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Effects of Cadmium and Phenol on Motility and Ultrastructure of Sea Urchin and Mussel Spermatozoa

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Abstract. Computer-assisted sperm analysis (CASA) was used to study the effects of Cd(II) and phenol on sperm motility of sea urchin and mussel. In parallel, ultrastructural changes of sperm induced by these two toxicants were also investigated and related to motility impairment. Spermatozoa of sea urchin were more sensitive than mussel spermatozoa to both toxicants. Sea urchin sperm motility showed a good dose-response relationship to Cd(II) levels as well as exposure time. Exposure to the two toxicants changed the size and shape of the midpiece, which might affect the balance of spermatozoa in their swimming. The plasma membrane became more convoluted, and such a change might affect the streamlining and integrity of spermatozoa and hinder their normal movement patterns. Most important, disorganization of mitochondrial membranes and cristae was observed, suggesting disruption of ATP supply for sperm movement. Cadmium also induced greater ultrastructural damages in sea urchin spermatozoa.

Sperm motility is one of the most important criteria in assessing sperm quality. Earlier studies have shown that motility of sperm was impaired on exposure to heavy metals (Young and Nelson 1974; Holland and White 1980; Earnshaw *et al.* 1986). The recent advent of image analysis techniques, especially computer-assisted sperm analysis (CASA), provides a rapid, quantitative, and objective assessment of sperm motility (WHO 1992; Young *et al.* 1995; Moore and Akhondi 1996; Kime *et al.* 1996). The CASA system has been employed to study effects of heavy metals on sperm motility of fish (Ebrahimi *et al.* 1995; Kime *et al.* 1996), and results of these studies have shown that motility of catfish sperm was decreased after exposure to 100 ppm cadmium or 2,000 ppm zinc for 24 h.

It is conceivable that impairment of sperm motility by pollutants may be ascribed to structural damage or biochemical/physiological alterations. This is especially important as earlier studies have shown that concentration of metals and other pollutants accumulated in spermatozoa of fish and molluscs could be several times/orders of magnitude higher than the surrounding water (Mann *et al.* 1988; Cope *et al.* 1994; Gümgüm *et al.* 1994; Kime *et al.* 1996), and spermatozoa may therefore experience a much higher concentration of pollutants than ambient water. However, only scant information is available concerning the effects of toxicants on ultrastructural damages of spermatozoa (Mottet and Landolt 1987; Earnshaw *et al.* 1986; Burgos *et al.* 1997). Indeed, impairment of motility in human sperm has been related to inherited ultrastructural defects in sperm tail (Ryder *et al.* 1990). Thus, information on sperm ultrastructure changes in response to toxicant exposure may help to explain the mechanisms involved in impaired sperm motility.

The grazing activity of sea urchins plays an important role in controlling the structure of subtidal macrophyte communities in temperate and Australian coastal waters (Pringle *et al.* 1982; Fletcher 1987). The short-spine sea urchin (*Anthocidaris crassispina*) is abundant in tropical and subtropical coastal waters and probably plays a similar role (Thompson and Wu 1981). The green-lipped mussel (*Perna viridis*) is abundant in intertidal regions of Asia Pacific (Siddall 1980). Cadmium and phenol are two important pollutants in industrial discharges. Impairment of sperm motility of these two species by Cd(II) and phenol may reduce reproductive success of these two important species, which in turn could lead to major ecological consequences. The objectives of the present investigation are to (1) investigate the effects of cadmium and phenol on motility and ultrastructure of spermatozoa of the short-spine sea urchin (*A. crassispina*) and the green-lipped mussel (*P. viridis*); (2) relate observed changes in sperm motility to changes in ultrastructure of sperm after exposure to cadmium and phenol, to explain the impairment of sperm motility from a cytopathological point of view; and (3) examine the feasibility of using sperm motility as a criteria in assessing the toxicity of complex industrial effluents.

Materials and Methods

Specimen Collection, Induced Spawning, and Toxicant Exposure

During the spawning season of the short-spine sea urchin *A. crassispina* and the green-lipped mussel *P. viridis* (May to August) (Lee 1986; Chiu 1988), these two species were collected from subtidal water (5–10 m below surface) at Kat O and the intertidal zone at Tap Mun, Hong Kong, respectively. Both Kat O and Tap Mun are pristine sites,

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free from agricultural and industrial discharges. Animals were induced to spawn after being kept for less than 24 h in aquarium (22°C, 30‰ salinity, with aeration). *A. crassispina* was induced to spawn by injecting with 1 ml 0.5 M KCl through the peristomal membrane (Nacci *et al.* 1985). Spawning of *P. viridis* was induced by exposing the mussels to 10^{-2}M serotonin (5-hydroxy-tryptamine) (Sigma) (Ram *et al.* 1993). Gametes released from each individual were examined under light microscope for confirmation of sex and screening of sperm activity. Spermatozoa from four to six individuals showing active sperm movement were pooled as a common sperm stock for all later experiments.

