4. DISCUSSION

4.1. MORPHOLOGICAL STUDIES

The shells of *Hemifusus* are fusiform, large and heavy as in other neogastropods. The shells are so large and the animal can be withdrawn into it completely (Ponder, 1973). The shells of male are usually smaller and narrower than the females. This fact has been shown in the case of *Buccinum undatum* by Hallers (1979). The columellar plates are absent in the study animal which are usually present in Fasciolaridae and Turbinellidae. The operculum is elongated and nuncleus is terminal. The above mentioned characters of shell and operculum confirms with the findings of Satyamurty (1960), Oliver (1975) and Kira (1962) on gastropods. The males and females can be differentiated by the presence of penis in males and pallial oviduct in females. The penis of *H.pugilinus* is flattened and slightly recurved which resembles the penis of *H.tuba* (Pelsenner, 1906). As in *Murex, Mitrids* and Volutids the hypobranchial gland immobilizes all the particles which tend to enter into it (Grynfelt, 1911 and Ponder, 1973). The foot is well developed and aids in locomotion of the animal along
with the lubrication of mucous secreted by the mucous glands of the foot as seen in the family Buccinidae (Ponder, 1973). The monopectinate ctenidium of *H. pugilinus* resembles that of *Mitridae* and *Volutidae* (Ponder, 1973), likewise the bipectinate osphradium resembles that of *Natica sp.*, and *Murex sp.*, and *Oliva sp.*, (Hulbert and Yonge, 1937; Sampath, 1985).

4.2. ANATOMICAL STUDIES

The general organization of *H. pugilinus* resembles other neogastropoda. The pleurembolic type of proboscis has also been noted in the *Cancellariacea* (Graham, 1966) and *Columbarium sp.* and *Colozea sp.* (Ponder, 1973a). The racchiglossate type of radula with 3 teeth in each row has been recorded in other families of neogastropods and the tendency towards the reduction of radular teeth in the families of the other neogastropods was reported by Ponder (1973). The salivary glands are similar to those present in other families of neogastropods. They show more resemblances to the salivary glands of *Volutidae* (Ponder, 1970), *Marginellidae* (Ponder, 1970a) and *Vexillidae* (Ponder, 1973). The gland of Leiblein is only a simple caecum and extends towards the posterior
oesophagus. This condition coincides with the findings of Vanstone (1894) in Buccinacea and Voluteacea. In *H. pugilinus* increased volume of digestive gland with the reduction of Leiblein gland resembles other muricids. The stomach is a simple sac and it has been reduced to small size due to its carnivorous habit. This trait has also been noted in *Nucella sp.*, (Graham, 1949), *Natica sp.*, and *Buccinum sp.*(Brock, 1936). Regarding the feeding mode of life *H. pugilinus* coincides with the description of Colton (1908) and Carriker (1951) for the American genus *Busycon*.

The single large kidney resembles the post torsional right one of other neogastropods (Perrier, 1889). The penis of *H. pugilinus* resembles *Muricids* and *Buccinids*. The male genital duct has the seminal vesicle which is formed from the coiled upper vas deferens modified to store the sperm (Fretter, 1941). Marcus and Marcus (1959) stated the presence of closed type of prostate gland in all families of the order neogastropods. In the female ovary the period of rest or resorption is indicated by the formation of yellow brown inclusions (Kostitzine, 1934). The more secretion of hypobranchial gland in female may provide the embedding medium for the egg masses (Gersch, 1936). Like all other neogastropods the capsule
gland forms most of the pallial oviduct. The bursa copulatrix shows resemblance to that of *Oliva sayana* (Marcus and Marcus, 1959), *Oliva oliva* (Sampath, 1985) and *Nassa dorsata* and *N.stolata* (Thivakaran, 1984), but is in contrast to the *Alicithoe sp.*, where the capsular gland is rather small than the albumen gland (Ponder, 1970).

**4.3. INFLUENCE OF ENVIRONMENTAL FACTORS ON THE POPULATION DISTRIBUTION OF *H.pugilinus***

It is well documented that nutrients play a vital role in the productivity of many aquatic ecosystems (Nilson and Nilsson 1978; Richardson *et al.*, 1978 ; Stauffer, 1991). Nitrites and other nutrients are regarded as important limiting factors for various aquatic organisms.

