CHAPTER II

RESPIRATION
INTRODUCTION

Whenever and wherever pollution takes place it affects the living organisms. In highly developed industrial areas air pollution is more common, under such condition if climatic conditions are not suitable, suffocation may takes place. Recently during the Iran-Iraq war, thousand tonnes of crude oil find their way in the Ocean, ultimately polluted the water and finally affect the aquatic food and feeding organisms.

Oxygen is a fundamental factor for the life processes of every living organisms, oxygen is necessary to provide energy for life. All living plants and animals depend upon the oxygen to continue their metabolism. Respiration is the energy producing physiological activity. Due to increase in the crop production, there is an increase in the use of several insecticides, pesticides, which due to careless handling, run-off from agriculture; get their way in open waters. The freshwater crab get affected by aquatic pollution and changes in physiological aspect takes place. The first indication of the physiological disturbance due to pollutant is reflected by the oxygen consumption, since any cumulative effect of pesticide in aquatic ecosystem alters the food web.

Pesticides are extensively used to eradicate disease vectors, inhabiting the aquatic ecosystem and also for the control of pest in agriculture. These pesticides cause
respiratory distress in non-target aquatic biota, which could
treat a physiological imbalance. (Diwan and Nagabhushanam, 1972;
Duke and Dumes, 1974; Nimmo et al., 1975; Bhagyakakshi, 1981;
consumption has been used to determine the effect of toxicant on
overall metabolism of exposed animals (Cairns and Scheir, 1964;
Weinwood and Johanson 1974). Vernberg et al.; (1974) have
observed the change in respiratory activity of fiddler crab, Uca
pugilator exposed to mercury, sublethal concentrations of
several insecticides have been shown to enhance respiration of
invertebrates (dela Cruz and Nagvi 1973 and Leffler, 1975) and
freshwater fishes (Weinwood and Johanson, 1974).

Respiration is the most important for understanding
physiological action of toxicant. Respiratory metabolism shows
reflections of extrinsic and intrinsic factors. Changes in oxygen
consumption have been measured as a response to toxicants.
(Chinnaya, 1971) estimated the effect of heavy metals on the
oxygen consumption on the shrimp Caridina rajadhari.

The availability of molecular oxygen to the cells by the
process of respiration can fulfill the cellular functions, the
energy requirement for survival of living organisms. The
constant need for oxygen is one of the most evident feature of
animal life because the energy for animals is provided by
aerobic oxidation of nutrients. Various workers have recorded
he changes in respiratory rates in the crustaceans after exposure to the different pesticides. Solski (1968) has investigated oxygen consumption of *Gammarus pulex*, under various concentrations of different herbicides. Gangshettiwar (1986) studied the alterations in respiration in the freshwater prawn, *Macrobrachium lamerii* when exposed to phenol. Jaiswal (1980) observed alteration in the rate of oxygen consumption of freshwater prawn, *M. kistnensis* after exposure to naphthalene.

The gills play a most important role in exchange between external and internal median, thus have a importance in respiratory process when the toxic contaminants are water born. The gills are the site of damage which can be easily analysed. Many workers have observed the gill damage due to toxicants (Fenzeng, 1971; Fowler, 1972; Olsen and Harrel, 1973 and Blanton and Robinson, 1973). Excessive mucous was produced on the gills when fishes were exposed to heavy metals (Varanasi et al., 1975) changes in the respirationatory activity have been used as the sensitive indicators of stress in animals exposed to pollutants (Schaumburg et al., 1967; Anderson, 1971; and Sharp et al., 1979). The relationship between the respiratory activity of animals and pollution has been studied in fishes (Belding, 1929; Ellis, 1937 and Jones, 1947).
Respiration is a vital phenomenon of life, changes in the environment both internal as well as external needs, oxygen in the form of energy. The activities of animals can be measured in terms of oxygen uptake. The relationship between the respiratory activity of animals and pollution has been studied considerably in aquatic animals (Robert; 1972, Davis, 1973 and Percy, 1977).

The metabolic rate of the poikilothermic animals is measured in terms of oxygen consumption, which is influenced by a number of factors, such as temperature, salinity, hydrogen ion concentration, oxygen tension etc. of the medium, chemicals and various insecticides.

