

## Chapter 21 Amblyopsidae: Cavefishes

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The name Cavefish implies habitation in a subterranean environment, and this small family (Amblyopsidae, Cavefishes; order Percopsiformes, Trout-perches) does possess a number of specializations that allow them to feed in the dark, even though not all species live in caves. Amblyopsids are unique to eastern North American fresh waters. Most species occupy waters of limestone belts to the east or west of the Mississippi River or to the north or south of the Ohio River between the southern limit of Pleistocene glaciation and the northern extension of the Cretaceous Mississippi Embayment (Figs. 21.1–21.3). Developmental and adult features, corroborated with DNA sequences of multiple genes, support a sister-group relationship between Amblyopsidae and Aphredoderidae (Pirate Perches). The Cavefishes have left no fossil record, but the estimated age of the most recent common ancestor of Amblyopsidae was 12.2 mya in the Miocene (Niemiller et al. 2013a).

The family name, Amblyopsidae, is from the Greek roots *ambly-* and *-opsis* meaning dull or dim sight in reference to poorly developed vision associated with the subterranean life of the cave-dwelling species and nocturnal activities of the surface dwellers. The family is unique in

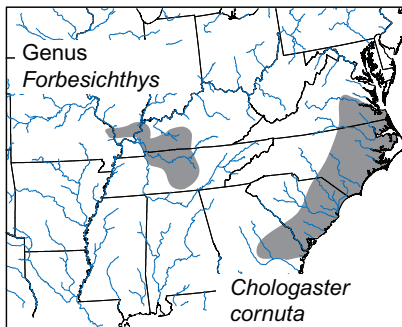


Figure 21.1. Geographic ranges of *Forbesichthys* and the Swampfish, *Chologaster cornuta*.

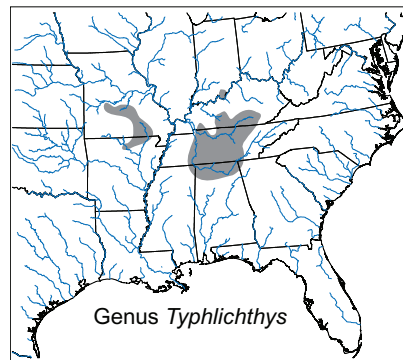


Figure 21.2. Geographic range of *Typhlichthys*.

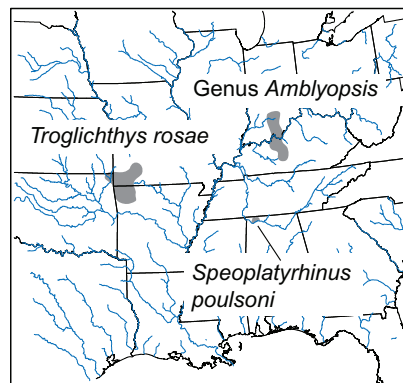


Figure 21.3. Geographic ranges of *Amblyopsis*, the Ozark Cavefish, *Troglitchthys rosae*, and Alabama Cavefish, *Speoplatyrhinus poulsoni*.

having one species (Hoosier Cavefish, *Amblyopsis hoosieri*) that broods the embryos and young in the gill chambers, a reproductive mode unknown in any other fish species in the world. The Amblyopsidae also contain the rarest fish, and probably the rarest vertebrate in North America, the Alabama Cavefish, *Speoplatyrhinus poulsoni*. The anus and urogenital openings of adult Cavefishes are jugular in position but migrate there from the more normal position in front of the anal fin. This transpositional change is known in only one other freshwater fish family, the Pirate Perches,



Plate 21.1. Swampfish, *Chologaster cornuta* (© Emily S. Damstra).



Plate 21.2. Spring Cavefish, *Forbesichthys agassizii*.



Plate 21.3. Southern Cavefish, *Typhlichthys subterraneus*.



Plate 21.4. Alabama Cavefish, *Speoplatyrhinus poulsoni*.



Figure 21.4. Swampfish, *Chologaster cornuta* (photograph from Big Pine Tree Creek, Kershaw County, South Carolina, in December by and used with permission of Dave Neely).

which is the sister family to Cavefishes. All are small, usually between 6–11 cm TL, and most are nocturnal or occupy environments (i.e., swamps or caves) generally inaccessible to humans. Two of the Cavefishes are federally protected as Threatened or Endangered.

The six genera placed in Amblyopsidae form a transformational series from adaptation to darkly stained surface waters to living in eternal lightless subterranean environments. The Swampfish, *Chologaster cornuta*, is strictly a surface water inhabitant (epigeal) (Fig. 21.4); the spring Cavefishes (*Forbesichthys*) are facultative headspring and cave occupants (stygothiles); and the remaining four genera (*Amblyopsis*, *Speoplatyrhinus*, *Troglichthys*, *Typhlichthys*) are obligate cave dwellers (stygothites). Since their discovery by W. T. Craigie in 1842 (in Mammoth Cave, Kentucky) and description by DeKay (1842), amblyopsids have elicited considerable interest from the scientific community. They represent one of the best-documented models of cave adaptation for any known group of cave organisms (Eigenmann 1909; Woods & Inger 1957; Poulson 1963). Information on life history, however, lags far behind that on conservation and phylogeography (e.g., Kuhajda & Mayden 2001; Graening et al. 2010; Niemiller et al. 2012, 2013e). We cannot overemphasize how little we know about the lives of Cavefishes in their natural settings; nearly all the natural history data are from a single source (Poulson 1963, 1964, 1985, 2001ab; summary by Niemiller & Poulson 2010).

Studies of regressive phenotypes (reduction or loss of eyes, depigmentation, hypertrophied tactile structures, many changes in brain parts and size) have focused on obligate cave organisms with putative surface ancestry. The most common argumentation scheme is that extant stygothites have descended from ancestors preadapted to, and that could exploit, the cave environment due either to nocturnal habits or to possession of highly developed nonvisual sensory systems. Others argue that accidental entry into caves led to permanent entrapment or that cave animals are dying lineages that seek refuge in caves. Historically, these hypotheses lacked experimental support (Green & Romero 1997).

Contemporary, more technical studies hypothesize degeneration of structures (e.g., eyes) is caused by the accu-

mulation of selectively neutral mutations and indels (insertions or deletions of bases in the DNA) in genes responsible for the development, structure, and function of eyes or pelvic fins. These mutations are free to accumulate because of the relaxation of selective constraints and are ultimately fixed in a population through genetic drift (i.e., certain alleles might disappear completely thereby reducing genetic variation). Over enough time, a character is destined to disappear if not maintained by purifying selection. A second hypothesis considers regressive traits may be driven by direct or indirect selection in aphotic environments if eye or pelvic fin degeneration is associated with increased fitness. Natural selection may act directly to reduce or eliminate eyes or pelvic fins in cave habitats because having such structures is deleterious or their development and maintenance are energetically costly in energy-limited subterranean habitats. Keep in mind these primary hypotheses when thinking about so-called regressive evolution.

## DIVERSITY AND DISTRIBUTION

### Taxonomic Changes

The most comprehensive morphological study of Cavefishes (Woods & Inger 1957) recognized three genera and five species. Several nominal species were placed in synonymy because diagnostic external features were lacking or considered indistinct. Subsequent study and new categories of data (e.g., allozymes, Swofford et al. 1980; Swofford 1982; CNS staining, Parenti 2006; DNA sequence, Niemiller et al. 2012; Chakrabarty et al. 2014) demonstrated cryptic species, the need for resurrection of species placed in synonymy, or the description of species new to science (Cooper & Kuehne 1974). We recognize here six genera and nine species as well as numerous lineages of *Typhlichthys*, the latter especially for conservation purposes (Table 21.1).

### Inter- and Intraspecific Variation

Several taxa of Cavefishes show evidence of divergence and circumscribed distinct genetic lineages. Putative cryptic

Table 21.1. Scientific and common name (state of occurrence) of the Cavefishes, Amblyopsidae, in alphabetical order by species. The common names Karst Cavefish and Eyeless Cavefish are coined here for communication purposes.

| Scientific and Common Name (state of occurrence)   | References   |
|--|--|
| <i>Amblyopsis spelaea</i><br>Northern Cavefish (Kentucky)  | Niemiller et al. 2012; Page et al. 2013  |
| <i>Amblyopsis hoosieri</i><br>Hoosier Cavefish (Indiana)   | Niemiller et al. 2012; Chakrabarty et al. 2014   |
| <i>Chologaster cornuta</i><br>Swampfish (Coastal Plain, Virginia to Georgia)   | Page et al. 2013   |
| <i>Forbesichthys agassizii</i><br>Spring Cavefish (Kentucky, Tennessee)  | Niemiller et al. 2012  |
| <i>Forbesichthys papilliferus</i><br>Karst Cavefish (Illinois, Kentucky, Missouri)   | Forbes 1882; Layne & Thompson 1952   |
| <i>Speoplatyrhinus poulsoni</i><br>Alabama Cavefish (Alabama)  | Cooper & Kuehne 1974; Kuhajda & Mayden 2001  |
| <i>Troglichthys rosae</i><br>Ozark Cavefish (Arkansas, Missouri, Oklahoma)   | Parenti 2006; Page & Burr 2011; Niemiller et al. 2012                                    |
| <i>Typhlichthys eigenmanni</i><br>Eyeless Cavefish (Arkansas, Missouri)  | Parenti 2006; Niemiller et al. 2012  |
| <i>Typhlichthys subterraneus</i><br>Southern Cavefish<br>(Alabama, Georgia, Kentucky, Tennessee) + ≥10 putative genetic lineages east of Mississippi River; possibly another lineage in Arkansas | Swofford 1982; Niemiller & Fitzpatrick 2008; Graening et al. 2010; Niemiller et al. 2012 |

species (≥10) related to the Southern Cavefish (Fig. 21.5), *Typhlichthys subterraneus*, were identified with multi-locus genetic data (Niemiller et al. 2012, 2013a). Some of these lineages (species) may be sympatric in northern Alabama (J. W. Armbruster pers. comm.). Vagility is presumably low in *Typhlichthys*, and subterranean geographic isolation has played a prominent role in speciation in this genus. The two species of *Amblyopsis*, the Hoosier Cavefish, north of the Ohio River in Indiana and the nominotypical Northern Cavefish, *Amblyopsis spelaea*, south of the river in Kentucky (Niemiller et al. 2013d; Chakrabarty et al. 2014) appear to exhibit little intraspecific genetic variation. Morphological studies of *Forbesichthys* have relied on either meristic (counting of serial parts) features, color only, or a combination of both with some straight-line measurement data (Forbes 1882; Woods & Inger 1957; Clay 1975). Some of these workers considered the Karst Cavefish, *Forbesichthys papilliferus*, as a species distinct from the Spring Cavefish, *Forbesichthys agassizii*, and probably restricted to southwestern Illinois and one location in southeastern Missouri. Clay (1975) and later Niemiller et al. (2013a) in a multi-locus DNA analysis recognized the two as full species. At issue are a lack of samples from throughout the geographic ranges of the two recognized forms. At least four lineages, closely demarcated by hydrological units, were allocated to the Ozark Cavefish, *Troglichthys rosae*, but no formal taxo-

nomic descriptions were made (Noltie & Wicks 2001; Graening et al. 2010). Even the Swampfish, *Chologaster cornuta*, shows evidence of isolation and intraspecific variation judging from an analysis of enzyme products (Swofford 1982). An available name (*Chologaster avitus* Jordan & Jenkins in Jordan 1889) could apply to more northern populations in the Virginia area.

### Geographic Ranges

The Cavefishes are endemic to the south-central United States (Woods & Inger 1957; Lee et al. 1980; Berra 2001; Page & Burr 2011; Table 21.1). This is the only fish family in the world where most of its species are restricted to limestone caves and springs. All other cave-adapted fishes are usually single representatives of more speciose families whose representatives are principally surface-dwelling.

The eight spring and cave-inhabiting species occur exclusively in or near limestone karst formations in northeast Oklahoma, southern Missouri, northern Arkansas, southern Illinois, southern Indiana, central and western Kentucky, south-central Tennessee, northern Alabama, and northwest Georgia (Figs. 21.1–21.3). The monotypic genus *Chologaster* (i.e., Swampfish) occurs only on the Atlantic Coastal Plain. The species ranges from the Chickahominy River (James River drainage), Virginia, to Alta-



Figure 21.5. Southern Cavefish, *Typhlichthys subterraneus*, from Drowned Rat Cave (upper), Pulaski County, and L&N Railroad Cave (lower), Barren County, Kentucky. Genetic analyses revealed the Southern Cavefish comprises  $\geq 10$  distinct and highly isolated lineages likely representing multiple cryptic species (Niemiller et al. 2013b) (courtesy of © 2014 Danté Fenolio / www.anotheca.com).

maha River drainage, Georgia (Page & Burr 1911; Figs. 21.1 and 21.4). The genus *Forbesichthys* is known only in cave-spring systems in the Green River system, southcentral Kentucky, and westward across the Shawnee Hills to southeastern Missouri and south to the Elk River system, southern Tennessee (Page & Burr 2011; Fig. 21.1).

*Typhlichthys* spp. show a disjunct pattern of distribution (Fig. 21.2). The Southern Cavefish occurs east of the Mississippi River primarily in the Cumberland and Interior Low Plateaus from northcentral Kentucky to northwestern Georgia and northern Alabama to the upper Coosa River system with a single population in the Ridge and Valley Province, northwest Georgia. West of the Mississippi River, the Eyeless Cavefish, *Typhlichthys eigenmanni*, occurs in caves of the Ozark Plateau, southern Missouri and northeastern Arkansas (Page & Burr 2011; Niemiller et al. 2012, 2016).

The genera *Amblyopsis*, *Troglichthys*, and *Speoplatyrhinus* are all relatively range restricted (Fig. 21.3). *Amblyopsis* spp. inhabit cave systems of south-central Indiana south to the Mammoth Cave area, central Kentucky (Page & Burr 2011). The Mammoth Cave system is one of few karst regions where three species (Northern Cavefish, *Typhlichthys subterraneus* lineage, Spring Cavefish) of am-

blyopsids are syntopic (co-occur) (Niemiller & Poulson 2010). The Ozark Cavefish is confined to subterranean waters of the Springfield Plateau, southwestern Missouri, northeastern Oklahoma, and northwestern Arkansas in the Missouri, Arkansas, and upper White River drainages (Page & Burr 2011; Figs. 21.3 and 21.6). The Alabama Cavefish occurs only in Key Cave, Lauderdale County, Alabama (Fig. 21.7). A unique lineage of *Typhlichthys subterraneus* is syntopic with the Alabama Cavefish in Key Cave but was only discovered after many visits to the cave (Kuhajda & Mayden 2001).

Most amblyopsid species (excluding the Swampfish) are hypothesized to be limited in the southern extent of their range by the Cretaceous shoreline of the Mississippi Embayment (Woods & Inger 1957). To the north, the southern extent of the Pleistocene glacial boundary may be the limiting factor. Only one species has a range that crosses a major barrier (i.e., Mississippi River). The Karst Cavefish occurs primarily east of the Mississippi River with only one population west of the river (McDonald & Pflieger 1979; Fig. 21.1). *Amblyopsis* has sister-species on the north (Hoosier Cavefish) (Fig. 21.8) and south (Northern Cavefish) (Fig. 21.9) sides of the Ohio River; *Typhlichthys* has Southern Cavefish east of the Mississippi River and Eyeless

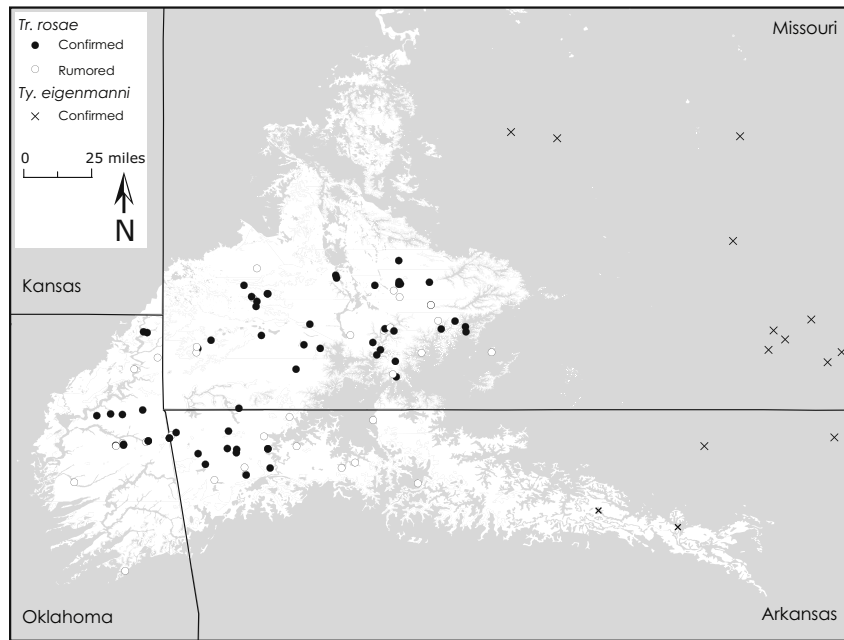


Figure 21.6. Distribution of confirmed sites (black circles) and unconfirmed sites (white circles) for the Ozark Cavefish, *Troglolithys rosae*, and confirmed sites (x) for the Eyeless Cavefish, *Typhlichthys eigenmanni*. The sites are shown in relation to the surface expression of Mississippian Period carbonate bedrocks of the Springfield Plateau (lighter area) in Missouri, Kansas, and Arkansas. The Ozark Cavefish is confined to this physiographic province, and the Eyeless Cavefish is a complex of taxa that comprises one to four distinct lineages in the region (Graening et al. 2010) (redrawn from Graening et al. 2010).

Cavefish (*Typhlichthys eigenmanni*) west of the river. Ranges of Ozark Cavefish and Eyeless Cavefish are restricted by geologic and hydrologic connections based on the stratigraphic layer inhabited by the species (Noltie & Wicks 2001).

### Cavefishes as Non-natives

In the 1950s, John G. Weise, then a graduate student at Southern Illinois University Carbondale, removed 36 live Karst Cavefish from the Pine Hills Research Natural Area, Union County, and transported them to a cave spring in Adams County, Illinois, near Quincy College (now Quincy University) far north of the glacial boundaries. The transplanted population did not survive the introduction in March 1954, and the precise locality where Weise (1957) transplanted the individuals is unknown. He had hoped to establish a reproducing population for research near his home institution, but he subsequently suggested the fish washed downstream and were eaten by predators. This is the only example of a Cavefish being transplanted in nature (Fuller et al. 1999).

## PHYLOGENETIC RELATIONSHIPS

### Higher and Intrafamilial Relationships

Early suggestions of relationships of amblyopsids to other families (e.g., Starks 1904; Regan 1911b) or intrafamilial

relationships (Woods & Inger 1957; Fig. 21.10) occurred before phylogenetic methods and analyses were developed and before all species were formally recognized and diagnosed. In an analysis of muscles and bones, amblyopsids were not related intimately to members of Cyprinodontiformes (Killifishes) (as previously believed) but that they were similar in many features to *Aphredoderus* (Pirate Perches) and *Percopsis* (Trout-perches) (Rosen 1962a). This work was the beginning of a long-shared association of amblyopsids with the two other families in the Percopsiformes. The work also suggested some gadiforms (Cods) and ophidiiforms (Cusk-Eels) might be related closely to amblyopsids. For example, members of the family Carapidae (Pearlfishes, order Ophiidiformes) also have their anus in a jugular position (Rosen 1962a). A later attempt to clarify the relationships of percopsiforms, cyprinodontiforms, and gadiforms (Gosline 1963) was again mostly unsuccessful and plagued by the lack of an explicit methodology to determine monophyletic groups. A landmark study of teleost classification included all three percopsiform families together (Greenwood et al. 1966). And his analysis of branchiostegals and associated structures arrived at an identical conclusion (McAllister 1968). Others questioned the monophyly of Percopsiformes (Rosen 1985; Patterson & Rosen 1989) and suggested that *Aphredoderus* + Amblyopsidae is a more likely relationship that can be supported by physical traits (i.e., removal of Percopsidae and addition of a new name *Aphredoderoides* encompassing *Aphredoderus* + Amblyopsidae).



Figure 21.7. A shallow pool (*upper*) within the type locality cave of the Alabama Cavefish, *Speoplatyrhinus poulsoni*. A unique lineage of the Southern Cavefish, *Typhlichthys subterraneus*, also occurs in the cave. A researcher (*lower*) searches the crystal clear waters of a narrow passage within the cave for Cavefishes (photographs by BRK).

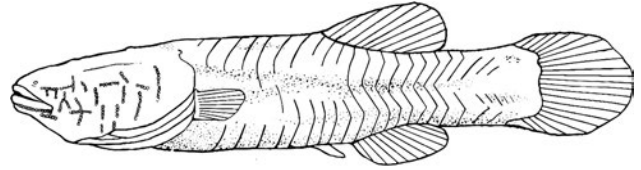


Figure 21.8. Illustration of the holotype of the Hoosier Cavefish, *Amblyopsis hoosieri* (INHS 106675, 75 mm SL) (original drawing by Nathan Coussou; redrawn from Chakrabarty et al. 2014). This species was subsumed for about 170 years under the Northern Cavefish, *Amblyopsis spelaea*, until genetic, geographic, and morphological evidence revealed it as a distinct species (Chakrabarty et al. 2014).

