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Response of Fouling Brown Mussel, *Perna perna* (L.), to Chlorine

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Abstract. *Perna perna* (L.), the edible brown mussel, is very widely distributed in the tropical and subtropical regions and is commonly found in rocky shores. Apart from being a candidate for commercial cultivation, *P. perna* is also a common pest organism in cooling water systems of coastal power stations. Therefore, a lethal and sublethal response of this mussel to commonly used antifouling biocides is of considerable interest to the industry. Mortality pattern (LT_{50} and LT_{100}) and physiological activities (oxygen consumption, filtration rate, foot activity index, and byssus thread production) of different size groups (9–34 mm shell lengths) of *P. perna* were studied in the laboratory under different residual chlorine concentrations (0.25, 0.50, 0.75, and 1.00 mg/L for sublethal responses and 1, 2, 3, and 5 mg/L for mortality). Results showed that exposure time for 100% mortality of mussels significantly decreased with increasing residual chlorine concentration. For example, mussels of 9 mm size group exposed to 1 mg/L chlorine residual took 384 h (16 days) to reach 100% mortality, whereas those exposed to 5 mg/L chlorine took 84 h (4 days). The effect of mussel size on mortality was significant between 1 mg/L and 5 mg/L residual chlorine, with larger mussels showing greater resistance than smaller ones. For example, at 2 mg/L residual chlorine, 9 mm and 34 mm size group mussels took 228 h (10 days) and 304 h (13 days), respectively, to achieve 100% mortality. All size groups of *P. perna* showed progressive reduction in physiological activities, when chlorine residuals were gradually increased from 0 to 1 mg/L. Reduction in physiological activities was strongly correlated with the residual level. A comparison of present data with data available for other common fouling organisms suggests that *P. perna* is relatively less tolerant to chlorine than *Perna viridis* (L.) and *Brachidontes striatulus* (Hanley), which also cause fouling problems in tropical coastal waters.

The brown mussel *Perna perna* (Linnaeus) (Syn *Perna indica*, *Perna picta*) is an important mussel species widely distributed in tropical and subtropical regions (Siddall 1980; Vakily 1989; Hicks *et al.* 2001). The reported range of *P. perna* includes India; Sri Lanka; Madagascar; the east (from central Mozambique to False Bay) and west (from Luderiz Bay north into the Mediterranean from Gibraltar to the Gulf of Tunis) coasts of Africa; the Atlantic coasts of Brazil, Uruguay, and Venezuela; and the West Indies (Berry 1978). *P. perna* is a potentially important species from the fisheries point of view because of its rapid growth rate that makes it suitable for commercial cultivation (Kuriakose 1980; Vakily 1989). However, it also causes severe biofouling in power plant cooling systems (Rajagopal *et al.* 1995a, 1996). In recent years, *P. perna* has attracted much attention because of extension of its range of distribution to nonnative areas, such as the Gulf of Mexico. This mussel species is thought to have been introduced to this area via ballast water release (Hicks and Tunnel 1993) and constitutes a potential threat to shipping safety in places such as the Gulf of Mexico (Hicks and Tunnel 1995; Hicks and McMahon 2002).

The ability of the mussel to be distributed in wide-ranging geographical regions is related to its ability to withstand considerable variations in environmental conditions, such as temperature and salinity. Such characteristics can also make the species difficult to control, especially when they become pests (Claudi and Mackie 1994; Jenner *et al.* 1998; Hicks and McMahon 2002). In cooling water systems of coastal power stations, mussels are the most dominant organisms (Jenner *et al.* 1998). In an earlier study, Rajagopal *et al.* (1996) reported that in the condenser cooling system of Madras Atomic Power Station (MAPS) on the east coast of India, *Perna viridis*, *P. perna*, and *Brachidontes striatulus* were the major fouling organisms. MAPS has been using chlorination as an antifouling method in the sea water cooling system. The chlorination regime was intermittent initially and because this did not control the fouling very effectively (1–2 mg/L residual at outfall for 1 h, once in 8 h), the mode of application was changed to continuous low dosing (Rajagopal *et al.* 1991). Presently, the power station has been using continuous chlorination (about 0.5 mg/L residual at outfall) with occasional shock dose treatment (up to 2 mg/L residual at outfall) during breeding seasons of important fouling organisms (Rajagopal *et al.* 1996). Given the fact that no information exists on the lethal and sublethal

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effects of chlorine on this mussel species, it was considered worthwhile to generate this data by exposing the mussels to a range of chlorine concentrations. It may be noted that there are published reports on the response of other common tropical fouling mussels, such as *P. viridis* and *B. striatulus* to chlorine (Rajagopal *et al.* 1995b,1997).