The freshwater crab, *Barytelphusa guerini* is a major component of freshwater fauna. It is observed that little work has been done on respiration of freshwater animals, exposed to the pesticides. Respiration is an important parameter in determining the physical state, as metabolic rate is reflected by respiration. Hence the present investigation was, undertaken to study the effect of endosulfan (organochlorine) exposure on the respiratory physiology of freshwater crab, *Barytelphusa guerini*. 
MATERIAL AND METHODS

The crabs, *Barytelphusa querini*, used in the present investigation were collected from the Godavari river, at Paithan, near Aurangabad. They were maintained in the laboratory in plastic troughs containing two litre of tap water, fed with earthworm. Water was changed once a day. The crabs were acclimatized to the laboratory conditions for 24 hours before experimentation. Only healthy and active crabs were used for experimental purpose. The crabs were not fed one day prior to the commencement of the experiment. Crabs belonging to different size groups were used in the investigation.

Dissolve oxygen content of water sample was determined, before and after the period of experimentation following the Winkler's method. From the difference between the initial and final oxygen content in the sample, the total oxygen consumed by the crabs was calculated. The experiment of each case was reported thrice and the quantity of the oxygen consumed was calculated in relation to the unit wet weight of the crab and the values thus obtained are expressed as the rate of oxygen consumption in ml/gm wet wt. hour/litre (ml/g/h/l) at NTP. Experiments were carried out with the crabs of definite weight.
Effect of 4.857 ppm (LC$_{50}$/24 hrs.) concentration of endosulfan exposure on oxygen consumption of crab, *Parytelphusa querini*.

A batch of ten crabs were exposed to 4.857 ppm (LC$_{50}$/24 hrs.) concentration of endosulfan. The oxygen consumption was measured after 1, 4, 8, 12, and 24 hrs.

Effect of different concentrations of endosulfan for different time intervals on oxygen consumption of crab, *B. querini*.

In this experiment, three concentrations of endosulfan were used i.e. 3.568 ppm (LC$_{50}$/48 hrs), 2.599 ppm (LC$_{50}$/72 hrs.) and 2.183 ppm (LC$_{50}$/96 hrs.).

Three batches of crab, each comprising ten individuals were exposed to the above mentioned concentrations. The rate of oxygen consumption of crabs was measured after 48, 72 and 96 hours.

Effect of prolonged exposure to low concentration of endosulfan of oxygen consumption of crab, *B. querini*:

In this set of experiment crabs were exposed to sublethal concentration of 0.3569 ppm (1/10th of LC$_{50}$ of 48 hrs) of endosulfan to study the sublethal effects of endosulfan on respiration. The oxygen consumption studies were made after 10,
20 and 30 days respectively. Estimation of oxygen consumption was made by Winkler's method as modified by Strickle and Person (1968). The rate of oxygen consumption is expressed in terms of ml/gm/hr/lit.

In all the above experiments appropriate control groups were maintained simultaneously and control groups were treated in the same way as the experimental group except that they were not exposed to endosulfan.
RESULTS AND OBSERVATIONS

The freshwater *Barytelphusa guerini* shows variations in oxygen consumption when exposed in lethal and sublethal concentrations of endosulfan.

**Effect of 4.857 ppm concentration of endosulfan exposure on oxygen consumption of crab, B. guerini,**

In the present investigation, it was observed that in the endosulfan exposed crabs the rate of oxygen consumption increased upto 12 hours of exposure period ($P \leq 0.05$) when compared to the control group (Table 1 and Fig. 1). However, after 12 hrs. of endosulfan exposure the rate of oxygen consumption of crabs gradually decreased, when compared to control group.

**Effect of different concentration of endosulfan for different time intervals on oxygen consumption of B. guerini:**

The crabs exposed to 4.857 ppm, 3.568 ppm, 2.599 ppm, 2.183 ppm of endosulfan concentration of 24, 48, 72 and 96 hrs. respectively showed a sharp fall in oxygen uptake in all the concentrations used compared to control group. The rate of oxygen consumption for 24, 48, 72 and 96 hrs. decreased (26% to 80%). Maximum respiratory activity was exhibited at the highest concentration (4.857 ppm in 24 hrs, 0.037 ml of
Table 1

Effect of endosulfan on respiratory rate of *B. guerini* after 1, 4, 8, 12 and 24 hrs. of exposure. Values are expressed as mean (ml of oxygen/consumed/hr/gm Wt./lit.) ± S.D., n = 3.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Exposure periods</th>
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<tbody>
<tr>
<td></td>
<td>1 hrs</td>
</tr>
<tr>
<td>Control</td>
<td>0.048 ± 0.0100</td>
</tr>
<tr>
<td>Experimential</td>
<td>0.055 ± 0.002</td>
</tr>
<tr>
<td>NS</td>
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<td>-14.58%</td>
<td>12.69%</td>
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</table>
Fig. 1: Oxygen consumption of freshwater crab, B. guerini after exposure to 4.857 ppm concentration of endosulfan.

