CHAPTER 4

STRESS STUDIES

INTRODUCTION

Mother nature has provided immense natural resources for human beings who worship them. Water is one such natural resource, which is a basic requirement for man, without which no life can exist on earth.

The oceans and seas provide immense amount of both living and non-living marine wealth. So also, man's reliance on the source of energy has never been greater. Whether it be tapping the tides for electricity, drilling the sea-bed for oil or trawling the continental shelf for turbot, we are demanding more and more from a finite resource, and unless exploitation is properly managed we could do irreparable damage. Good management can only be based on sound knowledge.

As we know, oceans and seas cover 71% of the earth's surface and yet they seem very remote. With limited resources on terrestrial environment, man has switched his attention towards the ocean for his requirements. Industrialization and urbanization has no doubt improved the standards of living of man, but in the process, the crucial environment balance established by nature over a millennium has been inadvertently disturbed. Man made activities have increased the flux of many pollutants into the marine environment. Sufficient reports are available in recent years to understand that there are definite limits to man's abuse of the oceans and in particular, many enclosed semi-enclosed seas and coastal areas. Man often neglects his environment and the resultant damages are gradual and slow and hence goes unnoticed until a large-scale damage is felt.
Marine pollution is currently a problem of great concern. We are well aware that knowingly or unknowingly our coastal environments are being polluted, ensuing a great concern on our marine wealth. Already many coastal areas have become either unproductive or unmarketable for a variety of finfish, shellfish and other marine living resources due to indiscriminate entry of domestic and industrial pollutants through the dumping of wastes. The main reason for this is that seas and oceans are regarded as a vastness pool with an infinite capacity to absorb natural and man-made pollutants. The “Environmental Safety” is not being considered in parallel with the industrial advancement.

Indian ecosystem has received some major setbacks because of haphazard industrial and urban development. Of the country’s 304 million hectares, 50% are subject to ecological degradation, and of the 14 major rivers including the Ganga, which provide nearly 85% of the country’s drinking water are all polluted (Govind, 1989). The pollutants of diverse origin including domestic sewage, agriculture runoffs and industrial effluents degrade the water quality threatening the life of biota living therein.

Pollutants like heavy metals, hydrocarbons, detergents, pesticides, radioactive wastes etc have received great attention because they are potentially toxic to many aquatic organisms. Toxic pollutants can exert detectable effects on living organisms at the sub cellular or cellular level, at the tissue, organ or whole organism’s level or at the population and community levels of organization.

Heavy metals are a unique class of toxicants, since they cannot be broken down to non-toxic forms. Once they contaminate the ecosystem, they remain a potential threat for many years. Some metals like Fe, Cu, Zn, Co, Mn, Sc, Cr, Ni, V, As, and Sn are of biological interest because they act as micronutrients. The non-
essential metals like Ag, Al, Be, Cd, Hg, Pb, Sb, and Ti has no established biological function and are regarded as important contaminants in the aquatic environment (Dasilva, 1978). A special characteristic of heavy metal chemicals is their strong attraction to biological tissues and the slow elimination of these chemicals from biological systems (Ochme, 1978). Thus, the heavy metals move from one trophic level to the other through bio-concentration and biomagnification by the biological systems. Once absorbed in to the body, metals are capable of reacting with a variety of binding sites.

In marine environment, it has been recognized that a greater potential hazard exists in estuarine and inshore areas than in the open ocean because of their proximity to sites of industrial and domestic activity resulting in concentration of specific pollutants by run off or by biological activities of the inhabiting organisms. The deleterious effects of pollutants including heavy metals on living resources are much more evident in the estuarine and coastal areas. These ecosystems particularly the estuaries serve as the important feeding and nursery ground and "passage zone" for a large number of commercially exploitable and cultivable aquatic organisms. Coastal and estuarine regions are the chief recipients of man made and natural pollutants.

Therefore, heavy metal contamination of the coastal and estuarine environment has received much attention in recent years. Hence, the information available is fragmentary and scattered. There is an urgent need of a thorough knowledge of the pollutants, their entry, source, nature of toxicity on biota, monitoring of the ecosystem, remedial measures and management, legislation etc to save the environment.
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The average concentration of mercury in seawater is usually quoted as 0.03 ppb (Goldberg, 1963), and surface seawater having more than 0.2 ppb have been considered to be contaminated with mercury. Copper in near shore water is about 1.5 ppb and values above 3.0 ppb probably reflect anthropogenic additions (Schmidt, 1978). It reaches the marine environment from mines and a large number of industrial processes like artificial fibre production, agriculture chemicals, wood preservatives, antifouling and anticorrosion paints etc. Cadmium is a highly toxic metal with no biological function (Varma and Katz, 1978). Natural levels of cadmium in near shore waters range from <0.01 to 0.41 ppb (Preston, 1973). Cd reaches the marine environment through dumping of wastes from plastics, phosphate fertilizers, sewage, sludge etc. The normal concentration of Zn in open seawater is 3 μg/l (IMCO et al., 1976). Though zinc is an essential element, extremely high concentration may prove to be toxic to the marine and estuarine animals.
MATERIAL AND METHODS

In the present study, two essential metals (Cu and Zn) and two non-essential metals (Hg & Cd) were selected, since these metals are widely considered as the most common pollutants in the marine environment.

The selection of an organism for toxicity studies is very important and will depend on a number of factors such as:

1. It should be widely distributed and available in good number throughout the year.
2. It should be sensitive to the toxicants and to the environmental factors.
3. It must be ecologically and economically important.
4. It should be culturable in laboratory.
5. It should be in good condition, free from diseases and parasites.

The pearl spot, *Etroplus suratensis*, an ecologically important species of Karwar coast was selected for the present study. The present investigation was carried out under the following heads:

1. Acute toxicity of heavy metals on the juveniles of *Etroplus suratensis*.
2. Effect of heavy metals on the oxygen consumption rate of juveniles of *Etroplus suratensis*. 
ACUTE TOXICITY

Test Species

Specimens of *Etroplus suratensis* were collected from the fishing areas of Kali estuary with the help of cast nets, drags nets or gill nets. They were brought to the laboratory in a bucket filled with water taken from the same site. Later they were introduced into a large tank of 500 liters capacity containing filtered seawater. Care was taken to maintain the same salinity (20 ppt) as that of the original environment. Aeration was provided and the fishes were acclimatized to laboratory condition. During acclimation, fishes were fed with pelleted feed containing mixture of prawn and wheat, once a day. Every day 90% of water was exchanged from the stocking tank and all the necessary care was taken to keep the tank free from serious mechanical or visual disturbances. Only the healthy fishes were used for experimental purpose.

