CHAPTER 4
IMPACT OF TREMATODE INFECTION ON REPRODUCTION

INTRODUCTION

The functional anatomy and histology of reproductive system of gastropods has been studied by number of workers. Hubendick (1945) and Harry and Hubendick (1964) studied the functional anatomy and histology of *Lymnaea limosa*. Hoff (1940) worked out the anatomy of the snail, *Ferrissiatarda*. Duncan (1960) has made a comparative study of the reproductive systems of four families of Basommatophora: Physidae (*Physafontinalis*), Lymnaeidae (*Lymnaea peregra*), Planorbidae (*Planorbariuscorneus*) and Ancylidae (*Ancylusfluviatilis*).

Wealth of information is available on the reproductive system and reproductive cycle of pulmonates. In the snail *Biomphalariaglabrata* (De Jong Brink, 1969), *Lymnaea stagnalis* (Plesch et al. 1971) and *Bulinustruncatus* (De Jong Brink et al. 1979), it has been observed that the reproductive tract could be differentiated into hermaphrodite part, including gonad (ovotestis), hermaphrodite (ovotesticular) duct and male and female reproductive tracts. These studies reveal that depending upon the nature of their secretory products, 9 different cell type in the female part and 7 in the male component were identified in the snail *Biomphalariaglabrata*, 11 different cell types in the female part and 7 in the male part of *L. stagnalis* and in *B. truncatus* 13 different cell types in the female part and 12 in the male part could be identified. There is some correlation with concentration of histochemicals to the secretory activity of these cell types (Sindou 1991).

With the advent of interest of the biologist in the role of molluscs as hosts of helminthes parasites beginning with Swammerdam (1737), the
importance of molluscs as carriers of pathogenic parasites has been gradually recognized. Fascioliasis is a worldwide zoonotic disease (WHO, 2006; Bargues et al. 2007 and Mas-Coma et al. 2007) caused by the liver fluke *Fasciola hepatica* and *Fasciola gigantica* (Mas-Coma et al. 2005). Fascioliasis caused high economic losses in the animal husbandry industry (Mas-Coma et al. 2009). Human fascioliasis has been reported in 51 different countries from continents (Esteban et al. 1998). An effective method to reduce the incidence of fascioliasis is to control the population of vector snails, thereby breaks the life cycles of these flukes or by reducing the reproductive capacity of snails (Agrawal and Singh, 1988; Singh and Singh 2009 and Jigyasu and Singh, 2010). Earlier investigation have shown that the reproductive capacity of snails vary from one season to other (Jigyasu and Singh, 2010; Maat et al. 1983 and Wayne, 2001).

It is a well-established fact that the density of conspecific individuals in the environment is a critical ecological factor that can affect growth, survivorship and fecundity of individual and the consequent dynamics of populations (Thomas and Benjamin 1974; Brown et al. 2002, and Lande et al., 2002). Such factors become importance for controlling host parasite system where the presence and the magnitude of population regulatory factors are crucial for disease dynamics and the design of effective long term control strategies (Feng and Milner, 2002).

Schistosomiasis is a parasitic disease caused by digenetic trematodes, belonging to the Schistosomatidae family. It is currently endemic in 74 developing countries, infecting an estimated 200 million peoples (Vennervald and Dunne, 2004 and Taylor and Karim, 2003). Schistosomiasis caused by *Schistosomamansoni* is a major public health problem and repeated exposure can lead to liver damage and anemia,
particularly in children (Taylor and Karim 2003). It is primarily found in rural areas in tropical and sub-tropical countries and infects humans and other vertebrates using representative of the family Planorbidae in particular. Fortunately, till to date there is no report of having any single case of Schistosomiasis from India.

The observation of Wesenberg-Lund (1934) on the exceptional size served by parasitized specimens of certain species of *Lymnaea* much attention has been paid to gigantism and the inhibition of reproduction in snails infected by the trematode larvae. Wright (1966) and Cheng (1967) have recently reviewed the literature on the pathogenesis of helminths in molluscs. Hodasi (1972) found that the ovotestis of infected snails degenerated and atrophied even though this organ was rarely invaded by the parasites. However, very few attempts have been made specifically on the potential role of parasites in the evolution of host life histories (Minchella, 1985 and Hochberg et al., 1992). Parasites could be important because several of them are known to influence key life history trait such as survival, growth and fertility (Minchella, 1985 and Jaenike, 1992).

Minchella (1985) while studying molluscs trematode associations, considered two possibilities: fecundity compensation and gigantism. Adult snails infected with schistosome have been shown to partially compensate for future parasite-induced fertility losses by an increase in egg production following parasites exposure (Minchella and LoVerde 1981). Molluscs infected with trematodes may also show either increases or decreased growth rates compared to uninfected individuals and their fecundity partially or completely inhibited by the parasites (Minchella, 1985). Ballabeni (1995) observed parasite induced gigantism in the hermaphrodite *L. peregra*, as a host adaptation to the trematode parasitic infection. So called ‘gigantism’ has been considered either an non-adaptive side effect of infections (Sluiters, 1981 and Souza, 1983) or an
adaptation benefiting the parasite, because it may enhance host survival and consequently prolong the duration of parasite reproduction (Baudoin, 1975).

Many relationships are compatible in the sense that, both the host snail and the larval trematode successfully perpetuate their species, but an individual infected snail may suffer marked pathological effects (Wright, 1966 and Cheng, 1967). These effects may not directly cause death and indeed infected snail may live longer and grows faster than uninfected ones in laboratory but under natural conditions it is possible that infected snails have a lowered tolerance of adverse environmental conditions. Even though infected snails can survive extensive damage to their tissue, their reproductive capacity is often completely or partly destroyed. For example, 2 or 3 weeks after infection with *F. hepatica* egg production by *L. truncatula* ceased entirely and was not resumed (Hodasi, 1972). The total egg production of snails infected at two weeks was depressed to a more 3% of that achieved by uninfected snails during their life time. Hodasi (1972) found that the ovotestis of infected snails degenerated and atrophied even though this organ was rarely invaded by the parasite.