A morphological analysis of paracanthopterygian fishes (sensu Patterson & Rosen 1989) incorporated fossils and relied on 47 osteological characters (majority-rule consensus, PAUP program; Murray & Wilson 1999). The resultant phylogeny indicated that amblyopsids are monophyletic and sister to selected gadiform and ophidiiform taxa, all united as a monophyletic Anacanthini; the genera *Percopsis* and *Aphredoderus* were living sister taxa but shared much closer relationships to several fossil genera; Percopsiformes was monophyletic and includes seven fossil genera and two living genera (*Aphredoderus* and *Percopsis*); amblyopsids form their own order Amblyopsiformes; Anacanthini is sister to Percopsiformes; and the genus *Chologaster* (herein *Chologaster* + *Forbesichthys*) is sister to *Typhlichthys* and these two genera are sister to *Amblyopsis*. In part because of its rarity in collections (only nine known specimens), *Speoplatyrhinus* was not included in the analysis.

A study of gill-arch musculature across numerous teleostome taxa (Springer & Johnson 2004) disagreed with the phylogeny of Murray & Wilson (1996). As in other work, the Percopsiformes were monophyletic and included the three families as Rosen (1962a) originally conceived (Springer & Johnson 2004). Eight characters united Percopsiformes (sensu Rosen 1962a), and the jugular anus and segmented premaxilla were evidence that *Aphredoderus* was sister to Amblyopsidae, and these two families sister to Percopsidae. In an analysis of gill-arch data (Springer & Orrell 2004), Amblyopsidae + Percopsidae were sister to Aphredoderidae. The gill-arch characters have a specialized nomenclature unfamiliar to anyone who does not study the details of gill-arch anatomy in fishes and hence are not elaborated here. Only *Chologaster* (= *Forbesichthys*) *agassizii* was used as a representative of the Amblyopsidae.

Well-resolved phylogenies based on molecular data sets of multiple genes and >1,400 fish taxa clearly show that



Figure 21.9. Lateral (*upper*) and close-up (*lower*) views of the Northern Cavefish, *Amblyopsis spelaea*, from Websters Cave, Breckinridge County, Kentucky, in April (courtesy of © 2014 Danté Fenolio /www.anotheca.com).

Percopsiformes is monophyletic and comprises the Percopsidae, Aphredoderidae, and Amblyopsidae (Betancur-R. et al. 2013ab, 2017; Near et al. 2012b, 2013). The sister-group of Cavefishes is Pirate Perches; the Trout-perches are sister group to Cavefishes + Pirate Perches (Dillman et al. 2011; Niemiller et al. 2012; Fig. 21.11). The order Percopsiformes shows an affinity with Gadiformes, Zeiformes (Dories), and Polymixiidae (Beardfishes) or are closely related to Stylephoridae (Tube-Eyes) (Dillman et al. 2011; Betancur-R. et al. 2013ab, 2017; Near et al. 2013; Fig. 21.11). Notably, all the clades that may be potential sister taxa to Percopsiformes are principally marine and some occur only in deep oceanic environments. Additional taxonomic sampling should aid in clarifying the sister-group of Percopsiformes.

In a formalized Linnaean classification system, overly simplified here, the Percopsiformes are actinopterygians (Ray-finned Fishes), euteleosts (True Bony Fishes), and still part of acanthomorphans (Spiny-rayed Fishes). The presumed close relatives of Percopsiformes comprise >1,370 species in 36 families; only about 20 species are confined to fresh water; 13 (+ numerous genetic lineages) are percopsiforms (in part, Betancur-R. et al. 2013ab, 2017; Nelson et al. 2016).

Variability of 19 genetic loci using nuclear encoded allozymes within and among 39 amblyopsid populations was examined (Swofford 1982; Fig. 21.10C, D). At the time, this was the most thorough geographic sampling for genetic data ever accomplished for this family. Considerable population data are available in Swofford's thesis (see genetics



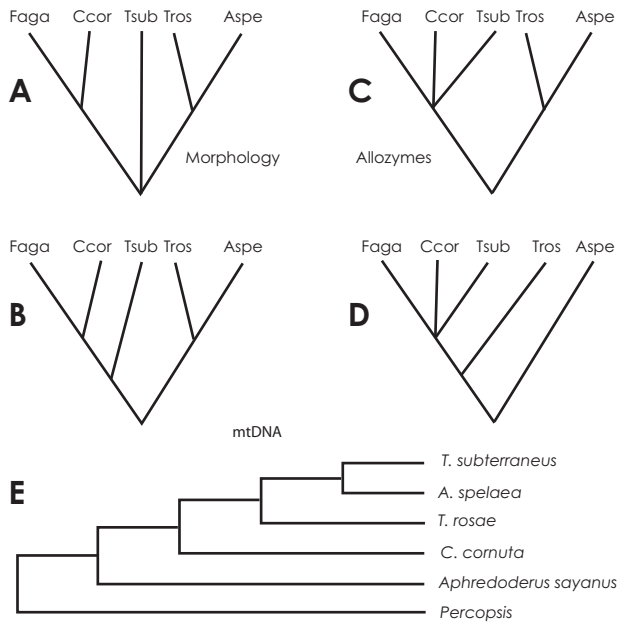


Figure 21.10. Former hypotheses of the interspecific relationships of the Cavefishes, Amblyopsidae, from (A and B) morphological examinations of Woods & Inger 1957, (C and D) 19 genetic loci using nuclear encoded allozymes within and among 39 amblyopsid populations (Swofford 1982), and (E) mtDNA (Niemiller & Fitzpatrick 2008; Near et al. unpublished data) (redrawn from Niemiller & Poulson 2010). Key: Faga = *Forbesichthys agassizii*; Ccor = *Chologaster cornuta*; Tsub = *Typhlichthys subterraneus*; Tros = *Troglichthys rosae*; and Aspe = *Amblyopsis spelaea*. *Speoplatyrhinus poulsoni* is not included in any analysis, and *Forbesichthys agassizii* is not included in the mtDNA analysis.

section), but his final conclusions can be summarized in two different phylogenetic statements (Fig. 21.10CD). The Alabama Cavefish was not included in Swofford's data set.

In a radically different phylogeny based on DNA sequences, the Northern Cavefish was resolved as part of a clade, including the other cavefish species exclusive of the Ozark Cavefish (Bergstrom 1997). The lineages containing the Ozark Cavefish and Eyeless Cavefish may have diverged about 8 mya.

Using the mtDNA ND2 gene and all taxonomic representatives of percopsiform fishes except *Amblyopsis* spp., two fully resolved phylograms and one chronogram (estimated ages of families and nodes) supported monophyly (never really questioned) of Amblyopsidae and *Troglichthys* as the sister to all other Cavefish genera (Dillman et al. 2011; Figs. 21.11 and 21.12). The Swampfish was the sister genus of the remaining spring- or cave-inhabiting genera.

Representatives of all percopsiform taxa and several unique populations of the stygobitic forms resulted in

consistent tree topologies using one mitochondrial and eight nuclear genes (including rhodopsin) (Niemiller et al. 2013a; Fig. 21.13). Age calibrations in these chronograms indicated that the common ancestor for Amblyopsidae was during the Miocene (12–13 mya) and that the origin of Percopsiformes was early Paleocene (60 mya). Consistent features of the least falsified phylogram were *Chologaster cornuta* was always resolved as the sister taxon to all other Cavefish genera; *Troglichthys rosae* was always sister to the spring- and cave-inhabiting species; the genera *Forbesichthys* and *Amblyopsis* were sister taxa; and the genera *Typhlichthys* and *Speoplatyrhinus* were sister taxa. The traditionally recognized *Amblyopsis rosae*, referred to here as *Troglichthys rosae*, is clearly not the sister taxon of *Amblyopsis spelaea* (Fig. 21.13). These contemporary phylogenies make much better geographic and hydrologic sense (Figs. 21.11–21.13), assuming the history of surface and subterranean drainages played a role in speciation.

## Evolutionary Considerations

Previous hypotheses regarding the evolution of life history traits in Cavefishes were constrained by progressive or regressive evolution scenarios. For example, the species with the greatest loss (i.e., regressive evolution) of eye structures or pigment was considered (sensu Poulson 1963) the most cave adapted in the family. The perceived transformation in morphological traits from epigeal to stygophile to stygobite is hypothesized to be ordered. A molecular phylogeny with temporal calibrations of all named taxa, except Northern Cavefish and Hoosier Cavefish, demonstrated that the reconstructed percopsiform ancestor had eyes and pigment (Dillman et al. 2011). In contrast, the ancestor for the amblyopsids is reconstructed with high likelihood values as eyeless and devoid of pigment (Fig. 21.12).

## FOSSIL RECORD

The present geography and environments occupied by amblyopsids probably precludes fossilization. No credible fossil specimens are known for this family (Smith 1981a; Cavender 1986; Wilson & Williams 1992). The common ancestor of Percopsiformes dates at least to the Paleogene (late Cretaceous) or early Paleocene, 60–70 mya (Dillman et al. 2011; Near et al. 2013; Niemiller et al. 2013d; Figs. 21.11 and 21.13). The estimate for divergence of Aphredoderidae and Amblyopsidae occurred in the early

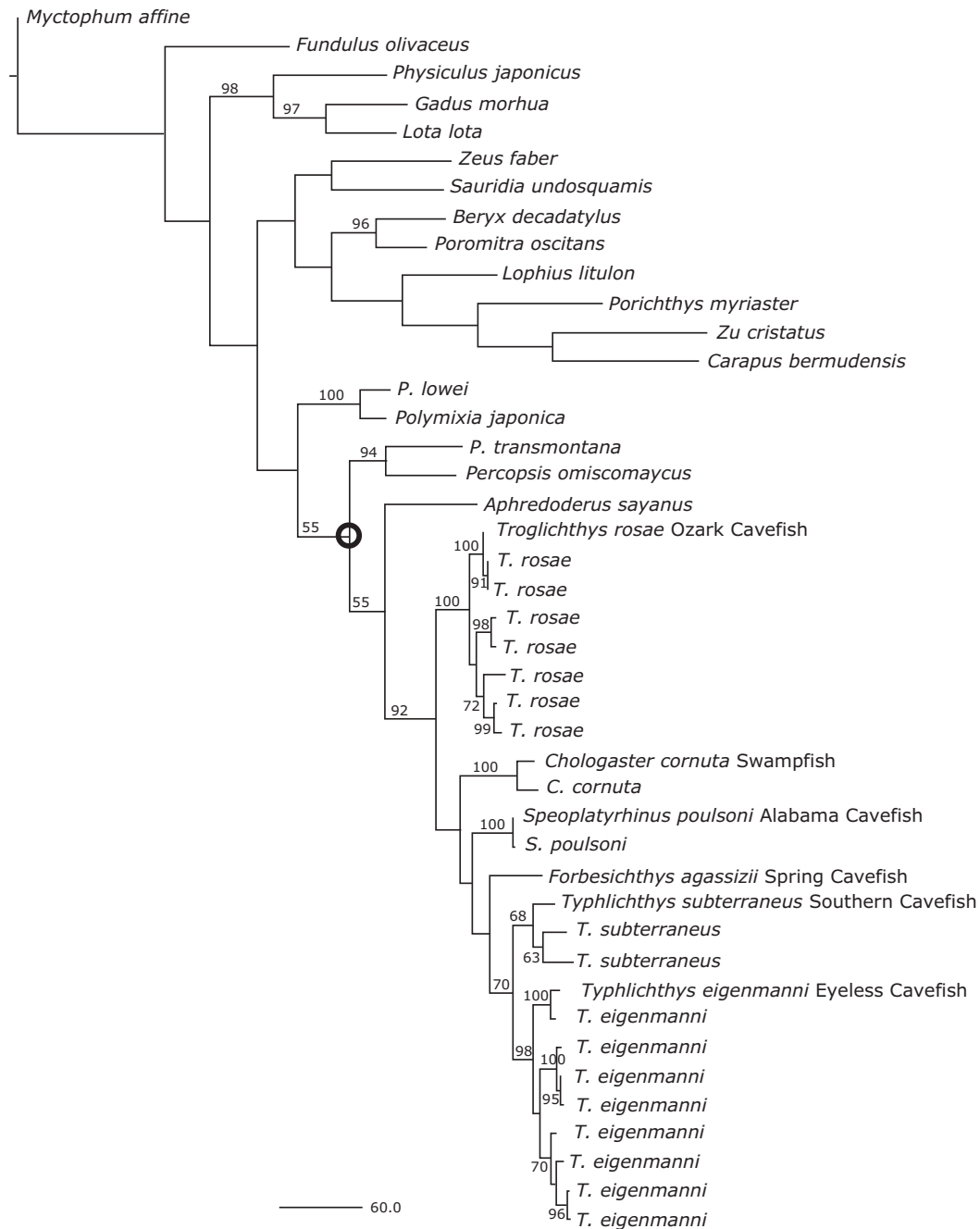


Figure 21.11. Phylogram for the monophyly and phylogenetic relationships of the Trout-perches, Percopsiformes (indicated by a circle), including the Cavefishes, Amblyopsidae, using ND2 gene sequences. Bootstrap support for recovered nodes is shown above the branches. The Northern Cavefish, *Amblyopsis spelaea*, or Hoosier Cavefish, *Amblyopsis hoosieri*, were not included in the analyses (redrawn and modified from Dillman et al. 2011).

Eocene (about 48–56 mya). The most recent common ancestor of amblyopsids is Miocene (12.2 mya) in age (Niemi et al. 2013a). An earlier date for the most recent common ancestor, which was based on a single mitochondrial gene, was Miocene (20 mya) in age (Dillman et al. 2011).

## MORPHOLOGY

### General Morphology

Cavefishes are small (adults from 6.3–11 cm TL) with tiny or rudimentary eyes, a strongly protruding lower jaw (more terminal in Alabama Cavefish), a flattened head,

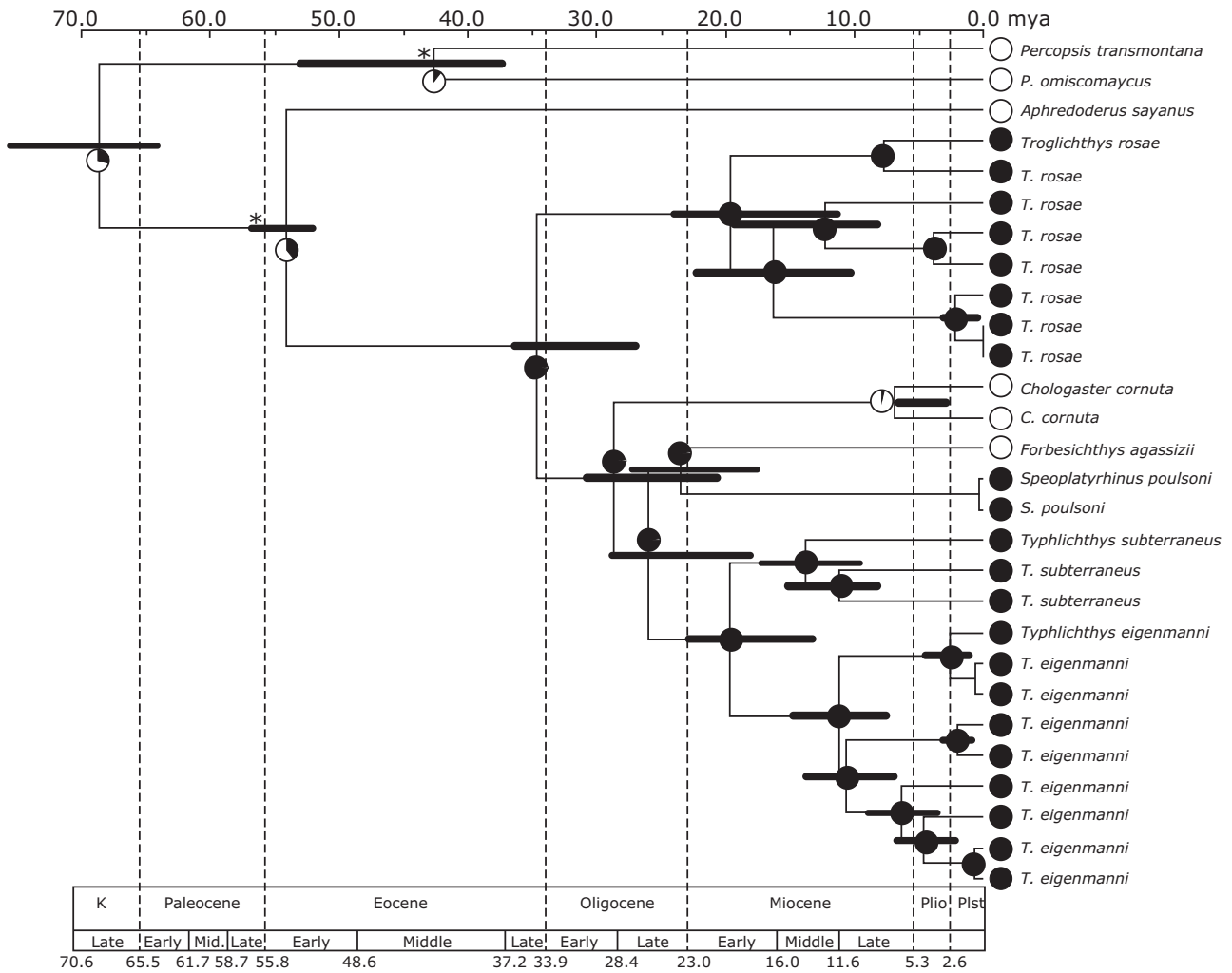


Figure 21.12. Scaled penalized likelihood (PL) chronogram showing age of nodes and confidence intervals ( $\pm$ SD; solid bars) for the Trout-perches, Percopsiformes, including the Trout-perches, Percopsidae, Pirate Perches, Aphredoderidae, and Cavefishes, Amblyopsidae. Pie charts at nodes show proportional likelihoods of the character state of eyed and pigmented (white) or eyeless depigmented (black). Calibrated nodes for PL analysis are shown with an asterisk. The Northern Cavefish, *Amblyopsis spelaea*, or Hoosier Cavefish, *Amblyopsis hoosieri*, were not included in the analyses (redrawn and modified from Dillman et al. 2011).

and tubular anterior nostrils (Figs. 21.4 and 21.14–21.16). The stygobitic genera have vestigial eyes under the skin and have a vestigial lens or no lens at all, lack ciliary muscles (i.e., muscle controlling lens shape), and have reduced or absent eye muscles; eye parts are variable within and between individuals (Fig. 21.17). No eye structures have been found in the Alabama Cavefish. The tubular anterior nostrils direct water intake to a complex olfactory rosette. The anus and urogenital openings are located between the branchiostegal membranes (in adults). The feature of having the anus and urogenital openings located between the branchiostegal membranes is significant phylogenetically because these features start out in the normal posi-

tion at the anterior insertion of the anal fin but migrate forward to the jugular position similar to the Pirate Perch. All species in the family have small, thin, embedded, and imbricate (overlapping) cycloid scales; the circulars are broken posteriorly by secondary radii and anteriorly by primary and secondary radii (Woods & Inger 1957). Annuli develop and are used to age individuals (Poulson 1963), but ages are neither precise nor accurate. The lateralis system and sensory papillae (neuromasts) are in rows on the head, body, and caudal fin (Fig. 21.14). The pelvic fins are tiny or absent. The single dorsal fin has 0–2 weak spines and 7–12 rays and lies slightly in front of the anal fin. The anal fin has 0–2 weak spines and 7–11 rays; the

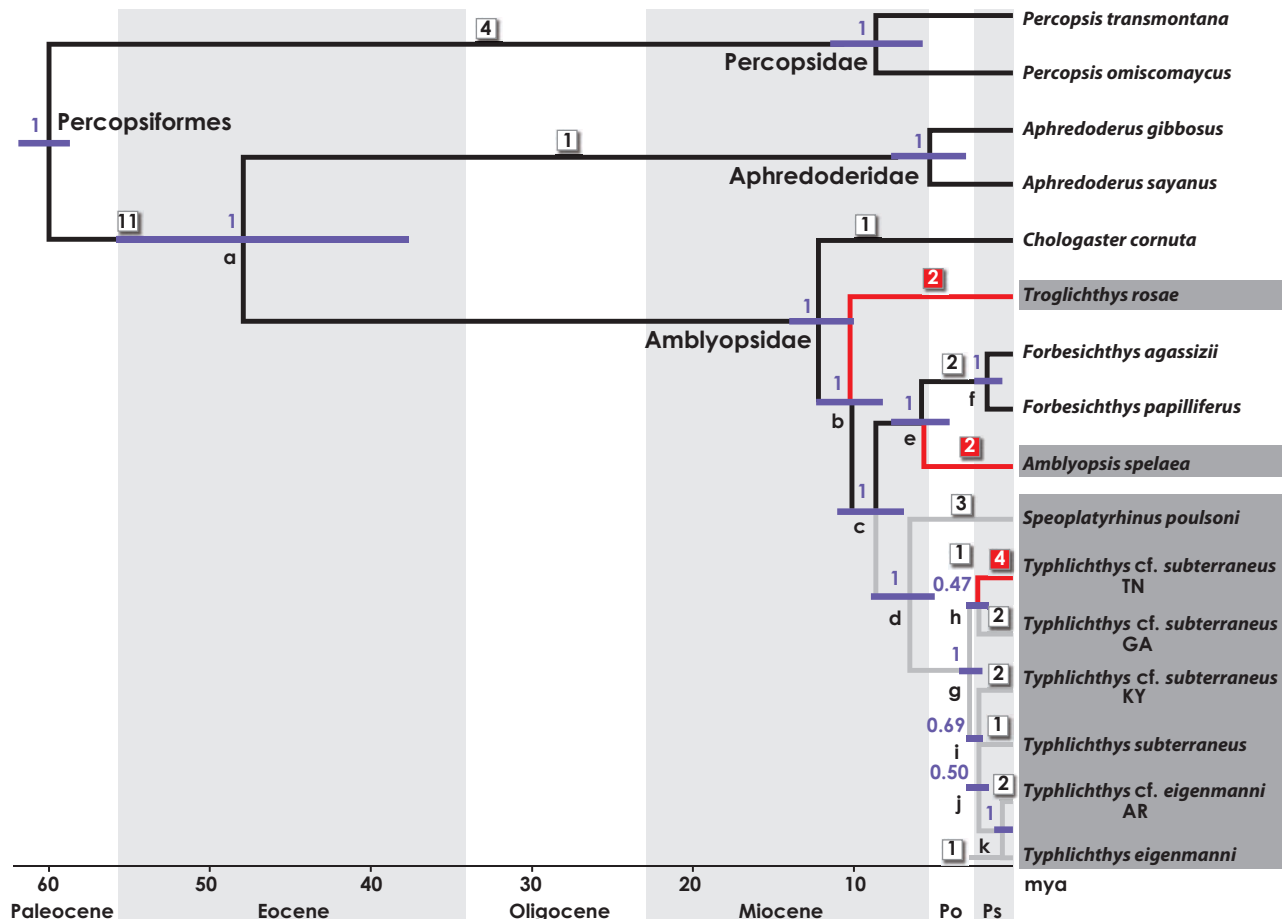


Figure 21.13. Chronogram of Trout-perches (order Percopsiformes), including the Trout-perches, Percopsidae; Pirate Perches, Aphredoderidae; and Cavefishes, Amblyopsidae. The chronogram was inferred from a multi-locus divergence time analysis from sequencing of a 798 base pair section of the rhodopsin gene. Blue bars at nodes represent 95% highest posterior density intervals of age estimates. Clade posterior probabilities are given by blue numbers next to nodes. Cave lineages are indicated by dark gray tip labels. Branches in black are reconstructed as surface and gray branches are reconstructed as cave based on a parallel evolution model. Nonsynonymous substitutions in rhodopsin (white or red square) are mapped above branches. Lineages with loss-of-function mutations in rhodopsin are indicated by red branches and red squares. The Northern Cavefish, *Amblyopsis spelaea*, included samples of the Hoosier Cavefish, *Amblyopsis hoosieri* (redrawn from Niemiller et al. 2013a).

pectoral fin, 9–12 rays; the caudal fin, 9–16 branched rays; branchiostegal rays, 6; pyloric caeca on stomach, 2–4; lateral line absent or incomplete. The myodome, a post-orbital skull cavity housing eye muscles that is present in many teleost fishes, is lost in all species. The palatine and vomer are toothed, and the premaxilla segmented. The gill rakers are blunt or absent. An air bladder is present. The postcleithrum, a pectoral girdle bone often bearing a spine associated with the pectoral fin, is present in some species but absent in *Amblyopsis*, *Troglitchthys*, and *Speoplatyrhinus*. The orbitosphenoid, basisphenoid, and suborbital shelf (bones of the eye orbit) are absent. Verte-

brae number 27–35. General physiognomy, eye size and loss, degrees of pigmentation, sensory pore patterns, and fin shapes show general transformations of morphology with major environments occupied (i.e., swamps, springs, caves) (Figs. 21.14 and 21.15).

