PHYSIOLOGICAL ASPECTS
INTRODUCTION

Time Course (Whole Animal)-Oxygen Consumption:

Animals respond to the environmental stress including pollution stress in their own way. These responses of animals subjected to such stress fall under two categories: (i) Immediate or short term responses immediately following the transfer of animal into the medium that exerts stress and (ii) long term responses after prolonged exposure of the animal over days or even weeks to the stress is observed (Kinne, 1958, 1964 a., Parvatheswara Rao 1968 a). Short term responses would be in the form of abrupt rise or fall in the respiratory activity and the long term responses involve a gradual stabilization of the respiratory rate.

Alterations in the respiratory activity serve as sensitive indicators of stress in fish exposed to the pollutants (Schaumberg et al., 1967; Anderson et al., 1974 a; Sellers et al., 1975). Fish have the ability to adapt to the pollutants. This is supported by the various evidences in the literature, in that a conceptional model of the possible effects of pesticides and other poisonous substances are proposed by John Couch on biological systems (Duke and Dumas, 1974). This model proposes that a pesticide could be considered to possess an adverse effect if it temporarily or permanently alters the normal steady state of a particular biological system to that level as to render the homeostatic mechanism incapable of maintaining an acceptable altered steady rate. An acute dose of a pesticide can alter a biological system outside the normal range of variations. In due course of time, the system could return to the normal state without suffering the effects that would last for a longer period. Coppage and Duke (1972) reported the capacity of a fish population to compensate for the effect of a pesticide malathion when it was aerially applied to control mosquito vectors of Venezuelan Equine Encephalomyelitis.
It has been evidenced that fish possesses the capacity to adapt to the alien atmosphere rendered by the toxicant. The increased resistance-time to lethal ammonia levels was shown by the rainbow trout, *Salmo gairdneri*, after one day of exposure at half the lethal level, the response disappeared after three days of exposure (Lloyd and Orr, 1969). Same type of the phenomena has been reported for the responses in the growth of rainbow trout (Dixon and Leduc, 1981) and also in the case of Cichilid, *Cichlasoma limaualatum*, as reported by Leduc (1966 a). In both the instances, the initial depression of specific growth rate disappeared after 10 and 14 days of cyanide exposure. Sub-lethal studies that involve time course experiments are designed to determine if a pesticide has an effect on concentration less than those that are lethal to the organism and utilise such criteria as growth, function of oxygen consumption, enzyme systems etc., and a great deal of work has been turned out on these lines of approach during the past few years with reference to the chronic as well as partial chronic exposure of sub-lethal concentrations of pesticides in marine organisms including fishes. (Vernberg and Vernberg, 1974; Vernberg et al., 1977).

A steady supply of oxygen is essential for the maintenance of life. Oxygen plays a vital role in number of oxidative reactions. The availability of oxygen imposes limitations on distribution and survival of animals. The amount of oxygen consumed by an intact animal reflects its total metabolic rate and hence the energy output (Proser 1973, Lehninger, 1979). The events involved in energy metabolism can be viewed through biological, biophysical and molecular perspectives to unravel many secrets of nature specifically in the synthesis of biochemical energy namely ATP (Lehninger, 1979).
The physiological status of an animal would be appropriately assessed by the rate of oxygen uptake. The rate of oxygen consumption is generally influenced by several factors such as temperature, body size, season, time of the day, oxygen tension and so on (Ramesh Babu and Venkateswara Rao, 1985), and in aquatic animals, in addition, it is also influenced by salinity and pH of water (Proser, 1973). Powers (1922) made the first comprehensive study on the toxicity of a wide range of substances on *Carassius auratus* and investigated the effects on the physiology of respiration in fishes.

