CHAPTER 2: BIOCHEMICAL STUDIES

2.1 INTRODUCTION:

The tropical regions of the world have got fluctuating environmental conditions. Most of the organisms from these regions have distinct patterns of breeding and reproductive cycles (Giese, 1959). Several methods are currently been employed in determining reproductive cycles of both aquatic as well as terrestrial invertebrates. Various methods of studying the reproductive states of marine invertebrates have been summarized by Giese et.al. (1959), includes spawning, number of larvae, appearance of ripe gametes, and brooding. The most useful method to note is that of determining the component/body weight ratios. These ratios are collectively known as body component indices, give an overall view of the entire reproductive state of an organism and coupled with determination of various biochemical constituents. These determinations enable us to trace the accumulation and mobilization of organic metabolite reserves, associated fluctuations in the body component indexes and in turn certainly their correlation with annual reproductive cycle.
Seasonal changes in body component indexes have been shown to reflect the fluctuations in constituents in several species of gastropods. The seasonal change in biochemical compositions are more pronounced in animals which have cyclic reproductive periodicities and broadcast fertilization, since a great deal of energy is to be channalized to gonads during reproduction owing to indispensability of vast number of gamete production.

Among the temperate organisms the reproductive cycles of a variety of prosobranch gastropod species have been worked out. Emerson (1965) has estimated polysaccharide levels in seven species of West Coast intertidal prosobranch snails. Seasonal variations in carbohydrate, protein and lipid contents in the edible winkle, *Littorina littorea* have been observed by Williams (1970). The reproductive biology of two West Coast prosobranch species, *Thais cannaliculata* and *Thais emarginata*, has been investigated into detail while using histological and histochemical techniques by Houston (1971) The reproductive physiology of another intertidal prosobranch species *Thais lamellosa* has been studied through the seasonal changes in the body component indexes and simultaneously changes in the protein, carbohydrate and lipid contents of different body components of the snail (Stickle, 1973 and 1975). Seasonal variations in
biochemical composition during reproductive cycle of *T. lamellose* have been observed by Lambert and Dehnel (1974) and seasonal patterns in the biochemical constituents and body component indexes of the muricid gastropod, *Thais haemostoma*, been analyzed by Beisle and Stickle (1978).

The reproductive phenomenon of an organism is a high energy demanding one when compared with other physiological processes. For example, 34% of the prespawning soft body weight of female was lost during aggregation and capsule decomposition in *T. lamellosa* (Stickle, 1973). Many animals meet the metabolic expense of spawning by drawing on reserve materials accumulated during non-reproductive period.

Seasonal changes in biochemical constituents in relation to reproductive cycle of marine invertebrates such as molluscs (Giese, 1969), echinoderms (Giese, 1966), crustaceans (Pillay and Nair, 1973) and bivalve molluscs (Nagabhushanam and Mane, 1973 and 1975; Nagabhushanam and Dhamne, 1979 and Nagabhushanam and Talikhedkar, 1977) have been investigated. Among the molluscs, members of the class Amphineura received much attention from the point of seasonal changes in different biochemical constituents (Giese and Araki, 1962; Nimitz and Giese, 1964; Giese and Hart, 1967 and Lawrence and Giese, 1969). Biochemical variations associated with reproductive cycles of amphineuran
chitons, prosobranch gastropods and marine bivalves are extensively worked out than other animals from marine environment (Giese, 1969; Blackmore, 1969; Webber, 1970; Nagabhushanam and Mane, 1973; Lambert and Dehnel, 1974; Stickel, 1975 and Belisle and Stickel, 1978).

Ansel (1974a, b and c) investigated in detail, the seasonal changes in biochemical composition of three genera of bivalve mollusks viz, *Abra alba, Chlamys septemradiata* and *Lima hians* from the Clyde sea area.

