Turk J Zool
25 (2001) 117-125
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Excretory Products of Green Mussel *Perna viridis* L. and their Implications on Power Plant Operation

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Received: 07.10.1998

Abstract: Excretion in marine animals is considered an important factor in assessing physiological status. *Perna viridis*, a widely distributed bivalve species, was found to be the dominant (>70%) organism in the seawater intake tunnel of Madras Atomic Power Station (MAPS). The excretion patterns (ammonia, nitrite, phosphate and fecal matter production) of three different size groups (4-5 cm, 6-7 cm, and 8-9 cm shell length) of *P. viridis* were studied in the laboratory at different salinities (15, 20, 25, 30 and 34 ppt). The amount of excreted products was positively related to the size of the mussels. At ambient salinity, the slope (b) values of the regressions against shell length were 1.5 for ammonia and 0.3 for nitrite, nitrate, phosphate, and fecal matter. Lowering of salinity resulted in a reduced rate of excretion for all the parameters studied except for ammonia. Ammonia excretion in all the size groups increased as the salinity was lowered up to 25 ppt; thereafter, the excretion rate was reduced and completely stopped at 15 ppt salinity.

Key Words: *Perna viridis*, salinity, excretion, ammonia, nitrite, nitrate, phosphate, fecal matter.

Perna viridis L.'nin Boşaltım Ürünleri ve Enerji Santrallarının İşletimi ile İlişkisi

Özet: Deniz hayvanlarının boşaltımı fizyolojik durum değerlendirmesinde önemli bir olgu olarak kabul edilmektedir. Madras Nükleer Santrali'nin (MAPS) deniz suyu giriş tünelinde hakim (>%70) organizmanın, geniş dağılım gösteren bivalv türü olan *Perna viridis* olduğu bulunmuştur. *P. viridis*'in üç farklı büyüklük grubu (kabuk uzunluğu 4-5 cm, 6-7 cm ve 8-9 cm) çeşitli tuzluluk değerlerinde (15, 20, 25, 30, 34 ppt) araştırılmıştır. Otuzdört ppt (ambient) tuzlulukta kabuk uzunluğuna karşı reğresyon eğim değerinin amonyak için 1,5 olduğu bulunurken nitrit, nitrat, fosfat ve dışkı için 0,3 olduğu belirlenmiştir. Amonyak dışında araştırılan bütün parametreler için tuzluluğun azalması boşaltım oranının azalmasına sebep olmuştur. Bütün büyüklük gruplarında tuzluluk 25 ppt'ye kadar azaldıkça amonyak boşaltımı yükselmiştir; tuzluluk 25 ppt'den 15 ppt'ye kadar azaldıkça amonyak boşaltımı azalmıştır; ve tuzluluk 15 ppt'ye varınca amonyak boşaltımı durmuştur.

Anahtar Sözcükler: *Perna viridis*, boşaltım, amonyak, nitrit, nitrat, fosfat, dışkı

Introduction

Nitrogenous compounds are one of the major metabolic byproducts of all animals (1). These byproducts formed inside the cells have to be eliminated since their accumulation is toxic to the organism themselves. Ammonia constitutes 60-90% of the total nitrogenous excreta in bivalves (2). It forms the major end product of protein and aminoacid catabolism in *Mytilus edulis* (3). Seawater is used as a condenser cooling medium in many electricity generating power stations. Generally water is drawn from the sea through an underwater tunnel. Sessile organisms growth in this tunnel and often interfere in the smooth operation of power plants. This process of growth of the attached organism on the artificial substrata is called biofouling. The excretion of ammonia by these biofouling organisms may have adverse impact on organisms present in the receiving water body to which the cooling water is ultimately discharged. Moreover, it will also have adverse implications on the treatment measures followed to prevent biofouling. Generally, in the cooling water systems of power plants, chlorination is practiced to minimize fouling growth. Ammonia excreted by these foulants interacts with chlorine and forms chloramines.

These chloramines have much lower biocidal efficiency than chlorine alone (4,5). In addition, the presence of ammonia may accelerate the corrosion of copper-based alloys when its concentration exceeds 1ppm in the cooling water (6). Furthermore, low concentration of ammonia produced by the fouling animals is reported to induce settlement of oyster larvae (7,8).

