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Effects of Organic Pollution on an Appalachian Cave: Changes in Macroinvertebrate Populations and Food Supplies

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ABSTRACT.—Density of troglobitic crustaceans and levels of potential food sources were compared in reference pools and pools polluted by septic system effluent in Banners Corner Cave, Virginia. Data from four physicochemical variables indicated slight to high pollution in five pools compared with two unpolluted pools. Polluted pools had high levels of conductivity, nutrients and fecal coliforms. Highly polluted pools also had decreased dissolved oxygen levels. Isopods (*Caecidotea recurvata*) were absent from highly polluted pools. Highest isopod density (up to $74.6/m^2$) occurred in slightly and moderately polluted pools. Amphipods (*Stygobromus mackini*) were absent from all polluted pools. Fungal biomass was negligible in all pools. Bacterial biomass accounted for a greater proportion of sediment total organic matter (TOM) in polluted pools, but there was little overall increase in TOM in polluted pools. Results of a laboratory growth experiment suggest that isopods can use bacteria from septic effluent as a food. However, presence of coarse particulate matter, not organic enrichment by septic system effluent, was the most likely cause of high isopod density in some pools. Septic system effluent may provide additional food to the aquatic community in Banners Corner Cave, but generally was damaging to the system.

INTRODUCTION

Karst groundwater communities are almost entirely dependent on energy sources from the surface. Although a few cave communities depend on chemoautotrophic production (Sarbu *et al.*, 1996), imported organic matter is the only source of energy that can be exploited by groundwater fauna in karst aquifers and caves (Ginet and Decou, 1977). Low food level appears to have been a factor in the evolution of cave organisms (Poulson, 1963; Culver, 1982) and in structuring cave communities by competition for food (Culver, 1982). However, food availability, importance of food type and trophic interactions in caves have received little attention (Culver, 1985).

Potential energy sources in caves include particulate organic matter (POM), dissolved organic carbon, bacteria and protozoans entering from surface water (Culver, 1985). Feeding ecology of the higher trophic levels in caves has been studied (Culver, 1973; Poulson, 1963), but trophic interactions of macroinvertebrates, primarily isopods and amphipods, are not well understood. Clayey mud, bacteria, fungi, POM and live prey have been implicated as potential foods for cave macroinvertebrates (Magniez, 1975; Kostalos and Seymour, 1976; Hobbs III, 1978; Dickson, 1979; Culver, 1985), but the amounts of each food type available and the specific diets of most species remain unknown. Size of POM also may be an important consideration in suitability of food resources (Culver, 1985). The contribution of biofilms, especially fungi, to detritivore nutrition has been demonstrated in surface stream communities (Bärlocher and Kendrick, 1981), but not in cave systems.

Organic pollution may damage groundwater communities by disrupting trophic interactions or by direct toxicity (Notenboom *et al.*, 1994). Septic systems, in particular, are common sources of groundwater pollution in karst. This is due to prevalence and poor performance of septic systems in thin-soiled karst areas. Groundwater pollutants from septic system effluent may include high levels of nutrients and organic material, human pathogens and toxic materials from household waste.

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This study was designed to examine three factors: (1) the availability of food (total organic matter (TOM), bacterial biomass and fungal biomass) in sediments of seep-fed cave pools; (2) the ability of the troglobitic isopod *Caecidotea recurvata* to use various foods and; (3) the effects of organic pollution on food availability and populations of *C. recurvata* and *Stygobromus mackini* (a troglobitic amphipod).

SITE DESCRIPTION

Banners Corner Cave is located in the Clinch River basin in Russell County, Virginia. Holsinger (1966) first examined the effects of organic pollution by septic systems on the aquatic community in the cave. Organic material from the septic systems may have supplemented food supplies causing high density of *Caecidotea recurvata* and the planarian *Phagocata gracilis* in cave pools. Several houses and a school overlying the cave are the suspected sources of groundwater pollution. Mill Creek sinks approximately 45 m in front of the cave entrance in a sinkhole. Water from the creek reappears in the cave 175 m from the entrance and then exits the cave through submerged conduits at a siphon. The water apparently resurges on the surface 1.5 km to the NE at Big Spring. During heavy flooding the creek overflows into the cave entrance. This occurred once during this study in February 1994.

Seven seep-fed pools on three distinct vertical levels in the cave were used in this study. The pools are relatively shallow (4–24 cm deep) and range in surface area from 0.5 to 6.7 m^2 (Simon, 1994). They include all pools previously examined by Holsinger (1966) and were tentatively designated as reference or polluted pools based on preliminary water quality tests. Two reference pools (R1 and R2), not affected by septic system effluent, and five polluted pools (P1, P2, P3, P4 and P5) receiving septic system effluent were studied. The position of the pools relative to the sources of water and septic system effluent determined the water quality of each pool (Fig. 1).

Pools P1 and R1 lie in a passage separate from the other pools. These pools receive water from seepage along the East passage wall and drip from the ceiling. Pool P1 is a fairly large rock-bottomed pool with no sediment. Sediment in Pool R1 is sandy mud.

