# Effect of Phenthoate 50% EC on Oxygen Consumption of Freshwater Fish *Catla catla*

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Abstract: Generally pesticide concentrations are toxic which may be lethal (or) sub lethal concentration in aquatic environment. Lethal concentration cause death of the organisms directly, but the sub lethal concentrations are too low to cause rapid death directly. Oxygen consumption of an animal is the important physiological parameters to assess the toxic stress because it is a valuable indicator of energy expenditure in particular and metabolism in general. Exposure to sub lethal concentrations is reported to increase respiratory activity, resulting in increased ventilation and hence increased uptake of the toxicant. Although, toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete under toxic stress. Hence it is useful to have an insight in to the histological analysis. The extent of severity of tissue damage is a consequence of the concentration of the toxicant and is time dependent. Also, the severity of damage depends on the toxic potentiality of a particular compound or pesticide accumulated in the tissue. The total oxygen Consumption is one of the indicators of the general well being of the fish. Hence the differential oxygen Consumption can be used as bio-indicator to evaluate the basic damage inflicted on the animal which could either increase (or) decrease the oxygen uptake changes in certain vital tissues like gill, liver and kidney of the Indian major Carp Catla catla exposed to sublethal concentrated (1/10 of 96 hrs LC 50).

Keywords: Respiratory activity, toxicity, lethal concentration, catla catla

#### 1. Introduction

Living organisms have the ability to adopt themselves to the changes in the environmental parameters such as temperature, humidity, oxygen supply or a pesticide stress (Hoar, 1976). The rate of oxygen consumption is considered as a reflection of the total metabolism and metabolic rate of the aquatic organisms. The water current flowing around the gills; carries the toxicants directly, before all other internal organs (Jagadeeson *et al.*, 1999). The fact that increasing use of contaminating chemicals in many industrialized nations of the world makes the development of ecotoxicity measurement techniques an absolute necessity (Brando *et al.*, 1992). The first step is the acute toxicity test on fish in order to show the potential risks of these chemicals (OECD, 1993).

Behavioral responses represent an integrated response of fish species to toxicant stress (Kane *et al.* 2005). Changes in spontaneous locomotors activity and respiratory responses are sensitive behavioral indicators of sub-lethal exposure in fish (Scherer, 1992). A guide covering some general information on methods for qualitative and quantitative assessment of the behavioral responses of fish (locomotors activity, feeding, and social responses) during standard laboratory toxicity tests to measure the sub-lethal effects of exposure to chemical substances and a guide covering information on methods to measure and interpret ventilatory behavioral responses of fish to pollutants are available (ASTM, 2008).

The early symptoms of acute pesticide poisoning are the alteration/failure of respiratory metabolism (Holden, 1973).

The changes in oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental stress factors (Rama Murthy, 1988) because, it reflects the energy expenditure and, ultimately, the food requirements. (Grinwis *et al.*, 1998), stated that the metabolic rate of a fish is usually measured by their rate of oxygen uptake from water ( $MO_2$ ); and  $MO_2$  is a criterion that has been suggested as an index of sub-lethal effect on fish and one that, if altered, may directly limit a fish's aerobic performance.

The changes in the respiratory activity of fish have been used by several investigators as indicators of response to pesticides (Chebbi and David, (2009a); Patil and David (2008); Veeraiah and Durga Prasad, (2001); Luther Das *et al.*,(2000). Changes in oxygen consumption have been measured as a response to toxicants (Jen and Tung, 2010). The oxygen consumption is a useful measure of sub-lethal effects because energy processes being disturbed are indicators of overall physiological state and of pesticide poisoning leading to respiratory distress (Rama Murthy, 1988) which is used to assess the toxic stress. The respiratory rate provides a critical index of environmental suitability for survival.

The oxygen consumption is not often used as a bioindicator of pollution associated stress in biological early warning systems. Respiratory responses were found to be less sensitive, but also could be successfully used in bioassay testing of treated industrial and municipal effluents, before they are discharged into receiving waters. Gill ventilation frequency and coughing rate are intimately associated with respiratory demands and gill irritation or blockage (Scherer *et al.* 1986).