Test Media

Test stock solution (1000ppm) of copper, mercury, zinc and cadmium were prepared by dissolving analytical reagent grade CuSO$_4$.5H$_2$O, HgCl$_2$, ZnSO$_4$.7H$_2$O and CdCl$_2$.2.5H$_2$O respectively. The stock solutions were prepared as outlined in APHA (1980) using double distilled water. Actual concentration of stock solutions were verified against known standard and double distilled water blanks using a Perkin - Elmer model 2380 Atomic spectrophotometer units air-acetylene flame for Hg and graphite furnace with a deuterium arc corrector for Cd, Cu and Zn (Martin et al., 1981). Test Solution was prepared by appropriate volumetric dilution of Stock solution with distilled seawater (Martin et al., 1981).
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Test containers

Glass troughs of 3 litres capacity were used as test containers for acute toxicity tests. All glass containers were acid-washed in 10% HCl for 24 hours and then thoroughly rinsed in deionized water prior to use (Ahsanullah and Arnoft, 1978).

Acute toxicity tests: -

Short-term acute toxicity tests were conducted according to APHA (1980) for a period of 26 hours on juveniles of the *Etroplus suratensis*.

The test animals were collected from the stocking tanks using scoop net and were transferred carefully in to glass troughs of 3 litres capacity prior to the experiment. Ten number of healthy test animals (actively swimming) were sorted and introduced into each of the test container. The size of fish varied from 4.5 to 6.0 cm (Average. 5.2 cm) and their weights ranged from 10 to 20 gms. (Average 15gms). The fishes were starved during the bioassay for 2 days prior to start of the experiment. Slow aeration was provided to maintain adequate dissolved oxygen concentration.

A preliminary range finding test was conducted to decide the test concentrations. The experiments were conducted in triplicates and a control (also in triplicates) free from toxicants was run simultaneously. Six to seven concentrations of each metal was tested during the experiment.

The physico-chemical parameters of seawater maintained during the experiment were as follows: 

\[ \text{Temp} = 28.5 \pm 1.0^\circ C \]

\[ \text{Salinity} = 33.0 \text{ ppt} \]
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\[ pH = 8.0 \pm 0.1 \]
\[ \text{Dissolved oxygen} = 5.0 \pm 0.4 \text{ mg/l.} \]

Eighty percent of the test solution was changed after 24 hours (Wong et al., 1993). The test solution was removed using a thin polythene tube with a 0.125 mm mesh affixed to the openings to prevent removal of the animals. Fresh test solutions were then added to the test containers.

Observation was done every 3 hours for 96 hours bioassay studies. The dead animals were removed periodically and mortality was recorded in terms of percentage. Death was assumed when fishes were immobile and showed no response even after gentle prodding.

**RESPIRATORY METABOLISM**

*Test species*

For respiratory metabolism, fishes from the same acclimated stocking tanks were utilized. Only healthy specimens without any symptoms of diseases were selected for the experiment. The size of the fish varied from 4.5 cm to 6 cm (Avg. 5.2 cm) and the weights ranged from 10-20 gms (Avg. 15 gms) respectively.

*Test Solutions*

Stock solutions of metals (Hg, Cu, Zn and Cd) were prepared as explained earlier for acute toxicity tests. The stock solution were diluted accordingly with distilled water and added to the experimental containers to get the desired test concentrations (Martins et al., 1981).

*Test concentrations*

The test concentrations were decided based on the 24 hour LC\textsubscript{50} values of toxicant. The concentration tested were close to one-tenth of the 24 hour LC\textsubscript{50}. 
value of the respective metals, which was found to be sub lethal for a 24-hour period. The concentrations of Hg tested on juveniles were 0.25 ppm, 0.45 ppm and 0.65 ppm. Cu was tested at 1.35 ppm, 1.55 ppm and 1.75 ppm. The concentrations of Zn tested were 7.1 ppm, 7.35 ppm and 7.6 ppm. Cd concentrations tested were 24.5 ppm, 28.5 ppm and 32.5 ppm respectively. Controls (free from toxicants) were run simultaneously along with the experiments. Three replicates were run for each concentration as well as for control.

Measurement of oxygen consumption

The test animals were starved during the experimental period. Seawater filtered through Whatman 42 filter paper was used for the study and the physico-chemical parameters of the experimental seawater were

- Salinity = 33.5 ppt
- Temp = 28 ± 0.5°C
- pH = 8.0 ± 0.05 and
- D.O = >4 ml/l

Oxygen consumption studies were conducted in respiration bottles (acid washed) of 1 litre capacity made up of glass. The bottles were provided with an outlet to draw samples periodically for the estimation of dissolved oxygen content. The oxygen in the bottles was near to 100% air saturation at the beginning of the experiment. Fishes were separately introduced into each of the respiration bottles containing the metal solution. The bottles were held in a water bath at 28.0°C throughout the duration of the experiment. The surface of the test media was sealed using liquid paraffin to prevent the diffusion of atmospheric oxygen (Baby Menon, 1986).

Ten ml of the water sample was drawn for the estimation of dissolved oxygen content (Kuttyamma, 1980) at every 6, 12, 18, and 24 hours. The dissolved
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Oxygen content was estimated titrimatically by Winkler's method (Strickland and Parsons, 1975). The difference between the initial and final oxygen content was considered as the oxygen consumed by the fish. Finally, the rate of oxygen consumed was expressed as ml O$_2$ gm body wt/hr. The oxygen consumption values of control animal was taken as 100 percent (normal rate).

Data analysis for acute toxicity

Cumulative percentage mortality was determined for each metal concentration of the mortality experiment. The lethal concentration (LC$_{16}$, LC$_{50}$ and LC$_{84}$), 95% confidence limits (for LC$_{50}$) and slope function were estimated statistically by probit analysis (Litchfield and Wilcoxon, 1949).

Data analysis for Respiratory metabolism

The data obtained was subjected to analysis of variance (two factor randomized complete block design) test to determine the statistical significance of effect of heavy metals on the oxygen consumption by the method described by Snedecor and Cochran (1967).
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RESULTS

Acute toxicity bioassay studies were conducted for 96 hours on juveniles of *Etroplus suratensis*. Mortalities were recorded in animals exposed to toxic conditions of Hg, Cu, Cd, and Zn; however, no mortality was recorded in control.

**Acute toxicity of heavy metals on juveniles of *Etroplus suratensis***

The cumulative percentage mortality of juveniles exposed to Hg, Cu, Zn, and Cd are given in Table 50 & 51 and the LC50 values along with their 95% confidence limits and slope functions are summarized in Table 52. The toxicity curve of Hg, Cu, Zn, and Cd to juveniles is depicted in Fig. 66.