Aliquots were sampled from the sperm stock and spermatozoa of each species were exposed to 1, 5, and 10 ppm Cd(II) (included 50 ppm Cd(II) for *P. viridis*) and 10, 50, 100, and 500 ppm phenol. An appropriate amount of sperm stock was mixed with an appropriate amount of Cd(II) or phenol stock solution (in filtered sea water) to achieve the desired concentrations (with 20 ml per treatment, sperm density ca. $\times$ 10^7/ml). Control with the same sperm density was prepared by mixing an appropriate amount of sperm from the same sperm stock with filtered sea water. Subsamples of sperm were taken at 0-, 30-, and 60-min intervals from each treatment for analyses of motility and ultrastructure. Pre-exposure of spermatozoa to test waters or toxicants for up to 60 min is commonly employed in sea urchin sperm bioassays (Dinnel *et al.* 1989; Dinnel 1995). Spermatozoa of mussels have a sufficient energy supply and can swim actively for hours in sea water (Crawford *et al.* 1992; Levy and Couturier 1996).

Motility Study

CASA was carried out using a CRISMAS sperm motility analysis system (Image House A/S, Denmark). Sperm movement was recorded using a CCD camera (PULNiX TM-710) attached to an Olympus BH-2 microscope with a phase-contrast objective lens at 20 $\times$ 10 magnification. Software parameters were set according to the manufacturer's instructions (Gade-Nielsen 1995). Images of cell aggregates or debris were filtered from video analysis using the GIPS object feature analysis provided by the CRISMAS system.

Three replicates of sperm suspensions (500 µl each) were prepared for each treatment at time intervals of 0, 30, and 60 min and mounted on clean, concave glass slides. For each sample, sperm motility was recorded for 10 s in five microscopic fields (50–100 sperm density per field) providing a total of 15 estimates (3 replicate $\times$ 5 fields) for each treatment at each time interval. The following motion parameters of individual spermatozoa were assessed: (1) curvilinear velocity (µms^{-1}) (VCL), the time-average velocity of the sperm head along its actual trajectory; (2) straight line velocity (µms^{-1}) (VSL), the time-average velocity of the sperm head along the straight line between its first detected position and its last position; and (3) average path velocity (µms^{-1}) (VAP), the time-average velocity of a sperm head along its spatial average trajectory, this trajectory is computed by smoothing the actual path according to algorithms of the CRISMAS system.

Ultrastructural Study

One milliliter of sample was taken from each treatment at 0, 30, and 60 min and placed inside a tissue culture well lined with a 0.1% poly-L-lysine–coated Thermanox coverslip and containing 1 ml of 5% glutaraldehyde in 0.2 M cacodylate buffer (pH 8.0). After 30 min of settlement, the Thermanox coverslip with attached sperm were rinsed in buffer and further fixed for 3 h in 2.5% glutaraldehyde with 1% tannic acid and 0.25 M sucrose in 0.1 M cacodylate buffer (pH 8.0). Fixed spermatozoa were then washed gradually in the same buffer containing a decreased concentration (0.25 M, 0.125 M, 0.0625 M) of sucrose then finally to a buffer with no surcose (10 min in each). Washed samples were postfixed in 2% aqueous OsO_4 for 2 h. Osmicated samples were rinsed in buffer and distilled water, dehydrated in a graded series of ethanol solutions, transferred to acetone, and infiltrated gradually in Spurr's resin before embedding. Ultrathin sections were stained in uranyl acetate and lead citrate and examined under a JOEL 100SX transmission electron microscope at 80 kV.

For each treatment group, 100 longitudinally sectioned sperm were examined. The thickness of the midpiece was also measured (at 10,000$\times$ magnification). The morphology of spermatozoa sampled from the seawater control at the same time interval served as reference for comparing the toxic effects. Any consistent ultrastructural change observed in the treatment (with $>$50% occurrence) but not the control (with $<$5% occurrence), was considered an abnormality induced by the toxicants.

Statistical Analysis

At each incubation period (0, 30, and 60 min), analysis of variance (ANOVA) was used to test the null hypothesis that different levels of cadmium/phenol treatment do not cause significant quantitative changes in the motion parameters (*i.e.*, VCL, VSL, and VAP) of sperm. The motility data were arcsine-transformed prior to ANOVA to achieve homogeneity of variances. Where treatment group means were significantly different, pair-wise comparisons between the control and individual treatment groups were carried out using a Dunnett's test. Percentages of spermatozoa with different thicknesses of midpiece were compared using a χ^2 test (Zar 1996).

Results

Motility Study

Spermatozoa of sea urchin and mussel in seawater controls (0 ppm) remained active after 60 min. VCL of control sea urchin sperm (110–130 µms^{-1}) (Figures 1a, 2a) was generally higher than that of the control mussel sperm (70–110 µms^{-1}) (Figures 3a, 4a), but VSL and VAP of control mussel sperm (30–50 µms^{-1} and 60–80 µms^{-1}, respectively) (Figures 3b, 3c) were slightly higher than that of the control sea urchin sperm (ca. 20 µms^{-1} and 40–60 µms^{-1}, respectively) (Figures 2b, 2c). Changes in VCL, VSL, and VAP of sea urchin and mussel spermatozoa generally had similar trends within the same treatment (Figures 1–4).