### Pigmentation

Only Swampfish and *Forbesichthys* spp. have obvious pigmentation (yellows, tans, and browns) (Figs. 21.4 and 21.16); the other three genera are essentially white, translucent, or colorless (some individuals have a few embed-

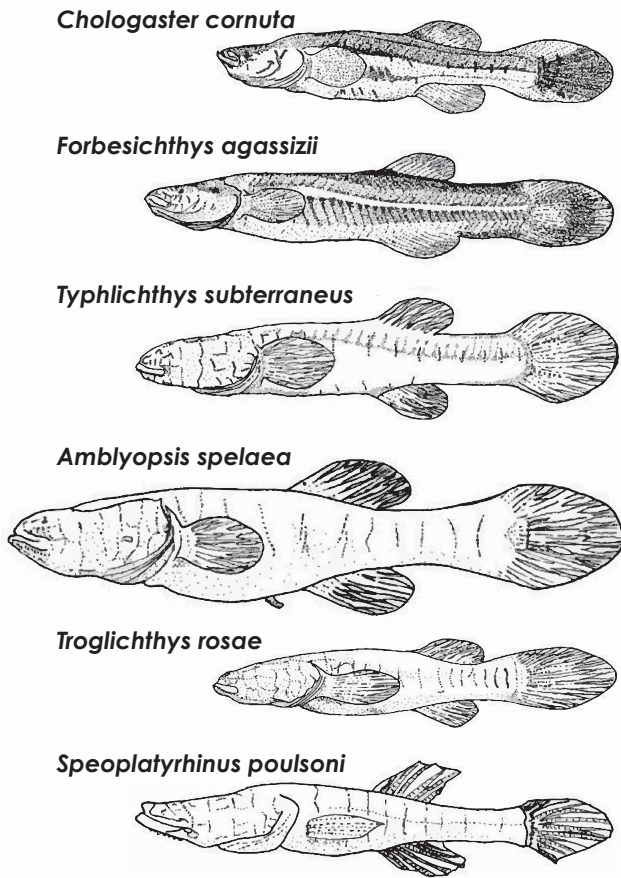


Figure 21.14. The Cavefishes, Amblyopsidae, include epigean (top), stygophilic (second from top), and stygobitic (lower four) species that exhibit a range of stygomorphy. The fish illustrated are drawn to relative scales of average-sized adults. Note the different physiognomy (body form), pigmentation or lack thereof, sensory pore patterns, and fin shapes (redrawn from Niemiller & Poulson 2010).

ded, microscopic melanophores) or have some pinkish (even red) color near the gill area from blood pigments (Figs. 21.5, 21.9, 21.18, and 21.19). When kept in a lighted environment for  $\geq 3$  months, individuals of *Amblyopsis* (normally unpigmented to the unaided eye) developed a dusky color on the flanks, caudal peduncle, and overlying the myosepta (Woods & Inger 1957). *Amblyopsis* spp. have only a few less pigmented melanophores than the *Forbesichthys* spp., but its melanophores have less melanin and are more fully dispersed. Pigmented melanophores occur in Eyeless Cavefish, Southern Cavefish, Ozark Cavefish, and Alabama Cavefish, but they are more reduced than those of Hoosier and Northern Cavefishes (Eigenmann 1909; Cooper & Kuehne 1974). Young Hoosier and Northern Cavefishes have a few punctate pigmented melanophores with little melanin, and these are lost or decrease

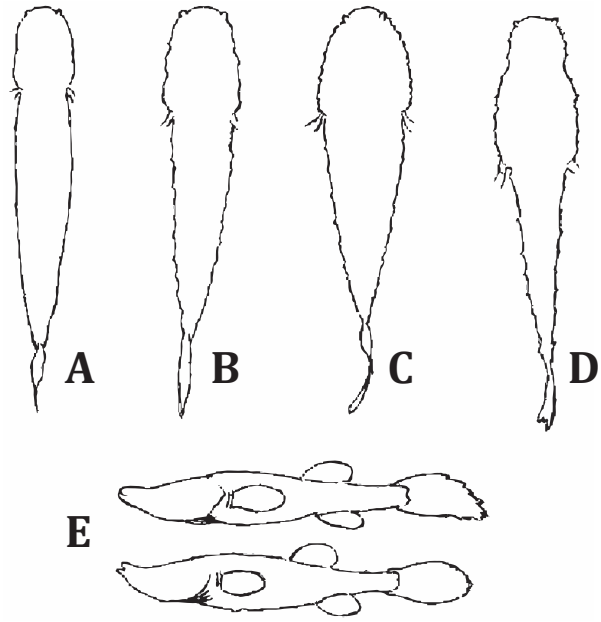


Figure 21.15. Scale drawings of the body shapes and relative head sizes of Cavefishes, Amblyopsidae. With increasing cave adaptation heads become relatively larger and bodies smaller. Dorsal views from left to right are the (A) Spring Cavefish, *Forbesichthys agassizii*; (B) Southern Cavefish, *Typhlichthys subterraneus*; (C) Northern Cavefish, *Amblyopsis spelaea*; and (D) Alabama Cavefish, *Speoplatyrhinus poulsoni*. These are neotenic trends as suggested by the side views (E) of a 48 mm SL Alabama Cavefish (upper) and a juvenile 13 mm SL Northern Cavefish (lower) (redrawn from Niemiller & Poulson 2010).

in amount with size and age even if young are kept in a lighted environment (Eigenmann 1909). Drawings of pigmented melanophores demonstrate only small amounts of melanin are present in species of *Amblyopsis* and Ozark Cavefish (Niemiller & Poulson 2010:200, fig. 7); pigment cells in Alabama Cavefish are closer in morphology and density to those of *Typhlichthys* spp. but are unique (less linear, less restricted to the myosepta) (Cooper & Kuehne 1974).

### Gill Membranes

Gill membranes and potential space to brood embryos vary among Cavefishes. The gill membranes range from being tightly connected (Swampfish, Karst Cavefish, and Spring Cavefish) with little room in the gill chambers to hold embryos or young (Rohde et al. 1994) to rather broadly spacious in Alabama and Hoosier Cavefishes. Only in the latter species has embryo brooding and carrying of young in the gill chambers been confirmed (Eigenmann 1909).



Figure 21.16. Lateral view (upper) of the stygophilic Karst Springfish, *Forbesichthys papilliferus*, and a closeup view (lower) of the head of the species. Note the tubular nostrils, which are typical in all Cavefishes, Amblyopsidae (courtesy of © 2014 Danté Fenolio / www.anotheca.com).

Fins

The fins of Cavefishes show several instances of loss or uniqueness. Only Northern and Hoosier Cavefishes have pelvic fins (with 0–6 rays, modally 4) (Figs. 21.8 and 21.9). Neoteny may explain loss of the pelvic fins in the remaining taxa (Figs. 21.14, 21.16, 21.18, and 21.19) with a relaxation of this trait in *Amblyopsis* spp. (Armbruster et al. 2016). The majority of researchers that counted fin rays in amblyopsids reported only rays and no spines in the dorsal and anal fins (e.g., Woods & Inger 1957; Etnier & Starnes 1993; Niemiller & Poulson 2010). Nelson (2006) reported 0–2 weak spines in the dorsal and anal fins of amblyopsids (repeated in Page & Burr 2011; Nelson et al. 2016). The Alabama Cavefish is unique in the family in lacking branched caudal-fin rays and having incised membranes along the tips of the rays (Cooper & Kuehne 1974; Fig. 21.18).

Skeleton and Muscles

Studies of the skeleton, gill-arch muscles, and jaw musculature were undertaken to search for phylogenetically infor-

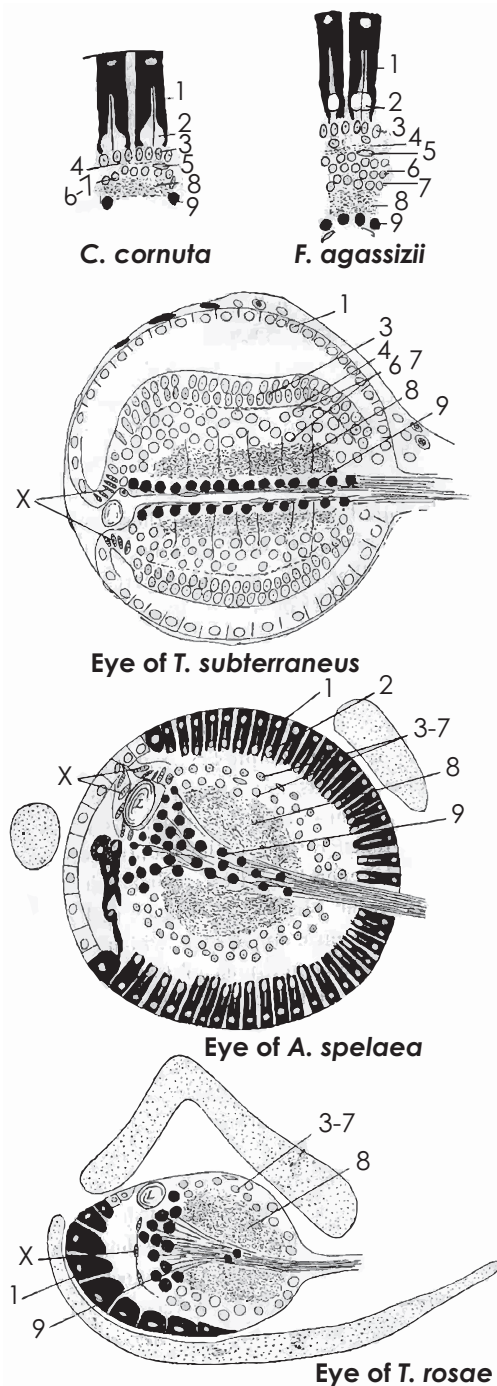


Figure 21.17. Scale drawings of the vestigial eyes of Cavefishes, Amblyopsidae, with the retinas of Swampfish, *Chologaster cornuta*, drawn to the same scale (thus complete eyes would be about 10 times as large). The degenerate lens or its parts are designated with an “X.” The vestigial eye muscles of the Northern Cavefish, *Amblyopsis spelaea*, are not shown. Numbers of the retinal layers are identified: 1. Pigment epithelium; 2. Rods and cones; 3. Outer nuclear layer; 4. Outer plexiform layer; 5. Horizontal cells; 6–7. Inner nuclear layer; 8. Inner plexiform layer; and 9. Ganglion layer (redrawn from Niemiller & Poulson 2010).

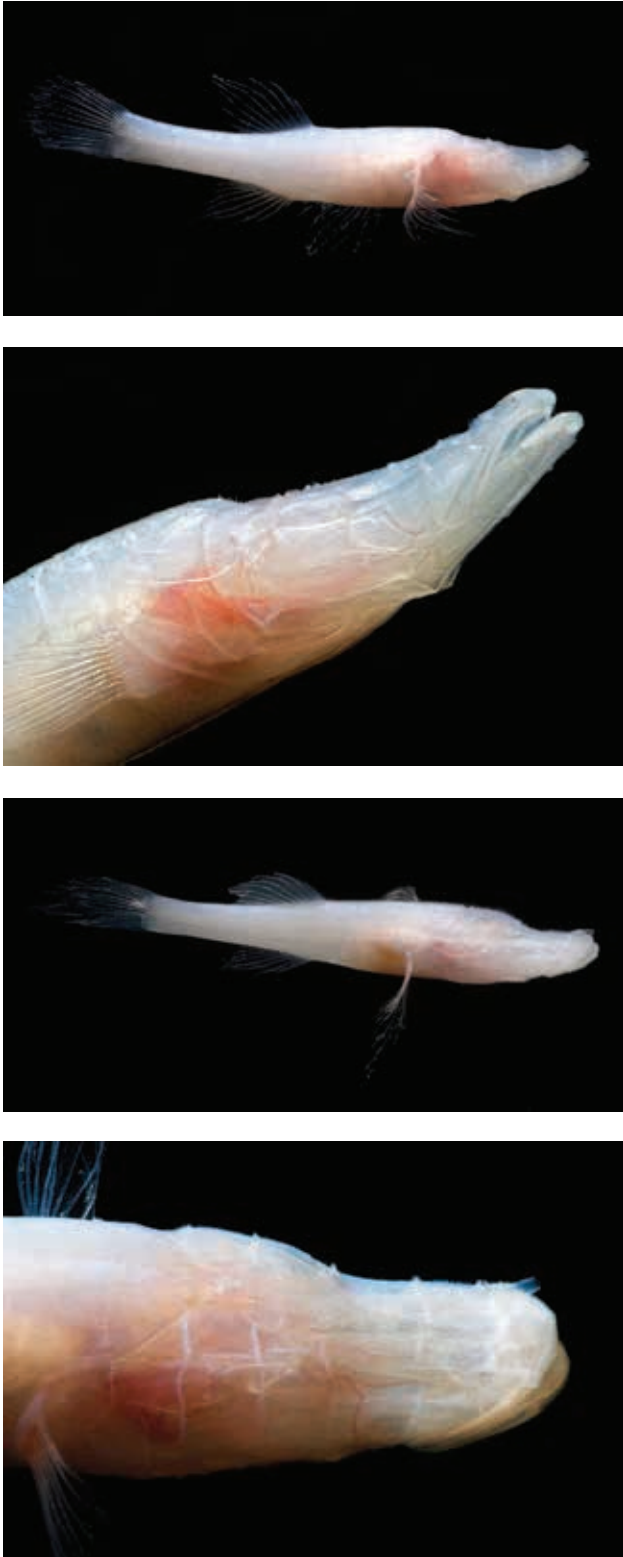


Figure 21.18. Lateral (upper) and close-up (upper middle) views of an adult Alabama Cavefish, *Speoplatyrhinus poulsoni*, and similar views of a juvenile Alabama Cavefish (lower middle and lower). Through allometric growth during ontogeny, the species undergoes changes in body shape (e.g., degree of head flattening). The species is restricted to only one cave (Fig. 21.7) (courtesy of © 2014 Danté Fenolio / www.anotheca.com).



Figure 21.19. An adult (upper) and juvenile (lower) of the Ozark Cavefish, *Troglichthys rosae* (courtesy of © 2014 Danté Fenolio / www.anotheca.com).

mative characters but rarely for descriptive purposes only. Detailed anatomical studies are unavailable for any species of Amblyopsidae, and illustrations of an entire amblyopsid skeleton are not published. For example, Starks (1904) described the skeleton of the Northern Cavefish, apparently from one specimen, but included no illustrations. Photographs and illustrations are available for scales (Woods & Inger 1957); the premaxilla, maxilla, jaw suspension, preoperculum, jaw muscles, shoulder girdle, caudal skeleton, dorsocranium, basicranium, and flank scales (*Forbesichthys agassizii* as *Chologaster*, Rosen 1962a); the upper jaw suspension, caudal skeleton, and opercular apparatus (*Amblyopsis*, Rosen 1962a); the pharyngobranchial apparatus and caudal skeleton (*A. spelaea*, Rosen 1962a); the branchiostegals (*Amblyopsis*, McAllister 1968); dorsal and posterior views of gill-arch muscles (*Forbesichthys*, Springer & Johnson 2004); and rather primitive and incomplete drawings of skulls (*F. agassizii* as *Chologaster*, and *A. spelaea*, Gosline 1963). The most phylogenetically informative characters were those associated with gill arches, the caudal skeleton, the branchiostegal rays, and the skull (Rosen 1962a; Gosline 1963; McAllister 1968). Major conclusions from examination

of internal morphology consistently showed the same shared-derived traits with other families (Aphredoderidae, Percopsidae) of the order Percopsiformes (Springer & Johnson 2004). Examination of cleared and stained specimens from every genus led Armbruster et al. (2016) to conclude that discovery of phylogenetically informative characters within the Cavefishes is complicated due to convergence and morphological conservation. They asserted that evolution of traits in the amblyopsids is likely a result of neoteny and convergence of traits.

### Sense Organs: General Trends

Amblyopsids are often compared based on their sensory traits in relation to the primary environment they occupy (i.e., from swamps, to springs, to caves). For example, comparing similar-sized Spring Cavefish (a stygophile) and Northern Cavefish (a stygobite) helps place often complex anatomical structures and their functions into the context of presumed cave adaptation (Fig. 21.20). Such compari-

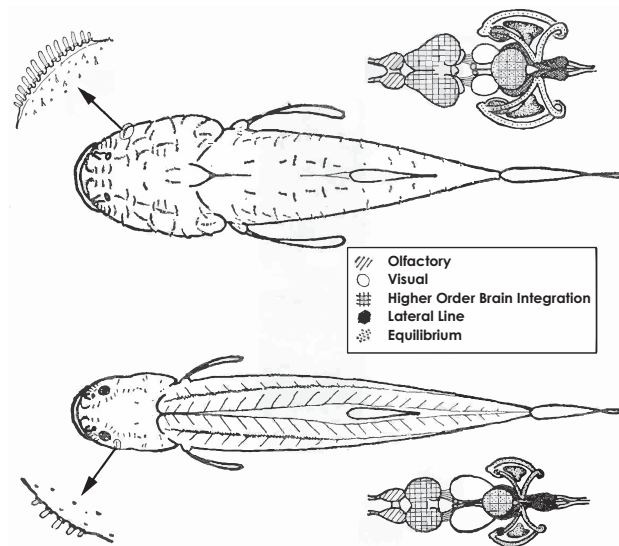


Figure 21.20. Scale drawings contrasting the stygobitic Northern Cavefish, *Amblyopsis spelaea* (upper), with its stygophilic relative the Spring Cavefish, *Forbesichthys agassizii* (lower). Compared with the Northern Cavefish, each of the lateral-line rows in the Spring Cavefish has fewer neuromasts, they are less elevated above the skin, and their associated brain centers (lateral line) are less hypertrophied, but the Spring Cavefish has external eyes and larger optic lobes (visual). In addition, the presumed tactile receptors (shown by the enlarged lateral-line rows) are smaller and fewer in kind than for the Northern Cavefish. Note also the difference in semicircular canals and otoliths (equilibrium); however, the olfactory lobes of the brain do not differ in size (Poulson & White 1969) (redrawn from Niemiller & Poulson 2010).

sons provide insights into eye loss, other sensory structure enhancement, and depigmentation. General trends include that with nocturnal habits and increasing cave adaptation, increases occur in olfactory rosette development, telencephalon size, cerebellum volume, semicircular canal size (dynamic equilibrium receptors), otolith size (static equilibrium receptors), and neuromast number (Poulson 1963). Most of these trends are observable when brains of a representative of *Chologaster*, *Forbesichthys*, *Amblyopsis*, and *Speoplatyrhinus* of about equal size are carefully compared (Figs. 21.20 and 21.21). These trends also make intuitive sense because hypogean species of Cavefishes live in an eternally lightless environment and have no visible eyes but clearly have enhanced sensory perception as evidenced by more skin surface neuromasts, larger olfactory organs, and a more pronounced development of equilibrium structures. For example, the epigeal Swampfish has just over 20 neuromasts in rows 2–4, but the hypogean Northern Cavefish has nearly 40 neuromasts in the same region. Similarly, in similar size specimens, the mean optic lobe mass in Swampfish is 2.12 g and 0.94 g in Ozark Cavefish.

