

The oxygen Consumption rate of an air breathing Catfish, *Heteropneustes fossilis* (Bloch.) in fresh an lethal & Sub lethal concentration of methyl parathion

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ABSTRACT

The opercula movement initially increased at 24 hr. of exposure in lethal & sub lethal concentrations, possibly to compensate the increased physiological activities & gulping air at the surface may be a protective behavior or due to more demand of higher oxygen level during exposure periods & gradually, the opercula movement decreased in both at 24 & 96 hrs of exposures may be due to pesticide induced physiological abnormalities. The oxygen consumption rate in both lethal & sub lethal concentration initially increased followed by decline depended on concentrations & exposure periods. The declines were statistically significant at 168hrs. in 6.70 ppm (50.47%) at 1440 hr in 4.5 ppm (58.41%) and 2160 hrs in 2.25 & 0.75 ppm (57.00 & 64.95% respectively). When normal value were considered as 100% O₂ uptake rate of the control fish. The initial increase observed, may be due to stress caused by pesticide on fish, making it active to combat stress & decline in later stages may be due to lowering down of energy requirements which can be considered as adaptive & even strategic.

Keywords: Metabolic, Concentration, Metabolism, Accumulation, Physiological

Introduction :

Fry (1971) has stated that oxygen consumption rate in fish can be considered as an index for denoting the intensity of metabolism. Changes in respiratory behavior & metabolic rate of pollutant induced fishes have drawn the attention of several biologist (Choudhary et al 1993; Patil & David, 2008; Anifasusan et al, 2010; Neelima et al, 2016), but their results are much conflicting as some have reported increased (Anderson et al 1947; Natarajan & Rajuli, 1983) while others observed a decline (Roy & Munishi 1988; Patil & David, 2008) whereas, Singh et al (2010) have reported either increase or decrease in their respiratory rate to variety of pesticides. Kumari et al, 2011 & Neelima et al (2016) have stated that oxygen consumption of the fish reflects the basal metabolic status & is one of the indicators of the general health and/ or well being of the fish and further stated that pesticides are indicated to cause respiratory distress or even failure by affecting respiratory centres of the brain or the tissues involved in breathing. Considering the above mentioned facts, the present study

has been conducted to evaluate the effect of lethal & sublethal concentrations of methyl parathion on the oxygen consumption rate of a freshwater airbreathing cat fish, *Heteropneustes fossilis* (Bloch) along with control fish.

Materials and Methods:

Healthy & living specimens of *Heteropneustes fossilis* (Bloch), commonly known as "Singhi" of 38.92 ± 3.14 gm. Wt. group & 18-21 cm in length were procured from local fish pond which had no connection with any type of effluents. In the laboratory, fish were treated for 15 minutes in 1% Aq. Soln. of potassium permanganate to remove any dermal infection, followed by proper wash (at least twice) in tube well water (ground water), thereafter, fishes were transferred to large tubs with sufficient ground water. The fish were provided tub fix/ chopped goat's liver at least three hours prior to change of water at every twenty four hours. The fish were acclimatized at least for ten days prior to start the experiment. The organophosphate pesticide, methyl parathion commercial grade manufactured by plant

remedies Pvt. Ltd, B-51/54 phase-industrial area, Hazipur has been used which constituted technical grade Methyl parathion 2.5%ww(based on 80% ww). Beconite clay 20% & shoap stone, 77.5%. The desired concentration of methyl parathion was prepared by dissolving known amount in minimum amount of ethanol & they mixed well with known quantity of distilled water as stock solution of methyl parathion. The Lc_{50} value of methyl parathion for *H. fossilis* has already been explored earlier which has been recorded to be 7.46 ppm. The ventilation rate or opercula frequencies of at least five fish of each concentrations as well as control at 24 & 96 hrs were counted using a magnifying glass by visual observation for 5 minutes and average per fish per minute were noted. To determine the oxygen uptake rate of *H. fossilis* in lethal & sub lethal concentrations, 0.9, 0.6, 0.3 & 0.1 of 96 hr Lc_{50} value (i.e 7.46 ppm) were selected which were 6.70, 4.50, 2.25 & 0.75 ppm and O_2 uptake rate at 8, 24, 48, 96, 168, 360, 720, 1080, 1440, 1800 & 2160 hrs were measured individually by continuous flow glass respirometer. The Winkler's lode metric method (APHA, 1990) was followed to measure the dissolved oxygen content in the water. The routine oxygen consumption rate of the fish during different condition was measured by estimating the difference between O_2 content of inlet water & out-let water at a specific time. At least five fish for each concentration at selected time intervals were used and the average were noted.

