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Seasonal variation in oxygen : nitrogen ratio of *Soletellina diphos* of Bhatye estuary, Ratnagiri coast, India

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Abstract

In present investigation, oxygen consumption rate (OCR) and ammonia excretion rate (AER) of estuarine clam *Soletellina diphos* was estimated seasonally. The OCR in clams was higher in all size groups during winter season, while in summer lowest OCR was observed. Smaller clams consumed more oxygen than medium and large sized groups, respectively. AER was maximum during winter season, while minimum during summer season. The OCR and AER in small size clams were higher than medium and large sized clams. The maximum O: N ratio value (5.16) was observed in small size clams during summer followed by medium (3.27) and large size (2.66) clams, while minimum O:N ratio value was recorded in post-monsoon for small (3.23), medium (2.01) and large size (1.77) clams respectively. The smaller clams showed higher O:N ratio than medium and large size groups respectively. In present study, the O: N ratio was used as a tool to identify the stress or impact of seasonal variation on the natural population of clams from Bhatye estuary at Ratnagiri.

Key words

Soletellina diphos, Oxygen consumption, Ammonia excretion, Oxygen-Nitrogen ratio

Introduction

Clams are sessile organisms and can't move from unfavorable to favorable environment. Animals are physically, physiologically or metabolically adapted at unfavorable environment. The studies on physiological and metabolic activity of marine organisms are useful to assess their adaptation mechanisms especially to habitat condition (Stead and Thompson, 2003). The respiration, excretion and energy balance in poikilothermic marine organism as well as their distribution in natural habitat are under control of environmental conditions (Tang *et al.*, 2005; Resgalla *et al.*, 2007). The organism adjust their metabolic activity under unfavorable environment by adaptation processes, the adaptive mechanism is basically depend on metabolic rate of animals. The metabolic rate in organism is reorganize under both, short and long term variation in environmental conditions (Hochachka and Somero, 2002; Portner, 2002 a, b). Respiratory limitations of an organism under changing environmental conditions are responsible for shifting of habitat by that organism (Jansen *et al.*, 2007).

For physiological or metabolic studies, the scientist has been given more attention towards the Lamellibranchs species (Peck and Conway, 2000). Several attempts were made to study the respiration rate in molluscs and other invertebrates from all parts of world. The physiological, metabolic and energetic studies in filter-feeding bivalves have been well documented in *Yoldia hyperborean* (Stead and Thompson, 2003), *Pinctada mazatla* (Pedro *et al*., 2004) *Acesta excavata* (Jarnegren and Altin, 2006), *Mytilus spp. and Macoma balthica* (Jansen et al., 2007) and *Katleysia opima* (Kamble and Muley, 2009).

In aquatic organisms, ammonia excretion helps in elimination of wastes and conservation of useful metabolites for growth, maintenance and reproduction. Nagabhushanam and Mane (1991) reported that the rate of nitrogen excretion by bivalve molluscs was extremely variable during seasonal changes in nutrient storage and utilization of reserve. Respiration and excretion rates are used to understand the metabolic or physiological activity of an animal (Heilmayer and Portner, 2004).

Present investigation revealed the detailed account on seasonal variation in oxygen consumption, ammonia excretion as well as O: N ratio of *Soletellina diphos.* 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

Animal collection and maintenance: The selected clams *Soletellina diphos* Linn. were collected from Bhatye estuary (16^o 51'N, 73^o 15'E) near Ratnagiri city. After collection, clams were cleaned and washed with the sea water and grouped in to three size group's viz. small (7.5-8.5 cm), medium (9.5-10.5 cm) and large (12.5-13.5 cm) followed by 48 hrs acclimatization under laboratory conditions. In all the seasons (summer, monsoon, post-monsoon and winter) same procedure was followed for animal collection and their maintenance in laboratory. For experimental work only healthy clams were selected and tested.

