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# IMPORTANCE OF LABORATORY-RAISED SNAILS IN HELMINTHOLOGY WITH LIFE HISTORY NOTES ON *GYRAULUS PARVUS**

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## INTRODUCTION

FROM the standpoint of a parasitologist, snails are of considerable importance. The parasitologist is vitally interested in classification, anatomy, physiology, ecology, and food relations of these invertebrates since they play a very important part in the life history of trematodes and probably other parasites. The unique relationship of the snails to trematodes is, no doubt, unparalleled in the animal kingdom.

The food of snails and their food habits may be important in various ways, as shown in the following examples. It is known that the eggs of some kinds of trematodes must be eaten by snails before the eggs will hatch. Joyeux (1929) found that *Lymnaea vulgaris* and *L. palustris* acquire the cysticercoids of *Hymenolepis collaris* by eating cyclops and are capable of transferring the larval stages to the duck, the final host. This suggests the possibility of snails serving as transfer agents for other cestodes and even nematodes.

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Bishop and Hart (publication in press) find that larvae and pupae of the mosquito, *Aedes dorsalis,* are consumed by *Lymnaea palustris* (Müller) and that these snails are an important factor in keeping down the number of mosquitoes.

Life history studies are rapidly occupying the attention of the helminthologists throughout the world, and this phase will soon be on a par with taxonomic investigations in the group. The snail is almost indispensable in life history work on trematodes.

## Importance of Snails in Helminthology

A snail in almost all the life histories of digenetic trematodes plays the part of the first intermediate host in which larval stages are found. In order to do accurate work on the larval phases it is necessary in many cases to have snails of various ages which are free from infection. A dearth of information on the larval stages involving the snail as a host is apparent in the life history work which has been done on trematodes. I dare say, that this situation is owing to the lack of information which exists in the field of conchology and to the lack of patience and time on the part of the helminthologist.

Before leaving the subject of the importance of the snail it must be mentioned that there is a definite economic consideration in this connection. The writer is prompted to add these few statements after reading a paragraph on "Economic Importance of Gastropods" (Baker, 1928). Baker states that snails are not of direct economic significance. He discusses, however, their importance as food for fish. Snails are important not only in this connection, but many of the fish living in bodies of water harboring snails are heavily parasitized with larvae, juveniles, and adults of trematode parasites. The juveniles and adults are usually of minor importance in fish as well as in other vertebrate groups. The larvae, metacercariae, resulting from the metamorphosis of cercariae received from a snail by a second intermediate host often are of economic importance. This is, perhaps, more true

of fishes, as second intermediate hosts, than of any other vertebrate group.

The cercariae usually gain entrance into the fish by penetrating the covering tissues, and then migrate to the part of the body where they normally continue their growth. The number which penetrates in some cases is so great that the pigmented cysts of the parasites may actually change the color of the fish, and when the fish carries such numbers they have a direct bearing on the growth of the host. The flesh of some kinds of fresh water game fish is so well permeated with large encysted larval trematodes that people refuse to eat them, designating them as "wormy" or "grubby." Control measures for this condition have yet not been devised, and in some lakes the existing condition is actually one of major importance. Heart, liver, and kidneys of fish are sometimes so heavily parasitized with metacercariae as to cause death. Heavily parasitized fish are believed to be so weakened that they are unable to resist changes in environmental conditions which are ordinarily withstood by those less heavily infested.

Blochmann (1910) has actually proved that the penetration of a few cercariae of certain kinds into a small fish will kill it very quickly. Just how extensive and important this is as a cause of death is still an open problem. Szidat (1927) is of the opinion that the penetration by cercariae may be responsible for the killing of large numbers of fish in the Kurisches Haff, a great area of shallow water in East Prussia.

The problem of the importance of the snail in human disease in tropical and semi-tropical countries is one of long standing. It is so well known that it need not be mentioned here. The reader is referred to *Human Helminthology* by Faust for information concerning this subject.

## Rearing *Gyraulus parvus* (Say)

The full grown snails can be kept in a number of different ways, all of which are equally successful. For the study of individuals they can best be kept in two-ounce shell vials.

If necessary, four or five can be kept in the same vial. Their habit of climbing out of the water and drying up is the greatest difficulty which has been encountered with them. For a long time it was thought that some adverse condition of the water was responsible for their behavior but after experimentation it was found that the food supply was a more general cause. These little snails will not feed on fresh lettuce but thrive and eat greedily of decayed lettuce. They are even selective in the matter of decayed lettuce, preferring the deep green decay which results from those leaves containing large quantities of chlorophyll.