Pools R2, P2, P3, P4 and P5 lie together on two connected levels and receive water from the W side of the cave. Sediment in these pools is clayey mud. Water enters Pool R2 by seepage along the SW passage wall and overflows into Pool P2. Water from Pool P2 flows down a 2-m flowstone wall into Pool P3. Water enters Pool P4 along the floor at the base of the NW passage wall. Overflow from this pool travels ca. 3 m along the floor to Pool P5. Pool P5 experienced the greatest fluctuations in water level and was dry or reduced by approximately one-third during sampling in September and October 1992. The water level of Pool P5 was slightly reduced during summer months of the following year, but the pool was never totally dry.

An effluent was observed entering the cave on seven different occasions. The effluent, which smelled strongly of sewage, flowed down a flowstone wall next to Pool P2 (Fig. 1) for approximately 20 min. Some of the effluent entered Pool P2, but most was diverted into Pool P3. Pools P4 and P5 apparently are influenced by a smaller, but more constant source of septic effluent. The frequency at which the effluent enters the cave and the number and size of the septic systems contributing to the effluent are not known.

After flooding in February 1994, Pools P1, P4 and P5 contained large amounts of organic debris (leaves, grass and other detritus) that had washed into the cave. Pool P5 also contained a large piece of wood at one end. The only other food sources evident in the pools were bodies of crickets, millipeds and other terrestrial animals that occasionally fell into the pools.

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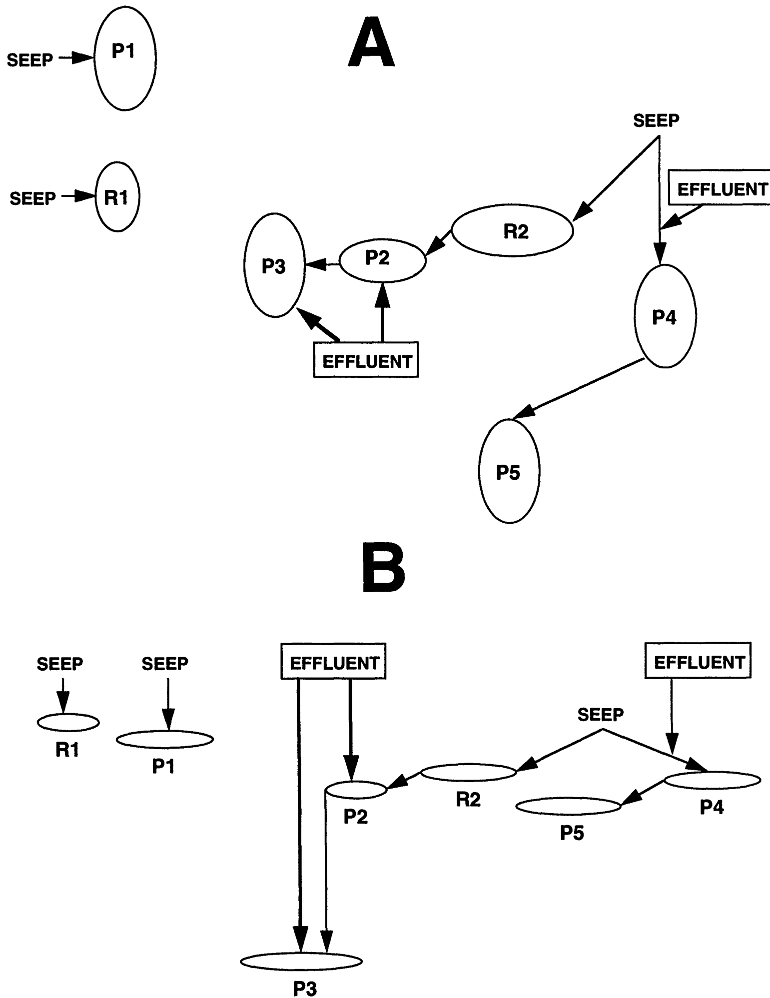

FIG. 1.—Plan view (A) and elevational view (B) of water flow routes between pools in Banners Corner Cave (P = polluted pools, R = reference pools). Distances are not to scale

METHODS

Water chemistry.—Water samples were collected from all pools approximately monthly from August 1992 to February 1994 (14 mo total). Dissolved oxygen (DO) was measured in the field with a YSI Model 54A oxygen meter. Conductivity, pH, alkalinity, hardness and levels of chloride, nitrate-nitrogen and sulfate were measured from water samples returned to the laboratory. Water samples were collected in bottles washed with Alconox, rinsed 10 times with tap H_2O, 10 times with distilled H_2O and finally 5 times with deionized H_2O to remove any traces of nutrients. Water samples were placed on ice and processed within 24 h after collection. Conductivity and pH were measured with a YSI model 32 conductivity meter and an Orion model 399A pH meter, respectively. Alkalinity and hardness were determined according to Standard Methods (APHA, 1985). Chloride, nitrate-nitrogen and

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sulfate levels were measured with a Dionex Ion Chromatograph. The effluent was collected on four dates and examined for conductivity and levels of chloride and nitrate-nitrogen. Differences in mean levels of each parameter between pools were tested with a one-way ANOVA and Tukey's multiple comparison procedure (SAS, 1991).

