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Accumulation of Cd by the Marine Sponge *Halichondria panicea* Pallas: Effects upon Filtration Rate and Its Relevance for Biomonitoring

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The marine demosponge *Halichondria panicea* Pallas, is a cosmopolitan species occurring in coastal waters with varied conditions of light, current, salinity and turbidity. *H. panicea* has a leuconoid structure and is composed of siliceous spicules and spongin fibers. Sponges are important members of many shallow water marine benthic communities, but comparatively little is known of their trace metal biology.

Sponge architecture is constructed around a system of water canals and the physiology of the sponge is largely dependent on the currents of water flowing through their bodies. The volume of water pumped by a sponge is remarkable, *ca.* 100-1200 ml h^{-1} g^{-1} (Vogel 1977; Riisgård *et al.* 1993). This large volume of water passing through the body of a sponge means that most cells are in direct contact with the external medium. Many sponges are able to accumulate trace metals and are highly tolerant of such pollutants (Bowen and Sutton 1951). This has led to the proposal that a "sponge watch" program be initiated supplementary to the existing "mussel watch" program (Patel *et al.* 1985). In view of the large volume of water passing through the bodies of sponges such as *H. panicea*, the suitability of this species as a biomonitoring organism was further investigated.

This study describes the accumulation strategy of the demosponge *H. panicea* exposed to dissolved cadmium (Cd) and the effect of Cd upon sponge filtration rate.

MATERIALS AND METHODS

Sponges *H. panicea* were collected by scuba diving during March 1992 from a depth of 4 m off the coast of Bøgebjerg Strand, Fyn, Denmark. The sponges were growing in association with red and brown macroalgae attached to large flat stones. Salinity was ca. 20‰ and the water temperature 8 ± 1°C. Care was taken to ensure that the sponges were always kept submerged during collection

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and all subsequent handling. They were returned immediately to the laboratory and separated from the accompanying macroalgae and other debris.

Pieces of sponge tissue, having an approximate volume of 4-8 cm^3 (wet volume - by displacement), and containing at least one osculum were cut from healthy looking intact colonies and all non-sponge material (e.g., sand and invertebrates) removed. Excised sponge pieces were individually suspended on 10cm long nylon threads (Barthel and Theede 1986) and kept in 10 l of seawater (15°C) in acid-washed plastic aquaria prior to use.

Individuals were selected for experiments such that all subsequent groups contained sponges of a similar size (range 4 to 8 cm^3). Each group was exposed for 14 d to one of a number of Cd concentrations in 5 l of filtered (0.45 μm) dilute seawater (collected from the Marine Station at Bøgebjerg) about 20‰ salinity at 15°C±1°C under a 12 h light:dark regime, with continuous aeration. The sponges were not fed throughout the experiment. The water was changed every second day to maintain the declared Cd concentrations. Cadmium was added from a standard stock solution (1000 μg Cd l^{-1}) of $CdCl_2$ (Analytical grade, Merck, FRG). Five groups of eight individual sponges were exposed to concentrations of ca. 0.045 (control)(Magnusson and Westerlund 1980), 10, 31.6, 56.2 or 100 μg Cd l^{-1}. The sponges were checked daily and dead or dying individuals (sticky and black-grey in color) removed.

After 14 d exposure sponges were removed, rinsed briefly in double-distilled water and freeze-dried in individual plastic bags for 72 hours to constant weight. Dried weighed sponges were manually homogenized and digested in 65% HNO_3 (Analytical grade, Merck FRG) at 100°C; subsequently the temperature was slowly increased to 150°C, and maintained until the digests evaporated to dryness. The residues were then redissolved in 3.0 ml of 0.2% HNO_3. Digests were analyzed for total cadmium, zinc and copper by flame atomic absorption spectrophotometry (AAS) (Perkin-Elmer 2380), using background correction where appropriate. Analytical quality was checked against and in good agreement with standard marine reference material (NRC Canada TORT-1 lobster hepatopancreas). All metal concentrations quoted are μg g^{-1} dry weight. Statistical analyses for differences in mean metal concentrations was by Analaysis of Variance (ANOVA) and linear regression (adapted from Sokal and Rohlf 1981).

