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CHANGES IN THE O:N RATIO OF LAMELLIDENS MARGINALIS AFTER EXPOSURE OF ACUTE TOXICITY TESTS OF CADMIUM IN DIFFERENT TIME HOURS IN DIFFERENT SEASONS

SHAIKH YASMEEN
Department of Zoology, Dr. Rafiq Zakaria College for Women, Dr. Rafiq Zakaria Campus II, Navkhanda, Jubilee Park, Aurangabad (M.S.) India
*Corresponding Author: Email-

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Abstract- In the present study the oxygen: nitrogen ratio (O:N ratio) was carried out in *Lamellidens marginalis* of three respective groups i.e. control, Lc_0 and Lc_{50} in different seasons viz., summer, monsoon and winter. The oxygen consumption and ammonia excretion was higher in winter than monsoon and summer after 96 hrs. O:N ratio was calculated by using atomic equivalents. In *Lamellidens marginalis* O:N ratio was higher in Lc_{50} groups (17.57) followed by control (13.63) and Lc_0 (10.00) groups respectively in summer season. In monsoon the O:N ratio was maximum in Lc_{50} group (31.00) followed by Lc_0 (24.67) and control (15.56) groups respectively. In winter the O:N ratio was maximum in Lc_{50} (33.65) than Lc_0 (25.37) and control (19.63) groups respectively.
Keywords: *Lamellidens marginalis*, Oxygen Consumption, Ammonia Excretion, O:N ratio.

Introduction

Seasonal changes in oxygen uptake and ammonia excretion in the gastropod *Concholepas concholepas* was reported by Navarrao and Tarjjos (1994). The energy utilized in oxygen uptake and ammonia excretion was significantly different depending on the season and temperature (Navarrao and Torjjos, 1994). Review of literature reveals information on O:N ratio of marine molluscs from India. The review of literature reveals that Hawkins et al (1986) reported on O:N ratio of *Perna viridis* and *Perna indica* from Cochin backwater where impact of entrophication are generally most pronounced, and recently Mathew and Menon (1993) reported heavy metals stress induced variation in O:N ratio in *Perna indica* and *Donax incanalus*. O:N ratios are useful for assessing the relative contribution of protein to toal catabolism. In bivalve several workers have studied nitrogenous execratory products and the report show that although ammonia is the dominant product, large amount of amino nitrogen are lost, and there is a small but significant amount of urea also excreted by some species (Bayne 1976 b). Further rate of nitrogen excretion by bivalve molluscs are extremely variable, which is surprising in view of the marked seasonal changes in nutrient storage and utilization of reserves (Nagabhushanam and Mane, 1991). When environmental factors are changed due to natural or anthropogenic activities, the physiological responses of an organism also changes. Comparative studies indicate positive relationship between water temperature and resting metabolic rate (Luxmoore, 1984; Clarke, 1991 and Peck and Conway, 2000). Bhagade (2005), studied the oxygen consumption and ammonia excretion from different sized groups of green mussel, *Perna viridis*. Many workers has estimated O:N ratios and filtration rates with fluctuation in temperature (Morton, 1971 and Reeders and De Vaate, 1990). Bayne and Widows (1978) has reported O:N ratio between 20 to 24^{0}C and measured the biochemical changes.

Material and Methods

The rate of respiration and ammonia excretion from each group viz., control, Lc_0 and Lc_{50} were analyzed and O:N ratio was determined on the basis of atomic equavalents. The bivalves were collected randomly from Kutluq lake, Daulatabad near Aurangabad (Maharashtra State) and brought to the laboratory in all three seasons. They were cleaned off and measured the length and weight of each individual.

The rate of respiration and animals in different seasons was determined in specially prepared glass jars of one liter capacity and jar were air-tightened with rubber corks and has inlet and outlet connections of rubber tubes. The bivalves individually placed in the respiratory jar were provided with a continuous flow of reservoir water till they fully opened and extended the visceral organs. Once they opened their values, the flow of water was stopped and the tubes were tightly clipped without leaving any air-bubbles in the jar. The sample of water from it was drawn for determination of initial oxygen content and ammonia. After 1 hour, water from experimental jars were carefully siphoned out in stoppard bottles and

oxygen was determined. For the determination of oxygen content five animals from control, Lc_0 and Lc_{50} groups were used of individual animal.

