Oecologia (Berl.) 44, 8–12 (1979)

Oecologia

Tissue Metal Concentrations in Two Crayfish Species Cohabiting a Tennessee Cave Stream

Gary W. Dickson, Linda A. Briese and John P. Giesy, Jr.
Savannah River Ecology Laboratory, P. O. Drawer E, Aiken, SC 29801, USA

Summary. Concentrations of Cd, Cr, Cu, Fe, Mg, Mn, Pb, Zn, Ca and K were examined in tissues of the troglobitic (obligatory cave-dwelling) crayfish *Orconectes australis australis* and troglophilic (facultative cave-dwelling) species *Cambarus tenebrosus*. These two species cohabit a stream in Merrybranch Cave, located in rural White Co., Tennessee. Tissue concentrations of essential metals did not exhibit any trends between species. In contrast, Cd and Pb concentrations were found to be significantly greater in *O. a. australis* for almost all of the tissues analyzed. The higher Cd and Pb concentrations in *O. a. australis* are thought to be due to the increased longevity of this troglobitic species. Because of the toxicity of Cd and Pb, chronic exposure to relatively low concentrations of these metals could cause changes in mortality, fecundity or behavior in aquatic organisms possessing long life spans. The bioaccumulation of metals from low level, non-point sources is discussed in relation to life history strategies.

Introduction

Closely related organisms may exhibit different physiological strategies under identical environmental conditions. This occurs in freshwater crayfish (Decapoda: Astacidae) in situations where troglobitic (obligatory cavernicole) and troglophilic (facultative cavernicole) species exist syntopically in cave streams or pools.

Troglobitic crayfish possess adaptations which allow them to successfully exploit the relatively stable and food-poor cave environment. These include lower metabolic rates than surface or troglophilic forms (Burbanck et al., 1948; Eberly, 1960; Jegla, 1964; Weingartner, 1977; Caine, 1978; Dickson and Franz, in press); loss of eye structure (Mellon, 1977) and body pigmentation (Wolfe and Cornwell, 1964); increased foraging efficiency through sensory enhancement (Cooper, 1969); and the presence of K-selected life history traits which include low growth and reproductive rates, larger size at hatching and increased longevity (Hobbs, 1973; Cooper, 1975; Franz, 1978). In contrast, troglophilic crayfish are less specialized than troglobitic species with populations occurring in both epigean and hypogean areas. They are considered to represent more recent invasions from surface habitats into subterranean systems. Troglophilic forms have exploited subterranean habitats through an increase in foraging range and a slight reduction in growth and fecundity compared with conspecific surface populations (Weingartner, 1977). Because of the specialized nature of cave organisms and their habitat interactions, it is important to understand the possible implications of man-induced environmental perturbations. In the present study, tissues of both a troglobitic and troglophilic crayfish cohabiting a cave stream were examined to gain insight into the influence of physiological and life history differences on the concentration of metals in crayfish tissues.

Methods and Materials

Samples of the troglobitic crayfish *Orconectes australis australis* (Rhoades) and the troglophilic species *Cambarus tenebrosus* Hay were collected with baited traps and dip net from syntopic populations in a stream in Merrybranch Cave, White Co., Tennessee. This location was chosen because of the relatively large populations of crayfish, allowing limited sampling with minimum population disruptions. Samples of the troglobitic isopod *Caecodotea alabamensis*, which probably serves as a food source for these crayfish, were also collected. After capture, crayfish and isopods were placed individually into polyethylene bags and transported to the laboratory in liquid nitrogen (−196° C). Cave stream water samples were taken in polyethylene bottles and returned to the laboratory at 0° C. Samples were maintained at −4° C prior to the analysis.

Prior to dissection, carapace length (mm) and sex were recorded for each crayfish. Specific tissues and structures (carapace, gill, hepatopancreas, gastrolith, green gland and dorsal tail muscle) were dissected from each crayfish and placed individually into polyethylene bottles. Dissection was conducted under a laminar flow hood with polyethylene dissecting tools to minimize contamination.

Tissue samples, along with whole isopods were lyophilized for 2–3 days and dry weights were determined to the nearest 0.01 mg. After lyophilization, samples were digested in Teflon (TFE) digestion tubes with one or five mls redistilled HNO_3 to give an approximate acid to tissue ratio (v/w) of 25 to 1 (Thorp et al., 1979).