In preparing a vial it is filled to within an inch of the top with water, then with a pair of forceps the snail and a piece of decayed lettuce the size of a penny are added. The vial with the snail can then be put on a shelf and left for two days without attention. It is possible that the snail will live until the water has all evaporated, deriving its nourishment, after exhausting the supply of lettuce, from a growth of fungus and bacteria which collects on the sides of the container. A growth of this white material one-fourth of an inch thick and covering the entire shell has been seen at times on the snails kept under such conditions. At times this growth does no harm, but it is not always innocuous and for that reason it is better by far to care for the snails every day. This is a simple procedure. The vial should be held under a tap. The running water will cause the snail to retract, release it attachment, and drop to the bottom. The lettuce and waste from the snail will be stirred up and if poured out immediately and carefully, everything but the snail will be removed. If filled with water, and lettuce is added, the vial needs no attention for another day. These snails can be kept collectively in the same way with equal success in evaporating dishes except that it is advisable to cover the dish with a glass plate. The same method of cleaning can be used. The size of the container does not seem to make any difference; the ones used were eight inches in diameter and three inches high. They were filled

slightly more than half full of water. There are other ways of handling the snails, but the methods described have given excellent results.

## Enemies

When the snails are kept collectively in evaporating dishes there is a tendency to neglect them by adding food only when necessary. This has been done several times with dishes each containing five hundred snails. Curiosity was aroused when after four or five days the shells of the majority of the snails in the dish were found to be clean and empty. After a careful examination a small leech, very nearly transparent, and inconspicuous when flattened out against the container, was suspected of doing the damage, and was watched. The manipulation of the next victim by the leech was quite remarkable. Its technic consisted of picking up the snail, wrapping itself around it, and devouring the soft parts completely. This incident prompted a very careful examination of future snail collections. Incidentally, this was a method of obtaining perfectly clean shells of all sizes. In doing some experimental work with dragonflies, genus *Sympetrum,* a rather unusual enemy of the snail was discovered. It was found that the nymphs ate the snails greedily. They would catch the walking snails and eat the foot, but the part in the shell they were unable to reach.

## Eggs

Eggs were obtained from snails which were kept in the laboratory all winter at a room temperature of 60° F. In February some of the snails were taken to a room of 70° F. The snails laid eggs while kept at either temperature. Laying began the middle of February and was continued until the middle of March. The snails lay eggs over a period of a few days, and each snail produces a number of egg masses. All eggs on which records were kept were deposited during the night. Whether they lay eggs during the day is yet to be determined. The egg masses never contain more than four

eggs, masses of two or three are the most common but those of one or four are also found. The snails are not discriminating in the place of deposit of eggs for they are found on the sides of the container, on the snail excrement, on the decaying lettuce, and even on the shell of the living snail. A linear arrangement of the eggs in masses of three or four has not been observed. The egg case consists of an almost transparent gelatinous enveloping layer with a thickness of about one-tenth of a millimeter where it is in contact with the vial. The size of the cases can best be determined by examining the figures. The egg capsules are usually in contact with each other and may be pressed so closely together as to produce straight sides. The eggs are tiny white specks in the capsules, and not more than one egg has ever been found in a capsule. Under the microscope they look opaque and granular. They average 135 μ in diamater and tend to be round. In the limpet, *Ancylus fuscus*, Clapp (1921) found one capsule with five eggs. According to this author one of the five underwent normal development for a time but died before hatching.

After a number of egg masses had been deposited in the vials the snails were removed without disturbing the eggs. The water was not changed and the eggs were allowed to hatch. Before the eggs hatched a small amount of fungus and bacterial growth accumulated in the vials.

## Early Development

The embryonic development of *G. parvus* is exceedingly rapid. Ciliated embryos more than twice as large as the egg and somewhat elongated can be seen rotating in the capsules on the third day. Data were taken on a large series of eggs kept in the laboratory at room temperature, and the majority of snails escaped from the egg cases on the seventh day, none required more than eight days, and some hatched on the sixth day. The rapidity of early development seems to be very much like that of the limpet *A. fuscus*, since Clapp (1921) saw rotating embryos on the fourth day. He says, however,

that the limpets did not escape from the capsule until the seventeenth or eighteenth day, which makes the embryonic period for the limpet almost three times that of *G. parvus*.

## Rearing

The shell of the snail at the time of hatching is not more than one-half millimeter in diameter. The snails crawl up the sides of the container where they can be seen as little white specks. In order to raise these small snails without difficulty and with only occasional attention, cultures resembling small ponds were prepared. Moist chambers, such as have been described, were filled to within an inch of the top with water, a handful of pebbles was piled on one side, and two dead maple leaves (other kinds of leaves could, no doubt, be used) were placed in the dish. The snails were added at the same time. The dish was kept covered.

