The Subterranean Aquatic Fauna of the Greenbrier Karst

19

Daniel W. Fong and David C. Culver

Abstract

The Greenbrier Karst harbors 16 species of stygobionts known from 92 caves, and six of these caves are type localities of ten of the species. The fauna is dominated by crustaceans and especially amphipods of the genus *Stygobromus*, and they primarily occupy vadose streams and the epikarst, but are notably absent from phreatic waters. *Stygobromus spinaus* is the most widely distributed, found in 59 caves. The segmented worm *Trichodrilus culeri* and the salamander *Gyrinophilus subterraneus* are both endemic to single site. The amphipod *Gammarus minus* is the most intensely studied species, but the basic biology of the other species is little known. The Organ Cave system holds the record in species richness (8) and in terms of type locality (3 species) and deserves a coordinated effort for protection.

19.1 Overview of the Subterranean Aquatic Fauna

Most research on the subterranean aquatic fauna is focused on species that show troglomorphy, morphological features associated with the aphotic environment, such as elongated appendages and reduced or complete loss of eyes and body pigment compared to related surface species. The ecological category of stygobiont consists of species that complete their entire life cycles in subterranean waters with no part of their life cycles occurring in surface waters. Thus, species that are only mildly troglomorphic or even non-troglomorphic are considered to be stygobionts as long as they fit this ecological criterion. Parallel to the situation with the terrestrial fauna (see Chap. 18), both stygobionts and non-stygobionts commonly occur in subterranean waters of the Greenbrier Karst.

D.W. Fong (✉)
Department of Biology, American University, 4400 Massachusetts Avenue NW, Washington, DC 20016, USA
e-mail: dfong@american.edu

D.C. Culver
Department of Environmental Science, American University, 4400 Massachusetts Avenue NW, Washington, DC 20016, USA

19.2 Non-stygobionts

Non-stygobionts include a whole range of species, from those that passively wash into cave streams from surface waters from episodic events such as storms and rapid snow melt, to those that continually infiltrate cave streams from sinking streams and from the epikarst through ceiling drips, to those that actively colonize cave waters during parts of their life cycles. These species occupy subterranean waters along a continuum of residence times, spanning from only hours to days to months to seasons and possibly years. Our knowledge of them necessarily correlates with their residence times because longer residence times increase their chances of being noticed by biologists. There have been few studies of non-stygobionts at a regional scale, however, and the following summary necessarily includes much speculation and very broad treatment of these organisms, especially ones with shorter residence times.

Species with short residence times, from hours to days, are soft-bodied benthic organisms washed into cave streams due to surface storm events, because a majority of them are unlikely to survive for long the severe physical disturbance of the substratum during such events, and those that survive rapidly become prey to resident species or starve due to insufficient or inappropriate resources. These include many

W.B. White (ed.), *Caves and Karst of the Greenbrier Valley in West Virginia*,
Cave and Karst Systems of the World, https://doi.org/10.1007/978-3-319-65801-8_19

species of insect groups with aquatic larvae, such as mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), dragonflies (Odonata), and some true flies (Diptera), as well as species of aquatic invertebrate groups such as flatworms (Platyhelminthes) and segmented worms (Annelida), among others. All of them are common and abundant components of the benthic fauna in surface streams, but are rarely recorded from cave streams in the Greenbrier Karst for several reasons. First, the harsh physical condition of cave streams during and immediately after flood events that transport these species into caves also prevents most cavers and biologists from entering caves. Second, their short residence times decrease their chances of being studied by biologists. Third, most biologists ignore them anyway because they are obviously part of the surface fauna and thus are considered uninteresting to study.

Species with longer residence times, from days to weeks to possibly months, are ones with more robust bodies and more likely to survive the physical disturbance of storm events and thus persist in subterranean waters for some duration. These include species of crayfish (Cambaridae) and fish such as minnows (Cyprinidae), sculpins (Cottidae), and sunfish (Centrarchidae) as well as some frogs (Ranidae). Emaciated fish and frogs are occasionally observed by cavers deep into cave systems weeks to months after a storm event. Ultimately, they all die from starvation and their bodies benefit scavengers and predators of the scavengers. Whether their body mass is an important energy source sufficient to maintain higher population sizes or longevity or both of the permanent cave stream fauna is unclear (Fong 2011). Some soft-bodied organisms may better survive the physical process of being washed into cave streams depending on the route of entry. For example, storms flushing water from low-gradient intermittent surface streams may cause only mild disturbance to the substratum, allowing a larger proportion of soft-bodied organisms to survive the event. Some of these may persist for weeks to months if appropriate resources are available in subterranean waters. Examples are caddisflies in the family Philopotamidae, known as finger-net caddisflies because their larvae spin and live within a silken net up to 1 cm in diameter by 4 cm in length. These nets function as filters that trap fine particulate organic matter, and this organic matter and the associated microbes serve as food for the larvae. Under rocks and pebbles in the upper level streams of some caves in the Greenbrier Karst, such as Organ Cave and The Hole, dense patches of these finger-shaped nets may be found throughout the year, although most are empty and only a small fraction is occupied. It is unknown if the empty nets indicate larvae that had successfully metamorphosed into flying adults or larvae that died from starvation and had since decomposed because the quality and quantity of the available organic matter are insufficient, or both, although it is not uncommon to encounter adult caddisflies in upper level cave stream passages in the Greenbrier Karst. Interestingly, in a group of European caddisflies in the family Limnephilidae the larvae grow in temporary surface waters, but adults migrate into caves and are considered troglophiles (Salavert et al. 2008). Some snails in the family Physidae are also not uncommon in upper level cave streams in the Greenbrier Karst. These snails are grazers on the epilithic biofilm, a matrix of algae and bacteria coating rocks in surface streams, and may persist in cave streams for some duration by surviving on the biofilm but without the algae in cave streams.

A number of species continuously colonize and exhibit lengthy residence times in subterranean waters, measured in seasons and years, and most are considered stygophiles. Some have large body sizes and thus are obvious components of the aquatic fauna (Fong et al. 2012). The more charismatic ones are salamanders, such as larvae and especially adults of the Spring Salamander, *Gyrinophilus porphyriticus*, the Dusky Salamander, *Desmognathus fuscus*, and the Cave Salamander, *Eurycea lucifuga*, although adults of the Cave Salamander are mostly terrestrial. These amphibians use subterranean waters as refuge from extremes of epigean temperature and aridity and for reproduction, where they also prey on invertebrates such as annelid worms as well as amphipod and isopod crustaceans. Another obvious stygophile is the crayfish *Cambarus bartonii*, a common inhabitant of cave streams and pools, where it is probably a top predator. A number of species appear to constantly rain down into vadose passages from the epikarst through ceiling drips and other routes, and these include many copepod crustaceans (Pipan and Culver 2005). These species are minute in size and difficult to observe and collect. They colonize rimstone pools and cave streams and pools and may persist for long periods; their ultimate fate is unclear, however, as the vadose passage is not their primary habitat.

