Introduction:

Studies on events during reproductive cycle of animals are not merely confined to the morphological and anatomical changes in the gonads and the associated ducts during various stages of the cycle. In fact they are just the outward manifestation of several biochemical changes that are taking place in an orchestrated manner not merely in these structures but also in several other tissues and organs. Further, during an annual cycle many animals invertebrates and vertebrates particularly of temperate region show a clear cut feeding phase and the reproductive phase alternating with each other. Echinoderms are one such group where such phases are distinct even in several tropical forms (Pearse, 1965, Anderson, 1966, Ferguson, 1975a & b; Scheilbling and Lawrence, 1982; Walker, et al., 1987; Jayashree, 1988 and Tyler and Billett, 1987). Not withstanding whether such phases are clear cut or not, organs like the gut, glands—the pyloric case of asteroids associated with the gut, and the body wall of holothuroids besides performing their routine functions, also act as the storage organs of nutrients to be transported to the developing gonads during their latter annual cycle (Krishnan, 1968; Lawrence, 1976, Masin, 1980, Jayashree

In contrast to our fairly good knowledge on organic components like proteins, aminoacids, lipids, carbohydrates etc., in different tissues of holothuroids during different stages of the gonadal cycle, it is but limted on inorganic components (Siddiqui, 1988 and Li-jing et al., 1989). Little is known about the role, if any, of the inorganic components in the reproductive physiology of echinoderms. In the present work an attempt is made to carry out estimation of few inorganic components viz. sodium, potassium, calcium, phosphorus, magnesium and chlorides in the gonads, gut and the body wall of H. atra to know if variations in their levels in these tissues during the reproductive cycle offer any clue to their role in this phenomenon.

By using the conversion factor of Tyler and Billett (1987), the caloric values of gut, body wall and gonads were determined corresponding with the bio-chemical estimations.

In some holothuroids, the main organic components, proteins, lipids and carbohydrates have been estimated during different phases of the reproductive cycle by some
workers (Taniwaka and Yashitoni* 1955; Taniwaka*, 1966; Boolootian 1966; Krishnan 1968, Lawrence 1972; Prim et al., 1976 and Sibuet and Lawrence 1981). In holothurians different body components such as gut, body wall, gonads and perivisceral fluids are known to store the nutrient materials (Fish 1967; Krishnan; 1968, Prim and Lawrence 1976, Walker 1982). The body wall seems to be the storage organ in Stichopus (Taniwaka*, et al., 1955), Holothuria scabra (Krishnan, 1968) Pentacta pygmae (Jesperson and Lutzen 1971), Synapta hydriforos (Prim et al., 1976), Holothuria leucospilota (Jayashree 1988) and Eostichopus regalis (Walker et al., 1987). Besides the body wall, the intestine is the storage organ in Neopentadactyla mixta (Smith, 1981). While the gut alone is the storage organ in Cherbonniera utriculus (Tyler et al., 1987), in Eumolpadia violacea no organ accumulates nutrients reserves available for one another particularly for the gonad. The storage of nutrients seems to take place directly in the ovary (Feral and Magniez 1985).

Russo* (1926) was the first to study the biochemical aspect of the breeding cycle in the echinoid, Paracentrotus

* Cited in Boolootian (1966)
lividus. He observed that the testes were the storage organs for the nitrogen to be utilized during the spermatogenesis. A large amount of glycogen was demonstrated in the gonads and as such they were considered to be the storage organs of glycogen in *Echinus esculentus* (Stott*, 1931).

Quantitative estimations of proteins, lipids and carbohydrates of the body wall and gonads in *Leptosynapta galliennei* and *Eumolpadia violacea* (Feral and Magniez 1985) have been carried out. Similar studies have been made on the body wall and gut of *Eostichopus regalis* (Mc.Clintock et al., 1990), body wall alone of *Actinopyga agassizi*, *Synapta hydriforms* and *Pentacta pygmaea* (Prim et al., 1975), *Holothuria atra*, *H. difficilis*, *H. glaberima* and *H. leucospilota* (Lawrence and Guille 1982) and on the ovary and the testes of *Oneirophanta mutabilis*, *Deima validum*, *Benthodytes sordida* and *Psychropotes longicauda* (Tyler and Billett 1987).

The studies on the biochemical composition of the various tissues of the Indian echinoderms are limited to a few species. The total proteins, lipids, glycogen and fatty

* Cited in Boolootian (1966)
acids of the gut, body wall and gonads have been studied in only one species of the sea urchin *Stomopnuestes variolaris* from Madras (East coast of India) (Giese et al., 1964), only two species of asteroids viz. *Oreaster hedamanni* (Rao 1965), and *Astropecten polyacanthus* (Bellary 1989), and a holothurian *Holothuria leucospilota* (Jayashree 1988).

In the present work quantitative estimations of proteins, lipids and carbohydrates in the gut, body wall and the gonads during different stages of the reproductive cycles of *Holothuria atra* have been carried out and results have been compared with those found in other species.

Histochemical studies with reference to the biochemical compositions of various tissues and cells of organisms offer a limited scope for probing compared to the biochemical studies. One must admit. But so far as localization of various chemical substances therein to attribute functional roles to them is concerned, histochemical methods are the most suitable tools.

Histochemical studies on the reproductive cycles of holothorian species and echinoderms at large are but a few (Immers, 1960; Anderson, 1968; Chia, 1968; Krishnan, 1968; Bai, 1970; Nimitz, 1976; Bellary, 1989; Schoenmakers, 1984;
Smiley 1988; Holland and Kubota, 1975; Tyler and Gage, 1983; Tyler and Billett, 1987; Pain et al., 1982; Tyler et al., 1982; Tyler et al., 1984). Further as far as we are aware of the literature such studies concurrent with biochemical studies have been made only in *Astropecten polyacanthus* and the findings of the two have been compared (Bellary 1989). We have made an attempt to examine the levels of proteins, lipids and carbohydrates (glycogen) in the ovarian tubules of *H. atra* during different phases of oogenesis by employing appropriate histochmical techniques. The levels of these metabolites have been assessed by intensities of the concerned reactions. The results have been compared with the ones obtained from biochemical studies. Distribution of acid mucopolysaccharides forms another interesting aspect of echinoderm histochemistry. Such work has been carried out on a few echinoderm species (Nakano and Ohashi, 1954, Pasteels et al., 1958; Perlman et al., 1959; Immers, 1960; Holland and Kubota, 1975; Pain et al., 1982a and 1982b; Desai and Bellary 1989; Tyler and Gage 1983; Tyler et al., 1985a and 1985b; Tyler and Billett, 1987).
MATERIALS AND METHODS:

A. ORGANIC COMPONENTS:

Preparation of Samples:

From specimens of both sexes of *M. atra* large pieces of the gut, body wall and the gonads were taken in sample tubes containing Sea water (S = 35%, s) from Binaga beach of Karwar coast (Karnataka, India) on a monthly schedule and were transported in frozen condition to the laboratory where they were freeze dried and preserved in an airtight disiccator at -20°C. till further use.

The freeze dried pieces of tissues were defatted by using 2:1 Chloroform: Methanol with Soxhelt apparatus for quantitative studies of proteins, carbohydrates and inorganic substances.

Estimation of proteins and carbohydrates:

The defatted tissues were separately powdered using pestle and mortar. 1 mg of defatted powder sample was mixed with 10 ml. of 10% concentration saline, kept for stirring for about 2 hrs, then centrifuged at 3000 rpm/30 mins. The supernatant was used for protein and carbohydrates estimations. Extraction of individual tissues were carried out separately.
Total Proteins:

Multiplying total nitrogen value with 6.25 has given us the total protein value. Nitrogen value of individual tissues were estimated by microkjeldahl method.

Soluble Proteins:

Soluble proteins estimation of the gut, body wall and gonads were carried out according to the method of Lowry et al., (1951) using BSA as standard.

Insoluble Proteins:

By deducting soluble protein content from total protein content has been given us the value of insoluble protein of individual tissues.

Estimation of Lipids:

The freeze-dried sample was powdered by using pestle and mortor. Known powdered sample was taken and mixed with 100 ml. 2:1 chloroform: methanol, according to Folchis et al., (1957) method in an Soxhelt apparatus. Then the mixture was heated for about 2 hrs. and filtered. The filtrate was used for lipid estimations. The lipid content was estimated by gravimetric method.
Estimation of Carbohydrates:

The carbohydrates content of sample were estimated by the phenol sulphuric acid method of Dubois et al., (1956) using glucose as standard. The levels of the main biochemical components are expressed in % dry weight. Any variations in the contents in individual tissues and between the tissues during different phases of the reproductive cycle of the animal of each sex have been tested by applying appropriate statistical formulae for their significance. The findings are presented in the form of tables, graphs and histograms.

B. INORGANIC COMPONENTS

Preparation of samples:

The body wall, gut and gonads of both sexes of the sea-cucumber were freeze dried and ground to a fine powder.

Procedure:

To estimate the inorganic constituents 1gm of powdered sample was digested using acid mixture containing conc. HNO₃, H₂SO₄ and perchloric acid (60%) in the ratio of 10:1:4, filtered the mixture through acid washed filter paper and made the volume to 50ml with 6N HCl. The
filtrate was preserved for the analysis of some elements described.

Estimation of sodium and potassium:

Diluted 0.5 ml. of digested solution into the 50 ml. volume and then fed to the flame photometer (Digital MK III Systronics) and noted the readings and calculated the percentage of sodium and potassium by the following equation.

\[
\% \text{ K}^+ \text{ and Na}^+ = \frac{\text{volume made}}{\text{wt. of sample}} \times \frac{\text{volume made}}{\text{ml. of the digested soln. taken}} \times \frac{\text{ppm of K and Na from standard curve}}{10,000}
\]

\[\text{ppm} \times \frac{\text{volume made after digestion}}{\text{Sample taken}} \times \frac{\text{Volume made}}{\text{ml. of aliquot taken}}\]

\[
\% = \frac{\text{FPR} \times 100}{10,000}
\]

Estimation of Ca\(^{++}\) and Mg\(^{++}\)

To estimate calcium and magnesium, 10 ml. of digested sample was fed to Atomic Absorption Spectrophotometer (model
AA-630) and noted down the readings for calcium and magnesium of the gut, body wall and gonads during different stages of the reproductive cycle of H. atra.