Dissolved chemicals gain entry largely through the respiratory surface of aquatic animals (Bright and Ellis, 1989). Hence, the first physiological function to be affected is oxygen consumption. The variations of it can be used as sensitive indicators of environmental stress (Sellars et al., 1975, Mahajan and Dheer, 1980; Prasad 1986). The metabolic rate has been measured by determining the oxygen consumption which provides information on the ability of the fish to extract oxygen from pesticide polluted water. The oxygen consumption under pesticide toxicity showed significant decrease resulting in hypoxic condition (Fergusson et al., 1966, Rao and Mane, 1979, Srinivasa Murthy et al., 1981). The effect of permethrin, cypermethrin and fenvalerate on respiration in the workers of insects *Atta sexdens redbipilosa* and *Atta laevigatta* was studied by Takahashi and Heblingberaldo (1987), and found that the rate of oxygen consumption in these insects has increased during a certain period of time. A significant drop in the rate of oxygen consumption in *Cyprinus carpio* exposed to both fenvalerate and cypermethrin was observed by Mallareddy (1987). Changes in the level of respiration and ions in the tissues of fresh water fish, *Labeo*
rohita under fenvalerate stress was studied by Mallareddy et al., (1992). All the above investigations indicate that oxygen consumption, a sensitive indicator of stress in fishes exposed to pollutant is suppressed considerably.

While assessing the influence of any stress on metabolism of animals, measurement of oxygen consumption, for very obvious reasons, has been employed more extensively than any other metabolic parameter. This type of programmed study also offers clue to the basic damage inflicted on animals which could either increase or decrease the rate of oxygen consumption. Such an event may lead to succession of chain reactions in metabolic pathways leading to activation or inhibition of their physiological functions. With this perspective, an attempt has been made to study the effect of deltamethrin on the rate of oxygen consumption at sublethal exposure periods in the fresh water fish, Labeo rohita.

Opercular Activity:

The rate of opercular movements indicate the respiratory efficiency of the animal. It indicates the rate of flow of water over the respiratory surface. The respiratory apparatus in a teleost fish comprises the buccal cavity, pharynx, gills and gill covering or operculum. The pulsating movements of mouth and operculum constitute opercular activity. (Chandy, 1970). At first, during the inspiratory phase, the buccal cavity expands allowing the water to enter into the buccal cavity, while the external gill opening is closed by the operculum. During expiration the pharynx contracts, forcing the water across the gills, and then to outside through operculum which is opened now. The mechanism constitutes the opercular activity. Radhaiah and Jayanth Rao (1990) reported a decrease in the opercular movement with an increase in
the concentration of fenvalerate in fish, *Tilapia mossambica*. Opercular movements can be regarded as an indicator for heat resistance in fishes (Thiede, 1965).

Some preliminary studies made in invertebrates like fresh water mussel showed an inhibition in ciliary activity during the malathion exposure (Kabeer Ahammad et al., 1979). It is reported that *Tilapia mossambica* subjected to malathion, methylparathion and phosphomidon showed a correlation between the decreased opercular activity and reduced oxygen uptake (Bashamohidden and Onnurappa, 1986).

A perusal of such studies indicated that information on the opercular activity of teleost fishes in relation to pollutants especially, the synthetic pyrethroids is scanty. The literature available on opercular activity in fishes is also confined to temperature, where the process of resistance - adaptation has been reported in *Rhadeus amarus* (Kunnemann, 1973). In this fish, the heat resistance was determined by an increase in the temperature (1°C/minute) beginning from adaptation temperature like 10°C and 20°C until cessation of opercular activity. Hence the opercular movement can be considered as an indicator of the environmental stress including the pollution stress. Keeping these factors as background, an attempt has been made to understand the impact of the pyrethroid deltamethrin on the opercular activity of the fish *Labeo rohita*. 
Results:

Time course (Whole Animal) Oxygen Consumption:

The data on the rate of whole animal consumption (O$_2$ml/h) in *Labeo rohita* exposed to sublethal concentration of deltamethrin, during the periods of 24 h, 48h, 72h and 96h, including the control medium (medium without deltamethrin) are presented in the table: 6 and fig. 4. The results indicate that in fish exposed to sublethal concentrations of deltamethrin oxygen consumption of Rohu increased by 24h of exposure and then declined during 48h, 72h and 96h of exposure and reached nearer to the control value by 96h of exposure.

