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Effect of Cadmium Body Burdens in Adult *Crassostrea virginica* on Fecundity and Viability of Larvae

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Metal studies with marine invertebrates, bivalves in particular, have dealt primarily with bioaccumulation. Several studies have reported metal toxicity to embryo-larval development (BRERETON et al. 1973; CALABRESE et al. 1973; WATLING 1978). Another study, that of GREIG et al. (1975), dealt with the transport of cadmium from adult oysters to their gametes. With the exception of the study by ZAROOGIAN et al. (1979), no studies have been concerned with the effect of metal body burdens in marine bivalves on embryo-larval success.

Since marine bivalves in general and Crassostrea virginica in particular are good accumulators of metals, we wanted to determine if embryos from parents with high concentrations of cadmium in soft tissues are more sensitive to cadmium toxicity than those from parents with low tissue cadmium concentrations. In addition, this study was designed to investigate the effect of selected cadmium treatments on larval growth.

MATERIALS AND METHODS

Adult oysters (C. virginica) were treated for a minimum of 33 weeks with ambient (control), 5 and 15 ug Cd/kg seawater as reported by ZAROOGIAN (1980).

During the natural spawning period, gametes were obtained from experimental oysters by methods similar to those described by LOOSANOFF & DAVIS (1963). Approximately 10 oysters were removed once weekly from each of the 3 test treatments and placed in individual glass dishes (10 x 20 x 6 cm) containing artificial seawater (ZAROOGIAN et al. 1969) and immersed in a temperature-controlled water bath. The oysters were spawned by alternating the water temperature between 18 and 28°C. Manipulation of the water temperature continued until a male and female from each treatment had spawned. After the eggs had settled to the bottom of the spawning dishes, they were transferred by pipet to approximately 1 L of seawater containing ambient, 5 or 15 ug Cd/kg. All possible cross-fertilizations between treatments were obtained by mixing 1 or 2 mL of the appropriate sperm suspension with the selected egg-seawater treatments. The procedure followed is outlined in ZAROOGIAN et al. (1979).

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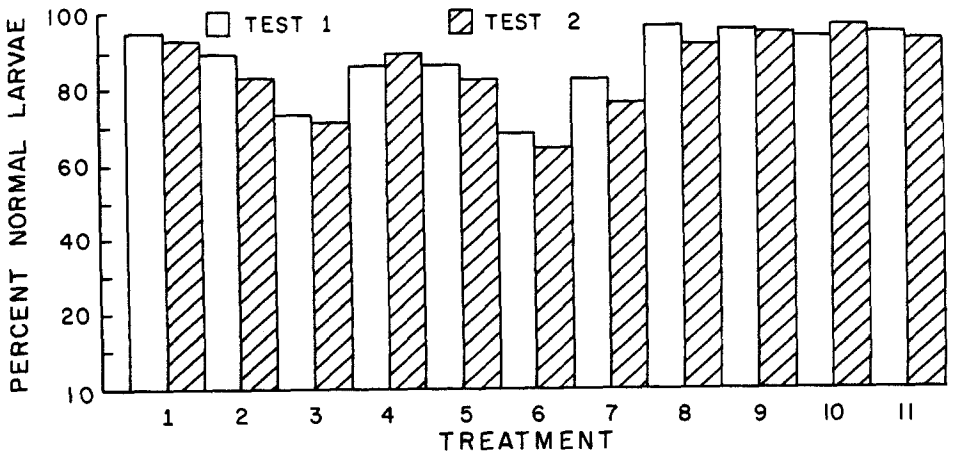

Figure 1. Percent normal larvae in the respective treatments after 48 h incubation at 20-22 °/oo S seawater. Larvae were obtained from adult C. virginica treated with ambient, 5 or 15 ug Cd/kg seawater for 35 (test 1) or 37 wk (test 2) at ambient temperature and salinity.

normally. As much as 29% of the embryos from control parents failed to develop or developed into abnormal larvae upon incubation for 48 h in 15 ug Cd/kg seawater (Fig. 1).

Oysters treated with 5 ug Cd/kg seawater for 37 wk contained total soft tissue cadmium concentrations of 91 ug/g dry weight. This tissue cadmium concentration appeared to have no effect on the viability of gametes, since embryos from these oysters produced normal straight hinged larvae when reared in ambient seawater for 48 h. Incubation of embryos from 5 ug Cd/kg treated oysters in 5 ug Cd/kg seawater for 48 h produced 14% abnormal larvae (Fig. 1). However, upon an additional 24 h incubation, a minimum of 95% of the embryos developed into normal larvae.

As much as 24% of the embryos resulting from oysters treated with 15 ug Cd/kg for 37 wk (total soft tissue Cd concentrations of 270 ug/g dry wt.) were abnormal after incubation for 48 h in ambient seawater. Thirty-six percent of the embryos from parents treated with seawater containing 15 ug Cd/kg developed into abnormal larvae when incubated for 48 h in seawater containing 15 ug Cd kg (Fig. 1). The data indicate that gametes from oysters treated with 15 ug Cd kg are stressed, since 24% of the embryos from adults in this treatment were abnormal after 48 h incubation in ambient seawater. In addition, higher mortality was recorded with these same embryos upon 48 h incubation in 15 ug Cd/kg seawater than with embryos from control oysters treated similarly. Under the conditions of this study, it appears that seawater containing 15 ug Cd/kg has a greater impact on embryonic development than on gametogenesis.

