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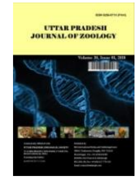

MODULATION IN RESPIRATORY DYNAMICS OF THE ESTUARINE CLAM, *Katelysia opima* (Gmelin) UNDER SUBLETHAL AND LETHAL STRESS OF CADMIUM CHLORIDE

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AUTHOR'S CONTRIBUTION

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The present study was conducted to find out effect of lethal and sublethal concentrations of $CdCl_2$, 2½ H_2O for 96 hrs. Static Bioassay Tests were conducted to find out LC_0 and LC_{50} values for summer, monsoon and winter of 2018/19. The data was analyzed by Finney's probit chart analysis method to calculate LC_{50} and 95% lower and upper fiducial limits. These predetermined values for cadmium chloride concentrations were used for exposure to LC_0 and LC_{50} group of clams. Experiments were conducted during summer, monsoon and winter by maintaining control group of clams. Estuarine water parameters like temperature, pH, salinity, rainfall and oxygen saturation were recorded. In the present study, it was found that, cadmium chloride has significant influence on rate of oxygen consumption. During summer, clams from LC_0 and LC_{50} group were treated with 0.8 and 1.3 ppm cadmium chloride, while in monsoon these values were 1.1 and 1.6 ppm respectively. During winter, clams from LC_0 and LC_{50} group were exposed to 1.0 and 1.8 ppm cadmium chloride respectively. These differences in subletal and lethal concentrations were attributed to seasonal estuarine water parameters. Clams from control group consumed more oxygen in winter and less in monsoon. During summer, clams from LC_0 group showed 3.01, 2.14, 4.98 and 7.64% significant increase at the end of 12, 24, 36 and 48 hrs respectively. Clams showed significant decrease up to the end of 96 hrs. Clams from LC_{50} group showed 3.61 and 6.0% increase at the end of 12 and 24 hrs, After 36 h, clams exhibited significant decrease in oxygen uptake. Moreover similar pattern of oxygen consumption was observed in winter. During monsoon clams from LC_0 group showed significant increase at the end of 12, 24, 36 and 48 h and further decrease up to end of 96 hrs. Clams from LC_{50} group showed 0.98% non-significant increase at the end of 24 hrs, while significant decrease at the end of 12, 36, 48, 60, 72 and 96 hrs. In the present study it was found that changes in oxygen consumption in clams from control group were attributed to highly labile estuarine water parameters and breeding season of clams, while, alterations in oxygen consumption of LC_0 and LC_{50} group are moreover attributed to cadmium chloride stress.

Keywords: *K. opima*; cadmium chloride; LC_0; LC_{50}; oxygen consumption; estuarine water parameters.

1. INTRODUCTION

Bhatye estuary provides valuable resources like commercially important crustaceans, fishes and molluscs. Amongst molluscan fishery, clam fishery is prominent and 15.14% population along the bank of Bhatye estuary is engaged in clam fishery [1]. During lean period of open sea fishery, it provides protein

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rich food and livelihood to local population. Thus, clam fishery in Bhatye estuary plays a pivotal role in rural livelihood and constitute a socio- economic entity From past 20 years this legacy has been declining. Rapid increase in population and industrialization along the bank of Bhatye estuary has started threatening the quality of estuarine water and life of estuarine biota. One of the recent trends is construction of tourist resorts along the bank of estuary and backwaters. Lot of waste, sewage, untreated domestic and industrial effluents is continuously dumping in estuarine water. This waste is impregnated with biological and chemical load [2]. Most of bivalves are comparatively stationary in nature or slow movers. They can`t move rapidly in clean environment [3]. Most of these contaminants accumulate in sediment which is feeding and breeding ground of clams. Many researchers worked on bioaccumulation capacity of heavy metals and its impact on physiological processes in marine bivalves [4,5,6,7,8]. *K. opima* has great capacity of cadmium accumulation and it showed variance of cadmium accumulation in soft tissues [2]. Due to their bioaccumulation capacity, these metals are affecting physiological processes in clams and Clams also returning back to the consumers. Many researchers have stressed the need of indicator organisms which could be employed to monitor environmental contamination by heavy metals [9,10,11,12] and they believed that, bivalves are perhaps the best indicator over a wide variety of environmental conditions. Clams are good bioindicators of heavy metal pollution. They indicate environmental and pollutant stress by their altered behavioural pattern. Kumbhar, Sanjay [2] found that clams tried to avoid unfavorable condition of estuarine water by increasing shell valve closure period and also showed same behavioral pattern during the heavy metal stress. Clams closed their shell valves for considerable period and secreted lot of mucus. Such behavioral patterns are related with oxygen consumption, and hence it was decided to employ this tool to find out intensity of cadmium pollution in estuarine environment. Measurement of oxygen consumption is a very important tool to assess the toxicity stress on aquatic organisms, since it is also an index of energy expenditure to fulfill the demands of environmental and biological alterations [13]. Utilization of oxygen is, therefore, a direct measurement of degree of activity, food conversion and heat production [14]. The process of respiration is sensitive to whole array of environmental and biological alterations. Considering commercial importance, bioaccumulation capacity of heavy metals and its impact on physiological processes, suitability as a bioindicator and respiratory array of clams, it was decided to study cadmium stress by modulating respiratory dynamics of *K. opima.*

