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Relationship Between Physiochemical Factors and Distribution of Stygobitic Crayfishes in Southeastern Caves

Genevieve R. Spanjer[1,2,*] and Martin L. Cipollini[1]

Abstract - This study was conducted to determine the relationship between water chemistry and presence of stygobitic (cave-obligate) crayfishes (Cambaridae) in Tennessee, Alabama, and Georgia. We analyzed nine chemical factors in water samples from twenty caves, twelve of which contained stygobitic crayfish and eight in which none were found. A multiple analysis of variance using principal components scores suggested that absence of crayfish was associated with lower dissolved oxygen, higher ammonia, and higher water temperature. Caves with externally originating streams supported no stygobitic crayfishes, and the chemical factors of the water in these caves were more variable.

Introduction

Crayfishes (freshwater members of the infraorder Astacidea) are found in various lentic and lotic aquatic systems worldwide and are considered moderately sensitive to pollution or alterations in water chemistry (Pennak 1989). The southeastern United States is home to the US's greatest crayfish diversity (Taylor et al. 1996), including about 30 stygobitic species and subspecies from three genera (*Cambarus*, *Procambarus*, and *Orconectes*) occurring in Tennessee, Alabama, and Georgia (TAG) (Hobbs 2000, Hobbs et al. 2003). Two stygobitic crayfish species are reported from the counties we surveyed. *Cambarus hamulatus* Cope is reported from Jackson County, AL, and Marion and Franklin Counties, TN, and *Orconectes australis australis* Rhoades is reported from Jackson County, AL, and Franklin and Van Buren Counties, TN. No stygobitic crayfish have been reported from Dade or Catoosa Counties, GA (J. Buhay, Provo, UT, pers. comm.; Hobbs et al. 2003).

Unlike epigean (aboveground) environments, caves completely lack light and may have relatively constant air and water temperatures (except during flooding). Like some epigean environments, cave ecosystems depend largely on allochthonous food sources. The scarcity of food in caves may have been a selective pressure on populations in caves and resulted in specialized adaptations to this environment (Hobbs 1992).

The distribution of stygobitic organisms may reflect island biogeography (MacArthur and Wilson 1967) because caves function as isolated "islands." Extensive migration between such islands may be difficult (Hobbs 2000). Therefore, species may evolve within a single cave or cave system. However, Culver et al. (1973) found that aquatic species are less isolated

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than terrestrial species because they may migrate between caves via hydrological connections. Culver et al. (2000) reported that overall endemism was high, as 61% of known cave-obligate (aquatic or terrestrial) species and subspecies in the 48 continental states of the US and 44% of aquatic cave-obligate species and subspecies were endemic to a single county.

Stygobitic crayfishes feed on allochthonous organic debris (Hobbs 1974), predate on cave isopods and amphipods, and exhibit adaptations as a consequence of life in the dark. These adaptations include lack of pigment, reduced or absent eyes and eye function, a slender body form, and attenuated appendages, the last of which compensate for lack of visual senses (Hobbs 2000, Hobbs et al. 1977). Loss of pigment and eyes that are functional in complete darkness may be an evolutionary result of selection for efficiency (Hobbs et al. 1977). Stygobites tend to exhibit "K-selected" characteristics (late maturity, small population size, low reproductive rates, extended lifespans; Hobbs et al. 1977), potentially making them more susceptible to disturbance than epigean species. While the lifespan of epigean crayfish is about two to three years, some stygobitic individuals may live for several decades (Taylor et al. 1996).

Most stygobitic organisms are highly sensitive to changes in water chemistry and susceptible to contaminants in surface and ground water (Culver et al. 2000). Mathews et al. (1977) found that mortality of the stygobitic crayfish *Orconectes australis australis* increased with increasing chlorine levels (with acclimated crayfish showing higher tolerance), but overall tolerance of stygobites to various water chemical variables remains little known. We designed this study to determine if water temperature, water chemistry, or stream source relate to presence or absence of stygobitic crayfish while further documenting their distribution in TAG. We predicted that caves with an influent stream source (originating outside the cave) would be less likely to support stygobitic crayfish than those with an effluent stream source (originating inside the cave) because surface streams have a greater likelihood of variability since their water chemistry and temperature are affected by surface conditions (Hobbs 1992).

