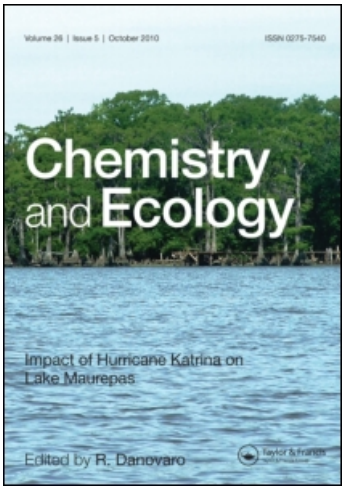

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Response of mussel *Brachidontes variabilis* to chlorination

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Brachidontes variabilis is a common fouling mussel species in cooling water systems of tropical coastal power stations. However, there are hardly any data available on the response of *B. variabilis* to chlorine, a commonly used antifouling biocide. Therefore, lethal and sublethal responses of this mussel to chlorine are of considerable interest to the industry. The response of mussels in terms of mortality pattern (LT_{50} and LT_{100}) and physiological activities (oxygen consumption, filtration rate, foot activity and byssus thread production) in different size groups (with shell lengths of 7–24 mm) of *B. variabilis* was studied in the laboratory under different chlorine concentrations (0.25, 0.50, 0.75 and 1.00 mg l^{-1} for sublethal responses and 1, 2, 3 and 5 mg l^{-1} for mortality). The results showed that the exposure time for 100% mortality of mussels decreased significantly with increasing chlorine concentration. However, mussel size was not a determinant of its chlorine tolerance: all size groups tested (with shell lengths of 7–24 mm) took comparable exposure times to reach 100% mortality at a given chlorine concentration (1–5 mg l^{-1}). All size groups of *B. variabilis* showed a progressive reduction in physiological activities such as oxygen consumption, filtration rate, foot activity and byssus thread production, when chlorine residuals were increased from 0 to 1 mg l^{-1}. The data generated in the present work are compared with similar data available for other tropical fouling mussel species to see how far relative chlorine toxicity could have influenced the relative distribution of the mussels inside the seawater intake tunnel of a power station at Kalpakkam in India. It is shown that in this insufficiently chlorinated system, the relative distribution of *Brachidontes striatulus, B. variabilis* and *Modiolus philippinarum* reflects the relative tolerance of the species to chlorine.

Keywords: Mussel fouling; *Brachidontes variabilis*; Chlorine; Mortality; Physiological activities

1. Introduction

Cooling water systems of coastal power stations, if not properly treated, invariably have problems arising from biofouling. Chemical control techniques involving injectable biocides are widely used to control biofouling in such systems. For example, chlorination has been the most commonly used fouling control method in industrial cooling water systems since more than five decades [1–4]. Chlorine as a biocide is effective against a variety of fouling organisms including bacteria, algae, fungi and invertebrates [3, 5]. Its advantages include relatively low

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costs, flexibility (being available in gaseous, liquid and solid forms), ease of dosage and broad spectrum of activity [6, 7]. However, there are several disadvantages associated with the use of chlorine as an antifouling agent in once-through cooling systems [8]. Chlorine by-products (e.g. trihalomethanes, halophenols, halo acetic acids) are known blacklist compounds and can be potential pollutants of receiving waters [9]. Power stations in many countries are, therefore, required to ensure that their cooling water effluents do not contain any detectable amounts of chlorine (see Jenner *et al.* [3] for legislation and regulatory controls on the use of biocides in power station cooling waters). Thus, it has become imperative to generate data on the optimum level of chlorine required to control the various species for efficient biofouling control.

Brachidontes variabilis (Krauss) is a mussel species widely distributed throughout the Indo-Pacific region [10–14]. *B. variabilis* (syn. *Brachidontes pharaonis*) has also recently invaded the Mediterranean Sea through the Suez canal [15]. *B. variabilis* typically occurs on protected shores [13] and sheltered estuarine environments [16], and is also associated with mangroves [17]. On estuarine shores without mangroves, it attaches to rocks and often nestles among encrusting oysters [18]. It has also been reported as an important fouling organism in cooling conduits of power stations [19]. For example, Rajagopal *et al.* [20, 21] reported a large-scale occurrence of *B. variabilis* and other mussel species, *Brachidontes striatulus* (Hanley), *Modiolus philippinarum* (Hanley), *Perna viridis* (L.) and *P. perna* (L.) in the cooling water conduits of the Madras Atomic Power Station (MAPS), on the east coast of India. *B. variabilis* contributed 6% of the total mussel densities [5]. This largely mussel-dominated community developed in spite of intermittent chlorination being practised at the power station [5]. It is possible that the relative distribution of these three mussels is related to the relative toxicity of chlorine to them. In an earlier paper [22], we showed that two closely related mussels, *Perna viridis* (L.) and *P. perna* (L.), were present among the same fouling community in accordance with their relative sensitivity to chlorine. *P. viridis*, which is more tolerant to chlorine [23], was four times as abundant as the relatively more sensitive *P. perna* [22]. Therefore, we hypothesized that the decreasing order in which *B. striatulus, M. philippinarum* and *B. variabilis* were observed in the fouling community was a reflection of the relative sensitivity of the respective species to chlorine. There are several published reports available on the response of other common tropical fouling mussels such as *P. viridis, P. perna, B. striatulus* and *M. philippinarum* to chlorine [4, 22–24]. However, there are hardly any data on the response of *B. variabilis* to chlorine. The objectives of this study, therefore, were: (1) to determine the mortality of *B. variabilis* on exposure to different chlorine concentrations, to assist utilities in planning chlorine regimes for controlling *B. variabilis*; (2) to ascertain if *B. variabilis* exhibited size-dependent variation in survival in chlorine bioassay that could be exploited for biofouling control; (3) to understand the physiological response of *B. striatulus* under chlorinated conditions; and (4) to compare the toxicity response of *B. variabilis* with that of other coexisting mussels to see how chlorine levels could have modulated the relative distribution of the species inside the cooling circuit, vis-à-vis that in the natural environment. It is expected that the results will help plant operators to optimize application of chlorine, so that maximal control could be achieved in a cost-effective and environmentally acceptable manner [3, 19, 25, 26].

