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MORTALITY CURVES OF BLIND CAVE CRAYFISH (ORCONECTES AUSTRALIS AUSTRALIS) EXPOSED TO CHLORINATED STREAM WATER*

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Abstract

Chlorinated stream water toxicity was tested on the blind cave crayfish *Orconectes australis australis)* from Merrybranch Cave, White County, Tennessee. An undisturbed natural cavern, Merrybranch was formed between two strata of sandstone having a mean elevation of 354 meters (MSL). Test water was collected from a subterranian stream in the cave supporting the hypogean crayfish population, and transported to the laboratory. No chlorinity was detected in the underground stream water.

In the laboratory, cave water was chlorinated with sodium hypechlorus solution at various concentrations of total residual chlorine, combined residual, and free chlorine content as measured by Standard Methods titration procedure. Thirty-six crayfish, six crayfish per test solution, were subjected to a three day acclimation period at chlorine concentrations ranging from 0.21-1.50 mg./l. total residual chlorine, 0.20-0.30 mg./l. combined residual chlorine, and 0.01-1.20 mg./l. free chlorine; and then subjected to a 24 hour time-to-death (hourly) bioassay at the following chlorine water dilutions (mg./l.): (1) 7.45 total residual, 0.45 combined residual, and 7.00 free, (2) 3.39 total residual, 0.39 combined residual, and 3.00 free, (3) 2.85 total residual, 0.35 combined residual, and 2.50 free, (4) 2.30 total residual, 0.30 combined residual, and 2.00 free, (5) 1.96 total residual, 0.21 combined residual, and 1.75 free, and (6) control. Fluctuations within these concentrations ranged from ± 0.20 free chlorine. All test solutions and a control were delivered by an Esvelt serial diluter. In addition, a 24 hour time-to-death (hourly) bioassay was conducted at the same dilutions on crayfish not acclimated to chlorine.

These test demonstrated that crayfish mortalities generally increased with increasing concentrations of chlorine in both bioassays, while acclimated crayfish tended to be more tolerant than non-acclimated ones.

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Introduction

The toxicity of chlorinated stream water was tested on blind cave crayfish *(Orconectes australis australis)* from Merrybranch Cave, White County, Tennessee (Fig. 1). Merrybranch Cave is an undisturbed natural cave formed in Banger limestone between two strata of sandstone-Penington Formation (upper strata) and Hartselle Formation (lower strata). The mean elevation of the cave is 1160 feet (354 meters) MSL. This composite formation origanated during the Mississippian Period (Wilson, 1962).

Increased use of chlorine and recent studies of residual chlorine toxicity in aquatic systems have emphasized the need for close scrutiny of the effects of present water-treatment procedures and industrial uses. Chlorination of sewage-disposal effluents is extensively utilized to either reduce the risk of pathogenic bacteria gaining access to a river or stream into which the effluent is discharged or to prevent the decomposition of organic matter in the area of the discharge (Allen et al., 1946). The City of Cookeville sewage-disposal system in Putnam County, Tennessee (neighboring White County-site of Merrybranch Cave) has caused severe stress on the aquatic ecosystem of Pigeon Roost Creek for approximately 8 km. downstream