Once the digenean miracidia penetrated into the tissue of a compatible snail host triggers a series of events, the parasite chemically manipulate the snail host's neuroendocrine system to divert resources towards development and reproduction by polyembryony (De Jong-Brink, 1995). Host specificity tends to be strict and host range narrow (Reversat et al. 1991; Pretson and Southgate 1994). *Plagiorchis elegaun* (Rudolphi 1802) is uncommon it can establish patent infection in both *Lymnaea stagnalis* and *Stagnicola elodes* (Williams, 1963 and Blankespoor, 1970). These parasites can castrate both of these compatible snail hosts, *Helisoma* sp. and *Physa* sp. Such destruction and alteration of gonadal tissues is a common feature of digenean infections (Hurd, 1990)
and is the consequence of the parasites interference with the host’s neuroendocrine system.

Most of the available information regarding trematode parasitic infections and its effects on reproduction of host snails is based on laboratory experimental evidences by Dreyfuss et al. (1966) on *Lymnaea truncatula*. Hodasi (1972) on infected by *F. hepatica* (Wilson and Denison, 1980; Rondelaud and Barthe, 1980 and Moukrim and Rondelaud, 1992) observed epithelial necrosis and variable follicle atrophy under the pressure of free rediae can be noted until 6 week p.e.(post exposure) at 20\(^{0}\) C afterward, epithelial reconstitution and restoration of gonad activity develop progressively. Some events of egg laying have been noted after week 8 p.e. in groups of *L. truncatula* shedding cercariae of *F. hepatica* (Dupperon, 1994). Zakikhani and Rau (1998) studied impact of *Plagiorchis elegans* (DigeneaPlagorchidae) infection on reproduction on *Biomphalaria glabrata*.

Like in other hermaphroditic pulmonate snails (Gastropoda, Pulmonata) male and female reproductive cells of *L. acuminata* are produced in the hermaphroditic gland glandular hermaphroditic ovotestis. The ovotestis of *L. truncatula* is a single organ, the structure of which is described as grapes like or vesicular (Jackiewicz, 1959; De Jong-Brink, 1969; De Jong-Brink et al. 1981, and Odiete, 1981). It has been shown that oviposition in snails are induced by neuroendocrine hormone caudo-dorsal cells (CDCs) in the cerebral ganglion (Geraerts and Bohlken, 1976 and Takeda, 1977).

Parasitic castration of molluscan hosts of digenetic trematodes is defined as the total or partial reduction of gameteformation (Malek and Cheng, 1974; Pearson and Cheng 1985). This phenomenon, originally reported by McCrady (1873), has been recorded by numerous investigators (Coelho 1954, Etges and Gresso, 1965; Hosier and
Goodchild, 1970; Hurst, 1927; Malek, 1952, McClelland and Bourns, 1969 and Najarian, 1961). All articles about parasitic castration due to digeneans, are related with the action of larval worms. Although the phenomenon is known to occur in parasitized molluscs, the information relative to the number of trematode species that can caused parasitic castration remains sparse.

Histological changes occurring in snails with active infection have already been reported in the *Galba truncatula- Fasciola hepatica* model under laboratory conditions (Rondelaud and Barthe, 1978, 1980, and 1983). In these snails four organs i.e. the albumen gland, digestive gland, gonads and kidney showed an epithelial necrosis followed by reconstitution (Sindou et al. 1991).

The successful establishment of a parasite within its host is associated with remarkable alterations of the host metabolism, fecundity and survival. Apart from the many constraints of parasite infection the infected host suffers significant loss energy for growth and reproduction, the competition for energy and nutrients is a complex process with the parasite physiology being intimately associated with the physiology of host. Besides the energy relationship also a strong spatial interaction exists within the snail trematode system (Wilson and Denison, 1980; Schwanbeck et al. 1986 and Gerard et al. 1993). Gerard and Theron (1995) proposed two different patterns of spatial integration of the parasites: (1) An integration by ‘substitution’ where the volume of parasites occupy a space normally devoted to host’s organs and (2) an integration by ‘addition’ where the volume of parasite is added to that of the host organs. Spatial investigation by ‘substitution’ usually results in the partial replacement of the digestive gland- gonadal complex.
The histopathological effect differs considerably among the investigated snail trematode systems: (1) Partial reduction of the digestive gland and partial or total inhibition of gonad growth and egg production by alteration in host’s hormonal balance (*Biomphalaria glabrata*/*S. mansoni*; e.g. Bayne and Loker, 1987) (2) Partial reduction of the digestive gland and complete destruction of the gonads (*Littorina littorea*/*Saxatilis* five specimens of trematodes, Rees, 1936; James 1965; *Galba truncatula*). Pelseneer (1906, 1928) was first to report that the penis of *Littorina littorea* infected with progenitor stages of *Cercaria emasculans* was greatly reduced in size when compared with that of uninfected snails. W. J. Rees (I.c.) reported the same phenomenon in the same snail when infected by rediae of *Cercaria himasthlaecunda* or by rediae of *Cercaria lophocerca*. However, Rees revealed that these trematode larvae actually devoured mollusc gonads and attributed the reduction in size of external genitalia to this destruction, since it is known that the degree of genitalia development is directly correlated with gonadal function. Woodard (1934) also reported a reduction in size of the external genitalia of parasitized *Goniobasis laqueata* (Say).

Recently, snail population density effects on growth, reproduction and survival of the planorbid snail *B. alexandrina* exposed to *S. mansoni* studied under laboratory conditions (Mangal et al. 2010).

