

compared the repertoire of social behaviors of cave crayfish to those previously reported with interacting sighted crayfish. The blind crayfish did not exhibit behaviors usually associated with visual displays and posturing. Additional investigations were conducted to determine how cave-adapted blind crayfish responded to novel territories of various sizes. We also used the cave crayfish and to examine their responsiveness to stimuli while monitoring their heart rate as a measure of an internal state. Heart rate is a reasonable measure of the responsiveness of blind cave crayfish to given stimuli even in the absence of observable behavioral changes. This enables the observer to determine if an individual is responsive to and making an assessment of particular cues. Alterations in the crayfish internal physical state, such as when the animal autotomizes its chelipeds, will cause the larger sized animals to tail flip when before they would not. Comparing adult crayfish in an epigeal species to a cave species revealed that the cave crayfish are more likely to tail flip to a given stimulus. Neural modifications in the cave crayfish visual and chemosensory structures were also examined. Troglotic crayfish have a disorganized neuronal ganglion within the eye stalk. In addition, neurons associated with olfaction that arise in the central brain are more numerous in cave crayfish, suggesting that they have more neural processing devoted to olfaction, as an adaptation to cave life.

CAVE DIPLURA OF KENTUCKY

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The world's first named cave campodeid dipluran, *Campodea cookei* [now placed in the genus *Litocampa*] was described from a specimen from Mammoth Cave in 1871 by A.S. Packard. This species is widespread in the caves of southern Kentucky, Tennessee, and southwestern Virginia. *Plusiocampa jonesi* [now also placed in the genus *Litocampa*], originally described by B. Condé in 1949 from a specimen collected in Dunbar Cave in northern Tennessee, has also been found in neighboring Christian County, Kentucky. In eastern Kentucky there is an undescribed species that is closely related to *L. cookei*. This new species is known from caves in Pulaski and Rockcastle counties. Another new species of *Litocampa* has been found in the extreme eastern Letcher County, Kentucky. These specimens appear to be the same as a species first discovered in Rye Cove in Scott County, Virginia. This currently presents a zoogeographic range that is difficult to explain. In all, 64 collections of campodeid diplurans from 44 Kentucky caves were examined. As for the japygid diplurans, *Japyx subterraneus* [now placed in the genus *Metajapyx*] was described by A. S. Packard from Little White Cave [White's Cave, Jr.], near Mammoth Cave, in 1874. The only other record of a japygid from a cave in Kentucky is an unidentified species from Barnes Smith Cave in Hart County.

NEW GENUS AND SPECIES OF CAMPODEID DIPLURAN FROM CAVES IN EASTERN TENNESSEE

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A new genus and species of campodeid dipluran has been identified from Tuckaleechee Caverns and four neighboring caves in Blount County in eastern Tennessee. The new species displays troglomorphic characters indicating a highly adapted cavernicole, with possible affinities to the *henroti* species group of the cave-inhabiting *Litocampa* of Tennessee, Georgia, and Alabama. Morphologically the new genus is somewhat intermediate to the *Plusiocampa* of the Mediterranean region and the *Litocampa* of North America and elsewhere. However, its affinity appears to be closer to the *Litocampa*, from which it is possibly derived. Since the *Plusiocampa* may have evolved from ancestral *Litocampa* as well, the similarity of the new genus to *Plusiocampa* is believed to be an example of parallel evolution.

ANALYSIS OF rRNA GENE SEQUENCES TO STUDY DIVERSITY OF MICROORGANISMS IN MAMMOTH CAVE

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Geochemical factors in streambeds inside Mammoth Cave cannot account fully for the observed rates of limestone dissolution. Microbial effects such as production of acids and acid-forming gases are implicated, but attempts to culture cave bacteria for their identification have had limited success. In this study we use 16S rRNA gene sequences as a means for identifying bacteria in cave

sediment without the need to culture environmental bacterial strains. DNA was extracted directly from cave sediments and bacterial 16S rRNA genes were selectively amplified by PCR. PCT products were ligated into the pGEM cloning and sequencing vector and circular molecules, thus produced were used to transform *E. coli* cells to create a cave clone library. Plasmid DNAs carrying 16S rRNA gene sequences from cave bacteria were isolated and used as templates for automated DNA sequencing. Sequences were compared to online databases and closest genetic matches to the cave bacteria were tabulated. Genetic matches to the cave bacteria include most notably soil inhabitants associated with the atmospheric and aqueous nitrogen cycle, plus some exotic strains and ecological red flags. Clues to the mechanisms by which bacteria promote cave formation are emerging, and applications of biotechnology as a tool for cave research are becoming apparent.

REEVALUATION OF THE TAXONOMIC STATUS OF AN "ALBINO" SCULPIN (ACTINOPTERYGII: COTTIDAE) FROM SUBTERRANEAN WATERS OF THE GREENBRIER RIVER DRAINAGE OF WEST VIRGINIA.