Biochemical investigations on freshwater gastropod molluscs are sparse (Meenakshi, 1954 and 1956; Chatterjee and Ghose, 1973, Diwansingh 1974 and Azmatunnisa, 1974) Morill *et al.*, (1964) observed that in pond snail *Lymnaea palustris*, there is an increase in the weight of albumen gland and it coincides with increased protein content of the gland. Brahmanahdam and krishnamoorthy (1973) observed protein degradation and 14 C amino acid incorporation rates into the foot muscle, proteins of pond snail, *pila globosa* during aestivation. Comparative account on biochemical analysis and the activity of an enzyme saccharidase in two gastropod mollusks, *Gyraulus piscinarum* (water snail) and *Xeropicta vestalis* (land snail) has been given by Jacob Ishay et al (1976). Nagabhushanam and Dummalod (1984) investigated annual biochemical changes in relation to reproductive activity of the freshwater planorbid

Considerable attention has been paid on reproductive behavior and breeding cycles and associated biochemical changes in a number of terrestrial gastropod molluscs. Water relations are of paramount importance in the life of terrestrial pulmonates specially slugs and are extensively studied by Howes and Wells (1934) and Warburg (1965). During active life, terrestrial gastropods continually lose water by desiccation as reflected by weight loss. Investigations regarding biochemical studies, seasonal changes in total polysaccharide were reported in the garden snail, *Helix Pomatia* (Von Brand, 1931). Anderson et al. (1968) made some cytochemical observations on the *Helix* spermatozoa. Chemical composition of egg capsule and total poly-saccharide of content of the reproductive system of the land snail, *Ariophanta ligulata* in the formation of egg capsules was investigated by Ranga Rao (1963 a and b). Carbohydrate metabolism of the slug, *Agriolimax columbianus* with special reference to the distribution of glycogen in the reproductive system was
studied by Meenakshi (1968). In further studies, Meenakshi and Scheer (1969) have shown that galactogen content of albumen gland of *A. columbia* varies seasonally. Presence of glycogen was noticed in various gastropod species such as in *Achatina fulica* (Ghose, 1963) in *A. columbia* (Meenakshi and Scheer 1969), in *A. ligulata* (Ranga Rao 1963a and b), in *Biomphalaria glabrata* (Correa et al 1967) and in *Semperula maculata* (Nanaware, 1974). Kulkarni (1981) estimated the glycogen and lipid content from the hepatopancreas and reproductive system of the land slug. *Laevicaulis alte*, shows correlation with breeding activity of the slug.

Circadian rhythmicity in total proteins and carbohydrate contents of various body components like, central nervous tissue, mid-gut gland and foot, muscles of *L. alte* was noticed by Kumar *et.al.*, (1981). Very recently, much work has been done on various physiological processes of gastropod molluscs such as seasonal changes in physiological activity of the land snail *Helicella vestalis* (Mohamed and Mounir 1982), glycolytic enzyme binding and metabolic control during aestivation of the snail *otala lactea* (Brooks and Kenneth, 1990) and physiological and hormonal mechanisms of albumen gland activity regulation in Pulmonata (Grygon and Arnold, 1994).
Whitwan and Kenneth (1991) while investigating the phenomenon of aestivation in the land snail *O. lactea* revealed that the enzyme phosphofructokinase plays an important role in the regulation of aestivation and also during anoxic conditions. The carbohydrate activity was determined in the digestive tract of edible snail *Helix lucorum* in relation to age and its physiological state by Flari and carrier (1992). Gonadal organic metabolites of the land snail *cerastus moussonianus* are under the control of environmental factors like photoperiod and temperature (Magre and Kulkarni, 1993).

Considerable literature on proteins of the reproductive tract and whole body of the hermaphrodite molluscs is available. But very few attempts have been made on the ovotestis of hermaphrodite molluscs. Ramasubramaniam (1979) made qualitative and quantitative studies on the proteins of the reproductive glands and egg envelops of the land snail *A. fulica* during preparatory and spent phase of reproductive activity, while working on same lines on *L. alte*, Nagabhushanam and Kulkarni (1971), have shown that changes in total protein content closely paralleled lipid and carbohydrate and in turn these changes are in correlation with the growth and maturation of the slug. It has been observed that during process of production of egg capsules at the time of spawning by the mollusc
*cominella maculosa* stored structural proteins are being utilized (Flower, 1973).