2. Materials and methods

2.1 *Experimental animals*

Mussels for the experiments were collected from the jetty piers of MAPS, situated at Kalpakkam (12° 33′ N and 80° 11′ E) on the east coast of India. In Kalpakkam, *B. variabilis* grows at the rate of 6 mm (shell length) per month to 24 mm in a year. The breeding season of

B. variabilis has been observed between April and November [27]. The experimental mussels were collected during non-spawning period (December to March) of *B. variabilis*, as spawning activity may weaken the mussels [28]. The mussels were gently removed from the concrete substratum by cutting their byssus threads using a pair of scissors and immediately transferred to the laboratory. Seawater collected from the field site was used to acclimate *B. variabilis* under laboratory conditions (mean ± S.D.; 34.1 ± 0.2‰ salinity, 29.3 ± 0.4 °C temperature, 6.1 ± 0.5 mg l^{-1} dissolved oxygen and 8.1 ± 0.1 pH). Mussels acclimated for at least 48 h in the laboratory were used for each experiment.

2.2 *Mortality experiments*

Three size groups of *B. variabilis* (shell length in mm ± S.D.; 6.5 ± 0.4, 17.1 ± 1.1 and 23.6 ± 1.4) were tested at four different chlorine concentrations (1, 2, 3 and 5 mg l^{-1}). Residuals used in the present study were so chosen as to include levels normally used in the power-station cooling water circuit [5]. The continuous chlorination generally employed in power stations uses low (i.e. less than 0.5 mg l^{-1}) chlorine residuals, while other modes of chlorination such as intermittent chlorination, shock-dose chlorination, soak chlorination and targeted chlorination use higher residuals, sometimes as high as 5 mg l^{-1}, for a relatively short duration [3, 5, 26].

Seawater 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 (mean ± S.D.; 34.4 ± 0.5‰), temperature (29.2 ± 0.4 °C), dissolved oxygen (6.1 ± 0.6 mg l^{-1}), pH (8.1 ± 0.1), seston (24.5 ± 3.4 mg l^{-1}), chlorophyll-a (2.1 ± 0.2 mg l^{-1}) and flow rate (80.3 ± 4.2 ml min^{-1}) did not show any considerable variation during the course of the experiments. In preliminary experiments, comparable mortality responses were observed between fed (mixed algal culture) and non-fed *B. variabilis*, when exposed to chlorination. Similar observations were also reported earlier for *P. viridis* [23], *B. striatulus* [24] and *P. perna* [4]. Hence, *B. variabilis* used in the present study 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.* [24]. Seawater 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 No. 73351), an appropriate mix of the two was employed to maintain the desired chlorine concentration in a 5 l glass beaker, with an outlet at the 4.5 l mark. Mixing of the water was facilitated by the use of aerators. After 2 d of acclimation, six randomly picked mussels were introduced into the experimental tanks containing seawater of known chlorine concentration. The levels of total residual chlorine were monitored at the outlet at 30 min intervals. The measurements were carried out using the iodometric and DPD methods [7]. Mortality was assessed at 6 h intervals. The criterion for mortality of mussels was a shell valve gape with no response of exposed mantle tissues to external stimuli [24]. Dead mussels were immediately removed from the tank. The number of dead animals in each experiment was recorded, along with their shell lengths and total weights for each observation event. The same experiment was repeated three times for each size group and chlorine concentration (6 mussels in each experiment × 5 chlorine concentrations (including control) × 3 size groups × 3 replicates = 270 mussels).

2.3 *Sublethal responses*

Oxygen consumption, filtration rate, foot activity and byssus thread production of different size groups of *B. variabilis* were also studied at five different chlorine residuals (control, 0.25, 0.50, 0.75 and 1.00 mg l^{-1}). Experiments were run exactly as detailed above; the only

difference was that the mussels were left for 24 h for foot activity and byssus thread production, for 3 h for filtration rate studies and for 1 h for oxygen-consumption studies.

