4. DISCUSSION

4.1 Population studies of *P. viridis* in relation to environmental factors

The present investigation indicated that all the stations have abundance of individuals during post monsoon, summer and pre-monsoon season, but the number was very low during monsoon season. The maximum number may be because of favourable environmental conditions like nutrients, abundance of phytoplankton. Jayabal (1984) and Kalyanasundram (1988) have suggested that *M. meretrix, M. casta* and *Katelysia opima* were abundant during pre-monsoon, and post monsoon, *Cerithidea cingulata* were abundant during pre-monsoon, post-monsoon and summer seasons at high water mark, at the same time during monsoon, they were found minimum due to heavy floods.

The present study indicate that *P. viridis* has two peak breeding, the first one start from the end of the March to May first week, and the second one in August to September. During that time the stations which have higher salinity, suitable habitat and abundant food material influence the diverse population and also help in breeding. Kalyanasundram (1988) reported that the gonad of bivalves have extended spawning period from March to September. Arularasan and Kasinathan (2007) also observed similar results in Vellar estuary.
The present result indicates that decrease in population during monsoon may be due to unfavourable environmental conditions. Jayabal and Kalayani (1984) have suggested that the decrease in bivalve landing during monsoon might be due to the adverse condition of fishing. Arularasan et al. (2007) reported that ecological factors such as rainfall, temperature and salinity play a vital role in the distribution of species which determine the species composition, population density, species diversity, species richness and evenness of the habitat. He also observed that diversity of mollusk on the artificial rocky shore (Tranquebar) mainly depends upon the nature of substratum, ageing of rocks, seaweed distribution, tidal amplitude wave action and water parameters. Station 1 show relatively high population density followed by station 2 and station 3, the higher number in station 1 might be due to the suitable substratum and favourable environmental conditions for the study animal. Similarly Rajagopal (1991) observed that the abundance and growth of mussel larvae in the coastal water is linked to sufficient food availability which enhances the chance of larval development and successful settlement.

When compared to first year, second year show lower percentage of total individuals in all the stations, particularly males dominate over females during the study period. The percentage contribution of the sexes appeared to be skewed in favour of males, which represent an average for two years of the samples. van Erkom
Schurink and Griffith (1991) stated that ecological significance of the biased sex ratio has been considered to reflect the greater reproductive effort of female, which produce much larger gametes than males per unit of reproductive output. Thompson (1979) observed that the higher caloric value of eggs relative to sperm, gamete release in females leads to a stronger decline in body weight.

The Chi-square values were significant in most of the months. The overall sex ratio of males and females was significant (p< 0.05) and it further indicated that the distribution was heterogeneous for both years (Tables 4 to 6).

ANOVA was performed to know if there are any significant differences among the stations, months and years of the *P. viridis* population density. It indicated highly significant monthwise and stationwise variations (p<0.05) in respect of immature, male, female and spent population of *P. viridis*, but, variations were not significant between years (Table 14).

**4.1.1 Hydrographical studies**

The correlation matrix between various environmental parameters at stations 1, 2 & 3 are given in tables 7 to 12. Analyses of variance for different environmental parameters are given in table 14.
(a) Rainfall

Rainfall is found to be an important cyclic phenomenon and the ecological parameters in marine and estuarine systems show a distinct pattern of variation all around the study period. Rainfall in India is largely influenced by two monsoons: the southeast monsoon on the west coast, and the northeast monsoon on the southeast coast (Kannan, 1980). During the present investigation, the maximum rainfall was recorded during the northeast monsoon period (November) and minimum during post monsoon and summer in all the stations during the study period. Maruthanayagam and Subramanian (1999) have also reported that the bulk of rainfall occurs in the southeast coast of India during northeast monsoon season. Seasonal changes in rainfall influence the density of lower invertebrates in intertidal areas (Odum, 1963). The distribution and relative abundance of *P. viridis* is not found uniform throughout the study period and is to be decreasing in monsoon period in all the stations perhaps due to the heavy rains and inflow of fresh water from the adjoining area. Three way ANOVA showed significant (p<0.05) monthwise variation.

(b) Temperature

Temperature is commonly considered the most important single ecological factor in the coastal and estuarine ecosystem which can
influence the distribution of marine animals (Hedgpeth, 1957 and Kinne, 1967).

During the present investigation second year showed less number of *P. viridis* in all stations which could be attributed to temperature fluctuation. Similar reports were proposed by Prabhahar *et al.* (2011) in Vellar estuary and Portonovo coastal waters. Statistical analysis showed a positive correlation of temperature with salinity in all the stations and analysis of variance showed significant monthwise, stationwise, yearwise, differences (p<0.05).

(c) *Salinity*

Salinity is considered to be the basic and prime factor among the environmental variables in the marine environment which influences greatly the dynamic situation of the estuarine and coastal waters by the inflow of fresh water and the prevailing temperature (Srilatha *et al.* 2012). Underwood (1979) reported that salinity plays a major role in the horizontal distribution of intertidal animals. Intertidal molluscs adapt themselves to changes in water salinity caused due to increase in evaporation during hot summer and decrease during heavy monsoon floods.

