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RESPONSE OF BROWN MUSSEL, *PERNA INDICA*, TO ELEVATED TEMPERATURES IN RELATION TO POWER PLANT BIOFOULING CONTROL

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Abstract—1. Results of a study on lethal and sublethal responses of different size groups of the tropical brown mussel, *Perna indica*, when exposed to different temperatures are presented.

2. Exposure to a temperature of 38°C showed 100% mortality of 9 mm size group mussels in 120 min.

3. Mortality was dependent on age (size) of the mussels, young ones being more susceptible than older ones.

4. All size groups showed a progressive reduction in physiological activities such as filtration rate, foot activity and byssus thread production when temperature was increased from 30°C.

5. This study suggests that heat treatment is an attractive alternative to chlorination for mussel fouling control in tropical power stations.

Key Word Index: Power station; *Perna indica*; fouling control; heat treatment; mortality; filtration rate; foot activity; byssus thread production

INTRODUCTION

For the economical and safe operation of a nuclear power plant, an uninterrupted supply of cooling water to the condensers is essential (Neitzel *et al.*, 1984). However, in seawater-based cooling systems, marine life associated with the incoming water can interfere with the smooth operation of the power plant (Rajagopal, 1991). Extensive growth of bio-fouling in the seawater cooling circuits can cause a reduction in flow rates (Jenner and Janssen-Mommen, 1993) and blockage of condenser tubes (Imbro and Gianelli, 1982).

Madras Atomic Power Station (MAPS) is situated at Kalpakkam (12°33'N and 80°11'E), on the east coast of India. MAPS experienced a serious flow blockage in 1987 due to the extensive growth of mussels, *Perna indica* Kuriakose and Nair and *Perna viridis* (L.), in the cooling water system (Rajagopal *et al.*, 1991a) and this frequently resulted in station outages (Nair, 1990). Chlorination is used at MAPS to control mussel fouling. However, killing of

established mussel communities, such as those in the MAPS tunnel, requires continuous doses of chlorine (Rajagopal *et al.*, 1991b). This leads to the question of environmental safety, as chlorine has been reported to be toxic to non-target organisms (Brungs, 1977; Smith and Kretschner, 1984; Jenner, 1985). Moreover, the failure of chlorine to control mussel settlement and growth, despite its continuous use in cooling waters, has been reported from various power stations (Jenner, 1983; Strauss, 1989). However, this is likely to be a failure in the application regime.

Heat treatment for mussel control in power plants has received some attention in recent years (Wright *et al.*, 1983; Claudi and Mackie, 1994). In this method, the heated effluent from power plants, instead of being discharged, is recirculated through the cooling conduits. Therefore, it is highly economical and is increasingly employed in new plants (Jenner, 1982). However, the method also has certain problems in that the application involves a certain level of production penalty for the power plant. Moreover, the heat treatment method would require major design modifications of the cooling system for already operating plants; it is often expensive or technically difficult (Burton and Liden, 1978). A few

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power plants in The Netherlands and U.S.A. have been successfully using heat treatment as a means to control mussel fouling (Graham *et al.*, 1975; Jenner and Janssen-Mommen, 1993). Heat treatment also has potential for the control of mussels in power stations in tropical waters, but there are hardly any data on the response of the brown mussel, *P. indica*, one of the most important fouling species in Indian coastal power stations (Rajagopal *et al.*, 1991a), to heat treatment. Therefore, experiments have been carried out to study lethal and sublethal responses of brown mussels to varying degrees of heat treatment. It is expected that the results will allow optimization of the techniques, so that maximum control could be achieved with a minimum of financial cost and environmental impact.

The present study focuses on the responses of different size groups of *P. indica* to different temperatures.

MATERIALS AND METHODS

The response of *P. indica* to different temperatures was studied using mussels collected from the coastal waters near the MAPS intake area. In the laboratory, the mussels were acclimated in seawater aquarium tanks ($34.1 \pm 0.6\%$ salinity and $29.8 \pm 0.7^\circ\text{C}$ temperature) for 2 days. Based on the shell length (Rajagopal, 1991), mussels were sorted out into five size groups *viz* 9 mm (1 month old), 18 mm (3 months), 25 mm (6 months), 31 mm (9 months) and 36 mm (12 months).