In recent years several attempts have been made to study the biochemical nature of the pulmonates, but their functional significance remained uncertain. Saleuddin and his associates (1990) have elucidated that Ferritin plays an important possible role in reproduction of the garden snail *Helix aspersa*.

The amount of total lipids in eight species of snails has been analyzed by Stickle and Emerson (1966). Attempts have been made by Van Der Horst (1973 and 1974 a and b) and Oudejans and Van Der Horst (1974) on biosynthesis of saturated and unsaturated fatty acids and their metabolism in the land snail, *Cepaea nemoralis* under different physiological conditions such as normal anoxic, hibernating and aerobic and anaerobic conditions. Invariability in the lipids and other fatty acids in the pulmonate land snail, *C. nemoralis* during annual course of cycle and phospholipids contents of the snail was investigated by Van Der Horst and Zandee (1973) respectively.

Recently, protein synthesis during aestivation in snail *H. aspersa* were investigated by Julian Pakay et.al.,(2002). It is reported that the rate of
protein synthesis is decreased in hepatopancreas, foot muscles in aestivating snails.

After going thoroughly the literature available in the field of pulmonate gastropods, no work has been done on the present land snail, *Macrochlamys petrosa*, it act as a minor pest on gardens, agricultural and horticultural plants. In view of the Paucity of the information about annual reproductive cycle and associated biochemical reserves, the present probe was undertaken to investigate the biochemical parameters like glycogen, proteins and total lipids in different body components like hepatopancreas albumen gland, gonad, mantle and foot, during prereproductive, reproductive, postreproductive and aestivating cycle of the snail.

The present chapter includes seasonal changes in total glycogen, proteins and total lipids i.e. Pre-reproductive, Reproductive and Post-reproductive in different body components of the land snail *Macrochlamys* investigated. The different body components subjected for biochemical studies are foot, mantle, hepatopancreas-gonadal complex and albumen gland.
2.2 MATERIAL AND METHODS:

Monthly collection of *M. Petrosa* was made during the year June, 1999 to May 2000 from different localities in and around the city, Aurangabad. Immediately after bringing to the laboratory, they were transferred to the glass or plastic troughs having sufficient moist soil. They were fed once in a day on various garden plant leaves and carrot during course of investigation period. For the biochemical investigation healthy normal sized snails were selected. In every month 150 to 200 animals were dissected out after breaking the shell in order to collect their soft body components like hepatopancreas, albumen gland, gonad, mantle and foot. At least three unknown assays of every tissue were used in order to calculate the average value. Essential statistical methods like average values and standard deviation were calculated.

**Glycogen**:

The glycogen in the tissue was estimated by the method of Kemp *et al.*, (1954). A known amount of tissue was taken and homogenized in 5 ml of T.C.A. solution (5% Trichloroacetic acid + 0.1 % silver sulphate
solution) and was kept in boiling water bath for 15 minutes. The contents were then cooled to room temperature and the original volume was restored while adding T.C.A. solution. After getting centrifuged at 3000 rpm for 10 minutes, 1 ml of supernatant was taken and 5 ml of conc. H$_2$SO$_4$ was added. Again it was kept in boiling water bath for 5-6 minutes and cooled to room temperature. The optical density was recorded using 520 mm wave length on the spectrophotometer. The amount of glycogen value was calculated by multiplying the glucose value by the factor 0.927. The result is expressed as mg/100mg of dry weight tissue. Pure D- Glucose (A.R.) was used as a standard glucose.