Ajmalkhan and Natarajan (1981) stated that in tropical waters, salinity is known to play a key role in the distribution of marine organisms in the near shore and estuarine region where fluctuations in
salinity are well pronounced. Salinity at the study areas was high during summer and pre monsoon seasons and low during the monsoon season during both years. Reduction in salinity in the study area is perhaps due to continuous flow of fresh water throughout the year. Similar results were observed by Nair et al. (1984), Mistra et al. (1990), Rajasegar (1998), Arularasan (2002), Jonas Gunasekaran (2003), Kesavan et al. (2007) and Palpandi (2011).

This is further evidenced by the negative correlation obtained between salinity and rainfall. But, salinity showed a significant positive correlation with temperature. Analysis of variance also showed a significant (p<0.05) monthwise, stationwise, and yearwise variation in respect of salinity.

(d) \textit{pH}

The pH is a reflection of many biological and chemical processes occurring in natural waters (Sreenivasan, 1974; Sadd, 1978). Trivedy and Goel (1984) reported that the pH of water undergoes drastic changes with time due to exposure to biological activity and temperature. The pH of natural waters mainly depends upon the carbonic system and interactions between carbonates and bicarbonates.
Generally, fluctuations in pH values during different seasons of the year is attributed to factors like removal of carbon dioxide by photosynthesis through bicarbonate degradation, dilution of sea water by fresh water influx, reduction of temperature and decomposition of organic matter as suggested by Zingde et al. (1987). In the present investigation pH concentration indicates the alkaline nature of water in the study area. The statistical analysis also revealed that pH had significant positive correlated with temperature and salinity, and negatively corrected with rainfall and dissolved oxygen. Further, ANOVA showed a significant monthwise, stationwise (p<0.05) variation whereas yearwise variations were not significant.

(e) **Dissolved oxygen**

The dissolved oxygen concentration is an index to study the productivity of an environment. The dissolved oxygen is very essential for the respiratory metabolism of all aquatic animals and it favours the solubility and availability of many nutrients of the animals and are therefore increasing the productivity of the aquatic ecosystem (Wetzel, 1975).

In the present study, the dissolved oxygen concentration was low during summer and high during monsoon months in all the stations. Hung (1972) opined that temperature is a major factor controlling oxygen saturation in water. Solubility of oxygen in water is inversely
proportional to temperature (Carpenter, 1966). The low dissolved oxygen concentration observed during summer may be attributed to the higher salinity of the water, higher temperature and less inflow of freshwater coupled with biological processes such as consumption of available oxygen by the organisms for respiration and active decomposition of organic matter during summer. It is well known that the temperature and salinity affect the dissolution of oxygen (Vijayakumar et al. 2000). The low summer values could be attributed to the lesser input of fresh water and reduced agitation and turbulence of the coastal and estuarine waters. High dissolved oxygen during the monsoon season might be due to the high oxygen content of the fresh water flowing in to the study area accompanied by low temperature and low salinity. Such an inverse relationship observed in the present study was also reported by Maruthamuthu (1988), Rajakumar (1995), Stella (1995), Rajasekar (1998), Santhanam (1998) and Ashok prabu (1999) from Vellar estuarine system; Arularasan (2002), Paul Ravindran (2003) Christy Ponni (2007) and Sankar et al. (2010) from Tranqubar coastal waters. The dissolved oxygen in the present study positively correlated with rainfall and negatively correlated with temperature and salinity. ANOVA indicated significant yearwise, monthwise and stationwise (p<0.05) variations.
4.1.2 Nutrients

Environmental conditions in most of the tropical aquatic ecosystem are largely governed by marked seasonal changes induced by the monsoonal cycle (Indra, 2005). Understanding of the hydrography and nutrient cycling in the river water become complicated by the continuous mixing of water masses with different physico-chemical properties (Odum, 1982). Nowadays due to the modern technology and development and their impact induces changes in aquatic medium. Karlson et al. (2002) reported that subsequent increase in nutrient loads produces an ecological impact over biological communities. Nutrients are considered to be the most important parameters in the marine and estuarine environment, influencing growth, reproduction and metabolic activities of biotic components. Distribution of nutrients is mainly based on the season, tidal conditions and freshwater flow from land source.

(a) Phosphate

The observed high monsoonal phosphate value might be due to the regeneration and release of phosphorus from bottom sediment into the water column by turbulence and mixing (Murugan, 1989; Saravanakumar et al. 2008 and Sankar et al. 2010). More over the bulk of weathering of rocks and soluble alkali metal phosphates in the upstream area are carried into the estuaries (Govindasamy et al.
The addition of super phosphates applied in the agricultural fields as fertilizers and alkyl phosphates used in households as detergents can be other sources of inorganic phosphate during the monsoon season (Anbazhagan, 1988; Ragothaman and patil, 1995; Das et al. 1997 and Bragadeeswaran et al. 2007). The variation may also be due to the processes like adsorption and desorption of phosphates and buffering action of sediment under varying environmental conditions (Rajasegar, 2003).

The statistical analysis also revealed a significant positive correlation with rainfall and dissolved oxygen, and negatively correlated with temperature, salinity and pH. ANOVA showed a significant monthwise, stationwise and, significant yearwise variations (p<0.05).