Perna viridis L. is the dominant fouling organism in the underwater tunnel of the Madras Atomic Power Station (MAPS), Kalpakkam, and has been reported to constitute >70% (410 tonnes) of the total foulants (570 tonnes) lodged inside the tunnel (9). It has been reported that the concentrations of ammonia and suspended matter in the sea water at the intake point of MAPS (Figure 1) are lower than in the forebay shaft (6). This suggests that as the water passes through the tunnel it undergoes changes due to the activities of the inhabiting fouling organisms (10). The physiological activities of marine organisms chiefly depend on hydrographic parameters such as temperature and salinity. Kalpakkam coastal waters show considerable fluctuation in salinity (between 22-35 ppt) throughout the year. Here, the seawater temperature fluctuates between 26 and 32°C oven a period b one year". It is thought to be important to have a database on the interrelationship between excretion in bivalves and a widely fluctuating hydrographic parameter, namely salinity.

In this study, a series of experiments were carried out to monitor excretion in mussels of 3 different sizes at varying salinities. This was done by monitoring the production of ammonia, nitrite, nitrate, phosphate and fecal matter by mussels of different sizes at different salinity regimes.

Materials and Methods

Perna viridis was collected from the sea water intake gates of MAPS and acclimatized in a 100-capacity fiberglass tank for two days (salinity 34 ± 0.5 ppt; pH 8.3 ± 0.1; and temperature 29 ± 0.5ºC). Epizoic organisms were carefully removed and the old byssus threads were cut off with scissors. The mussels were fed with a mixed diatom culture dominated by *Nitzschia closterium* and *Amphora bigibba* with a cell density of 18, 22 x 10^5 cells/ml. About 15 L of this culture solution was added to the tank. Mussels were allowed to feed for two days before they were used for the following experiments.

Experiments on excretion were conducted at five different salinities: 34 (ambient), 30, 25, 20, and 15 ppt. The responses of the 3 size groups of mussels (group 1: 4-5 cm; group 2: 6-7 cm; group 8-9 cm in shell length) to different were measured by monitoring ammonia, nitrite, nitrate and phosphate excretion and fecal matter production.

Nitrogen, phosphate and fecal matter production

Aged seawater from the storage tanks in the laboratory was filtered through GF/C (pore size 1µ) filter paper to remove suspended particles. All assays were carried out at 29 ± 0.5ºC. To 1 L of filtered seawater in a beaker, 1 mussel of a particular size group was added. All experimental sets were mildly aerated. As per the guidelines given by Chen et al.,(12) aliquots of water samples were drawn from the beakers after 24 hrs and the contents of ammonia, nitrite, nitrate and phosphate were estimated following standard analytical methods (13). The sensitivity of the methods are 0.1, 0.05, 0.01 µg at N/L and 0.03 µg at P/L for ammonia, nitrate, nitrite and phosphate, respectively. The fecal pellets were removed by a pasteur pipette with the help of a hand lens and transferred to a clean aluminium foil platform, rinsed with distilled water, dried at 80ºC for 24 hrs and weighed in a micro-balance (±0.01 mg) (14). Each experimental set was run in five replicates. For each salinity and size group one control set was run (n=5). Controls were filtered seawater without mussels. These control readings were used to estimate the excretion by the animals.

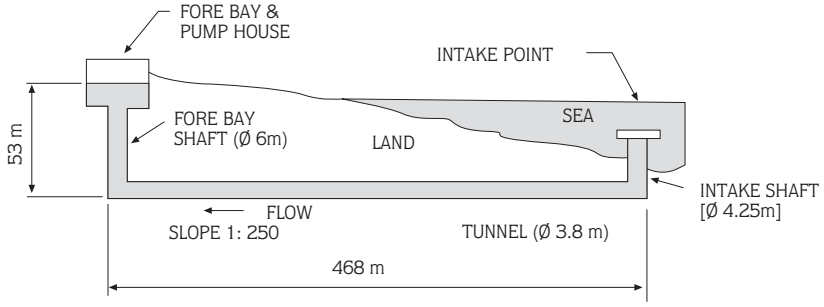

Fig. 1. Diagram of Madras Atomic Power Station cooling water system showing water intake, sub-seabed tunnel and forebay pump house.

