

Effect of salinity, starvation and feeding on ammonia excretion of a mollusc *Babylonia spirata* (Neogastropoda: Buccinidae)

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The rate of ammonia excretion of adult *Babylonia spirata* increased at salinity 31×10^{-3} in higher exposure time, whereas, in juveniles the rate of excretion was high at all the exposure periods of 31×10^{-3} salinity. In general, the rate of excretion in both adult and juvenile increased with the decrease of salinity. The rate of ammonia excretion during starvation and after feeding increased with extension of period and the 'r' values were statistically significant.

The spiral babylon, *Babylonia spirata* is an important commercial mollusc¹ occurring in intertidal zones. The gastropod is a continuous breeder and is abundantly available in all the seasons except monsoon (October-December), hence the production of metabolites by dense population may represent a significant method of recycling nutrients in intertidal zones. Numerous studies²⁻⁵ have shown the potential importance of invertebrates in nutrient cycling in aquatic environment. Ammonia excretion, however, represents only one pathway in which nitrogenous compounds can be lost to the environment. The contribution of ammonia from benthic communities is four to six times the contribution from river run off and sewage input in Narragansett Bay⁶.

Gastropods are common in the intertidal regions and has been commercially exploited for meat, shell and operculum¹. The present study examines the effect of salinity, starvation and feeding on the excretion of ammonia in *B. spirata* in order to elucidate, further, the factors which affect the potential role of macroinvertebrates in the regeneration of nutrients in marine environment.

Materials and Methods

Juvenile and adult molluscan species *Babylonia spirata* (Neogastropoda: Buccinidae) were collected from Annappanpettai located north of Parangipettai (lat. $11^{\circ}29'N$; long. $79^{\circ}46'E$), on the southeastern

coast of India. Experimental gastropods were brought to the laboratory and transferred to plastic tanks containing filtered seawater for acclimatization for a period of 5 days. No food was provided throughout the above period.

The rates of ammonia excretion in juvenile and adult *B. spirata* were determined at 3 different salinities 31, 33 and 35×10^{-3} . After acclimatization, animals were placed individually into plastic tanks with 2000 ml filtered seawater of respective salinity. Ten animals were tested in each salinity. Control tanks were set up without animal and kept under same conditions. All the experimental tanks were continuously aerated. Water samples were removed from control as well as experimental tanks at 24 hours interval and assayed for ammonia⁷, nitrate and nitrite⁸.

For the analysis of rate of ammonia excretion of starved *B. spirata*, the acclimatized animals were starved for 15 days before the start of experiments. In the case of feeding experiments, test animals were fed with a bivalve *Meretrix meretrix* until they abandoned the food. These fed gastropods were introduced immediately into the experimental tanks. The experimentals were fed with the same diet in between the experiments and the unfed material was removed immediately. In both the experiments 10 of each juveniles and adults were tested individually and all the experiments were conducted as previously described.

Table 1—Ammonia excretion of adult and juvenile *B. spirata*

Test salinity ($\times 10^{-3}$)	Mean $\pm$ SD of ammonia excretion ($\mu\text{g/g/24 h}$)				
	Period (h)				
	24	48	72	96	120
Adults					
31	2.265 $\pm$ 1.17	3.51 $\pm$ 2.07	4.553 $\pm$ 2.11	9.892 $\pm$ 2.41	10.030 $\pm$ 2.03
33	2.324 $\pm$ 1.31	3.948 $\pm$ 2.73	4.681 $\pm$ 3.27	8.354 $\pm$ 1.59	9.822 $\pm$ 1.28
35	4.596 $\pm$ 1.19	6.484 $\pm$ 1.39	7.354 $\pm$ 1.56	8.529 $\pm$ 1.53	9.233 $\pm$ 1.79
Juveniles					
31	29.465 $\pm$ 26.11	44.297 $\pm$ 34.2	50.489 $\pm$ 33.8	60.750 $\pm$ 36.8	100.596 $\pm$ 37.4
33	9.687 $\pm$ 6.22	12.863 $\pm$ 4.93	18.805 $\pm$ 8.98	28.601 $\pm$ 12.42	57.369 $\pm$ 32.2
35	13.022 $\pm$ 8.38	18.071 $\pm$ 9.42	38.124 $\pm$ 24.3	45.748 $\pm$ 26.3	81.419 $\pm$ 27.8

Table 2—Correlation coefficient values of rate of ammonia excretion of adult and juvenile *B. spirata*

Table 2—Correlation coefficient values of rate of ammonia excretion of adult and juvenile <i>B. spirata</i>						
Parameter		Test salinity (10^{-3})				
		31	33	35		
Correlation between rate of ammonia excretion and time duration at different salinities	Adult	0.819*	0.785*	0.787*		
	Juvenile	0.615	0.678*	0.739**		
		Time intervals (hours)				
		24	48	72	96	120
Correlation between rate of ammonia excretion and salinity at different time intervals	Adult	-0.156	0.502*	0.479**	-0.258	-0.006
	Juvenile	-0.427*	-0.447**	-0.268	-0.228	-0.219

* $P < 0.05$; ** $P < 0.01$

At the end of each experiment, the gastropod shell was broken and the soft tissues were separated and blott dried and the wet weights were noted. The amount of ammonia produced by each individual was calculated by subtracting the total values of ammonia, nitrate and nitrite in experimental tanks from the control values. The final results are expressed as μg ammonia/g wet weight/24 hours. All the data were analysed using regression analysis.