(b) **Nitrate**

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The primary sources of nitrate in water system usually come from inorganic fertilizer and animal manure (Nolan, 2002). Other source of contamination includes deposits from airborne nitrogen compounds emitted by industry and automobiles. In developing countries, inorganic fertilizers, septic tanks and domestic animal manure from feedlot are the common form of nitrate contamination (Nair, 2010). The recorded highest monsoonal nitrate value in the study area could be mainly due to the organic materials received from
catchment area during ebb tide as reported by Senthilkumar et al. (2002), Jeevanandham (2007) and Ashokprabu et al. (2008). Similarly Jagadeesan et al. (2011) reported that monsoonal months have relatively higher nitrate and silicate concentration than other seasons by the reason of nutrient rich water inputs from the adjacent agricultural land and mangrove litters through irrigational canals and water way. Another possible way of nitrate input could be through oxidation of ammonia form of nitrogen to nitrite formation (Rajasegar, 2003).

This is further evidenced by the positive correlation obtained between rainfall and phosphate. But, nitrate showed a significant negative correlation with temperature, salinity, dissolved oxygen and pH. Analysis of variance also showed a significant (p<0.05) monthwise and stationwise, and a significant (p<0.05) yearwise differences in respect of nitrate.

(c) **Silicate**

Relatively lower values of silicate were observed during summer and post monsoon which could be due to uptake of phytoplankton (Gouda and Panigrahy, 1991; Raman et al. 1990; Ashokprabu et al. 2008 and Saravanakumar et al. 2008) for their biological activity. The silicate content was higher than that of phosphate and nitrate and are recorded high during monsoon which could be due to large influx of freshwater derived from land drainage carrying silicate leached out
from rock and also from the bottom sediment (Govindasamy et al. 2000; Rajasegar, 2003; Jeevanandam and Asokan, 2010; Damotharan et al. 2010), the removal of silicates by absorption and co-precipitation of soluble silicate with humic compounds and iron (Rajasegar, 2003). Patra et al. (2010) stated that silicate content is high in the area where water circulation is less and water become stagnant there. More or less similar result was observed in the present investigation, because station 3 showed higher silicate content than other stations which is related to high human activities such as fishing, boating and tourism.

Silicate showed a positive correlation with rainfall, dissolved oxygen, phosphate and nitrate, but, negatively correlated with temperature, salinity and pH. ANOVA also showed a significant (p<0.05) monthwise, seasonwise and yearwise variation.

(d) Calcium

Calcium is an important micronutrient of aquatic environment. Generally calcium content is higher in marine and estuarine environment. They may result from the leaching of soil and other natural sources or many come from man-made sources such as sewage and some industrial wastes. Calcium is usually one of the most important contributors to hardness (BIS, 1991). Sen Gupta and Sugandhini (1981) reported that calcium content was high in Arabian Sea and Mandovi and Zuari estuaries. It serves an important role in the
health of water bodies. Present investigation show high concentration of calcium during summer and low during monsoon. The highest concentration during summer is mainly due to high degree of evaporation and also due to neritic water dominance. The high level of calcium was also observed by Palanichamy and Balasubramanian (1989) in Vellar estuary and Sugandhini et al. (1982) in west coast of India. Lowest concentration during monsoon could be attributed to heavy fresh water inflow as reported by Arularasan (2002) Christy Ponni (2007), Jeevanandham and Asokan (2010) in Tranquebar coastal waters and Soundarapandian et al. (2009) in Uppanar estuary of Cuddalore.

The statistical analysis also revealed that calcium had a significant positive correlation with temperature, salinity and pH, and also significantly negatively correlated with rainfall, dissolved oxygen, phosphate, nitrate and silicate. Three way ANOVA showed that calcium had significant monthwise, stationwise and yearwise (p<0.05) variation.

4.2 Morphological studies

Linnaeus (1758) first described *P. viridis* while, von Ihering (1901,1907) and particularly Juke-Browne (1905) discussed hinge and ligament structures and muscle scars as basis for establishing the taxonomic hierarchy of the Mytilidae. The close taxonomic relationship
among the genera Mytilus, Perna, Choromytilus, and Aulacomya were briefly described by Soot-Ryen (1952).

4.2.1 Shell

The shell is typically bilaterally symmetrical, with the hinge lying in the sagittal plane; the ligament and byssus are compared of aragonite (Kennedy et al. 1969). Along the hinge line of the shell there are often a number of hinge teeth which prevent the valves for moving totally relative to one another. The arrangement of these teeth is often important in identifying bivalves (Barrett John and Yonge, 1958). In juvenile Perna, the definitive ligament appears along the posterodorsal shell margin and subsequently expands obscuring the primary and secondary lateral hinge teeth. Seed (1968) reported that shell shape and thickness are characters of little taxonomic value in mytilidae. In older specimens of all species, abrasion of the external anterior surface removes the periostracum exposing the white to pink outer shell surface (Siddal, 1980).

4.2.2 Muscle

Visible on the inside of most empty bivalves’ valves is a shiny line that runs parallel to the outer margin of the shell and often connects the adductor muscle scars. The shell is actively closed using a adductor muscle or muscle attached to the inner surface of both valves. The portion of the muscles is often clearly visible on the inside
of empty valves as a muscle scar. The discontinuous nature of the retractor muscle scar is one of the definitive characters of the genus Perna. The muscle structure was described by Abbud (1969) in *P. pernaja* and compared with that of *Mytilus edulis*, reported by White (1937). It is usually extrinsic muscle of three types: 1. Adductor muscle, 2. Foot muscle and 3. Mantle muscle (intrinsic muscle).