2.4 *Oxygen consumption*

The oxygen consumption was determined following the method of Bruijs *et al.* [29]. A closed glass respiratory chamber (750 ml), placed inside a double-walled glass beaker (to minimize any temperature changes), was filled with Millipore (0.45 μm) filtered seawater (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 concentrations (including control) × 3 size groups × 12 replicates = 900 mussels). Control measurements were performed using the same set-up, but without mussels. The oxygen content of the water was determined at the start and end of each run (1 h) by Winkler's method [30]. 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^{-1} h^{-1}.

2.5 *Filtration rate*

Filtration rate was measured following the method described by Coughlan [31]. The method is based on the absorption of neutral red by mussels from ambient water. Altogether, 540 mussels were used for filtration rate studies (6 mussels per experiment × 3 size groups × 5 chlorine concentrations (including control) × 3 replicates = 270 mussels). The rate of filtration was calculated using the following equation from Coughlan [31]:

$$\mathrm{FR} = \frac{V}{nt} \log \frac{C_0}{C_t}$$

where V = volume of the test solution; n = number of animals used in the experiment; t = time (h); C_0 = initial concentration of the dye; C_t = concentration of the dye at time t; FR = the rate of filtration (ml h^{-1} mussel^{-1}). Note that the value is not the true filtration rate (F) because it is not possible, using this method, to show that particles are removed by the gills with 100% efficiency (see Riisgård [32] for more details).

2.6 *Foot activity index*

For foot activity index, six mussels were kept in 3 l of seawater and left undisturbed for 24 h. Every 10 min, the number of mussels with the foot extended outside the shell was noted [4, 33]. No attempt was made to follow the foot activity of individual mussels. For each experiment, the foot activity of all mussels was analysed, and the percentage foot activity index was calculated (6 mussels per experiment × 5 chlorine concentrations (including control) × 3 size groups × 3 replicates = 270 mussels).

2.7 *Byssus thread production*

Byssus thread production was determined following procedures outlined by Van Winkle [34] and Rajagopal *et al.* [23]. After 48 h of acclimation, one mussel was placed in a 1 l glass beaker containing 0.75 l of seawater of known chlorine concentration (1 mussel per experiment × 5

chlorine concentrations (including control) × 3 size groups × 12 replicates = 180 mussels). By using only one mussel per container, there was no need to code the mussels, and any problems of counting threads (when mussels clump, which they invariably did) were prevented [24]. The byssus threads produced by mussels were counted after 24 h and expressed as threads mussel^{-1} d^{-1} [34].

2.8 *Statistical analysis*

A three-way ANOVA was used to analyse data for the effects of chlorine concentration on the mortality of different size groups of *B. variabilis* [35]. The variables of interest were residual chlorine concentration and mussel size. The third factor was used to test for possible block effects caused by the use of different experimental tanks. Before analysis, the 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 [35]. The data obtained on mortality of various mussel species at different chlorine doses were subjected to probit and regression analysis, yielding the statistic LT_{50} [36]. The differences in physiological activity (oxygen consumption, filtration rate, foot activity index and byssus thread production) between control and experimental mussels (0.25, 0.50, 075 and 1 mg l^{-1} residual chlorine) were compared by Student's *t* tests after Bonferroni corrections for multiple pairwise comparisons [35]. The post-hoc 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). All analyses were performed using a Statistical Analysis Systems package [37].

3. Results

3.1 *Mortality*

The cumulative mortality of *B. variabilis* exposed to different chlorine levels is presented in figure 1. The exposure time required for 100% mortality of *B. variabilis* decreased significantly

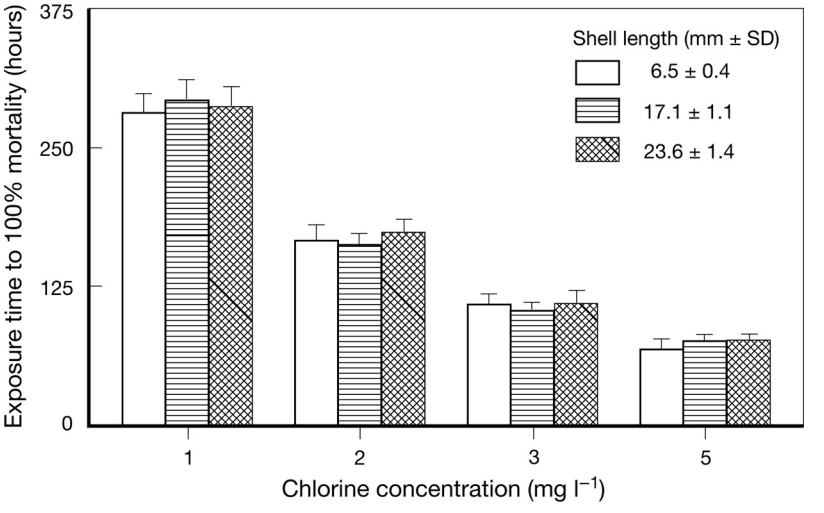

Figure 1. Exposure time required for 100% mortality of different size groups of *Brachidontes variabilis* at different chlorine concentrations. Data are expressed as mean ± S.D. (n = 18). Mortality was monitored at 6 h intervals. The criterion for mortality of mussels was shell valve gape with no response of exposed mantle tissues to external stimuli.

