STUDIES ON THE BIOACCUMULATION AND EFFECT OF ENVIRONMENTAL STRESS ON THE GREEN MUSSEL, *Perna viridis* (Linnaeus, 1758)

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Dedicated to
My
Beloved Mother

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CERTIFICATE

Certified that the dissertation entitled **"STUDIES ON THE BIOACCUMULATION AND THE EFFECT OF ENVIRONMENTAL STRESS ON THE GREEN MUSSEL Perna viridis (Linnaeus, 1758)"**, is a record of independent bonafide research work carried out by **Mr. Rajat Kumar Varshney** during the period of study from March, 2002 to September 2004 under our supervision and guidance for the degree of **Master of Fisheries Science (Mariculture)** at the Central Marine Fisheries Research Institute, Cochin, and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship, or any other similar title.

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I hereby declare that this thesis entitled **"STUDIES ON THE BIOACCUMULATION AND EFFECT OF ENVIRONMENTAL STRESS ON THE GREEN MUSSEL, *Perna viridis* (Linnaeus, 1758)"** is based on my own research and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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सारांश

शंबुओं की समुद्री संवर्धन प्रणालियों में, मुख्यतः प्रारंभिक अवस्था में विभिन्न पर्यावरणीय दुष्प्रभाव, विशेष रूप से वो दुष्प्रभाव जो लवणता में होनेवाली परिवर्तनशीलता और निर्जलीकरण से होती है, का सामना करना पडता है । अनुकूलित अवस्था में और नियन्त्रित अवस्था में निम्न लवणताओं में सीधे भरकर शंदु बीजों की उत्तरजीविता का अध्ययन किया गया । बडे (25 से 40 मि मी) और छोटे (10 से 25 मि मी) बीजों का LC_{50} मूल्य क्रमशः 14 और 21 ppt थे । परिवहन के बाद परिवेशी लवणता में बिना अनुकूलन करके ससीधे निर्जल रखने पर बडे बीज निम्नलवणताओं (LC_{50} - 32 ppt) को सहने में असमर्थ देखे गये थे । अतिजीवित और नियंत्रित, दोनों बीजों का प्रतिबंधी सूचक तुल्य थे । विभिन्न घण्डों में बीजों को निर्जन अवस्था में रखकर और इनके उत्तरजीविता के मूल्यांकन करके प्रत्यादान अवधि में बीजों पर निर्जलीकरण से होनेवाले दुष्प्रभाव का अध्ययन किया । इस प्रकार 12 से 24 घण्डों तक खुले रखे बीजों में प्रत्यादान अवधि के 30 घण्डों बाद कुल मृत्युत 6 - 26% के बीच में थी और इसके बाद यह स्थिर हो गयी थी । 26 से 30 घण्डों तक का निर्जलीकरण प्रत्यादान अवधि के 30 घण्डों बाद 42 से 96% तक की उच्च ममृत्युता दिखायी जब कि 30 से 48 घण्डों तक के निर्जलीकरण अवधि में 100% मृत्युता दिखायी पडी थी । जैव संचयन और बेचनेवाले शंबुओं की गुणता पर विचार करके कृषि के ज़रिए संग्रहित और प्रकृति से प्राप्त शंबुओं के मांस में निहित ज़िंक, काड्मियम, लेड और कॉपर जैसे धातुओं के स्तरों के स्तर के विश्लेषण करके तुलना किया । इन ट्रेस धातुओं के जैव संचयन का परिमाण प्राकृतिक संस्तरों से संग्रहित शंबुओं में zn> cu>pb>cd था तो तल से दूर खाडी और ज्वारनदमुख कुंडों में संवर्धित शंबुओं में धातुओं के परिमाण क्रमशः zn>pb>cu>cd थे । प्राकृतिक संस्तरों के शंबुओं के आकार के आधार पर, ज़िंक (R^2=0.7869) और लेड (R^2 =0.7266) के संचयन में सकारात्मक सहसंबध दिखाया पडा, परन्तु तल से दूर स्थित फार्मों के शंबुओं ने नकारात्मक संबध दिखाया। संग्रहणयोग्य आकार (>60मि मी) के शंबुओं में बहुत ही निम्न या नहीं के बराबर cd और cu मूल्य के साथ zn, cd, pb और cu का संचयन आकार से संबध नकारात्मक था । प्राकृतिक संस्तरों और फार्मों से संग्रहित विभिन्न आकार के शंबुओं में गाढ़ धातुओं का संचयन असंगत था ।

ABSTRACT

Environmental stress, particularly stress due to salinity variation and desiccation is encountered in mussel mariculture systems especially in the initial phase of stocking in the farms. The survivorship of mussel seed on conditioning and direct stocking in different salinities, under controlled conditions was studied. The LC_{50} value for large (25 to 40mm) and small size seed (10 to 25 mm) were estimated as 14 and 21 ppt respectively indicating the sturdiness of larger seed. The larger sized seed were less tolerant to lower salinities (LC_{50} = 32 ppt) when exposed directly without conditioning in ambient salinity. The condition indices of the surviving seed were comparable to that of the control. Stress related to desiccation was studied by exposing the seed for various durations and the survivorship in the recovery period was evaluated. In seed exposed for 12 and 24 hours of desiccation, mortality ranged between 6 and 26% during the recovery period at the end of 30 hours and was stabilized thereafter. Desiccation for 26 to 30 hours resulted in very high mortality ranging from 42 to 96% at the end of 30 hours of the recovery time while, above 30 hours and up to 48 hours of desiccation, none of the seed survived. Considering the significance of bioaccumulation and quality of the mussels marketed, the levels of heavy metals such as zinc, cadmium, lead and copper in farmed and naturally occurring mussels were analysed and compared. The order of magnitude of bioaccumulation of the trace metals was Zn>Cu>Pb>Cd in the mussel collected from the natural bed while in the mussel cultured in the off-bottom system in bay and estuarine pond were Zn>Pb>Cd>Cu and Zn>Pb>Cu>Cd respectively. Positive correlation with size was observed in the accumulation of zinc (R^2 = 0.7869) and lead (R^2 = 0.7266) in the natural bed mussels, while in the off bottom farms the accumulation of Zn, Cd, Pb and Cu were negatively related to size with very low to nil values of Cd and Cu in harvestable size (> 60mm) mussels. Gender based bioaccumulation of heavy metals was inconsistent in different size groups of mussels collected from the natural bed and in farmed systems.

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INTRODUCTION

1. INTRODUCTION

Environmental stress is an inescapable aspect of life in aquatic ecosystem, which is constantly under the threat of visible biotic and abiotic factors. In the aquatic realm, there is much greater physico-chemical and biological variability than what exists in the terrestrial environment. These unpredictable changes cause stress in the animals. Stress has been defined in various ways, always in physiological terms. Bayne (1975) has defined stress as "a measurable alternation of a physiological (or behavioural, biochemical, or cytological) steady-state which is induced by an environmental change and which renders the individual (or population or community) more vulnerable to further environmental changes." More recently Dhert *et al* (1992) has defined stress as "The reaction of an organism by a disturbed physico-biological balance to an abnormal impact of Environment".

Every species is adapted for life under a particular range of conditions. A change in environment, beyond the limits to which the species is adapted, leads to disturbance in their growth and to the death of the organism. Immediate response to any stress occurs over a time span of few seconds to few hours. The animals are affected not only by the magnitude of parameters changes, but also rate of environmental changes.

Molluscs (Phylum: Mollusca) are found in marine, brackish water and fresh waters ecosystems. They include diverse groups of animals such as clams, mussels, oysters, scallops, abalones, conchs, squids and octopus etc. The cultivation of molluscs is an old tradition. After fish, molluscs are the most commonly cultivated marine organisms in the world. Oysters and mussels represent about 90 % of the molluscs that are cultivated.

The commercially important bivalves are more in the intertidal region, which is prone to wide environmental fluctuations. However the shells provide protection for the bivalve species to protect their tissues from abrasive

physical forces such as wave action than those inhabiting sheltered environments. The shell can also provide valuable protection for the tissues from other environmental stress, e.g., salinity fluctuation, temperature, predators, parasites, pathogens, pollutants, chemicals, aerial exposure and other hidden environmental stress factors. A sedentary mode of life restricts bivalves to a particular habitat where environmental stress cannot be avoided by migration and under these circumstances they resort to behavioral avoidance mechanism.

Although bivalves living in estuarine habitats are well adapted to variations in salinity and temperature, abiotic factors such as heterogeneity of the sediment, salinity stress, increased exposure to air and variations in temperature due to the location on the tidal flat, can give rise to spatial heterogeneity of bivalve growth. It has been shown in recent years that the physiological condition of bivalves could be related in part, to the delicate balance between pro oxidant forces and anti oxidant defenses.

One of the basic principals in aquaculture is that the farm site must be assured in terms of suitability and capability. Suitability entails an assessment of viability in forms of socio-economic, resource use, infra structural and marketing factors. Capability involves an assessment of the site in terms of environmental (bio-physical) parameter i.e. temperature, salinity, food availability, sediment load, chemicals, heavy metals and predators, parasites etc.

The spatial and temporal structure of environmental variability (intensity, spatial extent, temporal persistence, predictability) has important consequences for how that variability is perceived and responded to by the individual organism.

Among all environmental stressors, salinity is considered as a master stress factor, which initiates structural and functional responses. The change in salinity affects the animal as in the maximum relative proportions

e.g. solutes, coefficient of absorption, saturation of dissolved gases, density and viscosity of the medium (Kinne, 1971). If rate of salinity change is intense, it leads to end point of life i.e. mortality.

Desiccation is another factor which causes stress both in the natural as well as is in the farming systems. Green mussel being widely distributed in inter-tidal region, experiences repeated episodes of exposure to air, due to strong tides, which regularly causes periods of dryness and aerial exposure. The cumulative affect of air exposure stressors is observed as reduction in responsiveness of the mussel.

Mussels are subjected to a variety of physical and environmental conditions during farming activities. Some conditions leads to stress and reduced performance or quality. In India, the green mussel, *Perna viridis* (Linnaeus, 1758) is cultured along the Kerala and Maharashtra coasts. Mussels have almost the same protein content per weight as beef meat, but only one quarter of the calories. It is a source of protein, iron and zinc for immunity booster and mental alertness. Mussels are also used for water purification and for extraction of chemicals and medicines.

The adoption level of mussel farming in India has increased in the recent years. The mussel farms are mostly located in the estuarine ecosystems, which are highly productive but subject to high variation in salinity. Salinity levels drop to nearly freshwater condition during certain months. The mussel seed is collected from the coastal intertidal and sub tidal regions for stocking in the estuarine farms. Since the seed, which are exposed to high salinities in their natural habitat, are transplanted to areas of lower salinities, the problem of stress on the seed due to salinity variation arises and farmers may have to face problems such as low attachment of seed on rope due to mortality.

Related to the mussel farm is another aspect : the proximity of seed collection centers with the farm site. Occasionally, the seed collection

centers are far from the collection centers and have to be transported to the farm site covering a transit period of 2 to 8 hrs. Earlier studies related to seed transportation have shown that dry method of transport is better than the wet method. In the dry method seed is subjected to desiccation prior to seeding. Desiccation for prolonged periods can cause stress and can lead to low survival and poor quality of the seed. Under this context, it is essential to have information on the mortality due to desiccation. In this study, attempts have been made to simulate the conditions of desiccation in lab for varied duration and evaluate the mortality during the subsequent immersion period.

The green mussel is a filter- feeder having the capability to accumulate heavy metals from the surrounding medium. On one side, these heavy metal accumulations affect the metabolic activity, physiological stress and cause the reduction of growth rate while on the other they are harmful to the consumer. To safeguard the consumer, quality of the marketed mussel has to be ensured prior to marketing

The United Nation conference on human environment in 1972 held at Stockholm accepted the Gesamp definition of marine pollution namely, "The introduction by man, directly or indirectly, of substances or energy into the marine environment resulting in such deleterious effect as harm to living resources, hazards to human health, hindrance to marine activities including fisheries, impairment of quality for use of sea water and reduction in amenities". Efforts are made globally to avoid critical damage to the marine environment and its resources. The concept of the use sentinel organisms as indicators of pollution in coastal waters has been developed and bivalves are considered as the most reliable sentinel organisms owing to their mode of existence, tolerance reactions, metabolism, growth and reproduction. The term "**Mussel Watch**" is significant in this context.

Recently the utilization of mussel has increased considerably with in India. From total production < 10,000 tonnes in 1996 from fishing in natural bed, it has increased to nearly 15,000 tonnes in 2002 (CMFRI Annual

Report, 2002). A similar trend has been observed in the production from mussel farming. The production from farming was negligible in 1995 increased 1,250 tones in 2002 (Modayil, 2003). These facts imply increased consumption of the resource. Under these circumstances, it is pertinent to evaluate the quality of mussel in terms of bioaccumulation of heavy metals. With this basic concept of consumer safety, attempt was made to evaluate the level of four heavy metals *viz.* zinc, copper, lead and cadmium in the mussels.

The dissertation entitled **"Studies on the bioaccumulation and effect of environmental stress on the green mussel, *Perna viridis*, (Linnaeus, 1758)** "is an effort to solve the problem directly faced by the mussel mariculture industry in a tropical environment.

The main **objectives** of the study are

1. To study the effect of salinity variation in small (10-25mm) and large seed (25-40 mm) in terms of mortality, condition index and total lipid content.
2. Mortality of un-conditioned seed in varied salinities.
3. Effect of desiccation on mussel seed and evaluation of mortality during recovery/immersion period.
4. Bioaccumulation of trace metals *viz.* zinc, copper, lead, and cadmium in different size groups of mussels.
5. Study the level of bioaccumulation of trace metals in mussels from natural bed (on-bottom) and off-bottom systems.

The main aim of this study is to provide information for improving mussel-farming husbandry. The results of the study will be useful to farmers to plan their mussel farming protocol especially with respect to viability of seed. The bioaccumulation studies are expected to provide information necessary for consumer safety and market development.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

One of the first problems in bivalve culture with regard to environmental stress was encountered at the beginning of the 20th century. Based on studies on the American oyster *Crassostrea virginia*, Prytherch (1924) asserted, "the rapid decline of this valuable industrial production had been brought about by constant depletion of the oyster beds sustainability because of various environmental factors." It was already evident that an environmental effect on this species was concerned with the natural reproduction of the species.

Mussels are an important group of bivalves, which support important fisheries in the coastal areas around Southeast and Southwest coasts of India and elsewhere in the world. They have emerged as much demanded commercial market food product worldwide (Guerra and Roch, 1994). Because of their mode of life and filtration capacity, their abundance and growth is dependent on several environmental factors. Salinity is one of the factors, which affects almost all stages starting from egg to adult.

In the phylum Mollusca, there is vast literature on salinity and its ecophysiological impacts on the animals studied (Bayne, 1976). The effect of reduced salinity on filtration, growth, osmoregulation and ecology has been studied in *Mytilius edulis* (Bohle,1972;Gilles,1972; Davenport,1979.). Considerable work has been done on oyster also. Loosanoff (1963) studied the behaviour, when transferred from low salinity to high saline water in *Ostrea virginica.*

The salinity tolerance of Indian bivalves such as *Katlysia opima* (Ranade and Kulkarni, 1969); *Saccostrea cucullata* (Nagabhusham and Bidarkar,1975); *Villorita cyprinoiodes* (Nair and Shynamma,1975); *Meritrix casta* (Salih,1978); *Sunetta scripta* (Thamputran *et al.*,1982); *Perna viridis*, *Meritrix meritrix* and *Crassotrea madrasensis* (Sundaram and Shafee,1989); *Paphia*

latrisulca (Mane and Dhamne,1980); *Paphia malabarica* (Ram Mohan,1993) and *Saccostrea cucullata* (Kripa,1998).

Studies were also conducted on the rate of filtration and growth with respect to different environment parameters of *M. casta* (Durve, 1962; Salih, 1978). Mane (1980) carried out study on adaptation of *Katelysia opima* to salinity fluctuations. Nagabhusanam and Bidarkar (1975) studied the salinity range 2.5ppt to 35ppt for *Crassostrea cucullata*. The variation in salinity tolerance in different size groups of *M. casta* has been studied (Salih, 1978).