Hg was found to be highly toxic when compared to all the other metals tested. At lowest concentration i.e. 0.02 ppm, no mortality was observed up to 84 hours. At the concentration of 0.50 ppm, 10% mortality was noticed in 48th hour (Table 50). However, at a concentration of 0.9 ppm, 50% mortality was observed during 60th hour. From Table 50 it is clear that only concentration of 0.65 ppm and higher recorded 50% or more mortality after 96 hours of exposure. 100% survival was seen up to 24 hours in the concentration of 0.65 ppm and 60 hours in the concentrations of 0.35 ppm respectively. 100% mortality was observed at 0.9 ppm over 96-hour period. Hg was found to be 3.0 times more toxic than Cu and 14.8 times more toxic than Zn. When compared to Cd, Hg was 57 times more toxic. The 96-hour LC50 value of Hg on juveniles was 0.5 ppm (Fig. 66a) whereas the LC16 and LC84 values were 0.24 ppm and 0.80 ppm respectively (Table 52).

Cu was highly toxic to fish at 1.90 ppm killing all the fishes in 96 hours. However, no mortality was recorded at 1.30 ppm Cu up to 60 hours. (Table 50).
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The 96-hour LC50 value of Cu was 1.52 ppm (Fig 66b) and the corresponding LC16 and LC84 values were 1.37 ppm and 2.0 ppm respectively (Table 52). Cu, which was found next to Hg in its toxic effects on *Etroplus suratensis* was 4.8 times more toxic than Zn. Zinc was not toxic at 7.10 ppm and did not cause any mortality in this concentration over a 96 hours period. (Table 51). The 96-hour medium lethal concentration of Zn on fish was 7.43 ppm (Table 52 and Fig 66c). All fishes were recorded dead at the concentration of 8.0 ppm and above in 96 hours. Zn was found to be more toxic than Cd on fish.

Cd, which was found to be least toxic for fish among the tested metals, did not cause any mortality up to 84 hours in the concentration of 20 ppm. However, 100% mortality was observed at 50 ppm Cd in 96 hours (Table 51). The 96 hour LC50 value of Cd on fish was 28.5 ppm (Fig. 66d) and the corresponding LC16 and LC84 values were 19.8 and 46.8 ppm respectively (Table 52).

The toxic effects of the tested heavy metals on fish were in the following order:

Hg > Cu > Zn > Cd

Respiratory metabolism

The effects of four heavy metals namely, Hg, Cu, Zn, and Cd on the oxygen consumption rate of juvenile *Etroplus suratensis* were studied for a period of 24 hours. The percentage mortality of *E. Suratensis* exposed to metals are represented in Tables 53, 55, 57 and 58 and Figures 69 to 70.

Effect of Hg

Hg was found to depress the oxygen consumption rate of fish (Fig 69). At 0.25 ppm, the consumption was 10.51% less than that of the control at the 6th hour.
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The consumption decreased further and was 29.84% less than the control by the end of the 24th hour. In the highest concentration tested (0.65 ppm), the consumption was 25.12% lesser than that of the consumption in control at the 6th hour, which subsequently dropped by 46.63% below the control at the 24th hour. (Table 53).

Analysis of variance test (Table 54) showed a significant difference over the tested concentrations. Among the tested concentrations, 0.25 ppm was found to be highly significant with respect to rate of oxygen consumption (Critical Difference at 1% = 0.14). A Significant difference was obtained between the hours too. However, 6th hours was found to be highly significant over the other durations (Table 54).

Effect of Cu

The mean oxygen consumption rate of fishes exposed to Cu is presented in Fig 68. Oxygen consumption decreased with time as well as with concentration. In the lowest Cu concentration tested (1.35 ppm), Oxygen consumption at the 6th hour was 12.76% below the control value. The Oxygen consumption was less by 27.98% below the control value at the 24th hour (Table 55) at 1.35 ppm Cu. In the highest concentration tested (1.75 ppm) Oxygen consumption decreased with time and by the end of 24th hour the consumption dropped by 49.89% below the control.

A significant difference was obtained statistically between time as well as between concentration (Table 56) when subjected to statistical analysis. Control was significantly different from the tested concentrations. Among the tested concentrations 1.35 ppm was found to be highly significant (Critical difference at 1% = 0.13). Among the durations 6th hour was found to be highly significant (Critical difference at 1% = 0.13).
Effect of Zn

The mean \( \dot{O}_2 \) consumption rates of fish exposed to Zn is given in Fig 69. In the lowest Zn concentration tested (7.1 ppm), \( \dot{O}_2 \) consumption was 7.48 \% less than the control value at the 6\textsuperscript{th} hour. It continued to decrease with time and by the end of 24\textsuperscript{th} hour the consumption was 26.72\% less than the control (Table 57). At 7.6 ppm Zn, oxygen consumption was 42.09\% below the consumption in control at the 24\textsuperscript{th} hour.

Analysis of variance test (Table 58) showed a significant difference between concentrations and time. Control was found to be highly significant when compared to the tested concentration. Among the tested concentration 7.1 ppm was found to be highly significant (Critical Difference at 1\% = 0.15). Between times 6\textsuperscript{th} hour was found to be highly significant (Critical Difference at 1\% = 0.15).

Effect of Cd

Fig 70 gives the mean respiration rate of fishes exposed to sub lethal concentration of Cd. Oxygen consumption decreased on exposure to Cd with increase in concentration as well as with time. At 24.5 ppm, the decrease was 30.63 \% less than the control value at the 24\textsuperscript{th} hour. In the highest concentration of Cd tested (32.5 ppm), oxygen consumption decreased considerably by the end of 24\textsuperscript{th} hour and it was 45.25\% less than control value (Table 59).

Statistical analysis showed a significant difference between concentration and time (Table 60). Control was found to be highly significant over the tested concentrations. 24.5 ppm was found to be highly significant (Critical Difference at 1\% = 0.11\%). Between duration 6\textsuperscript{th} hour was found to be highly significant (Critical Difference at 1\% = 0.11\%).
DISCUSSION

The impact of pollutants on marine environment is more acute and its deleterious effect on living resources is much more evident in coastal and estuarine areas than the open ocean. Among the different pollutants reaching the marine environment, heavy metals, which are non-degradable, toxic substances, pose a serious threat to the biota living therein, particularly to the early life stages of various organisms.

Results of this study confirm that the heavy metals Mercury, Copper, Zinc and Cadmium are toxic to *Etroplus suratensis*, an ecologically and economically important estuarine fish of Karwar.