Seasonal changes seem to influence the density of invertebrate population in general (Odum, 1963) with the accumulation of information on the distribution of fauna in oceans differences in habitats at different seasons are increasingly evident. The present study reveals that the distribution and abundance of *H.pugilinus* of Tranquebar coast are not much influenced by the environmental parameters, as these animals are bottom living.
Regarding rainfall the south east coast receives rainfall mainly due to the north east monsoon. Quasim (1980) stated that the monsoonal rainfall which can bring about profound changes in the hydrography due to flood and runway water, influences the tropical waters.

In the present study area the population distribution of *H.pugilinus* appears to the pressure dependent. Relatively higher animals were collected during December. Similar observations were made by Anandhakumar (1986); (Paul Ravindran, 2003) on *Rapana rapiformis*. The male and female population density was influenced by different environmental factors such as temperature, salinity, dissolved oxygen and pH. The population density of *H.pugilinus* showed significant variations (p < 0.05) between 6 and 10 fathom lines. It is interesting to note that in both the two depths, the female populations were dominating the males.

Temperature is an important factor in the coastal environment which influences the distribution of fauna. Prevalence of higher temperatures at 6 fathom and 10 fathom lines during summer and lower during monsoon season could be attributed to
the fluctuation in the air temperature caused by the seasonal changes in the incoming solar radiation (Bhattathiri, 1987).

During the present study dissolved oxygen content showed lesser values in summer and higher values in monsoon in both the years at 6 fathom and 10 fathom lines. The same trend was noticed in both years. Such a trend may be due to heavy rain. The dissolved oxygen content was found to be inversely related to temperature and salinity of the water. Similar observations were earlier documented by Jegadesan (1974) in Coleroon waters; John (1980) and Rajakumar (1995) in Portono waters.

Salinity is one of the important factors which influences the functional physiology and reproductive activity of the organisms (Kinne, 1971). In the present study the salinity is low during monsoon and high during summer seasons at both the levels and during both the years of study. This feature was earlier reported from other parts of the Indian coasts (Suresh et al., 1978; Nair et al., 1983; Harikantra and Parulekar, 1990; Martin Devaprasath, 2002; Paul Ravindran, 2003; Paul Jeevanandham and Christy Ponni, 2007).
H.pugilinus has been recorded throughout the study period along Tranquebar coast where the salinity ranged from 27.50% (Nov. 2009 at 10 fathom) to 33.68% (June 2008) at 6 fathom). H.pugilinus of Parangipettai coastal waters occur in the salinity range of 22.1% and 36.5% (Anandha Kumar, 1986). Rapana rapiformis has been reported to live in the salinity range of 25.03% and 38.92% in Tranquebar coastal waters (Paul Ravindran, 2003). The major role played by salinity as a factor controlling the horizontal distribution of intertidal animals was highlighted by Underwood (1979). In tropical waters, salinity, coupled with temperature seem to play a key role in the distribution of animals where fluctuations in salinity are well explained by Ajmalkhan and Natarajan (1981).

The hydrogen ion concentration (pH) remained almost in alkaline condition (8.11 to 9.76) in the study area throughout the study period and this observation is in confirmity with those of earlier works by Rajakumar (1995); Soekendarsi et al., (1996); Paul Ravindran (2003); Paul Jeevanandham and Christy Ponni (2007).
During the study period atmospheric temperature and surface temperature varied between monsoon and summer and it seems to influence the distribution of the study animal. The distribution of study animal *H. pugilinus* shows minor differences between 6 fathom and 10 fathom lines. In general, the population was relatively high in the 10 fathom line. From this it is regarded that this animal prefers more depth. The ecological factors too do not show any remarkable variation between 6 and 10 fathom lines. This fact has been well documented by Dehadri and Bhargava (1972); Jegadesan (1974) and Anandha Kumar (1986).

Regarding their migration in the 6 and 10 fathom line, relatively high number of animals were collected during monsoon and the more rain fall received by that season of both years (2008 and 2009) might be attributed to such variations. From the above facts it is clear that the migration of *H. pugilinus* to various depths is caused by the factor other than ecological parameters. As mentioned elsewhere perhaps it may be the food availability or in relation to the reproductive habit.
Intertidal snails adapt themselves to changes in salinity caused by evaporation and rainfall during summer and monsoon respectively (Hyman, 1967). In the present investigation *H. pugilinus* is found to have high tolerance to salinity and has a wide distribution limit. The present study reveals that the female population of the study animal is relatively high when compared to males. The density of both sexes is low during summer when compared to other seasons. This observation is in conformity with the earlier reports by Tagore (1989) in *Thais bufo* and *T. biserialis*, Stella (1995) in *C. virgineus*, Rajakumar (1995) Paul Ravindran (2003) in *R. rapiformis* in the South east coast of India.