Behavioral modification is one of the most sensitive of environmental stress and may directly affect survival (Eisler, 1979). Available literature on bivalve behavioral response to stress is limited, but studies (Perkin, 1979;Eisler, 1979; Olla *et al.*, 1983) carried out in the recent past indicate the sub lethal effect on bivalve behavior can give some insight into the observed physiological changes. Mortality is an end point that can be readily recognized and quantified; hence the standard assay for acute toxicity testing of environmental stresses in aquatic medium or concentration that causes 50% mortality over a standard period of time. It is evident that death is a very crude index of stress in the environment, and that sub lethal effects can be induced at much lower levels than the LC_{50}. While not directly resulting in death, sub lethal effects can affect survival through effects on behaviors, growth, physiology, and reproduction (Bayne *et al.* 1978,1979, ;Viarengo *et al.*, 1980; Lowe *et al.*, 1982; Calabrese *et al.*, 1984).

Numerous workers have studied the relation of condition index of various species of bivalves from boreal and temperate coastal waters and its seasonal variation and relationship to level of available food and the annual reproductive cycle (Baird, 1958, 1966; Westley 1961; Walne 1970; Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Gabbott and Stephenson, 1974; Dare 1976). Walne (1970) has made detailed investigations on the seasonal changes in dry weight condition index and glycogen content .

Studies have demonstrated that the lipid levels and composition of marine bivalves clearly reflect the biochemical and environmental conditions of seed development (de Moreno *et al.*, 1980; Napolitano *et al.*, 1992; Fernandez-Reiriz *et al.*, 1996; Soudant *et al.*, 1998). With regard to the influence that such period of starvation on lipids, some authors have noted a drop in triacylglycerols of seeds of different marine bivalves (Fraser, 1989; Caers *et al.*, 2000).

The consequences of starvation on different fatty acids in marine bivalves have been highlighted in the work published by Langdon and Waldock (1981), who studied the essential fatty acids PUFAs n-3 and n-6 of oyster seed *Crassostrea gigas*. Various authors have suggested that the seasonal variations observed in the levels of total lipids, neutral lipids and fatty acids of various species of marine invertebrates is intimately related to the environmental factors (Perry *et al.*, 1979; Langdon and Newell, 1990; Chu *et al.*, 1990; Galap *et al.*, 1999).

Byssal threads are formed by glands located in the posterior part of the foot and consists of conchiolin, a molecular complex with protein component (collagen- type fibrils) tanned by an aromatic component (quinone). These byssal threads provide " an admirable means of attachment, tough, yet yielding and capable of repair and modification with changing circumstances (Yong, 1949)". The ability to produce and maintain these mooring lines is of importance in determining which habitats can be occupied. The rate of thread formation can be measured and is an informative index of activity (Reish and Agers, 1968). Environmental factors can influence byssus formation in the mussel *Mytilus edulis* (Yong,1985).

Exposure to air, mechanical agitation and acclimatization can influence the byssus formation (Van Winkle, 1970). Several abiotic factors such as salinity (Glaus, 1968; Van Winkle, 1970; Allen *et al.*,1976), temperature

(Glaus 1968, Allen *et al.*, 1976), water velocity (Maheo, 1970; Van winkle 1970; Price, 1982) and oxygen tension (Widdows and Bayne, 1971) have been observed to affect byssus formation in bivalves. Circadian and tidal rhythms also have significant affect as secretion of byssal threads (Martella, 1974). Influence of sediment grain size (Meadows and Shand, 1989) and salinity on byssal thread formation in *M. edulis* and *Glukensia demissa* has been studied (Plec and Alexender, 1999)

Information of the seed source and its performance in mussel farming has been the subject of the research in recent years (Dickie *et al.*, 1984 Fuentes *et al.*, 1992; Peterson and Beal, 1989; Rawson and Hilbish, 1991; Fuentes *et al.*, 1998; Perez Camacho *et al.*, 1995; Babarro *et al.*, 2000). Fleury *et al.* (1997) have conducted a preliminary study of the behaviour of reseeded juvenile great scallops of three sizes. Apart from the sources of seed, the method of seed transport affects the survival of bivalve seed (Maguire *et al.*, 1999).

Boyden (1972) studied exposure to air in two species of Cardium (*Cerastoderma)* the common cockle. During transport the bivalve seed is subjected to desiccation. Studies on the physiological and biochemical responses of desiccation in bivalve species have been well documented (Widdows *et al.*, 1979; Demer and Guderley, 1994) but not specifically with respect to transport. Marsden and Weatherhead (1998) have reported on the effect of aerial exposure on the oxygen consumption by the New Zealand mussel *Perna canaliculatus*. Survivorship of aerially exposed zebra mussel *Dreissena polymorpha* at varied temperatures has been studied (Paukstis *et al.*, 1999). Sadok *et al.* (1999) have given account of the effect of aerial exposure and reimmersion with reference to nitrogen metabolism *in M. edulis*.

Effect of aerial exposure on clams (Ali and Nakamura, 2000) and gastropods (Pearson *et al.*, 2000) has been studied. Studies on stress on *Perna viridis* in India are limited. Effect of thermal and salinity stress on the cardiac

activity has been studied by Bhat and Desai (1988). The variation in filtration and clearance rates depending on the salinity has been reported by Rajesh *et al.*,(2001).

The capacity of bivalve molluscs to accumulate potentially toxic heavy metals in their tissues, far in excess of environmental levels, is well known and has become the focus of an increasing numbers of studies (Manley and George, 1977; Phillips, 1976,1977; Bryan and Gibbs, 1983). Most of the evidence for absorption of metals and their radionucleotides from the solution seems to involve passive diffusion of the metal, probably as uncharged soluble complexes, down gradients created by absorption at the surface and binding by constituents of the surface cells, body fluids, and internal organs (Bryan, 1979; Carpene and George, 1981; Simkiss, 1983).

The most important source of heavy metal in bivalve molluscs is from suspended particles and sediments (Bryan, 1976, 1979). Tissue distribution of heavy metals as a result of bioaccumulation is typically uneven and in some cases shows a high degree of organ specificity (Bryan, 1973; Bryan and Gibbs, 1983; George and Pirie, 1983; Viarenngo *et al.*, 1980 a,b, 1981; Carmichael, 1980; Calabrese *et al.*, 1984) Cunningham (1979) gave a comprehensive review on the factors affecting accumulation, distribution, and loss of metals from tissues due to intrinsic and extrinsic factors.

The effect of heavy metals on the enzyme activity has been studied (Dixon and Webb, 1967). Also the effect on the function of several cellular constituents such as membranes (Rothstein, 1959), lysosomes (Moore, 1977), and mitochondria (Corner and Sparrow, 1956; Kleiner1974; Zaba and Harris, 1978; Akberali and Earnsaw, 1982; Akberali *et al.*,1984) have been made. Observations have been made on heavy metals and their inhibitory effects on physiological processes, e.g., ciliary's activity of the gills, oxygen consumption (Brown and Newell, 1979; Manley, 1983; Calabrese *et al.*, 1984; Martin *et al.*,

1984), heart rate (Scott and Major, 1972) and byssus synthesis (Martin *et al.*, 1975;Davenport, 1977).

Considerable work has been done on the toxicity in bivalves and trace metal content in the coastal waters along the Indian coast but studies on bioaccumulation in bivalves are limited. Heavy metal load in the mussels *Perna viridis* and *Perna indica* has been studied (Pillai *et al.*, 1986, Krishna Kumar, *et al.* 1998). Toxicities of selected heavy metals in *Perna viridis* (Lakshman and Nambisan, 1986) and *in vitro* effect of heavy metal ions as myofibrillar Ca^{++}ATPase activity in *P. viridis* (Nambudiri,1986) have studied. Combined toxicity effect of two heavy metals like silver and copper and mercury and cadmium on the green mussel has also been evaluated (Menon 1986 a, b.). Hawkins *et al.* (1986) have compared the metabolic response of *Perna viridis* and *Perna indica* to reduction of oxygen tension and salinity as indicator of pollution. Prakash *et al.* (1994) have reviewed the metal in marine waters and bivalves from the coastline of India.

Apart from using the green mussel *P. viridis* as the sentinel organism, studies have been made on other bivalves such as the edible oyster *Crassostrea madrasensis* (Rajenderan and Kurian 1986; Senthilnathan, 1986; Pillai *et al.*,1986). The toxic load in the clam *Villorita cyprinoids* (Pillai *et al.*, 1986) and *Meritrix casta* (Rajan *et al*;1986; Shanthi *et al.*,1986) has been studied Nair and Nair (1986) have reported on the seasonality of trace metals in bivalves. Lakshman and Nambisan (1986) have reported on bioaccumulation and depuration of trace metals by the green mussel *P. viridis.*

Accumulation of trace metals in different coastal regions has been studied during last three decades. The metals levels in the bivalves of Cochin area have been reported by Sankaranarayan *et al* (1978); Lakshman and Nambisan(1986) and Pillai *et al.*(1986). The trace metal in oyster along the Tamil Nadu coast have been reported by Pillai *et al.* (1986) and Santhinathan *et al.*

(1986). Trace metal content in the three species of bivalves along the Karnataka coast has been reported by Krishna Kumar *et al.* 1998. Balaji and Rao (2000) have studied the size related accumulation of heavy metals by *Mytilopsis salleli* at Vishakapatnam harbour.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 TEST ANIMALS FOR STRESS STUDIES

Seed of the green mussel *Perna viridis* (Linnaeus) for experiments on salinity and desiccation stress studies (3.4.1 to 3.4.5) described below were collected from the natural bed (Fig.1.) at Kollam Bay at Kollam, Kerala along the southwest coast of India. Seed were also collected from the spat collectors such as roof tiles and nylon net suspended from the raft suspended in the bay for studies under the NATP project on Mussel Mariculture of CMFRI (Fig.2).

Seed mussels were collected during October to December 2002 and adult mussels for experiment 3.4.6 to 3.4.8 during March to May 2003. Care was taken not to damage the foot or other internal organ while detaching the mussel seed from substrata. Mussels were cleaned of epibionts, detritus and fouling organisms and washed well with fresh seawater. The cleaned seed were transported under moist condition without water by road. The transit period was 4 hours, but the total exposure period from the time of collection to stocking in the hatchery of CMFRI at Cochin was 7 hours. Part of the collected seed was used immediately for experiment (3.4.2) conducted to evaluate the mortality when the seed were exposed to different salinities with out conditioning. Rest of the seed was stocked in 5 liter tanks in 32ppt salinity with good aeration. Dead animals were periodically removed and complete water exchange was provided daily for 3 days. These conditioned seed were used for experiments 3.4.1, 3.4.3, 3.4.4, and 3.4. 5. Before stocking, the length of the seed was measured to nearest 0.01mm by digital Vernier calipers. Based on the length measurement the seed were graded into two

Fig.1. Mussel seed settled in the intertidal area in Kollam Bay

Fig.2. The mussel farm : the raft moored at Kollam Bay. The spat settlers were placed in this raft and mussels for analysis of heavy metal accumulation were also collected from this.

size groups, Group 'A' of 25 to 40mm length (average) and group 'B' of 10 to 25mm length (average).

3.2. LOCATION OF SITES FOR SAMPLE COLLECTION OF ANALYSIS OF HEAVY METALS

Mussel samples for studying bioaccumulation (3.4.6 to 3.4.8) were collected from Kollam ($8^0$45'- $9^0$28'N and $76^0$28'- 77 ^{0}17'E) along the south west coast of India. Mussels samples from the fishery at Kollam bay and from the off bottom farm (raft) in the same area were used for analysis of heavy metals. The fishery was based on the mussel population in the artificial semi enclosed bay with a permanent opening to the Arabian Sea. The depth at the farm site was between 2.5 to 3 m.

Samples were also collected from the mussel farm in a shrimp pond at Dalavapuram in Ashtamudi Lake. The mussel ropes in this farm were suspended horizontally between stakes above half a meter from the pond bottom.

3.3. ALGAL CULTURE

Pre-sterilized transparent plastic buckets of 10-liter capacity were used for mass culture of *Chaetoceros caliciterans*. These were filled with filtered seawater after addition of appropriate concentration of nutrient media as Walne medium. [1ml/lit A and B solution and 0.5 ml/lit silicate for *Chaetoceros*]. Each bucket was filled up to 6 liters with the addition of 10% good inoculums of *Chaetoceros* and placed near the window in natural light. The algal cell density was measured using haemocytometer daily.

3.4. EXPERIMENTAL PROCEDURES

The description of the experiments conducted and the methodology for trace metal analysis is given below

3.4.1. *Effect of salinity on the mussel seed survival: mortality after conditioning*

Experiments were set up at marine hatchery complex, CMFRI, Cochin, to assess mortality of two size groups of seed of green mussel *Perna viridis* (Fig.3). The animals were considered dead if it did not close the valve or were not responsive to physical stimuli.

The conditioned seed were exposed to various salinities between 5 and 50 ppt, taken at 5 ppt intervals i.e. 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50ppt. All the exposures were conducted in triplicate at a stocking density of 50 animals in 3 liters of sea water of required salinity in four liter plastic troughs. Experimental animals were fed with *Chaetoceros calicetrans* @ 50ml day^{-1} and uniform aeration was provided. At the end of every 24 hours the mortality was recorded and the dead animals were removed.

The lower salinity levels were prepared by diluting the seawater with required amount of aged, filtered tap water. Desired salinity levels from the known higher salinity was prepared by using the formula

$$V = \frac{\text{Required salinity} \times 100}{\text{Known higher salinity of seawater}}$$

Fig.3. Experimental set up in CMFRI Hatchery Complex at Cochin

Fig.4. The trace metal analyser - 757 VA Computrace (Metrohm, Switzerland).

Where, V is volume of seawater of known salinity that should be taken to diluted with fresh water to make 1 liter solution of desired salinity. The salinity of the prepared medium was checked by refractrometer.

Higher concentrations were made by dissolving required amount of common salt in 35ppt seawater, which was allowed to settle, filtered and used for the study (Alagarswami and Victor, 1976; Mane and Tilkedhar, 1976).

3.4. 2: Effect of salinity on mussel seed: mortality without conditioning

To assess the effect of combined effect of transportation and salinity the mussel seed were directly exposed to different salinities. The mussel seed were directly exposed to the salinity levels as in 3.4.1 without conditioning. The experimental procedure regarding stocking density, rearing condition, feed and observation were made as for 3.4.1

3.4.3. Effect of Salinity variation on condition index

The condition index of the surviving mussel seed in salinities 15 to 45 in 3.4.1 was found out. Ten mussels from each salinity treatment were taken. The total weight of mussel meat dried at 80°c over night in a hot air oven of each sample was taken in an electronic balance. The shells were dried and weighed. The condition index was calculated using the following formula (Walne and Mann, 1975).

$$\text{Condition index} = \frac{\text{Dry meat weight (g)}}{\text{Dry shell weight (g)}} \times 1000$$

3.4. 4. Effect of salinity on the total lipid content of mussel seed

To assess the quantity of lipid in seed mussels exposed to various salinity, live and healthy green mussels were taken from the surviving seed in 3.4.1. Total lipid was isolated by Choloroform methanol estimation method of extraction method (Folch *et al.*,1975). About 0.5g of dry tissue (pooled) were homogenized well in 7.5 ml of chloroform: methanol (2:1v/v) in pestle and mortar. The extraction step was repeated for the residues. The filtrate from the two extraction were transferred to a 100ml separating flask, and 2ml of distilled water was added, shaken well and allowed to stand for overnight at room temperature. Lower chloroform layer was collected in a beaker and evaporated to 5ml in a vacuum desiccator. Total lipid of the sample thus prepared was quantitatively estimated by Sulphophosphovanillin method (Barnes and Blackstock, 1973). 0.5ml of lipid extract was taken and dried in vacuum over silica gel in desiccators. To the dried sample, 0.5 ml of concentrated sulphuric acid was added and shaken well. The tubes were than plugged with non-absorbent cotton wool and heated at 100°C in a boiling water bath for exactly 10 minutes. The test tubes were rapidly cooled to room temperature under running tap water, washed and 0.2ml of this acid digest was taken in a separate tube, 5ml of vanillin reagent was added and kept undisturbed for half an hour. The absorption level and developed colour was measured at 520nm. 0.2ml of the cholesterol (8mg of cholesterol in 4ml of chloroform: methanol (2:1v/v) was taken as Standard 0.2ml and 0.2ml of chloroform was taken as blank.

Total lipid content (mg g^{-1} Dry wt.) =

$$\frac{\text{OD of unknown (sample) x vol. of extract (ml)}}{\text{OD of known X Dry weight of sample (mg)}} \times \mu g \text{ of standard}$$

3.4. 5. Effect of desiccation on survival of mussel seed.

