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OXYGEN CONSUMPTION, AMMONIA EXCRETION AND O: N RATIO OF FRESHWATER BIVALVE, *LAMELLIDENS MARGINALIS* DURING WINTER SEASON WITH SPECIAL REFERENCE TO BODY SIZE

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ABSTRACT: 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 size is related to oxygen consumption, ammonia excretion and O: N ratio in Freshwater Bivalve Mollusc *Lamellidens marginalis* species in an attempt to know how size specific changes affect their metabolism. The freshwater bivalve molluscs with specific size i.e. small (77-79 mm in shell-length) and large (90-93 mm in shell-length) were selected for experimental work from Bhima River at Siddhatek on December and January during winter. The adult bivalve molluscs with small size reported high value in oxygen consumption and O: N ratio but ammonia excretion was low value in small sized bivalves compared to large ones. The results are discussed in the glow of metabolic processes in fresh-water bivalve molluscs.

KEYWORDS: Mollusc, *Lamellidens marginalis,* aquatic animals, ammonia excretion, oxygen consumption.

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INTRODUCTION

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) [1]. In *Octopus vulgaris* (Petza et al. 2006) and *Octopus maya* [2] 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 ([1]. 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 [3]. 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 [4]. The daily rhythms of oxygen consumption in the *Mytilus galloprovicialis* studied by [5]. 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 [6]. 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 [7, 8]. [9] Stated that 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.*

[10] Observed increased oxygen consumption and ammonia excretion linear with increase in weight and decreases with period of starvation in *Abalone sulculus diversicular.* According to [11] reported that oxygen consumption and ammonia excretion of bivalve is a function of body weight. Excretion rate varies between species of bivalves, as well as with individual size, temperature, stage in reproductive cycle and food availability [12, 13, 14, and 15]. 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* reported by [16].Review of literature reveled that, very little information was available on fresh water bivalve molluscs from India, [17] reported O:N ratio on *Perna viridis* and *Perna indica* from Cochin backwaters and recently [18] 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 Godavari River and scarcity of information on O: N in fresh water bivalves, the present study was undertaken on *Lamellidens marginalis*.

Present study revealed the detailed account on oxygen consumption, ammonia excretion and O: N ratio of *Lamellidens marginalis* on winter season. This approach would help in monitoring the environmental quality and taking appropriate remedial control measures, where the population of bivalves is affected beyond the critical level.

MATERIALS AND METHODS

During the experimental period the samples of freshwater bivalve molluscs, *Lamellidens marginalis* were collected from Bhima River at Siddhatek Taq. Karjat, Dist. Ahmednagar, about 95 km from Ahmednagar city during December 2014 and January 2015 in winter season. The bivalves were divided into two different sizes i.e. small size 77-79 mm in shell length and large size 90-93 mm in shell length. The samples were collected during 4.00 to 6.00 pm at the time of collection. 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, hardness (in terms of carbonates) and dissolved oxygen contents were determined of the water on the habitat as well as tap water were determined during experiment.The rate of oxygen consumption determined by Winkler's modified technique [19] and ammonia excretion by phenol- hypochlorite method [20]. The rate of oxygen consumption 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 content and 50ml water sample in Eryelene's Mayer flask for determine 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 [21, 22]. Rate of oxygen consumption 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.

RESULTS AND DISCUSSION

During study period, the physico chemical parameters like temperature, pH, hardness and dissolved oxygen content of habitat water of bivalve *Lamellidens marginalis* and the experimental water (tap water), were determined during experiment (Table 1). The temperature of water of collection sites was (18.1 – 20.8ºC) on December and (17.5 – 20.3ºC) on January and also temperature of the tap water was (19.6 – 23.0ºC) on December and on January it was (18.6 – 21.8ºC). pH was found on December (7.92-8.10) and on January (8.10 – 8.38) in habitat water and also in tap water was (7.55 – 8.50) on December and on January (7.50 – 7.70). The hardness of water was found on December (96.0-104.30 ppm) and on January (95.80-105.20 ppm) also in tap water was (334.00 – 340.40 ppm) on December and (274.0 – 280.6 ppm) on month of January. The dissolved oxygen contents of the water was on December (4.8620-4.6220 mg/lit/hr) and on January (6.2178– 6.5132 mg/lit/hr) whereas in tap water was (3.7620 – 3.7620 mg/lit/hr) on December and on January (4.8177– 4.8177 mg/lit/hr).