The following cross-fertilizations and treatments were performed to determine the effect of cadmium on gametogenesis and embryological development:

1 - $\frac{♀C \times ♂C}{A}$ 2 - $\frac{♀C \times ♂C}{5}$ 3 - $\frac{♀C \times ♂C}{15}$ 4 - $\frac{♀5 \times ♂5}{5}$

5 - $\frac{♀5 \times ♂5}{A}$ 6 - $\frac{♀15 \times ♂15}{15}$ 7 - $\frac{♀15 \times ♂15}{A}$ 8 - $\frac{♀C \times ♂5}{A}$

9 - $\frac{♀C \times ♂15}{A}$ 10 - $\frac{♀5 \times ♂C}{A}$ 11 - $\frac{♀15 \times ♂C}{A}$

The denominator represents the seawater treatment in which the embryos were held.

C = control; 5 = 5 ug Cd/kg; 15 = 15 ug Cd/kg; A = ambient

Approximately 1 h after fertilization, eggs for the larval growth and survival study were transferred to 1-L pyrex beakers containing the appropriate seawater treatment (ambient, 5 or 15 ug Cd/kg) to give a final concentration of 1 egg/mL. There were two replicates of each treatment condition. The developing embryos were held in an incubator at 20°C for 48 h. The resulting larvae were collected on a nylon screen and examined microscopically after 48 h incubation to determine the percent of abnormal shell development (WOELKE 1961). The larvae were then resuspended in fresh test media, fed equal amounts of the alga Monochrysis lutheri, and held in the incubator at 20°C. Subsequent examinations for growth and mortality in addition to resuspension in fresh test media and feeding were performed every 48 h for 3 wks. With the exception of cross-fertilizations and treatments numbered 5 and 7 above, the crosses and treatments were identical to those used in the embryo development study.

RESULTS

Despite cadmium concentrations as high as 270 ug/g dry weight in the total soft tissues, oysters spawned heavily. It appears that cadmium concentrations this high do not affect the fecundity of C. virginica.

A minimum of 93% of the embryos resulting from control parents developed into normal larvae in 48 h when incubated in ambient seawater. Seventeen percent abnormal larvae were observed when embryos from control oysters were incubated in 5 ug Cd/kg seawater. However, incubation for an additional 24 h under the same conditions resulted in 94% of the embryos developing

In order to determine if male or female gametes are selectively affected by total soft tissue cadmium concentrations obtained with the respective cadmium treatments, crosses 8 through 11 were performed. The data suggest that neither male nor female gametes are affected selectively by cadmium during gametogenesis. No less than 92% of the embryos developed normally when either gamete from oysters treated with 15 ug Cd/kg were crossed with the appropriate control gamete and incubated in ambient seawater. Therefore, it appears that embryonic development is affected only when both gametes are from oysters treated with 15 ug Cd/kg.

When larvae were reared for 3 wks, no significant differences ($P = 0.05$) in the mean length of larvae were obtained among the various treatments in each test.

DISCUSSION

Unfortunately, we were able to perform only two tests during the spawning period, since synchronization of spawning among the oysters from the three treatments was not always possible. Because only two tests were performed and due to the variability in the data, strict interpretation is difficult. However, the data allow certain generalizations. It appears that seawater cadmium concentrations of 5 ug/kg are not toxic to oyster embryos but cause enough stress to delay development into larvae. CALABRESE et al. (1973) indicated that growth retardation of larvae would prolong the pelagic stage and increase their susceptibility to predation, disease, and dispersion which would result in less recruitment into the population.

CALABRESE et al. (1973) reported a 48 h LC_{50} value of 3.8 ppm for cadmium toxicity to C. virginica embryos. We did not determine LC_{50} concentrations, but our study suggests that a lower LC_{50} value for cadmium may be more appropriate. When WATLING (1978) exposed 5 day-old Crassostrea gigas larvae to 20 ug Cd/L seawater for 7 days, she obtained 50% mortality. The 30% control mortality that she observed after rearing C. gigas larvae for 12 days is the same that we experienced with control larvae after 3 wk rearing in ambient seawater. We observed the highest mortality (55%) when larvae from parents treated with 15 ug Cd/kg were reared in 15 ug Cd/kg seawater for 3 wk. Although the highest mortality was recorded in this treatment, those larvae that survived, grew to lengths not significantly different from controls.

GREIG et al. (1975) reported that the amount of metal transferred from adult to egg is fairly constant and not dependent on the amount of metal available in the adult oyster. Consequently, the same effect could be observed with both cadmium treatments if cadmium content had an effect on gametogenesis and subsequent embryonic development.

The data indicate that cadmium tissue concentrations of 270 ug/g dry weight do not affect the fecundity of adult C. virginica nor the viability of larvae. Treatment of adult oysters with seawater containing 15 ug Cd/kg creates a stress on gametes severe enough to effectively reduce embryonic development and recruitment into the population.

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