Furthermore, an experimental set with animals in a salinity of 34 ppt (ambient) was taken as the reference set to compare the effects of salinity alterations on excretion. Seawater of different salinities was prepared by dilution with distilled water. Salinity was determined by the Mohr-Knudson method (15). Mussels were not fed during the experiment. The data were analyzed by multivariate analysis (two-way ANOVA) (16). Because the rates of production of excretory products at 15 ppt were below detectable limits for all size groups, this salinity was excluded from the statistical analysis. In order to monitor the length-weight relationship in these mussels, the length and soft-body dry weight of 150 organisms were measured. The dry weight of the soft tissues was determined by weighing in a Mettler micro-balance after drying in an oven at 80ºC for 48 hrs. Length was measured using a vernier calipers.

Results

Length-weight relationship

The result of the power function regression analysis between shell length and soft tissue dry weight in these mussels showed a significant positive relationship (Figure 2).

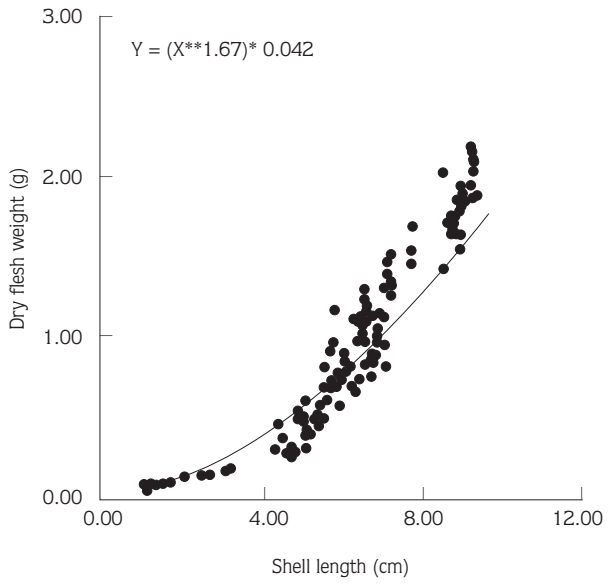

Figure 2. Power function of shell length and dry meat weight relationship of *P. viridis*.

Ammonia excretion

A positive relationship between ammonia excretion and size of animals was observed in all the salinities tested (Figure 3, Table 1). Maximum ammonia excretion was observed at 25 ppt for groups 2 and 3, while for group 1 maximum excretion was observed at 20 ppt. When the salinities increased and/or decreased beyond these levels (25 and 20 ppt for the respective size groups), the ammonia excretion decreased in all three size groups (Figure 3). Comparison with the individuals put in ambient sea water (reference set) showed that the salinity had a definite bearing on the ammonia excretion in these mussels irrespective of their size (Mann-Whitney U test p=0.009, Table 3). The rate of ammonia excretion in different size groups varied with salinity (Figure 3), thereby showing the significance of size and salinity changes in ammonia excretion in these mussels (Table 2).

Nitrite and Nitrate excretion

The results of linear regression analysis of nitrite and nitrate contents in the mussel excreta against shell length showed that they are directly proportional to the size of the mussels (Table 1). Nitrite and nitrate excretion decreased as the salinity was lowered from 34 ppt to 15

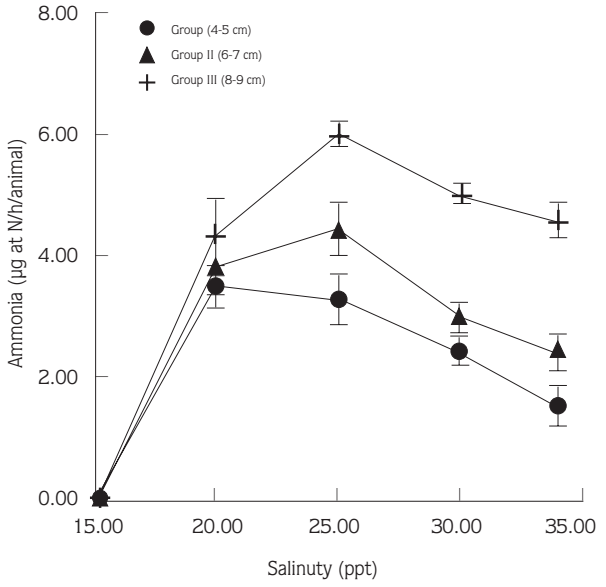

Figure 3. Ammonia excretion by three size groups of Perna viridis at different salinities. Symbols represent the mean of five observations. Vertical bars indicate the mean standard deviation. At 15 ppt the below detectable concentration is plotted as zero..

ppt. At 15 ppt the concentrations of nitrite and nitrate excretion were below detectable limits (Figs 4 & 5). The nitrite and nitrate excretion rates at lower salinities were significantly lower than those of the animals put in ambient salinity (Mann-Whitney U test p = 0.009, Table 3). Two-way ANOVA showed that salinity and mussel size significantly regulated excretion in this species (Table 2).