with increasing chlorine concentration (chlorine dose effect, $F_{(4,265)} = 384.52,\ P < 0.0001$). For example, mussels in the 24 mm size group exposed to 1 mg l^{-1} chlorine residual took 288 h to reach 100% mortality, whereas those exposed to 5 mg l^{-1} chlorine took 27 h (Tukey's test, $P < 0.0001$). No significant differences were found between replicate experiments (replicates: $F_{(2,267)} = 0.53,\ P > 0.05$), and there was no mortality in any of the control tanks. The three size groups (7, 17 and 24 mm shell length) of *B. variabilis* showed 100% mortality at similar exposure times at chlorine concentrations of 1–5 mg l^{-1} (mussel size effect: $F_{(2,267)} = 0.48,\ P > 0.05$). For example, at 2 mg l^{-1} residual chlorine, 7 mm and 24 mm mussels took 168 h and 174 h, respectively to achieve 100% mortality (Tukey's test, $P < 0.0001$). The time to 50% mortality (LT_{50}) of *B. variabilis* was investigated by probit and regression analysis and also shows a significant chlorine dose effect (ANOVA, $P < 0.0001$) and no size effect (ANOVA, $P > 0.05$) on LT_{50} of *B. variabilis* (figure 2).

For comparison, 100% mortality data of other coexisting mussel species *B. striatulus* [24], *M. philippinarum* [19], *P. viridis* [23] and *P. perna* [22] are given in figure 3. Test methods and mortality determinations used for these mussel species were similar to those used for *B. variabilis.* The exposure times required for 100% mortality of *B. variabilis* at different chlorine concentrations were much shorter than those required for *B. striatulus, M. philippinarum, P. viridis* and *P. perna* (figure 3).

3.2 *Oxygen consumption*

In control experiments, mussels in the 24 mm size group showed a maximum oxygen consumption of 0.82 ml O_2 $mussel^{-1}$ h^{-1} (figure 4). The oxygen uptake of *B. variabilis* at different chlorine levels showed a progressive decline as the chlorine concentration increased from 0 to 1 mg l^{-1}. For example, 17 mm mussels showed a decrease in oxygen consumption from 0.71 O_2 $mussel^{-1}$ h^{-1} in the control to 0.03 ml O_2 $mussel^{-1}$ h^{-1} at 1 mg l^{-1} residual chlorine ($t = 23.715$, d.f. $= 22$, $P < 0.001$). There was a significant size-dependent variation in the oxygen consumption of *B. variabilis* (size effect: $F_{(2,897)} = 79.05,\ P < 0.001$), with larger mussels showing a higher consumption.

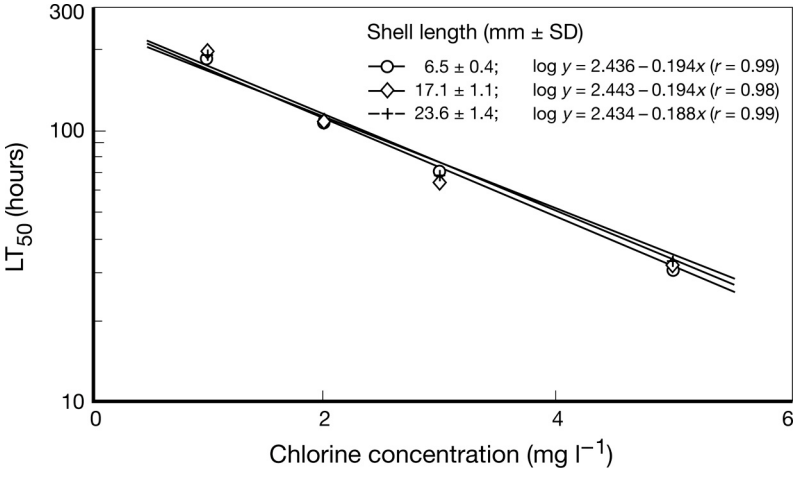

Figure 2. Time required for 50% mortality (LT_{50}) of different size groups of *Brachidontes variabilis* at different chlorine concentrations (after probit and regression analysis).