Effects of Cadmium on Motility

A good relationship between sperm motility and exposure concentration as well as exposure time is demonstrated by the sea urchin *A. crassispina.* Although a positive promotion in VCL, VSL, and VAP was evident shortly after exposure to 1 ppm Cd(II) (Figures 1a–c), all three velocity parameters became lower than their respective control after 60-min incubation, and a statistically significant decline was observed for VSL (Figure 1b) and VAP (Figure 1c). Upon exposure to higher levels of Cd(II) ($\geq$5 ppm), significant reduction of sperm motility occurred earlier and the extent of reduction increased with exposure time (Figures 1a–c).

For the mussel *P. viridis*, no significant reduction in sperm motility was observed after exposure up to 50 ppm Cd(II) for 60

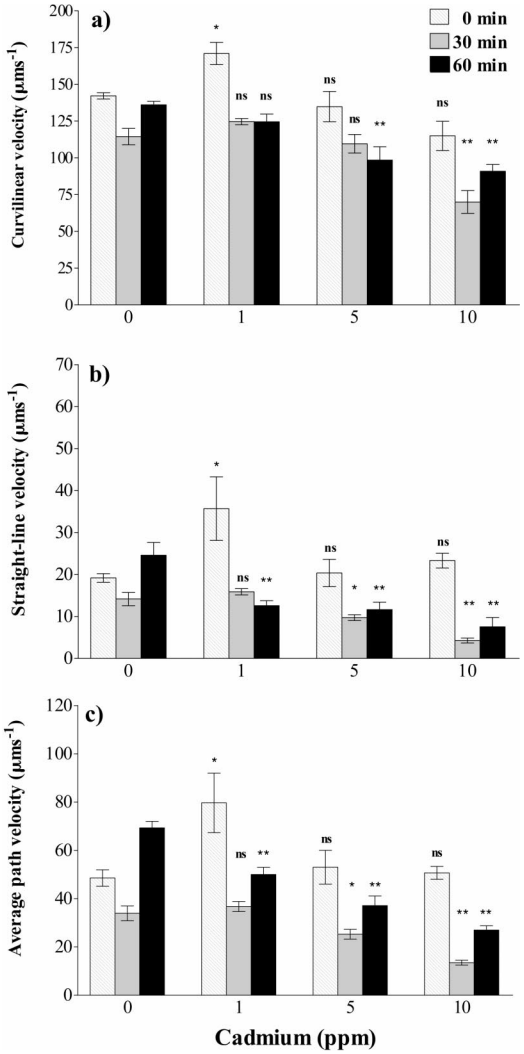

Fig. 1. Changes in (a) curvilinear velocity (VCL); (b) straight-line velocity (VSL); and (c) average path velocity (VAP) of the sea urchin *Anthocidaris crassispina* spermatozoa after exposure to cadmium for up to 60 min. Values (mean ± SEM of 3 replicate × 5 fields) significantly different from the control (0 ppm) for the same incubation period are indicated by asterisks *$p < 0.05$; **$p < 0.01$

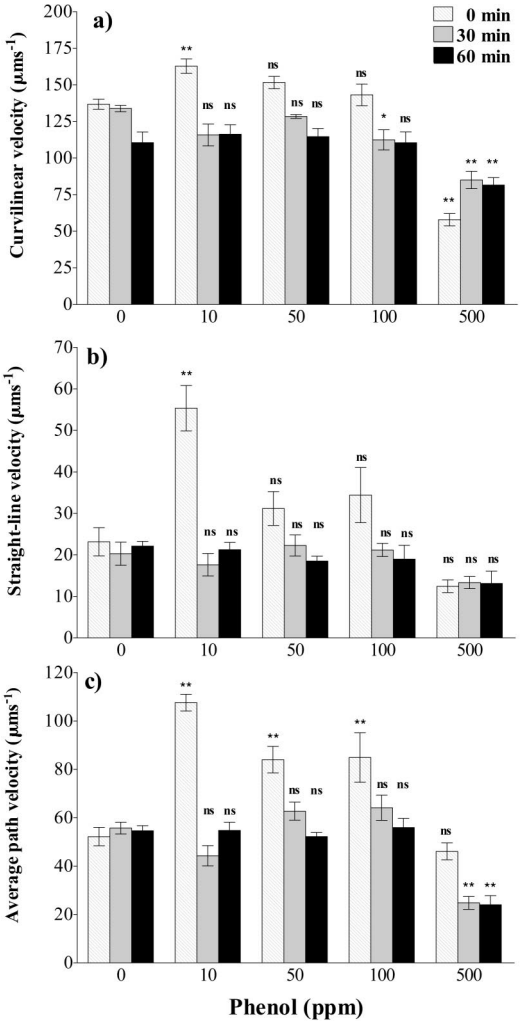

Fig. 2. Changes in (a) curvilinear velocity (VCL); (b) straight-line velocity (VSL); and (c) average path velocity (VAP) of the sea urchin *Anthocidaris crassispina* sperm after exposure to phenol for up to 60 min. Values (mean ± SEM of 3 replicate × 5 fields) significantly different from the control (0 ppm) for the same incubation period are indicated by asterisks *$p < 0.05$; **$p < 0.01$

min, except significant reductions in all the three motility parameters were observed at 1 and 10 ppm immediately after exposure (Figures 3a–c).