Phosphate excretion

In all the salinities tested, a positive relationship was observed between mussel size and phosphate excretion (Table 1). The level of phosphate excretion was found to be reduced as the salinity was lowered (Figure 6). This was also true with all the size groups of mussels tested (Table 2).

Table 1. Correlation coefficient analysis of excretory rates of various size groups of mussels *P. viridis* at different salinities.

Rate of excretion/ Production vs Size	Salinity (ppt)	a	b	r	p
Ammonia	20	3.439	0.39	0.83	0.0001
	25	2.800	1.5	0.9	0.0001
	30	1.700	1.6	1.0	0.0001
	34	1.200	1.5	0.9	0.0001
Nitrate	20	0.008	0.03	0.8	0.005
	25	0.308	0.1	1.0	0.0001
	30	0.044	0.1	0.5	0.0001
	34	0.300	0.3	0.9	0.0001
Nitrite	20	0.008	0.03	0.8	0.005
	25	0.031	0.1	1.0	0.0001
	30	0.440	0.1	0.6	0.0001
	34	0.300	0.3	0.9	0.0001
Phosphate	20	0.008	0.03	0.8	0.005
	25	0.308	0.1	1.0	0.0001
	30	0.044	0.1	0.6	0.0001
	34	0.030	0.3	0.9	0.0001
Fecal matter	20	0.400	0.7	0.9	0.0001
	25	0.400	0.8	0.9	0.0001
	30	0.300	0.3	0.9	0.0001
	34	0.300	0.3	0.9	0.0001

Table 2. Results of two way ANOVA analysis of excretory rates of various size groups of *P. viridis* with changing salinity.

Excretion	Source	df	Sum square	Mean square	F	P
	Size groups	2	54.7	27.4	701.4	0.0001
Ammonia	Salinity	3	24.6	8.2	149.6	0.0001
	Size x Salinity	6	9.1	1.5	27.7	0.0001
	Size groups	2	0.7	0.3	137.6	0.0001
Nitrate	Salinity	3	3.9	1.3	423.0	0.0001
	Size x Salinity	6	0.6	0.1	33.3	0.0001
	Size groups	2	0.4	0.2	148.1	0.0001
Nitrite	Salinity	3	1.4	0.5	500.0	0.0001
	Size x Salinity	6	0.2	0.036	38.7	0.0001
	Size groups	2	0.1	0.031	15.5	0.0043
Phosphate	Salinity	3	0.2	0.1	160.1	0.0001
	Size x Salinity	6	0.1	0.001	24.4	0.0001
	Size groups	2	7.4	3.7	104.4	0.0001
Fecal matter	Salinity	3	14.8	4.9	184.0	0.0001
	Size x Salinity	6	3.7	0.6	22.7	0.0001

Table 3. Results of Mann Whitney U-test analysis of excretory products of three size groups of *P. viridis* exposed to three salinities. Concentration of each excretory product at a certain salinity was compared with the concentration of the excretory product obtained in ambient salinity (34 ppt).

Excretory Products		Size Groups		
		4-5 cm	6-7	8-9 cm
		P	P	P
Ammonia	20	0.009	0.009	0.009
	25	0.009	0.009	0.009
	30	0.009	0.009	0.009
Nitrite	20	0.009	0.009	0.009
	25	0.009	0.009	0.009
	30	0.009	0.009	0.009
Nitrate	20	0.009	0.009	0.009
	25	0.009	0.009	0.009
	30	0.009	0.009	0.009
Phosphate	20	0.014	0.025	0.014
	25	0.009	0.009	0.009
	30	0.009	0.009	0.009
Fecal Matter	20	0.347	0.754	0.754
	25	0.009	0.009	0.009
	30	0.009	0.009	0.009

Fecal pellet production

The relationships between egestion rate and size of mussels at different salinity regimes are given in Table 1 and Figure 7. Fecal matter production was directly related to mussel size and salinity (two-way anova p = 0.0001, Table 2). However, little or no change in fecal pellet production took place when the salinity ranged between ambient (34) and 30 ppt.