4.2.3. Mantle

The mantle forms a thin membrane covering the animal's body and extending out from it in flaps or lobes. In *P. viridis* the mantle lobes are secreted by the valves and the mantle crest secretes the ligament, byssus threads and teeth (Encyclopædia Britannica, 2012). The mantle is attached to the shell by a continuous narrow row of minute mantle retractor muscles. In many bivalves the mantle edge fused at the posterior end of the shell to form two siphons. Through which water is inhaled and expelled for respiration and suspension feeding. Morton (1987) observed the functional morphology of the organs of mantle cavity of *P. viridis*.

4.2.4 The foot and byssus

The foot is the cylindrical body, thickly muscled and flat in the region ventral, which presents a series of shallow folds, parallel to each other. It has a groove of the byssus which ends in a small cavity near the apex of the foot; the foot is marked by pigmentation of brown.
Similar type of foot was studied in various Bivalves (Narchi, 1974) in *Petricola typica* and White (1937) in *Mytilus edulis*. The byssus is secreted in the form of lamellae (Boutan, 1895). The byssus comprises a major axis of about 1mm thick, variable length, which part of a large number of secondary filaments. Each strand has at its distal end, a retention device which helps the animal fixed to the bedrock.

**4.3 Anatomical studies**

**4.3.1 Digestive system**

The digestive system of typical bivalves consists of an oesophagus, stomach, and intestine. The arrangement of alimentary canal of *P. viridis* is very similar to described by Narchi and Galvao-Bueno (1997).

Most of the bivalves are filter feeders, using their gills to capture particulate food such as phytoplankton from the water. The tentacles are covered in mucus, which traps the food, and cilia, which transport the particles back to the labial palp, these then sort the particles, rejecting those that are unsuitable or too large to digest and covering others to the mouth (Man and Mollusc, 2012). The structure of the gills varies considerably, and can serve as a useful means of classifying bivalves into groups (Barnes and Robert, 1982). The end of the crystalline style which is exposed to the stomach and releasing a variety of digestive enzymes into the stomach (Kristensen, 1972). The
structure and the digestibility of the bivalve crystalline style were studied by Yonge (1926) and Reid and Betsy Sweeney (1979). Carnivorous bivalves have reduced style and a Chitinous gizzard that helps grind up the food before digestion. In \textit{P.viridis} gut is similar to the gut of filter feeding bivalves (Barnes and Robert, 1982).

4.3.2 Respiratory and Circulatory system

\textit{P.viridis} is efficient filter feeders which feed phytoplankton being the main source of food; they feed by actively filtering particles from the water, which pass into and out of the mantle cavity through the frilled siphons (Sallih, 2005). Bivalves have an open circulatory system that bathes the organs in hemolymph. The heart has three chambers (two auricles and one ventricle) situated dorsally, in front of the posterior retractor muscles (Barnes and Robert, 1982).

4.3.3 Nervous system

The nervous system consists of a nerve network and a series of paired ganglia. In \textit{P.viridis} the cerebro pleural ganglion is on either side of the oesophagus. It controls the sensory organs while the pleural ganglion supply nerves to the mantle cavity. The pedal ganglions, which control the foot, are at its base, and the visceral ganglia which are under the posterior adductor muscle (Zebra Mussel Research Program, 2012). All the bivalves have light-sensitive cells that can
detect a shadow falling over the animal (Zebra Mussel Research Program, 2012).

4.3.4 Reproductive system

Sexes are separate in *P. viridis*. But some bivalves show hermaphroditism. Coe (1943) observed that the majority of pelecypods have separate sexes with an occasional hermaphrodite making its appearance. Gonad occurs as a massive paired structure. During the process of maturation the sex cells occupied much of the gonad tubules which had expanded considerably, at this stage the colour differentiation over gonad and mantle become apparent. In *P. viridis*, two separate gonoducts lead to the mantle cavity; distinct streams of gametes are visible in spawning adults. This is in contrast to *M. edulis* which has a single gonoduct opening (Field, 1922). In tropical region, the reproductive cycles of many marine bivalves have been correlated with salinity fluctuations (Rao, 1951 and Durve, 1965). In general, the observations of present study with respect to anatomical features resemble those of Nagabhushanam *et al.* (1975) and Mane and Nagabhushanam (1983) in *P. viridis*.

4.4 Histological studies

Histological description of tissue sections represents a major technique in research field. From the histological examination, it is clear that all the selected vital organs viz., mantle has a layer of
epithelium, connective tissue and muscle fibers. Labial palp reveals
the presence of ridges and grooves which are covered with a ciliated
epithelium. The digestive gland shows digestive tubules and
diverticula. The gill shows filaments and ciliary epithelial cells. The
ovary consists of numerous follicles with ova and the testis has
numerous lobules with spermatozoa. It indicates that cellular
organization of various tissues of *Perna viridis* is more or less similar to
details reported earlier by Morton (1987), Mane and Nagabhushnam
(1983) in Ratnagri, Selvam (1992) in Vellar estuary and Rao and
Krishnakumari (1975) in *Perna viridis*, especially their histology more or
less resemble that of *P.perna* (Narchi and Galvao Bueno, 1983) and
*Mytilus edulis* (Field, 1922) and other bivalves Yonge (1926, 1957).

