International Journal of Engineering, Science and Mathematics
Vol. 7 Issue 4, April 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijesm.co.in, Email: ijesmj@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

EFFECT OF PARASITISM ON REPRODUCTIVE SYSTEM OF FRESHWATER SNAILS (Lymnaea and Bellamya) in MEERUT REGION

Dr. Anjula Jain

Department of Zoology, D.N. (P.G.) College, Meerut, 250002 (UP)

ABSTRACT

Snails are known to serve as an intermediate host for several species of larval trematodes. Snails were studied to understand the reduction of fecundity of the hosts which is a characteristic commonly exhibited in parasitic infections. Snails were collected during the monsoon period of July, August and September from various water bodies like ponds, pools, ditches, lake reservoirs, rivers (Garh Ganga) and crop fields in and around the Meerut region. They were collected in laboratory containers and beakers and maintained in aquariums and fed ad libitum with hydrilla. Approximately 150 snails were collected with different species like *Bellamya bengalensis, Lymnaea luteola* and *Lymnaea acuminata* in the Meerut region. The occurrence of the larval trematode parasite was found. The effect of parasitism on reproductive system of snails under study revealed that the gonadial tissue was invaded by cercariae. The damage to the gonadial tissue resulted in the lower fecundity rate of the host. The larval trematodes affect the reproductive system, the egg laying capacity, i.e., the fecundity of the host will also be affected.

Keywords- Reproductive System, Bellamya, Lymnaea, cercaria

Introduction:

It was well known that trematode infection has an inhibiting effect on molluscan reproduction. **Wesenberg,-Lund, (1931)** was first to report the invasion of the gonads of molluscan host by the larval trematodes. He reported that the ovotestis of Succinea putris Linn., was destroyed by the larvae of Leucochloridium macrostoma, but it regenerated the tissue after severe atrophy. Later on **Rees, (1936)** reported that in Patella vulgata infected with the rediae of Cercaria patella, the gonads were invaded and the ovarial germinal epithelium was reduced to a thin layer of flattened cells or was entirely consumed by the parasites. She also reported in 1936 that the rediae of Cercaria of Himasthala secunda, actually devoured the gonadial tissue of Littorina littorea and she attributed the reduction in size of the hosts external genitalia to the inhibition of the normal function of the gonad. The other notable workers in this field are **Rees, (1936); Rothschild, (1938); Cheng and Cooperman,(1964); Cheng and Burton, (1965); Lively, C.M.(1987); Hechinger, R.F. et al., (2008).**

Since the larval trematodes affect the reproductive system, the egg laying capacity i.e., the fecundity of the hosts will also be affected **Fried, B. (2001); Kube,S. et al., (2006)** reported a loose of fecundity in a population of snail caused by larval trematode. Such a

drastic reduction in the egg laying capacity of different molluscan hosts during different larval trematode infections have been reported by many authors viz., **Pesigan et al., (1958); Sluiters et al., (1980); Cooper et al., (1994); Jokela and Lively (1995).** In general, the infected snail faces a premature death due to the physiological state of starvation as a result of parasitism and the pathogenecity caused by the parasites. But **Bourns, (1974)** has reported that uninfected *Lymnaea stagnalis* died earlier than the infected snails as they lost more nutrients along with their enormous egg production.

In this investigation, the author has taken up the freshwater snail, *Lymnaea luteola* and *Bellamya bengalensis* for observing the changes in the gonads during different larval trematode infections viz., *Amphistome cercaria, Echinostome cercaria, Xiphidio cercaria* and *Furcocercus cercaria.*

Material and Method:

Fresh water snails were collected from various water reservoirs of Meerut region. Snails were collected and identified by the method suggested by **Subba Rao (1989).** The identified snails were found to be *B.bengalensis* and *L.luteola.*

The snails were removed from the shell and their gonads were removed and fixed in Bouin's fluid for 6 hours and then transferred to 70 % alcohol, dehydrate through ascending grade of alcohol, clear in xylol and then embedded in paraffin wax (M.P. 58°C) and 5mm paraffin embedded sections were made.

