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Effects of Protracted Cadmium Exposure on Gametes of the Purple Sea Urchin, *Arbacia punctulata*

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Gametes and larvae of sea urchins and more specifically Arbacia punctulata have been used extensively in embryological studies and toxicity bioassay testing (Burgess et al. 1993; Dinnel et al. 1989; Jonczyk et al. 1991; Meador et. al 1990; Lee and Xu 1984; Kobayashi 1971 and 1980; and Ringwood 1992). Most of the experiments and bioassays have used the fertilized eggs of different sea urchin species and measured abnormal growth, malformations, or changes in the rates of growth as a function of contaminant exposure. Guida et al. (1980) demonstrated that cupric ion activities of $<10^{-10.5}$ M caused reductions in the rates of growth of Arbacia punctulata larvae and caused incomplete or malformed pluteal skeletons. These effects occurred at cupric ion concentrations that were in the same ranges as some measured in the more contaminated estuaries in the northeastern U.S. (Sunda et al. personal communication, NMFS, Beaufort Laboratory). Sunda and coworkers also used sea urchin embryonic development to test potential trace metal toxicity in water samples collected from those same estuaries, and demonstrated toxicity potentially attributable to dissolved trace metals in the water column.

The purpose of these experiments was to determine if protracted sublethal exposure of sexually mature sea urchins to dissolved cadmium in sea water would affect the viability of eggs and sperm, and whether it would affect fertilization and embryonic development and ultimately the larvae. The results of the experiments support the hypothesis that spermatogenesis and oogenesis were affected by cadmium exposure.

MATERIALS AND METHODS

Adult sea urchins (Arbacia punctulata) were collected from the vicinity of Beaufort, N.C., and maintained in flowing sea water. Eighty urchins were selected at random, with 40 placed in filtered sea water and 40 placed in sea water containing 8.9×10^{-7} M (100 ppb) cadmium. Both the exposed and unexposed animals were maintained in 7001 tanks for 14 d. At the end of the

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exposure period the urchins were depurated for 24 h in flowing seawater. Both groups of urchins were fed on sea lettuce (Ulva lactuca) ad libidum. Water samples were collected every other day to monitor cadmium concentration which varied ±15%. Cadmium was measured in the water samples using flame atomic absorption spectrophotometry (AAS). Cadmium measurements also were made on the isolated gametes, but the detection limits were below those for flame AAS (<0.05 ppm).

Determination of sex and collection of gametes was accomplished by electric shock (Harvey 1956). Eggs and sperm were held no more than 30 min to avoid loss of spermatazoan motility (Timourian and Watchmaker 1970). A minimum of 3 females and 2 males were used for each replicate (exposed and unexposed). Females were spawned into 200 ml of filtered seawater, and males were spawned into finger bowls where the sperm concentrate was the diluted with 10 ml of filtered seawater. Fertilization was accomplished by adding 3 drops of sperm suspension to each aliquot of eggs.

A diagram of the experimental design is shown in Fig 1. The design consisted of collecting eggs and sperm from exposed and unexposed urchins, and then mixing the sex products to give four treatment groups (1. male and female exposed: 2. male exposed and female unexposed: 3. male unexposed and female exposed; and 4. male and female unexposed). There were four replicates for each treatment which contained approximately 1,000 eggs each. The experiment was conducted twice using two different groups of sea urchins. In the first experiment there were two replicates per treatment and in the second, four replicates per treatment. Since both experiments used the same design protocols, the data from all six replicates were pooled for analysis.

Subsamples of eggs/larvae were collected from each replicate at three sampling times over a period of two days (4-6, 24, and 48 h). From each subsample 100 eggs or larvae were randomly selected and scored for normal development. At 4-6 h the eggs were scored for fertilization and normal cell division, at 24 h prism and pluteal stages, and at 48 h for pluteus. The 24 and 48 h samples were fixed in 10% buffered formalin to facilitate counting. At 24 h abnormalities were determined by the presence or absence of a protruding oral lobe and arm buds as described by Harvey (1956) and Okazaki (1975). At 48 h, samples were taken to determine percentage of normal pluteal skeletal development. A normal plutal skeleton is V-shaped with triaxon spicules, normal gut, and mouth opening. Abnormalities were determined by the absence or malformation of triaxon spicules, gut development, intestines, mouth opening and shape of the pluteus. The criteria for abnormal larvae were determined using the protocol developed by Guida et al. (1980).