Thus from the above histological observation it could be inferred
that it is used as a tool to evaluate the health of *Perna viridis* from the
study area and is more or less similar to *Perna perna* and *Mytilus
edulis*.

### 4.5 Bio-chemical studies

#### 4.5.1 Protein

Proteins are fundamental bimolecules in all aspects of cell
structure and function. Protein occurs in the body in the form of amino
acids and other metabolites, which serve as building blocks of the
body.
In the present study the protein level of *P. viridis* showed significant variation throughout the year in different organs like whole animal, mantle, gill, adductor muscle, digestive gland, and gonad of male and female. Among the stations the seasonal variation in the protein concentration of the mussel was related to their natural reproductive cycle, food availability and climatic changes. Thus *P. viridis* showed two spawning peaks, one is during post monsoon and another one is during pre monsoon.

The protein level is a typical and general indicator that reflects the resting spawning cycle (Mohan and Kalyani, 1989). According to Lee (1986) the protein maxima and minima correspond to the development / spawning and regression/ resting phases respectively. According to Mane and Nagaphushanam (1983) the protein content remained fairly high throughout all seasons and decreased only in the spawned gonad. Similar result was observed in the present study. John (1980) has observed an increase in protein content in *A. rhombea* from June to August which was attributed to the spawning activity. Jayabal (1984) also reported a similar trend of high levels of protein during maturation period and a decreased level during spawning.

In the present investigation, male and female showed slight variation in protein level in all the stations. A similar report was made by Jayabal (1984) and Jonas Gunasekaran (2003) for *Meretrix casta*. 

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ANOVA revealed significant stationwise variation in the protein level in the gill and mantles (p< 0.05). All organs showed seasonwise significant variation (Tables 24 & 25). The paired sample statistics of protein showed a significant (P<0.05) variation between male and females of *P.viridis* in all the selected organs (Table 30).

### 4.5.2 Carbohydrate

Carbohydrates in the tissues of animals exist as protein bond sugars and glycogen. Protein bound sugar is the best energy procedure of the body of the living organism (Vijayavel *et al.* 2007). In the present study, carbohydrate values were higher than that of lipid and lower than that of protein value. The seasonal fluctuation in the carbohydrates content is directly related to the reproductive cycle (Fraga, 1958). In *P.viridis* such a variation in carbohydrate value is related various metabolic activities. According to Ansary *et al.* (1981), the carbohydrate of molluscs is mainly composed of glycogen and changes in the carbohydrate level may be due to the accumulation of glycogen at different stages like gametogenesis and spawning.

In monsoon (October-November) low values in carbohydrate levels were mainly due to low temperature and unfavourable conditions causing stress to the mussels. An earlier study (Gabbot and Bayne, 1973) stated that stress in mussel results in utilization of carbohydrate reserve and a decline in the rate of reaction of ammonia- N. He also
stated that the natural gametogenic cycle in bivalves is closely linked with cycles of glycogen storage and subsequent de Nova synthesis of lipids during vitellogensis at the expense of the stored glycogen.

Carbohydrate values fluctuated between the organs in relation to maturity of gonads, climatic changes, and availability of food. Ansell et al. (1964) reported that gonad and digestive gland shows respectively high percentage of carbohydrate and fat than any other organs. Durve and Bal (1961) reported that glycogen content was related to gonad development and increased during the period of active gametogenesis.

Stationwise variation in the carbohydrate level may be due to differences in water depth and other chemical features of water and so on. Similar reports were made by Jonas Gunasekaran (2003) in Tranguebar coastal waters. The present study showed slightly higher carbohydrate content in females than males, which may be related to reproductive cycle.

ANOVA revealed no significant stationwise variation in carbohydrate in all the selected organs but, seasonwise (except gill) all organs showed significant differences (Tables 26 & 27).

A paired sample statistics of carbohydrate in the male and female of *P. viridis* showed a significant variation in all the selected organs *(p<0.05)* (Table 31).
4.5.3 Lipid

Lipid content is an essential organic constituent of the tissues of all animals, and plays a key role in energy metabolism. Lipid is the best energy producer of the body next to carbohydrate. Lipid also provides food supply, when carbohydrate levels are low (Gabbot and Bayne, 1973; Pazos et al. 1996, 1997). The lipid composition of molluscs can be affected by external factors such as fluctuations in the environmental conditions and qualitative and/or quantitative changes in food availability, or by internal factors such as sexual maturation (Gardner and Riley, 1972 and Bonnet et al. 1974).

According to Qasim et al. (1977), and Parulekar et al. (1982) the period of low lipid content coincided with post-spawning seasons and it could be attributed to depletion of energy resources for spawning activity in the mussels. Similar result is reported by John (1980) on *Anadara rhombea*, Balasubrahmanian (1984) on *M.casta* and Jayabal on *M.meretrix*. Mane and Nagabhushanam (1983) reported that the fat content sharply dropped in the spawned gonad. Another probable cause for low lipid values could also be due to initiation of gametogenesis and utilization of energy reserves for development of gametes.
In general the gonad and digestive gland contain relatively high level of lipid which is perhaps due to the breeding performance and storage of the organs cited here.