Physiological procedure: Oxygen consumption rate (OCR) was determined by developing glass apparatus in the laboratory resembling the Galtsoff and Whipple apparatus (1930). Estimation of OCR rate was determined by glass respiratory jars having 1 liter capacity, wrapped it with black papers to provide natural habitat background for clams as they live 45 to 60 cm deep into mud. The selected clams of respective sizes were placed in the jar and continuous flow of filtered seawater was supplied through inlet till clam fully opened their siphon and extended the visceral organ. Initial sampling was done before animal placing for oxygen and ammonia estimation. And after one hour, once again the sampling was done to estimate the OCR and AER. On each attempt, water sample is siphoned out in stoppered bottle having capacity of 300 ml. Oxygen content from water sample was estimated by modified Winkler's method (APHA, 2005). Simultaneously, 10 ml of water sample was used from same jar for estimation of ammonia by phenol-hypochloride method (Sallorzano, 1969).

The oxygen consumed by clams was expressed in (O_2 $ml^{-1}hr^{-1}g^{-1}$) and converted in O_2 $mgl^{-1}hr^{-1}g^{-1}$ by multiplying with the conversion factor (1.428). Ammonia excreted by clam was expressed in $\mu gl^{-1}hr^{-1}g^{-1}$. The O:N ratio was statistically calculated from atomic equivalents of oxygen consumption and ammonia excretion using formula as suggested by Widdows (1985).

At the end of experimentation, the animals were removed from shell, blotted to remove the excess water and weighed in electronic balance having range from (0.001 mg). Each experiment on oxygen consumption and ammonia excretion rate was repeated 3 times and observations were calculated for arithmetic mean and standard deviation.

Results and Discussion

In the present study, the OCR in *S. diphos* varied with size groups and seasons. The highest value of OCR was found in winter and lowest in summer season for all size groups. The OCR started increasing from monsoon season and increased up to winter and after that it declined in the summer season (Table 1). Under varying temperature and nutritive stress, the body conditions were altered and resulted in a rapid decline in oxygen consumption. Such seasonal variability in oxygen consumption rate of clams

Table 1 : Seasonal variation in oxygen consumption and ammonia excretion rates in *Soletellina diphos*

Season	Clam size (cm)	Wet weight (g)	Oxygen consumption rate ($mgl^{-1}hr^{-1}g^{-1}$)	Ammonia excretion rate ($\mu gl^{-1}hr^{-1}g^{-1}$)	Atomic equivalent O	Atomic equivalent N	O : N ratio
Winter	Small (7.5-8.5)	30.907	0.234 ± 0.012	0.053 ± 0.002	0.0146	0.0037	3.87
	Medium (9.5-10.5)	60.79	0.116 ± 0.016	0.045 ± 0.009	0.0072	0.0032	2.22
	Large (12.5-13.5)	86.44	0.080 ± 0.008	0.039 ± 0.008	0.0050	0.0027	1.80
Summer	Small (7.5-8.5)	12.96	0.149 ± 0.016	0.025 ±0.011	0.0093	0.0018	5.16
	Medium (9.5-10.5)	67.137	0.086 ± 0.014	0.023 ± 0.018	0.0054	0.0016	3.27
	Large (12.5-13.5)	73.409	0.034 ± 0.011	0.011 ± 0.022	0.0021	0.0008	2.66
Monsoon	Small (7.5-8.5)	13.264	0.175 ± 0.009	0.033 ± 0.015	0.0109	0.0023	4.58
	Medium (9.5-10.5)	67.200	0.095 ± 0.005	0.029 ± 0.008	0.0059	0.0021	2.78
	Large (12.5-13.5)	86.74	0.053 ± 0.004	0.022 ± 0.022	0.0033	0.0016	2.07
Post-monsoon	Small (7.5-8.5)	39.23	0.186 ± 0.013	0.0505 ± 0.011	0.0116	0.0036	3.23
	Medium (9.5-10.5)	92.32	0.098 ± 0.010	0.0429 ± 0.007	0.0061	0.0030	2.01
	Large (12.5-13.5)	132.98	0.056 ± 0.018	0.0279 ± 0.024	0.0035	0.0019	1.77