The Chi-square analysis reveals that within the two fathom lines there exist significant monthly variations between the males and females (p < 0.05).

**NUTRIENT CONTENTS AND THE POPULATION DISTRIBUTION OF *H. pugilinus***

The seasonal distribution of nutrients and various abiotic and biotic processes also influence nutrient cycle in different coastal environments (Choudhry and Panigrahy, 1991). In the present study, seasonal distribution of phosphate is relatively high in
concentration during monsoon and low during summer seasons. During land run off following rains and the flushing of industrial and domestic effluents and the influx of river water could have brought heavy loads of nutrients into the sea (Ramaraju et al., 1987). The monsoon might be responsible for higher concentrations of phosphate in the sea water was observed by many researchers (Jayaraman and Seshappa (1957), Reddy and Sankaranarayana (1968) and Radhakrishnan (1982). In the present study the higher concentration was recorded at both 6 fathom and 10 fathom lines, (monsoon) lower concentration was recorded during summer. Similar results were reported by Santhakumari (1975) and Ramadhas (1977) from Vellar estuary.

The high concentration of nitrate was observed during monsoon from the waters of both 6 and 10 fathom lines. High concentration of nitrate may also occur due to bacterial reduction of nitrate and oxidation of ammonia by nitrifying bacteria (Ayyakkannu, 1996). Various reports indicate that the high concentration of nitrate in water is due to the flushing of pollutants from the harbour water into near shore waters (Sarma et al., 1988). In the present study minimum concentrations of nitrate are observed
during summer period both at 6 fathom and 10 fathom lines of both years. High rates of denitrification and the net effects of the biological and biochemical processes (Vinithkumar et al., 1999) could have lowered the nitrate concentration during the summer and premonsoon seasons.

The silicate concentration is high during monsoon period both 6 and 10 fathom lines. A similar trend of higher silicate concentrations during monsoon has been observed in Visakapattinam coast (Sarma et al., 1988) and in Kalpakkam coast (Nair et al., 1983), which might be due to upwelling of nutrient laden bottom. The minimum concentration of silicate is observed during summer at both 6 and 10 fathom lines in both the years of study. A drop in silicate concentration during summer was also reported by Chandran and Ramamoorthi (1984) from Vellar estuary, Jegadeesan (1986) from the Coleroon estuary and Ayyakkannu (1996) from the Cuddalore backwaters.

In the present study, seasonal distribution of calcium is characterized by relatively high concentrations during the summer seasons both at 6 and 10 fathom lines for both the years. The high concentration of calcium during summer could be attributed to the
discharge of freshwater with land derived materials. According to Sujatha Mishra et al., (1993) higher value of calcium for bottom layer of sea could be explained to the extraction of calcium from the surface water by animals forming calcareous shells. A reasonable solution for the increase in calcium content might be due to resolution of shells of animals. The minimum concentration during monsoon could be mainly due to utilization of large quantities of calcium by the molluscs and Vertebrates (Nair et al., 1983).

Chloride is one of the important factors which influences the functional physiology and reproductive activity of organisms (Kinne, 1971), thereby affecting floral and faunal abundance. In the present study the maximum chloride values were recorded during summer both at 6 and 10 fathom lines in both the years and the minimum during monsoon period. The lowest value observed during monsoon season might be due to high dilution of coastal waters as a consequence of massive freshwater input into the sea through the rivers. The high chloride content during summer could be due to low rain fall, decreased fresh water inflow, land drainage and rise in temperature. These findings are in conformity with the reports of Nair et al., (1983) and Harikantra and Parulekar (1989).
4.4. BIOCHEMICAL STUDIES

Protein

In general the protein values are higher in females than males. Thus the gonad seems to serve as a storage organ of protein in *H. pugilinus*. It has also been reported by Giese (1969) that protein is a predominant organic constituent in molluscs. Further, the females have higher percentage of protein content than males in all the seasons. A comparison of the protein content in the various body parts of *H. pugilinus* shows higher values in gonad followed by intestine, liver, foot and mantle in the present study.