ANOVA revealed a significant seasonwise difference in the lipid level of gill (P<0.01), but, the differences are not significant in the rest of the organs (Tables 28 & 29). A paired sample statistics of lipid in male and female of *P. viridis* showed a significant variation in all the selected organs (p< 0.05) (Table 32).

4.6 Trace metal analysis

Heavy metals usually present in trace amounts in natural waters, but many of them are toxic even at very low concentrations. The present investigation shows that the contamination level of heavy metals in estuarine, coastal waters and mussel tissues are mainly related to local and regional sources. The maximum allowable concentration for water samples and mussel samples are given in the table 33.

4.6.1 Iron

Among the metals analyzed, Fe level is higher in all the stations and iron accumulation is highest among the metals studied. According to Phillips (1980) the total iron in oceans is estimated to be $4110 \times 10^7$ metric tons, contributed by geological processes and man induced
activities. The present study indicates that station 1, has high level of iron content than stations 2 and 3. It was also inferred that Fe concentration in coastal waters is a function of fresh water input and is greatly influenced by riverine waters (Rivonker and Parulekar, 1998), and also due to various anthropogenic sources.

Further, the mussel samples (different soft tissue) have higher level of Fe content than water samples in all the stations which clearly indicates the tendency of organism in accumulating higher concentration of Fe in soft tissue and it might be due to the major role played by this essential metal in catalyzing various enzymatic activities (Kamaruzzaman et al. 2011).

Among different organs, particularly digestive gland followed by whole animal tissue and gill showed high level of accumulation than other soft tissue. Nicholson and Szefer (2003) reported that Fe level in soft tissues and byssus of *P. viridis* from Hong Kong costal waters were 97.2–354 µg /g/dry weight and 230–2318 µg /g/dry weight respectively. This could be due to its being a major and essential element required for the normal metabolic mechanisms and for byssal formation and attachment (Yap and Tan, 2007), and byssal is a super-accumulator of Fe when compared to the soft tissue. The present investigation shows that digestive gland act as a storage organ which contains highest amount of Fe when compared to other organs.
ANOVA shows significant stationwise and organwise variations in respect of iron concentration (Table 39). And it also shows a highly positive correlation with Mn and Cr in station 2, and Mn in stations 1 and 3 (Table 38).

4.6.2 Manganese

Manganese is an essential element for the formation of bone and connective tissues and for metabolism of carbohydrate and lipids in animals as well as in human. The main sources of manganese are production of steel, nonferrous alloys, dry cell batteries, chemical industry and fungicides.

The present result showed that maximum Mn level is present in station 1 and minimum in station 3 in water samples, but the level exceeds the international standard unit (0.1mg/l). The highest level of Mn is present in the digestive gland of *P.viridis* and lowest level in adductor muscle. In all the stations the tissue level does not exceed the permissible limit. The higher level of Mn was also reported in intertidal mussel in Karnataka coast by Sasikumar *et al.* (2006), and he also noted that Mn concentration differs from site to site.

ANOVA shows a significant (p<0.05) organwise difference. However, differences are not significant between stations (Table 39). Manganese positively correlated with Cr in station 2, and Cu, Cr, and Ni in stations 1 and 3 (Table 38).
4.6.3 Zinc

In the present investigation Zn shows second highest value among the metals. Station 1 sows higher level of Zn than stations 2 and 3 in mussel samples. Zinc is a rare metal in nature but it is commercially one of the most important metals in the world (Hietanen et al. 1988). It is also an important trace element for the organism as a cofactor for approximately 300 enzymes involved in nearly all aspects of metabolism (Vallee and Auld, 1990). Hashim et al. (1994) reported very low values of dissolved zinc in Arabian Gulf (Bahrain). They suggested that, unlike other heavy metal such as Pb, Cd, Ni, the Zn is essential for metabolic process through it may be toxic beyond concentration. According to Berrow and Webber (1972) the sewage and city waste were the major source of Zn in the aquatic environments. The high level of Zn recorded in station 2 followed by station 1 in coastal waters could be attributed to its proximity to sewage, agricultural runoff, zinc coated pipes, effluent from Thermal power plant and small fishing activities in the sea near the study area.

Among the soft tissue gill accumulated high level of Zn when compared to other organs. A similar result was observed by Yap et al. (2006) in gills of \textit{P.viridis} in Peninsular at Malaysia. When compared to water samples the mussels showed high level of accumulation. Like Fe, Zn concentration in the mussel were higher and comparable with
similar studies reported by Sangaranarayanan et al. (1978) and Krishnakumar et al. (1998), Zingde et al. (1976), Rajendiran et al. (1988), Radhakrishnan (1993), Tewari et al. (2000, 2001) and Sasikumar et al. (2006). Concentrations of Zn in the present study are clearly below the limit set by WHO (1982), FDA (2001) and MFR (1985) for sea food.

ANOVA indicated that there is a significant organwise variation (Table 39). Zn shows a positive correlation with Cd in station 2, Cr in station 3 and Cr and Pb in station1 (Table 38).

