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BODY SIZE DEPENDENT RATE OF OXYGEN CONSUMPTION, AMMONIA EXCRETION AND O:N RATIO OF FRESH WATER BIVALVE, *LAMELLIDENS MARGINALIS* DURING SUMMER SEASON.

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ABSTRACT

The study of molluscan animals is called Malacology and the study of molluscan shell is called as conchology. The scaling of metabolic rates with body mass is one of the best known and most studied characteristics of aquatic animals. We studied here how body size is related to rate of oxygen consumption, ammonia excretion and O: N ratio in Freshwater Bivalve Mollusc *Lamellidens marginalis* species in an attempt to know how body size specific changes affect their metabolism. The freshwater bivalve molluscs with specific body size i.e. small (76-79 mm in shell-length) and large (90-94 mm in shell-length) were chosen for experimental work from Bhima River at Siddhatek on April and May during summer. The adult bivalve molluscs with small body size reported high value in rate of oxygen consumption and O: N ratio but rate of ammonia excretion was low value in small body sized bivalves compared to large ones. The results are discussed in the flush of metabolic processes in fresh-water bivalve molluscs.

Figure: 00 **References: 38** **Tables: 03**
KEY WORDS: Ammonia excretion, Oxygen consumption, *Lamellidens marginalis*, Summer.

Introduction

The study of molluscan animals is called Malacology and the study of molluscan shell is called as conchology. 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. 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)[19]. In *Octopus vulgaris*[28] and *Octopus maya*[30] from total ingested energy (100%), U ranging from 2 to 14% and R between 23 and 68%. 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[19]. Rate of oxygen consumption in these animals 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[29]. 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[31]. The daily rhythms of oxygen consumption in the *Mytilus galloprovicialis*[34]. Also oxygen consumption is dependent on various environmental factors and endogenous regulation of reproduction is main synchronizers of the rhythm. Many authors have showed 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[7]. The body weight or 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[21]. In 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*[6].

Workers[33] 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[15] 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[12, 18, 11, 09]. 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. The rate oxygen consumption and ammonia excretion showed linear correlationship with body weight, seasonal changes in oxygen uptake and ammonia excretion in the gastropod, *Concholepas concholepas*[25].

Review of literature reveled that, very small information was available on fresh water bivalve molluscs from India, Researcher[16] reported O:N ratio on *Perna viridis* and *Perna indica* from Cochin backwaters and recently[23] 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 scarcity of information on O: N in fresh water bivalves, the present work was undertaken on *Lamellidens marginalis*.

Present work revealed that, the detailed account on oxygen consumption, ammonia excretion and O: N ratio of *Lamellidens marginalis* on season of summer. 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.

Materials and Methods

During the experimental period the samples of freshwater bivalve molluscs, *Lamellidens marginalis* were collected from banks of Bhima River at Siddhatek, about 95 km from Ahmednagar city during month of April 2015 and month of May 2015 in the season of summer. The bivalve molluscs were divided into two different sizes i.e. small size 76-79 mm in shell length and large size 90-94 mm in shell length. The samples were collected during 4.00 to 6.00 pm at the time of collection. Then immediately after arrival at the laboratory, the animals washed under tap water then the shells of the bivalves were brushed and again washed with freshwater in order to remove the mud, algal biomass and other fungal waste materials. Then the animals were divided into two groups of specific sizes i.e. small and large sized 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 like temperature, pH, dissolved oxygen and hardness (in terms of carbonates) contents were determined of the water on the habitat as well as experimental water were determined during experiment.The oxygen consumption rate determined by Winkler's modified technique[14] and rate of ammonia excretion by phenol- hypochlorite method[35].

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 size specific 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 oxygen consumption and ammonia excretion obtained for the same individual and finally the O: N ratio was established[5,38]. Oxygen consumption rate was expressed in mg O_2/l/h/gm body weight and ammonia excretion rate was expressed in mg NH_3-N/l/h body weight.