Before use, all Teflonware and glassware was soaked for 24 h in 1% Acationox (Scientific Products) and rinsed three times in deionized water (Milli-Q Water Purification System, Millipore). Teflonware was then leached 4–6 h in heated (130° C) 50% redistilled HNO_3 prior to storage in 10% redistilled HNO_3. Immediately before usage, digestion tubes were again rinsed twice in deionized water. Polyethylene bottles were soaked 24 h in 10% HCL and rinsed twice in deionized water.

Cadmium, Cr and Pb were measured by flameless atomization with a Perkin-Elmer Model 306 atomic absorption spectrophotometer equipped with an HGA-2100 flameless atomizer and deuterium background continuum. Calcium, Fe, Mg and Zn concentra-

0029-8549/79/0044/0008/$01.00

Table 1. Total metal concentrations in Merrybranch Cave stream water

Cd (μg/l)	Cu (μg/l)	Fe (μg/l)	Pb (μg/l)	Zn (μg/l)	Ca (mg/l)	K (mg/l)	Mg (mg/l)	Na (mg/l)
0.2	8.0	128.7	2.3	1.6	37.3	1.6	6.1	1.5

tions were measured by flame atomization and K by flame emission with an Instrumentation Laboratories Model 351 atomic absorption spectrophotometer. Lanthanum chloride was added to both samples and standards to increase sensitivity for Ca determinations. Copper and Mn concentrations were measured by either flame or flameless atomization depending upon sample concentrations. Matrix interferences were evaluated by standard additions and measurements at non-absorbing adjacent analytical wavelengths and eliminated by variation in charring and atomization parameters. Because of a persistent matrix interference due to Na, Pb concentrations were measured using standard additions techniques even though much of the interference was eliminated by NH_4OH additions. Standards were made from Fisher certified atomic absorption standards (Fisher Scientific). To evaluate possible contamination, reagent blanks were used throughout all preparation and analytical procedures. Reagent grade HNO_3 was re-

Table 2. Mean concentrations of trace metals in the isopod *C. alabamensis* and the crayfish *O. a. australis* and *C. tenebrosus* collected in Merrybranch Cave. Isopod values represent pooled, whole body concentrations while individual tissue concentrations are listed for crayfish

Species	Mean dry weight concentration (standard deviation)									
	Cd (μg/gm)	Cr (μg/gm)	Cu (μg/gm)	Fe (μg/gm)	Mg (μg/gm)	Mn (μg/gm)	Pb (μg/gm)	Zn (μg/gm)	Ca (mg/gm)	K (mg/gm)
Caecodotea alabamensis (Isopoda)										
$n=2$[a]	2.4 (1.3)	–	115.7 (13.0)	4,434.3 (1,835.0)	564.8 (576.6)	147.0 (25.0)	24.5 (4.9)	23.6 (1.1)	88.0 (9.7)	–
Orconectes a. australis (Decapoda)										
Tissue										
Carapace $n=13$	0.5 (0.7)	1.9 (1.1)	66.6 (43.1)	94.4 (71.4)	44.9 (29.6)	288.2 (742.4)	5.7 (8.4)	35.4 (23.8)	179.6 (60.4)	5.1 (1.1)
Gill $n=13$	1.7 (1.4)	3.4 (4.0)	351.8 (254.3)	327.8 (608.9)	21.0 (22.8)	148.8 (332.2)	23.2 (29.3)	36.9 (39.7)	19.4 (15.4)	21.6 (20.7)
Gastrolith $n=3$	1.2 (1.0)	2.1 (0.6)	75.1 (65.0)	82.8 (52.1)	184.8 (48.8)	77.3 (26.6)	48.0 (42.4)	23.8 (21.9)	346.3 (55.8)	4.8 (0.5)
Green Gland $n=3$	1.8 (1.2)	1.4 (1.5)	254.2 (175.6)	76.4 (23.0)	230.0 (181.4)	96.5 (73.6)	28.0 (23.1)	30.0 (25.6)	17.7 (8.9)	9.4 (4.3)
Hepatopancreas $n=13$	3.6 (4.0)	0.9 (1.3)	584.9 (769.4)	68.1 (72.1)	21.0 (21.7)	235.8 (195.3)	8.3 (15.8)	106.6 (97.9)	7.5 (5.7)	12.1 (4.7)
Muscle $n=13$	0.4 (0.2)	2.7 (2.9)	71.9 (50.0)	34.9 (41.1)	7.9 (2.3)	12.6 (15.1)	1.2 (1.3)	91.3 (51.5)	4.5 (3.0)	17.3 (2.9)
Cambarus tenebrosus (Decapoda)										
Tissue										
Carapace $n=7$	0.1 (0.02)	0.5 (0.2)	38.6 (68.7)	61.1 (61.3)	76.7 (50.1)	121.4 (74.5)	0.6 (0.4)	27.0 (7.9)	217.6 (53.9)	2.1 (0.7)
Gill $n=7$	0.5 (0.3)	2.0 (2.8)	236.6 (126.2)	115.0 (71.0)	15.9 (8.4)	36.2 (34.1)	1.1 (1.3)	125.0 (44.8)	10.5 (10.1)	10.1 (3.8)
Gastrolith $n=4$	0.1 (0.03)	1.2 (0.5)	9.9 (6.7)	16.2 (18.3)	1,372.4 (1,195.3)	117.6 (150.4)	1.9 (1.2)	23.6 (22.1)	421.7 (251.7)	4.7 (3.4)
Green Gland $n=5$	0.3 (0.2)	0.4 (0.4)	84.5 (49.0)	69.1 (35.4)	39.8 (28.1)	86.2 (113.1)	5.1 (6.8)	62.4 (71.5)	7.2 (4.2)	8.2 (4.4)
Hepatopancreas $n=7$	2.4 (1.3)	0.5 (0.7)	188.4 (265.2)	64.3 (71.2)	5.5 (1.0)	182.8 (232.5)	0.1 (0.1)	309.9 (271.5)	3.8 (4.5)	7.8 (4.5)
Muscle $n=7$	0.1 (0.04)	3.1 (3.0)	37.3 (32.4)	7.4 (5.1)	7.3 (5.0)	4.7 (5.3)	0.5 (0.7)	127.4 (66.6)	2.6 (1.6)	13.4 (5.7)