### Sensory Organs: Lateral-line System

The lateral-line system is highly modified in Cavefishes and was earlier thought to be a series of tactile organs. The numerous lines of pores or ridges on both the head and body are a part of the lateral-line system that is sensitive to water movement. Sense organs, referred to as neuromasts, consist of several sensory cells with cilia embedded in a gelatinous cupula (Fig. 21.22). Unlike the stygophilic Spring Cavefish, the stygobitic taxa have fully exposed neuromasts (Eigenmann 1909; Poulson 1963). In *Amblyopsis*, neuromasts show alternate right-left orientation and the neuromast cupulae have their central and denser area asymmetrically placed in the swollen apex (Fig. 21.22). Both *Troglichthys* and *Amblyopsis* apparently have fewer neuromasts than *Typhlichthys*; all their neuromasts in a particular ridge are oriented in one direction, and their cupulae are symmetric and tapered toward the apex (Poulson 1963). Enhancement of mechanoreception in fishes that live in caves appears to be the most common adaptive strategy (Soares & Niemiller 2013) in species around the world and is clearly a primary sense in amblyopsids (Fig. 21.23).

### Sensory Organs: Vision

Only Swampfish and *Forbesichthys* spp. have visible eyes or eye spots; the remaining genera have no visible eyes at



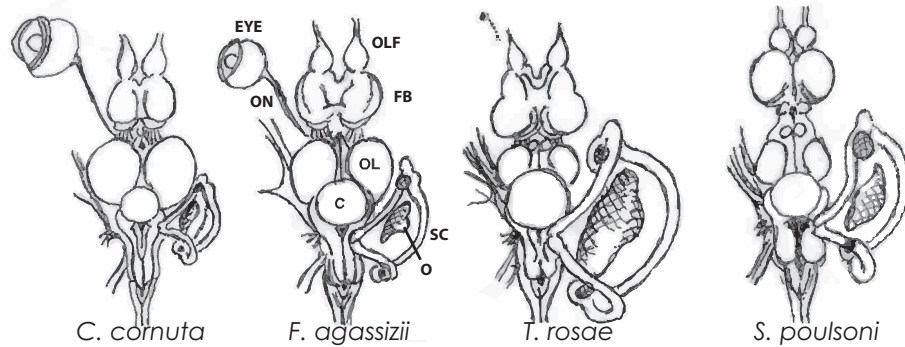


Figure 21.21. Drawings of eyes and optic nerve (ON) (where present), brains, and semicircular canals with otoliths in the Swampfish, *Chologaster cornuta*; Spring Cavefish, *Forbesichthys agassizii*; Ozark Cavefish, *Troglichthys rosae*; and Alabama Cavefish, *Speoplatyrhinus poulsoni* (redrawn from Niemiller & Poulson 2010). The Swampfish has the largest eyes and optic lobes (OL) and smallest forebrain (FB), cerebellum (C), semicircular canals (SC), and otoliths (O). The Spring Cavefish has intermediate brain proportions. The Ozark Cavefish has small optic lobes and the largest forebrain, cerebellum, semicircular canals, and otoliths, but shows the convergent proportions also seen in the Southern Cavefish and Northern Cavefish. The optic lobes of the Alabama Cavefish are even smaller than the other species, but its cerebellum and semicircular canals are not as hypertrophied. Interestingly, no trends are apparent in relative size of the olfactory lobes (OLF) at the front of the brain.

the skin surface. *Forbesichthys* spp. have smaller eyes and optic lobes (Fig. 21.21) and a thinner retina than Swampfish. Ciliary muscles are lacking in *Forbesichthys* spp. (Eigenmann 1909), so unlike those of Swampfish, they probably do not form images. *Forbesichthys* spp. apparently can discriminate light intensity (Eigenmann 1909; Poulson 1963). The lens of *Amblyopsis* is lost as an embryo, has a retina with pigment but no outer reticular layer, and has some eye muscles (Eigenmann 1909). *Troglichthys* retains a degenerate lens, a retina with pigment, and lacks rods and cones. In contrast, the hypogean Southern Cavefish does not lose its lens, has a retina with an outer reticular layer but no pigment, and has no eye muscles (Fig. 21.17). No trace of an eye can be found in Alabama Cavefish either by ordinary dissecting procedures or by clearing and staining (Cooper & Kuehne 1974). The eye does not function to form images in the hypogean Cavefishes but may serve as a light detector (Eigenmann 1909).

In a study of percopsiform phylogeny and the eye functionality of Cavefishes, Niemiller et al. (2013a) examined the repeated loss of selective constraint in the visual photoreceptor gene rhodopsin. The stygophilic genus *Forbesichthys* was consistently nested within the other stygobitic taxa (Fig. 21.13). A maximum-likelihood ancestral state reconstruction supported the hypothesis that eye functionality has re-evolved in *Forbesichthys* from a cave-dwelling ancestor. An inference of re-evolution, however, might be an artifact of extinction of surface lineages. The repeated loss of functional constraint of rhodopsin in

Cavefishes is compelling, because at least three cave lineages have independently accumulated loss of function mutations. Rather than re-evolution of eye functionality, Niemiller et al. (2013a:745) hypothesized that retained functionality of rhodopsin is likely due to recent subterranean colonization and the “stochastic nature of mutation accumulation, rather than unknown pleiotropic functions.” These same authors concluded that several lines of evidence support multiple, independent subterranean colonization events and losses of eye functionality. Even for a phylogeny with high likelihood, the authors stated directly that “ancestral reconstructions can occasionally produce strongly supported yet misleading results (2013a:745).”

### Sensory Organs: Hearing

Caves and other subterranean habitats impose constraints on sensory systems. Not only are stygobitic Cavefishes blind, they also have apparently lost a significant portion of their hearing range (Niemiller et al. 2013c). Stygobitic and surface Cavefishes shared the same audiogram profile at low frequencies, but only surface Cavefishes (i.e., Spring Cavefish) could hear frequencies  $>800$  Hz and  $\leq 2$  kHz. The ambient noise measured in aquatic cave and surface habitats was of high intensity with peaks near 1 kHz for streams underground, suggesting no adaptive advantage in hearing in those frequencies. In addition, stygobitic Cavefishes (Northern and Southern Cavefishes) had lower saccular

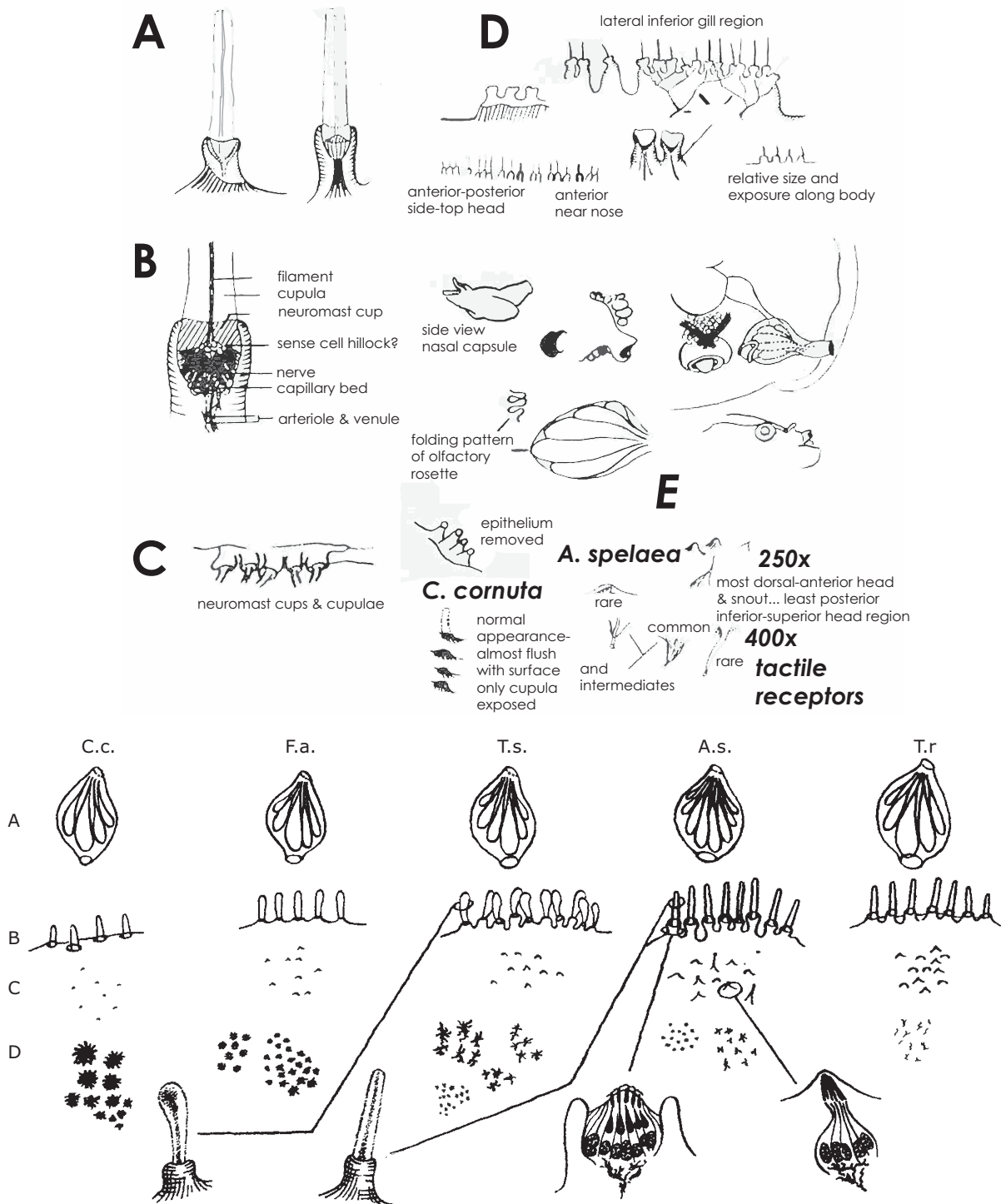


Figure 21.22. Drawings of the sensory system anatomy and melanophore conditions of Cavefishes, Amblyopsidae. (upper) Typical neuromast anatomy (A, B) of the Northern Cavefish, *Amblyopsis spelaea*; variation in tactile receptors of the Northern Cavefish (C right); the differences in exposure of neuromasts in Spring Cavefish, *Forbesichthys agassizii* (D) and Swampfish, *Chologaster cornuta* (C left); and the olfactory apparatus of the Spring Cavefish (E). (lower) Sense organs and melanophore condition in the Swampfish (C.c.), Spring Cavefish (F.a.), Southern Cavefish (T.s.), *Typhlichthys subterraneus*, Northern Cavefish (A.s.), and Ozark Cavefish (T.r.), *Troglichthys rosae*. (A) Olfactory sacs with olfactory rosettes; (B) exposure of neuromast ridges and orientation of cupulae; (C) tactile receptors (possibly modified neuromasts); and (D) range of melanophore condition in different populations. Drawings are to scale within each category (redrawn from Niemiller & Poulson 2010, upper, and Poulson 1963, lower).

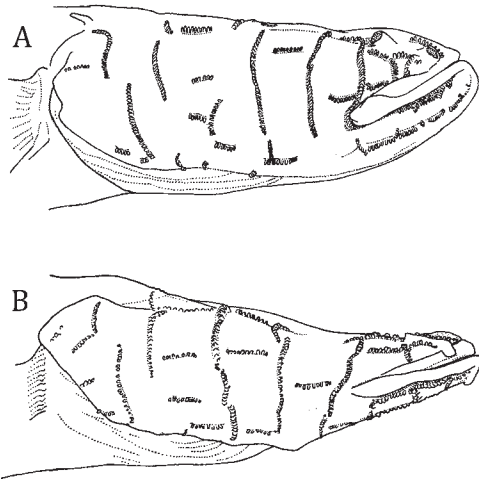


Figure 21.23. Comparison of head size and neuromast placement in the (A) Southern Cavefish, *Typhlichthys subterraneus*, and (B) the Alabama Cavefish, *Speoplatyrhinus poulsoni*. Note the relatively shovel-nosed shape and longer head of the Alabama Cavefish, which are likely neotenic trends. Both species show similarity in lateral-line ridges and stitches; each stitch has from 4 to  $\leq 39$  neuromasts (original drawings by John Cooper; redrawn from Niemiller & Poulson 2010).

hair cell densities compared with their surface relative (Spring Cavefish). These traits may have evolved in response to the loud high-frequency background noise found in subterranean pools and streams (Niemiller et al. 2013c). The mechanism (i.e., neutral loss versus selection) underlying hearing remains to be understood.

### Sensory Organs: Static and Dynamic Equilibrium Receptors

Similar to other actinopterygians, Cavefishes have semicircular canals and otoliths (Figs. 21.20 and 21.21). The sensory organs associated with each are modified neuromasts. The semicircular canals detect rates and directions of movement, and the otoliths detect body position even at rest (Niemiller & Poulson 2010). In epigeal fishes, the otoliths (static equilibrium) and the semicircular canals (dynamic equilibrium) act in concert with vision. Amblyopsids (except Swampfish) have hypertrophied (enlarged) semicircular canals, otoliths, and the cerebellum brain integrative area for these two systems (Figs. 21.20 and 21.21). Why the hypertrophy of these organs? Niemiller & Poulson (2010) speculated the relatively large head in stygobites was selected in part to increase surface area for the lateral-line sensory system. In turn, the increased head size results in less inertial change when turning and requires an increase in semicircular canals to detect movement.

### Evolutionary Considerations for Functional Morphology

As a family, Cavefishes show trends toward adaptation to lightless environments, and this is reflected in their general morphology. With increasing cave habitation increases occur in head width and length (exemplified in the extremely flat and elongate head of Alabama Cavefish), pectoral fin length, and mass/unit length (Poulson 1963; Figs. 21.15, 21.18, and 21.23). A wider and longer head provides more surface area for development of skin surface neuromasts and other lateral-line-like detectors (i.e., tiny conical sense organs). Longer pectoral fins perhaps function in turning, paddling, and stopping with more precision and power. The obligate cave dwellers (e.g., *Amblyopsis* spp.) live longer and grow larger than their surface counterparts, and in Hoosier Cavefish, carry the embryos and young in the gill chambers. These life-history traits may explain the greater weight achieved.

### GENETICS

#### Karyology

Karyotypes of both species of *Forbesichthys* and Northern Cavefish had  $2n = 24$  chromosomes and similar karyotypes (i.e., arm lengths, centric positions). Two populations of Southern Cavefish from Cave City, Kentucky, and Thomason Cave, Alabama, had  $2n = 46$  chromosomes, including a pair of large metacentric chromosomes. Karyotypes are unavailable for other amblyopsids (Ted Uyeno in Niemiller & Poulson 2010).

#### Intraspecific Genetic Variation and Phylogeography

An unpublished allozyme study (19 gene loci, 39 populations) revealed a trend of decreasing genetic variability with increasing cave inhabitation. Samples of *Typhlichthys* populations displayed the most pronounced genetic isolation (nearly every cave population examined had fixed differences), but little gene flow was evident even among epigeal populations of Swampfish (Swofford 1982). These same data revealed Northern Cavefish had greater between-population levels of genetic variability than did Ozark Cavefish. In contrast, individuals of Northern and Hoosier Cavefishes varied little north (Indiana) and south (Kentucky) of the Ohio River barrier. Extreme divergence

in two populations of Spring Cavefish in central Tennessee raised the possibility of an undescribed species.

Analyses of mtDNA sequence data revealed substantial phylogeographic structure in Ozark Cavefish (as *Amblyopsis*). Four genetically differentiated geographic forms of Ozark Cavefish were revealed: northwest Arkansas, Illinois River; southwest Missouri, White River; southwest Missouri, Neosho River; and northeast Oklahoma, Neosho River (Bergstrom 1997; Noltie & Wicks 2001). Minimal interpopulational gene flow was evidenced in Ozark Cavefish, and several populations constituted isolated, discrete endemic units.

Mitochondrial and nuclear gene sequences in 62 populations of *Typhlichthys* spp. distributed both east and west of the Mississippi River (Niemiller & Fitzpatrick 2008; Dillman et al. 2011; Niemiller et al. 2012), strongly supported the recognition of  $\geq 10$  genetically distinct cave lineages (Fig. 21.24). The authors also removed from synonymy the name *Typhlichthys eigenmanni* for populations west of the Mississippi River—a decision we follow here.

Niemiller et al. (2013b) made a case for description of these isolated lineages as species for both scientific and conservation purposes. In most cases, these lineages are endemic to a single or just a few hydrological subsystems making them more vulnerable to extinction from habitat degradation or contamination of groundwater. For example, lineage M is restricted to a small (38 km<sup>2</sup>) area of the upper Cumberland River watershed (Fig. 21.24), Pulaski County, Kentucky—a distinctive population first identified by Cooper & Bieter (1972). The population size was estimated to be 88 (Niemiller et al. 2013b). The populations now identified as *T. eigenmanni*, Eyeless Cavefish, also show considerable genetic and probable reproductive isolation, and a case can be made for recognition of  $>1$  taxon west of the Mississippi River (Niemiller 2012). The genus *Typhlichthys* has the widest range of the stygobitic taxa; estimates of when they differentiated differ widely ranging from the middle Miocene (Dillman et al. 2011) to the late Pliocene and early Pleistocene (about 3.6–1.8 mya) (Niemiller et al. 2012).

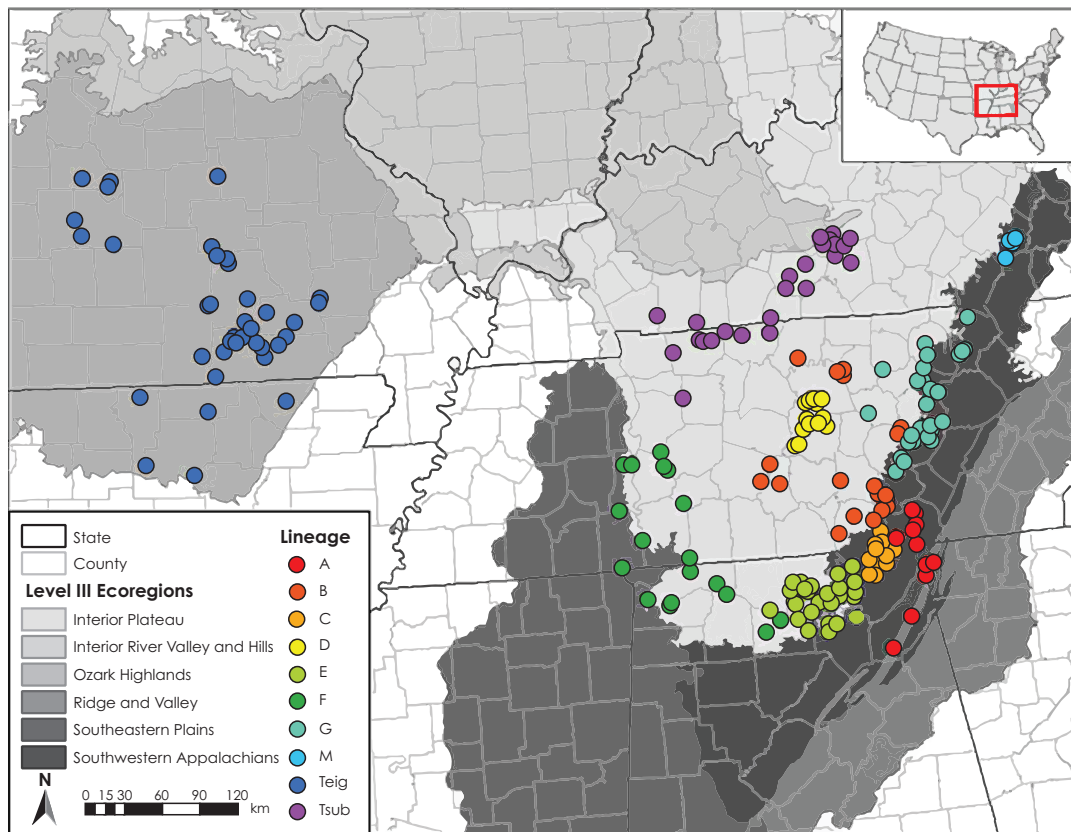


Figure 21.24. Distribution of the Southern Cavefish species complex (*Typhlichthys* spp.) based on 240 documented occurrences in six ecoregions across six states of the Interior Highlands of the United States. Cryptic lineages, which were assigned using genetic data from 62 of the populations, are color-coded (Teig, *Typhlichthys eigenmanni*, Eyeless Cavefish; Tsub, *Typhlichthys subterraneus*, Southern Cavefish).