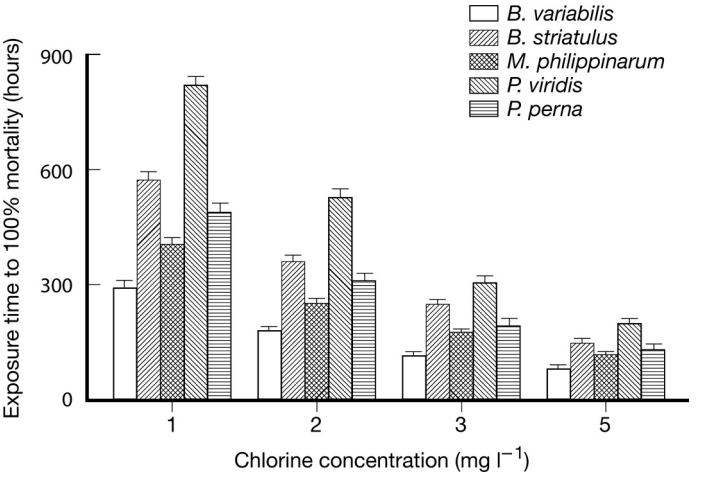

Figure 3. Comparison of exposure times to reach 100% mortality of *Brachidontes variabilis* along with published results for *Brachidontes striatulus* [19], *Modiolus philippinarum* [4, 19] *Perna viridis* [23], and *Perna perna* [22] at different chlorine concentrations (all studies from India). Test methods and mortality determinations were similar in all chlorine studies of different species.

3.3 *Filtration rate*

B. variabilis showed a maximum filtration rate in control experiments (figure 4). However, the filtration rate decreased significantly with increasing chlorine concentrations in all size groups of mussels tested (chlorine dose effect: $F_{(4,266)} = 52.09,\ P < 0.0001$). Data also show a clear size-dependent variation in filtration rate (size effect: $F_{(2,267)} = 95.16,\ P < 0.0001$). As the size increased, a progressive increase in filtration rate was observed.

3.4 *Foot activity index*

The highest foot activity index (69%) was measured in control experiments with 7 mm mussels (figure 4). At increased concentrations of chlorine, the foot activity index of the 9 mm size group tended to decrease (39% at 0.25 mg l^{-1} residual chlorine), reaching a very low average level of 2% at 1 mg l^{-1} of residual chlorine ($t = 27.385$, d.f. $= 34,\ P < 0.001$). A similar pattern was evident for other size groups of mussels as well (figure 4). Moreover, a significant size-dependent variation in foot activity index was observed in the control experiments (size effect: $F_{(2,267)} = 9.28,\ P < 0.001$). 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^{-1} chlorine residuals.

3.5 *Byssus thread production*

In control experiments, mussels of 7 mm size group produced 64 threads mussel day^{-1} (figure 4). The byssus thread production of *B. variabilis* showed a progressive decline as the chlorine concentration increased. The byssus thread production was also significantly different in mussels of different sizes (size effect: $F_{(2,177)} = 8.49,\ P < 0.001$); the smaller mussels showed a higher byssus production. The foot activity index and byssus thread production of