The fact that *P. perna* was able to thrive successfully in the cooling circuit in spite of chlorination raises the following questions: (1) Is the chlorine tolerance of *P. perna* comparable to that of *P. viridis* and *B. striatulus*? (2) Are there size-related differences: in the tolerance of *P. perna* to chlorine (as in the case of the latter two mussels)? (3) How does *P. perna* respond physiologically under chlorinated conditions? Development of an antifouling strategy for *P. perna* would require that these questions be answered by way of careful experimentation. In the present study, we attempt to find answers using mussels collected from the site in laboratory bioassays by subjecting different size groups of them to a range of chlorine concentrations. The results are compared with data (collected using comparable techniques) for coexisting mussel species, such as *P. viridis* and *B. striatulus*.

Materials and Methods

Experimental Animals

In Kalpakkam coastal waters (12°33′N and 80°11′E), *P. perna* grows to 9 mm shell length in a month to 36 mm in a year. The breeding season of *P. perna* has been observed between May to October (Rajagopal 1991). Mussels for the experiments were collected from the jetty piers of MAPS. The experimental mussels were collected over the entire nonspawning period (November to March) of mussels. The mussels were gently removed from the concrete substratum by cutting their byssus threads using a pair of scissors. In the laboratory, the mussels were acclimatized in sea water (mean ± SD; 34.3 ± 0.3% salinity, 29.6 ± 0.5°C temperature, 5.9 ± 0.7 mg/L dissolved oxygen, and 8.0 ± 0.2 pH) for 14 days. For each experiment, the animals were randomly picked up from this stock.

Mortality

Three size groups of mussels (shell length in mm ± SD; 9.6 ± 0.3, 25.4 ± 0.9, and 34.1 ± 1.8) were tested at five different chlorine concentrations (control, 1, 2, 3, and 5 mg/L). The sizes were chosen in such a way that the mussels were approximately 1 month, 6 months, and 1 year old. The chlorine concentrations generally used in power station cooling circuits depend on the mode of chlorination used (Rajagopal *et al.* 1996). Though continuous chlorination generally employs low (0.5 mg/L or less) chlorine residuals, other modes of chlorination (such as intermittent chlorination, shock dose chlorination, soak chlorination, and targeted chlorination, up to 5 mg/L) use higher residuals (Rajagopal *et al.* 1996; Jenner *et al.* 1998). Residuals used in the present study were chosen to include levels used at MAPS during the earlier intermittent dosing regime. Moreover, because we wanted to compare the mortality data of *P. perna* with the data already available for *P. viridis* and *B. striatulus*, we employed comparable chlorine residuals.

Sea water collected from the coastal waters was used for the experiment, after a day's storage. Factors that may change the response of mussels—such as salinity (34.1 ± 0.4‰), temperature (29.4 ± 0.6°C), dissolved oxygen (6.2 ± 0.7 mg/L), pH (8.1 ± 0.1), seston (22.3 ± 3.9 mg/L), chlorophyll *a* (2.3 ± 0.3 mg/L), and flow rate (80 ± 3 ml/min)—did not show considerable variation during the course of experiments. In preliminary experiments, comparable mortality responses were observed between fed (mixed algal culture) and starved mussels exposed to chlorination. Similar observations were also reported earlier for *P. viridis* (Rajagopal *et al.* 1995b). Hence, the mussels used here were not fed during the course of the experiment.

The experiments were conducted in continuous once-through flow systems, following the procedures outlined by Rajagopal *et al.* (1997). Sea water was stored in a 150-L aquarium tank, and chlorine stock solution prepared from bleaching powder was stored in a 2-L volumetric flask. Using a peristaltic pump (Buchler Instruments, Port Lee, NJ, model 73351), an appropriate mix of the two was employed to maintain the desired chlorine concentration in a 5-L glass beaker, having an outlet at the 4.5-L mark. Mixing of the water was facilitated by the use of aerators. After 14 days of acclimation, six mussels were introduced into the experimental tanks containing sea water of known chlorine concentration. The mussels were not allowed to attach in the experimental tanks before chlorine exposure (Rajagopal *et al.* 2002a). The levels of total residual chlorine were monitored at the outlet at 30-min intervals. The measurements were done using the iodometric and DPD methods (APHA *et al.* 1976; White 1999). Mortality was assessed at 6-h intervals; death of the experimental mussels was confirmed when their shell valves were agape and no response was observed when the exposed mantle tissue was stimulated with a needle. Dead mussels were immediately removed from the tank. The number of dead animals in each experiment was recorded along with the shell lengths and total weights for each observation event. The same experiment was repeated four times for each size group and chlorine concentration. Altogether, 360 mussels were used for the mortality experiments (6 mussels in each experiment × 5 chlorine doses including control × 3 size groups × 4 replicates = 360 mussels).