[a] Each sample group consisted of six individuals

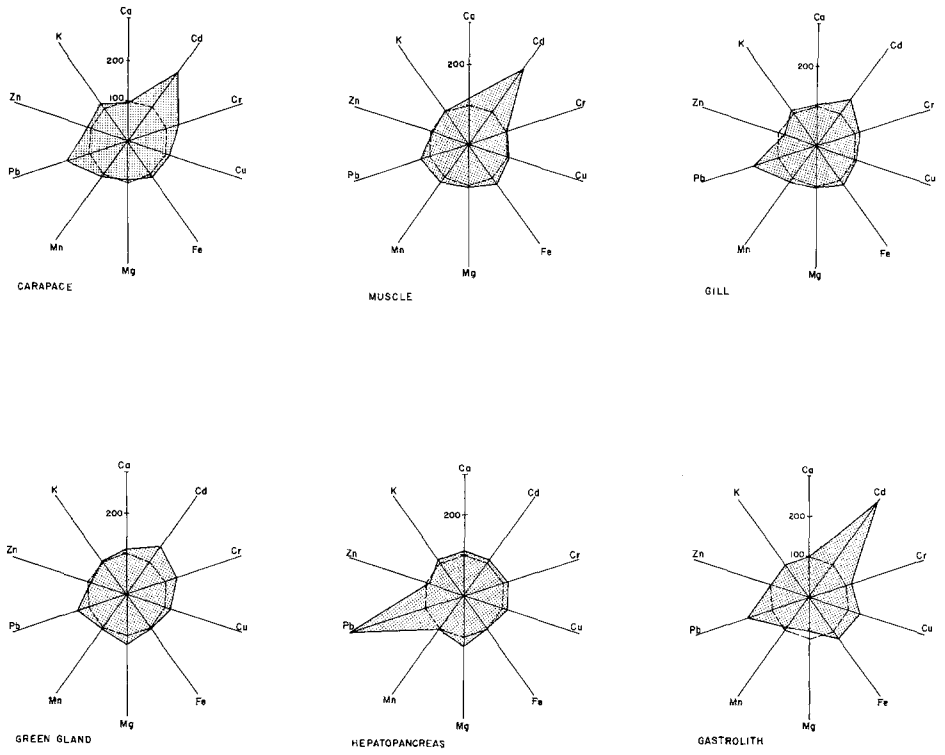

Fig. 1. Profiles of relative differences between metal concentrations in *O. a. australis* and *C. tenebrosus* tissues. The area enclosed by the broken line represents *C. tenebrosus* concentrations (100%). The point where the shaded area intersects axis denotes the percentage metal concentration in *O. a. australis* relative to *C. tenebrosus*

distilled in Teflon before use and NH_4OH was prepared by isothermal distillation in Teflon.