19.3 Stygobionts

Ultimately, along the same continuum, stygobionts are species with residence times measured at evolutionary rather than ecological scales. A majority of the subterranean aquatic fauna in the Greenbrier Karst is collected from cave streams, and caves streams are the main habitat of most stygobionts known in the area. Stygobionts are also collected from the epikarst (Culver and Pipan 2009), and some stygobionts collected from upper level cave streams originate from the epikarst (Fong and Culver 1994). The fauna of epikarst is understudied in the Greenbrier Karst and in North America in general, however, and more species may be discovered from the epikarst in the future. No species in the

Greenbrier Karst is known from phreatic waters, although several species occur in phreatic waters in the nearby Shenandoah Valley in Virginia (Holsinger and Culver 1988; Hutchins et al. 2010). The lack of phreatic stygobionts in the Greenbrier Karst reflects a similar pattern in karst areas of the Appalachians in general. Although stygobionts are well known from hypotelminorheic habitats (see Culver and Pipan 2014), which are shallow aquifers perched above the local water table, such habitats, however, are unknown in the Greenbrier Karst. Large springs or groundwater resurgences are common in the Greenbrier Karst (see Jones 1973, 1997), and stygobionts are occasionally collected from such habitats, probably having been passively flushed out from subterranean waters during high discharge. These resurgences harbor large populations of the amphipod crustacean *Gammarus minus* and are the origins of stygobiotic and troglomorphic populations of *G. minus* in many cave systems in the Greenbrier Karst (see Culver et al. 1995 and below).

19.4 Diversity Patterns

There are 16 species of stygobionts known from 92 caves within the Greenbrier Karst (Fong et al. 2007) (Table 19.1, Fig. 19.1). They include one flatworm (Platyhelminthes), two segmented worms (Annelida), two snails (Mollusca), and one salamander (Chordata). The other nine species are arthropods in the subphylum Crustacea (orders Malacostraca and Maxillopoda), including one copepod (Cyclopidae), one isopod (Asellidae), one crayfish (Cambaridae), one amphipod in the genus *Gammarus* (Gammaridae), and five amphipods in the genus *Stygobromus* (Crangonyctidae). The stygobiotic fauna of the Greenbrier Karst is therefore dominated by arthropods (nine of 16 species or 56%), and amphipods are dominant among the arthropods (six of nine species or 67%), while all but one of the six amphipod species (83%) belong to the genus *Stygobromus*. Thus, amphipod species in one genus, *Stygobromus*, account for almost one-third (31%) of all stygobionts in the Greenbrier Karst.

The frequency distribution of number of stygobiotic species per cave is given in Fig. 19.2. The distribution is strongly skewed to the left as is typical of species richness data. 49% of the caves (45) harbor only one species, and 23% (21 caves) are inhabited by two species, with 10% (9 caves) and 11% (10 caves) housing three and four species, respectively. Organ Cave has the highest number of species at eight, followed by The Hole with seven, then by General Davis Cave and McClung Cave at six each, while Benedicts Cave, Buckeye Creek Cave, and Fuells Fruit Cave each harbor five species. The locations of caves with five or more species are given in Fig. 19.3, and all six are located in Greenbrier County at or near the edge of the Greenbrier Karst. A map of species richness at a regional scale using 10 km × 10 km grids is given in Fig. 19.4. It mirrors the pattern of individual caves with five or more species shown in Fig. 19.3 and shows high species richness in central Greenbrier County and in southern Greenbrier County at the Organ Cave plateau and areas near General Davis Cave.

19.5 Distribution

The number of caves occupied by each of the 16 stygobionts is given in Fig. 19.5. The most widely distributed species is the amphipod *Stygobromus spinatus* (Fig. 19.6), occurring in 59 of the 92 caves. This species is ubiquitous in aquatic habitats within its range, found in both epikarst (Holsinger 1978) and small headwater cave streams (Fong and Culver 1994), but with a body size at only 5 mm it is rarely noticed. The isopod *Caecidotea holsingeri* ranks second in terms of distribution, occurring in 32 caves, but unlike *S. spinatus*, it is rarely associated with epikarstic habitats and is commonly found on the underside of rocks in cave streams (Fong et al. 2007) and frequently in hygropetric habitats, the thin film of water on flowstone (Sket 2004). The amphipod *Stygobromus emarginatus* inhabits 22 caves in the Greenbrier Karst and many more beyond. It is easily observable as a large white amphipod, at almost 10 mm in length, and is abundant in some headwater cave streams. Knapp and Fong (1999) used a mark–recapture technique to estimate its population size at 10–14 per linear meter along a 300-m-long first-order stream in Organ Cave and present evidence suggesting that, despite its relatively large body size, its primary habitat is the epikarst rather than the vadose stream.

The next three species in ranks of distribution are the snail *Fontigens tartarea*, the crayfish *Cambarus nerteius*, and the flatworm *Macrocotyla hoffmasteri*, found in 17, 16 and 15 caves, respectively. The snail, *Fontigens tartarea*, at only 1–2 mm in size is difficult to see and its basic biology is unknown. It has an extensive range outside of the Greenbrier Karst, and given its low potential for dispersal, it is likely a cryptic species complex. The other snail, *F. turritella*, is similar to *F. tartarea* in size, but has a puzzling distribution. It is found in two caves in Greenbrier County and in one other cave about 250 km to the northeast. The outlier may be a different species. The Greenbrier Cave Crayfish, *Cambarius nerterius* (Fig. 19.7), is the only stygobiotic crayfish in the Greenbrier Karst. Its range overlaps that of the related stygophile *C. bartonii*, and it is difficult to distinguish between them because of similar morphologies. Any large, white flatworm, up to 1.2 cm in length when stretched out, found gliding along rocks in cave streams in the Greenbrier Karst is probably *Macrocotyla hoffmasteri*, a predator on smaller organisms such as juvenile amphipod and isopod crustaceans. We have little knowledge of cave flatworms,

Table 19.1 List of sixteen species of stygobionts known from caves in the Greenbrier Karst arranged by higher taxonomic grouping

Class	Order	Family	Species	Abv	Sites
Trepaxonemata	Neoophora	Dendrocoelidae	*Macrocotyla hoffmasteri*	Mh	15
Oligochaeta	Lumbriculida	Lumbriculidae	***Stylodrilus beattiei***	Sb	3
			Trichodrilus culveri	Tc	1
Gastropoda	Neotaenioglossa	Hydrobiidae	***Fontigens tartarea***	Fta	17
			Fontigens turritella	Ftu	2
Maxillopoda	Cyclopoida	Cyclopidae	*Rheocyclops virginiana*	Rv	9
Malacostraca	Isopoda	Asellidae	***Caecidotea holsingeri***	Ch	32
	Amphipoda	Crangonyctidae	***Stygobromus emarginatus***	Se	22
			Stygobromus mackini	Sm	2
			Stygobromus pollostus	Sp	6
			Stygobromus redactus	Sr	1
			Stygobromus spinatus	Ss	59
		Gammaridae	*Gammarus minus*	Gm	10
	Decapoda	Cambaridae	***Cambarus nerterius***	Cn	16
Amphibia	Caudata	Plethodontidae	***Gyrinophilus subterraneus***	Gs	1

Abv is the abbreviation for each species used in Fig. 19.5. Sites indicate the number of caves where each species has been collected from within the Greenbrier Karst. Ten species with type localities within the Greenbrier Karst are identified in bold type. Species taxonomic authorities, distribution maps, and names of caves occupied are given in Fong et al. (2007)

however, because preservation and identification of flatworms are technically challenging, and systematic collection and studies of flatworms in North America have not been made for several decades.