Table 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	December	18.1 – 20.8	7.92 – 8.10	4.8620-4.6220	96.0 – 104.3
		January	17.5 – 20.3	8.10– 8.38	6.2178-6.5132	95.80– 105.2
2	Experimental Water	December	19.6 – 23.0	7.55-8.50	3.7620-3.7620	334.0 – 340.4
		January	18.6 -21.8	7.50-7.70	4.8177-4.8177	274.0 – 280.6

Table 02: Oxygen consumption, rate of ammonia excretion and O: N ratio of *Lamellidens marginalis* on December during Prewinter

	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	77	12.175	0.2223	0.3157	0.0046	4.6	0.0197	0.000329	59.8784
	II	77	12.067	0.1884	0.2675	0.0045	4.5	0.0167	0.000321	52.0249
	III	78	10.866	0.1693	0.2404	0.0045	4.5	0.0150	0.000321	46.7290
	IV	79	11.391	0.1948	0.2766	0.0043	4.3	0.0173	0.000307	56.3518
	V	78	11.146	0.1675	0.2379	0.0041	4.1	0.0149	0.000293	50.8532
					0.2676 ±0.0317	**0.0044 ±0.0002**				**53.1676 ±5.0816**
Large Size	I	92	19.050	0.1080	0.1534	0.0076	7.6	0.0096	0.000543	17.6796
	II	93	17.502	0.1176	0.1670	0.0072	7.2	0.0104	0.000514	20.2335
	III	90	21.040	0.0926	0.1315	0.080	8.0	0.0082	0.000571	14.3608
	IV	90	15.312	0.1308	0.1857	0.0084	8.4	0.0116	0.0006	19.3333
	V	91	17.824	0.1109	0.1575	0.0071	7.1	0.0098	0.000507	19.3294
					0.1590 ±0.0198	**0.00766 ±0.000546**				**18.1873 ±2.3294**

Table 3 : Oxygen consumption, rate of ammonia excretion and O: N ratio of *Lamellidens marginalis* on January during Post winter

	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	79	14.998	0.1010	0.1434	0.0036	3.6	0.0090	0.000257	35.0195
	II	78	15.038	0.1062	0.1508	0.0035	3.5	0.0094	0.00025	37.6000
	III	79	15.883	0.0982	0.1394	0.0033	3.3	0.0087	0.000236	36.8644
	IV	79	17.120	0.1058	0.1502	0.0038	3.8	0.0094	0.000271	34.6863
	V	78	15.200	0.1104	0.1568	0.0040	4.0	0.0098	0.000286	34.2657
					0.1481 ±0.0068	**0.00364 ±0.00027**				**35.6872 ±1.4588**
Large Size	I	94	26.262	0.0783	0.1112	0.004	4.0	0.0070	0.000286	24.4755
	II	92	25.615	0.0854	0.1213	0.0038	3.8	0.0076	0.000271	28.0443
	III	93	27.015	0.0821	0.1166	0.0040	4.0	0.0073	0.000286	25.5245
	IV	92	26.467	0.0828	0.1176	0.0039	3.9	0.0074	0.000279	26.5232
	V	96	29.019	0.0905	0.1285	0.0043	4.3	0.0080	0.000307	26.0586
					0.1190 ±0.0064	**0.00400 ±0.000187**				**26.1252 ±1.3161**