This experiment was conducted to determine the effect of aerial exposure on survival of mussel seed. The conditioned seed of mussel were placed outside the rearing tank without water to various time periods ranging from 2 to 48 hours. Groups of 50 nos. of mussel seed (20- 40mm shell length) were selected and exposed for different duration such 2, 4, 6,8,10,12,14,18,24,30 and 48 hrs . At the end of these time intervals the number of dead seed was recorded and the surviving seed were placed in ambient salinity (32ppt). Their survival was recorded for 30 hours at 6-hour intervals i.e. after 6hr, 12hr, 18hr, 24hr, 30 hr, 36 hr, and 48 hr. During aerial exposure or desiccation, animals were not fed but during the second phase *Chaetoceros calcitrans* was provided as feed @ 25ml/tub at 12 hour intervals. The number of dead animals during the recovery period was noted and the percentage mortality calculated. The behaviour of the animals and the formation of byssus was observed and taken as an indicator of stress.

3.4. 6: Trace metals (zinc, cadmium, lead, and copper) accumulation in mussel collected from different systems.

Green mussel samples collected from three areas such as natural bed in Kollam Bay, from suspended raft (off-bottom) in the Bay and from a mussel farm in shrimp pond in estuarine system in Ashtamudi Lake were collected and transported to the lab at CMFRI, Headquarters at Cochin.
The mussels were stored in the deep freezer after collection till the analysis.

3.4.6.1 Sample preparation and acid digestion of tissue sample:

10 numbers from each size groups of mussels of same size were taken. Each animal was rinsed with distilled water. Soft part of the animal in

its shells was rinsed with distilled water. The steps wise procedure followed for heavy metal analysis is given below:

1. Tissue was pooled, homogenized with a sharp scissors and mixed well in a clean watch glass
2. 2.5 grams (wet weight) of each of the above tissue sample was transferred to a digestion tube of Macro-Kjeldhal digestion system.
3. Ultra pure grade of HNO_3 and H_2O_2 were used for sample digestion (Martin and Flegal, 1975; Dalziel and Baker, 1983; Krishna Kumar *et al.*, 1990). 20ml of mixture digestion HNO_3 and H_2O_2 in1:1 ratio was added to the sample in the tube covered with watch glass and kept overnight.
4. Then the sample was placed for digestion for two hours at 105°c in Macro-kjeldhal digestion unit.
5. Digested sample was filtered by passing through Whatman no.40 filter paper into a 100 ml volumetric flask, made up to the mark with ultra pure water.
6. 1 ml sample was again diluted to 10 ml by adding ultra pure water, then 1 ml KCl buffer was added to sample, pH was adjusted to 4.6 with help of dil. HCl and NaOH.
7. The prepared sample was analysed using 757 VA Computrace (Metrohm, Switzerland) (Fig.4.) according the method No. AB231_1_Det of Zn, Cd, Pb, Cu. The final concentration of heavy metals in $\mu g\ g^{-1}$ wet wt. was calculated using the formula

$$\text{Concentration of heavy metal } (\mu g\ g^{-1} \text{ wet wt.}) = \frac{\text{VA 757reading x 100}}{\text{Weight of tissue sample}}$$

3.4.7. Variation in trace metal content relative to size groups

Based on the length measurements, the mussels collected from the natural bed in Kollam Bay, from the suspended (off-bottom) raft in the bay and from the mussel farms in a shrimp pond in the estuarine

system of Ashtamudi Lake were segregated in three groups viz. 40-50mm, 60-70mm and 80-90mm size. The mussels in these groups were pooled and analyzed for the presence of heavy metals by the method described in 3.4.6.1

3.4.8. Trace metal content in male and female mussels collected from three systems

The mussels collected from the three systems were segregated into male and female based on the colour of the gonad and microscopic analysis of the gonad smear. Light orange or deep red coloured gonads were identified as females and light cream colored gonad as male and analyzed for the presence of heavy metals by the method described in 3.4.6.1

3.5. STATISTICAL ANALYSIS

The LC_{50} values for the large and small seed in different salinities after conditioning and without conditioning and for desiccation were estimated by the Probit Analysis method in the SPSS software. The correlation value (R^2) for bioaccumulation of heavy metals with size was calculated.

RESULTS

4. RESULTS

4.1: Effect of salinity on mussel seed after conditioning

The mortality of the two length ranges, Group 'A' large seed (25 to 40 mm) and Group 'B' small seed (10 to 25mm) when exposed to 5,10, 15, 20, 25, 30, 35, 40, 45, and 50ppt salinities after conditioning at room temperature and ambient salinity (32 ± 1.50 ppt) showed a similar pattern. On the first day after immersion, there was no mortality in both the groups of mussels. Mortality in lower salinities, 5 to 15ppt and higher salinity, 50ppt started from the second day onwards. Complete mortality was observed in 5, 10 and 50ppt salinities by fifth and sixth days respectively (Table 1). In 15 to 20 ppt, the percentage mortality varied for both these groups(Figs. 5, 6 and 7). In salinities 20 to 35ppt, all the small size seed survived while in the large group, low mortality ranging between 4 to 10% was observed. In higher salinities between 40 to 45ppt the mortality ranged between 8 to 16%. In general, it was observed that larger size seed were more tolerant to lower salinity. The seed had formed byssus threads and were attached to the rearing container from the first day onwards. From day 9 onwards the surviving mussel seed showed healthy behaviour. The LC_{50} value for large seed was 14 while for smaller seed it was 21 ppt (Table 2, Fig. 8).

4.2 . Effect of salinity on mussel seed without conditioning

The mortality of mussel seed (25 to 40 mm) when directly exposed to different salinities such as 5,10,15,20,25,30,35,40,45, and 50ppt salinities, without conditioning was higher in all the salinities (Fig.9 and 10). Mortality was observed from day 1 onwards, ranging from 6 to 36% with highest mortality in 50ppt (Table 3).

Table 1. Mortality of two size groups of seed of *Perna viridis* when exposed to different salinities after conditioning

A = 25 to 40 mm seed : B = 10 to 25 mm seed

Sl. No.	Salinity (ppt)	Size group	Mortality in numbers (n= 50)									Total mortality (%)
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	
1	5	A	0	6	20	18	6	0	0	0	0	100
		B	0	18	10	12	10	0	0	0	0	100
2	10	A	0	5	10	19	16	0	0	0	0	100
		B	0	12	20	10	8	0	0	0	0	100
3	15	A	0	1	1	2	1	5	3	2	4	38
		B	0	15	3	2	1	1	1	1	1	50
4	20	A	0	1	1	2	1	3	8	7	5	56
		B	0	1	0	0	0	0	0	0	0	0
5	25	A	0	0	0	0	0	0	0	0	2	4
		B	0	0	0	0	0	0	0	0	0	0
6	30	A	0	0	0	0	0	1	0	0	0	2
		B	0	0	0	0	0	0	0	0	0	0
7	35	A	0	1	0	0	0	0	2	0	0	6
		B	0	0	0	0	0	0	0	0	0	0
8	40	A	0	0	0	0	0	2	0	1	0	10
		B	0	0	0	0	3	1	0	0	0	8
9	45	A	0	0	0	3	2	1	0	0	0	12
		B	0	0	2	3	1	0	0	2	0	16
10	50	A	0	28	12	10	0	0	0	0	0	100
		B	0	32	15	3	0	0	0	0	0	100

Table 2. LC_{50} values related to for different stress factors in the seed of *Perna viridis* – Results of Probit Analysis

Sl.no	Seed	Experiment details	LC_{50}
1	Length 25 to 40 mm	Conditioned seed	14 ppt
2	Length 10 to 25 mm	Conditioned seed	21 ppt
3	Length 25 to 40 mm	Unconditioned seed	32 ppt
4	Length 25 to 40 mm	Desiccation	24 hrs

Fig.7. Comparison of mortalities of large and small size seed of Perna viridis when exposed to different salinities

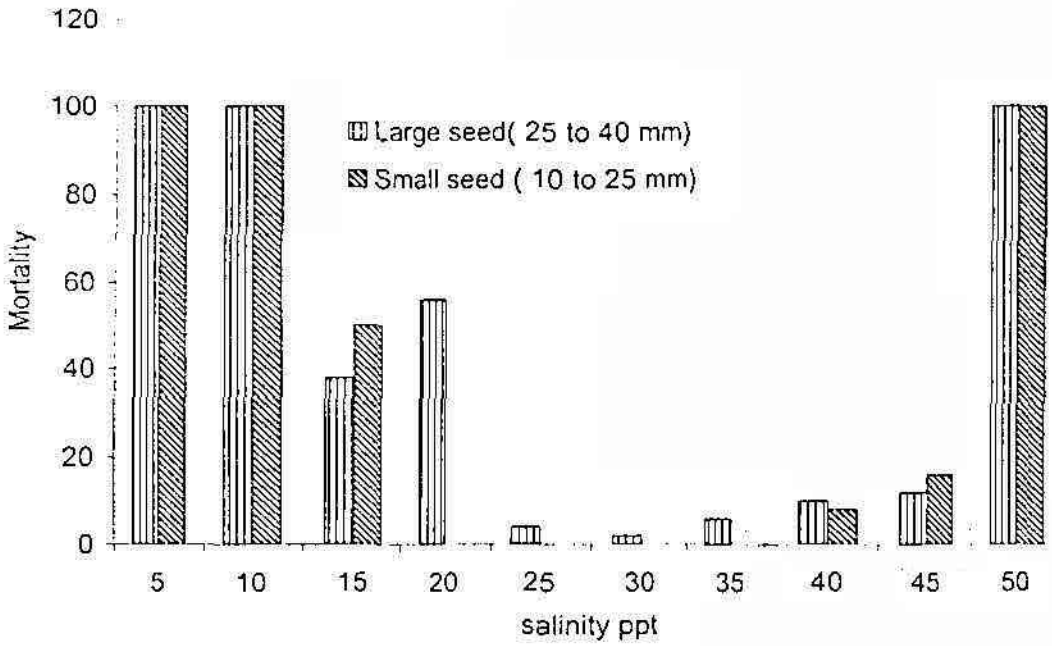
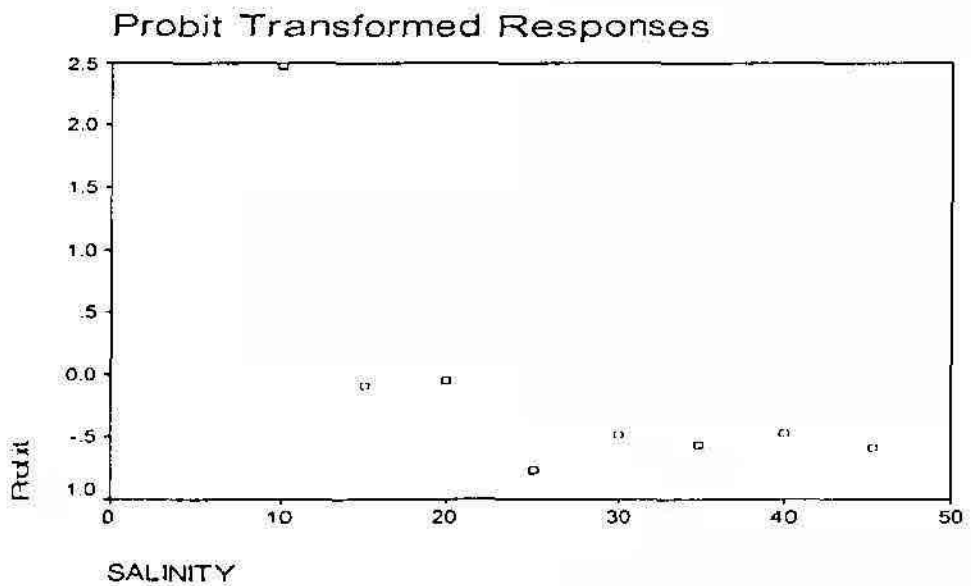


Fig.8. Probit transformed responses – LC_{50} value of large size seed of mussel



By the third day 100% mortality was observed in 5ppt while for the acclimatized seed 100% mortality was only on day 5. In 10 and 50ppt salinities also complete mortality was observed (Fig 9). In 15 and 20ppt salinities, the increase in mortality was 2.75 and 2.2 times to that observed when seed were transferred after acclimatization. Mortality in 25 to 40ppt mussel was 22% to 32% when transferred directly, which was 7 to 22 times higher than that observed when the seed were conditioned prior to exposure to salinity variation. Byssus formation and attachment were observed only from day 3 onwards compared to early byssogenesis in 3.4.1.The LC_{50}, values of unconditioned seed of the same size was 32 ppt (Table 2)

4.3. Effect of salinity variation on condition index

Condition index of the surviving mussel seed in all salinities except 45ppt, was lower than that of control mussel seed (Table 4). In 45ppt salinity it was slightly higher (Fig. 11). Though the condition index was lower, the seed were healthy as indicated by the strong byssal attachment to the substrata.

4.4: Effect of salinity variation on total lipid content

The total lipid content of the mussel seed surviving in different salinities showed wide variation (Fig. 12). The total lipid content of large seed was 210.9± 3.6 mgg^{-1}body weights while in the smaller size seed it was 161.2±9.3 mgg^{-1}body *weight (Table 4). After exposure to various salinities the total lipid content of* large seed was lower than the control in all salinities, except 15 and 45ppt ranging from 131.07±0.5 to 202.9±1.9 mgg^{-1}. The highest total lipid content was 268.49±1.5 mgg^{-1}*in the mussel seed surviving in 15ppt.*

Table. 3. Mortality of seed of *Perna viridis* when exposed to different salinities without conditioning

	Duration	SALINITY (ppt)									
		5	10	15	20	25	30	35	40	45	50
1	Day 1	20	18	10	8	6	18	14	26	14	36
2	Day 2	54	32	16	14	14	22	22	32	18	60
3	Day 3	100	56	20	30	18	22	26	32	22	74
4	Day 4	100	76	28	34	22	22	28	32	28	98
5	Day 5	100	92	40	38	22	22	28	32	28	100
6	Day 6	100	100	44	44	22	22	28	32	28	100

Table 4.Effect of salinity on the condition index (CI) and total lipid (TL) content of mussel seed after exposure to different salinities

A = 25 to 40 mm seed B = 10 to 25 mm seed			
Salinity (ppt)	CI	TL-A	TL-B
15	139 ± 3.6	268.49 ± 1.5	251.93 ±7.6
20	100 ± 1.5	207.05 ± 2.3	167.61 ±16.0
25	109 ± 3.4	131.07 ± 0.5	96.535 ±2.8
30	112 ± 8.1	202.97 ± 1.9	197.05 ±7.9
35	137 ± 8.5	160.15 ± 7.6	148.26 ±6.2
40	95 ± 4.1	129.12 ± 2.2	90.03 ±3.7
45	151 ± 4.8	242.87 ± 6.9	226.3 ±14.9
Control	148 ± 0.4	210.94 ± 3.6	161.24 ±9.3

Table 5. Mortality of mussel seed during desiccation for different duration and after immersion in ambient salinity.

