Vol. 1, 315–321, 1979 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published December 31

Effects of Ammonia and Ammonium on Tolerance and Byssogenesis in *Perna viridis*

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ABSTRACT: Individuals of the commercially important green mussel *Perna viridis* (Kuriakose and Nair, 1976) were exposed to different concentrations of ammonia (NH_3) and ammonium (NH^+_4), to determine their reactions with reference to tolerance and byssogenesis. Eight concentrations of ammonia, ranging from 2.75 to 33.00 mg l^{-1}, and 7 concentrations of ammonium, ranging from 5.00 to 75.00 mg l^{-1}, were tested. Young individuals in the size range 20 to 24 mm shell length, collected from an unpolluted rocky shore, were used as test organisms. The 96-h lethal concentration causing 50 % mortality (LC 50) was 7.60 mg l^{-1} for NH_3 and 13.00 mg l^{-1} for NH^+_4. For ammonia, the difference between LC 50 values at 48 and at 96 h is drastic. In sublethal concentrations *P. viridis* retarded or suspended pedal-gland activity. A sharp reduction in the number of threads produced occurs at 5.50 and 8.25 mg l^{-1} of ammonia. At higher concentrations the byssus threads secreted displayed reduced rates of tanning and hardening. The difference in the number of byssus threads produced at various ammonia concentration was highly significant. Compared to controls the discs developed at the tips of the byssus threads did not possess normal adhesive properties.

INTRODUCTION

This paper is part of an investigation of the ecology of waters off Mangalore (Arabian Sea), which receive effluents comprising mainly urea and ammonia from a fertilizer complex. *Perna viridis* (Syn. *Mytilus viridis*) forms extensive beds on the rocky shores along the coast of Mangalore. This species is regularly exploited by local fishermen. The analysis of its tolerance to various concentrations of ammonia and their effect on byssogenesis can help to elucidate potential influences of ammonia on essential life processes of this commercially important bivalve.

Information on the effect of NH_3 and NH^+_4 on the life processes of marine organisms is scarce (for a review consult 'Marine Ecology' Volume III: Kinne, 1976, pp. 80–93). Working on the effects of ammonium nitrate on fertilization and early development of *Chromytilus meridionalis* (Brown, 1974) found that concentrations as low as 0.5 ppm retard early development. Currie et al. (1974) obtained similar results. Brown and Currie (1973) determined the tolerance of *Bullia digitalis* to solutions of $NH_4 NO_3$ in natural sea water.

MATERIAL AND METHODS

The green mussel *Perna viridis* (Kuriakose and Nair, 1976) was collected from an unpolluted region at Someswara rocky shore (12°47'N; 74°51'E). All individuals were obtained during low tide from the subtidal belt. They were transported to the laboratory and kept unfed in large polythene trays, in aerated sea water for 24 h before commencement of the experiments. Young mussels in the size range 20 to 24 mm were used for the experiments. In a single set of experiments only members of the same population were examined.

Liquor ammonia, containing 25 % free ammonia was the source of molecular ammonia (NH_3) and ammonium sulphate was the source of ammonium (NH^+_4). Mortality tests were conducted over 96 h. Inability to close the valves upon mechanical stimulus and valve gaping of 5 mm, were the criteria used to define death. To check the possibility of revival, those mussels considered dead by the above criteria were exposed to normal control conditions for 24 h. Dead individuals were removed from the experimental media at 12 h intervals. The experimental vessels were cylindrical glass troughs of 5 l capacity containing 4 l filtered sea water. Ten mussels were exposed to each concentration (Table 2 and 3). Byssus-thread formation was calculated as the number of threads secreted by one mussel. Readings were taken at 12 h intervals (at 0600 and 1800 h). Since the majority of the threads formed developed adhesive discs, these discs were counted and then the whole byssal mass (stem and threads) was cut-off flush with the valves, and the

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mussels were left in the test solution. In assessing the percentage of mussels which had attached, only the presence of byssus threads with adhesive discs was taken into consideration. It was found that cutting off the threads does not affect further byssogenesis.