**Proteins :-**

Total protein content was determined by the method of Lowry *et. al.* (1951), 1 % tissue homogenate was prepared in 10 % TCA and centrifuged at 3000 rpm for 15 minutes. Then to the residue a known volume of I N sodium hydroxide was added to 1.0 ml of aliquot, 5 ml of reagent containing copper sulphate, sodium carbonate, sodium potassium tartarate and sodium hydroxide was added. This reaction mixture was kept at room temperature for 10 minutes. Then 0.5 ml of diluted (1 N) Folin-phenol reagent was added and after the colour is developed was read at 620
nm on spectrophotometer after half an hour. Simultaneously a blank was also run with distilled water. The amount of protein was calculated from the standard, graph paper and represented as mg protein /100 mg dry weight of tissue.

**Total lipids:**

The total lipid content in various body components was estimated by the method of Barnes and Blackstock (1973). A known amount of tissue was taken, homogenized in 5 ml Folch’s mixture i.e. chloroform-methanol in the ratio 2:1. The reaction mixture was boiled for 5 minutes in boiling water bath. After getting cooled at room temperature centrifuged for 10 minutes at 3000 rpm. The supernatant was collected into another tube and the same was dried through evaporation, 1.0 ml con. $\text{H}_2\text{SO}_4$ was added and kept in boiling water bath for 15 minutes and cooled to room temperature. Then 5 ml Vanilline reagent was added. After 15 minutes the optical density was recorded using 660 nm wave length in the spectrophotometer. The results are expressed as mg /100mg dry weight tissue.

**2.3 OBSERVATION AND RESULTS:**

Laboratory and field observations
Monthly observations were made in laboratory acclimated snails. *M. petrosa* (See chapter Reproductive biology). Number of egg capsules, laid down by per snail was calculated during reproductive period of the snail i.e. from August-September 1999. Maximum number of egg capsules was laid during peak breeding period i.e. in the month of August and September, 1999. Then there starts decline in the rate of egg laying from October to early November. Early October onwards at these snails become more active and their feeding rate is increased. This period may be the preparatory period of snails to undergo aestivation.

Field observation also reveals that from the month of November there starts the gathering of snails at the aestivation state. At the time of undergoing aestivation, the edge of the aperture is seased by mucous secretion. These snails remain under aestivating state from December to May. When the environmental conditions are favourable such as ample of rains fall in environmental temperature and increase humidity in the air that forces snails to emerge out from aestivation condition. No snail was present at the site of alive in the month of June, as there starts regular rains in Marathwada Region. After emergence from normal aestivation period, there is increased rate of feeding and snails of all size group gets scattered in the fields. But egg laying starts from August and maximum number of
eggs of animal was being laid in the month of September and continues up to early October.

From above field and laboratory observation it can be concluded that peak breeding period of snail *M. pestrosa* is from August to September. From late October the egg laying is completely stopped. Specimens of various size groups measuring from 1.5 to 1.7 cm. shell diameter starts gathering at aestivation site in the month of November. There snails remain under aestivating condition up to June or till onset of monsoon so October is the month of preparation to undergo aestivation.

The amount of various biochemical contents viz. glycogen, proteins and total lipids are estimated monthly in different body tissues of snail *M. petrosa* and results are depicted in the form of Tables I to IV. The body components selected for biochemical studies are foot, mantle, hepatopancreatic gondal complex and albumen gland of the snail. The results are expressed in mg/100 mg. of dry weight tissue of the snail body.

Seasonal variation in the water percentage in the whole body of *M. petrosa* is estimated monthly and results are shown in Table. I Annual reproductive cycle of the snail shows four periods i.e. pre-reproductive period from June to July; reproductive period from August to September; post-reproductive period from month of October November and from
December onward snails undergo aestivation. The water percentage varies from 68.62 to 85.30%.

After comparing the water percentage of whole body of the snail, *M. petrosa* it is evident that maximum percentage of water is present in reproductive period (August to Sept.) is (85.30 ± 1.900) and minimum in prereproductive period (June and July) is 68.62 ± 0.341). It is observed that the water percentage decreases in post-reproductive period (October to November) i.e. (73.59 ± 1.022) and again starts increasing before undergoing aestivation. It is observed that during aestivation condition snails sealed their opening with mucous secretion, to prevent the loss of water. The snails remain under aestivating state from December to June. During this period the water percentage of body is estimated is (77.40 ± 0.748).