Air exposure / Desiccation				Mortality After immersion							
	Hours	Number exposed	% Mortality	Mortality in numbers							%mortality
Sl.				6 hrs	12 hrs	18 hrs	24 hrs	30 hrs	36 hrs	48	%
1	2	50	0	0	0	0	0	0	0	0	0
2	4	50	0	0	0	0	0	0	0	0	0
3	6	50	0	0	0	0	0	3	0	0	6
4	8	50	0	0	3	2	0	1	0	0	12
5	10	50	0	0	0	2	0	0	0	0	4
6	12	50	4	2	0	0	0	3	0	0	14
7	14	50	0	2	0	4	3	2	0	0	22
8	16	50	0	2	1	2	1	4	0	0	20
9	18	50	6	0	0	0	0	0	0	0	6
10	20	50	8	0	0	0	4	3	0	0	22
11	22	50	6	4	4	0	0	0	0	0	22
12	24	50	4	2	1	0	0	8	0	0	26
13	26	50	12	0	4	5	2	4	0	0	42
14	28	50	18	4	2	7	5	8	0	0	70
15	30	50	24	7	9	6	8	6	0	0	96
16	36	50	38	4	11	9	3	4	0	0	100
17	40	50	64	12	2	4	0	0	0	0	100
18	48	50	100	0	0	0	0		0	0	100

Fig.9.Cumulative mortalities of large seed (25 to 40 mm) of *Perna viridis* exposed to different salinities without conditioning

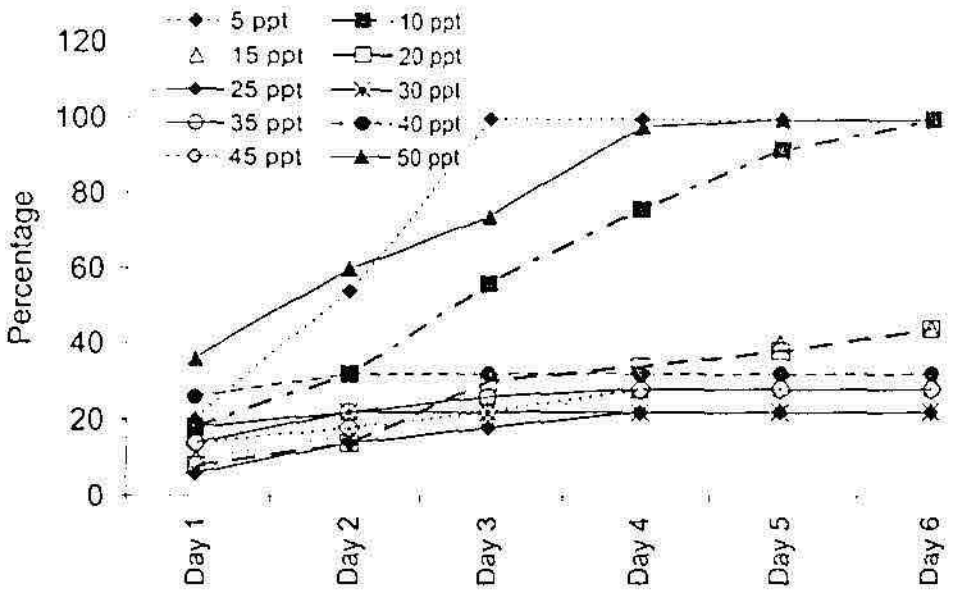


Fig.10. Comparison of mortalities of seed of *Perna viridis* in different salinities on Day -6 :
Direct immersion (DIR) vs immersion after conditioning (ACC)

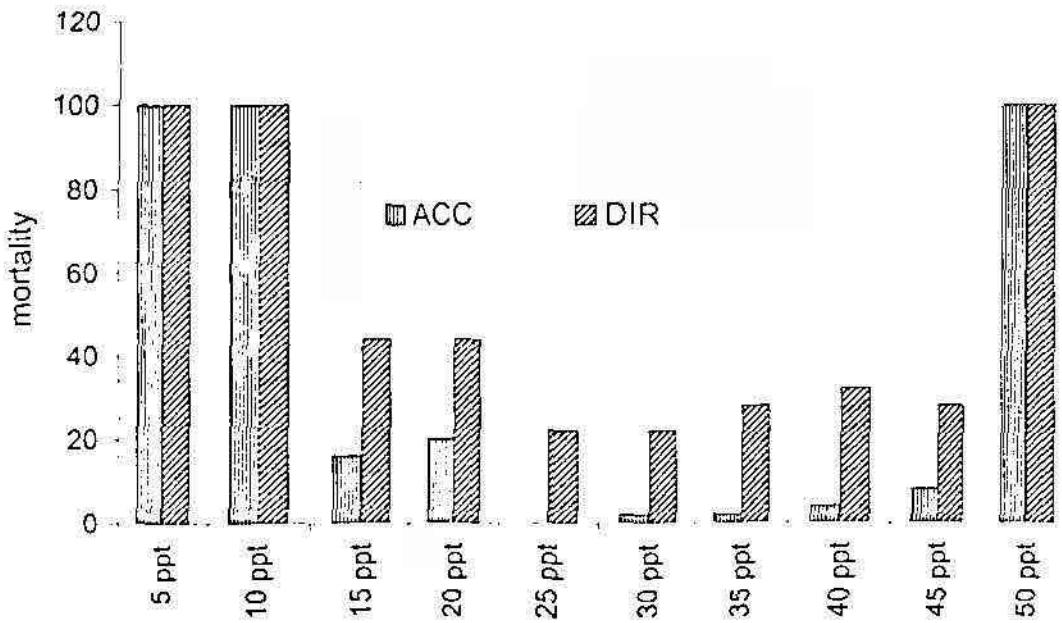


Fig.11. Condition index of *Perna viridis* exposed to different salinities

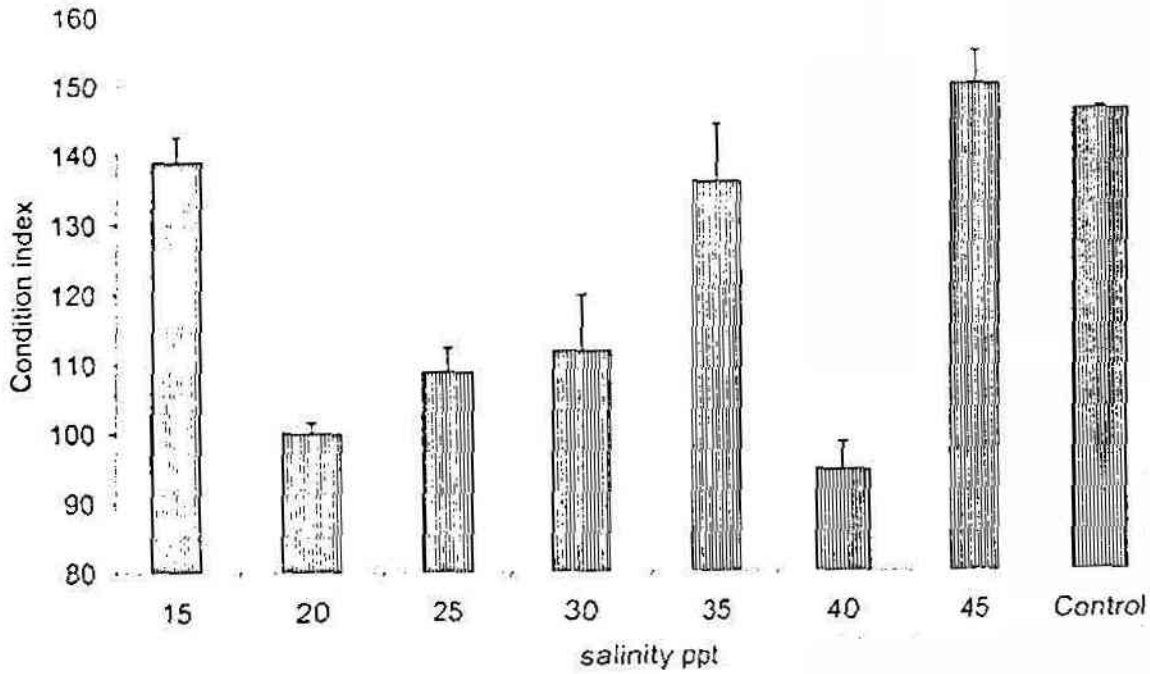
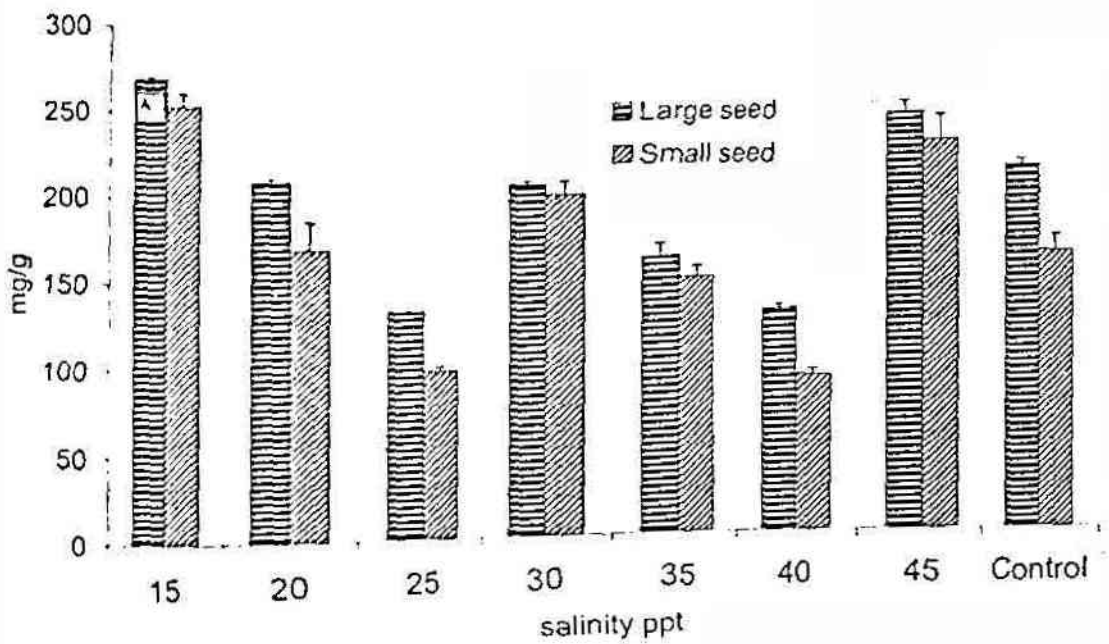


Fig.12. Total Lipid content (mg /g) in different size groups of *P viridis* exposed to different salinities



The total lipid content of smaller seed exposed to different salinities also varied from that of control samples. The lipid content was low in 40ppt (90.03 ±3.7 mg g^{-1}) and in 25ppt (96.5±2.8 mgg^{-1}) and high, 251.93 ±7.6 mg $g^{-1}$and 226.3±14.9 mg g^{-1}*in the mussel seed exposed to 15 and 45ppt salinities.*

4. 5. Effect of desiccation on survival of mussel seed

It was observed that up to 4-hour exposure to air, all the seed survived; there was no mortality either during air exposure or after immersion (Table.5). During 6 to 10 hours of air exposure, there was no mortality during the desiccation period, but 4 to 14% of the seed suffered mortality with 30 hours of immersion (Fig. 13). In seed exposed for 12 and 24 hours of desiccation, total mortality ranged between 6 and 26% during the recovery period at the end of 30 hour and was stabilized thereafter. Desiccation for 26 to 30 hours resulted in very high mortality ranging from 42 to 96% at the end of 30 hours of the recovery time. Above 30 hour up to 48 hours of desiccation, none of the seed survived. 100% mortality occurred during the desiccation period during 48 hours exposure.

4.6: Trace metal content in mussel samples collected from three sites

Among the trace metals Zn, Cd, Pb, and Cu, concentration of zinc was highest in the mussel samples collected from three systems viz. natural bed on-*bottom (Bay); off-bottom* (Bay) and estuarine pond (Table 6). While the concentration of Zn was 64.966μg g^{-1}wet weight in the mussel collected from natural bed of Kollam bay, in samples collected from the raft, Zn concentration was 38.893 μg g^{-1}wet weight. In the mussels cultured in the estuarine farm, concentration of Zn was 53.117μg g^{-1} wet weight which was higher than the in the mussel cultured in raft.

Fig.13 Mortality of seed mussels during various periods of dessication and at the end of 30 hrs after immersion

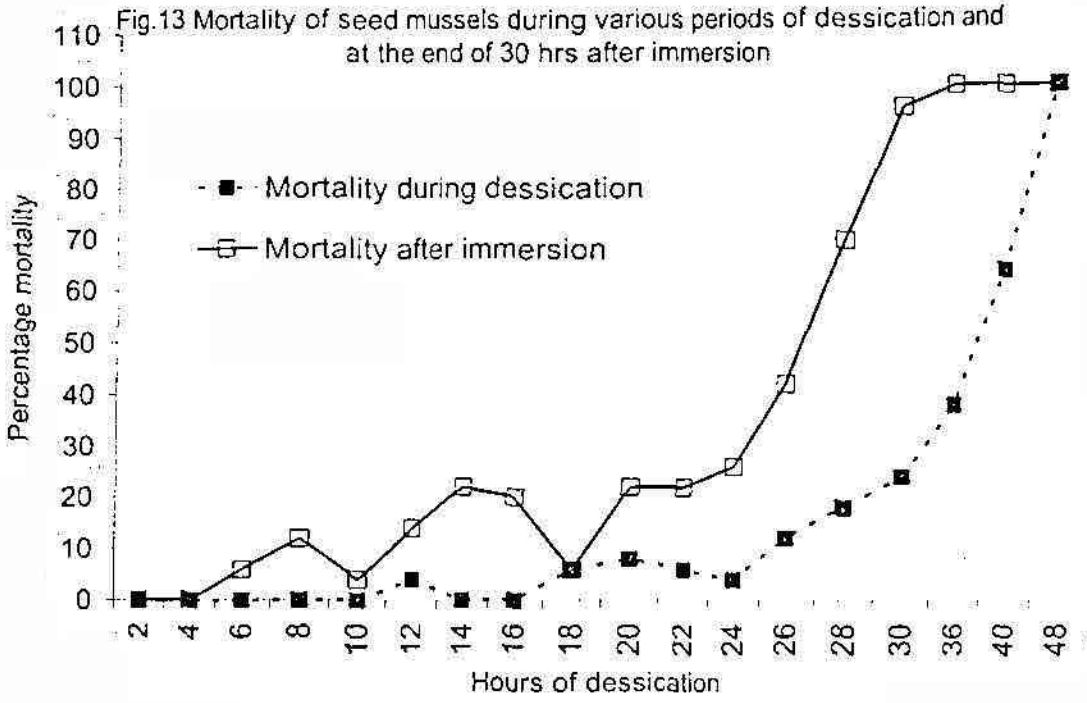
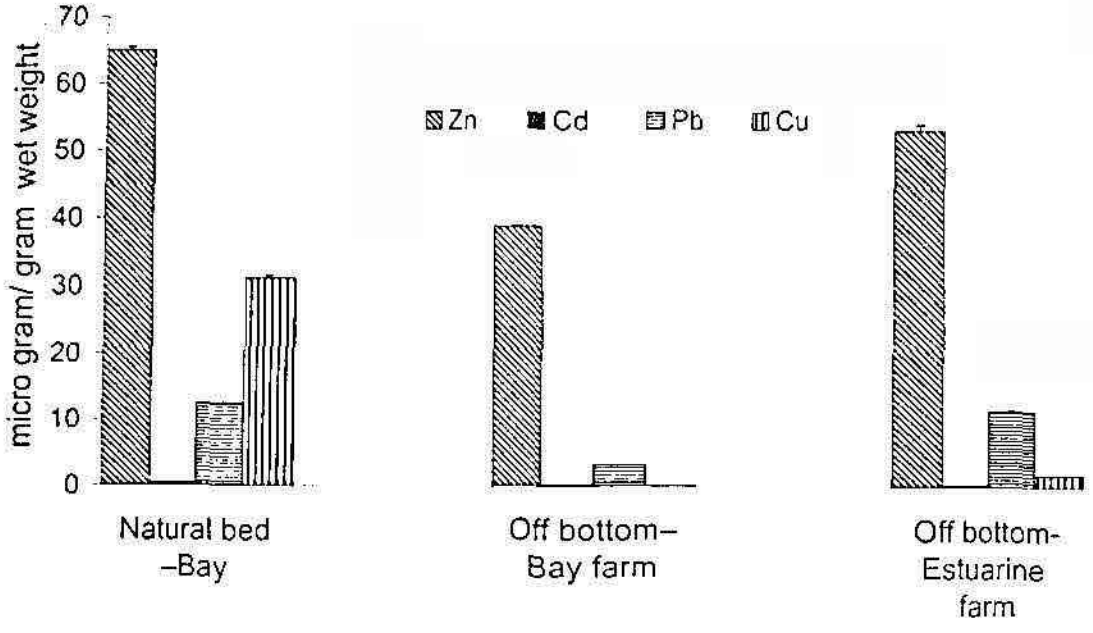


Fig.14. Average concentration of trace metal (micro gram/gram) in the mussel samples collected from three systems



The order of magnitude of bioaccumulation of the trace metals was Zn>Cu>Pb>Cd in the mussel collected from the natural bed while in the mussel cultured in the off-bottom system in bay and estuarine pond were Zn>Pb>Cd>Cu and Zn>Pb>Cu>Cd respectively.

Accumulation of copper and lead were very low compared to Zn. In the mussel collected from natural bed copper content was more, 31.183 ± 0.433μg g^{-1} wet weight. In the same area, the concentration of Cu was 0.03 ± 0.003 μg g^{-1} in the mussel collected from (off-bottom) raft. Low Cu concentration, 1.9943± 0.17μg g^{-1}wet weight was observed in the estuarine pond mussel samples.