Materials and Methods

Cave selection and determination of presence/absence of crayfish

From 20 June to 30 September 2001, we sampled water one time each from 20 caves in six counties: Jackson County, AL; Franklin, Marion, and Van Buren Counties, TN; and Catoosa and Dade Counties, GA (Fig. 1). Some caves were pre-selected based on prior observations of presence or absence of stygobitic crayfish. Crayfishes were not identified to the species level. Identification in the field is very difficult and collection of specimens was not feasible considering the protected status of some species. While identification of crayfish species could be useful information, our purpose was to examine factors potentially relating to distribution of stygobitic crayfish as a group, rather than any particular species. In each cave, at least two individuals searched thoroughly for stygobitic crayfish, looking in riffle and pool sections, under rocks and ledges, in stream sections with a variety of substrate types, and in puddles separated from the flowing stream. In smaller caves, the entire accessible length of the stream was searched. In larger caves, portions of the stream approximately equal to the size of smaller caves were explored. Epigean crayfish were also recorded when noticed.

Water collection and analysis of abiotic factors

In each cave, we collected one water sample from an undisturbed pool section of the stream deep enough to submerge a 500-mL amber Nalgene bottle. The bottle was capped while underwater to exclude all air. Samples were taken from areas containing stygobitic crayfish, from sections resembling crayfish habitat in other caves (pool section, non-bare rock bottom, within dark zone), or, when possible, from sections containing epigean crayfish or other aquatic organisms.

Water and air temperatures were taken onsite using a mercury thermometer. Dissolved oxygen (DO) was tested onsite using a LaMotte Winkler Kit (azide modification of Winkler Method; LaMotte 2004). Samples were refrigerated until analysis for the remainder of the chemical parameters, which occurred within the time frame appropriate for each factor (Hach 1989). We used a Hach EC10 pH meter to determine pH, and a Hach CO 150 conductivity meter to measure total dissolved solids (TDS) and conductivity. We used Hach manual standard methods 8038, 8204, 8203, and 8171

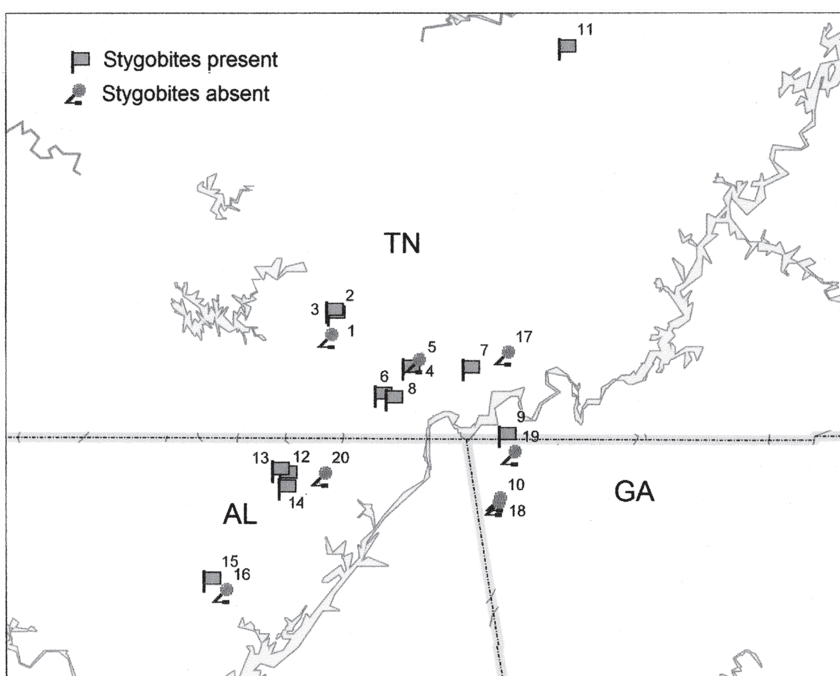

Figure 1. Location of caves sampled for the presence of stygobitic crayfish. See Table 1 for cave names and additional information.

