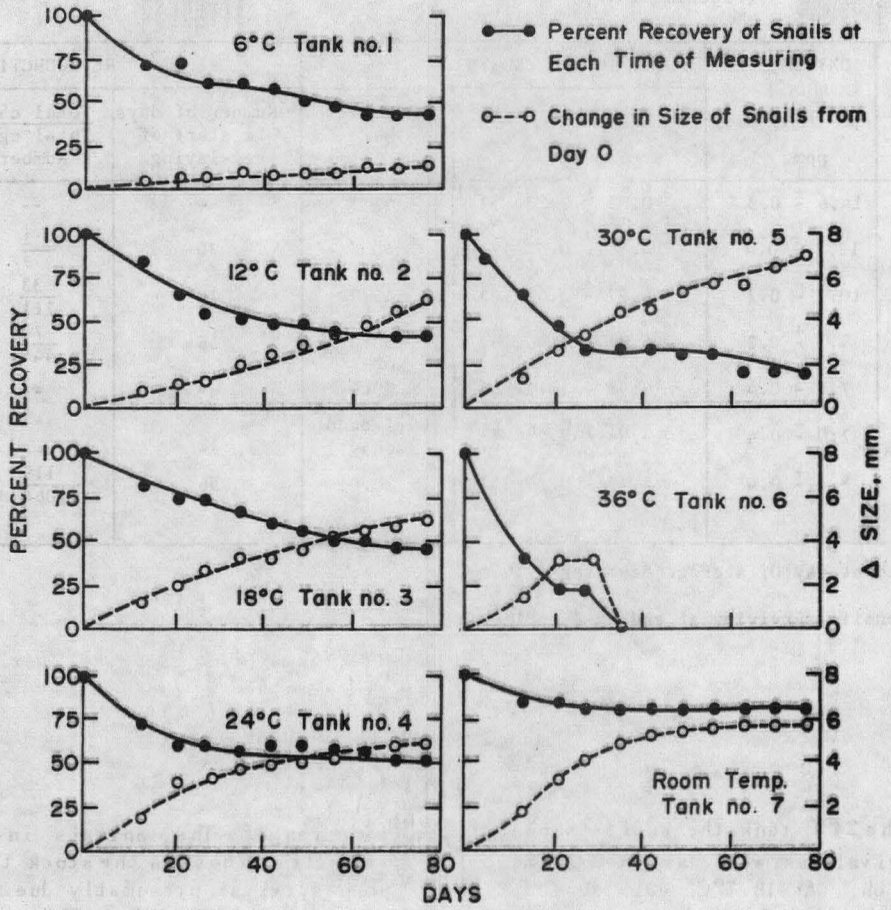


Figure 35. Growth and survival of Helisoma anceps maintained for 77 days in tanks at 6°C intervals between 6° and 36°C; room temperature (26°C) served as control. Experiment 10.

not encouraging and reflected the poor maintenance experienced in the 12°C group.

Gonad tissues of *Helisoma anceps* maintained at 6°C intervals (Experiment 10) were sectioned to determine the degree of development after remaining in cultures at temperatures ranging between 6° and 34°C for 77 days. The 12°C tank actually was held closer to 15°C (see Table 12) but did show good egg and sperm development (Plate V, Figure 20). A few eggs were even produced after 70 days in culture but only half of them were viable (Table 12). Unfortunately, the tissues from the 18°C tank were not available for sectioning but the egg-laying was good, starting at the 70th day, with relatively high (80%) viability. The 24°C cultures, both the control (Plate V; Figure 21) and the 24°C test cultures (Plate V; Figures 22a and 22b) showed good egg and sperm production as well as large numbers of viable eggs. When the culture was maintained at 30°C, growth (Table 12) was better than usual but the gonads (Plate V; Figures 23a and 23b) do not appear to be normal, nor were any viable eggs produced. At 34°C all of these snails were dead by the 27th day. In summary, *H. anceps* developed best in cultures around 24°C and they tend to do best in cool rather than warm (approaching 30°C) water. Since this species tends to be common in the northern regions of the Great Lakes area, the tendency to do better in moderately warm water is reflected by these tests.

TABLE 13. Summary of data for Helisoma anceps at 2°C intervals, studied for 84 days (11/17/70 - 2/9/71); 30 specimens per tank. (Experiment 14)

TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
		Total change $\pm$ 2 $S_{\bar{x}}$	Survival	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability
°C	ppm	in mm	%			%
16.8 ± 0.5	13.4 ± 0.4	6.25 ± 0.17	26.7	77	3/33	100
18.7 ± 0.4	12.6 ± 0.3	6.34 ± 0.02	13.3	--	--	--
20.2 ± 0.3	11.99 ± 0.3	5.19 ± 0.75	13.3	--	--	--
21.9 ± 0.05	11.36 ± 0.03	7.07 ± 0.72	13.3	--	--	--
24.1 ± 0.01	10.52 ± 0.01	6.83 ± 0.11	13.3	70	9/82	95.12
25.8 ± 0.01	9.7 ± 0.02	6.41 ± 0.07	46.7	70	2/19	68.42
27.7 ± 0.1	9.4 ± 0.01	7.24 ± 0.26	23.3	--	--	--
26.0 ± 0.04 (room)	9.8 ± 0.01	8.63 ± 0.28	10.0	70	2/7	100

*1-8 days old at day 0.

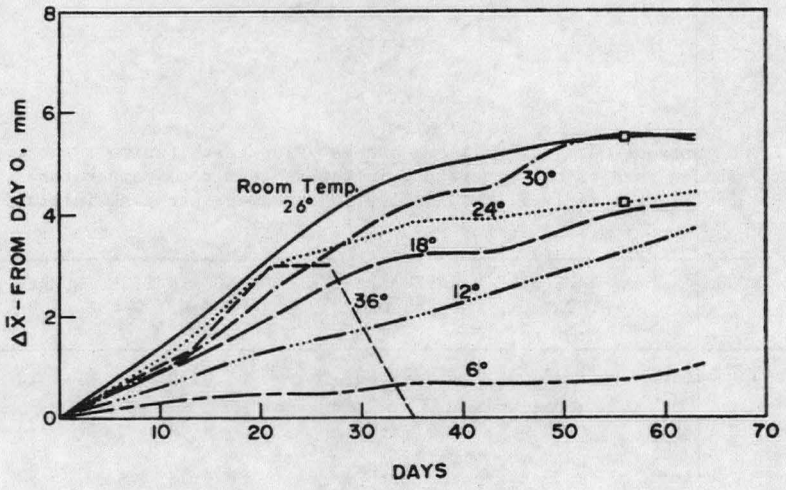


Figure 36. Growth of Helisoma anceps maintained for 63 of 77 days in tanks at 6°C intervals ranging from 6° to 36°C; growth was best in the warmer water with a fairly wide spread between 18° and 30°C. Egg-laying (squares) took place only at 24° and 26°C. Experiment 10.

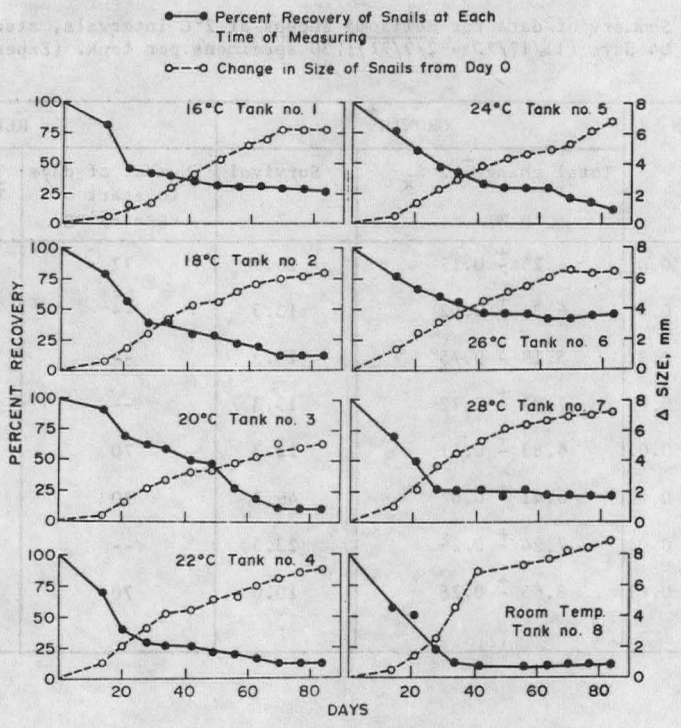


Figure 37. The growth and survival of Helisoma anceps maintained for 84 days in tanks with 2°C intervals of temperature ranging between 16° and 28°C; room temperature (26°C) served as control. Experiment 14.

TABLE 14. Summary of data for Helisoma anceps "Plop test" (animals subjected to sudden changes of temperature in tanks, from room temperature), studied for 21 days (4/14/71 to 5/5/71), 30 specimens per tank initially.

TEMPERATURE °C	OXYGEN ppm	AVERAGE SIZE* AT DAY 0 mm	AVERAGE SIZE AT DAY 21 mm	CHANGE IN SIZE FROM DAY 0 mm	NUMBER ALIVE AT DAY 21	SURVIVAL %
6.39 ± 0.09	16.64 ± 0.06	1.33	1.43	0.10	12	40%
12.26 ± 0.67	14.68 ± 0.30	1.46	2.02	0.56	21	70%
21.86 ± 0.34	10.62 ± 0.12	1.42	3.88	2.46	26	86.7%
30.50 ± 0.04	7.82 ± 0.01	1.41	3.29	1.88	21	70%

*Age at day 0: 3-14 days.

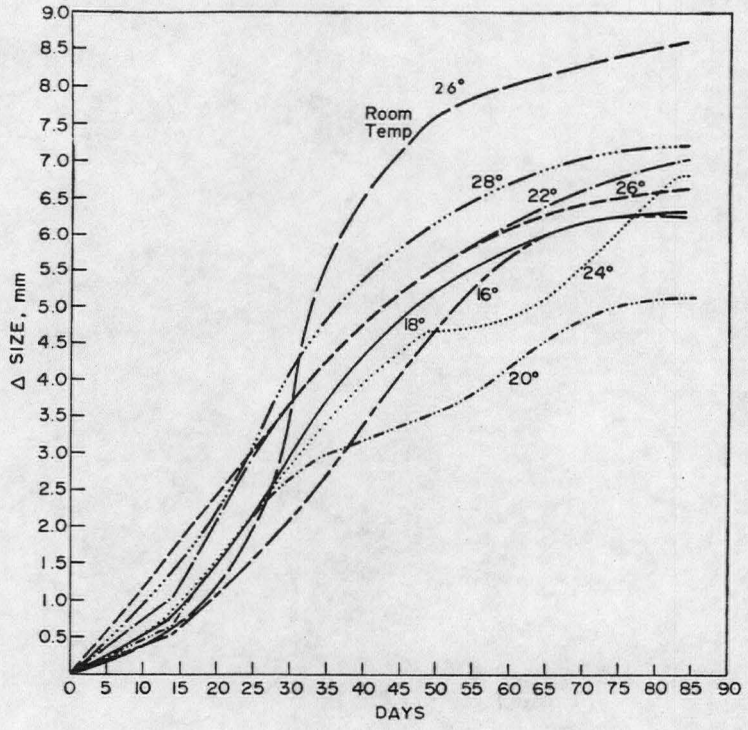


FIGURE 38. Growth of *Helisoma anceps* maintained for 84 days in tanks at 2°C intervals from 16° to 28°C; the control was at room temperature (26°C). Experiment 14.

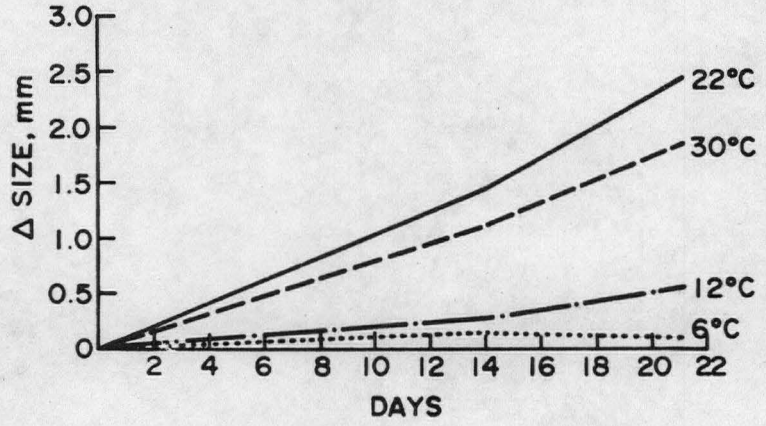
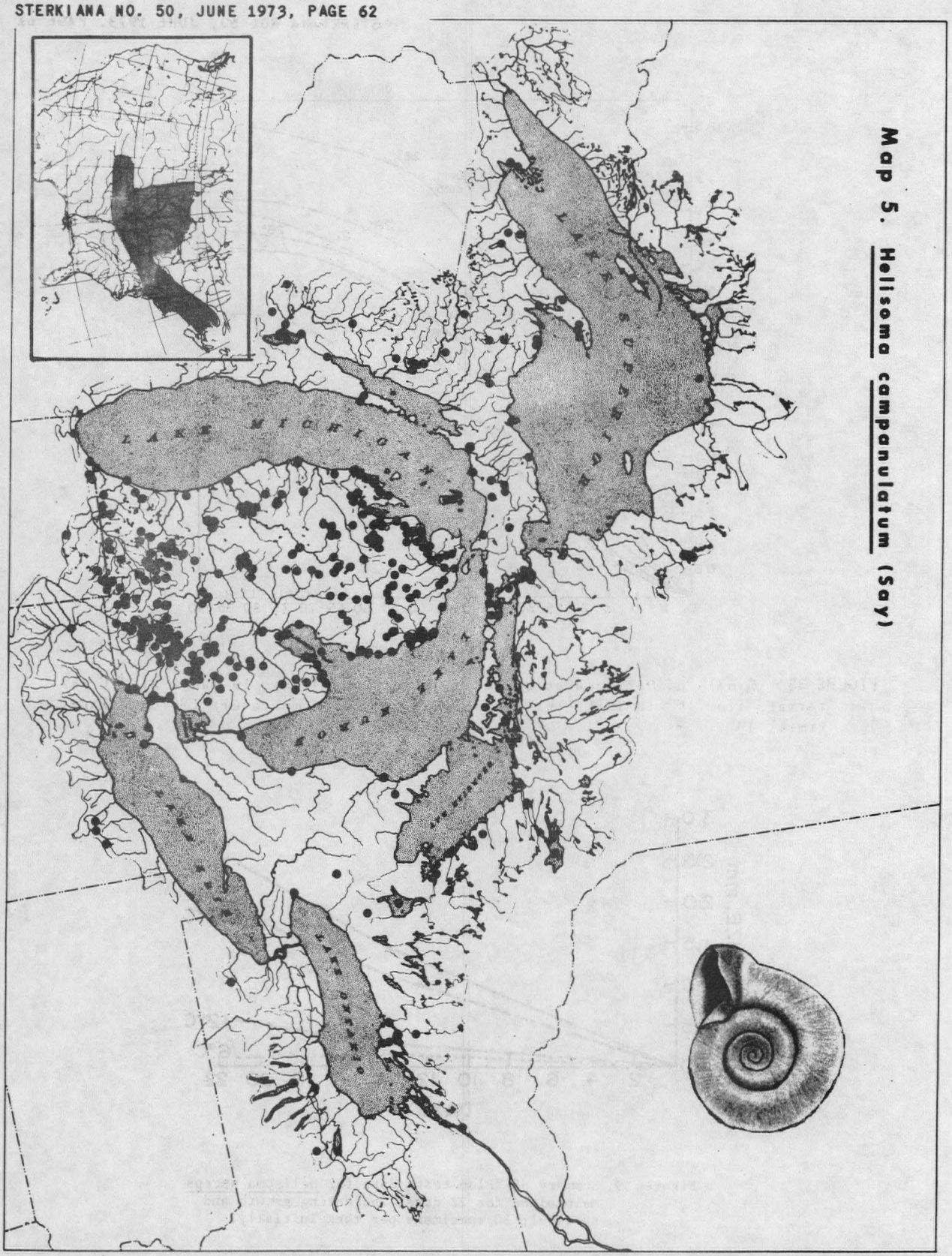
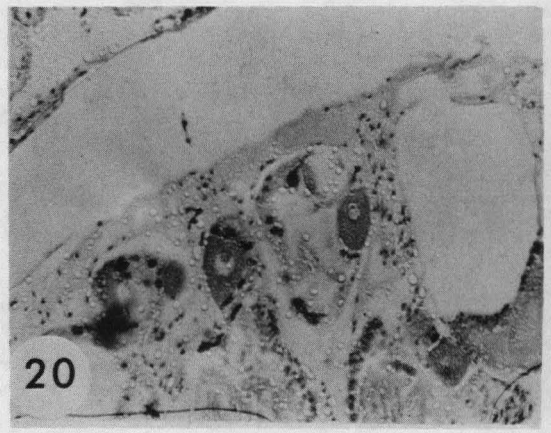
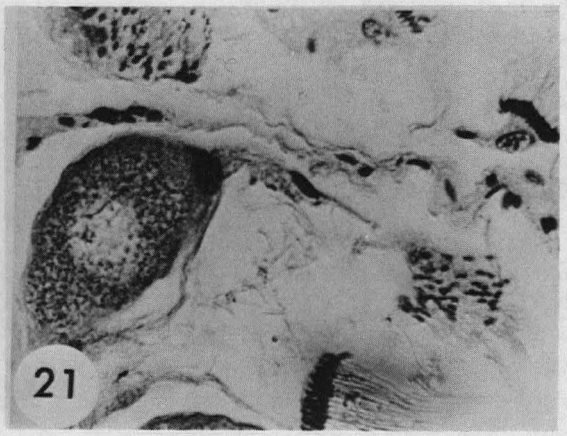


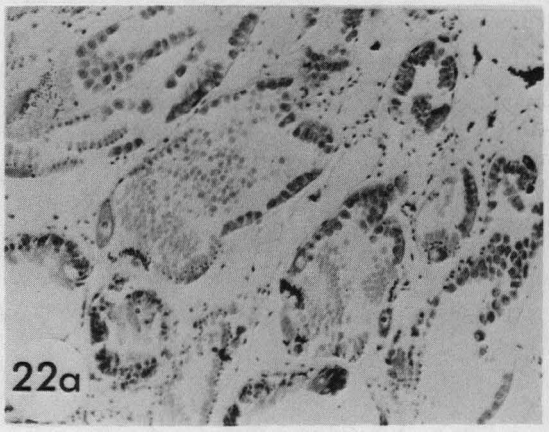
Figure 39. Summary of "Plop test" data for Helisoma anceps maintained for 22 days, indicating growth and survival; 30 specimens per tank initially.