The rate of respiration from each group was determined on 24, 48, 72 and 96 hrs, accordingly to Winkler's azid modification method, and the results were expressed in *O_2 / mg / g / wet weight / lit / h*. At the same time 50ml of water sample from the chamber was processed for analysis of ammonia by phenol phenate method (Widdows, 1985) O:N ratio by atomic equivalents was statistically calculated from the replicate of two determination of each animal. The rate of oxygen consumed to nitrogen excreted is calculated in atomic equivalents:

1. Multiply the rate of oxygen consumption in *ml-O_2h^{-1}* by 1.428 convert into *mgO_2h^{-1}* and then divide by the equivalent weight (16).
2. Divide the rate of nitrogen excretion in *mgNH4.Nh^{-1}* by atomic weight (14).

$$O:N = \frac{mlO_2 \times 1.428}{16} : \frac{mgNH_4Nh^{-1}}{14}$$

Results

O:N ratio:

Seasonal variations in the rate of oxygen consumption and ammonia excretion (O:N ratio) by *Lamellidens marginalis* revealed differences in ratios in different seasons. (Table and fig 1 to 3).

Summer:

In control group O:N ratios revealed high value of O:N ratio at 48 hr. (50.05) followed by at 24, 72 and 96 hr (45.40, 21.80 and 13.63 respectively). In Lc_0 group high value of O:N ratio was at 48 hr (76.38) followed by 24, 72 and 96 hr (70.50, 13.89 and 10.00 respectively). In Lc_{50} value showed highest 24 (21.55) followed by 96, 48 and 72 hr (17.57, 17.29 and 13.85 respectively). In Lc_0 group when compared to control group showed high value of O:N ratio at 48 hr (76.38) and 24 hr (70.50) and low value at 72 hr and 96 hr (13.89 and 10.00). O:N ratio revealed high value of O:N ratio in Lc_{50} group at 96 hr (17.57) and low value at 24, 48 and 72 hr (21.55, 17.29 and 13.85 respectively) when it was compared to control group. Where as the ratio showed high value at 96 hr (17.57) and low value at 24, 48 and 72 hr (21.55, 17.29 and 13.85 respectively) with the oxygen consumption and ammonia excretion observed in the above respective group when compared with the Lc_0 group.

Monsoon:

In control group showed highest value the ration was at 72 hr (197.30) followed by 74.26, 22.68 and 15.56 at 48, 24 and 96 hrs respectively. In Lc_0 group there was high value at 72 hr (169.89) followed by 106, 25.83 and 24.67 at 48, 24 and 96 hrs. in Lc_{50} the ratio was high at 48 hr (87.84) followed by 31.00, 72 and 25.00 at 96 hr, 72, and 24 hr. In Lc_0 group compared to control group highest value was at 48 hr (106.7) followed by 25.83 at 24 and 24.67 at 96 hr and lowest value showed at 72 hr (169.89). In Lc_{50} group showed O:N ratio highest from 48 hr (87.84) followed by 96 hr (31.00) and 24 hr (25.00) and lowest value showed at 72 hr (27.12). When compared to Lc_0 group Lc_{50} group showed highest value from 96 (31.00) and lowest value from 48, 72 and 24 hr (87.84, 27.12 and 25.00 respectively with oxygen consumption and ammonia excretion observed in the above groups).

Winter

In control group highest value showed at 72 hr (105.98) followed by 48, 96 and 24 hr (74.50, 19.63 and 23.85 respectively). In Lc_0 group highest value from 48 hr (123.0) followed by 72, 96 and 24 hr (11.50, 96 and 24.30) respectively. In Lc_{50} group showed highest value at 48 hr (86.44) followed by 96, 72 and 24 hr (33.65, 29.95 and 25.75 respectively). In Lc_0 group with compared to control group the highest value from 45 hr (123.0) followed by 42, 96 and 24 hr (11.50, 25.37 and 24.30 respectively). On other hand compared the control group Lc_{50} showed highest value from at 96, 48 and 24 hr i.e. (33.65, 86.44 and 25.75) and low values at 72 hr (29.95). In Lc_{50} group showed highest values at 24 hr (25.75) and low values at 48, 96 and 72 hr respectively with the oxygen consumption of ammonia. Excretion observed in the above respective group with compared with Lc_0 group.

Discussion

In the present study experiment on the freshwater bivalve *Lamellidens marginalis* was performed in three respective groups i.e. control, Lc_0 and Lc_{50} in different seasons. The oxygen consumption and ammonia excretion (O:N) rates were higher in winter than monsoon and summer.

The O:N ratio is an index of protein utilization in energy metabolism. Increased protein catabolism is indicated by high level of ammonia excretion and decline in oxygen : nitrogen ratio (Bayne, 1973) and thus changes in the rates of nitrogen excretion and best understood in the contest of physiological energenetics and nitrogen balance, when related to overall metabolic by means of the oxygen : nitrogen ratio. A decrease in oxygen consumption with partial closing the shell-values also shown by Galtsoff (1964) and attributed to a decrease in rate of water transport.