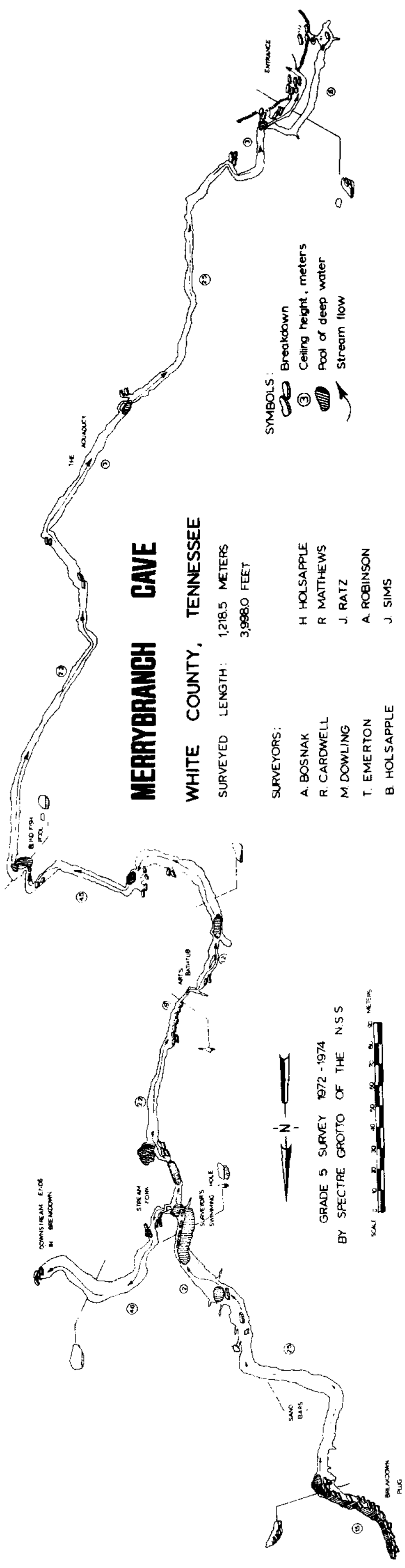

Fig. 1. Map of Merrybranch Cave, White County, Tennessee. Blind cave crayfish, *Orconectes australis australis*, used in test were from stream sections of this cave.

of the effluent before entering a cave stream system emptying into the Falling Water River (Smithson, 1974). Aquatic cave systems have been given little attention to date as to damages concured by chlorinated effluents entering into their water sheds. Industrial processes resulting in chlorinated effluents include the bleaching of paper and textiles, cooling waters to which chlorine has been added to reduce the growth of slime in cooling towers, and effluents from many other processes in which a cheap disinfectant or oxidizing agent is required (Merkens, 1958). The toxicity of chlorine on aquatic organisms has been investigated by many researchers whose findings have been reviewed by W. A. Brungs (1973) There is agreement that chlorine is a highly toxic substance, causing severe ecological stress to aquatic organisms at concentrations presently being discharged from municipal and industrial users.

Materials and methods

Collections of the blind cave crayfish, *Orconectes australis australis,* were made from Merrybranch Cave during October, 1974. Dip nets were used to collect this hypogean species from the stream water in the cave. Test water was also collected on site from the underground stream supporting the crayfish population. A Deming self-priming utility pump (model 3310) powered by a Sears power plant, 1400 watt alternater (model 32 AV 3200 4N) was used to transport water from the cavernous stream to holding containers for transport to the laboratory. The subterranian stream was free of chlorine, as determined by DPD ferrous titrametric and colorimetric methods (Standard Methods for the Examination of Water and Wastewater, 1971). Stream water was used so that the natural chemical characteristics would be tested under the influence of various added concentrations of chlorine, consequently duplicating subjected natural conditions as closely as possible. Chemical and physical parameters were taken of the cave stream water previous to being disturbed. These were for chlorinity, hardness, conductivity, pH, total alkalinity, dissolved oxygen, and temperature (Table 1). A Wildlife Supply Company Field Water Chemistry Kit was utilized in these test. All test were by titrametric procedure described by Standard Methods for the Examination of Water and Wastewater (1971).

In the lab, cave water was chlorinated with sodium hypochlorous solution at various concentrations of total

Table 1. Water chemistry of Merrybranch Cave Stream System (October, 1974)