Table 1. Concentrations of ammonia (NH_3) in various test solutions and their respective pH values. pH of control sea water = pH 8.05

NH_3 (mg l^{-1})	pH
1.375	8.225
2.750	8.325
5.500	8.475
8.250	8.600
11.000	8.700
16.500	8.850
22.000	9.000
33.000	9.200

RESULTS

The amount of the molecular form of ammonia increases conspicuously in the pH range of 8.0 to 9.5. The water temperature also plays a marked role in dissociation of NH_3 from its source. Waters of high temperature and high pH will therefore contain a high amount of the molecular form of ammonia, irrespective of the total ammonia concentration (Fig. 1). During the present study, the pH of the sea water varied between 8.0 and 8.3, the temperature between 28.0 ° and 30.0 °C. The addition of liquor ammonia greatly affected the pH of the test solution. Thus, an increase in NH_3 concentration from 1.375 to 33 mg l^{-1} altered the pH from 8.225 to 9.200. The increase in pH was directly proportional to the increase in NH_3 concentration (Table 1).

MORTALITY

Lethal Effects of Ammonia

Death prevailed in 5.5 and 8.25 mg l^{-1} NH_3 after 48 h exposure. 100 % mortality occurred before 96 h at 16.5 and 22.0 mg l^{-1}. No mussels kept at 33.0 mg l^{-1} survived beyond 36 h (Table 2; Fig. 2).

ET 50 values worked out from the results obtained on mortality are presented in Figure 2. It is clear from this

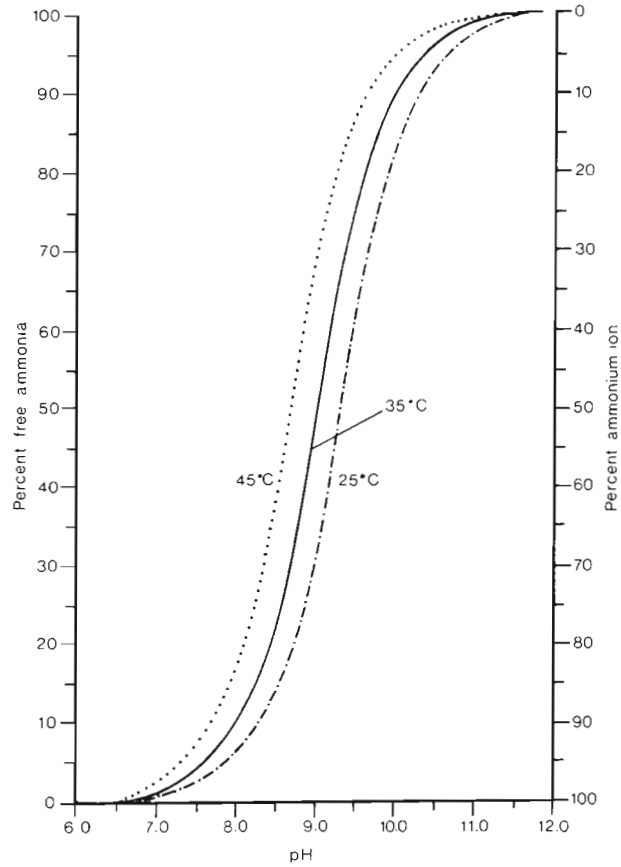

Fig. 1. Per cent free ammonia as a function of pH and temperature. (After Wuhurmann, 1947)

Table 2. *Perna viridis*. Cumulative percentage mortality of individuals exposed to different NH_3 concentrations

NH_3 Concentration (mg l^{-1})	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
2.75	0	0	0	0	0	0	0	0
5.50	0	0	0	20	20	20	20	20
8.25	0	0	0	20	20	20	40	50
11.00	0	0	0	20	40	40	60	100
13.75	0	0	0	40	40	60	80	100
16.50	0	0	20	40	40	80	100	
22.00	0	40	60	100				
33.00	0	60	100					

Table 3. *Perna viridis*. Cumulative percentage mortality of individuals exposed to different NH_4^+ concentrations

NH_4^+ Concentration (mg l^{-1})	Time (h) 12	24	36	48	60	72	84	96
Control:	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
10	0	10	20	20	20	20	20	20
15	0	20	60	60	70	70	70	80
20	0	30	50	80	80	90	90	90
50	0	20	90	100				
75	0	30	100					