Estimation of phosphorus:

To estimate phosphorus 50 ml. sample was taken in a tube and 0.05 ml. phenolphthalein was added as an indicator. At the point when red colour developed, 5N $\text{H}_2\text{SO}_4$ solution was added dropwise to discharge the colour. Now 8.0 ml. of combined reagent (50 ml. 5N $\text{H}_2\text{SO}_4$, 5 ml. potassium antimonyl tartarate soln. 15 ml. ammonium molybdate soln. and 30 ml. ascorbic acid soln.) was added and mixed thoroughly for 10 minutes and measured at the absorbance 880 mm. in the Spectrophotometer 20D of Milten Roy make.

Calculation:

$$\text{mg P/L} = \frac{\text{Mg p} \times 1000}{\text{ml. sample}} \times \frac{1}{\text{df}} = \frac{1}{10,000} = \% \text{ p = ppm x 2}$$

Estimation of chloride

The chloride content of the sample was determined by Standard method (Apha et al., 1980).
Procedure:

10 ml of sample was titrated against standard AgNO₃ (0.0141N) solution. Potassium chromate was used as an indicator. The appearance of brick red colour was considered as an end point.

Calculations:

Chloride =
\[
\frac{\text{AgNO}_3 \times \text{normality of AgNO}_3 \times \text{At. wt. of chlorine}}{\text{Vol. of the sample}}
\]

C. CALORIC CONTENTS:

Materials:

The calorific contents of the tissues i.e., the gut, the body wall and the gonads in both the sexes have been determined by using the conversion factors of 17.15J/Mg. for carbohydrate, 39.55J/Mg. for lipid, and 23.64J/Mg. for protein (Brody*, 1945 and Tyler and Billett, 1987), and multiplying with corresponding biochemical estimations.

* Cited in Tyler and Billett (1987)
D. HISTOCHEMICAL TECHNIQUES:


Deparaffinised sections of ovaries were stained with mercury bromophenol blue (MBB) for studying the proteins in general as described below. The paraffin wax was removed from the sections of the ovaries by keeping in xylene with two changes each of 30 minutes duration. Then they were washed thoroughly in absolute alcohol and were stained for 15 to 20 minutes in mercury bromophenol(MBB) (10 gm. HgCl₂ and 100 mg. bromophenol blue in 100ml 95% alcohol). Then they were washed in 0.5% acetic acid (3 changes) for 20 minutes and further washed briefly in water and phosphate buffer (P.H.7.0) for 3 minutes each. After the routine dehydration in ethyl alcohol grades and clearing in xylene, the preparations were mounted in Canada balsam.

Periodic and Schiff (PAS) reaction (after Mc manus: cited in Pearse, 1968):

For the localization of carbohydrates the sections were deparaffinised and processed in ethyl alcohol grades and brought to water. This was followed by oxidising the
section in 0.5% aqueous periodic acid for 4-5 minutes and washed in distilled water. Then they were treated with Schiff's reagent for 20 to 30 minutes and rinsed in sodium metabisulphite (10 ml. N HCl + 10 ml. \( 10\% \text{ Na}_2\text{SO}_4 \) + 200 ml. distilled water) solution thrice. After this they were washed in running water for 10 minutes dehydrated in alcohol grades, cleared in xylene and finally mounted in canada balsum. The sections were examined under the microscope. Carbohydrates stained pink or purple.

Detection of Glycogen:

Best's carmine method (Pearse 1968b):

For the detection of glycogen sections of the ovaries were deparaffinized and processed as usual in alcohol grades and brought to water. Then they were stained with Best's carmine solution [10 gm. Best's carmine powder + 100 ml distilled water boiled and cooled and then added 20 ml. strong ammonia soln. (Sp.gr.0.88)] for 20 minutes. After differentiation with Best's carmine differentiator for 20 minutes, the sections were dehydrated cleared in xylene and mounted in euparol. Bright red colour indicated the localization of glycogen.
Localization of acid mucopolysaccharides:

Toludine Blue method: (Karmer and Windrum: cited in Pearse 1968b)

The deparaffinised sections of the ovaries and testes were passed through the alcohol grades and were brought to water. They were then stained with alcoholic toludine blue (0.1% toluidine blue in 30% alcohol) for 15 to 20 minutes. Then the preparations were dehydrated, cleared in xylene and mounted in euparol. Purple or violet colouration (β-metachromasia) indicated the localization of acid mucopolysaccharides. Some sections were incubated at 37°C for 3 hours in 0.1% hyaluronidase medium prepared in 0.85% saline and stained with alcoholic toluidine blue(0.1%) and processed as described above. These preparations served as controls.

ALCIAN BLUE 8GS METHOD (Steedman, 1950; cited in Barka and Anderson, 1963):

For the localization of the acid mucopolysaccharides alcian blue 8GS method is considered as a more specific method and hence it served as a positive control method to confirm the findings by the toluidine blue method. Hence in this work localization of the acid mucopolysaccharides has
also been studied by alcian blue 8Gs method. The details are as under.

Sections of the ovaries and testes were deparaffinized and brought to water. Then they were stained with 0.1% alcian blue (prepared in 0.01N HCl) for 15 minutes. After staining they were briefly rinsed in distilled water, cleared in xylene and finally mounted in euparol. Green colour indicated the localization of the acid mucopolysaccharides.

DEMONSTRATION OF LIPIDS

Sudan Black B (SBB) method: (After Mc Manus 1946 cited in Pearse 1968b):

This method was adopted to demonstrate the total lipids in the ovaries. The deparaffinised sections of the ovaries were passed through ethyl alcohol grades and brought to 70% alcohol and were stained in saturated sudan black B (SBB) (prepared in 70% alcohol) for 30 minutes at room temperature. After briefly rinsing in 70% alcohol the sections were immediately washed thoroughly in running water for 10 minutes and finally they were mounted in glycerol jelly. The bluish black colour indicated the distribution
of lipids. Phospholipids are stained light grey while the neutral lipids are stained dark bluish black. Some sections were pretreated with chloroform and methanol mixture (2:1v/v) at 60°C for 2 hours and were subsequently stained with sudan black B and processed as described above. They served as controls.

Oil Red O (ORO) Method: (Lillie 1944 cited in Pearse in 1961)

Neutral lipids are stained brick red in colour. The smears of the ova processed in ethyl alcohol grades and brought to water. They were then stained for 30 minutes in freshly filtered 0.5% ORO soln. (prepared in 98% isopropanol). After a brief differentiation in 60% isopropanol they were washed in water and mounted in glycerol jelly. Red coloured droplets showed the distribution of neutral lipids.
RESULTS:

ORGANIC COMPONENTS:

FEMALE:

Biochemical changes associated with ovary:

A. Proteins:

a1. Soluble Proteins:

There was an increase in the level of soluble protein from the regeneration phase to the maturation phase of the ovary (6.6 ± 0.36% to 19.2 ± 0%). During the partial spawning phase there was a drop in the level from 19.2 ± 0% to 13.1 ± 0.61%. During the second maturation phase of the ovary again there was an increase in the level of soluble proteins (13.1 ± 0.6% to 21.7 ± 1.15%). During the total spawning phase and post spawning phase soluble protein level finally decreases to 4.0 ± 0% (Table 6 and Histogram 1).

a2. Insoluble Proteins:

There was an increase of insoluble proteins in the ovary from regeneration phase to maturation phase (18.47 ± 0.27 to 50.08 ± 0%). During partial spawning phase of ovary there was a drop in the level of insoluble proteins (40.8 ± 0.850). But again during the second maturation phase there was an increase in their level (52.54 ± 0.29).
During the total spawning and post-spawning phases the insoluble proteins level dropped down to 9.0 ± 0% (Table 6, Histogram 2).

a3. Total proteins:

The total proteins level of the ovary increased from regeneration phase to maturation phase (25.07 ± 0.5% to 70.01 ± 0%). Eventually the level dropped slightly during the partial spawning phase (53.9 ± 1.4%). During the second maturation phase again there was an increase in the protein level from 53.9 ± 1.4 to 73.16 ± 1.4%. Subsequently during total spawning and post-spawning phases it dropped to 13.0 ± 0% (Table 6, Graph 5 and Histogram 3).

B. LIPIDS:

The total lipids level in the ovary showed an increase from the regeneration phase (19.3 ± 0.2) to maturation phase (7.8 ± 0%) and a decrease in the level during partial spawning phase (3.7 ± 0.1%) during the second maturation phase an increased level in the lipids was observed (7.52 ± 0.5%). At the total spawning and post-spawning phases a decrease in the level was observed (1.5 ± 0%) (Table 6, Graph 6 and Histogram 4).
C. CARBOHYDRATES

The carbohydrates level in the ovary during regeneration phase was 2.08 ± 0.1% and it rose to 3.12 ± 0.15% during the growth phase. It was 6.4 ± 0% during maturation phase and 5.9 ± 1.5 during the partial spawning phase. During the second maturation phase of the ovary the carbohydrate level was 6.31 ± 0.21 and finally at the total spawning and post spawning phases it dropped to 2.76 ± 0% (Table 6, Graph 7 and Histogram 5).