The data were analysed with univariate ANOVA's to test for differences among treatment means for each of the three sampling times. Additionally, Dunnett's procedure was used to compare all treatments against the control, and single

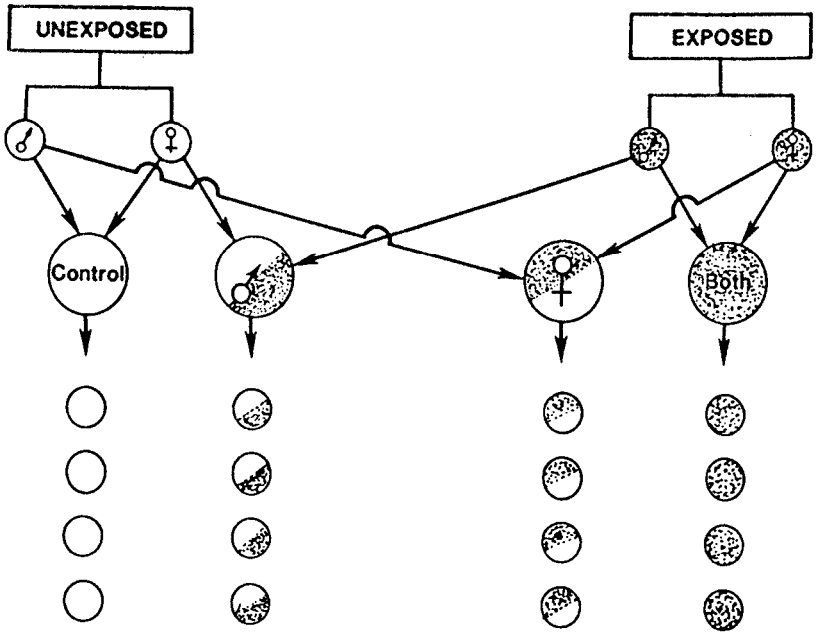

Figure 1. The experimental design for the determination of the effects of cadmium exposure on the viability of male and female purple sea urchin gametes.

degree of freedom contrasts were used to further examine the pattern of response to treatments.

RESULTS AND DISCUSSION

The exposure of sexually mature male and female purple sea urchins to 8.9 X 10^{-7} M (100 ppb) cadmium dissolved in seawater had significant effects on the ability of sea urchin gametes to produce normal larvae. ANOVAs showed that all three treatments significantly affected the larval development at 4-6 and 48 h relative to the controls ($P<0.05$), but not at 24 h. The results also showed that male gametes were affected to a greater degree than the females (Table 1).

Four to six hours after mixing the gametes, significant differences were seen in successful fertilization and early cell division between the treatment groups. The percentage fertilization of the unexposed control urchins eggs was high, >95%, and significantly higher than that of all of the other three treatments ($P<0.05$) (Table 1). The two treatments that involved exposed males were not significantly different from each other ($P>0.05$), but were between 18 to 20% lower than the controls. Effects observed among the nonviable eggs included both the lack of fertilization and abnormal cell division.

After 24 h of development all of the viable embryos had progressed to the prism stage. A majority of the controls and exposed female treatment had developed

Table 1. Percentages of sea urchin eggs and larvae that developed normally at three different times after fertilization. Either eggs or sperm of individuals were exposed either to 100 ppb cadmium or not exposed to cadmium. Each mean consists of six replicate subsamples of 100 individuals. Treatment groups are: CONTROL: neither exposed; BOTH: male and females exposed; MALE: male exposed, female not; and FEMALE: Male not, female exposed (* = significantly different from the control P<0.05).

	Sampling Times After Fertilization			
	(4-6 hr)	(24 hr)		(48 hr)
	24 Cell	Prism	Pluteus	Pluteus
Treatments				
CONTROL (N=6)	96.3 ±1.2	35.8 ±5.8	60.7 ±6.3	96.3 ±1.2
BOTH (N=6)	78.2* ±1.5	31.8 ±6.3	34.5* ±3.2	54.2* ±1.4
MALE (N=6)	75.7* ±2.1	30.8 ±2.4	23.0* ±3.3	53.2* ±1.3
FEMALE (N=6)	90.8* ±0.8	38.0 ±3.2	5l.0 ±2.9	81.3* ±2.3

to the early pluteus stage (Table 1). There were no significant differences among treatments in the percentage reaching the prism stage, but there were significant differences among those reaching pluteus. The percentage that had developed to the pluteus was significantly higher in the control and exposed female treatment groups (P<0.05) than in either of the treatments involving exposed males. The lower percentages of larvae reaching pluteus in the two treatment groups involving exposed males also suggests a reduced rate of development.