Fig. 2: Oxygen consumption of freshwater crab, B. guerini after exposure to different concentration of endosulfan for different time periods.
Fig. 3: Oxygen consumption of freshwater crab, *B. guerini* after exposure to low concentration (0.3569 ppm) of endosulfan for different time periods.
Table 2

Effect of lethal concentrations (4.857, 3.569, 2.599 and 2.483ppm) of endosulfan on respiration of fresh water crab, *B. querini* after 24, 48, 72 and 96 hrs. of exposure. Values are expressed in mean (ml. of oxygen consumed/hrs/gm. wt/lit) ± S.D., n = 3.

<table>
<thead>
<tr>
<th>Control</th>
<th>Exposure periods for acute</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>72 hrs</td>
<td>96 hrs</td>
<td></td>
</tr>
<tr>
<td>.048 ±</td>
<td>.037 ±</td>
<td>.025 ±</td>
<td>.0198 ±</td>
<td>.01 ±</td>
<td></td>
</tr>
<tr>
<td>.0100</td>
<td>.0015</td>
<td>.003</td>
<td>0.003</td>
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<tr>
<td>P/0.01</td>
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<td>P/0.001</td>
<td>P/0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26%</td>
<td>50%</td>
<td>60.4%</td>
<td>80%</td>
<td></td>
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</tr>
</tbody>
</table>
Table 3

Effect of sublethal concentrations, 356 ppm (1/10th of LC$_{50}$ value of 48 hrs.) of endosulfan on respiration of B. querini after 10, 20 and 30 days of exposure. Values are expressed as mean (ml. of oxygen consumed/hr/gm wt./lit.) ± S.D., n = 3.

<table>
<thead>
<tr>
<th>Periods of exposure</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td>.048 ±</td>
<td>.037 ±</td>
</tr>
<tr>
<td></td>
<td>.0100</td>
<td>.0015 NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.91%</td>
</tr>
<tr>
<td>20 days</td>
<td>.051 ±</td>
<td>.033 ±</td>
</tr>
<tr>
<td></td>
<td>.0017</td>
<td>.0015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35.29%</td>
</tr>
<tr>
<td>30 days</td>
<td>.049 ±</td>
<td>.03 ±</td>
</tr>
<tr>
<td></td>
<td>.0081</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.33%</td>
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</table>
oxygen /gm wet wt/hr/litre). The decrease in oxygen consumption was found to be significant statistically at $P \leq 0.01$ or $P \leq 0.001$. The continuous decrease in oxygen uptake may be due to failure of respiratory metabolism. (Fig. 2 Table 2).

**Effect of prolong exposure to low concentration of endosulfan of oxygen consumption of crab, *S. querini*:**

In the experimental groups, the rate of oxygen consumption of crabs decreased gradually after 10, 20, and 30 days of exposure period when compared to the control group. The decrease in the rate of oxygen consumption of crabs after 20 and 30 days of endosulfan exposure is statistically significant ($P \leq 0.01$, $P \leq 0.05$) but the decrease in the rate of oxygen consumption after 10 days of endosulfan exposure is statistically nonsignificant (Table 3, Fig. 3).

At sublethal exposure, the crabs showed a highly significant decrease in the rate of oxygen uptake. The observation denotes the failure of crabs to compensate with the stress of toxicant as the period of exposure increased continuously.
DISCUSSION

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of gills for their energy supply, damage of these vital organs causes a chain of destructive events which ultimately causes respiratory distress.

In fresh water crab, *B. querini* gill damage was observed after exposure to the pesticide, endosulfan (Organochlorine).

The pollutants act as physiological stressor as do the altered environmental parameters (Newell 1973). The pollutants affect the freshwater organism ultimately obstruct the respiratory mechanism to find out the effect of pesticide on respiration are difficult because such type of effects and changes vary from pesticide to pesticide, and from species to species and also one experimental condition to anothers.

