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Acute Toxicity and Accumulation of Zinc in the Crayfish, *Orconectes virilis* (Hagen)

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Zinc produces acute toxicity to freshwater organisms over a range of concentrations from 90 to 58,100 µg Zn/L; with the range of acute median effect concentrations being similar for freshwater fish and invertebrates (U.S. EPA 1980). Sources of zinc include electroplating, smelting and ore processing and drainage from both active and inactive mining operations (Weatherley et al 1980; Wentz 1974). Davies and Woodling (1980) noted that zinc was the primary toxicant to salmonids in a stream contaminated by metal mine drainage, as did Wentz (1974) in a survey of Colorado river basins.

The capacity to regulate internal zinc concentrations in decapod crustaceans has been described by Bryan (1968). Studies with the crayfish Austropotambius pallipes (Bryan 1967a) suggested a relatively high degree of tolerance to zinc by this animal. The only other available information on zinc toxicity to the crayfish has been provided by Boutet and Chaisemartin (1973).

The present study is designed to describe the toxicity of zinc to the crayfish Orconectes virilis over a 2-wk exposure period. In addition, whole animal and tissue analyses were performed on the test organisms and compared to the results of Bryan (1964, 1967a, 1968).

MATERIALS AND METHODS

Adult crayfish were collected from the inlet to Horsetooth Reservoir west of Fort Collins, Colorado (40°31'5"N, 105°10'0"E). Animals were acclimated for 2 wk at 18°C in the laboratory using dechlorinated tap water of drinking water quality, supplied by the City of Fort Collins. A 12-h photoperiod was used and the crayfish were fed trout chow ad libitum. Crayfish were not fed 48 h prior to testing; only intermolt crayfish were used.

Toxicity tests were conducted using two proportional diluters based upon the design of Mount and Brungs (1969). The diluters were calibrated to deliver a series of 5 concentrations of zinc plus a control, with 90% of the volume in each tank being replaced every 8 h. The diluters were set up to run as duplicates for each

test; animals were exposed for 2 wk. A stock solution was prepared by dissolving zinc sulphate ($ZnSO_4 \cdot 7H_2O$) in distilled water. Zinc concentrations were checked every three days as well as before and following each experiment with an atomic absorption spectrophotometer (Perkin-Elmer Model 306). Quality assurance for the atomic absorption analyses was maintained by using a Fisher Certified Standard (1000 ppm, A.C.S. specifications) to prepare the standard curve, the running of duplicate samples and the use of a reagent blank of deionized/distilled water analyzed every six samples. Final zinc concentrations expressed as means for the 2-wk period were determined to be 130, 63.3, 26.8, 12.2, 5.2 mg Zn/L (+/- 3.0 mg Zn/L).

A total of 12 experimental tanks, including duplicates, were partitioned and 6 crayfish were placed in each tank (12 per test per concentration) following equilibrium of the zinc concentrations. The tests were repeated three times so that a maximum of 36 crayfish were exposed at each concentration. Crayfish were selected for the toxicity tests from the acclimation tanks over a range of body sizes in order to determine the effects of body size on acute toxicity. In addition, crayfish were distributed in the tanks in an effort to obtain approximately equal numbers of males and females.

Water chemistry parameters were checked every three days during the experiment as well as prior to and following each test. The following water chemistry values were deemed to be important: temperature 18°C, pH 7.1, hardness 26 mg $CaCO_3$/L, alkalinity 15 mg $CaCO_3$/L, dissolved oxygen 8.0 mg/L. A Hach Kit was used to make the measurements. The values reported are the mean for each factor over the three tests.

Mortality data from the duplicate tanks were combined before the LC50 determinations were performed. The 2-wk LC50 value was calculated, using the method of Litchfield and Wilcoxon (1949). The 95% confidence limits were also calculated along with the LC50 value.