**Glycogen:**

The amount of glycogen present in different body components during annual reproductive cycle including aestivation is shown in Table II. From the table it is clear that the amount of glycogen present varies from 16.56 to 20.67 mg/100 mg. in foot, 11.02 to 17.22 mg/100 mg. in mantle, 16.57 to 26.24 mg./100 mg. in hepatopancreatic gonadal complex and 06.00 to 08.63 mg/100 mg. in albumen gland.
After comparing the glycogen content in various body components of the snail, *M. petrosa* it is evident that maximum amount of glycogen is present in hepatopancreatic gonadal complex (26.24 ± 1.15) and minimum in the albumen gland (06.00 ± 0.80) Hepatopancreatic gonadal complex and albumen gland are the important body components which directly contribute in reproduction of the snail. After emerging out the aestivating condition in the month of June there is an accumulation of the glycogen in tissue. Glycogen accumulation is maximum in pre-reproductive period (26.24 ± 1.15) which is prematuring phase of the snail. The glycogen content of the hepatopancreatic gonadal complex was minimum in post-reproductive period (16.57 ± 1.40). Again from the month of late November there start accumulation of glycogen. In foot and mantle there is continuous depletion of glycogen during month of aestivation i.e. April and May. However in these months there is light increase in glycogen contents of hepatopancreatic gonadal complex. The maximum amount of glycogen content in albumen gland is estimated in pre-reproductive period (08.63 ± 1.22) and minimum in post-reproductive period (06.00 ± 0.80).

**Proteins:**
Variation in total proteins of different body tissue is summarized in Table III. After comparing data of protein content of foot muscles minimum amount is noticed in post-reproductive period (33.16 ± 1.23) and maximum was present in pre-reproductive period(46.31 ± 1.14) The protein values of mantle tissue coincides with that of foot muscle and the trend of seasonal variation in protein values, similar to that of foot minimum being observed in post-reproductive period(33.81 ± 1.23).Comparatively very high amount of proteins are present in hepatopancreatic gonadal complex (67.23 ± 1.19 ) in the pre-reproductive period. Albumen gland proteins varies from maximum being observed in pre-reproductive period (58.87 ± 0.21 ) and minimum (30.11 ± 0.61 ) in month aestivation period.

**Lipids:**

The amount of total lipids estimated in different body part of the snail M. petrosa is mentioned in the Table IV. There is no much significant differences in total lipid, contents of the tissue such as foot and mantle of the snail. In foot maximum amount being observed in reproductive period (12.38 ± 1.38) and minimum value is present (8.31 ± 0.99) in aestivation period i.e. in April. The maximum lipid contents of mantle are (10.09 ± 1.44) in post-reproductive period and minimum (8.02 ± 1.32) in aestivation period. In hepatopancreatic gonadal complex lipid content was maximum
in pre-reproductive period (19.86 ± 2.77) and minimum is (12.79 ± 1.18) in post-reproductive period. Lipid content of albumen gland, minimum is noticed in aestivation period (4.38 ± 0.75) and maximum (12.63 ± 0.75) in pre-reproductive period.

2.4 DISCUSSION:

There are several methods currently employed in determining reproductive cycles in invertebrates. One of the most useful to date is that of determining body component/ body weight ratios. These ratios are collectively known as body component indexes allow for an overall view of the entire reproductive state of an organism and coupled with determination of various biochemical constituents, enable one to trace the accumulation and mobilization of organic reserves associated with reproductive cycle.

The reproductive cycles of a variety of temperate species of prosobranch gastropods have been very well established. Houston(1971) made use of histological and histochemical techniques to study the reproductive biology of two west coast species T. canaliculata and T. emarginata. Seasonal cycles in T. lamellose have been determined using the index method (Stickle, 1973; Limbert and Dehnel,1974) other
prosobranchs which have also been studied include *Fuscitriton oregonesis* by Stickle and Mrozek (1973) *Haliotis cracheroidii* by Webber (1970) and Webber and Giese (1969) and *Littorina littorea* by Williams (1970).