One of 4 individual sponges (6 to 8 cm^3) was exposed to a Cd concentration of 100, 200, 500 and 1000 μg Cd l^{-1} in 500 cm^3 of filtered seawater in 1 l acid-washed beakers at 15°C with continuous aeration. Filtration rates were measured as the volume of water cleared of flagellate cells (*Rhodomonas* sp., almost spherical cells, ca. 6.5 μm in diameter) which are retained by the sponges with 100% efficiency (Reiswig 1971)(see Riisgard *et al.* 1993). One hr before the clearance experiment the sponges were suspended in the center of each beaker. Flagellate cells were added (usually about 7,000 cells ml^{-1}) and the reduction in number of particles as a function of time was measured. Water samples (15ml)

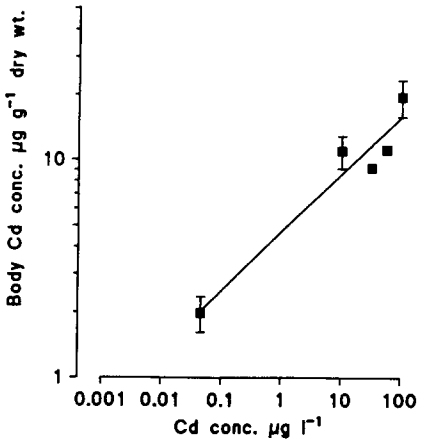

Figure 1. Mean total Cd concentrations ($\mu g\ g^{-1} \pm$ SD) of *H. panicea* exposed for 14 d to a range of dissolved Cd concentrations ($\mu g\ l^{-1}$). Equation of the regression line fitted by least squares is log (y) = 0.267 (log x) + 0.668: $P<0.01$ for all exposure concentrations. Error bars where not shown are contained within the data points.

were removed every 10 min and the particle concentration of the sample counted with an electronic particle counter (Elzone 180), taking the mean value of three measurements, each using 0.25 ml of the sample, as a standard. The rest of the sample was returned to the experimental beaker to avoid a significant reduction in seawater volume (V) during the experiment.

Filtration rate (F) was determined from the exponential reduction in algal cell concentration as a function of time (verified as a straight line on a semilog plot), using the formula $F = (V/t)\ \ln (C_0/C_t)$ where C_0 and C_t were the algal concentrations at time 0 and time t, respectively, calculated from the regression equation. The filtration rate was calculated before and after the addition of Cd to the beakers and clearance rates determined within periods of 40 min.

RESULTS AND DISCUSSION

Sponge mortality during the 14 d exposure was <1 %. Mean body Cd concentrations of sponges exposed to the range of dissolved Cd concentrations were significantly raised above that of the control group ($P<0.01$) (Fig. 1). There was a significant correlation between mean body Cd concentrations and dissolved Cd concentrations at all exposure concentrations ($P<0.01$). From Fig. 1 it was possible to calculate a mean accumulation rate of Cd for each exposure concentration. There was a significant correlation between the Cd accumulation rate and Cd concentration ($r^2=0.76$; all groups tested together by ANOVA; $P<0.001$).

There were no significant changes ($P>0.05$) in either body zinc or copper

concentrations in *H. panicea* with exposure to Cd (grand means 53.5 μg Zn g^{-1}, SE 2.8 and 6.7 μg Cu g^{-1}, SE 0.25, respectively).