Concentration of lead was almost same, 12.63±0.057μgg^{-1}wet weight and 11.693μg g^{-1}wet weight in the mussel samples collected from natural bed and off-bottom pond farm. The same metal in the mussel samples collected from raft were much lower, 3.237μg g^{-1} wet weight.

Accumulation of cadmium was negligible, 0.443±0.007μg g^{-1} wet weight, 0.067±0.00 μg g^{-1} wet weight and 0.293±0.00μg g^{-1} wet weight in the mussel samples collected from natural bay bed, bay raft, and estuarine pond off-bottom culture respectively.

4. 7: Variation in trace metal content relative to size groups

It was observed that the concentration of Zn and Pb increased with the size of mussel, with higher concentration in larger size animals in the natural bed and in the estuarine pond (Table 7, Fig. 15). The concentration of Zn was 33.369±0.01μg g^{-1}wet weight in 40-50 mm mussel while in 60-70mm and 80-90mm size groups of natural bed concentration were 34.84±0.33μgg^{-1}wet weight and 126.67±0.95μg g^{-1}wet weight respectively. In estuarine pond farm also the concentration of Zn increased from 38.56μg g^{-1}wet weight in mussel of <35mm to

Table 6. Average trace metal content (mean ± s.d) in $\mu g\ g^{-1}$ wet weight in *Perna viridis* collected from different systems

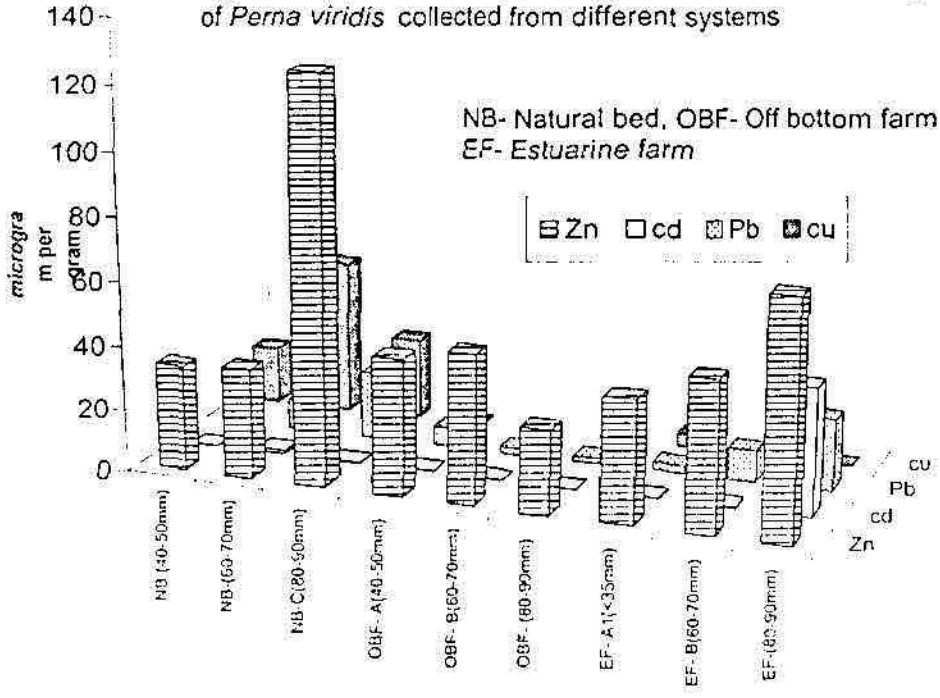
Source of sample	Zn		Cd		Pb		Cu	
Natural bed –Bay	64.99	±0.457	0.443	±0.007	12.63	±0.057	31.183	±0.433
Off bottom– Bay farm	38.893	±0.147	0.067	±0.000	3.237	±0.013	0.030	±0.003
Off bottom- Estuarine pond farm	53.117	±0.880	0.293	±0.000	11.693	±0.093	1.943	±0.017

Table 7. Trace metal content (mean ± s.d) in $\mu g\ g^{-1}$ wet weight in different size groups of *Perna viridis* collected from different systems

Natural bed –Bay								
size	Zn		Cd		Pb		Cu	
A(40-50mm)	33.369	±0.09	0.00	±0	6.350	±0.050	18.38	±0.110
B(60-70mm)	34.840	±0.33	1.14	±0.02	9.740	±0.030	49.20	±0.780
C(80-90mm)	126.69	±0.95	0.19	±0	21.78	±0.090	25.97	±0.410
Off bottom– Bay farm								
	Zn		Cd		Pb		Cu	
A (40-50mm)	43.11	±0.16	0.20	±0.00	5.99	±0.03	0.08	±.010
B (60-70mm)	46.97	±0.24	0.00	±0.00	2.06	±0.01	0.01	±0.00
C (80-90mm)	26.60	±0.04	0.00	±0.00	1.66	±0.000	0.00	±0.00
Off bottom- Estuarine farm								
size	Zn		Cd		Pb		Cu	
A1(<35mm)	38.56	±0.00	0.00	±0.00	2.400	±.010	4.36	±0.05
B(60-70mm)	41.31	±2.30	0.48	±0.00	9.960	±0.16	1.47	±0.00
C(80-90mm)	73.48	±0.34	0.40	±0.00	22.72	±0.11	0.00	±0.00

47.31±2.3 μgg^{-1}wet weight and 73.48±0.34$\mu g\ g^{-1}$wet weight in the 60-70mm and 80-90mm mussel samples respectively. However in the off-bottom cultured mussel in the bay, the concentration of Zn was low, 43.11±0.04μgg^{-1}wet weight in 80-90mm mussels while it was high 46.97mg/g in 60-70mm mussels.

Fig.15.Comparison of trace metal content in different size groups of Perna viridis collected from different systems



The concentration of copper showed variation in different size groups of mussels in the three systems. However Cu did not show any increasing trend with size. In the off-bottom cultured system the concentration of copper reduced with increase in size and became negligible in large size mussels. Cadmium was negligible in all the size groups and did not show any trend in accumulation.

The r^2 values of the relationship between different size groups and bioaccumulation of Zn, Cd, Pb, and Cu in mussels collected from the different systems is given in the Table. 8 and depicted in the Figs. 16. 1 to Fig. 16.12. The r^2 was *positive and significant for Zn in the natural bed* (r^2 =0.7869) and the

estuarine farm (r^2 = 0.5357). In the off-bottom farm in the bay it was negative (r^2=0.6445). The bioaccumulation of lead with increasing trend relative to size of mussel was observed in natural bed (r^2 = 0.7266). In the off-bottom farm in the bay (r^2 =0.7001) and in the estuarine pond farm (r^2 =0.8174) bioaccumulation of lead showed a negative relationship with size.

4.8: Trace metal content in male and female mussels collected from three systems

Trace metal content in the same size group of male and female mussels collected from three different location/systems was compared (Fig. 17). It was observed that the Zn concentration was similar in the male and female in the 40-50 and 60-70mm size groups of animals collected from the natural bed at Kollam Bay (Table 9). However in the large size group 80-90mm, the concentration of Zn was higher 135.568±0.44μg g^{-1}wet weight in female mussel than in males, 117.821μgg^{-1}wet weight. However, in the same size group at the estuarine farm the Zn content was higher 88.33±0.48μgg^{-1}wet weight in male mussel while in the female mussels it was 58.622±0.19μgg^{-1}wet weight. Copper concentration was higher 66.649±0.34μgg^{-1}wet weight in the female than the male 31.746±0.22μgg^{-1}wet weight in the mussel (40-50mm) collected from the natural bed at Kollam Bay. In the higher size group (80-90mm) mussel copper contents was higher (33.774μgg^{-1}wet weight) in females than males (18.169μgg^{-1}wet weight). In the same location, Cu was very low in both male and female mussels collected from the suspended raft. In the estuarine farm, 80 to 90 mm size male and female mussel did not have any accumulation of copper while in the 60-70 mm mussels, copper content more, 2.948 μgg^{-1}wet weight in male and while in females it was negligible.

Content of lead was slightly higher in the males of size 60-70mm mussel collected from natural bed bay (12.048 ± 0.06μgg^{-1}wet weight), than in the female, 7.437±0.01μgg^{-1}wet weight. However in the higher size 80-90mm the

content of lead was more (27.724±0.09μgg^{-1}wet weight) in females than in male (15.825±0.09μgg^{-1}wet weight). In the mussel samples collected from the off-bottom farm at the Kollam Bay the concentration of lead in the 60-70mm size group was only slightly higher (2.510±0.01μgg^{-1}wet weight) in the male than in the females (1.614±0.02μgg^{-1}wet weight). In the higher group, (80-90mm), there was no variation in the concentration of lead.

In the mussel sample collected from the farm in the estuarine pond concentration of lead was higher 18.566±0.26μgg^{-1}wet weight and 30.635±0.23μgg^{-1}wet weight in the males of the 60-70 and 80-90 size groups than in the females, 1.360±0.058 μgg^{-1}wet weight and 14.803 ±0.110 μgg^{-1}wet weight of same size.

Table 8. : Regression values of the relationship between size and trace metal concentration at different sites.

Sl. No.	Source	Trace Metal	Equation	Regression value (R^2)
1	Natural bed Bay	Zn	Y = 2.5422 x -101.58	0.7869
2	Natural bed Bay	Cd	Y =-0.0048 x +0.8625	0.0075
3	Natural bed Bay	Pb	Y = 0.4129 x -14.198	0.7266
4	Natural bed Bay	Cu	Y= 0.0632 x +29.444	0.0032
5	Off-Bottom raft Bay	Zn	Y = -0.7489 x +87.629	0.6445
6	Off-Bottom raft Bay	Cd	Y = -0.0056 x +0.4123	0.5871
7	Off-Bottom raft Bay	Pb	Y = -0.1335 x +11.522	0.7001
8	Off-Bottom raft Bay	Cu	Y = -0.0498 x +3.7478	0.652
9	Estuarine farm	Zn	Y = 0.6674 x +14.914	0.5357
10	Estuarine farm	Cd	Y = -0.0034 x +0.2509	0.743
11	Estuarine farm	Pb	Y = -.0782 x +7.5057	0.8174
12	Estuarine farm	Cu	Y= -0.0301 x +2.3068	0.8103