Effects of Phenol on Motility

For sea urchin spermatozoa, significant decreases in VCL (Figure 2a) and VAP (Figure 2c) were observed when exposed to 500 ppm phenol at almost all time intervals. However, VSL was not affected (Figure 2b). A positive promotion of VCL, VAP, and VSL was also evident immediately after exposure to lower concentrations of phenol (10–100 ppm) (Figure 2a–c). For mussel, a significant decrease in sperm motility was generally observable at 500 ppm of phenol at 30 min. At lower concentrations of phenol (10–100 ppm), increases in VCL, VSL, and VAP were also observed (Figures 4a–c).

Ultrastructural Study

Sperm morphology of *A. crassispina* and *P. viridis* has been described in two of our earlier studies (Au *et al.* 1998; Reunov *et al.* 1999). Specimens in these two studies were collected from the same pristine sites and therefore should represent ''normal'' condition. Morphology of both sea urchin and mussel spermatozoa in the controls and at time 0 for all treatments was normal.

A. crassispina: The normal sea urchin sperm head is comprised of a conical-shape nucleus and an apical acrosome, the sperm midpiece consists of an annular mitochondrion situated underneath or beside the nucleus, *e.g.*, the 30-min control (Figure 5A). Upon exposure to 10 ppm Cd(II) for 30 min (Figure 5B), the midpiece became less electron-dense and more swollen compared to the 30-min control (Figure 5A). In the

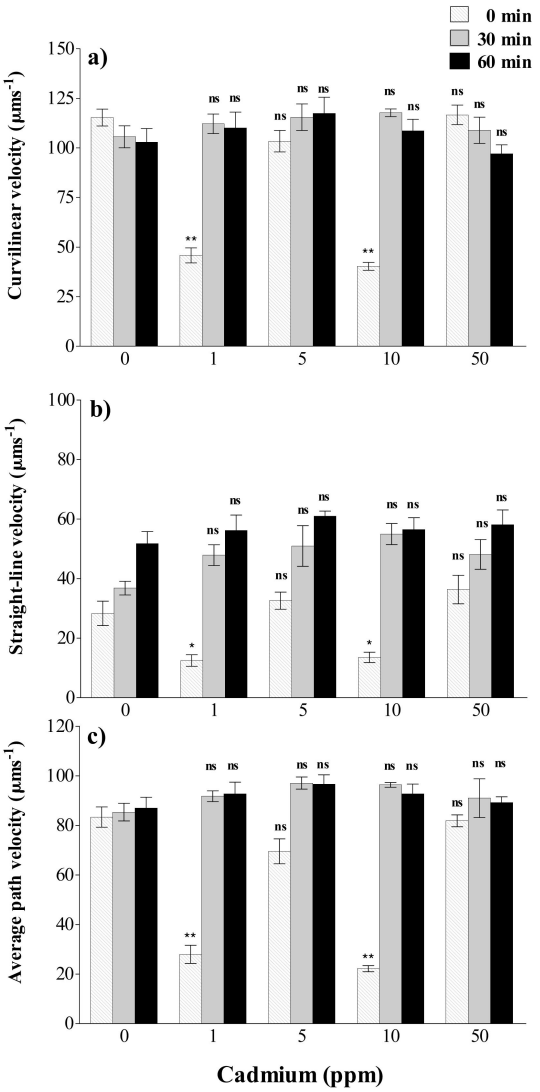

Fig. 3. Changes in (a) curvilinear velocity (VCL); (b) straight-line velocity (VSL); and (c) average path velocity (VAP) of the mussel *Perna viridis* sperm after exposure to cadmium for up to 60 min. Values (mean ± SEM of 3 replicate × 5 fields) significantly different from the control (0 ppm) for the same incubation period are indicated by asterisks *p < 0.05; **p < 0.01