This study has confirmed that the marine sponge *H. panicea* was able to accumulate Cd from seawater in direct proportion to the ambient Cd concentration. It is known that strategies of trace metal accumulation vary between individual sponges, among species and between metals (Patel *et al.* 1985; Bowen and Sutton 1951). Patel *et al.* (1985) measured mean body Cd concentrations of 68 and <0.1 μg Cd g^{-1} (below detection limits) in two species of siliceous sponges, *Spirastrella cuspidifera* and *Prostylyssa foetida*. A previous study undertaken on *H. panicea*, reported a Cd concentration of 0.85 μg Cd g^{-1} (Leatherland and Burton 1974) compared with 2 μg Cd g^{-1} for *H. panicea* from this study. Such wide variation in reported sponge Cd concentrations is a likely consequence of the geographical variation in ambient seawater Cd concentrations and changes in ambient salinities, acting in combination, with changes in species physiology and the differential availability of Cd to sponges from food and seawater. Freshly collected Danish sponges (ambient seawater Cd concentration *ca.* 0.045 μg l^{-1}; Magnusson and Westerlund 1980) had a Cd concentration factor (CF) of *ca.* 42200 (where CF is the ratio of the concentration of the element in the organism to that in the ambient water). Sponges exposed to the lowest cadmium concentration (10 μg Cd l^{-1}) and the highest cadmium concentration (100 μg Cd l^{-1}) had a CF of 1102 and 197, respectively.

The choanocytes lining the water canals of *H. panicea* are able to retain suspended particles down to 0.2 μm. Any metals associated with or complexed to such ingested particles may be digested intracellularly, eventually residing as food vacuoles in amoebocyte cells. Therefore, although sponges pump large volumes of water through their bodies, Cd may also be available bound to ingested "food" particles. Although, the sponges used in this study were not supplied with additional food, feeding may still have taken place and so extrapolation of our results to a field situation is severly limited.

In an attempt to relate the experimental values to a field situation it is possible to estimate the time taken by a sponge to accumulate a given amount of Cd. Such a calculation makes the assumption that the physico-chemical factors affecting Cd uptake and accumulation are identical in the field to those in the laboratory, which is not necessarily true. If we consider a "standard" sponge with a volume of 8 cm^3 as used by Riisgård *et al.* (1993), these authors have shown a correlation between volume (V) and dry weight (dw), thus;

$$dw = -0.162 + 0.139*V$$

gives a dry weight of 0.95 g for a sponge of 8 cm^3. These authors also found a correlation between the weight of a sponge and its filtration rate at a certain temperature (t). Thus, for a sponge with V = 8 cm^3, then its filtration rate would be estimated from;

$$F = -11.77 + 3.12*t$$

which gives a filtration rate of 16.3 ml min^{-1} at a temperature of 9°C (the mean annual water temperature in the Storebælt). As *H. panicea* is intolerant of anaerobic conditions it needs to pump water continuously through its aquiferous system (Barthel and Theede 1986). Thus, if we assume a constant filtration rate, our "standard" sponge would filter;

$$16.3 \text{ ml min}^{-1} * 1440 \text{ min day}^{-1} * 1 \text{ L } 1000 \text{ ml}^{-1} = 23.5 \text{ l day}^{-1}$$

The seawater from Storebælt has a Cd concentration of 0.045 μg l^{-1} (Magnusson and Westerlund 1980), resulting in the sponge accumulating 1.05 μg Cd d^{-1}. If we assume that the retention of Cd in these sponges is 100%, then the time taken for our standard sponge to reach the Cd concentration measured in our control sponges (2 μg g^{-1}) would be about 2 days. Alternatively, if we extrapolate using the regression line from Fig. 1 this gives us an accumulation rate of 0.4 μg Cd d^{-1} for sponges in Danish seawater (0.045 μg Cd l^{-1}), the time taken for a standard sponge in this instance to reach background concentrations would be about 5 days.

The utility of these estimates (2 and 5 days, respectively) is rather superficial. The concentration of Cd in the body of a sponge at any moment in time would be an integration of the time the sponge has been in contact with the surrounding medium and any depuration processes. The sponges used for experiments in this study had an estimated age of about 7 to 8 months (Barthel 1986). The fact that, despite this considerable time period, the sponges sampled had a mean body Cd concentration of only 2 μg g^{-1} would indeed suggest a much lower rate of accumulation in the field than those calculated. The high rates of Cd accumulation calculated are correlated to the artificially high Cd concentrations the sponges were exposed to in the laboratory and cannot necessarily be extrapolated to actual field situations; the complexities of metal speciation and binding occuring in natural seawater may overrule any such simple calculations.

