EFFECTS OF MERCURY, CADMIUM, AND LEAD SALTS ON REGENERATION AND ECDYSIS IN THE FIDDLER CRAB, *UCA PUGILATOR*

Crabs are capable of autotomizing injured limbs at a preformed breakage plane and subsequently regenerating them. The regenerating limb bud grows in a folded position within a layer of cuticle, and unfolds when the animal molts. The length of regenerating limb buds is generally expressed in terms of "R-value" (Bliss 1956) which is length of limb bud × 100/carapace width. Such a regeneration index is useful for comparisons of crabs of different sizes. Since regeneration always terminates with a molt, the presence of regenerating limbs can affect the timing of ecdysis, and factors which influence ecdysis will also affect regeneration. For example, removal of eyestalks, a source of molt-inhibiting hormones, is a standard way of inducing precocious molting. Such animals will regenerate missing limbs rapidly, but will generally die at ecdysis. Skinner and Graham (1972) have shown that multiple autotomy, producing many regenerating limb buds, can cause accelerated regeneration, also leading to precocious molt.

Heavy metals as pollutants of the marine environment are of great concern. These chemicals are released as a result of industrial processes and tend to be toxic and to accumulate in organisms. Their toxicity to Crustacea has been studied by Corner and Sparrow (1957), Wisely and Blick (1967), Eisler (1971), Vernberg and Vernberg (1972), and O'Hara (1973).

This paper reports on the effects of mercury, lead, and cadmium on regeneration in the fiddler crab, *Uca pugilator*. With its estuarine intertidal habitat, this crab is likely to be subject to heavy metal pollution in industrial areas.

Materials and Methods

Fiddler crabs were collected in July and August from Accabonac Harbor, near East Hampton, N.Y., and brought into the laboratory. Autotomy of one chela and six walking legs was induced by pinching each merus with a hemostat. Immediately after autotomy, crabs were placed in solutions of $Pb(NO_3)_2$ (Reagent grade, Fisher Scientific), $HgCl_2$ (Reagent grade, Fisher Scientific), or anhydrous $CdCl_2$ (Reagent grade, Matheson, Coleman and Bell) at concentrations of 0.1 or 1.0 mg/liter of the metal ion. Crabs were maintained in groups of 10 in 1-liter glass aquaria in 200 ml of filtered seawater (30‰ salinity, room temperature 25°C). Twice weekly the aquaria were washed out and redosed. (In a similar static experimental design, Jackim et al. (1970) determined that the loss of metal ion from solution over a 96-h period was 0% for cadmium, 26% for mercury, and 79% for lead.) Crabs were fed twice weekly with Purina Fly Chow[1]. In all experiments, groups were arranged to have the same mean carapace width and to have equivalent distribution of males and females (5/5).

The growth of limb buds was measured twice weekly under a dissecting microscope with a calibrated ocular micrometer. In all cases, the first walking leg was measured as a representative limb. Values thus obtained were converted to R-values, and the means for each group were compared by the use of the *t*-test. Times of molting were recorded for all animals. Limb buds reached R-values of about 20 just prior to ecdysis.

Whole crabs were analyzed for mercury, cadmium, and lead following 2 wk of exposure to 0.1 mg/liter. Five crabs were used for each assay, which was done by New Jersey Department of Health personnel, using atomic absorption spectrophotometry.

Results

In experiment 1, crabs (mean carapace width 15 mm, range 13-16 mm) were exposed to 0.1 mg/liter of lead, cadmium, and mercury. Ten crabs were in each group, total biomass about 11 g. Cadmium had a retarding effect on regeneration (Table 1) although most individuals had molted by 28 days. The majority of controls molted by 21 days, and the rest completed ecdysis by 24 days. Mercury and lead had no retarding effect.

This experiment was repeated with crabs of a somewhat smaller size (13 mm carapace width, range 11-14 mm). Although cadmium again retarded regeneration, the retardation was less and was not always statistically significant (Table 1). These crabs reached ecdysis at the same time as controls (21 days). No effects of lead or mercury were seen.

In experiment 2, crabs were exposed to lead, mercury, and cadmium at concentrations of 1.0 mg/liter. Carapace width of crabs was 15 mm

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1.—R-values (mean ± standard error) of first walking legs of crabs after multiple autotomy and treatment with Pb, Hg, and Cd at 0.1 mg/liter.