Mortality

Four size groups of mussels were tested for 7 different target temperatures (38–44°C, at 1°C intervals). Preliminary experiments had shown that large mussels took about 3 h to attain 100% mortality at 38°C. Since 3 h is approximately the maximum practical duration for which heat treatment could be done in actual utility systems (Jenner, 1982), experiments below 38°C were not included. Factors which may affect temperature tolerance such as salinity and dissolved oxygen were kept as stable as possible in each experiment, 33.8–34.3% and 5.9–6.3 mg/l, respectively. The experiments were carried out using an ultrachriostat (ISSREF, cat. no. IRO 017), following procedures outlined by Sasikumar *et al.* (1992). The temperature was raised at the rate of 0.1°C/min from 30°C. The rate of raise was chosen as it corresponds to the rise rates that can be expected during heat treatment in power plants (Jenner, 1982). Mussels failing to respond to external stimuli by closing their shells, were considered dead (Wallis, 1975).

Sublethal responses

Filtration rate, foot activity and byssus thread production of different size groups of mussels were also studied in an effort to understand the sublethal effects of temperature on mussels. Each experiment was run using 10 replicates for each size group and temperature.

Filtration rate was measured following the method described by Coughlan (1969). The method is based on the absorption of neutral red by mussels from ambient water and rate of filtration was calculated using the following equation (Coughlan, 1969):

$$m = \frac{M}{n \cdot t} \ln \frac{C_0}{C_t}$$

where, M is the volume of the test solution, n the number of animals used in the experiment, t the time in hours, C_0 the initial concentration of the dye, C_t the concentration of the dye at time t and m the rate of filtration in ml/h/mussel.

For foot activity, at every 10 min for 12 h, a note was made of the number of mussels with the foot extended outside the shell following procedures described by Holmes (1970). For each experiment, all foot activity readings were analyzed and percentage foot activity was calculated. Rate of byssus thread production was determined following the methods outlined by Winkle (1970) and Allen *et al.* (1976). Byssus threads produced by each mussel were counted after 12 h and expressed in threads/mussel/day.

Statistical analysis

The differences in physiological activity between control (30°C) and experimental mussels were compared by 't' tests (Sokal and Rohlf, 1981). A *posteriori* multiple comparisons of differences between size groups were done using Student–Neuman–Keuls (SNK) tests along with one-way ANOVA (Wilkinson, 1989).

RESULTS

Mortality

Significant size-dependent variation in the response of mussels was observed at all target temperatures (ANOVA, $P < 0.0001$; Table 1), large mussels showing more tolerance than small mussels. At 44°C, 100% mortality was achieved after 5 min in all size groups of mussels.

Filtration rate

The filtration rate of mussels showed a progressive decline at the higher temperatures (Table 2). For

Table 1. Time to reach 100% mortality for various size groups of *Perna indica* to different temperatures

Temp (°C)	Exposure time (hours)				df	F	P
	Size of the mussels (mm ± SD)						
	8.9 ± 0.6	18.3 ± 1.0	25.5 ± 1.7	36.0 ± 2.0			
38	119 ± 17	134 ± 20	151 ± 28	173 ± 25	143	36.944	***
39	62 ± 12	87 ± 16	104 ± 19	121 ± 15	143	92.544	***
40	43 ± 9	62 ± 18	72 ± 15	94 ± 10	143	88.948	***
41	26 ± 7	44 ± 10	54 ± 16	63 ± 12	143	65.986	***
42	14 ± 6	24 ± 5	30 ± 8	33 ± 11	143	41.122	***
43	6 ± 2	10 ± 4	13 ± 4	14 ± 6	143	25.833	***
44	0 ± 0	0 ± 0	2 ± 1	5 ± 2	143	32.160	***

Values are expressed as mean ± SD ($n = 36$). One-way ANOVA followed by Student–Newman–Keuls (SNK) tests was used to determine whether differences between size groups were significant.

***Significant at $P < 0.001$.

example, mussels in the 12 mm size group showed a filtration rate of 30 ml/h/mussel at 30°C, reducing to 22 ml/h/mussel at 32.5°C and to 7 ml/h/mussel at 35°C. This pattern of sharp decrease between 32.5 and 35°C was observed in all the size groups tested. A significant size-dependent variation in filtration rate (SNK tests, $P < 0.05$) was observed; the large mussels showed a higher filtration rate.