Discussion

Ammonia is the major excretory product (60 to 90%) of molusks (17). While this is the case for the snail *Nacella* sp., the value of 88.8% reported by Clarke et al. (1) is slightly higher than those reported by Nicol (18) for the rest of the gastropods, bivalves and cephalopods. The excretion of ammonia nitrogen may be regarded as the rate of protein and aminoacid catabolism (3). Diffusion is the principal mode of ammonia excretion in marine invertebrates since its concentration in the body fluids is considered to be higher than that in the outside environment (19). In the present study, ammonia excretion increased significantly (p = 0.0001) with the size of the mussels, i.e., with the dry body weight of the mussels (Figure 2). These results of ammonia excretion are comparable with earlier observations in other related

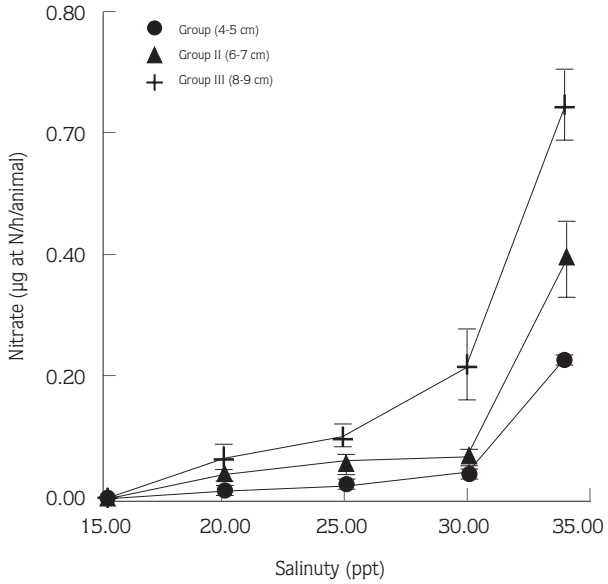

Figure 4. Nitrite excretion by three size groups of *Perna viridis* at different salinities. Symbols represent the mean of five observations. Vertical bars indicate the mean standard deviation. At 15 ppt the below detectable concentration is plotted as zero.

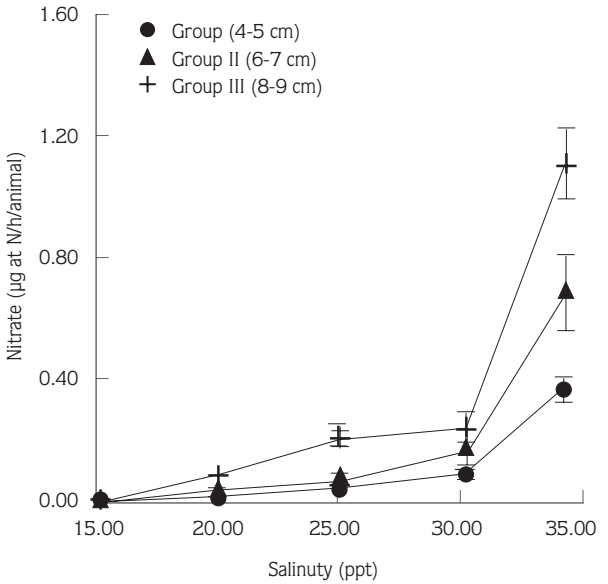

Figure 5. Nitrate excretion by three size groups of *Perna viridis* at different salinities. Symbols represent the mean of five observations. Vertical bars indicate the mean standard deviation. At 15 ppt the below detectable concentration is plotted as zero.