(Hach 1989) and a Hach DREL/2000 spectrophotometer to measure ammonia nitrogen, calcium hardness, alkalinity, and nitrate as nitrogen, respectively. We measured DO saturation using a standard nomograph (Horne and Goldman 1994) and calculated un-ionized ammonia from total nitrogen ammonia using a standard table (Thurston et al. 1979).

Data analysis

We included the variables mentioned above for each cave in a principal components analysis (PCA). This analysis allowed for the description of overall relationships between water quality parameters and the presence or absence of stygobitic crayfish. Principle components were extracted as linear combinations of the raw data for the above parameters with maximum variance rotation using the statistical package SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL). Factor loadings were calculated by correlating the original variables with each factor extracted. Factor loadings indicate the strength of association of each water quality parameter with each factor.

To test statistically for differences in water quality between caves containing and lacking stygobitic crayfish, we used the PCA scores as dependent variables in a multiple analysis of variance (MANOVA), using presence of stygobitic crayfish as the independent variable (factor). We used PCA components instead of raw data because many of the variables tested were chemically related and thus correlated statistically. The use of principle components avoids problems of collinearity among the numerous independent variables. The MANOVA technique thus tested for overall statistical differences between the two sets of caves.

Results

We observed at least one stygobitic crayfish in 12 caves. In nine of these, epigean crayfish were also observed. In three caves, only epigean (no stygobitic) crayfish were observed (Table 1). No stygobitic crayfishes were found in the four caves with an influent stream source.

Water chemistry overview

For every variable except nitrate ($N\text{-}NO_3$), ranges of values were greater for caves without stygobitic crayfish than for caves with these crayfish present (Table 2). Dissolved oxygen was at or near saturation in all caves. Total and un-ionized ammonia levels were greater for caves without stygobitic crayfish than for those with stygobitic crayfish. A single cave without stygobitic crayfish (Howard's Waterfall Cave) had levels of several chemicals that differed greatly from the remainder of sites. However, for each physiochemical parameter, the mean value for caves without stygobites did not change significantly when this cave was excluded (two-tailed t-tests; $P \geq 0.3$ for each).

Water chemistry PCA analysis

About 80% of the variation in abiotic factors among the caves was accounted for by the first three principal components of the PCA (Table 3).

The first component segregated caves predominantly along a gradient from values representing high pH, ammonia, hardness, alkalinity, TDS, and conductivity to low values for those parameters. The second component was positively related to temperature and ammonia, and negatively related to DO. The third component was positively related to nitrate nitrogen values and negatively related to pH. None of the other extracted components were deemed statistically significant, since their eigenvalues were < 1.0 (they explained less of the overall variation than did the raw variables).

Table 1. Name, location, stream source, and presence or absence of stygobitic and epigean crayfish in each cave during this study. # = number on map (Fig. 1)

#	Cave	County, state	Stygobitic crayfish observed?	Epigean crayfish observed?	Stream source
1	Buckets of Blood	Franklin, TN	No	Yes	Effluent
2	Walker Spring	Franklin, TN	Yes	Yes	Effluent
3	Wet	Franklin, TN	Yes	*	Effluent
4	Bible Spring	Marion, TN	Yes	No	Effluent
5	Catacomb	Marion, TN	No	*	Influent
6	Gourdneck	Marion, TN	Yes	Yes	Effluent
7	Owen Spring	Marion, TN	Yes	*	Effluent
8	Shakerag	Marion, TN	Yes	Yes	Effluent
9	Whiteside	Marion, TN	Yes	Yes	Effluent
10	Howard's Waterfall	Dade, GA	No	No	Influent
11	Cueva Guapa del Norte	Van Buren, TN	Yes	Yes	Effluent
12	Jess Elliot	Jackson, AL	Yes	Yes	Effluent
13	Tate	Jackson, AL	Yes	Yes	Effluent
14	Bluff River	Jackson, AL	Yes	Yes	Effluent
15	Limrock Blowing	Jackson, AL	Yes	Yes	Effluent
16	Isbell Spring	Jackson, AL	No	*	Effluent
17	"Keener, Wright"	Marion, TN	No	No	Effluent
18	Sitton's	Dade, GA	No	Yes	Effluent
19	Lost Creek	Catoosa, GA	No	*	Influent
20	Upper Tumbling Rock	Jackson, AL	No	Yes	Influent