During the tests, dead crayfish were removed and the time of death recorded as well as the sex and wet weight of each animal. The crayfish were frozen for whole animal and tissue zinc analyses. At the conclusion of each test the surviving crayfish were sexed and weighed and frozen for subsequent zinc analysis. Crayfish used for whole animal zinc analysis were placed in 250 ml flasks and freeze dried for 48 h. The dried animals were then ground into a fine powder using a mortar and pestle. A 0.25 g sample of each animal was digested in concentrated HNO_3 in a 60°C water bath for 48 h. Following digestion each sample was diluted with deionized/distilled water and analyzed using the atomic absorption spectrophotometer (Perkin-Elmer 306). Duplicate analyses were performed on each animal in determining the whole animal zinc concentrations. The gills, hepatopancreas, abdominal muscle, carapace and intestine were excised from those crayfish not used for the whole animal analysis and freeze dried for 48 h. The

tissues were treated and analyzed using methods similar to those used for whole animal determination. Quality assurance consisted of identically prepared samples of National Bureau of Standards Standard Reference Material 1577 (Bovine Liver) for each group of samples analyzed. In addition, reagent blanks, consisting of deionized/distilled water, and zinc standards using a Fisher Certified Standard (1000 ppm, A.C.S. specifications) were prepared fresh for each group of samples analyzed. The data were analyzed by regression analysis and ANOVA procedures and significance determined at the $P<.05$ level.

RESULTS AND DISCUSSION

Mortality data of O. virilis to zinc over a 2-wk exposure period are presented in Table 1. The 2-wk LC50 for O. virilis was 84 mg Zn/L, with 95% confidence limits of 58.3 - 121 mg Zn/L. The LC50 values for periods of time less than 2 wk were not calculated, because 50% mortality was not achieved at any of exposure concentrations within less than 10 days. In fact, 100% mortality was never reached even at the highest exposure concentration (130 mg Zn/L) during the 2-wk exposure period. Mortality significantly increased with time over the exposure period, and demonstrated a significant, positive correlation with exposure concentration (r^2 =0.94). There was no effect of body size or sex of the organism on mortality of the crayfish to zinc.

The state of Colorado's current zinc standard at a hardness within the range used in this study is 50µg/L (Wentz 1974), while the National Criteria is 180µg/L (U.S. EPA 1980). It is evident that the levels of zinc which were toxic to the crayfish over the exposure period of this study greatly exceeded the recommended levels. However, surveys of river basins throughout Colorado have found zinc levels which are within the range found to be toxic to the crayfish. Wentz (1974) reported zinc concentrations as high as 99.0 mg/L in streams within the Platte River Basin, while Roline and Boehmke (1981) found zinc levels up to 79.0 mg/l in areas of the Arkansas River Basin. In addition, other sampling sites within Colorado's river basins yielded relatively lower zinc concentrations then those mentioned above. However, these levels were also greater than the current national and state zinc standards (Wentz 1974; Roline and Boehmke 1981). Subsequent chronic and sublethal studies of the effects of zinc on the crayfish are needed to determine the response of the organism to these high zinc levels.

It appears from the LC50 value (84 mg Zn/L for 2 wk) derived from this study, that O. virilis is highly tolerant to zinc. The only comparable data of zinc toxicity to crayfish are the 96-h LC50 values for Austropotambius pallipes and O. limosus (Boutet and Chaisemartin 1973). The 96-h LC50 values for these two crayfish species were 52 and 58 mg Zn/L, respectively. However, the water hardness was not given so that a direct comparison with this study is not possible. Chronic toxicity data for A. pallipes and O. limosus revealed that the 30-d LC50s for fed crayfish decreased only slightly to 44 and 48 mg Zn/L, while unfed crayfish had LC50s of 22 and 27 mg Zn/L, respectively. In addition, Bryan (1967a)

Table 1. Mortality data for Orconectes virilis exposed to zinc over a 2-wk exposure period (S.E. = standard error).

Mean Exposure Concentration (mg Zn/L)	Total Number of Crayfish Exposed	Total Number of Crayfish Dead	Mean Number Dead per Test ± S.E.
130.0	36	22	7.3 ± 0.003
63.3	35	15	5.0 ± 0.000
26.8	35	8	2.7 ± 0.003
12.2	35	2	0.6 ± 0.047
5.2	34	1	0.3 ± 0.144
Control	36	0	----------

while studying zinc regulation in the crayfish A. pallipes, noted that crayfish could not survive indefinitely at zinc concentrations between 20 and 100 mg Zn/L. Bryan stated further that the crayfish was relatively impermeable to zinc and suggested that death was likely related to high adsorption of zinc to the gill surfaces with subsequent absorption by the gills.

