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Size-specific variation in the rate of oxygen consumption, ammonia excretion, and O: N ratio of freshwater bivalve lamellicorns marginalis during the season of monsoon

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ABSTRACT

Malacology means the study of molluscan animals and also conchology means the study of molluscan shell. Body mass is one of the best known and most studied characteristics of aquatic animals on scaling of metabolic rates. We studied here how size specific variation in the rate of oxygen consumption, ammonia excretion and O: N ratio in Freshwater Bivalve Mollusc Lamellidens marginalis species in an attempt to know how size specific variation affects their metabolism. The freshwater bivalve molluscs were chosen for experimental work from Bhima River at Siddhatek on August and September for the period of monsoon season with body size i.e. small (75-79 mm in shell-length) and large (90-93 mm in shell-length). In current work reported that the rate of oxygen consumption and O: N ratio was high in the small body sized bivalve mollusc but rate of ammonia excretion was low in small body sized bivalves compared to large ones. The results are discussed in the flush of metabolic processes in fresh-water bivalve molluscs.

Keywords: *size specific, variation, oxygen consumption, ammonia excretion, O: N ratio, and monsoon.*

1. INTRODUCTION

Malacology means the study of molluscan animals also conchology means the study of molluscan shell. The mollusca, a word meaning 'soft', includes a variety of invertebrate animals, with soft unsegmented body having a slippery skin and commonly sheltered in a hard calcareous shell of their own secretion. The scientist Lucas (1993) stated that, Food ingestion, ammonia excretion, and oxygen consumption rates are the key elements of bioenergetic models because they reflect the energy ingested (I), the energy lost as nitrogen (U), and the physiologically useful energy (R). Bioenergetic models are commonly used to estimate growth or consumption in aquatic animals and are very useful for estimating how types of food modulate the destination of ingested energy. In fact, energetic models allow us to estimate food digestibility, important data for balanced food designs (Lucas 1993). In *Octopus vulgaris* (Petza et al. 2006) and *Octopus maya* (Rosas et al. 2007) from total ingested energy (100%), U ranging from 2 to 14% and R between 23 and 68%. Rate of consumption of oxygen in these bivalves are influenced by activity, body size, stage in the life cycle and time of the day, in addition to by previous oxygen experience and genetic background (Prosser, 1973). The scientist S. S. Taware (2011) stated that, the metabolic rate by measuring oxygen consumption rate of *S. diphos* in relation to the various environment factors like body size, body weight, temperature, salinity air exposure, starvation and diurnal rhythm. The daily rhythms of oxygen consumption in the *Mytilus galloprovicialis* studied by Slatina (1991). Also the rate of oxygen consumption is dependent on various environmental factors and endogenous regulation of reproduction is main synchronizers of the rhythm. The researchers, Bishop et al., (1983) stated that, ammonia in general is a major nitrogenous excretory product of bivalves and there occurs a profound difference in loss of nitrogen between different sizes and seasons. The body size of the bivalve mollusc is an important parameter, which influencing the pattern of metabolic responses. In bivalve molluscs, the relationship between the rate of ammonia excretion and the body size can be variable due to a disproportionate reliance of protein catabolism for energy production (Mangesh Jadhav et al. 2012). Bayne and Scullard (1977) stated that, in the bivalve molluscs the relationship between ammonia excretion rates and body size can be variable due to a disproportionate reliance on protein catabolism for energy production by small individuals and O: N ratio was shown to vary considerably with in complex interactions with the season, temperature and ration in *Mytilus edulis.*

Segawa (1991) observed that, increased oxygen consumption rate and ammonia excretion rate linear with increase in weight and decreases with period of starvation in *Abalone sulculus diversicular.* According to Gonzalo and Cancino (1988) reported that, oxygen consumption and ammonia excretion rate of bivalve molluscs is a function of body weight. Excretion rate varies between species of bivalves, as well as with individual body size, temperature, stage in reproductive cycle and food availability (Dietz, 1985; Lauritsen and Mozley, 1989; Davis et al, 2000; Christian and Berg 2000). The changes in the relationship between excretion rate and body size may be explained in part by seasonal changes in the synthesis and utilization of nitrogenous compound as substrates for energy metabolism.