Sublethal Responses

Oxygen consumption, filtration rate, foot activity index, and byssus thread production of different size group of mussels were also studied at five different chlorine residuals (control, 0.25, 0.50, 0.75, and 1.00 mg/L). Experiments were run exactly as detailed; the only difference was that the mussels were left for 24 h for foot activity index and byssus thread production, for 3 h for filtration rate studies, and for 1 h for oxygen consumption studies.

Oxygen Consumption

Oxygen consumption was determined following the method of Bruijs *et al.* (2001). A closed glass respiratory chamber (750 ml), placed inside a double-walled glass beaker (to minimize temperature changes), was filled with Millipore (0.45 μm) filtered sea water (500 ml) previously aerated to 100% oxygen saturation. Five animals of a particular size group were placed together in the chamber for each measurement. In each experiment, 12 replicate measurements were taken (5 mussels in each experiment × 5 chlorine doses including control × 3 size groups × 12 replicates = 900 mussels). Control measurements were performed using the same setup but without mussels. The oxygen content of the water was determined at the start and at the end of each run (1 h) by Winkler method (Strickland and Parsons 1972). The amounts of oxygen used by the animals were taken as the average differences in oxygen concentration between the measurements with animals and the controls. Oxygen consumption was expressed in ml O_2/mussel/h.

Filtration Rate

Filtration rate was estimated (as clearance rate) following the method described by Coughlan (1969). The method is based on the rate of removal of neutral red by the mussels from ambient water (for details see Rajagopal *et al.* 1997). Altogether 270 mussels were used for filtration rate studies (6 mussels per experiment × 3 size groups × 5 chlorine concentrations including control × 3 replicates = 270 mussels). The equation provided by Coughlan (1969) was used for calculation:

$$m=[M/(n\cdot t)] \log (C_o/C_t) \quad (1)$$

where M = the volume of the test solution; n = the number of mussels used; t = the time in hours; C_o = the initial concentration of the dye; C_t = the concentration of the dye at time t; and m = the volume water filtered (ml/mussel/h). It may be noted that the value is not true filtration rate (F), because by using this method it cannot be shown that particles are removed by the gills with 100% efficiency (see Riisgård 2001 for more details).

Foot Activity Index

Six mussels were placed in 3 L of sea water and left undisturbed. Every 10 min, a note was made of the number of mussels with the foot extended outside the shell (Rajagopal *et al.* 1997). Each experiment lasted 24 h. No attempt was made to follow the foot activity of individual mussels. For each experiment, all foot activity readings were analyzed, and percentage foot activity was calculated (foot activity index). The same experiment was repeated three times for each size group at each chlorine concentration (6 mussels per experiment × 5 chlorine concentrations × 3 size groups × 3 duplicates = 270 mussels).

Byssus Thread Production

Rate of byssus thread production was determined following procedures outlined by Van Winkle (1970). After acclimation, one mussel was placed in a 1-L glass beaker (outlet at 0.5-L mark) containing 0.5 L of water of known chlorine concentration (1 mussel per experiment × 5 chlorine concentrations including control × 3 size groups × 10 replicates = 150 mussels). Byssus threads produced by mussels were counted after 24 h and expressed in number of threads/mussel/day.

Statistical Analysis

A two-way ANOVA was used to analyze data for the effects of chlorine concentration on the survival time of different size groups of *P. perna* (Zar 1984). The variables of interest were residual chlorine concentration and mussel size. Before analysis, survival time was log-transformed for homogeneity. Differences between mean values of survival time for each group were tested by Tukey's pairwise multiple comparison test (Zar 1984). The data obtained on mortality of various size groups at different chlorine doses were subjected to probit and regression analysis, yielding the statistic LT_{50} (Litchfield and Wilcoxon 1949). The differences in physiological activity between control and experimental mussels (0.25–1 mg/L residual chlorine) were compared by Student t-tests after Bonferroni corrections for multiple pairwise comparisons (Zar 1984). The differences in sublethal responses of different size groups of mussels at various chlorine concentrations were tested by two-way ANOVA (chlorine dose effect and mussel size effect) followed by Student-Newman-Keuls (SNK) multiple comparison tests (Zar 1984). All analyses were performed using a Statistical Analysis Systems package (SAS 1989).