In handling these fragile little snails it is best to use a dropper. If possible it is better to make one's own droppers in order to have them with openings of suitable size and thickness of wall at the tip end. In collecting the snails the receptacle is held up to the light, the dropper with the bulb compressed is inserted into it, and the snails are forced from the side of the container with the tip of the pipette. As soon as the snails are released the bulb is slightly relaxed and the snails are drawn into the dropper. With a little practice one can recover twenty or thirty, one after another without removing the pipette. In transferring the snails they are set free just under the surface of the water above the dead leaves so that they drop upon the leaves which form their immediate food supply. These small snails will not eat fresh lettuce. They thrive on dead leaves, and the amount they eat as evinced by the excrement is astonishing. They are perfect skeletonizers; the leaf pulp is entirely removed but the vascular bundles, veins, and veinlets remain intact so that a perfect skeletonized leaf is left. After a period of growth of about six or seven days they change their food habits somewhat and take some decaying lettuce. This food should be added in

small amounts when necessary. If fed on decaying lettuce when first hatched they eat it for a day or so and then crawl out of the water and die.

In a few days fifty or sixty snails in a container produce a large amount of excrement, and it is necessary to remove this at least weekly. It is best removed with a wide-mouthed pipette having a large bulb. By running the mouth of the pipette over the bottom of the dish the waste can be drawn into the opening and be removed. The few snails crawling on the bottom will "hug" tightly and resist moderate suction of the bulb. The leaves to which most of the snails are attached can be shifted with a pair of forceps without detaching the snails. After the excrement has been removed the amount of water in the dish should be replenished. The water in the dish may become the color of weak coffee owing to the leaves, but it should not be replaced by fresh water as long as it is clear. Sometimes the water is cloudy after a fresh culture is prepared, but it usually clears up in a day or two.

Sometimes in the course of several days the leaves become covered with a thick, slimy, whitish growth which the snails will not eat. In this event it is best to prepare another culture. The snails should then be removed from the old container to a watch glass, washed in fresh water several times, and transferred to the new culture.

## Growth

The growth of the snails is exceedingly rapid; in from fifteen to seventeen days they are usually half-grown, and fully grown in from four to five weeks. The older the snails the greater is the variation in size. In the culture there are generally found some individuals showing no growth and some which are exceptionally large, with the majority intermediate. In two weeks after they have completed their growth they produce eggs.

If it is desired, the snails may be removed to shell vials after they are half grown and fed on decayed lettuce. Dead leaves are not recommended for the vials because they

usually become covered with a heavy growth, such as has been already described, which starves the snails.

### General Considerations

Compressed air should not be bubbled into the water containing the snails. Pond water should not be used since one invariably introduces algae or fungi which sooner or later destroy all the snails. It has been impossible to keep the young or grown snails in numbers any length of time in water containing algae. Tap water has been found to be better than any other. Pond water, tap water, filtered tap water, and cistern water have been tried.

The tap water in Ann Arbor is not chlorinated, and the analysis of the water used in this work was as follows: pH 7.2, oxygen less than 1 part per million, free carbon dioxide 15 parts per million, phenolphthalein alkalinity none, methyl orange alkalinity 300 parts per million. It is generally thought that snails are not sensitive to water conditions. My experimental work with snails has convinced me that this is a problem which is well worth investigating. Additional evidence as to the importance of the composition of the water is found in an excellent paper by Crabb (1929) on *Lymnaea stagnalis appressa.*

These small snails are well adapted for physiological, embryological, anatomical, and other investigations since the newly hatched and young snails are very nearly transparent. The author was surprised to find that it took no more than twelve to fifteen minutes for food to pass through the digestive system of a day-old snail.

Snails differ very decidedly in their response to handling and vibration. Upon handling or upon jarring *Gyraulus parvus* retracts only temporarily, if at all, and for this reason this species is much to be preferred to other snails with which the author has had experience. These little fellows are always hungry and are always moving.

It is hoped that this work on one of our smallest North American snails may stimulate some of the younger concholo-

gists in the direction of life history work and general ecological considerations.

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REFERENCES CITED

BAKER, F. C. 1928. The Fresh Water Mollusca of Wisconsin. Wisc. Geol. and Nat. Hist. Sur., Bull. 70, pt. I, 494 p., 28 pls.

BISHOP, S. C. & R. C. HART. Notes on some natural enemies of the mosquito in Colorado. Jour. N. Y. Ent. Soc. (Accepted for publication in 1931.)