Temperature (Air, °C)	11.5
Temperature (Water, °C)	10.5
Total residual chlorine (mg/l)	<0.00
Conductivity (μMHOS)	28
pH (standard units)	8.9
Total alkalinity (mg/l $CaCO_3$)	167
Dissolved oxygen (ppm)	5.1
Total hardness (mg/l $CaCO_3$)	242.4

residual chlorine, combined residual chlorine, and free chlorine content as measured by DPD ferrous titrametric and colorimetric methods (Standard Methods for the Examination of Water and Waste Water, 1971). The same chemical and physical parameters were taken for the stock solution as was tested on the natural cave stream water and by the same chemical methods (Table 2). The stock solution and dilution water were maintained at 10.5°C (± 0.5°C), the same temperature as the natural cave stream water in Merrybranch Cave, by a Blissfield continuous flow coil cooling agitator (model BHL 1038). Thirty-six crayfish, six crayfish per test solution, were subjected to a three day acclimation period (72 hours) at chlorine concentrations ranging at 0.21-1.50 total residual chlorine, 0.20-0.30 mg./l. combined residual chlorine, and 0.01-1.20 mg./l. free chlorine (Table 3), and then subjected to a 24 hour time-to-death (hourly) bioassay at chlorine water concentrations ranging from 1.96-7.45 total residual chlorine, 0.21-0.45 combined residual chlorine, 1.75-7.00 free chlorine, and a control as presented in table 4. All test solutions and the control were delivered via a continuous flow Esvelt serial diluter (Esvelt & Conners, 1971). During the acclimation period one mortality occured per dilution (14% mortality). No crayfish mortalities occured in the control. In addition, a 24 hour time-to-death (hourly) bioassay was conducted at the same dilutions on crayfish not acclimated to chlorine. Test chambers for holding the crayfish at various test solution concentrations and controls consisted of thirty-six 500 ml. vacuum filter flask. The use of this type holding apparatus for the crayfish allowed for the test and control solutions to enter the flask through a glass tube projecting near the bottom of the flask from a one-hole stopper. The continuous flow test solution entering the bottom of the flask would then displace the older solution out the vent. Thus, the crayfish were always subjected to well oxygenated water at 10.5°C (± 0.5°C). The solution in the flask was replaced every 10 minutes. In order to simulate natural conditions as much as possible, black plastic sheeting was framed over the flask to block out all light except during hourly checks. Any response to photosensitivity as described by Larimer (1966) was therefore considerably reduced.

The effects of chlorine on mortality rates were analyzed statistically by Litchfield's (1949) method of log-probit transformations for dose-effect mortality curves. This method is recommended by Sprague (1969) in his recent review stressing the need for a uniform method of testing and statistically evaluating toxicological measurements of pollutant toxicity to fish. Mortalities below 10% and above 90% were not used in this test, as recommended procedure for a more exacting logprobit toxicity curve

Table 2. Water chemistry of chlorinated stock water utilized for 24 hours continous flow bioassays

Temperature (Water, °C)	10.5 (± 0.5)
Total residual chlorine (mg/l)	7.45 (± 0.22)
Combined residual chlorine (mg/l)	0.45 (± 0.02)
Free chlorine (mg/l)	7.00 (± 0.20)
Conductivity (μMHOS)	28
pH (standard units)	8.9
Total alkalinity (mg/l $CaCO_3$)	167
Dissolved oxygen (ppm)	5.0 (± 0.5)
Total hardness (mg/l $CaCO_3$)	242.4

Table 3. Chlorine water concentrations (mg/l) subjected to cave crayfish for a three day (72 hours) acclimation period

Dilutions	Chlorine water concentrations (mg/l)		
	Total residual	Combined residual	Free
1	1.50 (± 0.22)	0.30 (± 0.02)	1.20 (± 0.20)
2	1.20 (± 0.22)	0.28 (± 0.02)	0.92 (± 0.20)
3	0.87 (± 0.22)	0.25 (± 0.02)	0.62 (± 0.20)
4	0.54 (± 0.22)	0.22 (± 0.02)	0.32 (± 0.20)
5	0.21 (± 0.22)	0.20 (± 0.02)	0.01 (± 0.20)
6	<0.00	<0.00	<0.00

Table 4. Chlorine water concentrations (mg/l) subjected to unacclimated and acclimated cave crayfish in 24 hours (time-to-death) continuous flow bioassay