Review of literature reveled that, very less information was available on fresh water bivalve molluscs from India, Howkins *et; al.* (1986) reported O:N ratio on *Perna viridis* and *Perna indica* from Cochin backwaters and recently Mathew and Menon (1993) reported heavy metal stress induced variation in O:N ratio in *Perna indica* and *Donax incarnates*. Considering the abundant distribution of bivalve molluscs along the banks of Bhima River and the scarcity of information on O: N in fresh water bivalves, the present work was undertaken on *Lamellidens marginalis*.

The current work revealed that, the detailed account on rate of oxygen consumption, rate of ammonia excretion and O: N ratio of *Lamellidens marginalis* on monsoon season. This approach would help in monitoring the environmental quality and taking appropriate remedial control measures, where the population of bivalve molluscs is affected beyond the critical level.

2. MATERIALS AND METHOD

During the research period, the samples of freshwater bivalve molluscs, *Lamellidens marginalis* were collected from Bhima River at Siddhatek Tahsil- Karjat, about 95 km from Ahmednagar city during month of August 2015 and month of September 2015 in the monsoon season. The bivalves were divided into two different sizes 75-79 mm in shell length and 90-93 mm in shell length in small and large size respectively. The bivalve molluscs were collected during 4.00 to 6.00 pm at the time of collection. After collection of bivalves, immediately after arrival at the laboratory, the animals washed under running tap water and then the shells of the bivalves were brushed and again washed with running tap water in order to remove debris material from the shells. Then the animals were divided into two groups i.e. small and large sized groups and then animals were allowed for defaecication or depuration (not acclimatization) for 12 – 13 hours in laboratory conditions under constant aeration. Each group consists of 10 animals for experiment.

The physico-chemical characteristics of water on the habitat as well as experimental water were determined during experiment like temperature, pH, dissolved oxygen and hardness (in terms of carbonates) contents were determined of the water.

The rate of oxygen consumption and ammonia excretion determined by Winkler's modified technique (Golterman et al., 1978) and phenol- hypochlorite method (Solarzano, 1969) respectively. The oxygen consumption rate of individual animal was determined in specially prepared brown coloured respiratory jars of one liter capacity. The jars were fitted with rubber corks having an inlet and outlet of glass tubes connected with rubber tubes and clips. Individual animal was placed in each jar and constant flow of water was given through the inlet to flow through the outlet for 2.0 minutes. The flow of the water was cut down slowly without disturbing the animals.

After one hour, water from the respiratory jar was carefully siphoned out in a stoppard reagent bottle of 125 ml capacity for determine oxygen consumption content and 50ml water sample in Eryelene's Mayer flask for determine rate of ammonia excretion. The flesh of the individual animal was then taken out carefully from the shell and blotted on the filter paper to remove excess water. This flesh was then weighed to obtain the wet-weight of the five individual bivalves.

Every five individual animals of each group were used and mean of triplicate water samples were estimated for each group. The statistical analysis was done to express final data. The atomic equivalent values of oxygen and nitrogen were calculated on the basis of values of rate oxygen consumption and ammonia excretion obtained for the same individual and finally the O: N ratio was calculated (Widdows, 1978; Bayne and Newell, 1983). Oxygen consumption rate was expressed in mg O_2/l/h/gm body weight and rate of ammonia excretion was expressed in mg NH_3-N/l/h body weight.