Results

The mean $\pm$ SD and correlation coefficient values of the rate of ammonia excretion of adult and juvenile *B. spirata* exposed to salinities are given in Tables 1 and 2 respectively. The adult *B. spirata* exposed to 31×10^{-3} salinity showed increased rate of

excretion at higher exposure periods such as 96 and 120 hours (Table 1). Table 2 shows that, in all the salinities, the rate of ammonia excretion increased with increase in time and the correlation coefficient values (r) were statistically significant ($P < 0.01$). The correlation between rate of ammonia excretion and salinity at different time intervals reveals that, the rates of excretion after 48 and 72 hours increased with increase in salinity and the ' r ' values had the statistical significance. But after 96 and 120 hours, the rate declined with increase in salinity and ' r ' values showed negative correlation.

In juveniles the rate of excretion was high at all the exposure periods at 31×10^{-3} followed by 35 and 33×10^{-3} salinities (Table 1). As in adults, the rate of excretion of juveniles also increased with time duration. However, the statistical significance was

Table 3—Rate of ammonia excretion of adult and juvenile *B. spirata* during starvation and after feeding

Hours of exposure	Mean $\pm$ SD of ammonia excretion ($\mu\text{g/g/24 h}$)	
	Adult	Juvenile
During Juvenile		
24	2.032 $\pm$ 1.6	1.228 $\pm$ 0.64
48	3.676 $\pm$ 1.1	10.429 $\pm$ 4.2
72	5.876 $\pm$ 1.7	15.479 $\pm$ 8.5
96	10.724 $\pm$ 4.6	19.077 $\pm$ 10.1
120	12.732 $\pm$ 4.3	29.195 $\pm$ 12.9
	$r=0.803^{***}$	$r=0.769^{***}$
After feeding		
24	2.274 $\pm$ 0.92	9.214 $\pm$ 4.1
48	5.758 $\pm$ 1.32	13.289 $\pm$ 6.9
72	8.530 $\pm$ 2.4	24.245 $\pm$ 8.18
96	10.377 $\pm$ 2.4	36.049 $\pm$ 12.8
120	13.477 $\pm$ 3.7	43.146 $\pm$ 10.9
	$r=0.823^{***}$	$r=0.823^{***}$

*** $P<0.001$

found only in 33 ($P<0.05$) and 35×10^{-3} ($P<0.01$) salinities. The correlation at different time duration in relation to salinity (Table 2) shows only negative correlation at all the time duration and the ' r ' values of 24 and 48 hours were statistically significant.

Table 3 depicts the rate of ammonia excretion of adult and juvenile *B. spirata* during starvation and after feeding. From the results it is clear that, in both the cases the rate of excretion increased with extension of starvation period and the ' r ' values were statistically significant ($P<0.001$), whereas in fed animals, as in starvation the excretion rate increased with duration and the ' r ' values are statistically significant.

Discussion

In marine gastropods, ammonia forms the bulk of the excreted nitrogen⁹ and factors such as body size¹⁰, temperature^{11,12}, salinity¹³ and nutritional state¹⁴ are known to influence ammonia excretion. In the present study the rate of excretion was examined only in 31, 33 and 35×10^{-3} salinities, since the normal behaviour such as locomotion and feeding of juveniles as well as adults were reported between 27-35 and $31-35 \times 10^{-3}$ salinities respectively¹⁵.

In the present study, the rates of ammonia excretion of juvenile and adult *B. spirata* were high at 31×10^{-3} salinity. The large amount of ammonia excreted at low salinity may be due to the changing ionic conditions of the test media. Allan & Garrett¹⁶ observed large amount of ammonia production in first few days following transfer of *Mya* sp. to seawater of lower salinity and suggested that the production of large amount of ammonia is related to the loss of free amino acids and taurine to meet the animals changing physiological requirements to the new external ionic condition. By transferring an animal from its natural medium to either a hypo or hyperionic medium causes a physiological stress or shock¹⁷. An increase in ammonia excretion, as salinity decreased, was also noted in *Mya arenaria*¹⁶, *Thais lappillus*¹³ and *Nuttilus edulis*¹⁸. This was explained by either an increased catabolism of the amino acids involved in osmotic regulation^{12,19} or polypeptide synthesis¹⁷. It was suggested that the increase in ammonia excretion in response to dilution might have been due to increased sodium input^{20,21}. Bartberger & Pierce²² observed an increase in both hemolymph ammonia concentrations and external ammonia excretion in *Modiolus demissus* at low salinity. They further suggested that, during low salinity acclimation free amino acids are released intact from the cell into the hemolymph. The subsequent transitory increase in hemolymph ammonia concentrations and external ammonia excretion rates were probably due to degradation of the effluxed amino acids by some specific organs of the body.