2. MATERIALS AND METHODS

Estuarine water samples were collected by dipping plastic jar below 30 cm. It is collected in clean and deionised cans. These water samples were filtered with 0.45 cm filter paper. Filtered water samples were used for detection of dissolved oxygen, temperature, pH and salinity. Rainfall data was obtained from Government Meteorology Department. Concentration of cadmium in estuarine water was analyzed by Atomic Adsorption Spectrophotometer (Perkin - Elmer Model 3030, USA). Clams of median size (4.0 to 4.8 and 3.8 to 4.8 cm in length) were collected and brought to the laboratory. Clams were acclimatized for 96hrs. Acclimatized clams were used to conduct bioassay tests and further experiments. The toxicity tests were repeated three times and LC_0 and LC_{50} values were determined for each season. Clams were exposed to predetermined concentrations of cadmium chloride in different season. Oxygen consumption experiments were performed in specially designed glass jars of one liter fitted with rubber tubes. The marked clams were kept for one hour. Dissolved Oxygen was determined by Winkler's method .The rate of oxygen consumption of LC_0 and LC_{50} groups along with control after every 12 hrs time interval was determined. The experiments were repeated for three times during summer, monsoon and winter season.

3. RESULTS

Estuarine water was analyzed for detection of temperature, salinity, pH and oxygen saturation. During summer, monsoon and winter, average temperature was 26.9, 26.1 and 25.5°C respectively. At Ratnagiri, the rainfall was maximum in the month of July (34.39) but later on it decreased. There was scanty rainfall (0.006) in winter and no rainfall in summer. Maximum average salinity was recorded in summer (35.2). It was 30.36 mg/l in winter. Minimum average salinity was recorded in monsoon season (5.2). The average pH was 8.1, 7.29 and 8.5 in summer, monsoon and winter respectively. The average dissolved oxygen was 3.5, 5.0 and 4.3 in summer, monsoon and winter respectively. During monsoon estuarine water showed maximum saturation of oxygen (5.0). It was minimum (3.5) in summer. The present study it was observed that, during summer, cadmium concentration in water was below detectable level. In monsoon and winter average concentration of cadmium was 0.005 and 0.001 µg/l respectively.

Table 1. Estuarine water parameters of Bhatye Estuary, Ratnagiri (2018 – 19)

Experimental period	Water temperature (°C)	Rainfall (mm)	Salinity (mg/l)	pH	Dissolved oxygen (ml/l)	Cadmium (µg/l)
Summer (April-May)	Avgas- 26.9	-	35.2	8.1	3.5	B.D.L.
Monsoon (July- August)	26.1	34.39	5.2	7.29	5.0	0.005
Winter (Nov- Dec)	25.5	5.2	30.36	8.5	4.3	0.001