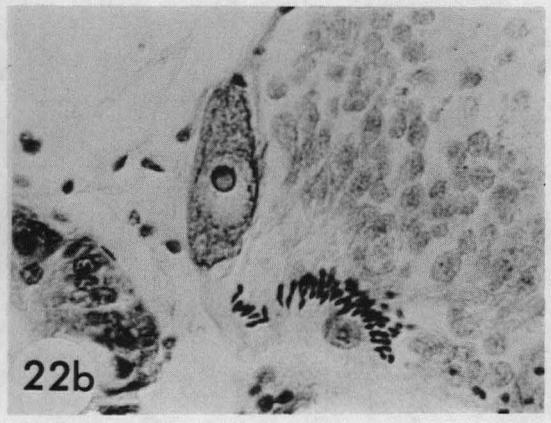


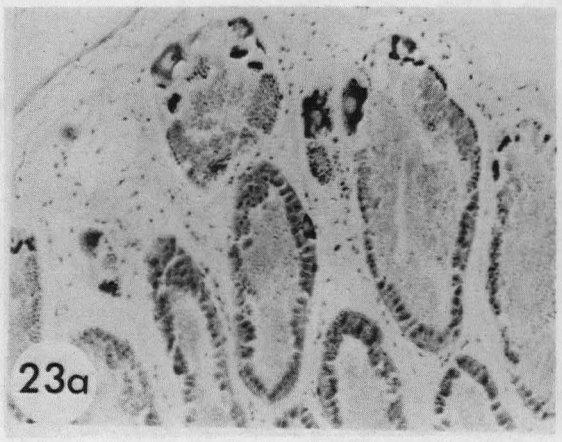
Map 5. Hellsoma campanulatum (Say)











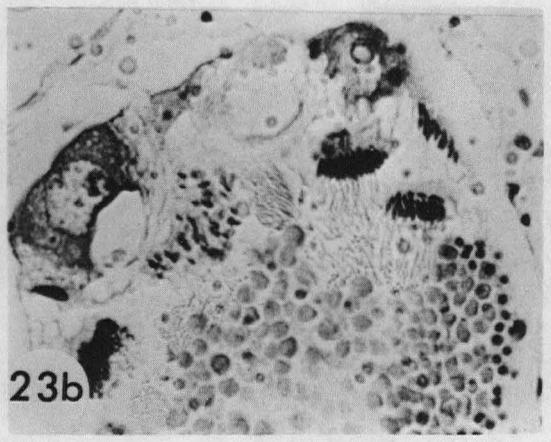


PLATE V. Gonad tissue sections from Helisoma anceps cultured for 77 days at different temperatures.

Figure 20. 15°C (X70)
Figure 21. Room temperature (X400)
Figure 22a. 24°C (X70)
Figure 22b. 24°C (X400)
Figure 23a. 30°C (X70)
Figure 23b. 30°C (X400)

Helisoma campanulatum (Say)

This is one of the most common snails in the thousands of lakes throughout the Great Lakes region, yet very little information of a basic nature has been published relating to it. It is always amazing to find a virtual mat of dead shells of this snail covering the windswept parts of the beaches, but it is often difficult to find the live animals when surveying areas of the lake itself. Our stock came from a small lake (Crooked Lake) in southern Michigan where they were collected in shallow water under an extended dock. More studies are needed on the ecology of this snail in the lake habitats. Within its very wide distribution range (Map 5) at least a half dozen varieties have been recognized. An evaluation of this variation in nature is long overdue.

Studies to measure growth and reproduction (Experiment 9) were first made at 6°C intervals over a period of 99 days (Table 15 and Figure 40). It will be noted that the data are similar to those for *Helisoma trivolvis* which grew and reproduced best in the temperature range between 24° and 30°C. Egg-laying started first in the control tank (room temperature or 25°C). While culture may have been somewhat influenced by the presence of chironomids, the pattern is nevertheless reasonably definite and the information from the 2°C interval study (Experiment 15) corroborates these data. Also, as in the case of other planorbids studied, maximum growth occurred under warmer conditions (about 30°C) while survival was better around 24°C (Figure 41).

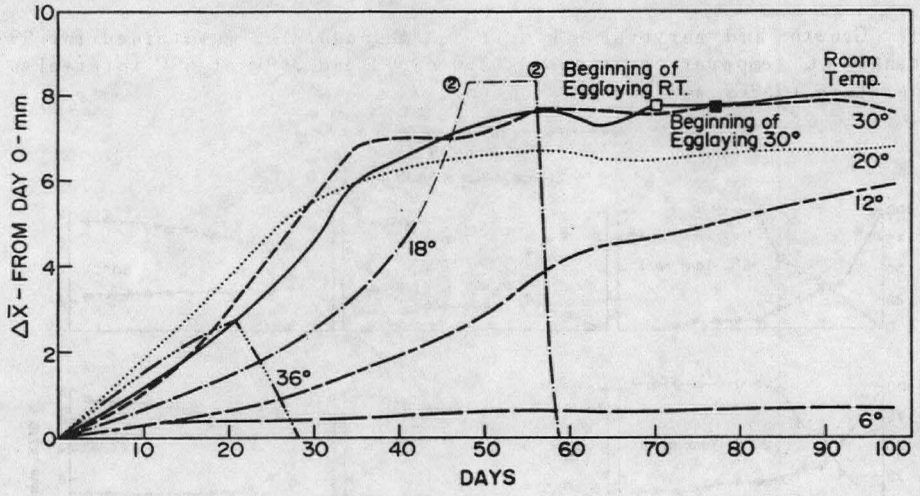


FIGURE 40. Growth and reproduction of Helisoma campanulatum maintained for 99 days in tanks ranging from 6°C to 36°C at 6°C intervals; egg-laying started on 77th day at 30°C but survival was better at 24°C.

When *H. campanulatum* was stressed for 84 days at 2°C intervals between 18° and 34°C, survival was relatively poor (Table 16 and Figures 42 and 43). It was difficult to determine the reason for the high mortality but the tanks that showed poor survival developed what may have been a noxious algal growth. For example, when a new tank was established at 22°C to replace the one in which only 2 snails were alive after the first 14 days, there was less difficulty and almost half of the snails survived. Again, in the 24°C tank on day 35 only one snail was living (see Figure 42) and a replicate was established. The losses remain unexplained, but when the work was re-done the tests were successful.

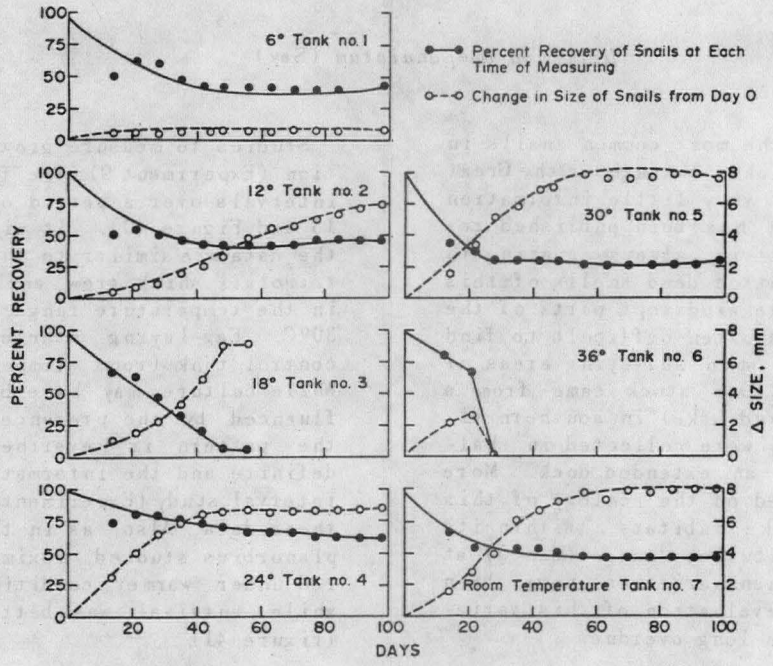


FIGURE 41. Growth and survival of *Helisoma campanulatum* maintained for 99 days in tanks at temperatures ranging between 6° and 36°C at 6°C intervals; room temperature (25°C) was used as control.

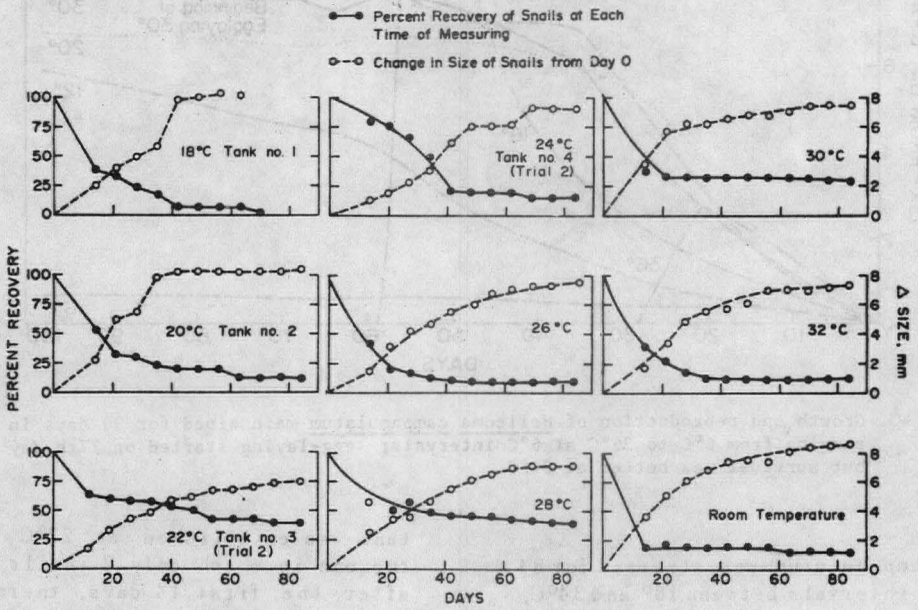


Figure 42. Growth and survival of Helisoma campanulatum maintained in tanks for 84 days in temperatures of 2°C intervals ranging between 18° and 32°C; 26°C was the control. Experiment 15

Whether viewed in terms of the 6°C series of tests or the 2°C series, the pattern is similar to that of the other species of planorbid snails tested, i. e., growth in the warmer range is reasonably good but survival is poor; reproduction appears limited with a range between 20°C and the mid-twenties. The optimum temperature both for growth and reproduction usually is around 25°C.

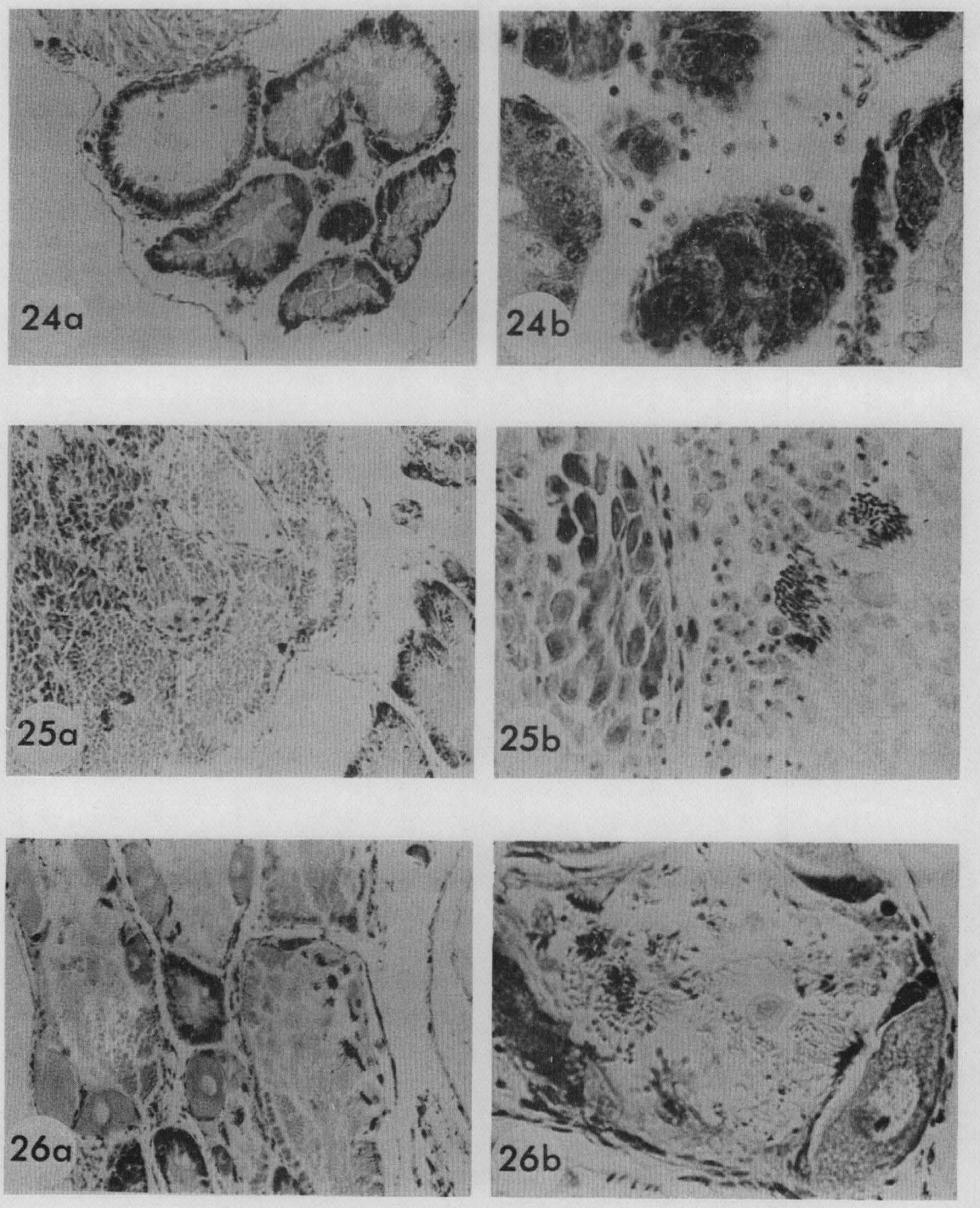


PLATE VI. Gonad tissue sections from Helisoma campanulatum cultured for 99 days at different temperatures.

Figure 24a. 6°C (X70)
Figure 24b. 6°C (X400
Figure 25a. 12°C (X70)
Figure 25b. 12°C (X400)
Figure 26a. 18°C (X70)
Figure 26b. 18°C (X400)

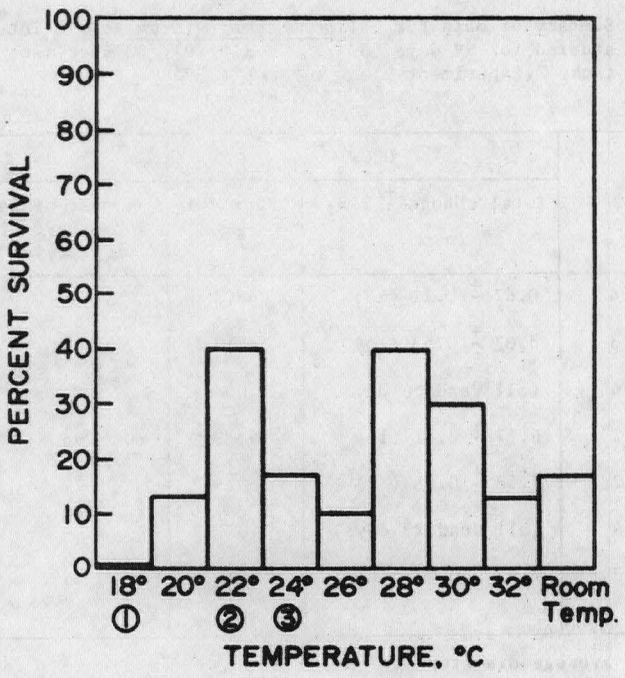


Figure 43. Survival of Helisoma campanulatum maintained for 84 days in tanks at temperatures ranging between 18° and 32°C at 2°C intervals; this indicates relatively low survival in culture. (Experiment 15)

At 6°C only an undifferentiated mass of tissue (Plate VI; Figures 24a and 24b) was seen. At 12°C the oogenesis was in an early stage, but well developed sperm were in evidence (Plate VI; Figures 25a and 25b). However, no egg-laying took place (see Table 15). At 18°C both eggs and sperm (Plate VI; Figures 26a and 26b) were well developed but the snails failed to survive and there were no eggs. In a subsequent series eggs did appear (Table 16) in the 20°C tank, which indicates a normal pattern in that temperature range. At 24°C gonad production (Plate VII; Figures 27a and 27b) was good, and viable egg production (Table 15) appeared to be at its best. At 30°C there was also evidence of normal gonad activity, but fewer eggs were laid and viability was only half as good as in the 24°C range. The tank maintained as a control at room temperature (about 24°C) proved to be the best in gonad development (Plate VII; Figures 28 and 29) as well as in the high production of eggs that were viable.