The results also reveal that, both in adult and juvenile *B. spirata*, the rate of ammonia excretion increases during starvation and after feeding and the increases were statistically significant. The ammonia excretion in *Thais lappillus* increased throughout starvation period, indicating the catabolism of protein for energy during long term starvation¹⁴.

In the present study, the increased ammonia excretion after feeding was not due to the feeding material used for the experiment because it contains only a small amount in their body ($1.044 \pm 0.5 \mu\text{g}$). After feeding *Tetraselmis* sp. and *Mutulus edulis* displayed an increase in ammonia excretion²³. *Nassarius* sp. and *Buccinum* sp. also exhibit a

transient peak of ammonia excretion after a feed¹⁴. Takahashi & Ikeda²⁴ found a correlation between both ammonia and phosphorus excretion and the rate of feeding *Euphausia pacifica* and *Metridia pacifica* which were fed with phytoplankton. They also suggested that the ammonia excretion by population of aquatic consumers will depend on the feeding level which, in turn, may be related to the abundance of food resources.

The changing environmental salinities and nutritional state of the animals affect the metabolic responses of aquatic organisms which in turn may influence the processing and turnover of nutrients in marine environment. Further study to assess the significance of ammonia release in nutrient regeneration in marine ecosystem will be of great use.

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References

- 1 Ayyakkannu K, *Phuket Mar Biol Spec Publ No.* 13 (1994) 53.
- 2 Cockcroft A C & McLachlan A, *Mar Biol*, 96 (1987) 343.
- 3 Cockcroft A C, Webb P & Wooldridge T, *Mar Biol*, 99 (1988) 75.
- 4 Cockcroft A C, McLachlan A, *J Mollus Stud*, 56 (1990) 469.
- 5 Mc Carthy J J & Whittledge T E, *Fish Bull US*, 70 (1972) 395.
- 6 Hale S S, in *Mineral cycling on southeastern ecosystems*, edited by F G Howell J B Gentry & M H Smith, ERDA symposium series, (National Technical Service, US Department of Commerce, Springfield, USA) 1975, 291.
- 7 Solorzano L, *Limnol Oceanogr*, 14 (1969) 799.
- 8 Strickland J D H & Parsons T R, *Bull Fish Res Bd Can*, 167 (1968) 1.
- 9 Huges R N, *A functional biology of marine gastropods*, (Croom Helm, London) 1986, 233.
- 10 Johannes R, *Science*, 146 (1964) 923.
- 11 Mace A M & Ansell A D, *J Exp Mar Biol Ecol*, 60 (1982) 275.
- 12 Emerson D N, *Comp Biochem Physiol*, 29 (1969) 1115.
- 13 Stickle W B & Bayne B L, *J Exp Mar Biol Ecol*, 58 (1982) 1.
- 14 Crisp M & Gill C W, *J Mar Biol Ass UK*, 61 (1981) 381.
- 15 Jamila Patterson, Shanmugaraj T & Ayyakkannu K, *Phuket Mar Biol Cent Spec Publ No.* 13 (1994) 185.
- 16 Allan J A & Garrett M R, *Comp Biochem Physiol*, 39A (1971) 633.
- 17 Sppargaren D H, Richard P & Ceccaldi H J, *Comp Biochem Physiol*, 72A (1982) 127.
- 18 Bayne B L, *J Mar Biol Ass UK*, 53 (1973) 39.
- 19 Haberfield E C, Haas L & Hanman C S, *Comp Biochem Physiol*, 52A (1975) 501.
- 20 Mangum C P, Silverthorn S V, Harris J L, Towle D W & Krall A R, *J Exp Zool*, 195 (1976) 129.
- 21 Pressley T A, Graves T S & Krall A R, *American J Physiol*, 241 (1981) 370.
- 22 Bartberger C A & Pierce S K Jr, *Biol Bull*, 150 (1976) 1.
- 23 Bayne B L & Scullard C S, *J Mar Biol Ass UK*, 57 (1977) 371.
- 24 Takahashi M & Ikeda T, *Fish Res Bd Can*, 32 (1975) 2189.