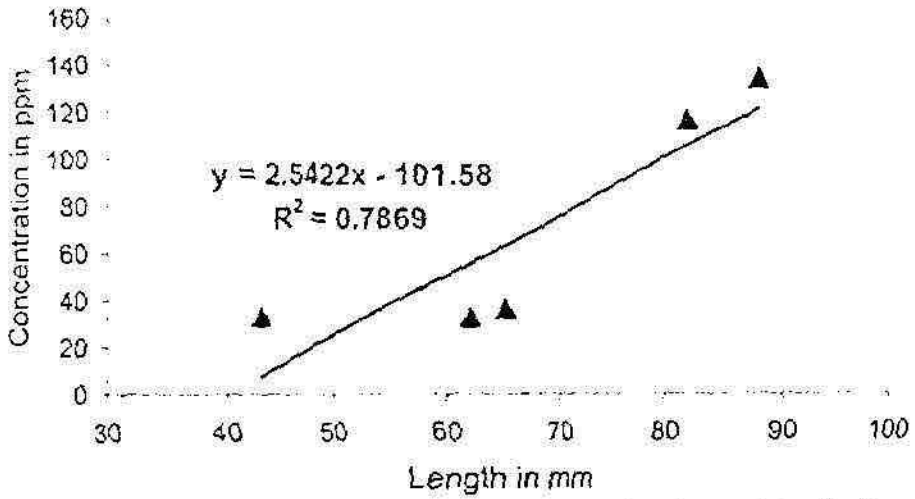


Fig.16.1.Concentration of Zinc in mussels- Natural bed in Bay

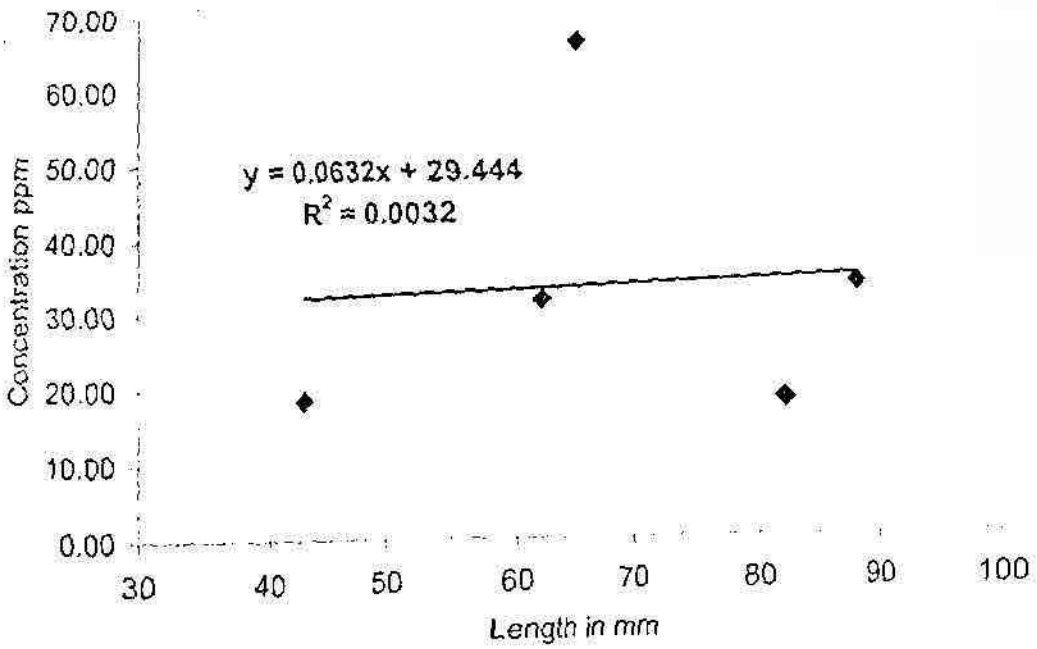


Fig.16.2. Concentration of copper in mussels- natural bed -bay

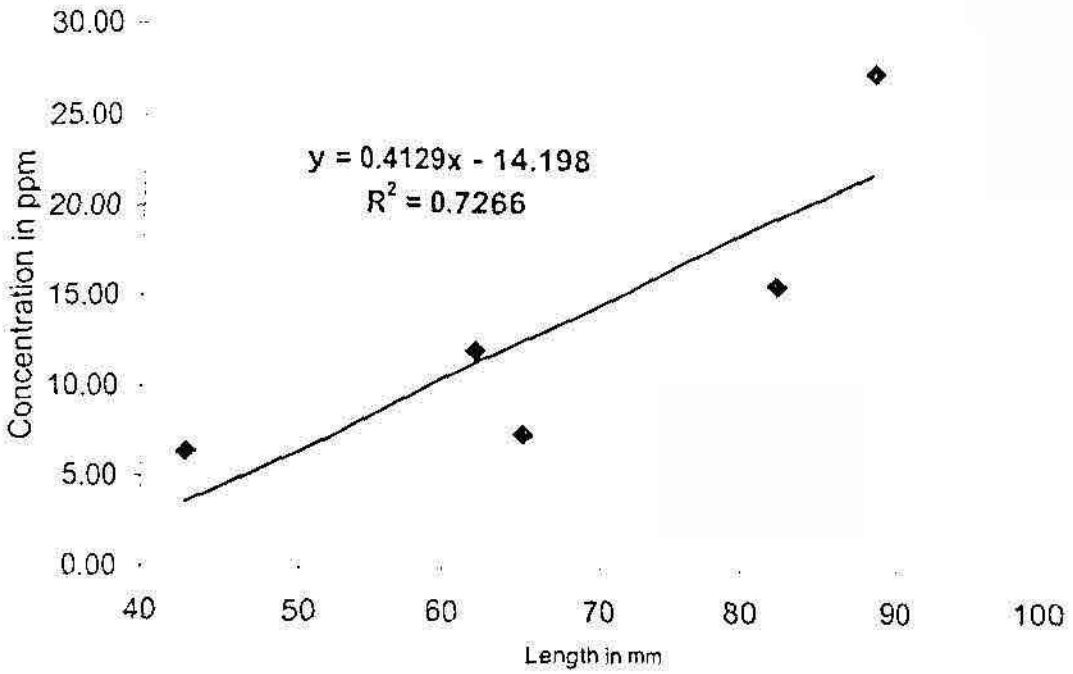


Fig.16.3. Concenntration of Lead in mussels- natural bed in bay

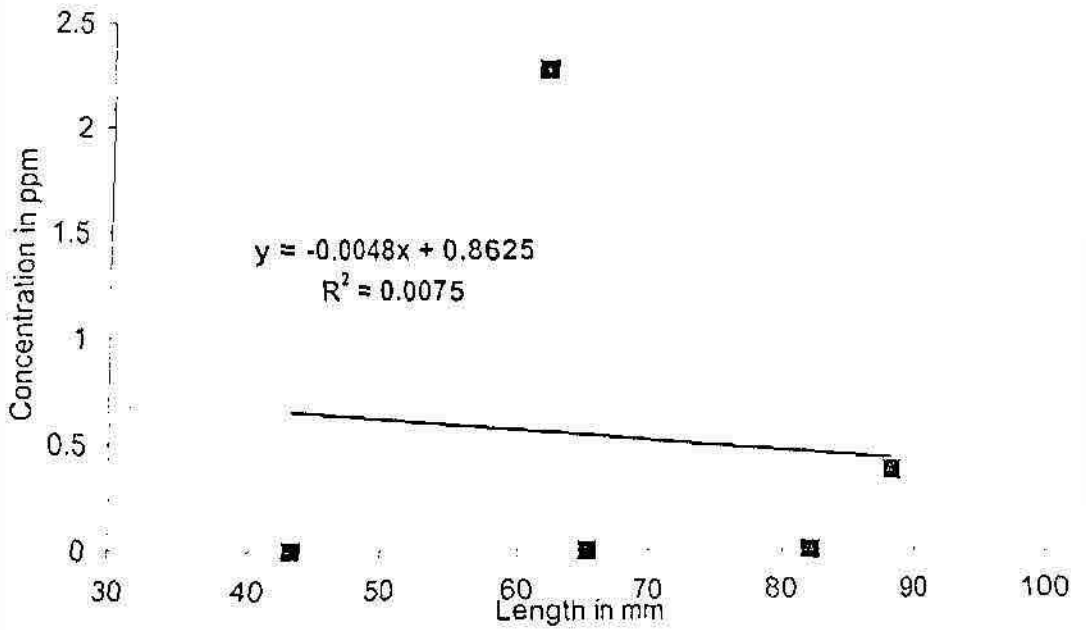


Fig.16.4. Concentration of Cadmium in mussels- Natural bed bay

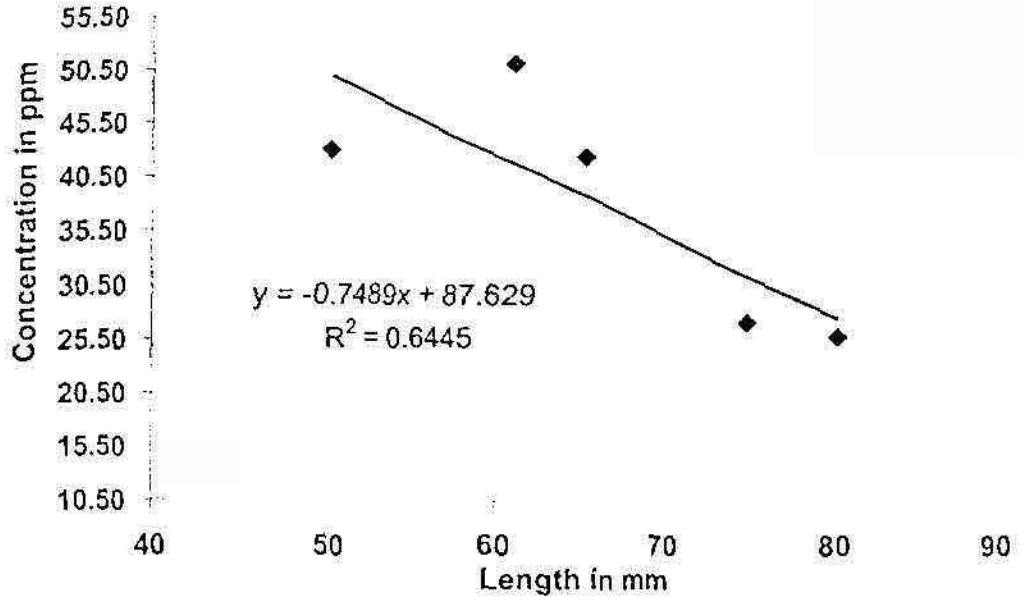


Fig.16.5.Concentration of zinc in mussels collected from off *bottom farm in Kollam bay*

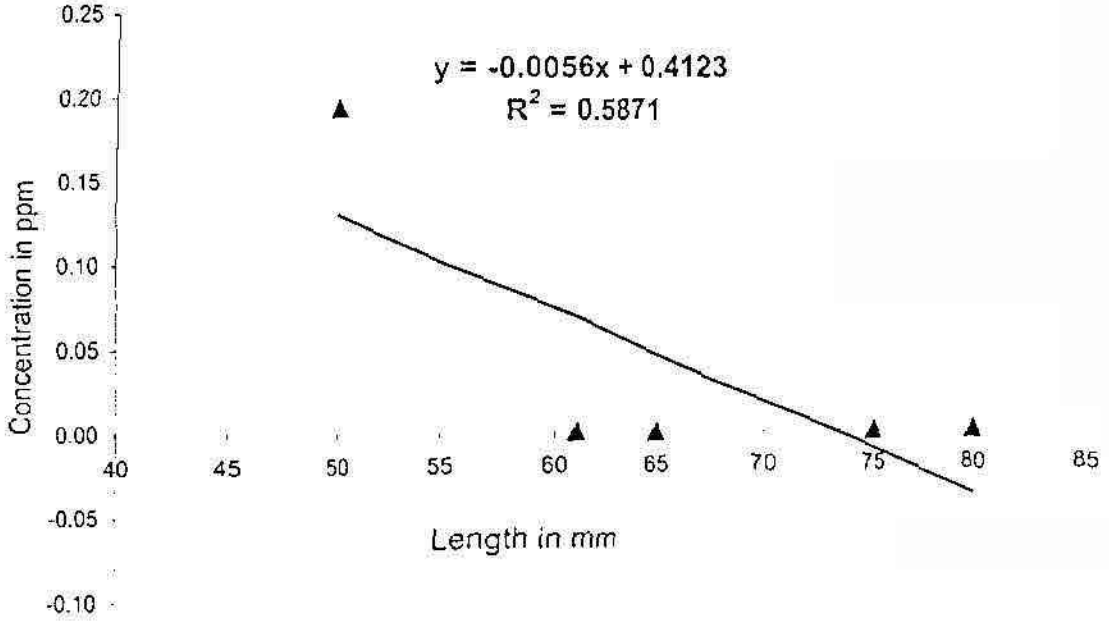


Fig.16.6.Concentration of cadmium in mussels collected from off bottom farm in *Kollam bay*

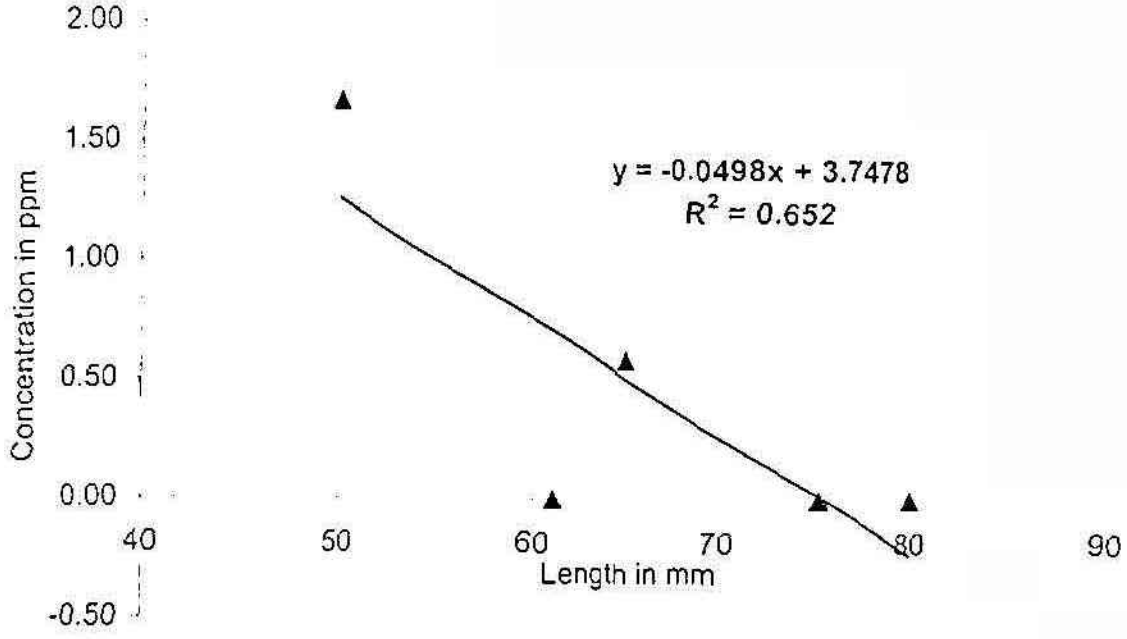


Fig.16.7.Concentration of copper in mussels cultured in off bottom farm in Kollam Bay

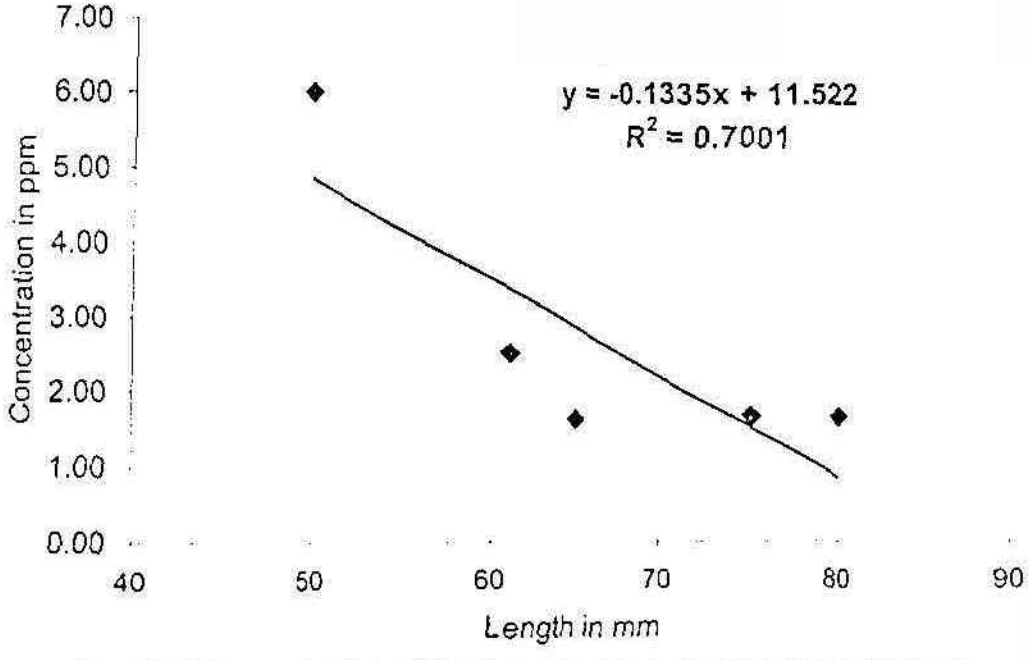


Fig.16.8.Concentration of lead in mussels collected from off botom farm in Kollam Bay