Observation:

The frequent surface breaking (3-4 times/ minutes) and faster opercula movements were recorded in all the fish exposed to different concentrations of methyl parathion at the beginning of the experiment to few hours depending on concentrations of the pesticide and thereafter, a decline was observed in different concentrations depended on concentration & exposure periods. The fish exposed to 24 & 96 hours of exposure to different concentrations have been summarized in Table-1. This showed in increase upto 7.9%, 9.61 & 3.35% in 4.05, 2 & 6.8 ppm concentrations which gradually declined from 8.8 ppm (-5.80%) with maximum decline at 19.3 ppm (-40.38%). The declines were significant ($P < 0.05$) 14.8 PPM (-28.85%) & $P < 0.01$ in 19.3 ppm (40.38%) when compared with that of normal values 100 % at 24 hr of exposure. All fish died in 25.1

ppm. Concentration, whereas at 96 hr. of exposure, the gradual decrease has been observed in all concentration which were recorded statistically significant ($P < 0.050$) in 5.2 ppm (-30%) and $P < 0.01$ at 6.8 & 8.8 ppm (-42.00% & -52.00% respectively) all fish died in 11.4 ppm onwards.

To determine the oxygen consumption rate, five fish for each selected conditions i.e 6.70, 4.50, 2.25 & 0.75 ppm alongwith control without considering their sex. Thus 0.9th, 0.6th, 0.3rd, & 0.1st of 96 hr Lc_{50} (i.e. 7.46 ppm) were used for this experiment as lethal & sublethal concentrations. Their average weight was also noted. The oxygen consumption rate in a continuous flow glass respirometer was recorded at 8, 24, 48, 96, 168, 360, 1080, 1440, 1800 & 2160 hrs of exposure were noted & summarized in Table-2 and taking normal value of O_2 -uptake rate as 100% the percent utilization of toxicant induced fish has been presented in table-3 in normal conditions (i.e. control fish), the oxygen uptake rate of average 383 gm fish at 26^oc was recorded to be 2.140.19 ml O_2 /I/hr. accordingly, the value of O_2 -uptake rate of 1 gm & 1 kg fish were calculated to be 0.055 ml O_2 /I/hr respectively. In lethal concentration (6.70 ppm) the oxygen uptake rate initially increased up to 12.15 & 0.47% at 8 & 24 hrs of exposures respectively, followed by a decline which as statistically significant ($P < 0.05$) at 96 & 168 hrs. (34.81 & 49.53%) respectively. In sub lethal concentration i.e 4.50 & 2.25 ppm, the O_2 -uptake rate initially slightly increase followed by a decrease from 24 hr onwards. Which were recorded statistically significant ($P < 0.05$) at 1440 hr in 4.50 ppm and at 1800 hrs with maximum decline at 2160 hrs. of exposure (42.99%) in 2.25 ppm concentration. Whereas, the decline was noted ($P < 0.05$) at 2160 hr of exposure in 0.75 ppm (35.04%) concentration when compared with that of the overall average normal value of control fish (i.e 14 ml O_2 /I/hr oxygen consumption.) in other words, taking the overall average value of O_2 -uptake rate of the fish in control condition as 100 % the fish exposed to lethal concentration (6.70 ppm) showed 112.15, 100.47, 65.42 & 50.47% at 8, 24, 48, 96 & 168 hrs. of exposures respectively. Whereas in lower concentrations the O_2 -uptake rate after an intial increase upto 24 hrs gradually declined with maximum at 4.50 ppm (58.41%) at 2160 hrs in 2.25 & 0.75 ppm (57.00% & 64.95%) respectively.

Table: 1
Ventilation rate per minute in H. fossils exposed to selected to selected concentration of methyl parathion at 24 & 96 hr of exposure with control

		24 hrs of Exposure Value	Ventilation Rate per minute		
			96 hr. Exposure % Change	Value	% Change
		Wt. of Fish	38±3gm wt. groups		
Control	Average	52±3	100%	50±4	100%
4.0	0.60	56±4	+7.69	42±3	-16.00
5.2	0.72	57±3	+9.61	35±3	-30.00
6.8	0.83	54±3	+3.35	29±2	-42.00
8.8	0.94	49±4	-5.80	24±2	-52.00
11.4	1.06	42±3	-19.23	-	-
14.8	1.17	37±3*	-28.85	-	-
19.3	1.28	31±3**	-40.38	-	-
25.1	1.40	-	-	-	-

“*”=p<0.052 “**”=p<0.01

Table -2 :-
Oxygen Consumption rate of H. Fossilis exposed to lethal & sublethal concentration of methyl parathion all selected hours of exposures.