The fishes showed signs of distress initially, when introduced to the assay containers. The initial stress was highly noticeable in the higher concentrations of the tested heavy metals.

The effect of heavy metals on different aquatic organisms is often complex and difficult to interpret. These metals are relatively toxic at low concentration and effect the survival of fishes and other aquatic organisms. The biological effect of heavy metals in the aquatic environment is adverse mainly due to the complex nature. Many responses have been investigated in bioassays with fish (Warren et al., 1967). Toxicity of most pollutants varies with water characteristics and fish species, no safe concentration is likely to be applicable to all water bodies. For this reason, many investigators feel that a safe concentration should be established for each body of water and each pollutant of concern.
Poisons especially salts of heavy metals exert a depressive action on fish through physical accumulation. Mercury and its compounds (HgS, HgSO₄, HgNO₃) are toxic for fish and other aquatic organisms. In soft water, a concentration of 0.01 mg/l is lethal for fish (Metelev et al., 1983). Somsiri et al. (1982) reported 24hrs LC₅₀ value of 5.98 ppm on the fish *Tilapia nilotica*. On the same fish, he reported 48hrs LC₅₀ value of 3.8 ppm and 72hrs LC₅₀ value of 3.71 ppm respectively. Yuxin and Zhihui, (1989) reported 48hrs LC₅₀ value of 0.0034 ppm on *Moina mongolica*. A higher 96hr LC₅₀ of 0.67 ppm Hg was reported by Gaikwad, (1989) on *Entroplus maculatus*. In the present study, the 96-hour LC₅₀ value of Hg on juveniles was 0.50 ppm (Table 52 and Fig 66 a).

A comparison of the present results with that of other workers as mentioned above, gives a clear indication that the juveniles of *Entroplus suratensis* may be highly sensitive to the heavy metal mercury.

In the present study, copper was also found to be highly toxic but only next to mercury as is evident from (Table 50 and Fig 66 b). The 96hr LC₅₀ value of copper obtained in the present study was found to be much lower and does not agree with that of Gaikwad, (1989) who reported higher 96hr LC₅₀ value of 1.83 ppm Cu on *Entroplus maculatus*. The LC₅₀ values of Cu were found to vary from species to species. Mohapatra (1993) reported 96hr LC₅₀ value of 21.8 ppm of Cu on *Liza parsia*. The same author has reported a higher value of 96hr LC₅₀ to be 73.4 ppm, 63.9 and 58.3 ppm on *Tilapia nilotica*. Jackim et al. (1970) had reported 48hr LC₅₀ value of 1.0-3.3 ppm of Cu on *Pleuronectes flesus*. On *Fundulus heteroclitus* he reported a value of 3.2 ppm Cu for the same 48hr LC₅₀. Andros and Garten (1980) studied effects of copper on Northern squam fish and found that LC₅₀ value for 96hr was 18.0 ppm.

The study also indicates that zinc and Cd were also toxic to the fish. Bradly and Sprague (1985b) reported incipient LC₅₀ value to be 3.07-6.69 (4.53) ppm on
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*Salmo gairdneri.* Mohapatra (1993) has reported 96hrs LC\textsubscript{50} value of 13.7ppm of Zn on *Liza parsia* and 60.3ppm of Zn\textsubscript{aq} H\textsubscript{2}O on the same fish. On *Salmo gairdneri*, Lovegrove and Eddy (1982) reported LC\textsubscript{50} value of 2.0ppm of Zn. Toxic effects of Zn were also studied on *Tilapia nilotica* for 24hr, 48hr and 78hr. The values were found to be 88.3ppm, 74.8ppm and 65.6ppm respectively (Som\textsc{masiri} et al., 1982). Similarly Sen et al (1991) has reported 96hr LC\textsubscript{50} value of 23.7 Zn on *Channa punctatus*. In the present study, the 96hr LC\textsubscript{50} value was found to be 7.43ppm.

The above cited comparisons show the usefulness of *E. suratensis* as a highly sensitive test organism for heavy metal. Toxicity studies of heavy metals may thus vary from species to species and the tolerance level of different species to a particular metal may vary to a considerable extent. Sehgal and Pandey (1984) studied the effect of CdCl\textsubscript{2} on *Lebistes reticulates* and found the LC\textsubscript{50} value for 96hrs to be 250.00ppm. Mc Carty (1978) tested Cd on gold fish and found 96hrs LC\textsubscript{50} to be 2.13ppm. Saxena and Subba Rao (1982) studied the acute toxicity of the same metal and found LC\textsubscript{50} for 96hr to be 25.0ppm. The 96hr LC\textsubscript{50} value for Cadmium was found to be 28.2ppm in the present study.

Thus the variation in resistance to a particular metal in different species may be due to various reasons. The metallothioneins (MTS) present in organism are a group of low molecular mass, cystine-rich metal binding polypeptides. Olafson and Thompson (1974) were first who reported this (MTS) in an aquatic species.

According to them, the metallothioneins are small (6000-7000 Dalton), Suphydryl rich proteins whose free thiol groups readily bind the heavy metals. Khan et al (1988) opined that the acquired tolerance of organisms to heavy metals can result from increased synthesis of metallothioneins. Thus, the quantum of (MTS) synthesized may vary from one species to the other resulting in variation in their resistance to metals.
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The tolerance developed by any organism to the pollutant may also depend on the environment in which they lived earlier. The organisms that have lived in a slightly contaminated environment might have developed resistance to thrive in an environment having higher pollution levels. Several investigators have demonstrated that tolerance to a pollutant increases on previous exposure to low sub lethal concentrations (Brown, 1976, Saliba and Kozycz, 1976, Kraus, 1986). Bryan and Hummerstone (1971) also opined that the polychaete worm *Nereis diversicolor* living in an environment chronically polluted with copper, developed a tolerance to copper toxicity.

The present findings show that the survival of organisms in metal contaminated water was inversely proportional to the concentration of the metal solution. Toxic levels of a particular heavy metal may vary from species to species. However, Ahsanullah and Williams (1991) have shown that the metabolic regulation do not take place in the non-nutrient metals like Cd, though they reported metabolic regulation of nutrient metals of Cr, Cu and Zn. Also in a natural environment, not only metals, but also ligands (whether water-soluble or liquid soluble) are expected to be present, and these may also alter toxicity of a metal.