Dilutions	Chlorine water concentrations (mg/l)		
	Total residual	Combined residual	Free
1	7.45 (± 0.22)	0.45 (± 0.02)	7.00 (± 0.20)
2	3.39 (± 0.22)	0.39 (± 0.02)	3.00 (± 0.20)
3	2.85 (± 0.22)	0.35 (± 0.02)	2.50 (± 0.20)
4	2.30 (± 0.22)	0.30 (± 0.02)	2.00 (± 0.20)
5	1.96 (± 0.22)	0.21 (± 0.02)	1.75 (± 0.20)
6 (Control)	<0.00	<0.00	<0.00

Table 5. Median lethal concentration (LC50), standard error equivalents of the LC50 (± S.E.), and slope functions (S) of the mortality curves tested by log-probits statistics

Crayfish treatment	LC50 (±S.E.) (mg/l) Residual chlorine	S	LC50 (±S.E.) (mg/l) Free chlorine	S
Unacclimated	2.70 (± 1.26)	1.51	2.25 (± 1.41)	1.72
Acclimated	3.39 (± 2.63)	1.69	3.00 (± 2.10)	3.21

(Shepard, 1955). The arithmetic value which is equivalent to the deviation of the distribution has been termed the slope function ('S') by Litchfield (1949) (Table 5).

The median lethal concentration (LC50) for a curve together with its 95% confidence limits and slope function adequatly define a curve. In order to compare one curve with another, Bliss (1935) showed that when a dose-effect mortality curve, in which tolerances of the test subjects with respect to a stimulus were normally distributed, was logarithmic, the variances of the tolerance for a number of closely related stimuli were often nearly equal. This equality of variances is shown in the probit analysis by parallelism of the probit regression lines (Finney, 1974). Litchfield (1949) provides a rapid test for parallelism by calculating a function known as the slope function ratio ('SR'). Once a test for parallelism is completed and the curves are found to be parallel, a second value known as the potency ratio ('PR') is computed to determine whether or not the LC50 values being compared are significantly different. The slope function ratios, potency ratios, and their 95% confidence limits have been included in this paper inasmuch as they may have value for future investigators (Table 6).

Table 6. Comparison of mortality curves: Slope function ratios (SR), potency ratios (PR), and their 95% confidence limits

Curves compared	SR (mg/l)	PR (mg/l)
Total residual chlorine (acclimated crayfish) vs Total residual chlorine (unacclimated crayfish)	1.12 (0.90-1.40) $P < 0.95$	1.33 (0.85-2.11) $P < 0.95$
Free chlorine (acclimated crayfish) vs Free chlorine (unacclimated crayfish)	1.07 (0.58-1.98) $P < 0.95$	1.33 (0.85-2.11) $P < 0.95$

Results and discussion

The mortality of cave crayfish, *Orconectes australis australis,* as expected increased as concentrations of chlorine increased. Probit analysis calculations for the median lethal concentration (LC50) at 24 hours of exposure time are presented in Table 5. The LC50 increased for acclimated crayfish as compared to the LC50 of unacclimated crayfish subjected to the same chlorine concentrations.

Test data tend to indicate that cave crayfish, *Orconectes australis australis,* are more tolerant of chlorine toxicity after having been subjected to acclimation at sub-lethal concentrations of chlorine, than when no acclimation was administered. In both acclimated and unacclimated crayfish, mortality rates showed positive correlation to increases in concentrations of chlorine. The test for parallelism for each chlorine treatment showed that both curves (Figs. 2 and 3) were parallel ($P < 0.05$). Therefore, we may assume that no other interactions except those of the various chlorine concentrations were operating in this experiment. No significant difference ($P > 0.05$) was found between the potency ratios of the two treatments at the 95% confidence level. However, at the 90% confidence level the potency ratios of the two treatments do differ