Biochemical changes associated with the body wall

A. PROTEINS

a1 Soluble proteins:

The Soluble proteins level in the body wall was maximum (20.33 ± 1.2%) during the regeneration phase of the ovary. During the subsequent stages of ovarian cycle up to the partial spawning the soluble proteins level dropped to 7.07 ± 1.92%. Again during the second maturation phase the soluble proteins level in the body wall increased slightly (8.0 ± 1.2%). During the total spawning and post-spawning phases the soluble proteins level in the body wall finally dropped down to 6.0 ± 0% (Table 6, Histogram 1).
a2. Insoluble Proteins:

The insoluble proteins level was maximum in the body wall during regeneration and growth phases (44.03 ± 1.1%) and (36.21 ± 0.4%). This level decreased to 16.28 ± 0% and to 16.58 ± 2.4% during the maturation and partial spawning phases respectively. During the second maturation phase again there was a slight increase (18.58 ± 1.5%) which was rather insignificant. At the total spawning and post-spawning phases it again dropped down to 14.0 ± 0% (Table 6, Histogram 2).

a3. Total Proteins:

The total proteins level in the body wall was maximum (64.36 ± 2.2%) during the regeneration phase of the ovary. During the subsequent stages of the ovarian cycle including the maturation phase the level kept on dropping to the final level 22.98 ± 0%. During the partial spawning phase there was slight increase in the total protein content (26.51 ± 2.7%). At the complete spawning and post-spawning phases the level was minimum (20.0 ± 0%) (Table 6, Graph 5 and Histogram 3)
B. LIPIDS:

The lipids level in the body wall was highest during regeneration phase of the ovary (19.36 ± 1.0%), which showed a steady drop during the subsequent stages as follows. During the growth phase (15.30 ± 0.80%), maturation phase (15.41 ± 0%), partial spawning phase (14.51 ± 0.3%); it was 11.95 ± 0.2% during the second maturation phase. At the total spawning and post-spawning phases it was 8.55 ± 0% (Table 6, Graph 6 and Histogram 4).

C. CARBOHYDRATES:

The carbohydrates level in the body wall was 1.80 ± 0.11 during the regeneration phase and 2.30 ± 10.16 and 1.70 ± 0.0 during active growth and maturation phases. During the partial spawning phase it was 2.35 ± 0.3%. During the second maturation and total spawning it was 1.92 ± 0.20 and 1.55 ± 0.0% respectively (Table 6; Graph 7 and Histogram 5).
Biochemical changes associated with gut:

A. Proteins:

a1. Soluble Proteins:

Much variation was not observed in the gut soluble proteins. During regeneration phase of the ovary it was 6.83 ± 1.19% ;7.63 ± 1.13% during growth phase. At the maturation phase it was 6.5 ± 0% and it was 5.9 ± 0.09 during partial spawning phase. During the second stage of maturation it was 7.5 ± 0.5. At the total spawning and post-spawning phases it was 2.18 ± 0% (Table 6<sup>a</sup> Histogram 1).

a2. Insoluble Proteins:

In any of the stages, the insoluble proteins of the gut did not show much variations. During regeneration phase it was 6.07 ± 0.7% and at the growth phase it was 4.15 ± 1.13 and during maturation phase it was 5.5 ± 0%. At the partial spawning phase it was 5.02 ± 0.2%. During the second stage of maturation it dropped down to 2.06 ± 0.26%. At the total spawning and post-spawning phases it was 2.02 ± 0% (Table 6<sup>a</sup> and Histogram 2).

a3. Total Proteins:

There was no much variation in the total protein gut
level during any of the above said stages. During the regeneration phase of the ovary it was $12.90 \pm 1.4\%$ and a slight decrease during the growth phase ($11.75 \pm 1.6\%$). During maturation phase it was $12.0 \pm 0.1$ and during the partial spawning phase it was $10.92 \pm 0.12$. During the second stage of maturation it was observed as $9.56 \pm 0.75\%$. At the total spawning and post-spawning phases it was $4.2 \pm 0.0\%$ (Table 6, Graph 5 and Histogram 3).

B. LIPIDS:

During the different phases of ovarian cycle no much variation in the level of the lipid in the gut was noticed. However, during regeneration phase it was $0.9 \pm 0.02\%$ and $0.89 \pm 0\%$ during the second maturation phase. At the total spawning and post-spawning phases it was $0.88 \pm 0\%$ (Table 6, Graph 6 and Histogram 4).

C. CARBOHYDRATES:

The carbohydrates level of the gut during the regeneration phase of the ovary was $1.25 \pm 1.2\%$ and during growth phase it was $1.08 \pm 0.16\%$. At the maturation and partial spawning phases there was an increase in the carbohydrate level of the gut ($2.4 \pm 0\%$ and $2.63 \pm 1.6\%$). During the second stage of maturation it dropped down to
0.71 ± 0.36% and at total spawning phase it was 0.98 ± 0%
(Table 6, Graph 7 and Histogram 5).

ORGANIC COMPONENTS:

MALE:

Biochemical changes associated with testes:

A. Proteins:

a1. Soluble Proteins:

The soluble proteins level showed an increase in the testes, from regeneration phase to maturation phase (4.63 ± 0.8% to 14.0 ± 0%). During the partial spawning phase there was a drop in the level of soluble proteins (12.29 ± 0.05%). During the second stage of maturation there was again increase in the level of soluble protein (14.89 ± 1.5%). At the total spawning and post-spawning phases the soluble protein level in the testes dropped down to 1.06 ± 0% (Table 7, Histogram 1).

a2. Insoluble Proteins:

During the regeneration, growth and maturation phases of the testes the insoluble proteins level was maximum (17.15 ± 0.83% to 47.21 ± 0%). But during the partial spawning phase it dropped down to 41.20 ± 1.4%. During second stage of maturation the insoluble proteins level in
the testes increased to $47.05 \pm 1.7\%$. At the total spawning and post-spawning phases it dropped down to $9.32 \pm 0\%$ (Table 7, Histogram 2).

a3. Total Proteins:

The total proteins level of the testes increased from regeneration phase ($21.79 \pm 1.0\%$) to maturation phase ($61.21 \pm 0\%$). During partial spawning phase there was a slight drop in the total proteins level ($53.5 \pm 1.5\%$). Again during the second stage of maturation the total proteins level increased ($61.95 \pm 3.2\%$). At the total spawning and post-spawning phases there was a steep drop in total proteins content of the testes ($10.38 \pm 0\%$) (Table 7 Graph 5 and Histogram 3)

B. LIPIDS:

During the regeneration phase lipids level in the testes was $1.5 \pm 0.12\%$, but during maturation phase the level increased to $6.0 \pm 0.5\%$. In partial spawning phase it depleted to $2.8 \pm 0.1\%$. During the second stage of the maturation again the lipids level increased ($5.9 \pm 1.5\%$). At the total spawning and post-spawning phases it decreased to $1.02 \pm 0.0\%$ (Table 7 Graph 6 and Histogram 4)
C. CARBOHYDRATES:

The carbohydrates level of the testes did not show much variations. During the regeneration phase it was $(1.07 \pm 0.14\%)$, during the maturation phase it was $(2.25 \pm 0\%)$ and it was $1.38 \pm 0.2\%$ during the partial spawning phase. During the second stage of maturation a rise in the level was observed $(2.52 \pm 0.3\%)$. At the total spawning and post-spawning phases it was $1.5 \pm 0\%$ (Table 7, Graph 7 and Histogram 5).

Biochemical changes associated with body wall:

A. Proteins:

a1. Soluble Proteins:

The soluble proteins level was maximum during the regeneration phase of the testes $(19.36 \pm 0.46\%)$. It dropped to $14.66 \pm 0.24\%$ during growth phase and to $14.10 \pm 0\%$ during maturation phase. During further phases it has gradually dropped down to $9.3 \pm 0.8\%$, $9.25 \pm 0.25\%$ and $8.1 \pm 0\%$ respectively (Table 7, Histogram 1).

a2. Insoluble Proteins:

The level of insoluble proteins was maximum in the earlier stages of the testes cycle only to drop down
subsequently. In regeneration phase it was $36.1 \pm 3.9\%$, $29.43 \pm 1.1$ during the growth and at maturation phases it was $29.62 \pm 1.9\%$. During partial spawning phase it was $20.1 \pm 1.9\%$. During the second stage of maturation it was almost the same. At the total spawning and post-spawning phases it was $17.0 \pm 0\%$ (Table 7, Histogram 2).

a3. Total Proteins:

The level of total proteins of the body wall was higher during regeneration phase ($55.46 \pm 4.1\%$). This depleted to $43.72 \pm 0.1$ at maturation phase and to $29.4 \pm 2.6\%$ during partial spawning phase. During the second maturation stage the total proteins level was $29.55 \pm 0.9\%$. At total spawning and post-spawning phases it was $25.9 \pm 0\%$ (Table 7, Graph 5 and Histogram 3).

B. LIPIDS:

During the regeneration phase of testes cycle the lipids level of the body wall was higher $17.31 \pm 0.13\%$. It showed a depletion during maturation phase to $13.25 \pm 0\%$. During partial spawning phase it was $12.35 \pm 0.2\%$. During the second stage of maturation the lipid level further dropped to $10.90 \pm 0.4\%$. At the total spawning and post-
spawning phases it dropped to 6.80 ± 0% (Table 7, Graph 6 and Histogram 4)

C. CARBOHYDRATES:

The carbohydrates level during the different stages of testes did not show much variations. During the regeneration phase it was 1.53 ± 0.14% and 2.15 ± 0% during maturation phase. During partial spawning phase its level was 2.27 ± 2.2%. Further during the second stage of maturation it was 1.97 ± 2.1%. At the total spawning and post-spawning phases its level was 1.68 ± 0% (Table 7; Graph 7 and Histogram 5).

Biochemical changes associated with gut:

A. Proteins

a1. Soluble Proteins:

The soluble proteins level of the gut was 6.16 ± 0.6 and 4.42 ± 0.29% during the regeneration phase and the growth phase. At maturation phase it was 6.27 ± 0% and was 8.0 ± 0.19% during the partial spawning phase. During the second stage of maturation it was 6.25 ± 1.75%. At the total spawning and post-spawning phases it was 5.8 ± 0% (Table 7; Histogram 1).
a2. Insoluble Proteins:

Not much variations were observed in the levels of insoluble proteins in the gut. During regeneration phase the level was $4.06 \pm 1.3\%$ and during growth phase it was $5.28 \pm 0.53$. During the maturation phase it was $3.73 \pm 0\%$ and it was $5.24 \pm 0.34\%$ during the partial spawning phase. During the second stage of maturation the level was $7.75 \pm 2.25\%$. At the total spawning and post-spawning phases it was $6.2 \pm 0\%$ (Table 7, Histogram 2).

a3. Total Proteins:

The total proteins level in the gut was almost steady throughout the gonadal cycle. It was $10.17 \pm 1.0$ during the regeneration phase, $9.71 \pm 0.32$ during active growth phase and $10.0 \pm 0\%$ during the maturation phase. During the partial spawning phase it showed slight rise $13.24 \pm 0.15\%$. During the second stage of maturation it still rose to $14.0 \pm 0.4\%$. At the total spawning and post-spawning phases it dropped to $12.0 \pm 0\%$ (Table 7, Graph 5 and Histogram 3).