The effect of temperature and nutritive stress on the O:N ratio vary seasonally and depend on the relative levels of carbohydrate, lipid and protein, reserves. Changes in the O:N ratio therefore afford a useful measure of the degree of stress experienced by the animals. Many authors have quoted that ammonia in general is a major nitrogenous excretory product of bivalves and there occurs a profound differences in loss of nitrogen between sized and seasons (Bishop et. al., 1983). Fit and Coon (1992) stated that actual concentration of NH_3 was associated with the surface for the oysters Crassostrea virginica and Crassostrea gigas, Hopkins (1934, 1936) and Pederson (1947) believed the mantle to be the primary respiratory site while the gills pump food and oxygen bearing water.

Cockroff (1990) reported that nitrogen excretion of *Donex serra* and *Donax sordidus* under laboratory and field conditions showed slight difference between species in ammonia and amino acid content exposure at low tide. The excretion rate in the *L. corrianus* was reduced much in the monsoon than summer despite the fact that the temperature of the habitat water got reduced (Meena, 1997). Low of O:N ratio is generally indicative of a stressed condition (Bayne and Newell, 1983; Widdows, 1985). Accordingly to Grant and Thorpe (1991) *Mya arenaria* exhibited a significant decrease in oxygen consumption and increase in ammonia excretion during turbidity treatment compared to control one.

Mathew and Menon (1993) reported that in *Perna indica* and *Donax incarnatus* O:N ratio was influenced due to stress of the heavy metals like Hg, Cu and Cd. The value of ratio in summer showed that the bivalves had more protein substrate utilization than the value of ration in winter. It is possible that in a period of post monsoon, the bivalve build up the body reserve and there by increased the O:N ratio. The ratio, by atomic equivalents, of oxygen consumed to nitrogen excreted can provide an index of the balance in the animal's tissues between the rates of catabolism of protein, carbohydrates and lipid substrates. Changes in the rates of nitrogen excretion are best understood, in the context of physiological energetic and nitrogen balance, when related to overall metabolic rate by means of oxygen : nitrogen (O:N) ratio.

Physiological responses of an organisms to changes in the environmental character due to natural and / or anthropogenic factors can be used as a measure of the quality of the environment (Widdow et. al., 1980; Widdow, 1985).

The authors further stated that variations in the O:N ratio of animals exposed to heavy metals probably indicate that catabolism processes were affected and the rate which this had occurred could not be predicted based on the above parameter. Prins and Small (1989) observed that high concentrations did not influenced the nitrogen budgets of the mussels *M. edulis* and the cockle, *Cestoderma edule*. Novarro and Torrijos (1994) determined a seasonal fluctuation in oxygen consumption, ammonia excretion in predatory gastropod, *Concholepas coneholepas*.

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Table 1*- Changes in the O:N ratio of L. marginalis after exposure of acute toxicity tests of cadmium in different time hours in different seasons.*

Time	Body size	Summer			Monsoon			Winter		
		Control group wet wt = 18.5	**Lc_0 group wet wt = 16.5**	**Lc_{50} group wet wt. = 17.5**	**Control group wet wt = 17.5**	**Lc_0 group wet wt = 15.5**	**Lc_{50} group wet wt. = 16.5**	**Control group wet wt = 14.5**	**Lc_0 group wet wt = 12.5**	**Lc_{50} group wet wt. = 13.5**
24 hrs	90-100 mm	45.4	70.5	21.55	22.68	25.83	25	23.85	24.3	25.75
48 hrs		50.05	76.38	17.29	74.26	106.87	87.84	74.5	123	86.44
72 hrs		21.8	13.89	13.85	197.3	169.89	27.12	105.98	115	29.95
96 hrs		13.63	10	17.57	15.56	24.67	31	19.63	25.37	33.65