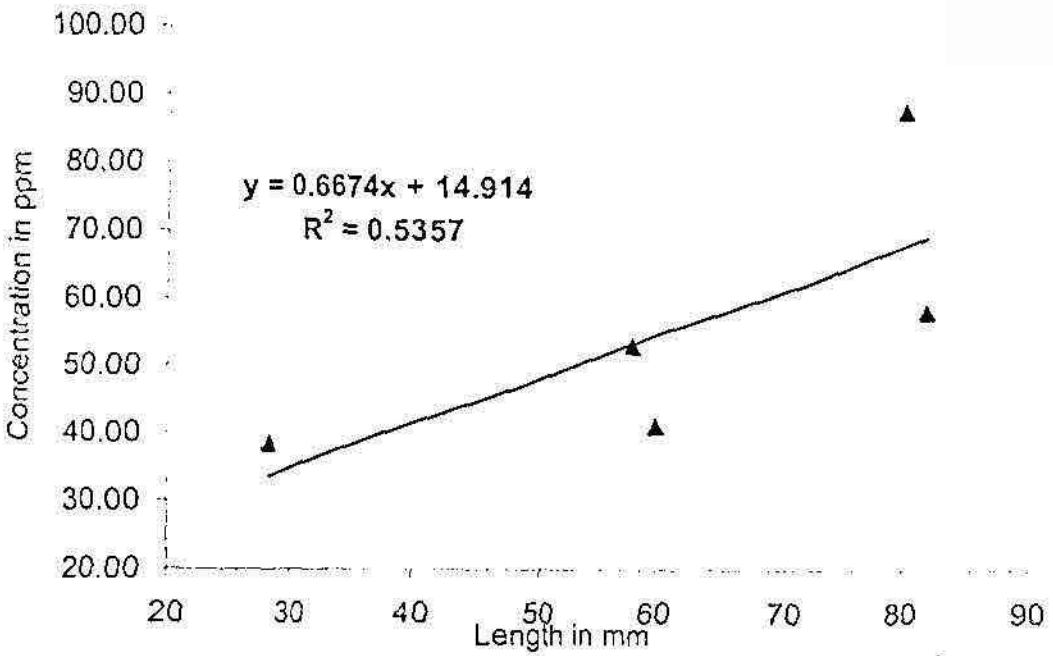


Fig.16.9. Concentration of zinc in mussels collected from estuarine farm

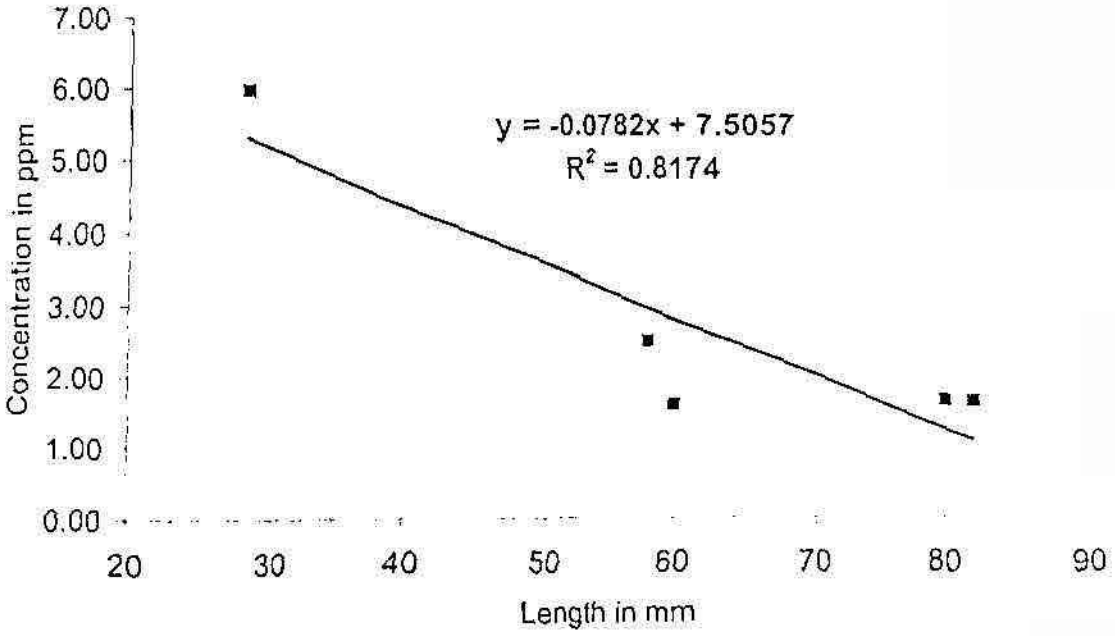


Fig.16.10. Concentration of lead in mussels collected from estuarine farm

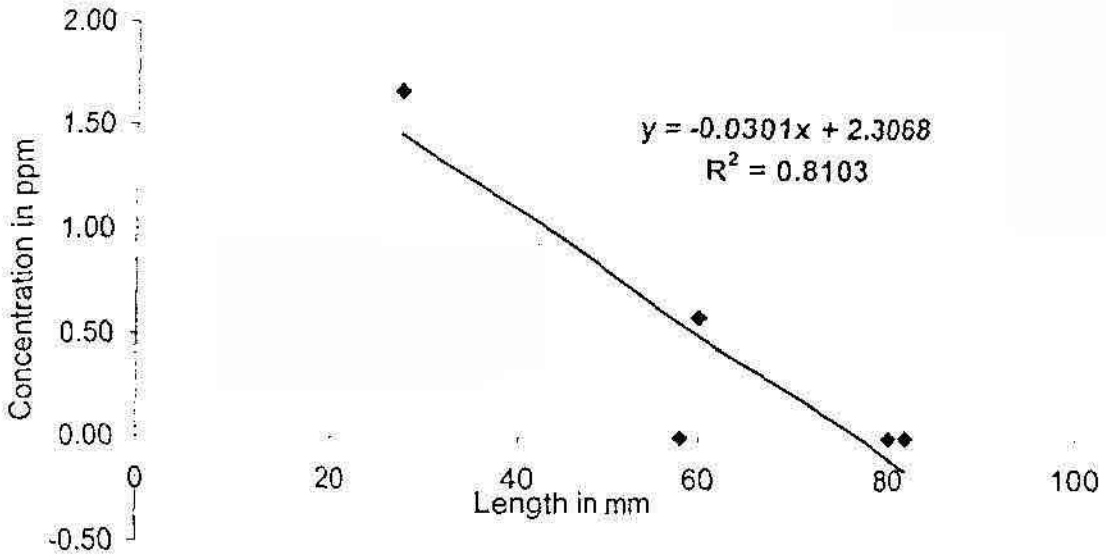


Fig.16.11.Concentration of copper in mussels collected from estuarine farm

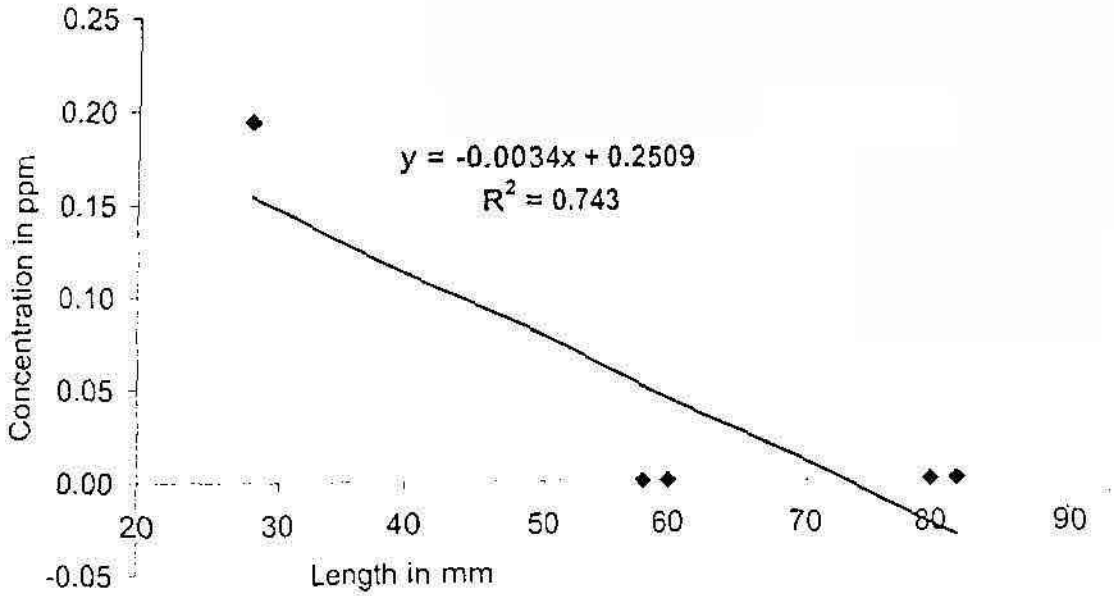
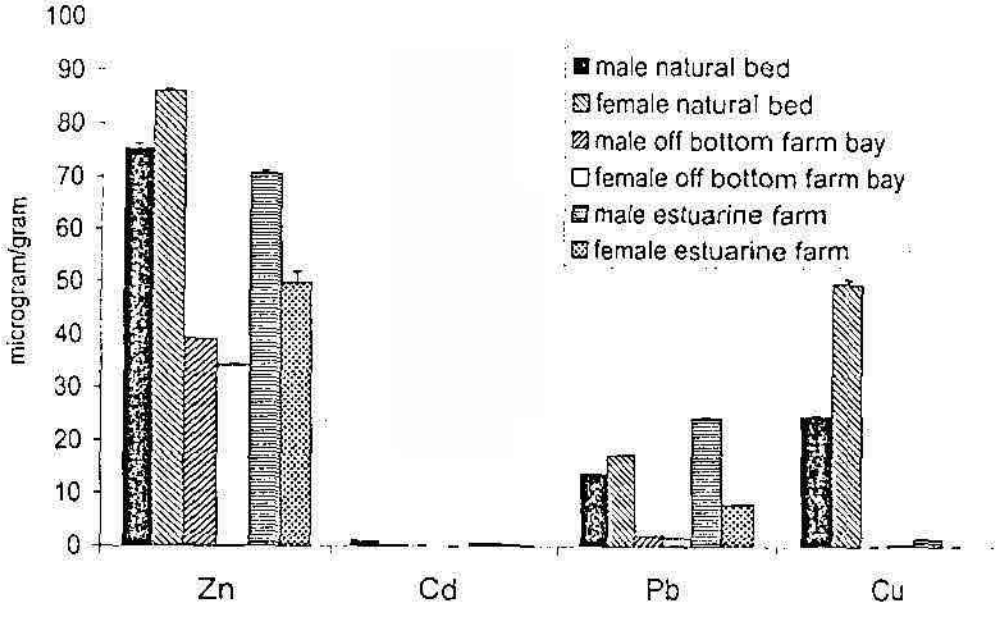


Fig.16.12. Concentration of Cadmium in mussels collected from estuarine farm

Table 9. Trace metal content in $\mu g\ g^{-1}$ wet weight in different size groups of male and female *Perna viridis* collected from three different systems

	Zn		Cd		Pb		Cu	
On bottom – Natural bed Bay								
Male - (40-50mm)	33.36	±0.09	0.00	±0	6.35	±0.05	18.38	±0.11
Female (40-50mm)	33.37	±0.09	0	0	6.35	±0.05	18.38	±0.11
Male- (60-70mm)	33.04	±0.28	2.29	±0.02	12.05	±0.06	31.75	±0.22
Female- (60-70mm)	36.64	±0.39	0.00	±0	7.44	±0.01	66.65	±1.34
Male - (80-90mm)	117.82	±1.46	0.00	±0	15.83	±0.09	18.17	±0.31
Female- (80-90mm)	135.57	±0.44	0.38	±0.00	27.72	±0.09	33.77	±0.52
Off bottom - Farm – Bay								
Male - (40-50mm)	43.11	±0.16	0.18	±0.00	5.49	±0.03	1.62	±0.01
Female (40-50mm)	43.113	±0.16	0.195	0.00	5.992	±0.03	1.673	±0.01
Male- (60-70mm)	51.373	±0.16	0.000	±0.00	2.510	±0.01	0.000	±0.00
Female- (60-70mm)	42.559	±0.31	0.000	±0.00	1.614	±0.02	0.591	±0.01
Male - (80-90mm)	27.222	±0.04	0.000	±0.00	1.667	±0.00	0.000	±0.00
Female- (80-90mm)	25.984	±0.03	0.000	±0.00	1.654	±0.01	0.000	±0.00
Off – bottom Pond/ Estuary								
Male - (40-50mm)	37.25	±0.10	0.00	±0.00	2.13	±0.01	4.15	±0.05
Female (40-50mm)	38.560	±0.10	0.000	0.00	2.400	0.01	4.360	±0.05
Male- (60-70mm)	53.307	±0.47	0.478	±0.00	18.566	±0.26	2.948	±0.01
Female- (60-70mm)	41.320	±4.12	0.080	±0.00	1.360	±0.06	0.000	±0.00
Male - (80-90mm)	88.333	±0.48	0.556	±0.00	30.635	±0.23	0.000	±0.00
Female- (80-90mm)	58.622	±0.19	0.236	±0.01	14.803	±0.11	0.000	±0.00

Fig. 17.Comparison of the trace metal (micrtogram /gram) in male and female mussels collected from three sites



In the natural bed in the bay, the concentration of Cadmium was 2.288±0.02μg g^{-1}wet weight in males of 60-70mm size group while in the females cadmium was not present. However in the large group 80-90mm in the same site cadmium was not detected in the males while in the female it was 0.380 ± 0.001μgg^{-1}wet weight. Concentration of cadmium was negligible in both male and female mussels collected from the off-bottom culture system at Kollam Bay.

In the mussel collected from estuarine pond, cadmium content was more, 0.478 ± 0.002μgg^{-1}wet weight and 0.556μgg^{-1}wet weight in males of 60-70mm and 80-90mm males while in the females of the same size group, the values were lower, 0.80 ± 0.002μgg^{-1}wet weight and 0.236 ± 0.008μgg^{-1}wet weight respectively. In the smaller size < 35mm it was not present in both male and females.

DISCUSSION

5. DISCUSSION

Tolerance to variation in salinity is a characteristic feature of euryhaline animals. In the present study it was observed that both large and small size mussel seed can survive in salinities from 15ppt to 45ppt. Along the Kerala coast, during the location testing programme by CMFRI mortality of the seeded mussels was observed at a site in Vemband lake when the salinity at the farm site remained low, <10ppt for more than 3 days due to river runoff (Kripa, personal communication). Similarly very low levels of survival or complete mortality of mussel seed in 10 to 28ppt has been reported from Sri Lanka (Indrasena and Wanninayke, 1994). The brown mussel *P. perna* seed (0.5 to 20mm) collected from a reef area showed 100% survival in a lagoon where the salinity was 25ppt but suffered 100% mortality at a site with salinity variation 10 to 28ppt.

Nagabhushanam and Bidarkar (1975) while comparing the salinity tolerance of *S. cucullata* in summer and monsoon seasons observed 100% survival in salinities above 14ppt in the former and 7.5ppt in the latter respectively. They also observed a lower lethal salinity value for this species during these periods: it was 8.6ppt in summer and 2.5ppt in monsoon. This shows that with conditioned submergence of these oysters in low salinities, the tolerance level increases which is an adaptation to survive in the low salinity condition (Ranade and Kulkarni, 1969). The lower salinity ranges for other Indian bivalves are between 10 to 15ppt for *Meretrix meretrix* (Sundaram and Shafee 1989); small size *M. casta*, 15ppt (Salih, 1978); *Marcia opima* 14ppt (Ranade and Kulkarni, 1969); *Sunetta scripta* 15ppt (Thampuran *et al.*, 1982). Lower tolerance range extends below 10ppt for *V. cyprinoides* up to 0.87ppt (Nair and Shynamma, 1975) and *C. madrasensis* up to 7% (Sundaram and Shafee, 1989).

Bivalves inhabiting coastal areas which have comparatively increased (>15ppt) lower salinity tolerance level are *Paphia laterisulca* 19.2ppt (Mane and Dhamne, 1980); *Sunetta scripta*: 20-25ppt (Thampuran *et al.*, 1982) and *Donax cuneatus* 22ppt. Sundaram and Shafee (1989) observed the tolerance level of *P. viridis* as 17ppt which is almost similar to the values obtained in the present studies.

Tolerance to salinity variation has been found to be related to size and age (Manzi and Castanga, 1989) of the bivalves. In the present studies the upper limit was same, 45ppt for both the size groups, beyond which there was complete mortality. However small size seed was found to survive better in lower salinities with 100% survival while for the larger seed the survival rate was lower. However the LC 50 values were lower for the larger seed than the smaller indicating that the larger seed is better when the salinity of the farm site is less between 15 to 20 ppt. Observations where the smaller size groups being more tolerant than large one have been observed in other Indian bivalves like *V. cyprinoides*, *M. casta* and *S. cripta* (Nair and Shynamma, 1975; Salih, 1978; Thampuran *et al*, 1982). Contrary to this, in *Paphia malabarica* large clams were more tolerant to low salinity conditions (Ram Mohan1993).

In nature, organisms are subjected to a variety of environmental factors acting together and they respond to the total resulting stimulus rather than to single environmental variable. In addition, the animal's response is not in terms of individual physiological rates but rather as a whole organism.

In present study, it was observed that the mortality started very early and was higher in unconditioned seed when compared to the conditioned seed. The effect of transportation /desiccation combined with stress to variation in salinity must have had a synergistic effect. During mussel farming the farmers collect the seed from areas of high salinity (>30ppt) and after a transport period of 4 to 5 hours seed them on ropes and expose them to estuarine salinities

ranging between 20 to 32ppt. Under these circumstances, the seed is not conditioned resulting in mortality. From the present study it can be concluded that if the transit period is high it will be preferable to condition the seed in 30 to 32ppt to reduce the mortality if the ambient salinity at the farm site is lower (between 15-20ppt) than the collection site. Such conditiong is usually practiced in shrimp and finfish farming and have become a standard protocol.

A biological system does not work in isolation with its environment conditions, a combination of physiological, biochemical and behavioral tests can give a more complete picture of an individual organism's reaction to their environmental stress effects. The combined role of endogenous and exogenous factors in modulating individual responses will depend on the nature, amplitude and frequency of environmental changes. However, direct responses and survivals vary according to potentially synergistic interaction between separate environments variables (Newell, 1979).

Condition index has been used as measure of quality and health of farmed mussel (Okumus and Stirting, 1998). Numerous workers have demonstrated that the condition of the various species of bivalves from boreal and temperate coastal water varies seasonally and is related to the level of available food and the annual reproductive cycle (Baird, 1958, 1966; Westley 1970; Walne, 1970; Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Gabbott and Stephenson, 1974; Dare, 1976). In detailed field studies, Walne (1970) has shown that seasonal changes in dry weight condition index and glycogen content are started that the condition of adult bivalves declines when the temperature is high and the food levels are low (Walne, 1966; Bayne and Thompson, 1970; Gabbott and Walker, 1971; Gabbott and Bayne, 1973; Gabbott and Stephenson, 1974). The differences in condition which occur seasonally are large enough to be considerable importance to shellfish harvests and processors, and knowledge of the timing of these differences is invaluable for the

development of optimal harvesting schedules aimed at maximizing yields from both natural and cultivated shellfish stocks.

Babarro *et al.* (2000) have evaluated the CI of seeded mussel as a physiological basis for the performance of mussel seed. Weight increase rates for the seed from collector ropes were higher than those for the seed from rocky shore, and the growth rate variations during the cultivation period were associated with the environmental parameters measured (chlorophyll a and temperature). The origin of the seed was also found to be a significant factor. The condition index (C.I) of the seed from collector ropes was significantly greater than that of the rocky shore seed at the beginning of the cultivation time. However the authors found that both mussel seeds showed a similar C.I. after 70 days. In the present study the condition index of the survived mussel seed in different salinities were different from the control. But their behavioral performance as seen by byssus attachment indicated their capability to survive even in varied salinities.

There have been few studies on mechanical and chemical detection in bivalve molluscs regarding environmental stress. From the literature, it is apparent that among invertebrates there are no common mechanisms for registering environmental changes. Studies on certain mobile species such as gastropods molluscs (Bland ford and Little, 1983), crustaceans (Gross, 1957; Lagerspetz and Mattila, 1961; Mclusky, 1970; Thomas *et al.*, 1981), and annelids (Janson, 1962) indicate that, when subjected to a choice of different salinities, these animals are capable of detecting and discriminating salinity levels. In sedentary species such as bivalve molluscs, the underlying basis of the salinity detection has been investigated by exposing animals to artificial sea water of differing ionic and osmotic composition and observing the effect on behavior (Davenport, 1979, 1981; Akberali and Davenport, 1981). In both mobile and sedentary species, salinity detection depends on the concentration of the particular ions (Barnes, 1939; Davenport, 1981; Akberali and Davenport, 1982; Black, 1983), the osmotic pressure of the medium (Davenport, 1972; Bettison

and Davenport, 1976; Blandford and Little, 1983), or to the combination of both (Barnes and Barnes, 1958).The metabolic rate showed an exponential relationship to body size and salinity. The increased salinity of the media is considered as salinity stress in winter and summer seasons. In these seasons, the animal withdraws itself into the shell or closes the valves and shifts to anaerobic metabolism to tide over the abnormal conditions in the environment.