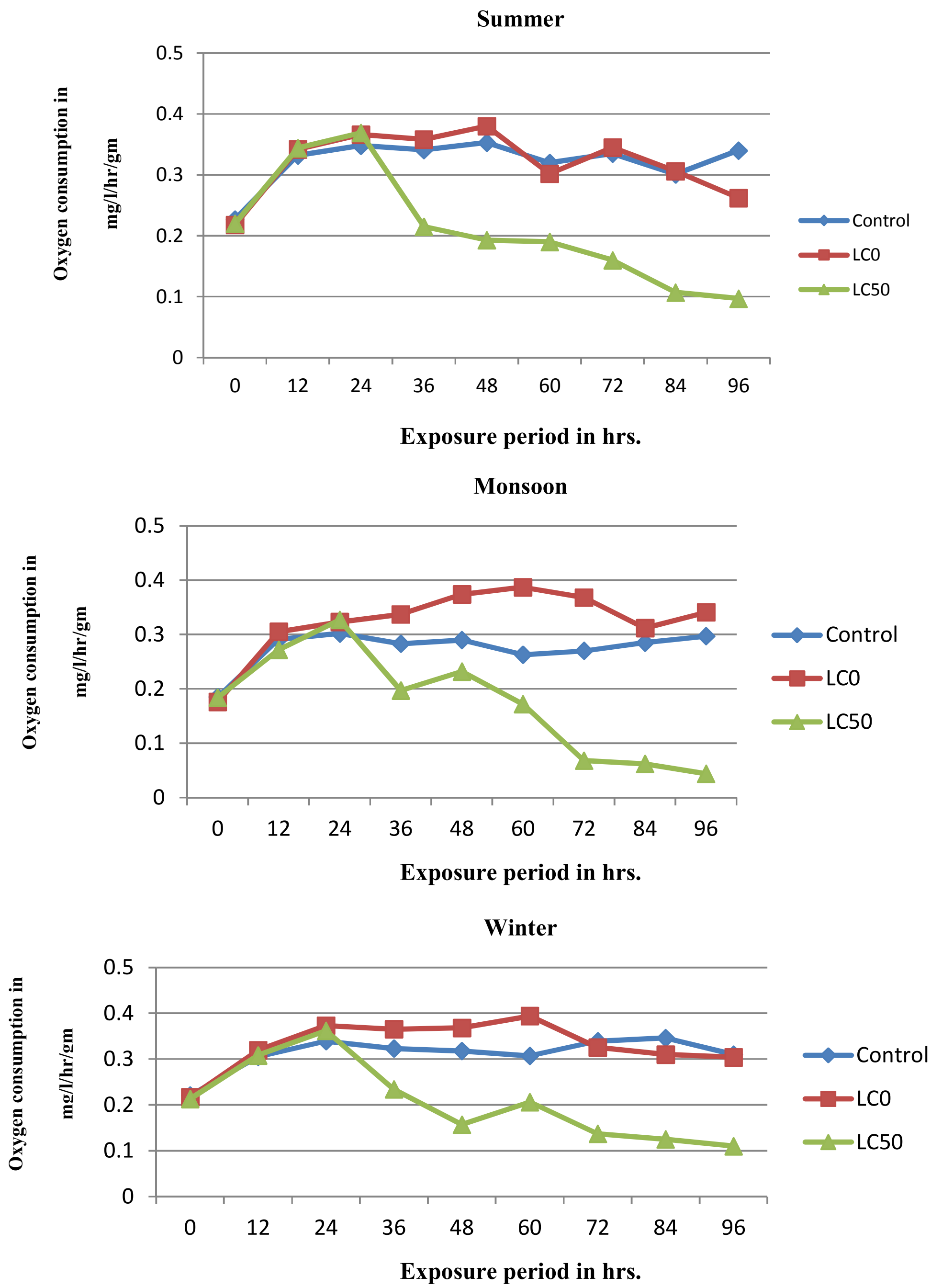

Fig. 1. Rate of oxygen consumption (ml/l/hr/gm wet wt) in *K. opima* exposed to different concentrations of cadmium chloride after acute exposure

Table 2. Rate of oxygen consumption (ml/l/hr/gm wet wt) in *K. opima* exposed to different concentrations of cadmium chloride after acute exposure