Diawara et al. (2003) observed the development of tissue lesions caused by *F. hepatica* in naturally infected *G. tuncatula*, collected from the oasis Of Tozur (South- Western Tunisia). Little information is available on the histological changes occurring in the viscera of the snails. The intensity of lesions in the four visceral organs was weaker and developed more slowly (Sindou et al., 1991). Practically lesions in the other organs such as accessory sexual organs of freshwater snails have never been studied.
A majority of authors agree that host parasite interaction can be regarded as a strategy which benefits the parasites; trematode larvae need energy for development and nutrient directed towards host reproduction are not available for parasite. Disfunction of the albumen gland seems to play an important role in *L. stagnalis* infected by *T. ocellata*, larval trematodes after galactogen synthesis and reactivity of snail hormone to the albumen gland (Joose and Van Elk, 1986; Bayomy et al., 1989). Schistosomes induce production of peptide schistosomin that was observed in the hemolymph of parasitized snails at the time of cercarial maturation. It interferes with the host’s neuroendocrine systems including the actions of the dorsal body hormone and calfluxin on the albumen gland and egg laying (Hordijk et al., 1991 and De Jong-Brink et al., 1995). ‘Schistosomin’ a peptide produced by the central nervous system of *L. stagnalis* when infected by *T. ocellata* acts directly of AG synthetic activity by inhibiting the action of one of the reproductive hormones on its target organ at the receptor level (De Jong-Brink et al., 1988 a and b; Schalling et al., 1991). Theron and Gerard (1994) reported reduction of the size of the ovotestis of the infected *B. glabrata* are confirmatory to the results of Crews and Yoshino (1989). The effects of the trematode infection in the growth rates of hosts ASO were little or partially documented in the *S. mansoni/B. glabrata* combination (Meier-Brook, 1981). More information is available concerning the *T. ocellata/L. stagnalis* combination, for which Sluiters et al. (1980) showed that the weights of the male and female ASO (at 154 days post infection) were very small in highly productive infections compared to those of control snails.

The present chapter deals with the pathological lesions caused to reproductive organs of the snail, *Lymnaea acuminata*. Field infected an endemic lymnaeid snail *L. acuminata* collected from different water
bodies around the city Aurangabad in order to investigate, possible impact of larval trematode infection on size difference, mortality rate, egg laying and number of egg capsules present in egg-clutch per strip, gonad-histomorphology and alteration in the neurosecretory activity of the brain or cerebral ganglion compared to non-parasitized snails of the same locality.
MATERIAL AND METHODS

Collections of snails, *L. acuminata* was done during infection period i.e. from September to November of the study period. After getting the snails to the laboratory, were washed with tap water in order to remove mud particles and algal threads grown on the shell if any. Food material consisting of aquatic vegetation, *Spirogyra, Hydrilla* and other aquatic plants was provided, *ad libitum*.

Different sized animals shell lengths ranging from 5±1mm, 10±1mm, 15±1mm and 20±1mm were sorted out and maintained separately in the dechlorinated tap water under laboratory conditions. Observations were made after 24 hrs. of laboratory acclimation. Naturally field infected snails were identified while observing under binocular microscope, from the point of cercarial release. Infected snails show release of cercaria, which is evident under binocular microscope.

**Size dependent egg-laying activity of the infected snail *L. acuminata* during patency period**

After getting identified infected snails of different size groups i.e. 5±1mm, 10±1mm, 15±1mm and 20±1mm shell length were sorted out and triplica of each size group were maintained individually in 250 ml capacity beakers in order to study egg-laying activity of the different sized animals during period of patency. Food material was provided *ad libitum* consisting of hydrophytes collected from snail’s habitat. Egg capsules in the form of gelatinous strip or sting are laid on the leaves of aquatic plant which serves as food of snail. Every 24 hrs.interval egg strips were collected and their length was measured with the help of scale in all sized group animals. An average length in triplicate of three species of each size group was calculated. A batch of all different sized groups of
non-infected snails were maintain simultaneously. Also simultaneously number of egg capsules present in each egg string or strip was counted and an average number of egg-capsule per sting was calculated for each size group of infected and non-infected snails.

**Effect of parasitic infection on egg-laying activity in percent during period of patency in *L. acuminata***

In order to study the egg laying behavior in infected and non-infected snail of *L. acuminata*, 25 infected snails of irrespective size were selected and maintained individually in 250 ml capacity separate beakers with sufficient dechlorinated tap water. Simultaneously non-infected were sorted out and maintained in separate beakers as control, in order to compare egg laying behavior. Food material was provided *ad libitum* consisting of hydrophytes collected from snail’s habitat. Egg capsule in the form of gelatinous strip or string are laid on the leaves of aquatic plant which serves as food of snail.

After 24 hrs.of patency period interval, snail water was observed individually in both infected and non-infected snail beakers in order to know egg laying activity. Number of snails showing egg laying activity was calculated in both infected and non-infected groups. Knowing this number percentage of snails showing egg laying was calculated for both parasitized and non-parasitized host snails for total patency period of 7 days.

**Cytomorphological alterations caused by trematode parasitic infection in the hermaphrodite gonad and other accessory sexual organs of *L. acuminata***

To find out histopathological lesions caused by larval trematodes to the gonad and other sexual organs VIZ albumen gland, prostate gland and
reproductive tract of snail, *L. acuminata*, and these body components of both infected and non-infected ones were dissected out and fixed in Bouin’s fixative for 24 hrs. After fixation, tissues were subjected for normal histological study. Tissues were dehydrated while passing through increasing grades of ethyl alcohol. After getting cold impregnation process, tissue blocks were prepared in 58-60°C M.P. molten paraffin wax. With proper orientation of tissues blocks got treated and serial sections were cut at 7 to 9µm thickness with the help of rotary microtome of Weswox optic Company. Sections were spread on micro slides manually over the spirit lamp. Precaution was taken that the water temperature over the slide was not allowed to rise more than 50°C.