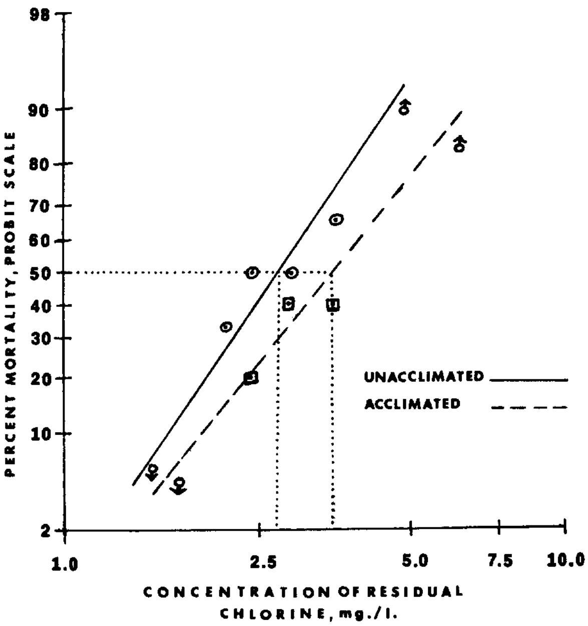

Fig. 2. Estimation of the median lethal concentration (LC50) for 24 hours of exposure time to residual chlorine. The LC50 for 24 hours of exposure time to unacclimated blind cave crayfish is 2.70 mg./l.; the LC50 for 24 hours of exposure time to acclimated blind cave crayfish is 3.39 mg./l.

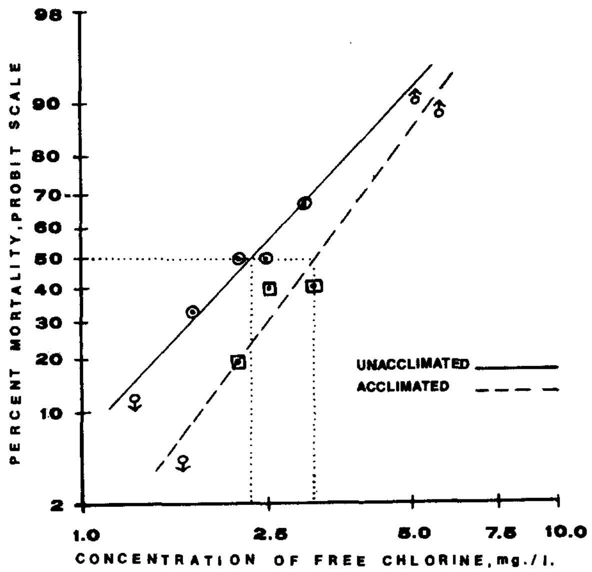

Fig. 3. Estimation of the median lethal concentration (LC50) for 24 hours of exposure time to free chlorine. The LC50 for 24 hours of exposure time to unacclimated blind cave crayfish is 2.25 mg./l.; the LC50 for 24 hours of exposure time to acclimated blind cave crayfish is 3.00 mg./l.

significantly ($P < 0.10$). The slope function ratios, potency ratios, and their 90% confidence limits are presented in table 7.

Summary

The simple food chain involved in this cave system, as well as most cave systems, restricted the removal of more specimens for test purposes. In a simple food chain, the removal of any link in the chain causes more stress on the ecosystem than in complex food chains (Odum, 1971).

Moreover, evidence is presented on the tolerance of these organisms to total residual chlorine at concentrations which do fall into normal municipal and industrial use concentrations in below effluent stream stressed zones. Free chlorine is by far the major toxic component of the total residual chlorine toxicity in this test as introduced into the natural cave stream water. This is due to the low organic level carried in the cave stream system. Such conditions result in very low combined chlorine concentrations.

From these results, it is suggested that further studies, particularly multivariate studies, be conducted to determine the possible effects of the interactions of chlorine effluent toxicity and other potentially lethal and sublethal pollutants on cave crayfish when introduced into cave systems.

Table 7. Comparison of mortality curves: Slope function ratios (SR), potency ratios (PR), and their 90% confidence limits

Curves compared	SR (mg/l)	PR (mg/l)
Total residual chlorine (acclimated crayfish) vs Total residual chlorine (unacclimated crayfish)	1.12 (0.90-1.40) P < 0.90	1.26 (0.95-1.68) P < 0.90
Free chlorine (acclimated crayfish) vs Free chlorine (unacclimated crayfish)	1.07 (0.58-1.98) P < 0.90	1.33 (1.00-1.77) P < 0.90

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