Foot activity

At 30°C, using the 9 mm size group, a highest foot activity of 47% was registered (Table 3). The reduction in foot activity was very sharp between 32.5

and 35°C. Data also show a clear size-dependent variation in foot activity at 30 and 35°C. However, no foot activity was observed at 37.5°C for all size groups.

Byssus thread production

A sharp decrease was recorded in the byssus thread production at 35°C (Table 4); indicating again that 32.5–35°C is the range of temperature within which physiological activities are critically affected in the brown mussels. Size-dependent variation in the rate of thread production was observed, with the rate of thread production decreasing with increasing shell

Table 2. Filtration rate of various size groups of mussels at different temperatures

Temp (°C)	Filtration rate (ml/h/mussel)					df	F	P
	Size of the mussels (mm ± SD)							
	9.0 ± 0.9	18.7 ± 2.1	24.8 ± 4.4	31.5 ± 3.2	35.4 ± 3.7			
30.0	29.2 ± 2.7	30.2 ± 2.7	32.3 ± 3.4	33.1 ± 1.8	31.2 ± 3.7	49	2.878	*
32.5	22.2 ± 1.8***	31.6 ± 3.2 ^{NS}	30.2 ± 3.8 ^{NS}	31.5 ± 4.0 ^{NS}	35.2 ± 2.7 ^{NS}	49	22.538	***
35.0	7.1 ± 2.2***	8.1 ± 2.0***	9.7 ± 1.1***	9.8 ± 1.1***	10.0 ± 1.8***	49	6.070	***
37.5	2.2 ± 0.4***	3.3 ± 0.8***	3.5 ± 0.9***	4.0 ± 0.2***	4.5 ± 0.9***	49	23.486	***

Values are presented as mean ± SD ($n = 10$). Differences between control (30°C) and experimental mussels are compared by 't' tests and *post hoc* multiple comparisons of differences between size groups were done using one-way ANOVA and Student–Newman–Keuls (SNK) tests.

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{NS}, not significant.

Table 3. Foot activity of various size groups of mussels at different temperatures

Temp (°C)	Foot activity (%)					df	F	P
	Size of the mussels (mm ± SD)							
	9.3 ± 0.3	18.4 ± 0.9	24.2 ± 1.9	31.0 ± 2.1	36.2 ± 4.9			
30.0	46.9 ± 8.4	40.8 ± 7.0	39.0 ± 7.4	35.3 ± 7.2	35.2 ± 6.4	49	4.308	**
32.5	32.4 ± 7.0***	35.1 ± 5.1*	35.0 ± 6.2 ^{NS}	30.7 ± 6.5 ^{NS}	27.9 ± 5.1*	49	2.536	^{NS}
35.0	4.5 ± 3.6***	8.3 ± 3.1***	8.6 ± 4.0***	9.3 ± 4.7***	9.1 ± 3.1***	49	2.684	*
37.5	0 ± 0***	0 ± 0***	0 ± 0***	0 ± 0***	0 ± 0***	—	—	—

Values are presented as mean ± SD ($n = 10$). Statistical analysis as in Table 2.

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{NS}, not significant.

Table 4. Byssus thread production of various size groups of mussels at different temperatures

Temp (°C)	Byssus thread production (threads/mussel/day)					df	F	P
	Size of the mussels (mm ± SD)							
	9.1 ± 0.5	18.7 ± 1.7	24.5 ± 2.0	31.7 ± 4.9	35.8 ± 3.2			
30.0	15.4 ± 7.0	13.9 ± 5.5	13.7 ± 5.3	9.8 ± 7.4	8.4 ± 3.4	49	2.649	*
32.5	10.3 ± 7.7 ^{NS}	8.9 ± 3.8*	8.4 ± 6.0*	8.4 ± 5.8 ^{NS}	7.2 ± 5.6 ^{NS}	49	0.386	NS
35.0	0 ± 0***	1.9 ± 0.5***	1.4 ± 0.7***	1.7 ± 0.5**	3.1 ± 1.4***	49	22.034	***
37.5	0 ± 0***	0 ± 0***	0 ± 0***	0 ± 0***	0 ± 0***	—	—	—

Values are presented as mean ± SD ($n = 10$). Statistical analysis as in Table 2.