Exposure to pesticides produces respiratory disturbances and reduces oxygen uptake in several animals like fishes (Hiltibran 1974; Rath and Mishri, 1979, Jauch 1979; Rao et al., 1980; Natarajan 1981; Rao et al., 1981), crab *B. cunicularis* (Nagabhushanam and Diwan, 1972) and *Paratelphusa jacouemontii* (Kulkarni and Kamat, 1980) and molluscs (Costa 1965; Scott and Major 11972 and Akarte, 1985).
Survey of related literature revealed that the rate of oxygen consumption of animals initially increased when the animals are exposed to different pollutants. Vernberg et al. (1978) have observed that the metabolic rate of both male and female crab, *Uca pugilator* was initially high when the crabs were exposed to polychlorinated biphenyls. The initial increase of oxygen consumption in a marine pulmonate, *Onchidium verruculatum* has been observed following exposure to NaPcP and DDT pesticide (Deshpande and Nagabhushanam and Hanumante et al., 1980). Similarly, Crandall and Goodnight (1962) have shown that the fish fry increase their oxygen consumption following exposure to NaPcP. Initial increase but later decrease in oxygen consumption was observed in *P. merguiensis* when exposed to malathion (Manikumar, 1986).

Some workers have reported increase in the oxygen consumption of animals following exposure to petroleum hydrocarbons. Khan et al., (1987) have observed an initial increase in oxygen consumption on exposure to petroleum hydrocarbon. Neff et al., (1976) have observed an initial elevation of respiration rate of shrimp *Mysidopsis almyra* on exposure to petroleum hydrocarbon. It has been shown that benzene, a soluble hydrocarbon stimulates the respiration of fish (Brocken and Bailey 1973 and Strushsakar et al. 1979).
In the present study, it was observed that the rate of oxygen consumption of crab, *B. quercinus*, is increased after exposure to lethal concentration of endosulfan. The rate of oxygen consumption increased up to 12 hrs. of experiment and then there is decline in oxygen consumption rate in lethal and sublethal level, compared to control. The increase in oxygen consumption was therefore exhibit a new steady state of metabolism to compensate physiologically with the stress of endosulfan pesticides.

Changes in the metabolic process after pollutant exposure have always been used as indicators of stress. The initial increase in the rate of oxygen consumption observed in the present study can be the first response of the animal to pollutant stress and might be an indicator of new steady state of metabolism to compensate the enhanced physiological activity.

Rice *et al.* (1979) have suggested that part of the increased oxygen is utilized by the exposed animals, to support the enhanced physiological activity in metabolizing and eliminating the pollutants such as possibility can hold good for *B. quercinus* also in the present study.

The decline of oxygen consumption rates after an initial increase and after subsequent prolonged exposure might be the result of the onset of poisoning. This decline in oxygen
consumption after an initial increase depending upon the concentration increased. This decrease may be because of failure of crabs to compensate for the new steady state of metabolism due to the stress of endosulfan (Organochlorine) as the exposure periods of endosulfan concentration increased.

This is in agreement with the findings of Laughin and Neff (1980) in the crabs, *Rhithropanopeus harrisii* exposed to some aromatic hydrocarbon and in the fish *Tilapia mossambica* after exposure to some commercial pesticides (Bash *et al*., 1984). Bodkhe (1983) has also reported a similar increase in freshwater crab, *B. cunicularis* exposed to sevinol. Manikumar (1986) reported decrease in oxygen consumption when *P. meriquensis* was exposed to lethal concentration of mercuric chloride, initially.

Reddy *et al.* (1986), reported that the decrease in oxygen consumption after initial elevation may be due to the increased flow of pesticide molecules into the body of prawn or accumulation of pesticide as a function of time in the prawn, *P. indicus* exposed to phosphomidon.