4.6.4 Copper

Most interestingly, among the eight different tissues, gonad (male) and gill tissue (female) shows the higher level of Cu accumulation indicating its suitability for use in identifying Cu bioavailability more efficiently than the other soft tissues. When compared to water samples in all the stations soft tissues of P.viridis show high level of Cu accumulation. The higher Copper level in the different soft tissues of the bivalve might be due to the various mechanisms which included homeostatic processes in the body in response to varying metabolic demands and entrapment of Cu under certain conditions by additional mucilage production/extrusion by the animal (Pyatt et al. 2003). In the present study Cu level in tissues does not exceed the maximum permissible level (WHO, 1982). But, in water
samples it exceeds the National Environmental Board Report (1994). According to Chang and Wong (1992) the Cu resembles or even overlaps with the role of iron in the metabolism of molecular oxygen in molluscs, because the oxygen carrying pigment of molluscs’ blood is not hemoglobin but, Cu containing Cuproprotein hemocyanin. He also reported that the higher level of Cu is used for the formation of shell greening in *P. viridis*.

Probably the induction of metallothionein in the tissue distribution of metals of *P. viridis* could explain the present results. Viarengo *et al.* (1985) also confirmed that metallothioneins played a fundamental role in the accumulation of Cu and Cd in the gill and digestive gland cells of *Mytilus galloprovincialis*.

The ANOVA shows a significant (p<0.05) stationwise and organwise variations (Table 39). Copper is positively correlated with Pb and Ni at station 1, Pb in station 2 and Cr, Pb, and Ni in station 3 (Table 38).

**4.6.5 Chromium**

In the present study Cr concentration is high in mussel sample than water sample. Among the different soft tissues, digestive gland had high level of Cr than other organs. When compared to station 2 and 3, station 1 showed higher accumulation in both samples. According to Sasikumar *et al.* (2006) Cd and Cr concentrations in
mussels are site dependent. The high level of Cr in station 1 might be related fishing, tourism activity and influence of Uppanar estuary.

Analysis of variance showed significant stationwise and organwise variations in respect of Cr (Table 39).

4.6.6 Cadmium

Among the eight metals Cd was accumulated in very low level in tissues as well as in water samples of all the stations. Station 1 show high level of Cd in mussel sample than stations 2 and 3, likewise station 3 show high level of Cd in water than stations 1 and 2. According to Schulz-Baldes (1974), blue mussel take up Cd in dissolved form. In the present investigation gonad showed high level of Cd when compared to other organs and P.viridis has been used as an indicator of Cd pollution (Chidambaran, 1996), It appears to regulate metals in its tissues to greater degrees than oysters by means of mucus secretion (Sze and Lee, 1995) and metalloprotein production (Yang and Thompson, 1996).

Further, ANOVA, showed a significant organswise (P< 0.05) and stationswise difference (Table 39).

4.6.7 Lead

Lead concentration is high in both samples in station 1 than stations 2 and 3. When compared to water sample, (P.viridis) soft
tissues such as gills show higher level of accumulation than other tissues. The non essential Pb that is bound to metallothioneins (Viarengo et al. 1985) and to a few allozyme get lost because of local adaptations to contamination (Yap et al. 2004). Presence of too much of Pb in the water may decrease hemoglobin production and enzyme activities in fishes are causing anemic disorders (ASEAN, 1999). According to Dumalagan et al. (2010) Lead (Pb), the highly defected metal in sea water could not accumulate well in the soft tissues of mussel. Further, the present study shows that the Pb concentration in water samples as well as in soft tissues of mussels from three sampling areas is below detection limit of National standard level for coastal waters (1994), Moore (1991) and WHO (1982) and FDA (2001) permissible level for sea food.

The analysis of variance shows a significant (p<0.05) stationwise and organwise difference (Table 39) and it positively correlated with Ni in stations 1 & 3 (Table 38).

### 4.6.8 Nickel

The concentration of nickel in the earth crest is about 0.08%. It is mainly used for production of stainless steel and other nickel alloys. Important source of nickel are combustion of coal and oil for heat and power generation, incineration of waste and sewage sludge, steel manufacture and electroplating (WHO, 1990), fertilizer, automobiles in
addition to nickel mines (Duke, 1980; Moore and Ramamoorthy, 1984). Though nickel is essential in trace quantities and play vital role in metabolism, at higher concentrations it has deleterious effects (Calabrese et al. 1977; Eisler and Henneky, 1977). Toxic role of nickel to different aquatic organisms (Khangarot and Ray, 1987; Ali Khan and Stroch, 1990; Ali Khan et al. 1990) including snails (Gupta et al. 1981; Khangarot et al. 1982) is well documented, due to its ability to accumulate in various body tissues (Sadiq and Alam, 1989; Bardeggia and Ali Khan, 1991).

Sasikumar et al. (2006) stated that the level of toxic trace metals like Pb, Cd, Ni and Cr in the whole tissue of *P. viridis* were low thought the coast of Karnataka and within the safe limits. Nickel concentration in *P. viridis* of the present study are lower and comparable with similar studies reported by Tewari et al. (2001) in Gujarat Macha in dry weight of *P. viridis*, Radhakrishnan (1993) in Calicut and Cochin.

ANOVA shows a significant (p<0.05) organwise and stationwise difference (Table 39).