Pesticides enter into the body of fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases (Anderson and Premdas, 1982). The severity of distress may lead to respiratory failure by affecting the respiratory centers of the brain or the tissue involved in breathing ('O' Brien, 1967). The pesticides and the resultant metabolites are reported to cause respiratory distress or even failure of activity by affecting

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the other related tissues, gill and brain (O'Brien, 1967). In the gill epithelium, the tissue will have intracellular and extracellular exchange of ions related to oxygen and chloride shift (Thosar and Lonkar, 1994). Any xenobiotic chemical having the capacity to alter this structure will affect the oxygen carrying capacity of hemoglobin and reduction in oxygen uptake. In the earlier reports, it was mentioned that the damage observed in fish gill exposed to toxicant was due to impairment in the respiratory metabolism (Rama Murthy, 1988 and Jayantha Rao, 1982). This is also due to the fact that any pollutant including pesticide should enter into the fish, mainly through the gills only (Vijayalakshmi and Tilak, 1996; Tilak and Yacob, 2002; Tilak and Marina Samuel, 2001; Tilak *et al.*, 2005).

The metallic pollution and pesticides of different classes like organochlorines, organophosphates, carbamates and other new generation ones affect the fish in oxygen consumption in sub lethal concentrations. Skidmore (1970) reported that zinc reduced the efficiency of oxygen transport across the gill membrane, so that the fish dies of hypoxia. This evaluation was done by using cannulation technique. Respiratory responses to lethal concentrations increase the ventilation volume and symptoms of organophosphate intoxication suggesting that the effect on respiratory surface were lethal in fish. The death has been variously ascribed to the respiratory failure (Handerson and Wolly, 1970).

Hence, in the present study an attempt has been made to study the effect of sub-lethal and lethal concentrations of Phenthoate 50% EC on oxygen consumption for 22 h at each 2 hours interval to the Indian major carp, *Catla catla* (Catla).

# 2. Material and Methods

The experiment on the oxygen consumption of the fish *Catla catla* was carried out in a respiratory apparatus developed by Job (1955). The fish was brought from a local fish farm and acclimatized to the laboratory conditions in well aerated water for 7 days. The water used for acclimatization and experimentation was the same as used in the toxicity experiments (Table I.). During this period, the fish were regularly fed, but the feeding was stopped for two days prior to the experiment. The fish measuring 6 to  $7 \pm \frac{1}{2}$  cm in length and 6 to  $8 \pm \frac{1}{2}$  gm in weight, all the precautions laid down on recommendations of the toxicity tests to aquatic organisms are followed (Rao and Mane, 1978). For finding 96 h LC<sub>50</sub>, static bioassay experiments was set by using the toxicant Phenthoate 50% EC insecticide.

#### 1) Description of respiratory chamber: The apparetus used for the measurement

The apparatus used for the measurement of whole animal oxygen consumption is a wide mouthed bottle which is called a respiratory chamber (RC). Its mouth was fitted with a four holed rubber stopper (S) and through one of the holes a thermometer (T) was passed to know the temperature of the medium in the respiratory chamber. From the remaining three holes three glass tubes were passed whose outer ends were fitted with rubber tubes. These three tubes served as delivery tubes and are designated as  $T_1$ ,  $T_2$  and  $T_3$ respectively. They were fitted with pinch locks  $P_1$ ,  $P_2$  and  $P_3$ .  $T_1$  was connected with the reservoir (R) and through this water could be drawn (inlet) into the respiratory chamber. T<sub>2</sub> was atmospheric tube; useful for testing the air tightness of the respiratory chamber. Through the T<sub>3</sub> tube (outlet) water samples from the respiratory chamber were collected for estimation of dissolved oxygen. The respiratory chamber was coated black to avoid photochemical reactions and to keep the animal activity at normal during the experiment.

# 2) Setting up of the Apparatus

Only one fish was introduced into each respiratory chamber and was filled with water drawn through  $T_1$  from the reservoir. After checking the air tightness pinch lock  $P_2$  was closed and pinch lock  $P_3$  was opened slightly so that a very gentle and even flow of water was maintained through the respiratory chamber. This was continued for 15 minutes to facilitate the animal in returning to a state of normality from the state of excitement, if any, due to the handling and also to allow the animal to adjust to the darkness in the chamber (acclimatization).