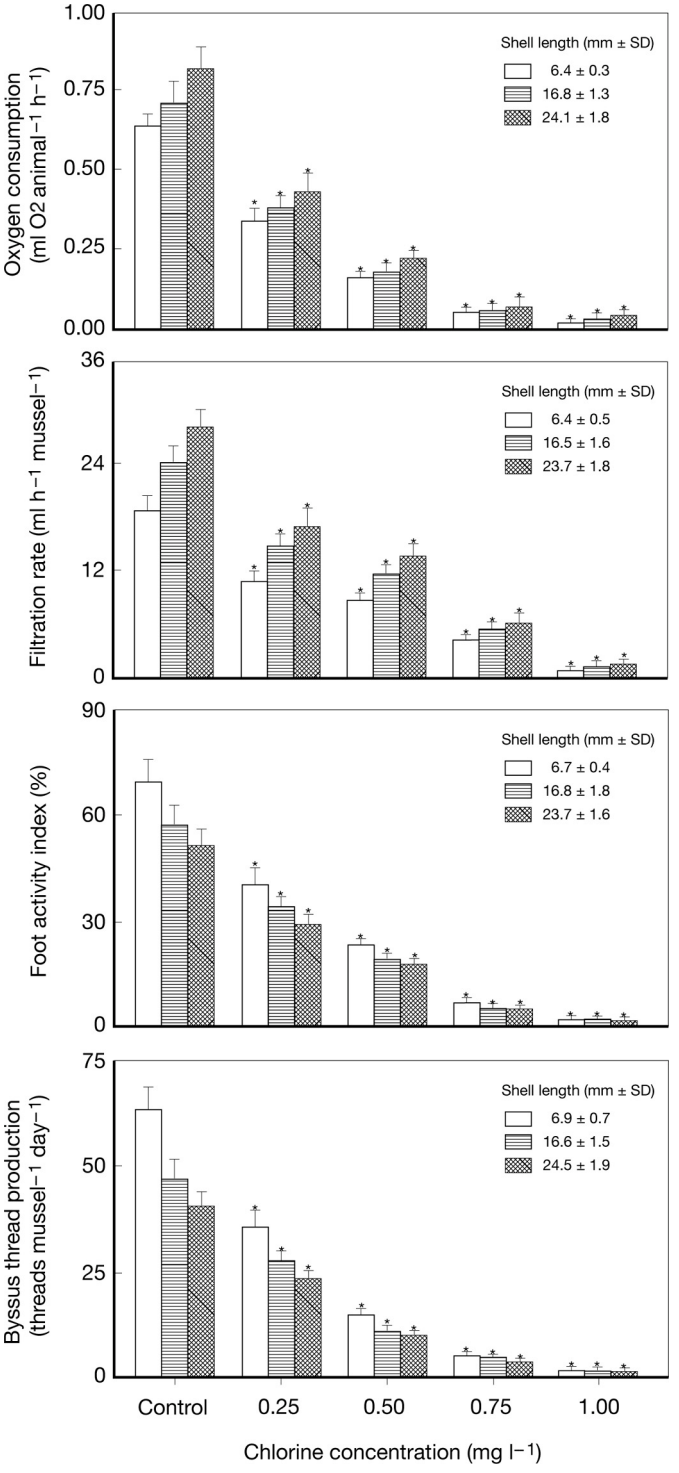

Figure 4. Oxygen consumption, filtration rate, foot activity index and byssus thread production of different size groups of *Brachidontes variabilis* at different chlorine concentrations. Data are expressed as mean ± S.D. (n = 12–60). Differences between control and experimental mussels (0.25–1.00 mg l^{-1}) were compared by Student's t tests after Bonferroni's adjustment for multiple pairwise comparisons. * $P < 0.001$.

Table 1. Relationship between chlorine concentration on lethal (100% mortality) and sublethal (oxygen consumption, filtration rate, foot activity index and byssus thread production) responses of different size groups of *Brachidontes variabilis* subjected to different chlorine concentrations.

Response of *B. variabilis*	Size groups (shell length in mm ± S.D.)	Number of mussels	Regression parameters	r	P
100% mortality	6.5 ± 0.4	72	$y = 361.292\,e^{-0.357x}$	0.98	0.0001
	17.1 ± 1.1	72	$y = 361.292\,e^{-0.357x}$	0.95	0.0001
	23.6 ± 1.4	72	$y = 361.292\,e^{-0.357x}$	0.97	0.0001
Oxygen consumption (ml O_2 animal^{-1} h^{-1})	6.4 ± 0.3	240	$y = 0.410\,e^{-0.428x}$	0.95	0.0001
	16.8 ± 1.3	240	$y = 0.455\,e^{-0.468x}$	0.94	0.0001
	24.1 ± 1.8	240	$y = 0.520\,e^{-0.528x}$	0.96	0.0001
Filtration rate (ml h^{-1} animal^{-1})	6.4 ± 0.5	72	$y = 33.889\,e^{-3.284x}$	0.94	0.0001
	16.5 ± 1.6	72	$y = 44.210\,e^{-3.225x}$	0.95	0.0001
	23.7 ± 1.8	72	$y = 53.297\,e^{-3.324x}$	0.95	0.0001
Foot activity index (%)	6.7 ± 0.4	72	$y = 140.473\,e^{-4.147x}$	0.98	0.0001
	16.8 ± 1.8	72	$y = 105.972\,e^{-3.971x}$	0.99	0.0001
	23.7 ± 1.6	72	$y = 95.851\,e^{-4.001x}$	0.97	0.0001
Byssus thread production (threads mussel^{-1} day^{-1})	6.9 ± 0.7	48	$y = 99.693\,e^{-3.945x}$	0.99	0.0001
	16.6 ± 1.5	48	$y = 72.433\,e^{-3.757x}$	0.98	0.0001
	24.5 ± 1.9	48	$y = 59.556\,e^{-3.692x}$	0.99	0.0001

Note: Correlation coefficients are indicated where they are significant. x = chlorine concentration (mg l^{-1}): y = mortality (h).

B. variabilis 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 *B. variabilis* showed a progressive reduction with increasing chlorine concentration (figure 4). The sublethal responses of *B. variabilis* are strongly correlated with different concentrations of chlorine ($0.94 < r < 0.99;\ P < 0.0001$; table 1). In all size groups, the physiological activities of *B. variabilis* showed a decrease in about 96% (as compared with control) at 1 mg l^{-1} residual chlorine (figure 5). Even though there was a significant size-dependent variation in oxygen consumption, filtration rate, foot activity and byssus thread production of *B. variabilis* at different chlorine concentrations (figure 4), the percentages of reduction were not significantly different for different size groups (figure 5).