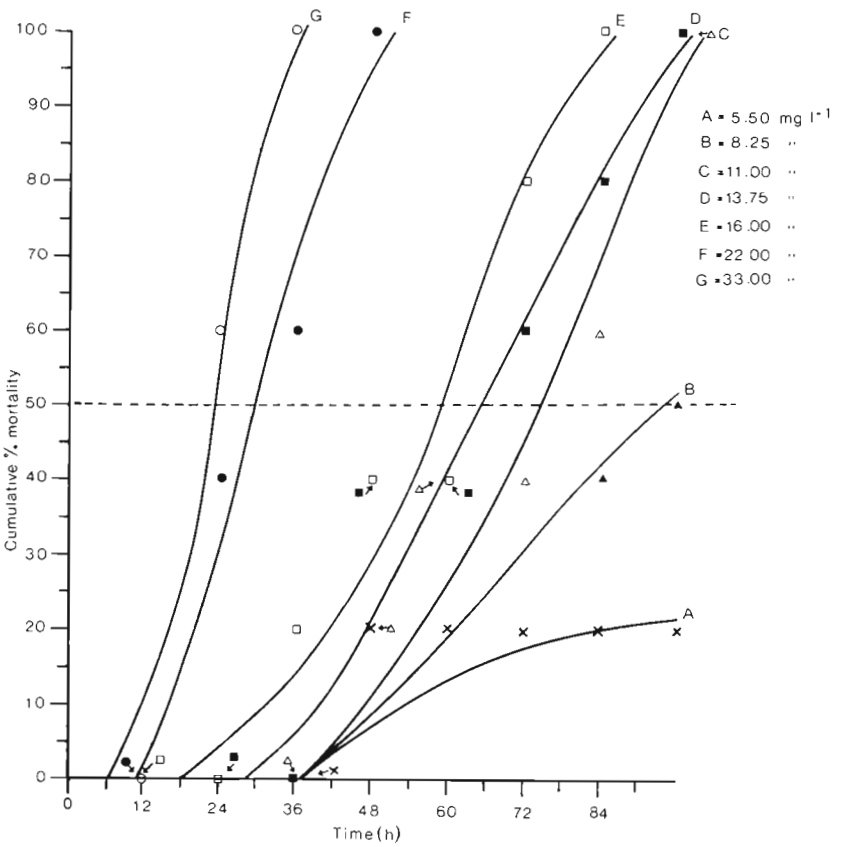

Fig. 2. *Perna viridis*. Mortality of individuals exposed to different NH_3 concentrations

Table 4. *Perna viridis*. Average number of byssus threads secreted by individuals exposed to various NH_3 concentrations. D = dark; L = light

NH_3 Concentration (mg l^{-1})	Time (h) D 12	L 24	D 36	L 48	D 60	L 72	D 84	L 96
Control:	46.2	40.4	54.2	47.0	54.0	34.6	76.0	47.6
2.75	30.4	32.2	37.2	25.0	44.5	23.4	68.6	41.0
5.50	9.6	7.6	11.4	12.0	15.4	9.0	26.4	16.0
8.25	1.2	0	1.0	0	0.4	0	1.4	0
11.00	0	0	0.6	0	1.0	0.4	0	0
13.75	0	0	0.6	0	0	0	0	0
16.50	0	0	0	0	0	0	0	0
22.00	0	0	0	0	0	0	0	0

figure that a conspicuous difference in time exists for the incidence of 50 % mortality between concentrations of 16.5 and 20.0 mg l^{-1}. The lethal concentration causing 50 % mortality (LC 50) in a given length of time values were derived for different concentrations. Figure 2 shows that the 48-h LC 50 is 14.0 mg l^{-1} the 96-h LC 50, 7.6 mg l^{-1} (Fig. 3). The 5 % confidence limits varied between 13.0 and 15.5 mg l^{-1} for 48 h and between 7.2 and 7.8 mg l^{-1} for 96 h.