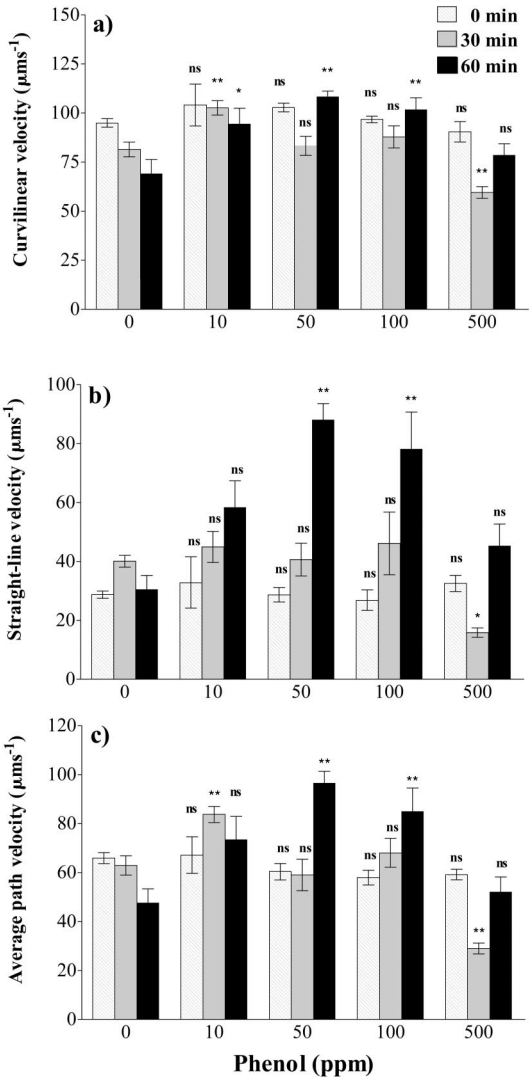

Fig. 4. Changes in (a) curvilinear velocity (VCL); (b) straight-line velocity (VSL); and (c) average path velocity (VAP) of the mussel *Perna viridis* sperm after exposure to phenol for up to 60 min. Values (mean ± SEM of 3 replicate × 5 fields) significantly different from the control (0 ppm) for the same incubation period are indicated by asterisks *p < 0.05; **p < 0.01

control, over 50% of the sperm had a midpiece ≤1 μm thick and only <10% had a midpiece >1.2 μm (Figure 6). The percentage of sperm with a thicker midpiece increased significantly when exposed to 5 ppm Cd(II) and higher concentrations ($\chi^2 = 8.79$) (Figure 6). After 60 min exposure to 5–10 ppm Cd(II) (Figures 5C–E), swelling of mitochondria subsided, but disruption of mitochondrial organization, loss of cristae, and a more electron-lucent matrix were observed. In addition, the plasma membrane that covered up the sperm cell became more convoluted, vesicles of varied sizes were formed over cell surface (Figures 5C, 5D). Such features were uncommon among the 60-min control spermatozoa (Figure 7A). The integrity, size, and shape of acrosome in the Cd(II)-treated sperm appeared to be less affected (Figure 5D).

After exposure to 500 ppm phenol for 30 min (Figure 7B), the midpiece became irregular in shape, less rigid, with less cristae and a more electron-lucent matrix, and the plasma membrane was convoluted when compared to the 30-min control (Figure 5A). After exposure for 60 min (Figure 7C), sperm morphology was basically similar to the ones at 30 min (Figure 7B), except that unusual membranous structures were also found in the mitochondrial matrix.

P. viridis: Head morphology of the green-lipped mussel spermatozoa was very different from that of the sea urchin spermatozoa. The normal mussel sperm head is comprised of a funnel-shaped acrosome sitting on a barrel-shaped nucleus, and the sperm midpiece is made up of a ring of five mitochondria situated underneath the nucleus, *e.g.*, the 60-min control (Figure