The tolerance of O. virilis to zinc may also be due in part to prior exposure to low concentrations of zinc in the water. The dechlorinated tap water used to acclimate the crayfish and also used as the dilution water for the experiments was found to have zinc levels below the detection limits of the atomic absorption spectrophotometer (<0.5µg Zn/L). However, analysis of water samples from the collection sites along the inlet to Horsetooth Reservoir, revealed zinc concentrations of 1.0 mg Zn/L. This suggests the possibility that the crayfish from Horsetooth Reservoir had become adapted to low levels of zinc. The occurrence of an increased tolerance to zinc as a result of previous exposure has been reported for rainbow trout (Davies and Woodling 1980), for the flagfish (Rahel 1981), and for chironomid larvae (Wenstral et al. 1978). This acquired tolerance to zinc could have caused an increase in the organism's tolerance to higher zinc levels, producing the high LC50 value in this study. It remains to be determined exactly how much of the tolerance described in this study is a consequence of adaptation to zinc and how much is inherent in the organism.

The whole animal zinc concentrations were directly related to and significantly affected by the concentration of zinc in the water. The mean whole animal zinc concentrations and the standard deviations for each exposure concentration are presented in Table 2. Regression analysis of whole animal zinc concentrations versus exposure concentrations produced a correlation coefficient of $r=0.87$ and a coefficient of determination of $r^2=0.76$. The whole animal zinc concentrations of the exposed crayfish were all significantly different from the concentration of zinc in the control animals as well as from each other, except for the concentrations at 26.8 mg Zn/L and 63.3 mg Zn/L. Neither the body size nor the sex of the crayfish had a significant effect on the

Table 2. Mean whole animal zinc concentrations ± standard deviation for Orconectes virilis and the respective concentration factors. N = 17 crayfish per concentration.

Mean Exposure Concentration (mg Zn/L)	Mean Whole Animal Zinc Concentration (µg/g)	Concentration Factors
130.0	346.2 ± 106.8	2.7
63.3	189.2 ± 57.5	3.0
26.8	175.8 ± 60.6	6.6
12.2	63.5 ± 22.8	5.2
5.2	29.3 ± 14.3	5.5
Control	66.8 ± 5.7	---

amount of zinc accumulated by the animal; correlation coefficients ranged from .032 to .187 for these factors.

The concentration factors for zinc are presented for each exposure concentration in Table 2. Concentration factors are defined as the ratio of whole animal zinc concentration to exposure concentration. The concentration factors for zinc were determined by first subtracting the mean zinc levels found in the control organisms from the mean zinc level at each exposure concentration and then dividing this value by the respective exposure concentration. The concentration factors are maintained at approximately the same level at exposure concentrations 5.2 to 26.8 mg Zn/L. This suggests that the crayfish is capable of accumulating zinc body levels in proportion to the concentration present in the water. The subsequent lower concentration factors at the two highest exposure concentrations may either be due to an increased excretion of zinc by the crayfish, or decreased uptake as a result of the toxic effects of zinc at these high ambient levels.

The gills are the main site of zinc uptake from the water. Appreciable levels were found in the gills at all exposure concentrations and increased as the concentration in the water increased ($r=0.87$) (Table 3). The observed increase is probably due in large part to adsorption of zinc to the gill surfaces, with absorption being dependent on the availability of proteins to which the zinc may bind (Bryan 1967a, 1968). The slow penetration of zinc across the gills, coupled with the tight binding of zinc to blood proteins are two main reasons for the low toxicity of this metal to the crayfish. The carapace is another site for zinc adsorption and concentrations of zinc were found to increase as external concentrations increased ($r=0.82$), although to a lesser extent than in the gills. Since no evidence is available to indicate that zinc can be absorbed through the exoskeleton, it is assumed that the zinc adsorbed to the carapace is not a significant factor in determining internal zinc levels.

