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The Effects of Lead on Oxygen Uptake in the Crayfish, *Orconectes virilis* (HAGEN)

Richard V. Anderson*
Department of Biological Sciences, Northern Illinois University, DeKalb, Ill. 60115

The continual increase in the occurrence of trace metals in our environment has led to an increase in the number of investigators studying the effects of trace metals on the constituents of the environment (LELAND et al., 1974). This is particularly true of the aquatic system since accumulation of trace metals occur through runoff or direct dumping of sewage effluents into waterways. Lead has become particularly important due to its relative toxicity and increased environmental contamination via automobile exhaust and highway runoff (LAXEN and HARRISON, 1977). Many investigations have been directed toward assaying the concentrations of the trace metals in organisms (LEVY and CROMROY, 1973; GALE et al., 1973; MATHIS and CUMMINGS, 1973; ANDERSON, in press). Some have been concerned primarily with determination of lethal concentrations of various metals (JONES, 1938; SPRAGUE, 1969; WARNICK and BELL, 1969). Those that have worked with sublethal effects have usually delt with various species of fish, consequently there is little information on the effects of sublethal metal concentrations on invertebrates. Therefore, the purpose of this study was the investigation of the effects of sublethal lead concentrations on the oxygen uptake of the crayfish, *Orconectes virilis*.

Methods

One hundred-twenty small crayfish, carapace length 25 to 30 mm, and wet weight 11 to 13 g, were collected from the Kishwakee River in northern Illinois. The crayfish were placed in holding tanks and acclimated to the laboratory lighting and feeding routines. After two weeks 30 crayfish were placed in each of four aquaria with 40 liters of water. Sufficient lead acetate was dissolved in the tanks to result in the follow lead concentrations; 0.5 ppm, 1.0 ppm, and 2.0 ppm. A control without lead addition was the forth treatment. The solutions were changed every 5 days in order to maintain the lead concentration at approximately the indicated values.

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*Present address: Department of Zoology and Entomology and Natural Resource Ecology Laboratory, Colorado State University, Ft. Collins, Colorado 80523

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Every 10 days for 40 days the oxygen consumption of 5 crayfish from each tank was determined. Each individual was placed in a glass jar with 450 ml of oxygen saturated water, 9 ppm oxygen, which was closed with an air tight seal. The oxygen content of the water was taken with a Beckman oxygen meter one and two hours after the crayfish had been placed in the water. The concentration of the oxygen utilized was determined on the basis of the oxygen concentration in 3 control jars which has no crayfish in them.

After the oxygen utilization had been determined the crayfish were prepared for analysis of lead content. They were dissected into general body regions; gills, viscera, muscle, and exoskeleton. Each sample was placed in a preweighed crucible and dried at 100°C for 24 hours. The dried samples were then ashed in a muffle furnace at 450°C for 8 hours. The resulting ash was dissolved in a 2:1 nitric acid, distilled water solution which was added to the crucibles. This solution was filtered through ashless filter paper into 25 ml volumetric flasks and brought to volume with distilled water. The aqueous samples were analyzed for lead using a Varian Techtron atomic absorption spectrophotometer at a wave length of 217.0 nm (EWELL and GIDLEY, 1962). The sensitivity of analysis was 0.16 $\mu g/\ell$ Pb. Correction for sample preparation losses, spectrophotometric background absorption, and proportional matric effects were made using blanks, standard additions, and by bracketing between the non-specific wave lengths 210.0 and 239.4 nm.

Due to an apparent lack of significant differences in oxygen consumption between control and experimental groups of crayfish after 30 days the ventilation volume of the crayfish was determined on the last sampling date. A method similar to that described by THOMAS (1954) and LARIMER (1961) was used. A small water tight chamber was constructed using 6 mm plastic sheets. This chamber was divided into two sections with another sheet of plastic through which a short 10 cm by 2.5 cm glass tube was inserted near the bottom. Onto one end of this tube a piece of latex was secured. A small hole was cut in the center of the latex covering the tube entrance. Two drains were placed in the bottom of the tank in each section to maintain an equal water level in each side. A crayfish was placed through the hole in the latex covering until its body anterior to the cheliped stalks was in the glass tubing. The rubber formed a water tight seal around the body of the crayfish. Water was pumped by the crayfish from the side of the tank with the section of the crayfish posterior to the cheliped stalks across the gill surfaces exiting anteriorly and consequently into the other section of the tank. The ventilation volume could thus be determined by measuring the overflow collected from the drain in the anterior section of the tank. The crayfish chelipeds were removed 2 weeks prior to determination of their ventilation volume. This was necessary in order to place them in the glass tubing and to maintain a tight seal. It should be noted that care must be taken when inserting the crayfish through a hole in the latex. It must be small enough to insure a complete seal around the