Fecal coliform levels were determined in the laboratory by the multiple tube fermentation technique (APHA, 1985). Samples were collected on seven dates in 500-ml nalgene bottles that were washed as previously mentioned and autoclaved. Most probable numbers (MPN's) of fecal coliforms and 95% confidence intervals were calculated from series of three dilutions with five replicates for each dilution. Mean MPNs were compared with a Kruskal-Wallis test with multiple comparisons (Hollander and Wolfe, 1973).

Food resource measurements.—Sediment samples were collected from Banners Corner Cave during May 1994. Each pool was divided into grids of numbered squares roughly 0.25 m $\times$ 0.25 m for use in food resource measurements and mark-recapture trials. Glass marbles were placed at the corners of each square in the grids for reference points. Three 25-cm^3 sediment samples were collected randomly from the top 5 cm of the substrate in each square of the grids and then mixed. After mixing, two 1-cm^3 subsamples were preserved: one in 5 ml methanol for fungal biomass estimates and the other in 5 ml 5% buffered formalin for bacterial biomass estimates. The subsamples were kept at 4 C until processing. The remaining sediment was frozen for determination of sediment TOM.

Ash-free dry mass (AFDM) was used to estimate sediment TOM. The frozen sediment was thawed, ground, dried at 60 C for 48 h and stored in a desiccator. Weight before and after combustion at 550 C for 60 min was determined. TOM was transformed by arcsin of the square root and differences in mean levels between pools was tested with a one-way ANOVA and Tukey's multiple comparison procedure (SAS, 1991).

Bacterial biomass was measured using the acridine orange direct count method (Hobbie *et al.*, 1977). Preserved sediment was homogenized and diluted with filter-sterilized H_2O to 1 ml sediment/1 liter final concentration. Four ml of the diluted sediment were mixed with 0.4 ml 0.01% acridine orange and filtered onto a 0.2 μm black polycarbonate membrane filter. The filters were mounted and examined by epifluorescence microscopy. Counts of rods and cocci were made in 15 randomly selected fields of each filter. Photographs from one sample at each pool were enlarged and rods and cocci were digitized to determine average cell volume at each pool (Bratbak, 1985). Bacterial density and mean cell volume were converted to biomass using a conversion factor of 2.2×10^{-13} g of C/μm^3 (Bratbak, 1985). Contribution of bacterial biomass to sediment organic matter was determined as percentage of TOM accounted for by bacterial carbon. Differences in mean bacterial biomass and percent contribution to total TOM between pools were tested using a one-way ANOVA and Tukey's multiple comparison procedure (SAS, 1991).

Fungal biomass was determined by extraction of ergosterol from sediments (Newell *et al.*, 1988). Sediment was refluxed for 30 min in 5 ml of saponification solution (4% KOH in 95% EtOH). The sample was cooled, filtered and mixed with 10 ml of distilled H_2O and 10 ml of pentane. The pentane fraction was collected and the saponification solution was treated two more times each with 5 ml of pentane. The pentane was allowed to evaporate overnight and the film containing the ergosterol was redissolved in 4 ml of methanol. Ergosterol was measured with a Waters HPLC fitted with a reverse phase column.

Laboratory growth experiment.—Growth rates of *Caecidotea recurvata* fed CPOM, FPOM and bacteria were determined in the laboratory. *Caecidotea recurvata* collected from Banners Corner Cave were kept in a darkened environmental chamber at 13 $\pm$ 1 C (ambient cave temperature) until use. During the experiment, isopods were kept in 8 cm $\times$ 10 cm $\times$ 10 cm plexiglass boxes at 13 $\pm$ 1 C. Each box contained one isopod, 10 g of homogenized

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sediment from Pool R1 and 250 ml of filtered (0.45 μm) water from Big Spring. Because young *C. recurvata* are difficult to culture and addition of sediment increases their survival (possibly by aiding in removal of molts), low organic sediment was provided in each treatment. Pool R1 was chosen as a sediment source because it is a reference pool with sediment of low organic content.

Maple leaves, which are commonly washed into Appalachian caves, were conditioned for 5 wk in the laboratory and used for CPOM and FPOM. Leaves were collected after abscission and stored for less than 6 mo before use. Dried leaves were placed in filtered (0.45 μm) water from Big Spring with a leaf pack collected from Mill Creek and aerated for conditioning. CPOM treatments consisted of addition of three leaf disks, 1.6 cm in diam, to each box. For FPOM, leaves were ground and filtered through a 1 mm^2 mesh sieve. Approximately 10 g of leaf material was added to each of the FPOM treatment boxes. *Escherichia coli* (ATCC #9637) was used for bacterial treatments because it is easily cultured and was present in polluted pools in high numbers. *Escherichia coli* was cultured in T-soy broth and centrifuged. The supernatant was drained and the pellet of bacteria was resuspended in distilled H_2O and centrifuged. The resulting pellet was mixed with a small amount of distilled water and approximately 10 g of bacteria was mixed with the sediment in the bacteria treatment boxes. Control boxes contained sediment and water only. Food sources were replenished three times during the experiment to maintain surplus food supplies. Filtered water was added when necessary to compensate for evaporation. Loss of water to evaporation exceeded 25% of the total volume in only two of the bacterial treatment boxes which were not used in calculations.