Sections were stained with Harry’s haematoxylin stain and eosin as a counter stain. After getting cleared in xylene, were mounted in DPX (DeparaffinizedXylo). Serial sections of gonad and other accessory sexual organs such as albumen gland, prostate gland and reproductive tract were observed under microscope and histological structure of these organs of infected and non-infected snails was described and compared.

**Alteration in the neurosecretory profiles of cerebral ganglion of *L. acuminata* due to larval trematode infection**

Cerebral ganglion of naturally infected host and non-infected snail were dissected out and fixed in the Susa’s sublimate or fixative for 24 hrs. After fixation the tissues were post-treated with Lugol’s iodine for mercury removal from the tissues. The yellow colour of iodine persist to the tissue indicating complete removal of mercury from the fixative. Later on, tissues were dehydrated while directly passing through 70% alcoholic grade to 100% ethyl alcohol. Following usual method of microtechnique, sections were obtained at 4 or 5 µm thickness with rotary
microtome of Wesvox. Sections were stained with Ewen’s paraldehyde Fuchsin Staining method modified by Bergman, after getting prepared paraldehyde Fuchsin crystals in the laboratory.

Serial sections of cerebral ganglion (brain) of the snail were observed under microscope and changes in the neurosecretory profiles both infected and non-infected snails reported.
OBSERVATIONS AND RESULTS

Size dependent parasitic infection on egg-laying behavior of the snail, *L. acuminata* during period of patency

Egg strip laid by infected and non-infected snails with number of egg capsule present in each strip is shown in the table 17. From the table it is clear that the size of the egg-strip laid by small sized snail (5±1mm shell length) is smaller than the egg strip of large sized snail. Also, the number of egg capsules present is directly proportional to the size of egg string laid by non-parasitized snail. The egg strips of infected snails are smaller in size compared with non-infected one. The number of egg capsules present per string is most significantly less; even sometimes egg strips are without egg capsules in them. That time it is in the form of simple gelatinous mass. Egg strips are abnormal in their structures in infected snail. Normal sized (20±1mm shell length) healthy snails lay the gelatinous egg-strips of size 15±0.15 mm length, having 45±5 egg-capsules in each strip.

Impact of parasitic infection on egg-laying activity of the snail *L. acuminata* during patency period

Number of snails showing egg-laying activity in percent during patency is depicted in the table 18 and 19. Normal egg laying activity during 7 days of patency period ranges from 55 to 70% snails shows egg capsules in the form of egg strings. But in infected snails, the egg laying capacity is decreased due to larval trematode parasitic infection. During early phase of patency only 20 % shown egg-laying activity but at the end of patency period again egg laying capacity of the snails significantly reduced to 2 % compared with 55 % egg laying was present in non-parasitized snail. (See table 18).
Histopathological lesions caused by trematode larval forms in hermaphrodite gonad and associate glands in *Lymnaea acuminata*

**Histomorphology of Non-infected hermaphrodite gonad:**

Non-parasitized gonad of normal sized (20±2 mm shell length). Snails show well developed follicles with normal cytoarchitecture of hermaphroditic gonad. These hermaphroditic gonad looks like bunch of grapes. Histological observations reveal normal gametogenic process. Various stages of spermatogenesis and oogenesis are evident (See plate 8). Gonadal follicles are filled with vitellogenic ova with earlier stages of oogenesis. Populations of all stages of oogenesis VIZ oogonial cells, primary and previtellogenic secondary oocytes, and vitellogenic ova are almost equal indicating simultaneous proliferation, maturation and released of mature ova within the ovotestis of the snail.

**Histomorphology of Larval trematode infected gonad (ovotestis)**

Naturally infected snails gonad (ovotestis) is completely filled by the fully developed rediae with various stages of cercarial development in them (See plate 9). Normal cytoarchitecture of the gonad is completely changed. The process of gametogenesis got hampered in infected snails. Arrangement of hermaphroditic follicles got affected. Bunch of grapes like arrangement totally disturbed. No any sign of such type of gonadal follicle organization is observed in histological pictures of infected snail’s gonad. Few hermaphrodite follicles show development of already proliferated ova in earlier stages of oocyte development and some spermatogenic mass here and there within the lumen of follicles (See plate 8). During peak patent period, there is tremendous damage to the gonad. Total necrosis in the gametogenic tissue is observed. In heavily infected snails, the gonadal tissue can be observed with a great difficulty,
may due to feeding of redia on gonadal tissue. Very few intact follicles shows empty spaces after cercarial release in them. The infection free gonad shows large empty space surrounded by some hepatopancreatictubules (See plate 9)

**Histomorphology of Non-infected albumen gland**

Albumen gland is normal moderate in size with single lobe having shape of human heart. Histologically, it is made up of irregular shaped tubules or acini. The secretory epithelium is a single celled thick encloses lumen at the centre. Albumen secretory cells are of single type, cuboidal in shape. Ductules of these tubules join to form single albumen duct which opens in the region of Carrefour, a region where opens hermaphrodite duct and reproductive tract gets bifurcated into male and female ducts. Secretory product of albumen gland cells stored within the lumen of alveolar follicles of albumen gland. (See plate 10).

**Histomorphology of Infected Albumen Gland**

In parasitized infected snails, there is destruction in the normal structure of the albumen gland acini. There is an entry of parasitic pathogens through albumen duct. Due to heavy infection rediae are found invaded there by causing enlargement within the albumen duct. May be due to parasitic infection derangement within the follicular arrangement together with eosinophilia is developed with in the tissue. (See plate 10).

**Histomorphology of Non-infected Prostate Gland**

Prostate gland of non-infected snail is having regularly arranged, elongated, prostatic follicles with tall, columnar single celled thick prostatic epithelium. Small ductule gets originated from each prostatic follicles and join to a common prostate duct to carry prostatic secretion.
These are a basal nucleus in the columnar cells of prostatic epithelium. (See plate 10).