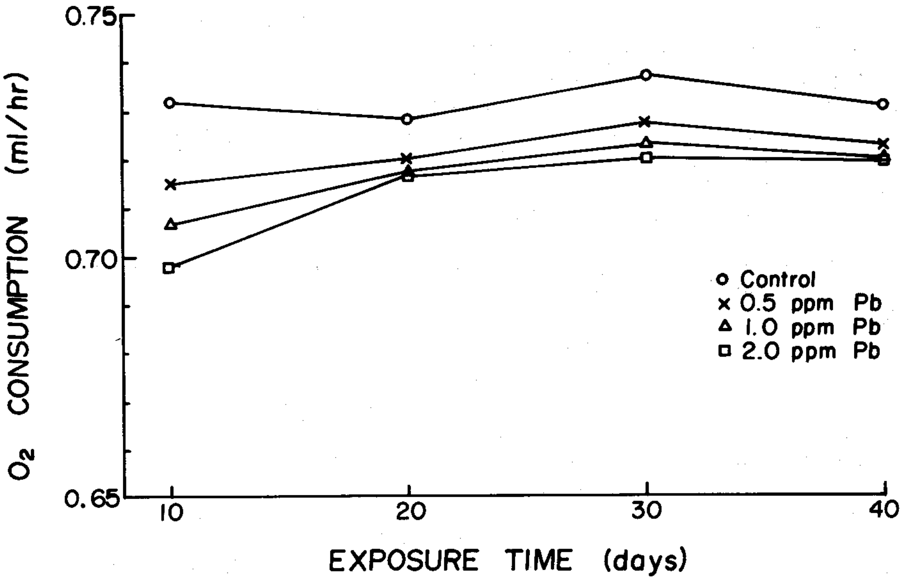

Figure 1. The oxygen consumption of crayfish kept in water with different lead concentrations for a period of 40 days. Each point is the mean of five determinations.

crayfish but not so small that it decreases the volume of the gill chamber. The ventilation volume of 5 crayfish from each of the tanks were determined on each sampling date.

Results

As indicated by Figure 1, the oxygen consumption between treatments on the last 3 sampling periods were not significantly different (t-test, p 0.05 for all significance testing). The first sampling period did show some significant differences with the tank with the highest Pb concentration having the lowest oxygen consumption rate. The oxygen consumption of crayfish from the control tank was always slightly higher than that of the crayfish in leaded water.

Analysis of body parts (Figure 2) showed a marked increase in the Pb found both in the gills and exoskeleton as water concentration of Pb and time of exposure increased. There was some increase in the Pb concentrations in muscle and viscera, however, there were no significant differences in concentrations eithor between treatments or sampling times. Five crayfish were dissected into the indicated body parts and analyzed at the start

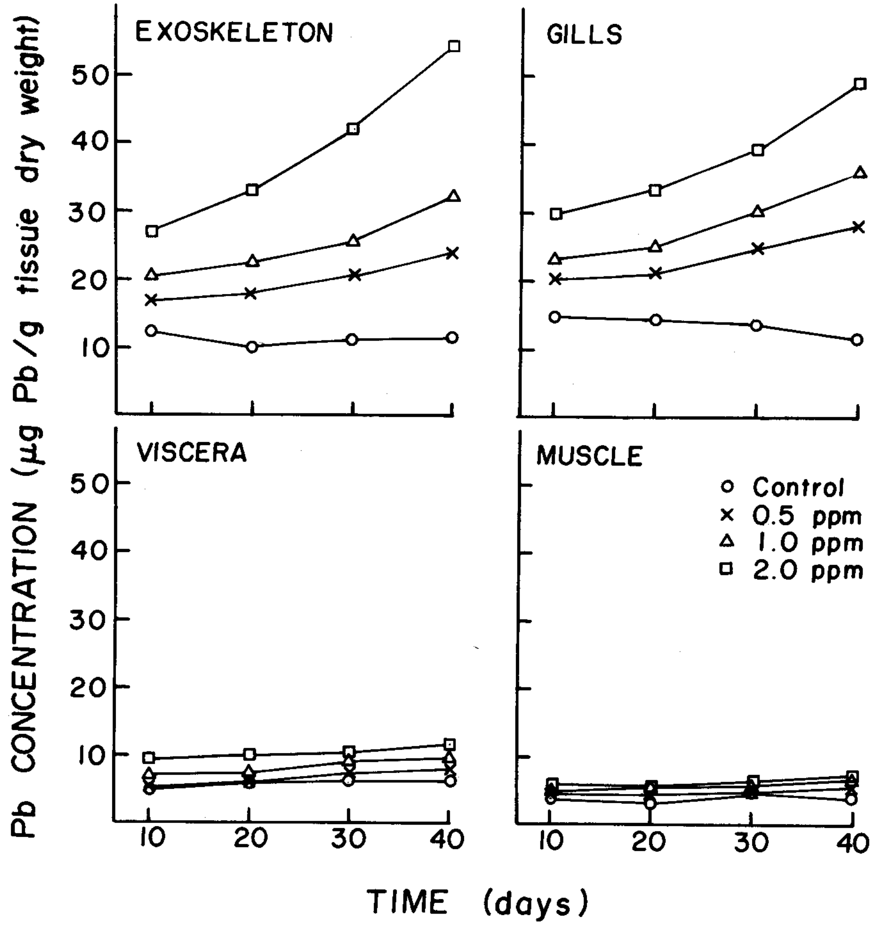

Figure 2. Lead concentration in body parts of the crayfish at each sampling time. Each point is the mean of five samples.

of the experiment to determine the background Pb concentration. The values obtained were similar to those found in the control tank after 10 days.