Results

Mortality

The applied chlorine concentration and actual measured chlorine concentrations (in parentheses; mean ± SD) at the experimental tanks were as follows: 1 mg/L (1.01 ± 0.01, n = 2,397), 2 mg/L (2.02 ± 0.04, n = 1,634), 3 mg/L (3.01 ± 0.03, n = 1,216), and 5 mg/L (4.99 ± 0.05, n = 1,059). The cumulative mortality of *P. perna* exposed to different chlorine levels is presented in Figure 1. The exposure time required for 100% mortality of all size groups of *P. perna* significantly decreased with increasing chlorine concentration (chlorine dose effect, $F_{(4,355)} = 510.71$, $p < 0.0001$). For example, mussels in the 9 mm size group exposed to 1 mg/L chlorine residual took 384 h to reach 100% mortality, whereas those exposed to 5 mg/L chlorine took 84 h (Tukey's test, $p < 0.0001$). The three size groups (9, 25, and 34 mm shell length) of *P. perna* showed 100% mortality at significantly different exposure times between 1 and 5 mg/L chlorine concentrations (mussel size effect: $F_{(2,357)} = 188.19$, $p < 0.0001$), with larger animals more resistant than smaller ones (Table 1). For example, at 3 mg/L residual chlorine, 9 mm and 34 mm mussels took 126 h and 186 h, respectively, to achieve 100% mortality (Tukey's test, $p < 0.0001$). No significant differences were found between replicate experiments (replicates: $F_{(3,356)} = 0.79$, $p > 0.05$) and no mortality occurred in control tanks. The time to 50% mortality (LT_{50}) of *P. perna* was investigated by probit and regression analysis and also shows that significant chlorine dose effect (ANOVA, $p < 0.0001$) and size effect (ANOVA, $p < 0.0001$) on LT_{50} of *P. perna* (Figure 2).

Oxygen Consumption

Oxygen uptake of *P. perna* at different chlorine levels showed a progressive decline as the chlorine concentration increased from 0 to 1 mg/L (Figure 3). For example, the 25 mm size group showed a decrease of oxygen consumption from 1.1 ml O_2/mussel/h in the control to 0.2 ml O_2/mussel/h at 1 mg/L residual chlorine ($t = 16.471$, df = 22, $p < 0.001$). There was a size-dependent variation in the oxygen consumption of *P. perna* (size effect: $F_{(2,897)} = 203.54$, $p < 0.0001$), with larger mussels showing higher consumption (Figure 3).

Filtration Rate

In control experiments, mussels in the 35 mm size group showed a maximum filtration rate of 40 ml/mussel/h (Figure 3). The filtration rate decreased significantly with increasing chlorine concentrations in all size groups of mussels tested (chlorine dose effect: $F_{(4,266)} = 93.84$, $p < 0.0001$). Data also show a clear size-dependent variation in filtration rate (size effect:

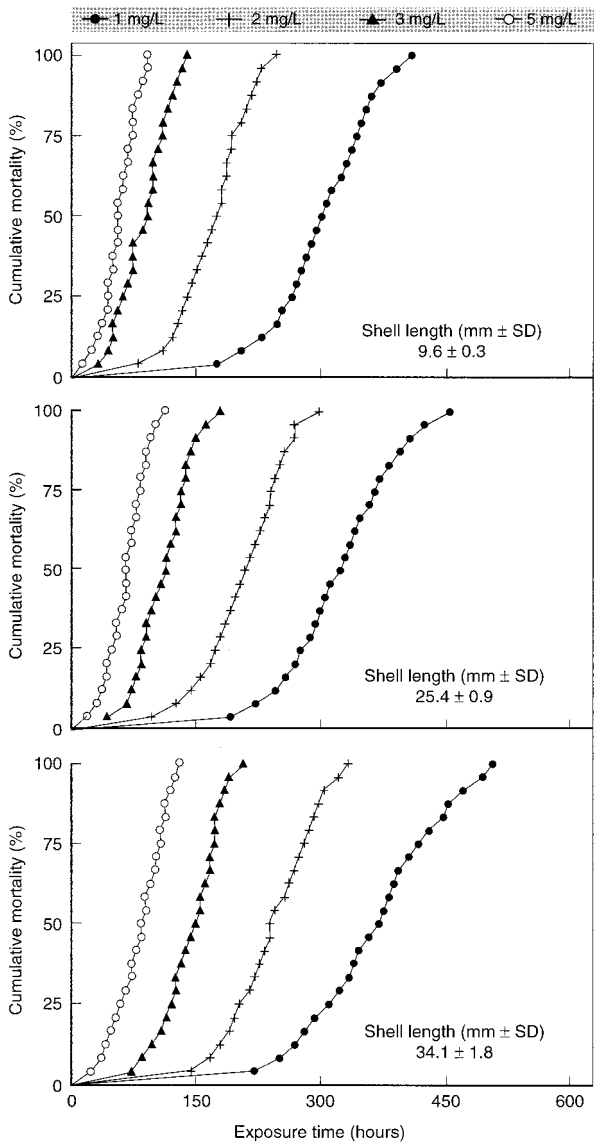

Fig. 1. Cumulative mortality (%) of different size groups of *Perna perna* at different chlorine concentrations. Twenty-four mussels were used at each chlorine dose. Mortality was monitored at 6-h intervals. The criterion for mortality of mussels was shell valve gape with no response of exposed mantle tissues to external stimuli

$F_{(2,267)} = 211.03$, $p < 0.0001$). As the size increased, a progressive increase in filtration rate was observed.

Foot Activity Index

The highest foot activity index (58%) was measured in control experiments with 9 mm mussels (Figure 3). At increased concentrations of chlorine, the foot activity index of 9 mm group tended to decrease (40% at 0.25 mg/L residual chlorine), reaching a very low average level of 5% at 1 mg/L of residual chlorine ($t = 21.094$, df = 34, $p < 0.001$). A similar pattern was evident for other size groups of mussels as well (Table 1). Moreover, significant size-dependent variation in foot activity index was observed in the control experiments (size effect: $F_{(2,267)} = 14.71$, $p < 0.0001$). As the size increased a progressive decrease in foot activity index was observed. However, the size-dependent response was not significantly different between 0.75 and 1 mg/L chlorine residuals (see Figure 3).

Byssus Thread Production

In control experiments, mussels of the 9 mm size group produced approximately 18 threads/mussel/day (Figure 3). The byssus thread production of *P. perna* showed a progressive decline as the chlorine concentration increased. The byssus thread production was also significantly different in mussels of different sizes (size effect: $F_{(2,147)} = 10.33$, $p < 0.0001$); the smaller mussels showed higher byssus production. As in the case of foot activity index, a size-dependent variation in the rate of byssus thread production was not significantly different between 0.75 and 1 mg/L residual chlorine (SNK tests, $p > 0.05$). Foot activity index and byssus thread production of *P. perna* were strongly correlated (Spearman rank correlation test, $r = 0.94$, $p < 0.0001$) at different chlorine concentrations.

Oxygen consumption, filtration rate, foot activity index, and byssus thread production of *P. perna* showed progressive reduction with increasing chlorine concentration (Figure 3). In all size groups, physiological activities of *P. perna* showed a decrease of about 90% at 1 mg/L residual chlorine. The sublethal responses of *P. perna* are strongly correlated with different concentrations of chlorine ($0.94 < r < 0.99$; $p < 0.0001$).

Discussion

The present results clearly indicate significance of mussel size in the tolerance of *P. perna* to chlorine concentrations. Larger mussels exhibit greater tolerance when compared to smaller animals. Similar results have been reported for *P. viridis* and *B. striatulus* (Rajagopal *et al.* 1995b, 1997). Accordingly, this indicates the control of these three species of mussels is best achieved when they are young. Consequently, the dead mussels would be small enough to pass through the cooling system, without blocking the condenser tubes. Continuous low-dose chlorine regimes (< 1 mg/L) targeted against mussel spat (during breeding season) were successfully employed against *Mytilus edulis* at the Maasvakte power station, Rotterdam, and the Dunkerque power plant, French North Sea coast (Jenner *et al.* 1998); against *P. viridis* at the MAPS, Kalpakkam (Rajagopal *et al.* 1996); and against *Dreissena polymorpha* at the Monroe power station, Lake Erie (Kovalac *et al.* 1993). In most cases, chlorine treatments against mussel spat were practiced for the maximum period of 2 months (Jenner *et al.* 1998). However, establishment of mature communities containing larger mussels would make it difficult for a given dose of chlorine to bring about satisfactory results. For example, higher chlorine doses (between 3 and 5 mg/L) were continuously used for nearly 4 months to remove well-established mussel communities (mostly dominated by *P. viridis*) at MAPS in 1988 (Rajagopal *et al.* 1996). The present data show that the chlorine tolerance of the 9 mm size group *P. perna* (in terms of time to