Estimation of protein in egg masses was done by Lowry's method (1951). Carbohydrate estimation was done by Carral's method (1956).

Result:

The gonad of *Lymnaea luteola* is composed of a large number of acini. It is enveloped by a thin layer of connective tissue within which are found pigment granules and blood vessels. Each acinus is also covered by a thin sheath *viz.*, Ancel's layer consisting of squamous epithelium and a thin network of connective tissue fibres.

The histological studies on the infected snails revealed that during light infections the larval trematodes attack the hepatopancreas and gonads. The gonads may be invaded by the parasites, either in earlier or in the later stages of infection depending on the type of infection. During heavy infection the gonadial tissues were found to be sloughed off by the rediae.

During heavy infection of Amphistome cercaria, the digestive gland is completely packed with rediae and cercariae and the tunica propria of the gland is severely ruptured. Rediae are found invading the reproductive organs of the host. But in E.cercarial infection, they were found predominantly in the ovotestis and the albumin gland than in the other regions of the body.

Rediae and cercariae were also found in the lumen of the hermaphroditic duct which is easily recognized by its possession of bud like protrusions on its surface. The columnar epithelium making up the wall of the oviduct was mostly sloughed off in the heavily infected snails.

Mechanical damage is more than physiological effects on the gonadial issue. The albumin gland, secretes a material which form the egg membranes, is a large structure composed of a large number of branching tubules which are separated by thin layers of connective tissue containing blood spaces. This gland is easily recognizable by the presence of large numbers of albumin droplets in them. Many rediac and released cercariac can be seen in the albumin gland adjacent to it since a large number of these cells did not include albumin droplets or in some poorly developed droplets. The digestive gland

sections **(Fig.-1. A, B, C)** stained with Bromophenol blue for basic proteins also showed slight stain in this gland of the infected snails. The digestive gland sections stained with **PAS (Fig.-2. A, B, C)** revealed that the infected snails have less amount of glycogen than normal.

The male reproductive system is not much affected by the parasites, but during heavy infections the number of sperm cells decreased in number. In the infected snails, though the number of sex cells is reduced, the Spermatogenesis and Oogenesis are still represented by all the stages. The decrease in the number of sex cells was affected by a transport of the sperm out of acini and by a reabsorption of the oocytes by their nurse cells. In heavy infections the gametogenesis is inhibited absolutely. Though more sperms were produced, no ova production could be seen.

	Carbohydrates ± S.D.	**Proteins ± S.D.**	**Size of the egg masses (Range) cm.**
Normal *(L.luteola)*	0.839 ± 0.081	0.233 ± 0.081	2.6-3.2
Infected with *E.Cercaria*	0.325 ± 0.028	0.141 ± 0.023	1.6-2.4
Normal *(B.bengalensis)*	0.872 ± 0.127	0.258 ± 0.026	2.5-3.4
Infected with *Amphistome cercaria*	0.347 ± 0.042	0.151 ± 0.028	1.7-2.6
X.cercaria	0.361 ± 0.061	0.176 ± 0.031	1.9-2.4
F.cercaria	0.378 ± 0.057	0.164 ± 0.013	1.9-2.5

Table: The levels of carbohydrates, proteins and the size of the egg masses of L.luteola during different larval trematode infections. Values of carbohydrates and proteins are expressed in gm% of the wet tissue weight Mean ± S.D. of 20 estimations. The size of the egg masses expressed in cm.

The data on the egg production i.e., fecundity of the snails during different larval trematode infections have been presented in the form of table. The controls show a continuous oviposition varying from 65-101 eggs per snail/week. During *E.cercaria*l infection there was not much decline in the egg production during the first four weeks of post miracidial exposure. From the fifth week onwards, the egg production showed two-three fold decrease and laid no eggs after 9th week. In *L. luteola* infected with *A. Cercaria* and *X.cercari*a also the egg production showed a three fold decrease. In Furcocercus

cercarial infection, though there was a decrease it was not that acute as in the case of other two infections studied. The results are based on the snails collected from natural habitat, the exact time after initial miracidial infection and the intensity of infection (with how many miracidia they are infected) are not known. The results show that the snails are heavily infected with *A .cercaria, X.cercaria* and *F.cercaria,* hence the egg laying capacity during these infections was minimum. These results were compared with the experimentally infected L.luteola with the miracidia of Echinostoma revolutum.