The ventilation volumes between crayfish in the four treatments are significantly different. As the Pb content of the water increased the ventilation volume greatly increased (Figure 3).

Discussion

There tended to be an acclimation to the lead concentrations in the test tanks. The oxygen consumption for all metal concentrations became approximately equal after the first 20 days of

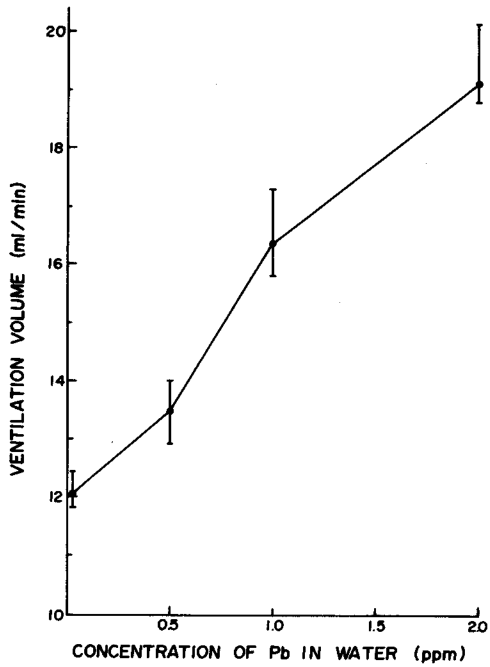

Figure 3. The ventilation volume of the crayfish as a function of the Pb concentration in the water. Each point is the mean of five determinations and the bars represent the range of values found.

exposure. That there was some type of physiological compensation was postulated due to the indication of increasing lead concentrations in the gills with increasing water concentrations and time. It was found by CARPENTER (1930), JONES (1964), and SPARKS et al. (1972) that fish exposed to relatively high concentrations of trace metals developed a film on their gill surfaces which decreased the efficiency of oxygen uptake and if prolonged would lead to death. A similar responce in crayfish, if uncompensated, should lead to a continually decreasing oxygen uptake. It was found, however, that the crayfish were compensating for the postulated decrease in gill efficiency by increasing the amount of oxygenated water passing over gill surfaces. Since the water in the test tanks was maintained at a saturated oxygen level the increased ventilation volume may have resulted in the similar values of oxygen consumption obtained after 20 days of exposure to the Pb. It should be noted that the ability of the crayfish to compensate in this way would be of relative short term value if it were not for the fact that ecdysis in crayfish results in the loss of gill surfaces and the exoskeleton which also results in the loss of the mucoid film as well as the accumulated Pb (ANDERSON and BROWER, in press). This may be particularly important at sublethal metal concentrations since continued accumulation may result in toxic body burdens and a continually deteriorating gill efficiency. As indicated by LARIMER and GOLD (1961), testing decreasing oxygen concentration in water, crayfish compensate for a short time but eventually were only able to maintain themselves by reducing activity and metabolism. It was noted that the crayfish used in the oxygen consumption tests became increasingly less responsive and remained motionless in their containers toward the end of the tests. The ventilation volume found in the control group of crayfish was similar to that reported by WIENS and ARMITAGE (1961), LARIMER and GOLD (1961), and MCMAHON et al. (1974). These investigators found that when oxygen tension in the water decreased ventilation volume increased. This response would be similar to decreasing the gills uptake efficiency.

The Pb concentrations fould in the body part analysis was higher than that reported by ANDERSON and BROWER (in press). The water concentrations used in this experiment, although not lethal, were much higher than those at the field stations of the previous authors. The high Pb concentration used in this experiment was above that found in two-thirds of the 730 rivers investigated by DURUM and HEM (1972), most of the rivers having about 1 ug/ℓ of Pb. The high exoskeleton concentration indicates that it may act as a sink for lead and that the cycling of Pb is initially through uptake across gill surfaces. This type of relationship was found by MARTIN (1973) in iron uptake by lobsters.

In conclusion, increasing concentrations of Pb will cause some decrease in the ability of *O. virilis* to take up oxygen. The crayfish was able to acclimate to the metal concentration by compensating for decreased gill efficiency through increasing its ventilation volume. Crayfish tend to take up lead primarily in the exoskeleton and gills. Concentration in these body parts increased as environmental concentration and time increased.

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