The tolerance *Argopecten purpuratus* (Chilean scallop) to conditions of decreasing salinity, showed that clearance rate was higher and similar at 30 and 27ppt, decreasing significantly at the lower salinities. Oxygen uptake increased with decreasing salinity from 30 to 24ppt, showing the lowest value at the extreme condition of 18ppt. A similar pattern was presented by the excretion rate, which also increased within the range 30-24ppt, to show a reduction with decreasing salinities.

Studies have demonstrated that the lipid levels and composition of marine bivalves clearly reflect the biochemical and environmental conditions of seed development (de Moreno *et al.*, 1976 ; Napolitano *et al.*, 1992; Fernandez-Reiriz *et al.*, 1996; Soudant *et al.*, 1996). Dhert *et al.* (1992) considered stress tests to be invaluable in testing the nutritional requirements of aquaculture species at various stages of their development and established a standard stress test to determine the of seed survivability, in which they used elevated salinity as a stressor.

With regard to the influence that such period of starvation usually exercises on lipids, some authors have noted a drop in triacylglycerols of seeds of different marine bivalves (Fraser, 1989; Caers *et al.*, 2000). The consequences of starvation on different fatty acids in marine bivalves have been highlighted in the work published by Langdon and Waldock (1981), who studied the essential fatty acids PUFAs n-3 and n-6 of oyster seed *Crassostrea gigas*. Various authors have suggested that the seasonal variations observed in the

levels of total lipids, neutral lipids and fatty acids of various species of marine invertebrates is intimately related to the environmental factors (Perry *et al.*, 1979, Langdon and Newell, 1990 Chu *et al.*, 1990; Galap *et al.*, 1999). The total lipid of the survived seed in varied salinities were different from the control, indicating that the physiological condition were affected by the salinity changes. However the adaptability of mussel seed could be assessed by the survival after day 9.

Mussels are intertidal animals and it has been observed that the height at which individuals are located on the shore can affect the respiration rates. Both *M. edulis* and *M. californianus* from higher regions on the shore showed lower ventilation rates than mussel lower down (Bullock, 1965; Moon and Pritchard, 1970).

The ability to endure desiccation is due, at least in part, to the mussel tolerance of increased osmoconcentration of the body fluids. Kuenzler (1961) and Lent (1968) reported that *M. demissus* opens its valve during exposure to air. The median survival time of *M. demissus* in air is proportional to the amount of oxygen present (Lent, 1968)

In the present study it was observed that the mussel seed could survive well (with 26% mortality) up to a exposure period of 24 hours beyond which the mortality increases drastically. This endurance is seen in the intertidal regions of the Indian coast. Studies have shown that the survivorship of mussel is directly related to the length of aerial exposure (Paukstis *et al.*, 1999). Experiments on the nitrogen metabolism during aerial exposure of *M. edulis* have shown that there is reduction in ammonia efflux rate during emersions (Sadok *et al.*1999). The authors have related this as the energy –saving process of the mussel to tide over air exposure.

Byssus thread formation has been found to be related to the environmental factors (Yong, 1985). Though the actual number of threads formed has not been counted it was observed that the mussel seed attached the rearing

container immediately in the optimum salinity range. The comparatively poor attachment in the unconditioned seed in the first two days of experiment also indicated that byssus formation is related to stress.

The concentration of Zn, Cd, Pb, and Cu in the mussel samples analysed during the period varied markedly within the same animal. Zn was the dominant metal in all the mussel samples. It has been proven that the concentration of heavy metals present in an aquatic environment increases with proximity to areas of high industry. Hence, concentrations are higher in coastal and estuarine waters than in the open ocean. However, although the severity of the concentration is relevant to the toxic effects to the organisms in these ecosystems, other factors also influence the "biochemical availability of the metals to the organism." for example, the heavy metals may occur in aquatic systems in "multiphase states", establishing a complex equilibria between sediment and aqueous phases. Soluble, colloidal and particulate forms all have the potential to exist at one point in time and this may affect the possibilities of their being metabolized by different organisms. The presence of other "natural" metals (e.g. Na, K, Mg and Ca) may also compete with the presence of the toxic metal.

Bioaccumulation studies conducted along the Kerala coast have indicated similar trend in other bivalves like oyster and clams (Pillai *et al* 1986). High level of Zn concentration in *C. madrasensis* from Kollam (1574ppm) and Cochin (2450 to 1250ppm) has been reported by Sankaranarayana *et al.*, 1978. Bhatt and Desai (1998) have found lower levels (171 to 403ppm) in *C.gryphoides* collected from Bombay coast. Zinged *et al.*, (1976) have reported Zn concentration in *Crassostrea* species between 323-2800ppm.

Along the Karnataka coast Krishna Kumar *et al.*, (1998) found Zn concentration in *P. viridis* (11.1 to 70.5 ppb), *C. cucullata* (31 to 703 ppb wet wt.) and *Meretrix casta* (11.1 to 34.5 ppb wet wt.) to be higher than Cu, Cd and Pb.

In present study, Zn concentration was found to be higher in the mussels collected from the natural bed. Pillai *et al.*, (1986) found Zn concentration (>48ppb dry wt.) in *P. indica* collected from the sea shore in contrast to off-bottom raft (<44ppb dry wt.).

Studies conducted on bioaccumulation in relation to water-column have showed that concentrations of Zn, Cd, Pb, and Cu in the cultured mussel, *Perna canaliculatus* varied with the depth (Nielsen, 1974). Contrary to this, Philips (1976) observed that mussel in deeper water (>3m) had lower concentration than in the upper layers. However these observations pertain to mussel cultured in the column water and not in the sediment (on bottom). The concentration in the mussel may be higher either due to age or due to the higher trace metal load in the sediments than in the water.

The distribution of trace metals in the sediments follows a similar pattern to those in water column and is controlled by the formation of trace metal enriched clay, silt and organic particles in the backwater and their subsequent redistribution in the sediments water movement and bioturbation (Rajendran and Kurian,1986).The mussel in the natural bed are exposed to trace metals in the sediments which is usually higher than in water and hence the higher concentration of trace metals may be directly linked to the environment.

Zn concentration in the mussels of the same size group also varied between locations. In the present study, the highest concentration was observed in the mussel collected from natural bed. Among the off-bottom collected mussel more accumulation was in the mussel near to the substrate i.e. where the depth was shallow. In the estuarine pond mussel 50 cm above the level while in the raft they were upto 3 meter above bottom. These variations can also be due to the salinity profile, flushing of water or trace metal levels in the environment. Concentration of trace metals in bivalves have been found to vary with salinity (Bryan, 1973; Philips, 1976; Lakshmanan and Nambishan,1986.) Low salinity did

not affect the net uptake of Zn by mussels, but increased the net uptake of Cd and decreased that of Pb (*Bryan*, 1973; *Phillips*, 1976).

The uptake kinetics of copper in the mussel *M. edulis* is distinct from those of other metals in these organisms. Scott and Major (1972) reported that *M. edulis* takes up copper rapidly from solution after several days, either by increased secretion of mucus or possibly via feces.

Concentration of cadmium, chromium, copper, lead, and nickel in *P. viridis* in mariculture zones of Malaysia was studied and it was observed that indicated a two-fold increase in the mean levels of the Cd in 1997 when compared to levels in 1990(Chiu,2000). However levels of other metals in 1997 were lower by 32-39% for copper but the greatest reductions were recorded for lead 51 to 75% and the authors have related it to the increased use of lead free petrol.

Comparison of bioaccumulation by male and female mussel, *Mytilus trossulus* and *Crenomutilus grayanus*, the oyster *Crassostrea gigas* and the scallop *Mizuhopecten yessoensis* exposed to sublethal concentration of heavy metals have shown that female gonads had a significantly higher initial concentration microelement than male gonads. However the concentration of copper and zinc increased in males more than females, except in the zinc content in the *M. trossulus*. In the present study Zn and Cu concentration in the natural bed was mussel higher in the larger females than the males while in the smaller size groups it was almost similar. Contrary to this, at the estuary site concentration of zinc, copper cadmium and lead were more in larger males than the females. However in the same size groups in the off-bottom cultured mussel in the bay such difference in male- females were not found. The soft tissue of females of *Perna viridis* showed highest concentration of Fe>Co>Zn>Pb>Ni>Cu>Mn>Cd>Hg followed by males and in determinates. The

biomagnification of all heavy metals was maximum in gonad (Krishnan Nambishan and Lakshman, 1986).

The rate of bioaccumulation has been related to the both environmental factors and the physiological process of the animals (Abrahm *et al*, 1988). It is well known that heavy metals can inhibit the activity of many enzymes (Dixon and Webb, 1967) and affect the function of several cellular constituents such as membranes (Rothstein, 1959), lysosomes (Moore, 1977), and mitochondria (Corner and Sparrow, 1956; Kleiner 1974; Zaba and Harris, 1978; Akerbali and Earnsaw, 1982; Akerbali *et al.*,1984). With respect to tissues of marine organisms, and bivalves in particular, it has been reported that heavy metals exert inhibitory effects on physiological processes e.g. ciliary activity of the gills, oxygen consumption (Brown and Newell, 1972; Manley, 1983; Calabrese *et al.*, 1984; Martinella *et al.*, 1974), and heart rate (Scott and Major, 1972), byssus synthesis (Martin *et al.*, 1975; Davenport, 1977).

The variation in accumulation of Zn in male and female collected from the same habitat may be due to the variation in physiological process such as filtration rates. However in different environments, the variation can due to the turnover rates. Trophic levels difference in trace metals have been observed (Benny and Ayyakkanu, 1992) suggested that the trace metals content in primary production may different in the two natural habitats. Reinfelder *et al.* (1997) have found that the Assimilation Efficiencies (AE) and turnover rates of certain trace metals (Ag, Cd, Se, and Zn) was related to the proportion of each metals in the cytoplasmic fraction of the ingested phytoplankton, their studies also indicted that 80% of the elements in the prey algae's cytoplasm assimilated . The ratio of AE:k (k= physiological turnover constant) which is proportional to the consumer-prey trace-element bioaccumulation factor (concentration in consumer : conservation in prey) was generally greater for Cd, Se, and Zn than for Ag, Ni, and Co. The ratio of AE:k was low for *M. edulis* indicating that this organism is relatively

inefficient in accumulating important elements such as Ag, Cd, Zn from ingested phytoplankton.

Cortesao *et al.*, (1986) have also observed higher content of Cu, Zn, Pb, and Cd in the sediments of a coastal lagoon in Algarve (South of Portugal) and they have also related it to anthropogenic sources in the lagoon. Schulz-Baldes (1973) found that the Pb concentration in small size *M. edulis* was significantly higher than larger mussels.

In the present study variation in concentration of Cu, Zn, Pb, and Cd with size of mussels were not significant in the off bottom cultured mussels in the bay. Contrary to this, concentration of Zn was very high in the samples from the natural bed. This variation may be related to the age of the mussel rather than the size. In the natural bed the large mussels (80-90mm) may be more than one year while in the culture systems the age of mussel is known to be less than one year.

Increase in the concentration of Zn has been observed to increase with the size of the mussel in the natural bed and in the estuarine farm while Cu, Zn, Pb, and Cd concentration decreased with increase in size of mussels in off bottom farm in the bay. Cd, Pb and Cu contents in the estuarine mussel also decreased with size. Similar observation have been made by Balaji and Rao (2000) who found concentration of Cu, Zn, Pb, and Cd in the tissues of *Myilopsis sallei* to decrease with increasing in size. In *M. edulis*, Boyden (1974, 1977) and Philips (1976) have also observed decreased concentration Cu, Zn, Pb, and Cd with increasing size.

In *M. edulis* similar trend has been observed in accumulation of lead (Schulz-Baldes, 1973). In *C. commercialis* Mackey *et al.* (1975) have reported decreasing concentration of copper and zinc with increasing size.

Accumulation of cadmium has been observed to be independent of weight in *M. edulis* and *Ostrea* sp. (Boyden, 1974, 1976) and in *M. sallei* (2000) while in *C. commercialis* the concentration decreased with increasing weight (Mackay *et al.*, 1975). Pillai *et al* (1986) have observed that seasonal variation in the accumulation of Zn in *P. viridis* but variations in the concentration with size have been found to be inconsistent. Similarly, the concentration of copper and lead decreased with increasing size in the off bottom cultured mussel and were absent in large group mussels. The higher concentration of metals in smaller size is probably because of the rapid metabolic activity and to uptake of metals. Aoyama *et al* (1976) has proposed that growth may dilute metal concentration in organisms if tissue is added faster than its retentions. Apart from this, metallothioneins in invertebrates which are related to metal retention are more in younger individuals than older ones (Lukyanova, 1982).

SUMMARY

6. SUMMARY

The salient points of the experiments conducted on stress related to salinity and desiccation and the analysis trace metals in mussel samples collected from three different systems such as natural bed, off bottom farm in bay and from a shrimp pond in an estuary are given below

1. In 20 to 35ppt salinities 100% survival of small size seed (10 to 25 mm) was observed while in the large group (25 to 40 mm), low mortality ranging between 4 to 10% was observed. In 15 to 20 ppt, the mortality was lower for the large seed than the small size seed. Complete mortality was observed in 5, 10 and 50ppt salinities for both size groups of mussel seed.

2. The mortality of mussel seed when directly exposed to different salinities without conditioning was higher. In 15 and 20ppt, the increase in mortality was 2.8 and 2.2 times to that observed when seed were transferred after acclimatization. Mortality in 25 to 40ppt was 22% to 32% which was 7 to 22 times higher than when the seed were conditioned prior to exposure to salinity variation.

3. The LC_{50} value for 25 to 40 mm and 10 to 25mm size seed was stimated as 14 and 21 ppt respectively. The LC_{50} values of unconditioned seed of the former size group was higher, 32 ppt.

4. Condition index of the surviving mussel seed in all salinities except 45ppt, was lower than that of control mussel seed. However, the seed were healthy as indicated by the strong byssal attachment to the substrata.

5. In the seed exposed for 12 and 24 hours of desiccation, mortality was low (6 and 26%) and while desiccation for 26 to 30 hours resulted in very high

mortality (42 to 96%) during the immersion or recovery phase. Above 30 hours of desiccation, none of the seed survived. The LC_{50} value for desiccation was estimated as 24 hrs.

6. The order of magnitude of bioaccumulation of the trace metals was Zn>Cu>Pb>Cd in the mussel collected from the natural bed while in the mussel cultured in the off-bottom system in bay and estuarine pond were Zn>Pb>Cd>Cu and Zn>Pb>Cu>Cd respectively.

7. Concentration of Zn was high (64.966μg g wet weight) in the mussel collected from natural bed at Kollam bay, while at the same site the Zn content was nearly half (38.893 $\mu g\ g^{-1}$wet weight) in the off bottom cultured mussels. In the mussels cultured in the estuarine farm, concentration of Zn was 53.117$\mu g\ g^{-1}$ wet weight which is higher than the in the mussel cultured in raft in the bay.

8. Accumulation of cadmium was negligible in the mussel samples collected from natural bay bed, off bottom farm in bay and estuarine pond .

9. Positive correlation with size was observed in the accumulation of zinc (R^2 = 0.7869) and lead (R^2 = 0.7266) in the mussels collected from natural bed, while in the off bottom farms the accumulation of Zn, Cd, Pb and Cu were negatively related to size with very low to nil values of Cd and Cu in harvestable size (> 60mm) mussels.

10. Gender based bioaccumulation of heavy metals was inconsistent in different size groups of mussels collected from the natural bed and in farmed systems.

CONCLUSION.....

Points for improvement of mussel mariculture husbandry

- The study indicated that farmers can use seed of size 10 to 45 mm for stocking at a site with salinity ranging between 20 to 35 ppt. However if the salinity is between 15 to 20 ppt then it is better to use seed of size 25 to 40 mm than smaller size seed.
- If the salinity at the farm site is less than the ambient salinity at the seed collection point, then it is better to acclimatize the seed in higher salinity before stocking.
- If the seed collection centers are situated away from the farm site it is better to plan the seed collection from a point such that the entire transit period is less than 24 hrs to ensure better health of the seed. Above this, the farmers will lose more than 50% of the seed collected.
- Better quality of the mussel with respect to heavy metal accumulation was seen in off bottom cultured mussels which were more than 50 cm above the substrate. Hence it is better to adopt off bottom culture methods than on bottom.

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