For the determination of maximum permissible level of metals and thereby to protect marine life, NAS/NAE (1973) recommended applying an application factor of 0.01 to the 96hrs LC50 values for the most sensitive indigenous organism. However, the only drawback of this recommendation is that there is no mention about a specific life stage of organisms. An application factor when applied to some adult LC50 data produce derived safe concentrations, where as the same concentration may cause acute lethality to their corresponding larvae. Thus, an application factor applied to acute toxicity tests is only a temporary solution to the problem of pollution and in assessing water quality (NAS/NAE, 1973).
Respiratory Metabolism

Rate of oxygen consumption seems as a useful parameter to assess the stress of organisms, as this indicates the energy expended by them to cope up with the changing environmental conditions. Heavy metal contaminants can either depress or elevate the rate of oxygen consumption in marine organisms. This study has demonstrated heavy metal-induced alterations of oxygen consumption in the juveniles of *Etroplus suratensis*.

During the present study, fishes initially showed signs of distress when introduced to metal concentrations. It showed significant reduction in oxygen uptake (Fig 67-70) when exposed to sub lethal levels of all the tested metals namely Mercury, Copper, Zinc and Cadmium. The reduction in gill permeability caused by the heavy metals may lead to a drop in oxygen consumption. Sultana and Devi (1995) attributed the same reason for the fluctuated respiratory response in *Mystus gulio* exposed to Copper and Zinc.

The present study is in congruence with Skidmore (1970) who observed decreased oxygen uptake in rainbow trout, and with Tort et al (1982) who also recorded suppression in respiration by zinc in the dogfish. Steed and Copeland (1967) examined the effects of exposure of industrial effluents on the respiratory rates of shrimps and concluded that their effects on the respiratory rate of fish were seemed to be highly variable. Studies on this aspect with *Labeo rohita* by Hingorani et al (1979) showed that the rate of oxygen consumption of the fish decreased which an increase in the consumption of the effluents. The present study also agrees with the experiments of Gadkari (1981) who reported lowered oxygen consumption rate in *Lebistes reticulates* exposed to cadmium. James (1990) noticed that Copper declines the oxygen consumption of *Oreochromis mossambicus*. The results also corroborates with Jones (1947) who showed suppression in respiration of
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*Gastrosteus aculeatus* subjected to mercury and copper toxicity. Mercury was reported to be a respiratory depressant by Venkatasubbiah et al (1984) in the freshwater mussel *Lamellidens marginalis*.

However, in contrast to the results of the present study, Brown and Newell (1972) found that zinc had no effect on the oxygen consumption of *M. edulis*. The drastic suppression in oxygen consumption with an increase in metal concentration and exposure period may be due to considerable damage to the gill tissue and the formation of mucus layer over it. This in turn may cause decreased flow of water on to the gill surface leading to reduced uptake of oxygen. Gills, which are vital part, are highly sensitive to and environmental alterations. It has been confirmed that accumulation of metal ions within the gill tissue may cause adverse effect on the gill function (respiration, osmoregulation etc). (Verberg and Hara, 1977; Thurberg et al., 1973). The intimate contact of gill with water borne pollutants may result in an early damage to this vital part, thereby resulting in a decreased respiratory surface area. This in turn lowers the diffusing capacity of gills (McFadden, 1965; Skidmore, 1970; Mathiensen and Bratfield, 1973; Hughes, 1980; Cairns et al., 1981).

Physiological, histological and ultra structural studies on exposure of animals to metal toxicant have also shown the disruption of the gill cells (Eisler and Gardner, 1973; Papanathanassiou, 1986). Some workers have also opined that mitochondria is also one of the most important targets of heavy metals in cells (Akberali and Earnshaw, 1982; Viarengo, 1985).

The reduction in oxygen uptake may also appear to be as a protective measure to ensure that there is a low intake of toxic substances from the environment by the organisms. In the present study, it has been observed that
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Oxygen consumption varies invariably with concentration and time. The decreased respiratory rates at lower concentrations as against the higher concentrations by the fish in the present study may indicate the efforts taken by the animal for its survival in a less toxic environment.

Thus in the present study all the four metals were found to inhibit respiration in the fishes. Thus, it may seem apparent that the ability to adapt may vary with the different life history stages within a species. Also, the metal induced changes in respiration is complicated and as such alterations differ with metals, species, experimental conditions, etc (Thurberg et al., 1973; Sultana and Devi, 1995).
Table 50: Cumulative percentage mortality of pearl spot exposed to Hg & Cu

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<th>Metal</th>
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Table 5: Cumulative percentage mortality of juveniles exposed to Zn & Cd

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<th>Metal</th>
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Table 52: Lethal concentrations of heavy metals to *Etroplus suratensis* for 96 hour with 95% Confidence limit and slope function

<table>
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<tr>
<th>Metals</th>
<th>Duration of exposure (hrs)</th>
<th>LC 84 (ppm)</th>
<th>LC 50 (ppm)</th>
<th>95% confidence limits</th>
<th>( \frac{LC}{10} )</th>
<th>Slope</th>
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<tbody>
<tr>
<td>Hg</td>
<td>60</td>
<td>1.05</td>
<td>0.85</td>
<td>(1.023-0.705)</td>
<td>0.58</td>
<td>1.3503</td>
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<tr>
<td></td>
<td>72</td>
<td>0.92</td>
<td>0.55</td>
<td>(0.565-0.534)</td>
<td>0.27</td>
<td>1.0772</td>
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<tr>
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<td>84</td>
<td>0.83</td>
<td>0.52</td>
<td>(0.502-0.380)</td>
<td>0.25</td>
<td>1.8592</td>
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<tr>
<td></td>
<td>96</td>
<td>0.80</td>
<td>0.50</td>
<td>(0.685-0.360)</td>
<td>0.24</td>
<td>1.8635</td>
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<tr>
<td>Cu</td>
<td>60</td>
<td>2.14</td>
<td>1.9</td>
<td>(2.0650-1.7447)</td>
<td>1.54</td>
<td>1.1794</td>
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<tr>
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<td>72</td>
<td>2.12</td>
<td>1.68</td>
<td>(1.8477-1.5286)</td>
<td>1.45</td>
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<tr>
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<td>96</td>
<td>2.00</td>
<td>1.52</td>
<td>(1.6538-1.3970)</td>
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<tr>
<td>Zn</td>
<td>60</td>
<td>8.10</td>
<td>7.65</td>
<td>(7.8377-7.4707)</td>
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<tr>
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<td>72</td>
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<td>7.507</td>
<td>(7.7070-7.3124)</td>
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<td>96</td>
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<td>7.43</td>
<td>(7.6358-7.2297)</td>
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<td>(34.430-23.58)</td>
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Table 53: Percentage oxygen consumption of E. suratensis exposed to Hg.

Control is considered as 100%.