The decrease of oxygen consumption in the endosulfan exposed crabs may be due to the change in gill structure. It is well known that the gills play an important role in the gaseous exchange in aquatic environment. In the present study, the
damage of gill structure of crab, *B. guerini* was also observed after exposure to endosulfan concentrations (See chapter III). Many workers have shown harmful effects of pollutants on histological structure of gill of crustaceans and fishes. Ghate and Mulherkar (1979) have observed damage in the gill surface of two freshwater prawns *Macrobrachium* and *Caridina* exposed to copper sulplate. Vernberg et al. (1974); Eller (1971), Koundinya (1978) and Dixon and Leduc (1981) have studied the histopathology of gills of animals exposed to different pollutants. Pollutants of any nature are known to be effective at sublethal concentrations, where they accumulate in low concentrations in tissues, thereby disturbing the normal histological build up. Gills are the respiratory organs, damage to these vital organs, ultimately causes respiratory distress. In the present study the histology of gills were studied, show damages in gills following to exposure to pesticides. After sublethal exposure the initial inhibition in oxygen consumption may be due to the continuous exposure of crabs to pesticides. The increased in oxygen may be an indication of stress on the crabs.

The rate of oxygen consumption of the crab *B. guerini* decreased after an initial increase in oxygen consumption. The decrease in oxygen consumption in the crab may be due to the failure of crab to compensate for the new steady state of the
Metabolism due to the stress imposed by organochlorine pesticide. A similar reason may be attributed to the prawn, *Caridina weberi* after exposure to methyl parathion for its steady decrease in oxygen consumption by Martin (1988).

Rao (1984) has shown the exposure of *Scylla serrata* to endocel (organochlorine) produced a severe gill damage. It has been observed by Singh and Sahai, (1984) the degeneration of respiratory lamellae and inflammation in the fish *Rashora* after treatment with BHC (organochlorine). Anderson (1970) has reported that the metabolic rate of *Salmo salar* was inhibited when exposed to different concentration of DDT.

Reduction in oxygen consumption and disturbances in respiration may cause due to pesticide exposure and due to its concentrations. Many workers have observed that the rate of oxygen consumption of animals decreased, when subjected to different pollutants. Vernberg *et al.* (1979) have observed that the rate of oxygen consumption of fiddler crab, *Uca pugilator* decreased periodically up to 28 days when the crabs were exposed to low concentration of mercury. Anderson (1971) has reported that the metabolic rate of *Salmo salar* decreased when exposed to low concentration of DDT. The decreased oxygen consumption has been shown by Nagabhushanam and Diwan (1972) in crab, *B. cunicularis*, Chinnaya (1971) in *Caridina rajadhari*,
Bhagyalakshmi (1981) in Crab, *Oziotelphusa senex senex* and Natarajan (1980) in fish, *Channa striatus* after exposure to different pollutants at low concentration. In the present study it was observed that after prolonged exposure to endosulfan concentration, the rate of oxygen consumption of crab, *B. guerini* decreased gradually (may be due to the inhibition of oxidative phosphorylation).

The respiratory failure may occur due to effect of pollutants and also due to effect of pollutants on hormones leading to death of animal. O'Brien (1967) shows that in the animal exposed to organophosphate compounds death occurs due to asphyxiation which is caused due to the respiratory failure. Crandal and Goodnight (1963) suggested that prolonged exposure to fish to low level of heavy metal pollution subjects them to stress, which causes a hormonal imbalance ultimately leading to variety of internal pathological changes. In the present investigation there is possibility of similar changes in haematological status of crab, *B. guerini* due to endosulfan (organochlorine) exposure.

Some workers have shown the variation in oxygen consumption of animals exposed to endosulfan (organochlorine) pesticide. Subhadradevi (1985) reported a decrease in oxygen consumption in crab, *Oziotelphusa senex senex* exposed to lethal and sublethal concentrations of endosulfan resulting in greater oxygen demand.
On sublethal exposure the animal might have stepped up hemocyanin synthesis to meet the increased demand for oxygen. Vijayakumari et al. (1987) reported a drastic decrease in hemolymph copper and as such in hemocyanin concentration upon exposure to lethal concentration of endosulfan. Sambasivarao (1984) has shown the exposure of *Scylla serrata* to endosulfan (organochlorine) produced a severe gill damage. It has been observed by Singh and Sahai (1984) the degeneration of respiratory lamellae and inflammation in the fish *Rasbora* after treatment with BHC (organochlorine). Anderson (1970) has reported that the metabolic rate of *Salmo salar* was inhibited when exposed to different concentration of DDT. In the present study the decrease in oxygen consumption may be due to serving in all synthetic activities of the animal.

In conclusion, it may be said from present investigation that under the effect of uniform stress both an elevation and a suppression in the rate of oxygen consumption can be observed.