Values are mean of three observations ± S.D.

may be due to alterations in the physiological cost of metabolism, seasonal gametogenic cycle, use of different substrate for energy metabolism and balance between metabolic active and inactive materials in the mantle and non-mantle tissues (Bayne and Thomson, 1970). In the present study, small size clams showed more oxygen consumption rate than medium and large sized clams. Yatian *et al.* (1999) has reported, the rate of oxygen consumption in scallop was inversely proportional to size of animals on the basis of wet weight and size of clam. Similar results were obtained by Chandran and Damodaran (2000) in *V. cyprinoides* and Kamble and Muley (2009) in *K. opima.* Sandip (2006) reported the oxygen consumption rate in pearl oyster *P. fucata* was significantly increased with increasing shell length and dry weight. Similar observation was observed in *Yoldia hyperborean* by Stead and Thompson (2003).

In excreta, nitrogen is a major component and it can be more readily estimated than mucus. Excretion rate used as an indicator of stress in animals (Smaal and Widdows, 1994). Ammonia is foremost excretory part of the protein catabolism in aquatic organism (Brockington, 2001). In experimental study, the AER also varied considerable throughout seasons and in different size groups of clams similar to OCR. The AER was found maximum during winter and minimum in summer season, identical trend were observed at all sized clams (Table 1). In the present study, despite the fact that maximum excretion rate was observed during winter whereas, it was observed minimum for *Y. hyperborean* (Stead and Thompson, 2003). Ammonia excretion rate in bivalves strongly fluctuates with seasons and other factors also responsible to decrease or increase the excretion rate are water temperature, food concentration, feeding activity, growth and gonadal development in particular season (Stead and Thompson, 2003). The smaller clams excreted more ammonia than the medium and large sized clams respectively. A similar relationship was observed in *C. farreri* and *K. opima* (Yang *et al.,* 1998; Kamble and Muley, 2009).

The respiration and excretion are major components of bioenergetics in bivalve and such physiological energetics are studied by means of the oxygen: nitrogen (O: N) ratio. The studies on physiology and energetics of commercial important bivalve species under environmental condition has been reported by Hutchinson and Hawkins (1992), Navarro *et al.* (1998), Pedro *et al.* (2004) and Tang *et al.* (2005). The O:N ratio measures balance between breakdown of protein and catabolism of carbohydrates and lipids. The ratio of O:N indicates the proportion of lipid and carbohydrate relative to protein which breaks down for energy metabolism (Stead and Thompson, 2003). In bivalve species, the metabolic activities are increased up to certain level, beyond which they suddenly decreased (Wang *et al.,* 2002).

In summer O: N ratio was observed maximum (5.16) while minimum in post-monsoon seasons (1.77). According to size groups, the higher values of O:N ratio was recorded in smaller clams followed by medium and large clam respectively. The results of O:N ratio obtained in present study was close to those obtained in *K. opima* (Kamble and Muley, 2009). On the basis of seasonal comparison, the O:N ratio varied considerable throughout seasons and maximum value was found in summer season, from summer it started to decline and reached lower level in post-monsoon and was increased from winter season (Table 1). The low O: N ratio indicates a relative increase in use of protein as an energy yielding substrate. Minimum O: N ratio is associated with an increase in ammonia-excreta due to an increased use of protein as an energy substrate. High values of O: N ratio indicates the increased catabolism of carbohydrate and lipid (Bayne, 1976) and low values of O: N ratio indicates protein metabolism (Mace and Ansell, 1982). Thus it is suggested that energy accumulates in the form of lipid and carbohydrate which fluctuates with physiological activities induced by seasonal environmental changes. The environment condition regulates the physiological and metabolic activities of animals. Therefore, the seasonal study is necessary to test the physiological index of an organism.

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