4. Discussion

Though chlorination is the most commonly used mussel fouling control measure in industrial cooling water systems [3], the environmental release of water containing chlorine residuals in recipient water bodies is tightly regulated in most countries [8, 38]. This has led power utilities to use chlorine residuals that are barely sufficient for effective biofouling control [19, 25]. A total residual chlorine level of 1 mg l^{-1} is normally used for mussel control in Europe and North America during breeding periods, while during non-breeding periods, considerably lower chlorine levels (0.2–0.5 mg l^{-1}) are used [1, 3, 39, 40]. The actual concentrations of chlorine used in a power station cooling water system depends on several factors including targeted fouling species, water quality and the mode of dosing employed: exomotive, intermittent, shock-dose or targeted chlorination [3, 26, 39]. MAPS has been using chlorination as an antifouling method in the seawater cooling system since 1983. The chlorination regime was intermittent initially (1–2 mg l^{-1} residual at outfall for 1 h, once in 8 h), and since this did

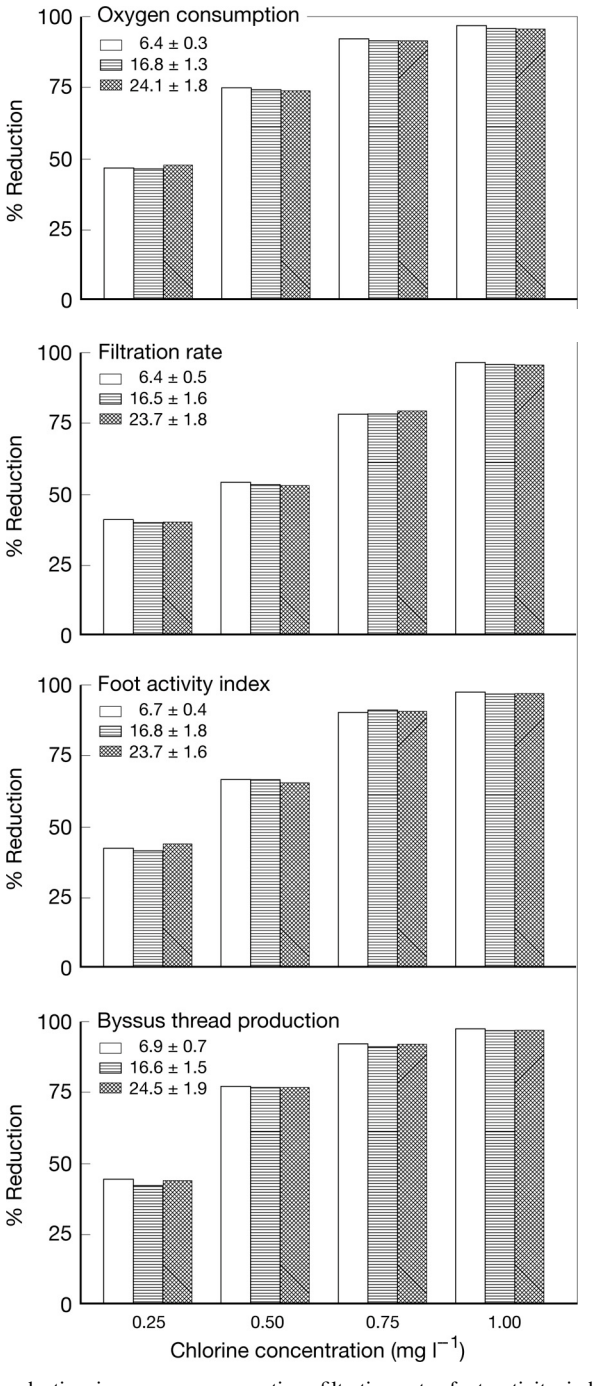

Figure 5. Percentage reduction in oxygen consumption, filtration rate, foot activity index and byssus thread production of different size groups of *Brachidontes variabilis* at different chlorine concentrations when compared with control experiments.

not control the fouling very effectively, the mode of application was changed to continuous low dosing [19, 20]. It is necessary therefore that we collect data on the response to chlorine of a wide variety of organisms that are generally encountered in cooling water systems [5, 22–24, 41, 42]. Given the fact that no information exists on the lethal and sublethal effects of chlorine on *B. variabilis*, it was considered worthwhile to generate these data by exposing the mussels to a range of chlorine concentrations. Chlorine concentrations ranging from 1 to 5 mg l^{-1} for the mortality experiments of *B. variabilis* were used. The relatively higher concentrations used in the present mortality experiments are justified because one of our objectives was to compare the chlorine toxicity of *B. variabilis* with that of other important tropical mussels, for which comparable data exist. The present studies show that the time to 100% mortality of *B. variabilis* was 288 h at 1 mg l^{-1} and 27 h at 5 mg l^{-1} chlorine concentration (figure 1). The exposure time required for 100% mortality appears to be much lower than that reported for other coexisting tropical mussel species *P. viridis* [23], *P. perna* [22], *B. striatulus* [24] and *Modiolus philippinarum* [4]. The difference in tolerance among these species could be due to the different degrees of their metabolic adaptations [43]. However, no published results are available for *B. variabilis* to facilitate comparison with the present study. Based on the results of this study, it seems that *B. variabilis* would succumb more easily to a given dose of chlorine, when compared with *P. viridis, P. perna, B. striatulus* and *M. philippinarum.* From a practical perspective, it would appear that the chlorine regime targeted against *P. viridis* (the most dominant mussel species on the east coast of India) would also eliminate *B. variabilis*, as it is more sensitive to chlorine than *P. viridis.*