BLOCHMANN, F. 1910. Sterben von Aquarienfischen durch Einwanderung von *Cercaria fissicauda* LaVal. Centralbl. f. Bakt. I Abt., Orig., 56 (Heft 11): 47–49.

CLAPP, W. F. 1921. Eggs and young of the river limpet, *Ancylus fuscus* Adams. Occ. Papers Boston Soc. Nat. Hist., 5: 5–10, 1 pl.

CRABB, E. D. 1929. Growth of a pond snail, *Lymnaea stagnalis appressa,* as indicated by increase in shell-size. Biol. Bull. 56: 1: 41–63.

DAWSON, J. 1911. The Biology of *Physa.* Behavior Monogr. 1: 5: 12-p., 10 figs.

FAUST, E. C. 1929. Human Helminthology. Lea and Febiger, Philadelphia. Pp. i–xxii, 1–616, 297 engravings.

HUBBS, C. L. 1927. The related effects of a parasite on a fish. Jour. Parasit. 14: 75–84.

JOYEAUX, CH. 1929. Procédé pour rechercher les cysticercoides des petits crustacés. Ann. de Parasit. 7: 112–115.

SZIDAT, L. 1927. Über ein Fischsterben im Kurischen Haff und seine Ursachen. Zeitschr. f. Fisch. u. deren Hilfswiss., 25: 83–90, 5 text figs.

## Explanation of Plate

Fig. 1. Camera lucida drawing of three egg capsules in an egg case.

Fig. 2. Camera lucida drawing of four egg capsules in an egg case.

Fig. 3. Camera lucida outline of an embryo three days old in the egg capsule.

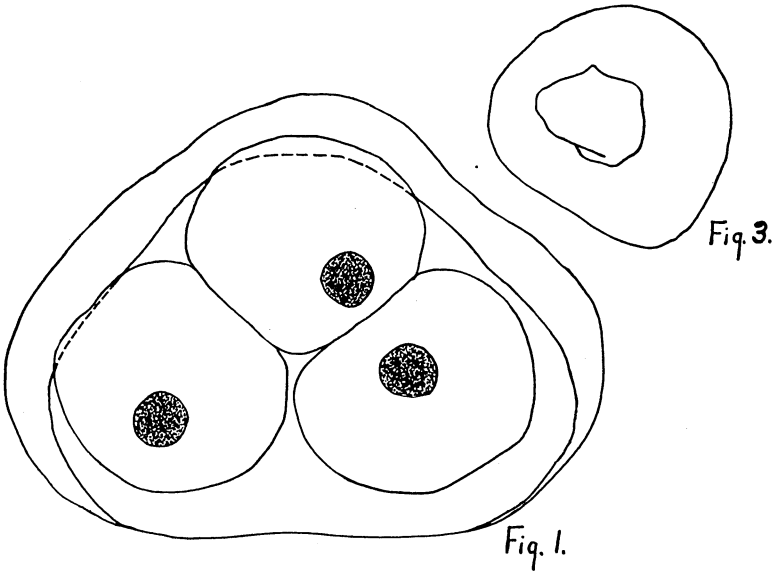
Fig. 3.
Fig. 1.

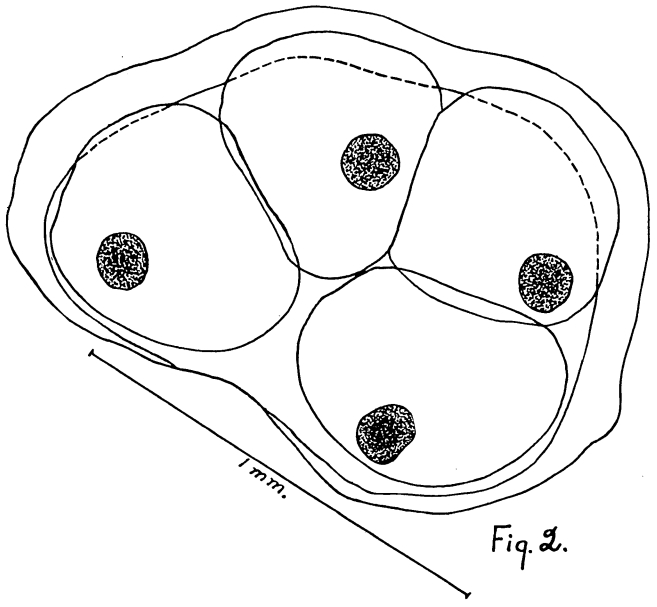
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Fig. 2.