The results of the chemical analysis of the egg masses are presented in the **(Table).** The results show that the eggs of normal snails have more amount of carbohydrates and proteins ($P<0.001$).

The Oocytes of normal snails showed strong acid and alkaline phosphatase activity. But no difference was observed in the enzyme activity in the oocytes of infected snails.

Discussion:

The histological studies revealed that during hyper infections of *E. cercariae* and *A. cercaria* the reproductive system of *L.luteola* was invaded by the rediac and cercariae. Though the actual process of invasion was not observed, the heavy concentration of rediac and cercariae in the ovotestis and in the albumin gland strongly suggests that these two are the main organs of the reproductive system affected by parasitism. The albumin gland is usually the first part of the reproductive system to be invaded by the parasites, may be due to the storage of protein content in them. In the infected snails it has been observed that the protein content of this gland is depleted. Since the tunica propria of ovotestis and albumin gland, the ancel's layer of acini are ruptured the rediae might have penetrated into these organs from the hepatopancreas. From the results it is also clear that the E.cercaria and A. cercaria favour the female reproductive system than the male reproductive system. This may be due to the different physiological conditions of the male and female tracts like pH etc. **Cheng and Cooperman, (1964)** reported that the male tract may not be compatible to the parasites as in the case of Helisoma trivolvis infected with Glypthelmins pennsylvaniensis. The drop in the number of sperm may be due to the destruction caused to the germinal epithelium of the acini and this causes a decrease in the sperm production.

In *L.luteola* the shape of the acini is more irregular when compared with that of members of Planorbidae. In *E.cercarial, A.cercarial* and *X. cercarial* infections, the acini are found to be occupied completely by the parasites. The granules of parasite excreta were also visible. The degenerating oocytes which are light yellow in colour could be seen on the walls of the cach acini. This condition was similar to that found in *Lymnaca stagnalis* under the influence of radiation and starvation, where the oocytes and sperm were reabsorbed by the sertoli cells and nurse cells respectively **Joosse et al., (1986).** This shows that the physiological state of the snails during starvation is almost same as that of the physiological state of the infected snails. Most of the damage caused was primarily mechanical, resulted by the pressure exerted by the parasites and the 'autophagy' by the rediae. But the damage done to the albumin gland is physiological too, since the protein content is found to be depleted in the droplets.

During *E. cercarial* infection, the gonads of *Lymnaea* are not affected. These cercariae develop from sporocysts and do not have rediae in their life cycle. The pathological effects on the hepatopancreas, during infection are comparatively less. Though it has been reported by many workers (ref. cited) that rediae invade the gonadial tissue, sporocysts are also reported to invade the gonads, **Cheng and Cooperman, (1964).** Thus the invasion of gonads by the sporocysts of *X.cercariae* is not a surprising phenomenon.

The drop in the egg production or fecundity of the snails during parasitism is due to invasion of the gonadial tissues. During *F.cercarial* infection, though the gonads are not affected by parasitism, the physiological state of starvation resulted due to parasitism has affected the oviposition.

Along with the depletion of the egg production, the size of the egg masses have also decreased. The number of eggs laid by the infected snails showed a decreasing trend as the time progressed which was due to the increasing pathogenicity occurring in gonads and non-availability of sufficient nutrients for the developing oocytes. **Najarian, (1961)** found that the egg laying capacity of *Lymnaea luteola* infected with *Schistosoma haematobium* showed a three fold reduction in the mean eggs laid per clutch, more than a two fold reduction in the mean clutches laid per snail per day and an eight fold decrease in the mean eggs laid per day compared with control snails. Genetic heterogenecity can also be attributed as one of the factors responsible for the fluctuations among the individual means of the eggs per cluster in the normal *Lymnaea*. In the infected snails, the variation in the egg out put primarily depends on the type of infection and secondarily on the intensity of infection. In light infections, when the self cure of the host is possible it may start oviposition within a short time, after losing infection as in the case of starved Lymnaea stagnalis **Joose et al., (1968)**. The results reveal that the eggs of normal snails have more amount of carbohydrates and proteins ($P>0.001$). As the number of eggs laid by the infected snails are few, the nutrient drain was also less. Previous studies by other authors, **Bourns, (1974)** have shown that the nutrient drain in the infected snails was not only limited to the egg masses but also occur along with emerged cercaria.