Data analyses were conducted with an IBM 360-195 computer using the Statistical Analysis System (Barr et al., 1976) and FORTRAN programs. Metal concentrations were log transformed before statistical analyses because of positive skewness (Giesy and Wiener, 1977). The tissue concentrations of metals in the two species of crayfish were examined by profile analysis with tests of significance made with Wilk's criterion (Morrison, 1967, p. 141).

Results

Ambient temperature, pH and dissolved oxygen of the stream waters at the time of sampling were 12° C, 7.7 and 9.3 mg/*l*, respectively. However, stream conditions are dependent on surface hydrologic factors and do vary slightly (Mathews et al., 1977). Trace metal concentrations in stream water (Table 1) were low and did not indicate point source contamination from mineral deposits or human activity.

In almost all instances, tissues of the troglobitic species *O. a. australis* contained higher metal concentrations than the troglophilic *C. tenebrosus* (Table 2, Fig. 1). Statistical comparison of tissue metal concentrations between the two crayfish species (Table 3) indicate the presence of significant differences in many of the pairings. Significantly greater metal concentrations in *C. tenebrosus* were only observed for Zn in gill and hepatopancreatic tissues. Greater concentrations of the potentially toxic metals Cd and Pb were measured in *O. a. australis* tissues (Table 2, Fig. 1). As one example (Fig. 1), *O. a. australis* carapace contained approximately 200% of the Cd and 150% of the Pb found in *C. tenebrosus* carapace. Significant differences (profile analysis, $P<0.05$) were found between tissue concentrations (carapace, gill, hepatopancreas and muscle) of Ca, Cd, Cr, Fe, Pb and Zn in the two species.

Table 3. Statistical comparison of metal concentrations[a] in tissues between *O. a. australis* and *C. tenebrosus*. Analyses conducted utilizing one way ANOVA

	Cd	Cr	Cu	Fe	Mg	Mn	Pb	Zn	Ca	K
Carapace	***	***	**	*	NS	NS	NS	NS	NS	***
Gill	***	NS	NS	NS	NS	NS	***	***	NS	**
Gastrolith	***	NS	**	*	NS	NS	***	NS	NS	NS
Green Gland	***	NS	NS	NS	NS	NS	*	NS	NS	NS
Hepato-pancreas	NS	NS	***	NS	**	NS	***	**	**	***
Muscle	***	NS	**	***	NS	**	**	NS	*	*

[a] Log transformed

* $P<0.10$; ** $P<0.05$; *** $P<0.01$

No major trends were observed in linear ratios between body size and tissue metal concentrations of either species. This could be due in part to the low variability in carapace length (cm) of the crayfish collected (*O. a. australis*, $\bar{X}=2.20$, SD=0.38, $N=13$; *C. tenebrosus*, $\bar{X}=3.86$; SD=0.39, $N=7$). Sexual differences in tissue metal concentrations were not examined statistically because of the small sample sizes and evidence from a previous crayfish study (L.A. Briese, unpublished data) indicating the lack of sexual influence in metal concentrations.

Discussion

Compared with other hard water systems (Kubota et al., 1974; McIntosh and Bishop, 1976; Atchison et al., 1977; Stenner, 1978), metal concentrations in Merrybranch Cave (Table 1) are representative of a relatively non-polluted aquatic habitat. Because of the rural location of this cave stream it is assumed that no significant introduction of metal pollutants has occurred prior to this study. Even under these conditions, *O. a. australis* tissues contain metal concentrations as high as those reported by Anderson and Brower (1978) in the crayfish *Orconectes virilis* collected from an industrially-polluted river.

The relative importance of metal uptake vectors (i.e., water or food) were not analyzed in this investigation. Studies involving aquatic invertebrates indicate that the uptake of non-essential metals such as Cd and Pb involves both food and water vectors (Enk and Mathis, 1977; Giesy et al., 1979). In a study of cave crayfish, Weingartner (1977) determined that over 80% of the ingested food of the troglobite *Orconectes inermis inermis* and the troglophile *Cambarus laevis* were isopods and amphipods. Based on Weingartner's observations, the species examined in this study are thought to obtain metals primarily through ingestion of the isopod *C. alabamensis* and exposure to water.