B. LIPIDS:

The lipids level of gut did not show much variations during the different phases of testes cycle. During the
regeneration phase it was $0.80 \pm 0.03\%$, $0.85 \pm 1.1\%$ during growth phase and was $0.71 \pm 0\%$ during the maturation phase. During the partial spawning phase the lipids level was $0.76 \pm 1.5\%$. In second stage of maturation it was $0.8 \pm 0.3\%$. At the total spawning and post-spawning phases the lipid level in the gut was $0.75 \pm 0\%$ (Table 7; Graph 6 and Histogram 4).

C. CARBOHYDRATES:

The carbohydrates level in gut during different stages of testes cycle were $0.85 \pm 0.02\%$ during the regeneration phase and $2.2 \pm 0\%$ during the maturation phase. During partial spawning phase the level was $2.65 \pm 1.4\%$. During the second stage of maturation it was $1.06 \pm 0.11\%$. At the total spawning and post-spawning phases it was $1.01 \pm 0.0\%$ (Table 7, Graph 7 and Histogram 5).

INORGANIC COMPONENTS:

Female:

Variations in sodium concentration in the ovary, body wall, and the gut during the different stages of reproductive cycle of *H. atra* are as under.
A. Ovary:

During the regeneration phase of the ovary, the concentration of sodium was 2.04%; during the active growth phase it was 2.97% and during the maturation phase 3.0%. During the partial spawning phase the concentration was 2.42%. During the second maturation phase it was 2.25% and at total spawning phase it 1.2%. (Table 8).

b. Body wall:

The concentrations of sodium in the body wall during the above said different phases of the ovary were 1.79%, 2.12%, 2.62%, 2.67%, 1.74%, and 1.3% respectively.

c. Gut:

The concentrations of sodium in the gut during the above said different phases of the ovary were 2.5%, 3.07%, 8.2%, 2.51%, 3.22%, and 2.7% respectively.

Male:

Variations in sodium concentration in the testes, body wall and the gut during the different stages of reproductive cycle of H. atra are as under.
a. **Testes:**

During the regeneration phase of the testes concentration of sodium was 2.07%, during the active growth phase it was 0.9% and during maturation phase 3.5%; during partial spawning phase the concentration was 1.23%. During the second maturation phase it was 0.9% and at total spawning phase it was respectively (Table 8).

b. **Body wall:**

The concentrations of sodium in the body wall during the above said different phase of gonadal cycle were 2.72%, 2.27%, 2.14%, 3.17%, 2.54%, and 1.0% respectively.

c. **Gut:**

The concentrations of sodium in the gut during the above said different phases were 2.2%, 3.1%, 5.2%, 2.1%, 3.02 and 2.71% respectively (Table 8).

**Female:**

Levels of potassium concentration in the ovary, body wall and the gut during the different stages of the reproductive cycle of *H. atra* are as follows.
a. Ovary:

The levels of potassium in the ovary during the regeneration phases was 0.53%, during active growth phase it was 0.37% and during the maturation phase 0.08. during the partial spawning phase the concentration was 0.03%, and during the second maturation phase it 0.50% and at total spawning phase it was 0.06%.

b. Body wall:

The levels of potassium in the body wall during the above said different phases of the ovary were 0.25%, 0.12%, 0.16%, 0.08%, 0.065% and 0.7% respectively. (Table 8)

c. Gut:

The levels of potassium in the gut during the above said different phases of the ovary were 0.66%, 0.26%, 0.01%, 0.05%, 0.13% and 0.1% respectively (Table 9).

Male:

Variations in potassium concentrations in the testes, body wall and the gut during different stages of the reproductive cycle of H. atra are as described below.
a. **Testes:**

The concentrations of potassium in the testes during the regeneration phases was 0.67%, and during active growth phase it was 0.71%, and during maturation phase 0.13%. During the partial spawning phase the concentration was 0.11% and during second maturation phase 0.10%, and at total spawning it was 0.35% (Table 8).

b. **Body wall:**

The concentrations of potassium in the body wall during the above different phases of the testes were 0.53%, 0.2%, 0.06%, 0.11%, 0.06%, and 0.2% respectively (Table 8).

c. **Gut:**

The concentrations of potassium in the gut during the above different phases of the testes were 0.5%, 1.7%, 0.01%, 0.05%, 0.11% and 0.1% respectively (Table 8).

**Female:**

Variations in calcium concentrations in the ovary, body wall and the gut during the different stages of reproductive cycle of *H. atra* are as given below.
a. Ovary:

The concentrations of calcium in the ovary during the regeneration phase was 0.1%, during the active growth phase 0.9%, and at maturation phase 0.99%. During partial spawning phase it was 0.87%, and during the second maturation phase 0.79% and at total spawning phase it was 0.45% (Table 8).

b. Body wall:

The concentrations of calcium in the body wall during the above said different phases of the ovary were 2.3%, 2.5%, 1.88%, 1.2%, 1.7% and 1.1% respectively. (Table 9)

c. Gut:

The concentrations of calcium in the gut during the above said different phases of the ovary were 1.21%, 1.31%, 0.85%, 0.92%, 1.38% and 1.0% respectively (Table 8).

Male:

Variations in calcium concentrations in the testes, body wall and the gut during the different stages of the reproductive cycle of H. atra are given below.
a. Testes:

The concentrations of calcium in the testes during the regeneration phase was 0.92%, during active growth phase 0.84% and at maturation phase 0.87%. During the partial spawning phase 0.6% and at second maturation phase it was 0.5% and at total spawning 0.32% (Table 8).

b. Body wall:

The concentrations of calcium in the body wall during the above said different phases of the reproductive cycle were 2.0%, 2.1%, 1.98%, 1.0%, 1.5%, and 1.2% respectively. A gradual decline in its level during the gonadal cycle is quite evident (Table 8).

c. Gut:

The concentrations of calcium in the gut during the above said different phases of the reproductive cycle were 1.2%, 1.31%, 0.95%, 0.9%, 1.4%, and 1.02% respectively (Table 8).

Female:

Variations in phosphorus concentrations in the ovary body wall and the gut during the different the stages of the reproductive cycle H. atra are as under.
a. Ovary:

The concentrations of phosphorus in the ovary during the regeneration phase was 1.7%, during active growth phase 0.67% and at maturation phase 0.9%. During the partial spawning phase 0.81% and at second maturation phase it was 0.6% and at total spawning 0.4% (Table 8).

b. Body wall:

The concentrations of phosphorus in the body wall during the above said different phases were 0.23%, 0.69%, 0.09%, 0.5%, 0.06%, and 0.01% respectively (Table 8).

c. Gut:

The concentrations of phosphorus in the gut during the above said different phases were 0.63%, 0.21%, 0.73%, 0.55%, 0.54%, and 0.5% respectively (Table 8).

Male:

Variations in phosphorus concentrations in the testes, body wall and the gut during the different stages in the reproductive cycle H. atra are as under.
a. **Testes:**

The concentrations of phosphorus in the testes during the regeneration phase was 1.3%, during active growth phase 1.4% and at maturation phase 1.68%. During the partial spawning phase 1.59% and at second maturation phase it was 1.37% and at total spawning 1.0% (Table 8).

b. **Body wall:**

The concentrations of phosphorus in the body wall during the above said different phases of the testes were 0.13%, 0.39%, 0.02%, 0.245, 0.085 and 0.9% respectively (Table 8).

c. **Gut:**

The concentrations of phosphorus in the gut during the above said different phases of the testes were 0.59%, 0.18%, 0.6%, 0.4%, 0.51% and 0.42% respectively (Table 8).

**Female:**

Variations in magnesium concentrations in the ovary, body wall and the gut during the different stages of the reproductive cycle of *H. atra* are as described below.
a. Ovary:

The concentrations of magnesium in the ovary during the regneration phase was 1.3%, during active growth phase 1.29% and at maturation phase 1.8%. During the partial spawning phase 1.2% and at second maturation phase it was 1.77% and at total spawning 0.6% (Table 8).

b. Body wall:

The concentrations of magnesium in the body wall during the above said different phases of the ovary were 2.0%, 2.0%, 1.9%, 1.64%, 1.9%, and 1.0% respectively. (Table 8)

c. Gut:

The concentrations of magnesium in the gut during the above said different phases of the ovary were 0.96%, 0.79%, 0.88%, 0.60%, 0.97%, and 0.61% respectively (Table 8).

Male:

Variations in magnesium concentrations in the testes, body wall and the gut during the different stages of the reproductive cycle of H. atra are as described below.
a. Testes:

The concentrations of magnesium in the testes during the regneration phase was 0.9%, during active growth phase 0.89% and at maturation phase 0.96%. During the partial spawning phase 0.5% and at second maturation phase it was 1.0% and at total spawning 0.71% (Table 9). The above said different phases was 0.91%, 0.89%, 0.96%, 0.5%, 1.0% and 0.71% (Table 8).

b. Body wall

The concentration of magnesium in the body wall during the above said different phases of the testes were 1.8%, 1.71%, 1.0%, 1.02%, 1.9% and 0.94% respectively (Table 8).

c. Gut:

The concentration of magnesium in the gut during the above said different phases of the testes were 0.9%, 0.8%, 0.91%, 0.84%, 0.6% and 0.63% respectively (Table 8).