Exposure Hour (Hr)	Concentration PPM	Av. Weight fish gm	Oxygen			
			mlO ₂ /hr	mlO ₂ /gm/hr	mlO ₂ /kg/hr	% change
1	2	3	4	5	6	7
8hr	Control	40.20	2.12±0.19	0.052±0.004	52.74±4.72	-
(log 0.90)	6.70	39.76	2.40±0.22	0.060±0.005	50.36±5.50	+12.15
	4.50	37.54	2.16±0.18	0.057±0.005	57.34±4.79	+0.93
	2.25	35.48	2.17±0.23	0.061±0.006	61.16±6.48	+1.40
	0.75	41.00	2.24±0.20	0.055±0.005	54.63±4.89	+4.67
24hr	Control	39.80	2.16±0.21	0.054±0.005	54.27±5.024	-
	6.70	38.38	2.15±0.18	0.056±0.005	56.01±4.69	+0.47
Log1.38	4.50	38.96	2.12±0.21	0.054±0.005	54.41±5.39	-0.93
	2.25	36.70	2.09±0.24	0.057±0.006	56.95±6.54	-2.34
	0.75	41.10	2.18±0.19	0.053±0.004	53.04±4.62	+1.87
48hr	Control	38.25	2.15±0.16	0.056±0.004	56.21±4.18	-
(Log 1.68)	6.70	40.18	1.60±0.20	0.040±0.005	39.82±4.98	-25.33
	4.50	38.76	2.08±0.23	0.053±0.006	53.56±5.93	-280
	2.25	37.90	2.06±0.20	0.054±0.005	54.35±5.28	-3.74
	0.75	39.00	2.14±0.17	0.055±0.004	54.87±4.36	0.00
96hr	Control	37.64	2.08±0.15	0.055±0.004	55.26±3.98	-
(Log 198)	6.70	39.50	1.40±0.19	0.035±0.005	35.00±4.81	-34.81
	4.50	37.84	1.89±0.17	0.050±0.005	49.95±4.49	-1168
	2.25	38.66	2.02±0.20	0.052±0.004	52.25±5.17	-5.61
	0.75	40.28	2.10±0.18	0.052±0.004	52.13±4.47	-187

168hr	Control	40.40	2.16±0.20	0.053±0.005	53.47±4.83	-
(Log 2.22)	6.70	38.72	1.08±0.15	0.028±0.004	27.89±3.87	-4953
	4.50	39.64	1.61±0.21	0.041±0.005	40.61±5.30	-24.77
	2.25	39.12	1.76±0.20	0.045±0.005	44.99±5.11	-17.76
	0.75	41.00	2.00±0.18	0.047±0.004	48.78±4.39	-6.54
360 hr	Control	39.98	2.20±0.22	0.056±0.005	56.42±5.50	-
(log 2.56)	6.70	-	-	-	-	-
	4.50	38.00	1.56±0.20	0.041±0.005	41.05±5.25	-27.10
	2.25	37.64	1.70±0.15	0.045±0.004	45.16±3.95	-20.56
	0.75	36.68	1.94±0.19	0.053±0.005	52.89±5.18	-9.34
720 hr	Control	38.20	2.16±0.20	0.056±0.005	56.54±5.23	-
log 2.86	6.70	-	-	-	-	-
	4.50	39.64	1.47±0.19	0.037±0.003	37.08±3.53	-31-31
	2.25	37.78	1.66±0.17	0.044±0.005	43.94±4.50	-22.43
	0.75	38.30	1.88±0.16	0.048±0.004	48.30±4.18	-13.55
1080 hr	Control	39.00	2.18±0.17	0.055±0.004	55.89±4.25	-
Log 3.03	6.70	-	-	-	-	-
	4.50	40.00	1.38±0.17	0.034±0.004	34.50±4.25	-35.51
	2.25	38.74	1.60±0.14	0.041±0.004	41.30±3.61	-25.23
	0.75	37.58	1.75±0.16	0.046±0.004	46.57±4.26	-18.22
1440 hr	Control	38.06	2.12±0.22	0.053±0.005	53.05±5.50	-
(Log 3.16)	6.70	-	-	-	-	-
	4.50	-	-	-	-	-
	2.25	40.12	1.29±0.15	0.032±0.004	32.15±3.74	-39.72
	0.75	40.24	1.56±0.19	0.039±0.005	38.77±4.72	-27.10
1800hr	Control	37.98	2.10±0.17	0.055±0.004	55.29±4.48	-
(log 3.25)	6.70	-	-	-	-	-
	4.50	-	-	-	-	-
	2.25	40.12	1.29±0.15	0.032±0.004	32.15±3.74	-39.72
	0.72	38.96	1.48±0.17	0.038±0.004	37.99±4.36	-30.84
2160 hr)	Control	40.00	2.14±0.19	0.053±0.005	53.50±4.75	-
(Log 3.33)	6.70	-	-	-	-	-
	4.50	-	-	-	-	-
	2.25	37.55	1.22±0.16	0.032±0.004	32.49±4.26	-42.99
	0.75	37.70	1.39±0.18	0.037±0.005	36.88±4.77	-35.04