The differential distribution of *B. variabilis* when compared with *B. striatulus* and *M. philippinarum* in the seawater intake system of MAPS appears to be related to the relative chlorine tolerance of this species. Among the three closely related mussel species, spat settlement of *B. striatulus* was consistently high numerically (59%), followed by *B. variabilis* (29%). *M. philippinarum* (12%) was the least abundant among the three in Kalpakkam coastal waters [19]. However, inside the cooling conduits of MAPS, which is characterized by relatively high flow rates and chlorine residuals, we observed a change in the relative distribution of the three mussel species. While *B. striatulus* (78%) was numerically more abundant, *M. philippinarum* (16%) consistently settled in excess of *B. variabilis* (6%). This observation indicated the importance of flow regimes and chlorine residuals in species selection among the three mussel species. It appears that mussel species which are more tolerant to chlorine have a selective advantage over mussels which are least tolerant to chlorine. However, there is a need for further studies of how other environmental factors such as water velocity and food availability influence their relative distribution in such habitats.

Experiments were also conducted to ascertain the effects of chlorine administered at low levels (0.25–1 mg l^{-1}) on the physiological activities such as oxygen consumption, filtration rate, foot activity index and byssus thread production of *B. variabilis.* The most important effect of low-level continuous chlorination is a decrease in respiration (oxygen consumption), feeding rate (filtration rate) and foot activity, the last leading to a reduction in the number of byssus threads. The present data clearly indicated that *B. variabilis* was able to sense the presence of chlorine at levels as low as 0.25 mg l^{-1} and responded by reducing the physiological activities by 41–49% (figure 5). If chlorination is carried out on a continuous basis, mussels do not get an opportunity to compensate for the loss incurred due to reduced food intake and oxygen consumption [19]. Under such circumstances, a significant decline of the growth rate could be expected [6]. A similar suppression of foot activity and reduction in byssus strength in chlorinated mussels have been reported by Rajagopal *et al.* [44], who observed that mussels chlorinated at 0.2 mg l^{-1} residual level required 36–52% less force to detach than unchlorinated mussels. Rajagopal *et al.* [44] also estimated that the 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. The present physiological data show that continuous dosing at a residual level of at least 1 mg l^{-1} is necessary to force the *B. variabilis* to close their shells, without allowing a recovery phase. Therefore, it is desirable to maintain such residual levels during peak settlement periods of *B. variabilis* to prevent fresh colonization.

One important observation in the study is that mussel size does not seem to be a factor influencing chlorine sensitivity of *B. variabilis*. This is unlike in other tropical mytilid mussels that we have studied so far (refer to [19] for review). The rates of physiological activities also showed percentage reduction values that were comparable within different size groups. This means that, unlike in other coexisting mussel species, adult mussels of *B. variabilis* do not enjoy the benefit of increased chlorine tolerance, when compared with their recently settled counterparts. In fact, lethal and sublethal levels of chlorine affect the entire *B. variabilis* population to the same extent. In other mussel species, chlorine tolerance was significantly increased with increasing mussel size. Rajagopal *et al.* [4, 22–24] have reported that the chlorine tolerance of 1-month-old *P. viridis, P. perna, B. striatulus* and *M. philippinarum* (in terms of time to reach 100% mortality) at a chlorine concentration of 1 mg l^{-1} is 23–31% less than that of the 12-month-old size groups. This shows that the chlorine tolerance levels between small and large mussels increase with increasing mussel size. This is probably related to the mode of action of chlorine on the animals [3]. Unlike at relatively low chlorine residuals (below 1 mg l^{-1}), where mussels open and feed on and off, higher chlorine residuals cause the mussels to shut their shells off completely, forcing them to obtain energy by anaerobiosis [6]. It would be interesting to study the relative susceptibility of different size groups of various mussel species to anaerobiosis and its implications.

5. Conclusions

(1) At 1 mg l^{-1} chlorine concentration, *B. variabilis* (7–24 mm shell length) takes about 288 h to reach 100% mortality.
(2) In *B. variabilis*, the size (*i.e.*, age) of the mussel does not seem to be a determinant of its chlorine tolerance. Small (7 mm shell length) and large (24 mm shell length) mussels take a comparable time to reach 100% mortality.
(3) *B. variabilis* showed a significant size-dependent variation in physiological activities. However, the values for percentage reduction (as compared with control) were not significantly different for different size groups.
(4) Data are presented to show that *B. variabilis* is able to sense the presence of chlorine at levels as low as 0.25 mg l^{-1} and respond by reducing the physiological activities (by about 41–49%).
(5) The relative distribution of *B. variabilis* vis-à-vis other mytilid and modiolid mussels inside the cooling water system of an operating power station was found to be related to its relative sensitivity to chlorine.

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