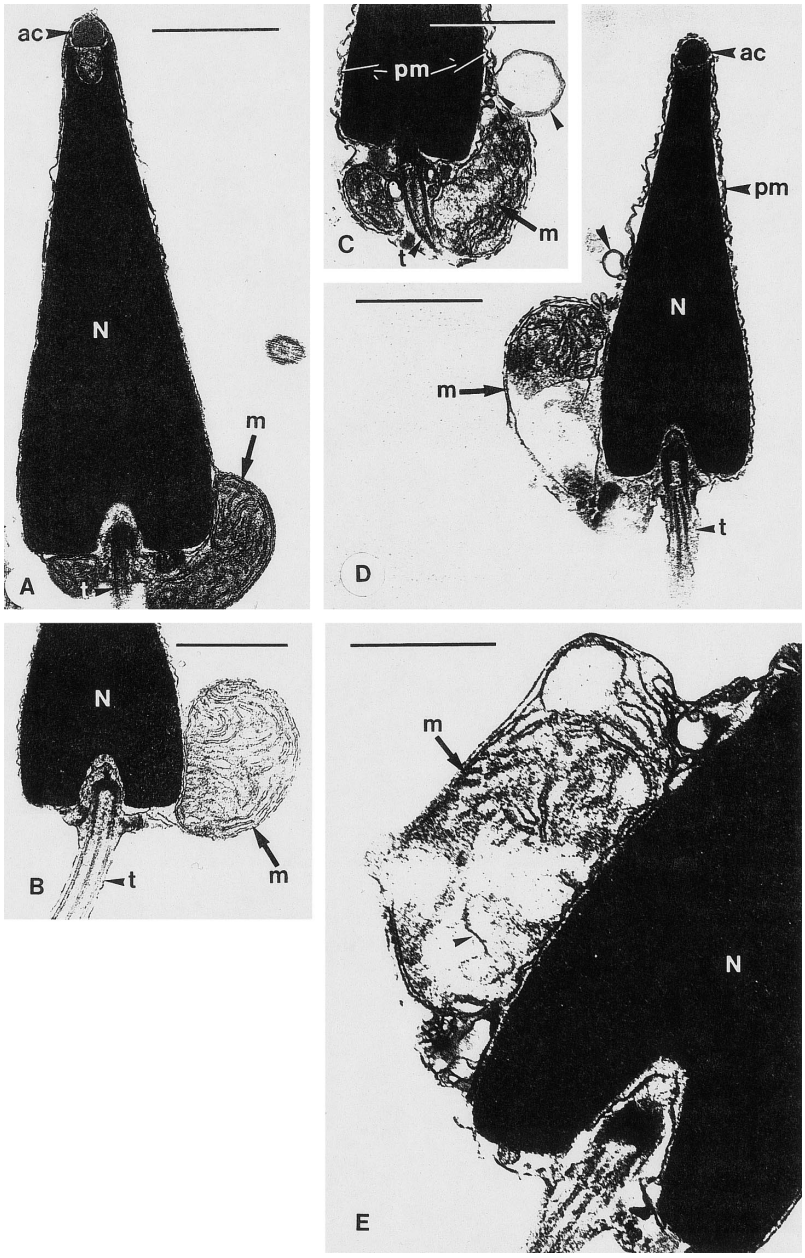

Fig. 5. *Anthocidaris crassispina.* (A) Control sperm incubated in sea water for 30 min. The midpiece is made up of a single, annular mitochondrion (m) situated underneath the conical-shaped nucleus (N), the matrix of mitochondrion is electron-dense and consists of many cristae. (B) Sperm incubated in 10 ppm Cd(II) for 30 min. The mitochondrion (m) is more round in shape compared to the control (A). (C) Sperm incubated in 5 ppm Cd(II) for 60 min. The plasma membrane (pm) is convoluted, vesicles of varied sizes (arrowheads) are present over the cell surface, and the matrix of mitochondrion (m) is electron-lucent. (D and E) Sperm incubated in 10 ppm Cd(II) for 60 min. (D) The plasma membrane (pm) is convoluted and vesicle-like structures are present over cell surface (arrow head). (E) Higher magnification at mitochondrion (m) shows disorganization of mitochondrial membranes and crista (arrowhead), the mitochondrial matrix appears ''empty.'' ac = acrosome, L = lipid, m = mitochondiron, N = nucleus, pm = plasma membrane, t = tail. Scale bars = 1 μm (except for F, scale bar = 0.5 μm)

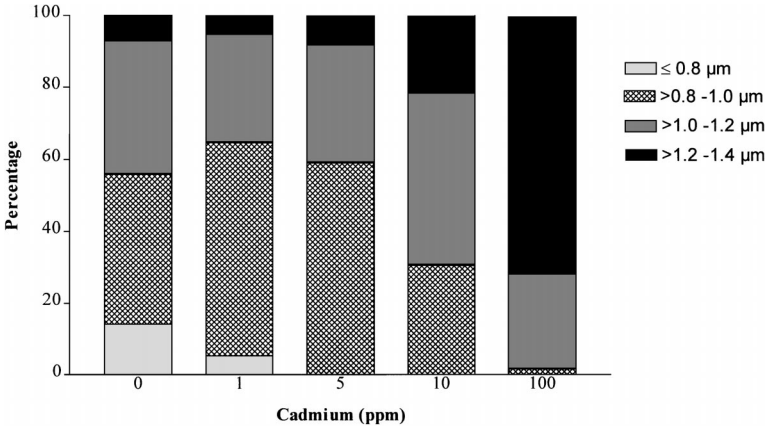

Fig. 6. Thickness of midpiece in *Anthocidaris crassispina* spermatozoa (n = 100) after exposure to various concentrations of cadmium for 30 min

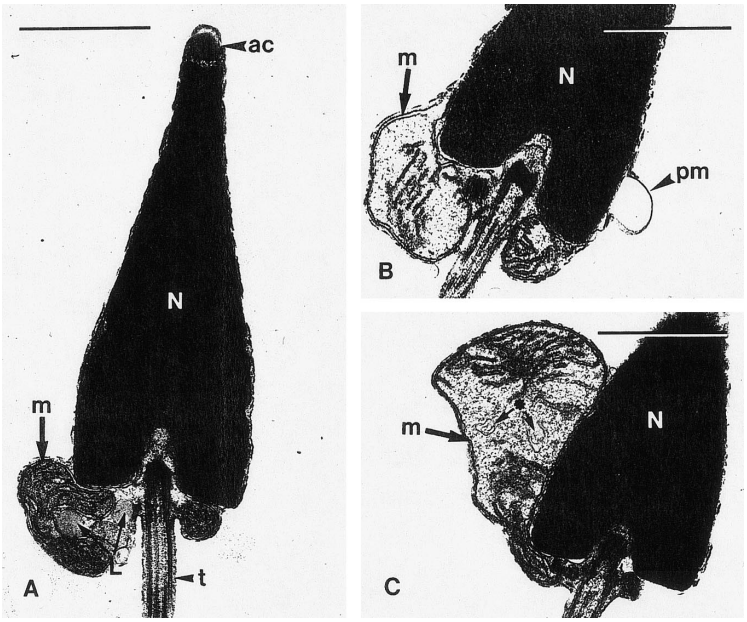

Fig. 7. *Anthocidaris crassispina.* (A) Control sperm incubated in sea water for 60 min. (B) Sperm incubated in 500 ppm phenol for 30 min. Slight convolution of plasma membrane (pm) is evident; the mitochondrion (m) is irregular in shape, density of mitochondrial cristae is lesser, and the matrix is more ''empty'' compared to the control (A). (C) Sperm incubated in 500 ppm phenol for 60 min. The mitochondrion (m) is irregular in shape, membranous structures (*), which are absent in the control sperm (A), are found in the matrix

8A). After exposure to 50 ppm Cd(II) for 60 min (Figure 8B), the sperm morphology was basically similar to the 60-min control (Figure 8A), except that convolution of the plasma membrane was occasionally found. After exposure to 500 ppm phenol for 60 min, mild convolution of plasma membrane, slight deformation of mitochondria, and loss of cristae were observed (Figure 8C). Otherwise, the ultrastructure was not much different from the control (Figure 8A).