Though the nutrient drain of the eggs of normal snail is more than that of infected snail, it is not harmful for the host to lead, for its premature death, since it has plenty of reserve food material. It has been observed under laboratory conditions that the fresh water snails, *L.luteola* infected with larval trematodes died much earlier than the normals. During ovarial infection, the host died, after 10 weeks of miracidial infection. The naturally infected L.luteola died after 8 *(A.cercaria)* and 9 weeks *(X. cercaria, F. cercaria).*

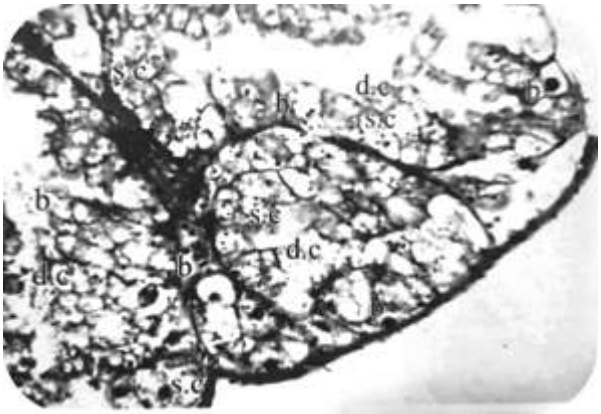
The author's results do not agree with the results of **Bourns, (1974)** in which he reported that the normal snails died earlier than the infected snails. In the infected *L. luteola* though the nutrient drain was comparatively low, the overall effect, i.e. including that of larval trematodes has a profound effect on the host to lead to its premature death.

In addition to the depletion in egg production, the viability of these eggs of the infected snails has been reported to be very low by **Pesigan et al., (1958)** and aberrations such as polyembryony by **Etges and Gresso, (1965)** will occur. In the present study, since the nutrients present in the infected eggs are lower than the normal eggs, it may lead to inhibition of normal development of these eggs. Polyembryony has not been observed in the present study,

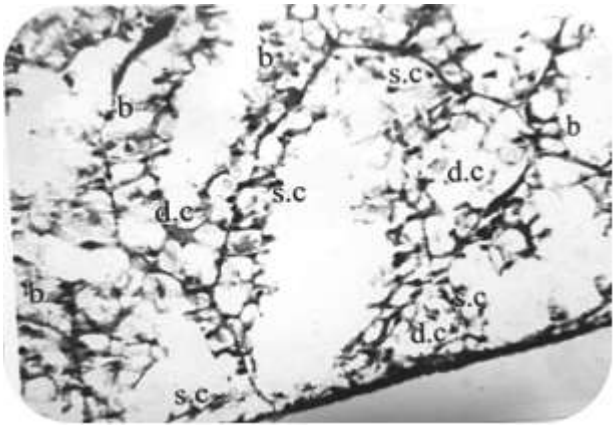
The differential nutrient drain presented here in might account for some of the differences in the viability of eggs and longivity exhibited by the two groups of snails. It can be concluded that the parasitic (larval trematodes) castration inhibits the oviposition of *Lymnaca luteola,* **Hechinger, R.F.et al., (2008).**

Fig. 1. Bromophenol blue preparations of T.S. of the digestive gland of normal uninfected snails and infected snails

(A) T.S. of the digestive gland revealing deeply stained moderately coarse protein granules in the digestive cells (d.c.) of uninfected snails These inclusions appear in the secretory cells (s.c.) as strongly coloured fine granules. In the secretory bodies (b), these components did not exhibit any reaction with mercuric bromophenol blue.