An examination of one mitochondrial and four nuclear genes on 72 individuals and 16 populations of *Amblyopsis* from across its geographic range in Indiana and Kentucky (Niemiller et al. 2013d) revealed two major clades corresponding to samples north and south of the Ohio River. These two clades represented different taxa with the populations north of the Ohio River (in Indiana) designated as *A. hoosieri* (Chakrabarty et al. 2014). Populations of *Amblyopsis* were estimated to have diverged at 0.53 mya in the Pleistocene similar in timing with the estimated date of formation of the modern course of the Ohio River (Niemiller et al. 2013d). Interestingly, *Amblyopsis* spp. persisted in at least two distinct periglacial refugia, including one north of the Ohio River near the southern glacial maximum during the Pleistocene and one south of the river, rather than isolation in a single, more southern refugium, followed by recent northward colonization.

Genetic sequence data indicated differentiation in *Forbesichthys* that appears to correspond with the names *Forbesichthys agassizii* (originally from a well near Lebanon, Tennessee) and *F. papilliferus* (described from Union County, Illinois, probably from the springs along the Mississippi River bluffs in what is now referred to as the Pine Hills Research Natural Area). In phylogenies with complete taxonomic sampling, *Amblyopsis* is consistently resolved as the sister taxon of *Forbesichthys*. The common ancestor for these two genera diverged 4.95 mya during the early Pliocene (Niemiller et al. 2013ad). Diversification within these genera occurred during the Pleistocene; Spring Cavefish and Karst Cavefish diverged during the early Pleistocene (1.49 mya). Samples from across the range of *Forbesichthys* are available in frozen tissue collections, and we expect to see thorough studies of phylogeography on this genus in the near future.

## PHYSIOLOGY

### Temperature Tolerance

Metabolic rate and spontaneous activity of all species of amblyopsids (Alabama Cavefish unstudied) acclimate to a wide range of temperatures. Caves in which amblyopsids occur fluctuate in temperature because of spring and autumn floods, winter snow melts, and occasional warm summer flooding events. In the Pine Hills Research Natural Area, southwestern Illinois, the springs in which Karst Cavefish occur only vary in water temperature from 11–15°C (Weise 1957). Spring Cavefish (from Rich Pond, Kentucky) and Southern Cavefish acclimated similarly in the

laboratory to water temperatures of 5, 10, 15, 20, and 25°C even though Spring Cavefish experience a much wider temperature regime in their Rich Pond habitat (7–26°C) than do Karst Cavefish in their relatively constant spring environments (Hill 1969b).

Each amblyopsid species shows acclimated metabolic rates and spontaneous activity that are essentially the same from 5 to 25°C (Niemiller & Poulson 2010). The process of acclimation takes 1–3 weeks depending on the temperature difference. Initially, fish taken from water varying from 5 to 15°C showed a reduced metabolic rate and activity, and fish taken from water ranging from 15 to 25°C showed an elevated metabolic rate and activity (Niemiller & Poulson 2010).

### Oxygen Requirements

Spring Cavefish from Rich Pond, Kentucky, were maintained in a laboratory setting designed to study reactions of fish to water of various oxygen concentrations (Hill 1968). Experimental fish preferred high over low oxygenated water; however, fish did not discriminate between dissolved oxygen concentration intervals of  $\leq 2$  mg/liter, whether the two concentrations were relatively high or low. Caves inhabited by Spring Cavefish have lower oxygen concentrations than do their epigeal (e.g., springs) counterpart habitats. Interestingly, the fish spent significantly more time in dark, low oxygenated (0.6 mg/liter) water than in lighted high oxygenated water (8.0 mg/liter). Hill (1968) suggested that among the intrinsic factors involved in Spring Cavefish moving from springs to caves included a preference for darkness (less metabolic activity) and high oxygen concentrations.

Poulson (1963) routinely made measurements of dissolved oxygen at least twice a year from his study sites. Swampfish occurred in water with 4–11 mg/liter of dissolved oxygen; *Forbesichthys* spp. in 9.56–10.2 mg/liter in caves and 7.3–11.8 mg/liter in springs; Southern Cavefish in 7.7–10.8 mg/liter; *Amblyopsis* sp. in 8.5–11.9 mg/liter; and Ozark Cavefish in 9.3–10.2 mg/liter. These data are unavailable for Alabama Cavefish.

### Salinity Tolerance

Swampfish were captured from sites with salinities  $\leq 5$  ppt in North Carolina but no occurrences are known in brackish water (Niemiller & Poulson 2010). Jenkins & Burkhead (1994 citing an unpublished report of F. J. Schwartz) used the term brackish water based on the

5 ppt value. The other amblyopsids are considered primary freshwater fishes and are geographically distant from marine environments and have been since their time of origin (see phylogenetic relationships section, fossil record section, and phylogeography subsection, genetics section). Groundwater pollution from oil brines and mining could eventually test the salinity tolerance of the stygobites, but data are presently unavailable.

### Metabolic Rates

All amblyopsids have lower metabolic rates than comparably sized epigeal fishes. For example, even the swamp-dwelling Swampfish has an estimated standard metabolic rate 0.92 times that of the Goldfish, *Carassius auratus* (Poulson 1963). Poulson (1963) described a gradient of metabolism for Cavefishes (excluding the Alabama Cavefish). He observed a gradual reduction in standard, active, and routine metabolic rates from surface (Swampfish) to spring (Spring Cavefish) to cave habitats (*Amblyopsis* > *Typhlichthys* > *Troglichthys*).

Metabolism in the Ozark Cavefish varied seasonally in Logan Cave, Arkansas. Significantly different relationships between mass and metabolic rate (i.e., oxygen consumption) occurred among seasons with positive relationships during summer and autumn and negative relationships during winter and spring (Adams & Johnson 2001). The positive slopes observed during summer and autumn may be related to the presence of Gray Bats (*Myotis grisescens*), which supplied a novel food, guano, and associated bacteria and invertebrate fauna.

In the most contemporary research across amblyopsids, the strongest correlates in metabolic rate were reduction in ventilation frequency and volume > brain metabolic rate > gill surface area (Poulson 2001b). Muscle metabolic rate or histological indices of thyroid activity showed no reductions that would explain the overall decreases in total gill surface area from surface-dwelling to facultative to obligate cave-dwelling.

### Swimming

Average swimming velocity appears to be correlated with cave occupation; however, activity level, defined as the percentage of time spent swimming, did not (Poulson 1963). Apparently, stygobites (e.g., Northern and Ozark Cavefishes) move a greater percentage of their body length with each pectoral-caudal-fin stroke than do stygophiles: 4.6 strokes / body length in *Forbesichthys*, 7.1 in Southern

Cavefish, 0.8 in Northern Cavefish, and 1.8 in Ozark Cavefish. The greater overall swimming velocity of the stygobites allows them to cover more distance to search for food in a food-poor environment. Also, the stygobites have an increased refractoriness to disturbance when compared with their epigeal relatives.

### BEHAVIOR

Few scientists in either aquatic biology or speleology have ever seen a hypogean Cavefish in its natural habitat. Behavioral observations in a cave setting have been limited to brief periods of time in a harsh, lightless environment. Since the 1960s, numerous university laboratories and museum research institutes attempted to create an aquatic karst environment in an artificial setting. The published data from these settings are limited to essentially one researcher (Poulson 1963). Even the astounding observation of gill-chamber brooding of embryos and young in the Hoozier Cavefish was made over a century ago (reviewed by Eigenmann 1909), and no detailed descriptions or photographs of this unique phenomenon are available since then. As judged from aquarium observations, Eigenmann (1909) and later Poulson (1963) quantified and demonstrated the unique behavioral traits of Cavefishes. Here, the focus is primarily on phototaxis (response to light) and blindness; thigmotaxis (orientation by touch), barrier avoidance, and spatial memory; feeding behavior; and aggression.

#### Phototaxis and Blindness

The Swampfish, an epigeal species primarily inhabiting acidic to circumneutral (pH range 5.6–7.8) swamps, is nocturnal, negatively phototactic (avoids light), and more inactive than its habitat associates. Compared with other co-occurring species, its eyes, optic tectum (midbrain), and optic lobes are reduced. The flattened head and superior mouth of the Swampfish (Fig. 21.4) may allow for bottom-dwelling and stalking of prey (Poulson 1963). The species has anteriorly directed tubular nostrils that serve as intake tubes, which confer directional discrimination of chemical cues. The olfactory rosette and olfactory lobes of the brain are well developed indicating a reliance on chemical cues for information received in a nocturnal environment (Figs. 21.21 and 21.22). The brain also has hypertrophied tactile and lateral-line nuclei, and the skin has numerous tactile receptors indicating additional adaptations for nocturnal living.

The Spring Cavefish and Karst Cavefish, like the Swampfish, are nocturnal and negatively phototactic in epigeal situations. *Forbesichthys* spp. have relatively smaller eyes and optic lobes (Figs. 21.17 and 21.21). The eyes of Spring Cavefish lack ciliary muscles (Eigenmann 1909), so unlike Swampfish, they probably do not form images. When eyes are ablated (removed), the negative phototaxis of Spring Cavefish is reduced, but eye ablation produced no obvious change in swimming or feeding behavior (Eigenmann 1909). Compared with Swampfish, the cerebellum, otoliths, and semicircular canals in *Forbesichthys* spp. are larger (Fig. 21.21) and may be compensations for loss of visual cues normally important for equilibrium responses (Poulson 1963). On *Forbesichthys* spp., the neuromasts are more exposed, tactile receptors more numerous, and although the olfactory lobes are similar in size, the olfactory rosettes are larger when compared with *C. cornuta* (Fig. 21.22). In Spring Cavefish, positive thigmotaxis (orientation by touch) takes precedence over photonegative behaviors (Poulson 1963; Hill 1969a). Because of these features and concomitant behaviors, Spring Cavefish are often inactive during the day and may either be associated with cover when found on the surface or may move underground when surface cover is inaccessible.

The obligate hypogean genera *Troglichthys*, *Typhlichthys*, *Amblyopsis*, and *Speoplatyrhinus* culminate the trend of increasing development of semicircular canals, otoliths, neuromasts, and tactile receptors and decreasing development of optic lobes and eyes, pigmented melanophores, embedded scales, and certain cranial nerves (Eigenmann 1909; Poulson 1963; Cooper & Kuehne 1974; Figs. 21.17, 21.21, and 21.22). These morphological changes are reflected in reduced or no phototaxis, moderate thigmotaxis (see thigmotaxis, barrier avoidance, and spatial memory subsection, this section), a learned awareness of surroundings, and a heightened sensitivity to prey movements.

### Thigmotaxis, Barrier Avoidance, and Spatial Memory

Putative tactile receptors were quantified and are concentrated on the head, body, and lips and under the mouth of most Cavefishes (Fig. 21.22); Alabama Cavefish apparently lacks these receptors entirely. Aquarium observations and designed studies revealed that Karst Cavefish is the most thigmotactic amblyopsid (Weise 1957; Poulson 1963). The Karst Cavefish approached barriers without hesitation, initiated avoidance at an average of 2.7 mm from the barrier, and stopped or darted away when it contacted the

barrier. The species may have rather poor spatial memory and poor obstacle avoidance because of its relatively underdeveloped neuromast system and a presumably weak central nervous system. When resting under rocks in springs, Karst Cavefish are often in groups touching each other (GLA and BMB pers. obs.).

*Typhlichthys* spp. are also thigmotactic. Individuals hesitated when first approaching an unfamiliar barrier, initiated avoidance at an average of 27 mm, and contacted the barrier while turning. On subsequent approaches the avoidance response averaged about 47 mm, the individual then turned parallel to the barrier at an average of 8 mm, and contacted the barrier a little less often than on the initial approach. The species apparently perceives distances, and contact with the barrier is associated with strong thigmotaxis (Poulson 1963).

Ozark Cavefish and *Amblyopsis* spp. are less hesitant when approaching a barrier than individuals of *Typhlichthys* spp. They initiated avoidance at an average of 13 mm distance from the barrier and avoided the barrier more consistently than *Typhlichthys* spp. Both continued to initiate avoidance at greater distances in subsequent approaches to the barrier. Spatial memory apparently disappeared after about 10 approaches (Poulson 1963). Nothing is known about these behaviors in Alabama Cavefish.

In aquaria and in the field, Swampfish rest amongst dense vegetation during the day and individuals are often found touching each other. Despite relatively low numbers of tactile receptors and superficial neuromasts, Swampfish are strongly thigmotactic (Neimiller & Poulson 2010).

### Feeding Behavior

Observations of feeding behavior in a laboratory setting revealed different prey capture behaviors among Cavefishes (Poulson 1963); some of these differences are probably a reflection of the variation in their sensory capacities. Swampfish reacted to small, live food at 0–10 mm from the prey item; its thigmotactic response is apparently uncoordinated and undirected, especially when touched on the body by a prey item. Spring Cavefish began capture movements at about 20–30 mm from some swimming prey items, or it passed over the prey, backed up, and initiated capture movements when the prey moved or if the prey touched any part of its body. Southern Cavefish oriented toward living prey at about 30–40 mm from the item and homed in on prey items; capture was initiated at about 10 mm from the object. *Amblyopsis* spp. oriented to prey at distances similar to

*Typhlichthys*; it also homes in on its prey. Cupulae in the neuromasts of the two hypogean taxa studied apparently move at 20–40 mm distance from a living prey item (Fig. 21.22). This strongly suggests detection of prey movement by non-optic sensory structures.

All amblyopsids (except for lack of data on Alabama Cavefish) use their lateral line and touch receptors in a complementary way; they captured the same live prey by a combination of lunge and snatch, unlike many fishes that use a gape and suck mode of feeding. In Karst Cavefish, Weise (1957:199) noted that “an amphipod is taken by a vicious sidewise jerking of the head and is immediately swallowed.” Individuals of *Amblyopsis* spp. also jerked and snatched as they captured large prey items.

### Aggression

In the laboratory, overt aggressive behaviors among hypogean amblyopsids varied considerably among the species even though all were frugal energetically (Bechler 1981, 1983). Swampfish showed non-agonistic behavior toward conspecifics; in Ozark Cavefish only tail-beating and submissive acts were observed. *Forbesichthys agassizii* and *Amblyopsis* sp. engaged in relatively intense, complex agonistic bouts with conspecifics. Southern and Ozark Cavefishes engaged in simpler, shorter, less intense bouts. Tail-beating was the most frequent behavior of any of the species studied (Alabama Cavefish not included). Neither Poulson (1963) nor Bechler (1983) observed agonistic behavior in the field. In *Amblyopsis*, *Forbesichthys*, *Troglichthys*, and *Typhlichthys*, Bechler (1983) documented two behaviors in all species: freeze and escape. This is the first time that a freeze behavior was observed in any hypogean fish species (Niemiller & Poulson 2010) and may be a response by recently hatched fish to avoid cannibalism (probably filial cannibalism, eating own young). This behavior would be a purposeful response since Cavefishes use only their lateralis system to detect other individual fish and prey. Fish that can perceive they are losing in an agonistic encounter might freeze more often than swimming away as an escape response.

Scarcity of food may be a primary selective force in amblyopsids that influences the intensity and kinds of agonistic behavior. In general, metabolic rate and fecundity decrease in cave populations of amblyopsids, longevity increases, and swimming efficiency improves. These adaptations confer an advantage of energy conservation, at least on the hypogean species, which serves to reduce selection

pressures produced by a scarce food supply, ultimately allowing for a reduction in overt intraspecific agonistic behavior (Poulson 1963).

### REPRODUCTION

Critical aspects of reproduction (e.g., reproductive mode, timing, reproductive capacity, and critical spawning habitat) are incomplete or remain a mystery for amblyopsids. Although several authors suggested all Cavefishes (e.g., Woods & Inger 1957; Poulson 1963) exhibit gill-brooding behavior because of the jugular position of the anus and urogenital opening, this is documented only in the Hoosier Cavefish (Eigenmann 1909).

### Age and Size at Sexual Maturity and Spawning Frequency

The ages of Cavefishes are estimated primarily by examining length-frequency diagrams or examining scales for annual marks (Poulson 1963; Ross & Rohde 2003). No studies to date used otoliths in aging or raised known-age fish in aquaria from embryo to adult. Thus, the ages of Cavefishes, especially as it relates to reproduction, are not accurately known and lack precision. Because of its perceived rarity, no age or maturity data is available on Alabama Cavefish. Swampfish and *Forbesichthys* spp. probably mature at one year of age and  $\leq 20$  mm SL (Table 21.2). In southern Illinois, individuals of *Forbesichthys*  $> 43$  mm SL were sexually mature; first reproduction was 12 months, and 100% of adult females were capable of spawning each year (Poulson 1963). The stygobitic species may be mature by two or three years of age and about 40–45 mm SL. Mean age at first reproduction for Southern Cavefish was estimated at 22–24 months and 33–40 mm SL with only 50% of the population breeding in any given year (Poulson 1963); the time between reproductive cycles was 1–2 years (Poulson 1963, 1964). The Northern Cavefish reproduces for the first time at 36–48 months of age (about 40 mm SL) with 2–3 years between reproductive cycles (Poulson 1963, 1964); only about 10% of females are estimated to breed each year (Poulson 1963; Table 21.2). For Ozark Cavefish, an estimated 20% of the females in the population breed in any given year. Interpretation of published size and age data indicates that an individual female of the stygobitic species does not spawn each year once maturity is achieved (Table 21.2).



Table 21.2. Life-history attributes for Cavefishes, Amblyopsidae, compiled from Eigenmann (1909), Weise (1957), Poulson (1963, 1964), Poulson & White (1969), Ross & Rohde (2003), Niemiller & Poulson (2010) and references therein.

|  | <i>Chologaster cornuta</i>   | <i>Forbesichthys</i>                                 | <i>Amblyopsis</i>   | <i>Typhlichthys</i>                           | <i>Troglichthys rosae</i> | <i>Speoplatyrhinus poulsoni</i>                                  |
|--|--|--|---|---|---------------------------|--|
| Number of extant species                         | 1  | 2  | 2   | 2 (>10 lineages)                              | 1                         | 1  |
| Primary or secondary fresh water                 | Primary fresh water  | Primary fresh water                                  | Primary fresh water   | Primary fresh water                           | Primary fresh water       | Primary fresh water  |
| Maximum size recorded in length                  | 6.8 cm TL  | 8.4 cm TL  | 11 cm TL  | 8.6 cm TL                                     | 6.2 cm TL                 | 7.2 cm TL  |
| Maximum age                                      | 2 years  | 2–3 years  | 10 years  | 4 years in the wild; 10 years in captivity    | 4–5 years                 | 7–8 years  |
| Age and size at first reproduction               | 1 year; 23–55 mm TL  | 1–2 years; 34–44 mm TL                               | 3–4 years, possibly older; 45–56 mm TL                            | Usually 2 years; 33–40 mm TL                  | 3–4 years; 44–52 mm TL    | Unknown  |
| Iteroparous versus semelparous                   | Iteroparous in North Carolina; suggested semelparous in parts of range         | Iteroparous  | Iteroparous, but a given female may spawn only once every 3 years | Iteroparous; spawning occurs every 1–2 years  | Iteroparous               | Unknown  |
| Fecundity estimates from ovarian counts          | 6–98, mean 25  | 80–285, mean 152                                     | 61–70, mean 69.5  | mean 49.8                                     | mean 23                   | Unknown  |
| Mature egg diameter                              | 1.1–1.8 mm   | 1.5–2.0 mm suggested                                 | 2.3 mm  | 2.3 mm  | 2.2 mm                    | Unknown  |
| Egg deposition sites                             | Unknown  | Unknown  | In gill chambers of adult female                                  | Unknown                                       | Unknown                   | Unknown, but gill chamber capacity large enough to brood embryos |
| Clutch size in nest                              | Probably no nest built   | Probably no nest built                               | About 70 held in gill chamber of female                           | Probably no nest built                        | Unknown                   | Unknown  |
| Range of nesting/spawning dates and temperatures | March–April; 13–22°C   | December–March; about 18–22°C                        | February–April, but also March–November; 8–17°C                   | Spring months; 10–14°C                        | Unknown                   | A May-captured female with mature ova; probably <13°C            |
| Habitat of spawning sites; average water depth   | Unknown, but probably similar to general habitat, perhaps <i>Sphagnum</i> moss | Probably in cave streams rather than surface springs | In cave waters; similar to general habitat                        | In cave waters; perhaps at considerable depth | Unknown                   | Unknown, but in cave waters                                      |
| Incubation period; larval type at hatching       | Unknown; probably protolarva   | Unknown; protolarva                                  | About 28 days at 22°C; protolarva                                 | Unknown                                       | Unknown                   | Unknown  |
| Mean size at hatching                            | At least 5–6 mm TL   | About 6.5 mm TL                                      | About 5.0 mm TL; 10 mm TL leave gill chamber                      | Unknown                                       | Unknown                   | Unknown  |

(continued)

Table 21.2, continued

|                            | <i>Chologaster cornuta</i>   | <i>Forbesichthys</i>   | <i>Amblyopsis</i>   | <i>Typhlichthys</i>  | <i>Troglichthys rosae</i>   | <i>Speoplatyrhinus poulsoni</i>   |
|----------------------------|--|--|---|--|---|---|
| Parental care              | No known parental care   | No known parental care   | Female carries (and functionally protects) embryos and young in gill chambers                             | No known parental care   | No known parental care  | No known parental care  |
| Major dietary items        | Amphipods, chironomids, and cladocerans  | Amphipods, chironomids, copepods, and oligochaetes; filial cannibalism   | Isopods, amphipods, crayfishes, and copepods; filial cannibalism  | Trichopterans, tendipedids, cladocerans, isopods, crayfishes, and copepods   | Copepods, cladocerans, isopods, and crayfishes; filial cannibalism            | Unknown   |
| General year-round habitat | Roadside ditches; ponds, sloughs, swamps; often <i>Sphagnum</i> moss; acidic water, tannin-stained | Springs, but under cover during day; caves; rarely washed out into streams and swamps                              | Strictly caves; flowing cave streams or pools; often near rock ledges                                     | Caves, rarely springs; often in large underwater lakes at considerable depth | Cave waters in Arkansas, Missouri, and Oklahoma; typically in caves with bats | Clear, cool water of Key Cave, Lauderdale County, Alabama; gray bats supply guano food source |
| Migratory or diadromous    | Probably live out entire life near place of birth  | Some movement of young out of cave openings into springs; adults move into caves at night and perhaps for breeding | Little migration known in caves, although tagged adults known to move some distances within a single cave | Considerable movement probably takes place, but not truly migratory          | Unknown   | Unknown   |
| Imperilment status         | Currently stable over historic range   | Currently stable over historic range   |   | Currently stable over historic range, but may be a species complex           | Federally threatened  | Federally endangered  |

## Sexual Dimorphism

Distinguishing between sexes in Cavefishes is difficult using external features, except when females are visibly carrying ripe eggs that can be seen through the translucent skin of the belly (Fig. 21.25). Males of the Swampfish develop a fleshy Y-shaped protuberance on the upper jaw; mature females may have small bumps there (Ross & Rohde 2003; Marcy et al. 2005; Figs. 21.26 and 21.27). These unusual structures perhaps have some function in reproduction or chemoreception, but both suggestions are speculative. The male Swampfish appendage is not noticeable until about 23 mm SL, and by 25 mm SL males are

easily sexed using this feature; gender can also be determined by color through the ventral body wall—white for males, yellow for females (Ross & Rohde 2003).