The next species in rank in term of distribution is the amphipod crustacean *Gammarus minus* (ten caves). It is the most intensively studied stygobiont in the Greenbrier Karst, and its biology is summarized in a separate section below. Three species, the copepod *Rheocyclops virginiana* (nine caves) and two amphipods *Stygobromus pollostus* (six caves) and *S. redactus* (one cave within the Greenbrier Karst, but also occur in a few caves outside the area) are dwellers of the epikarst. All of them are minute in body size and difficult to observe, and they were collected only opportunistically rather than systematically. For example, mature individuals of *S. redactus* reach only 2 mm, and those from Parlor Cave were collected from water in caver's heel prints, the only standing water in the cave (Fong et al. 2007). A systematic survey of the epikarst fauna at the regional scale using techniques pioneered by Pipan (2005) should lead to a better understanding of their distribution. Again, we know little of their basic biology. Although the amphipod *Stygobromus mackini* occurs in only two caves in the Greenbrier Karst, these sites are located at the extreme northern edge of its large range which extends to the southwest for over 400 km. This species is mainly found in water associated with the epikarst, such as drip pools and very small first-order streams, with females being much more common than males (Hoslinger 1978).

At the opposite extreme in terms of distribution, only two species, the segmented worm *Trichodrilus culveri* and the salamander *Gyrinophilus subterraneus*, are single-site endemics. The restricted distributions of *T. culveri* and of the other segmented worm *Stilodrilus beattiei* which occurs in three caves in the Greenbrier Karst are most likely due to the lack of systematic collections since the 1970s (see Cook 1975), and further work should yield expanded ranges of extant species and additional species. The West Virginia Spring Salamander, *G. subterraneus*, is the only stygobiotic vertebrate in the Virginias and one of only four species of stygobiotic salamanders in the family Plethodontidae east of the Mississippi River. It is endemic to General Davis Cave in Greenbrier County, where it co-occurs with the closely related Spring Salamander *G. porphyriticus*. The larva is confined to the cave stream while the adult is equally adept on land and in the water (Fig. 19.8), and they act as the top predator in the cave. The status of *G. subterraneus* as a distinct species was questioned almost immediately after its description (Besharse and Holsinger 1977) because of the high degree of morphological variability in both *G. porphyriticus* and *G. subterraneus* (Blaney and Blaney 1978), and this situation remains unresolved (Niemiller et al. 2010). Interestingly, there are occasional reports of albino specimens of *G. porphyriticus* larvae deep in caves (e.g., Brandon and Rutherford 1967). It is unknown if these albino larvae will metamorphose into albino adults or if albinos are more likely than non-albinos to remain and reproduce within cave systems. These observations, however, suggest that

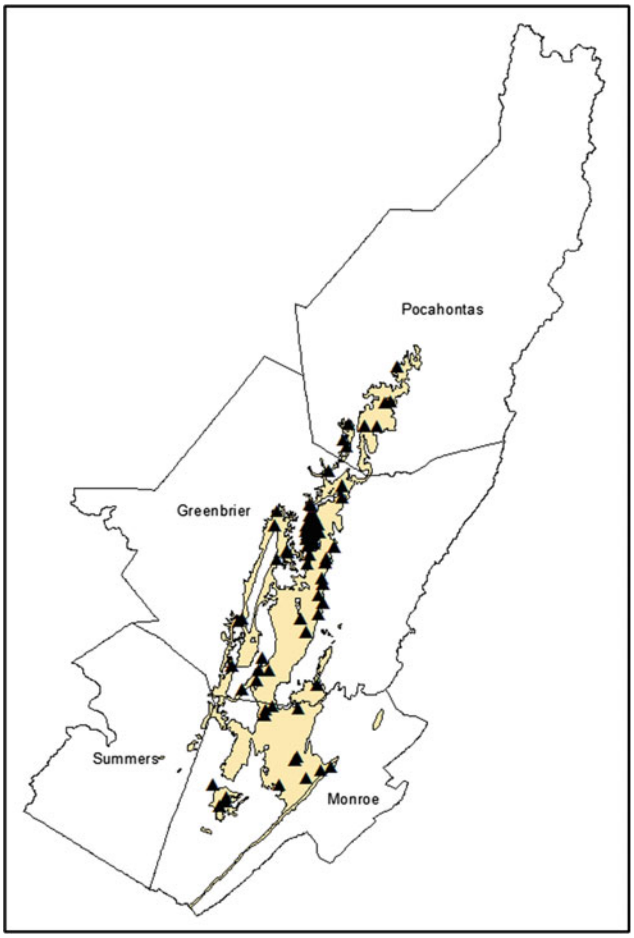
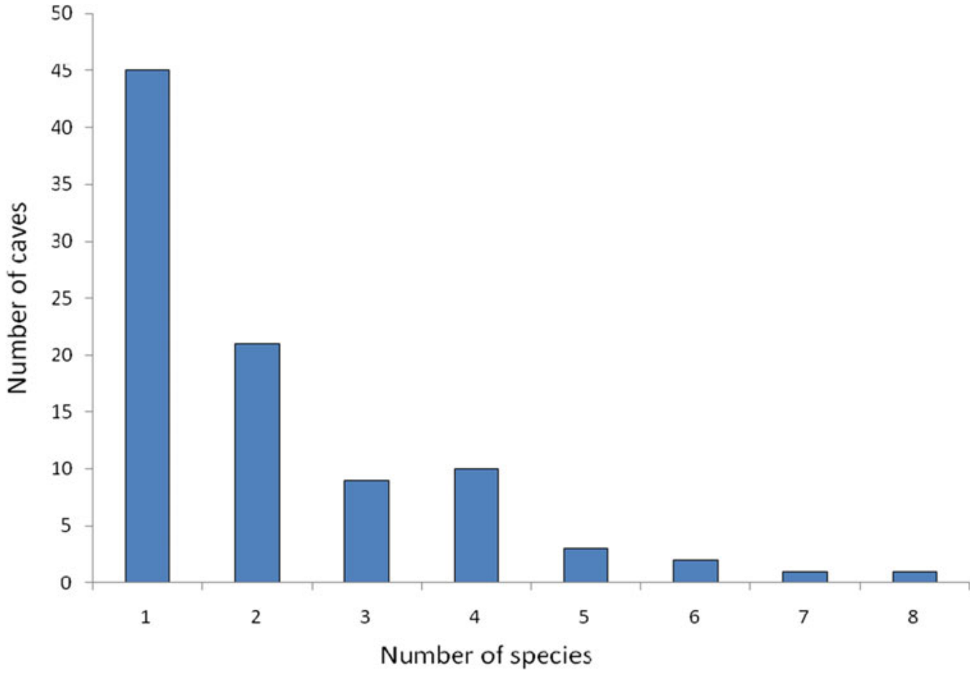


Fig. 19.1 Locations of the 92 caves harboring stygobionts in the Greenbrier Karst

stygobiotic variants are evolving in some of the stygophilic *G. phorphyriticus* populations in the Greenbrier Karst, a potential case study of speciation in action.