Organisms from the tropical areas also have a distinct pattern of breeding and reproductive cycles (Gises, 1959). The present snail *M. pestrosa* emerges out of the aestivating conditions in the month of June after onset of monsoons. Rate of feeding is more during June and July. From the month of August starts egg laying. The process of egg laying is continued through the months August to early October. Then undergoes aestivation in the month of late November. These snails remain under aestivating condition from late November to May is the period of dormancy. The amount of stored biochemical constituents in the body is greatly influenced body activities and breeding of the animal. The snails *Macrochlamys* feeds actively after emerging out of aestivation. But when breeding starts from July, these organisms are more active but show reduced rate of feeding. At the same time energy required during reproductive period is high when compared with that of nonreproductive period.

Variations in biochemical constituents in relation to reproductive cycles have been observed in some marine invertebrates such as decapods.
crustaceans (Pillay and Nair, 1973) bivalve mollusks (Nagabhushanam and Dhamne, 1979 and Nagabhushanam and Talikhedkar, 1977) These studies show that changes in biochemical reserves are always related with reproductive cycle and changes in environmental conditions. The present land snail is a minor pest of garden plants found distributed throughout the tropical country India, where the environmental conditions fluctuate greatly. May be due to this reason, *M. pestrosa* show distinct pattern of biochemical storage and utilization, during annual cycle of the snail.

The process of reproduction is a very high energy demanding. It is well established fact that energy is stored in the form of biochemical reserves to meet the demands of reproduction in marine invertebrates (Giese, 1969). Studies on these lines are scanty about the terrestrial pulmonate gastropod metabolic reserves and their use during annual course of reproductive cycle. The present snail, *M. pestrosa* has a distinct phase of gonadal maturation i.e. gametogenic and egg laying during annual course of reproductive cycle, as evidenced by monthly changes in gonad cytology of the snail (See reproductive biology chapter).