TABLE 15. Summary of data for Helisoma campanulatum at 6°C intervals, studied for 99 days (3/4/70 - 6/10/70); 30 specimens per tank. (Experiment 9)

TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
°C	ppm	Total change ± 2 $S_{\bar{x}}$ in mm	Survival %	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
6.6 ± 0.2	13.7 ± 0.4	0.67 ± 0.21 (13)*1	43.3	--	--	--
12.9 ± 0.3	11.8 ± 0.3	5.87 ± 0.53 (14)	46.7	--	--	--
18.1 ± 0.4	9.8 ± 0.4	(all dead,56 days)	0	--	--	--
23.5 ± 0.7	8.7 ± 0.2	6.77 ± 0.31 (19)	63.3	98	7/44	90.0
29.2 ± 0.2	7.7 ± 0.2	7.58 ± 0.68 (9)	30.0	77	3/11	54.5
34.5 ± 0.9	6.5 ± 0.4	(all dead,28 days)	0	--	--	--
24.9 ± 0.4 (room)	8.1 ± 0.3	7.93 ± 0.27 (14)	46.6	56	97/835	95.7

*1-3 weeks old at day 0; average diameter 1.47 mm.

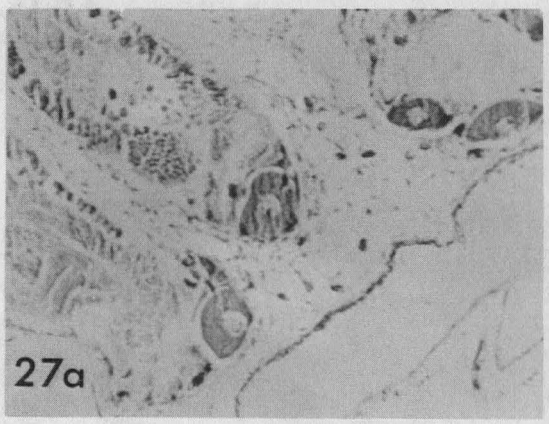
*1Number of snails surviving at end of experiment.

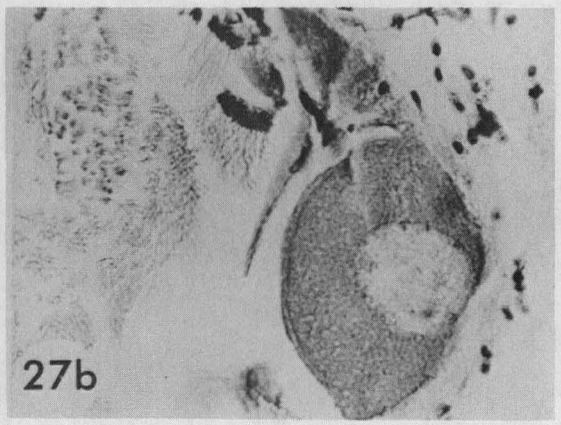
TABLE 16. Summary of data for Helisoma campanulatum at 2°C intervals, studied for 84 days (1/13/71 - 4/7/71); 30 specimens per tank. (Experiment 15)

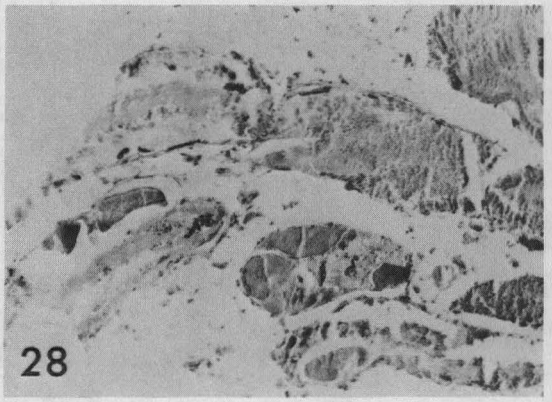
TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
°C	ppm	Total change ± 2 $S_{\bar{x}}$ in mm	Survival %	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
18.88 ± 0.17	11.57 ± 0.11	--*1	0	--	--	--
20.41 ± 0.06	11.32 ± 0.05	8.34 ± 0.04	13.3	77	5/72	94.4
22.27 ± 0.17	11.11 ± 0.9	5.99 ± 0.08	40.0	--	--	--
24.02 ± 0.02	9.86 ± 0.01	7.32 ± 0.17	16.7	--	--	--
25.74 ± 0.02	9.47 ± 0.01	7.54 ± 3.5	10.0	84	2/16	100.0
27.62 ± 0.03	8.73 ± 0.02	7.13 ± 0.10	40.0	--	--	--
29.31 ± 0.01	8.54 ± 0.01	7.69 ± 0.02	30.0	--	--	--
31.22 ± 0.02	7.81 ± 0.04	7.40 ± 0.76	13.3	--	--	--
26.24 ± 0.04 (Room temp.)	9.43 ± 0.04	8.56 ± 0.52	16.7	70	11/125	85.6

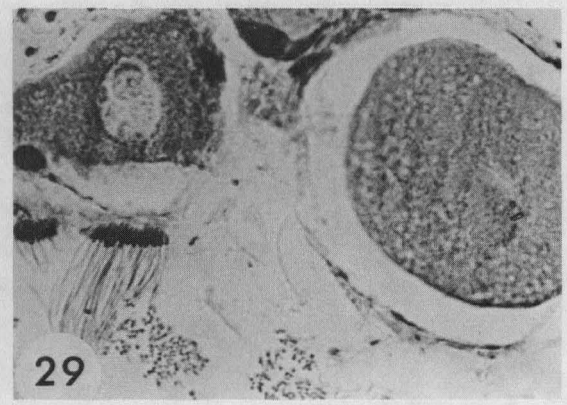
*7-18 days old at day 0

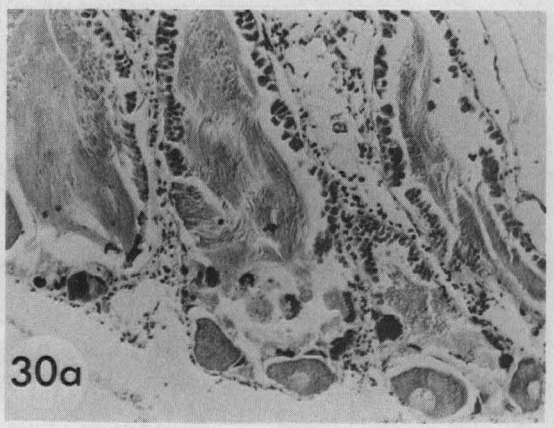
*118°C tank: 1 alive at day 70 (3.3% survival); 8.55 ± 0.00 mm total change.











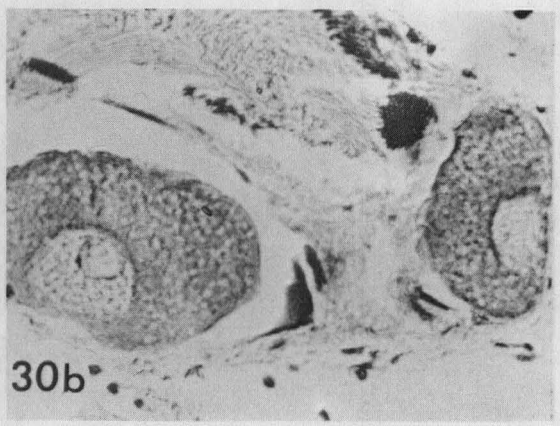


PLATE VII. Gonad tissue sections from Helisoma campanulatum cultured for 99 days at different temperatures.

Figure 27a. 24°C (X70)
Figure 27b. 24°C (X400)
Figure 28. Room temp. (X70)
Figure 29. 24°C (X400)
Figure 30a. 30°C (X70)
Figure 30b. 30°C (X400)

Physa gyrina Say

Physid Snails. While *Physa* as a group ranks among the most common and widespread of all freshwater snails (Map 6), it is difficult to find data on their tolerances to the temperature extremes which they experience in nature. DeWit (1955), studying the life history of *Physa fontinalis* near Amsterdam, Netherlands, measured the influence of temperature on the rate of growth and noted especially how temperature influenced hatching and the mortality of young. He also found that when the animals failed to feed and grow in the winter the mean temperature was usually less than 6°C; this was shown in our laboratory tests undertaken later as well. He correlated the growth and reproductive patterns with the seasons but the definite ranged of temperature were not given.

In the Ann Arbor region, another DeWitt (1955) compared the ecology and life history of a local American pond snail, *Physa gyrina*, in widely separated colonies--those in southern Michigan with ones 300 miles north at the University of Michigan Biological Station on Douglas Lake. He observed (1955: 41) ovipositing 'in April when the water had risen to at least 10°C.' Again, these data can be compared with our laboratory tests. While the pattern in the life cycle of this snail in its various temporary and permanent habitats is now established, it is evident from these studies, as well as our own, that the physids tolerate a far wider range of temperatures than other pulmonate groups. This tolerance will also be indicated in the data obtained from studies reported here.

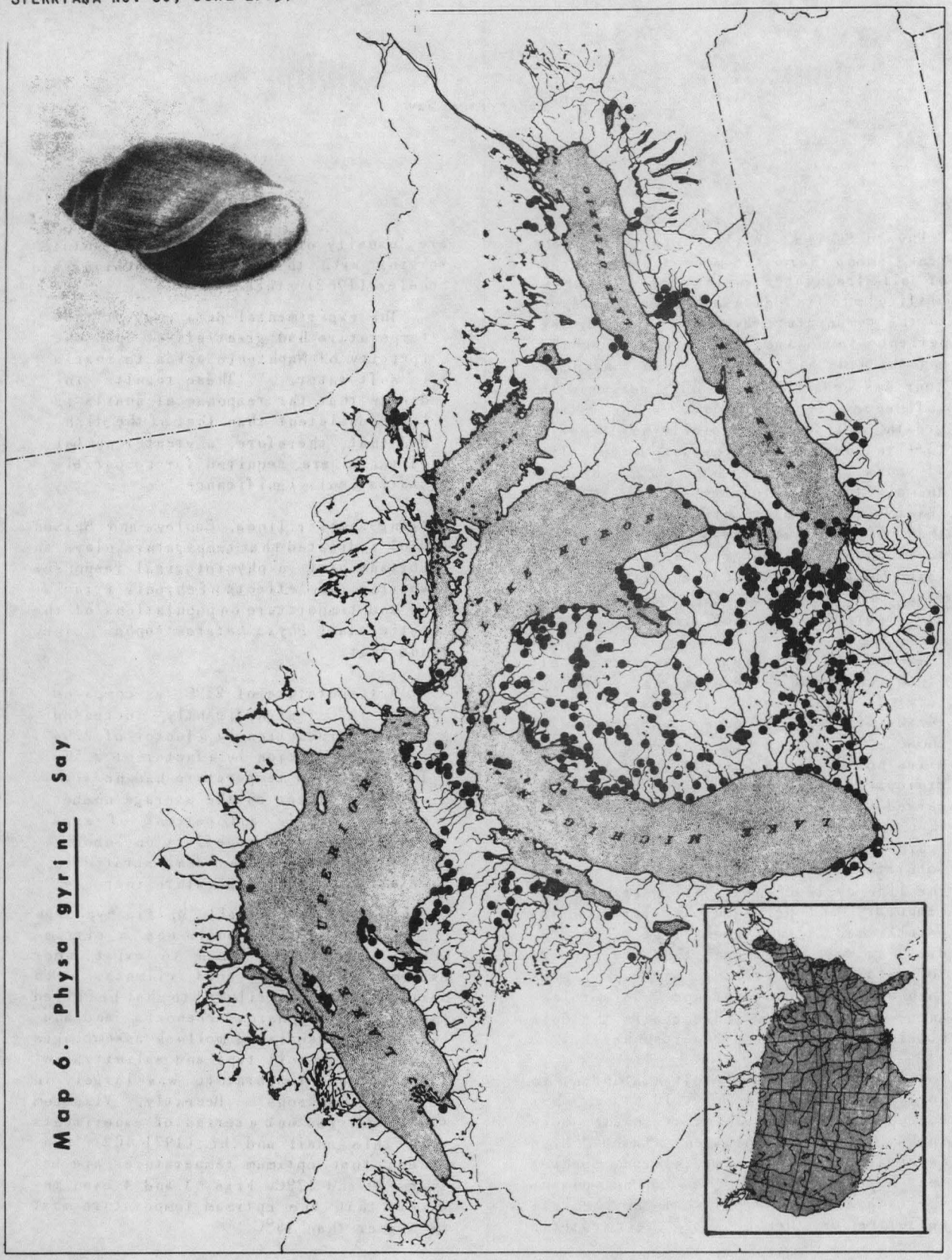
The physid snails are often abundant in polluted waters. Wurtz (1956), in his study of the relation of freshwater mollusks to stream pollution, stated *'Physa heterostropha* is the most tolerant species that has been found.' He did not pursue the effects of the physical and chemical pollution on them because their effects are 'usually direct, and usually absolute.' Working with this same snail, Cairns and Scheier (1962) stated:

> 'The experimental data suggest that temperature had great effect upon the toxicity of Naphtenic acids to snails in soft water...' 'These results indicate that the response of snails is less consistent than that of the fish, and that, therefore, a greater number of tests are required for comparable statistical significance.'

Along similar lines, Cooley and Nelson (1970) indicated that temperature plays an important part in physiological responses involving the 'effects of chronic irradiation and temperature on populations of the aquatic snail *Physa heterostropha.*' They found that:

> 'A temperature of 25°C, as compared with 15°C, significantly increased capsule production by a factor of 2.04 and egg production by a factor of 2.50. The increased temperature had no significant effect on the average number of eggs/capsule and percent of eggs hatched. No adverse effect on laboratory populations was demonstrated to result from 10°C temperature increase,'

Another physid snail, *Aplexa hypnorum* L., common to woods pools has a circumpolar range and is known to exist under extremely rigorous cold climates. Den Hartog (1963) contributed to what he called the *'Aplexa hypnorum* coenosis' and analyzed the associated mollusk assemblages in relation to soil type and salinity; information on temperature was largely in terms of seasons. Recently, Vlasblom (1971) carried out a series of experiments with this snail and he (1971:102) 'concluded that optimum temperatures are between 10 and 17°C. Figs. 3 and 4 even indicate that the optimum temperature must be higher than 15°C.'



Map 6. Physa gyrina Say

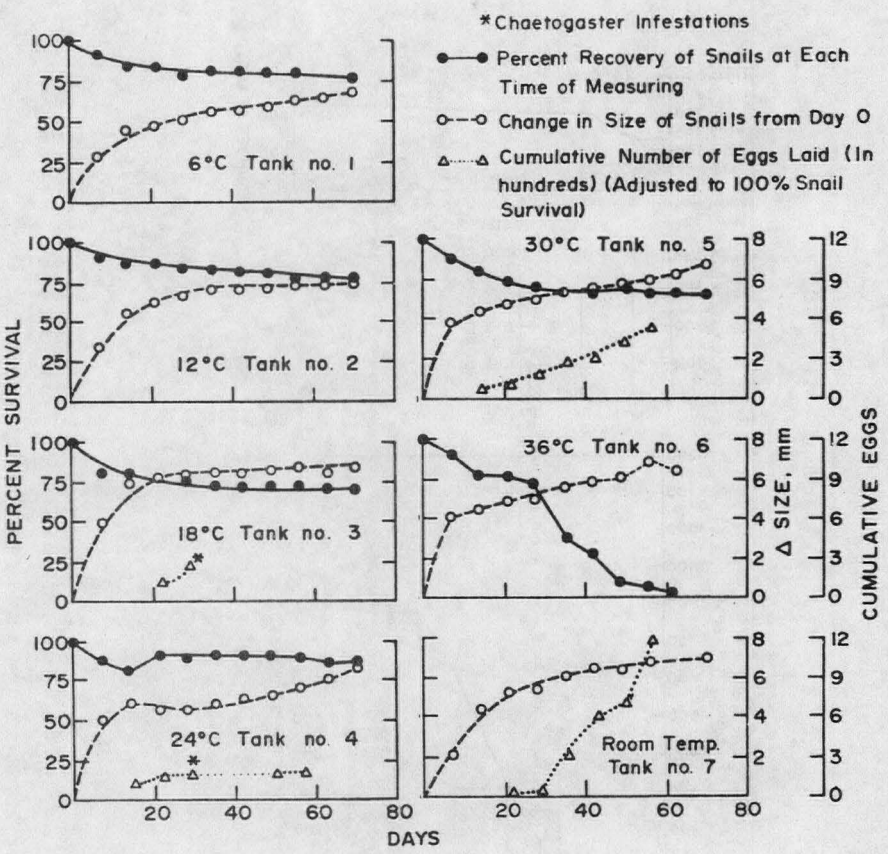


Figure 44. Growth, survival and cumulative number of eggs laid (adjusted to 100% snail survival) of Physa gyrina, maintained in tanks with temperatures set at 6°C intervals between 6° and 36°C for 70 days; room temperature (27°C) served as control. Experiment 7.