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We reexamined the single known specimen of a depigmented sculpin from Buckeye Creek Cave in the Greenbrier River drainage of West Virginia. This specimen has previously been considered to represent an aberrant albino individual of the banded sculpin, *Cottus carolinae*. The specimen differs from all known species of *Cottus* in the possession of a frenum. In addition, the specimen differs from epigeal mottled sculpin (*Cottus bairdi*), as well as both epigeal and troglomorphic populations of Kanawha sculpin (*Cottus* sp. cf. *carolinae*) in meristic counts, body shape, prickling, development of the cephalic lateralis system, and other characters. This suite of character differentiation is inconsistent with the hypothesis that the specimen is an albino displaying pleiotropically induced anomalies associated with albinism. We urge cavers in the New River drainage, and especially the Greenbrier Valley, to be alert for additional specimens of this unique member of the Appalachian ichthyofauna.

EXPERIMENTAL ANALYSIS OF METABOLIC ADAPTATION OF *COTTUS CAROLINAE* IN RESPONSE TO PHOTOPERIOD AND FOOD AVAILABILITY

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Variation commonly exists between organisms inhabiting epigeal (surface) and hypogean (cave) environments due to the differences between the two habitats. As organisms move from surface to cave environments, they adapt to cave conditions: constant darkness, relatively constant temperatures year-round, low food availability, and high humidity. Fish adapted to cave life often experience reductions in pigmentation, eye size, pelvic fin ray count, and metabolic rate. The focus of this study was to understand metabolic changes in cave-adapted *Cottus carolinae* (banded sculpin) in response to photoperiod and food availability. Metabolic rates of sculpin were measured after brief acclimation to laboratory aquaria. Soon after initial metabolic measurement, sculpin were placed into one of four treatments for a period of eight weeks before final metabolic tests were run. Treatments included: 1) 24 hours dark, low food availability; 2) 24 hours dark, high food availability; 3) 12 hours light; 12 hours dark; low food availability; and 4) 12 hours light; 12 hours dark, high food availability. Results indicated no statistically significant differences existed in fish as a result of photoperiod, food availability, or the interaction of the two. Also, no significant differences existed between laboratory measurements and field measurements from cave and surface environments. However, many variables were identified that may have influenced fish metabolism in the laboratory. Further study is necessary to determine the influences of photoperiod and food availability on metabolism of banded sculpin.

UNPARALLELED EVOLUTION: BLINDNESS, DEPIGMENTATION, AND SCALELESSNESS DO NOT RUN HAND IN HAND AMONG TROGLOMORPHIC FISHES

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Anecdotal evidence have suggested in the past that blindness, depigmentation, and simplification or loss of scales may be an example of parallel evolution among troglomorphic hypogean fishes. We investigated the level of blindness, depigmentation, and scalelessness among 423 families of fishes. Among those with troglomorphic features, blindness was categorized as eyes

present, eyes sunken, microphthalmic, and eyes not visible; pigmentation level was categorized as fully pigmented, mostly pigmented, mostly depigmented, and totally depigmented/albino. For all families of fishes, scalelessness was categorized as "have scales," "do not have scales," or "mixed" (some species have scales, some do not). We could not find reliable information on the scales for 22 of them, usually small, little known families, none of them with hypogean representatives. Of the rest, 257 (64.0%) have scales, 117 (29.2%) do not have scales, and 27 (6.7%) were mixed. There are 18 families of fish with troglomorphic representatives. Of those, seven (38.8%) families have scales, seven (38.8%) do not, and four (22.2%) contain both scaled and scaleless species. Our results suggest that levels of blindness, depigmentation, and scalelessness is different even among species of the same family and that simplification and/or loss of scales are common features among troglomorphic fishes, but that the lack of scales in the family as a whole cannot be considered a preadaptive feature. Different phylogenetic histories, selective pressures, and genetic independence governing these features account for the explanation of these results.

ONE EYE BUT NO VISION: TROGLOMORPHIC *ASTYANAX FASCIATUS* (PISCES: CHARACIDAE) WITH REGENERATED EYES DO NOT RESPOND TO LIGHT

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One of the most intriguing questions in evolutionary biology is the degree to which behavior can be viewed as a consequence of morphology. We explore this issue by examining behavior associated with the loss of phenotypic structure and its presence, using responses to light by characid blind cave fish, *Astyanax fasciatus*, that are eyed and eyeless. Our experiments examine subjects that are epigeal (eyed surface) and troglomorphic (blind cave) forms. We compare their photoresponsiveness with blind cave fish with restored eyes. These are produced transplanting the lens from an epigeal fish into the optic cup of a blind cave form. The lens from the surface fish stimulates growth and development of the eye, restoring optic tissues lost during cave fish evolution. Fish were placed in an aquarium with one half illuminated with dim or bright white light or infrared light, the other half dark. Their photoresponsiveness was examined by scoring their presence in the illuminated or dark half. Our results strongly suggest that both the blind subjects and those with restored eyes are indifferent to the illumination whereas the surface forms are not. Deactivation of the genes responsible for scotophilic behavior and/or lack of appropriate neurological connection may account for these results.