The results of rate of oxygen consumption, ammonia excretion and O: N ratio was also determined (Table 2 and 3). The rate of oxygen consumption of individual animal in small size were ranged from 0.2379 – 0.3157 mg O_2/l/h (on December), 0.1394 - 0.1568 mg O_2/l/h (on January), and in large size were ranged from 0.1315 – 0.1857 mg O_2/l/h (on December), 0.1112 – 0.1285 mg O_2/l/h (on January), during winter season. The ammonia excretion of individual animal were ranged from 0.0041-0.0046 µg NH_3-N/l/h (on December) and 0.0033-0.0040 µg NH_3-N/l/h (on January) in small size and 0.0071-0.0084 µg NH_3-N/l/h (on December) and 0.0038-0.0043 µg NH_3-N/l/h (on January) in large sized animal during winter season. The calculations of O: N ratio after determining the atomic equivalent of oxygen and nitrogen were ranged from 46.7290-59.8784 (on December) and 34.2657-

37.6000 (on January) in small and 14.3608-20.2335(on December) and 24.4755- 28.0443(on January) in large sized animal.

The values of rate of oxygen consumption were 0.2676±0.0317 mg O_2/l/h (on December) and 0.1481±0.0068 mg O_2/l/h (on January) in small sized bivalve and 0.1590±0.0198 mg O_2/l/h in (on December) and 0.1190±0.0064 mg O_2/l/h (on January) in large sized bivalves. The rate of ammonia excretion in small and large sized animal were 0.0044±0.0002 µg NH_3-N/l/h (December), 0.00364±0.00027 µg NH_3-N/l/h (January) in small sized and 0.00766±0.00546 µg NH_3-N/l/h (December), 0.00400±0.000187 µg NH_3-N/l/h (January) in large sized animals respectively. The O: N ratio showed higher values 53.1675±5.0816 and 35.6872±1.4588 on December and January respectively in small sized bivalve and lower values 18.1873±2.3294 and 26.1252±1.3161 on December and January respectively in large sized bivalves during winter season.

DISCUSSION

In the present work on freshwater bivalve, *Lamellidens marginalis (*Lamark) from Bhima River at Siddhatek, during winter season, the rate of oxygen consumption was more in small sized bivalves as compared to large sized ones. The rate of ammonia excretion found more in large sized bivalves. The rate of oxygen consumption increased in small 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 relatively more glycogen storage [23]. The metabolic processes of animals are considerably affected by their body sizes or length. Small clam have more respiratory rates than medium and large clam in *K.* opima [24]. Also in *S. diphos* more respiration rate in small shell length than medium, large and old sized group of clams [4].The metabolic rate 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 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 [24]. This generalization applies in both intra-specific comparisons between bivalve molluscs of different sizes as well as inter-species belong to same species or different.

In the present study on *Lamellidens marginalis*, the size specific oxygen consumption followed a general trend of acceptance i.e. higher values of oxygen consumption for smaller sized bivalves than larger sized. Similar result was found by [25] in *V. cyprinoides*, [26] in *K. opima*, [27] in *Indonaia caeruleus*, *[*7, 8] in *Lamellidens marginalis* and [28] in *Soletellina diphos*. [24] and [29] 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 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 sized bivalve particularly during winter because it is known that, the oxygen uptake was mainly dependent on reproductive condition of bivalves in winter season. The energy utilization in oxygen consumption and ammonia excretion was significantly different, which depending on size, season and temperature but season being important factor which affect the overall fitness of the animal [16, 7, and 8].

Many authors have shown that, the ammonia in general considered as major nitrogenous excretory product of bivalves and there occur profound difference in loss of nitrogen between different sizes and seasons [30, 31]. In the present study on *Lamellidens marginalis*, the rate of ammonia release showed more increase large sized bivalves on December and January during winter seasons, because it is known that small size bivalves catabolise different biochemical substrates to varying degrees, according to season [22, 32]. Also similar result was found by [27] in *Indonaia caeruleus* and [8]in *Lamellidens marginalis*.

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 [33].

In *Thias Lapillus* [34], the O: N ratio did not alter with size that is exponent for rate of oxygen conception and ammonia excretion against body weight. However, in *Mytilus* the O: N ratio varied considerably with size and complex interaction with season and temperature [9]. [29] 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 sized bivalve animals than the large sized bivalve animals. Also similar result was of found by [27] in *Indonaia caeruleus* and [7, 8] in *Lamellidens marginalis*.

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

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