Female:

Variations in chloride concentrations in the ovary, body wall and the gut during the different stages of the reproductive cycle of H. atra are as follows.
a. Ovary:

The concentrations of chloride in the ovary during the regeneration phase was 0.86%, during active growth phase 0.91% and at maturation phase 0.99%. During the partial spawning phase 1.1% and at second maturation phase it was 1.55% and at total spawning 1.53% (Table 8).

b. Body wall:

The concentrations of chloride in the body wall during the above said phases of the ovary were 1.71%, 1.94%, 2.0%, 2.41%, 2.54%, and 2.7% respectively (Table 8).

c. Gut:

The concentrations of chloride in the gut during the above said phases of the ovary were 1.17%, 1.23%, 1.4%, 1.23%, 1.29%, and 1.2% respectively (Table 8).

Male:

Variations in chloride concentrations in the testes, body wall and the gut during the different stages of the reproductive cycle of H. atra are as follows.
a. Testes:

The concentrations of chloride in the testes during the regeneration phase was 0.73%, during active growth phase 0.83% and at maturation phase 0.88%. During the partial spawning phase 0.93% and at second maturation phase it was 1.16% and at total spawning 1.15% (Table 8).

b. Body wall:

The concentrations of chloride in the body wall during the above said different phases of the testes were 1.75%, 1.91%, 2.0%, 2.10%, 2.34% and 2.6% respectively (Table 8).

c. Gut:

The concentrations of chloride in the gut during the above said different phases of the testes were 1.04%, 1.16%, 1.25%, 1.54%, 1.82% and 1.60% respectively (Table 8).

A. Gonad caloric values:

a. Proteins caloric value:

During the regeneration phase of the testes and the ovary the caloric values of proteins were 515.11 cal/gm and 592.65 cal/gm respectively. During active growth phase the
values increased to 684.14 cal/gm and 638.75 cal/gm. During the maturation phase there was a further increase to 1447.00 cal/gm and 1655.03 cal/gm respectively. A depletion was observed during partial spawning phase (1264.74 cal/gm and 1274.19 cal/gm). Again during the second maturation phase another increase was noticed (1464.4 cal/gm and 1729.50 cal/gm) which dropped finally to 245.38 cal/gm and 307.32 cal/gm during the total spawning phase (Table 9).

b. Lipids caloric value:

During the regeneration phase of the testes and the ovary the lipid caloric values were 59.32 cal/gm and 76.33 cal/gm respectively. Subsequently there was a rise 126.56 cal/gm and 158.20 cal/gm during the active growth phase. During maturation phase again there was a further increase to 237.30 cal/gm and 280.80 cal/gm, while during the partial spawning phase the values dropped to 110.74 cal/gm and 146.33 cal/gm respectively. During the second maturation phase again an increase was noticed (233.34 cal/gm and 40.34 cal/gm), and in the course of total spawning the values dropped to 40.34 cal/gm and 59.32 cal/gm respectively (Table 9).
B. Caloric values in body wall:

The body wall caloric value also fluctuate seasonally with an inverse relationship with the gonad index (G.I.) and also with the biochemical variations in the body wall and gonads. The caloric values of the body wall of the male and female of *H. atra* are 1311.07 cal/gm and 1521.47 cal/gm during regeneration phase, 1042.28 cal/gm and 1263.32 cal/gm during the active growth phase, 1033.54 cal/gm and 543.14 cal/gm during the maturation phase, 695.56 cal/gm and 626.46 cal/gm during the partial spawning phase. At the total spawning phase the caloric values of the body wall were 593.64 cal/gm and 472.80 cal/gm for proteins, and 684.61 cal/gm and 765.88 cal/gm, 578.22 cal/gm and 605.11 cal/gm, 524.03 cal/gm and 609.46 cal/gm, 488.44 cal/gm and 573.87 cal/gm, 431.60 cal/gm and 472.62 cal/gm and 268.94 cal/gm and 338.15 cal/gm for lipids during the above said phases of the testes and ovary respectively (Table 9).

The protein caloric value in the body wall was high in the regeneration phase of both sexes v.i.z. the males and females respectively. (M:1311.07 cal/gm and F:1521.47 cal/gm), correspondingly in the gonads it was less (M:515.11 cal/gm and F:592.65 cal/gm), in maturation phase the caloric
value was higher in the gonads (M:1447.0 cal/gm and F:1655.03 cal/gm), and in the body wall it was lower (M:1033.54 cal/gm and F:543.24 cal/gm). Likewise in partial spawning phase there was a decline in the caloric value in the gonads (M:1264.74 cal/gm and F:1274.19 cal/gm). Again in the second maturation phase there was an increase in the caloric value of the gonads (M:1464.49 cal/gm and F:1729.50 cal/gm). Finally during the total spawning phase it declined to M:245.38 cal/gm and F:307.32 cal/gm respectively. Similarly, the caloric values of lipid also showed seasonal fluctuations corresponding to values of the gonad index and also to the biochemical variations in the gonads. Compared to the caloric values of proteins those of the lipids are decidedly lower in H. atra as observed by us. Likewise the caloric values of carbohydrates compared to those of proteins are also lower, and they do not show any definite relationship with those in the other two tissues during the different phases of the gonadal cycle of H. atra. Hence, the proteins form the major contributors to the caloric values, in H. atra of Karwar coast.
HISTOCHEMICAL OBSERVATIONS:

A. PROTEINS:

Mercury Bromophenol Blue (MBB) Reaction:

The occurrence of proteins is indicated by the blue colour in the tissues. The ovarian wall showed an intense MBB +ve reaction as evident by blue colour. The cytoplasm of small oocytes showed a moderate blue colouration. The nucleus, nucleolus and chromatin were also MBB +ve in reaction. As the oocytes increased in size the blue colour increased in intensity. The oocytes in the post spawning phase of the ovaries showed a moderate MBB +ve reaction in their cytoplasm. The vitelline membrane in the larger oocytes also showed a MBB +ve reaction (Figs. 48 and 49).

B. LIPIDS:

Sudan Black B Reaction:

The vitelline membrane and cytoplasm of the young oocytes showed a mild bluish grey colour reaction showing the presence of phospholipids; but matured oocytes were densely packed with deep bluish black coloured droplets indicating accumulation of neutral lipids in them (Figs. 50 and 51). Section of ovaries pretreated with a hot mixture of chloroform and acetone followed by staining with SBB
showed only traces of bluish black colouration and served as control (Fig. 52).

Oil Red O Reaction:

The smears of younger oocytes showed a mild ORO +ve reaction around the nucleus and while the matured ones showed a strong ORO +ve reaction around the nucleus indicating an increasing deposition of neutral lipids with the growth of the oocytes forming the lipid part of yolk (Fig. 53).

C. CARBOHYDRATES:

Periodic Acid Schiff's (PAS) Reaction:

The ovarian wall showed an intense PAS +ve reaction. The cells of the germinal epithelium were basophilic and showed a weak PAS reaction. In the growing oocytes the cytoplasm was acidophilic and showed a moderately intense PAS +ve reaction. The mature oocytes showed an intense PAS +ve reaction. This increasing intensity in the PAS reaction with the growth of the oocytes indicated increasing deposition of carbohydrates in the growing oocytes. Albeit, quantity wise carbohydrate deposition is far less, when compared with proteins deposition (Figs. 31-33).
Best's Carmine Reaction:

The Best's carmine +ve pink reaction in tissues indicates presence of glycogen therein. The ovarian wall and the cytoplasm, nuclei and nucleoli of the young or matured oocytes showed only traces of Best's carmine reaction which indicated the presence of glycogen in these tissues only in traces (Fig 34). The muscles of the body wall on the other hand showed an intense reaction indicating a rich amount of glycogen therein (Fig. 35). This preparation served as a positive control.

D. Acid Macopolysaccharides:

Toludine blue and alcian blue staining:

The muscular layer of the ovarian wall, the underlying connective tissue in the genital haemal sinus and also the basement membrane of the germinal epithelium of the ovary showed a strong β-metachromasia and alcianophilia with alcoholic toludine blue and alcian blue respectively. While the very young vitellogenic oocytes did not show either of such reactions, the vitellogenic as well as mature oocytes did show strong β-metachromasia and alcianophilia in the vitelline membrane (Figs. 36, 44 and 45). Further, strongly
β-metachromatic granules appear in the cytoplasm of the former and increase in number with their growth (Figs. 37-40). Metachromasia was abolished in sections treated with hyaluronidase prior to staining with toludine blue (Fig. 41). Sections of ovary in the post-spawning phase also showed β-metachromasia in the vitelline membrane of some oocytes that have failed to get released.

Sections of testes during the growth and mature phases showed strong β-metachromasia and alcianophilia in the muscular and connective tissues of the wall and also in the basement membrane of the germinal epithelium. Further, the cells of the germinal epithelium and also the spermatogonial cells showed orthochromasia with toludine blue and no alcianophilia with alcian blue. But spermatids and the sperms did yield positive results with both these stains (Figs. 42, 43, 46 and 47). Owing to an extremely regressed condition, testis tubules in the post-spawning phase could not be studied.

Discussion:

A number of biochemical studies have been carried out on many higher invertebrates including crustaceans, molluscs and echinoderms to evaluate their nutritional values.
(Ansell***, 1972 and Bergman* 1949). Among the echinoderms, only in two echinoids and a few asteroids biochemical compositions of different tissues have been studied in detail during the reproductive cycle. Following are some of the details. In *Paracentrotus lividus* for the first time free and bound amino nitrogens were detected in the maturing testes (Russo** 1926). The author concluded that the testes were storage organs for nitrogens utilised during spermatogenesis. In *Echinus esculentus* large amounts of glycogen were detected in the gonadal tissues and hence the gonads were to be considered the storage organs of glycogen (Stott** 1933). In *Pisaster ochraceus* and *P. giganteus* total proteins, carbohydrates, lipids and non-proteinous nitrogen (NPT) in the gonads have been studied (Greenfield et al., 1958). In *Oreaster hedemanni* the quantitative estimation of proteins, lipids (including the fatty acids) and glycogen of the gonads have been recorded (Giese et al., 1964). In *Asterias vulgaris* the total proteins, lipids, carbohydrates, (glycogen), amino acids, and the nucleic acids RNA and DNA in the testes have been studied. (Smith and Walker, 1986).