Chemical	Days 7	10	14	17	21
Carapace width 15 mm:					
Controls	1.8 ± 0.3	7.3 ± 0.6	12.0 ± 0.7	18.4 ± 0.8	60% molt
Pb	2.8 ± 0.5	10.2 ± 0.7	14.7 ± 1.1	20.1 ± 0.6	80% molt
Hg	2.3 ± 0.2	8.8 ± 1.2	13.8 ± 1.3	17.7 ± 0.8	70% molt
Cd	1.0 ± 3.3	3.3 ± 0.7*	8.6 ± 1.2*	11.0 ± 1.3*	13.5 ± 1.5
Carapace width 13 mm:					
Controls	4.8 ± 0.4	10.6 ± 1.0	17.7 ± 1.0	20.2 ± 0.7 40% molt	70% molt
Pb	4.2 ± 0.4	9.2 ± 0.9	17.7 ± 0.8	18.2 ± 0.9 50% molt	90% molt
Hg	3.9 ± 0.7	9.4 ± 0.9	16.2 ± 1.1	17.9 ± 0.5 30% molt	60% molt
Cd	3.5 ± 0.6	8.0 ± 1.0	14.2 ± 1.1*	17.0 ± 0.8* 0% molt	70% molt

*P = 0.05 or less.

(range 14-16 mm). At this concentration, cadmium retarded regeneration to an even greater extent. This concentration of mercury was usually toxic, and the data obtained were from four crabs which survived the duration of the experiment. Regeneration did not take place in these crabs (Table 2). The cadmium, however, was not toxic, and all crabs survived, the majority (60%) completing ecdysis by 28 days. There was no mortality in lead, cadmium, or clean water in any of the experiments. The majority of controls molted by 24 days. A second group of crabs (carapace width 13 mm, range 12-14 mm) was exposed to cadmium and mercury at 1.0 mg/liter. Lead was not used in this experiment. Because of the high mortality in mercury in the previous experiment, 20 crabs were exposed to mercury. By the 17th day, the number surviving in mercury was reduced to eight, the same percentage as survived the previous experiment. The amount of growth in these crabs, though slight, was nevertheless much greater than in the previous experiment. Likewise, the retardation in cadmium was not as striking as in the earlier experiment (Table 2). The majority of controls molted by 21 days, whereas the majority in cadmium molted by 28 days. After 2½ wk, the eight crabs remaining in mercury were transferred to clean water, which was then changed daily, but they did not show evidence of recovery within 4 wk after return to clean water, during which time no significant growth occurred.

Residue analysis revealed that the crabs exposed for 2 wk to 0.1 mg/liter of mercury had absorbed 0.026 ± 0.001 ppm; those exposed to 0.1 mg/liter of cadmium had absorbed 0.50 ± 0.10 ppm; and those exposed to 0.1 mg/liter of lead had absorbed 2.04 ± 0.55 ppm.

Discussion

Retardation of regeneration was a specific effect of cadmium at both 0.1 and 1.0 mg/liter. At 0.1

TABLE 2. — R-values (mean ± standard error) of first walking legs of crabs after multiple autotomy and treatment with Pb, Hg, and Cd at 1.0 mg/liter.

Chemical	Days 7	10	14	17	21	24
Carapace width 15 mm:						
Controls	4.2 ± 0.4	8.0 ± 0.6	13.1 ± 1.0	15.9 ± 0.9	18.1 ± 0.3	70% molt
Pb	2.8 ± 0.6	6.2 ± 0.7	11.4 ± 1.0	14.5 ± 1.2	17.6 ± 0.7	70% molt
Hg	0*	0*	0*	0*	0.01 ± 0.01*	0.01 ± 0.01*
Cd	0.3 ± 0.2*	2.2 ± 0.8*	4.3 ± 1.2*	5.6 ± 1.5*	8.3 ± 2.5*	7.6 ± 2.3* 20% molt
Carapace width 13 mm:						
Controls	4.6 ± 0.5	10.2 ± 0.7	15.7 ± 0.9	18.0 ± 0.6	70% molt	90% molt
Hg	1.0 ± 0.6*	1.5 ± 0.8*	1.6 ± 0.8*	12.1 ± 1.0*	2.7 ± 1.1*	2.9 ± 1.1*
Cd	3.5 ± 0.2	6.8 ± 0.6*	11.5 ± 1.3*	13.8 ± 1.5*	16.0 ± 2.0*	50% molt

[1]Returned to clean water.
*P = 0.05 or less.

mg/liter, mercury was not toxic and did not have an effect on the growth of limb buds. At 1.0 mg/liter, mercury caused almost total inhibition of limb growth, but also proved lethal to 60% of the crabs. Therefore, the inhibition of regeneration may not be a specific effect of the mercury, but just an indication of the toxicity of the metal to the crabs. In this light, *Uca* is seen to be much more resistant to mercury than the porcelain crab, *Petrolisthes armatus*, in which the 96 h LC_{50} (mean lethal concentration) was 0.050-0.064 ppm (Roesijadi et al. 1974). With long-term exposure to mercury, however, *Uca* can tolerate only 0.18 ppm (Vernberg and O'Hara 1972). In the present study, cadmium might have shown a greater effect than mercury at 0.1 mg/liter because it was absorbed to a much greater extent than the mercury. It is possible that exposure to mercury at levels between 0.1 and 1.0 mg/liter could inhibit regeneration without causing mortality. Despite the high amounts absorbed, lead had no effect on regeneration rate.