<table>
<thead>
<tr>
<th>Conc (ppm)</th>
<th>Duration (hrs)</th>
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<tr>
<td></td>
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<td>Control</td>
<td>4.38</td>
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<tr>
<td>0.25</td>
<td>89.49</td>
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<tr>
<td>0.65</td>
<td>81.27</td>
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<td>1.45</td>
<td>74.88</td>
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Table 54: Analysis of variance. Oxygen consumption of Pearl spot exposure to mercury.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor A</td>
<td>3</td>
<td>5.600919</td>
<td>1.866973</td>
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<tr>
<td>Factor B</td>
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<td>1.283169</td>
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<tr>
<td>Error</td>
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<td>0.354556</td>
<td>0.039395</td>
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</tbody>
</table>

C.D. at 1%: Factor A = 0.14 |
Factor B = 0.14

Factor A = Concentration
Factor B = Duration
Table 55: Percentage oxygen consumption of *E. suratensis* exposed to Cu.
Control is considered as 100 %

<table>
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<tr>
<th>Conc (ppm)</th>
<th>Duration (hrs)</th>
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<th>12</th>
<th>18</th>
<th>24</th>
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<td>73.34</td>
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<td>64.56</td>
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<td>61.04</td>
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Table 56: Analysis of variance of *E. suratensis* exposed to copper

<table>
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<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
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<td>Factor A</td>
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<td>8.10882</td>
<td>2.70294</td>
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<td>Factor B</td>
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<td>0.52232</td>
<td>0.17411</td>
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<td>0.15626</td>
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C.D. Factor A Factor B
at 1% 0.13 0.13

Factor A = Concentration
Factor A = Duration
Table 57 Percentage oxygen consumption of *E. suratensis* exposed to Zinc
Control is considered as 100 %

<table>
<thead>
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<th>Conc (ppm)</th>
<th>Duration (hrs)</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
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<td>82.71</td>
<td>76.81</td>
<td>69.64</td>
<td>65.72</td>
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<td>72.19</td>
<td>67.21</td>
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<td>57.91</td>
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Table 58: Analysis of variance Oxygen consumption of *E. suratensis* exposed to Zinc

<table>
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<th>Source of Variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
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<td>4.99903</td>
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<td>Factor B</td>
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<td>0.81822</td>
<td>0.27274</td>
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<td>Error</td>
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<td>0.24452</td>
<td>0.02717</td>
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</table>

C.D. at 1%

Factor A = Concentration
Factor A = Duration

See table 53 and figure 33.
Table 59 Percentage oxygen consumption of *E. suratensis* exposed to Cadmium
Control is considered as 100%

<table>
<thead>
<tr>
<th>Conc (ppm)</th>
<th>Duration (hrs)</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>81.9</td>
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<td>65.15</td>
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<td>54.75</td>
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</table>

Table 60: Analysis of variance Oxygen consumption of Pearlspot exposure to Cadmium

<table>
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<th>Source of Variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
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<td>7.27723</td>
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<td>52.7686</td>
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<td>1.08163</td>
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<td>Error</td>
<td>9</td>
<td>0.41372</td>
<td>0.04597</td>
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</table>

C.D. Factor A Factor B at 1%

Factor A = Concentration
Factor B = Duration
Fig 66 Toxicity curve of Hg, Cu to the fish *Etroplus suratensis*.

(a) Time to 50% mortality for Hg.

(b) Time to 50% mortality for Cu.
Fig 66: Toxicity curve of Zn and Cd to the fish, *Etroplus Suratensis*.

(C)

Time to 50% mortality

(D)

Time to 50% mortality
Fig. 67: Rate of O₂ consumption (ml/l O₂/g/hr) of *Etroplus suratensis* exposed to Mercury exposure

Concentrations (ppm)
Fig. 68: Rate of Oxygen consumption (ml/l O$_2$/g/hr) of *Etroplus suratensis* exposed to Copper.
Fig. 69: Rate of oxygen consumption (ml/l O₂/g/hr) of *Etroplus suratensis* exposed to Zinc concentrations (ppm) exposure.
Fig. 70: Rate of Oxygen consumption (ml/l O₂/g/hr) of *Etroplus suratensis* exposed to Cadmium exposure concentrations (ppm)
CHAPTER 4.1

DISEASE STUDIES

INTRODUCTION

Fish diseases and parasites have been less studied until last decade, since aquaculture activity was restricted to extensive and semi-intensive culture systems. At low stocking density, the water quality remains undisturbed and the incidence of infection is less. Secondly, the detection of disease is equally difficult at low densities in large water bodies.

The advancement in intensive culture techniques, obviously invite quick environmental deterioration leading to the outbreak of diseases resulting in heavy mortality. The high stocking density further increases the chances of mortality as a consequence of en masse infection. Further, the fish is invariably transported either in the form of seed or brood stock, which may carry the localized diseases and parasites and spread all over. Therefore, it is essential to study the localized diseases and parasites of at least cultivable species so that therapeutic and prophylactic measures could be developed.

The two reasons why there are few reports on the disease and parasites of *E. suratensis* than those of other cultivable species are: 1) Its distribution is restricted to only peninsular India and Sri Lanka and no importance has so far been given to the study of its diseases, since the culture itself is practiced in traditional way. 2) Probably the thick coat of scales, which protect it from various external injuries and infections. In the present study, the common diseases and parasites from the natural population and from the laboratory culture tanks are described.
MATERIALS AND METHODS

Specimens of *Etroplus suratensis* were examined for both external and internal parasites during the course of this study. Observations on parasites were carried out on specimens ranging in size from 85 mm to 165 mm in the months of March to June 1999 and the parasites were identified as per Gussev (1963); Babu and Raj (1985); Kabata (1985). External parasites found in the gill region, dorsal and ventral body surfaces were removed from the host fish. Fishes were also maintained in the laboratory culture tanks for diseases studies. In some specimens "fin rot" disease was observed and the etiology was investigated.

The infected specimens were treated with nitrofurazone (Colomi and Paperna, 1983) and the complete cure with the regeneration of the fin was observed in a period of 7-10 days. Other contagious infection observed in the laboratory reared specimens was of monogenean in gill filaments. In this case, only symptoms could be recorded, but the therapy could not be performed as the specimen died in a couple of days. The specimens were also examined to investigate the incidence of an isopod parasite.
DISEASES

Epizootic ulcerative syndrome (EUS): EUS is a serious disease, which has been spreading across South and Southeast Asia since 1980. The disease is characterized by large hemorrhagic necrotizing ulcers extending deep into the tissues on a wide variety of wild and cultured fish species, leading most invariably to death (Roberts et al., 1986; Lilley et al., 1992). Etiology of the outbreaks of EUS remains a mystery. Although viruses, bacteria and fungi have been isolated prior to 1994 (Roberts et al., 1973; 1992; 1993; Lilley et al., 1992). However, in more recent studies the fungus *Aphanomyces* is found to be associated with the disease (Roberts et al., 1994; Vishwanath et al., 1997).