Fifteen isopods were randomly assigned to each of the three treatments and the control. Initial lengths, measured from the anterior margin of the cephalothorax to the posterior margin of the abdomen, ranged from 3.0 mm–6.0 mm and were not significantly different between treatments (ANOVA, $P > 0.86$). Some mortality occurred in each treatment leaving 11, 10, 7 and 12 individuals in the CPOM, FPOM, bacteria and control treatments, respectively. Final length was measured after 90 days and daily growth rates were compared with a Kruskal-Wallis test with multiple comparisons (Hollander and Wolfe, 1973).

Isopod and amphipod density.—Mark-recapture trials were conducted quarterly (May 1993, August 1993, November 1993 and February 1994) to determine seasonal isopod density in each pool. Isopods were marked with two stains mixed in water from Big Spring. Animals were stained for 1 h with a 10 ppm solution of methylene blue or a 15 ppm solution of neutral red. The stains marked isopods reliably (up to 10 h) with no observed impairment in preliminary laboratory and field tests. These stains also have been used with other crustaceans (Smith and Present, 1983; Howard, 1985). In each mark-recapture trial, three capture periods were used with Bailey's (1951, 1952) triple catch method to estimate population sizes. Population sizes and standard errors were calculated as

$$N_1 = \frac{M_1(n_1 + 1)m_{02}}{(m_{01} + 1)(m_{12} + 1)}$$

$$S.E. = \sqrt{N_1^2 - \frac{M_1^2(n_1 + 1)(n_1 + 2)(m_{02} - 1)m_{02}}{(m_{01} + 1)(m_{02} + 2)(m_{12} + 1)(m_{12} + 2)}}$$

(where M = # of animals marked, n = # of animals examined for marks, m = # of marked individuals recaptured and subscripts indicate time 0, 1 or 2). All isopods were removed from a grid square until no isopods could be found within 3 min. Squares were examined sequentially until the entire grid had been sampled. The captured isopods were counted, marked with methylene blue and released into their respective squares. The process was

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TABLE 1.—DO, conductivity and fecal coliform levels for 14 mo (mean, standard deviation and coefficient of variation). Fecal coliform levels are mean of 7 dates. Values for the effluent (EFF) are from 4 samples

	DO (mg/l)			Conductivity (μmhos)			Fecal coliforms (#/100 ml)		
Pool	Mean	SD	C.V.	Mean	SD	C.V.	Mean	SD	C.V.
R1	10.3	1.1	10	258	79	31	13	28	209
R2	9.9	1.2	12	332	60	18	10	18	187
P1	10.1	1.2	12	511†	164	32	759	707	93
P2	7.2†	2.4	34	439†	154	35	25,896†	59,399	229
P3	6.4†	3.5	55	388	144	37	4195†	6020	144
P4	5.9†	2.9	50	392	105	27	656	1051	160
P5	8.3	1.8	22	364	97	27	670	737	110
EFF	nd	nd	nd	793	189	25	nd	nd	nd

† Significantly different from reference pools
nd = no data available

repeated a second time 2 h after the first release. Unmarked isopods were captured, marked with neutral red and released. Marked isopods from the first capture were counted but not collected. Two h after the second release, all marked isopods from captures 1 and 2 and all unmarked isopods were counted.

Population size estimates and standard errors were divided by the pool's total area (digitized from photographs) to estimate isopod density. In some cases, capture rate was too low for Bailey's formula to provide a population size estimate. In these cases, total number of unmarked isopods captured during the trials was used to calculate density. Presence of gravid females (indicated by a marsupium) was noted during each mark-recapture trial.

Photographs were taken of all isopods captured during the first capture period of each mark-recapture trial. The photographs were digitized by hand on a Jandel digitizer tablet to determine size-class distributions of isopods in each pool. Total length, from the anterior margin of the cephalothorax to the posterior margin of the abdomen, was measured and recorded. Seasonal and annual mean length were compared by pool using a one-way ANOVA with Tukey's multiple comparison procedure (SAS, 1991). Annual mean length was determined from combined data of all seasons.

Amphipod density was determined from captures because staining proved unreliable in laboratory and field tests. Amphipods were removed with isopods during the first capture period. Immediately before the isopods were released after the first staining, the grids again were sampled for amphipods. Total number of amphipods collected in the two captures was divided by pool area to determine amphipod density. Presence of gravid females was noted during the trials.

RESULTS

Water chemistry.—Polluted pools, in general, had lower mean levels of DO and increased conductivity compared to reference pools (Table 1). Mean DO levels in Pools P2, P3 and P4 were significantly lower than reference pools and Pool P1 ($P < 0.05$). Pool P1 had significantly higher mean conductivity than both reference pools, and Pool P2 had significantly higher conductivity than Pool R1 ($P < 0.05$). Conductivity in the effluent was significantly higher than all pools ($P < 0.05$). Variation in DO and conductivity also was greater in polluted pools. DO values below 2 mg/liter were common in Pools P3 and P4.