In view of their wide range of temperature tolerance, the physids hold a unique position among mollusks studied. Beams and Lindborg (1967) cite Precht (1958) in Prosser's book on physiology, where adaptation to temperature among the poikilotherms may be (1) 'resistance adaptation' or (2) 'capacity adaptation' and that the former is more common in nature than the latter. As for capacity adaptation, the *Physa anatina* studied in spring-fed, hot water pools near Montezuma, New Mexico, had become adapted to the point where they withstood temperatures for one hour as high as 43°C. Beames and Lindborg (1967: 14) observed:

'In summary, *P. anatina* is capable of 'capacity resistance adaptation' to changes in its environmental temperature. The animal should be a very useful experimental animal for determination of the mechanisms of temperature adaptation in poikilotherms.'

As will be shown later, the physid snails do show far greater plasticity both in their growth and reproduction when subjected to a wide range of temperatures.

A more definite study on the tolerances to heat of two common and widespread American species of *Physa* was undertaken by

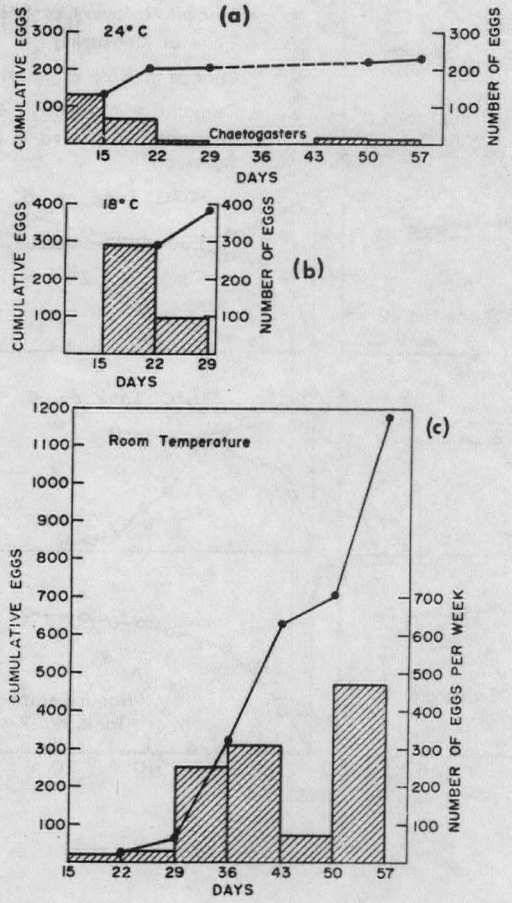


FIGURE 45. Curtailment of egg production of *Physa gyrina* due to *Chaetogaster* infestation at 24°C (a) and 18°C (b); for comparison, the more normal and high production at room temperature (27°C) is also given (c). Experiment 7.

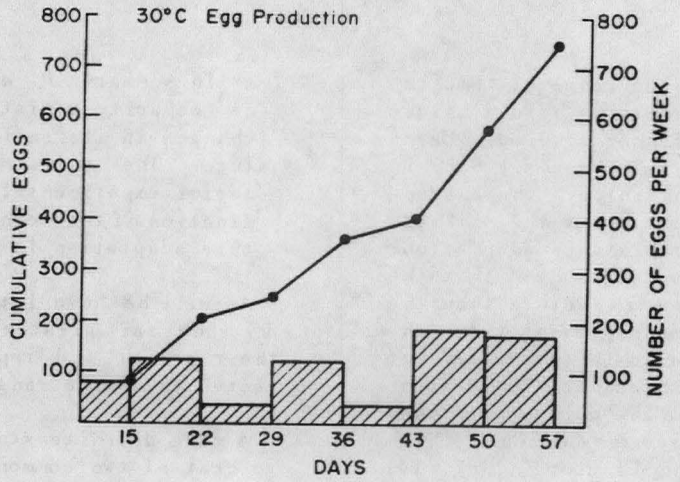


Figure 46. Eggs of Physa gyrina produced in the 30°C tank over a 6-week period showing the fluctuations in the number produced from week to week. Experiment 7.

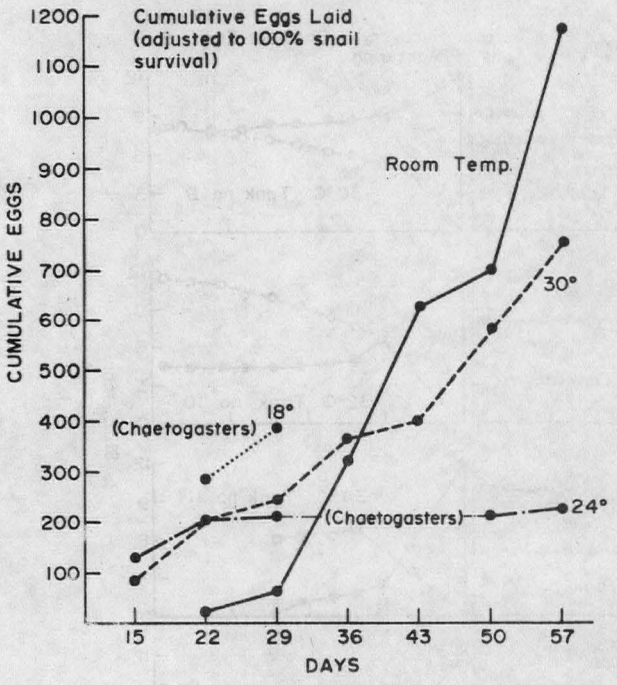


Figure 47. Comparison of Physa gyrina eggs produced in the 30°C tank with the number (adjusted to 100% survival) at room temperature (27°C) over a 6-week period. The 18° and 24°C tanks were infested with Chaetogasters and egg production was not normal. Experiment 7.

Clampitt (1970) in his work on the comparative ecology of *Physa gyrina* and *Physa integra*. He acclimatized both species to average room temperatures (23°-25°C) and then heated their aquaria in a 4-hour period to 40°C; in a second set he heated the water to 35°C. He found (1970: 144; Figure 14):

> 'In both sets of experiments, *P. gyrina* consistently tolerated the high temperature conditions for a longer period than did *P. integra*. At 40°C (Fig. 14a), the range for the former species, until 50% mortality occurred, was 7-10 hours; for the latter species, only 5-10 hours. Considering the results of each experiment as a unit, the difference between species is significant (P .01). At 35°C (Fig. 14b), both species survived many times longer than at 40°C; 50% mortality was reached in 11-13 days (264-312 hours) in *P. gyrina* and in 5.7 - 8.7 days 136-208 hours) in *P. integra*. Again the difference between species is significant (P .005). Differential tolerance to high temperatures may therefore be a factor helping to explain the presence of *P. gyrina* in ponds and the absence of *P. integra* from such habitats.'

The snails used in our study (Experiment 7) were somewhat older (3-4 weeks of age) than generally used for stocking test tanks. A slump in production of young necessitated the mustering of these animals. The temperatures in the six experimental aquaria again were maintained at 6°C intervals between 6° and 36°C. Since these *Physa* were somewhat older at the start of the tests it was not surprising that egg-laying began on the 14th day (Table 17; Figures 44, 45, 46, 47) in both the 24° and 30°C tanks. The snails in the control tank (about 27°C) showed the greatest growth and the best survival. It is clearly shown that *Physa gyrina* has an unusually wide range of tolerance with exceptional ability to survive; reproduction, as well as egg viability, was normal between 18° and 30°C with an optimum at 24° C. An experiment to measure growth and egg production at 2°C intervals within a range between 14° and 34°C was also made (see Experiment 13).

The experimental tanks in Experiment 7 became infested with chaetogasters. While they are usually considered to be commensals, they appear to have caused a decrease in egg production. This hindrance was especially noticeable in the 18°C and 24°C tanks and became acute two to three weeks after egg-laying started. Their presence in the stock aquaria may also have caused the slump in production of young previously mentioned.

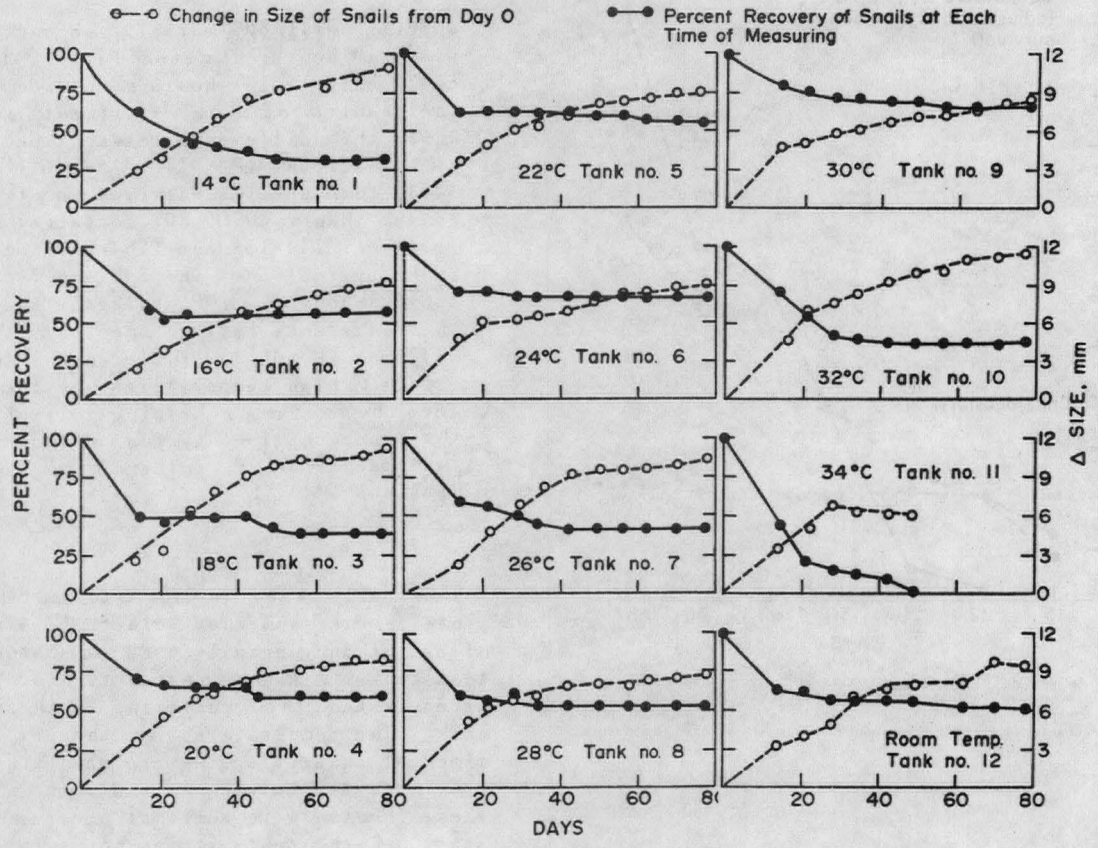


FIGURE 48. Growth and survival of *Physa gyrina* maintained for 77 days in tanks with 2°C temperature intervals ranging from 14°C to 34°C; room temperature (25°C) served as control. The lower temperatures are tolerated better than the highest (34°C). Experiment 13.

Growth and egg production of *Physa gyrina* were measured in tanks at 2°C intervals (Figure 50) in a range from 14° to 34°C (Experiment 13). The tests were fortunately free of the chaetogasters that adversely influenced the tanks previously maintained at 6°C intervals. On the whole, survival was good and varied from 33% in the 14°C tank to 66% in both the 24°C and 30 C tanks (Table 18; Figures 48 and 49). Adjustment to temperature and dissolved oxygen are shown for the 14°C tank (Figure 51) and for the 16°C tank (Figure 52). In this experiment, reproduction was high (Figure 53) with an average of 15.4 eggs per case produced in the 30°C tank and 39.2 eggs per case in the 14°C tank. It is evident that egg production was better in the colder temperatures where viability was 98% in the three coldest tanks and 24.9 eggs or more per case were produced. While 30°C was about the upper limit for all of the other pulmonate snails tested, some of the *Physa gyrina* in the 34°C tank remained alive for 49 days; however, only a few eggs were produced at such a high temperature. In growth the greatest amount was 11.6 mm in the 32°C tank, indicating that the warm water induces growth but hinders reproduction. In decreasing order showing greatest change in size, the other temperatures were 14°C, 18°C, 26°C and room temperature; these data are summarized in Table 18.

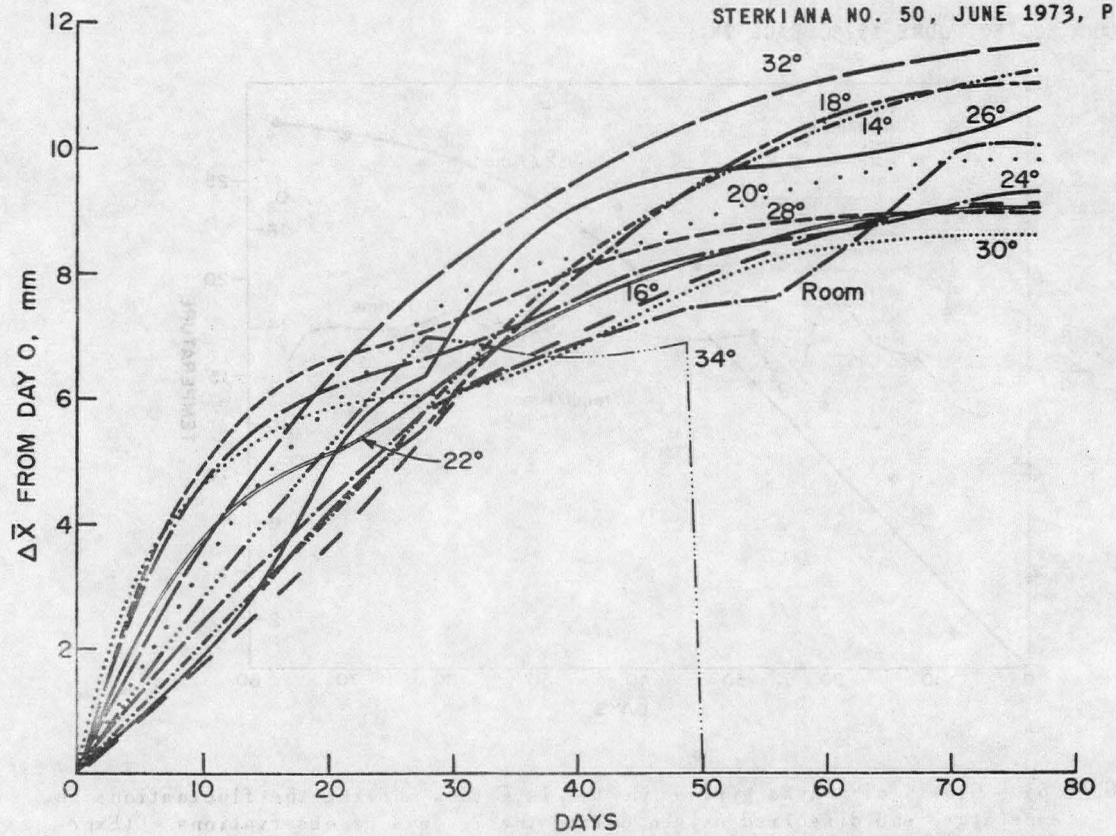


Figure 50. Growth of Physa gyrina maintained in tanks with temperatures set at 2°C intervals between 14° and 34°C; room temperature (25°C) served as control. P. gyrina, in contrast to the lymnaeids and planorbids tested, can maintain itself in an unusually wide range of temperatures. Experiment 13.

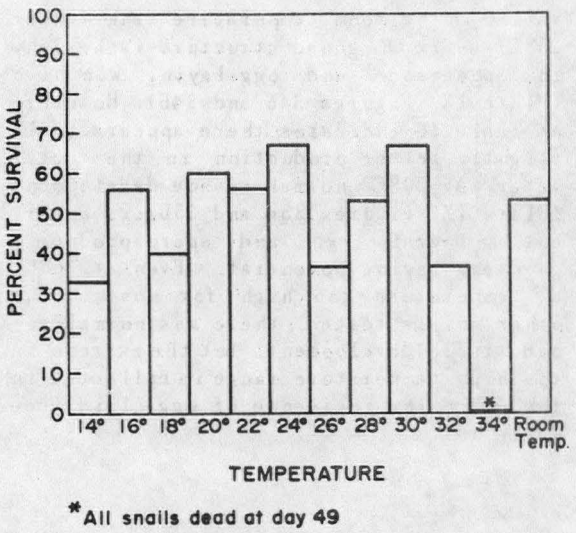


Figure 49. Survival of Physa gyrina maintained in tanks at 2°C temperature intervals between 14° and 34°C for 77 days. Except for the extreme 34°C temperature, survival throughout this range was relatively good. Experiment 13.

In the sections made of the gonads of *Physa gyrina* (Plate VIII; Figures 31-36), it was surprising but interesting to observe that this species could reproduce and maintain itself under a wider range of temperatures than any of the other species or groups tested. Even at 6°C, while no eggs were laid (see Table 17), there was already good gonad development. This 6°C interval experiment was concluded after 70 days but from the appearance of the gonad tissues (Plate VIII; Figures 31a and 31b) it could be presumed that egg and sperm development, as well as egg-laying, may have come later and still be normal.