The whole animal oxygen consumption was increased by 31.034% during 24h of exposure. Then the whole animal oxygen consumption gradually reduced by 29.655% on 48h of exposure and then by 15.517% on 72h of exposure period. By the 96h of exposure the oxygen consumption was further decreased to a minimum level of 3.793%. All the results except the 96h of exposure periods are significant.

Opercular activity:

The data on the rate of opercular activity (10/t) in *Labeo rohita* exposed to sublethal concentration of deltamethrin during the exposure periods of 24h, 48h, 72h and 96h including the control medium is presented in the table:7 and fig.5. The results indicate that in fish exposed to sublethal concentration of deltamethrin the opercular activity of Rohu increased by 24h of exposure and then decreased during 48h, 72h, and 96h, of exposure periods. The opercular activity was increased by 22.418% on 24h exposure, and then gradually declined by 8.866% on 48h of exposure and then by 1.285%
on 72h of exposure period. On 96h of exposure, the opercular activity further decreased by -2.015% and returned almost to normal and all the results are significant.

Discussion:

**Time-course (Whole Animal) Oxygen Consumption:**

The physiological status of an animal would be approximately assessed by the rate of oxygen uptake. Hence, the first physiological function to be affected is oxygen consumption during sublethal intoxication. The variation in respiratory activity (rate of oxygen consumption) has been used as a sensitive indicator of fish exposed to pollutants in general (Schaumberg et al., 1967; Anderson, et al., 1974a; Sellers et al., 1975; Bayne et al., 1980; Mahajan and Dheer, 1980; Prasad, 1986). The rate of oxygen consumption is generally influenced by several factors such as temperature, body size, season, time of the day, oxygen tension and so on, (Ramesh Babu and Venkateswara Rao, 1985) and in aquatic animals in addition to above, it is influenced by salinity and pH of water (Proser, 1973).

Since fish breath the water in which they live, changes in the chemical properties thereof may be reflected in the animal's ventilatory activity (Sellars et al., 1975). Toxicants gain entry through the gills of aquatic animals and affect primarily their structure and function (Tovell et al., 1975). Mechanism of toxicant uptake is through gills probably through pores by simple diffusion and then absorbed through cell membranes (Opperhuizen et al., 1985).

The results indicate the whole animal oxygen consumption increased by 31.034% by 24h of exposure. This initial increase may be due
to increased locomotory activity arising out of the animal's tendency to escape from the new medium which is a stress medium and this phenomenon is called "Escape Reaction" of the animals as suggested by Potts, (1954) and Gross, (1957). Thereafter, the whole animal oxygen consumption gradually decreased and reached almost the normal level by 96h of exposure. Earlier studies revealed that pesticide exposure in fishes generally resulted in the suppression of oxygen consumption (Hunter et al., 1967). David (1992) reported decrease in oxygen consumption by the whole fish, Labeo rohita, when exposed to sublethal concentration of fenvalerate. Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in Labeo rohita (Sridevi, 1991) and Tilapia mossambica (Reddy and Yellamma, 1991b). Several authors reported similar decline in whole animal oxygen consumption in different species of fishes exposed to pesticides (Kabeer Ahammad et al., 1981, Rangaswamy, 1984). Malla Reddy (1987) also reported significant drop in the rate of O₂ consumption in Cyprinus carpio exposed to both fenvalerate and cypermethrin.

These results obtained on oxygen consumption indicate that Labeo rohita undergo compensating mechanisms leading to homeostasis by varying the metabolic rate during deltamethrin exposure and ultimately these variations in oxygen consumption could be attributed to and befitting into the compensating mechanisms proposed by John Couch (1974) in his conceptional model wherein the pesticide like deltamethrin of the present investigation could cause a physiological system (O₂ consumption) to oscillate outside its normal range of variations, mostly suppressive, yet with time, the system could show the indications of its return to the normal state by way of recovery from suppression without suffering from lasting effects. Such
compensatory mechanism was demonstrated in few of the other fish species (Leduc, 1966a; Lloyd and Orr, 1969; Coppage and Duke, 1972; Couch, 1974; Dixon and Leduc, 1981; Dickson and Sprague, 1981).