Little is known about the distribution of stygobionts within cave systems because most collection records associate species occurrences with a cave name only and provide no more information. Knowledge on fine-scale species distribution within cave systems is needed for a deeper understanding of the ecology of stygobiotic fauna. For example, Fong and Culver (1994) examined the fine-scale distribution of five crustacean species within the complex Organ Cave system (Stevens 1988). They found that while three crangonyctid amphipods and one asellid isopod are more abundant at first-order headwater streams and decrease in abundance at higher-order lower level streams, the opposite is true for a gammarid amphipod. They suggested that the pattern is explained by different routes of colonization of the cave system, with the crangonyctid amphipods colonizing from the epikarst and the gammarid colonizing from downstream originating from the resurgence. Furthermore, although all of the water from the Organ Cave systems exits at a single resurgence, there is a subterranean divide that partitions the water in the system into a western and an eastern drainage (Stevens 1988). While the gammarid is abundant in the eastern drainage, it is absent in the western drainage, and the crangonyctids are present in both. They explained the pattern by suggesting that the western drainage was a separate system with a different resurgence in the past and subsequently merged with the eastern drainage by a subterranean stream capture event. The gammarid is absent from the western drainage either because

Fig. 19.2 Frequency distribution of caves by number of species



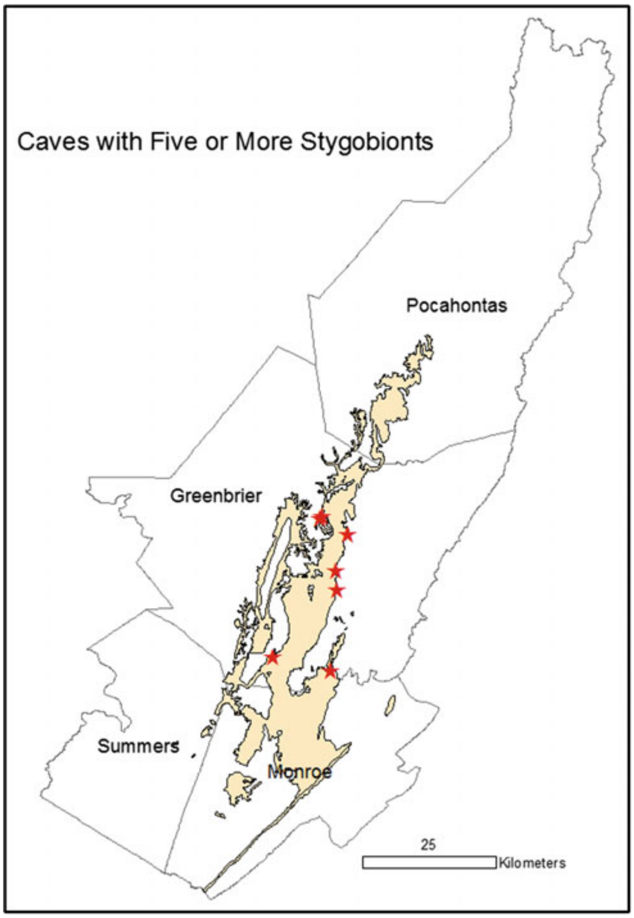


Fig. 19.3 Map of caves with five or more stygobionts each. All six caves are located at or near the contact between the Greenbrier Karst and non-carbonate rocks

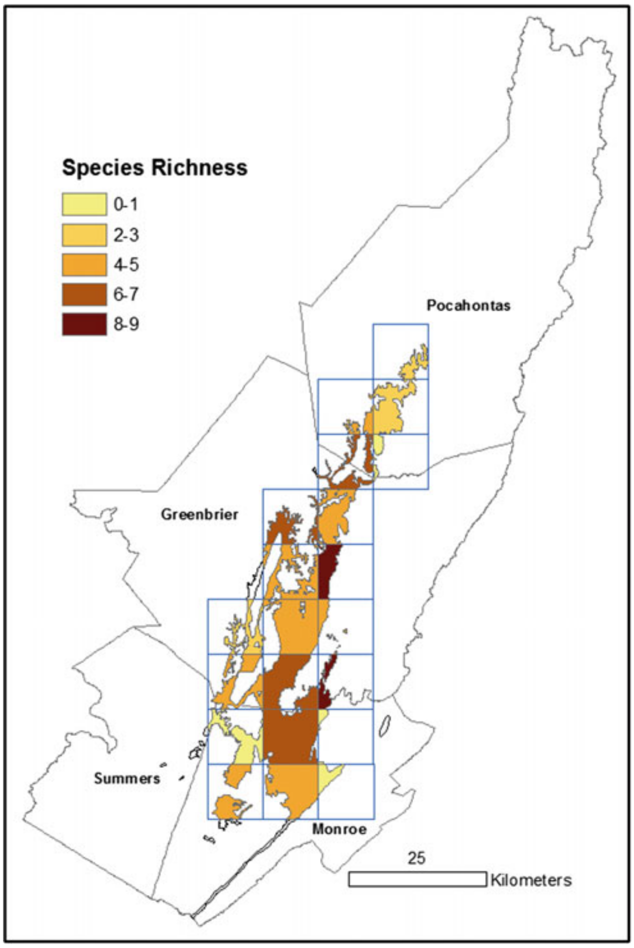


Fig. 19.4 Map of species richness at a regional scale using 10 km × 10 km grids

it colonized and adapted to the eastern drainage prior to the stream capture, or the hydrogeological setting of the stream capture physically prevented the possibility of the gammarid to travel upstream into the western drainage, or both.

19.6 The Case of *Gammarus Minus*

The most intensively studied stygobiont in the Greenbrier Karst is the amphipod *Gammarus minus* (see overview in Culver et al. 1995 and Fong 2012). Here we summarize the results of some of the studies focused on this species that contributed to a better understanding of the evolution and ecology of stygobionts. It is a common and abundant inhabitant of karst springs and cave streams, often reaching densities of 15–20 per m^2 in cave streams and an order of magnitude higher in karst springs. Populations in springs and in most cave streams are not troglomorphic. Troglomorphic populations are found only in large cave systems in Greenbrier and Monroe Counties in the Greenbrier Karst and in Tazewell County in Virginia. Troglomorphic populations show only remnants of eyes, elongated appendages, and reduced body pigmentation compared to non-troglomorphic populations (Fig. 19.9). Shoemaker (1940) considered these troglomorphic populations to be a distinct variety and named it *tenuipes*. Recent genetic evidence indicates such a designation is unwarranted (e.g., Carlini et al. 2009), and we use the term *Gammarus minus tenuipes* simply as a convenient label for the troglomorphic populations. A map of the locations of non-troglomorphic cave populations and of *G. m. tenuipes* is given in Fig. 19.10, which shows that all *G. m. tenuipes* sites are situated at or near the eastern edge of the Greenbrier Karst.

Evidence strongly indicates that *G. m. tenuipes* populations originated through colonizing cave streams at some time in the past by *G. minus* dwelling at the spring where the cave water resurges, and that *G. m. tenuipes* populations in different drainages are each independently derived from a different spring population. Populations of *G. m. tenuipes* on average have lower within population variation in the

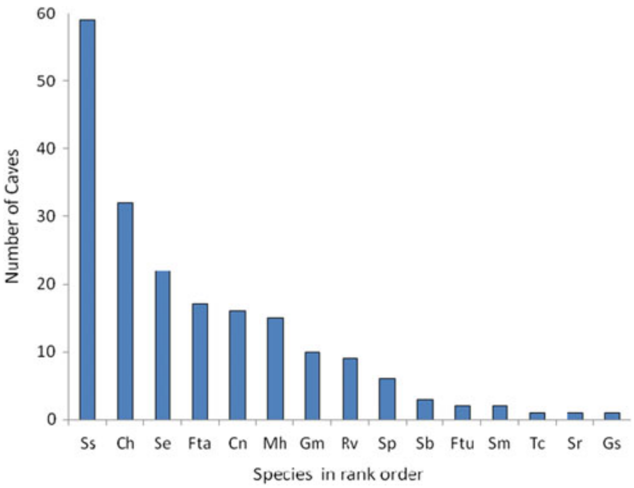


Fig. 19.5 Number of caves occupied by each species arranged by rank order. See Table 19.1 for the key to abbreviated species names

Fig. 19.6 *Stygobromus spinatus*, the most widely distributed stygobiont in the Greenbrier Karst. This ovigerous female, carrying eight developing embryos in a ventral marsupium, was 4 mm in length. Photograph by Michael E. Slay. Used with permission

sequences of the COI and ITS-1 genes than do spring populations, and are also on average more closely related to hydrologically proximate spring populations than to *G. m. tenuipes* populations in other drainages even though they differ substantially in morphology from sister spring populations (Kane et al. 1992; Carlini et al. 2009). *G. m. tenuipes* populations also show much lowered effective population sizes as measured by levels of codon bias compared to surface populations of the same drainage (Fig. 19.11; Carlini et al. 2009), indicating severe genetic bottlenecking as a result of reduced population size during colonization of the cave habitat.