At both dose levels of cadmium and the higher concentration of mercury, the retarding effects were greater the first time the experiment was performed (July) than the second (August). Since these crabs normally molt in August, it is probable that they have higher titers of ecdysone at that time, and their progress toward ecdysis cannot be inhibited to the same extent. A similar seasonal difference in sensitivity to cadmium was seen in the shrimp *Paratya tasmaniensis*, which showed a threefold higher LC_{50} value in mid-October than in early July (Thorp and Lake 1974).

Thurberg et al. (1973) have found that cadmium reduced the level of oxygen consumption in the crabs *Carcinus maenas* and *Cancer irroratus*. A reduction of oxygen consumption of the gills of the mud crab, *Eurypanopeus depressus*, exposed to cadmium was found by Collier et al. (1973). Reduced metabolism may be responsible for the retardation of regeneration of the crabs in cadmium. Cadmium has been found to inhibit oxygen consumption and metabolism of fishes (Thurberg and Dawson 1974; Jackim et al. 1970) and has similarly been found to retard fin regeneration in fishes (Weis and Weis in press).

In this sort of study it is difficult to extrapolate laboratory findings to the field. In nature, metals would tend to be concentrated in the sediments more than the water, and it would be primarily from the sediments that these estuarine intertidal crabs would pick up the metals. Crabs would not normally be subjected to the loss of many appendages. Loss of a single limb is not particularly debilitating to a decapod. Should many limbs be lost, however, the crab's locomotion would be impaired, and it would be at a disadvantage. It would therefore be advantageous to regenerate the lost limbs as quickly as possible. Crabs which could not regenerate as quickly could be more subject to predation, and the toxic heavy metal pollutant would then be passed on to higher trophic levels.

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NOTES ON THE EARLY DEVELOPMENT OF THE SEA RAVEN, *HEMITRIPTERUS AMERICANUS*

Egg and larval characteristics of the sea raven, *Hemitripterus americanus* (Gmelin), are distinctive. The species ranges from Labrador to Chesapeake Bay but is nowhere abundant (Bigelow and Welsh 1925). Notes on the fertilized eggs (Bean 1897), newly hatched larvae (Warfel and Merriman 1944), and juveniles larger than 45 mm (Huntsman 1922; Bigelow and Welsh 1925; Bigelow and Schroeder 1936) have been recorded. However, there is no available information dealing with specimens between 12 and 45 mm in length. The present paper attempts, in part, to bridge this gap in previous observations of these larvae.

Methods and Materials

A cluster of nearly 90 eggs was found on the rocky shore of Montauk Point, N.Y. The eggs were collected at the level of the high tide mark at 0930 h on 9 November 1974. They were placed in an open system seawater aquarium at the marine station of Southampton College. In mid-December half of the eggs were transferred to laboratory facilities at the Academy of Natural Sciences of Philadelphia, where they were held in artificial seawater (7°C, 32‰) with a controlled photoperiod of 10.5 h light and 13.5 h darkness. Crude but effective temperature control was achieved by placing the covered rearing container in a water bath. The water bath and rearing container were then placed in a refrigerator. The temperature of the water bath was maintained with a thermostatically controlled aquarium heater. A 7½-W light bulb, controlled by an electric timer, was suspended above the rearing container. Moderate aeration kept the eggs in motion. After hatching, the larvae were maintained in similar conditions but without aeration. The strong current resulting from aeration appeared to be detrimental to the fragile larvae. When the yolk was nearly absorbed, the larvae were presented with food in the form of *Artemia* sp. nauplii and small pieces of *Palaemonetes* sp. and *Littorina* sp. flesh. Only three specimens could be induced to eat the pieces of flesh by placing the food in their mouths. Eventually one specimen ate the *Artemia* sp. nauplii unassisted.

Measurements were made on live material. Egg diameters were measured with dial calipers. Total lengths (TL) of the larvae were measured through a dissecting microscope using an ocular micrometer. Myomere counts were made with the aid of two Polaroid[1] HN 38 × 0.3 inch filters placed above and below the larvae and used in conjunction with a dissecting microscope and substage lamp. Final identification of the larvae was based on a comparison of the largest reared specimen in this study and the specimens collected in the Gulf of Maine by Joanne and Wayne Laroche. All 36 preserved specimens were preserved in 5% buffered Formalin and deposited in the Department of Ichthyology, Academy of Natural Sciences of Philadelphia (ANSP 131947).

Descriptions

Egg and Embryo

Some of the peripheral eggs in the cluster had

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