The disease was noted first in Sri Lanka in 1987, around the lower reaches of the Kelani River. It then spread very rapidly within a number of water bodies, affecting over 20 species of freshwater and brackish water fish in the South-Western Zone (Costa and Wijeyaratne 1989). Since then the disease has recurred annually, affecting a variety of water bodies in the Dry and Wet zones.

The fish species affected with EUS in the wild stock of brackish water fish of Karwar area were Mugil cephalus, Arius sp., Ambasssis commersoni, Diodon sp., Therapon jarbua, Puntius sp., Glossogobius sp, Scatophagus sp, Siganus sp and Eptoplos suratensis. Lesions with necrotic tissue and red patches were observed all over the body of *E. suratensis* (Plate D). The intestine was empty and the visceral parts were ulcerative. A similar outbreak of disease has been also reported earlier from different parts of India (Anon., 1991; Kumar et al., 1991) and from Asian Pacific region (Anon., 1981; Rodgers and Burke, 1981; Coates et al., 1984; Roberts et al., 1986; Anon., 1988; Pathiratne et al., 1984). The signs recorded in the infected fish were similar to those of EUS, reported earlier. The pattern of prevalence of epizootic (1989 in North-Eastern states of India, 1990 in ...
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Sri Lanka and 1991 in Kerala) indicates that the disease first erupted in the north-
eastern states and later spread down south along the east coast, followed by an
upward trend along the west coast of India.

In Karwar the incidence of EUS was less compared to other states. The
prevalence of the disease coincided with the onset of the south-west monsoon,
when the prevailing salinity in the brackish water area was almost freshwater. The
primary etiological agent is still an enigma; however, the preliminary findings
indicate the possible role of virus in the epizootic (Anon, 1991; Kumar et al., 1991).
In Karwar waters, the prevalence of disease was only for a period of one month and
latter subsided by the end of monsoon with simultaneous increase in salinity. In
natural waters no prophylactic and control measures can be adopted, though, in
culture systems the application of lime at the rate of 600 kg/f in three installments
has been recommended (Kumar et al., 1991).

**Fin Rot Diseases:** Fin rot was observed to be the most frequently occurring
disease in *E. suratensis*, when reared in captivity. *E. suratensis* is generally found in
a shoal of 5-25 numbers in natural waters. Initially, it seems to be territorial when
stocked and reared in small culture tanks, and needs to be provided with sheltered
hiding place, probably because of its timid nature. Hiding places further increased
the territorial instinct and the fish developed nibbling habit and damaged each
others caudal fin which later aggravated to a fin rot disease. Brightsingh et al
(1981) also reported the same disease, when they tried to rear it in an aquarium.
However, the primary cause of infection was not assigned.

**Clinical Signs:** Initially, the caudal fin membrane is eroded which slowly
progressed towards the caudal peduncle and in a couple of days the fin ray
membrane, part of the dorsal, anal and pectoral fin started eroding (Plate E). The
swimming behavior of the fish got affected. It became inactive with folded fins and preferred to hide at the bottom.

Brightsingh et al (1981) reported the dominance of *Pseudomonas, Vibrio, Aeromonas, Bacillus* and *Corynebacterium* as an associated flora with fin rot disease of *E. suratensis*. Mahoney et al (1973), Karunasagar et al (1986 and 1988) and Kumar et al (1986) reported the specific pathogenicity of the bacteria causing fin rot diseases. *Pseudomonas, Micrococcus, Alcaligenes, Vibrio, Arthrobacteria* and *Cytophaga* are reported to be the associated flora of *E. suratensis* caught from the backwaters of Kerala (Surendran and Iyer, 1985). Similar associated bacterial flora has been reported from *Sardinella longiceps* and *Rastrelliger kanagurta* (Karthiyan and Iyer, 1967; Surendran and Iyer, 1976).

These studies indicate that *Pseudomonas, Vibrio, Aeromonas* and *Cytophaga* are the most common microbes in marine and brackish water environment and in the fish species, though the incidences of fin rot disease in natural waters is rather low. Brightsingh et al (1981) inferred that probably dense bacterial population and environmental stress are the pre-requisites of epizootics. In a study of fin rot in brook trout (*Salvelinus fontinalis*), Bullock (1968) suggested that the primary cause of bacterial invasion is the lesion resulting from nutritional deficiency, injury and other predisposing factors. Thus, injury by nibbling, transportation or handling is the primary cause paving the way to the opportunistic *Vibrio, Pseudomonas* and *Aeromonas* resulting in fin rot disease.

In culture systems, a commercial antibacterial agent sold under the trade name of "Furacin" can be applied (Coloni and Paperna, 1983). This commercial preparation consists of 0.2% of nitrofurazone w/w, an active anti-bacterial agent. It is water soluble and self content with emulsifier. Furacin can be applied in a fish...
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tank of about 500 litres water capacity at the rate of 0.4 to 0.5 mg/l of nitrofurazone for three successive doses on alternate days.

Colomi and Paperna (1983) reported that nitrofurazone cannot control the systemic infection; however, it has a practical value as a general disinfectant. It does not interfere negatively with tissue re-generation (Kubota and Hagita, 1963). Thus, the fin rot disease in *E. suratensis* may be a superficial epidermal infection, which could have further led to systemic infection. The fast regeneration of fin could be an added advantage of the use of nitrofurazone as a therapeutic agent.

**PARASITES**

The different parasites recorded during the present study were 1) *Lernea sp*, *Trichodina sp.* (Ciliate), *Ancyrocephalus sp.* (Monogenea), *Caligus* (Copepoda) and *Cymothoa krishnai* (Isopoda).

*Lernea species*: *Lernea sp* are among the most harmful parasites of cultured freshwater fishes, both in temperate and tropical waters. They are the largest tissue-invading copepods. The parasite was found attached to the body; the head was embedded in the body muscle and can reach visceral organs with the help of much branched tentacle like things on the head. Only the black body and greenish ovarian sacs were visible outside. The parasites could be seen on both sides of fish and more infestations were on the dorsal side. Some fishes were infested by more than one *Lernea sp*. In severe cases of infestations, more than 18 parasites were observed on the fish. Generally, the parasite was observed on the dorsal side of the head region.