3. RESULTS

The table No. 01 represents the physico chemical parameters like temperature, pH, dissolved oxygen and hardness content of habitat water of bivalve mollusc, *Lamellidens marginalis* and the experimental water (tap water), were determined during study period. The water temperature of habitat was (26.2 – 28.8ºC) and (24.7 – 28.3ºC) on August and September month respectively and also (27.5 – 29.2ºC) and (25.3 – 28.1ºC) temperature of the experimental water on August and September respectively. The pH of habitat water was found on August (8.21 -8.94) and on September (8.68 – 8.86) and also in experimental water was (6.50 – 6.60) on August and on September was 6.00 – 7.05. The dissolved oxygen contents in the habitat water was (5.9958-6.5225 mg/lit/hr) and (6.2541– 6.5102 mg/lit/hr) on August and September respectively also in experimental water was (5.8453 – 5.9265 mg/lit/hr) on month of August and (5.8937 – 6.2213 mg/lit/hr) on month of September. The hardness of water was found (105.4 – 112.6 ppm) and (96.0-109.8 ppm) on August and September respectively in habitat water and also (384.2 – 390.0 ppm) and (334.6 – 342.6 ppm) in experimental water during monsoon season on August and September respectively.

Table no. 01: Physico - chemical Parameters of Habitat water and Experimental Water (Tap water) used in laboratory

Sr.No.	Season	Month	Temperature (ºC)	pH	Dissolved Oxygen content (mg/l/h)	Hardness (ppm)
1	Habitat Water	August	26.2 – 28.8	8.21 – 8.94	5.9958-6.5225	105.4 – 112.6
		September	24.7 – 28.3	8.68 – 8.86	6.2541-6.5102	96.0 – 109.8
2	Experimental Water	August	27.5 – 29.2	6.5-6.60	5.8453-5.9265	384.2 – 390.0
		September	25.3 – 28.1	6.0-7.05	5.8937-6.2213	334.6 – 342.6

In table No. 02 and 03 shows the results of rate of oxygen consumption, ammonia excretion and O: N ratio in monsoon season during research work. The oxygen consumption rate in small body size of individual animal was ranged from 0.1333 – 0.2181 mg O_2/l/h (on August), 0.1599 - 0.2050 mg O_2/l/h (on September) and 0.0926 – 0.1308 mg O_2/l/h (on August), 0.1208 – 0.1444 mg O_2/l/h (on September) in large body size during monsoon season. The ammonia excretion of individual animal were ranged from 0.0027-0.0043 µg NH_3-N/l/h (on August) and 0.0032-0.0034 µg NH_3-N/l/h (on September) in small body size and 0.0038-0.0042 µg NH_3-N/l/h (on August) and 0.0023-0.0027 µg NH_3-N/l/h (on September) in large body sized animal during summer season. The calculations of O: N ratio after determining the atomic equivalent of oxygen and nitrogen were ranged from 60.9121-45.74.2081 and 62.0087- 79.4760 on August and September in small sized animal and 35.6667-43.9114 and 58.5492- 66.3212 on August and September in large body sized animal. The values of rate of oxygen consumption were 0.2705±0.0486 mg O_2/l/h (on August) and 0.2746±0.0267 mg O_2/l/h (on September) in small body sized bivalve and 0.1816±0.0071 mg O_2/l/h in and 0.1866±0.0123 mg O_2/l/h on August and September in large body sized bivalves respectively. The rate of ammonia excretion in animal were 0.0036±0.000671 µg NH_3-N/l/h (August), 0.0033±0.000 µg NH_3-N/l/h (September) in small body sized and 0.00402±0.000164 µg NH_3-N/l/h (August), 0.00256±0.000167 µg NH_3-N/l/h (September) in large body sized animals respectively. The O: N ratio showed higher values 65.8651±5.4940 and 72.7644±6.5100 on month of August and September respectively in small body sized bivalve and lower values 39.6542±3.1468 and 63.7879±3.21399 on month of August and September respectively in large body sized bivalves during monsoon season.

Table No. 02: Oxygen consumption, rate of ammonia excretion and O: N ratio of *Lamellidens marginalis* on August during monsoon