# 3) Collection of the initial and final samples

After allowing the animal to settle in the chamber, the initial sample was collected from the respiratory chamber through  $T_3$ . After the collection of initial sample, the respiratory chamber was closed by closing  $P_3$  first and then  $P_1$  after one hour. The next sample was collected from the respiratory chamber. Likewise, other samples were also collected at the end of each hour for a total of 22 hours period of the experiment.

Along with the experimental fish chamber, one respiratory chamber without fish (control) was maintained as control. The control serves to estimate initial amount of oxygen, at each experimental period i.e 2h.

The experiment was conducted with sub-lethal and lethal concentrations of Phenthoate 50% EC to fish *Catla catla*.

The amount of dissolved oxygen consumption was calculated per gram body weight per hour.  $O_2$  consumed by fish/gram body weight/hour =  $\alpha - \beta \times N$  of hypo x 8 x 1000 Vol. of the sample taken x Correction factor x Wt. of the fish x Time interval for each sample

 $\alpha$  = hypo rundown before exposure

 $\beta$  = hypo rundown after exposure

# 3. Results and Discussion

The comparative data on the whole animal oxygen consumption of control and experimental fish, calculated per gram body weight in sub-lethal and lethal concentrations of Phenthoate 50% EC commercial grade for *Catla catla* was given in the Tables.1. The results of the experiments and control values are graphically represented by taking time on X-axis and the amount of oxygen consumed per gram body weight on Y-axis, Fig.1.

Oxygen consumption measurements provide a robust indicator of whole animal stress and concomitant water quality. Respiratory rate is the basic parameter, and serves as

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one of the indicators of environmental stress. Effect of insecticides on the oxygen consumption of mollusk has been studied by Agarwal (1978) he was observed effects of Endrin on certain fresh water gastropods, Rao and Mane ( 1978) noted that the effects of Malathion on survival and respiration of Mytilus gallanoprovincialis. Moorthy et. al. (1984), reported changes in the respiration and ionic constituents in tissues of freshwater mussel exposed to Methyl parathion, Thosar and Lonkar (1994), studied the effect of Metasystox on the oxygen consumption of Vivipara bengalensis. Rohankar and Kulkarni (2005) reported alteration in oxygen consumption in freshwater snail Bellamya bengalensis during pesticide exposure. Lonkar (2012) reported changes in oxygen consumption rates of mollusk Indoplanorbis exustus exposed to sub-lethal concentrations of insecticide Tricel.

Disturbance in oxidative metabolism was reported earlier under cypermethrin toxicity in *Tilapia mossambica* (David *et al.*, 2003). Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (Magare and Patil, 2000). Secretion of mucus over the gill curtails the diffusion of oxygen, which may ultimately reduce the oxygen uptake by the animal (Grinwis *et al.*, 1998); or the membrane functions are disturbed by a changed permeability (Hartl *et al.*, 2001), oxygen uptake rate would rapidly decreased. Kalavathy *et al.* (2001) reported that the dimethoate is efficiently absorbed across the gill and diffuse into the blood stream resulting in toxic stress to fish.

In sub-lethal concentrations of Phenthoate 50% EC commercial grade; it was observed that fish Catla catla showed tendency of increase in oxygen consumption during the initial time of exposures i.e. 1 to 6 hours and a gradual decrease was observed during the subsequent period of study. The presence of sub-lethal concentration of toxicants is inevitable. In such a case, the fish Catla catla was more sensitive to toxicant. The toxicant stress in oxygen consumption along with depletion in oxygen in aquacultures practices make them less fit and reduction in growth due to lack of proper metabolism. The data as per Table.1, the fish was in more stress during first hour and later they are showing signs of recovery. That recovery is evident as the toxicant exposure is increased in time, during 22 h experiment.