By 48 h, all viable larvae had reached the pluteus stage in all of the treatment groups, with the control having the highest percentage of normal plutei (Table 1). The treatments involving exposed males once again had significantly lower percentages of normal larvae than either of the other two treatments (P<0.05). Although the percentage of normal plutei for exposed females was higher than either of two male groups, they were still significantly lower than the controls

($P<0.05$). The percentages of abnormal larvae among the treatments having exposed males were significantly higher ($P<0.05$), but the percentages were virtually the same. The most common type of larval malformation was the lack of complete development of the pluteal skeleton (i.e., triaxon spicules). Even though the exposed females had significantly reduced normal development, once again normal development was higher than in the treatments containing exposed males.

These experiments showed that protracted exposure of prespawning sea urchins to cadmium dissolved in seawater significantly affected the development and survival of progeny. It also demonstrated significant differences in the sensitivities of adult male and female urchins and the viability of their sex products. Such a differential sensitivity suggests that the cadmium was interacting directly in situ with maturing spermatozoa and oocytes, but that the effects were more pronounced on the sperm. Since the exposed urchins were depurated in flowing noncontaminated seawater for 24 h prior to stripping the gametes, the interactions between the gametes and the cadmium had to occur prior to fertilization. Such an inference is further supported by the fact that no cadmium was added to the filtered seawater used to culture the larvae.

Previous investigations examining the effects of metals on larval sea urchins at our laboratory have demonstrated that environmentally relevant cupric ion concentrations can affect development (Guida et al. 1980). In those experiments the fertilized eggs were maintained in cupric ion-buffered media from fertilization through the late pluteal stage. Elevated cupric ion concentrations caused decreases in the rate of embryonic development and also interferred with the development of the calcified spicules in the pluteal stages. The mechanism of interferrence by copper with calcium metabolism may have involved either inhibition of calcification or interference with transport. Experiments on the effects of cupric ion on calcium uptake by juvenile flounder showed that copper inhibited uptake, but removal of copper from the medium restored the uptake of calcium (Dodoo et al. 1992). In the current experiments, there were apparent differences in rates of development. Embryos derived from exposed males had a slower rate of development relative to the exposed females and controls. Since no cadmium was added to the culture media, it appears that the observed effects were related to cadmium bound to some biochemical or structural component of the gametes, and more pronounced effects occurred with the males. In these experiments, there was inhibition of normal spicule formation, but the mechanism of interference with calcium metabolism is unclear.

In the toxicological literature there are references to gonadal damage from cadmium to other species of sea urchins. In Stongylocentrotus intermedius, Khristoforova et al. (1984) and Lipina et al. (1987) demonstrated that cadmium concentrations of 0.5 to 1.0 ppm caused cellular damage to ovaries and testes in these urchins. In another experiment, exposure to a dissolved cadmium concentration of 0.1 ppm for 40 days did not cause observable cellular damage,

but the fertilized eggs of exposed individuals did not develop normally. They did not attempt, however, to separate the sex products of exposed male and female urchins. Lucu et al. (1991) also demonstrated that longterm exposures to low levels of cadmium caused abnormal development in the sea urchin, Sphaerechinus granularis. In addition, Lipina et al. (1987) showed that small amounts of cadmium could be detected in the nuclei of oogonia and spermatids of exposed animals. The urchins used in our experiments probably also had cadmium associated with the mature sex products, but due to limitations in our analytical abilities at the time, we were unable to measure significant concentrations of cadmium in either shed eggs or sperm.

Our experiments have shown that cadmium appears to have the capability of affecting embryonic development when only adults are exposed for extended periods of time. The message derived from these experiments is that cadmium has the capability of altering development through exposure of the parents, and even at low concentrations it could affect reproductive success.

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