In the 12°C culture the tissues (Plate VIII; Figures 32a and 32b) with eggs and sperm looked healthy and normal but again it is possible that there was not enough time allowed for egg-laying. The 18°C tank specimens were inadvertently lost so tissues for that series are not available. At 24°C (Plate VIII; Figures 33a and 33b) the gonads appeared fully functional and,

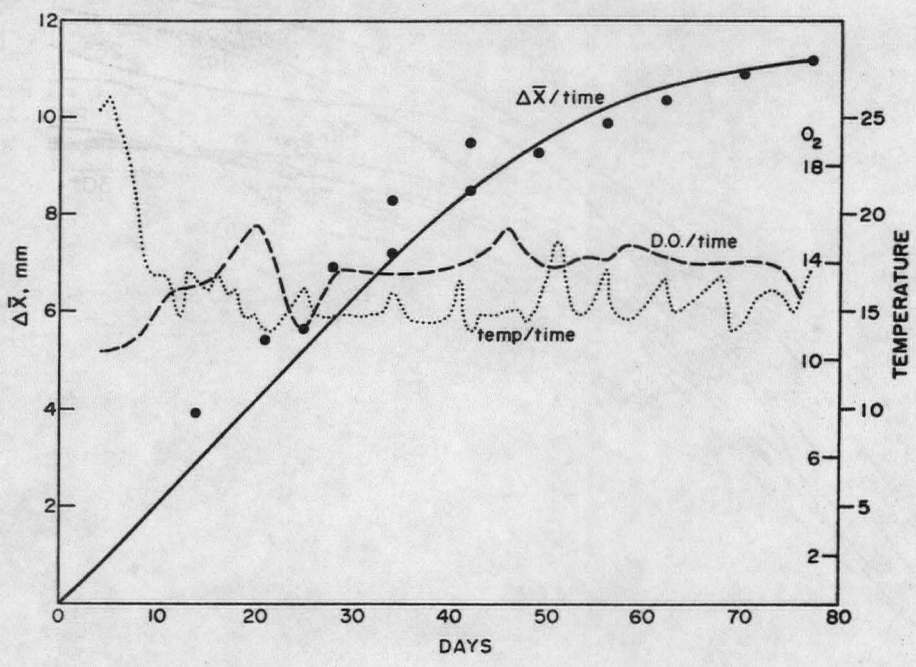


FIGURE 51. Growth of *Physa gyrina* in the 14°C tank showing the fluctuations in temperature and dissolved oxygen during the 77 days of observations. (Experiment 13).

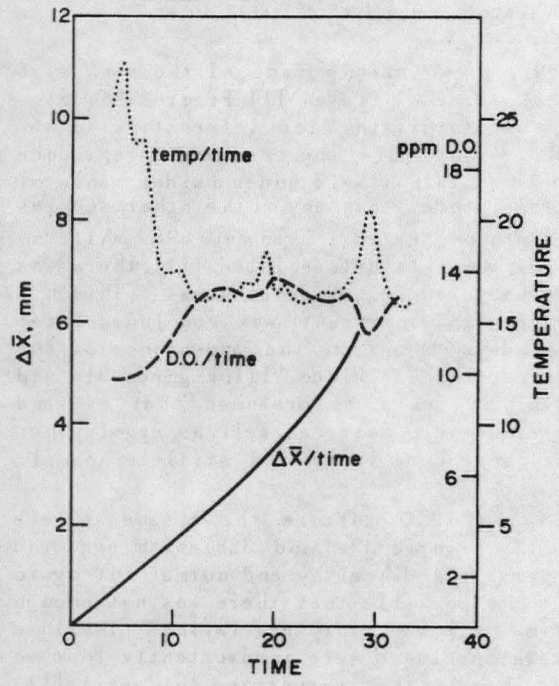
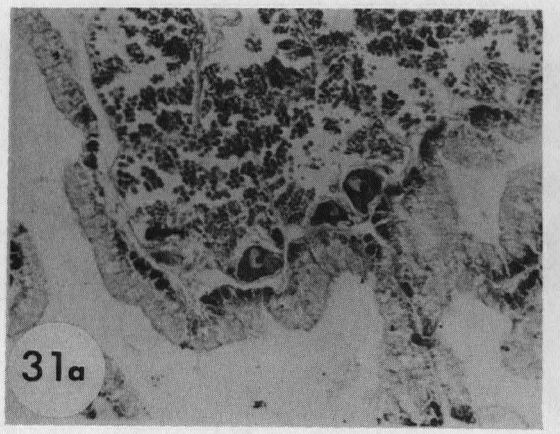
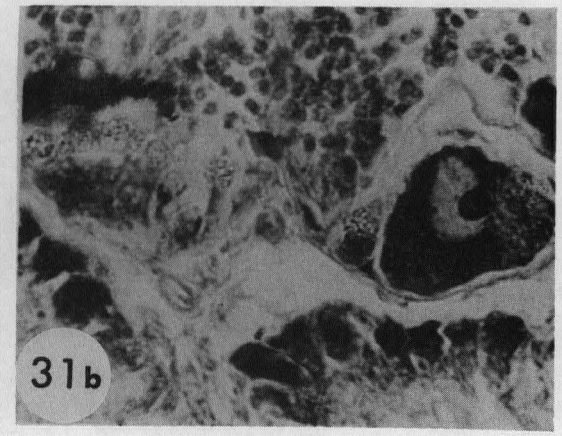
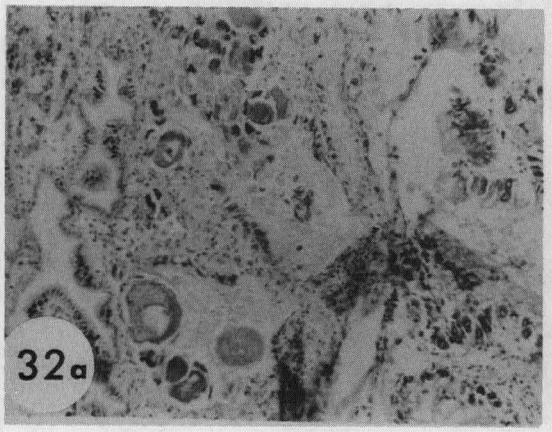


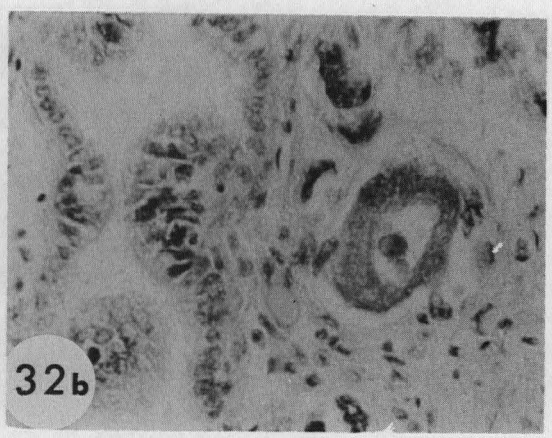
Figure 52. The adjustment of temperature, dissolved oxygen and growth of Physa gyrina over a 35-day period in the 16°C tank. Experiment 13.

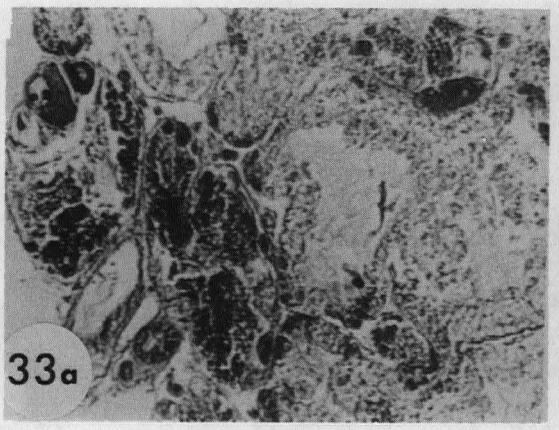
as Table 18 (Experiment 13) shows, large quantities of viable eggs were produced at this temperature. The same situation prevailed in the room temperature tank (about 25°C) where the gonad structure is healthy in appearance and egg-laying was high (Plate IX; Figures 34a and 34b). However, as Table 18 indicates, there appears to be slightly better production in the cooler water. At 30°C, normal tissue development (Plate IX; Figures 35a and 35b) is apparent and again eggs and sperm produce a good egg-laying potential. Even at 36°C, a temperature too high for most of the other snails tested, there was normal gonad tissue development, but the extreme in the high temperature range is reflected in the very low incidence of eggs laid (see Table 18).











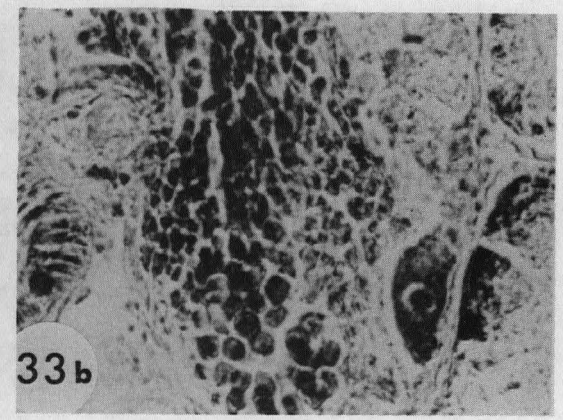


PLATE VIII. Gonad tissue sections from Physa gyrina cultured for 70 days at different temperatures.

Figure 31a. 6°C (X27.5)
Figure 31b. 6°C (X400)
Figure 32a. 12°C (X27.5)
Figure 32b. 12°C (X400)
Figure 33a. 24°C (X70)
Figure 33b. 24°C (X400)

TABLE 17. Summary of data for Physa gyrina at 6°C intervals, studied for 70 days (1/9/70 - 3/20/70); 30 specimens per tank (Experiment 7).

TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
		Total change ± 2 $s_{\bar{x}}$	Survival	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability
°C	ppm	in mm	%			
6.7 ± 0.2	12.4 ± 0.3	5.4 ± 0.6	76.7	--	--	--
12.2 ± 0.4	10.8 ± 0.4	5.9 ± 0.6	76.7	--	--	--
18.1 ± 0.4	9.7 ± 0.3	6.7 ± 0.5	70.0	22	14/293	98.0
24.1 ± 0.2	8.3 ± 0.2	6.6 ± 0.3	86.7	14	16/204	98.0
30.2 ± 0.1	7.4 ± 0.1	6.7 ± 0.6	66.7	14	42/537	99.4
36.0 ± 0.3*1	6.6 ± 0.3	5.8 ± 0.4*2	0	--	--	--
27.8 ± 0.5 (room)	7.0 ± 0.4	7.0 ± 0.5	100.0	21	71/1175	100.0

*3-4 weeks old at day 0.

*1Only one snail left at 59 days; used for sectioning.

*28 snails at 42 days - last date for statistical use.

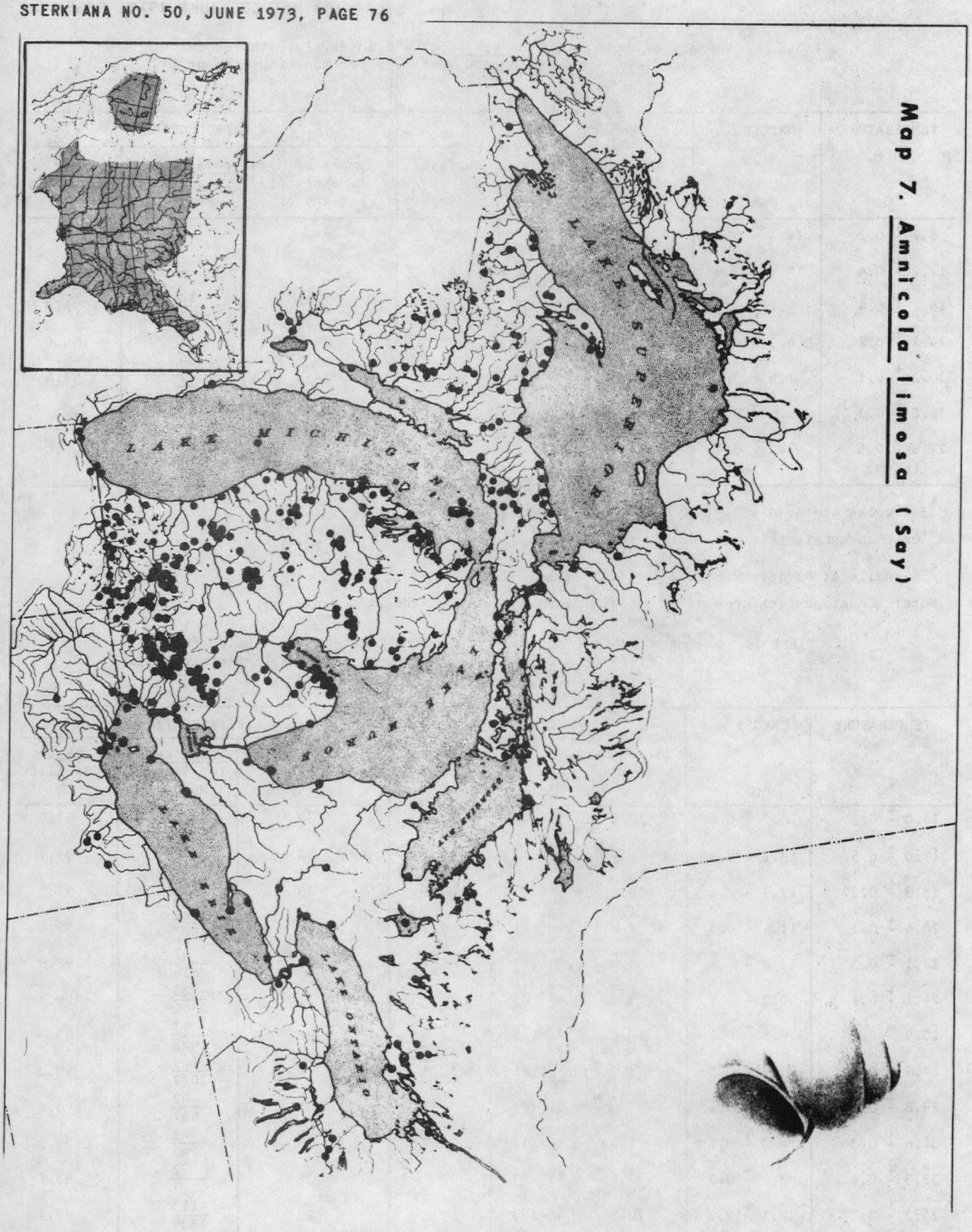
Note: Chaetogasters present at varying times in 12°, 18° and 24°C tanks.

TABLE 18. Summary of data for Physa gyrina at 2°C intervals, studied for 77 days (10/22/70 - 1/7/71); 30 specimens per tank (Experiment 13).

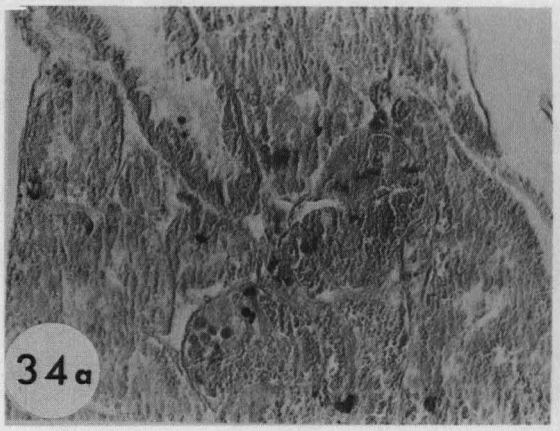
TEMPERATURE	OXYGEN	GROWTH*		REPRODUCTION		
		Total change ± 2 $s_{\bar{x}}$	Survival	Number of days to start of egg-laying	Total cases / Total eggs Number	Egg Viability %
°C	ppm	in mm	%			
15.0 ± 0.3	13.9 ± 0.4	11.2 ± 0.8 (10)*1	33.3	34	60/2353	97.5
17.0 ± 0.5	13.1 ± 0.3	9.9 ± 0.6 (17)	56.7	34	45/1116	99.7
18.3 ± 0.3	12.9 ± 0.2	10.9 ± 0.6 (12)	40.0	28	146/4313	97.5
20.4 ± 0.3	11.6 ± 0.3	9.8 ± 0.5 (18)	60.0	21	3198/3780	99.0
22.1 ± 0.3	11.3 ± 0.3	9.0 ± 0.5 (17)	56.7	28	47/816	98.9
24.1 ± 0.1	10.3 ± 0.2	9.3 ± 0.4 (20)	66.7	21	133/2396	99.5
25.9 ± 0.1	9.9 ± 0.1	10.6 ± 0.8 (11)	36.7	28	67/1482	97.4
27.9 ± 0.1	9.3 ± 0.2	8.9 ± 0.7 (16)	53.3	21	109/2022	99.2
29.8 ± 0.1	8.7 ± 0.1	8.6 ± 0.5 (20)	66.7	40	42/647	95.8
31.6 ± 0.3	8.5 ± 0.2	11.6 ± 0.9 (11)	36.7	28	42/743	56.5
33.3 ± 0.4	8.2 ± 0.2	7.0 ± 0.7 (3)	0 (day 49)	28	2/7	85.7
25.2 ± 0.2 (room)	10.1 ± 0.2	10.0 ± 0.4 (16)	53.3	28	113/1839	98.9

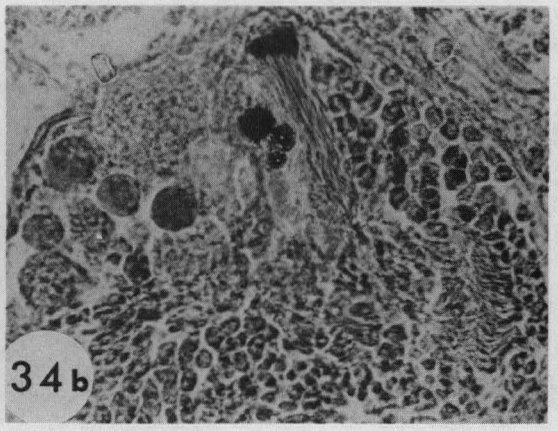
*Age at Day 0: 1-14 days.