Similarly when a comparison is made between the effects of sub-lethal concentrations of Phenthoate 50% EC on *Catla catla* decreased in oxygen consumption was observed. Under lethal concentration of Phenthoate 50% EC a significant increase is found in the initial stages of exposure i.e., 1-6 hrs in *Catla catla*. Hence, it has sensitivity on toxic stress as a result of more oxygen consumption. Shereena *et al* (2009) reported that fish exposed at 24 hours of treatment showed elevated levels of oxygen consumption. The values reported were  $0.544\pm0.04$ ,  $0.450\pm0.04$ ,  $0.393\pm0.02$  and  $0.279\pm0.06$  ml/g/hr in Dimethoate concentrations of

0.15ppm, 0.2 ppm, 0.3 ppm and 0.6 ppm respectively. The minimum consumption was observed at 0.15ppm whereas the maximum was at 0.6ppm. At 72 hours of exposure, the values showed a decline in the rate of oxygen consumption. Concentrations of 0.15ppm, 0.2 ppm, 0.3 ppm and 0.6 ppm depicted a reduction in the oxygen consumption of  $0.544\pm0.03$ ,  $0.474\pm0.03$ ,  $0.393\pm0.07$  and  $0.279\pm0.04$  ppm/hr respectively. The oxygen consumption was higher in 0.15 ppm than the other concentrations.

In controls also, the rate of oxygen consumption was gradually decreased and this can be attributed to the starved conditions and the reduced metabolic rates of the starved fish. In exposed fish, the reduction in oxygen uptake can be correlated to the extent of damage of gill epithelium. Throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased mucus secretion, higher ventilation volume, decrease in oxygen uptake efficiency, laboured breathing and engulfing of air through the mouth when exposed to Phenthoate 50% EC.

The increased oxygen consumption at initial stages of exposure i.e 6 h in the present study is in agreement with in therapar exposed to DDT, dimethoate and carbaryl in which an elevation in oxygen uptake is observed during initial stages of exposure i.e., 1-4 hours followed by decrease in subsequent hours. Rao *et al.*, (1980, 1981) observed that increasing concentrations of endosulfan decreased the oxygen consumption in *Macrognathus aculeatum*, whereas oxygen consumption was first stimulated and then inhibited in *Catla catla* 

Table. 1: The amount of oxygen consumed in mg/g body weight/hr of the fish *Catla catla* exposed to sub-lethal and lethal concentration of Phenthoate 50% EC Commercial grade.

 Table 1: The amount of oxygen consumed in mg/g body

 weight/hour of the fish Catla catla exposed to sub-lethal and

 lethal concentration of Phonthoate

lethal concentration of <b>Phenthoate</b>				
Control	Sublethal	%	Lethal	%
		change		change
$0.836 \pm 0.003$	$0.828{\pm}0.004$	0.956	$0.831 {\pm} 0.003$	0.598
$0.818 \pm 0.003$	$0.871 {\pm}\ 0.005$	-6.479	$0.806{\pm}0.004$	1.466
$0.792 \pm 0.004$	$0.934{\pm}0.003$	-17.92	$0.782 \pm 0.004$	1.262
$0.776 \pm 0.003$	$0.766 \pm 0.003$	1.288	$0.756 \pm 0.003$	2.577
$0.745 \pm 0.003$	$0.697 {\pm} 0.004$	6.442	$0.665 \pm 0.003$	10.73
$0.705 \pm 0.003$	$0.668 {\pm} 0.004$	5.248	$0.636 \pm 0.003$	9.787
$0.701 \pm 0.003$	$0.656 \pm 0.004$	6.419	$0.596 \pm 0.003$	14.97
$0.687 \pm 0.003$	$0.636 \pm 0.003$	7.423	$0.548 \pm 0.004$	20.23
$0.658 \pm 0.004$	$0.606 \pm 0.007$	7.902	$0.512 \pm 0.006$	22.18
$0.546 \pm 0.007$	$0.526 \pm 0.003$	3.663	$0.50{\pm}0.003$	3.144
$0.506 \pm 0.003$	$0.486 \pm 0.002$	3.952	$0.466 \pm 0.003$	7.905
$0.496 \pm 0.004$	$0.468 {\pm} 0.004$	5.645	$0.446 \pm 0.004$	10.08
$0.447 \pm 0.003$	$0.427 \pm 0.003$	4.474	$0.396 \pm 0.004$	11.40
	$\begin{array}{c} \text{Control} \\ \hline 0.836\pm 0.003 \\ 0.818\pm 0.003 \\ 0.792\pm 0.004 \\ 0.776\pm 0.003 \\ 0.745\pm 0.003 \\ 0.705\pm 0.003 \\ 0.701\pm 0.003 \\ 0.687\pm 0.003 \\ 0.688\pm 0.004 \\ 0.546\pm 0.007 \\ 0.506\pm 0.003 \\ 0.496\pm 0.004 \end{array}$	Control         Sublethal           0.836±0.003         0.828± 0.004           0.818±0.003         0.871± 0.005	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$