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TABLE 2.—Cl^- and NO_3^--N levels for 14 mo (mean, standard deviation and coefficient of variation)

	Cl^- (mg/l)			NO_3^--N (mg/l)		
Pool	Mean	SD	C.V.	Mean	SD	C.V.
R1	1.3	0.5	37	1.7	0.9	53
R2	1.5	0.6	38	2.3	0.6	24
P1	27.6†	25.7	93	2.1	1.1	53
P2	13.2	18.3	139	3.6	1.8	51
P3	7.5	7.7	103	3.3	1.4	42
P4	16.4	10.7	65	9.7†	6.3	66
P5	12.7	9.8	78	8.2†	5.8	71
EFF	44.8†	39.6	88	7.8	5.6	71

† Significantly different from reference pools

Levels of chloride and nitrate-nitrogen were higher and more variable in polluted pools (Table 2). Pools P4 and P5 had significantly higher mean levels of nitrate-nitrogen than all other pools ($P < 0.05$). Pool P1 had a significantly higher mean level of chloride than both reference pools and Pool P3 ($P < 0.05$). Maximum levels of chloride and nitrate-nitrogen in the effluent were 96.2 and 15.0 mg/liter, respectively. Mean level of chloride in the effluent was significantly greater than all other pools except P1 ($P < 0.05$). Although pool P1 had some indication of septic system pollution, it never experienced heavy organic loading or low DO levels. Alkalinity, hardness and pH of all pools were similar and were not useful indicators of pollution. Pools P2, P3 and P5 had consistently poor water quality (especially low DO and high nitrate-nitrogen) and were considered highly polluted. The other polluted pools had better water quality and were considered moderately polluted.

Mean levels of fecal coliforms were 1–3 orders of magnitude higher in polluted pools than in reference pools (Table 1). Mean MPNs for Pools P2 and P3 were significantly higher than reference pools ($Z\alpha = 3.0$, $Z_{R1/P2} = 3.8$, $Z_{R1/P3} = 3.6$, $Z_{R2/P2} = 3.9$, $Z_{R2/P3} = 3.7$). One sample collected from Pool P2 immediately after the effluent entered the cave had an MPN of $\geq$160,000 coliforms/100 ml.

Food resource measurements.—Organic matter in sediments was low in all pools; 19.1–86.9 μg AFDM/g sediment (Table 3), but was present in greater amounts in polluted pools. TOM in Pool R1 was significantly lower ($P < 0.05$) than all other pools. TOM in Pool R2 was significantly lower ($P < 0.05$) than Pool P4 only.

Bacterial biomass was significantly lower in reference pools than in polluted pools (P <

TABLE 3.—Total organic matter (TOM), bacterial biomass and contribution of bacterial biomass to TOM in sediments (May samples). Standard deviations are in parentheses. Means with the same letter are not significantly different ($P < 0.05$)

Site	n	Total organic matter (μg AFDM/g sed.)	Bacterial biomass (μg C/g sed.)	% bacterial biomass
R1	6	19.1 (5.4) C	0.06 (0.05) C	0.4 (0.3) D
R2	23	67.3 (19.8) B	0.20 (0.05) C	0.3 (0.2) D
P2	14	72.0 (12.0) AB	0.74 (0.15) B	1.1 (0.3) B
P3	15	82.4 (17.8) AB	1.60 (0.54) A	1.9 (0.5) A
P4	23	86.9 (14.0) A	0.64 (0.26) B	0.7 (0.2) C
P5	21	74.0 (21.5) AB	0.54 (0.19) B	0.8 (0.3) BC

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TABLE 4.—Isopod density (#/m²) from mark–recapture trials. One standard error in parentheses

Pool	Spring May 1993	Summer August 1993	Fall November 1993	Winter February 1994
R1	0.0	0.0	2.1[a]	0.0
R2	3.0 (1.9)	2.1[a]	0.5 (0.4)[b]	0.0
P1	27.5 (10.8)	7.3 (4.7)	74.6 (32.8)	26.7 (13.4)
P2	1.0[a]	1.0[a]	1.0[a]	1.0[ab]
P3	0.0	0.0	0.0	0.0
P4	18.2 (6.8)	1.0 (1.0)	0.0	0.0
P5	43.6 (11.7)	5.2 (2.7)	5.9 (2.0)[b]	7.0 (3.4)[b]

[a] Density calculated from total isopods present
[b] Gravid females were present

0.05) (Table 3). Pool P3 had significantly higher bacterial biomass than all other pools ($P < 0.05$). Bacterial biomass and TOM were not highly correlated for all pools combined ($r = 0.48$). The contribution of bacterial biomass to sediment TOM was significantly higher in pools polluted by septic effluent ($P < 0.05$) (Table 3). Fungal biomass was negligible in all pools tested. No ergosterol was detectable in sediments from any pools.

Laboratory growth experiment.—All food treatments led to higher growth rates than the control. Isopod growth rates for FPOM and CPOM were significantly greater than the control ($Z\alpha = 2.5$, $Z_{control/FPOM} = 4.2$, $Z_{control/CPOM} = 2.9$). There were no significant differences among food treatments. Highest growth rates were obtained from isopods fed FPOM (45 μm/day, SD = 12), followed by CPOM (36 μm/day, SD = 16), bacteria (33 μm/day, SD = 14) and control (14 μm/day, SD = 5). Leaf discs were usually skeletonized within 3 wk.