The protein values are high during monsoon season. It could be due to intense proliferation of gonad and the low protein value in summer may be due to the spawning activity. Similar observations have been made in *Thais sp.* (Tagore, 1989 and Christy Ponni, 2007).

Carbohydrate

In the present study carbohydrate values are high in the gonad during monsoon but low in summer when the gonad is in ripe stage. Krishnakumari (1985) has reported that the carbohydrate
level in *Cerithium rubus* varies between 4.85 and 1.89%. It has varied from 0.84 to 3.04 in *Pythia plicate* as reported by Shanmugam (1987). Relatively high carbohydrate levels (5.31%) in *Littorina quadricentus* and 4.96% in *Nodilittorina pyramidal* have been reported by Thivakaran (1988). In *Thais biserialis* the reported value for carbohydrate was 13.6% and 15.2% in *Thais bufo* (Tagore, 1989 and Christy Ponni, 2007). In the species presently studied carbohydrate content is high when compared to other gastropods. Ansell *et al.*, (1973) has reported that in molluscs generally the carbohydrate reserves may be utilized under favourable conditions and the wide variation in this constituent in the tissue is due to the reasons cited above. Such variations in carbohydrate level may be related to prey availability and metabolic efficiency of the animals.

**Lipid**

The fluctuations in lipid values closely follow the protein level. Thivakaran (1988) has reported that the variation in lipid in *Littorina quadricentus* is (0.79%). Krishnakumari (1985) has reported that the lipid values range from 5.10 to 22.93% in males and from 4.90 to 24.19 in females of *Cerithium rubus*. In *Cellona*
the lipid values ranged from 0.8 to 10.75% (Suryanaryanan and Nair, 1976). Anandhakumar et al., (1986) has recorded 15.0-23.6% fat in *H.pugilinus*. Xavier Ramesh and Ayyakkannu (1992) has reported 2% of lipid in the foot muscle of *C.ramosus*. Tagore (1989) has reported that the lipid values ranged from 0.80 to 2.42% in males and from 0.84 to 2.68% in females of *Thais bisteralis* and *Thais bufo*. In the present study the maximum lipid content is found in liver during monsoon season. Similar observation has been made in *Thais lamelllose* and *C.virgineus* (Lambert and Dehnel, 1974 and Paul Jeevanandham, 2007). In most of the molluscs, the digestive gland acts as the storage organ (Owen, 1966; John, 1980; Paul Ravindran 2003). Tagore (1989) has reported that the digestive gland acts as the storage site in *Thais bistrialis* and *Thais bufo*.

Generally the fluctuations in the percentage values of protein, carbohydrate and lipid may either be due to ecological factors, that is the nutrient’s availability or due to the animal’s reproductive cycle (Blackmore, 1969; Giese, 1969; William, 1970). Umadevi (1985) has reported progressive increase and decrease in the levels of biochemical constituents and attributed maturation and spawning
activity of morula granulata for such variation. Similar observations have been made in *Pythia plicate* (Shanmugam, 1987) (*Littorina quadricentus* and *Rapana rapiformis* (Rajakumar, 1995) and Paul Ravindran (2003) *Nodilittorina pyramidalis* (Thivakaran, 1988) *Chicoreus virigineus*  (Paul Jeevanandham, 2007) *Thais bufo* (Christy Ponni, 2007) have studied the fluctuations in the biochemical constituents of both the species and are largely attributable to their reproductive and feeding activities.

The paired sample statistics (t-test) between male and female for biochemical constituents were found to be significant at 5% (0.05) level (Table 24, 25 and 26). In the present study the fluctuations in the biochemical constituents are largely attributable to the reproductive and feeding activities of the study animal, which is also supported by the results of the Analysis of Variance (ANOVA) (Table 27 to table 29) performed against various seasons.
4.5. TRACE ELEMENTS