Values are the mean of five observations: *Standard Deviation is indicated as*  $(\pm)$ , Value are significant at p < 0.05.

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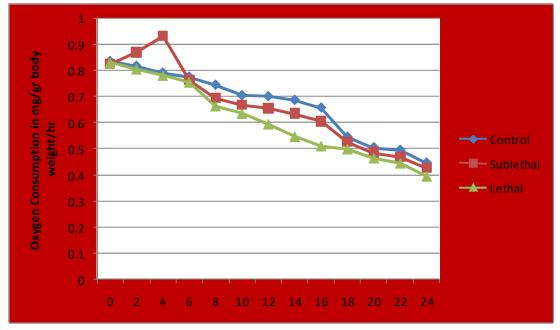


Figure 1: The amount of oxygen consumed in mg/g body weight/hr to the fish *Catla catla* exposed to sublethal and lethal concentration of Phenthoate 50% EC commercial grade

Male guppies exposed to 0.5 or 1 mg/l sodium pentachlorophorate consumed more oxygen than the controls (Crandall and Goodnight, 1962). Reduction in oxygen consumption was reported in Channa striatus exposed to organophosphate pesticide by Natarajan (1981), Carbamate treatment in Macropodus Carpanus Arunachalam and Palanichamy (1982), and to carbaryl exposure in C. punctatus by Anita Susan (1994), Vijayalakshmi (1994), Ramana Kumari (1999), Veeraiah (2001). The pesticides induced changes in oxygen consumption of the whole animal and also of its tissues were studied earlier by following investigators. Rath et al., (1980) observed that dichlorvos reduced the oxygen consumption of the excised tissues of gill, brain, muscle of Tilapia mossambica. In a study of Hiltibran (1974) on the effect of herbicides with substituted acetic, propionic and methyl groups on mitochondrial respiration, the number and position of the chloro groups substituted on the phenoxy moiety influenced the oxygen uptake. This effect may be further increased or decreased by different phenoxy compounds containing different acid analogs.

Effects of pyrethroid insecticides also revealed the decrease in oxygen consumption it might also be due to disruption of  $O_2$  binding capacity of respiratory pigment as evidenced by the decrease in RBC and Hb content in carp under cypermethrin toxicity (Bradbury *et al.*, 1987a). Decreased  $O_2$  uptake efficiency was also observed in another species, *Salmo gairdneri*, exposed to pyrethroid insecticides (Kumaraguru and Beamish, 1983; Bradbury *et al.*, 1987a). The change in the level of respiration and ions in the tissues of freshwater fish *Catla catla* under fenvalerate stress was studied by Reddy *et al.*, (1977) which supports the present findings. All the above investigations indicate that  $O_2$ consumption is a sensitive indicator of stress in fishes exposed to pollutant is suppressed considerably.

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their

energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress (Magare and Patil, 2000). Pronounced secretion of mucus layer over the gill lamellae has been observed during phenthoate stress. Secretion of mucus over the gill curtails the diffusion of oxygen (David *et al.*, 2002), which may ultimately reduce the oxygen uptake by the animal. In exposed fish, the reduction in oxygen uptake can be correlated to the extent of damage of gill epithelium (Tilak *et al.*, 2001a, 2005a). On the other hand, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. Kalavathy *et al.*, (2001) reported that the dimethoate is efficiently absorbed across the gill and diffused into the blood stream resulting toxic stress to fish.