		Exposure time								
		0 Hrs	**12 Hrs**	**24 Hrs**	**36 Hrs**	**48 Hrs**	**60 Hrs**	**72 Hrs**	**84 Hrs**	**96 Hrs**
Summer	Control	0.227 ±0.028	0.332 ±0.045	0.348 ±0.081	0.341 ±0.002	o.353 ±0.056	0.320 ±0.008	0.335 ±0.040	0.301 ±0.063	0.340 ±0.021
	LC_0	0.218 ±0.010	0.342 ±0.022 (3.01)	0.366 ±0.095 (2.14)	0.358 ±0.063 (17.04)	0.380 ±0.008 (7.64)	0.302 ±0.015 (-5.62)	0.345 ±0.080 (-2.98)	0.306 ±0.009 (-1.66)	0.262 ±0.013 (-22.94) **
	LC_{50}	0.220 ±0.093	0.344 ±0.046 (3.61)	0.369 ±0.051 (6.03)	0.215 ±0.043 (-36.95)	0.193 ±0.005 (-45.32) *	0.190 ±0.017 (-31.19)	0.160 ±0.032 (-109.37) **	0.107 ±1.031 (-64.45)	0.097 ±0.015 (-71.47) ***
Monsoon	Control	0.185 ±0.005	0.291 ±0.041	0.302 ±0.037	0.283 ±0.069	0.290 ±0.028	0.263 ±0.032	0.270 ±0.011	0.285 ±0.094	0.297 ±0.006
	LC_0	0.176 ±0.061	0.305 ±0.093 (4.81)	0.323 ±0.029 (6.95)	0.337 ±0.051 (19.08)	0.374 ±0.017 (28.96) *	0.387 ±0.021 (47.14) **	0.368 ±0.029 (36.29) **	0.312 ±0.042 (9.47)	0.341 ±0.007 (12.90)
	LC_{50}	0.184 ±0.062	0.272 ±0.06	0.307 ±0.028	0.197 ±0.025	0.232 ±0.018	0.172 ±0.016	0.068 ±0.024	0.062 ±0.030	0.044 ±0.014
Winter	Control	0.221 ±0.015	0.305 ±0.049	0.339 ±0.021	0.323 ±0.079	0.318 ±0.013	0.307 ±0.005	0.339 ±0.065	0.346 ±0.025	0.310 ±0.036
	LC_0	0.217 ±0.093	0.319 ±0.072 (4.59)	0.373 ± 0.026 (10.02)	0.365 ±0.038 (13.00)	0.368 ±0.030 (15.72)	0.394 ±0.018 (22.08) **	0.325 ±0.020 (-4.12)	0.310 ±0.010 (-11.61)	0.304 ±0.005 (-1.93)
	LC_{50}	0.213 ±0.026	0.308 ±0.055 (0.98)	0.362 ±0.031 (6.78)	0.234 ±0.063 (-6.98)	0.157 ±0.025 (-50.62) ***	0.206 ±0.090 (-32.89)	0.137 ±0.069 (-59.58) *	0.125 ±0.034 (-63.87) ***	0.110 ±0.015 (-64.51) **

Values in parenthesis are percent change, ± = S.D. of five animals
(= p < 0.05, * * = p < 0.01, *** = P < 0.001)*

In summer, clams from control group shower fluctuations in oxygen consumption between 0.227 to 0.353 ml/l/g/h from 0 to 96h. In LC_0 (0.8 ppm) group, rate of oxygen consumption fluctuated between 0.218 and 0.380 ml/l/g/h at the end of 48 h. At 60 h, clams consumed 0.302 ml/l/g/h, again at 72 h, it was increased to 0.345 ml/l/g/h. From 72 to 96 h, there was considerable decrease in rate of oxygen consumption from 0.345 to 0.262 ml/l/g/h. if this is compared with control, there was considerable decrease of 5.62, 2.98, 1.66, 22.94% ($p<0.01$) at the end of 60, 72, 84 and 96 h respectively. In LC_{50} (1.3 ppm) group, rate of oxygen consumption fluctuated between 0.097 and 0.369 ml/l/g/h from 0 to 96 h. There was considerable increase in uptake from 0.220 to 0.369 ml/l/g/h at the end of 24 h. From 36 h, there was considerable significant decrease from 0.215 to 0.097 ml/l/g/h at the end of 96 h. In LC_{50} group, as compared to control group, there was 36.95, 45.32($P<0.05$), 31.19, 109.37 ($p<0.01$), 64.45, and 71.47% ($p<0.001$) decrease at the end of 36, 48, 60, 72, 84 and 96 h respectively. On the other hand, there was non significant decrease of 3.61 and 6.03% at the end of 12 and 24 h respectively (Fig. 1) during monsoon, clams from control group showed fluctuations in rate of oxygen consumption between