Opercular Activity:

The opercular activity constitutes the inspiratory phase in which the buccal cavity expands allowing the water to enter into the buccal cavity, while the external gill opening is closed by the operculum, and the expiratory phase during which the pharynx contracts forcing the water across the gills and then to outside through the operculum which is opened now. The rate of opercular movements indicate the respiratory efficiency of the animals. The variations in the rate of opercular movements may be due to physiological and biological changes.

From the results obtained, it is observed that, there is an increase in opercular activity during 24h exposure. This could be towards a metabolic shift to derive more food and energy to meet the sublethal toxic stress (Ramakrishna, 1995) and this phenomenon was referred to as “Escape Reaction” as mentioned in earlier chapters. Anyhow, from 48h of exposure a decrease in the opercular activity was observed. As there is a correlation between the opercular activity and the rate of oxygen consumption, the same trend was observed in the earlier chapter i.e., the time course (whole animal) oxygen consumption. This correlation is because, when opercular movements are decreased, the rate of water flow on to the gill surface with in an unit time decreases and hence, the possibility for potential oxygen consumption with in an unit area and time is decreased. Similiar co-relation between the decreased opercular activity and reduced oxygen uptake in fish, Tilapia
*mossambica* subjected to malathion, methyl-parathion and phosphomidon was reported by Bashamohideen and Onnurappa (1986). The variation in the oxygen consumption and the rate of opercular movement was supported by a number of workers like Ramamurthy and Koundinya (1979) for Sevin and Sumithion, Sha Nawaz and Bashamohideen (1985) for malathion on other fishes. The decrease in oxygen uptake of gill resulted in histotoxic anoxic condition in which gill tissue not only suffered from 'oxygen debt' (Natarajan, 1983) but also loses the effective mechanism for removing Co$_2$ from the blood. The inhibition in oxygen consumption may be due to disintegration or rupture of respiratory epithelium and coagulation of mucus film over the gill surface (Jones, 1947). As a result, the absorption of oxygen by the gills from the external *milieu* is adversely affected. Therefore, in *Labeo rohita*, deltamethrin alters the opercular activity which in turn influences the oxygen uptake.
TABLE 6

Time course (whole animal) oxygen consumption (O₂ ml/g/h) of fish, *Labeo rohita*, exposed to sublethal concentration of deltamethrin (0.03 ppb) during different exposure periods. Each value is a mean of six measurements. The % change over control is given in parenthesis.

<table>
<thead>
<tr>
<th>Whole animal Oxygen consumption</th>
<th>Exposure periods (sublethal concentration of deltamethrin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Mean</td>
<td>0.290</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.020</td>
</tr>
<tr>
<td>% change</td>
<td>(31.034)</td>
</tr>
<tr>
<td>'t' test</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

S.D: Standard deviation. N.S: Not significant.
Opercular activity (10/t) of fish, *Labeo rohita*, exposed to sublethal concentration of deltamethrin (0.03 ppb) during different exposure periods. Each value is a mean of six measurements. The % change over control is given in parenthesis.

<table>
<thead>
<tr>
<th>Opercular activity</th>
<th>Exposure periods (sublethal concentration of deltamethrin)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>24h</td>
<td>48h</td>
<td>72h</td>
<td>96h</td>
</tr>
<tr>
<td>Mean</td>
<td>3.970</td>
<td>4.860</td>
<td>4.322</td>
<td>4.021</td>
<td>3.890</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.040</td>
<td>0.010</td>
<td>0.090</td>
<td>0.080</td>
<td>0.050</td>
</tr>
<tr>
<td>% change</td>
<td>(22.418)</td>
<td>(8.866)</td>
<td>(1.285)</td>
<td>(-2.015)</td>
<td></td>
</tr>
<tr>
<td>'t' test</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

S.D: Standard deviation.
Fig. 4. Percent change over control in time course (whole animal) oxygen consumption of fresh water fish, *Labeo rohita* at different exposure periods to sublethal concentration of deltamethrin.
Fig. 5. Percent change over control in opercular activity of freshwater fish, *Labeo rohita* at different exposure periods to sublethal concentration of deltamethrin.