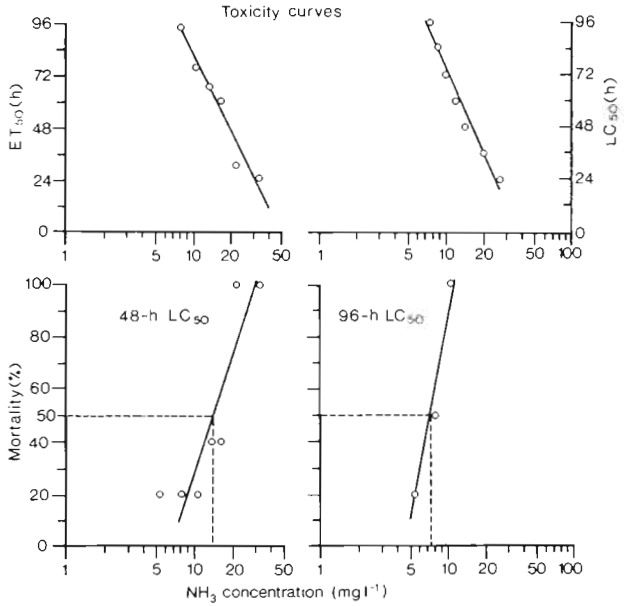

Fig. 3 *Perna viridis*. Median lethal concentrations (LC 50) for different NH_3-exposure times and toxicity curves

Lethal Effects of Ammonium

Ammonium sulphate at various concentrations was used so as to derive NH_4^+ values ranging from 5.0 to 75.0 mg l^{-1}. A slight reduction in pH occurred upon addition of $(NH_4)_2\ SO_4$; pH of the media ranged from 8.125 (5.0 mg l^{-1}) to 7.85 (75.0 mg l^{-1}).

Mortality data are presented in Table 3. 80 and 90 % of the test individuals were killed after 96 h exposure in 15.0 and 20.0 mg l^{-1} NH_4^+, respectively. 100 % mortality occurred in 48 h at 50.0 mg l^{-1} and in 36 h, at 75.0 mg l^{-1}. The ET 50 values, computed from mortality rates, show that 50 % of the test organisms died within 49.8 h at 15.0 mg l^{-1}, whereas 50 % died after 30 h at 75.0 mg l^{-1}. Curiously, the difference between the ET 50 values for 50.0 and for 75.0 mg l^{-1} is only 1.8 h (Fig. 4). The LC 50 values were derived from the mortality rates. The 48-h LC 50 was 15.5 mg l^{-1}; the 96-h LC 50, 13.0 mg l^{-1}. The 5 % confidence limits for the duration of 48 and 96 h range from 13.75 to 16.00 mg l^{-1} and 12.50 and 14.00 mg l^{-1}, respectively (Fig. 5).

BYSSOGENESIS

The process of byssus-thread secretion by some Bivalvia is referred to as byssogenesis. The rate of production, the nature of secretion, the structure of

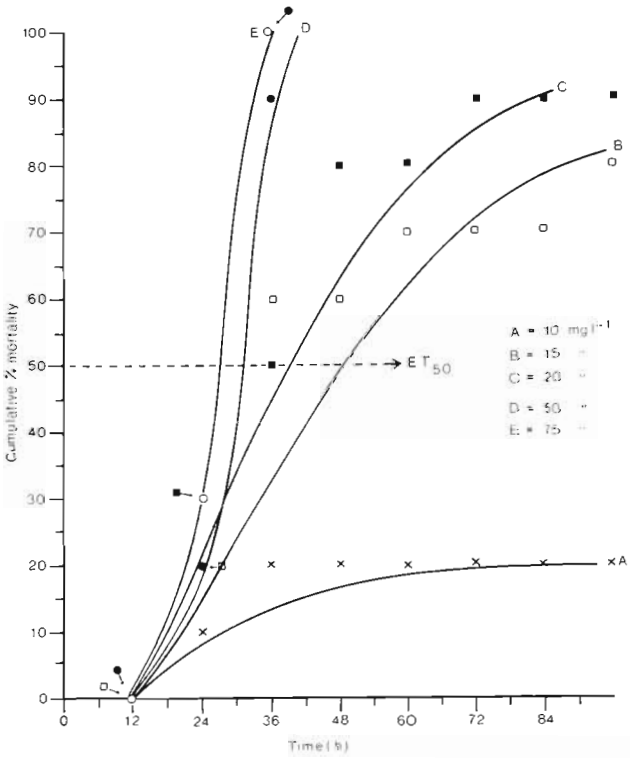

Fig. 4. *Perna viridis*. Mortality of individuals exposed to different NH_4^+ concentrations