Isopod and amphipod density.—Isopod density generally was highest in the spring, autumn and winter (Table 4). Reference pools had low isopod density relative to other pools. Isopods were present in Pool R1 only in Fall 1993. Pool R2 contained low density throughout the year, but isopods were absent in the winter trials. Isopod density was variable in polluted pools. Isopods were absent from heavily polluted pools (P3 and P4) and occurred at high density in Pools P1 and P5 (Table 4).

Isopod density dropped sharply in pools P4 and P5 between spring and summer samples. In months between spring and summer mark-recapture trials, DO in Pool P4 ranged from 2.1–3.3 mg/liter. Fifty-six dead isopods (including 10 gravid females) were found in Pool P4 in July. DO in Pool P5 also was low (4.9–6.5 mg/liter) during these months. After summer sampling, no isopods were found in Pool P4 and density in Pool P5 increased slightly. Percentage of captures on CPOM (wood) in Pool P5 ranged from 21–32% of total isopods captured in that pool. An additional 23–36% were captured within 0.5 m of the CPOM. Isopod density was not highly correlated with sediment TOM or bacterial biomass ($r = 0.32$ and -0.10, respectively). Gravid females were present in some reference and polluted pools in June, November and February (Table 4).

Mean seasonal isopod length was greater in Pools R2 and P1 in every season, but was significantly different only in spring and winter ($P < 0.0003$ and $P < 0.03$, respectively) (Table 5). Mean annual size also was greater in Pools P1 and R2 and significantly greater than Pool P4 ($P < 0.0001$). Annual size classes in Pools P1 and R2 had similar distributions with the majority of isopods in the 8- and 9-mm size classes (Fig. 2). Size classes in Pools P4 and P5 included a wide range of values and were skewed towards the smaller size classes (Fig. 2).

Amphipods were present in Pools P1, P2 and R2. Density was slightly higher in spring,

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TABLE 5.—Mean isopod lengths (mm) by season. One standard deviation in parentheses. Means with the same letter are not significantly different

Pool	Spring May 1993	Summer August 1993	Fall Nov. 1993	Winter Feb. 1994	Annual
R2	8.5 A	7.5 A	8.6 A		8.1 A
	(1.0)	(1.0)	(0.3)		(1.0)
P1	7.7 AB	7.3 A	8.8 A	8.4 A	8.1 A
	(1.1)	(1.1)	(0.9)	(0.9)	(1.2)
P4	6.5 B	7.3 A	8.1 A	7.2 B	7.2 AB
	(1.6)	(1.8)	(1.7)	(1.8)	(1.8)
P5	6.5 B	6.8 A			6.4 B
	(2.2)	(1.8)			(2.1)

autumn and winter in P1 and P2, but not in Pool R2 (Table 6). Lower autumn and winter density in Pool R2 may be attributable to the movement of amphipods into Pool P2. We observed amphipods moving between Pools P2 and R2 in autumn and winter when physicochemical and nutrient values indicated Pool P2 had not been polluted by the effluent recently. Gravid females were present in November and February (Table 6).

DISCUSSION

Water chemistry and food resources.—Polluted pools all had typical indicators of septic system contamination: low DO and high levels of chloride, nitrate-nitrogen, conductivity and fecal coliforms (Tables 1, 2). High nitrate-nitrogen levels, in particular, have been found in karst areas affected by septic systems (Wells and Krothe, 1989; Green *et al.*, 1990; Scanlon, 1990).

Effluent observed entering the cave contained a large amount of flocculent organic material. The added organic material and bacteria (evident in high coliform levels) from the septic systems increased both TOM and bacterial biomass in polluted pools (Table 3). Although bacterial biomass generally increases with sediment organic content (Findlay *et al.*, 1986a; Bott and Kaplan, 1985; Meyer, 1988), sediment organic content and bacterial biomass were not highly correlated in Banners Corner Cave. This low correlation showed differences in sediment organic matter alone were not responsible for differences in bacterial biomass between pools.

Fungi have been considered important in determining distribution of aquatic troglobites (Dickson, 1975, 1979; Dickson and Kirk, 1976). Seep-fed pools in Banners Corner Cave contained no detectable fungal biomass. Clayey mud may lack sufficient organic matter to support growth of fungal hyphae. Fungi do not appear to be major food sources in cave-pool sediments. However, fungi probably are important food sources in the presence of POM which provides a suitable substrate for fungi.

Macroinvertebrate responses to food supplies.—In some pools, food levels affected isopod density (Table 4). Absence of isopods in pool R1 and high isopod density in some polluted pools (P1, P4 and P5) can be explained by differences in food availability (Table 3). Food levels in sediments of reference and polluted pools were low (<9% TOM). However, pool R1 contained sandy mud almost entirely of mineral origin with very low organic content (<2% TOM). The other pools contained clayey mud which had higher organic content. Differences in amount of organic matter in these sediments may be due to greater amounts of organic material entering these pools in seep and flood waters. Low isopod growth rates on R1 sediments also show sandy mud is not suitable for maintaining cave macroinverte-