Table 1. Lethal (mortality) and sublethal responses (oxygen consumption, filtration rate, foot activity index, and byssus thread production) of different size groups of *Perna perna* at different chlorine concentrations

Chlorine concentration (mg/L)	Size of the mussels (mm ± SD)		
	9.6 ± 0.3	25.1 ± 0.9	34.8 ± 1.8
		Time to reach 100% mortality (h)*	
1	384 ± 12	440 ± 16	484 ± 24
2	228 ± 11	268 ± 13	304 ± 18
3	126 ± 7	162 ± 10	186 ± 12
5	84 ± 6	102 ± 8	120 ± 9
		Oxygen consumption (ml O_2/animal/h)	
	9.4 ± 0.5	24.7 ± 1.2	35.4 ± 2.1
Control	0.92 ± 0.07	1.13 ± 0.08	1.28 ± 0.09
0.25	0.65 ± 0.05	0.86 ± 0.06	1.01 ± 0.06
0.50	0.47 ± 0.04	0.69 ± 0.04	0.81 ± 0.05
0.75	0.18 ± 0.02	0.43 ± 0.03	0.48 ± 0.03
1.00	0.09 ± 0.01	0.19 ± 0.02	0.24 ± 0.02
		Filtration rate (ml/mussel/h)	
Control	25.7 ± 2.24	34.2 ± 2.77	39.4 ± 3.01
0.25	17.6 ± 2.11	26.1 ± 2.23	32.3 ± 2.18
0.50	15.3 ± 1.64	22.5 ± 1.76	24.6 ± 1.84
0.75	8.4 ± 0.91	16.5 ± 1.35	18.2 ± 1.26
1.00	2.9 ± 0.41	6.9 ± 0.64	8.4 ± 0.75
		Foot activity index (%)	
Control	58.2 ± 4.63	45.6 ± 3.85	35.4 ± 3.07
0.25	39.9 ± 3.74	35.2 ± 2.79	27.9 ± 2.33
0.50	24.2 ± 2.41	24.2 ± 1.90	16.2 ± 1.45
0.75	7.9 ± 1.07	10.1 ± 1.16	8.9 ± 0.98
1.00	4.9 ± 0.85	8.2 ± 0.67	5.6 ± 0.59
		Byssus thread production (threads/mussel/day)	
Control	17.5 ± 1.86	12.7 ± 1.41	10.3 ± 1.25
0.25	12.7 ± 1.03	9.9 ± 1.14	8.4 ± 0.83
0.50	6.7 ± 0.68	6.5 ± 0.62	5.8 ± 0.46
0.75	2.6 ± 0.34	2.9 ± 0.37	3.1 ± 0.41
1.00	1.1 ± 0.23	1.6 ± 0.18	1.3 ± 0.27

Data are expressed as mean ± SD (n = 10–60).
* No mortality occurred in control tanks.

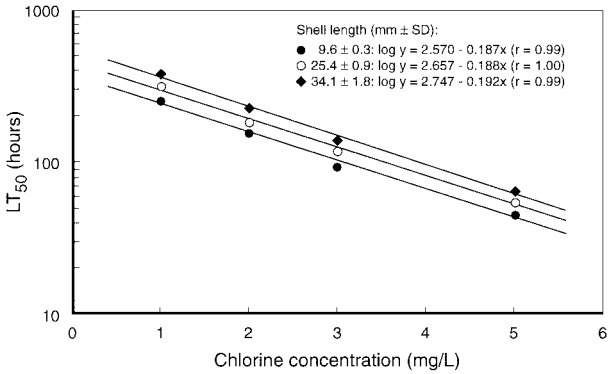

Fig. 2. Time required for 50% mortality (LT_{50}) of different size groups of *Perna perna* at different chlorine concentrations (after probit and regression analysis)

reach 100% mortality) at a chlorine concentration of 5 mg/L is 40% less than that of the 34 mm size group. However, at 1 mg/L residual, the equivalent difference is only 27%. It shows that the difference in tolerance levels between small and large mussels decreases as chlorine concentration is increased. This is probably related to the mode of action of chlorine on the animals (Jenner *et al.* 1998). Unlike at relatively low chlorine residuals, where mussels open and feed on and off, higher chlorine residuals cause the mussels to shut their shells off completely, leading to anaerobiosis (Lewis 1985). It would be interesting to study the relative susceptibility of different size groups of *P. perna* to anaerobiosis and its implications. It has been reported that this kind of size-related variation in tolerance levels is not observed in certain mussel species like *D. polymorpha* (Rajagopal *et al.* 2002b).