*Caves not exhaustively searched for epigean crayfish.

Table 2. Descriptive statistics for physiochemical variables of water from caves with and without stygobitic crayfish.

	Stygobites present		Stygobites absent	
Parameter	Mean ± SD	Min.–Max.	Mean ± SD	Min.–Max.
Air temperature (ºC)	15.7 ± 1.4	14.0–18.5	17.6 ± 2.6	14.5–23.0
Water temperature (ºC)	14.0 ± 0.8	12.0–15.0	16.3 ± 3.1	14.0–21.0
DO (mg/L)	9.6 ± 0.4	9.0–10.2	9.0 ± 0.8	8.0–9.9
pH	7.5 ± 0.2	7.3–7.9	7.3 ± 0.3	6.8–7.8
NO_3 (mg/L x 10^{-1} N)	3.4 ± 1.8	2.0–9.0	3.6 ± 2.1	2.0–8.0
NH_3-N (mg/L x 10^{-2} N) (total)	1.4 ± 0.9	0.0–4.0	18 ± 28.0	1.3–81.0
NH_3 (mg/L x 10^{-4} N) (un-ionized)	1.3 ± 1.0	0.0–4.0	18 ± 26.0	0.3–67.0
Hardness (mg/L x 10^2 $CaCO_3$)	1.2 ± 0.4	0.6–1.6	1.0 ± 0.7	0.2–2.4
Alkalinity (mg/L x 10^2 $CaCO_3$)	1.1 ± 0.4	0.5–1.5	0.8 ± 0.6	0.2–2.1
TDS (mg/L x 10^2)	1.1 ± 0.3	0.7–1.6	1.0 ± 0.6	0.2–2.0
Conductivity (µS x 10^2)	2.4 ± 0.6	1.5–3.2	2.0 ± 1.2	0.4–4.2

A 3-dimensional PCA graph (Fig. 2) depicting scores for each cave for the first three components showed caves with stygobitic crayfish clustered together, while those without them were more scattered. Moreover, segregation between caves with and without stygobitic crayfish can be seen along the second PCA axis (see MANOVA results below).

Multiple analysis of variance

The MANOVA analysis using components extracted from the PCA gave a Wilks' Lambda value of 0.561 ($F_{3,16} = 4.175$; $P = 0.023$) for the overall effect (differences between caves with and without stygobitic crayfish). Tests of between-subjects effects indicated that the second PCA component was significantly related to presence/absence of stygobitic crayfish (Table 4). Based upon the results of the PCA factor loadings for this component (Table 3), lower values for temperature, higher values for DO, and lower values for total and un-ionized ammonia were associated with cave streams containing stygobitic crayfish.

Discussion

Stygobitic crayfish survival may be limited to a relatively narrow range of acceptable values with regard to water chemistry and temperature. In our study, we sampled water from each cave only once, and the range of physiochemical values found in water from the group of caves not supporting stygobitic crayfish was greater than the range of values recorded from caves known to be inhabited by stygobitic crayfish (Table 2, Fig. 2). Additionally, we did not find stygobitic crayfish in any cave with an influent stream (Table 1), and water chemistry in this stream type is known to be more variable than in effluent streams because water chemistry is directly affected by surface conditions (Hobbs 1992).

Table 3. Component matrix for PCA with presence of stygobites as the independent variable.