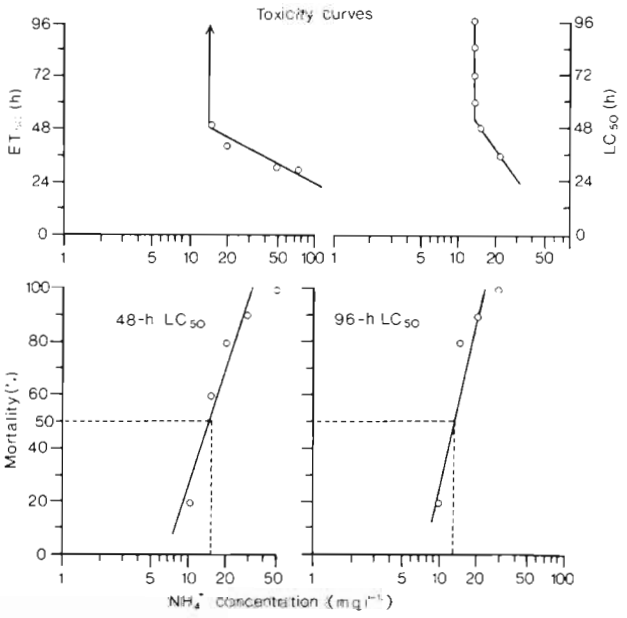

Fig. 5 *Perna viridis*. Median lethal concentrations (LC 50) for different NH_4^+-exposure times and toxicity curves

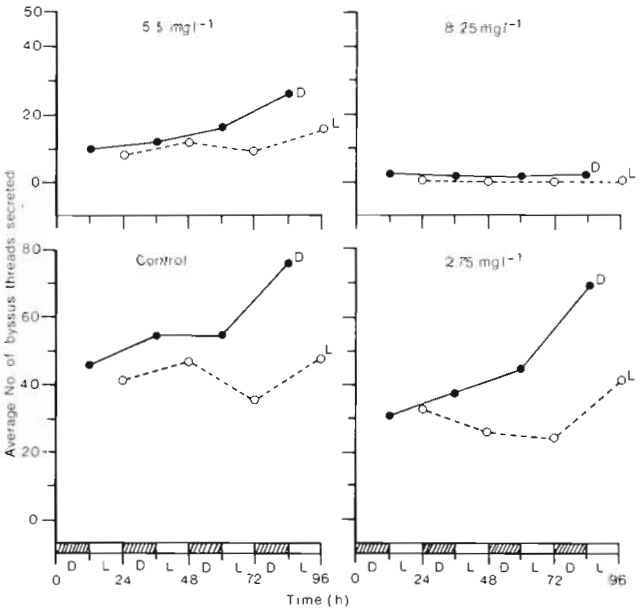

Fig. 6. *Perna viridis*. Rate of byssus-thread secretion by individuals exposed to different NH_3 concentrations

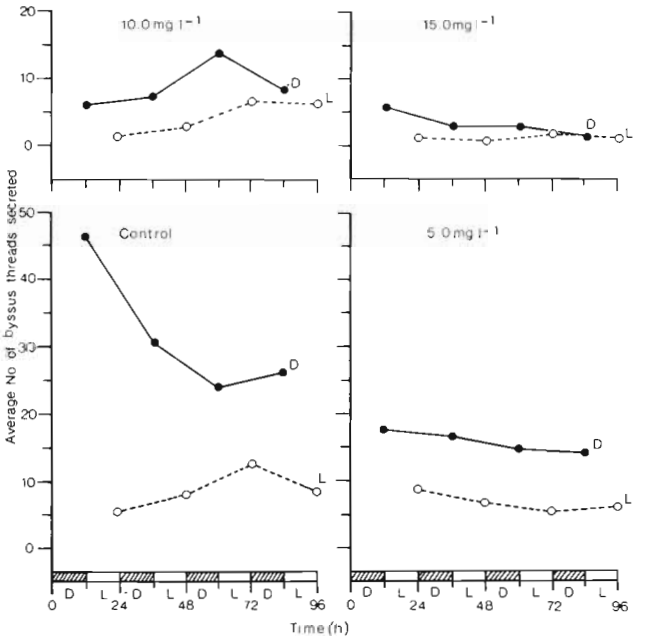

Fig. 7. *Perna viridis*. Rate of byssus-thread secretion by individuals exposed to different NH_4^+ concentrations