## Sex Ratios and Sex Brooding Embryos

Among all the Cavefishes, sex ratio estimates are only available for the Swampfish. In that species females ( $n = 240$ ) were always more numerous than males ( $n = 142$ ) in a North Carolina drainage ditch (Ross & Rohde 2003), a significant departure from the expected 1:1 ratio. Females were also larger than males. Apparently



Figure 21.25. (upper) Ventral close-up view of a female Spring Cavefish, *Forbesichthys agassizii*, showing yolked oocytes and blood pigment near heart through the body wall. Live specimen from Elm Spring, Pine Hills Natural Research Area, Union County, Illinois, 14 November 2000. (lower) Ventral view of live, mature male (above) and female (below) Spring Cavefish from Pocahontas Spring, Coffee County, Tennessee, 8 March 2013 (upper courtesy of GLA; lower courtesy of BRK).

only females of Hoosier Cavefish carry developing embryos in their gill chambers, but sample sizes are <10 and live individuals cannot be sexed (Eigenmann 1909).

Fecundity

Description of methods used to determine mature or ripe ova in any Cavefish species are too brief and inadequate for the determination of either annual or lifetime fecundity. In addition, with the exception of Swampfish, sample sizes are small, and the data reported is almost certainly misleading, especially when nonspecialists might consider that no further study is necessary. Mean annual fecundity was estimated as 25 mature ova / female (Table 21.2) (n = 156; Ross & Rohde 2003), which is much lower than another estimate of 93 ova/female (Poulson 1963). Likewise, both one and two egg-size classes were reported in ovaries of mature females. Poulson (1963) observed a single egg-size class with mature ova of 0.9–1.2 mm in diameter. In contrast, Ross & Rohde (2003) found two size classes of ova with mature ova of 0.8–1.9 mm in diameter

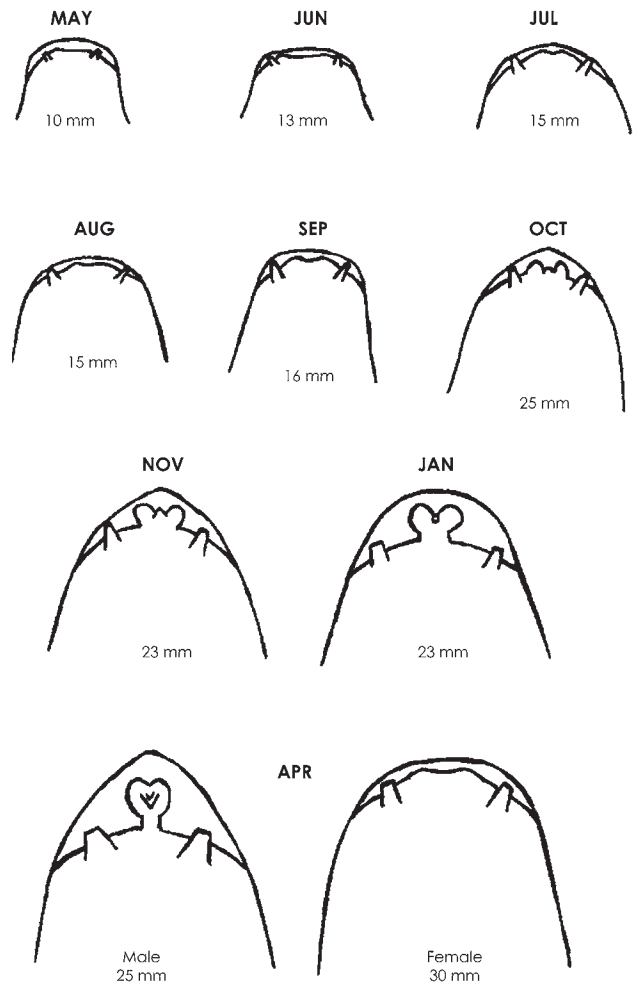


Figure 21.26. View of the dorsal surface of the snout of the Swampfish, *Chologaster cornuta*, illustrating the development of the snout appendage in the male. The April illustration compares both sexes (all lengths are SL) (redrawn from Ross & Rohde 2003).



Figure 21.27. A male Swampfish, *Chologaster cornuta*, showing Y-shaped snout appendage, which may be implicated in reproduction (Marcy et al. 2005) (courtesy of Dean Fletcher).

and immature ova of 0.5–0.8 mm in diameter. Observation of two size classes of ova indicates that 25 ova/female is probably a more accurate estimate of annual fecundity than 93. Mean fecundity estimates of mature ova is 152 for Spring Cavefish (Weise 1957; Ross & Rohde 2003). We assume here that these estimates refer to a clutch of ripe eggs ready to be released. Presumably mature clutch sizes in stygobitic taxa (except Alabama Cavefish) range from a mean of 23 to 50 (Poulson 1963; Table 21.2). In 10 females of Southern Cavefish, mean fecundity was about 50 ova/female and ova diameter was 2.0–2.3 mm (Poulson 1964 also reported a mean of 70 eggs/clutch) (Table 21.2). Seven female Northern Cavefish averaged 69.5 mature ova (Poulson 1963), and clutches apparently averaged 60 eggs (Poulson 1964). No clear distinction is apparent between mature ova and clutch size in Poulson's (1963, 1964) studies. Ozark Cavefish had a mean count of 23 ova/female (Poulson 1963). For comparison, four small (mean 30 mm SL) species of madtom Catfishes (*Noturus* spp.) had a mean clutch size of 37, which is within the range for the similar-sized stygobitic Cavefishes. Species of *Noturus* provide extensive parental care to the embryos and fry, perhaps similar (in both time and energy) to gill brooding in *Amblyopsis* spp.

### Gill Brooding and Parental Care

Numerous studies, encompassing extensive field effort, attempted to document the reproductive mode for amblyopsids with no success (Brown & Johnson 2001; Ross & Rohde 2003). Lack of observation of embryos or young in the gill chambers may imply a reproductive mode similar to that of the Pirate Perch (Poly & Wetzel 2003; Fletcher et al. 2004) or a slight deviation from transbranchial spawning (passage of gametes from the urogenital pore through the branchial and buccal oral cavities and finally, out of the mouth and into the substrate; see Burr & Warren, this volume). Functionally, the gill chambers of Swampfish, *Forbesichthys* spp., and perhaps Ozark Cavefish seem to be much smaller than *Amblyopsis* spp. and would not easily harbor many embryos or young for lengthy periods.

Hoosier Cavefish are unique among all fishes in exhibiting a reproductive behavior termed gill brooding. Amblyopsids have a jugular vent, meaning the opening of the oviduct and anus is located between the branchiostegal membranes (see morphology section; Fig. 21.25). Placement of the vent may facilitate gill brooding by allowing easy movement of eggs from the urogenital opening to the

gill chamber. How fertilization occurs is unknown (i.e., if it is before or after movement of eggs to the gill chamber), but the eggs, embryos, and then young are brooded in the gill chambers (Eigenmann 1909). We have seen individuals of Hoosier Cavefish with tiny young in the gill chambers but were unable to distinguish gender in a cave environment (GLA and BMB pers. obs.). This may benefit the young by allowing them to attain a larger size under adult protection. Large early life-history stages and parental care would increase survivorship in the energy-limited environment of the Hoosier Cavefish. Also, a larger propagule could withstand downstream drift and feed on copepods more effectively. Although some details of reproduction are understood for this species, little is known about the habitat requirements for spawning.

It was not until Eigenmann (1909) initiated in-depth studies of amblyopsids that gill brooding was discovered and carefully observed. He first reported that about 70 embryos occurred in a Hoosier Cavefish female's gill chamber, remaining there for about two months, until the yolk was nearly absorbed and the young fish had reached about 10 mm TL (contra Poulson 1963, who reported 4–5 months in the gill cavities). When a female is handled, young escape; because of this behavior, the species was earlier thought to be viviparous. Eigenmann, studying a cave in southern Indiana, found the first young in May, then in September, and others in June. In March 1898, 29 adults were captured, and four were females with embryos in their gill cavities (Fig. 21.28). One had 61 embryos in the branchial chamber, another 70; embryos in the other two females were not counted but were similar in number to the other two. Females brooding embryos can be distinguished by the distended gills; the females even retain dead embryos in the gill chamber until they disintegrate. Eigenmann felt that the breeding period was from about March through November, and probably throughout the year. He was unsuccessful in getting females to release embryos in the laboratory.

### Spawning Season and Reproductive Details: Swampfish

Swampfish spawn in North Carolina from March to late April (at water temperatures of 13.3–21.7°C) based on gonadosomatic indices (Fig. 21.29). The spawning season is short and to date reproductive behavior has not been observed (Ross & Rohde 2003). In an aquarium, however, Swampfish constructed a depression under a rock (Ross & Rohde 2003). Noticeable ovarian development occurs in the autumn preceding the reproductive season.



Figure 21.28. Hoosier Cavefish, *Amblyopsis hoosieri*, embryo (upper), larva at time of hatching (middle), and an older larva (lower). Note tactile structures (small raised nodules) on the older larva (redrawn from Eigenmann 1909).

Spawning Season and Reproductive Details: Karst Cavefish and Spring Cavefish

Weise (1957) and Hill (1969b) suggested species of *Forbesichthys* were dependent on underground spawning habitats to complete their life cycle. Reduced numbers of adults on the surface during summer through winter and appearance of larvae on the surface in late winter led to the conclusion that spawning occurs primarily underground in winter (Weise 1957). Weise (1957:200) found what he reported as gravid females from “practically every month of the year,” but the yellow color he observed through the body wall may have been in some instances fatty material. Others observed gravid females only during winter and early spring except for a single gravid female captured in June (southern Illinois) (Smith & Welch 1978). Courtship behaviors, including egg fertilization, remain unobserved. Female Karst Cavefish had a mean of 152 putatively mature ova (7 females); egg diameter was 1.5–2.0 mm (Weise 1957; Table 21.2).

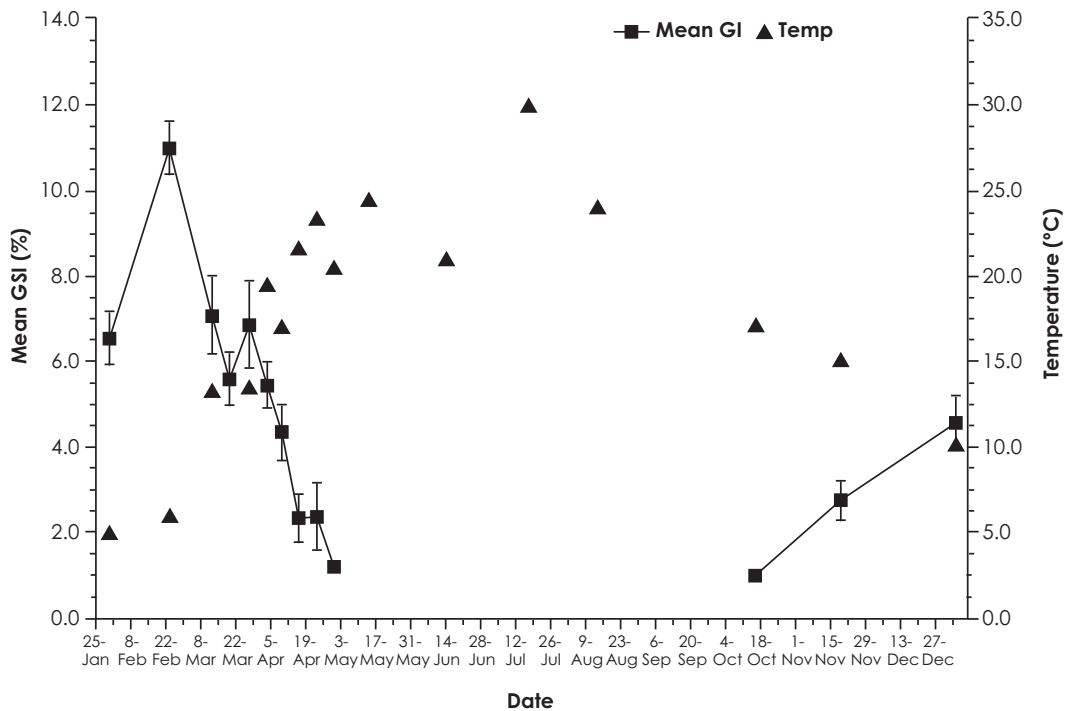


Figure 21.29. Mean female gonad somatic index (GSI) (squares) ( $\pm$  S.E.) and water temperatures (triangles) for each sample date of Swampfish, *Chologaster cornuta*, January 1977–January 1978, from a tributary of Black Swamp Creek, North Carolina. Ovaries were too small to calculate GSI from late spring through autumn (redrawn from Ross & Rohde 2003).

### Spawning Season and Reproductive Details: Southern Cavefish

Little is known concerning the reproductive cycle of the Southern Cavefish. Breeding may occur in spring based on the presence of spent females and abundance of small to medium larval fish in June and July (Poulson 1963). Many of the caves inhabited by Southern Cavefish are inaccessible during spring due to high water levels, but the increase in water volume may initiate reproduction. Most adult females are heavy with mature ova from October to December.

### Spawning Season and Reproductive Details: Northern Cavefish

Although here we treat the work of Poulson (1963, 1964) as focusing on Northern Cavefish, it is unclear from those papers whether the data are based on Northern or Hoosier Cavefishes. Reproduction in the Northern Cavefish may be induced by increased flows (i.e., spring rains) into karst habitats from February through April. Mature ova are present in females from January to March, and spawning occurs during high water from February through April. Early mortality is decreased by branchial embryo incubation, increased egg size, and a decrease in number of eggs/g of female (Poulson 1963).

In a census of two optimal Northern Cavefish habitats for seven reproductive seasons, reproducing females occurred during two seasons in one of these habitats and only once in the other (Poulson 1963). Of the 127 adult females, only 10 had embryos or young in their gill cavities. Reproduction evidently occurs when density of adults is high and number of young is low.

### Spawning Season and Reproductive Details: Ozark Cavefish

Ozark Cavefish exhibit mature ova from July to December with the largest number of gravid females occurring from October to December. In northwest Arkansas, presumed reproduction occurred from August to December (Poulson 1963; Table 21.2). An earlier reproductive season is suggested by the presence of young Ozark Cavefish in July and gravid females in late August (Adams & Johnson 2001). Mature ova range from 1.9–2.2 mm in diameter.

### Spawning Season and Reproductive Details: Alabama Cavefish

The holotype of the Alabama Cavefish is a 58.3-mm SL female captured in late May with ova (maturity stage not clarified) (Cooper & Kuehne 1974). Small young (12 and 15 mm SL) were observed from February and November (Kuhajda & Mayden 2001). From these limited data, a summer spawning period is surmised. The Alabama Cavefish has spacious gill chambers and, at least, functionally has the capacity to be a gill brooder. Other reproductive data are lacking.

### Embryo Characteristics and Development

Poulson & White (1969) compared mature eggs, larvae, and small young of Ozark Cavefish and Swampfish. The mature egg of Swampfish was 0.9 mm in diameter and that of *T. rosae* was 2.2 mm. Hatchlings of Swampfish were 3 mm TL and those of Ozark Cavefish were 5 mm; both have relatively large yolk sacs with the largest in Ozark Cavefish. Free-swimming larvae were 8 mm TL in Swampfish and about 12 mm in Ozark Cavefish. Embryos and early life-history stages of hypogean Cavefish taxa are apparently larger, contain more yolk, and attain a greater length at the free-swimming stage than their epigeal counterparts (e.g., Swampfish).

The yolk of the Hoosier Cavefish is translucent, of various tints of amber, and contains a large (1–1.2 mm) oil droplet. Development as a fertilized egg lasts about one month; in the laboratory, some embryos hatched in about 28 days (at 22°C), but in cold cave streams (12°C) hatching would occur several days later. The yolk at time of hatching is about 1.8 mm, the oil droplet about 1.0 mm. About one more month is spent in the gill chambers until all the yolk is absorbed. The young at hatching are about 5 mm TL and lie on their sides. Development is direct, and the young are about 10.0 mm TL about the time they leave the gill chambers (Eigenmann 1909; Fig. 21.28).

In Spring Cavefish, cycloid scale primordia first appear on the caudal peduncle along the lateral line between 3–4 weeks of life (Fig. 21.30). These primordia advance anteriorly along the lateral line, less rapidly dorsally and ventrally (Hill 1971). Complete squamation occurs between 11–12 weeks of age.

## ECOLOGY

### Habitat: Swampfish

Swampfish occur most frequently in acidic water heavily stained with tannins and humic acids (Marcy et al. 2005),

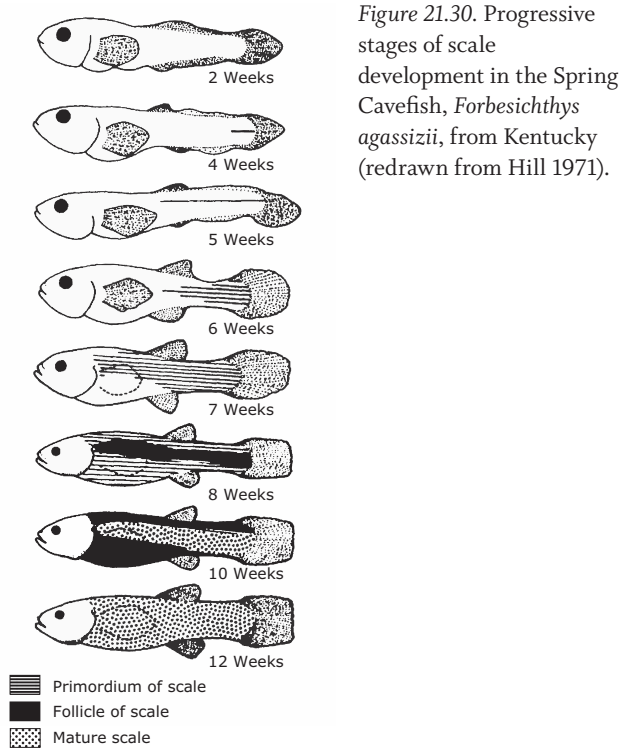


Figure 21.30. Progressive stages of scale development in the Spring Cavefish, *Forbesichthys agassizii*, from Kentucky (redrawn from Hill 1971).



Figure 21.31. An example of a lowland stream habitat in which the Swampfish, *Chologaster cornuta*, is found in the Croatan National Forest, Craven County, North Carolina (courtesy of Fritz Rohde).

but they also occupy clear streams. As the common name suggests this species is often captured in quiet water, including swamps, roadside ditches, ponds, and sluggish creeks, often in dense vegetation, especially *Sphagnum* moss (Ross & Rohde 2003; Fig. 21.31). In streams they occupy tangles of roots and debris in well-shaded reaches where the water temperature remains <23°C (Poulson 1963).

Habitat: Spring Cavefish and Karst Cavefish

Caves, springs, spring runs (Fig. 21.32), and spring seeps are characteristic habitats of species of *Forbesichthys*, species that bridge the gap between an epigean and hypogean existence. In caves, the species are associated with water temperature from 9.9–13.2°C; in springs, they occur at temperatures from 5.1–15.3°C (Poulson 1963). Occasionally, specimens are taken from streams, usually after a heavy rain has flushed them out from nearby caves or springs. Karst Cavefish are mostly subterranean, emerging at dusk and usually retreating underground 1–2 h before dawn (Smith & Welch 1978). *Forbesichthys* spp. often are referred to as facultative cave dwellers (stygophiles). In epigean habitats, they hide under large rocks, fallen trees, or other cover and are observed more easily as an active fish at night in spring heads and spring seeps. *Forbesichthys* spp. are distributed

sparingly throughout their range but tend to be locally abundant. The current distribution probably reflects historical surface movement of individuals and colonization of new habitats (Woods & Inger 1957; Hill 1969b). Large rivers or streams likely are sufficient barriers to movement among present-day populations. Even within a localized area (e.g., La-Rue Pine Hills Research Natural Area, southern Illinois), a mark-recapture study did not detect movement of Southern Cavefish among springs (Smith & Welch 1978). However, the presence of large numbers of young and adults on the surface during certain seasons implies a high likelihood that at least some migration among populations still occurs through surface dispersal.