* Cited in Krishnan (1968)
** Cited in Boolootian (1966)
*** Cited in Jayashree (1988)
Our knowledge on the general biochemical constituents of the holothurians is much less compared to that on the toxins. (Taniwaka et al., 1955; Taniwaka and Yamahita, 1955; Boolootian, 1966; Krishan, 1968; Lawrence, 1972; Prim et al., 1976; Sibuet and Lawrence, 1981).

In many echinoderm species the prodigious amount of nutrients utilized during intensive gametogenesis are retrieved from specialised extra gonadal storage organs or intra-gonadal cells. Many authors (Delaunacy, 1926b; Cuenot, 1948; Farmanforman et al., 1958; Cognetti and Delavault, 1962; Pearse, 1965a & b; Mauzey, 1966; Anderson, 1966; Ferguson, 1975a & b; Nimitz, 1976; Jangoux and Impe, 1977 and Bellary, 1989) have demonstrated that the pyloric caeca are the storage organs and show a reciprocal relationship with the gonads in asteroids from the point of nutrient storage. On the otherhand in Odontaster validus proteins, carbohydrates, lipids and total nitrogen are stored in the gonads as well as the pyloric caeca (Pearse, 1985).

In echinoderms the genital haemal sinus is considered to play a crucial role in atleast temporary nutrient storage.

* Cited in Boolootian (1966)
** Cited in Jangoux and Lawrence (1982)
*** Cited in Krishnan (1968)
and in the translocation of these nutrients to the developing gametes (Walker* 1982; Smiley 1988c; Smiley and Cloney 1985) a view contrary to that of Hassin (1978) who has shown that during the period of the gonadal activity the cucumber, Holothuria tubulosa if subjected to starvation, draws the nourishment from the body wall and not from the fluid of the genital hemal sinus.

In Echinaster spp. amino acids, carbohydrates and fatty acids in the gonads as well as pyloric caeca (Ferguson 1975 a, b and 1976) and proteins, carbohydrates and lipids in the gonads and pyloric caeca have been estimated (Scheibling and Lawrence 1982). In the above said asteroids the metabolites are first stored in the pyloric caeca and are subsequently transported to the developing gonads. In echinoids ovarian and testicular nutritive phagocytes act as storage-cells (Fugi, 1960a; Giese* 1961; Farmanfarmaian and Phillip* 1962; Holland* 1965b, 1967b; Holland* and Giese 1965; Lawrence et al., 1965). In crinoids the intra-gonadal accessory cells and the body wall play a role in the storage of nutrients (Chubb 1906, Harvey* 1930 and Holland and Kubota, 1975). In holothurians the wall of the gut,

* Cited in Jangoux and Lawrence (1982)
visceral peritoneum and the body wall act as nutrients storage organs (Krishnan 1968). In H. tubulosa and H. atra no change in the organic levels after starvation or during the reproductive cycle was observed (Lawrence 1972 and Masin 1978). Hence, Masin (1978) did not consider the haemal system as a reserve place and also pointed out that if starvation occurred during the period of gonadal activity of the body wall would have been mobilized.

Nutrient supply to the gonads during the periods of their activity has been investigated in most of the biochemical studies on holothurians.

In Eumolpadia violacea no reciprocal change in the amount of biochemical components between the ovary and the other body tissues was found, suggesting that no organ accumulates nutrient reserves available for another, particularly for the gonads. The storage of nutrient reserves seems to take place directly in the ovary (Feral and Magniez 1985). In Stichopus seasonal variations in the organic constituents of the body wall have been attributed to the demands by the animal during hibernation as well as during the gonadal activity (Tanikawa et al., 1955).

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* Cited in Boolootian (1966)
In *H. scabra* proteins and carbohydrates of the body wall are used for gametogenesis (Krishnan, 1968). In *Eostichopus regalis* the body wall contained higher levels of energy. The same is true of bathyal and abyssal holothuroids (Walker et al., 1987a & b, and Mc Clintock, 1990). The body wall acts as a storage organ in *H. leucospilota* (Jayashree, 1988) and *Pentacta pygmae* (Jesperson and Lutzen, 1971). Further, from the body wall these nutrients are supplied to developing gonads in *Synapta hydriformis* (Prim, et al., 1976). In *Neopentadactyla mixta* besides the body wall intestine also stores the nutrients for their subsequent transfer to the gonads (Smith, 1981). In *Cherbonniera utriculus* the direct transfer of energy in the form of metabolic nutrients from the gut to the developing gonads takes place (Tyler et al., 1987).

From our studies on *Holothuria atra* on the biochemical components of the gut, body wall and gonads it is evident that the biochemical components of only the body wall and the gonad show reciprocal relations during different phases of the reproductive cycle. This suggests that the body wall acts as the major nutrient reserve from where the latter are transported to the gonads during their growth and maturation phases (Graphs 5-7).
Mechanism of nutrient transfer from organs of ingestion or extra gonadal storage to the gonads have been thoroughly investigated only in the Asteroidea and Echinoidea. In Asterias rubens by autoradiographic studies provides evidence for a role of the haemal system in the transport of nutritive materials (Beijnink and Voogt 1984). Such studies have not been done in any other echinoderms so far.

Glycogen is always at low levels in various tissues of echinoderms in general, except in the gonads of Echinus esculentus (Stott, 1931) and in the longitudinal muscles of the body wall of holothuroids. But the same cannot be said of proteins and lipids. In different species of asteroids and holothuroids the levels of proteins and lipids vary antagonistically indicating their relative importance in the animals metabolic activities. Following is some information on this aspect.

In Pisaster ochraceus (Greenfield, 1958), Asterias rubens (Oudjeans, et al., 1979), Echinaster spp (Scheibling and Lawrence 1982) and Astropecten polyacanthus (Bellary, 1989) the proteins form the major components in the gonads and pyloric caeca. In Astropecten polyacanthus during the

* Cited in Boolootian (1966)
different phases of reproductive cycle compared to protein level the lipid level is less (28.2 ± 2 proteins and 6.88 ± 0.92 lipids) during the regeneration phase (Bellary, 1989). In *Pisaster ochraceus* and *P. giganteus* the lipid levels increase during gametogenic stages and decrease during the spawning and post spawning phases in accordance with accumulation and depletion of lipids in pyloric caeca (Greenfield, et al., 1958). In *Echinaster* spp also the same pattern of accumulation and depletion has been observed as in other asteroids (Ferguson, 1975, b and 1976). In *Asterias rubens* (Jangoux and Impe 1977; Oudejans and Sluis, 1979) *Asterias forbesi* and *Oreaster hedemanni* (Rao and Rahaman, 1968) the total lipid level increases rectilinearly in the ovaries up to maturation phase, this view has been corroborated by Scheibling and Lawerence (1981 and 1982).

In the holothuroids, *Oneirophanta mutabilis*, *Deima validum*, *Benthodytes sordida* and *Psychropotes longicauda* the insoluble protein in the dominant fraction in both ovary and testis (Tyler and Billette, 1987). Here one notices a bit of self contradiction by the authors Tyler and Billette (1987, p. 286) regarding the soluble and insoluble protein levels in the testes of species they have studied. In adult *Cucumaria curata* the protein content was high (Turner and
Rutherford, 1976). In Eostichopus regalis protein is the primary organic constituent of body wall (Mc Clintock, 1990). Protein is high in body wall and the gonads of Holothuria leucospilota (Jayashree, 1988).

In Cucumaria elongata lipid was an important food reserve and it declined during gametogenesis (Fish 1967). In Cucumaria planci the lipid level was 0.31. In Oneirophanta mutabilis, Deima validum, Benthodytes sordida and Psychropotes longicauda the soluble lipid value is high in the ovary (Tyler and Billette, 1987). In the ovaries of Actinopyga, Stichopus, Parastichopus, and Pentacta, the protein levels are 18.6%, 20.7%, 18.1% and 22.6%. Comparable to proteins the lipid level is low i.e., 1.5%, 5.1%, 7.5% and 1.5% respectively (Prim et al., 1978) and in the ovary and testes of Leptosynapta galliennei it is 94.6% and 39% respectively (Feral 1985). In Laetomogone violacea in both testes and ovary it is 291.4% and 116.8% (Walker et al., 1987), whereas the ovary of Eumolpadia violacea in July it was higher 13.9% comparable to proteins (9.2%) while lower in February 22.6% comparable to protein (26.2%) (Feral and Magniez, 1985) and in Labidoplax digita 10% (Lawrence and Guille 1982).
In *H. scabra* amount of lipid was the second most important factor and was utilised during gametogenesis (Krishnan, 1968). In *Holothuria difficilis* the lipid level was 2.71%. In *Holothuria glaberrima* it was 3.8%, *Holothuria grisea* 2.5% and *Holothuria hilla* 3.6% (Lawrence and Guille, 1982).

In *H. atra* the lipid levels did not change during starvation and the author concluded that contribution of intestinal lipids to the gonads development was undoubtedly small (Lawrence, 1972).

During our study the lipid levels in the gut, body wall and gonads of *H. atra* are low compared to proteins.

Our comparative studies on lipid levels in gut, body wall and gonads of *H. atra* also show an inverse relationship exists only between the body wall and gonads during the different stages of reproductive cycle. The level of lipids is higher in the females than the males in all these tissues. In both the sexes, the lipids are transported to the gonads probably from the body wall during the stages of gonadal activities.
Lawerence and Guille (1982) found no evidence of geographic distribution oriented variations in the biochemical compositions of the body wall and pyloric caeca of asteroids (9 species), the tests, spines and lantern of echinoids (13 species), the disc and arms of ophiuroids (11 species) and the body wall of holothuroids (17 species) including H. atra from Eniwetok Atoll of the tropical Pacific ocean. However, their studies do not cover the gonads. Information on the levels of proteins, lipids, carbohydrates in the gonads of some holothurian species from different geographical regions is available in the recent literature (Prim et al., 1985; Feral, 1985; Feral and Magniez, 1985; and Walker et al., 1987).

Following are some details of the same.

Indian ocean sub-temp. (49° 50' S and 69° 30' E).