**Histomorphology of infected Prostate Gland**

The regular arrangement of elongated prostatic follicles gets changed after parasitic infection. The columnar nature of secretory epithelium gets changed to cuboidal in shape. Penetration of rediae within the prostate gland is observed in the histological picture of the gland (See plate 10).

**Histomorphology of non-infected Reproductive Tract (Oviduct)**

Oviduct of non-infected snails, when studied after histological preparation shows regular arrangement of parenchymatous tissue epithelium. Lumen of the duct is having entry of foreign spermatogonia received at the time of copulation. Spermatheca is also found filled by foreign spermatozoa. (See plate 10).

**Histomorphology of infected Reproductive tract**

Due to trematode larval parasitic infection, the regular arrangement of myometrial parenchymatous epithelium got disturbed. There is entry of larval trematodes (rediae) within the lumen of reproductive tract. Rediae embedded reproductive tract shows necrosis within the oviduct tissue. Fully developed rediae are having various stages of cercarial development in them.

**Impact of larval parasitic infection on morphology of the Penial complex of the snail Lymnaea acuminata**

Naturally infected snails in nature with larval trematodes shows regressed condition of their accessory sexual organs such as glands,
reproductive tract and penial complex. There is reduction in the size of preputium and penis sheath. The penialrectractor muscles are poorly developed in the infected snail (See plate 7). It is represented as a vestigeal organ.

In non-infected host snail, these accessory sexual organs are quite normal in size compared with infected one. Retracted conditions of the preputium and penis sheath is not noticed in normal non-parasitized snail. The vas deferens is quite longer in its length (See plate 10).

**Effect of larval trematode parasitic infection on cerebral ganglionic neurosecretory and associated dorsal body cell activity**

Topographically, there are three types of neurosecretory cells in the cerebral ganglion of the snail, *L. acuminata* VIZ Mediodorsal cells (MDC); Caudodorsal cells (CDC) and Laterodorsal cells (LDC). MDC and LDC are Fuchsinophilic or Gomori negative or Phloxinophilic in nature from the point of their cytoplasmic inclusions (granular hormonal material) indicative of different chemical natured secretory material from that of the MDC and LDC neurosecretory hormones. Under light microscope the neurosecretory material (NSM) is observed in the form of NSM flakes. Associated with cerebral commissure on either side at the juncture of each cerebral ganglion and dorsal in position are epitheliod endocrine glands. Because of their position are called as medio-dorsal bodies (MDB). MDB are having only one single type of simple epithelial endocrine secretory cells; are involved in the secretion of medio-dorsal body hormone involved in the process of vitellogenesis. MDC and LDC are involved respectively in the regulation of oogenesis and spermatogenesis. The process of egg-laying is under the control of CDC hormone.
In non-infected snail, the secretory activity of MDC shows normal with simultaneous release of the NSM from their cytoplasm. Mediodorsal cells are more active from the point of their secretory activity, because egg laying process is observed during the study period (infection) in non-parasitized snails. Caudo-dorsal cell too actively involved in the synthesis and release of NSM from their cell cytoplasm (See plate 11), which is evident in having NSM in their cell perikaryon and axon process. Due to increased secretory activity of MDB cells, there is an intense staining affinity in the secretory cells. The process of egg laying is under the control of CDC hormone in non-infected snails.

Larval trematode infected snails shows clear cut changes in the secretory activity of the different neurosecretory cells profiles within the cerebral ganglion of the snail *L. acuminata*. Caudo-dorsal cell activity is reduced. Neurosecretory material is present an accumulation in the form of flakes. Their cell and nuclear diameter decreased. The epithelial cells of MDB show less secretory material in their cytoplasm which is evident by less staining affinity by the aldehyde Fuchsin stain. Reduced secretory activity of MDC and LDC may have affected the normal simultaneous maturation of male and female gametogenesis within the hermaphrodite follicles of the gonad and egg-laying activity of the snail. Reduced MDB cells synthetic activity indicative of no vitellogenic hormone synthesis by these cells.

One large sized neurosecretory neuron (giant) from the mediodorsal cell group shows as enhanced secretory activity of the infected snail. These Gomori-positive neurons of MDC group is designated as growth regulating giant neuron present in the cerebral ganglion. In infected snail, the secretory activity of this giant neuron is increased may be increased growth rate in parasitically infected host snail compared to non-infected one. Wherein there is an increased vacuolization within
cytoplasm of these neurons suggesting reduced secretory activity of the hormone principle in non-parasitized healthy normal healthy snail.
Table 17

Size dependent egg-laying activity of the trematode infected snail, *L.acuminata* during patency period.

<table>
<thead>
<tr>
<th>Snail category</th>
<th>Size of the snail in mm</th>
<th>Length of egg strip in mm</th>
<th>No. of egg present per strip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected</td>
<td>5±1</td>
<td>6±00.2</td>
<td>8±1</td>
</tr>
<tr>
<td>Infected</td>
<td>5±1</td>
<td>4±0.42</td>
<td>Nil</td>
</tr>
<tr>
<td>Non-infected</td>
<td>10±1</td>
<td>8±0.013</td>
<td>17±3</td>
</tr>
<tr>
<td>Infected</td>
<td>11±1</td>
<td>6±0.21</td>
<td>2</td>
</tr>
<tr>
<td>Non-infected</td>
<td>15±1</td>
<td>11±0.07</td>
<td>53±3</td>
</tr>
<tr>
<td>Infected</td>
<td>15±1</td>
<td>9±0.038</td>
<td>5±1</td>
</tr>
<tr>
<td>Non-infected</td>
<td>20±1</td>
<td>15±0.15</td>
<td>45±5</td>
</tr>
<tr>
<td>Infected</td>
<td>21±2</td>
<td>13±0.74</td>
<td>8±2</td>
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Table 18
Number of snails showing egg-laying in % during period of patency.