Results

During the period of study, the physico chemical parameters like temperature, pH, dissolved oxygen and hardness content of habitat water of bivalve *Lamellidens marginalis* and the experimental water (tap water), were determined during study and represented in Table-1. The temperature of water of habitat was (26.9 – 29.3ºC) and (30.2 – 33.9ºC) on April and May month respectively and also temperature of the experimental water was (26.1-29.0ºC) on April and on May it was (30.0 -33.7ºC). pH was found on April (8.20 -8.60) and on May (8.10 – 8.40) in habitat water and also in experimental water was (6.90 -7.10) on April and on May (6.75-7.48). The dissolved oxygen contents of the habitat water was on April (5.4523-5.7815 mg/lit/hr) and on May (5.7999-5.9935 mg/lit/hr) whereas in experimental water was (4.8553 – 4.8821 mg/lit/hr) on month of April and on month of May (4.7929-5.2929 mg/lit/hr). The hardness of water was found on April (98.2 - 105.0 ppm) and on May (102.0-106.8 ppm) in habitat water and also in experimental water was (258.0 – 263.2 ppm) and (270.0 – 276.0 ppm) on April and May respectively during season of summer.

TABLE-1: Physico-chemical Parameters of Habitat water and Experimental Water (Tap water) used in laboratory.

Sr. No.	Season	Month	Temp. (ºC)	pH	DO content (mg/l/h)	Hardness (ppm)
1	Habitat Water	April	26.9 – 29.3	8.20 – 8.60	5.4523 – 5.7815	98.2 – 105.0
		May	30.2 – 33.9	8.10 – 8.40	5.799 – 5.9935	102.0 – 106.8
2	Experimental Water	April	26.1 – 29.0	6.90 – 7.10	4.8553 – 4.8821	258.0 – 263.2
		May	30.0 - 33.7	6.75 – 7.48	4.7929 – 5.2929	270.0 – 276.0

TABLE-2: Oxygen consumption, rate of ammonia excretion and O:N ratio of *Lamellidens marginalis* on April during summer.

	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 (mgNH_3-N/l/h)	Ammonia excretion (µgNH_3-N/l/h)	Atomic equivalent of Oxygen	Atomic equivalent of ammonia	O:N ratio
Small Size	I	76	14.440	0.2422	0.3439	0.0066	6.6	0.0215	0.000471	45.6476
	II	78	14.010	0.2550	0.3621	0.0071	7.1	0.0226	0.000507	44.5759
	III	78	15.500	0.1973	0.2802	0.0058	5.8	0.0175	0.000414	42.2705
	IV	79	14.516	0.2200	0.3124	0.0071	7.1	0.0195	0.000507	38.4615
	V	79	14.995	0.2112	0.2999	0.0058	5.8	0.0187	0.000414	45.1691
					0.3197 ±0.033125	0.00648 ±0.000653				43.2249 ±2.9612
Large Size	I	93	22.700	0.1800	0.2556	0.0067	6.7	0.0160	0.000479	33.4029
	II	92	24.220	0.1620	0.2300	0.0071	7.1	0.0144	0.000471	30.5732
	III	91	18.500	0.2180	0.3096	0.0076	7.6	0.0193	0.0000543	35.5433
	IV	90	19.400	0.2120	0.3010	0.0079	7.9	0.0188	0.000564	33.3333
	V	90	18.600	0.2139	0.3037	0.0076	7.6	0.0190	0.0000543	34.9908
					0.2799 ±0.0353	0.00738 ±0.000476				33.5687 ±1.9350

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TABLE-3: Oxygen consumption, rate of ammonia excretion and O: N ratio of *Lamellidens marginalis* on May during summer.

	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 ($mgNH_3$-N/l/h)	Ammonia excretion (μgNH_3-N/l/h)	Atomic equivalent of Oxygen	Atomic equivalent of ammonia	O:N ratio
Small Size	I	78	13.060	0.2715	0.3855	0.0062	6.2	0.0241	0.000443	54.4018
	II	79	13.250	0.2737	0.3887	0.0062	6.2	0.0243	0.000443	54.8533
	III	76	12.080	0.3227	0.4582	0.0068	6.8	0.0286	0.000486	58.8448
	IV	77	12.480	0.2884	0.4095	0.0064	6.4	0.0256	0.000457	56.0175
	V	77	13.080	0.2918	0.4144	0.0065	6.5	0.0259	0.000464	55.8189
					0.4113 ±0.029107	0.00642 ±0.000249				55.9873 ±1.7316
Large Size	I	93	23.020	0.1693	0.2404	0.0048	4.8	0.0150	0.000343	43.7318
	II	93	22.890	0.1655	0.2350	0.0045	4.5	0.0147	0.000321	45.7944
	III	92	21.540	0.1646	0.2337	0.0047	4.7	0.0146	0.000336	43.4524
	IV	94	22.500	0.1612	0.2289	0.0047	4.7	0.0143	0.000336	42.5595
	V	90	20.010	0.1609	0.2285	0.0044	4.4	0.0143	0.000314	45.5414
					0.2333 ±0.004895	0.00462 ±0.000164				44.2159 ±1.3973