The contrasting physiological strategies of *O. a. australis* and *C. tenebrosus* are thought to represent the major source of species differences in tissue metal concentrations. Higher metal concentrations appear to be associated with the greater longevity of *O. a. australis*. The increased longevity of the troglobitic form, resulting from selection for lower metabolic rates and specific life history traits (K-selection), provides a longer period of exposure to metals than in the troglophilic species. Evidence from a long term study of *O. a. australis* in Shelta Cave, Alabama indicates that this species may attain life spans of over 100 years (Cooper, 1975). In contrast, troglophilic forms exhibit metabolic rates similar to surface crayfish (Weingartner, 1977), and would be expected to attain lifespans similar to crayfish in epigean populations. Although average ages of the two species examined in Merrybranch Cave could not be estimated, it was assumed that *O. a. australis* possesses a significantly longer lifespan than *C. tenebrosus* based on the previous investigations.

The essential metals (Cr, Cu, Fe, Mg, Mn, Zn, Ca and K) did not exhibit comprehensive species differences in tissue concentrations similar to those observed for Cd and Pb (Table 3, Fig. 1). This is probably due to the internal homeostatic control of essential metals through uptake and elimination processes (Liebsher and Smith, 1968). Distribution of some metals among tissues of the two species differed (Profile analysis; $P < 0.05$) and could result from temporal (i.e., molting cycle) or specific physiological differences.

In contrast to the essential metals, Cd and Pb concentrations were observed to be significantly greater in *O. a. australis* for almost all the tissues examined (Table 3, Fig. 1). Direct exposure to relatively high concentrations of Cd and Pb from point discharges are not the only danger posed to aquatic organisms by these toxic metals. The amount of Cd (Anon, 1975) and Pb (Patterson, 1965) is increasing in the environment on a global scale through the action of numerous non-point sources. Both of these non-essential metals tend to be bioaccumulated in various tissues and may be sequestered by protein binding (metallothioneins), rather than eliminated (Bryan, 1976; Brown, 1977). Fassett (1974) stated that Cd is accumulated continuously upon chronic exposure to this metal. The greater longevity of *O. a. australis* would explain the significantly higher concentration of Cd and Pb in tissues of this species.

The unique adaptations of troglobitic organisms may inhibit their ability to cope with chronic exposure to even low concentrations of toxic metals. Based on evidence of low metabolic rates in troglobitic crayfish, the biochemical expense of metallothionein production could divert energy from other important functions such as reproductive output. In addition, because of the increased longevity of these species, detoxification of metals by protein binding could decrease because of age-related factors in the formation of metallothioneins.

The long term accumulation of metals could potentially contribute to the deaths of aquatic troglobites, many of which are now considered to be of an endangered or threatened status. Another unique faunal group possessing long life spans (Turekian et al., 1975; Engemann, 1978), the abyssal invertebrates, could also face problems through long-term bioaccumulation of toxic metals. Future studies elucidating possible biochemical adaptations to metal burdens in troglobitic or abyssal organisms may allow a better understanding of this phenomenon.

Acknowledgments. This work was supported by Contract DE-AC09-76SR00819 between the University of Georgia and U.S. Department of Energy. Mr. A.D. Bosnak of Tennessee Technological University helped with sample collection and supplied much valuable information.

References

Anderson, R.V., Brower, J.E.: Patterns of trace metal accumulation in crayfish populations. Bull. Environ. Contam. Toxicol. **20**, 120–127 (1978)

Anon.: Cadmium in the environment: toxicity, economy, control. Environmental Directorate, Organization for Economic Co-operation and Development, 88 p. Paris 1975

Atchison, G.J., Murphy, B.R., Bishop, W.E., McIntosh, A.W., Mayes, R.A.: Trace metal contamination of bluegill *(Lepomis macrochirus)* from two Indiana lakes. Trans. Am. Fish. Soc. **106**, 637–640 (1977)

Barr, A.J., Goodnight, J.H., Sall, J.P., Helwig, J.T.: A Users Guide to SAS 76. Raleigh, N.C. SAS Institute, Inc. (1976)

Brown, B.E.: Uptake of copper and lead by a metal tolerant isopod *Asellus meridianus Rac.* Freshwater Biology **7**, 235–244 (1977)

Bryan, G.W.: Some aspects of heavy-metal tolerance in aquatic organisms. Soc. Expt. Biol. Seminar Series **2**, 7–34 (1976)

Burbanck, W.D., Edwards, J.P., Burbanck, M.P.: Toleration of lowered oxygen tension by cave and stream crayfish. Ecology **29**, 360–367 (1948)

Caine, E.A.: Comparative ecology of epigean and hypogean crayfish (Crustacea:Cambaridae) from northwestern Florida. Amer. Midl. Nat. **99**, 315–329 (1978)