Feral and Magniez (1985)

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Proteins %</th>
<th>Lipids %</th>
<th>Carbohydrates %</th>
</tr>
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<tr>
<td>Eumolpadia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>violacea</td>
<td>(July)</td>
<td>9.2</td>
<td>13.9</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>(Feb) 26.2</td>
<td>22.6</td>
<td>6.2</td>
</tr>
</tbody>
</table>
July and February months fall in the austral winter and summer respectively. An equal preference for protein dependent metabolism is evident from these figures.

Sub temperate region (49° 51'N and 12-14°W).
Walker et al., (1987)

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Proteins %</th>
<th>Lipids %</th>
<th>Carbohydrates %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laetomogone</td>
<td>Ovary</td>
<td>96.4</td>
<td>291.4</td>
<td>12.3</td>
</tr>
<tr>
<td>-violacea</td>
<td>Testes</td>
<td>210.2</td>
<td>116.8</td>
<td>11.4</td>
</tr>
<tr>
<td>Benthodytes</td>
<td>Ovary</td>
<td>67.4</td>
<td>117.6</td>
<td>6.2</td>
</tr>
<tr>
<td>sordida</td>
<td>Testes</td>
<td>170.1</td>
<td>144.6</td>
<td>19.0</td>
</tr>
</tbody>
</table>

However, in these species protein level is higher in the testes.

Roscof Brittany coast France (48° 0'N and 3.0° 0'W).
Feral (1985)

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Proteins %</th>
<th>Lipids %</th>
<th>Carbohydrates %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptosynapta</td>
<td>Ovary</td>
<td>68.9</td>
<td>94.6</td>
<td>11.9</td>
</tr>
<tr>
<td>galliennei</td>
<td>Testis</td>
<td>82.2</td>
<td>39.1</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Tropical/subtropical region (24° 41'N and 81° 11'W; and 27° 31'N and 82° 45'W). Prim et al., (1976).

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Proteins %</th>
<th>Lipids %</th>
<th>Carbohydrates %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinopyga agassizi</td>
<td>Ovary</td>
<td>18.6</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Synaptula hydriformis</td>
<td>Ovary</td>
<td>28.1</td>
<td>6.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Parastichopus californicus</td>
<td>Ovary</td>
<td>18.1</td>
<td>7.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Pentacta pygmaea</td>
<td>Ovary</td>
<td>22.6</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Stichopus japonicus</td>
<td>Ovary</td>
<td>20.7</td>
<td>7.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

H. atra Karwar coast. (14°18'N: 74°97'E)

<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>Proteins %</th>
<th>Lipids %</th>
<th>Carbohydrates %</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. atra</td>
<td>Ovary</td>
<td>73.16</td>
<td>7.50</td>
<td>6.31</td>
</tr>
<tr>
<td></td>
<td>Testes</td>
<td>61.95</td>
<td>6.00</td>
<td>2.25</td>
</tr>
</tbody>
</table>
The above mentioned data on the levels of proteins and lipids at least in the ovaries do show some geographical variations. As such our view differs from that of Lawrence and Guille (1982). In the species of tropical region or close to it the metabolic activities of the ovaries are more protein dependent in contrast to those in the temperate and sub-temperate regions which are more lipid dependent. With reference to amphibians Loft (1984; p.175) has opined that the winter accumulation of cholesterol-lipoidal material in the follicle cells of temperate species probably reflects a lowered metabolic activity and synthesis of estrogenic hormones during hibernation period with a consequent accumulation of precursor material v.i.z. the lipids. Further, Loft (1984; p.169) also states that during hibernation the mature oocytes are not inactive as indicated by increasing amount of smooth endoplasmic reticulum and lipid content. Added to this Smith et al., (1968 – vide Loft, 1984) have shown by isotopic and autoradiographic studies a lowered rate of protein synthesis in the oocytes of species Rana and Bufo. To conclude, it is evident that the ovarian metabolism is more lipid dependent in the temperate anurans.
Carbohydrates (glycogen) known for their instant supply of energy are stored in various tissues or organs in various animal groups. But so far as holothuroids in particular and echinoderms in general are concerned, these molecules are the least preferred ones, for the obvious reason that these animals are known for their sluggishness and their passive life never demands instant supply of energy. This is particularly so in holothurians.

Compared to the proteins carbohydrates also are the minor constituents in the gonads of Pisaster ochraceus, and P. giganteus (Greenfield et al., 1958), Oreaster hyndmanni, Asterias vulgaris (Smith and Walker 1986; and Rahman, 1968) and Echinaster spp (Ferguson, 1975b) the glycogen level does not vary during gonadal cycle; while in Asterias rubens (Jagnoux and Impe, 1977, Oudejans et al., 1979) it increased to a maximum during maturation stage.

It is only in Stichopus mollis that substantial deposits of glycogen are noticed in the longitudinal muscles of the body wall but not in the gonads (Freeman and Simon, 1964). In Actinopyga agassizi, Synaptula hydridimbus and Pentacta pigmaea the low levels of carbohydrates are reported in the body wall and gonads (Prim et al., 1976) In
Cucumaria elongata there was no evidence of glycogen in the body wall (Fish, 1967a).

In Holothuria leucospilota low levels of carbohydrates have been recorded (Jayashree, 1985). Low carbohydrate values could be due to the fact, the glycogen, the usual storage carbohydrate in many marine animals does not contribute much to the nutritional reserves in the body of holothurians (Raymont and Krishnaswamy, 1960, and Raymont and Canover, 1961).

Our present study on H. atra shows that the carbohydrates level remains low and fairly constant in the gut, body wall and gonads throughout the reproductive cycle. Compared to the proteins, glycogen in all these tissues of H. atra is decidedly insignificant as are the lipids too. These facts point out that in H. atra energy supply is mostly protein dependent at least as far as the reproductive events are concerned. Further, our findings corroborate the views of Prim et al., (1976), and Jayashree (1988).

Compared to the biochemical information on the major organic components viz., proteins, carbohydrates and lipids, information on the inorganic composition of different
tissues of holothuroids is limited to only two studies. Li-Zing et al., (1989) have determined eleven micro-elements in the tissues of Stichopus japonicus, while Siddiqui et al., (1988) have carried out estimations of micro and macro-elements of the sea-cucumber Actinopyga mauritiana along with a few different invertebrate species, viz. the coelentrate Aurelia sp., the crabs Portunus pelagicus, P. sanguinolantus and Scylla serrata, the gastropod Telescopium telescophium and the starfish Astropecten indicus. The macro-elements studied were sodium, potassium, calcium, magnesium and phosphorus, while the micro-elements studied were manganese, copper, iron, zinc, nickel, cobalt, lead, and cadmium. Since we could not get copies of the article by Li-Zing et al., (1989), despite our persistent efforts, we are unable to record here any more information regarding their findings on Stichopus japonicus. Eventhough due to the non-availability of copies of the articles by Siddiqui et al., (1988) also, we have recorded here some information available on their findings only from the abstract obtained from National Institute of Oceanography (NIO), Goa (India). In all the invertebrates studied by these authors iron and zinc concentration were the highest. Lead was detected only in the sea-cucumber A. mauritiana and the starfish A.
indicus. Nickel, cobalt and cadmium were present in the starfish A indicus and the coelenerate Aurelia sp. The authors mention that the concentration of these metals was high enough to be alarming. However in both these studies no mention has been made about the relationship of these inorganic metals with the reproductive cycles of any of the species examined.

In our present work an attempt made in that direction has yielded some results probably indicating their direct and indirect roles in the reproductive cycle of H. atra. Following are some of the details.

In the females Ca\textsuperscript{++} levels are high throughout the gonadal cycle except during the total spawning phase, a feature that may be correlated with vitellogenesis. It is shown in the case of Sea-urchin eggs Ca\textsuperscript{++} is essential to hold the blastomeres together during cleavages (Tyler and Tyler*, 1966). Our studies have also shown a high level of calcium in the ovaries (oocytes) of H. atra during the maturation phase. However, these levels are lower compared to the levels of Mg\textsuperscript{++}. During the spawning phases (I & II), an appreciable regression of Mg\textsuperscript{++} levels is noticed. Mg\textsuperscript{++} is

* Cited in Boolootian (1966)
closely associated with muscle phosphatases. As such a
considerable drop in the levels of Mg$^{++}$ during spawning
phase may be attributed to high utilisation of Mg$^{++}$ in the
increased activity of this enzyme that brings about muscular
contraction of the gonadal tubules.

In adult life of animals, the ratio of
calcium:phosphorus should be about 1:1.5. If either is
present in an inadequate amount the other is not utilized
properly even through it is present in normal quantity
(Kliener and Orten, 1962). In H. atra we notice in both the
ovary and testes this ratio to hold good in the gonadal
recrudescence stage. During rest of the stages however it
falls to almost 1:1.

In animals sodium is essential for contraction of
involuntary and voluntary muscles, while potassium ions
inhibit this process (Kliener and Orten, 1962, and Chatterjee
and Banerjee, 1966). In addition, carbohydrate (glycogen in
particular) metabolism is intimately related to potassium
level in the plasma of vertebrates. The relationship is
inverse. During the gonadal cycle of H. atra carbohydrate
level remaining lowest throughout, the role of K$^+$ in this
regard appears to have a limited significance. A gradual
but slight increase in the level of $K^+$ during the first gonadal maturation phase from 0.53% (regeneration phase) to 0.80% may be correlated with facilitating the muscular distension of the ovarian wall. Further, in regulating the contraction - relaxation process of the gonadal wall (muscles) both $Na^+$ and $K^+$ involve themselves and act in an antagonistic manner in such a way that from regeneration to 1st maturation phase the gonads bulge in volume, followed by a little shrinkage in individual tubules at partial spawning phase and once again increasing in volume during the maturation phase and $\sigma$ shrink finally during the total spawning phase. Since the gonadal samples were taken immediately after the spawning event, the levels of $Na^+$ and $K^+$ were still far from the expected ones allowing the tubules still remain in a bit relaxed state. The views expressed here a just suggestions that need experimental verification.