Table 5. *Perna viridis*. Percentage of mussels secreting byssus threads at 12-h intervals in various NH_3 concentrations. D = dark; L = light

NH_3 Concentration (mg l^{-1})	Time (h)							
	D 12	L 24	D 36	L 48	D 60	L 72	D 84	L 96
Control:	100	100	100	100	100	100	100	100
2.75	100	100	100	100	100	100	100	100
5.50	60	60	60	60	60	60	60	60
8.25	40	0	40	0	40	0	80	0
11.00	0	0	20	0	20	20	0	0
13.75	0	0	20	0	0	0	0	0
16.50	0	0	0	0	0	0	0	0
22.00	0	0	0	0	0	0	0	0

Table 6. *Perna viridis*. Average number of byssus threads secreted at 12-h intervals by individuals exposed to various NH_4^+ concentrations. D = dark; L = light

NH_4^+ Concentration (mg l^{-1})	Time (h)							
	D 12	L 24	D 36	L 48	D 60	L 72	D 84	L 96
Control:	46.3	5.4	30.3	7.9	23.7	12.5	26.0	8.3
5	17.4	8.4	16.2	6.5	14.2	4.9	13.8	5.7
10	6.0	1.2	7.1	2.8	13.8	6.6	8.0	5.9
15	5.2	0.9	2.4	0.2	2.4	1.6	8.8	0.5
20	3.6	0.8	1.0	0.2	0.5	0.3	0.3	0.5
30	0	0.2	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0

threads are important and useful criteria employed for assessing the activity of the bivalves concerned.

Ammonia

Results are presented in Table 4 and Fig. 6. Only at 2.75 and 5.50 mg l^{-1} did *Perna viridis* produce threads continuously. Byssus-thread production decreased in intensity as a function of increasing ammonia concentration. Individuals exposed to 16.5 and 22.0 mg l^{-1} did not produce any threads. In the 12-h interval, the rate of thread production was higher in darkness (1800 to 0600 h) than in light condition. The difference was statistically significant ($p = 0.05$). The results indicate that continuous exposure to the same concentration will not drastically affect pedal activity. However, the difference in the rate of byssus-thread secretion between the test concentrations employed was highly significant ($p = 0.01$). When the total number of byssus threads produced by all mussels exposed to different concentrations was analysed, a sharp decline occurred between individuals kept at 5.50 and 8.25 mg l^{-1} respectively. The 96-h LC 50 is 7.60 mg l^{-1}. However, when the percentage of mussels that have secreted byssus threads in different concentrations is taken into consideration, inactivity starts only from 13.75 mg l^{-1}, after 36-h of exposure (Table 5).

Ammonium

The intensity of byssus-thread secretion is presented in Table 6 and Fig. 7. In these tests, dark and light regimes influenced considerably the number of byssus threads produced at various ammonium concentrations. Secretion dropped significantly above concentrations at 10.0 mg l^{-1}. The mussels in higher concentrations produced a considerably lower number of threads.

The rate of production was negligible after 12 h in 15.0 and 20.0 mg l^{-1}. The mussels did not show any byssus production in 50.0 and 75.0 mg l^{-1}. Analysis of variance reveals that the variations between intervals were not statistically significant. However, the difference between concentrations was significant ($P = 0.01$). The total number of threads produced by any group of test individuals retained in each concentration for 96 h was also conspicuously different. Similarly, the percentage of test mussels capable of secreting byssus threads dropped clearly above 15.0 mg l^{-1} (Table 7). The differences between concentrations was significant ($P = 0.01$).