Within springs, Karst Cavefish use underground and surface portions of the habitat. Adults are more commonly found closest to the springhead; however, young-of-the-year can occur ≤120 m from the springhead in swamp-like habitat (Adams et al. 2001). Few data are available on the chemical and physical limitations of species of *Forbesichthys*. They occur primarily in springs and caves that exhibit a fairly stable water temperature of about 13°C (Poulson & White 1969). A study of oxygen preference of Spring Cavefish indicated optimal dissolved oxygen concentrations of ≥6 ppm (Hill 1968). In a comparison of five springs (two with and three without Karst Cavefish) to determine suitable habitat, the two Karst Cavefish sites varied tremendously, and no physical or chemical factors emerged explaining the current density or distribution of Karst Cavefish (Sharp & Bybee 1982).



Figure 21.32. As the common name implies, the Karst Springfish, *Forbesichthys papilliferus*, inhabits springs and spring seeps. (upper) The Karst Springfish is consistently found under rocks and debris in Elm Spring run within Pine Hills Research Natural Area, Union County, Illinois (April 2016). (middle) The species also occurs in the heavily vegetated Pocahontas Spring in Coffee County, Tennessee (8 March 2013), and (lower) the clear waters of Ramsey Barn Spring in Warren County, Tennessee (16 January 2014) (upper courtesy of Jeff Stewart; lower and middle photographs by BRK).

#### Habitat: Northern Cavefish and Hoosier Cavefish

The Northern Cavefish, a stygobite, is an inhabitant of cool (8.0–17.3°C), cave streams that have mixed mud-rock substrates in shoals and mixed sand-silt substrates in pools (Poulson 1963, but assignment to Northern or Hoosier Cavefishes is enigmatic). Northern Cavefish >35 mm SL inhabited primarily rocky, flowing cave stream habitats, but individuals <35 mm SL were found primarily in low velocity, shallow habitat over a sand and mud substrate.

#### Habitat: Southern Cavefish and Eyeless Cavefish

Similar to habitat of the Northern Cavefish, Southern Cavefish inhabit cool (10.2–13.7°C), lentic cave waters but also occur in pools of streams at or near the water table (Poulson 1963). The Eyeless Cavefish inhabits cave streams, the outlets of springs, and underground lakes where they are “sometimes washed out of springs and other subterranean habitats after heavy rains” (Pflieger 1997:232). Noltie & Wicks (2001) strongly suggested Eyeless Cavefish occur at great depths in caves, and this species is only observed in atypical habitats near the surface where effluent springs conduct water from depth to surface or near-surface outlet points. In a laboratory study, this species clearly showed strong affinity for cobble-sized particles and the interstitial spaces between them with some evidence of rheotaxis (orientation to current) and preference for deeper portions of the water column (Schubert & Noltie 1995). Interestingly, this is the only Cavefish species that experiences gas-bubble disease, the equivalent of the bends in human scuba divers (Schubert et al. 1993). This is another piece of evidence arguing for the great depth at which *Typhlichthys* spp. might reside in some caves. *Typhlichthys* spp. demonstrate some rheotactic responses, but somewhat less so than the Ozark Cavefish.

#### Habitat: Ozark Cavefish

Ozark Cavefish are restricted to the Springfield Plateau section of the Ozark Highlands (Fig. 21.6), an area underlain with highly soluble limestone bedrocks that apparently favored the formation of extensive subsurface drainage ways (see diversity and distribution section). This fish apparently rarely, if ever, ventures into surface streams having been captured only in caves, wells, and the outlets of springs (Pflieger 1997). Ozark Cavefish are most common in small cave streams over a rocky, rubble substrate (Poulson 1963). They occur frequently in caves that harbor maternity colo-



nies of the endangered Gray Bat. The species is somewhat rheotactic and demonstrates an attraction to low flows.

### Habitat: Alabama Cavefish

Despite searches of caves in the area, Alabama Cavefish are still known from only one cave, Key Cave, Alabama (Fig. 21.7). Key Cave is a multilevel maze in Tusculumbia Limestone of Mississippian age (323–359 mya) with entrances in the north bank of the Tennessee River. A large summer maternal colony of Gray Bats frequents the cave. Guano areas are inundated with water seasonally and enrich the cave waters with nutrients. Water in the cave is usually clear to 4.6–6.1 m, and the fish occurs at this depth at a temperature near 15°C. Alabama Cavefish occur in narrow pools with the Southern Cavefish and at least four other species of stygobitic crustaceans (crayfishes, shrimps, amphipods, isopods) (Cooper & Kuehne 1974; Kuhajda & Mayden 2001).

### Diet: Swampfish

Swampfish are thought to be nocturnal feeders because of their increased nighttime activity compared with other co-occurring fishes (e.g., Grass Pickerel, *Esox americanus*;

Bluespotted Sunfish, *Enneacanthus gloriosus*) and the high percentage of empty stomachs during the day (53% of 299 individuals); however, no nighttime samples were taken (Ross & Rohde 2003). Although remains of 14 invertebrate taxa were observed in stomachs, the dominant food was amphipods (60% of specimens), which accounted for 41% of food by number (Table 21.3). Chironomids and cladocerans also contributed significantly to the diet. Large Swampfish consumed a greater percentage of amphipods compared with small Swampfish (<30 mm SL), which consumed chironomids and amphipods in about equal percentages in most seasons (Table 21.3). The number of taxa and percentage occurrence and abundance of organisms in stomachs were greatest in late winter to early spring, decreasing substantially in summer and autumn. Cannibalism was not observed (Ross & Rohde 2003).

### Diet: Karst Cavefish and Spring Cavefish

Although data are limited, in two studies diets of the two species of *Forbesichthys* differed dramatically. The stomach contents of adult Karst Cavefish from Pine Hills Research Natural Area, Illinois (n = 75), were dominated by amphipods (*Gammarus* sp.) (Weise 1957). In contrast, the

Table 21.3. Percentage frequency and number of major food items by season and size group for Swampfish, *Chologaster cornuta*. Fish were captured during daylight from a tributary of Black Swamp Creek, North Carolina, January 1977–January 1978. Unidentified remains could not be counted (data from Ross & Rohde 2003).

| Food Items                 | Winter        | Spring        | Summer        | Autumn        |
|----------------------------|---------------|---------------|---------------|---------------|
|                            | %Freq. (%No.) | %Freq. (%No.) | %Freq. (%No.) | %Freq. (%No.) |
| <b>≤29 mm SL</b>           |               |               |               |               |
| Cladocera                  | 11.8 (15.4)   | 7.7 (4.1)     |               |               |
| Ostracoda                  | 11.8 (17.9)   | 2.6 (2.7)     | 9.1 (10.0)    |               |
| Amphipoda                  | 47.1 (28.2)   | 38.5 (24.3)   | 27.3 (30.0)   | 42.9 (37.5)   |
| Misc. Crustacea            | 5.9 (2.6)     | 5.1 (6.8)     |               | 14.3          |
| Diptera                    | 41.2 (28.2)   | 33.3 (40.5)   | 27.3 (40.0)   | 28.6 (25.0)   |
| Misc. Insecta              | 11.8 (5.1)    | 28.2 (18.9)   | 9.1 (5.0)     | 14.3 (12.5)   |
| Unident. items + nematodes |               | 5.1           | 27.3          | 28.6          |
| No. with food              | 17            | 39            | 11            | 7             |
| No. empty                  | 11            | 48            | 22            | 18            |
| <b>≥30 mm SL</b>           |               |               |               |               |
| Cladocera                  | 8.3 (4.4)     | 13.9 (15.5)   |               |               |
| Ostracoda                  | 4.2 (2.2)     | 2.8 (1.7)     |               |               |
| Amphipoda                  | 87.5 (64.4)   | 77.8 (58.6)   |               | 50.0 (50.0)   |
| Misc. Crustacea            | 12.5 (8.9)    |               |               |               |
| Diptera                    | 16.7 (13.3)   | 11.1 (12.1)   |               | 50.0 (50.0)   |
| Misc. Insecta              | 12.5 (6.7)    | 13.9 (8.6)    |               |               |
| Unident. items + nematodes | 4.2           | 5.6           |               |               |
| No. with food              | 24            | 36            |               | 2             |
| No. empty                  | 8             | 39            |               | 7             |

primary food item in the stomachs of young-of-the-year and adult Spring Cavefish in Rich Pond, Kentucky, was chironomids with copepods, oligochaetes, nematodes, and ostracods also fairly common. Adults captured in the cave also contained relatively high frequencies and total weight of young-of-the-year Spring Cavefish in their stomachs (Fig. 21.33). Over 99% of young-of-the-year Spring Cavefish in the cave had empty stomachs, but only 13% captured in surface habitats had empty stomachs, indicating that the downstream surface habitat is critical for survival of young (Hill 1969a). Whether variation in diet of the two species between the two sites reflects a contrast in food choice between species, simple opportunistic feeding, or a difference in availability of food items is unclear.

#### Diet: Southern Cavefish

Southern Cavefish diet comprises primarily (60–90% total volume) trichopterans, tendipedids, cladocerans, isopods, crayfishes, and copepods (Poulson 1963). In the laboratory, prey availability and consumption were uncorrelated (Schubert & Noltie 1995). In Sloans Valley Cave, Kentucky, eyed amphipods and an isopod formed part of the diet of the Southern Cavefish captured in November (Cooper & Beiter 1972).

#### Diet: Northern Cavefish

Northern Cavefish >45 mm SL fed primarily on the isopod *Asellus stygius* and the amphipod *Cragonyx gracilis* and to a lesser extent on the Mammoth Cave Crayfish, *Orconectes pelucidus*, and small Northern Cavefish. Those <45 mm SL principally ate copepods (Poulson 1963). A small salamander was regurgitated from a living specimen taken near Fort Knox, Kentucky (Clay 1975), and Putnam (1872) recorded one instance of predation on an eyed fish in Mammoth Cave. The Northern Cavefish forages along cave walls and ledges, on the substrate, and sometimes in mid-water. When feeding, the Northern Cavefish moves slower than the Spring Cavefish or the Southern Cavefish. It is, however, active 24-h a day and swims a greater distance before turning than the Spring Cavefish, thus sampling a wider area. Also, a well-developed neuromast system allows it to locate prey at considerable distances (20–45 mm) from its body (Poulson 1963).

#### Diet: Ozark Cavefish

The Ozark Cavefish diet is dominated by copepods (70–90% volume) with additional items, including small sala-

manders, crayfishes, isopods, amphipods, and young Ozark Cavefish (Poulson 1963).

#### Diet: Alabama Cavefish

No information on diet is available for the Alabama Cavefish. No data on stomach contents have been taken for the nine available museum specimens.

#### Age and Growth

Epigeal Swampfish have free-swimming fry with yolk absorbed by 0.6 months (about 4.5 mm SL) and the stygobites *Forbesichthys* spp. at one month (6.0 mm SL). The stygobitic species apparently grow slower and are free swimming by 2–6 months (8–8.5 mm SL in *Amblyopsis*). The first scales are formed by 1.5 months (7–18 mm SL) in Swampfish and 3–13 months (9–17 mm SL) in the stygobites. Complete migration of the vent takes 3–5 months (15–32 mm SL) in Swampfish and *Forbesichthys* spp., but 12–20 months (12–28 mm SL) in *Amblyopsis* spp. and Ozark Cavefish. *Typhlichthys* spp. apparently develop faster than the other stygobites because the vent has fully migrated by five months (10–23 mm SL). The first annulus forms at 7–10 months (21–48 mm SL) in Swampfish, *Forbesichthys* spp., and *Typhlichthys* spp. but takes 19–21 months (16–26 mm SL) in the other stygobites. First reproduction may be delayed in the stygobitic taxa to  $\geq 2$  years (about 40 mm SL) but occurs in the first year for Swampfish and *Forbesichthys* spp. (Poulson 1963; Niemiller and Poulson 2010; Table 21.2).

Fish aging is historically biased toward the reading of annual marks on scales and a few other structures (e.g., opercula) or the use of length-frequency histograms (for accuracy the latter requires huge sample sizes). Fisheries professionals agree that the ages of many fish species aged by scales in North America are underestimated (e.g., Campana 2001; Maceina et al. 2007). Estimates of ages made in the earlier literature (Poulson 1963) are misleading and underestimate the ages of nearly all species of Cavefishes.

Swampfish in a North Carolina ditch were estimated to live 26 months. In that habitat, a majority of their growth occurred in the first year (from means of 14–23 mm SL in eight months). Growth declined during the second year (means of 26–33 mm SL in 11 months; maximum about 37 mm SL) (Ross & Rohde 2003). Reduced growth rates also were correlated with summer season, possibly related to a decrease in food consumption.

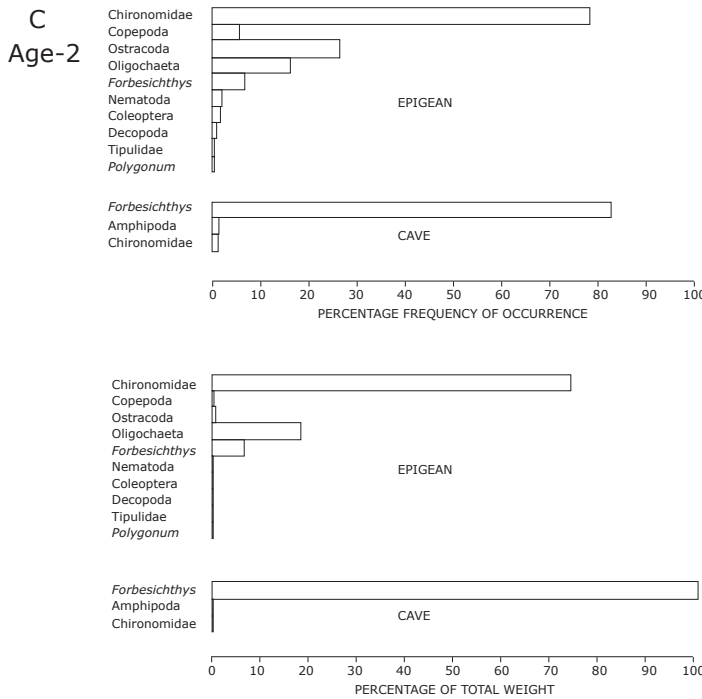
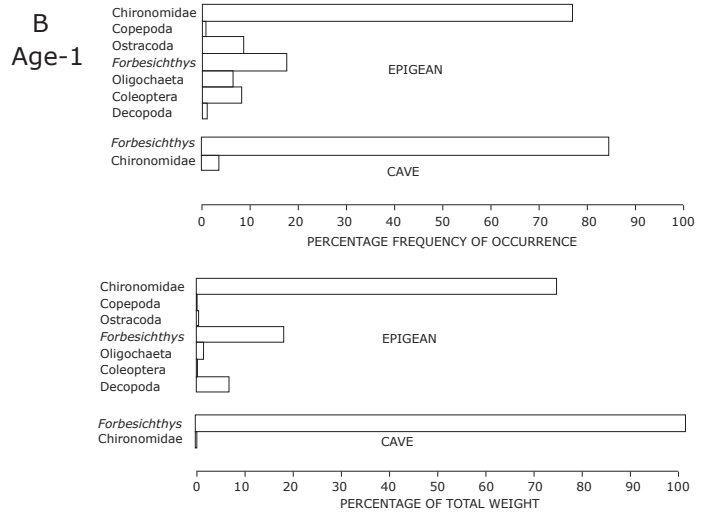
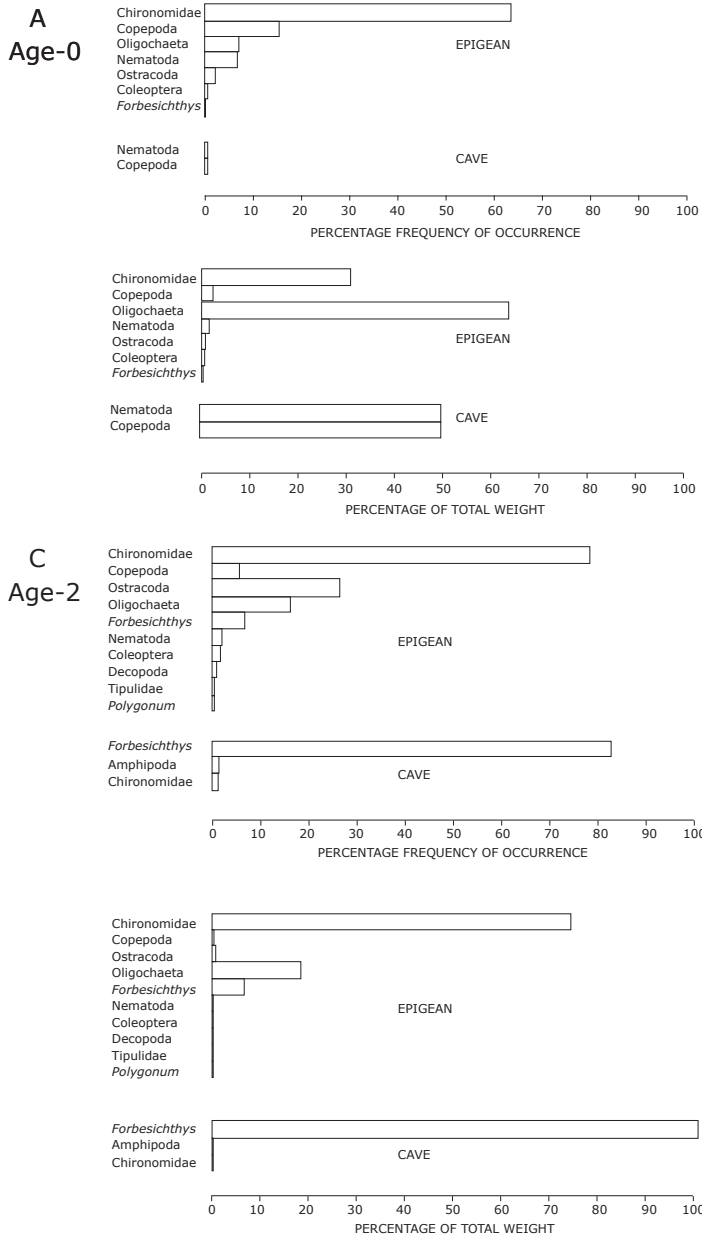


Figure 21.33. Percentage frequency of occurrence (above) and percentages of the total weight of food (below) of the principal food items in stomachs of epigean and hypogean Spring Cavefish, *Forbesichthys agassizii*, in three age groups: (A) age-0; (B) age-1; and (C) age-2 (n = 3,590). Note the high total weight and hence high cannibalism of Spring Cavefish in the cave diet of age-1 and age-2 individuals. In addition, of 1,097 age-0 individuals examined from the surface only 13% had empty stomachs. In contrast, 99% of 1,920 age-0 individuals in the cave had empty stomachs (redrawn from Hill 1969a).

Longevity estimates for *Forbesichthys* spp. vary among studies. Poulson (1963) estimated a maximum age of 28 months (38–61 mm SL); others suggested a maximum life span of three years (based primarily on a mark-recapture study at La Rue-Pine Hills Research Natural Area, southern Illinois) and attainment of 70 mm SL (Smith & Welch 1978; Hill 1969b). Overall growth was estimated to be 10–20 mm/year in southern Illinois, but growth at different ages is unknown (Smith & Welch 1978).

A maximum age of 50 months (45–62 mm SL) was estimated (Poulson 1963) for Southern Cavefish, but individuals lived for 10 years in aquaria (Noltie & Wicks 2001). Considering the number of cryptic lineages of *Typhlichthys*,

much is still to be learned about longevity and other aspects of natural history. Annual growth rates are unknown.

Hoosier Cavefish in southern Indiana was subject of considerable study by Eigenmann (1909), and a continuing study by William D. Pearson, University of Louisville, who conducted mark-recapture studies for ≥10 years in Donaldson Cave. Poulson (1963) estimated a maximum age of 73–84 months (76–85 mm SL) for *A. spelaea*. In contrast, preliminary estimates of age based on mark-recapture studies clearly demonstrated that Hoosier Cavefish live ≥10+ years in Eigenmann’s original study cave in southern Indiana (W. D. Pearson pers. comm.). However, annual growth rates are unknown.

Estimates of longevity of Ozark Cavefish are also in some disagreement. Scale-aged individuals of Ozark Cavefish were estimated to reach 57 months (about six years; Poulson 1963). In a mark-recapture study ( $n = 147$  fish) in Logan Cave, Arkansas, maximum persistence of tagged fish was 28 months, suggesting a maximum life span of 4–5 years (Brown & Johnson 2001). Growth averaged 0.6 mm/month with a maximum recorded growth of 6 mm/month and a maximum size of 65 mm TL. Most fish gained length during the April to October period when a maternity colony of Gray Bats occupied (and guano enriched) the cave. Interestingly, half of all tagged fish disappeared in  $\leq 3$  months from the study site. Death was the most likely explanation for loss of tagged fish, including those that emigrated out of the cave, although tag loss, hiding, and emigration almost certainly occurred.

At least three size classes of the extremely rare Alabama Cavefish occur in its only known locality, Key Cave, Alabama (Kuhajda & Mayden 2001). The largest size class was about 40–50 mm SL, the middle size class contained individuals around 30 mm SL, and the smallest individuals were 12–20 mm SL. The smallest individuals occurred on 2 February (12 and 15 mm SL) and on 18 November (15 mm SL). Specimens in the 20-mm range were seen on 25 January. The holotype, a female, captured 24 May, was 58.3 mm SL and was considered to be between 7 and 8 years old (Cooper & Kuehne 1974). Age data, however, are unvalidated. If longevity is inherited and *Typhlichthys* is the sister taxon to *Speoplatyrhinus* (Fig. 21.13) then its maximum age may be near the estimate made for Alabama Cavefish.