The increased oxygen consumption in the present study is also in agreement with the Reddy et al., (1977) in Channa striatus exposed to cypermethrin in which an elevation in oxygen uptake is observed during first two hours of exposure followed by decrease in subsequent hours. Similar trend was reported in Channa punctatus (Luther Das et al., 2000; Veeraiah and Durga Prasad, 1998) in Catla catla (Veeraiah, 2001) exposed to permethrin. Elevation of basal metabolic rate of rainbow trout exposed to permethrin was reported by Kumaraguru and Beamish (1983). Similarly earlier works revealed a significant reduction in oxygen consumption by many fish species under the toxic stress of aquatic pollutants such as insecticides Anita Susan (1994), Vijayalakshmi (1994), Ramana Kumari (1999), Veeraiah and Durga prasad (2001). Patil and David (2008), Tilak and Japamali (2009) and Marigoudar et al., (2009).

The effect of different carbamate insecticides on oxygen consumption has been studied by many researchers. According to Kumaraguru *et al.*, (1982) when methyl parathion and fensulfothion were exposed to freshwater fish *Mystus cavasius*, both pesticides at higher concentrations, affected the oxygen uptake of *M. cavasius*, with a

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progressive reduction of the oxygen consumption with increasing concentration of the pesticide. The highest concentration of methyl parathion that caused irreversible damage during the period of the respiratory observed significant change in total oxygen consumption of the freshwater fish Anabas testudineus exposed to disyston and furadan. Significant increase was noted after 3 hr of exposure in both the pesticides, their effects varied from one exposure period to another. In sublethal concentrations of ekalux oxygen consumption rate of the fish Lepidocephalichthys thermalis decreased by more than 40% with increasing concentration of pesticides was reported by Palanichamy et al., (1986). When Spangled perch, Leiopotherapon unicolor, were exposed to concentration of temephos, an organophosphorus insecticide observed an immediate reduction in ventilation rate and oxygen consumption, and also reduced heart rate during the second hour of exposure and concluded that effects of exposure to tempos correspond to cholinesterase inhibition in nerves supplying the respiratory musculature and the heart. Effect of multiple sublethal concentrations of Phosalone on whole animal and kidney oxygen consumption in freshwater fish Oreochromis mossambicus, caused a significant change indicating the presence of hypoxic condition in the biosystem during the toxicity of phosalone was reported by Malla Reddy et al., (1988).

Vijayalakshmi and Tilak (1996), reported that monocrotophos and Fenvalerate severely effected the epithelial cells of the gill in fish Catla catla. Tilak et al., (2001) also reported that gill architecture was severely affected in the fish Labeo rohita exposed to chlorpyrifos. Kumaraguru et al., (1982) reported that the gill is the target organ for synthetic pyrethroid toxicity in fish. The respiratory metabolism was impaired and damage was also in the gills of fish exposed to the pesticides (Hughes and Perry, 1976; Rama Murthy, 1988; Anita Susan, 1994; Vijaya Lakshmi, 1994; Veeraiah, 2001; Ramana Kumari, 2001). Devi Swetharanyam (2000) observed an initial increase in oxygen consumption in sublethal and median lethal concentrations followed by a decline in subsequent hours and lethal dosed fish. Bharathi et al., (2001), observed that the decreased rate of oxygen consumption with the increase in the test concentrations of mercuric chloride also consequently decreased metabolic rate.

Studies on organochlorine pesticides by Devi Swetharanyam (2000) observed enhancement in the oxygen consumption rate initially in the fishes of sub-lethal and median lethal exposures to endosulfan might be due to a sudden response of the fish to the impending toxicity of endosulfan However the declining respiratory rates recorded in the subsequent periods in sub-lethal and median lethal exposures suggest that the fish could not succeed in their attempts of boosting oxidative metabolism. According to the effects of treatment with sub-lethal concentrations of organochlorine pesticides such as lindane, lorsban, chlordane and DDT to shrimp larvae Penaeus vannamei increased the larval respiratory rate. The analysis of data from the present investigation indicates a considerable effect of Phenthoate 50% EC on oxygen consumption in selected fish in lethal as well as sublethal concentrations. The present study revealed alterations in the oxygen consumption of Catla catla exposed to sub lethal and lethal concentrations of carbamate insecticide. Variation in the oxygen consumption in Phenthoate 50% EC exposed fish was probably due to impaired oxidative metabolism and pesticide induced stress.

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