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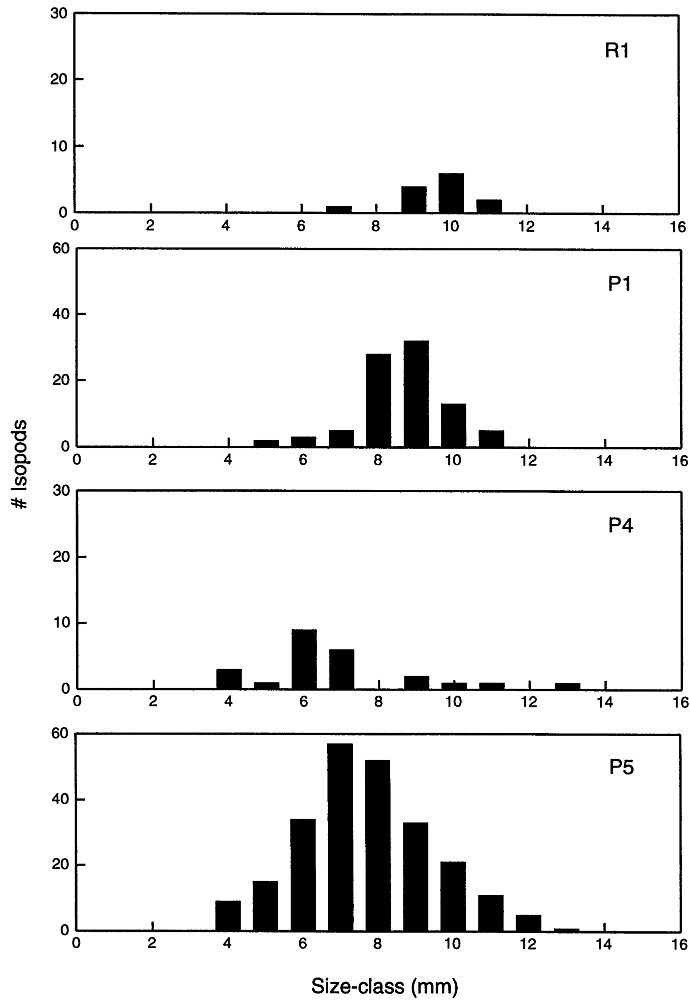

FIG. 2.—Isopod size-class distributions for all seasons combined

TABLE 6.—Seasonal amphipod density (#/m²)

Pool	Spring May 1993	Summer August 1993	Fall November 1993	Winter February 1994
R2	5.6	7.3	4.5[a]	6.3[a]
P1	1.5	0.8	3.1	1.5
P2	0.0	0.0	18.0	22.0

[a] Gravid females were present

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brates. Absence of isopods due to pollution in Pool R1 was unlikely; this pool had no physicochemical or bacteriological indication of pollution.

Presence of CPOM is the likely cause of high isopod density in pools P1 and P5. Only these two pools contained CPOM. Pool P1, which was slightly polluted and had no sediment, had the highest isopod density (Table 4). Differences in TOM and bacterial biomass were not large between Pools R2 and P5 (Table 3); however, these pools had very different isopod density (Table 4). Highest isopod density in Holsinger's (1966) study (60.9/m^2) occurred in a clean pool with a large amount of CPOM present. Dickson and Kirk (1976) found high density (200/m^2 and 300/m^2) of *Caecidotea recurvata* in shallow pools in Spangler Cave, Lee Co., Virginia, that had organic detritus present. Distribution of other aquatic troglobites has been correlated with POM (Dickson, 1975, 1979; Poulson, 1992). The majority of isopods captured in Pool P5 (52%–68%) during this study was on or within 0.5 m of the wood present in the pool. This is probably a combination of thigmotactic behavior and use of the wood and the associated biofilm as a food source.

POM is a high-quality food source, as shown by higher isopod growth rates with POM than with sediment alone. Leaf material and microbial films both probably contributed to isopod nutrition. Growth rates observed in this study are close to that obtained by Magniez (1981). In his study, *Caecidotea recurvata* grew roughly 31 μm/day when kept at 11–12 C with boiled elm (*Ulmus campestris*) leaves and clayey mud. This value falls between the control and treatment growth rates in our experiment. The higher growth rates in our growth experiment may be due to differences in temperature and leaf conditioning. Nutritional importance of POM and associated microbial films have been demonstrated for other crustaceans (Kostalos and Seymour, 1976; Bärlocher and Kendrick, 1981). FPOM may be easier for smaller isopods to handle and provide more surface area for microbial colonization; however, the increase in palatability appears minimal. Findlay *et al.* (1984, 1986b) demonstrated that fungal and bacterial biomass on POM was insufficient to meet the carbon needs of an epigean isopod. POM alone may fulfill a significant portion of *C. recurvata*'s energy needs. Leaves washed into Banners Corner Cave after flooding usually had several isopods feeding on them and the leaves were skeletonized within a few weeks. Presence of large amounts of POM could provide food capable of sustaining high density of isopods. Aquatic macroinvertebrates in caves typically can use a wide range of food sources (Ginet, 1960; Hobbs, 1973; Magniez, 1975; Culver, 1985). As with other troglobites, *C. recurvata*'s diet may be very broad. We have observed *C. recurvata* ingesting clayey mud, leaves, wood and bodies of crickets and millipeds. Magniez (1981) also reports *C. recurvata* skeletonizing leaves and feeding on wood. FPOM, CPOM and bacteria-enriched sediment resulted in nearly equal growth rates. Even isopods fed only organically poor sandy mud sediments alone exhibited some growth.