Aspiras et al. (2012) compared the levels of expression of four genes, hedgehog, pax6, sine oculus and dachshund, involved in the developmental pathway of arthropod eyes in

Fig. 19.7 *Cambarus nerterius*, the only stygobiotic crayfish in the Greenbrier Karst. It is only mildly troglomorphic, but has never been found outside caves. This specimen was about 8 cm in body length. Photograph by Horton H. Hobbs III. Used with permission

Fig. 19.8 Larva (*top*) and adult (*bottom*) of the West Virginia Spring Salamander, *Gyrinophilus subterraneus*, the only stygobiotic vertebrate in the Greenbrier Karst. The snout-vent lengths (SVL) of larvae and adults reach up to 9 and 11 cm, respectively. Photographs by Danté Fenolio. Used with permission

Fig. 19.9 Specimen of *Gammarus minus* from a surface spring (*top*) and of *Gammarus minus tenuipes* from a cave (*bottom*). Both are mature males. The *tenuipes* specimen is about 10 mm in body length. The two specimens are shown at the same scale. Photographs by Michael E. Slay. Used with permission

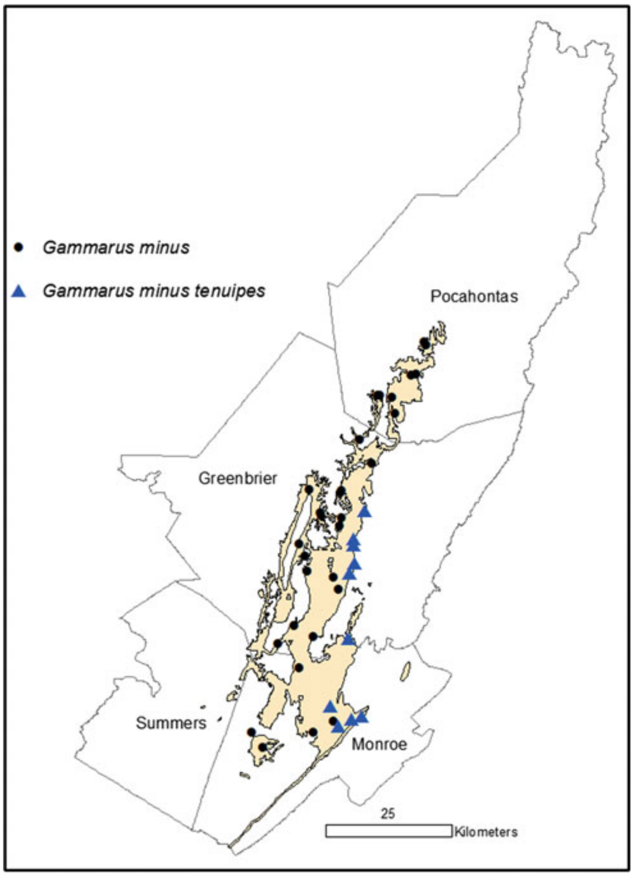


Fig. 19.10 Map of non-troglomorphic cave populations (*black dots*) of *Gammarus minus* and of troglomorphic populations or *Gammarus minus tenuipes* (*blue triangles*) in the Greenbrier Karst. All *G. m. tenuipes* locations are at or near the eastern edge of the Greenbrier Karst

three sister pairs of surface *G. minus* and *G. m. tenuipes* populations. They found a parallel reduction in expressions of only one of the genes, hedgehog, in the *G. m. tenuipes* populations compared to surface populations, but not in the other three genes upstream of hedgehog in the pathway (Fig. 19.12). Their results mirror that of Yamamoto et al. (2004), who showed that hedgehog-related genes are also involved in eye reduction in the cavefish *Astyanax mexicanus*. The conclusion is that selection may target similar genes governing eye development in a vertebrate and an invertebrate during adaptation to the subterranean environment. The implication is that the genetic mechanism behind convergent morphological adaptation among diverse species may be simpler than expected.

Carlini et al. (2013) compared DNA sequence variation and levels of expression in two paralogs of the gene for opsin, a protein that functions in phototransduction and is responsible for photosensitivity, and also in three sister pairs of surface *G. minus* and *G. m. tenuipes* populations. They discovered little sequence variation as well as little difference in synonymous to non-synonymous ratios of amino acid substitutions in the opsin genes among populations, indicating no loss of functional constraint of opsins in the *G. m. tenuipes* populations. They did detect a parallel reduction in opsin expression in the *G. m. tenuipes* compared to surface populations. The conclusion is that functions of the opsins in darkness were likely maintained by selection through pleiotropy because opsins serve an important but unknown function or functions unrelated to vision. Thus, reduction in opsin expression may be advantageous in darkness up to a point, beyond which further reduction is selected against because of the pleiotropic effects. The implication is that loss of optic structure and reduction to loss of optic function may be governed by different genetic mechanisms.

Although surface populations of *G. minus* are ecologically categorized as detrivores, consuming decaying plant material but deriving their nutrition from the associated microbes (Kostalos and Seymour 1976), the food niche of *G. m. tenuipes*is usually assumed to be similar to surface populations. MacAvoy et al. (2016) compared the food niche among surface *G. minus* and *G. m. tenuipes* populations using stable isotopes of carbon and nitrogen. They show that