	Animal number	Size of the animal (mm)	Weight of animals (gms)	Oxygen consumption (ml/l/h/gm)	Oxygen consumption (mg/l/h/gm)	Ammonia excretion (mgNH3-N/l/h)	Ammonia excretion (µgNH3-N/l/h)	Atomic equivalent of Oxygen	Atomic equivalent of ammonia	O:N ratio
Small Size	I	79	09.214	0.1851	0.2628	0.0031	3.1	0.0164	0.000221	74.2081
	II	75	07.905	0.2054	0.2917	0.0039	3.9	0.0182	0.000279	65.2330
	III	78	09.307	0.2181	0.3097	0.0040	4.0	0.0194	0.000286	67.8322
	IV	77	11.783	0.1333	0.1893	0.0027	2.7	0.0118	0.000193	61.1399
	V	79	11.570	0.2105	0.2989	0.0043	4.3	0.0187	0.000307	60.9121
					0.2705 ±0.0486	0.0036 ±0.000671				65.8651 ±5.4940
Large Size	I	92	19.050	0.1080	0.1835	0.0039	3.9	0.0115	0.000279	41.2186
	II	93	17.502	0.1176	0.1718	0.0042	4.2	0.0107	0.0003	35.6667
	III	90	21.040	0.0926	0.1857	0.0041	4.1	0.0116	0.000293	39.5904
	IV	90	15.312	0.1308	0.1897	0.0038	3.8	0.0119	0.000271	43.9114
	V	91	17.824	0.1109	0.1775	0.0041	4.1	0.0111	0.000293	37.8840
					0.1816 ±0.0071	0.00402 ±0.000164				39.6542 ±3.1468

Table No. 03: Oxygen consumption, rate of ammonia excretion and O: N ratio of *Lamellidens marginalis* on September during Monsoon

	Animal number	Size of the animal (mm)	Weight of animals (gms)	Oxygen consumption (ml/l/h/gm)	Oxygen consumption (mg/l/h/gm)	Ammonia excretion (mgNH3-N/l/h)	Ammonia excretion (µgNH3-N/l/h)	Atomic equivalent of Oxygen	Atomic equivalent of ammonia	O:N ratio
Small Size	I	77	12.565	0.2025	0.2876	0.0034	3.4	0.0180	0.000243	74.0741
	II	78	14.220	0.1599	0.2271	0.0032	3.2	0.0142	0.000229	62.0087
	III	77	13.300	0.1994	0.2831	0.0034	3.4	0.0177	0.000243	72.8395
	IV	76	13.250	0.2002	0.2843	0.0033	3.3	0.0178	0.000236	75.4237
	V	79	13.200	0.2050	0.2911	0.0032	3.2	0.0182	0.000229	79.4760
					0.27464 ±0.026757	0.0033 ±0.000				72.7644 ±6.5100
Large Size	I	91	19.921	0.1318	0.1872	0.0026	2.6	0.0117	0.000186	62.9032
	II	90	19.800	0.1271	0.1805	0.0027	2.7	0.0113	0.000193	58.5492
	III	90	20.400	0.1208	0.1715	0.0023	2.3	0.0107	0.000164	65.2439
	IV	93	20.800	0.1444	0.2050	0.0027	2.7	0.0128	0.000193	66.3212
	V	91	22.814	0.1330	0.1889	0.0025	2.5	0.0118	0.000179	65.9218
					0.18662 ±0.012343	0.00256 ±0.000167				63.7879 ±3.21399

4. DISCUSSION

In the present research work carried out on freshwater bivalve mollusc, *Lamellidens marginalis* (Lamark) from Bhima River at Siddhatek, for the period of monsoon season, oxygen consumption rate was more in small body sized bivalves as compared to large sized bivalve molluscs. Also ammonia excretion rate found more in large sized bivalves than the small body sized bivalves. The rate of oxygen consumption was increased in small body sized animals because small sized bivalve individuals with relatively little glycogen reserves, which increases very much their protein catabolism, whereas larger ones to a great extent on their moderately more glycogen storage (Bayne, 1973). The metabolic processes of animals are considerably affected by their body sizes or length of body. Small sized clam have much more respiratory rates than medium and large clam in *K.* opima (Mane U.H, 1975). Also in *S. diphos* more respiration rate in small sized bivalves than medium, large and old sized group of clams (S.S. Taware et al., 2012).The rate metabolism is strongly dependent on body size, it is necessary to introduce weight specific correlation comparison between animals of different sizes. It is known that weight specific or size specific rate of oxygen consumption is lower in larger organisms than in smaller ones. The oxygen consumption in clams is inversely proportional to the size of organisms, when calculated on the basis of wet weight of the clam (Mane U.H, 1975). This overview applies in both intra-specific comparisons between bivalve molluscs of different body sizes as well as inter-species belong to same species or different species.