In general carbohydrates are the major source of energy for vital activities of the organisms. Glycogen is the chief carbohydrate reserve of the tissues and glucose as a major circulating sugar in the blood and other
Carbohydrate metabolism can be divided into two aerobic and anaerobic phases depending upon the oxygen supply which reflects the physiological makeup of the organ systems or the physiological alterations brought by intrinsic and extrinsic environmental conditions (Dudeja et al., 1980). May be because of above noted fact in the present land snail, *M. pestrosa* the glycogen content of hepatopancreas and gonadal complex there is continuous depletion in the glycogen content during normal course of aestivation i.e. from the month of November to December slight increase in glycogen content is in the month of March and April i.e. aestivation period. The stored glycogen is the only source of energy during aestivation and reproductive period of *Pila virens* (Meenakshi, 1956) and of *Patella* (Berry and Munday, 1959). There is an increase level of glycogen in almost all tissues of *M. petrosa* studied in the month of June and July which is the peak gametogenic activity of the snail, Recently Kulkarni and Shinde (1992) while working on freshwater gastropod snail, *I. exustus* reported increased level of glycogen during per breeding and hibernating period of the snail which are in agreement with present observations on land snail., *Macrochlamys*. According to Blackmore (1969) in *Patella* and Webber (1970) in *Heliotis*, the polysaccharide content varies directly with feeding. Since the present snail *M. pestrosa* is active but with reduced rate of
feeding during peak breeding period i.e. August and September, may be due to release of egg-capsules and reduced feeding caused drastic fall in the glycogen content of HGC, which are directly involved in breeding activity of the snail. Similar type of results has been obtained by Lambert and Dehnel (1974), while studying seasonal variations in biochemical composition of the intertidal Prosobranch snail *T. lamellose*. According to them also *T. lammellosa* stops feeding during breeding season inspite of abundance availability of food material. Nagabhushanam and Dummalod (1984) reported total glycogen content of whole body reached to peak period to start of breeding and followed by steep fall during peak egg-laying period of the freshwater planorbid snail, *G. convexivsculus*. The increase level of glycogen in albumen gland during prebreeding months i.e. prior to start of egg laying may be due to increased synthetic activity induced by mating and copulation of the snail *M. pestrosa* as shown recently by Grygon and Arnold (1994), the most important activators of polysaccharide synthesis process in pulmonates are mating, copulation and egg laying. Meenakshi (1954) observed that there is a sudden decline in the galactogen content of the Indian gastropod *Pila* after oviposition and within a few weeks after egg laying, the galactogen contents was found to increase again.
Among the biochemical metabolites proteins are the chief organic components of cellular structure and organization. In the *M. petrosa* proteins are the major organic metabolites of all biochemical constituents. Maximum amount of protein reported in hepatopancreas gonadal complex of matured individuals in the month of July Stickle (1973) shown that most of the biomass lost from the female viscera mass of *T. lamellose* is deposited as capsule material. Stickle (Unpublished data) found that the egg capsule composition of *T. haemastoma* composed of 53% protein, 7% lipid and 2% carbohydrate when deposited at 30% salinity. Very few attempts have been made on the biochemical composition of hermaphrodite mollusks. Hyman (1967) while studying the freshwater as well as terrestrial pulmonates concluded that egg laying in freshwater pulmonates occur throughout the year. But the terrestrial pulmonates snails breed during rainy season of the year. The present land snail *M. pestrosa* during annual course of study on breeding behaviour, it has been observed that egg capsules are being laid during favourable environmental conditions i.e. ample of rain fall, increased humidity and fall in environmental temperature. Hajari (1983) studied the reproductive cycle of the terrestrial snail, *C. moussonianus* into two phases, such as preparatory phase and spent phase. During spent phase the highest contribution for the protein
was from reproductive tract. Similar type of observations have been made by Ramasubramaniam (1979) in another land snail *Achatina fulica*, wherein proteins of the reproductive glands are being utilized for preparatory and spent phase of reproductive activity of the snail. The amount of proteins in the HGC depleted drastically during intense breeding season of the snail *M. pestrosa* i.e. in the months of August and September, during these months which maximum number of eggs are being laid. These proteins may contribute in the formation of gametes and their subsequent release as shown by Nagabhushanam and Kulkarni (1971) in the slug *L. alte*, that seasonal variations in total protein content closely paralleled lipid and carbohydrates.

In recent years several attempts have been made to study the biochemical nature of pulmonates, but their functional significance remained uncertain. Saleuddin and his associates (1990) have elucidated that the specific protein Ferritin plays an important role in reproduction of the snail *Helix aspersa*.

Lipid storage has been reported in the digestive gland of gastropod, *Busycon* (Mendel and Bradley 1905), *Viviparus* (Rosen, 1932), *Marisa* (Dougherty, 1956) and *Helix* (Von Brand, 1931, Thiele, 1959). Shiganatus and Takeshita (1959) are of the opinion that after glycogen and proteins,
lipids are used as energy source. There is a considerable decrease in total lipids of the tissues, HGC, reproductive tract and albumen gland of the present snail, *M. pestrosa* during intense breeding season. Then there starts accumulation of lipids in these body components prior to undergo aestivation i.e. in the month of October and November. During intense breeding season, the process of vitellogenesis increased as number of vitellogenic ova during these months is increased. In this process large amount of lipids are being utilized hence there is low liters of lipids observed. Meyer and Meyer (1971) stated that since the biosynthesis of fatty acids and steroids required the large amount of energy. Hence the present snail *M. pestrosa* after emerging out of the aestivation, more active with increased feeding rate. Van Der Horst et al. (1973) observed invariability of composition of fatty acids and other lipids in the pulmonate land snail *C. nemoralis* during annual cycle. In *M. petrosa* a consistent decrease in lipid levels of tissues studied during the progress in the normal aestivating cycle of the snail.