	Component		
Parameter	1	2	3
Water temperature	-0.20	**0.81**	0.37
Air temperature	0.05	**0.89**	-0.12
DO	0.18	**-0.87**	-0.08
pH	**0.50**	-0.23	**-0.61**
NO_3	0.24	-0.04	**0.77**
NH_3-N (total)	**0.68**	**0.50**	-0.11
NH_3 (un-ionized)	**0.64**	**0.54**	-0.20
Hardness	**0.93**	-0.12	0.12
Alkalinity	**0.93**	-0.10	0.14
TDS	**0.93**	-0.10	-0.06
Conductivity	**0.94**	-0.11	-0.05
Eigenvalue	4.74	2.85	1.19
% variance	43.1	25.9	10.8
Cumulative % variance	43.1	70.0	79.8

Note: Factors with values ≥ 0.50 or ≤ -0.50 are in boldprint.

We found stygobitic crayfish only in caves with water ≤ 15 °C. We believe that temperature, which is important for growth and survival of many crayfish species, is one of the most important factors contributing to this distribution pattern (Table 3, Table 4). Taylor (1984) found that three species of epigean crayfish preferred temperatures ranging from about 20 to 26 °C in a lab setting, while Hobbs (1974) reported that a species found both inside and outside caves lived only in water ≤ 20 °C. Optimum growth ranges and lethal limits regarding temperature vary by species, but 10 °C is the lowest temperature at which many epigean species can grow (Biggs 1980, Brewis and Bowler 1983, Pratten 1980). While an increase in temperature has been positively correlated with crayfish growth (Aiken and Waddy 1992), crayfishes also have an upper temperature limit, and stygobitic crayfishes may be adapted to groundwater (lower) temperatures because they have evolved in subterranean environments.

H. Hobbs III (pers. comm., Springfield, OH) reports that he kept stygobitic crayfish at "room temperature" for years without apparent harm. These individuals did not reproduce, but other factors may have been involved,

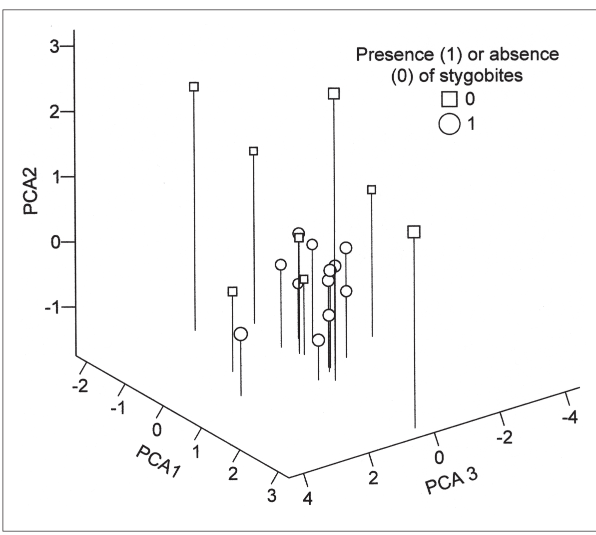

Figure 2. Plot of PCA scores for caves with and without stygobitic crayfish. PCA scores were based on the physiochemical parameters listed in Table 2. For interpretation of PCA factor loadings, refer to Table 3.

Table 4. MANOVA tests of between-subjects effects, indicating the relationship of each factor to the overall statistical distinction between caves with and without stygobitic crayfish. Factors with P ≤ 0.05 were significantly related to this difference.

Source	Factor	Type III SS	df	MS	*F*	*P*
Intercept	PCA1	0.01	1	0.01	0.01	0.944
	PCA2	0.32	1	0.32	0.53	0.478
	PCA3	0.01	1	0.01	0.01	0.933
Stygobite	PCA1	0.13	1	0.13	0.13	0.728
(presence/	PCA2	8.02	1	8.02	13.15	0.002
absence)	PCA3	0.19	1	0.19	0.18	0.675
Error	PCA1	18.87	18	1.05		
	PCA2	10.98	18	0.61		
	PCA3	18.81	18	1.05		

making the true link between temperature and stygobite distribution difficult to discern. In our study, caves with an external stream source had a higher mean water temperature and a wider range in water temperature than did caves with an internal stream source. It is possible that the amount of variability in water temperature may be more important than the mean water temperature when it comes to predicting the presence of stygobitic crayfish.