DIFFERENCES IN FEEDING BEHAVIOR, PREY SIZE, AND DIETARY COMPOSITION AMONG BANDED SCULPIN POPULATIONS IN PERRY COUNTY, MISSOURI

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In the early 1990s, unique populations of banded sculpin (*Cottus caroliniae*) were discovered in Missouri, showing troglomorphic adaptations typical of many cave species. Banded sculpin are traditionally crepuscular feeders. We were interested in investigating the differences in feeding behavior of these unique cave sculpin populations from typical surface populations. Stomachs were removed from samples collected for a previous study and analyzed for content. At the study sites, contents were flushed, using a non-lethal method, from the stomachs of fish found and taken to the laboratory for analysis. Initially, results indicated surface sculpin had an average stomach content weight ~6x that of the cave populations, while there was not a significant difference (ANOVA, $p < .05$) in total body weights. Surface sculpin total body weight was only 1.1 times the weight of the cave sculpin. Surface sculpin stomachs contained an average of 21% Diptera and 72% digested material. Organic debris (5%) was also found in the surface sculpin stomachs. In the cave sculpin, the stomachs contained 55% partially digested invertebrate material, 10% Amphipoda, and a higher percent (11%) of organic debris compared to surface sculpin. The cave sculpin stomachs also contained 70% acanthocephalon parasites occurrence, which were not found in the surface populations.

FRESHWATER OLIGOCHAETES (ANNELIDA) IN FINE SEDIMENTS OF CAVE STREAMS AND SEDIMENT CHEMICAL COMPOSITION

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We report on quantitative collections of aquatic oligochaetes from fine sediments of 8 cave streams in Illinois and Missouri, USA. Four of these streams were sampled monthly for one year. Eight annelid genera (*Haplotaxis*, *Dero*, *Pristina*, *Pristinella*, *Limnodrilus*, *Rhyacodrilus*, *Tubifex*, *Varichaetadrilus*) were collected. Some of the species identified are associated with more pristine conditions and others with organic enrichment. Measuring slide mounted specimens, we estimate the minimum volume of worms per unit volume of fine sediment in the cave streams. Examination of monthly samples did not reveal any statistically significant seasonal patterns in worm density or diversity. Sediment samples were analyzed for a variety of chemical constituents. We expected these would be positively correlated with the same constituents in water samples, but no such trend was detected for calcium, magnesium, sodium, and potassium. Metals in sediment samples showed some tendency to co-vary. For example, elevated iron levels in sediment samples were typically associated with increased lead and nickel levels in the sediments. Mercury was detected twice as often (6 of 12 monthly samples) in sediment samples from one wild cave that experiences heavy visitation as it was in three less frequented caves (3 of 12 monthly samples each). Lead was present in most sediment samples but only a few water samples, while Atrazine was detected in few sediment samples, but was more common in water samples.

STUDY OF A CONVERGENT CAVE BEETLE/CAVE CRICKET PREDATOR PREY SYSTEM

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Darlingtonia kentuckensis is a cave dwelling trechine beetle found in caves of the Cumberland Plateau (MP-II) (Barr 1985) that has evolved specialized foraging behavior that allows it to prey upon the eggs of the cave cricket, *Hadenocercus cumberlandicus*. *Neaphaenops tellkampfi*, a trechine beetle found in caves of the Pennyroyal Plateau (MP-I) in west-central Kentucky (Kane & Poulson 1976; Kane & Ryan 1983; Griffith & Poulson 1993), has also evolved similar specialized foraging behavior that allows it to prey upon the eggs of *Hadenocercus subterraneus*. The predator-prey interaction between *N. tellkampfi* and *H. subterraneus* has been previously studied (Kane and Poulson 1976; Griffith and Poulson 1993). Unlike the *N. tellkampfi/H. subterraneus* system, the dynamics of the *D. kentuckensis/H. cumberlandicus* system are unknown. Due to the fact that caves are similar in selective pressures but discontinuous in space, the comparison of *D. kentuckensis/H. cumberlandicus* to *N. tellkampfi/H. subterraneus* may give evidence for convergent evolution.

COMMUNICATIONS AND ELECTRONICS

CAVING LIGHT USING 24 SERIES/PARALLEL WHITE LEDs

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A caving light design using four parallel strings of six Nichia White LEDs in series does not need as many LEDs to match the strings as when they are all wired in parallel. The circuitry is based on the MAXIM 1698 integrated circuit, which has provisions for efficient dimming and switch mode operation using an external MOSFET. By carefully selecting the inductor and other components for low loss, it is possible to achieve efficiencies greater than 90% using surface mount components. The electronics and 24 LEDs are mounted on a 1.25 x 1.75 inch printed circuit board.

SIMPLE WHITE LED LAMPS FOR PRIMARY AND EMERGENCY LIGHTING

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An array of 24 (or more) LEDs can be mounted in a red "Easter Seals" headpiece along with a simple adjustable linear current source that can be powered from any 4-6 volt battery pack. The result is a waterproof lamp, using 4 AA alkaline batteries, which can be adjusted from very dim (25mA current, 100 hours life) to very bright (440mA, 3-4 hours life). The light pattern, with 20° half-beam width LEDs, is similar to a carbide cap lamp (with a large polished reflector), with a large bright area and plenty of side-light. The "rings" and sharp cutoff of halogen lamps are absent. In a 2-week test, the white, even