Overall average value of Control fish O₂ uptake rate

= 2.14±0.19 ml O₂/hr

= 0.055 ml O₂/gm/1/hr

“*”=PV0.05

= 55.58 ml O₂/kg/hr

Table -3
Percentage of Oxygen consumption rate of *H. fossilis* at selected hr. of exposures in different concentration of methyl parathions taking normal O₂ –uptake rate of control fish as 100 %

Concentration (PPM)	8 hr	24 hr	48 hr	96 hr	168 hr	360 hr	720 hr	1080 hr	1440 hr	1800 hr	2160 hr
1	2	3	4	5	6	7	8	9	10	11	12
Control	Overall average O ₂ uptake rate=2.14 ml/hr=100%										
	100	100	100	100	100	100	100	100	100	100	100
6.70	112.15	100.47	74.77	65.42	50.47						
4.50	100.93	99.07	97.20	88.32	75.23	72.90	68.69	64.48	58.41	-	-
2.25	101.40	97.66	96.26	94.39	82.24	79.44	75.57	74.77	67.29	60.28	57.00
0.75	104.67	101.87	100.00	97.66	93.46	90.65	86.45	81.77	72.90	69.16	64.65

Discussion:

It has been observed that the opercula movement raised at first in almost all pesticide induces fishes followed by a decrease progressively in lethal exposure compared to sublethal exposures. The initial increase in opercular movements observed may possibly compensate the increased physiological activities under stressful condition as suggested by Shivakumar & David, 2004. Gulping of air by fish at surface may be protective behavior which helps to keep the fish away from contact of toxic medium or may be due to more demand of higher oxygen level during exposure period. The amount of oxygen uptake by animal reflects its metabolic rate & consequently the energy yield. It is well known that pesticides can cause respiratory distress or even failure by affecting respiratory enters distress or even failure by affecting respiratory centers of the brain or tissue involved in breathing (O' Brien, 1967). It is also known that oxygen is necessary for many metabolic processes that are very important to aerobic life. Lee et al (1972) have reported a progressive but steady decline in O₂ uptake rate in *channa punctatus* exposed to organophosphate pesticide, whereas, Ray & Munshi (1988), Patil & David, (2008) & Neelima et al (2016) have a decrease in O₂ uptake

rate in different pesticides including organophosphate in different fishes and have stated that oxygen consumption of the fish reflects the basal metabolic status & is one of the indicator of the general health or well being of the fish. In the present study, the fish *H. Fossilis* exposed to both lethal & sublethal concentration of methyl parathion showed an initial increase followed by a gradual decline in O₂ uptake rate depended on concentration & exposure periods when compared with that of the normal value.

Conclusion:

The increase/decrease of oxygen uptake rate might be due to the capability of the fish to take atmospheric air directly or due to the formation of excess mucus on the gill surface and/or accumulation of pesticide in the gills or injury to it, might be the reasons for the rapid decline in O₂ uptake rate after a considerable period. However, changes in O₂ uptake rate consumption rate by the individual fish in same concentration are not of the same magnitude and vary according to the metabolic activity of fish. Further, the initial increase may also be due to over activities of the fish or due to some internal factors as suggested by (Crandall & Good night, 1963)

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