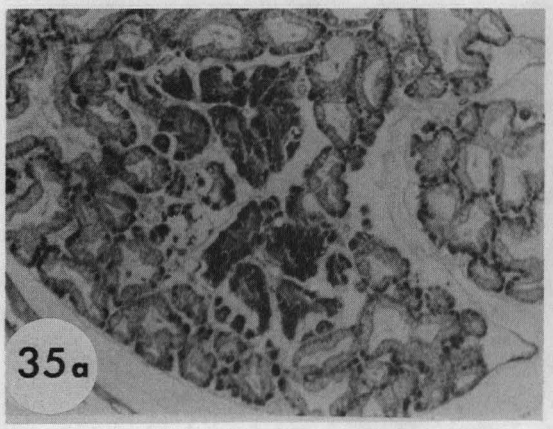
*1Number of snails surviving at end of experiment.

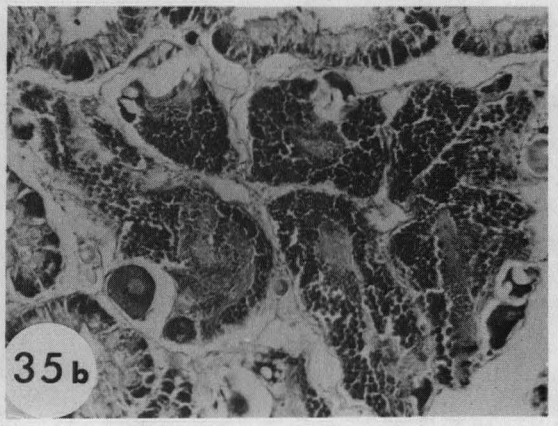


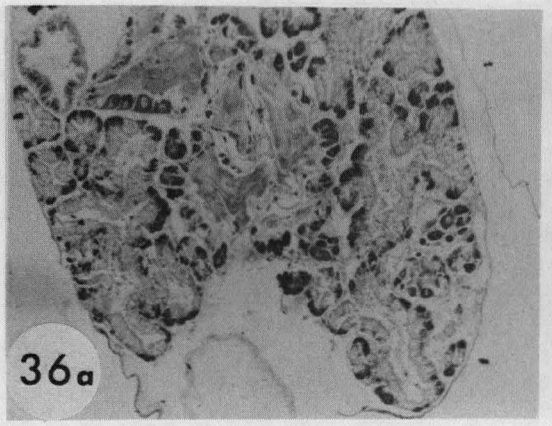
Map 7. Amnicola limosa (Say)











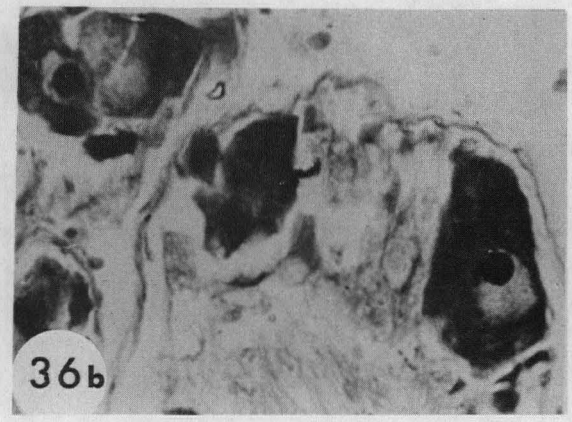


PLATE IX. Gonad tissue sections from Physa gyrina cultured for 70 days at different temperatures.

Figure 34a. Room temp. (X70)
Figure 34b. Room temp. (X400)
Figure 35a. 30°C (X70)
Figure 35b. 30°C (X400)
Figure 36a. 34°C (X70)
Figure 36b. 34°C (X400)

Amnicola limosa (Say)

As noted in a study by E. G. Berry (1943), these small operculates are among the most common of aquatic gastropods. *Amnicola limosa* has the widest distribution of all the New World amnicolas and also produces larger colonies than the other species of the genus. Its wide distribution in the Great Lakes region is shown in Map 7; its general range east-west extends from the Atlantic to Utah and north-south from Labrador to Florida.

A. limosa occupies a diversity of habitats such as creeks, rivers and most lakes. It is often present in large numbers on *Vallisneria* and *Elodea*, for example. The snails browse on the rich supply of diatoms and algae that cover these plants.

Culturing *Amnicola* and maintaining them in tanks for study purposes has problems not encountered with the pulmonates previously considered. Adult animals are only 4.5 mm long, so they were maintained in small trays that floated in the larger standard aquaria. Young snails from eggs (laid singly) in the stock aquaria were placed in trays of the 7 experimental tanks (30 per tank). Since the animals were so small, the age-range at the beginning of the experiment was wider (1-4 weeks of age) than preferred. Survival (Table 19; Experiment 8) was poor with less than 50% alive in the 6°C tank after 105 days; in the room temperature tank (24°C) only 8 snails (26.7%) survived the tests. Excess algal growth and chironomid flies were probably in part responsible for this poor maintenance. Growth as measured (Figure 54) indicated that 18°, 24° and room temperature (24°C) were best; in 30°C all were dead by the 21st day.

In contrast to Experiment 8, a more successful experiment (Experiment 11) with *Amnicola limosa* was completed (Table 20). The snails were maintained for 84 days. To compensate for poor survival the tanks were stocked with 40 rather than 30 snails at the start of the experiment. Ages of snails used were better, being 1 to 2 weeks old rather than 1 to 4 as in the previous test. These snails also were maintained in small plastic containers within the regular 8-gallon tanks. Precautions were taken to retain the snails and small petri dishes with algae were placed with them to serve as food.

As in Experiment 8, no eggs were produced and survival again was relatively poor. The young in this test were from eggs of snails recently brought to the laboratory from the field. In Experiment 8 the snails were second generation young. However, the cold (7.2°C) tank over the 84-day period had a survival of only 30%; in the 13.6°C and 18.7°C tanks it was even less (25%). An unexpected cracked heater in the 24°C tank electrocuted some snails to reduce the number from 34 to 19 at day 42; only one survived until day 70. In the 29.6°C tank, 9 were left at day 70 and they died before day 84. At 36°C only 25 lasted 2 weeks but by day 28 all were dead. The 35.3°C temperature started at 25.2°C and rose to 34°C by day 13; hence, the 2-week survival (Figures 55 and 56) was to be expected. Even in the room temperature (or control) tank survival was poor and only half of the original 40 specimens were alive at day 28; only 2 were left at the last day of the experiment. These tanks were also infested with chironomids.

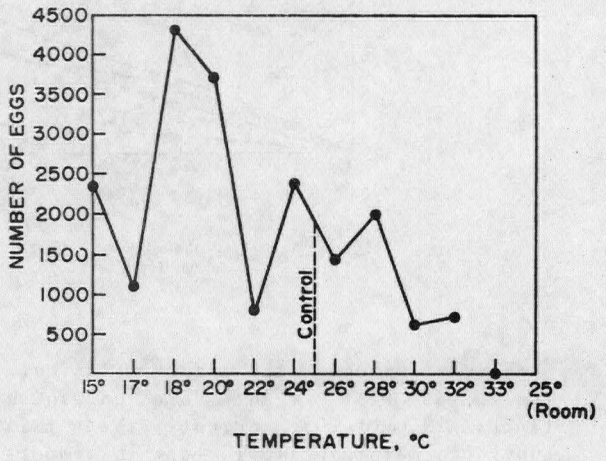


Figure 53. Egg production by Physa gyrina maintained for 77 days at temperatures ranging from 14°C to 34°C; room temperature (25°C) served as control. Production is highest in the colder ranges. Experiment 13.

TABLE 19. Summary of data for Amnicola limosa at 6°C intervals, studied for 105 days (2/26/70 - 6/11/70); 30 specimens per tank. Terminated without reproduction.(Experiment 8)

TEMPERATURE	OXYGEN	GROWTH*	
		Total change $\pm 2S_{\bar{x}}$	Survival
°C	ppm	in mm	%
6.7 ± 0.3	13.7 ± 0.4	0.56 ± 0.33 (14)**	46.7
13.5 ± 0.4	11.5 ± 0.4	0.52 (3)	10.0
18.6 ± 0.3	10.9 ± 0.3	2.04 ± 0.37 (11)	36.7
24.1 ± 0.2	8.6 ± 0.3	3.55 (4)	13.3
29.6 ± 0.3	6.9 ± 0.5	(all dead, 34 days)	0
35.0 ± 0.3	6.2 ± 0.3	(all dead, 21 days)	0
23.9 ± 0.4	8.9 ± 0.3	2.72 ± 0.25 (8)	26.7

*1-4 weeks old at day 0; average length, 1.02 mm.

**Number of snails surviving at end of experiment.

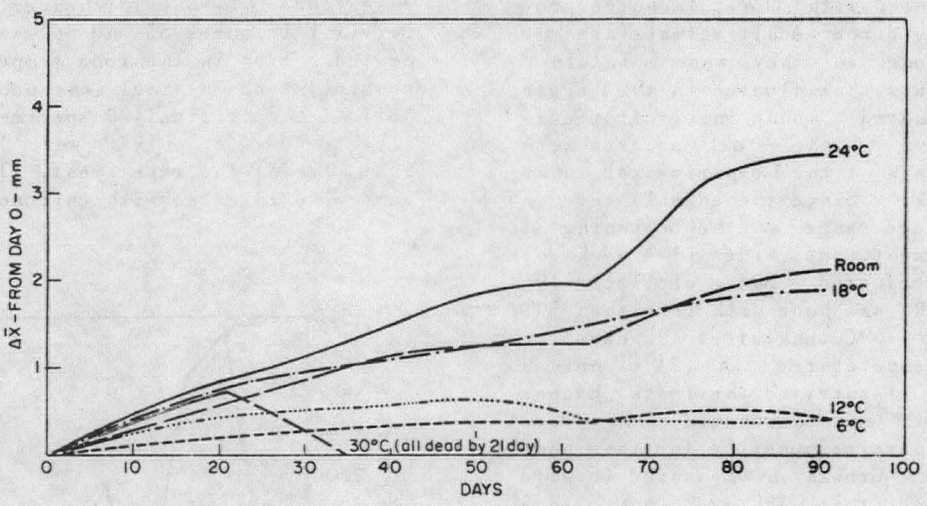


Figure 54. Growth of Amnicola limosa maintained in tanks at 6°C intervals of temperature between 6° and 30°C; room temperature (24°C) served as control; 30 specimens per tank were used. Survival in all tanks was relatively poor. Experiment 8.

The development of the gonads in *Amnicola limosa*, as well as the data on growth (Tables 19 and 20), indicate that this snail can maintain itself best in temperatures on the cool side, i.e., between 7° C and 24°C. In sections of the gonad of a female (Plate X; Figures 37a and 37b) some differentiation is shown at the end of 84 days in culture, but spermatogenesis was incomplete. The male at 18°C (Plate X; Figures 38a and 38b) has good spermatogenesis development but no mature sperm. Whether or not these tissues would develop

The data from these experiments do indicate that these small operculate snails survive better in cold water and that they do not manage to tolerate the warmer (30°C or higher temperatures. Both with *Amnicola* and the common stream operculate *Goniobasis*, much remains to be accomplished in learning how best to maintain them in the laboratory. Because these groups are so widespread and abundant, additional studies are needed and information on their adjustment to temperatures in natural waters is of importance.

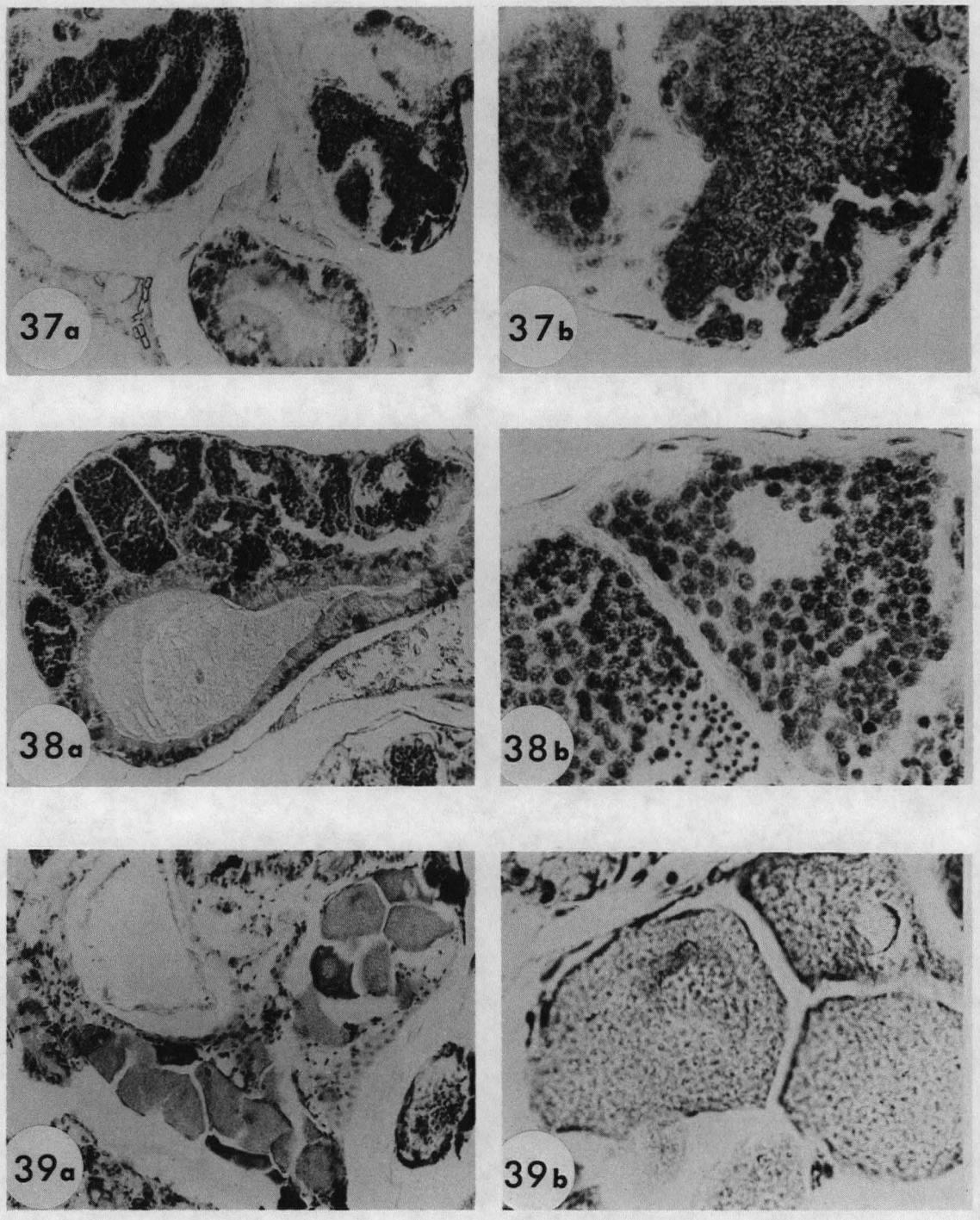


PLATE X. Gonad tissue sections from Amnicola limosa cultured for 84 days at different temperatures.