Increased bacterial biomass resulting from bacterial and carbon inputs from the septic systems may have increased isopod density in pool P4 during spring (Table 4). In spring, Pool P4 had high isopod density and higher bacterial biomass and TOM than reference pools. No other differences in food sources or habitat were evident to explain the distribution of isopods. If bacterial turnover rates are high and isopods can use bacteria as a food source, bacterial production could provide a significant energy source for isopods. Bacteria were considered a potential food source for *Caecidotea recurvata* by Holsinger (1966). In this study, growth rates of isopods fed *E. coli* show bacteria can sustain isopod growth almost as well as conditioned POM, at least for short-term periods. Systems such as Banners Corner Cave that periodically receive septic system effluent would have *Escherichia coli* and other nonnative bacteria present as food sources for macroinvertebrates. *Escherichia coli* are not native to groundwater systems, but it is likely isopods also can use indigenous

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bacteria as a food supply. These bacteria could be stimulated by organic carbon from organic pollution, providing additional food for macroinvertebrates. Although organic pollution may increase bacterial biomass, the combination of organic enrichment with tolerable changes in water quality did not occur often in this study. Consequently, the effects of increased food supply were negated by the disrupting effects of the effluent in most polluted pools.

Macroinvertebrate responses to pollution.—Isopod density in reference pools during this study was close to that of Holsinger's (1966) reference site (Chadwell's Cave, Tennessee: $6.0/m^2$). In polluted pools, septic system effluent reduced isopod density. Isopods and amphipods were absent in highly polluted pools (P2 and P3) and isopod density declined sharply in Pools P4 and P5 immediately after several episodes of very poor water quality (Table 5, 6). Reduction of isopod density in polluted pools with low DO levels (P3, P4 and P5) and presence of isopods in a polluted pool with high DO levels (P1) suggest reduction of DO was the major problem caused by septic system pollution. Damaging effects of organic pollution on groundwater fauna is not uncommon. For example, organic pollution of the Cedars karst system in Lee County, Virginia, caused the extirpation of troglobitic isopods (*Caecidotea recurvata* and *Lirceus usdagalun*) and amphipods (*Crangonyx antenatus*) (Culver *et al.*, 1992).

The effects of septic system pollution on isopod growth and reproduction are not clear. The trend of smaller isopods in polluted pools (Fig. 1) may be due to inhibition of growth caused by the effluent. Or, greater input of FPOM and bacteria to polluted pools may have provided additional food usable by smaller size classes. This additional food source may allow higher density of smaller isopods to coexist with larger isopods. Presence of gravid females in reference and moderately polluted pools (Table 4) showed moderate levels of pollution by septic effluent may not greatly affect reproduction. Timing of reproduction in Banners Corner Cave is consistent with other studies. In laboratory cultures, Magniez (1981) found that *Caecidotea recurvata* released eggs into the marsupium in March, July, September, October and November. Isopod young were released 70–80 days later. Isopod young would have been released in Banners Corner Cave roughly from September through April according to our data on presence of gravid females and a 70–80 day intramarsupial development period. Release would coincide with periods of highest levels of water flow and organic input to the cave. Because it was not possible to tell the exact stage of development of the young in the marsupium in the cave, reproductive timing estimates are inaccurate.

Stygobromus mackini may be very sensitive to septic system pollution. Amphipods were absent from all polluted pools, except Pool P2 when it had not been polluted recently. Absence of amphipods in polluted pools may reflect pollution of the epikarst (the area of fractured rock at the soil/bedrock interface). Stygobromid amphipods are strongly tied to the epikarst which may be their primary habitat (Fong and Culver, 1994). Because epikarst habitat lies closer to surface pollution sources it may be at greater risk to pollution. This habitat needs to be sampled directly to ascertain the effects of groundwater pollution on this component of karst groundwater ecosystems.

Caecidotea recurvata appears less susceptible to groundwater pollution than *Stygobromus mackini,* Isopods in general are pollution-tolerant (Aston and Milner, 1980; Pennak, 1989). *Caecidotea* sp. have been seen in other organically polluted caves (Horse Cave, Ky., Holsinger, pers. comm.) and *C. recurvata* was the first aquatic troglobite to return to the Cedars after organic pollution (Culver *et al.*, 1992). Differences in pollution tolerance between *C. recurvata* and *S. mackini* may be useful in biomonitoring for groundwater pollution. In this study, isopod and amphipod distributions were useful indicators of pollution, especially in pools with widely fluctuating water quality. *Caecidotea recurvata* and *S. mackini* were present

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in unpolluted or slightly polluted pools. In moderately polluted pools only *C. recurvata* was present. In highly polluted pools *C. recurvata* and *S. mackini* were absent. Use of groundwater fauna in addition to physicochemical measurements is being increased in groundwater pollution studies (*e.g.*, Plenet *et al.*, 1992; Poulson, 1992; Ward *et al.*, 1992). Information on general ecology and pollution tolerance of aquatic troglobites is needed if groundwater communities are to be used in groundwater biomonitoring.

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