Discussion

Overall, sea urchin spermatozoa were more sensitive than mussel spermatozoa to both cadmium and phenol. In addition, Cd(II) induced a greater inhibitory effect on sperm motility and ultrastructural damage than phenol in spermatozoa of *A. crassispina* and a good dose-response relationship was generally demonstrated between sea urchin sperm motility and Cd(II) levels as well as exposure time.

Impaired sperm motility in *A. crassispina* on exposure to Cd(II) may be explained by the associated ultrastructural changes. Our quantitative ultrastructural data showed that midpiece width increased with concentration of Cd(II) and was concomitant to a decline in sperm VCL, VSL, and VAP. The enlarged sperm midpiece (mitochondria) may affect normal movement of swimming sperm. Earlier studies using x-ray microanalysis on Cd(II)-treated rat livers and thyroids (Hulínská *et al.* 1989; Kawahara *et al.* 1990; Yoshizuka *et al.* 1991) showed accumulation of Cd(II) in swollen mitochondria. Swelling of mitochondria may be attributed to interaction of Cd(II) and mitochondrial membrane proteins, resulting in an increase of membrane permeability to cations and anions (Kendall *et al.* 1983; Viarengo 1985; Hemelraad *et al.* 1990; Kesseler and Brand 1994). We also observed plasma membrane convolution and disorganization of mitochondrial cristae in Cd(II)-treated sperm. We did not quantify the extent of convolution and disorganization, but such ultrastructural alterations became more apparent with an increase in Cd(II) concentration and time of incubation. The highly convoluted plasma membrane found in the Cd(II)-treated spermatozoa (≥5 ppm) may affect the streamlining and integrity of a sperm cell, which may further hinder normal sperm movements. Most important, the reduction in sperm motility at 10 ppm Cd(II) may well explained by the disorganization of mitochondrial membranes and cristae, which indicate disruption of normal respiratory processess and hence ATP supply for sperm movement. Results of other studies showed that Cd(II) was potent to prevent wide curvature flagellar beating in spermatozoa of rat and rabbit (Lindermann *et al.* 1991; Young *et al.* 1995), inhibit microtubule sliding in axoneme of bovine spermatozoa (Kanous *et al.* 1993), and induce microtubule depolymerization in cultured 3T3 cells (Li *et al.* 1993). Morphological changes in sperm tails were not apparent in the present study; it is uncertain whether or not similar mechanisms may also be applicable to the inhibitory effect of Cd(II) on motility of *A. crassispina* spermatozoa.

Phenol can easily penetrate cell membrane (Kordylewska 1980). Remarkable changes in ultrastructure have been observed in gastropod (*Limnaea stagnalis*) embryos after short-term (1–4 h) exposure to 1,000 ppm phenol (Kordylewska 1980). Observed alternations included changes in cell shape, swelling of nuclei, and destruction of membraneous complexes (*i.e.*, cell membrane, nuclear envelope, endoplasmic reticulum, and internal mitochondrial membranes); cytoplasmic matrix also became more granular, and the above ultrastructure changes have been related to impairment of cell respiration (Kordylewska 1980). Ultrastructural study of bivalve *Spisula solidissima* sperm demonstrated an irregular appearance of plasma membrane after exposure to gossypol, a phenolic compound (Burgos *et al.* 1997). Convolution of the plasma membrane was also observed in the sea urchin and mussel spermatozoa after phenol treatment (500 ppm). In addition, the noticeable changes in midpiece shape of sea urchin sperm may affect the balance of swimming sperm, thereby leading to decline in sperm motility. The biochemical mechanism by which phenol causes structural abnormality in cell mem-