<table>
<thead>
<tr>
<th>Patency period in days</th>
<th>Egg laying % in non-infected snail</th>
<th>Egg laying % in infected snail</th>
</tr>
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<tr>
<td>1</td>
<td>70</td>
<td>20</td>
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<tr>
<td>2</td>
<td>68</td>
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<tr>
<td>6</td>
<td>57</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 19
Showing numbers of egg-capssule per string by the non-infected and infected snail *Lymnaea acuminata* ±S.D.

<table>
<thead>
<tr>
<th>Egg-laying in days</th>
<th>Non-infected snail</th>
<th>Infected snail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>70±5</td>
<td>3±1</td>
</tr>
<tr>
<td>2 day</td>
<td>54±3</td>
<td>Nil</td>
</tr>
<tr>
<td>3 day</td>
<td>49±4</td>
<td>02</td>
</tr>
<tr>
<td>4 day</td>
<td>40±2</td>
<td>Nil</td>
</tr>
<tr>
<td>5 day</td>
<td>36±2</td>
<td>Nil</td>
</tr>
<tr>
<td>6 day</td>
<td>27±2</td>
<td>Nil</td>
</tr>
<tr>
<td>7 day</td>
<td>15±2</td>
<td>Nil</td>
</tr>
</tbody>
</table>
PLATE - 7

Egg strip laid by snail *Lymnaea acuminata*

Normal (non-infected snail)  Infected snail

Penial complex of snail *Lymnaea acuminata*

Normal (non-infected snail)  Infected snail
T.S. Passing Through Gonad of Snail *Lymnaea acuminata*.

Normal gonadal tissue (10x)  
Infected gonadal tissue (10x)

Normal ovarian tissue (40x)  
Infected gonadal tissue (10x)

C-Ceraria, FC-Follicle cell, GF-Graafian Follicle, GT-Gonadal Tissue,  
HF-Hermaphrodite Follicle, HP-Hepatopancreas, N-Nucleus,  
NCL-Nucleolus, OG-Oogonia, PVO-Pre vitellogenic ovum,  
R-Redia, SB-Sperm Bundles, SM-Spermatogenic Mass, VO-vitellogenic Ova
PLATE - 9

T.S. Passing Through Gonad of Infected Snail *Lymnaea acuminata.*

Infected gonadal tissue (10x)  
Infected gonadal tissue (40x)  
Infected gonad (Uterus) 40x  
Infected gonad (Redia) 40x

C- Cercaria, CT-Connective Tissue, EF-Empty Follicle,  
GT- Gonadal Tissue, R-Redia
T.S. Passing Through Accessory Sexual Organ of *Lymnaea acuminata.*

AG- Albumen Gland, AGF- albumen Gland Follicle, C- Cercaria,
CP- cercarial Parasite, F- follicle, FL- Follicular Lumen, L- Lumen,
MG- Muciparous Gland, PRD-Prostatic Duct, PRG- Prostatic Gland,
R- Redia, SC-Secretory Cell, SM- Secretory Material,
SPT- Spermatheca, UD- Uterine Duct, UW- Uterine wall
L.S. Passing Through Cerebral Neurosecretory Cells of *Lymnaea acuminata*.

Magnification (40x)

A & B- Neurosecretory Cell Types, CC- Cerebral Commissure, CDC- Cudo dorsal cell, LDC- Laterodorsal Cell, MBD- Mediodorsal body, MDC- Mediodorsal Cell, N- Nucleus, NA- Neuropile Area, V- Vacuole
DISCUSSION

In field populations of Lymnaeid snails, *Lymnaea acuminata* the prevalence and intensity of patent of larval trematode infections in particular of *Fasciola* and avainschistisome *Trichobilharzia* species was noticed during infection period. Parasitically infected snails were showing shedding of cercaria indicating polyembryony i.e. simultaneous development of cercaria and their release during period of patency. Snails infected in nature, when collected at random irrespective of their body size, trematode infection prevalence was high in large sized *L. acuminata* compared with small sized snails. Prevalence increased with host size in laboratory studies made by Rothschild (1941) and Rothschild and Rothschild (1939) a pattern suggestive of gigantism in the snail *Peringiaaulvae*.

Naturally parasitized snails of *L. acuminata* in nature, when compared with non-infected ones are having slightly large sized shell lengths indicative of parasitic induced shell growth in the snail. Similar type of enhanced growth of parasitized snails (gigantism) was reported in the prosobranch snails *Hydrobiaaulvae* both field (Gorbuschin 1997) and laboratory investigations (Rothschild and Rothschild, 1939 and Mouritsen and Jensen, 1994).

Increased in the size of the snail may be due to increased parasitic volume to accommodate more number of developing cercaria within the soft body tissues of the host snail. A spatial integration by addition of the parasite volume requires either some empty space within the snail host organ increase in the volume available. It means that the snail host has to attain a larger size by accelerating its growth rate (gigantism). Cheng (1971) and Joosse and Van Elk (1986) for instance reported that trematode infections in two freshwater snails caused an enhanced shell
sized rather than an increase in soft tissue growth seems to be considered as a process of selection of hosts with a particular physiological range (Kalbe et al., 1996 and 1997), or whether it only occurs because larger individuals have been exposed to infection to longer (Robson and William, 1971; Curits, 1997 and Jokela and Lively 1995). In the present study, infected snail host are with slightly larger sized shells than non-infected ones may be more likely due to second explanation mentioned above. Because, the mechanism of infection differs considerably among trematode species (ingestion of eggs in Notocolyliidae, Murrills et al., 1985; penetration by free living miracidia in Echinostomatidae Loos-Frank, 1967). However, further laboratory investigations are necessary to understand the underlying mechanisms in this regards.