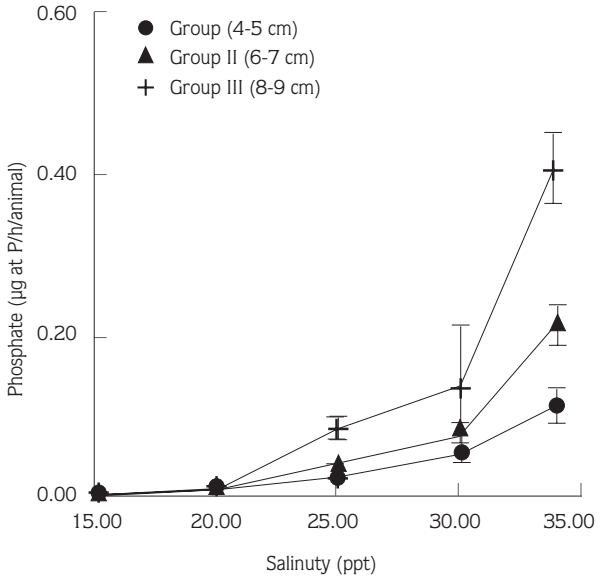

Figure 6. Nitrite excretion by three size groups of *Perna viridis* at different salinities. Symbols represent the mean of five observations. Vertical bars indicate the mean standard deviation. At 15 ppt the below detectable concentration is plotted as zero.

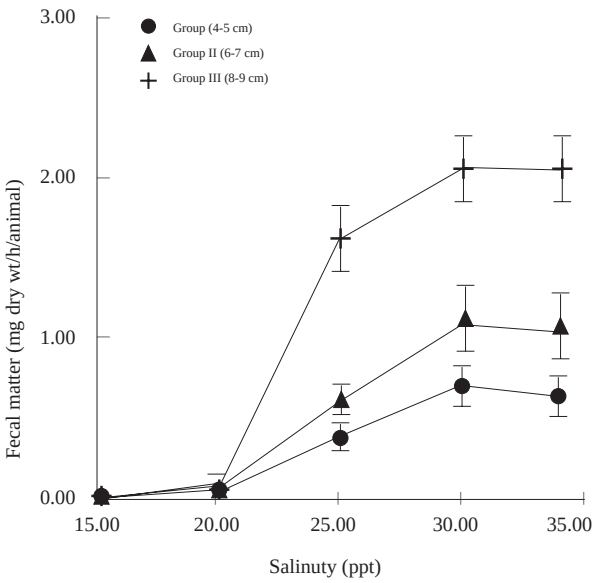

Figure 7. Fecal matter excretion by three size groups of *Perna viridis* at different salinities. Symbols represent the mean of five observations. Vertical bars indicate the mean standard deviation.

molluskan species such as *Mya arenaria* (20), *Donax vittatus* (21), *Mytilus californianus*17 and *Mytilus edulis* (22).

The higher rate of excretion in larger individuals could be attributed to the larger surface area for diffusion as compared to the smaller individuals. Ammonia excretion in green mussels was higher at low salinity regimes but decreased below detectable levels at 15 ppt. An increased rate of ammonia excretion in diluted sea water medium was also reported in *Macoma inconspicua*(19), *Mya arenaria* (23) and *M. edulis* (24). Decreased salinity leads to reduced osmolarity in animals, which in turn triggers the protein catabolism and results in a higher rate of ammonia excretion (25). However, as reported by Davenport (26), in unfavorable conditions, the tropical mussel M. edulis isolates itself from its surroundings by firmly closing its valves and resorting to anaerobic respiration. Sundaram & Shafee (27) reported that the green mussel P. viridis can withstand lowering of salinity by closing its valves for 2 days. Due to this closing behaviour, the physiological activities were lowered, thereby resulting in a low rate of excretion(28). This might be the process taking place at 15 ppt where excretory products suchas ammonia, nitrite, nitrate, and phosphate were below the detactable limit after 24 hrs of exposure.

Studies on the production of ammonia are of considerable economic significance in the context of power plants using sea water as a cooling medium. Our previous experiments showed that the forebay water of MAPS (Figure 1) have higher levels of ammonia as compared to the coastal waters at the intake area (6). This was attributed to the production of ammonia by the fouling organisms, especially by *P. viridis*, which largely colonized the interior of the sea water intake tunnel. As mentioned earlier, the mussel population constitutes a major component (>70%) of the fouling community in the intake tunnel (9). Ammonia excreted by these organisms interacts with the hypochlorous acid (HOCl) in the medium (formed as a result of chlorination) to form chloramines, which are described as less effective biocides than HOCl (4,5). Thus, the presence of ammonia makes chlorine a less effective biocide for the fouling organisms. Moreover, chloramines are more stable compounds than HOCl and are toxic to fishes at concentrations >0.1 ppm (29).