The results of rate of oxygen consumption, ammonia excretion and O: N ratio in summer season was also determined and represented in Table-2 and 3. The oxygen consumption rate of individual animal in small body size was ranged from 0.2802- 0.3621 mg O_2/l/h (on April), 0.3855- 0.4582 mg O_2/l/h (on May) and in large body size was ranged from 0.2300-0.3096 mg O_2/l/h (on April), 0.2285- 0.2404 mg O_2/l/h (on May) during summer season. The ammonia excretion of individual animal were ranged from 0.0058-0.0071 μg NH_3-N/l/h (on April) and 0.0062-0.0068 μg NH_3-N/l/h (on May) in small body size and 0.0067-0.0079 μg NH_3-N/l/h (on April) and 0.0044-0.0048 μg NH_3-N/l/h (on May) 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 38.4615-45.6476 and 54.4018- 58.8448 on April and May in small and 30.5732-35.5433 and 42.5595- 45.7944 on April and May in large body sized animal. The values of rate of oxygen consumption were 0.3197±0.03313 mg O_2/l/h (on April) and 0.4113±0.0291 mg O_2/l/h (on May) in small body sized bivalve and 0.2799±0.0353 mg O_2/l/h in and 0.2333±0.0490 mg O_2/l/h on April and May in large body sized bivalves. The rate of ammonia excretion in animal were 0.00648±0.000653 μg NH_3-N/l/h (April), 0.00642±0.000249 μg NH_3-N/l/h (May) in small body sized and 0.00738±0.000476 μg NH_3-N/l/h (April), 0.00462±0.000164 μg NH_3-N/l/h (May) in large body sized animals respectively. The O: N ratio showed higher values 43.2249±2.9612 and 55.9873±1.7316 on month of April and May respectively in small body sized bivalve and lower values 33.5687±1.9350 and 44.2159±1.3973 on month of April and May respectively in large body sized bivalves during summer season.

Discussion

In the present study on freshwater bivalve, *Lamellidens marginalis (*Lamark) from Bhima River at Siddhatek, during summer season, oxygen consumption rate was more in small body sized bivalves as compared to large sized bivalve molluscs. Ammonia excretion rate found more in large body sized bivalves then the small body sized bivalves. The oxygen consumption rate increased in small body sized animals because small individuals with relatively small glycogen reserves, which increases considerably their protein catabolism, whereas larger ones to a great extent on their moderately more glycogen storage[3]. The metabolic processes of animals are significantly affected by their body sizes or length. Small clam have more respiratory rates than medium and large clam in *K.* opima[20]. Also in *S. diphos* more respiration rate in small shell length than medium, large and old sized group of clams[31]. The metabolic rate is powerfully dependent on body size, it is necessary to introduce weight specific correlation comparison between animals of different sizes. It is known that weight 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[20]. This generalization applies in both intra-specific comparisons between bivalve molluscs of different body sizes as well as inter-species belong to same species or different.

In the present work on *Lamellidens marginalis*, the body size specific oxygen consumption rate followed a general trend of acceptance i.e. higher values of oxygen consumption rate for smaller body sized bivalves than larger body sized animals. Similar result was found in *V. cyprinoides*[8], in *K. opima*[17], in *Indonaia caeruleus*[1], in *Lamellidens marginalis*[22, 26] and in *Soletellina diphos*[37]. 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 individuals[20, 4]. It is also showed that, the energy flow through small individuals of species may be much greater than larger ones. The oxygen consumption rate showed significant increase in smaller body sized bivalve particularly during summer season because it is known that, the oxygen consumption was mainly dependent on reproductive condition of bivalves in the season of summer. 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[22, 25].

Many 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[24, 32]. In the present work 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[5,13]. Also similar result was found in *Indonaia caeruleus*[1] and in *Lamellidens marginalis*[22, 26].

The O: N ratios can provide indices of balance in animal tissues between the rate of catabolism of protein, carbohydrate, and lipid substrates. The changes in the nitrogen excretion (conversion of ammonia) 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 ratio produces an index of the relative amounts of protein, as compared to carbohydrates and lipids that are catabolized by the organism[2].

In *Thias Lapillus*[36], 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[6]. 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[4].

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 in *Indonaia caeruleus*[1] and in *Lamellidens marginalis*[21, 26].

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

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