Figure 37a. 6°C (X70)
Figure 37b. 6°C (X400)
Figure 38a. 18°C (X70), male
Figure 38b. 18°C (X400), male
Figure 39a. 18°C (X70), female
Figure 39b. 18°C (X400), female

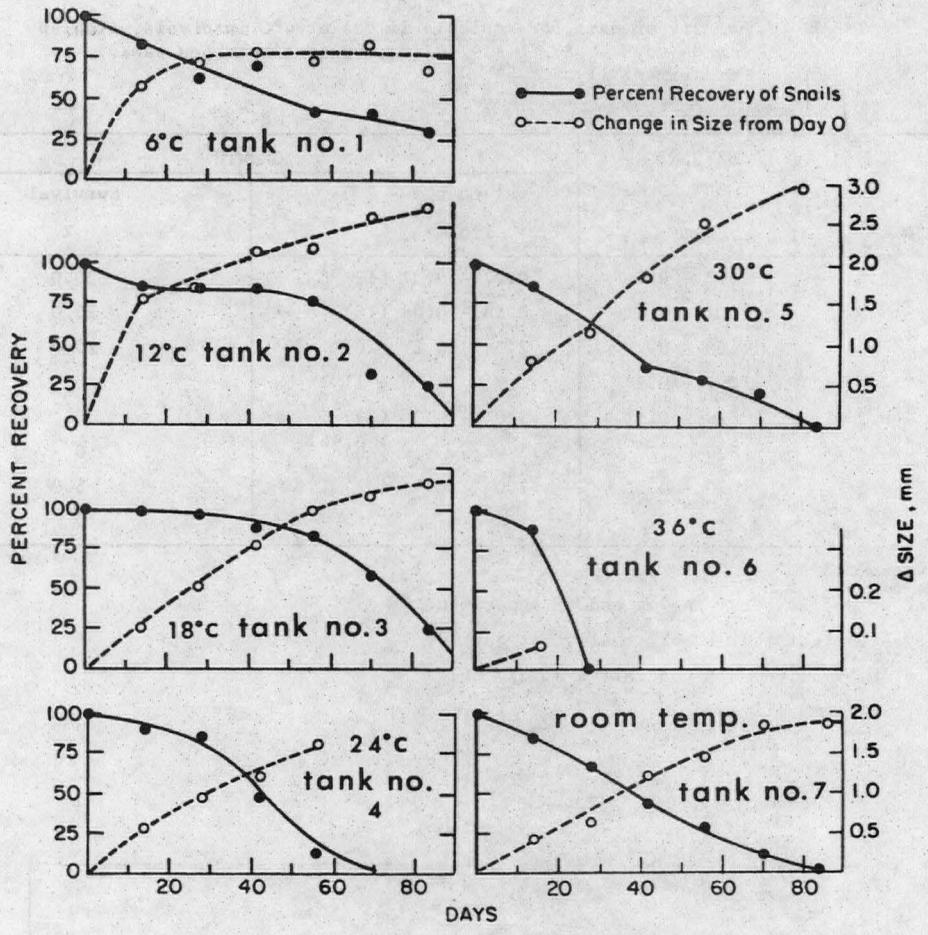


Figure 55. Growth and survival of Amnicola limosa maintained in tanks at 6°C intervals between 6° and 36°C, with room temperature (about 23°C) as control. Experiment 11.

completely over a more extended period in that relatively cool temperature remains an open question. At that same temperature the female (Plate X; Figures 39a and 39b) had well-developed eggs. It is possible that reproduction could take place at 18°C but in the cultures no eggs were laid. The section of a male gonad maintained at 24°C (Plate XI; Figures 40a and 40b) had an abundance of well-developed sperm; also, the female (Plate XI; Figures 41a and 41b) had well-developed eggs. Another male (the control at 24°C) had a normal and productive gonad with an abundance of sperm (Plate XI; Figures 42a and 42b). As also shown in the growth studies, *Amnicola limosa* appears to be quite sensitive to warm water to the point where its optimum range would be on the cool side and in the 18° to 24°C range. Its small size and its usual failure to produce eggs when cultured suggest that this snail will need more intensive work to establish good culture methods.

TABLE 20. Summary of data for Amnicola limosa at 6°C intervals, studied for 84 days (6/23/70 - 9/10/70); 40 specimens per tank. (Experiment 11)

TEMPERATURE	OXYGEN	GROWTH*	
		Total change $\pm$ 2 $S_{\bar{x}}$	Survival
°C	ppm	in mm	%
7.2 $\pm$ 0.6	15.3 $\pm$ 0.7	0.27 $\pm$ 0.07 (12)*1	30.0
13.6 $\pm$ 0.5	13.1 $\pm$ 0.6	0.55 $\pm$ 0.08 (10)	25.0
18.7 $\pm$ 0.6	11.3 $\pm$ 0.2	2.3 $\pm$ 0.1 (10)	25.0
24.1 $\pm$ 0.7	10.1 $\pm$ 0.2	1.3 (1)*2	0
29.6 $\pm$ 0.3	8.6 $\pm$ 0.3	3.0 $\pm$ 0.3 (9)*3	0
32.1 $\pm$ 1.8	8.4	(0)*4	0
23.0 $\pm$ 0.5 (room)	10.0 $\pm$ 0.5	2.0 (2)	5.0

*1-2 weeks old at day 0

*1Number of snails surviving at end of experiment.

*2On day 42, electrical shock; 1 alive at day 70.

*3On day 70, 9 alive, but none alive at day 84.

*4On day 28, none alive.

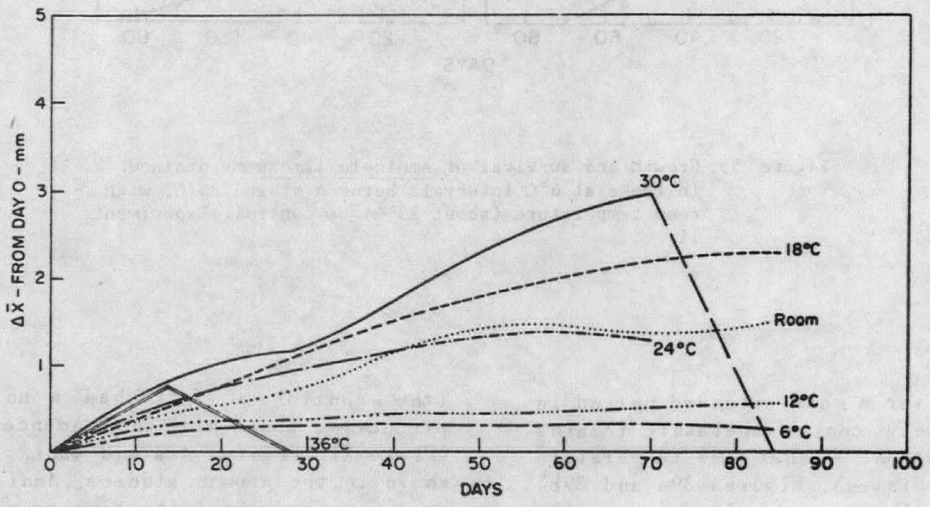


Figure 56. Growth of Amnicola limosa maintained in tanks at 6°C intervals of temperature between 6° and 30°C; room temperature (24°C) served as control; 40 specimens per tank were used. Growth was best between 18°C and 24°C. Survival in all tanks was poor. Experiment 11.

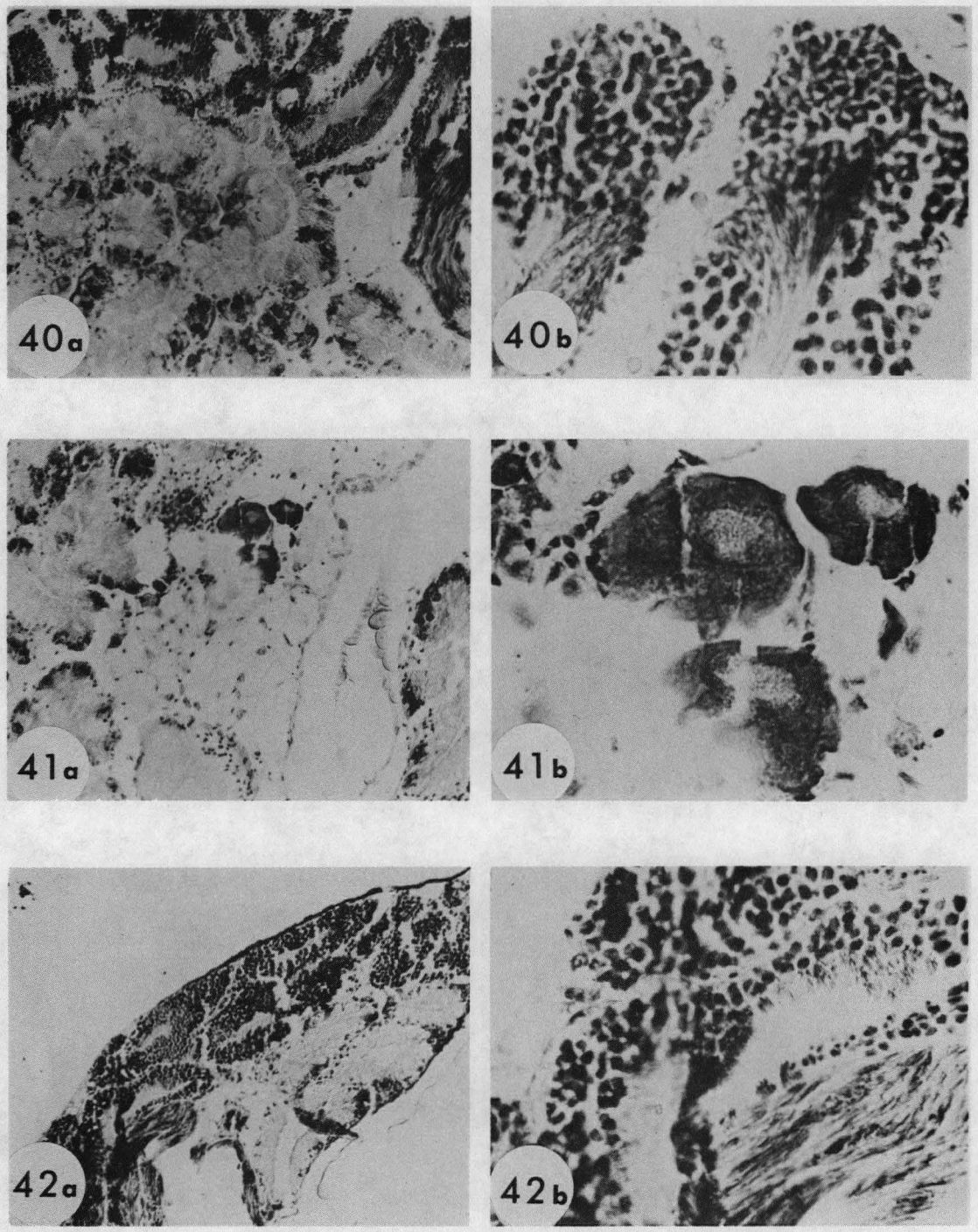


PLATE XI. Gonad tissue sections from Amnicola limosa cultured for 84 days at different temperatures.

Figure 40a. 24°C (X70), male
Figure 40b. 24°C (X400), male
Figure 41a. Room temp. (X70), female
Figure 41b. Room temp. (X400), female
Figure 42a. Room temp. (X70), male
Figure 42b. Room temp. (X400), male

DISCUSSION

From the data given both in the tables and the graphs, one can see that the snails tested do have definite patterns of behavior in their growth and in their reproductive processes. While some of these patterns were known for a few of the species previously studied, it has now been established that there are definite restrictions, even for the families, in relation to temperature stress. In a few instances these relationships were studied (e.g., the investigation by Vaughn (1953) working with *Lymnaea stagnalis*. He maintained them in a range, as follows: 3.4, 6.9, 11.5, 15.7, 20.1, 24.2, 28.1, 32.0 and 36.0°C; the control was 24.1°C.) His tests ran for 6 weeks; ours were maintained for 4 months or the time to run a complete life cycle with this species. In essence, besides discovering the range (between 11° and 32°C) at which this species can be maintained, Vaughn established that growth was best at 24°C, but at that temperature mortality was higher than when the snails were kept at 16° to 20°C, which range appears to be the best for their maintenance. This work essentially substantiates the observations of Noland and Carriker (1946), in their very definitive study on the biology of *Lymnaea stagnalis*.

Temperature conditions in nature have a profound effect on the pulmonate snails as shown in limnological investigations undertaken by Cheatum (1934). The pulmonates he studied in nature at Douglas Lake in northern Michigan were for the most part the same as those stressed in these laboratory tests. Since temperature has a profound effect on the amount of oxygen available to the pulmonates in nature, several lake-inhabiting species have an annual migratory cycle which brings them into deeper water during the winter and back on the shoals with the advent of spring. As the water warms, the number of trips which snails make to the surface for filling their pulmonary cavity with air increases markedly. Cheatum showed that at 1.7 cc of oxygen per liter the pulmonates he studied came to the surface for air three times more than when the amount was 6.4 cc per liter. With regard to respiratory acclimatization to temperature, Kaj Berg (1953), in testing an hypothesis by August Krogh, found:

> 'The oxygen consumption increases 1.9 times when the temperature of the experimental animals increases from 11°C to 18°C. The 'reversed acclimatization' found for *Ancylus fluviatilis* is wbout 1.4. It means that the observed respiration of the warm-water animals in relation to that of the cold-water animals is about 1.4 times greater than would be implied from the temperature difference between their habitats.'

Hurst (1927), in his studies of *Physa occidentalis*, found that those snails needed twice as much oxygen at 21°C as compared to the amount they used at 4°C.

The migration cycle among the 16 species of pulmonate snails studied by Cheatum appeared to be prompted in the autumn by the lowering water temperatures, with the reverse trend as the waters became warmer in the spring. Several critical questions remain unanswered, such as: What are the temperatures that stimulate this movement; do all of the species respond in the same way; what is the 'deeper' part of the lake; what is meant by 'active' in the 'deeper' areas; when the snails spend 3 to 4 months in an inactive state in 'deep' water under the ice, what determines the extent of the time they spend there? More recent studies indicate there is a question whether some of the pulmonates assumed to have such a migration pattern actually remain on the shoals throughout the winter. Both Wall, working in our laboratory (paper in press), and Clampitt, currently studying migration patterns of these snails in Douglas Lake, have reservations about the

extent and nature of these migrations. Wall has data taken over a three-year period in Lake Ann north of Interlochen, Michigan, indicating *Lymnaea emarginata* did not migrate to deeper water during any of the winter periods under investigation.

Among the snails tested in our experiments, two—*Helisoma trivolvis* and *Physa gyrina*—were frozen in ice by Cheatum and living specimens recovered later. It has been known that the circumpolar physid, *Aplexa hypnorum*, annually survives through severe frigid conditions in Siberia. The crux of the matter appears to be related to the suddenness with which the changes occur. Cheatum found that many snails do not survive if they are suddenly transferred from warm water (29°C) to cold (5.5°C). The same animals would survive if they were cooled gradually over a 24-hour period. More studies on the adjustment of animals to sudden changes in temperature are needed and this introduces the obvious need for 'plop tests' which were not possible in the time allotted for our studies. W. Russell Hunter (in Wilbur and Yonge, 1964) indicated that: 'In environments provided by fresh waters, physical, chemical, and biotic-trophic conditions vary more widely than in the sea. The temperature range within which some fresh-water molluscs can live practically corresponds to the absolute limits for metabolism in metazoan tissues ....' In his studies of the life cycles and intraspecific variation of four snails in Loch Lomond, W. Russell Hunter (1961) stated 'Variations in breeding are dependent both on environmental factors, water temperature being most important, and on endogenous causes involving the growth of the snails; ....' There are exceptions, perhaps more than realized, to the rule that lymnaeid snails often thrive better under 'cool' conditions. In the region of Australia, Boray (1964) studied *Lymnaea tomentosa* both in the field and in the laboratory. He discovered that the optimum temperature for that widespread host of fascioliasis was 26°C; the snails were able to survive 6 weeks in water at 36°C and 'they remained alive for 3 years at 2-5°C.' More recently Foster (1971), working on *Fasciola hepatica* in Oregon and studying the movement (sometimes called 'vagility') of *Lymnaea bulimoides*, noted its 'tolerance to cold is indicated by its ability to survive in the laboratory at a water temperature of 5°C for more than 3 months with or even without food. Movement is slowed considerably at this temperature.' Dean Arnold (1969) wrote about the development of problems that relate to thermal pollution and nuclear power in the Great Lakes. He stressed that temperature is very important both in relation to physical as well as biological processes. Such problems are apt to increase since it has been predicted that electric power demands will double every 8 years! He stated (1969: 134): 'Probably the most likely effect of heated discharges is an increase in biological production in the local surrounding area. This may take the form of 'blooms' of weeds or of blue-green and green algae, which are already a problem in Lakes Ontario and Erie and some areas of Lake Michigan.' One could add that the altering of temperature conditions would also undoubtedly eliminate or alter the mollusks that inhabit the lakes. These changes are not of small proportions since, in bio-mass, this group far outweighs many of the other invertebrate animals. For example, at one time thousands of *Lymnaea auricularia* introduced from Europe thrived on the dense emergent aquatic vegetation in western Lake Erie. Today the species is absent. If one were to add the mussel fauna, there would be no other group of animals that would be represented so much in biomass!

Mackenthum and Koup (1969) also stressed that 'temperature is a prime regulator of natural processes within the water environment .... depending on the extent of environmental temperature change, organisms can be activated, depressed, restricted, or killed.' Later, they state: 'Because of its capacity to determine metabolic rate, temperature may be the most important single environmental entity to life and life processes.'

Merriman (1970) studied the 'calefaction' (also known as 'thermal pollution') at the site of the nuclear power plant near the mouth of the Connecticut River. While the results have not been published, the point was made that (1970: 52): 'Where the calefaction of streams, rivers and lakes is concerned, what must be done is not only to squarely face the ecological problems that the rising demand for power are creating but also to accompany programs of

construction with programs of environmental research so that the most favorable possible conditions are achieved.'

Naylor (1965) wrote a very sound and comprehensive paper on 'Effects of Heated Effluents upon Marine and Estuarine Organisms.' He gave one of the best summary statements indicating the relatively narrow temperature range (roughly 12°C to 32°C) within which freshwater organisms must live. His succinct statement (1965: 77) is as follows:

> 'For freshwater organisms in general the normal population structure is maintained only up to a tolerance limit of about 32°C and extensive loss in numbers and diversity of organisms occurs above that temperature (Coutant, 1962). Some genera were shown to be more tolerant than others of temperatures of 40 - 43°C (Coutant, 1962). Cairns (1956) concluded that to maintain survival in temperate streams large areas should not be heated above 30°C for long periods. This conclusion is also supported by the results of work by Alabaster (1963) who showed that though coarse fish are attracted into water heated to about 26°C., temperatures above 30°C are avoided.'