DISCUSSION

Both nitrite ion (NO_2^-) and ammonia are highly toxic to a wide variety of marine organisms (Becker and Thatcher, 1973). Adult *Crassostrea virginica* have a median ammonia tolerance limit (96-h TLm) of 0.082 mg l^{-1} (Epifanio and Srna, 1975). For *Mercenaria mercenaria*, these limits range from 0.011 to 0.082 ml^{-1} (110 to 880 mg NH_4Cl l^{-1}) for ammonia. *C. virginica* and *M. mercenaria* are considered very tolerant. The present observations on the LC 50 values show that for NH_3, the 48-h LC 50 is 14.0 mg l^{-1} and for NH_4^+, 15.5 mg l^{-1} [57.2 mg l^{-1} of $(NH_4)_2 SO_4$] in *Perna viridis*. At the above NH_3 concentration, the pH ranged between 8.70 and 8.85, for NH_4^+, the pH was 8.07.

The present study demonstrates that the tolerance limits of *Perna viridis* for NH_3 and NH_4^+ are different and that comparatively higher concentrations (13.0 mg l^{-1}) of NH_4^+ could be tolerated up to 96 h. Individuals of *P. viridis* exposed to higher concentrations secreted considerable quantities of mucus and their pseudofaeces showed a gelatinous consistency. The biological consequences of ammonia accumulation have been reviewed in 'Marine Ecology', Volume III by Kinne (1976). In freshwater fishes, high ammonia levels can

Table 7. *Perna viridis*. Percentage of mussels secreting byssus threads at 12-h intervals in various NH_4^+ concentrations. D = dark; L = light

NH_4^+ Concentration (mg l^{-1})	Time (h)							
	D 12	L 24	D 36	L 48	D 60	L 72	D 84	L 100
Control:	100	100	100	100	100	100	100	100
5	80	100	80	100	89	89	90	100
10	60	25	70	45	67	78	70	80
15	50	12.5	30	10	34	23	20	20
20	40	12.5	10	10	11	11	10	10
30	0	6.25	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0

lead to hyperplasia of the gill epithelium. According to Currie et al. (1974), NH_4NO_3 concentrations up to 10 ppm. have no significant effect on fertilization of *Chromytilus meridionalis,* although embryonic development was retarded, even at values as low as 0.5 ppm. The asymptotic pattern of the ET 50 curve indicates that low NH_4^+ concentrations do not cause lethal effects, indicating that NH_4^+ is less toxic than NH_3.

Where *Perna viridis* has survived ammonia and ammonium exposure, byssus-thread formation tended to be affected negatively and the pedal activity dropped in concentrations just above the threshold levels. The decrease in the activity is more conspicuous in NH_3 than NH_4^+ exposed individuals.

The site of NH_3 and NH_4^+ entry could be the mantle, gill, gut or the adductor muscle. In general, the rate of NH_3 entry through the cell wall is quicker than that of NH^+_4. Due to increased dissociation of NH_3 at pH values above 8.2, maintenance of high pH in the sea water bathing the mantle and gills of *P. viridis* during exposure, will result in an increased NH_3 entry into the tissue. *P. viridis* is found to thrive well in sea water where the pH ranges from 7.8 to 8.6 (Rao and Menon, in press). Hence it may be assumed that, within the above range, pH is not a limiting factor to normal life processes of *P. viridis.* However, high pH resulting from the presence of NH_3 might lead to cellular damage.

At higher concentrations the byssus threads of *Perna viridis* showed variations in morphology. The most conspicuous changes were lack of hardening and inability of the adhesive disc to attach firmly. The rate of byssus-thread production is influenced by various environmental factors. These include water movement, salinity, the position occupied by the mussels in the intertidal region, dissolved oxygen concentration, temperature, oil, detergents and mercury salts (Reish and Ayers, 1968; van Winkle, 1970; Roberts, 1975). Improper development of adhesive discs under ammonia stress results in the mussels losing the capacity to attach byssally. During darkness, *Perna viridis* secretes more byssus threads than when exposed to light.

Acknowledgements. We are thankfull to Prof. H. P. C. Shetty, Director of Instruction, College of Fisheries for the facilities provided and the constant encouragement offered during the investigation. One of us (N. A. Reddy) is grateful to the Indian Council of Agricultural Research, for the award of a Junior fellowship during the tenure of which this work was carried out. We are grateful to the members of the Department of Fishery Hydrography. College of Fisheries for their assistance.

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This paper was submitted to the editor; it was accepted for printing on September 21, 1979.