Recent laboratory experiments have shown that the presence of ammonia induces settlement in oyster larvae that are competent to undergo metamorphosis by a mechanism involving pH-induced depolarization of nerve cells (7,8,30). Similarly, ammonia has been reported to

induce the settlement of the larvae of other invertebrates also (31). Furthermore, ammonia is known to accelerate the corrosion rate of copper-based alloys which are used as condenser tubes in seawater-cooled power plants. It is of interest to mention here that a concentration of 1 mg/L of ammonia can induce stress corrosion cracking in copper-based alloys (32). In the cooling water system of MAPS, the ammonia concentration at the forebay area varied between 1.14 and 12.0 µg-at/L during high-flow conditions over a period of year (6). Although this concentration in the forebay is much lower than the required concentration for stress corrosion craking, during stagnation or low-flow periods the concentration could be of a much higher magnitude to initiate corrosion. Moreover, ammonia concentration beneath the slime film formed in the condenser tube could be of much higher magnitude than in the water column, as reported by Satpathy et al. (33).

Chen et al. (12) described the excretion of nitrite and nitrate by animals as the intrinsic mechanism for detoxification of ammonia in the blood and maintaining ionic stability inside the animal. Decreased salinity resulted in decreased excretion rates of nitrite and nitrate in *P. viridis*. This has also been found to be the case for crustaceans like *Carcimus maenas* (34) and *Penaeus monodon* (12). Hammen et al. (35) reported 16.6% nitrogen excretion (other than ammonia) in the oyster *Crassorstrea virginica*. The nitrite and nitrate excretions in *P. viridis* were found to be a function of the body size of the organisms (Figs. 4 and 5). The inorganic nitrogen produced by these invertebrates is an important nutrient source for many micro algae (36-38) and could result in algal bloom in the receiving water bodies. Dense populations of mussels may be expected to contribute significantly to the nutrient budgets of inshore waters in terms of both of particulate and dissolved nutrients. A study by Kuenzler (39) on a population of *M. demissus* in a salt marsh ecosystem is a vivid illustration of the nutrient cycles and its influence on the energy budget of a natural mussel population.

Phosphate ions play an important role in euryhaline organisms, and the animals exhibit a tendency for retention of phosphate ions in response to decreased salinity (39). The excretion of phosphate ions by marine animals contributes to the phosphate levels in the water bodies. Richard et al. (38) reported that the increase in the phosphate level in the Wadden Sea is due to excretion by mussels from the mussel bed. In *Modiolus demissus* the phosphate excretion was reported to be directly proportional to the salinity (39). In our laboratory experiments we observed that phosphate excretion by green mussels is correlated well with size of the animals. Mussels have been reported to absorb phosphorus from the environment (38-40). Kuzenzler (39) reported that out of the total phosphorus absorbed by mussels, 83% was excreted as phosphate. Hence the rate of phosphate excretion might be influenced not only by the metabolism of the animal but also by the availability of phosphorus in the medium.

Fecal matter production is proportional to the size of the green mussel. Similar observations have been reported by Clarke (14) in *Nacella concinna*. The cooling circuit of MAPS is characterized by a high content of suspended solids in the forebay as compared to the intake point. This could be due to the fecal matter production by the foulants growing inside the underwater tunnel. The average concentration of suspended matter in the forebay is much higher than at the intake point (6). Suspended matter in the water causes siltation problems in the pumps, thereby increasing pump vibration and decreasing efficiency. In addition, bacterial populations get energy-rich particles in the form of suspended matter, which in turn may grow to form biofilms on the heat exchangers. These biofilms have been reported to have deleterious effects, such as reducing the heat transfer efficiency and increasing biocorrosison (41,42).

In conclusion, all the physiological parameters studied here are related to both the size of the animal and the salinity at which the animal lives. In a power plant cooling water system which uses sea water as coolant, ammonia production can be an important parameter to monitor so as to adopt an effective antifouling regime.

Acknowledgement

We express thanks our sincere to the Board of Research in Nuclear Science, Department of Atomic Energy, Government of India, for financial support. Thanks are due to Dr. S.V. Narasimhan Head, WSCL, Kalpakkam and Dr. K. Hariharan, Station Director, Madras Atomic Power Plant for facilities. We also thank Dr. V.P. Venugopalan WSCL for fruitful discussion during the course of this study.

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