As already mentioned, in the cooling water systems of MAPS, *P. perna* coexist with *P. viridis* and *B. striatulus* (Rajagopal *et al.* 1996). A comparison of the chlorine tolerance of these three species shows that *P. perna* is the most sensitive among the three (Figure 4), with *P. viridis* being the most tolerant. The pattern of relative tolerance remains the same at all the four chlorine concentrations tested. At higher chlorine

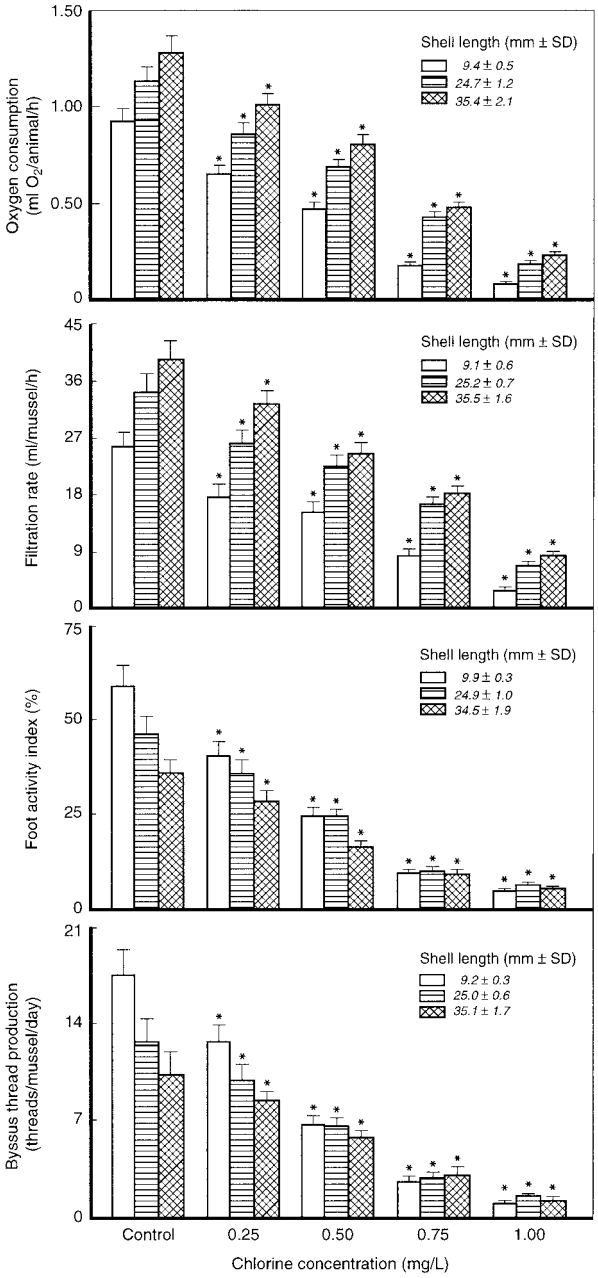

Fig. 3. The oxygen consumption, filtration rate, foot activity index, and byssus thread production of different size groups of *Perna perna* at different chlorine concentrations. Data are expressed as mean ± SD ($n = 10-60$). Differences between control and experimental mussels (0.25–1.00 mg/L) were compared by Student *t*-tests after Bonferroni's adjustment for multiple pairwise comparisons. *Significant at $p < 0.001$

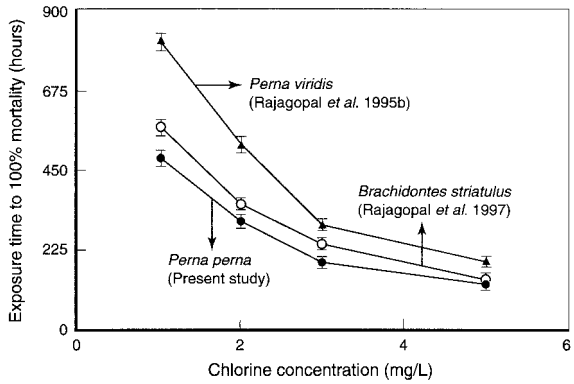

Fig. 4. Comparison of exposure times to reach 100% mortality of *Perna perna* (shell length in mm ± SD; 34.1 ± 1.8), *Perna viridis* (95.3 ± 1.58; Rajagopal *et al.* 1995b) and *Brachidontes striatulus* (25.4 ± 1.9; Rajagopal *et al.* 1997) at different chlorine concentrations. Mortality data are expressed as mean ± SD ($n = 24$ for chlorine). Test methods and mortality determinations were similar in all toxicity studies of species