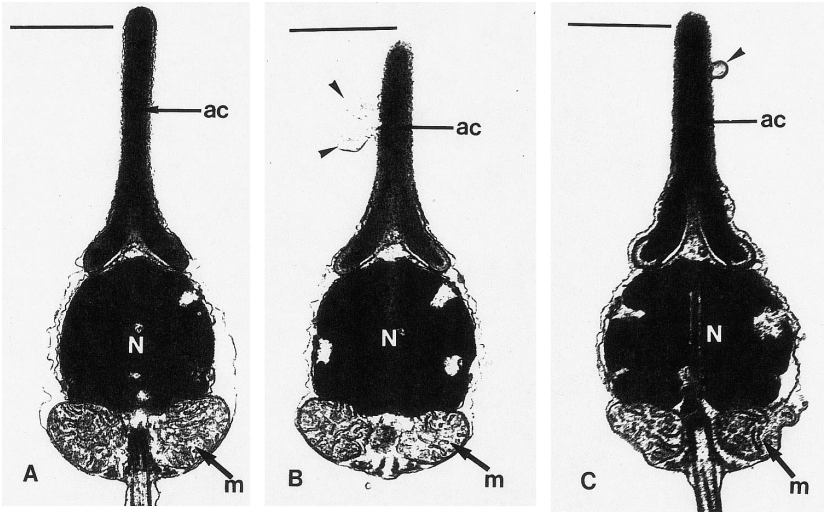

Fig. 8. *Perna viridis.* (A) Control sperm incubated in sea water for 60 min. The midpiece is made up of a ring of five mitochondria (m) situated underneath the barrel-shaped nucleus (N), which is capped by a funnel-shaped acrosome (ac). (B) Sperm incubated in 50 ppm Cd(II) for 60 min. The plasma membrane is convoluted (arrowheads). (C) Sperm incubated in 500 ppm phenol for 60 min. Slight deformation of mitochondria (m) and convolution of plasma membrane (arrowhead). Scale bars = 1 μm

branes/shape or changes in sperm motility, however, remains obscure.

Our quantitative ultrastructural data was limited to midpiece width, and we did not attempt to quantify the extent and degree of membrane convolution and distortion. Nevertheless, the present qualitative ultrastructural observation does provide useful information to relate the observed decline in sperm motility to ultrastructural changes.

The observed promotion of sea urchin and mussel sperm motility on exposure to low concentrations of Cd(II) and phenol could well be explained by hormesis, a stimulatory effect upon short-term or continuous exposure to subinhibitory levels of toxic agents/environmental stress that are toxic at higher concentrations (Laughlin *et al.* 1981). Hormesis induced by Cd(II) has been reported in growth of yeast (Stebbing *et al.* 1984), and reproduction of water flea (Bodar *et al.* 1988). However, instead of hormesis, a decrease in VCL, VAP, and VSL was observed in mussel spermatozoa shortly after exposure to 1 and 10 ppm Cd(II) (Figure 3); the reason for such a decrease remains difficult to explain.

Our experiments showed that the CASA system can serve as a quick, objective, and quantitative assessment on effects of pollutants on sperm motility, but sensitivity of sperm motility to the two reference toxicants tested was relatively low, especially for spermatozoa of *P. viridis*, albeit sensitivity may increase with an increase in exposure time ($>$60 min). The result of this study showed that spermatozoa of *A. crassispina* and *P. viridis* would remain active in sea water for more than an hour as no apparent retardation in motility of spermatozoa can be revealed with time in the seawater control (0 ppm Cd(II)/phenol). Likewise, morphology of spermatozoa remained intact after 60 min.

In fact, the inhibitory concentrations to sperm motility reported from other similar CASA studies were generally higher than realistic pollutant levels. For example, VCL, VAP, and VSL of freshly diluted catfish sperm was significantly decreased by Cd(II) at $\geq$1,000 ppm, Zn(II) at $\geq$2,000 ppm (Kime *et al.* 1996). Likewise, Young *et al.* (1995) showed that direct exposure of rabbit spermatozoa to 100 μM Cd(II) (ca. 11 ppm) for 4 h changed the motion pattern and lowered the VCL of sperm. The insensitivity of sperm motility to pollutants may be attributed to the relatively short period of exposure. Indeed, inhibitory concentration of Cd(II) decreased to 100 ppm if the catfish spermatozoa were pre-incubated with Cd(II) in extender for 24 h at 4°C (Kime *et al.* 1996). The present study aimed to relate the impairment of sperm motility to sperm cytopathology and found that induction of significant ultrastructural alterations only occurred at concentration inhibitory to sperm motility, but not at subinhibitory levels (*e.g.*, $\leq$1 ppm Cd(II) and $\leq$100 ppm phenol). We have extended our investigation to study chronic effects of Cd(II) on sperm quality of *A. crassispina* (Au *et al.* 1999). By exposing adult urchins to Cd(II) for 4 weeks during gametogenesis, the inhibitory concentration on sperm motility has lowered to 0.01 ppm Cd(II), which is close to the US EPA recommended criterion of 9.3 μg/L Cd(II) in salt water (US EPA 1994).

Previous fertilization studies showed that pre-exposure of sea urchins and mussels spermatozoa to Cd(II) (Pagano *et al.* 1982; Dinnel *et al.* 1989; Ringwood 1992; Bowen and Engel 1996) and phenolic compounds (Nacci *et al.* 1986; Ferrari *et al.* 1989; Anderson *et al.* 1994) reduced fertilization rate. Fertilization capacity of rat spermatozoa was correlated with a decline in VSL (Moore and Akhondi 1996). It is conceivable that a reduction in motility of urchin and mussel spermatozoa will lead to a subsequent decrease in fertilization rate. Experiments have now been undertaken in our laboratory to further establish the link between sperm motility and fertilization success in *A. crassispina* and *P. viridis.* Parallel studies have also being carried out to relate sperm motility to fertilization success of urchins exposed to Cd(II) and phenol during gametogenesis. The result of this series of studies would enable us to predict the effects of pollution on reproductive success and make extrapolation on population effects of these two ecologically important species.

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