Similar type of observations also have been made in different size classes of Hydrobiaventrosa (Probst and Kube, 1999) in Wilsmar Bay was similar to that observed in other areas (Ankel, 1962 and Lauckner, 1986) and to that observed in other gastropod- trematode systems (Pohley, 1976; Davey, 1983 and Jokela and Lively, 1995). Trematode prevalence increased with host size, a pattern suggestive of gigantism (Wesenberg-Lund, 1934 and Rothschild, 1936).

The effect of infection can however be influenced by the age of the host. The present snail at the end of patency period, shows the surviving population is only upto 40%. More than 60% mortality observed in infected snail population at the completion of patency period. This finding supports to the findings of high mortality rate during infection period (Chernin, 1960; Pan 1963 and 1965; Chu et al., 1966, Sturrock and Sturrock, 1970).

Normally, in L. acuminata egg laying is observed throughout the year. During October normal eggs are laid by non-infected snails. But due
to larval trematode infection had tremendous effects on reproductive behavior of the snails.

The parasitic castration of molluscan host by digenetic trematodes is known since 1873, and was first described by McCrady. In the present investigations it has been observed that fully matured rediae found invading in the hepatopancreas resulting in complete damage to the tissue ovotestis of the snail *L. acuminata*. More recently, many investigators have reported the phenomenon of castration (Sullivan et al., 1985; Pearson and Cheng, 1985) and reviewed by many investigators (Souza, 1983 and Minchella 1985). All the known reports on parasitic castration are related to larval stages of digenetic trematodes, but not by the action of an adult trematode. The present castration effect to the gastropod snail *L. acuminata* is only due to the normal stages of trematode parasites in particular at redial stages of infection to the gonad and hepatopancreas of the snail.

The effect of trematode infections on fertility and egg-laying has been reported previously. Najarian, (1961) observed a significant reduction in the average number of eggs laid per snail per day by *Bulinustruncatus* infected with *Schistosomahaematobium*. The present snail *L. acuminata* infected by *Fasciola hepatica* in nature shows changes in egg laying behavior during period of patency. There is a significant decrease in number of egg laying snails in percent during infection season (2%) compared with the non-infected shown (70%) snails egg laying activity under laboratory conditions. Again Sturrock (1966) reports that infection in *Biomphalaria pfrifferi* with *S. mansoni* results in complete sterility, but that some eggs were laid throughout life, if infection is establish before maturity. In the adult Lymnaeid intermediate host snail, naturally infected with trematode larvae at field also show egg-laying in the laboratory during period of patency (2-10% snails). The size of egg-
strip laid by infected snail is similar than the egg strip laid by normal non-parasitized snails. Also the number of egg capsules present within the egg strips laid by infected snails, are few in number compared to the normal one. The normal structure of egg strip is not maintained by parasitized snail’s egg-strip. The egg-laying capacity which is already decreasing due to parasitic infection comes to lowest level at the end of the patency period of the snail, *Lymnaea*. Infections with mother sporocysts of *Plagiorchis elegans* had a significant impact on reproductive output of *B. glabrata* exposed as juveniles or adults. The total number of eggs produced was reduced to approximately 7 and 13% of their respective control levels (Zakikhani and Rau 1998). Among immature *B. glabrata* infected with *Schistosoma mansoni* such effects on host reproduction are associated with the beginning of cercarial production (Thornhill et al., 1986), whereas immediate, prepatent effects have been reported from adult snails by Crews and Yoshino (1989).

Present observations on histopathological lesions resulted due to trematode pathogens imply that many of the explanations proposed in the literature for the adverse effect of trematode larvae infection on snail host reproduction are in agreement to some extent. It has been suggested that the effect has been attributed to partial destruction to the gonad and the reproductive tract caused by toxic substances (Neuhaus, 1940 and 1949); starvation atrophy (Rees, 1936 and Szidat 1941); mechanical pressure (Rees, 1936 and Cheng and Cooperman 1964) and destruction through consumption by rediae (Rees, 1936). During period of patency hepatopancreas of the snail, *L. acuminata* is penetraterd by redia with development and found embedded in the ovotestis may be responsible to cause derangement in the reproductive activity VIZ gamatogenic process and other related processes of the snail. It has been confirmed that fully developed rediae are having well developed digestive system may be
responsible for the destruction of gonadal follicles. The gonadal tissue may be consumed by these rediae since peak cercariogenesis was observed in the gonadal embedded rediae. Similar type of ingestion of gonadal (including gametes), lymphatic and or epithelial cell by parasites has been reported by Cooley (1962), and he further claimed that the rediae of Parorchis acanthus actively ingested gonadal cells of the gastropods, Thais haemastoma. A similar type of situation has been reported by Crews and Esch (1987) for Helisomaanceps infected with Halipegusoccidualis.

Sullivan et al., (1985) put forth hypothetic mechanism by which the parasites may inhibit the host gametogenesis, and considered to be primary and secondary effects. The first one, are related with an effect the gonads, the second on the host physiology and indirectly on reproductive success. Discussion by Cheng et al., (1973) the mechanism underlying parasitic castration are not understood but may be due to the mechanical (e.g. ingestion, abrasion or pressure) or chemical (e.g. secretion of lytic toxic or endocrinologically antagonistic molecules) effect of the parasites on the gonads. Chemical castration, in which molecules secreted by the parasites have an effect upon the gonad, may be either direct, with the parasite in the vicinity of the gonad or indirect, with the parasites located at the distance from the gonad. In order to make conclusive remark on the present parasitic castration which has been resulted in L. acuminata, need to be reviewed experimentally under controlled laboratory conditions.