concentrations (> 1 mg/L), mussels are forced to shut their valves and exist on stored food reserves and anaerobic respiration until energy resources are depleted or metabolic wastes reach toxic levels (Lewis 1985). It is also reported that many species of bivalves adapt to oxygen stress by reducing their metabolic rate, thereby reducing their metabolic demand and conserving energy stores (Bayne *et al.* 1976). Mortality in the long run would then result (apart from chlorine toxicity) from depletion of energy resources and respiratory acidosis (Methews and McMahon 1999). It is obvious that the differential mortality among different mussel species is likely to be due to the differences in the energy resources *vis-à-vis* metabolic requirements during anaerobiosis. The data imply that continuous chlorination would first eliminate *P. perna* in a system cofouled with *P. viridis* and *B. striatulus*.

In the present study, four types of physiological activities (all related to shell valve opening) have been monitored at four different residual chlorine concentrations. The concentrations chosen were relatively low (0.25–1.00 mg/L) to represent the concentrations used under low-dose continuous dosing regimes in power plant conditions (Jenner *et al.* 1998). Here again, the response of the mussels clearly indicates dose dependence. Foot activity index generally decreases with increasing size of the mussel because smaller mussels are normally more active (Bayne *et al.* 1976). However, at increasing chlorine concentration we see that the difference in foot activity index between different size groups diminishes, indicating a greater tolerance on the part of larger mussels to chlorine. A similar trend is also seen in the case of byssus thread production. Filtration rate in a given mussel species is correlated with surface area of gills; therefore, larger mussels generally have higher filtration rate (Jones *et al.* 1990). At increasing chlorine concentrations, this trend is also maintained (Figure 3).

The sublethal physiological responses of mussels may provide valuable data for planning chlorination strategies for mussel control (Bidwell *et al.* 1999; Rajagopal *et al.* 2002c). The most obvious effect of low-level continuous chlorination is to reduce respiration (oxygen consumption) and feeding rate (filtration rate) and to cause a depression of foot activity, leading to a reduction in the number of byssus threads. Apparently, a lower number of byssus threads per animal makes mussel attachment to substratum relatively weaker in chlorinated environments compared with that in a healthy environment (Jenner *et al.* 1998; Rajagopal *et al.* 2002a). In the literature, suppression of foot activity index and reduction of byssus strength in chlorinated *D. polymorpha, M. edulis* and *Mytilop-*

sis leucophaeata (Conrad) have been reported by Rajagopal *et al.* (2002c), who observed that mussels chlorinated at 0.2 mg L^{-1} residual level required 36–52% less force to detach than unchlorinated ones. Rajagopal *et al.* (2002b) also estimated that force required to detach *P. viridis* from a chlorinated cooling water system was 59–88% less than that for control mussels. Once the attachment is weakened, the likelihood of the mussel being washed away by flow is substantially increased, especially in cooling water systems. Earlier, Rajagopal *et al.* (1996) reported removal of 165 tons of mussels from the cooling circuits of MAPS using chlorination and suggested weakness in mussel attachment to be a probable cause for such large release under flow conditions. The present physiological data show that continuous dosing at a residual level of at least 1 mg/L is necessary to force *P. perna* to close their shells, without allowing a recovery phase. Therefore, it is desirable to maintain such residual levels during peak settlement periods of *P. perna* to prevent fresh colonization.

Conclusions

P. perna appears to be the least tolerant to chlorine when compared to other two cofoulants (*P. viridis* and *B. striatulus*). However, *P. perna* compares well with other two species in terms of size dependence of chlorine tolerance and chlorine effect on the oxygen consumption, filtration rate, foot activity, and byssus thread production. It is obvious from the present study that logically it is prudent to control mussel fouling in the initial stages of colonization, because these mussels are most sensitive when they are young. However, the residual levels to be administered depend on the most tolerant species. Therefore, to control a mussel fouling community containing *P. viridis*, *P. perna*, and *B. striatulus*, chlorine residuals are to be chosen based on the tolerance of *P. viridis*, which is the most tolerant among the three.

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