In the current work on the species, *Lamellidens marginalis,* the body size specific oxygen consumption rate followed a general trend of receiving i.e. higher values of oxygen consumption rate for smaller body sized bivalves than larger body sized animals. The similar results on body size specific was found by Chandran and Damodaran (2000) in *V. cyprinoides*, Kamble and Muley (2009) in *K. opima*, A. N. Vedpathak et al. (2011) in *Indonaia caeruleus*, Mangesh Jadhav, et al. (2012) and P.Ramanlal Gugale, et al. (2017, 2017) in *Lamellidens marginalis* and V.M. Lagade et al.(2013) in *Soletellina diphos*. Mane (1975) and Bayne (1976) stated that, body size in bivalves are important implication, hence, bivalve populations that are dominated by older and large individuals have a lowest value than those composed of small sized individuals. It is also showed that, the energy flow through small individuals of species may be much greater than larger ones. The rate of oxygen consumption showed significant increase in smaller body sized bivalve for the period of monsoon season because it is known that, the oxygen consumption was mainly dependent on reproductive condition of bivalves in the season of monsoon. The energy utilization in oxygen consumption and ammonia excretion rate was significantly different, which depending on size, season and temperature but season being important factor which affect the overall fitness of the animal (Navarro and Torrijos, 1994, Mangesh Jadhav, et al., 2012, P. Ramanlal Gugale, et al. 2017).

The several authors have shown that, the ammonia in general considered as major nitrogenous excretory product of bivalve molluscs and there occur profound difference in loss of nitrogen between different body sizes and seasons (Nagwanshi, 1996, Salve, 2007). In the present current on *Lamellidens marginalis*, the ammonia excretion rate showed more increase large body sized bivalves on April and May during summer season, because it is known that small body sized bivalves catabolise different biochemical substrates to varying degrees, according to season (Bayne and Newell, 1983; Gabbott, 1983). Also similar results was found by A. N. Vedpathak et al. (2011) in *Indonaia caeruleus* and Mangesh Jadhav et al. (2012) and P.Ramanlal Gugale, et al. (2017, 2017) in *Lamellidens marginalis*.

The O: N ratios can provide indices of balance in animal tissues between the rate of catabolism of carbohydrate, protein and lipid substrates. The changes in the conversion of ammonia (nitrogen excretion) are best understood in context of physiological energetic and nitrogen balance related to overall metabolic rate by means of O: N ratio. This ratio when calculated by atomic equivalents may be used to indicate the proportion of protein catabolise to carbohydrate and lipids. Atomic O: N ratios are linked to the availability of energy stores and the utilization of body protein. This O:N ratio produces an index of the relative amounts of protein, as compared to carbohydrates and lipids that are catabolized by the organism (Bhagde R. V. et al. 2005 and Ana Farias et al., 2009).

In *Thias Lapillus* (Strickle and Bayne, 1982), the O: N ratio did not change with body size that is exponent for oxygen consumption rate and ammonia excretion rate against body weight. However, in *Mytilus* the O: N ratio varied considerably with body size and complex interaction with season and temperature (Bayne and Scullard, 1977). Bayne (1976) stated that, if the amino acids which result from protein catabolism are dominated and the resultant ammonia excreted, carbon skeleton of amino acid are completely oxidized. Higher value of O: N ratio indicates increased catabolism of carbohydrates or lipids.

In the present work the O: N ratio was more in the small body sized bivalve animals than the large body sized bivalve animals. Also similar result was of found by A. N. Vedpathak et al. (2011) in *Indonaia caeruleus* and Mangesh Jadhav, et al. (2012) and P.Ramanlal Gugale, et al. (2017, 2017) in *Lamellidens marginalis*.

The O: N ratio increase or decrease in bivalve moluscs of different body sizes, noticed that the significant level could be due to the state of a gonadal development and level of metabolic activity of the molluscs in individual body size group.

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