Measurements of the filtration (clearance) rates of individual sponges defined within time intervals of 40 minutes are shown in Fig. 2. Comparison of the filtration rates for each individual with the mean filtration rate of that sponge throughout all time periods showed that sponges #1 and #2 (Cd concentrations 100 & 200 μg l^{-1}, respectively) had significantly reduced ($P<0.01$) filtration rates during the third time period (i.e., immediately following the addition of Cd) but were able to recover their filtration rates with time. Sponges #3 and #4 (Cd concentrations 500 and 1000 μg l^{-1}, respectively) had significantly higher filtration rates ($P<0.05$) immediately prior to the addition of Cd than at any time afterwards. Thus, at low Cd concentrations sponges were able to fully recover their filtration rates within 120 minutes. Higher concentrations of Cd, however,

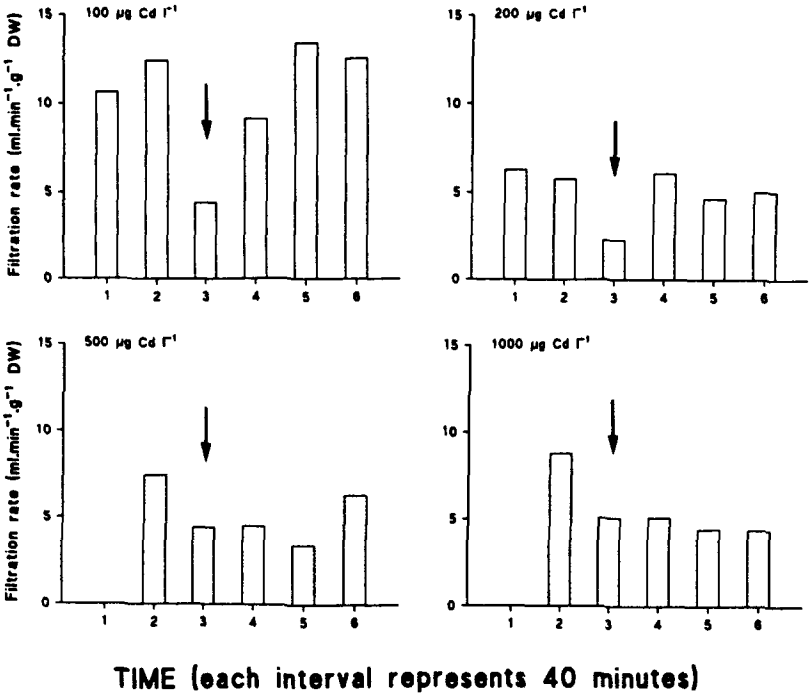

Figure. 2. *In situ* changes in filtration (clearance) rates of individual sponges prior to, and immediately following the addition of dissolved Cd (indicated).

brought about longer-term loss in sponge filtration rate. Fluctuations in sponge pumping rates are not uncommon, and considerable variation may be exhibited by the same individual. The relatively large variation in the initial filtration rates of the four sponges in this study may be due to variations in body size.

Sponges are intolerant of low oxygen conditions, and any prolonged cessation of pumping water through the aquiferous system has been shown to have a lethal effect on the sponges. Reduced water flow leads to the rapid development of an increased bacterial biomass in the canals and ostia, which subsequently leads to secondary bacterial infections resulting ultimately in sponge death (Hummel *et al.* 1988). Cadmium caused an immediate reduction in sponge filtration rate at all dissolved Cd concentrations. At lower Cd concentrations some recovery of the pumping mechanism was evident; however, at the highest Cd concentrations no recovery occurred within 120 minutes. It is likely that Cd had a direct toxic effect on the choanocyte cells, leading to impaired flagella activity.

Numerous organisms have been employed as biomonitors for trace metals in coastal marine environments. An ideal biomonitoring program should contain as wide a selection of organisms as is economically feasible. The work described suggests that the marine sponge *H. panicea* is a potentially useful biomonitor

organism for Cd pollution, and in view of the large volume of water passing through the bodies of sponges, such as *H. panicea,* they might prove useful in the monitoring of other heavy metals. It must be noted, however, that high concentrations of certain heavy metals may cause a reduction in sponge pumping capacity, or death resulting in the underestimation of the actual degree of contamination.

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