4.6.9 Bio-concentration factors (BCF)

Bivalves are well known for their ability to concentrate heavy metals in their tissue from environmental water. Substantial enrichment of heavy metals in bivalve’s mollusc has been reported (Eustace 1974;

It is observed that different tissues of any organism accumulate metals at different concentration and at different rates and the biological half-lives of metals at each type of soft tissues also differ from one another (Lakshmanan and Nambisan, 1989; yap et al. 2007). This phenomenon is due to the role played by the different parts of the bioindicator organisms, their metabolic rate and the physiological conditions of the internal body parts. This directly influences the distribution of metals in different body parts. It is also to be noted that metal contamination is very localized and closed to discharge point sources and molluscs inhabit in different microhabitats of intertidal areas where metal discharge is high due to various anthropogenic sources.

The digestive gland followed by gills and gonad are the major site for accumulation of most of the metals in the present study animal. Accumulation in the adductor muscle and siphon region is relatively low. Among the eight metals, Cr, Ni and Pb are accumulated as higher levels by P. viridis.

The results of the experiment clearly indicate the ability of muscle P. viridis to accumulate appreciable quantities of trace metals from the environmental water. The amount of metals in the tissue was
very much dependent on the site and the source of anthropogenic activities. The high concentrations of trace metals found in the gills tissues of the mussel is due to the filter feeding habit of the molluscs where by uptake occurs in this organ. Similar result was previously observed in bivalves in New Zealand (Brookes and Rumsby, 1965). The digestive gland is a storage organ, hence it accumulates higher amount of metals than other organs. Gupta and Singh (2011) reported that the digestive gland of bivalves is a target organ for accumulation of metals, furthermore, the lysosomes of the digestive cells are generally considered as target organelles. Gonad is also an important part for bio- accumulation of metals in the present study, the higher concentration in gonad indicates that female gonad tissues have metal concentrations in the gametes. This suggests that there is a difference in the role of uptake and release of metals between eggs and sperm (Burton et al. 1996). Because both genders feed on the same diet, the reason for the different growth rates resulting in differences in the concentrations of metals (Yap et al. 2006). Burton et al. (1996) suggest that the gender of individual mussels or groups of mussels may have important effects on the physiological parameters and biochemical composition of soft tissues.
4.7 Length-weight and allometric relationships

As an animal grows, the resultant increase in size, shape and volume can be measured as length and weight respectively. Analysis of its length against weight has become a standard practice in fishery studies (Ricker, 1973).

Kasinathan et al. (1987) also reported significant difference between length and weight and suggested that the difference appears to be due to the sexual maturity and also due to increase in size of both the sexes. Similar report was observed by Maruthamuthu (1988) in L.undulata from Tranquebar and Mandapam water, Shanmugam (1987) in P.plicata, Thivakaran (1988) in Littorina quadricentus and Nodilittorina pyramidalis, John (1980) in A.rhombea and Kalyanasundram (1988) in Katelysia opima. Ismail and Elkarmi (2000) reported that the increases in shell and body weight was slower in young (<20 mm in length) than older ones.

In the present study also a significant positive relationship exists between total length and weight in immature, male and female P.viridis. The co-efficient of correlation values obtained for the total length and weight of immature, male and female of P.viridis is more than 0.60 which indicates that a significant and high degree of positive correlation is between total length and weight of this species. From this it is evident that P.viridis obeys cube law and hence, it is clear that they
maintain their shape throughout their life span. Similar relationship was reported in the earlier study in *Clithon ovalaniensis* and *C. retifera* (Kanagasabai, 1985) and *T. brunneus* (Ramesh and Ravichandran, 2008).

Intra and inter specific comparison of growth can be made using the slope or exponent of the length-weight equation, which typically lies between 2.5 and 4.0 for most animals. When comparing two exponents, the animal with the higher slope value increases in height at a faster rate than the animal where the slope value is lower. When the slope of the length-weight relationship is equal to 3.0, weight is considered to be increasing as the cube of the length and indicative of isometric body growth (Ricker, 1958). When the slope is not equal to 3, growth is said to be allometry.

In the present study, the exponential value (b) for the relationship total length and weight of immature, male and female *P. viridis* was calculated as 2.545, 1.4117 and 1.4385 respectively.

Park and Oh (2002), while studying the length weight relationship parameters for 17 species (belonging to 11 families and 15 genera) of bivalves from coastal water of Korea, the correlation coefficient ($r^2$) for all species analyzed were found significant at $P. < 0.001$. Estimates of ‘b’ ranged from 2.44 in *Atrina (Servatina) pinnata*,

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japonica to 3.31 in Scaphora broughtonii. The mean of ‘b’ was 2.89 (± 0.212).

Presently, the relationship between the total length, total weight, shell weight, total tissue weight, height and width of *P. viridis* were studied in all possible combinations using the linear regression technique and correlation coefficient (Simpson et al. 1960; John, 1980; Shanmugam, 1996). The allometric relationship between shell length, height, shell weight, total tissue weight, width and volume to live weight can be used for monitoring the growth of their species in the natural population and some individuals of the same length show different width and height and these differences constitute shape variations.

In the present study, the relationship between Length-weight, Length-shell weight, Length-total tissue weight (immature, male, and female), Length-height, Length-width (immature), Total weight-total tissue weight (male), Shell weight -Total tissue weight (female) showed positive allometric relationship. All the other combinations analyzed recorded a negative allometric character which might be due to the difference in the growth rate between immature, male and female mussels. The relationship in *P. viridis* could indirectly be influenced by the local ambient environmental conditions, including food availability, feeding efficiency and population density (Rivonkar, 1991).