Chloride level in the gonads of the both the sexes in $H. atra$ as our results reveal shows a steady increase from the regeneration phase to the first maturation phase and then to the complete spawning phase. It is admittedly difficult to establish any direct relationships between the chloride levels and the physiology of gonads in $H. atra$ in
the absence of similar studies elsewhere in the field of invertebrate reproductive physiology. On the other hand, as far as we are aware there is solitary reference by Spies and Chappel (1984) to chloride level and ovulation in the non-human primate Cynomolgus macaques. Spies and Chappel (1984) observe "in Cynomolgus macaques increases in the concentrations of chloride ions within the cervical mucus give several days notice of impending ovulation. If the basic principles of physiological roles of various organic and inorganic components are the same in the gonadal physiology of animals irrespective of their taxonomic status, then a steady increase in the level of chloride ions in the gonads of H. atra during both the spawning phases may indicate the impending spawning by this animal also. Of course this may be taken as a suggestion at present that needs confirmation from similar studies on other animal groups.

Caloric values:

In their studies on the caloric values of many species of echinoderms including 19 species of holothuroids H. atra being one among them only body wall has been examined (Lawrence, 1985).
In the asteroid *Luidia clathrata* (Lawrence, 1973) on subjecting the specimens for starvation for one month, the resulting decrease in the size of the pyloric caeca is shown to have produced 2.048 Kcal. and the energy requirement over the period calculated from QO2 values is 2.112 K.cals. In the following asteroids the caloric values K.cals. of body wall and pyloric caeca are respectively 3.0 and 0.4 (*Asterina frigida*) and 87.9 and 70.2 (*Anasteria perriri*). In the following two ophuroids the caloric values of disc and arms are 0.138, 0.511, (*Amphiura antarctica*) and 0.138, 0.293 (*Amphiura filiformis*) in the tests, spines and lantern of the two echinoids studied, viz., *Echinometra lucunter* and *Colobocentrolus atratus* these values are 79.5, 93.7 and 14.2, in the former while they are 10.9, 11.3 and 0.8 in the latter (Lawerence and Kafri 1979).

In the holothurian, *Benthodytes sordida* (Tyler and Billett, 1987) the calorific content of whole ovary varies from 25.46 J mg\(^{-1}\). In *Deima validum* 28.68 J mg\(^{-1}\) (Tyler and Billett, 1987). The caloric values of the testes of the Psychroptid species (Tyler and Billett, 1987) is very similar owing to the very high protein levels.
The body wall caloric values in the species examined are as follows, Benthogone rosea (2.519 K cal g\(^{-1}\)), Paelopatides gigantea (2.903 K cal g\(^{-1}\)) Psychropotes longicauda (2.758 K cal g\(^{-1}\)). (Lawrence and Kafri, 1979), the highest in Actinopyga mauritiana (1475.6) and the lowest was Pentacucumis bovetensis (0.6) (Lawrence and Guille, 1982).

In species of the genus Holothuria, the body wall caloric values are as given in the parentheses like H. difficifis, (2.0), H. glaberrima (73.7) H. hilla (12.6) and H. leucospilota (103.8) (Lawrence and Guille 1982).

In H. leucospilota (Jayashree, 1988) the caloric values in the body wall fluctuate seasonally with the high caloric content corresponding to the high values in gonad index. The high caloric values were recorded during Aug 1985 (1010.48cal/gm) and during Apr (817.78 cal/gm), which was evidenced from the biochemical variations in the gonads and in the body wall.

In the H. atra of Eniewetak Atoll, Marshall Islands, the body wall energy content is 106.3 KJ Ind\(^{-1}\). (Eberts, 1978).
Our findings on the caloric values of H. atra of Karwar are in agreement with those by Jayashree (1988), in that the caloric values in the body wall and gonads fluctuate seasonally with high caloric content corresponding to the high values in gonad indices. In both species v.i.z. H. leucospilota and H. atra the major contributors to organic material and consequently to calories are the proteins.

The accumulation/depletion of proteins carbohydrates and lipids in various tissues during the reproductive cycle has been examined histochemically in the following species of asteroids v.i.z. Leptasterias hexactis (Chia, 1964 and 1968), Pisaster ochraceus (Mauzey, 1966), Patiria miniata (Nimitz, 1976), Bathyiaster vexillifera (Tyler, et al., 1982), Hymanaster membraneous and H. giannaeus (Pain, et al., 1982b), Brisinga endecacnemos and Freyella spinosa (Tyler et al., 1984), Asterias rubens (Schoenmakers, et al., 1981 and 1984), Plutonaster bifrons and Psilaster filholi and Pontaster lenuis (Pain et al., 1982a), Astropecten polyacanthus (Bellary, 1989). Similarly the echinoid species examined from this aspect are Hemicentrotus pulcherrinus (Nakuna and Ohashi, 1954), Echinus esculentus and Echinocardium cordatum (Immers, 1960) Paracentrolus lividus and P. milliaris, (Sverker, 1966),
Strongylocentrotus purpuratus (Chatlynne, 1969)  
Strongylocentrotus drobachiensis (Bal, 1970), Salmacis virgulata (Vivekraja 1979) and Heliocidaris erythrogramma and H. tuberculata (Laegdsgaard, et al., 1991). The authors have concluded that, there is an increased accumulation of proteins, lipids and carbohydrates as the vitellogenesis progresses.

Among the holothurians vitellogenesis has been studied by histochemical methods in a few species, and they are Holothuria scabra (Krishnan, 1968), Ypisolithuria talismani (Tyler and Gage 1983), Benthodytes sordida, Oneirophanta mutabilis, Psychropotes depressa and P. semperiana (Tyler and Billett, 1987) Cherbonneiera utriculus, Molpadia blakei (Tyler, et al., 1987) and Stichopus californicus (Smiley, 1988).

Krishnan (1968) has noted an increased protein deposition in both the ovary and testes of H. scabra as evidenced by an increased intensity of mercuric bromophenol blue (MBB) reaction. He accordingly has inferred that proteins formed the major organic component; the next in the order were the lipids and then the carbohydrates (glycogen). Our histochemical findings on H. atra are in
agreement with those of Krishnan (1968), and are well supported by our biochemical findings too. Similarly Krishnan (1968) suggested that in comparison to proteins, carbohydrates and lipids appeared to be stored in smaller quantities and glycogen seems to be in traces in gonads while present appreciably in the body wall. And it serves as potential source of energy. Our findings too corroborate Krishnan's (1968) views.

Acid mucopolysaccharides the polymers of hyaluronic acid and of chondroitins A, B and C (Kleiner and Orten, 1962 and Barka and Anderson, 1963) are components of cell coats and serve as a sticky cement (Conn and Stumpf, 1976). They also form an effective barrier in animal tissues against microbial infection (Oser, 1976). Hence it is reasonable to expect their occurrence in the extraneous coats/membranes of gametes of vertebrates and invertebrates. They also occur in the connective tissue of various animals. They occur in the yolk spherules, cortical granules and the jelly coat of the eggs of the echinoids Hemicentrotus pulcherrinus, (Nakuna and Ohashi, 1954) and Paracentrotus lividus (Pasteels et al., 1958, and Perlman et al., 1959), in the cortical region of oocytes of Echinus esculentus, Echinocardium cordatum and Psammachinus millarius (Immers,
1960), in the vitelline membrane of the oocytes of the crinoid Comanthus japonica (Holland and Kubota, 1975) and of the asteroid Hymeaster membranaceus (Pain et al., 1982b) and in the walls of testes and ovary, the sperms, the vitelline membrane and follicle cells of oocytes of the asteroid Astropecten polyacanthus (Desai and Bellary, 1989). Acid mulopolysaccharides have also been detected histochemically in the connective tissues of the ovarian wall and in the vitelline membrane of the oocytes of the holothuroids Ypsilothuria talismani (Tyler and Gage, 1983), Peniagone azorica (Tyler et al., 1985) and Benthodytes sordida. Psychroprotes longicauda, Diema validum and Oneirophanta mutabilis (Tyler and Billett, 1987) and Cherbonniera utriculus and Molpadia blakei (Tyler, et al., 1987). Excepting the holothuroids examined by Tyler and Billett (1987) and the asteroid Astropecten polyacanthus (Desai and Bellary, 1989), in none of the echinoderm species the testes and spermatozoa have been so far examined for the acid mucopolysaccharides. In our present study on H. atra presence of acid mucopolysaccharides has been demonstrated in the connective tissue in the wall of the ovaries and testes, in the cytoplasm of vitellogenic and mature oocytes and their vitelline membrane, the spermatocytes and
spermatids. Abolition of metachromasia in hyaluronidase treated in sections of the ovary of *H. atra* indicates that these acid mucopolysaccharides are of hyaluronic acid and/or chondroitin sulphates A and C types (Kleiner and Orten 1962, Barka and Anderson 1963). Our results also suggest that these acid mucopolysaccharides in the vitelline membrane of oocytes are derived from oocyte cytoplasm.
1. Only the proteins form the major biochemical component in the body wall and the gonads of both the sexes. Proteins and lipids show an inverse relationship in their accumulation/depletion between the body wall and gonads; this indicates that the body wall acts as the main nutrient reserve from where the nutrients are transported to the gonads during the reproductive cycle of _H. atra_.

2. Some inorganic elements studied in the present work, viz, Na⁺, K⁺, Ca²⁺, Mg²⁺, P³⁺ and Cl⁻ also show consistent variations in the above mentioned tissues during the different phases of reproductive cycle. Based on these observations the probable roles of them in the gonadal physiology of _H. atra_ have been proposed.

3. The caloric values of different biochemical components show variations similar to those of the above mentioned, during the different phases of reproductive cycle of _H. atra_.

4. Proteins, lipids and carbohydrates have been studied in
the ovaries of *H. atra* during the different phases of reproductive cycle by adopting histochemical methods also. While proteins and lipids show their increasing accumulation during the gonadal growth, glycogen in particular remains fairly constant throughout the different phases of its reproductive cycle.

5. On the basis of β-metachromasia with toludine blue and alcianophilia, acid mucopolysaccharides have been detected in the connective tissue in the wall of the ovaries and testes, in the cytoplasm of vitellogenic and mature oocytes and their vitelline membrane, the spermatocytes and spermatids.

6. Histochemical results on the ovaries are well supported by biochemical results.