The sensitivity of animals to temperature was reviewed by Stauber (1950) in a study to determine whether there were physiological species particularly among oysters and oyster drills. He also refers to Runnström (1929: 1936) who worked industriously to show that among marine animals of Europe there were physiological races which were noticeable through their spawning periods in which one group spawned only during the winter while forms in another group spawned throughout the year. In summary, Stauber (1950: 117) stated: 'The evidence for physiological species of oysters and oyster drills has been presented. The probability has been raised that other marine inshore invertebrates may also be shown to contain physiological varieties within the designation of the usually accepted morphological species. Finally, the practical importance of such information has been cited to demonstrate the need for additional observations and to stimulate the interest of others in this problem.' The existence of physiologically different races of oysters, *Crassostrea virginica*, was again established later by Loosanoff and Nomejko (1952).

Mollusks have been important in the interpretation of former ecological conditions; and this relationship was reviewed by F. C. Baker (1937). Snails are recognized as having 'northern' or 'southern' patterns of distribution; they are also understood to have sensitivity to degrees of moisture for interpreting whether conditions were moist or dry, as shown by studies of such operculate species as *Pomatiopsis* or *Hendersonia*. More recent applications involve the effects of the removal of the forest cover and the changes that occur when rivers are impounded. Wurtz (1969) indicated that the removal of vegetation over extended areas exposed large drainages to increased solar radiation and brought about a warming of natural waters. He cited conditions in Lake Erie which had a mean annual temperature of 50°F between 1918 and 1928, but that mean increased by 2°F between 1925 and 1930. While the animals were already subjected to wide ranges in temperature, it is known that during growth and reproduction such temperature changes may become critical.

Just as Wurtz (Ibid.) indicated that there was evidence for increased temperature in Lake Erie, an interesting corollary also appears in the lake region of Ann Arbor where about a half century ago it was relatively easy to obtain *Lymnaea stagnalis* in large quantities. In those days such investigators as E. D. Crabb (1929, in studies of genetics and embryology) and George R. LaRue, sponsoring parasitological work with his students, were finding *L. stagnalis* in several of the local lakes (Third Sister, Frains, etc.) within the lower part of the southern peninsula. It is no longer possible to find this snail anywhere in this region; the nearest good collecting grounds are some 200 miles north, starting about at the level of Houghton Lake (see Map 1). A likely explanation is indicated graphically in a diagram (Figure 57) and in a tabulated form below:

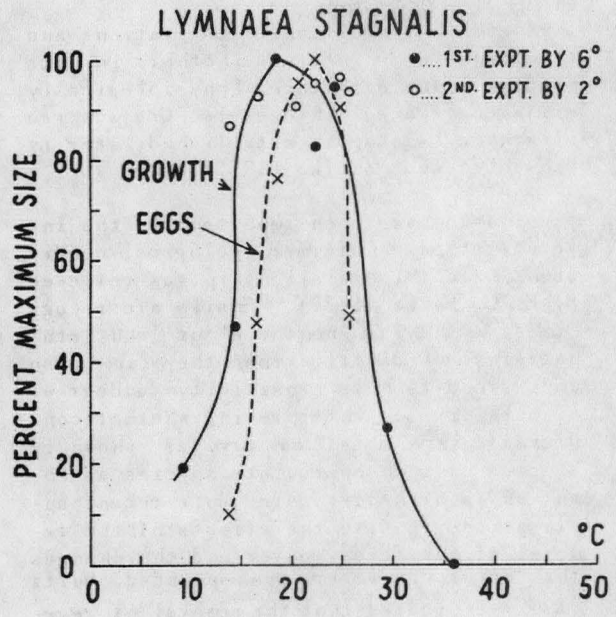


Figure 57. Diagrammatic comparison of growth and egg-laying among Lymnaea stagnalis snails in Experiment 1 (6°C intervals) and Experiment 3 (2°C intervals), indicating the sensitivity of this species to critical temperatures (18°C for growth and around 24°C for egg-laying).

Lymnaea stagnalis - First experiment using 6°C intervals

Tank Temperature	% of maximum size change	% maximum eggs
18.3	100.0	100.0
24.1	94.4	0
22.2 (control)	83.2	4.0
14.4	47.2	0
29.3	27.1	0
9.3	19.2	0
36	0	0

Lymnaea stagnalis - Second experiment, using 2°C intervals

Tank Temperature	% of Maximum Size Change	Tank Temperature	% Maximum Eggs
18.5	100.0	22.1	100.0
24.7	96.3	20.4	95.6
22.1	95.7	24.7	90.2
25.6 (control)	94.0	18.5	76.0
16.5	92.4	16.5	47.8
20.4	90.7	25.6 (control)	44.6
13.8	86.7	13.8	10.0

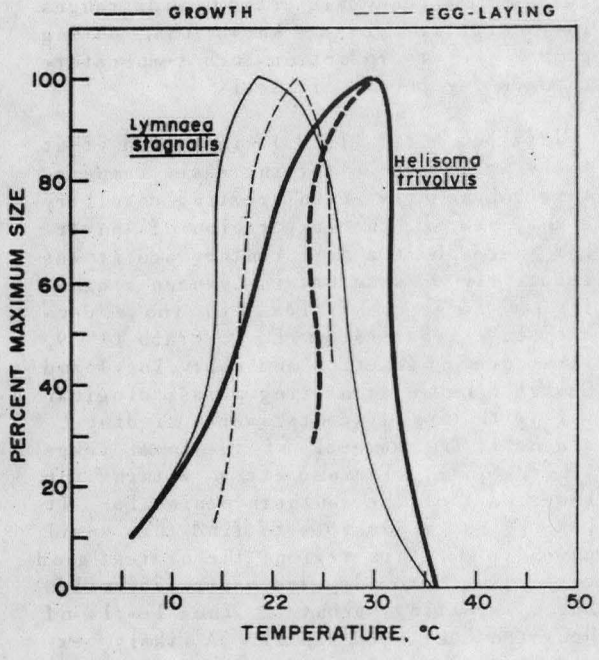


Figure 58. Diagrammatic comparison of growth and egg-laying, showing that Lymnaea stagnalis requires "cool" conditions while Helisoma trivolvis thrives in "warm" water.

The data from two experiments (1 and 3), when plotted on a percentage basis, indicate, as previously shown, that growth of *L. stagnalis* is best around 20°C, with the optimum for egg-laying only slightly higher. While the data given for the two experiments are on a percentage basis and may not be statistically valid, the diagram, nevertheless, gives a true indication of the relation of growth and reproduction to temperatures. In contrast to the relatively low optimal temperatures best suited for *Lymnaea stagnalis*, the tendency for *Helisoma trivolvis* (and for that matter some of the other large planorbids) to show preference for relatively warm conditions is shown in a similar and superimposed graph (Figure 58) and in the following tabulation:

Tank Temperature	% of Maximum Size Change	% Maximum Eggs
30	100.0	100.0
24.8 (control)	86.0	85.3
24	82.2	24.1
18	57.2	0
12	48.9	0
6	9.9	0
36	0	0

While, as yet, there is little basic information to substantiate the observation, it is possible that both the warmer waters and some eutrophication will tend to favor the *Helisoma* group so that they may become dominant species while the lymnaeids would disappear with the advent of an increasing amount of warmth in the heat budgets of bodies of water subjected to sources of increased solar or other radiation.

The tests designed to measure the sensitivity of animals to various temperatures during developmental stages may also be useful for interpreting the role temperature played during the glacial periods. Kearney (1968), in assessing post-glacial climatic conditions based on pollen analyses indicated that there were three periods: (1) increasing; (2) maximum; and (3) decreasing warmth. He was concerned about the land snails and indicated that 'very little comparative work has yet been done on the thermal tolerances of mollusca.' He also stressed the need for precise geographic ranges, experimental and observational work on thermal tolerances, and much more careful paleontological work. The use of mollusks as time markers and for measuring ecological conditions has been recognized by Pleistocene geologists, among them F. C. Baker (1937), Deevey (1949), Hibbard (1960), D. W. Taylor (1966), and others.

According to D. W. Taylor (1966: 9) the extinct snail, *Biomphalaria kansasensis* Berry, is 'one of the most significant fossil occurrences in southwestern Kansas.' As a generally recognized tropical or subtropical snail, it became extinct in that region in early Pleistocene. With the more precise information provided by the tests used here showing the need for warm conditions for growth and reproduction, the climatic conditions that must have existed can most certainly be assessed in a more positive way. Hibbard (1960) dealt with this problem in an article giving a better appreciation of North American climates during the Pliocene and Pleistocene. His speculations were based on extrapolations of climates that would account for the presence of giant land tortoises and crocodiles. With more precise information on tolerance as provided by experimental studies, similar temperature requirements can be postulated for many mollusks found so abundantly in many of the interglacial fossil beds.

Crisp (1957) also considered the limits of temperature within which marine animals could breed. He emphasized that as a function breeding differed according to the 'variation in temperature at different latitudes.' His studies on Arctic barnacles indicated that they did not reach a breeding condition if they were subjected to high temperature for prolonged periods. Essentially these observations substantiate those given here in which freshwater pulmonate snails grow better in 'warm' water (around 30°C) but often fail to show a normal reproductive potential. As already shown for some of the species of lymnaeids, Crisp found that among marine barnacles 'a prolonged period of high temperature prevents the animals from reaching the breeding condition.' The problem is accentuated if the heat is applied during the breeding period, a condition which appears complex as was indicated by Joosse (1964:95) who found in *Lymnaea stagnalis* 'a clear annual periodicity in the activity of the neurosecretory cells and of the C-cells.' Acclimated responses do occur but, as stated by Segal (1961): 'Seasonal acclimation is particularly difficult to demonstrate.' Where it is known to occur, it would be emphasized in *Physa* which seem to tolerate a far wider range of temperatures than any other mollusk so far tested.

In an attempt to measure the effects of the extremes of temperature on the cold side, McNeil (1963) studied *Lymnaea (Stagnicola) palustris* and *Physa propinqua* through several winters (1958 to 1961). He found that the former survived better 'attached to the cement lining of culvert walls and formed an epiphragm when the

water receded in late October.' The *Physa* survived best 'chiefly in the water of the culvert and at the bottom of the larger ditches.' The problem of survival of pulmonate snails in areas where winters are severe and where the mollusk groups are widely diverse needs far more attention than hitherto given it. In some cases the animals migrate, as indicated in studies by Cheatum (1934), Chernin (1967), Wall (in press) and Clampitt (in press).

Abrahamson (1972) also gave a broad and basic discourse on some of the ecological hazards produced by power plants. He stated: 'Over two-thirds of the total heat generated by a nuclear power station is discharged as waste into the local environment. Should this heat be introduced into lakes, rivers, or estuaries, serious and widespread changes can be expected.' In the same volume *(The Careless Technology)*, Cairns presented data of studies designed to assay the effects of heated effluents both in the Savannah River basin and in the Potomac drainage. While his data include a large number of species (including mollusks) found at different seasons, the monitoring showed all stations remained healthy in the Savannah study. He did state that 'all stations remained in the 'healthy' range from 1951 to 1960 according to the system proposed by Patrick (1949).' Perhaps the importance of the experience by this limnological team is best summarized in the following statement by Cairns (1972:845):

> 'These situations are not dramatic—no rivers were grossly damaged with accompanying fish kills; there were no spectacular confrontations between the various groups involved; and although the communities of aquatic organisms changed, the successional pattern appeared to be similar for the areas studied in each of the rivers (of course, there were considerable differences between the Savannah and Potomac rivers). The two factors that are of interest are (1) that a truly multidisciplinary group of administrators, plant waste disposal engineers, and ecologists from state regulatory agencies and a research organization worked successfully towards the common goal of preserving the river, and (2) the extensive use of river water was made over a number of years without interfering with other beneficial uses or degrading the aquatic community inhabiting the receiving waters.'

The problems relating to 'thermal pollution' or 'calefaction' were well defined by Cairns (1972) and he clearly stated the alternatives to those interested in maintaining a reasonable balance of conditions in natural ecosystems. It is not possible here to do justice to his thought-provoking analysis, but the following quotation seems appropriate as applied to the results of these snail-stressing studies:

> 'The rapidly increasing warming of surface waters by industrial uses of water is an example of the urgent need for basic social and policy changes so that proper management of the environment may occur. (Detailed discussions sre given of present problems and techniques of observing and analyzing changes in ecological patterns in heated waters). Electric power companies and all others using the environment for waste disposal have two alternatives: either to continue to increase stress on the environment and regard the resulting damage as a necessary product of civilization, or manage the environment so it serves the greatest number of beneficial uses. If we don't choose the second of these, we may soon have no choices at all. If we agree to manage the environment we must set up institutions to coordinate and implement the complex decisions on environmental uses and safeguards which must be made.'

In terms of the studies reported here with regard to the sensitivity to growth and reproduction of the snails studied, the degree of change the animals can tolerate is not great. Any change that would add as much as 10 C^{o} to the normal heat budgets these snails tolerate could be disastrous to the fauna. There are those who would argue that the added heat in the environment might be temporary and adjustments could be made later. Unfortunately, most natural conditions were established only over long periods of time, and significant changes in the number and composition of a fauna usually cannot be 'fixed up' regardless of how well the promise is intended.

Another factor in the data obtained by stressing these snails relates to the fact that in warmer water the animals invariably grow better and appear to be huskier specimens. From such observations it would seem reasonable to argue that adding heat to the habitat obviously produces more robust specimens. Unfortunately, what calipers show in larger size is countermanded by the accompanying failure by these same animals to produce offspring. The level, as shown previously, varies definitely with the groups studied so that lymnaeids tolerate warm conditions poorly, planorbids show a preference for warm water, and the physids seem most tolerant with a tendency to do better toward the cooler side.

An aspect of change that might bear watching relates to the shifts that can and often do take place when environmental conditions are altered. Many of the current problems in regions of the world plagued with human blood fluke (schistosomiasis) are directly related to discouraging and eliminating the appearance of the intermediate host snails which, for two of the most serious schistosomes, are planorbid snails. The *Biomphalaria* found as a fossil in Kansas (members of the group that make *Schistosoma mansoni* a serious disease in Puerto Rico, in other islands of the Greater Antilles and in South America) might get established in the United States if more semi-tropical conditions were available to those intermediate host snails. In this connection, there is also ample evidence—but not enough good data—to indicate important shifts in faunal assemblages at sites where temperature, eutrophication, industrial pollutants, etc. have changed the habitat conditions. In those situations the less tolerant species disappear and some of the more resistant ones remain and may even multiply. Such changes are well documented in places where *Physa* became a nuisance in clogging sieves, gumming up intake pipes, etc. The data given for *Physa* as compared with those obtained for *Lymnaea* and *Helisoma*, even in this one factor of temperature tolerance, shows that its widespread occurrence is directly related to its wider range of tolerance.

A further aspect relating to Public Health interests may be cited in connection with maintaining snails in culture; some intensive studies have already been undertaken with the genus *Pomatiopsis* in Michigan and its close oriental relative, the genus *Oncomelania*, that serves as host for Oriental Blood Fluke *(Schistosome japonicum)*. Responses of four *Oncomelania (nosophora, formosana, hupensis* and *quadrasi)* and two *Pomatiopsis (cincinnatiensis* and *lapidaria)* were measured both for their tolerances and their movements by H. van der Schalie and Lowell L. Getz (1963). Their observations (1963: 82) were summarized, as follows: when subjected to a temperature gradient ranging from 6°C to 36°C, the mean temperatures preferred by the 6 species tested varied from 21°C for *P. cincinnatiensis* and *O. hupensis* to 26°C for *O. quadrasi*. The range of selection was between 12°C and 30°C.

Movement was studied in relation to temperature (at 5, 10, 15, 20, 25, 30 and 35°C). In all species except one, little movement occurred before 20°C was reached; most movement occurred at 25°C. The exception, *P. cincinnatiensis*, displayed considerable movement at temperatures as low as 10°C and it was still very active at 30°C and 35°C. This adaptation is a necessity for a snail subject to wide extremes of temperature in the late fall when it must find its way to the top of the river bank where it hibernates.

These studies of growth and reproduction produced what may in the aggregate appear to be a confusing amount of temperature information. It would, perhaps, be useful to indicate for each of the species tested our results in terms of the optimum temperature for growth and survival, as well as for highest egg production. Such information could provide quick reference in situations, for example, where changes are suddenly brought about in nature by various development projects. It is summarized as follows:

FAMILY	GENUS AND SPECIES	OPTIMAL TEMPERATURES GROWTH AND SURVIVAL	EGG-LAYING
Lymnaeidae	*Lymnaea stagnalis*	20°C	22°C
	Lymnaea emarginata	18°	24°
Planorbidae	*Helisoma trivolvis*	28°	30°
	Helisoma anceps	26°	26°
	Helisoma campanulatum	24°	26°
Physidae	*Physa gyrina*	26°	20°
Amnicolidae	*Amnicola limosa*	24°	18°

SECTION VI. ACKNOWLEDGEMENTS

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NOTE. THE FOLLOWING PERTINENT AND TIMELY ARTICLE APPEARED AFTER THIS REPORT WAS PREPARED. THIS ARTICLE AND ITS REFERENCES WOULD BE INDISPENSABLE TO ANYONE INTERESTED IN PROBLEMS RELATING TO HEAT AND WASTE WATER DISCHARGES.

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