0.185 and 0.302 from 0 to 96 h. In LC_0 (1.1 ppm) group, clams showed fluctuations in rate of oxygen consumption between 0.176 and 0.387 ml/l/g/h. There was considerable decrease from 0.368 to 0.312 ml/l/g/h at the end of 84 h. Again rate of oxygen consumption was increased (0.341 ± 0.007 ml/l/g/h) at the end of 96 h. As compared to control, there was 4.81, 6.95, 19.08, 28.96 ($P<0.05$), 47.14 ($p<0.01$), 36.29 ($p<0.01$), 9.47 and 12.90% increase in oxygen uptake at the end of 12, 24, 36, 48, 60, 72, 84 and 96 h respectively. In LC_{50} (1.6 ppm) group, rate of oxygen consumption fluctuated between 0.044 and 0.327 ml/l/g/h. There was considerable increase from 0.184 to 0.327 ml/l/g/h at the end of 24 h. From 24 h, there was considerable decrease with minor fluctuations (Fig. 1). In winter, control group of clams showed fluctuations in the rate of oxygen consumption between 0.221 and 0.346 ml/l/g/h. In LC0 (1.0 ppm) group, clams exhibited fluctuations in the rate of oxygen consumption between 0.217 and 0.394 ml/l/g/h from 0 to 96 h. There was considerable increase from 0.217 to 0.394 ml/l/g/h from 0 to 60 h. From 60 h, there was considerable decrease up to 0.304 ml/l/g/h at the end of 96 h. As compared to control, there was 4.59, 10.02, 13.00, 15.72 and 22.08% ($p<0.01$) increase in oxygen uptake at the end of 12, 24, 36, 48, and 60 h respectively. There was a slight decrease of 4.12, 11.61 and 1.93% at the end of 72, 84 and 96 h respectively. In LC_{50} (1.8 ppm) group, rate of oxygen consumption fluctuated between 0.110 and 0.362 ml/l/g/h. There was considerable increase from 0.213 to 0.362 ml/l/g/h at the end of 24 h. It decreased from 0.362 to 0.157 ml/l/g/h at the end of 48 h, while at 60 h, it again increased to 0.206 ml/l/g/h. From 60 h onwards, it decreased to 0.110 ml/l/ is g/h at the end of 96 h. As compared to control group, there was considerable decrease of 27.55, 50.62 ($p<0.001$), 32.89, 59.58 ($P<0.05$), 63.87 ($p<0.001$), and 64.51% ($P<0.05$) at the end of 36, 48, 60, 72, 84, and 96 h respectively. On the other hand, there was nonsignificant increase of 0.98 and 6.37% at the end of 12 and 24 h respectively (Fig. 1).

4. DISCUSSION

The present study highlighted several areas where information on impact of cadmium toxicity on oxygen consumption in estuarine clams is scanty. The largest data gap is related to interrelations in estuarine parameters, oxygen utilization and cadmium chloride stress. To unveil these interrelations, respiratory dynamics was studied seasonally. During summer and winter, temperature was higher as compared to monsoon. During monsoon there was high influx of Kajali River. Pillai, et al. [15], observed that, influx of freshwater through rivers and intrusion of sea water through the lower reaches have profound influence o the distribution of temperature in backwater systems. It was observed that, temperature, oxygen saturation, salinity are interrelated parameters. Temperature was directly proportional to salinity and inversely proportional to dissolved oxygen, it affected oxygen concentration in water. It had propound effect on toxicity of cadmium chloride. This is in good agreement with observations of Mc Leod, [16]. The bioaccumulation and toxicity of metals increases with increase in temperature. The absorption and release of metals can also depend on temperature. This was established for mercury, methyl mercury and phenyl acetate in rainbow trout, *Salmo guardeneri*. Salinity is key trigger of other environmental characteristics. It depends on certain factors as, local precipitation, water influxes, mixing of seawater with fresh water and evaporation. In the present investigation, seasonal fluctuations were quiet well marked. Low salinity was observed during monsoon, it was due to heavy rainfall in the Basin of Kajali River, which caused dilution. Similar observations were recorded by many workers [17,18,19]. The oxygen saturation varied seasonally, in general, during monsoon, saturation was better than those of summer and winter. It can be attributed to turbulence, freshwater influx and low temperature. Similar observations were noted by Imley, A. J. [20]; Keller, A. E. [21] and Lee. H [22]. Variations in pH did not show significant seasonal pattern of fluctuations. However, the pH values were low during monsoon due to heavy influx of freshwater. Similar observations were noted by Chandran and Ramamurti [23] studying estuarine water parameters of Vellar estuary. The hydronomics of an estuary, in general, is very complex because of the interdependable variables like tide, river discharge, and density difference in the water masses in the estuary, salinity and temperature. The estuarine water parameters have important role in metabolism of clams and it directly or indirectly affect the opening and closing period of shell valves, filtration rate, feeding, growth and several physiological activities. The process of respiration is sensitive to whole array of environmental as well as biological variables. Oxygen consumption is useful measure to assess the sub lethal effects of xenobiotics, as energy processes serves as an indicators of overall physiological state [24].