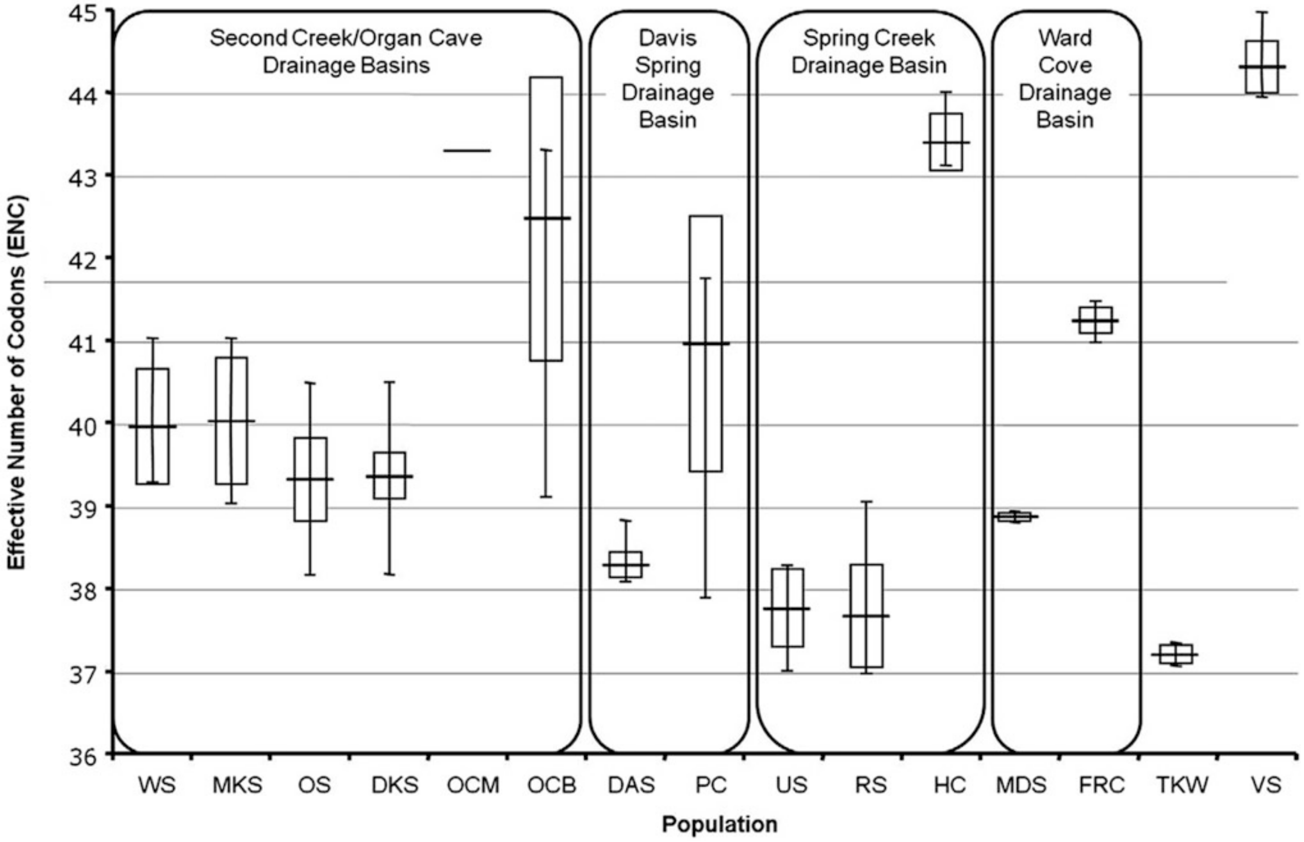


Fig. 19.11 Effective number of codons (ENC; Wright 1990) in COI sequences from 15 populations of *Gammarus minus*. High ENC indicates small effective population size, and low ENC indicates large effective population size. The mean (*horizontal line*), standard deviation (*box*), and range (*vertical bar*) of ENC are indicated for each population (except for OCM where all individuals were fixed for one sequence). Populations are grouped by drainage basin and habitat type (spring populations are designated by the letter *S*, cave populations by the letter *C*, and the single karst window population by the letters *KW*). The *TKW* and *VS* populations were the only sites sampled within their respective drainage basins and so are not grouped with any other population. Reproduced from Carlini et al. (2009). Used with permission

G. m. tenuipes populations clearly have a different nitrogen source than do surface populations, and that the change corresponds to a jump in trophic level, indicating that *G. m. tenuipes* acts as a predator on other invertebrates as well as a consumer of available detritus. Their results confirm the observations that *G. m. tenuipes* preyed on the isopod *Caecidotea holsingeri* in artificial streams in the laboratory (Culver et al. 1991) and that *G. m. tenuipes* preyed on other invertebrates in the field (Fong 2011). Broadening of food niche as an adaptation to the cave environment has also been demonstrated for subterranean amphipods in the Edwards Aquifer (Hutchins et al. 2014) and in a cave salamander (Fenolio et al. 2005) and is consistent with the general theory of expansion of the food niche in food poor environments.

19.7 Competition Among Cave Stream Invertebrates

Caves in the Greenbrier Karst have proved to be useful ecological laboratories for the study of interspecific interactions, just as they have proved to be useful evolutionary laboratories for the study of adaptation and natural selection. The cave streams in the Greenbrier Karst are dominated by four species: the amphipods *Gammarus minus, Stygobromus emarginatus*, *S. spinatus*, and the isopod *Caecidotea holsingeri.* This number of species is quite small compared to most surface communities and even some highly diverse cave stream communities in the Dinaric karst (Fišer et al. 2012), yet with enough species that a variety of combinations of species exist in different caves, making for natural

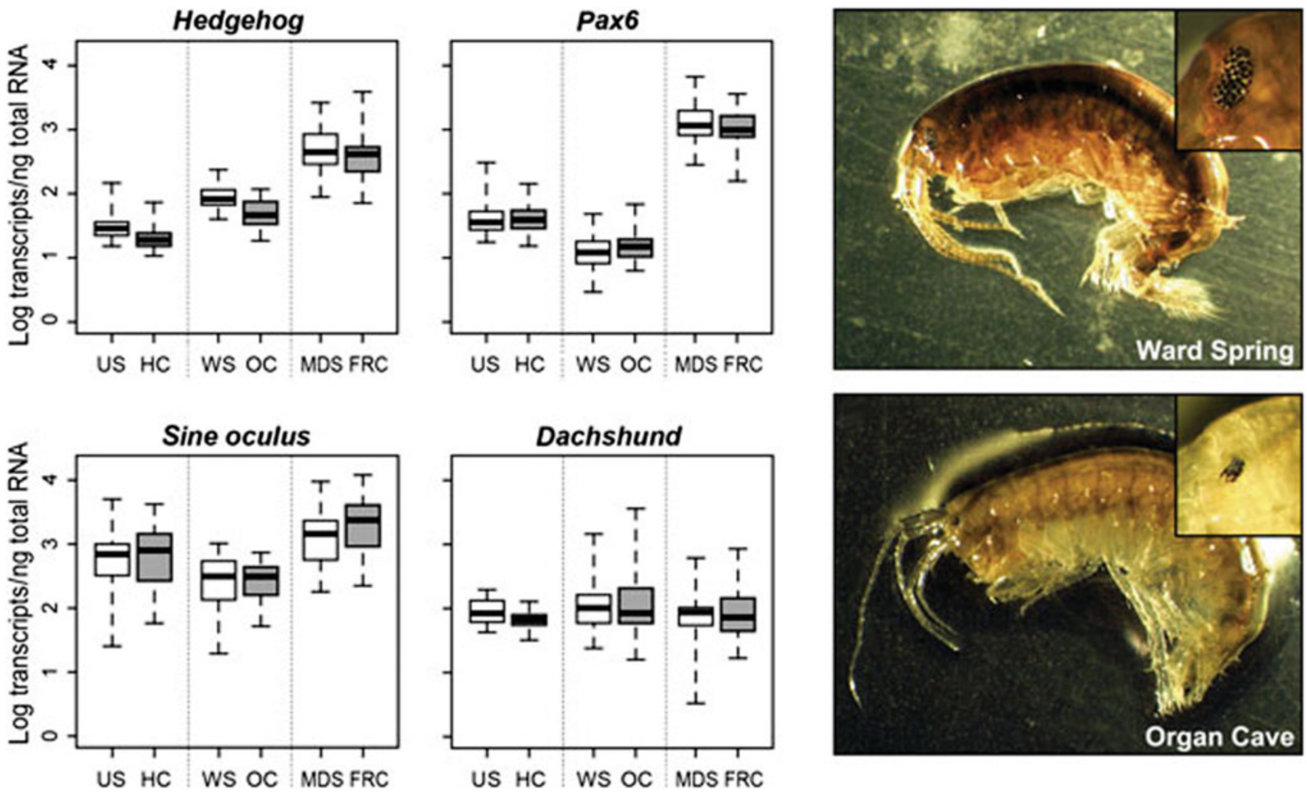


Fig. 19.12 Gene expression in the heads of adult *Gammarus minus*. **a** Hedgehog, **b** pax6, **c** sine oculus, **d** dachshund. *Bars* indicate the median value for each population, whereas boxes denote the upper and lower quartiles. Whiskers indicate the full range of sampled values. Boxes are *shaded gray* for cave populations and white for surface populations. The expression levels are significantly lower in cave compared to surface populations only in hedgehog. **e** An example individual specimen from Ward Spring with normal eye size for surface amphipods. **f** A troglomorphic specimen from Organ Cave illustrates reduced eye size. Insets in (**b**, **c**) show detail of the eyes at the same scale. Reproduced from Aspiras et al. 2012. Used with permission

experiments. In a community of four species, there are six pairs of possible interactions, whereas in a community of 10 species there are 45 such pairs. Because of the patchy nature of the habitat and the resulting patchy distribution of most species, nearby caves often have different species present. This allows these “natural experiments” to be used to study the effects of species additions and removals. The following account is adapted from Culver et al. (1991) and Culver (2012).