Because of caves' low biological and chemical oxygen demand and cool water temperatures, DO is usually not a limiting factor for crayfish survival (Hobbs 1992), and DO levels in all caves tested were well above the threshold of 1.0–1.5 mg/L reported by Biggs (1980). Un-ionized ammonia (NH_3) may harm crayfishes. Levels of 0.09 mg/L NH_3 can reduce growth, and 5.71 mg/L can kill some crustacean species (Chin and Chen 1987, Colt and Armstrong 1979). The lowest NH_3 level considered hazardous to most aquatic organisms is 0.02 mg/L (Department of Agricultural and Biosystems Engineering 1996), and the range of the values in this study was substantially lower (0–0.0067 mg/L). Total ammonia can also be harmful, and Lee et al. (1985) reported that concentrations of 0.5 to 1.0 mg/L total ammonia nitrogen (not just NH_3) can cause histological damage in *Procambarus clarkii* Girard. Our highest recorded total ammonia value (in Howard's Waterfall Cave, no stygobites present) was 0.81 mg/L, but high ammonia levels usually are not problematic when DO levels are sufficiently high (H. Hobbs III, Springfield, OH, pers. comm.), as they were in all study sites. Because stygobitic crayfishes are relatively understudied, their tolerance to various chemicals is not well known. It is possible that ammonia affects distribution among stygobitic crayfishes, but most likely only if levels exceed the values we recorded in this study.

Calcium, which is related to both alkalinity and hardness, is an important parameter for crayfishes, particularly during molting, because it is a major component of the exoskeleton. Our results show a correlation between higher alkalinity and hardness values and stygobitic crayfish presence (Table 3). In limestone caves, the calcium ion is very common and usually sufficient for crayfishes (McGregor et al. 1997), and we have no data indicating calcium was harmfully low in any of the sampled caves.

Pollution seemed a likely factor for crayfish survival at Howard's Waterfall Cave, and a potential factor in Upper Tumbling Rock Cave (neither site contained stygobitic crayfish). In the former, which is frequently visited by humans and near a highway, garbage was found around the cave and directly in the stream inside the cave. Additionally, even the undisturbed stream water was cloudy in appearance (unlike other caves sampled), and we saw no organisms in this stream despite its near-saturation DO and low temperature (14.5 °C). This stream had a total ammonia level more than three times greater than the next highest value and > 25 times higher than most values. Its alkalinity, hardness, conductivity, and TDS levels also deviated greatly from that of other caves sampled. In Upper Tumbling Rock Cave, much garbage was dumped uphill of the cave and its stream, though less was found within the cave. However, the presence of epigean organisms here, in combination with the occurrence of an external stream source, suggest that the latter, rather than pollution, was responsible for the absence of stygobitic crayfish.

Based upon frequency of precipitation, water level and chemical properties fluctuate seasonally (McGregor et al. 1997). Therefore, multiple water samples from different times of year should have been collected. Further, while this study examined factors potentially related to presence of stygobitic crayfish as a group, identification to the species level is recommended in future studies, as tolerance to various chemical parameters may vary by species.

For more insight into the ideal environment for stygobitic crayfish, testing of both biotic and additional abiotic parameters, such as turbidity and concentrations of heavy metals, should be conducted. Excess sediment can clog crayfish gills (Holdich 2003), and high levels of certain heavy metals, which may be present as a result of human influence, are harmful to crayfish (McGregor et al. 1997). Two additional factors potentially affecting stygobite distribution include predation from epigean fish or crayfish, particularly in caves with influent streams, and competition from epigean relatives. Also, a study including only caves with an effluent stream source would eliminate the factor of stream source evidently influencing water chemistry. Finally, lab or field experiments could address whether some caves with seemingly ideal conditions for stygobitic crayfish contain none because of chance alone.

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