At Ratnagiri, heavy rainfall was recorded in monsoon (34.39 mm/day).Rainy freshets brought heavy load of soil that impregnated with heavy metals like cadmium. It resulted in increase in concentration of cadmium in monsoon. It also showed the rapid loss of cadmium during summer and winter. In the present investigation concentration of cadmium in estuarine water was considered while quantifying sub lethal and lethal concentrations of cadmium chloride. Analysis of concentration of cadmium was in good agreement with findings of many workers, Fowler and Oregioni [25] suggested that seasonal maximum concentration appeared in the spring was due to high water run-off which increased the amount of available metals. Philips [26] proposed similar conclusion with zinc, cadmium, lead and copper. S. Ray [27] reported that, in open sea water cadmium occurs in very low concentrations, averaging about 40ng/l. Eaton and Boys et al. [28] observed 40 - 60 mg/l cadmium concentration in North Atlantic ocean. S. Ray [27] observed that cadmium concentration in coastal environment is normally higher due to weathering and anthropogenic activities. Estuarine water parameters are highly labile and are bound to influence the oxygen consumption correlated with estuarine environment. In the present study, it was found that clams from control group showed fluctuations in oxygen consumption, but fluctuations were more during monsoon. The salinity and temperature data (Table 1) shows that, during monsoon, when river enters the estuary, salinity decreases due to freshwater influx. It has been observed that, the rate of water filtration and metabolic activity decreases during monsoon because for the majority of period, values of clams remained closed in low salinity .In winter, oxygen consumption was less than in summer. It may be due to presence of optimum range of water parameters and physiological status of clams at that time. During summer, clams exhibited higher degree of oxygen consumption. It may be due to depleted oxygen content in water and cadmium induced stress. Isono, Raisuke et al. [29] report that, in Japanese little neck clam, *Ruditapes philippinarium,* the rate of oxygen consumption decreased markedly above 40°C. Clams suffer from thermal stress over 25°C and have significant mortality at around 34°C within a few days and no heat resistance over 40°C. Hare et al. [30] observed enhanced oxygen consumption rate in flat oyster *Ostrea edulis* during summer [31] observed increased rate of oxygen consumption during summer in the Antartic clam, *Laternula ellipitica,* Ranade, [32] studied hydronomics of Kalbadevi estuary, Ratnagiri and observed similar fluctuations in oxygen consumptions of estuarine clams. In the present study, clams from control group showed significant decrease in oxygen uptake in monsoon and also closed their shell valves for most of the time to avoid unfavourable estuarine water parameters. Similar observations were noted by Ranade [31]. As compared to control group of clams, LC_{50} group showed decreased oxygen consumption in three different seasons. This decrease in oxygen consumption is attributed to variance in the volume of water ventilated through the gills, caused by the intermittent closure and opening of shell valves. Here, the main factor which caused decrease in oxygen uptake was coagulation of mucus on gills due to cadmium stress. Coagulation of mucus causes reduction in effective transport of oxygen to internal tissues. Coagulation film anoxia adversely affects the absorption of oxygen from the ambient medium. Brown and Nevel [33] suggested that, reduced oxygen uptake maybe due to reduced energy requirement caused by suppression of ciliary activity. In the present study; considerable mucus secretion was found in 33 the clams from LC_0 and LC_{50} group. These fluctuations in oxygen uptake in *K. opima* are due to cadmium chloride stress and unfavourable estuarine water parameters.

5. CONCLUSION

The present investigation demonstrated that cadmium chloride is highly toxic to estuarine clam, *K.opima* and it had immense impact on oxygen consumption. It reveals interaction between seasonal estuarine water parameters and oxygen consumption. Experimental results showed that, clams from control group consume more oxygen in summer and less in monsoon. It is attributed to seasonal estuarine water parameters like temperature, pH, oxygen, saturation and salinity. Clams from LC_0 and LC_{50} group showed marked initial increase and later significant decrease at the end of 96 h. This trend was similar in all three different seasons. It is clear that these fluctuations were due to $CdCl_2$ stress and not due to seasonal estuarine water parameters.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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