Levels of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{NS}, not significant.

size of mussels. Foot activity and byssus thread production of mussels were strongly correlated ($r^2 = 0.86$, $P < 0.0001$) at different sublethal temperatures.

DISCUSSION

Experimental work on the thermal tolerance of *Mytilus edulis* L., along with field trials at the Redondo Beach power station, California (Fox and Coheran, 1957) showed that *M. edulis* could be controlled at a temperature of 43°C, maintained for 2 h once every 4 weeks during spatfall. The present studies showed that 100% kill of all size groups of brown mussels could be achieved by raising the temperature to 38°C and maintaining that temperature level for about 3 h (Table 1). At San Onofre power station (SONGS), California, heat treatment for 2 h at 38°C, once every 6 weeks is successfully used to control mussel fouling in the cooling conduits (Stock and Strachan, 1977). At Eems power station, The Netherlands, a temperature of 38°C is used for 1 h to ensure 100% mortality of *M. edulis*; it was also reported that this treatment is more economical and less damaging to the environment than continuous chlorination (Jenner, 1982). The average ambient seawater temperature at these power plants (SONGS and Eems) ranges from 18–21°C during summer (Stock and Strachan, 1977; Jenner, 1982) and to achieve 100% mortality of the mussels at 38°C, the intake water has to be heated by 17–20°C. It should be noted that the average intake water temperature at MAPS, Kalpakkam ranges from 27°C (winter) to 31°C (summer). Therefore, the energy required to raise the water temperature to the desired level (38°C) is correspondingly less (7–11°C). Thus, it is logical to presume that the heat treatment method reportedly successful at SONGS and Eems power stations would be more economical if practised in the tropics.

Mussels in general respond to sudden changes in temperature by closing their shells. Trueman and Lowe (1971) suggested that temperature sensitive receptors on the mantle cavity might be responsible

for shell closure in mussels. The period for which they remained closed could last for many days, with only short and intermittent periods of shell opening at sublethal temperatures (Widdows, 1973). However, at lethal temperatures, mussels are forced to shut

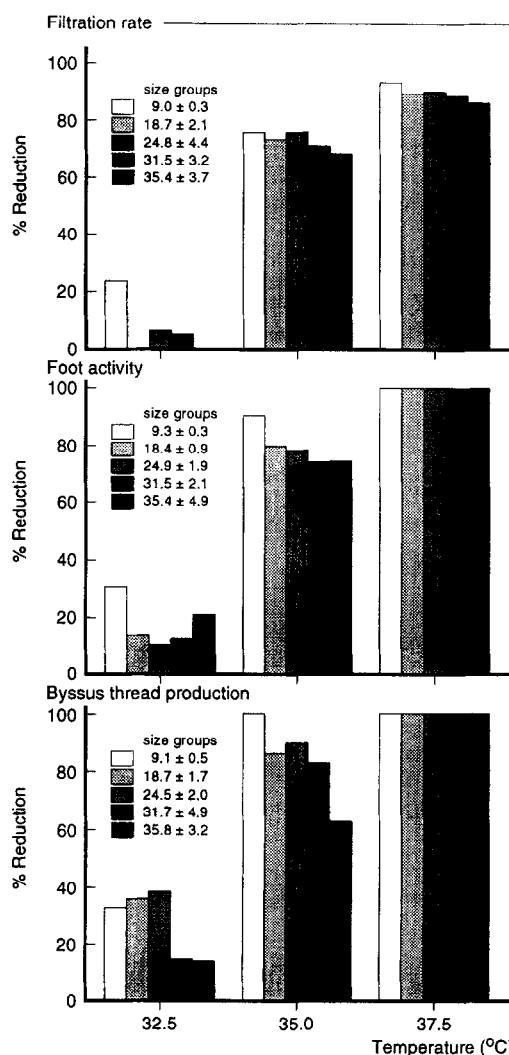


Fig. 1. Percentage reduction in filtration rate, foot activity and byssus thread production of various size groups of brown mussels at different temperatures when compared to 30°C (control).