In many cave streams in the Greenbrier Karst, there is an alternation between deeps (pools) and shallows (riffles). The amphipods and isopods are highly concentrated in riffles as a result of the concentration of food (especially leaf detritus), increased oxygen, and the absence of salamander predators, which live in pools. In this habitat, the three obvious kinds of interactions are as follows: (1) Species may compete for food, (2) species may compete for space (the underside of gravels), and (3) species may serve as food for other species. All can and do occur in particular situations, but the most universal (and easiest to analyze) is competition for space on the underside of riffles.

The basis for competition for space on the underside of riffles is that when any two individuals meet in a riffle, space is in short supply. It is very easy to observe the behavioral response to most encounters even in a small dish in the laboratory—one or both individuals rapidly move away (Culver 1970). More realistic laboratory experiments were done in a small artificial riffle, where the washout rate of individuals put in the riffle in various combinations could be measured. For competition for space in a riffle is approximately equal to the ratio of the washout rate of species i when species j is present to the washout rate of species i when species j is not present. The resulting estimates of competition can then be compared with field data on the amount of overlap among species in caves and within riffles within a cave. The greater the amount of overlap in the field should correspond with lower intensity of competition for space, because otherwise the species would not be in proximity.

In Organ Cave, when isopod and amphipod species co-occur, they occupy different sized rocks within a riffle, different riffles, or different streams. In this study, laboratory stream studies were combined with perturbation (addition and removal) experiments in the field. Three species pairs of the six possible species pairs were studied:

Stygobromus spinatus and *Caecidotea holsingeri*
Gammarus minus and *Caecidotea holsingeri*
Gammarus minus and *Stygobromus emarginatus*

Laboratory stream competition between *C. holsingeri* and *S. spinatus* was one-sided—the isopod always seemed to dislodge the amphipod. Superficially, the *Gammarus minus*–*Caecidotea holsingeri* pair seemed to compete for space: Fewer individuals remained in the artificial riffle when the other species was present. However, it turned out that *G. minus* was eating, rather than dislodging, *C. holsingeri*. Thus, this was predation rather than competition. In the field, a new interaction appeared. The presence of *G. minus* had a positive effect on the abundance of *S. emarginatus*, rather than the negative effect predicted from the artificial riffle experiments. Subsequent investigation showed that *S. emarginatus* was feeding on the fecal material of *G. minus*. Thus, the two species compete at the microscale (a single rock), but have a commensal relationship at the scale of a riffle.

Even though it initially appeared that these cave streams were dominated by simple competitive interactions for space, the reality was much more complicated, with both negative and positive effects. The teasing apart of these interactions was only possible because of the small number of species involved.

19.8 Protection of Stygobionts

Strategies for the protection of stygobionts necessarily focus on the nature of threats to the fauna and methods to eliminate or mitigate such threats as well as setting criteria for prioritizing sites for protection. Potentially severe threats arise from human activity (see Humphreys 2011) that may directly destroy subterranean habitats such as construction, quarrying, and other resource extraction practices using earth moving equipment. In the Greenbrier Karst, especially in the vicinity of Lewisburg, widespread development of home sites is the most immediate threat because of the increased potential for groundwater contamination from excess household and lawn chemicals and spillage of petroleum products from increased local traffic and delivery of supplies via trucks. The expansion of impervious surfaces associated with urban development also reduces groundwater recharge, which may severely curtail the input of energy into subterranean systems, especially via the epikarst. Fong (2011) suggests that disruption of input of photosynthetically based energy and nutrients in support of the subterranean fauna is a less apparent but continuous and insidious problem, and this is especially true for the Greenbrier Karst because chemosynthesis is unknown in the area. Fortunately, organizations such as the West Virginia Cave Conservancy and The Nature Conservancy are dedicated to protecting many cave entrances and immediately surrounding areas in the Greenbrier Karst (see a list of caves in Chap. 18). However, proper management of not only the entrance areas to caves but the entire recharge area of a cave system is necessary to ensure protection of stygobionts and troglobionts.

A way to set priorities among caves for protection of stygobionts is to emphasize cave systems that are type localities or contain relatively high numbers of species or both. In the Greenbrier Karst, ten caves meet these criteria: Six are type localities for a total of ten species, and seven caves house five or more species each (Table 19.2). Some of these caves are managed by conservancies. McClung and Benedicts Caves are managed by the West Virginia Cave Conservancy and General Davis Cave is owned and managed by The Nature Conservancy. Although this affords some degree of protection, the protected areas include only

Table 19.2 Caves with high stygobiont species richness ($Sp \geq 5$) or are type localities (type: number of type species) or both

Cave	Sp	Type
Organ	8	3
The Hole	7	–
General Davis	6	1
McClung	6	2
Benedicts	5	–
Buckeye Creek	5	–
Fuels fruit	5	–
Court street	4	1
Tub	2	2
Arbuckle	1	1

They are all located within Greenbrier County except for Tub Cave which is located within Pocahontas County the immediate vicinities of the cave entrances and leave the recharge zones unprotected. All of the other caves are also unprotected. Organ Cave stands out because it has the highest species richness at eight and is the type locality for three species. In addition, Organ Cave is also a hot spot in terms of terrestrial biodiversity, housing 13 troglobionts (Chap. 18). This is a large cave system with multiple entrances (Stevens 1988), and although the historic, commercial entrance is gated, all other entrances and all of its recharge area are unprotected and face increased pressure for development of housing and especially intensive poultry operations. At the other extreme is Arbuckle Cave, the type locality of its only stygobiont, *Stygobromus redactus*, and the only location of this epikarstic species within the Greenbrier Karst (the species occurs in only a few caves outside the Greenbrier Karst). Although this is a small cave, it also harbors 12 troglobionts and is the only known site of the beetle, *Horologion speokoites*, the primary habitat of which is likely also the epikarst (Chap. 18). The location of Arbuckle Cave, near the Greenbrier Valley Airport and surrounded by expanding housing development, makes its fauna extremely vulnerable and urgently needs protection. All of the remainder of the unprotected caves, except for Tub Cave, are located in Greenbrier County, and all are subject to threats associated with increased development and changing land use patterns. But it is clear, however, that the Organ Cave system is biologically unique in the Greenbrier Karst and especially deserving a coordinated effort for protection.

References

Aspiras, A.C., R. Prasad, D.W. Fong, D.B. Carlini, and D.R. Angelini. 2012. Parallel reduction in expression of the eye development gene hedgehog in separately derived cave populations of the amphipod *Gammarus minus*. *Journal of Evolutionary Biology* 25: 995–1001.

Besharse, J.C., and J.R. Holsinger. 1977. *Gyrinophilu ssubterraneus*, a new troglobitic salamander from southern West Virginia. *Copeia* 1977: 624–634.

Blaney, R.M., and P.K. Blaney. 1978. Significance of extreme variation to a cave population of the salamander *Gyrinophilus porphyriticus*. *Proceedings of the West Virginia Academy of Science* 50: 23.