In the present study an attempt has been made to study the distribution of trace elements in the mollusc *H.pugilinus* collected from the marine environment. The concentration of these trace elements shows fluctuations in different tissues. Bhat *et al.*, (1968) have reported that due to the industrial wastes, the level of Zn, Mn and Cu are moderately high in the summer season and progressively low in the monsoon season. Natarajan (1986) has reported that heavy metals are essential for living organisms and their concentration may either be moderate or low and Cu and Zn are essential for growth of the organisms. In various lamellibranches and temperate gastropods, it has been reported that the fluctuation of trace elements accumulation is due to flood water run-off, direct rainfall and discharge of industrial wastes (Krishnamoorthy, 1969; Kumaraguru and Ramamoorthy, 1978; Ireland, 1979; Ley, 1982). There is a progressive increase in the concentration of Fe, Zn, Mn and Cu in *H.pugilinus* during the study period. This indicates more or similar details as reported by Kasinathan and Thirumavalavan (2006) and Anandhakumar (1986) in Parangipettti coastal waters in *Babylonia spirata* and *H.pugilinus*. 
The females show higher accumulation of zinc. Indeed the values got for *H. pugilinus* are lesser than that for *Cerithium rubus* from Bombay coastal waters (Krishnakumari, 1985). Natarajan (1986) has noted that high concentrations of Zn and Mn affect the life of organisms in their behaviour. Their physiology is greatly disrupted and mortality occurs at higher concentration. It may account for the differential environment. The difference between the males and females is only little and this results coincide with the findings of Latouche and Mix (1982) in Bay mussels. However, the results clearly suggest that the concentration of trace elements is less.

### 4.6. BIO-ENZYMEOLOGICAL STUDIES

Enzymes are proteins which play a vital role in the physiology of living organisms. All the functions of an organism are regulated by enzyme and hormones, which are proteins. If any alteration takes place in the protein turnover, it may have an adverse effect on the important and complex groups of biological materials such as enzymes and hormones comprising the nitrogenous constituents of the body and food intake and thus performing
different biological events to maintain homeostasis of the cell. Therefore the protein content of a cell can be considered as a diagnostic tool to determine the physiological phases of a cell (Bowers and McComb, 1966). The liver which occupies the pivotal position in the body, plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of toxic substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign (Xenobiotic) compounds culminating in liver dysfunction (Devarshi, et al., 1986; Ambiga et al., 2007).

Acid phosphatase, a lysosomal enzyme (Aruna et al., 1979), plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis (Abou Donia, 1978). ALP is a membrane bound enzyme, found in hepatocytes (Shakoori et al., 1992). Alkaline phosphatase plays a critical role in protein synthesis (Pilo et al., 1972), shell formation (Timmermans, 1969), other secretary activities (Ibrahim, 1974) and transport of metabolites (Vorbrodt, 1959) in gastropods.
ALP and ACP activities have been studied in several organisms and the influence of heavy metals has been reported by Blasco et al., (1993). These enzymatic activities are involved in a variety of metabolic processes, such as molecule permeability, growth and cell differentiation and steroidogenesis. ALP is known to be involved in bone formation and in transport membrane activities. This enzyme is also involved in shell deposition in bivalves and gastropods (Mazorraa et al., 2002). In the blue crab, *Callinectes sapidus*, ALP is involved in the modulation of the Osmoregulatory response. The low levels found for ALP activity suggest that membranes are ineffective barriers to most molecular substances in this species (Lovett et al., 1994).

ALP and ACP catalyse the hydrolysis of various phosphate containing compounds and act as transphophorylases. ACP acts as marker enzymes for the detection of lysosomes in cell fractions and can be altered by the presence of xenobiotics (Cajaraville et al., 2000), whilst ALP are intrinsic plasma membrane enzymes found on the membranes of almost all cells.
4.7. HISTOLOGICAL STUDIES

The cellular organization of the various body parts of *H. pugilinus*, proves the same type of functional significance as encountered in other gastropods. Histological studies of the foot shows ciliated epithelial cells with cilia and the muscle fibres resembling that of other gastropods. Such observations have been already made by Hyman (1967) in gastropods. The mantle reveals the presence of a layer of columnar epithelium and glandular cells. Histology of the liver indicates a group of Acini with an outer basement membrane and glandular cells. The testis has numerous lobules. The ovary consists of numerous follicles and the spent ovary shows wrinkled and flat nature of ovarian follicles.

More or less similar observations have been made by Christy Ponni (2008); Paul Ravindran (2004); and Paul Jeevanandham (2007) in *Thais bufo*, *Rapana rapiformis* and *Chircoreus virgineus* respectively at Tranquebar coastal waters.
The histological studies of various organs in general, indicate more or similar details as reported by Stella (1995) in Parangipettai coastal waters in *Chicoreus virgineus* and other gastropods and thereby suggesting that the present study area is more or less similar to Parangipettai coastal waters, for the distribution of *H.pugilinus*. 