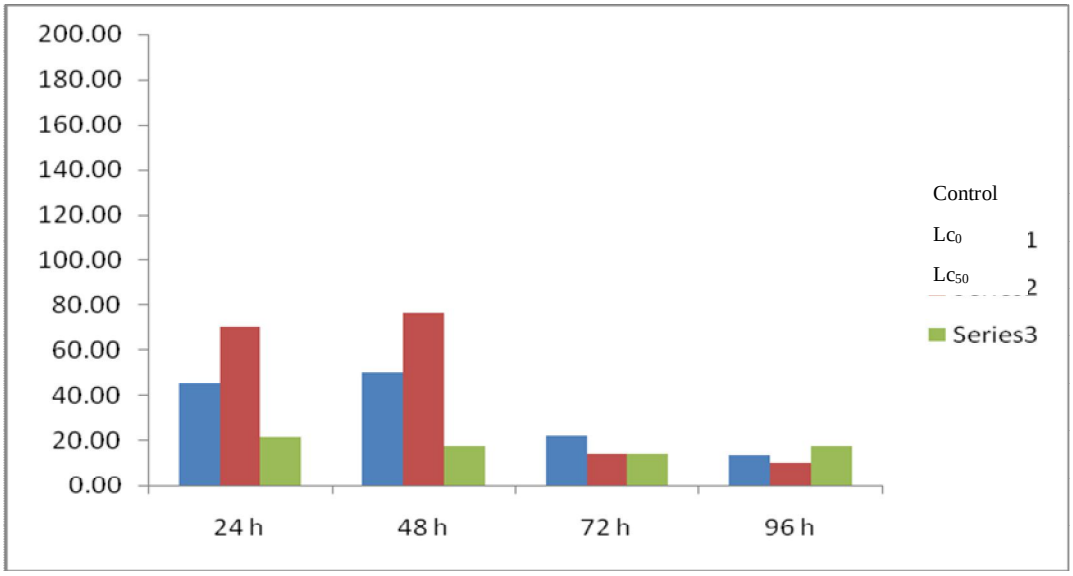

Fig 1 Ratio in summer

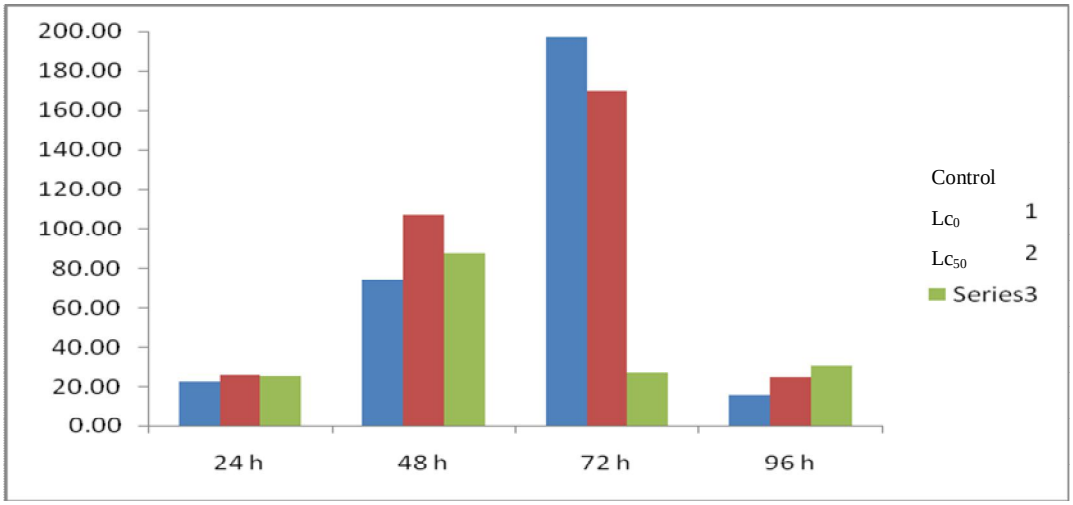

Fig 2 Ratio in monsoon

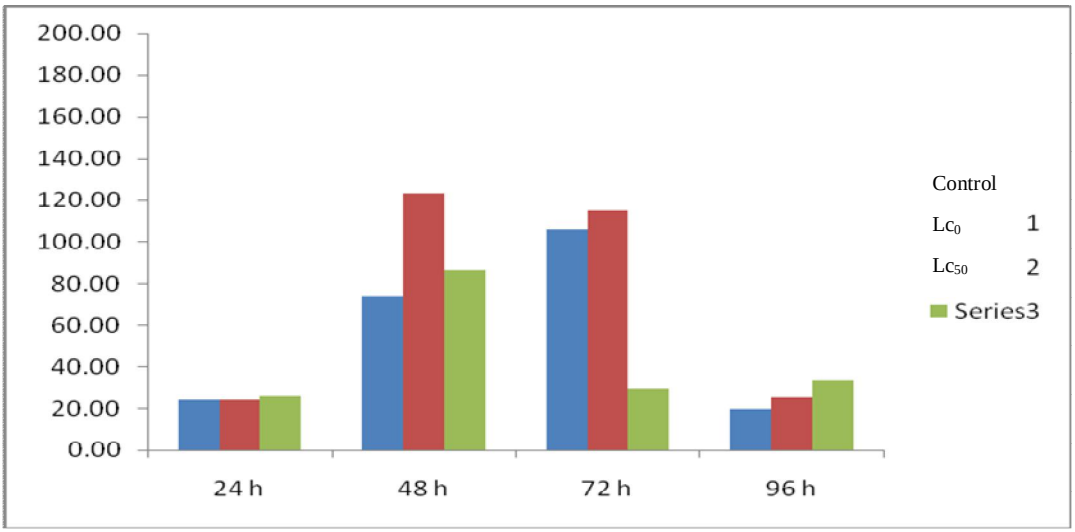

Fig 3 Ratio in winter