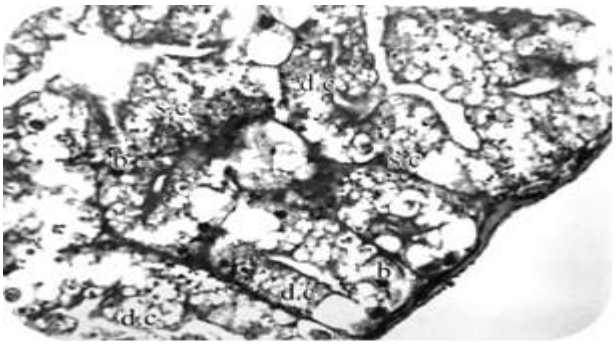


(B) Moderately stained protein inclusions in both digestive cells (d.c.) and secretory cells (s.c.) of a snail infected with cercariae but they have moderately coarse sizes in the digestive cells and fine granulations in the secretory cells.

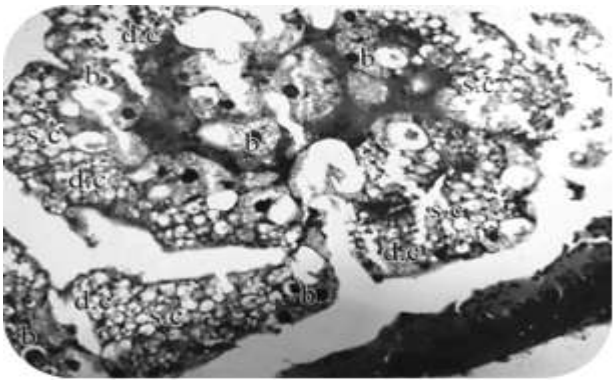


(C) Heavily infected snails protein particles exhibit a faint colouration in both the digestive cells (d.c.) and secretory cells (s.c.)

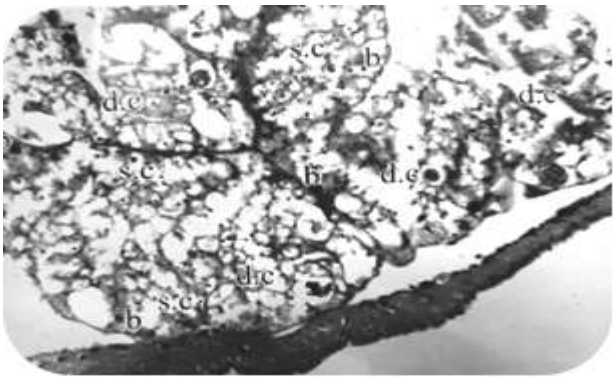
Fig. 2. Periodic Acid Schiff's (PAS) preparations of the TS. of digestive gland of normal snail and infected snail.



(A) Deeply stained coarse carbohydrate granules in the digestive cells (d.c.) of normally feeding snails. In the secretory cells (s.c.) these elements appear as strongly coloured fine particles. The secretory bodies (b) exhibit a strong reaction with PAS.



(B) In infected snails, strongly PAS positive carbohydrates content have occurred as coarse granules in the apical parts of the digestive cells (d.c.). The basal regions are crowded with moderately coloured fine particles. Moderately stained fine carbohydrate granules a scattered homogeously in the secretory cells (s.c.).



(C) In the digestive cells (d.c.) of snails heavily infected with cercarie carbohydrates are visualised as weakly stained fine particles hing in the apical portions, whereas they are moderately coarse in the basal regions. The secretory cells (s.c.) are occupied with weakly PAS reactive fine granules.

Conclusion:

The effect of parasitism on reproductive system of snails under study revealed that the gonadial tissue was invaded by cercariae. During light infections, the larval trematodes attack the hepatopancreas and gonads. The gonads may be invaded by the parasites, either in earlier or in the later stages of infection depending on the type of infection. During heavy infection, the gonadial tissues were found to be sloughed off by the rediae. The damage to the gonadial tissue resulted in the lower fecundity rate of the host. The larval trematodes affect the reproductive system, the egg laying capacity, i.e., the fecundity of the host will also be affected.