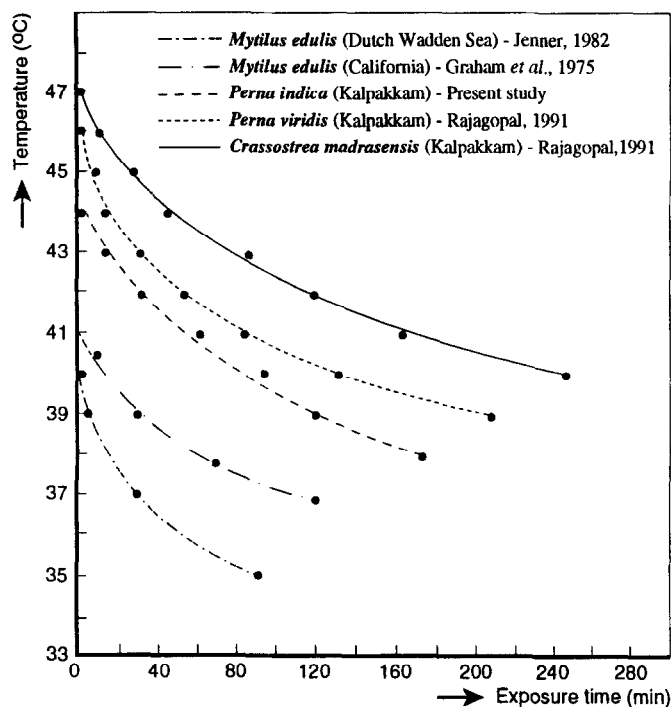


Fig. 2. Comparison of time to 100% mortality over a range of temperatures for the brown mussel, *Perna indica*, green mussel, *Perna viridis* and oysters, *Crassostrea madrasensis* at Kalpakkam along with published results for blue mussel, *Mytilus edulis* from Californian coastal waters (Graham *et al.*, 1975) and Dutch Wadden Sea (Jenner, 1982).

their valves and exist on stored food reserves and anaerobic respiration, until energy resources are depleted or metabolic wastes reach a toxic level (Bayne *et al.*, 1976).

In the present study, physiological activities such as filtration rate, foot activity and byssus thread production were significantly reduced ($P < 0.001$) when the temperature was over 32.5°C. Gonzalez and Yevich (1976) also observed that higher temperature causes a reduction in the feeding rate, which could be attributed to the degeneration of the gill filaments and histological changes in the stomach and intestine. Read (1962) demonstrated that the weight specific respiration rate of large mussels decreased appreciably at higher temperatures, the probable reason again being gill damage as mentioned above. Histological studies by Gonzalez and Yevich (1976) also showed that at higher temperatures the formation of byssus threads in *M. edulis* was weak and irregular. The present study has indicated that at higher temperatures (> 35°C) byssus thread production of *P. indica* is completely stopped. Moreover, a higher percentage of reduction of physiological activities was observed in young mussels (Fig. 1). It is well known that young mussels are more active than older ones and they have relatively high metabolic rates (Bayne *et al.*, 1976). Therefore, under stress, juveniles might react more rapidly than larger mussels.

The exposure time required for 100% mortality of *P. indica* to different target temperatures (Fig. 2) is shorter than that reported for *P. viridis* and *Crassostrea madrasensis* Preston (Rajagopal, 1991). The last two species are commonly found in backwaters and coastal waters (Rajagopal *et al.*, 1990) whereas *P. indica* is a marine species (Kuriakose, 1980). As there is greater temperature natural variation in backwaters compared with the marine environments, the temperature tolerance among these bivalves may be related to habitat differences (Bayne *et al.*, 1976; Wright *et al.*, 1983).

Comparisons of mortality between size groups show that large mussels are more tolerant than smaller ones. Wallis (1975) has suggested that metabolic rates which vary with age or size of the mussels are the causative factors for the differences. Thus, at any lethal temperature, smaller mussels will succumb more quickly than larger mussels. The significance of this observation is that heat treatment could be more economically employed following peak breeding periods when young brown mussels are encountered in the cooling conduits as the time required to kill them would be shorter.

Heat treatment appears to be a viable method for mussel control in tropical power stations. It would also be relevant to study its synergistic efficiency when used with other control methods such as

chlorination or osmotic shock. It is possible that this combination of treatments may result in mussel control at much lower temperatures and shorter exposure times than the results of the present study would indicate.

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