Cooper, M.R.: Sensory specialization and allometric growth in cavernicolous crayfishes. Proc. 4th Internat. Congress Speleol, Yugoslavia (1965), 203–208 (1969)

Cooper, J.E.: Ecological and behavioral studies in Shelta Cave, Alabama, with emphasis on decapod crustaceans. Ph. D. thesis, University of Kentucky (1975)

Dickson, G.W., Franz, R.: Respiration rates, ATP turnover and adenylate energy charge in excised gills of surface and cave crayfish. Comp. Biochem. Physiol. A. (In press, 1979)

Eberly, W.R.: Competition and evolution in cave crayfishes of southern Indiana. Syst. Zool. **9**, 29–32 (1960)

Engemann, J.G.: Indirect evidence shows deep-sea benthos may reach extreme ages as individuals. Amer. Zool. **18**, 666 (1978)
Enk, M.D., Mathis, B.J.: Distribution of cadmium and lead in a stream ecosystem. Hydrobiologia **52**, 153–158 (1977)
Fassett, D.W.: Cadmium. In: Metallic Contaminants and Human Health. (D.H.K. Lee ed.), New York: Academic Press 1974
Franz, R.: Ecological strategies of closely-related surface and troglobitic Florida crayfishes. Bull. Ecol. Soc. Amer. **59**, 70 (1978)
Giesy, J.P., Jr., Wiener, J.C.: Frequency distributions of trace metal concentrations in five freshwater fishes. Trans. Am. Fish. Soc. **103**, 729–735 (1977)
Giesy, J.P., Bowling, J.W., Kania, H.J.: Cadmium and zinc accumulation and elimination by freshwater crayfish. Arch. Environ. Contam. Toxicol. (1979) (submitted)
Hobbs, H.H., III.: The population dynamics of cave crayfishes and their commensal ostracods from southern Indiana. Ph. D. thesis. Indiana Univ. (1973)
Jegla, T.C.: Studies of the eyestalk, metabolism, and molting and reproductive cycles in cave crayfish. Ph. D. thesis. Univ. of Illinois (1964)
Kubota, J., Mills, E.L., Oglesby, R.T.: Lead, Cd, Zn, Cu and Co in streams and lake waters of Cayuga Lake basin, New York. Environ. Sci. Technol. **8**, 243–248 (1974)
Liebscher, K., Smith, H.: Essential and non-essential trace elements. Arch. Environ. Health **17**, 881–890 (1968)
Mathews, R.C., Jr., Bosnak, A.D., Tennant, D.S., Morgan, E.L.: Mortality curves of blind cave crayfish *(Orconectes australis australis)* exposed to chlorinated stream water. Hydrobiologia **53**, 107–111 (1977)
Mellon, De F., Jr.: Retention of oculomotor reflexes in blind cave-dwelling crayfish. Brain Res. **134**, 191–196 (1977)
McIntosh, A., Bishop, W.: Distribution and effects of heavy metals in a contaminated lake. Purdue Univ. Water Resources Research Center, Technical Report No. 85 (1976)
Morrison, D.F.: Multivariate Statistics Methods. New York: McGraw-Hill Book Co. 1967
Patterson, C.C.: Contaminated and natural lead environments of man. Arch. Environ. Health **11**, 344–360 (1965)
Stenner, R.D.: The concentration of cadmium, copper, lead and zinc in sediments from some caves and associated surface streams on Mendip, Somerset. British Cave Res. Assoc. **5**, 113–120 (1978)
Thorp, J.H., Giesy, J.P., Wineriter, S.A.: Effects of chronic cadmium exposure on crayfish survival, growth, and tolerance to elevated temperatures. Arch. Environ. Contam. Toxicol. **8**, 449–456 (1979)
Turekian, K.K., Cochran, J.K., Kharkar, D.P., Cerrato, R.M., Vaisnys, J.R., Sanders, H.L., Grassle, J.F., Allen, J.A.: Slow growth rate of a deep-sea clam determined by ^{288}RA chronology. Proc. Nat. Acad. Sci. **72**, 2829–2832 (1975)
Weingartner, D.L.: Production and trophic ecology of two crayfish species cohabiting an Indiana cave. Ph. D. thesis. Michigan State Univ. (1977)
Wolfe, D.A., Cornwell, D.G.: Carotenoids of cavernicolous crayfish. Science **144**, 1467–1469 (1964)

Received August 6, 1979