The abdominal muscle was the least variable tissue with regards

Table 3. Mean zinc tissue concentration ± standard deviations for Orconectes virilis. Values are expressed in micrograms of zinc per gram of tissue. N = 17 crayfish per concentration.

Mean Exposure Concentration (mg Zn/l)	Gill	Hepatopancreas	Abdominal Muscle	Carapace	Intestine
130.0	5976.6 ± 2412.0	638.3 ± 321.6	123.4 ± 20.1	122.6 ± 40.2	221.8 ± 59.2
63.3	2168.0 ± 1248.4	555.8 ± 258.3	100.2 ± 18.2	85.8 ± 27.7	213.5 ± 68.3
26.8	1628.1 ± 986.5	428.0 ± 248.8	94.9 ± 17.1	64.6 ± 28.3	124.2 ± 45.2
12.2	333.3 ± 145.5	212.6 ± 121.6	89.0 ± 14.1	36.1 ± 12.7	100.9 ± 59.9
5.2	154.5 ± 38.1	116.8 ± 66.1	83.6 ± 12.4	22.5 ± 6.8	86.9 ± 22.0
Control	41.7 ± 18.4	65.7 ± 34.2	61.8 ± 8.3	7.6 ± 1.6	50.9 ± 27.3

changes in the zinc concentration over the range of test concentrations used (Table 3). A similar relationship was found by Bryan (1964, 1967a) for the crayfish A. pallipes and the lobster H. vulgaris. The observed increases in muscle zinc levels may be a reflection of an increase in the amount of zinc bound to blood proteins rather than an increase in zinc levels in the muscle tissue itself (Bryan 1968). In addition, Bryan (1967b, 1968) found that in the lobster the deep abdominal flexor and extensor muscles contained about 15μg/g zinc, whereas the superficial abdominal flexor and extensor muscles contained about 100μg/g of zinc. Since no attempt was made to distinguish between deep and superficial abdominal muscles in this study, the samples analyzed probably contained both types of abdominal muscle, resulting in somewhat higher zinc concentrations than reported by Bryan (1964).

The zinc concentrations in the hepatopancreas were second only to the gills for each treatment (Table 3). Bryan (1968) found that the hepatopancreas acted as a storage site when excess zinc was absorbed from food or from the water. Zinc was shown to be removed from the hepatopancreas of the crayfish as a result of fecal production. This mechanism was found to be of much greater importance as a means for excretion of zinc in the crayfish than any other process (Bryan 1967a). The loss of zinc by this route depends upon whether the animal is feeding, since unfed crayfish produce some feces but with little or no zinc content (Bryan 1967a). Analysis of intestine revealed some fecal material present and undoubtedly contained some zinc, since zinc concentrations in the tissue showed a significant increase before leveling off at the highest exposure concentration (Table 3). Since O. virilis was not fed during the experiments it would be expected, based upon the results of Bryan (1967a), that excess zinc would be primarily stored in the hepatopancreas and levels in this tissue would tend to increase as exposure concentration increased. However, zinc concentrations in the hepatopancreas are not significantly different at the higher exposure concentrations (Table 3). These results suggest that the hepatopancreas had become saturated with zinc and probably reflects saturation of blood proteins with zinc as well. The saturation of the blood with zinc may also account for the leveling off of zinc concentrations in the other tissues at the higher exposure concentrations.

Under normal conditions, the crayfish appears capable of regulating zinc content through mechanisms for absorption across the body surface and loss via fecal production. This regulatory capability is maintained to some extent even when the organism is exposed to high concentrations of zinc in the water. Bryan (1967a) stated that an external zinc concentration of 1.0 mg Zn/L was equal to the concentration of zinc in the blood of the crayfish and only upon exceeding this level does the zinc concentration in the blood and internal tissues increase. It has been found in this study that O. virilis had been exposed to as much as 1.0 mg Zn/L prior to testing. This pre-exposure under

natural conditions may explain the relatively high tissue and whole animal zinc concentrations in the control animals compared to the results of Bryan (1967a). Furthermore, the regulatory mechanisms for zinc in O. virilis undoubtedly contributed to the relatively high tolerance to this metal observed in this study. This capacity to regulate zinc in the body probably affords the probably affords the crayfish a degree of protection where metal pollution is prevalent.

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