Naturally infected snail shows various types of pathological lesions in the gonad such as changes in the number of hermaphroditic tubules and totally disturbed process of gametogenesis and egg-laying process is suggestive of parasitic castration in the trematode infected host snail, L. acuminata. Inhibition of oocytes production and germinophagy are just few causes of the parasitic castration known to occur in snails and leading
to reduction of the host population and reproductive potential. Many authors contend that reduced fecundity of the infected snails is primarily a result of the direct parasitic infected damage to the gonad (Robson and Williams, 1971; Yoshino, 1975; Huffman and Fried, 1985; and Crews and Esch, 1987). Lapeta (2003) while studying effects of larvae of *F. hepatica* on activity of an enzyme lactate dehydrogenase in the reproductive gland (albumen) was due to toxic effects of the developing larvae. Moreover, the effects involve thickening of albumen gland tubules and reduction of their lumen at the initial stage of the invasion as a result of connective tissue hyperplasia. Similar types of changes have been observed in the albumen gland of the infected snails studied during period of patency. Album gland secretory cells were subjected to histolysis; their nuclei were displaced with drastic reduction in the lumen of the albumen gland tubules. Tall columnar secretory epithelium gets converted inactive cuboidal in nature because of the contraction and shortening of the secretory cells. Less attention has been paid on impact of larval trematode on accessory sexual organs of the parasitized host snail. Pelseneer was pioneer to report that the penis of the *Littorinalittorea* infected with progenitor stages of *Cercaria emasculaus* was greatly reduced in size with that of uninfected snails. Similar type of conditions was noticed in the present study on *L. acuminata*. Accessory sexual organs (ASO including glands) were reduced in size during patency period of the snail. Rees (1936) reported that same phenomenon in the snail *L. littorea* when infected by rediae of *Cercaria himasthalasecunda* or by rediae of *Cercaria lophocerca*. Rees revealed that these trematode larvae actually damaged mollusc gonad and attributed the reduction in size of external genitalia to this destruction, since it is known that the degree of genitalia development is directly correlated with gonadal function. May be due to gonadal atrophy in the snail, *L. acuminata*, resulted reduction in the size of
penial complex compared to normal non-infected snails. Woodard (1934) also reported a reduction in size of the external genitalia of parasitized *G. laqueta*. Theron and Gerard (1994) while investigating development of accessory sexual organs in *B. glabrata* during different growth period of the snail reported that the ASO in snails infected when immature remains at a juvenile developmental stage. When infections of snails occurs after sexual maturity, growth of the ASO is inhibited after 3rd week of parasitic infection, which corresponds to the decrease in egg-laying for the host (Pan 1965) and beginning of intrasporocystic cercarial production for the parasite (Theron 1981). The present findings on the impact of parasitism on reproduction is based on the snails infected in the nature, needs further experimentation in the laboratory to have more elaborated conclusions on the intermediate snail host, *L. acuminata*, which has a wide distribution in various water bodies in and around the city, Aurangabad (M.S.)

Topographically, three groups of neurosecretory cells are present with in the pond snail *L. acuminata* VIZ medio-dorsal cells (MDC) which are in association with mid-dorsal bodies, within procerebrum of the ganglia. Latero-dorsal cells (LDC) lateral in each ganglion and caudo-dorsal cell (CDC) in the caudal region of the ganglion. In the normal non-infected snails these cells shows changes in their secretion and release pattern with respect to reproductive activity of the snail. It is well established fact that the process of gametogenesis in particular oogenesis is under the control of MDC present in the cerebral ganglion and medio-dorsal bodies under the control of MDC neurosecretory activity regulated vitellogenesis with in developing ova in the ovotesticular follicles through their endocrine secretions.

The caudo-dorsal cell hormone regulates the process of egg-laying in *Lymnaea stagnalis* (De jong-Brink et al., 1995 and Geraerts et al., 1976). It has been hypothesized by Nassi (1979) that interfere with
synthesis or secretion of gonadotropic hormones that are elaborated by the central ganglion and believed to control gonadal maturation and gametogenesis in mollusc (Bayne 1976; Joosse, 1972 and Mortoja, 1972). A giant neuron from the medio-dorsal group of NSC in the cerebral ganglion of infected snail *L. acuminata* shows increased neurosecretory material within the cell perikaryon together with increase in cell diameter. There is a ceased gametogenic activity together with egg-laying stopped in the larval invaded snails. Normally, the general body growth if the snail under the control of giant neurons of the medio-dorsal cell group. Due to parasitic infection to the snail, there is slight enhanced growth rate of infected snails compared with non-infected one. May be because of this noted fact, there is an increased synthesis of NSM within medio-dorsal cells.

Parasitic castration in *L. acuminata* also must be due to alteration of the neurosecretory activity of the brain as reported earlier in *L. peregra* (Thompson, 1990). Lee Breton (1979) claimed that the duplication of germinal cells is stimulated by a mitogenic factor that is secreted by the ganglionic brain and Nassi (1979) has already pointed out interference with the synthesis or secretion of gonadotropins by the central ganglion believed to control the gametogenesis in molluscs. The NSC in close association with MDB’s of the parasitized snails are in inactivated condition may be due to disturbances in the normal gametogenic process of the snail, thereby resulting inhibited gonadotropic activity of these neuroendocrine centres. Ultimately, the medio-dorsal body cell activity gets reduced thereby affecting the normal egg-laying process in infected snail, *L. acuminata*. Conclusively, it has been shown that oviposition in snails are induced by neuroendocrine hormone synthesized by caudo-dorsal cells in the cerebral ganglion (Geraerts and Bohelken, 1976; Takeda 1977; Maat and Lodder 1980; Maat et al., 1982;
Singh et al., 2008). The present naturally infected snail in patency has a decreased hormonal secretion within caudo-dorsal neurosecretory cells with vacuolization in their cell perikarya (See plate 11) and are reproductively quiescent condition due to larval trematode parasitic infection.