References:

[1] Rees, W. J., (1936). The effect of parasitism by larval trematodes on the tissues of Littorina littorea (L). Proceedings of the Zoological Society of London 2, 357–68
[2] Wesenberg-Lund, C. (1931). Contributions to the development of Trematoda Digenea. Part I. The biology of *Leucochloridium paradoxum*. *D. Kgl. Danske Selsk. Skrifter, Nauturvidensk og Mathem*. Afd. 9, Række 4: 90– 142.
[3] Rothschild, M. I. R. I. A. M. (1938). Further observations on the effect of trematode parasites on Peringia ulvae (Pennant) 1777. *Novitates zoologicae*, *41*(2), 84-102.
[4] Cheng, T. C., & Cooperman, J. S. (1964). Studies on host-parasite relationships between larval trematodes and their hosts. V. The invasion of the reproductive system of Helisoma trivolvis by the sporocysts and cercariae of Glypthelmins pennsylvaniensis. *Transactions of the American Microscopical Society*, *83*(1), 12-23.
[5] Cheng, T. C., & Burton, R. W. (1965). Relationships betweenBucephalus sp. andCrassostrea virginica: Histopathology and sites of infection. *Chesapeake Science*, *6*(1), 3-16.
[6] Lively, C. M. (1987). Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature*, *328*(6130), 519-521.
[7] Hechinger, R. F., & Lafferty, K. D. (2005). Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1567), 1059-1066.
[8] Fried, B., Muller, E. E., Broadway, A., & Sherma, J. (2001). Effects of diet on the development of Schistosoma mansoni in Biomphalaria glabrata and on the neutral lipid content of the digestive gland–gonad complex of the snail. *Journal of Parasitology*, *87*(1), 223-225.
[9] Kube, S., Kube, J., & Bick, A. (2006). A loss of fecundity in a population of mudsnails Hydrobia ventrosa caused by larval trematodes does not measurably affect host population equilibrium level. *Parasitology*, *132*(5), 725-732.
[10] Pesigan, T. P., & Hairston, N. G. (1961). The effect of snail control on the prevalence of Schistosoma japonicum infection in the Philippines. *Bulletin of the World Health Organization*, *25*(4-5), 479.
[11] Sluiters, J. F., Brussaard-Wüst, C. M., & Meuleman, E. A. (1980). The relationship between miracidial dose, production of cercariae, and reproductive activity of the host in the combinationTrichobilharzia ocellata andLymnaea stagnalis. *Zeitschrift für Parasitenkunde*, *63*(1), 13-26.
[12] Cooper, L. A., Richards, C. S., Lewis, F. A., & Minchella, D. J. (1994). Schistosoma mansoni-relationship between low fecundity and reduced susceptibility to parasite infection in the snail Biomphalaria glabrata. *Experimental Parasitology*, *79*(1), 21-28.
[13] Jokela, J., & Lively, C. M. (1995). Parasites, sex, and early reproduction in a mixed population of freshwater snails. *Evolution*, 1268-1271.

[14] Bourns, T.K.R.: Carbohydrate and protein in*Lymnaea stagnalis* eggs and*Trichobilharzia ocellata* cercariae. J. Parasitol.**60**, 1046–1047 (1974)
[15] Subba Rao, N.V., 1989. Handbook of freshwater molluscs of India, Zoological Survey of India, Calcutta, pp 1-289.
[16] Lowry O.H., Rosebrough N., Farr A.L. and Randall (1951), Protiuen measurement with folin phenol reagent J. Bio. Chem.(193)pp165-285.
[17] Joosse, J., & Van Elk, R. (1986). Trichobilharzia ocellata: physiological characterization of giant growth, glycogen depletion, and absence of reproductive activity in the intermediate snail host, Lymnaea stagnalis. *Experimental parasitology*, *62*(1), 1-13.
[18] Najarian, H. H. (1961). Egg-laying capacity of the snail Bulimis truncaîus in relation to infection with Schistosoma haematobium. *Texas Reports on Biology and Medicine*, *19*(2).
[19] Etges, F. J., & Gresso, W. (1965). Effect of Schistosoma mansoni infection upon fecundity in Australorbis glabratus. *The Journal of Parasitology*, 757-760.
[20] Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-Macedo, L., Boch, C. A., ... & Lafferty, K. D. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, *454*(7203), 515-518.