In sum, contemporary mark-and-recapture studies to measure growth have advanced our understanding of the ages obtained by Cavefishes (reviewed by Niemiller & Poulson 2010 and references cited in this subsection). Estimates of ages in Swampfish (1–2 years) and *Forbesichthys* spp. (2–3 years) obtained by scales or length frequency classes agree with mark-recapture estimates. For all the stygobites, mark-recapture efforts indicate scales or length frequency underestimates age by 2–4 times. Hence, the potential lifespans are as long as 20–40 years for *A. spelaea*, 8–20 years for *T. rosae*, and 8–32 years for the other stygobites.

### Relative Abundance

The Swampfish, Spring Cavefish, and Karst Cavefish are common locally; the other six strictly hypogean species

are generally uncommon to extremely rare. In fact, few people have seen the Alabama Cavefish alive. In contrast, the Karst Cavefish can be seen at particular spring outlets anytime of the year or the day-night cycle within walking distance of a small parking space in the LaRue Pine Hills Research Natural Area, Union County, southwestern Illinois.

### Demography

Population estimates for Cavefishes are rare in the literature. Many of the estimates are based on census data with only a few examples of mark-recapture studies. Densities of Swampfish were estimated as 1.68 individuals/m<sup>2</sup> in a North Carolina ditch (Ross & Rohde 2003). Estimates for *Forbesichthys* spp. tend to be a little higher, ranging from 4.16 individuals/m<sup>2</sup> in Illinois to 4.34 individuals/m<sup>2</sup> in Missouri (Weise 1957; Adams et al. unpubl.). Densities for *Forbesichthys* spp. are highly variable in surface habitat and are highest during the winter (2.27 individuals/m<sup>2</sup>) and spring (4.34 individuals/m<sup>2</sup>) when young-of-the-year drift to the surface, and lowest during summer (0.44 individuals/m<sup>2</sup>) and autumn (0.5 individuals/m<sup>2</sup>) when most individuals move underground (Poulson 1963; Hill 1966; GLA pers. obs.).

In contrast to the Swampfish, Springfish, and Karst Cavefish, cave obligate members of the family tend to have lower densities. Southern Cavefish had densities ranging from 0.02 individuals/m<sup>2</sup> in Blowing Springs Cave, Tennessee, to 0.08 individuals/m<sup>2</sup> in Shelta Cave, Alabama (Niemiller & Poulson 2010) based on maximum observed using census data. Population estimates among Hoosier Cavefish localities are highly variable, and a single census method may dramatically underestimate actual population size. Pearson & Boston (1995) compared mark-recapture data to census data and found the census data only yielded 53% of the population size estimated by mark-recapture. The two caves (Donaldson and Websters Caves) for which mark-recapture data were compiled had estimated population sizes of  $187 \pm 38$  and  $154 \pm 38$ , respectively. These, however, were the only two caves for which estimates are available. Pearson & Boston (1995) indicated there are  $\geq 2,159$  Hoosier Cavefish in Indiana and that the actual number is likely higher, perhaps  $\leq 21,590$ . For Kentucky they estimated the population of Northern Cavefish was  $\geq 5,602$  but potentially  $\leq 56,000$  individuals.

Data for Ozark Cavefish are most complete in Logan Cave, Arkansas, where two mark-recapture studies were

conducted. Counts of individuals from 24 trips over two years ranged from 11 to 29 individuals; seasonal trends in abundance were not apparent (Brown & Johnson 2001). These data suggest comparing point in time data across years may give a false sense of potential long-term trends. In addition, long-term data are often lacking.

Alabama Cavefish abundance data dates to the 1960s and includes a total of 36 trips into Key Cave (Kuhajda & Mayden 2001). Individuals were seen on most trips into the cave, and numbers observed appeared to be fairly stable over time (ranging from 0.8 to 1.0 individuals/pool).

### Competition and Predation

Few fish species are competitors with or predators of Cavefishes in the cave environment. The Banded Sculpin (*Cottus carolinae*) may be the only exception to this, occurring in caves with the Karst Cavefish in Illinois and possibly competing with them for food. Central Mudminnows (*Umbra limi*) and adult Karst Cavefish prey on young Karst Cavefish (Gunning & Lewis 1955). Pirate Perch feed on Swampfish (Shepherd & Huish 1978), implying a high probability they would also consume Spring Cavefish. Besides the Pirate Perch, Swampfish occur with a diverse community of fishes, including many other piscivores that might prey on Swampfishes (e.g., Grass Pickerel, *Esox americanus*; Yellow Bullhead, *Ameiurus natalis*; several Sunfishes, Centrarchidae) (Ross & Rohde 2003). Although not documented predators of Spring Cavefish, Grass Pickerel likely eat them. Young salamanders (*Eurycea* spp.) appear in the stream at the same time as young Spring Cavefish and may serve as competitors for food at this young age. Adult Spring Cavefish are highly cannibalistic in Kentucky, particularly in the cave habitat where food is a principal limiting factor (Fig. 21.33). Cannibalism probably serves as a means of population control (Hill 1969a).

The Northern Cavefish is frequently cannibalistic; no other predators are known. In large cave systems where >1 species of Cavefish occurs (e.g., Mammoth Cave, Kentucky) they might possibly eat each other, especially the young-of-the-year. Filial cannibalism is highly possible, but DNA parentage data are unavailable.

### Parasites

Cavefishes are specialized hosts to tapeworms and a trematode. The tapeworm, *Proteocephalus chologasteri*, which inhabits the small intestine and pyloric caecae, was described

from Spring Cavefish from Rich Pond, Kentucky (Whittaker & Hill 1968). Whittaker & Zober (1978) described another tapeworm, *Proteocephalus poulsoni*, from *Amblyopsis spelaea*. A monogenetic trematode, *Gyrodactylus chologastris* was described from Swampfish and Spring Cavefish (Mizelle et al. 1969); the trematode occurs on the external surface of the fish. The population of Karst Cavefish in southern Illinois harbors an occasional external leech (Smith & Welch 1978). When Weise (1957) was conducting his studies, just over 70% of Karst Cavefish contained cestodes and other internal parasites (George Garoian pers. comm.).

The acanthocephalan, *Neoechinorhynchus cylindratus* parasitized four of 50 specimens of the Northern Cavefish from Under the Road Cave, Breckenridge County, Kentucky (Nickol & Whittaker 1978), a parasite that occurs in nature in a range of fish taxa (Hoffman 1999). This parasite was found in the intestine but was immature.

## CONSERVATION

Globally, 26 families of fishes are adapted to living in caves, and many are of conservation concern. For example, in a worldwide review, 63 of 104 species of subterranean fishes were recognized as imperiled by the International Union for the Conservation of Nature (IUCN) (Proudlove 2006, pers. comm.). The North American Cavefishes are no exception. Specific habitat requirements, generally low population densities, low reproductive potential among the stygobitic species, and the limited, often highly localized ranges of Cavefishes makes most of the group extremely vulnerable.

### Conservation Rankings

Eight of nine Cavefishes are considered imperiled by one or more conservation agencies or organizations (Table 21.4). The Alabama Cavefish is protected as Endangered and the Ozark Cavefish as Threatened under the U.S. Endangered Species Act; both species of *Amblyopsis* are Under Review (USFWS 2016c). Citing habitat destruction and restricted range as primary causes, the American Fisheries Society considered the Alabama Cavefish as Endangered, the Ozark Cavefish and both species of *Amblyopsis* as Threatened, and both species of *Forbesichthys* and *Typhlichthys* as Vulnerable (Jelks et al. 2008). The Southeastern Fishes Council had earlier assigned similar status rankings to these species, but *Forbesichthys* at that time was assessed as Currently Stable (Warren et al. 2000).

The IUCN considers the Alabama Cavefish as Critically Endangered and the Ozark Cavefish and both species of *Amblyopsis* and *Typhlichthys* as Near Threatened (IUCN 2016). States recognize five of the species as imperiled within their boundaries (Ozark Cavefish, Arkansas, Missouri, and Oklahoma; Hoosier Cavefish and Northern Cavefish, Indiana, Kentucky; *Typhlichthys* spp., Alabama, Georgia, Kentucky; Alabama Cavefish, Alabama; Karst Cavefish, Missouri) (Niemiller & Poulson 2010).

Within *Typhlichthys* a number of distinct genetic lineages were assessed for conservation rankings following IUCN criteria (Niemiller et al. 2013b). Of the 10 lineages considered (including the Eyeless Cavefish and Southern Cavefish *sensu stricto*), one was Critically Endangered (lineage M), four Endangered (C, D, E, G), one Near Threatened (Eyeless Cavefish), and four Vulnerable (A, B, F, and Southern Cavefish *sensu stricto*) (Fig. 21.24).

## Threats

Direct human intrusion in cave habitats has played some role in Cavefish imperilment, but the magnitude of that role is diminished by human-induced influences in the watershed and associated recharge area. Clay (1975) claimed that youthful spelunkers have reduced numbers of Cavefishes, apparently by taking them home where they quickly perished. Noltie & Wicks (2001) made the point that spelunker traffic, closure of cave mouths, and human removal of Cavefishes are peripheral impacts, and although not negligible, their importance pales in comparison with impacts that occur at the recharge-area scale. Managers, and the Cavefishes themselves, would be better served by focusing attention on threats to groundwater quality, through-ground infiltration of allochthonous nutrients and

silt, and the physical integrity of the karst itself. “It is to these ecosystem level changes that Cavefishes are potentially most susceptible” (Noltie & Wicks 2001:191).

Perhaps the greatest threat is pollution of the underground water system through water contamination. In Karst regions surface runoff passes swiftly into the subterranean channels with little or no adsorption, filtration, or degradation (White 2002; Butscher & Huggenberger 2009). The intensive use of agricultural chemicals adds to the other pollution hazards and creates dangers insurmountable by the unique amblyopsids (Clay 1975). In a southeastern Missouri cave stream system, 20 compounds originated primarily from agricultural sources, including two organochlorine insecticides, dieldrin and heptachlor epoxide, which were found at levels exceeding U.S. EPA criteria for the protection of aquatic life. Presence of legacy-use chemicals in cave streams may be a reflection of leaching of chemicals from sinkholes being used as trashcans for improperly discarding pesticides and herbicides (Fox et al. 2010).

In summary, researchers have identified several broad factors likely to limit or cause decline within Cavefish populations. Primary among these is the destruction or degradation of habitat from poor land-use practices (e.g., sedimentation) and alteration of surface drainage or hydrological manipulations. Others include overexploitation; disturbance by humans, either indirect or direct; introduced non-native species; and loss of genetic variation (Pearson & Boston 1995; Proudlove 2001, 2006).

## Case Studies

The Alabama Cavefish, federally Endangered, occupies only one cave (Key Cave, Alabama) and perhaps <100 in-

Table 21.4. Conservation status of Cavefishes, Amblyopsidae. E, Endangered; CE, Critically Endangered; CI, Critically Imperiled; T, Threatened; NT, Near Threatened; V, Vulnerable; CS, Currently Stable; S, Secure; AS, Apparently Secure; UR, Under Review. USFWS, U.S. Fish and Wildlife Service (USFWS 2016ab); AFS, American Fisheries Society, Jelks et al. (2008); SFC, Southeastern Fishes Council, Warren et al. (2000); IUCN, International Union for Conservation of Nature, IUCN (2016); NS, NatureServe (2016).

| Species  | USFWS | AFS | SFC | IUCN | NS |
|--|-------|-----|-----|------|----|
| <i>Amblyopsis spelaea</i> , Northern Cavefish        | UR    | T   | T   | NT   | V  |
| <i>Amblyopsis hoosieri</i> , Hoosier Cavefish        | UR    | T   | T   | NT   | V  |
| <i>Chologaster cornuta</i> , Swampfish               | —     | —   | CS  | —    | S  |
| <i>Forbesichthys agassizii</i> , Spring Cavefish     | —     | V   | CS  | —    | AS |
| <i>Forbesichthys papilliferus</i> , Karst Cavefish   | —     | V   | CS  | —    | AS |
| <i>Speoplatyrhinus poulsoni</i> , Alabama Cavefish   | E     | E   | E   | CE   | CI |
| <i>Troglichthys rosae</i> , Ozark Cavefish           | T     | T   | T   | NT   | V  |
| <i>Typhlichthys eigenmanni</i> , Eyeless Cavefish    | —     | V   | V   | NT   | AS |
| <i>Typhlichthys subterraneus</i> , Southern Cavefish | —     | V   | V   | NT   | AS |



Figure 21.34. (upper) Logan Cave in the Ozark Mountains of northwest Arkansas, now in a wildlife refuge and closed to the public, supports the federally protected, stygobitic Ozark Cavefish, *Troglichthys rosae*, and the federally protected endemic, stygobitic Benton County Cave Crayfish, *Cambarus aculabrum*. The cave is rated as the highest quality cave habitat in the Ozark Mountains. The spring, which flows the length of the cave, delivers 18,927 m<sup>3</sup> of crystal-clear water daily to Osage Creek (USFWS 2016ab). (middle) Mammoth Cave, by far the longest known cave system in the world (627 surveyed km, 1,600 km potential), supports three imperiled species of Cavefishes, Amblyopsidae: Southern Cavefish, *Typhlichthys subterraneus*; Northern Cavefish, *Amblyopsis spelaea*; and Spring Cavefish, *Forbesichthys agassizii* (Burr & Warren 1986; NPS 2016). Unlike many cave systems, the integrated set of subterranean drainage basins of Mammoth Cave, which covers >1,050 km<sup>2</sup>, is protected in part by having Mammoth Cave National Park at its core, a 214 km<sup>2</sup> area (NPS 2016). (lower) Tourists on a boat tour being conducted in Twin Caves, Spring Mill State Park, Indiana, to catch a glimpse of the imperiled Hoosier Cavefish, *Amblyopsis hoosieri*, and other cave fauna (photographs are in the public domain and courtesy of U.S. Fish and Wildlife Service, upper, National Park Service, middle, and Sjh123 at English Wikipedia, lower).

dividuals exist;  $\leq 10$  individuals (grand mean about 4 individuals/visit) were seen on any given single visit to the cave (Romero 1998; Kuhajda & Mayden 2001). The species is now ostensibly protected within a 428-ha recharge area in the Key Cave National Wildlife Refuge (Kuhajda & Mayden 2001), but the flow in the cave was drastically modified by impoundment of Pickwick Reservoir on the Tennessee River and the recharge area was threatened by construction of an industrial park (Kuhajda 2004). The Alabama Cavefish may be the rarest vertebrate in North America and one of the rarest in the world.

The Ozark Cavefish, federally Threatened, is highly localized in six counties in Missouri and in one county each in adjacent Arkansas and Oklahoma (Fig. 21.6). In

Missouri, the Ozark Cavefish occurred historically at 83 sites but in most of these the species has not been observed in  $\geq 20$  years. Many of these caves undoubtedly are interconnected and the actual number of separate locations may be much  $< 82$  (Graening et al. 2010). Conversely, caves are often poorly accessible, and certainly, other unexplored caves occur in the region that may support Ozark Cavefish. All Missouri populations are apparently small, six sites with  $\leq 3$  individuals observed. Two caves in northwest Arkansas, Cave Springs Cave and Logan Cave (Fig. 21.34), contain  $> 90\%$  of individuals. Once a marked Ozark Cavefish entered Logan Cave, it was reobserved in that cave for a relatively short time (about 3–7 months) before disappearing from samples, suggesting individuals

do not migrate upstream back into the aquifer but are lost to the surface. Logan Cave, now part of the Logan Cave National Wildlife Refuge in northwestern Arkansas (Fig. 21.34), supports  $\geq 100$ –150 individuals, and its aquifer and the population has remained relatively stable for several years (Brown & Johnson 2001). In a range-wide review, Graening et al. (2010) confirmed Ozark Cavefish presence in 32 caves, a decline of about 50% compared with 1990. The number of individuals observed across all sites (222), however, stayed fairly consistent, which they indicated may be due to an increase in population size within current populations. Variation in census techniques may have varied over time.

The ranges of the Hoosier and Northern Cavefishes combined has not changed appreciably since the latter was described in 1842 (the description included the range of the Hoosier Cavefish). Reliable records for Hoosier Cavefish cover 44 sites in southern Indiana, and for the Northern Cavefish, 21 cave locations in Kentucky since 1989 and in numerous other caves since the 1950s. Relatively large populations ( $\geq 350$  individuals) of the two species occur in two centers in each state: Blue Spring Cave and Spring Mill State Park caves, Indiana (Hoosier Cavefish) and Sinking Creek caves and Mammoth Cave, Kentucky (Northern Cavefish) (Pearson & Boston 1995; Fig. 21.34). Depending on reliability of actual counts and extrapolation of data, 5,600–56,000 individuals may occur over the two species entire range in Kentucky and Indiana. Human influences with the most adverse effects include sedimentation (e.g., from quarrying, agricultural practices), toxic wastes (e.g., sinkhole dumping, industrial effluents), alteration of surface drainages (impoundments that inundate cave passages), direct destruction of habitat (e.g., water withdrawal, sealing of openings), and over collecting (e.g., scientific and curio). A large and stable population still exists in the original cave (Spring Mill) studied by Eigenmann (1909), and where ongoing movement and longevity studies are being conducted (GLA pers. obs.; W. D. Pearson pers. comm.).

The genera *Forbesichthys* and *Chologaster* occur over the same geographic areas from which they are known historically. In some areas, large numbers of both species were captured or seen over the past 60 or so years, and presently, no compelling reason exists to consider either species for federal protection. A 2012 reconnaissance of the original study site (Rich Pond, Kentucky) for Loren G. Hill's work on Spring Cavefish, is now a large-scale aquaculture facility, and no longer supports a viable population of the species (BMB pers. obs.).

The genus *Typhlichthys* has a wide range in six ecoregions and six states (Fig. 21.24) with >242 documented populations (many identification and location errors in the literature now are corrected; that is, the species does not occur in Oklahoma, Kansas, or Indiana). In Missouri, for example, a population in one cave in Wayne County was estimated conservatively to have  $\leq 90$  individuals; this area is protected ostensibly by the USDA Forest Service (Pflieger 1997). After a fertilizer accident that leaked ammonium nitrate into the aquifer of the Maramec Spring, Phelps County, Missouri, cave animals emerged from the spring opening, and thousands of Eyeless Cavefish and other cave animals were discovered occupying the aquifer. This genus is a complex of distinct genetic lineages, likely representing several cryptic species (see genetics section and conservation rankings, this section; Fig. 21.24), but if conceived as only two species, *Typhlichthys* is more common than either species of *Amblyopsis*.

## COMMERCIAL IMPORTANCE

### Commercial Cave Development

Development of caves for commercial use can potentially impact Cavefishes due to increased light, nutrient input, and direct disturbance by foot traffic. During surveys for *Amblyopsis* spp. at least four caves inhabited by these species were also used for commercial tours, including two in Indiana (Fig. 21.34) and two in Kentucky (Pearson & Boston 1995).

### Aquarium-keeping

With required permits from state agencies, the non-protected species of Cavefishes can be kept in aquaria under proper conditions (e.g., water quality, light-dark regime, hiding places, appropriate food). Sometimes aquarium-keeping leads to discoveries of breeding behavior, feeding behavior, interactions among adults, and other sorts of natural history still unknown for amblyopsids. Clay (1975) noted that Cavefishes were captured and sold to biological supply houses, perhaps as curiosities.

### Limits to Scientific Inquiry

Cavefishes are not legally sold in the pet trade, and they do not reach a size or population density adequate for take as food or bait. The habitats of the hypogean species are difficult to access, often gated and locked, and federal and



state laws provide some protection from collecting for curiosity. Species protected as Endangered or Threatened by the U.S. Fish and Wildlife Service are protected under the Lacey Act, which makes it unlawful to import, export, transport, sell, receive, acquire, or purchase any wild animal subject to the Act (alive or dead, including parts, products, eggs, or offspring). Because of their rarity, we know of no examples in which Cavefishes are traded in commerce. All the species are extremely engaging research animals, but ethical, responsible scientists acquire proper permits before capturing and holding live Cavefishes in the laboratory for study.

## LITERATURE GUIDE

The unstinting field and laboratory efforts of Carl H. Eigenmann (Fig. 21.35), then at Indiana University, sparked years of research on cave vertebrates, especially Cavefishes, and culminated in his major work on the group (Eigenmann 1909). His election to the National Academy of Sciences is a worthy testament to his cutting-edge techniques and data analysis in the early 20th century. Anyone intrigued by Cavefishes and the history of science will be compelled to peruse this captivating volume. Much of the ecological work summarized here is based largely on the studies of Thomas L. Poulson (Poulson 1963, 1964, 1985, 2001ab; Poulson & White 1969; Niemiller & Poulson 2010), who completed his doctoral dissertation in 1960 on Cavefishes while attending the University of Michigan, Ann Arbor. Loren G. Hill conducted intensive studies of the life history of Spring Cavefish (e.g., Hill 1968, 1969b, 1971) and earned his doctoral degree in 1966 from the University of Louisville. Later studies have focused on the biodiversity, evolution, and conservation of Cavefishes, including numer-



Figure 21.35. Carl H. Eigenmann (1863–1927), a groundbreaking researcher and pioneer of the natural history and biology of Cavefishes, Amblyopsidae, from a photograph taken in 1915 (modified from Plate 37 in the *Annals of the Carnegie Museum* Volume 17, 1927).

ous papers by Matthew L. Niemiller and his associates (e.g., Niemiller et al. 2012, 2013bde and other papers cited herein). We encourage others to peruse these papers for a more complete understanding of what is presently known (and not known) on North America's unique family of Cavefishes.

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