*Trichodina sp*: Only one type of organism was recorded in five specimens from natural population. It was located in gill filaments, with low number (5-10 per gill
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arch) in all the specimens except in one where the population was in hundreds. The parasite was identified to be a ‘ciliate’. The generic level of identification was carried out following description mentioned by Kabata (1985). Hemispherical body and the distinctive internal ring of denticles placed this parasite under the order Peritrichida and the suborder Mobilina. The adoral spiral of approximately 360° and shape of the denticles, place this parasite under the genus Trichodina sp. The cell is roughly hemispherical, bell shaped and concave along its aboral surface; body cavity is completely surrounded by adoral cilia, marginal and lateral cilia.

Ancyrocephalus sp: About 7 specimens were observed to be infected in gills by the Ancyrocephalus sp, a member of monogene group. The intensity of infection was 1-5 numbers per gill arch. However, the same species infected the laboratory-reared specimens, where the intensity was 20-60 numbers per arch. The diagnosis could be done probably at a later stage when one or two specimens started dying every day. Delay in diagnosis restricted the use of therapeutic agents as the whole stock of more than 50 specimens died in few days.

The fish became inactive, remained at the water surface and started gasping, indicating respiratory stress. The fish could be caught easily. Overall colour of the fish turned dark. Opercula appeared to be somewhat open. The colouration of gills turned pink rather than the original brick red and the edge of the gill filaments had grayish colouration. The gill filament appeared clotted and necrotic.

The parasite was identified as per the key described by Tripathi (1957). Body elongated and sides almost parallel; anterior end truncate; two pairs of eye spots with the posterior overlapping the pharynx; three pairs of head organs present; haptor demarcated from the body by a constriction and broader than the length; two separate equal horizontal bars on the haptor which lies one above the other; vagina not prominent and is on the right side of the body; vitellaria from behind the pharynx to the end of the body. In preserved specimens the length of the
Ancyrocephalus sp. recorded from *E. suratensis* varied from 59.00-122.83μ and breadth 36.75 - 51.45μ.

*Caligus sp.*: Three copepod parasites were recorded from the branchial chamber of different hosts. The parasites were identified as *Caligus sp* as per Pillay (1963a & b) and Kabata (1985).

Unfortunately, all the three specimens were females bearing following characters: Cephalothorax covered dorsally by sub-circular sucker like shield; carapace broader than long with maximum width below the median line and two lateral incisions; anterior margin with fringed plates; lunules big, sub-circular; posterior margin of the thoracic zone slightly protruding beyond the tip of lateral zones; forth leg bearing segment narrower than the posterior margin of the cephalothorax and the genital segment; genital segment flask shaped and slightly longer than the abdomen; lateral setae on the posterior margin of the genital segment; abdomen single segmented and sub-triangular; five setae on each uropod, four prominent sub-equal in length with the fourth being the largest. Total length, 2.60 mm – 2.75 mm.

*Cymothoa krishnai* (Babu & Raj, 1985): Four pairs of isopod parasites were recorded from the buccal cavity and the bucco-pharyngeal cavity of different hosts. The parasites were identified as male and female of *Cymothoa krishnai* following standard key. The striking feature of the observation was that the parasites were recorded in the form of male and female pair in a single host. The female is distinctly bigger than the male.

**Female**: Body short, stout and obviate; cephalon triangular constricted at the antennal area; cephalon convex posteriorly with prominent posterio-lateral angle; cephalon sunk in the first peraeonite; eyes very small; first four peraeonites of sub-equal length; first peraeonite relatively long, anterio-lateral parts acute and posterior
margins slightly convex; second to fifth peraeonites are in increasing order of width, posterior margins convex; maximum width of parasite is at the fifth peraeonite; sixth and seventh peraeonites are short and narrow than the fifth with the posterior margin concave having the posterio-lateral processes extending up to the last pleonite; legs prehensile, arranged in a gradual increasing order of size and curved dactli; seventh leg is the largest and inwardly produced into a blunt process.

Pleon triangular, with the posterior margin read and the anterior immersed in the peraeon; a median ridge on the pleon; pleonite sub-equal in length, but arranged in increasing order of breadth; breadth of telson is double of its length; telson broader than pleon, uropods inwardly produced and shorter than the telson; both rami with apical setules.

Light brown pigment throughout the dorsal part of the body, with dark colouration at the cephalon, first peraeonite, at the base of pleon and proximal part of telson. Total length is 19.00 – 20.10mm.

**Male:** Body oblong, sides sub-parallel; cephalon triangular and sunk in the first peraeonite; eyes relatively large and conspicuous; first peraeonite; slightly long with anterio-lateral and posterio-lateral angles produced, posterior margin convex; peraeonites from second to fifth arranged in increasing order of length with straight margin; sixth and seventh peraeonites sub-equal, both with anterio-lateral and post-lateral angle, posterior margin convex; seventh peraeonite shorter than the sixth; pleon narrow and sunken in the peraeon; all pleonites sub-equal and placed in an increasing order; telson broader than the length ; uropods short with apical setule. Body uniformly coloured with brown pigment on the dorsal side, comparatively darker at the cephalon and pleon, total length, 7.00- 10.00mm.
DISCUSSION

Fin rot disease and *Ancyrocephalus species* infestation seem to be the major threats to the culture of *Etroplus suratensis*. Nitorfurazone can be used as prophylactic measure to control fin rot disease in culture systems, provided the diagnosis is done before it could attain the stage of systemic infection. EUS is a deadly disease causing mass mortality in short duration; however, the trend indicates that the stock gets immune after the first attack.

The extent of damage done by *Tricodina sps* could not be adjudged from the present study, since the host specimens were collected from the market. *Caligus sps* and *Cymothoa krishnai* were recorded in very low intensity, thus it does appear that the extent of damage to the standing stock may not be severe. Monogean parasites, *Ancyrocephalus etropli* and *Ceylonotrema colombensis* have been reported to infest the gill filaments of *Etroplus suratensis*(Gussev, 1963).

*Cymothoa indica* was reported to infest various fish species including *Etroplus suratensis* in Adyar estuary and fish farm (Pannikkar and Aiyar, 1937; Evangeline, 1963). Similarly *C. krishnai* has also been reported to infest various estuarine fish species in Pulicate lake, but not in *Etroplus suratensis* (Babu & Raj, 1985). *Trichodina sps*, *Caligus sps* and *C. krishnai* are the new records of *Etroplus suratensis* in the estuarine complex of Karwar. *Cymothoa krishnai* has also been reported from the Goa waters (Parveen, 1994).