Brandon, R.A., and J.M. Rutherford. 1967. Albinos in a cavernicolous population of the salamander *Gyrinophilus porphyriticus* in West Virginia. *American Midland Naturalist* 78: 537–540.

Carlini, D.B., J. Manning, P.C. Sullivan, and D.W. Fong. 2009. Molecular genetic variation and population structure in cave and surface populations of the freshwater amphipod *Gammarus minus*. *Molecular Ecology* 18: 1932–1945.

Carlini, D.B., S. Satish, and D.W. Fong. 2013. Parallel reduction in expression, but no loss of functional constraint, in two opsin paralogs within cave populations of *Gammarus minus* (Crustacea: Amphipoda). *BMC Evolutionary Biology* 2013 (13): 89.

Cook, D.G. 1975. Cave-dwelling aquatic Oligochaeta (Annelida) from the eastern United States. *Transactions of the American Microscopic Society* 94: 24–37.

Culver, D.C. 1970. Analysis of simple cave communities: niche separation and species packing. *Ecology* 51: 949–958.

Culver, D.C. 2012. Species interactions. In *Encyclopedia of Caves*, ed. W.B. White, and D.C. Culver, 743–748. Amsterdam: Elsevier/Academic Press.

Culver, D.C., and T. Pipan. 2009. *The biology of caves and other subterranean habitats*. Oxford, UK: Oxford University Press.

Culver, D.C., and T. Pipan. 2014. *Shallow subterranean habits: ecology, evolution and conservation*. Oxford, UK: Oxford University Press.

Culver, D.C., D.W. Fong, and R.W. Jernigan. 1991. Species interactions in cave stream communities: experimental results and microdistribution effects. *American Midland Naturalist* 126: 364–379.

Culver, D.C., T.C. Kane, and D.W. Fong. 1995. *Adaptation and natural selection in caves: The evolution of Gammarus minus*. Cambridge, MA: Harvard University Press.

Fenolio, D.B., G.O. Graening, B.A. Collier, and J.F. Stout. 2005. Coprophagy in a cave-adapted salamander: The importance of bat guano examined through nutritional and stable isotope analyses. *Proceedings of the Royal Society of London [Biology]* 273: 439–443.

Fišer, C., A. Blejec, and P. Trontelj. 2012. Niche-based mechanisms operating within extreme habitats: A case study of subterranean amphipod communities. *Biology Letters* 8: 578–581.

Fong, D.W. 2011. Management of subterranean fauna in karst. In *Karst management*, ed. P.E. Van Beynen, 201–224. Dordrecht, Germany: Springer.

Fong, D.W. 2012. *Gammarus minus* as a model system for the study of adaptation. In *Encyclopedia of caves*, ed. W.B. White, and D.C. Culver, 341–347. Amsterdam: Elsevier/Academic Press.

Fong, D.W., and D.C. Culver. 1994. Fine-scale biogeographic differences in the crustacean fauna of a cave system in West Virginia, USA. *Hydrobiologia* 287: 29–37.

Fong, D.W., D.C. Culver, H.H. Hobbs III, and T. Pipan. 2007. *The Invertebrate Cave Fauna of West Virginia*, 2nd ed., vol. 16. Barrackville, WV: West Virginia Speleological Survey Bulletin.

Fong, D.W., M.L. Porter, and M.E. Slay. 2012. *Cave life of the Virginias. A field guide to commonly encountered species*. Huntsville, AL: Biology Section of the National Speleological Society.

Holsinger, J.R. 1978. Systematics of the subterranean amphipod genus *Stygobromus* (Crangonyctidae), Part II: Species of the eastern United States. *Smithsonian Contributions to Zoology* 266: 1–144.

Holsinger, J.R., and D.C. Culver. 1988. The invertebrate cave fauna of Virginia and a part of East Tennessee: Zoogeography and ecology. *Brimleyana* 14: 1–162.

Humphreys, W.F. 2011. Management of groundwater species in karst environments. In *Karst Management*, ed. P.E. Van Beynen, 283–318. Dordrecht, Germany: Springer.

Hutchins, B., D.W. Fong, and D.B. Carlini. 2010. Genetic population structure of the Madison Cave Isopod, *Antrolana lira* (Cymothoida: Cirolanidae) in the Shenandoah Valley of the eastern United States. *Journal of Crustacean Biology* 30 (2): 312–322.

Hutchins, B.T., B.F. Schwartz, and W.H. Nowlin. 2014. Morphological and trophic specialization in a subterranean amphipod assemblage. *Freshwater Biology* 59: 2447–2461.

Jones, W.K. 1973. *Hydrology of Limestone Karst in Greenbrier County, West Virginia*. Morgantown, WV: West Virginia Geologic and Economic Survey Bulletin 36.

Jones, W.K. 1997. *Karst hydrology Atlas of West Virginia*, vol. 4. Charles Town, WV: Karst Waters Institute Special Publication.

Kane, T.C., D.C. Culver, and R.T. Jones. 1992. Genetic structure of morphologically differentiated populations of the amphipod *Gammarus minus*. *Evolution* 46: 272–278.

Knapp, S.M., and D.W. Fong. 1999. Estimates of population size of *Stygobromus emarginatus* (Amphipoda: Crangonyctidae) in a headwater stream in Organ Cave, West Virginia. *Journal of Cave and Karst Studies* 61: 3–6.

Kostalos, M., and R.L. Seymour. 1976. Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). *Oikos* 27: 512–516.

MacAvoy, S.E., A. Braciszewski, E. Tengi, and D.W. Fong. 2016. Trophic plasticity among spring vs. cave populations of *Gammarus minus*: examining functional niches using stable isotope and C/N ratios. *Ecological Research* 31: 589–595.

Niemiller, M.L., M.S. Osbourn, D.B. Fenolio, S.K. Pauley, B.T. Miller, and J.R. Holsinger. 2010. Conservation status and habitat use of the West Virginia Spring Salamander (*Gyrinophilus subterraneus*) and Spring Salamander (*G. porphyriticus*) in General Davis Cave, Greenbrier Co., West Virginia. *Herpetological Conservation and Biology* 5: 32–43.

Pipan, T. 2005. *Epikarst—a promising habitat. Copepod fauna, its diversity and ecology: a case study from Slovenia (Europe)*. Postojna-Ljubljana, Slovenia: ZRC Publishing, Karst Research Institute at ZRC-SAZU.

Pipan, T., and D.C. Culver. 2005. Estimating biodiversity in the epikarstic zone of a West Virginia cave. *Journal of Cave and Karst Studies* 67: 103–109.

Salavert, V., C. Zamora-Muñoz, M. Ruiz-Rodríguez, A. Fernández-Cortés, and J.J. Soler. 2008. Climatic conditions, diapause and migration in a troglophilic caddisfly. *Freshwater Biology* 53: 1606–1617.

Shoemaker, C.R. 1940. Notes on the amphipod *Gammarus minus* Say and description of a new variety, *Gammarus minus* var. *tenuipes*. *Journal of the Washington Academy of Science* 30: 388–394.

Sket, B. 2004. The cave hygropetric—a little known habitat and its inhabitants. *Archiv für Hydrobiologie* 160: 413–425.

Stevens, P.J. 1988. *Caves of the Organ Cave Plateau, Greenbrier County, West Virginia,* vol. 9. Barrackville, WV: West Virginia Speleological Survey Bulletin.

Wright, F. 1990. The ‘effective number of codons’ used in a gene. *Gene* 87: 23–29.

Yamamoto, Y., D.W. Stock, and W.R. Jeffery. 2004. Hedgehog signaling controls eye degeneration in blind cavefish. *Nature* 431: 844–847.